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Reproductive Ecology of Flowering Plants: Patterns and Processes



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Reproductive Ecology of Flowering Plants: Patterns and Processes

Rajesh Tandon • K. R. Shivanna •
Monika Koul
Editors

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Springer

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*Dedicated to
Professor H.Y. Mohan Ram*

Foreword

Higher plant reproduction is important as it sustains life on this planet. All cereals, millets, pulses, nuts, and oil seeds are products of plant reproduction. It is, therefore, no wonder that constant human endeavor has gone into understanding different aspects of flowering plant reproduction through field and laboratory experimentation. Plant reproductive ecology is comparatively a younger field. Yet, on account of its importance, it has attracted wide attention of biologists pursuing diverse fields.

Successful accomplishment of plant reproduction depends heavily upon compatibility of plants with biotic and abiotic components of the environment they live in. The importance has increased manifold following changes caused in environment by anthropogenic pressures.

A lot of information on reproductive ecology has been generated in recent years. For want of a journal or book solely devoted to this subject, it is scattered and, therefore, not handy to researchers pursuing this aspect of plant reproduction. This volume will fill this long-felt need.

Edited by three eminent biologists who have themselves contributed richly to unravel the mysteries of plant reproduction, the book comprises 16 chapters. Each chapter is authored by well-grounded author/authors in the subject.

The book makes for an absorbing read, as it presents systematic and lucid account of what reproductive biology is all about and in what way plant reproduction is linked with ecology. Students of botany and evolutionary biology will find latest information on floral symmetry, nectary, pistil, and secondary pollen presentation in the book. Plant breeders and crop geneticists will be enriched by Chaps. 8, 11, 12, 13, and 15. Chapters 2, 14, and 16 are of wide interest, focusing on the impact of climate change, the availability of pollinators and seed-dispersing birds, and the accomplishment of plant reproduction. As it is, the book will benefit a wide spectrum of biologists.

The book is well written, with suitable illustrations, wherever required, to help the readers. It will also help develop greater interest for plant reproductive ecology among biologists.

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January 3, 2020

A. K. Koul

Preface

Plant reproductive ecology has emerged as one of the most versatile and important fields of plant sciences. It answers two basic questions relevant to the survival of plant species: First, how do plants reproduce to sustain their populations? And second, how do plants strategize to escape reproductive failure? Considering the enormous diversity of plant species and their habitats, significant amount of variation is expected in the ecological processes that facilitate their reproductive success. Furthermore, the scales at which the processes become evolutionarily tenable also vary over successive generations of plants. Over the years, these aspects have been explored and understood at different levels of organization to answer specific questions. In recent years, the progress of the subject has been stimulated by growing interdisciplinarity to address the key global issues of ensuring future food security and conservation of biodiversity in the light of human-induced environmental changes, particularly climate change.

This is an area that has attracted a large number of laboratories around the world, and enormous new knowledge is being generated. The vast information generated in the subject is mostly scattered among journals. Though there are books by experts on the subject focusing on flowering plants, they largely deal with specialized areas of reproductive biology. The need for an updated, comprehensive book that does not represent conventional presentation of information has been felt time and again by reproductive ecologists. Although the information in this discipline is too vast to compile into a single volume, this book tries to highlight some of the fundamental processes and patterns linked to plant reproduction. The focus has been to synthesize and use the information that is available for greater understanding and interpretation of the ecological processes governing reproduction.

Reproductive ecological database broadly includes the details of flowering phenology, floral biology, pollination mechanism, pollen-pistil interaction, breeding system, and natural recruitment. Chapter 1, authored by the editors, is an overview on these key aspects and some of the noticeable dependencies associated with them. However, variations may arise depending on species. Chapter 2, authored by Saxena and Rao, highlights the potential of plant phenophases in understanding global climate change. After providing a conceptual background on the topic, it also highlights the key gaps in the knowledge and illustrates some pertinent examples on the issue.

Chapters 3 and 4, authored by K.R. Shivanna, briefly highlight the structural details of the pistil including that of the embryo sac in relation to its function. Besides the structural diversity of stigma, style, and ovary, these chapters also elaborate upon physiological and biochemical features that govern the dynamics during the pollen-pistil interaction, and provide information on extracellular substances that play a role in intercellular interactions at various levels prior to and during fertilization.

Pollination is an important event that decides the generation of a progeny. It is facilitated by both biotic and abiotic factors. Enormous literature has accumulated on various facets of pollination over the years. As is becoming clear by both field and laboratory studies, plant-pollinator interactions are governed and mediated by various cues. Chapter 5, authored by Sashidhran and Venkatesan, updates on current knowledge about floral volatiles (olfactory cues) as a functional trait for plant reproduction. It also discusses in detail an account of diverse functions of floral volatiles, their chemical nature and biosynthesis including the regulation of their emission by biotic and abiotic factors.

Chapter 6, contributed by Phukela et al., gives an overview of the distribution of floral nectaries along with a brief account on the genetic switches that regulate nectary development among flowering plants. By mapping the distribution of nectaries on angiosperm phylogeny, the authors trace the evolutionary history of nectaries across angiosperm lineages and discuss the role of nectary as a key functional trait in pollination.

Chapter 7, by R. Geeta and Eapsa Berry, provides a stimulating account on floral symmetry, the facet that is variable in angiosperms as polysymmetrical, disymmetrical, and monosymmetrical forms. The authors discuss reasons behind the variation and role played by ecological interactions of flowers with biota, especially with pollinators, which may exert selection to favor one or the other symmetry state. On the basis of phylogenetic analysis, the authors also look at the evolution of symmetry and how it may get compromised in plants.

Resource allocation and optimization strategies are important as these favor natural selection and maximize plant fitness to the prevailing biotic and abiotic stresses. Chapter 8, by Kumari et al., shows that resource allocation and optimization are regulated by life-history patterns and breeding system of the species to ensure the development of a viable progeny. The authors give information on various theories prevalent on resource allocation and partitioning and also discuss the methods used to quantify resources allocated for reproductive effort citing various examples from their research work.

Dioecy, characterized by the presence of distinct male and female plants, is relatively rare in flowering plants and is an important mechanism that is accompanied by obligate outcrossing. Chapter 9, contributed by Mangla et al., explains the role of various ecological factors which work in unison to shape dioecy in angiosperms. The authors discuss in detail the role of floral attributes, wind pollination, fruit type, clonality, and resource allocation which favor and help in the maintenance of dioecy.

The knowledge of floral attributes is important to understand the intricacy of reproductive mechanisms that are integral to establish successful plant-pollinator

interaction and maximize plant fitness. Chapter 10, authored by Barman et al., focuses on structural and mechanistic diversity of secondary pollen presentation among angiosperms. The significance of these methods mainly lies in the targeted deposition of pollen for successful mating. Chapter 11, authored by Kaur, dwells on the outbreeding patterns in angiosperms in detail and also highlights the diverse outbreeding packages that the flowering plants have evolved citing interesting examples. Moreover, it briefs on the plasticity in resource allocation in relation to the outcomes of sex differentiation in higher plants.

Sexual selection in plants is rarely discussed, as it has been more extensively investigated in evolution of animals for their better adaptability to choose traits for mate. Chapter 12, by Kaul and Raina, gives a succinct account on how sexual selection occurs in plants. After providing a brief historical perspective on the issue, it discusses various theories, conceptual developments, and roles of various traits associated with sexual selection in plants.

Apomixis is an asexual mode of reproduction through seeds wherein meiosis and fertilization are bypassed. Chapter 13, contributed by Sharma and Bhat, is a crisp and consolidated information on this very important topic. The authors trace the origin of apomixis citing various types and variations found in angiosperms, especially grasses. While earlier the apomictic lineages were considered to be a dead end, the authors indicate that current understanding treats them as a means of diversification of polyploid complexes in angiosperms.

Frugivore animals are known to have played a crucial role in the evolution of seed dispersal mechanisms. Chapter 14, by Sinu et al., provides an interesting account on the role of endozoochory through birds, bats, and fishes, especially in long-distance dispersal of seeds. It then discusses in detail about the complexity of ecological drivers that materializes the dispersal and points out the present-day threats to this important mutualistic interaction in the tropics.

The widespread global adoption of transgenic plants has been controversial and has raised concerns regarding the transgene flow. Chapter 15, authored by Lal et al., comprehensively deliberates on most of the pertinent aspects of transgene flow including the biosafety concerns. It also highlights the details of alien genetic elements introgressed into the genome of weedy and wild species through transgene flow from crop species and how they confer selective advantage in some species. The approaches that need to be adopted and the analytical methods and mitigation strategies to be followed to contain harmful impacts are explained. Furthermore, the chapter also discusses in detail studies carried out on some of the selected crop species such as maize, rice, and mustard.

Chapter 16 is on the present-day challenge posed by pollinator decline. Authored by the editors, K.R. Shivanna, Rajesh Tandon, and Monika Koul, this chapter highlights major concern over loss of pollinators, particularly in the tropics. It discusses the details of drivers for pollinator decline across the globe and points out as to how anthropogenic activities are the major culprits and the need for mitigation of the crisis. It also highlights the close linkage between the pollinator crisis and food and nutritional security of the growing population.

This book is a tiny tribute to Professor H.Y. Mohan Ram, one of the most passionate teachers of botany in India. He was an ardent propagator of botanical knowledge. We are sure that this volume would be of interest to teachers and researchers in both the basic and applied fields of plant sciences including agricultural scientists, foresters, and conservation biologists.

It is a privilege to thank all the contributors who have made this book a reality. We appreciate Yash Mangla, Vineet K Singh, Banisha Phukela, Arjun Adit, Sachin, and Manisha for their willingness to help during the finalization of this edited volume. Our warm appreciation goes to the Springer team for their constant support and comradeship.

New Delhi, Delhi, India
Bengaluru, Karnataka, India
New Delhi, Delhi, India

Rajesh Tandon
K. R. Shivanna
Monika Koul

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Monika Koul is presently working as an Assistant Professor in the Department of Botany, Hansraj College, University of Delhi. She did her Ph. D. in Botany from the University of Delhi in 2004. She is involved in teaching of Reproductive Biology of Plants and Ecology to the undergraduate students of Botany, Zoology and Life Sciences for last 14 years. Monika has published 12 research papers, written a number of popular science articles in magazines and contributed chapters in various books. She has also co-edited one book and written articles for national newspapers on topics related to environment and conservation. Monika has also been a resource person for teacher training workshops and participated in developing e-content in NCERT and CBSE. She is the recipient of Ramaseshan Fellowship and SRF from Current Science Academy, IASC Bangalore, India.

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Reproductive Ecology of Flowering Plants: An Introduction

1

Rajesh Tandon, Monika Koul, and K. R. Shivanna

Abstract

Sexual reproduction is vital to ensure variation and perpetuation in organisms. In flowering plants, the reproductive process is accomplished in four major phases. The first phase encompasses the development and organization of flowers, the second ensures pollination, the third includes events leading to fertilization and seed formation and the fourth is defined by dispersal of seeds and their recruitment in populations. Plants optimize reproductive success through interaction between these components of reproductive system and ecological factors. Longevity, vitality and sterility of sexual systems have been well understood in wild and crop species and studies on these aspects have unravelled interesting patterns in angiosperms. However, there is paucity of information on certain aspects such as how plant species modify their reproductive functions in varied habitats and how sexual systems respond to evolutionary pathways, and environmental cues. Reproductive ecological studies are currently trying to answer these questions, as reproductive processes operate in continuum with environmental variables and show specific responses and patterns. These studies also help in understanding the trade-offs and constraints that the angiosperms are always confronted with. The present chapter highlights some of the crucial aspects of reproduction covering phenology, floral biology, pollination ecology, breeding strategies and recruitment pattern. Elucidation of these processes is of immense importance in the management of forest ecosystems, recovery of threatened plant species and sustenance of yield in crops.

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Keywords

Phenology · Floral biology · Pollination ecology · Seed dispersal · Natural recruitment

Reproduction is a crucial step in the lifespan of organisms, which ensures their perpetuation as a distinct species. The success of the process is largely influenced by two components – the inherent biology of organisms and the ‘interactive’ ecological domain in which they grow and flourish. For plants, the second component becomes strongly decisive as it influences almost every step of reproduction. This is primarily due to immobile nature of plants, and therefore it is likely that reproductive success among them can be potentially constrained by any of the attributes of their multivariate environment in which they grow.

In flowering plants, the intricacy of the reproductive process, especially by sexual means, usually assumes a higher level by having numerous reiterative modules (*ramets*) in the same plant (*genet*). Such an organization of the plant body among angiosperms has fitness consequences – the prominent being the greater chance of self-interference. However, plants are equipped with devices and mechanisms to promote outcrossing (Lloyd and Webb 1986, Richards 1997). These devices (*barriers of selfing*) can be morphological, physiological or genetic in nature. In spite of the outbreeding advantages, there is no dearth of plants which follow selfing (~20% *autogamous*) in nature. In the eco-evolutionary context, outbreeding packages better competitive propagules than that from selfing. On the other hand, selfing offers stasis and assures survival (*reproductive assurance*). The questions arise: Why are a great majority of angiosperms inclined to *cosexuality* and bear both the genders either in the same flower (*bisexuality*) or on the same plant (*monoecy*)? One plausible explanation in favour of the predominance of cosexuality is that plants can avoid a separate investment for promoting male and female functions, by capitalizing on resources already allocated to attract the pollinators (Givnish 1980; Charlesworth and Charlesworth 1987a). However, if the barriers are not strong enough, the modularity in plant architecture and proximity of sexual organs and overlapping of their functions (*incomplete dichogamy*, Lloyd and Webb 1986) would increase the chances of self-interference with detrimental effects. *Inbreeding depression* (reduced fitness) and *ovule discounting* (clogging of the stigma with self-pollen) are the two adverse consequences of selfing in preferential outbreeders (Charlesworth and Charlesworth 1987b; Holsinger 2000; Barrett 2002). Studies in recent decades have shown that there is reasonable flexibility in predominant attributes, and shifting to alternative strategies is possible to favour perpetuation and survival (Shivanna 2015). For example, self-incompatibility (SI) in many plants is not absolute (Nasrallah and Nasrallah 2014), herkogamy and dichogamy may diminish if outcrossing fails (Kalisz et al. 2012), and plants may have mutualistic interaction with more than one type of pollinator (Johnson and Steiner 2000). Plants capitalize on such trait variations for adapting new mechanisms for the reproductive success when they experience shift in conditions and colonize new environments.

Though plants cannot move, their migration can be visualised in context of thinning of population sizes at unfavourable sites and their expansion at relatively salubrious conditions. Smaller population size is unstable and susceptible to extinction. The natural process of local extinction of a species in a population is gradual and directly happens due to failure in recruitment of new plants at regular intervals. The failure may happen at any stage from events leading to fruit/seed set or recruitment. Habitat fragmentation and consequent shortage of ecological safe sites further hamper the process of regeneration. The problem is more acute in the case of endemics which have narrow ecological amplitude. Ecological requirements in some species are too stringent to ensure reproductive success on a regular basis. How plants manage to recuperate under such threatened ecological suites can be suitably understood by investigating the species-specific reproductive strategies in relation to their surroundings.

The ecological approach to investigate reproductive biology of plants gives a perspective of populations and communities. One may look into the reproductive strategy of a species at different levels of organization from individual plants to their entire range of distribution. The information generated, in fact, strengthens the inferences by providing a wider perspective and reasoning on how developmental attributes are governed. For example, low seed or fruit set in a species may be due to self-incompatibility. Alternatively, limited fruit/seed set may also occur due to asynchronous flowering, occurrence of more than one co-flowering species, insufficient flowering to engage pollinators, insufficient pollination, heterospecific pollen transfer, pollinator's absence, poor availability of compatible mates, or even as a consequence of conflicts (*trade-off*) in allocation for resources to various functions (Larson and Barrett 2000; Morales and Traveset 2008; see Chap. 8). Further, vegetative reproduction through propagules (*bulbs, corms, tubers, root suckers, bulbils*) in many species including crops is an advantage to escape failure in sexual reproduction and help them survive through clonal expansion. Agamospermy (*apomixis*), another mode of asexual reproduction, harnesses the benefit of packaging clonality by seeds for distant dispersal and can be part of gene flow (see Chap. 13). All these dimensions in a way are decisive in determining the fate of progeny, composition of genetic structure of population, consequent gene flow pattern and many other processes that contribute to patterns on a larger scale for the species to maximize fitness. Reproductive ecological studies thus essentially incorporate 'elucidation of all aspects of reproductive events and their interactions with the biotic and abiotic components of the environment' (Shivanna and Tandon 2014). The list of microevolutionary processes driven by variability in heritable traits leading to different outcomes of mating patterns is far from complete as evolution is a continuous process. It justifies the continuous endeavour to unravel them in multifarious contexts. On account of brevity, we have highlighted a few of these.

1.1 Flowering Phenology

Life history stages are usually adaptive and are selected for features such as timing, duration and periodicity in relation to climatic factors (Rathcke and Lacey 1985). Flowering, a key life history event, is a subset of sequence of events (*phenoevents*) which responds to suitable signals from the environment; the other phenoevents include flushing of new leaves and their subsequent fall, fruiting and dispersal of *diaspores* (fruits or seeds) after maturation. The sequence of appearance of leaves before or after flowering can influence the manner in which flowers are presented for enhanced visibility and accessibility.

Recording various features of flowering pattern in a species over a period helps in identifying their discrete contribution to overall reproductive success (Richards 1997; Frankie et al. 1990). A species is considered to be in flowering if more than 50% of the individuals in the population are in bloom (Stiles 1978). Besides the above features, synchrony of flowering among the plants in a population has a significant influence on the mating pattern. Many a times, the inherent genetic variation among the individuals in a population may also cause out-of-phase blooming (Bullock and Bawa 1981; Opler et al. 1980). The flowering patterns may vary depending upon whether the plants flower en masse (*big bang, cornucopia, multiple bang*) or in extended phase (*steady state or modified steady state*) in a population (Gentry 1974). These patterns influence the constancy and foraging behaviour of pollinators. For example, in self-compatible *Strobilanthes kunthiana* (Acanthaceae), in the Western Ghats of India, flowering occurs in a big-bang pattern for short duration and after a regular gap of almost 12 years. The sudden enriched floral resource attracts *Apis cerana indica* which develops *constancy* and effects pollination. The plants are able to escape reproductive failure through gregarious flowering which enhances pollination efficiency and counters the effects of geitonogamy (Sharma et al. 2008). However, such a pattern is likely to differ with fluctuation in the intensity of flowering, as it is known to vary during different flowering seasons in the species. This may directly influence the relative proportion of the sired self- and cross-progeny. In bat-pollinated plants like *Oroxylum indicum* (Bignoniaceae), the long *steady-state* flowering (6–8 weeks) helps in establishing the fidelity to the pollinator type in different regions of their occurrence (Vikas et al. 2009). Bats are most conducive for pollination success as their trap-line foraging behaviour helps in ensuring outcrossing (*xenogamy*) in this obligate self-incompatible tree species. The flowers in *O. indicum* are short-lived and open acropetally in the long protruding and exposed inflorescence. Here, the direction of the opening of flowers within the inflorescence also becomes a crucial factor to permit the bat of only proper mass to be able to forage and fly away with ease. The reverse of this would be highly demanding for the constancy or even mutualism to establish. Similarly, the direction of the opening of flowers in the capitula of cheiropterous *Parkia* spp. (Fabaceae) has been shown to influence the presentation of nectar to the foragers on a spatio-temporal scale. In co-occurring bat-pollinated *Parkia* species, phenological separation through different timing of flowering has been shown to reduce competition for pollinators (Hopkins 1984).

As the phenological patterns are sensitive to climatic conditions, consistent minor changes in ambient temperature are likely to impact the phenological patterns (Easterling et al. 2000, Abu-Asab et al. 2001). The impacts would also cover community structure, composition and ecosystem functioning (see Chap. 2). Flowering stimulus is controlled and co-ordinated through external factors such as light, temperature and humidity. Photoperiod, quality and intensity of light all play a significant role in perception of flowering stimulus. Among these, increased ambient temperature is implicated to be the major causal factor in shifting the flowering phenology (Sherry et al. 2007). In the absence of systematic phenological records, it is difficult to ascertain the impact on natural vegetation. However, in species for which reliable records are available, significant shift in the onset of flowering dates has been recorded as in *Prunus jamasakura* by 7 days (Primack et al. 2009), in *Robinia pseudoacacia* by ~8 days (Walkovszky 1998) and in *Rhododendron arboreum* by 97 days (Gaira et al. 2014).

These observations on phenological patterns give information on the plasticity of key life history traits known and the putative developmental changes through epigenetic modifications that might follow (Hedhly et al. 2008). For example, the expression of *FLOWERING LOCUS C (FLC)* gene involved in the vernalization pathway is epigenetically regulated to induce flowering in *Arabidopsis thaliana*. The gene has been shown to successfully act as a quantitative tracer of the fluctuating temperature in *Arabidopsis halleri* (Shimizu et al. 2011). Such approaches may be employed for the focal species along the ecological cline to gather clues on the epigenetic changes that are accompanied by climate change-induced phenotypic variations.

1.2 Floral Biology

Flower is the fundamental unit of sexual reproduction. The timing of the opening of a flower (*anthesis*), the onset of stigma receptivity and the duration of pollen availability within a flower are the key features of floral biology. These attributes determine the longevity of the flower and correlate with the breeding and pollination mechanism.

For maximizing fitness, flowers may adopt various means to enhance export of pollen (*male fitness*), so that they may reach as many conspecifics as possible to ensure seed formation (*female fitness*) (Sutherland and Delph 1984). In order to achieve this, plant species optimize the timing of their functions in such a way that they coincide with the availability of mates and pollinators. Floral display (*colour, shape, size, olfaction*), their presentation in the plant (solitary or in an inflorescence; *enantiomorphy*), longevity, anthesis time, relative positions (*herkogamy*) and timing of maturation (*dichogamy*) of essential organs, presentation and type of rewards are some of the prominent floral functions that determine the potency of advertisement to attract pollinators (Waser and Price 1981; Mitchell et al. 2009 see Chaps. 5 and 7). Many of these traits are believed to be influenced through selection imposed by the pollinators (Parachnowitsch and Kessler 2010).

The timing of anthesis is important to understand floral biology and varies among taxa to exploit the pollinators according to their active period. Flowers that open during the daytime (*diurnal*) usually have display through colours of petals, while olfaction is the major cue for those that bloom during the night-time (*nocturnal*). The display size and arrangement of flowers further ensure the range up to which the floral cues may be captured by the potential pollinators during the day. Nocturnal flowers are predominantly white or dull in shades and are usually scented to attract pollinators in the night.

Usually the flowers open only once and senesce after pollination. However, in certain species, especially those with bowl or disc-shaped flowers, repeated cycles of opening and closure have been noticed (e.g. *Ipomoea*, *Silene saxifraga*). Such movements are attributed to physiological changes in response to light intensity in diurnals or by an increase in relative humidity among the nocturnal plants (see van Doorn and van Meeteren 2003). In temperate and arctic environment, temperature within the floral space conferred by its shape can strongly influence the visitation of pollinators (e.g. *Dryas integrifolia*, Rosaceae) and therefore the reproductive success (Kevan 1989, Dyer et al. 2006). In the tropical diurnal weed, *Volvulopsis nummularium* (Convolvulaceae), flowers open partly under lower temperature on rainy days and give an opportunity to the terrestrial snails (*Lamellaxis gracile*) to pollinate rather than to *Apis cerana indica*, which are more effective on bright sunny days (Sarma et al. 2007).

The blossom shape and size are perhaps the most co-adapted floral traits investigated in predicting the mutualistic partners (Faegri and van der Pijl 1979). Phenotypic matching between the flower and pollinators determines the legitimacy required in pollen transfer. Many examples have been cited in literature to demonstrate that how specialization is derived by the matching of phenotypic traits (Balducci et al. 2019). The best example is that of plants with long nectar spur and the matching mouthparts of nectar-feeding animals such as moths and bats (Darwin 1862; Muchhala and Thomson 2009). In orchids, including that of Darwin's *Angraecum sesquipedale*, the matching establishes as a result of directional selection for flowers with deep-seated nectar (Alexandersson and Johnson 2002). Similarly, *lock-and-key* mechanism has been suggested to be operational in the pollination success of *Oroxylum indicum*. In Thailand, the flowers of the species were shown to be essentially pollinated by *Eonycteris spelaea*, and in the similar manner by *Cynopterus sphinx* under Indian conditions (Gould 1978, Vikas et al. 2009).

Like the display size, longevity of flowers also has consequences on attracting pollinators and their frequency of foraging visits (Mathur and Mohan Ram 1978; Shivanna and Tandon 2014). It usually refers to the duration up to which flowers retain their freshness under field conditions (Primack 1985), but more precisely to the duration up to which the flowers are able to disperse or receive pollen (Shivanna and Tandon 2014). The duration may vary among species from few hours to 8 weeks, e.g. *Malvastrum coromandelianum* (2 h), *Tribulus terrestris* (4 h, pers. observ.), *Oroxylum indicum* (10 h, Vikas et al. 2009), *Kigelia pinnata* (2–3 days, Harris and Baker 1958) and orchids (up to 2 months, Schoen and Ashman 1995). Long-lived flowers can amply compensate for low pollinator visitation rates in

pollinator-limited environments to ensure sufficient receipt and promote pollen export (Arroyo et al. 2017). According to Ashman and Schoen (1994), floral longevity is a heritable adaptation that balances rates of pollen receipt and removal against the cost of floral maintenance which can be optimized by selection in nature.

The female and male phases in a flower are determined by the onset of stigma receptivity and anther dehiscence, respectively. The proximity, duration and overlapping of the two phases drive selection on the plant to dissuade and restrict self-interference. This may be achieved by separating the overlap either in space (*herkogamy*) or time (*dichogamy*) (Lloyd and Webb 1986; Webb and Lloyd 1986; see Chap. 11). Interestingly, both the modes are represented among the self-compatible as well as the self-incompatible plants (Takebayashi et al. 2006; see Kissling and Barrett 2013). This suggests that the traits are plastic and modifiable through minor developmental changes. Depending on the efficacy of the mechanism, either of the modes may be preferentially selected in a species (Webb and Lloyd 1986). For example, herkogamy has been demonstrated to be more effective than dichogamy in *Epimedium* (Berberidaceae), and it need not be necessarily accompanied by dichogamy to prevent self-interference in the species (Li et al. 2013). On the other hand, plasticity in the traits might be exploited by the species to adopt an alternate strategy while colonizing new environments. For example, in *Blackstonia perfoliata* (Gentianaceae) with minor temporal shift in the male phase, the breeding system has been shown to shift from delayed selfing under pollinator-rich conditions to competing selfing in recently established populations (Brys and Jacquemyn 2011). In *Utricularia praeterita* and *U. babui* (Lentibulariaceae), failure in cross-pollination (due to pollinator limitation) leads to loss in herkogamy (diminished herkogamy) at the later stages of opened flower, and the elongation of stamens causes autonomous delayed selfing in the species (Chaudhary et al. 2018).

The incidence of dichogamy among plants varies in the context of separation of sexual function within or between the flowers. *Protandry* (maturation of anthers prior to stigma) is more common (~66%) than *protogyny* (~33%) at the intrafloral level, and the reverse is true for the interfloral level with greater incidence (77%) of protogyny (Bertin and Newman 1993). Further, dichogamy besides preventing self-fertilization also prevents self-interference during the *progamic* (pollen-pistil interaction) phase and synchronizes pollen release and stigma receptivity in the different flower types.

1.3 Plant Sexuality

Sexual expression in a plant is the representation of the predominant phase of a particular gender. The condition may involve *temporal* or *spatial* separation of the exchange of gametes among the individuals in a population (see Renner 2014). It can be determined at the level of a flower, individual plant or the population (Shivanna and Tandon 2014). In contrast to the hermaphroditic plants with bisexual flowers (~70%), the proportion of plants with sexually dimorphic conditions is limited

(Barrett 2002). Only 6% of the taxa are known to exhibit dioecy, a condition which has been derived from cosexuality on numerous independent occasions (Westergaard 1958; Charlesworth 2002). Unlike taxa with temporal separation of gamete exchange, those with spatial separation mechanisms, especially dicliny, monoecy, trioeicy, androdioecy and gynodioecy, appear to have direct link with evolution of dioecy (Renner et al. 2007; Renner 2014). In spite of their low proportion, dioecious taxa have a considerable occurrence range and their distribution pattern correlates with some of the key ecological features (see Chap. 9).

Sterculia urens (Malvaceae) is a commercially important gum (Indian tragacanth) producing deciduous tree species. The *andromonoecious* condition in the tree species is attributed to a greater proportion of the male than the functionally female bisexual flowers (*cryptic monoecy*). The red anthers in the bisexual flowers serve as a means of pollinator attraction (Sunnichan et al. 2004). Cryptic monoecy may also be recorded among taxa with temporal separation of genders (e.g. *Acer oblongum*) (Yadav et al. 2016).

The temporal patterns allow the same individual to behave as male or female, and the proportion of individuals with different genders in the population influences their ratio. The sequence of sexual expression varies among taxa. For example, in Sapindaceae, most of the *Acer* species are known to exhibit temporal separation of genders (Renner et al. 2007). They are either *heterodichogamous* (two morphs that alternate with each other) or *duodichogamous* (the same morph behaves in the order as male, female and then again male); very few are dioecious. In oil palm (*Elaeis guineensis*, Arecaceae), the plants alternate in sex expression as male or female (*ontogenetic sex change*) in the population, and their ratio can significantly influence the yield (Tandon et al. 2001a). It has been noticed that the nutrient supplementation in the Tenera hybrids (dwarf variety) of the palm is essential for the first three years of their plantation to maintain the seasonal sequence of genders; otherwise the plants behave as male throughout their lifespan. Such gender plasticity is known to be partly determined by resource status in some taxa (Diggle 1993).

1.4 Pollination

Pollination ecology is the most extensively investigated aspect of reproductive biology in flowering plants. The dynamics of plant-pollinator interaction have been used to explain several micro- and macroevolutionary processes, ranging from elucidation of reproductive strategy of a given species to gene flow, mating system, specialization of pollination syndromes and pollinator-mediated speciation. Most of these studies suggest that these processes are ecologically driven, at times can be context dependent, often highly diffused and yet vital for the survival of plants (Mitchell et al. 2009; Harder and Barrett 2006; see Barrett 2008; Sletvold 2019).

With increase in the awareness over a period of time, standardization of protocols, application of molecular tools, genetic approaches and availability of powerful software tools, a gamut of information has been generated on the subject. The

information has paved the way to test theoretical generalizations. Based on the information, some of the noticeable trends that have emerged are cited below:

- Entomophily is the predominant mode of biotic pollination across the planet, and the bees (tribe Apidae) are the most efficient pollinators. Wind pollination is secondarily derived among insect-pollinated taxa (Bronstein et al. 2006; Waser and Ollerton 2006; Friedman and Barrett 2008; Culley et al. 2002; also see Chap. 9).
- The notion that *pollination syndromes* (suites of convergent floral traits among unrelated taxa adapted to attract a particular functional group of pollinators) are important for predicting the mutualistic partners may not always hold true. In a specialized mutualism, the pollinators may belong to one functional group rather than to just one species (Faegri and van der Pijl 1979; Johnson and Steiner 2000; Tandon et al. 2003; Fenster et al. 2004; Ollerton et al. 2009).
- Bilateral symmetry; the quality, quantity and location of rewards in a flower; and cues of attraction (visual as well as olfactory) promote constancy and pollination efficiency. Many of them are also effective in filtering the antagonists (Williams and Dodson 1972; Baker and Baker 1983; Irwin et al. 2004; Junker and Blüthgen 2010; Singh et al. 2014; Tiedeken et al. 2016).
- Because of the obligate reliance of nearly 90% of flowering plants on a range of taxonomically unrelated biotic pollinators, their foraging behaviour largely influences the gene flow and mating patterns on a spatio-temporal scale (Levin and Kerster 1969; Nason et al. 1998; Karron et al. 2009).
- Networks of plant-pollinator interactions are mostly nested due to the preponderance of generalists rather than the specialists. Generalists impart strength, diversity and stability to the networks through numerous links, enhanced connectedness and low modularity (Bascompte et al. 2003; Olesen et al. 2007). High modularity in the interaction network is a feature of enhanced specialization, as in Bignoniacae (Genini et al. 2010).
- Pollinators exert selection on floral traits to enable plants to adapt to new environments with suitable syndromes, resulting in speciation and diversification of taxa (*ecological speciation*) (Goodwillie et al. 2010; van der Niet et al. 2014; Dellingen et al. 2019). But the presence of antagonists like nectar robbers may mask the selection of the traits by pollinators, and many a times abiotic factors can also exert equal strength of selection (Harder and Johnson 2009; Caruso et al. 2019).
- Pollinator-mediated selection is driven through *geographical mosaics* of pollination landscape, spanned across the distributional range of a species (Van der Niet et al. 2014; Newman et al. 2014).
- Transitions in reproductive traits (e.g. floral colour, pollination syndromes, biotic and abiotic mode of pollination, self-incompatibility and sexual systems) are accompanied by modifications in the genetic systems and other related life history traits (see Barrett 2008).

In spite of its importance in sustaining crops, and conserving biodiversity and ecosystem services, pollination data from many regions, especially the tropics, is far from complete (Corlett 2004, Roubik et al. 2005, *see* Chap. 16). Considering that mutualistic interactions are diffuse and highly amenable to shifts, it is likely that the present trend of unstable climatic conditions may destabilize the fidelity of interactions. Intermittent reproductive failure in natural populations of plants is expected for a long period. Habitat modifications leading to fragmentation of vegetation would induce density-dependent demographic effects on mating patterns and trophic organization in plant communities.

1.5 Progamic Phase

The phase comprising events from pollination to fertilization is termed the progamic or the pollen-pistil interaction phase. Pistil is the key developmental innovation accompanied by angiospermy (*syncarpy*), the mechanism which concealed the ovules and the developing seeds to prevent predation. The mechanism also elaborated the maternal tissue for recognition or rejection of the male gametophytes (Endress 2001). Syncarpy is a predominant feature among angiosperms (~83%), while those with apocarpous pistils are usually seen among the basal angiosperms indicating that syncarpy evolved on multiple occasions (Endress 1982; Armbruster et al. 2002). Besides protection to ovules, the other advantages of syncarpy are attributed to greater precision in pollination, pollen-pistil interaction and efficient dispersal of seeds through adaptations of fruits (Armbruster et al. 2001). Pollen tube growth through the pistil serves as the site for competition among males and favours fertilization by most vigorous pollen resulting in better quality of seeds (Mulcahy and Mulcahy 1987, Shivanna 2003, 2011).

Innovation of pistil led to a significant shift in the manner the gametophyte selection occurs in flowering plants (*see* Chaps. 3, 4 and 12). The recognition and rejection of male gametes were taken over by somatic tissues of the pistil rather than by the gametes, as in the lower plants (Shivanna 2003; Bedinger et al. 2017). Flowering plants exhibit considerable diversity in the structural organization of the pistil (*see for the types* Heslop-Harrison and Shivanna 1977). The style primarily differs either by having a transmitting tissue tract (*solid-style*) or a stylar canal (*hollow-style*), while the stigma types differ on the basis of surface morphology and the ability to produce mucilagenous exudates once receptive (Chap. 3). The structural modifications in the pistil were superimposed by the physiological mechanisms that favoured outcrossing (Herrero and Hormaza 1996, de Graaf et al. 2001; Scutt et al. 2006). The attributes of the stigma surface usually correlate with the genetics of self-incompatibility; the sporophytic type (SSI) is associated with the dry type of stigma and gametophytic type (GSI) in most of the cases with the wet types (Shivanna 2003).

Another noticeable superimposition includes touch-sensitive nature of stigma, as seen in Lentibulariaceae, Scrophulariaceae, Bignoniaceae, Martyniaceae and Acanthaceae (Friedman et al. 2017; Chaudhary et al. 2018). The additional

mechanisms to prevent self-interference in the pistil can be transient and become effective only at the peak time of pollen presentation. For example, SI can be weak in the bud stage (Nasrallah et al. 2002, Shivanna 2003), and the sensitive lobes of the stigma may close permanently on single touch (Vikas et al. 2009). Such a developmental plasticity creates amenabilities for selfing to assume the role of reproductive assurance under pollinator or mate-limited environment as seen in *Utricularia*.

1.6 Breeding System and Mating Patterns

The breeding characteristics of flowering plants range from complete selfing (*autogamy*) to obligate outcrossing (*xenogamy*) (Bawa and Beach 1981; Richards 1997). In a population, the characteristics are driven by pollinator-mediated pollen flow and the extent of self-incompatibility among the conspecifics (Gan et al. 2013; Barman et al. 2018). Together these attributes generate a mating pattern which reflects the relative genetic contributions to subsequent generation (Wyatt 1986; Loveless and Hamrick 1984; Barrett 2003).

Self-incompatibility (SI), a feature common among plants with bisexual flowers (Bawa 1992, de Nettancourt 2001), is mainly a pre-fertilization barrier manifested through inhibition of pollen germination on the stigma or pollen tube growth in the style and differs *sensu stricto* from self-sterility (Barrett 1988). In the latter type, the fertilized ovules abort at various stages. Both the mechanisms lead to failure in seed set. Reproductive success in obligate SI plants (selfing rate < 20%, Zapata and Arroyo 1978, Schemske & Lande 1985) requires maintenance of a sufficient amount of allelic diversity and heterogeneity among the conspecifics. Delayed SI in which the pollen tubes are inhibited in the ovarian region has been reported in some species, and this is referred to as late-acting SI or LSI (Seavey and Bawa 1986; Gibbs and Bianchi 1999; Tandon et al. 2001b; Vikas et al. 2009).

In many plants SI is weak (*pseudo SC* or *partial SI*) and may permit some fruit set through selfing as well, making them preferential outbreeders (Bawa 1974, 1979; Tandon et al. 2001b; Good-Avila & Stephenson 2002). The extent of SI can be ascertained through usual bagging experiments (Shivanna and Tandon 2014). The approach has established that many plant species in the tropics are partially self-compatible or principally outcrossing (Bawa 1974; Tandon et al. 2001b; 2003). The relative proportions of selfed and crossed progeny sired are difficult to ascertain by routine bagging experiments alone; such studies need to be combined with suitable genetic markers to establish the extent of outcrossing rate (multi-locus t_m and single locus t_s), biparental inbreeding (t_m-t_s), correlation of paternity (r_s), gene flow pattern and even sexual selection by determining the pollen donors (Ritland and Jain 1981; Ritland 2002). Many tropical tree species (e.g. *Eucalyptus urophylla*, *Platypodium elegans*, *Shorea congestiflora*, *Warburgia ugandensis*) exhibit high ($t_m > 85\%$) outcrossing rates (Hamrick and Murawski 1990; Murawski and Hamrick 1991; Gaiotto et al. 1997; Muchugi et al. 2008). However, species with outcrossing rate between 50% and 80% belong to the mixed-mating category.

There has been a recent surge in studies demonstrating the increasing incidence of mixed mating among flowering plants. The case is more prevalent among the animal-pollinated species which show continuous variation in outcrossing index (Goodwillie et al. 2005). Variation may occur even within the same species and in different seasons and populations. Thus, the contributory variables for the success of mixed mating are context dependent (Schemske and Lande 1985; Holsinger 1991). There is a continuous debate on how such a strategy may evolve or be maintained in some species. The extent of biparental inbreeding (crossing between sibs), geitonogamy and inbreeding depression are some of the factors recognized that play an important role in the evolutionary maintenance of mixed mating (Uyenoyama 1986; Goodwillie et al. 2005). With the increase in biparental inbreeding, the genetic relatedness of the parents with the progeny also increases and promotes frequency-dependent selection to maintain intermediate outcrossing in a population (Uyenoyama 1986). Inbreeding depression, which plays a crucial role in mating system evolution, correlates with the selfing rates (Charlesworth and Charlesworth 1987b; Holsinger 1991; Winn et al. 2011). Geitonogamous selfing is an unavoidable feature common to plants with mixed-mating system as well as in those where autogamy is prevented (Eckert 2000). For example, the trees of *Wrightia tomentosa* (Apocynaceae) are self-compatible, but autogamy is not permissible due to architectural restrictions imposed in the flower – a feature conserved in the family for promoting outcrossing. However, geitonogamy mediated by the ‘settling moths’ has been shown to cause nearly 30% selfing among the trees (Barman et al. 2018). The other types of moths (hawkmoths) were responsible for xenogamy (~70%). The study has shown that besides the above-mentioned factors, pollination systems could also be crucial in driving and maintaining mixed mating in plant species.

The breakdown in self-incompatibility (SI) can set a course for the evolution of selfing, and such transitions have happened on numerous occasions among the flowering plants (Barrett 2002, Brandvain et al. 2014). Conditions that select for the evolution of self-compatibility include those which promote reproductive assurance and must be accompanied by gradual lowering in inbreeding depression (Shimizu and Tsuchimatsu 2015). Plants are believed to promote self-fertilization in harsher environments (Lloyd 1992). According to the *reproductive assurance hypothesis*, the selfing taxa are commonly associated with ephemeral and extreme environments where the availability of mates and pollinators both are limited (Cheptou 2012). Mixed mating can evolve into a complete selfing when plants colonize pollination-limited environment. However for the success of the shift in pollination, floral traits conducive for anemophily like small size of flowers with high pollen production should also evolve in synchrony (Shimizu and Tsuchimatsu 2015).

1.7 Dispersal and Natural Recruitment

Besides the pollen grains, seeds constitute the second important component of gene flow in angiosperms. Dispersal of seeds or fruits (*diaspores*) serves as an important link between the post-fertilization developmental events and the establishment of subsequent generation (*recruitment*). Like all flowers do not develop into fruits and all ovules do not develop into seeds, all seeds formed are not able to establish into a new plant. Such patterns emerge by developmental and environmental stochasticities working on the processes (Shivanna and Tandon 2014). Yet, it is important that the diaspores ought to be produced in optimal amount to ensure reasonable recruitment in the population (Stephenson 1981; Ghazoul and Sheil 2010).

For recruitment, it is the ecological framework which defines the operational success of a dispersal strategy and patterns. Like pollination, the success of dispersal also depends on a variety of dispersal modes – by animals (*zoochory*), wind (*anemochory*) and self-dispersal like explosive release of seeds (*autochory*) (van der Pijl 1982; Corlett 1998; Cousens et al. 2008; Shivanna and Tandon 2014). However, it is believed that some of the dispersal syndromes have proved insufficient to explain (i) the intricacies of long-distance dispersal mechanisms, including invasion, which may often involve more than one dispersal agents (see Chap. 14), (ii) range expansion of plant species and (iii) their fitness consequences on plants (Okubo and Levin 2001). In general, this inadequacy can be attributed to lack of data on the higher scales of quantification of dispersal patterns (Nathan and Muller-Landau 2000; Tackenberg 2003). Also, most of the reproductive biological studies are traditionally restricted up to the outcome of pollination studies and have not been effectively linked with dispersal. Therefore, the foundation of information on the dynamics of seed dispersal patterns is largely consolidated by ecological studies. These studies are focussed on the functional aspects of population growth, successional trends, resilience, reactions and community organization. This reliance on functional traits has retained its relevance even in current approaches as well. It has been emphasized that by considering the plant functional groups as analytical units of dispersal, it would be possible to distinguish and predict the components of the seed dispersal process of a focal species (Aslan et al. 2019).

As fruits and/or seeds are also the source of nutrition for animals, the dispersal mechanisms have evolved to escape predation and ensure the survival of a sizeable portion of the seed lot (Howe and Miriti 2004). The net recruitment in the community then eventually depends on the chances of occupying the ecological safe sites (*seed dispersal effectiveness, SDE*) available in the dispersal range of viable seeds (Wenny 2001; Schupp et al. 2010). Thus, the processes that contribute to the mortality of seeds during dispersal (*dispersal costs*) are *selected against dispersal* (Cody and Overton 1996; Clobert et al. 2001). The processes *selected for dispersal* may include kin competition and environmental heterogeneity (Auffret et al. 2017).

Like the pollination mutualism, dispersal mutualism is also generalized where more than one functional group of disperser may be linked to a plant species (Bascompte and Jordano 2007). With the involvement of different dispersers, variation in seed dispersal and recruitment pattern may arise due to the differential

assemblages and ability of the animals to remove seed crop across the distributional range of a plant species (Blendering 2017). In anemochorous species (e.g. *Acer*), besides the morphological adaptations, settling velocity, height of release, wind speed and turbulence contribute to the pattern. The pattern is usually leptokurtic with majority of seed dropping close to the source, and the frequency recedes with distance (Okubo and Levin 1989).

1.8 Scope of Plant Reproductive Ecology

Reproductive processes, as briefly discussed above, play a crucial role in governing the adaptability of plants to multivariate environment. Knowledge of these key processes can prove useful in providing useful inputs in addressing the present-day challenges, which directly or indirectly impact ecosystem functioning. Incidentally, most of the challenges have emerged from direct human interference. Anthropogenic factors such as habitat destruction, over-exploitation and extreme modifications in land-use patterns have downsized the natural populations of many plant species. By elucidating the reproductive strategies of plant species, it is possible to predict their survival capacity and to take appropriate measures for their conservation (Bernardello et al. 2001).

In the current scenario, conservation and sustainable utilization of germplasm have become the priority of the species-rich regions. Most of these regions are located in the tropics, where forests have high endemism (Kier et al. 2009), and are also the major source of fuel, fodder, timber and alternative medicines (Bawa et al. 2004). The identification of the key reproductive requirements and constraints of threatened plants can help in species recovery (Kwak and Bekker 2006). For example, in *Sterculia urens*, besides the pollination constraint in fruit set, poor seed germination in nature and a longer seedling establishment phase (2–3 years) in the forests of Central India have been identified as the main reproductive constraints in the recruitment phase of the species (Sunnichan and Shivanna 1998; Sunnichan et al. 2004). For conservation of trees with obligate SI and those that exhibit strong inbreeding depression, maintenance of heterogeneous population of conspecifics is essential for the reproductive success and sustained recruitment process (Elam et al. 2007; Jones and Comita 2008; Singh et al. 2018). *Spilanthes acmella*, an important medicinal plant, is an over-exploited species, and its propagation by seeds is also limited because of the heterozygous nature of plants due to protandry that prevents self-pollination. In vitro methods can be employed to recover threatened plant species, as it has been done in *Eremostachys superba* (Sunnichan and Shivanna 1998). The species has mainly declined due to habitat destruction. For reintroduction, it has been recommended that populations should be supplemented with plants from distant populations to maintain heterogeneity (Verma et al. 2007). The problem of failed seed germination may be overcome by giving them pre-treatments or making artificial seeds. Therefore, for recovery of endangered species, combining the in situ or ex situ conservation approach along with those which reclaim habitats could be a useful strategy.

Akin to phenological changes, pollen-pistil interaction has been found to be sensitive to rise in temperature (Zinn et al. 2010). Elevated temperature has been shown to adversely affect pollen germination (Flores-Rentería et al. 2018). Likewise, many plants of medicinal and economic relevance have low fecundity due to abnormalities in pollen meiosis such as *Seasamum indicum* (Kumar and Yadav 2012) and threatened Himalayan poppy (Singhal and Kumar 2008). *Tetracentron sinense*, an economically important endangered tree species, shows the restricted distribution and poor natural regeneration due to abnormalities in the process of anther dehiscence, tapetum degeneration and pollen grain maturation (Gan et al. 2012). In *Coptis teeta*, an endangered medicinally important Himalayan plant, poor pollen fertility has been found to be the major impediment in sexual reproduction of the species (Pandit and Babu 1998). In smaller populations, the unavailability of sufficient viable pollen leads to an imbalance in sex ratio which effects population build-up and increases the probabilities of extinction (Frankel et al. 1995). Lower pollen viability, germinability and abnormal pollen tube growth have been attributed to reduced fruit set and sparse population distribution in *Rauvolfia hookeri* in the Western Ghats (Ranjusha et al. 2013).

Loss of habitats to expand urban development and modification in the land-use patterns for the cultivation of cash crops are the major issues faced in some tropical regions. Many of the crops cannot sustain prolonged storage (e.g. oil palm, sugar-cane) and have to be processed within a short period of time to meet the commercial demand. Therefore, the processing units are also established along with the plantations. The immediate impact is seen in fragmentation of forests to smaller patches and consequent loss in the resilience of natural populations to recuperate. Disruption in the foraging range of pollinators due to fragmentation of a continuous habitat into smaller patches is the main reason for the drop in pollinator frequency in small populations. Moreover, such small populations with scattered individuals also fail to attract pollinators. Selective cutting causes microclimatic changes and decreases the amount of old and dead trees, which may threaten the persistence of many threatened species (Pykala 2007). Effects of genetic stochasticity such as inbreeding depression in small and fragmented populations of threatened plant species are also due to altered patterns of mating strategies and pollinator behaviour. In *Impatiens coelotropis*, a medicinally important and critically endangered species of South Western Ghats, reproductive stress is attributed to scattered and fragmented population distribution (Sreekala et al. 2008).

For recovering the forest corridors, rescuing dominant species to a viable population size can ensure a gradual build-up of the community and help in the re-establishment of key ecological associations. For example, mangroves are unique formations that regulate the ecosystem dynamics of coastal deltaic regions of the tropics (Upadhyay et al. 2002). These areas are also facing the vagaries of agricultural expansion. In such systems, true vivipary (seed germination when attached to the canopy of the parent plant) in some trees plays a crucial role in the recruitment process and regulates the dynamics of the entire ecosystem, while other species occur as associates (Solomon Raju 2013). Thus, by protecting the regenerative capacity of viviparous species in the forests, the mangrove ecosystem can be made

resilient to withstand the perturbations and species loss of associated species as well. Knowledge of species-specific key life history traits is thus crucial to rescue threatened communities. For this, the identification of key ecosystem services that link the reproductive diversity of native dominant plant species would be constructive in restoring ecosystems. A recent meta-analysis comprising 64 plant pollinator networks of temperate forests has revealed that ecosystem restoration results in a marked increase in pollinator species along with shifts in interaction patterns. Besides it had direct and positive effects on the total fruit production of native plants. Interactions in restored networks were more generalized than in unrestored networks, indicating a higher functional redundancy in restored communities (Kaiser-Bunbury et al. 2017).

Pollinators are the common requirement of crops as well as the forests, and the web of pollination systems is necessary for ecosystem stability. Biotic pollination is estimated to enhance global crop output by US \$ 235–577 billion annually (Potts et al. 2016). The unabated intensification of agriculture, which involves the uncontrolled application of fertilizers and pesticides, has adversely affected the communities of wild bees and consequently the pollination services as well (Klein et al. 2007, see Chap. 16). The effect is not only confined to the key social honeybees (*Apis mellifera* and *Apis cerana*) and their colonies but also the other crucial pollinators including butterflies, flies and many vertebrates. Global pollinator decline is a major ecological crisis, as the loss in pollinator diversity has been recorded from the protected areas as well (Hallmann et al. 2017). As social bees tend to move with resource availability, prolonging their stay through provisioning of resources in a relay manner can mitigate the problem of sustaining pollination services (Russo et al. 2013). For this appropriate wild plants can be intercropped along the borders of the agriculture fields that flower in succession with crops. Many native pollinators can be potential pollinators of crops and serve as a bridge between two successive social bee pollination episodes. However, it would be necessary to ensure that their brood sites are appropriately located with crop fields as well. By maintaining the diversity of pollinator communities, the yield of crops can also be sustained (Garibaldi et al. 2016). To contain further decline in essential pollination services, information is needed to identify pollinator species impacted, their distribution, the rate of declines and the consequences on ecosystem functioning.

Recruitment is an ecological process and is important for the propagation and perpetuation of species in natural environment (Chap. 14). Seedling recruitment involves optimal seed production, effective seed dispersal and establishment. It has also been well established that seedling recruitment relies on other biota and is a mutualistic interaction. Problems are encountered in hunted habitats where reduced zochory has resulted in low population density. Fruit and seed predation is an important interactive force in plant communities affecting the dynamics and spatial distribution of populations. Plant-animal interactions which occur during seed dispersal and predation play a crucial role in determining the success of recruitment and community structure. The mechanistic difference of seed production between the common and rare species can shed new light on species coexistence and community assembly. Long-term monitoring of both seed rain and seed predation is required for

understanding the ecological and evolutionary implications of species regeneration strategies in a species-rich forest community (Xu et al. 2015).

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Climate Change and Vegetation Phenology

2

K. G. Saxena and K. S. Rao

Abstract

The rate at which climate change is influencing the living organisms and ecosystem is considered as a major threat to sustaining the resources that are required for the human survival in the future. Environmental impacts on the life stages and functioning of organisms have been a major area of study over the last century. The information available from these studies has provided some insights to the impact of climate change on various phenophases of organisms. Individuals in a vegetation community being fixed to a location have to withstand the environmental variation as compared to animals which had the opportunity to move to favourable environments. Thus, plant phenology has greater potential value to understand the impact of climate change on organisms. Plant phenology studies traditionally provided information from ground-based studies. However, with the use of remote sensing technology and climate models, predicting the future plant community structure and functions also started. Validation of such model results using controlled condition experiments will lead to a greater understanding of the influence of climate change on the vegetation phenology. This chapter provides a summary of published information on the impact of climate change on the plant phenology.

Keywords

Phenophases · Climate change indicator · Biomes

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2.1 Introduction

There are many and divergent views about the magnitude and implications of global climate change, both among the researchers and decision makers. While all agree on perceptible impacts of climate change on species, communities and ecosystems, opinions differ about the nature and magnitude of these changes in different geographical regions and their impacts on humans. The literature is replete with studies on climate change impacts on structural and functional traits of organisms and their reflections in terms of decline in ecosystem functions influencing humans. Yet, indicators of climate change which could be easily understood by common people inducing activities enabling mitigation and adaptation over a short span of time are lacking (Werndl 2016).

Terrestrial plants being fixed to a location can persist only when they are able to withstand ambient climate changes, unlike animals with the ability to quickly move to favourable habitats. There is a clear relationship between climatic conditions and organismal life events. Phenology is the study of life-cycle events, the phenophases, which occur periodically (Lieth 1974). Vegetation phenology deals with the annual/seasonal life-cycle events of plants starting from the onset of the growth to senescence and their relationships with biotic and abiotic factors (Zhang et al. 2012; Zhao et al. 2013; Levin 2019). Although vegetation phenology research was set in the 1950s (Schnelle 1955), phenology-climate relationships were clearly established only by the early 1990s (Donnelly and Yu 2017). Thus, phenological changes are driven by climatic changes in many instances and can serve as sensitive markers or indicators of climatic changes (van Schaik et al. 1993; Wright and van Schaik 1994; Inouye 2008; Moore et al. 2015; Ford et al. 2016; Chmura et al. 2019).

Phenological studies are advantageous in that they can be carried out as long-term natural experiments and, thus, can be synchronised readily with climatic data (Myneni et al. 1997; Menzel et al. 2006). Many studies have shown a significant impact of global warming on photosynthetic activity, leaf longevity and length of the growing season (Chmielewski and Rötzer 2001; Chmielewski et al. 2004; Zhang et al. 2004; Donnelly and O'Neill 2013). These conclusions are based either on field-based or remote sensing-based studies or those that combine both the approaches (Fitchett et al. 2015; Tang et al. 2016; Fu et al. 2018; Daham et al. 2019; Laskin et al. 2019; Qu et al. 2020). The increase in temperature is reported to increase greenness of vegetation, postpone arrival of new flush of leaves and extend/prolong the growing season in most parts of the Northern Hemisphere (Myneni et al. 1997; Menzel and Fabian 1999; Zhou et al. 2001; CaraDonna et al. 2014), a conclusion also substantiated by simulation experiments (Matsumoto et al. 2003; Wolkovich et al. 2012; Gray and Brady 2016). This chapter provides a review of recent studies that used phenological aberrations as a putative indicator of climate change.

2.2 The Conceptual Framework

Phenological stages/changes are the outcomes of the interaction of intrinsic attributes of a species and their sensitivity to external environment. These traits comprise biotic potential, energy capture and management, nutrient uptake and metabolism, while insolation, temperature, humidity, wind, competition, resource availability and pollution represent the environmental attributes (Chuine 2010; Ibáñez et al. 2010; Zhao et al. 2013) (Fig. 2.1).

Spatial variations in plant phenology are largely the outcomes of low temperatures at high latitudes and water stress in desert and arid regions (Beaubien and Freeland 2000; Moser et al. 2010; Visser et al. 2010; Wilczek et al. 2010). However, the photoperiod-phenology relationships may vary with species and location (Borchert and Rivera 2001; Zhao et al. 2013). On a global or regional scale, growth potential is directly proportional to the growing season (Sparks et al. 2000; Miller-Rushing et al. 2007). The role of environmental drivers regulating plant phenology has been studied extensively (Kramer et al. 2000; Badeck et al. 2004; Cleland et al. 2007; Forrest et al. 2010; Forrest and Miller-Rushing 2010; Moore et al. 2015). Temperature and photoperiod are the primary determinants of intra-seasonal timing of phenophases (Wlegolaski 2001; Chmielewski et al. 2004). The diurnal temperature fluctuation and the number of frost-free days in the growing

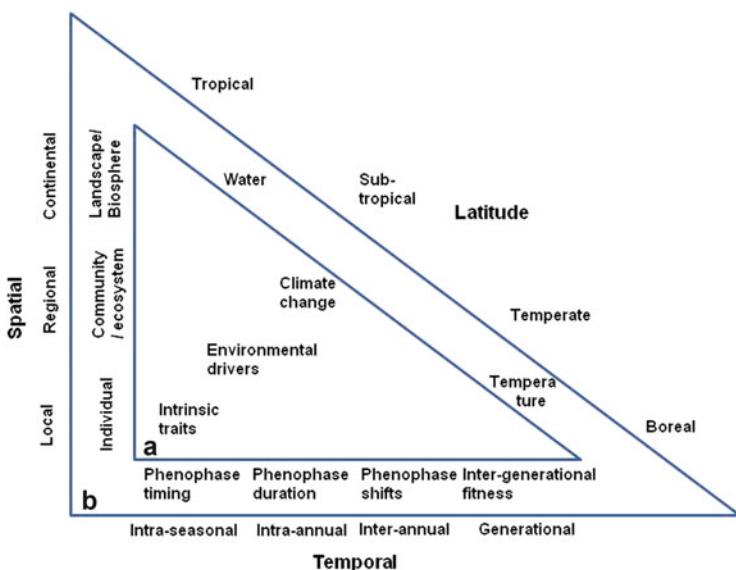


Fig. 2.1 Factors controlling plant phenology. Framework of phenology-climate interactions: (a) factors impacting plant phenology are shown in triangle (a) and scalar dimensions of phenology in triangle (b) (After Zhao et al. 2013)

season also seem to serve as the determinant factors for phenophases (Eppich et al. 2009), especially at high latitudes.

Precipitation regime may be crucial when studying pollen availability, flowering and fruit production (Galan et al. 2001; Crimmins et al. 2010). Soil properties (such as texture, moisture content, nutrient status), competition and pathogens/pests also invariably affect plant growth but affect phenology only when their interactions with primary determinants of phenology are significant (Winder and Cloern 2010; Ibáñez et al. 2010). Climatic changes progressing at intra-seasonal, decadal or century scale are directly manifested in phenological changes (Schwartz 1994). Responses to climate change may differ between annuals and perennials, primarily due to the duration of their exposure to them. Annuals and biennials may show more prominent impacts on population distribution. Perennials show both population distribution and performance of individuals encountering changes other than climate change during their long life spans.

Researchers have considered growth stages such as bud development, leaf development, shoot development, flowering, fruiting, fruit dispersal and senescence as the key events and monitored their timings of occurrence and duration in relation to the environmental conditions. A stage may be further subdivided into sub-stages for elaborate monitoring. The impact of rising greenhouse gas concentration has been evaluated on biomass production more than phenology (Ovaskainen et al. 2013). As greenhouse gases influence temperatures, they also influence plant phenophases (Gray and Brady 2016).

2.3 The Knowledge and the Gaps

Attention to changes in phenophases as surrogate indicators has increased over the last three decades. Scopus database included 27,523 documents with the keyword “phenology” from 1921 to 2019 and 10,213 documents on “climate change” as well. Similarly, one gets 259,673 documents containing the keyword “climate change” for the period of 1910 to 2019 and 16,361 “phenology” as well. Donnelly and Yu (2017) while reviewing the growth of publications in climate change and phenology in the *International Journal of Biometeorology* observed a substantial increase in the number of publications after 1990, with two issues in 2011 and 2014 dedicated to the theme (Donnelly and Yu 2017).

Direct ground-based observations are complemented/supplemented by satellite (Cleland et al. 2007; Fitchett et al. 2015; Adole et al. 2016; Bussotti and Pollastrini 2017; Cheng et al. 2018; Blundo et al. 2018) and herbarium (Jones and Daehler 2018; Love et al. 2019). Martinez-vilalta (2018) explored the possibility of reflection of climate change in tree rings. Mathematical modelling has been a widespread approach for predicting impacts of climate change on the phenology and ecosystem/community functions (Hudson and Keatley 2010; Zhao et al. 2013; Schmidt et al. 2014; Xia et al. 2015; Yang et al. 2017; Wang et al. 2019a).

The impact of warming on bud breaking is the most frequently studied event in climate change studies (Badeck et al. 2004; Bertin 2008; Penuelas et al. 2009;

Vitasse et al. 2011, 2018; Shen 2011; Shen et al. 2015; Fitchett et al. 2015; Parmesan and Hanley 2015; Adole et al. 2016; Gray and Brady 2016; Bussotti and Pollastrini 2017; Dahlin et al. 2017; Gerst et al. 2017; Scranton and Amarasekare 2017; Kumar and Chopra 2018; Maurya et al. 2018; Chmura et al. 2019; Gillison 2019; Wang et al. 2019a). The advancement of bud breaking event is not only a prominent indicator of the overall warming in high latitudes and high elevations but also of changes in the seasonality in tropical and equatorial regions. In a few studies carried out in tropical deciduous forests, the onset of rainy season and soil moisture conditions are explained as stronger drivers of bud break than temperature (Singh and Kushwaha 2016). There are arguments in favour of as well as against the stronger role of moisture than temperature in regulating phenological events in Tibet (Yu et al. 2010; Zhao et al. 2013; Yang et al. 2017; Wang et al. 2013; Shen et al. 2015; Wang et al. 2019b). However, the researchers from Europe predict a strong relationship between the snow melt and the bud break (Vitasse et al. 2011, 2018). The emergence and longevity of leaves though are important parameters in phenology, their relationships with climate change or shift in growing period are not yet sufficiently examined (Marques et al. 2004; Zhang et al. 2006; di Francescantonio 2017; Blundo et al. 2018).

Bertin (2008) concluded that (i) leafing and flowering, which are spring events, have typically advanced, with median advances of 4–5 days per degree Celsius; (ii) leaf colouring or leaf fall, which are autumn events, are now delayed; (iii) changes in phenological events along a latitudinal gradient are more variable than elevation gradient, especially in Scandinavia and Mediterranean Europe; and (iv) precipitation changes may counter warming-driven range shifts in response to warming in some areas. Wang et al. (2019a) concluded that phenology was influenced more by temperature than precipitation regimes. Dahlin et al. (2017) identified latitude, continental location and potential evapotranspiration as the best predictors of phenological timing in semi-arid and savanna-type ecosystems across the world.

CaraDonna et al. (2014) studied flowering of 60 species of a subalpine plant community and observed the expansion of the flowering season by more than a month. However, the initiation, peak and completion of flowering rarely shifted independently, which resulted in the diversity in phenological changes through time. Jones and Daehler (2018) discerned climate change impacts on plant phenology from herbarium specimens and concluded that the length of the flowering phase was a more robust statistic as compared to mean flowering day of the year or first-flowering day of the year. Park and Mazer (2019) concluded that the rate of sequential flowering (standardised by the absolute length of the flowering season in each region) increased among the relatively early-flowering but decreased among the late-flowering taxa with an increase in mean annual temperature (MAT). Zhang et al. (2018) indicated that extreme climate events shifted the peak flowering time in 12 species studied from Macao region.

Gerst et al. (2017), while studying the phenology of *Quercus* in North America, observed that within the growing season species from western and water limited ecosystem the phenoevents such as bud break, flowering tend to occur intermittently

through the season and each event was longer in sub-tropical than that of the species from temperate zone. Gordo and Sanz (2010) while investigating plant phenology in Mediterranean ecosystems concluded that warm and dry springs under a positive phase of North Atlantic Oscillation advanced the flowering, leaf unfolding and fruiting dates and lengthened the growing season. Climatic conditions over a few weeks before a phenophase had greater impact than the average conditions over months/year.

2.4 Climate Change-Based Vegetation Models and Plant Phenology

The use of phenology as surrogate indicators of climate change became popular with the use of regional and global vegetation models based on remote sensing technology (Shen et al. 2015; Tang et al. 2016; Scheffers et al. 2016; Scranton and Amarasekare 2017; Daham et al. 2019; Wang et al. 2019a). The growing season index model was made refined for precise prediction of vegetation phenology and tested in Iraq by Daham et al. (2019). Tang et al. (2016) identified the lack of species-level knowledge a barrier in developing community/ecosystem-scale models. Wang et al. (2019b) concluded the slowing down of changes in phenology during the warming hiatus that occurred between 1998 and 2012. Yu et al. (2010) found that strong warming in winter could slow the fulfilment of chilling requirements, which may delay spring phenophases. Such a phenomenon was pronounced in regions such as the Tibetan Plateau which experienced a rapid increment in temperature and had highly temperature-responsive vegetation.

Simulation studies by Zettlemoyer et al. (2019) indicated that warming-led non-native species were likely to flower earlier and more plastic to temperature than the natives. Flowering by non-native species turned more synchronous with other community members under warming. Early flowering was also associated with range expansion of non-native species. Such differences in both timing and plasticity of phenological responses indicate the aggravation of biological invasion in the face of climate change.

Altered phenology can influence the community structure (Cleland et al. 2007). Phenology also controls feedbacks of vegetation to the climate system by influencing albedo, canopy conductance and fluxes of water, energy, CO₂ and biogenic volatile organic compounds (Richardson et al. 2013).

We need a better understanding of the drivers of phenology in under-studied biomes (e.g. tropical forests) and of the role of photoperiod, even in well-studied biomes. We have only a qualitative understanding of phenology-mediated feedbacks between vegetation and climate (Fig. 2.2) and need to quantify these feedbacks.

Indeed, our understanding of the vegetation phenology–climate change relationship has substantially improved with the passage of time (Zang 2012). Yet, many phenometric details needed for precise climate change monitoring/predictions are lacking, and we need to have more intensive studies covering all eco-regions.

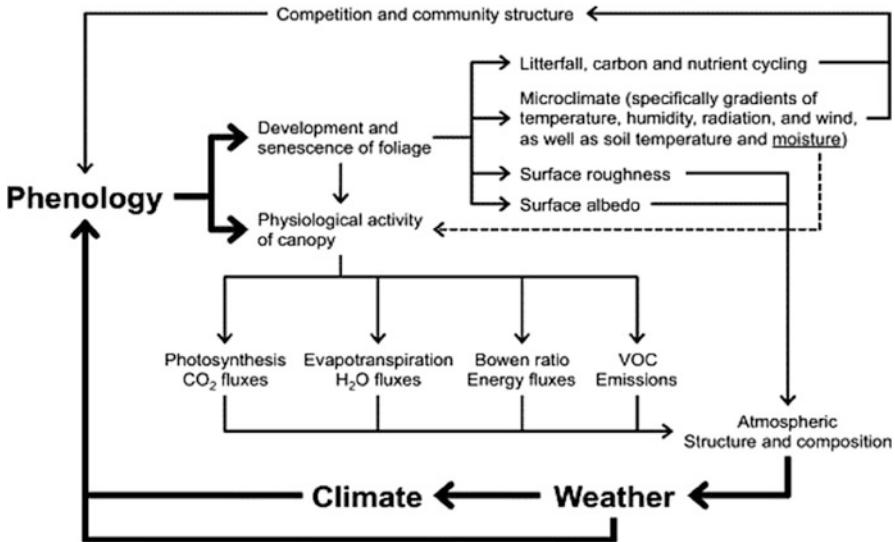


Fig. 2.2 The feedback linkages between vegetation and climate system which influence the vegetation phenology (From Richardson et al. 2013)

2.5 Ecological Implications

Predictive models used so far have not been able to give conclusive evidences to the pathways of adaptation of plants to climate change but showed some promise with the use of plant functional strategies such as Grime's C-S-R strategies (Parmesan and Hanley 2015). The sequence, frequency and duration of phenophases are related to the sensitivity of the organism to the environmental conditions occurring in the habitats they occupy. Intra- and inter-annual variations in the temperature and moisture are considered to be the major driving forces in the variation in phenophases of individual species in addition to the biotic interactions. Thus, it is important to understand the influence of climate change on the sequence, frequency and duration of phenophases of individual species and how this will influence the ecosystem structure and functional characters in various bioclimatic regions. Buitenwerf et al. (2016) reported that between 1981 and 2012, 54% of the global land surface undergone severe changes in phenology of vegetation. Herbaceous vegetation-dominant meadows showed an advancing start of the growing season (SOS), delaying end of the growing season (EOS) and increasing length of the growing season (LOS) in the eastern Tibetan Plateau which do not show moisture stress, but not in steppe and sparse herbaceous or sparse shrub regions in the western edges of the Tibetan Plateau where moisture stress is evident (Cheng et al. 2018; Suonan et al. 2019). Invasive shrub species under forest cover leafed out earlier and retained leaves longer than native species (Donnelly and Yu 2019), but no evidence

for local adaptation and epigenetic underpinning in native and non-native ruderal plants was found (Herden et al. 2019). Ettinger et al. (2018) indicated that a larger part of the variation in phenophase frequency and duration can be explained by the sequence of earlier events and by interphase durations. Thus, a shift in one phenophase will have cascading effects on those phases occurring later. Accurate forecasts of climate change impacts should therefore include multiple phenophases within and across years.

The reproductive success of a species is controlled by feedbacks from several biotic and abiotic factors (Richardson et al. 2013). The sensitivity of species to photoperiod and vernalisation requirements thus was a major area of research for understanding the reproductive ability of a species (Satake et al. 2013). Alpine plants have different temperature responses during their reproductive cycle, do not have constant thermal thresholds and heat use efficiencies to achieve the seed dispersal stage and can change their temperature sensitivity to flower along snowmelt gradients (Carbognani et al. 2018). Temperature variation strongly influenced the development rate of the pre-flowering period, and plants from later snowmelt areas flowered faster in comparison with plants from earlier snowmelt areas (Lluent et al. 2013). Plants in later snowmelt sites flowered at lower heat accumulation levels due to change in heat use efficiency along the snowmelt gradient (Matteodo et al. 2016). Higher species richness and species diversity of herbs were reported in early snowmelt microsites than in late snowmelt microsites of Himalaya (Adhikari et al. 2018). This indicates that early snowmelt in a warming climate due to climate change scenarios would promote species diversity and plant density – advanced and lengthened their vegetative and flowering phenophases.

Synchronous phenophases among species could be important for maintaining food webs and thus energy flows in an ecosystem. However, with climate change influencing the phenologies of various species differently, this synchrony of events in terms of sequence, frequency and duration is getting disturbed which is causing disturbance in the existing balance of structure and function in ecosystems. Burgess et al. (2018) indicated that increasing latitude delays phenology of all species, but more so for oak, resulting in a shorter interval between leaf emergence and peak caterpillar biomass at northern latitudes. Asynchrony found between peak caterpillar biomass and peak nestling demand of blue tits, great tits and pied flycatchers increases in earlier (warm) springs. They also reported that there is no evidence of spatial variation in the timing of peak nestling demand relative to peak caterpillar biomass for any species. Phenological mismatch alone is thus unlikely to explain spatial variation in population trends (Kharouba et al. 2018). Such trends seem to be occurring uniformly both in Northern and Southern Hemispheres (Chambers et al. 2013). Gilbert (2019) found temperature is a more parsimonious predictor of food web structure than latitude and thus suggests that food webs may be affected by a combination of biotic and abiotic conditions. The ecosystem stability and sequence of dominance by various species depend on the resource ratio hypothesis proposed by Tilman (1985). However, Linden (2018) suggests that adaptive and non-adaptive phenological synchrony is the major factor determining the stability of the trophic structure of any ecosystem. The effects of climate change-mediated asynchrony

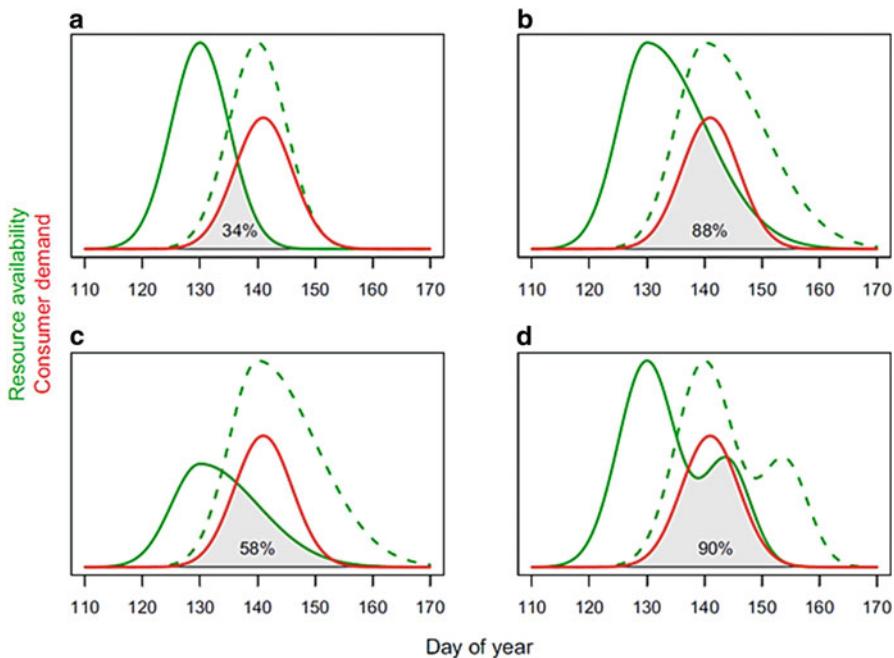


Fig. 2.3 Changes in phenological synchrony illustrated by the original phenology of resource availability (green dashed line; peak at day 140) have advanced with 10 days (green solid line; peak at day 130) under climate change scenario, while the consumer demand has stayed unchanged (red solid line; peak at day 141). Assuming no benefits of resource surplus, the intersecting area of the resource and consumer curves (grey shaded area) divided with total consumer demand (area under red curve) is a parsimonious parameter for measuring phenological match (the percentage reported). In scenario (a), both resource availability and consumer demand are symmetric and narrow; thus, phenological asynchrony strongly affects the resource supply. In scenario (b) a wide and right-skewed distribution of resource availability, asynchrony has a much smaller effect, and in scenario (c) changes in resource population abundance may prove to be controlling the asynchrony. The resource supply in nature may show multiple peaks, for example, corresponding to the phenology of several prey species (d), leading to a situation similar to that described in (b) (Adapted from Linden 2018)

between prey and predator systems could lead to ecosystem instability (Fig. 2.3) and could influence the population densities of univoltine and multivoltine species.

The ecosystem stability could be assessed by the functional stability of its energy flow or nutrient turnover which can control the net primary productivity (NPP). Solar radiation exerted stronger influence than temperature and precipitation on the NPP of alpine grasslands, but moisture played a more crucial role in alpine steppe (Zheng et al. 2020). Yu et al. (2019) concluded that temporal changes in precipitation distribution greatly influenced the rate of litter decomposition than the annual precipitation in tropical forest ecosystems. Though least understood, climate change is expected to increase the frequency and intensity of pollen allergy in several agroclimatic regions due to the lengthened growing season and longer pollen

viabilities (Ziska et al. 2011, 2019; Zhang et al. 2015). Atmospheric carbon dioxide is a major driver of total pollen concentrations beyond the effect of temperature, thus the relative contributions of global warming and carbon dioxide fertilisation on changes in the pollen season are linked (Hess 2019). Petruski et al. (2019) while studying the changes in phenology of *Cardamine concatenata* and *Erythronium americanum* over 111 years in the central Appalachians found that the plants at <500 m elevation bloomed earlier and demonstrated a stronger shift in flowering date over time than flowers at >1000 m elevations. Lower elevations, higher spring and winter temperatures and low amounts of precipitation were associated with earlier spring flowering. Urbanisation is inducing anthropogenic climate change due to the urban heat island effect on the urban vegetation. However, plant phenology showed stronger influence by the regional temperatures (Li et al. 2019).

2.6 Conclusions

The global concern of carbon management in various biomes in the climate change scenarios prompted researchers to look for comprehensive studies that provide viable indicators that could be used as phenometrics. The precision of the outcomes of the models depends on the accuracy of the studies on the relationship of environmental variables and their influence on various phenophases. Though the current understanding of temperature and moisture as the major drivers of bud break across the biomes from various latitudes provides some baseline, the need for more ecosystem-based studies from various latitudes is being felt by one and all.

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The Pistil: Structure in Relation to Its Function

3

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Abstract

The pistil, made up of the basal ovary that bears the ovules, elongated style, and terminal stigma, is the female reproductive organ of the flower. The female gametophyte, designated as the embryo sac, is located inside the ovule. The pistil is the recipient of the pollen and a conduit for the transport of the male gametes through the pollen tubes into the embryo sac. The most important outcome on the structure of the pistil in relation to its function is the presence of extracellular matrix (ECM) of heterogeneous compounds on the surface of the stigma and in the transmitting track of the pollen tubes along the style, irrespective of their morphological variations. These extracellular components play a crucial role during pollen-pistil interaction. Recent studies using molecular approaches have revealed subtle intercellular interactions among the clonal cells of the embryo sac, particularly the cells of the egg apparatus and the central cell during its differentiation to facilitate pollen tube entry and double fertilization.

Keywords

Central cell · Egg apparatus · Embryo sac · Extracellular components of style · Ovule · Solid and hollow styles · Stigma-surface components · Stigma types

3.1 Introduction

The pistil is the female reproductive organ of the flower and is unique to the flowering plants. It is made up of the basal swollen ovary that bears ovules, elongated style emerging from the tip of the ovary, and the terminal stigma. The female gametophyte, referred to as the embryo sac (ES), is located inside the ovules.

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Unlike gymnosperms, the other group of seed plants, in which the pollen grains are deposited at the tip of the ovules, in flowering plants, the pollen grains are deposited on the surface of the stigma following pollination. The stigma is thus the recipient of the pollen grains, and the style, which may exceed 10 cm in several species, is the conduit for the transport of the male gametes through the pollen tubes to the embryo sac. Although flowering plants were the last to evolve among the land plants, they became the most successful group and outdid all other land plants. The significance of such a circuitous prefertilization journey for the male gametes through the pistil on the evolutionary success of flowering plants has attracted the attention of evolutionary biologists since long (Whitehouse 1950; Mulcahy 1979). As discussed in Chap. 4, evidences indicate that the evolution of the pistil is one of the contributing factors for the evolutionary success of the flowering plants. The pistil performs the function of pollen screening by inhibiting incompatible pollen and promoting only the compatible pollen tubes to reach the ovules. Even among compatible pollen, the pistil selects the most vigorous pollen for fertilization by creating intense competition among them, thus increasing the fitness of the progeny.

Extensive studies have been carried out since the beginning of the twentieth century on the reproductive events such as the development of the male and female gametophytes, double fertilization, and the development of the embryo, endosperm, and fruits and seeds in a large number of species (Maheshwari 1950, 1963). However, in spite of the importance of the pistil in sexual reproduction, there were hardly any investigations on the structural details of the pistil in relation to its function until 1970s. Since then, detailed studies have been carried out on the structural and functional details of the pistil of a large number of species and many generalizations which play crucial roles in pollen germination and pollen tube growth have emerged. This chapter briefly highlights the structural details of the pistil, including that of the ES in relation to its function. Developmental aspects of the ovule and different types of embryo sacs are not included as there is not much new information on these aspects and are readily available in several books (Maheshwari 1950, 1963; Johri et al. 1992; Shivanna and Johri 1985; Shivanna 2003).

3.2 The Stigma

The stigma shows enormous morphological diversity. The first comprehensive studies on the structural details of the stigma were carried out at the Royal Botanic Gardens, Kew, London, in the mid-1970s. Based on the studies of over 1000 species, Heslop-Harrison and Shivanna (1977) categorized all the prevailing morphological variations of the stigma into five groups based on the presence or absence of the secretion and of the papillae on the receptive surface of the stigma (Table 3.1). In taxa characterized by the wet stigma, the surface is covered with a secretion, termed stigmatic exudate (Fig. 3.1a), at the receptive stage, which may be scanty or abundant. The exudate may be lipoidal as in members of Solanaceae and Leguminosae or aqueous as in members of Liliaceae. In the dry type, there is no

Table 3.1 Types of stigma (Based on Heslop-Harrison and Shivanna 1977; Shivanna 2003)

Dry stigma (without apparent fluid secretion on the surface during the receptive period)

Group I: Plumose – receptive surface dispersed on multiseriate branches

Group II: Receptive surface confined to differentiated stigma

A: Surface non-papillate

B: Surface papillate

1. Papillae unicellular

2. Papillae multicellular

a. Papillae uniseriate

b. Papillae multiseriate

Wet stigma (fluid secretion present on the stigma surface during the receptive period)

Group III: Receptive surface papillate

Group IV: Receptive surface non-papillate

Group V: Receptive surface covered with copious exudate in which detached secretory cells of the stigma are suspended

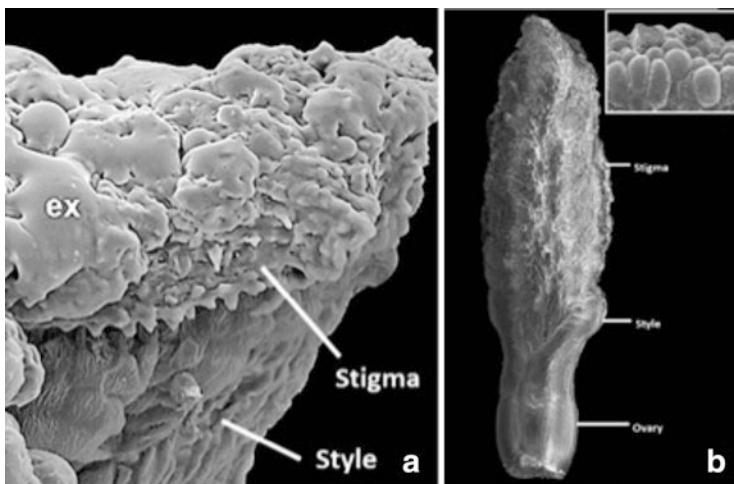


Fig. 3.1 Scanning electron micrographs of stigmas. (a) Wet stigma of *Utricularia*. The stigmatic surface is covered with exudates (ex). (b) Dry stigma of *Hippophae rhamnoides*. A part of the stigma is enlarged in the inset. (Photo credit: a, Anjali Chaudhary; b, Yash Mangla)

visible exudate on the stigma surface (Fig. 3.1b). The stigma surface may be covered with papillae (papillate) or smooth (non-papillate). Papillate stigmas are further divided on the basis of the nature of the papillae – unicellular or multicellular and uniseriate and multiseriate.

Studies of Heslop-Harrison and Shivanna (1977) also revealed that irrespective of the morphological variations of the stigma, extracellular matrix (ECM), secreted by the cells of the stigma, is invariably present on the receptive surface of the stigma. In

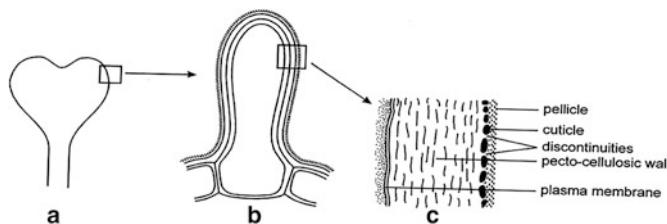


Fig. 3.2 Diagrammatic representation of the dry stigma to show the pellicle. (a) Outline of the stigma. (b) One of the papillae enlarged from the blocked region in a. (c) A part of the wall of the stigmatic papilla blocked in b enlarged to show the details. (After Shivanna 1977)

the wet stigma, ECM is in the form of the stigmatic exudate, and in the dry type, it is in the form of a thin extracuticular layer, termed pellicle, on the stigma surface (Fig. 3.2). ECM is highly heterogenous and is made up of proteins, carbohydrates, amino acids, and phenolics; it also responds to several enzymes such as esterases, phosphatases, and peroxidases (see Shivanna 2003). Subsequent studies, apart from confirming the generalization about the presence of the ECM on the receptive surface of the stigma of all the taxa so far investigated, have shown that the ECM plays a very important role in pollen-pistil interaction (see Chap. 4). Further, analyses of the structural details of the stigma (Heslop-Harrison and Shivanna 1977) indicated several correlations between the type of the stigma and the cytology of the pollen grains (two- or three-celled) as well as the genetics of self-incompatibility. Species with dry stigma are generally associated with sporophytic type of self-incompatibility and three-celled pollen. Species with wet stigma are associated with gametophytic type of self-incompatibility and may have two- or three-celled pollen. Members of Poaceae are exceptions in having dry stigma with gametophytic self-incompatibility and three-celled pollen.

3.3 The Style

The style is traditionally distinguished into the solid and hollow types. In the solid style, present in a majority of dicots, a core of transmitting tissue connects the stigma with the ovarian cavity, and in the hollow style, a canal, termed stylar canal, connects the stigma surface with the ovarian cavity (Fig. 3.3). The transmitting tissue of the solid style is made up of narrow, elongated cells, connected end to end by cell walls traversed by plasmodesmata, but separated laterally by massive intercellular spaces filled with ECM. The ECM of the transmitting tissue is also made up of heterogeneous compounds such as pectins, carbohydrates, proteins, glycoproteins, and often lipids. Pollen tubes invariably grow through this massive intercellular spaces filled with ECM and not through the cells of the transmitting tissue. In the hollow style, the stylar canal is lined with one or a few layers of glandular cells, termed the canal cells. The canal cells also secrete ECM which may fill the entire canal or form a layer on the inner tangential wall of the canal cells. In the former, the cuticle bordering the

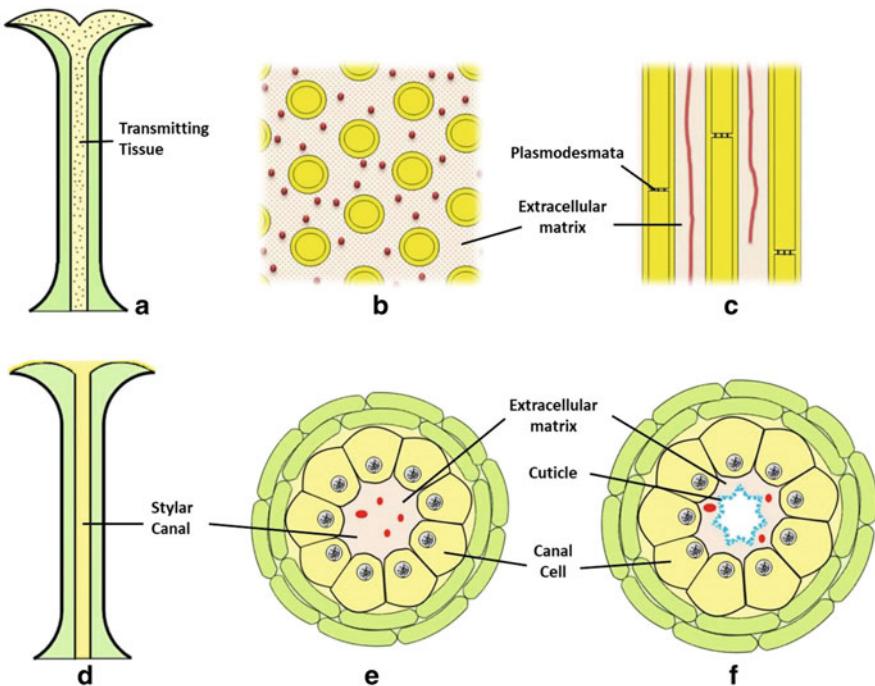


Fig. 3.3 Diagrammatic representation of solid (a–c) and hollow (d–f) styles. (a) Longitudinal sections of solid style with the continuous strand of transmitting tissue. (b, c) Transverse and longitudinal sections of a part of the transmitting tissue to show massive intercellular spaces filled with extracellular matrix. Elongated cells are connected through plasmodesmata in the transverse walls but not along the longitudinal walls. Pollen tubes (shown in red) are clear in the extracellular matrix. (d) Longitudinal section of the hollow style with a continuous stylar canal. (e, f) Transverse sections of the style showing a layer of canal cells. In E, the cuticle bordering the stylar canal is disrupted, and the canal is filled with extracellular matrix. In F, the cuticle remains intact, and the matrix accumulates between the canal cells and the cuticle. Pollen tubes are represented as red patches. (Modified from Shivanna 2003)

canal cells is disrupted, but in the latter, the cuticle is pushed toward the center of the stylar canal. Pollen tubes grow through the ECM filling the canal or forming a lining on the inner tangential wall of the canal cells. The glandular cells of the transmitting tissue (in solid styles) and canal cells (in hollow style) continue into the ovary along the placenta providing continuity in the path of the growing pollen tubes up to the ovules.

Papilionoid legumes, so far investigated, show a combination of features of solid and hollow styles (Ghosh and Shivanna 1982; Malti and Shivanna 1984; Lord and Heslop-Harrison 1984, see Shivanna and Owens 1989). The stigma is solid, but the style is hollow. A narrow stylar cavity develops in the upper part of the style, just below the stigma due to the degeneration of the central cells of the transmitting tissue; the cavity gradually enlarges further down, and the style becomes typically hollow bordered by one or a few layers of the canal cells. Thus, the upper part of the

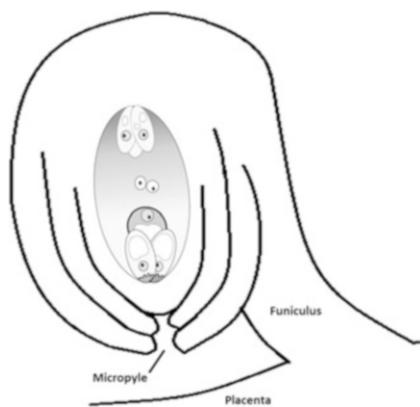
hollow style develops due to the cellular degeneration, and the lower part of the style seems to be the extension of the ovarian cavity. In some members, the lowermost part of the stylar canal is loosely filled with the papillae formed by the extension of the canal cells (Malti and Shivanna 1984).

3.4 The Ovule and Embryo Sac

The ovule houses the embryo sac (ES), the female gametophyte, which is the site of fertilization and development of the embryo, endosperm, and seed. A comprehensive account of ovule development in the light of molecular information, particularly in the model system *Arabidopsis thaliana*, is given by Skinner et al. (2004). Ovules arise from the placental region of the ovary. The ovule is essentially made up of one or two integuments, the nucellus and the ES. The nucellar tissue may be massive (crassinucellate) or confined to a few layers around the ES (tenuinucellate). The integuments do not cover the nucellus fully but leave a narrow passage at the tip called the micropyle, through which the pollen tube enters the ovule. Traditionally, six types of ovules are distinguished based on their orientation with reference to the placenta – orthotropous, anatropous, campylotropous, amphitropous, hemianatropous, and circinotropous (see Maheshwari 1950, 1963; Shivanna 2003). Only the anatropous ovule, the most common and extensively studied type in which the ovule is inverted thus bringing the micropyle closer to the placenta (Fig. 3.4), is discussed here.

The megasporangium mother cell differentiates in the hypodermal region of the nucellus at the micropylar region of the ovule; it is distinguishable from its neighboring cells by its large size and dense cytoplasm. It undergoes meiosis giving rise to four haploid cells or nuclei. There is great diversity in the structural details of the embryo sac based on the number of megasporangia/megasporangium nuclei taking part in ES development – monosporic, bisporic, and tetrasporic. The polygonum type of ES, discussed here, is the most common type and is reported in >70% of the flowering

Fig. 3.4 An anatropous ovule with Polygonum type of embryo sac



plants including those of Brassicaceae, Malvaceae, Leguminosae, Poaceae, and Solanaceae (see Yadegari et al. 2004). In the Polygonum type of ES, meiosis of the megasporangium mother cell results in a linear tetrad of four megasporangia. Of these, three micropylar megasporangia degenerate, and only the chalazal megasporangium develops into an eight-nucleate structure by undergoing three mitotic divisions of the microspore nucleus followed by cellular organization to give rise to seven-celled ES (Fig. 3.4). The ES is a polarized structure; the micropylar pole, located near the micropyle, contains the egg apparatus (with the egg and two synergid cells), and the opposite chalazal pole bears the three antipodal cells. The large central cell with two polar nuclei, one coming from the upper quadrat and the other from the lower quadrat, is highly vacuolated with its cytoplasm confined to a thin layer along the wall. Even the egg and the synergids are polarized; the nucleus in the egg cell occupies the chalazal end and the micropylar end is occupied by the vacuole, while in the synergids, the nucleus occupies the micropylar end and the vacuole occupies the chalazal end. The central cell is not very active metabolically, and the antipodals in most of the species are ephemeral and degenerate by the time of fertilization. The synergids develop extensive wall ingrowths at the micropylar region referred to as the filiform apparatus (FA). FA enormously increases the surface area of the plasma membrane. The synergids contain a large number of mitochondria and dictyosomes and, thus, are metabolically the most active cells of the ES. The cell wall of the egg and the synergids attenuates toward the chalazal pole; they are separated from the central cell by their respective plasma membranes. During the initial stages of the ES development, the polar nuclei of the central cell in *Torenia* and *Arabidopsis* occupy the central position. By the time of the pollen tube arrival, the central cell undergoes cellular reorganization, and the polar nuclei move to the micropylar region closer to the chalazal part of the egg apparatus (see Waterings and Russell 2004).

In recent years, significant progress has been made in understanding some of the molecular processes involved during ovule and ES development by using the techniques of molecular biology and genetics. Studies on ovule mutants particularly of the model organism, *Arabidopsis thaliana*, have contributed to our understanding of the role of many genes involved in ovule morphogenesis (see Gasser et al. 1998; Skinner et al. 2004). Although the ovule is morphologically a simple structure, a number of genes seem to be involved in its initiation and differentiation (Skinner et al. 2004). Retinoblastoma homolog RBR in *Arabidopsis* (Ebel et al. 2004) and indeterminate gametophyte in maize seem to be necessary to restrict nuclear proliferation in the ES; their respective mutants have been shown to give rise to supernumerary nuclei. Evidences indicate that the micropylar-chalazal polarity is established by the accumulation of an auxin at the micropylar region of the nucellar tissue during the initial stages of ES formation.

Han et al. (2000) were able to microinject water-soluble fluorescent tracers of various molecular weights into the central cell of the ES of *Torenia fournieri* during its developmental stages and follow the movement of the tracer. These studies showed that the movement of tracers depends on the developmental stage of the ES and the size of the tracer molecules. When the ES is young, fluorescent tracers up to 10 kDa were able to pass from the central cell into the cells of the egg apparatus.

However, the tracers with larger molecular weights remained confined to the central cell. As the ES matures, symplastic connectivity decreased, and only tracers <3 kDa could move from the central cell to the egg cell. These studies also revealed that the ES is symplastically isolated from the surrounding sporophytic cells of the ovule. In a study involving the identification and analysis of 31 new defective ES mutants in *Arabidopsis*, Christensen et al. (2002) identified a mutant gfa2 with a defect in the death of a synergid cell as well as a defect in the fusion of polar nuclei. In the mutant in which the synergid failed to degenerate before the arrival of the pollen tube near the ovule, there was no fertilization. Thus, the results clearly indicate that the *GFA2* gene product is required for synergid cell death prior to fertilization process.

Some information is also available on the mechanism of differentiation of egg and accessory cells of the ES. Groß-Hardt et al. (2007) screened MES-induced mutants in *Arabidopsis* and isolated *lachesis* (*lis*) mutants which formed supernumerary egg cells. In *lis* mutants, the synergid cells also differentiated into gametic cells. Thus, *LIS* prevents synergid cells to follow gametic cell fate. Until cellularization of the ES, there was no difference between the mutant and the wild type. After cellularization, the polarity of the synergids was reversed in the mutants; the nucleus of the synergids occupied the chalazal position similar to that of the egg cell, and their ability to attract pollen tube was compromised. Differences in the cellular characteristics of the central and antipodal cells were also observed in *lis* mutants (see Groß-Hardt et al. 2007).

Lawit et al. (2013) used laser-assisted cell ablation as well as complementary genetic cell ablation by means of barnase in developing ES in *Arabidopsis*. Ablation of egg cell changed the developmental fate of a synergid to become the egg- or embryo-like cell. It was also possible to manipulate the fate of somatic cells of the ovule to become the egg or cells similar to adventitious embryos. This may open an effective approach for engineering somatic apomixis, which is being attempted in different laboratories around the world to fix hybrid vigor which would greatly help the seed industry. Further, ablation of the egg cell resulted in the loss of the synergid's ability to attract pollen tube; this was associated with the reduction of LURE activity, responsible for pollen tube attraction (see Chap. 4, Susaki et al. 2015). Another interesting variation observed by these authors is a shift in synergid polarity with respect to the position of the nucleus.

3.5 Conclusions

The pistil performs the most important function of selecting only the compatible pollen grains for fertilization. The extracellular components on the stigma surface and along the path of the pollen tubes are involved in this function (see Chap. 4), although the molecular details are yet to be fully understood. The life of the embryo sac, although short and made up of a limited number of clonal cells, each cell/groups of cells differentiate to perform diverse functions through effective cell-cell communication and programmed cell death. Our understanding of the molecular details involved in differentiation of ES and its coordinated function has started emerging in

recent years and is largely the outcome of studies of a large number of mutants generated in *Arabidopsis* and maize. Hopefully, such integrated investigations in the coming years would lead to a fuller understanding of the structure of the stigma, style, ovule, and ES in relation to their function.

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Pollen-Pistil Interaction and Fertilization

4

K. R. Shivanna

Abstract

Pollen-pistil interaction covers the sequential events from pollination until the pollen tubes enter the ovules. During this interaction the pistil screens the pollen grains. Pollen of other species are inhibited at the level of pollen germination or pollen tube growth in the style. Conspecific pollen grains are recognized by the stigma and activate physiological processes to facilitate their germination and pollen tube growth. In self-incompatible species, even self-pollen are recognized and inhibited. Only compatible pollen grains which are most vigorous are selected for fertilization. Thus, there is a close dialogue between the gametophytic partners (pollen grains and pollen tubes) and the sporophytic partner (the pistil) during pollen-pistil interaction. Extracellular components present in and on the pollen wall and on the stigma and along the path of the pollen tube in the style are involved in pollen recognition and their subsequent promotion/inhibition. Some of the genes/gene products involved in these interactions have been identified in recent years in a few model systems.

After entering the ovary, pollen tubes are guided towards the micropyle of the ovule by the attractants secreted by the synergids of the embryo sac (ES). The nature of these attractants is known in some model systems such as *Torenia* and *Arabidopsis*. The pollen tube enters the ES through one of the synergids and discharges the two male gametes for fertilization. Some of the coordinated details amongst the cells of the ES in orchestrating pollen tube guidance, release of male gametes, double fertilization and cessation of the secretion of pollen tube attractants are discussed.

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Keywords

Embryo sac · Double fertilization · Pollen competition · Pollen germination ·
Pollen tube growth · Pollen tube guidance · Pollen screening

4.1 Introduction

Following pollination, pollen grains land on the stigma. If pollen grains are of the right type, they germinate on the stigma and the resulting pollen tubes grow through the tissues of the stigma and style and enter the ovary. In the ovary, each pollen tube enters an ovule and eventually the embryo sac (ES) and discharges the two non-motile male gametes. One of them fuses with the egg to give rise to the zygote, and the other fuses with the secondary nucleus located in the central cell to give rise to the primary endosperm cell. The occurrence of two fertilization events is another unique feature of the flowering plants. The zygote produces the embryo, and the primary endosperm cell produces the endosperm, a nutritive tissue for the embryo; the ovule develops into the seed and the ovary into the fruit, thus completing the events involved in sexual reproduction.

Pollen-pistil interaction covers the sequential events from pollen germination until the pollen tubes enter the ovules. During this phase, there is a continuous dialogue between the pollen grains and the stigma and later between the pollen tubes and the tissues of the transmitting tissue/canal cells. The evolution of the male gametophyte in the form of the pollen grain and the retention of female gametophyte inside the ovule made seed plants independent of water for fertilization, and this was the crucial step in the evolution of land plants. The evolution of the flower in angiosperms, the most successful group of land plants, made the receipt of pollen grains, particularly from other plants, more efficient and thus enabled them to increase genetic diversity which facilitated their ability to adapt to new environments. Intensive studies to understand the structural and functional details of pollen-pistil interaction were initiated only in the 1970s, and this area is presently an active field of integrated studies using the modern techniques of cell and molecular biology and genetics. There has been a considerable progress in understanding cellular interactions involved in double fertilization in recent years. This chapter covers largely the recent studies on pollen-pistil interaction and fertilization. The reader is referred to many earlier books for classical details (Maheshwari 1950, 1963; Johri et al. 1992).

4.2 Selection of Suitable Pollen Grains for Fertilization

Sexual reproduction involves the fusion of the male and female gametes. The site of fertilization is highly variable in different groups of plants. Broadly the fertilization may take place externally in the aqueous medium or internally inside the female reproductive organ. One of the fundamental requirements of sexual reproduction of

any organism is that fertilization has to take place between the gametes of the same species which is essential to maintain the integrity of the species. Plants and animals have evolved elaborate mechanisms to ensure fusion of gametes of the same species and to prevent fusion of gametes of different species. This requires the ability of the gametes of the same species to recognize each other and facilitate fusion. Gametes from different species fail to recognize each other and cannot fuse. In thallophytes, the motile gametes are released into the surrounding aqueous medium and come in direct contact with each other; if they belong to the same species, they establish recognition and fuse, and if they come from different species, they cannot establish recognition and thus cannot fuse. In bryophytes and pteridophytes, the female gametes are non-motile and are retained inside the archegonium; however, the male gametes are released into the surrounding aqueous medium. The male gametes, being motile, eventually reach the egg inside the archegonium; if it is from the same species, recognition is established between male and female gametes and fusion is ensured. In seed plants male gametes are not released into the surrounding medium, but male gametophytes (i.e. pollen grains), which act as vehicles for the transmission of male gametes, are released into the atmosphere. In gymnosperms, the archegonia containing the egg develop inside the ovule, a sporophytic tissue. Pollen grains are deposited in the pollen chamber by the wind and/or insects. Pollen grains produce short pollen tubes which pass through a few-layered sporophytic nucellar cells and release the male gametes near the archegonia. It is not yet clearly established whether the gamete recognition is established at the level of the gametes or pollen grains themselves are recognized and inhibited during their germination or passage of pollen tubes through a few-layered diploid nucellus ([Hagman 1975](#)).

In flowering plants, pollen grains are deposited on the stigma, and there is a massive interaction between the haploid pollen grains and later pollen tubes and the massive sporophytic tissues of the pistil during pollen-pistil interaction. As elaborated below, pollen grains are recognized during this interaction; incompatible pollen grains and/or pollen tubes are inhibited on the stigma or in the style before they reach the ovary. Thus the pistil screens the pollen grains and permits only the right type of pollen grains to enter the ovules and effect fertilization (see [Shivanna and Johri 1985](#); [Shivanna 2003](#)).

4.2.1 Rejection of Heterospecific Pollen

Pollen grains that land on the stigma following pollination are diverse in origin; they may belong to the same species (conspecific) or other species (heterospecific). Even when they are conspecific, they may come from the same plant as that of the pistil (self-pollen) or from other plants of the same species (cross-pollen). Therefore, heterospecific pollen grains need to be prevented from taking part in fertilization. The rejection of heterospecific pollen, in general, is passive as they are not recognized by the pistil. The pistil can be visualized as the home of the female partner with a series of locked doors. Pollen grains from the same species have the matching keys to open these locks. As the new species diverges from the parent

species during speciation, the new species becomes more and more distantly related; pollen of the new species lose more and more matching keys to the locks present in the pistil. Depending on the loss of specific keys to the locks located at different levels of the pistil, pollen grains are inhibited on the stigma itself or at different levels in the style. Such passive rejection is often termed as incongruity (Hogenboom 1975).

4.2.2 Rejection of Self-Pollen

Another requirement of sexual reproduction is that it should maintain some genetic diversity amongst individuals of the population. This is achieved by discouraging fertilization from self-pollen and encouraging the same by outcross pollen (from other plants of the same species). Plants have evolved a series of devices to achieve this objective. Self-incompatibility (SI) is one such outbreeding devise present in about half of the flowering plant species (de Nettancourt 1977; Shivanna 2003). SI is the inability of a hermaphrodite plant to effect fertilization upon self-pollination. In this system self-pollen grains are actively recognized by the pistil; following recognition the pistil activates physiological processes to inhibit self-pollen at the level of its germination or during pollen tube growth through the pistil. In a majority of the species, SI is controlled by multiple alleles, termed S_1, S_2, \dots, S_n at one locus. When the S allele of the pollen grain matches with that of the pistil, pollen inhibition is activated by the pistil. For example, the plant with S_1 and S_2 genotypes produces half the pollen with the S_1 allele and the other half with the S_2 allele. When they land on the pistil of the same plant, the interaction takes place between the haploid pollen and the diploid tissues of the pistil. Thus both types of pollen grains are recognized since the pistil has both the alleles and are inhibited. SI has evolved in lower groups of plants also. For example, in *Chlamydomonas* SI is based on + and – system. The interaction takes place at the level of the gametes; + gamete cannot fuse with another + gamete, and similarly – gamete cannot fuse with another – gamete. However, + gamete can fuse with the – gamete, although they may be coming from the same sporophyte. Thus in SI species, where interaction takes place at the gametic (haploid) level, fusion of only half of the self-gametes can be prevented. In flowering plants where the recognition of the pollen carrying self-gametes takes place at the level of the diploid tissues of the pistil, all self-pollen grains are inhibited. Thus, by the evolution of SI, flowering plants have become 100% outbreeders by the involvement of the pistil.

4.2.3 Screening of Conspecific Pollen for Quality

Apart from compatibility, the pistil also screens even compatible pollen grains for quality. Under optimal pollination conditions, the number of pollen grains deposited on the stigma is generally many times more than the number of ovules available for fertilization. Thus, all compatible pollen would not be able to find an ovule to

fertilize. Pollen grains have to compete with each other to succeed in fertilization (Mulcahy 1979, 1984). Under such a condition, pollen grains which are more vigorous (able to germinate early and produce faster growing pollen tubes) are able to reach the ovules earlier and effect fertilization; less vigorous pollen grains are eliminated in this competition. This is often referred to as sexual selection (Tejaswani et al. 2001). As cross-pollination takes place to a limited or greater extent in most of the species, there is a considerable genetic variability in the pollen population that land on the stigma on which pollen competition and selection can operate. There is no scope for such a competition amongst male gametes in lower groups of plants where recognition occurs at the level of the gametes. Any compatible gamete that is able to reach the female gamete earlier, irrespective of its vigour, would be able to achieve fertilization. The adaptive significance of such pollen competition in increasing the fitness of the progeny has been shown in several systems (see Shivanna 2003).

Flowering plants, although last to evolve about 110 million years ago, became the most successful and dominant group of land plants. It has been suggested that (a) evolution of the flower which made biotic pollination most efficient and (b) evolution of the pistil that enabled flowering plants to become the most efficient outbreeding SI systems and induced intense pollen competition even amongst compatible pollen have been considered to be the causative factors responsible for the evolutionary success of the flowering plants (Whitehouse 1950; Mulcahy 1979; see Shivanna 2003).

4.3 Pollen Viability and Stigma Receptivity

Viability of the pollen and receptivity of the stigma at the time of pollination are critical requirements for effective initiation of pollen-pistil interaction. Pollen viability is the ability of the pollen to perform its function of delivering functional male gametes into the ES on a compatible, receptive stigma. Generally, pollen grains show maximum viability soon after anther dehiscence. The period for which they retain viability after dispersal varies greatly depending on the intrinsic nature of the pollen species and the prevailing environmental conditions. In general, two-celled pollen grains retain viability for a longer period when compared to three-celled species (Brewbaker 1967; Shivanna 2003). Pollen of some three-celled species, especially members of Poaceae, lose viability rapidly, often within 30 min under dry conditions. On the other hand, pollen of many two-celled species such as members of Leguminosae and Solanaceae retain viability for several weeks even under field conditions. The possible causes for the loss of viability and various methods available to test pollen viability are discussed by Shivanna (2003) and Shivanna and Rajesh (2014).

Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen. In general, pollen viability and stigma receptivity are at their respective peaks in freshly opened flower. The period for which the stigma retains viability varies greatly, from a few hours to a few weeks (Losada et al. 2014).

Pollinated flowers initiate senescence within a day or two. Unpollinated flowers, however, tend to retain receptivity for much longer periods. For example, unlike pollinated flower, unpollinated flower of *Petunia* remains fresh and retains stigma receptivity for up to a week. Similarly, unpollinated flowers of several orchids are reported to remain fresh and retain stigma receptivity for several weeks/months.

In protandrous species the stigma becomes receptive several hours or days after pollen shedding, and in protogynous species the stigma becomes receptive several hours or days before pollen grains are shed. For various tests available for assessing stigma receptivity, refer to Shivanna and Rajesh (2014).

4.4 Recognition of Conspecific Pollen

As pointed out earlier, conspecific pollen grains have to be recognized by the pistil and activate physiological processes to facilitate pollen germination and pollen tube growth. Until the 1970s, we had no knowledge about the location and the nature of these recognition factors in the pollen or in the pistil. Initial studies on these lines were largely from the laboratory of Heslop-Harrison at the Royal Botanic Gardens, Kew (Knox and Heslop-Harrison 1970; Heslop-Harrison 1975; Knox et al. 1972, 1975; see Shivanna 2003). The studies showed that the pollen wall, intine as well as exine, and the pollen surface contain extracellular components (Fig. 4.1a, b) made up

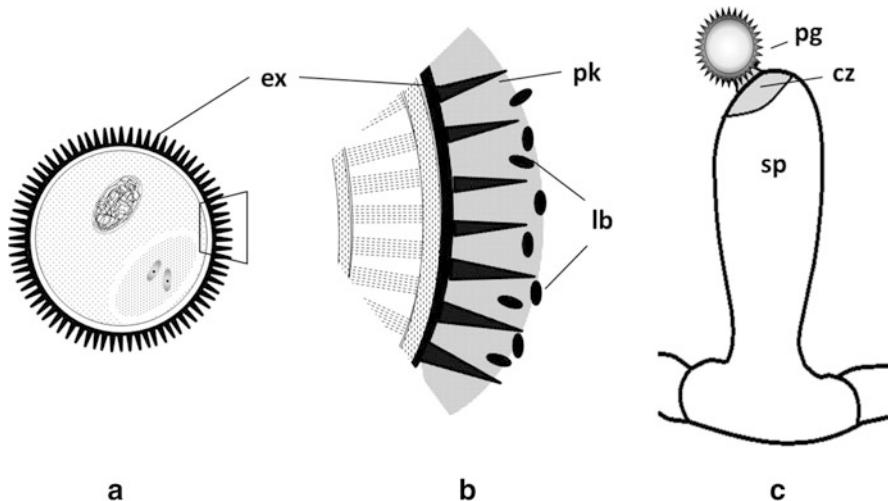


Fig. 4.1 (a, b) Pollen wall proteins. Sectional view of a pollen grain (a) and a part of the pollen wall blocked in (a) enlarged to show the details (ex exine, lb lipid bodies, pk pollen kitt). Stippled part on the inner side of exine is intine. Exine proteins are present between exine spines, and intine proteins are dispersed throughout the intine. (c) A pollen grain (pg) in contact with the stigmatic papilla (sp) of a dry stigma. Pollen lipids establish a contact zone (cz) with the pellicle on the surface of the papilla and provide a medium for the establishment of water gradient for pollen hydration and germination

of a number of heterogenous materials such as carbohydrates, lipids, proteins, glycoproteins including several enzymes, carotenoids and phenolics. The intine components are synthesized by the pollen cytoplasm and are incorporated into the intine during pollen development; thus they are gametophytic in origin. The exine proteins and pollen coat substances present on the pollen surface are synthesized in the surrounding tapetal cells in the developing anther and are incorporated into the pollen exine as pollen coat substances following the breakdown of the tapetum; thus they are sporophytic in origin. As discussed in Chap. 3, the stigma surface, irrespective of the morphological variations of the stigma, invariably contains surface components (Heslop-Harrison and Shivanna 1977; see Shivanna 2003). Subsequent studies have shown that pollen wall and stigma surface components play important roles in pollen-pistil interaction including pollen recognition followed by their promotion or inhibition (Shivanna 2003; Edlund et al. 2004; Sanchez et al. 2004; Zheng et al. 2018).

4.5 Promotion of Compatible Pollen

4.5.1 On the Stigma Surface

The most critical events that occur on the surface of the stigma are pollen adhesion, hydration, germination and pollen tube entry into the stigma. Pollen adhesion is more critical on the dry stigma when compared to that on the wet stigma. Many studies have shown that pollen adhesion on the dry stigma depends on the nature and amount of the components of the pellicle and the pollen coat substances. The presence of adhesive carbohydrates especially arabinogalactans has been reported on the surface of the stigmas of some species such as *Gladiolus* (Clarke et al. 1979). Amongst the pollen coat substances, lipidic and protein components seem to play a role in pollen adhesion. In *Arabidopsis*, the stigma has been shown to bind its own pollen with greater affinity than pollen from related species (Zinkl et al. 1999), and many mutants with defects in pollen coat substances with less adhesive ability have been isolated (Zinkl and Preuss 2000). In *Brassica*, pollen coat substances are released onto the stigma surface following pollination and form a meniscus (contact zone) at the interphase (Fig. 4.1c) facilitating effective interaction of the pollen coat and the stigma surface components (see Dickinson 1995). The meniscus, apart from facilitating pollen adhesion, is also involved in pollen hydration by sending appropriate signals for the movement of water from the stigma to the pollen. Pollen grains from which pollen coat has been removed fail to hydrate. Long-chain lipids seem to act as signals in pollen hydration (Preuss et al. 1993; Hulskamp et al. 1995b). In recent years aquaporins, water channel proteins, have also been implicated in pollen hydration (Ikeda et al. 1997; Sanchez et al. 2004; Di Giorgio et al. 2016). In species with the wet stigma, pollen adhesion is not critical; pollen grains effectively adhere to the exudate irrespective of the nature and quantity of the pollen coat substances.

Traditionally the stigma is considered to provide all the requirements needed for pollen germination (Fig. 4.2a) including inorganic components such as boron and

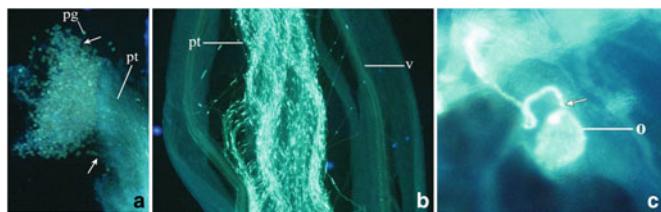


Fig. 4.2 (a–c) Fluorescence micrographs of pollinated pistil in *Wrightia tomentosa*. (a) Stigmatic region with profuse pollen germination (pg) and pollen tubes (pt) entering the style. (b) Stylar region showing a bundle of pollen tubes (pt); v is the vasculature. (c) Ovary region showing a pollen tube (arrow) entering the ovule (o). (Photographs credit: Chandan Barman)

calcium which are generally required for pollen germination. In *Vitis vinifera*, plants grown on boron-deficient soil contain hardly any perceptible amount of boron in the stigma, and such stigmas fail to promote pollen germination (Gartel 1974). The presence of high levels of calcium in the stigma and its transport to the pollen grains have been demonstrated (Bednarska 1989, 1991). In the dry type of the stigma, pellicle components seem to be involved in facilitating pollen germination. Washing the stigma with a detergent removes the ability of the stigma to support pollen germination (Knox et al. 1976). Treatment of the stigma with a lectin, which binds to specific carbohydrates of the pellicle, does not inhibit pollen germination but inhibits pollen tube entry into the stigma. In *Raphanus*, enzymatic digestion of the pellicle reduces pollen germination but inhibits pollen tube entry into the stigma. These results indicate that the pellicle components are composed of many components, some of them are involved in facilitating germination and others in pollen tube entry (see Shivanna 2003). Additional studies have shown the involvement of some of the specific components of the pollen wall and the stigma surface. Flavonoids of pollen extracellular matrix (ECM) are involved in pollen germination and pollen tube growth. Pollen grains from flavonoid-deficient mutants of maize (Coe et al. 1981) and *Petunia* (van der Meer et al. 1992) failed to function on the stigma of the flavonoid-deficient mutants. However, the addition of kaempferol to the pollen of the mutant stigma restored pollen germination and resulted in seed set (see Shivanna 2003).

Pollen tube entry into the stigma is another important post-pollination event. In dry stigmas, the cuticle which acts as a physical barrier has to be digested by the pollen tube by activation of cutinases. Several studies have shown the enhanced activity of esterases at the region of pollen tube entry, and it is likely to represent cutinase activity (Heslop-Harrison 1979; Sastri and Shivanna 1979). In the wet stigma type, however, cuticle is eroded during the secretion of the exudate and does not form a barrier for pollen tube entry into the extracellular matrix of the transmitting tissue. Long-chain lipids present on the stigma/pollen surface have also been shown to be involved in pollen hydration, germination and pollen tube guidance into the stigma.

4.5.2 In the Style

The style is a conduit for the growth of pollen tubes that carry the male gametes in their long journey to the ES located inside the ovule (Fig. 4.2b). To perform this function, there has to be a precise co-ordination between the pollen tube and the sporophytic tissues of the pistil. Following the entry of pollen tubes into the style, they are in direct contact with the extracellular matrix (ECM) of the transmitting tissue or the secretions of the canal cells during their growth through the style (see Chap. 3). Pollen tubes have to produce enormous amount of pollen wall material for them to travel for long distances, often >10 cm, before they could reach the ES. Obviously the amount of nutrients present in the pollen is totally inadequate to cope with pollen tube growth requirement (Heslop-Harrison and Heslop-Harrison 1987). The pistil provides the required nutrients for the growth of the pollen tubes. The uptake of carbohydrates from the pistil and their utilization for the synthesis of pollen wall material have been shown by labelled studies (Kumar and Hecht 1970; Kroh and Helsper 1974). In *Nicotiana* transmitting tissue-specific (TTS) proline-rich glycoproteins, encoded by two genes (TTS 1 and TTS 2), have been identified and implicated in pollen tube nutrition (Cheung et al. 1993, 1995; Wu et al. 1995). Some other ECM components have also been implicated in actively facilitating pollen tube growth by way of biochemical recognition-adhesion system analogous to migrating cells in animal systems (Sanders and Lord 1992; Lord 2001). Although the presence of proteins in the walls of the growing pollen tubes and their release during their growth are well documented, the details of their interactions are yet to be worked out (Rubinstein et al. 1995).

4.6 Pollen Tube Guidance

4.6.1 Stigma and Style

The pollen tubes, during their journey through the pistil, invariably grow along their predetermined path unidirectionally from the stigma to the ovary, irrespective of the orientation of the flower. Since long it was thought that there are some chemotropic factors that guide the pollen tube growth. In tobacco, the lipids present in the stigmatic exudate play a role in guiding the pollen tube growth into the stigma. Transgenic tobacco plants that do not produce stigmatic exudate do not support pollen tube entry into the stigma (Goldman et al. 1995). The application of lepidic exudate from the stigma of tobacco or petunia restores this function. However, the aqueous, carbohydrate-rich exudate of the lily stigma is unable to restore the function of pollen tube entry (Wolters-Arts et al. 1998). The application of vegetable oil such as olive oil (the fatty acid composition of which is similar to that of the stigma exudate) or trilinolein (a component of purified oil) was effective in restoring the pollen function on the transgenic stigma. Further studies showed that the lipids in the stigmatic exudate establish water gradient to supply directional supply of water from the stigma to the pollen grains, and the pollen tubes follow this gradient

(Wolters-Arts et al. 1998; Lush et al. 1998). Thus pollen tube entry into the stigma appears to be a hydrotropic rather than a chemotropic phenomenon.

In the dry type of the stigma, the lipids present in the pollen grain surface seem to have taken over the role of guiding the entry of pollen tubes into the stigma. Following pollination the pollen surface lipids come in contact with the stigma surface to form a contact zone (Fig. 4.1c) and provide a medium to establish directional water gradient for pollen hydration, germination and entry of pollen tubes. In *Brassica*, pollen grains from which surface lipids were removed failed to hydrate and germinate (Doughty et al. 1993). In *Arabidopsis*, pollen grains of mutants defective in the synthesis of long-chain lipids failed to hydrate and germinate on the stigma (Preuss et al. 1993; Wolters-Arts et al. 1998). However, the application of the triacylglyceride, trilinolein to pollen restored pollen function.

The factors that guide the pollen tubes in the style are not yet clear. Many studies during the 1960s indicated that calcium forms a gradient along the pistil and the pollen tubes chemotropically follow this gradient (Mascarenhas and Machlis 1962, 1964). However, subsequent studies did not support this concept (see Heslop-Harrison and Heslop-Harrison 1986; Shivanna 2003). A general consensus developed over the years, based on the structural features of the style, on unidirectional growth of the pollen tubes in the style even in the absence of a chemotropic gradient (Heslop-Harrison and Heslop-Harrison 1986). The transmitting tissue of the style with cylindrical, elongated cells with massive intercellular spaces filled with ECM provides a continuous path of least mechanical resistance for unidirectional growth of the pollen tubes. When once the pollen tubes enter the ECM of the transmitting tissue, they continue to grow from the stigma to the ovary. Some of the early experimental studies supported this concept (see Shivanna 2003). When pollen grains are deposited inside the stylar cavity of *Lilium* by making a window (Iwanami 1959), or in the transmitting tract of *Petunia* (Mulcahy and Mulcahy 1987), pollen tubes grow in both the directions, towards the ovary as well as the stigma. Similarly, when the ovarian end of the excised style of *Lilium* was pollinated, pollen tubes grew unidirectionally from the ovarian end towards the stigma (Ascher 1977).

Later studies in tobacco (Cheung et al. 1995) have shown that TTS proteins attracted pollen tubes that emerged from the cut end of the styles implanted in the medium. However, deglycosylated proteins were ineffective in attracting pollen tubes. TTS proteins display a gradient of increasing glycosylation in the transmitting tissue of the pistil from the stigma to the ovary. These results indicate that sugar gradient of TTS proteins may provide a chemotropic gradient for unidirectional growth of pollen tubes in the pistil. Thus, our understanding of the guidance factors at the level of the style is rather inconclusive.

There are some reports of a few other chemical components of the pistil involved in pollen tube guidance in some species (see Mizuta and Higashiyama 2018 and references therein). In *Lilium*, chemocyanin has been reported to attract pollen tubes in vitro (Kim et al. 2003). In *Arabidopsis*, plantacyanin, which is most abundantly localized along the transmitting tract, shows over 80% similarity to lily chemocyanin and has also been reported to promote directional pollen tube growth in the pistil (Dong et al. 2005).

4.6.2 Ovary

We have ample evidences to show that after the pollen tubes reach the ovary, the ES guides the precise growth of the pollen tube from the transmitting tissue of the ovary until its entry into the ovule (Fig. 4.2c) and the ES. The pollen tube enters one of the synergids through the filiform apparatus (FA) located at the micropylar end. Even in species with *Plumbago* type of ES in which synergids are absent, the egg cell itself develops filiform apparatus at the micropylar region through which pollen tube enters (Russell 1982). In many species such as cotton, one of the synergids degenerates before the arrival of the pollen tube; in such species the pollen tube invariably enters the degenerated synergid. In some other species, both the synergids remain intact and the pollen tube enters one of them through the FA; the synergid that receives the pollen tube soon degenerates.

There has been considerable progress in recent years in understanding pollen tube guidance in the ovary through the use of molecular genetic approaches at least in the model systems, *Arabidopsis*, *Torenia* and *Zea*. In *Arabidopsis*, detailed studies on pollen tube growth in the ovary have shown that pollen tubes, in general, grew directly towards ovules and entered the micropyle (Hulskamp et al. 1995a, b). However, in mutants defective in the development of ES, pollen tubes grew normally until they reached the ovary, but then they lost direction in the ovary and grew randomly. In a mutant in which ovules had normal or partial or no ES, over 90% of ovules with normal ES and only 28% of them with partial ES received pollen tubes, but none of the ovules with no ES received pollen tubes (Fig. 4.3a, b). Further studies using semi-in vitro techniques (in which the cut end of the pollinated style and unfertilized ovules were implanted in an agar medium) carried out in *Torenia* in which the micropylar part of the ES protrudes outside the ovule (Higashiyama and Hamamura, 2008; Higashiyama and Takeuchi 2015; Dresselhaus and Franklin-Tong 2013) as well as *Arabidopsis* (Hulskamp et al. 1995a, b; Kasahara et al. 2005) have shown that the components that attract pollen tubes to the micropylar part of the ES are secreted by the synergids. In semi-in vitro cultures, the pollen tubes that emerged through the cut end of the style grew towards the ovules and reached the filiform apparatus (FA) of the synergids (Fig. 4.4a, b). The ovules with immature ES or damaged ES or ES that has already received a pollen tube failed to attract pollen tubes. The ES also ensures that each ovule receives just one pollen tube. Pollen tubes seem to develop competence to respond to pollen tube attractants only after growing for some distance in the pistil (Higashiyama et al. 1998; Palanivelu and Preuss 2006). However, in several species pollen tube entry, double fertilization and seed development have been achieved by depositing the pollen grains directly on cultured ovules without any prior growth in the pistil. Whether such differences reflect variations in the species or any other unknown phenomenon needs further investigation. Subsequently, Higashiyama et al. (2001) were able to ablate each cell of the ES by a UV laser beam before ovule implantation. Ablation of the egg cell or central cell did not affect the attraction of pollen tubes, but ablation of both the synergids ceased attraction. These studies showed that one synergid cell is necessary to attract the pollen tube into the ES.

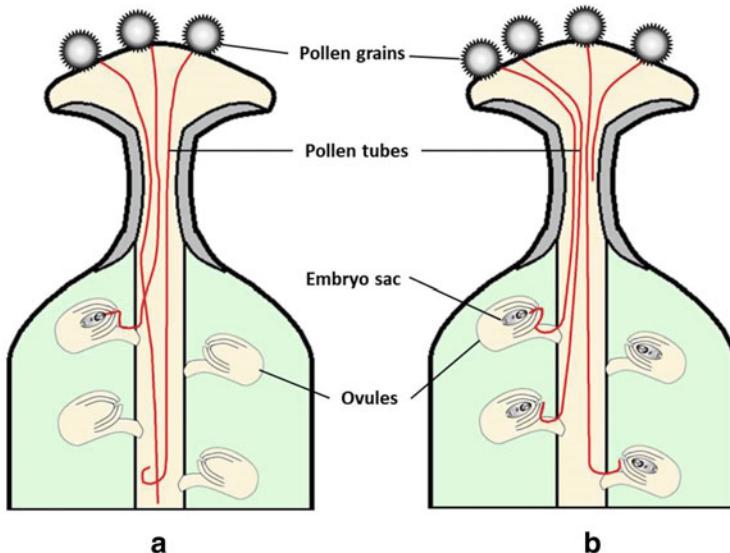


Fig. 4.3 Diagrammatic representation of the role of the embryo sac in attracting the pollen tubes from the transmitting tissue of the ovary to the micropyle of the ovule in *Arabidopsis thaliana*. (a) Mutant pistil in which many of the ovules lack the embryo sac; such ovules have not received pollen tubes. The ovule with the embryo sac has received the pollen tube. (b) Wild-type pistil in which the embryo sac is present in all the ovules and all of them have received the pollen tube. Many pollen tubes have lost direction and have grown randomly. (Based on Hulskamp et al. 1995b)

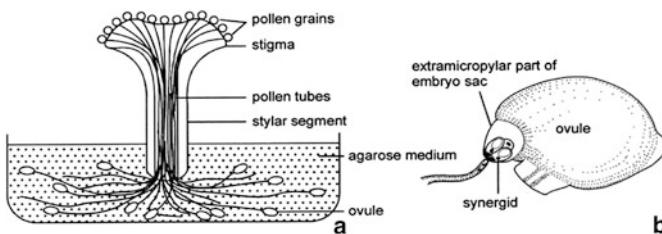


Fig. 4.4 Diagrammatic representation of pollen tube guidance using semi-in vitro pistil culture technique in *Torenia fournieri*. (a) Set up of the technique. Pollen tubes have emerged from the cut end of the pistil and have grown towards the ovules. (b) One of the ovules magnified to show the extramicropylar part of the embryo sac and the arrival of the pollen tube near the synergids. (Based on Higashiyama et al. 1998, modified from Shivanna and Rangaswamy 1997)

In *Arabidopsis* the transcription factor MYB98, expressed specifically in the synergid cells, plays an important role in the formation of filiform apparatus and in pollen tube guidance (Kasahara et al. 2005; Punwani et al. 2007, 2008). Further studies in *Torenia* and *Arabidopsis* have shown that defensin-like cysteine-rich peptides known as LUREs, secreted through the FA, act as pollen tube attractants (Okuda et al. 2009; Kanaoka et al. 2011). LURE genes are specifically expressed in

the synergids, and the peptides are secreted to the micropylar region (Okuda et al. 2009; Takeuchi and Higashiyama 2011). In *Torenia* two LURE genes (TfLURE1 and TfLURE2) and in *Arabidopsis* six duplicated LURE genes (AtLURE1.1–AtLURE1.6) have so far been reported. In maize, however, the EGG APPARATUS1 (ZmEA1) peptide has been shown to be the pollen tube attractant (Marton et al. 2005, 2012). Recent studies have indicated that both the central cell and the egg cell also indirectly control micropylar guidance of the pollen tube (Sasaki et al. 2015; Tekleyohans et al. 2017). The studies so far conducted on ovular guidance indicate considerable variability between species.

Pollen Tube Receptors Some information is available on pollen tube receptors that respond to synergid attractants. A number of receptor-like kinases located on the pollen tube tip seem to be involved in perceiving species-specific attractant peptides from the synergids. In *Torenia*, the ovular arabinogalactan sugar chain, called AMOR, has been shown to make pollen tubes fully competent for LURE1 and LURE2 peptides (Mizukami et al. 2016). In *Arabidopsis*, pollen tube receptors for LURE sensing include MDIS1 (MALE DISCOVERER 1), MIK (MDIS1-INTERACTING RECEPTOR-LIKE KINASE) and PRK6 (POLLEN RECEPTOR-LIKE KINASE6) (Cheung and Wu 2016; Higashiyama and Yang 2017; Takeuchi and Higashiyama 2016; Wang et al. 2016; Zhong et al. 2019). Pollen tube receptors sense LURE attractant peptide and guide the pollen tube towards LURE by regulating the cytosolic calcium gradient and the exocytosis of secretory vesicles at the growing tip of the pollen tube.

4.7 Double Fertilization

Double fertilization, involving the fusion of one of the sperms with the egg cell and the other with the central cell of the ES, was discovered by the end of the nineteenth century. Subsequent studies confirmed that double fertilization is a unique feature of flowering plants. There was hardly any progress in understanding further details associated with double fertilization until the middle of the twentieth century. This was largely because the ES, the site of fertilization, is deep-seated inside the ovule covered with the ovary wall and is inaccessible for routine experimental studies. However, because of the availability of a range of modern techniques of aseptic culture, cytochemistry and molecular biology and genetics, we have started understanding some of the molecular and cellular details of cell-cell interactions associated with fertilization and prevention of polyspermy. Most of the progress have been on the model system, *Arabidopsis*.

Soon after the pollen tube enters the synergid, the pollen tube ceases further growth and releases the male gametes through a rupture or a pore at the tip. Many evidences indicate that the arrest of pollen tube growth and release of the male gametes are controlled by the synergid (see Weterings and Russell 2004). In maize, a defensin-like ZmES4 mediates the pollen tube burst through the opening of the potassium channel KZM1 (Amien et al. 2010). The sperm cells move to the chalazal

end of the synergid. Cytoskeletal components of the ES guide the sperm cells to the egg cell and the central cell (Russell 1993; Lord and Russell 2002; Weterings and Russell 2004). One of the sperms eventually comes in contact with the plasma membrane of the egg cell and the other with that of the central cell. Their plasma membranes fuse at places to form bridges; the bridges gradually widen resulting in the entry of the contents of one of the male gametes into the egg cell and the other into the central cell. The nuclei of the male gametes migrate and align themselves with the respective egg and polar nuclei and eventually complete nuclear fusion. Some specific proteins involved in fertilization have been identified. Mori et al. (2006) have identified a novel protein, GCS1 (GENERATIVE CELL SPECIFIC 1), in *Lilium longiflorum* localized on the plasma membrane of the generative cell. In *Arabidopsis*, GCS mutant gametes fail to fertilize indicating that GCS seems to be a critical factor for fertilization. In *Arabidopsis*, small cysteine-rich EC1 (EGG CELL1) proteins accumulate in storage vesicles of the egg cell. These vesicles are exocytosed upon sperm arrival (Sprunck et al. 2012). The evidences indicate that the sperm responds to EC1 by redistributing GCS1 to the cell surface.

Soon after the male gametes are released in the synergid, AtLUREs, the pollen tube attracting signals in *A. thaliana*, decrease in the degenerated synergid. However, AtLURE 1 remains high in the persistent synergid. In this species (Palanivelu and Preuss 2006), ovules have been reported to release rapidly diffusible pollen tube repellents following pollen tube penetration, thus preventing the entry of additional pollen tubes. In the same system, Maruyama et al. (2015) have shown that the attraction of additional pollen tubes to the ES is prevented by the fused central cell and fertilized egg cell. The persistent synergid cell fuses with the primary endosperm cell soon after its nucleus starts dividing, and the cytoplasm of the synergid mixes with the endosperm cytoplasm about 5 h after fertilization causing rapid dilution of the pre-secreted pollen tube attractant in the persistent synergid cell. The zygote also contributes to the attenuation of AtLURE 1 production by inducing rapid disintegration of the nucleus of the persistent synergid via an unknown ethylene response pathway. Thus, the lack of attraction of additional pollen tubes seems to be due to the combination of attenuation of pollen tube attractants and release of pollen tube repellents. In maize (Antoine et al. 2001) and *Torenia* (Han et al. 2002), fertilization triggers a calcium influx in the zygote followed by an increase in cytoplasmic calcium.

4.8 Ovule Pollination and In Vitro Fertilization

Although stigmatic pollinations carried out since the 1940s clearly showed that the pistil inhibits incompatible pollen before reaching the ovary, they could not tell whether the ovule also has the ability to recognize and reject incompatible pollen. To understand this aspect, it was necessary to eliminate the stigma, style and ovary wall altogether and bring the pollen grains in direct contact with the ovules. This was achieved for the first time in *Papaver somniferum* (Kanta et al. 1962). Groups of ovules and pollen grains were cultured together on a nutrient medium, thus bringing

the pollen grains in direct contact with the ovules. Following ovule pollination, pollen germination, pollen tube entry into the ovules and double fertilization proceeded normally, and fertilized ovules developed into viable seeds. Subsequent studies showed that though this technique worked in a few systems (Rangaswamy and Shivanna 1969), it was not effective in several others because of the failure of the pollen tube entry into the cultured ovules. Eventually the technique of ovule pollination was modified; instead of culturing isolated groups of ovules and pollen grains together on the medium, the whole placenta intact along with the ovules was cultured by inserting only the pedicel into the medium. Pollen grains were then sprinkled on the ovule mass (Rangaswamy and Shivanna 1967; Zenkteler 1967). This modified technique, termed placental pollination, brought the pollen in direct contact with the ovules without causing any injury to the ovules and also prevented wetting of the ovules and pollen grains. Using this technique, it was possible to achieve fertilization and seed development *in vitro* (Fig. 4.5a–e). More importantly, this technique was effective in overcoming self-incompatibility in *Petunia* (Rangaswamy and Shivanna 1967) as well as interspecific incompatibility between *Melandrium* and related species (Zenkteler 1967). These studies showed that in flowering plants, the function of pollen recognition has been transferred to the pistil; the ovules and the egg cell have lost the ability to recognize pollen tubes/male gametes and seem to accept any male gametes that reach the embryo sac (Shivanna 1979, 2003; Shivanna and Johri 1985). The modified technique has been used by other investigators to understand some fundamental aspects of seed development (Balatkova and Tupy 1972; Balatkova et al. 1976) and to produce interspecific hybrids in *Nicotiana* and *Brassica* (Zenkteler 1990; Zenkteler and Bagniewska-Zadworna 2001).

Recently Zhong et al. (2019) showed that knockout of the entire *AtLURE1* gene family did not affect conspecific pollen tube guidance, indicating that *AtLURE1*-PRK6-mediated signalling is not a requirement for pollen tube entry and fertilization by pollen tubes of the same species. However, *AtLURE*-PRK6-mediated signalling accelerates conspecific pollen tube emergence from the septum when compared to pollen tubes of alien species. They also identified maternal peptides XIUQIU1-4, which attract pollen tubes of any other species of *Arabidopsis*. These studies also clearly indicate that unlike the pistil, which can reject all heterospecific pollen, the ovule has no ability to actively reject male gametes of alien species. This is in agreement with the earlier concept (Shivanna 1979, 2003) on the inability of the ovule/egg of flowering plants to recognize and reject incompatible male gametes; it seems to accept male gametes coming from homospecific as well as heterospecific species. Thus, the selection of the male partner in flowering plants is the prerogative of the maternal parent, and the egg does not seem to have any say in this selection.

The inaccessibility of the male and female gametes during fertilization has been a major limitation in understanding and manipulating fertilization in flowering plants. After achieving ovule pollination, the next logical step was to achieve *in vitro* fertilization using isolated egg and sperm cells as has been done in a number of animals including humans and a few lower plants (Schmid et al. 1994; Wilson et al. 1997; Gilbert 2000; Herberg et al. 2018). Isolation of gametes had to wait until the

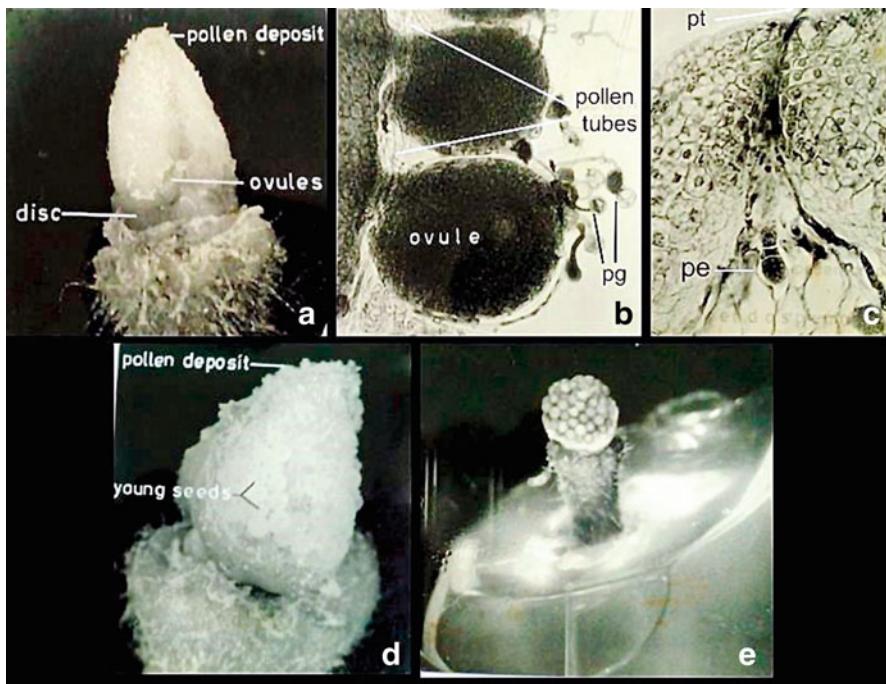


Fig. 4.5 Ovule pollination in vitro in *Petunia*. (a) Entire mass of ovules of a flower intact on the placentae after removing the stigma, style and ovary wall, pollinated with pollen mass before culture. (b) Free-hand transverse section of pollinated placenta 24 h after culture to show germination of pollen grains (pg) on the ovules and pollen tubes growing along the placenta. (c) A longitudinal microtome section of the ovule 6 days after culture to show pollen tube (pt) entry and development of the proembryo (pe). (d) A 10-day-old culture showing young developing seeds. (e) A 3-week-old culture showing mature seeds

technique of protoplast technology was standardized during the 1970s and 1980s. The progress in protoplast technology stimulated studies on the isolation of male and female gametes in different plant species. Isolation of male gametes from three-celled pollen grains was much simpler as it could be achieved by disrupting pollen grains to release the gametes followed by suitable centrifugation (Kranz et al. 2008; Kranz and Lorz 1993; Kranz 1997). However, it was not easy to isolate male gametes from two-celled pollen systems in which the gametes are formed during pollen tube growth in the style. The first success of isolation of male gametes from a two-celled pollen was achieved in *Gladiolus* by using a semi-in vitro technique (Shivanna et al. 1987). The stigma along with a part of the style, after suitable interval following stigmatic pollination, was excised and implanted in an agar medium. Pollen tubes continued growth and a large number of them emerged into the culture medium through the cut end of the style. The generative cell had completed division and formed the male gametes in all the emerged tubes. The pollen tubes were treated with suitable enzymes and male gametes were isolated.

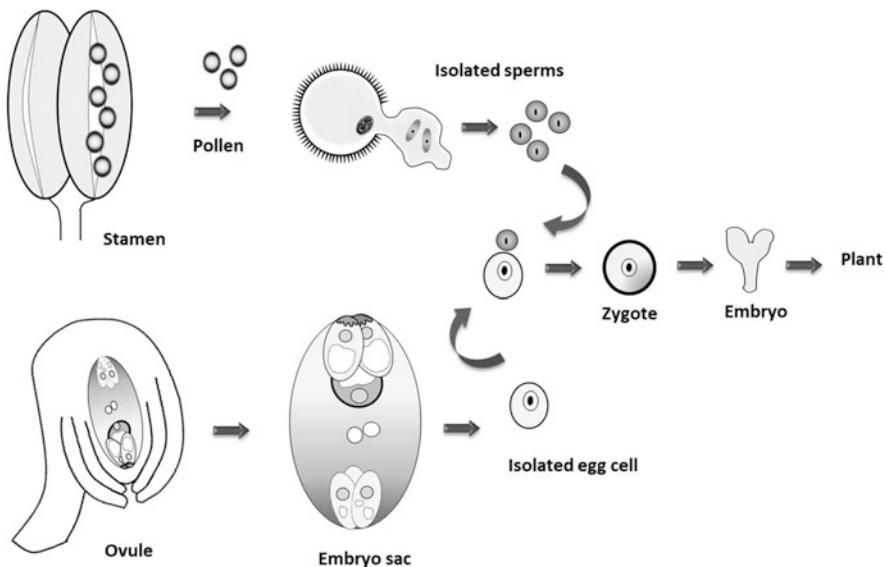


Fig. 4.6 Diagrammatic representation of the isolation of male and female gametes and in vitro fertilization. (Based on Kranz and Lorz 1993)

Subsequently, the technique has been used by others to isolate male gametes from other two-celled pollen systems (Tian and Russell 1997; Qui et al. 2004). Simultaneously, progress was made in isolating female gametes in a number of species. The first success of in vitro fertilization was reported in maize by Kranz and Lorz (1993) (Fig. 4.6). They could raise normal fertile plants from in vitro fertilized zygote. A number of studies are being made to understand several fundamental aspects such as details of gamete fusion, parthenogenesis and polyspermy, the establishment of polarity in the zygote and the genes involved in early embryogenesis, and considerable progress has been made (Okamoto 2011; Okamoto and Kranz 2005; Uchiumi et al. 2007; Ohnishi et al. 2014).

Attempts have been made to achieve in vitro fertilization in interspecific combinations also. These studies have shown that maize sperm fuses with the egg of related species such as wheat and sorghum and the zygote undergoes a limited number of divisions; maize sperm also fuses with even the egg of unrelated species, *Brassica*, but the zygote fails to divide (Kranz and Dresselhaus 1996). Isolated gametes of flowering plants seem to behave more like protoplasts and readily fuse in intra- and interspecific combinations.

4.9 Conclusions

There have been intensive studies on pollen-pistil interaction and fertilization during the last 30 years through the use of modern techniques. These studies have revealed a wealth of new information particularly on pollen tube guidance in the ovary and fertilization. So far the progress is confined to a few systems, largely *Arabidopsis*, *Torenia* and maize. Still there are many gaps in our understanding. Hopefully the gaps would be bridged soon and would result in a better understanding of the precise role of controlling factors during pollen-pistil interaction and double fertilization, the unique features of flowering plants.

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Olfactory Cues as Functional Traits in Plant Reproduction

5

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Abstract

Floral scents are important traits which mediate interactions within biotic communities. These volatiles perform diverse functions, ranging from attracting pollinators, repelling florivores or herbivores, as well as controlling the growth of pathogens. Therefore, floral volatiles are under constant selection to balance attraction and repellence in accordance with local communities and this has led to the evolution of highly complex volatile profiles. To date, more than 1000 volatile compounds have been identified from flowers, which ultimately ensure plant reproduction by attracting pollinators. Plant–pollinator interactions mediated by floral volatiles can range from highly generalized to extremely specialized systems. By evolving exclusive relationships with pollinators that are most abundant or efficient, plants increase reproductive success. On the other hand, attracting many kinds of insects by providing generic cues and rewards leads to generalization, where plants benefit by taking advantage of pollinator diversity. Such interactions of varied strength could be achieved through tailoring different floral volatile blends. Floral scents vary widely among plants but despite the variation, there are mainly three major classes of floral volatile organic compounds (FVOCs) based on their origin, function and biosynthesis. They are terpenoids, benzenoids or phenylpropanoids, and fatty acid derivatives. Along with these, certain flowers also produce unusual compounds, which attract pollinators by mimicking food or brood sources. The production, composition, quantity and timing of volatile emissions are tightly regulated by biotic and abiotic factors that help in fine-tuning the ecological interactions mediated by FVOCs. This chapter updates the current knowledge on these aspects and emphasizes the ecological importance of floral volatiles. Further, the various methods for collection and analyses of FVOCs are also described.

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5.1 Introduction

In the history of plant evolution, the origin of flower remains a mystery, although ~90% of the terrestrial plants are angiosperms. The benefits of cross-pollination and adaptation by selection clearly indicate adaptive evolution of floral function. Evolution of flowers is a result of selection on traits that optimize plant reproductive success (Schiestl and Johnson 2013) and diversification of floral traits is often hypothesized to have initiated co-evolutionary changes in pollinators and pollen dispersers that are rewarded with nectar and pollen. About two-thirds of all angiosperm plants depend on insects for pollination and the role of visual cues in pollinator attraction is well-studied (Lucas-Barbosa et al. 2016). In comparison, relatively less is known about olfactory signals mainly due to the complex nature of floral volatile organic compounds (FVOCs) as well as the lack of clear understanding of the sensory receptors of pollinators. Flowering plants provide both visual and olfactory cues that are used by the pollinators to discriminate and recognize flowers (Chittka and Wells 2004; Dobson 2006). Both visual and olfactory cues seem to play synergistic roles, in the process of host recognition. For instance, in the case of *Chelostoma rapunculi* bees, decoupled visual and olfactory cues were found to be less attractive than combinations of these two sensory modalities (Milet-Pinheiro et al. 2012). Though visual and olfactory cues could play a synergistic role, when given a choice between visually attractive, artificial rewarding model and non-rewarding mimic flowers, bumble bees were shown to use scents to identify the mimic, indicating that odour enhances colour discrimination (Kunze and Gumbert 2001). Similarly, several studies show that there are differences in learning performance of bees in response to visual and olfactory stimuli (Menzel 1999). From these reports, it is clear that although visual cues are essential to locate reliable food sources, whether a pollinator will make the choice to visit a flower depends on the presence of acceptable scent. Indeed, studies have shown that honey bees learn odour cues faster than visual cues and such associative learning confers fitness advantage (Wright and Schiestl 2009). In a study on honey bees, it was shown that they can associate odour with food (sucrose solution) within a single trial, indicating the potential advantage to a plant that emits scents in addition to visual cue and reward (Friedrich et al. 2004). The same study shows that repeated conditioning can induce stable memory. Insects, in general, can learn to associate general odours with food as evident from studies on *Drosophila melanogaster*, an important model for understanding olfaction and memory (Masek et al. 2015). Further, odour and visual cues complement each other and bimodal signals are known to be much more effective in pollinator attraction (Guo and Guo 2005; Leonard et al. 2011a, b; Katzenberger et al. 2013). Previous studies have also shown that colours are only perceived from a short distance, when the coloured object subtends a visual angle of 15° or larger (Giurfa et al. 1996). Thus, FVOCs emitted by the flowers are perceived before colour information is available to the animal. Taken together, scent alone can enhance plant–pollinator interactions but multimodal signals improve foraging efficiency and flower constancy (Kulahci et al. 2008). Flower constancy is the ability of a pollinator to visit flowers of the same species, thereby ensuring the pollen transfer

between conspecific flowers even in a generalized system, where pollinators visit flowers of different species (Chittka and Thomson 2001). Maintaining constant visitors to flowers depends on many factors, such as olfactory bias of pollinators, floral reward and presence of reliable odour cues.

Floral scents are, more often, not single compounds but emitted as complex mixtures of high chemical diversity. How then can a plant ensure constant visitors? Interestingly, insects are known to choose a flower based on key odorants, and modification of these key odours could have strong effect on pollinator visitation rates (Byers et al. 2014). Further, flowers of species belonging to different genera or families can be very similar in appearance and may not be well discriminated by pollinators (Burger et al. 2010; Milet-Pinheiro et al. 2012; Papadopoulos et al. 2013). In this context, specific compounds or unique ratios of common compounds make them highly specific cues that provide pollinators with an astonishing amount of information (Gong et al. 2015; Knudsen et al. 2006). To date, in flowers, 1700 floral volatile organic compounds (VOCs) have been identified from about 1000 flowering plant species (Knudsen and Gershenson 2006; Ramya et al. 2018). Flowering plants have species-specific odours that allow pollinators to distinguish between different plant species as well as between rewarding and non-rewarding flowers of the same species (Howell and Alarcon 2007).

Pollinators often show innate preferences for particular floral scents either as a result of co-evolution (Schaffler et al. 2015) or because flowers exploit pre-existing preferences of pollinators for certain volatiles that are important in other context (Heiduk et al. 2010; Schiestl and Dotterl 2012; Schiestl and Johnson 2013; Oelschlagel et al. 2014). Such “receiver bias” associations often result in specialized pollination system such as interaction between flowers which produce oil as a primary reward and oil bees that specifically collect oil from these flowers for building nest and as food for their larvae (Table 5.1) (Schaffler et al. 2015). The innate sensory preference for certain odours can be hypothesized to be a major reason for unrelated plants being pollinated by same pollinators leading to pollination syndromes. For instance, moth-pollinated flowers commonly emit lilac aldehyde and phenylacetaldehyde (Pettersson 1997; Dobson 2006) (Table 5.1). The exploitation of insect preferences is also often encountered in deceptive pollination where floral odours mimic insect pheromones or oviposition signals (Schiestl and Schluter 2009). Such deceptive pollination is common in many orchid species. In more generalized systems, an insect associates floral scents with reward, which facilitates host finding although these volatiles may not evoke positive responses prior to associative learning (Katzenberger et al. 2013; Wright and Schiestl 2009; Junker 2016). In such systems, flowers do not emit any unique blend of volatiles that evoke specific response in a single animal taxon and the pollinators also do not possess a specifically adapted olfactory system.

FVOCs could also function as plant defence against florivores, herbivores and pathogens (Muhlemann et al. 2014). Some volatile compounds found in floral scent also function in vegetative processes by attracting the natural predators of herbivores (Pare and Tumlinson 1997) or as airborne signals that activate disease resistance via the expression of defence-related genes in healthy tissues of infected plants and in

Table 5.1 Examples for FVOCs involved in attracting different species of animal pollinators

Plant	Compound	Pollinator	Reference
<i>Chiloglottis trapeziformis</i>	chiloglottone (2-ethyl-5-propylcyclohexan-1,3-dione)	<i>Neozeleboria cryptoides</i>	Schiestl et al. (2003)
<i>Drakaea glyptodon</i>	hydroxymethyl pyrazine	<i>Zaspilothynnus trilobatus</i>	Bohman et al. (2014)
<i>Ficus semicordata</i>	4-methylanisole	<i>Ceratosolen gravelyi</i>	Chen et al. (2009)
<i>Mimulus lewisii</i>	D-limonene; β -myrcene; (<i>E</i>)- β -ocimene	<i>Bombus vosnesenskii</i>	Byers et al. (2014)
<i>Ophrys speculum</i>	9-hydroxydecanoic acid	Scoliid wasp, e.g. <i>Campsoscolia ciliata</i>	Ayasse et al. (2003)
<i>Caladenia plicata</i>	(<i>S</i>)- β -citronellol; 2-hydroxy-6-methylacetophenone	<i>Zeleboria</i> spp.	Xu et al. (2017)
<i>Caladenia crebra</i>	2-(methylthio)phenol; 2-(methylthio) benzene-1,4-diol; 4-hydroxy-3-(methylthio) benzaldehyde	<i>Campylothynnus flavopictus</i>	Bohman et al. (2017)
<i>Cypripedium fargesii</i>	3-methyl-1-butanol; 2-ethyl-1-hexanol; 1-hexanol; benzyl acetate	<i>Cheilosia lucida</i>	Ren et al. (2011)
<i>Dracula lafleurii</i>	1-octen-3-ol; 3-octanone; 3-octanol	Zygothrica flies	Policha et al. (2016)
<i>Gastrodia similis</i>	ethyl acetate; ethyl 2-methylpropanoate; methyl 2-methylpropanoate	<i>Scaptodrosophila bangi</i>	Martos et al. (2015)
<i>Hydrocleys martii</i>	ρ -methylanisole	<i>Protodiscelis palpalis</i>	Carvalho et al. (2014)
<i>Mimulus cardinalis</i>	D-limonene	Hummingbird	Byers et al. (2014)
Floral-oil-secreting plants, e.g. <i>Lysimachia punctata</i>	diacetin; 1-hydroxy-1-phenyl-1-propanone	Oil bees, e.g. <i>Macropis fulvipes</i>	Schaffler et al. (2015)
<i>Satyrium pumilum</i>	dimethyl disulfide; dimethyl trisulfide; 2-heptanone; <i>p</i> -cresol; indole	<i>Sarcophaga redux</i> ; <i>Sarcophaga guillarmodi</i>	van der Niet et al. (2011)
<i>Adansonia digitata</i>	3-methyl butanal; dimethyl disulfide	Bats, e.g. <i>Eidolon helvum</i> ; <i>Rousettus aegyptiacus</i>	von Helversen et al. (2000)

(continued)

Table 5.1 (continued)

Plant	Compound	Pollinator	Reference
<i>Helicodiceros muscivorus</i>	Dimethyl disulfide; dimethyl trisulfide	Necrophilic insects, e.g. <i>Lucilia</i> spp.; <i>Calliphora</i> spp.	Stensmyr et al. (2002)
<i>Cimicifuga simplex</i> ; <i>Polemonium foliosissimum</i>	α - pinene; β - pinene	<i>Bombus</i> bees	Kost (2008)
<i>Rubus</i> spp.; <i>Ranunculus</i> spp.; <i>Chamaedora linearis</i>	(E)- β -ocimene	Drosophilid flies; nitidulid beetles; small beetles	Kost (2008)
<i>Nicotiana alata</i>	1,8-cineole; linalool	Hawk moths	Raguso et al. (2003)
<i>Silene vulgaris</i> ; <i>S. maritima</i>	lilac compounds	Noctuid and sphingid moths	Pettersson (1997)

the neighbouring plants (Dudareva and Pichersky 2000). The multifunctionality of FVOCs is attributed to their chemical diversity (Knudsen et al. 2006; Muhlemann et al. 2014). In earlier work by Pellmyr and Thien (2006), it was proposed that floral odours originated as plant defence to repel herbivores, an idea that has found considerable support in recent literature. For instance, it is known that (E)- β -caryophyllene emitted by *Arabidopsis thaliana* flowers acts as an antibiotic (Huang et al. 2012) and is an aversive odour to *Metrioptera bicolor*, a devastating florivore (Junker et al. 2010). Another well-known example is the “push-pull” pollination strategy employed by cycads and specialist pollinator *Cycadothrips* (Terry et al. 2007). Thrips are obligate mutualists of cycads and reside in the male cones, which provide pollen for adults and larvae. During the day, the plants heat up and emit million-fold-increased concentration of volatiles to drive thrips out of male cycad cones. In the interim, the female cone lures these thrips by emitting low concentration of the same volatile blend resulting in pollination. By end of the day, male cone cools down, volatile emission rate decreases and this in turn attracts the thrips again and this cycle goes on. This system thus provides an important clue to the path of the evolution of plant odours from being an insect repellent to an attractant especially for pollinators (Terry et al. 2007). Apart from variation in function, FVOCs also show tremendous variation with changes in environmental factors such as temperature, light availability and CO₂ changes.

This chapter aims to provide an update on current knowledge about FVOCs as a functional trait for plant reproduction. The chapter begins with an overview of the diverse functions of floral volatiles followed by the chemical nature and biosynthesis of different volatile components. Next, we discuss the regulation of FVOC emissions by biotic and abiotic factors. Future directions in floral volatile research are examined after describing various methods for collection and analyses.

5.2 Diverse Functions of FVOC

Plant–insect interactions are among the most diverse interactions on this planet and floral scent is an important trait that mediates this association. Plants, in general, exhibit high diversity in eco-physiological traits and their functions that in turn affects community dynamics and ecosystem functioning. An eco-physiological trait like floral scent can be considered adaptive as there is a direct correlation to plant fitness. Floral traits like color, scent or shape might converge or diverge among plant assemblages due to selection imposed by pollinator and this pollination-driven selection is well-studied. However, the functional diversity and regulation of floral scents is still poorly understood. For instance, why do flowers emit a bouquet of different chemicals when only a few of them actually convey the message to pollinators? What controls the emission rate and frequency? Is there any trade-off associated with pollinator attraction and herbivory? Here, the multifunctionality of FVOCs in the context of plant interactions is presented followed by discussion on the regulation of FVOC emission by biotic and abiotic factors.

5.2.1 Pollinator Attraction

The primary function of FVOC is believed to be pollinator attraction to improve reproductive success (Raguso et al. 2003; Raguso 2008; Schiestl and Dötterl 2012; Ramya et al. 2018). Floral odours are known to impact foraging behaviour of pollinators such as bumble bees, honey bees, moths and stingless bees. Bees convey information to their nest mates in the hive in the form of dance behaviour and it is well known that the probability of bee dance after returning from a potential food source is higher when scents are present. In a study by Reinhard et al. (2004), it was shown that if bees were trained at a feeder with a scent and this scent was blown into the hive, it induced other bees to recall navigational memory associated with that scent. Further, bees flew in the direction of the scent even in the absence of feeders, indicating the pivotal role of floral scent on foraging success and efficiency. Although floral odours attract several kinds of pollinators, in general, plant–pollinator interactions can range from highly generalized to extremely specialized partnerships (Waser and Ollerton 2006). In generalized systems, insect association with floral scents are solely based on rewards, which facilitate host finding, although these volatiles may not evoke positive responses prior to associative learning (Ings et al. 2009; Gomez et al. 2015). In these systems, flowers do not emit specific volatiles to attract a single taxon, nor do the animals possess specialized olfactory receptors. For example, in *Apis mellifera*, cognitive processing of the floral volatiles and other stimuli helps to exploit resources and also benefits plants due to conspecific pollen transfer (Junker 2016). Since natural selection favours signals that utilize preferences and behaviour of pollinators that are locally abundant, pollinator perception is a key factor that selects for floral signals like odour leading to specialized pollinator mutualisms. A pollinator response to floral odors could be based on an innate, adaptive or associative preference for a chemical odour. Many floral

fragrance compounds are often similar to insect-related compounds. For example, when floral fragrance and insect volatiles were compared, an extensive overlap was found and of the 71 floral volatiles identified from 15 angiosperm families, at least one insect family produced 89% of these as a pheromone or allomone (Schiestl 2010). Interestingly, the greatest overlap was reported for aromatic compounds followed by monoterpenes, although monoterpenes are often reported from vegetative tissues. The study corroborates that pre-existing bias for chemical compounds could be an important selective force for unrelated plants converging on similar floral fragrances and pollinators (Schiestl 2010). Sensory preference of pollinators can also be learnt or associated to rewards leading to extreme mutualisms. For instance, hawk moths that primarily forage on bat-adapted flowers can learn to shift to moth-adapted flowers when they become abundant locally through olfactory conditioning (Riffell et al. 2008). Such learning could also cause flower constancy, leading to mutualistic interactions. However, establishing a constant visitor would require high rewards and honest signals. For example, the obligate mutualism between floral-oil-secreting plants and oil-collecting bees is mediated by floral scent, and behavioural experiments on European *Macropis fulvipes* females with no prior experience revealed that olfactory cues are more crucial than visual cues to locate their host plants (Dotterl and Vereecken 2010). Further, diacetin and 1-hydroxy-1-phenyl-1-propanone present in oil extracts are suggested to attract these bees (Table 5.1). Interestingly, similar chemicals are found in floral oil plants throughout the world, thus providing an honest, reliable and private channel of valuable information to the specialized pollinators (Schaffler et al. 2015).

FVOCs can also be dishonest signals in cases where the plants possess no reward for pollinator service. Several hypotheses have been put forward to explain such pollination deception, of which the theory of cross-pollination is most acceptable. According to this hypothesis, the longer a pollinator spends on a flower, the possibility of self pollination increases and minimizing the probing or visitation time by means of no reward could be beneficial as it reduces the negative consequences of geitonogamy (Jersakova et al. 2006). Deceptive pollination can also lead to specialized flower pollinator system. The Orchid family is well known for such mechanisms and has unusually high occurrence of non-rewarding flowers compared to other plant families. The mechanisms of deception in orchids include generalized food deception, food-deceptive floral mimicry, brood-site imitation, shelter imitation, pseudo antagonism, rendezvous attraction and sexual deception (Jersakova et al. 2006). Among these, food deception is the most common mechanism (reported in 38 genera) followed by sexual deception (18 genera). For example, green-winged orchid (*Anacamptis morio*) produces a scent that attracts the plant's main pollinators, queen bumblebees, which associate the odour with nectar. These orchids, however, do not produce nectar and the queen does not receive a food reward for her pollinating efforts. It is estimated that around 1000 orchid species perform sexual deception, in which flowers attract pollinators by producing scents that mimic sex pheromones. The orchid *Chiloglottis trapeziformis* is an example of this form of deception where it releases a compound identical to a sex pheromone (chiloglottone) normally produced by female of its pollinator, the thynnine wasps

(*Neozeleboria cryptoides*), and thereby lures male pollinator (Table 5.1). The males are attracted to mate with the flower, and in this process they pick up pollen from the flower and transfer it to a second plant, again lured by the pheromone-mimicking odour (Schiestl et al. 2003). Another example is of *Ophrys* flowers, which emit volatile compounds in similar proportions to the sex pheromones produced by virgin *Andrena nogroaenea* females (Schiestl et al. 1999). Males who confuse *Ophrys* flowers for receptive females learn the odour profiles of specific flowers following an unfulfilling mating attempt. The information conveyed by FVOCs depends on its amount, composition and context of their emission. Pollinators respond to this varied information by eliciting distinct behaviours. Long-distance emission of volatiles helps to guide pollinators to flowers particularly in moth-pollinated flowers, which are searched out and visited at night. To meet this need, volatiles are usually produced at a higher intensity since flowers are not readily visible at night. For example, the moth-pollinated *Petunia axillaris* and *Silene latifolia* emit higher amounts of volatiles when compared to the flowers of the plants of the same genus like *Petunia integrifolia* and *Silene dioica* which are pollinated during the day by bees (Ando et al. 2001; Waelti et al. 2008). Unlike long-distance emissions which are used for guiding pollinators, short-distance emissions usually trigger landing, feeding and reproductive behaviour of the pollinators.

FVOCs are usually emitted as a complex mixture of varying chemical diversity that change depending on several factors ranging from plant species, geographic location, and presence of herbivores or pathogens as well as environmental factors. The extraordinary complexity of odour blend from flowers and its variation makes one wonder how these diverse chemicals can be correlated to visits by particular insects in nature. The degree of specialization mediated by floral odours point towards the existence of special channels of information exchange, as in the case of oil-collecting bees. Indeed, several such examples do exist. For instance, in highly specialized fig–fig wasp mutualism, a species-specific attraction to specific ratios of volatile compounds released by receptive figs was reported (Proffit and Johnson 2009). Several attempts have been made to correlate FVOC profiles with pollination syndromes with limited success. A few generalizations are known. For example, plants relying on moths for pollination predominantly release some acyclic terpene alcohols, their corresponding hydrocarbons, benzenoid alcohols, esters and small amounts of some nitrogen compounds (Knudsen and Tollsten 1993; Dobson 2006), and bat-pollinated species mostly emit 3-methyl butanal and sulphur-containing volatiles (von Helversen et al. 2000) (Table 5.1). In general, moth-pollinated flowers have a sweet, pleasant odour and within such flowers, sphingophilous flowers can be distinguished by the presence of oxygenated sesquiterpenes from phalenophilous flowers (Knudsen and Tollsten 1993). Bird-pollinated flowers are usually scentless but trace levels of odours like (Z)-hexenol, hexanol, 6-methyl-5-hepten-2-one have been reported (Knudsen et al. 2004). It is interesting to note although the floral volatile blend could be composed of several compounds, only a few compounds are reported to be actually detected by pollinators (Table 5.1). This indicates that the function of different chemicals in the FVOC could be synergistic, additive or unique.

5.2.2 As a Repellent

Flowers are delicate plant organs that lack physical barriers such as a highly lignified cell wall or cuticle like other plant parts. This makes them highly susceptible to pathogens and florivores. Due to their high moisture and nutrient content, they carry higher densities of microorganisms than other aerial plant surfaces (Johnson and Stockwell 1998). These microorganisms are known to colonize different floral parts like sepals, petals, carpels, stamens, pollen, and nectaries (Aleklett et al. 2014). Different cell structures and topography along with discrepancy in chemical profile of volatiles and exudations of sugars provide distinct habitats for both epiphytic and endophytic microbes. In general, microbes are abundant in the nectary since it offers higher humidity and is a rich source of nutrients. Other parts like sepals and petals, which differ in their physical environment, contribute to the colonization of a diverse array of microorganisms that have different humidity and temperature optima (Aleklett et al. 2014). Presence of yeast in nectar might actually attract pollinators by modifying nectar quality and scent, as shown in the case of *Delphinium nuttallianum*, a short-lived perennial montane herb. Here, the authors manipulated yeast density in nectar and studied the effect on pollinators. Their study revealed that presence of yeast causes enhanced pollen donation, thereby increasing male plant fitness, highlighting the importance of microbes in such interactions (Schaeffer and Irwin 2014). However, presence of bacteria/fungi has shown to be detrimental to plant–pollinator interactions. In a study conducted by Vanette et al., it was clearly shown that nectar-inhabiting bacteria, not yeast, weaken plant–pollinator interactions by altering nectar pH and glucose concentration (Vanette et al. 2012). Further, honey bees are also reported to avoid flower nectar colonized by specific bacterial species as their presence altered nectar chemistry (Good et al. 2014). Spores of floral fungal pathogen *Microbotryum violaceum*, when deposited on mature flowers of family Caryophyllaceae do not cause any immediate effect on flowers; instead, it grows into the plant meristem and destroys developing pollen mother cells (Schafer et al. 2010). *Erwinia amylovora*, a bacterial pathogen, infects the hypanthium (where nectar is secreted) of the plants in the family Rosaceae (Farkas et al. 2011). The pathogen then enters into the inner floral tissues via the nectar-secreting stomata causing fire blight disease (Farkas et al. 2011). Along with floral pathogens, certain animal pathogens are also transmitted by shared use of flowers. For example, bumble bee pathogen *Critidium bombi* is horizontally transmitted between hosts through flowers (Durrer and Schmid-Hempel 1994). In some interesting examples of pathogen–vector associations, pathogens mimic floral scents to trick insects into dispersing it. Classic examples of such interactions include *Monilia vaccinii-corymbosi*, the cause of blueberry mummy disease, where infected leaves produce high quantities of cinnamyl alcohol and aldehyde attractive to bees thereby transmitting the disease (McArt et al. 2016) and the case of pseudo flower transformation by the rust fungus, *Puccinia arrhenatheri*. Here, fungal spores resemble flowers and also emit attractive floral scents that is consumed by foraging insects (Raguso and Roy 1998).

How does a plant deal with this wide array of pathogenic floral microbes? Interestingly, many FVOCS have antimicrobial and antifungal activities and such effects of essential oils from floral tissues is well known (Bakkali et al. 2008) and their mode of action is generally believed to impact integrity and permeability of bacterial/fungal membranes. For instance, the floral volatile (*E*)- β -caryophyllene emitted from the stigma of *Arabidopsis thaliana* flowers is known to have an inhibitory effect on the growth of *Pseudomonas syringae* pv. *tomato* DC3000, a pathogen of Brassicaceae (Huang et al. 2012). They also observed that Brassicaceae plants lacking (*E*)- β -caryophyllene emission displayed denser bacterial growth on their stigmas and reduced seed weight compared to wild-type plants. Interestingly, de novo synthesis and emission of terpene upon pathogen attack is documented in several reports (Hammerbacher et al. 2019). Another well-studied antimicrobial volatile terpenoid is linalool, which is also effective as pesticide for control of ticks and fleas as it reversibly inhibits acetylcholinesterase (Duman et al. 2010). In a recent study on bee-pollinated *Penstemon digitalis*, it was shown that nectar volatile, linalool, could reduce bacterial growth (Burdon et al. 2018). Interestingly, fumigation of flowers by antibiotics is reported to reduce floral scent emission (Penuelas et al. 2014), indicating the key role of phyllospheric microbiota in regulating floral emission. Studies that evaluate the actual impact of volatile chemicals on plant pathogens show mixed results. For instance, in a study conducted by Junker et al. (2011), it was found that FVOCS inhibited the growth of certain bacterial strains originated from leaves but the same volatile blend failed to inhibit the growth of bacterial strains isolated from flowers, which shows that these strains might have potentially adapted to the FVOCS. Overall, more studies are needed to characterize and evaluate the role of individual compounds and FVOC blend on different microorganisms to understand their functional significance.

Apart from microbes, several insects are known to cause floral damage. Such insects are a major threat since they reduce the reproductive success either by feeding on flowers, displacing potential pollinators and/or altering flower morphology (McCall 2008; Sober et al. 2010). For example, ants show aggressive behaviour towards pollinators and sometimes feed on floral structures and also act as nectar thieves. Interestingly, FVOCS can function as chemical deterrents against ants. Many common European ants are deterred by volatiles emitted from temperate flowers and pollen (Willmer et al. 2009). Although ants can be problematic for flowers, they are excellent bodyguards against herbivores and several plants possess extra-floral nectar as an indirect defence strategy to attract ants (Heil 2011). Such myrmecophytic plants establish mutualistic or facultative relationships with ants. How do these plants maintain their flowers and pollinators? How can they balance pollinator attraction and herbivore repulsion? One solution that plants have evolved is temporal regulation of volatile emission as seen in the example of the Ant-*Acacia* system, where volatiles from inflorescences repel resident ant-guards for only a few hours, allowing pollinators free access (Willmer and Stone 1997). In case of *Acacia constricta*, activity of ants and pollinators activity patterns peaked at different times of the day, thus resolving the conflicts between ants and pollinators temporally (Nicklen and Wagner 2006). Apart from ants, other insects are also

known to feed on flowers. Common volatiles like linalool and (*E*)- β -caryophyllene are known to elicit aversive behaviour in facultative florivore, *Metrioptera bicolor* (Junker et al. 2010). Since pollinators and florivores can be attracted to same compounds, plants have sophisticated mechanisms to attract as well as hide from or repel florivores simultaneously. One way plants achieve such opposing functions is to exploit the chemistry of the complex volatile mixtures, where each chemical might elicit a different response, for example, in *Petunia*, some compounds like eugenol and benzylbenzoate control florivores while methylbenzoate is attractive to pollinators. Using transgenic plants, distinct roles of each compound in the mixture were evaluated (Kessler et al. 2013); however, whether their roles change in a mixture or in different communities is unclear. *Polemonium viscosum* flowers that are well protected against ants by its narrow nectar tube emits less 2-phenylethanol, an ant repellent when compared to flowers of the same species with larger nectar tube, which are prone to floral damage by ants. This example shows how the same compound (2-phenylethanol) acts as attractant as well as repellent in a dose-dependent manner. Interestingly in this study, it was also shown that higher emission of this compound reduces bees visitation suggesting an ecological cost to defence (Galen et al. 2011). However, some plants might intentionally reduce pollinator visitation. For example, *Pieris rapae* butterflies are repelled by floral scent of *Osmanthus fragrans* (Omura et al. 2000). This might be a plant strategy to repel other insects that may be either nectar thieves like butterflies or those that become voracious herbivore larvae once the adult deposit their eggs on the plant.

Spatiotemporal volatile emission is another mechanism by which flowers achieve some control over mutualists versus antagonist. In a fascinating study using wild tobacco plants that emit benzylacetone (BA) as major floral fragrance, it was shown how a single volatile compound can serve two different functions. Using transgenic plants, the authors show that *Nicotiana attenuata* can prevent floral damage by emitting BA before dusk as the florivores (beetles) search for new hosts during early evening and remain on the host in the night (Kessler et al. 2008). The Orchidaceae family have been extensively explored as they are excellent models in specialized plant-pollinator studies. For example, *Dichaea pendula* exclusively depends on orchid bees for pollination and the weevils are major florivores of this plant. In a report by Nunes et al. (2016), it was shown that floral scent of *D. pendula* was composed of 99% 2-methoxy-4-vinyl phenol, an unusual volatile that was attractive to both bees and weevils. This indicates that floral volatiles evolution is driven more by pollinator attraction rather than anti-florivory, since florivory was not a major constraint for fruit set in this example. This is further corroborated by a recent finding that presence of non-pollinators is also not terribly detrimental as in the case of buckler mustard, *Biscutella laevigata*. Here, the crab spiders camouflage themselves on these flowers to hunt for pollinators. Both bees and spiders are attracted by the floral signal, (*E*)- β -ocimene and the trade-off associated with spider attraction is mitigated by the fact that the spiders also feed on florivores. In fact, the study reports that florivore-infested flowers emit higher amounts of ocimene, demonstrating an indirect defence strategy mediated by FVOCS (Knauer et al. 2018).

Taken together, volatiles can function as attractant as well as repellent and the detrimental effect of attracting florivores could be a major trade-off in evolution of these signals. How plants solve the dilemma is still debated. This could be possibly achieved by (a) employing single compound with multiple functions. For example, linalool is attractive to bees and butterflies while it repels ants. (b) Different compounds in the volatile blend perform different functions. In *Cucurbita moschata*, for example, three volatile compounds from the flower profile were tested and it was found that 1,2,4-trimethoxybenzene was attractive to both herbivore and pollinator, indole was attractive only to herbivore, while (*E*)-cinnamaldehyde was attractive only to pollinator (Andrews et al. 2007). (c) Spatiotemporal regulation of volatile emission to enhance the function of these scents and/or (d) indirect attraction of predators of florivores. Overall, floral scents are under selection to balance attraction and repellence in accordance with local communities and can be predicted to evolve highly complex profiles.

Research on inter-plant communication via volatiles is fascinating and most of the evidence for this comes from upregulation of defence-related genes upon exposure to herbivore-induced plant volatiles from neighbouring plants (Karban et al. 2014). However, the role of FVOCs in plant – plant communication is relatively less studied. Since floral volatiles and herbivore-induced plant volatiles are chemically similar, FVOCs could also convey reliable, ecologically relevant information about the mating environment (Raguso 2008; Caruso and Parachnowitsch 2016). Nevertheless, there is not much direct evidence to show that plants exchange information using exclusive floral volatile blends. A few reports show the effect of individual floral volatiles on other plants under laboratory conditions; however, it is not clear if this is true in nature. Since some volatile compounds are unique to flowers, it can be hypothesized that floral scents are also part of inter-plant communication signals, especially under conditions such as high temperature, higher densities of florivores and/or pollinators. This is speculated in the case of gynodioecious plants where females perceive the frequency of hermaphroditic neighbours and respond by adjusting their floral traits. For example, female great blue lobelia (*Lobelia siphilitica*) open more flowers per unit time when they are rare relative to hermaphrodites. It is observed that female great blue lobelia plants adjust their rate of flower opening even when hand-pollinated. This suggests that they do not use pollen receipt as cues to the frequency of hermaphroditic neighbours (Rivkin et al. 2015). This information on whether the environment contains many or few hermaphrodites might be perceived based on the floral volatiles emitted by the neighbours. Ashman et al. (2005) clearly demonstrated that female and hermaphroditic flowers emit different volatiles in gynodioecious species (Ashman et al. 2005). This study was conducted on gynodioecious wild strawberry (*Fragaria virginiana*) and they found that anther specific volatile, 2-phenylethanol is produced by hermaphrodite flowers. This suggests that female plants could use floral volatiles as a cue to know the presence of their hermaphroditic neighbours.

5.3 Biosynthesis of FVOC

FVOCs are a complex blend of low molecular weight lipophilic molecules that have low boiling points (Dudareva and Pichersky 2006) and are produced in specialized organs like osmophores, glandular trichomes, hairs or unspecialized epidermis (Effmert et al. 2006; Widhalm et al. 2015). FVOCs are classified based on their origin, function and biosynthesis as terpenoids, benzenoids/phenylpropanoids and fatty acid derivatives. Additionally, certain unusual FVOCs are also reported (Wong et al. 2017). For example, sulphur- and nitrogen-containing compounds derived from amino acid metabolism are known to attract pollinators by mimicking food or brood sources. Examples of these include dimethyl disulfide, dimethyl trisulfide, indole, pyrazines and trimethyl amines, which are mainly emitted by sapromyiophilous flowers (Juergens et al. 2006). Some miscellaneous cyclic compounds are also known to be emitted by flowers whose biosynthetic origin is not clear, for example, 5,5-dimethyl-1,3- cyclopentadiene produced by the flowers within the genus *Vaccinium* (Forney et al. 2012). Likewise, Annonaceae and Araceae plants produce 4-methyl-5-vinylthiazole, which is a nitrogen and sulphur-containing compound reported to attract its scarab beetle pollinators (Maia et al. 2012). Biosynthetic pathways of such unusual compounds that are limited to certain taxonomic group are yet to be investigated. Here, biosynthesis of major classes of floral volatiles that are universally distributed throughout the flowering plants are presented.

5.3.1 Terpenoids

Terpenoids are the dominant class of floral volatiles, of which more than 556 volatile compounds are reported (Muhlemann et al. 2014). Extensive literature is available on this abundant class of volatiles. All terpenoids originate from C5 precursors, namely isopentenyl diphosphate (IDP) and dimethyl allyl diphosphate (DMADP) (McGarvey and Croteau 1995) (Fig. 5.1). IDP and DMADP are produced from two independent pathways, the mevalonic acid (MVA) and the methylerythritol-phosphate (MEP) pathway (Vranova et al. 2013). Monoterpenes (C10) and diterpenes (C20) are formed through MEP pathway (Knudsen and Gershenson 2006), which occur in the plastids (Hsieh et al. 2008), whereas sesquiterpenes are synthesized by the MVA pathway distributed between the cytosol, endoplasmic reticulum and peroxisomes (Simkin et al. 2004; Pulido et al. 2012) (Fig. 5.1). Interestingly, in snapdragon flowers, sesquiterpenes are formed through MEP pathway instead of MVA pathway (Dudareva et al. 2005). MVA pathway comprises six enzymatic reactions starting from the condensation of three molecules of acetyl-CoA while the MEP pathway begins with the condensation of D-glyceraldehyde 3-phosphate and pyruvate and consists of seven enzymatic reactions (Muhlemann et al. 2014).

Terpenoids are produced from prenyl diphosphate precursors as a result of condensation reaction mediated by prenyltransferases. Head-to-tail condensation

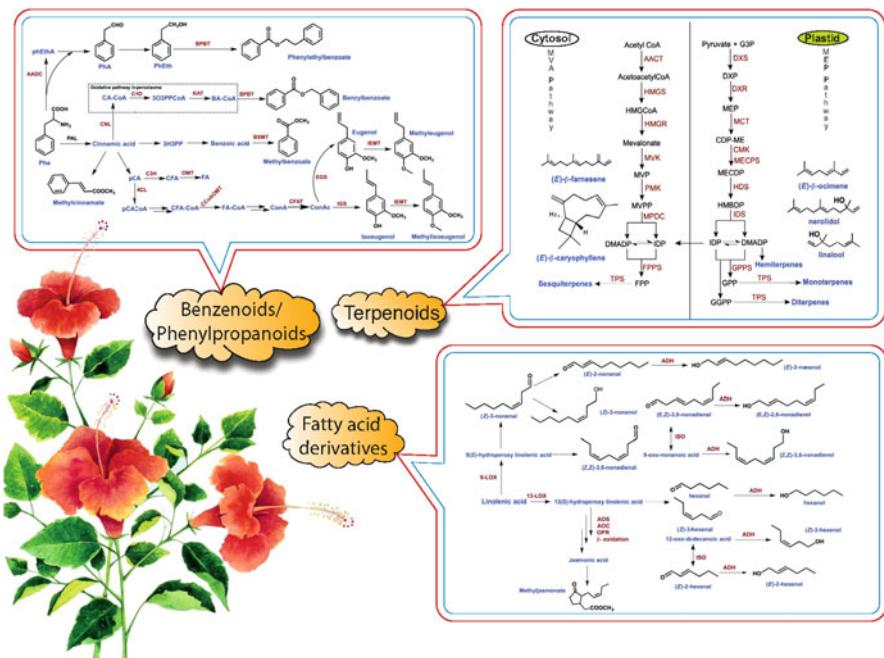


Fig. 5.1 Biosynthesis of floral volatile organic compounds (FVOC). FVOCs are classified into benzenoids/phenylpropanoids, terpenoids and fatty acid derivatives (Flower image credit Dhara Mehrotra©)

Benzenoid/phenylpropanoids derivatives are synthesized from phenylalanine. Abbreviations: *AADC* aromatic amino acid decarboxylase, *BA-CoA* benzoyl-CoA, *BPBT* benzoyl-CoA: benzylalcohol/2-phenylethanol benzoyltransferase, *BSMT* benzoic acid/salicylic acid carboxyl methyltransferase, *CA-CoA* cinnamoyl-CoA, *CFA* caffeic acid, *CFA-CoA* caffeoyl-CoA, *CFAT* coniferyl alcohol acetyltransferase, *CHD* cinnamoyl-CoA hydratase-dehydrogenase, *C3H* *p*-coumarate-3-hydroxylase, *ConA* coniferyl alcohol, *ConAc* coniferyl acetate, *4CL* 4-coumaroyl-CoA ligase, *EGS* eugenol synthase, *FA* ferulic acid, *FA-CoA* feruloyl-CoA, *IEMT* isoeugenol O-methyltransferase, *IGS* isoeugenol synthase, *3H3PP* 3-hydroxy-3-phenylpropionic acid, *KAT* 3-ketoacyl-CoA thiolase, *3O3PP-CoA* 3-oxo-3-phenylpropionyl-CoA, *OMT* *O*-methyltransferase, *PAL* phenylalanine ammonia lyase, *pCA* *p*-coumaric acid, *pCA-CoA* *p*-coumaroyl-CoA, *PhA* phenylacetaldehyde, *Phe* L-phenylalanine, *PhEth* 2-phenylethanol, *PhEthA* phenylethylamine. **Terpenoid derivatives** are synthesized by the cytosolic mevalonic acid (MVA) and the plastidial methylerythritol phosphate (MEP) pathways. Abbreviations: *AACT* acetyl-CoA acetyltransferase, *CDP-ME* 4-diphosphocytidyl-2-C-methyl-D-erythritol, *CMK* 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase, *DMADP* dimethylallyl diphosphate, *DXP* 1-deoxy-D-xylulose 5-phosphate, *DXS* DXP synthase, *DXR* 1-deoxy-D-xylulose 5-phosphate reductoisomerase, *FPP* farnesyl pyrophosphate, *FPPS* FPP synthase, *G3P* glyceraldehyde 3-phosphate, *GGPP* geranylgeranyl pyrophosphate, *GPP* geranyl pyrophosphate, *GPPS* GPP synthase, *HDS* 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase, *HMBDP* (E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate, *HMG-CoA* 3-hydroxy-3-methylglutaryl-CoA, *HMGR* HMG-CoA reductase, *HMGs* HMG-CoA synthase, *IDS* isopentenyl diphosphate synthase, *IDP* isopentenyl diphosphate, *MCT* 2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase, *MECPD* 2-C-methyl-D-erythritol 2,4-cyclodiphosphate, *MECPs* MECPD synthase, *MVK* mevalonate kinase, *MPDC* mevalonate diphosphate decarboxylase, *MVP* mevalonate 5-phosphate, *MVPP* mevalonate 5-pyrophosphate, *PMK* phosphomevalonate kinase, *TPS* terpene synthase. **Fatty acid derivatives** are synthesized from linolenic acid. Abbreviations: *ADH* alcohol dehydrogenase, *AOC* allene oxide cyclase, *AOS* allene oxide synthase, *ISO* isomerase, *9-LOX* 9-lipoxygenase, *13-LOX* 13-lipoxygenase, *OPR* 12-oxophytodienoate reductase

of one DMADP with one IDP molecule through the action of GPP synthase produces the precursor of mono and diterpenes, geranyl pyrophosphate (GPP) and geranylgeranyl pyrophosphate (GGPP) in plastids (Fig. 5.1). The universal precursor of all the sesquiterpenes, farnesyl pyrophosphate (FPP) is produced in the cytosol as a result of sequential head-to-tail condensation of two IDP and one DMADP molecules (Nagegowda et al. 2008). In the final step, terpene synthases (TPS) as well as cyclases act on FPP and GPP resulting in the production of a vast variety of volatile terpenoid compounds (Cane 1999; Wise and Croteau 1999). Terpenoids from either of these pathways can further undergo hydroxylation, dehydrogenation, acylation or other chemical reactions, thereby increasing their volatility and altering their olfactory properties (Dudareva et al. 2004). TPS genes are highly diversified throughout the plant kingdom (Bohlmann et al. 1998; Chen et al. 2011). Many of the known TPS are promiscuous and can synthesize multiple products from a single prenyl diphosphate precursor (Degenhardt et al. 2009). For example, 20 different sesquiterpenes in the floral volatile blend of *Arabidopsis thaliana* are synthesized by only two terpene synthases (Tholl et al. 2005). This variation and plasticity of terpene formation by TPS is mainly because these enzymes can easily acquire new catalytic properties with minor structural changes (Tholl and Lee 2011).

Monoterpene like linalool, limonene, myrcene and (*E*)- β -ocimene, as well as sesquiterpenes such as farnesene, nerolidol and caryophyllene, are common constituents of floral scent. Other than mono, di and sesquiterpenes, certain flowers also emit irregular terpenoids (C8 – C18), which are formed by additional three-step modification including dioxygenase cleavage, enzymatic transformation and acid-catalysed conversion. For example, an irregular C12 terpenoid is synthesized in flowers of *Rebutia marsoneri* by oxidative removal of a C3 side chain from a C15 intermediate (Feng et al. 1993).

So far, extensive work has been done to understand terpenoids biosynthetic pathways; however, further studies are needed to understand the regulation of terpenoids metabolism, specifically on regulatory factors which control genes involved in the terpenoids synthesis (Cheng et al. 2007).

5.3.2 Benzenoids/Phenylpropanoids

Phenylpropanoids and benzenoids are the second largest class of floral volatile compounds. These molecules are derived from the aromatic amino acid phenylalanine (Phe) (Dudareva et al. 2013). The components are further grouped into three classes based on their carbon skeleton: phenylpropanoids (with a C6-C3 backbone), benzenoids (C6-C1) and phenylpropanoids-related compounds (C6-C2)—24% of all described phenylpropanoid/benzenoid compounds constitute phenylpropanoids-related compounds (C6-C2) (Knudsen and Gershenson 2006). Of all other phenylpropanoids-related compounds, biosynthesis of phenylacetaldehyde and 2-phenylethanol are known so far. Genes and enzymes involved in their biosynthetic pathways are isolated and well characterized (Hirata et al. 2012). For example, phenylacetaldehyde is formed in roses via two alternative routes. In the first route,

phenylacetaldehyde is formed by decarboxylation-amine oxidation reaction with the help of phenylacetaldehyde synthase (Kaminaga et al. 2006). The formation of phenylacetaldehyde in the second route is by the deamination of Phe by an aromatic amino acid aminotransferase followed by decarboxylation of the phenylpyruvate intermediate (Sakai et al. 2007; Farhi et al. 2010; Hirata et al. 2012). In Petunia petals, phenylacetaldehyde is produced through the action of phenylacetaldehyde synthase similar to the first pathway in roses. The second component, 2-phenylethanol, is formed from phenylacetaldehyde by the action of phenylacetaldehyde reductase (Sakai et al. 2007).

Benzoid (C6-C1) and phenylpropanoid (C6-C3) biosynthesis starts with the deamination of Phe to *trans*-cinnamic acid (CA) catalysed by L-phenylalanine ammonia-lyase (PAL) (Fig. 5.1). Further, benzenoid formation occurs by shortening of the propyl side chain of CA by two carbon units. This process proceeds via a β -oxidative pathway, a non- β -oxidative pathway or a combination of both (Boatright et al. 2004; Orlova et al. 2006) (Fig. 5.1). Recent researches in petunia flowers have given a clear picture of the β -oxidative pathway that is localized within plastids. The pathway begins with the activation of CA to cinnamoyl-CoA followed by hydration, oxidation and cleavage, resulting in the formation of benzoyl-CoA (van Moerkercke et al. 2009; Klempien et al. 2012; Qualley et al. 2012). In non- β -oxidative pathway, benzaldehyde is oxidized to benzoic acid by a NAD⁺-dependent benzaldehyde dehydrogenase (Long et al. 2009) (Fig. 5.1). However, the enzymatic reactions leading to the formation of the key intermediate benzaldehyde remains unknown.

Biosynthesis of phenylpropanoids shares the initial steps with the lignin biosynthetic pathway up to the formation of coniferyl alcohol. This is followed by the acetylation of coniferyl alcohol to coniferyl acetate by an acyltransferase from the BAHD superfamily (Dexter et al. 2007). Further, coniferyl acetate is converted to phenylpropanoids such as eugenol and isoeugenol by eugenol and isoeugenol synthases (Koeduka et al. 2006, 2008) (Fig. 5.1). Like volatile terpenes, the diversity of phenylpropanoid/benzenoid compounds in flowers is also increased by modifications such as methylation, hydroxylation and acetylation of direct scent precursors. This enhances the volatility or olfactory properties of the scent compounds.

5.3.3 Fatty Acid Derivatives

Fatty acid derivatives are less common representatives of floral volatile compounds. These are derived from the unsaturated C18 fatty acids, namely linolenic and linoleic acid. Biosynthesis of these compounds begin with stereo-specific oxygenation of fatty acids catalysed by the lipoxygenase (LOX) to produce 9 and 13-hydroperoxy intermediates (Schaller 2001) (Fig. 5.1). These intermediates can be acted upon by either allene oxide synthase (AOS) or hydroperoxide lyase (HPL) enzymes. The HPL pathway leads ultimately to the production of fatty-acid-derived volatile compounds. AOS converts 13-hydroperoxy intermediate to an unstable epoxide, which is then subject to cyclization followed by a reduction and a series of

cyclization reactions to form jasmonic acid (JA), an important phytohormone in plant defence against herbivores and necrotropic pathogens (Muhlemann et al. 2014) (Fig. 5.1). On the other hand, HPL converts both 9 and 13-hydroperoxy intermediates into volatile C6 and C9 products. These C6 and C9 aldehydes and alcohols, commonly known as green leaf volatiles (GLVs), are indicators of general plant tissue damage and also act as baseline defence components (Hammerbacher et al. 2019). However, they are also important constituents in the floral volatile bouquet of several plant species such as carnation and wild snapdragon (Schade et al. 2001; Suchet et al. 2011).

Thus, floral odours are composed of specific mixtures of the above-mentioned classes of volatile compounds which are produced from more than one biochemical pathways. These volatile blends which are species specific play a crucial role in the fine-tuning of plant–pollinator interactions both in specialized and generalized systems. However, together with the specific blend of volatiles, regulated emission of these scents helps in increasing the efficiency of these interactions.

5.4 Regulation of FVOC

5.4.1 Spatiotemporal Regulation

Flowers have evolved several regulatory mechanisms to increase the efficiency of pollination. Regulation of floral volatile emission spatially and temporally is one such strategy, which aids successful reproduction. For example, FVOC emission is often restricted to certain floral tissues and it is regulated developmentally and temporally. Petals are the major sites for floral volatile synthesis. These volatiles are synthesized and released to the atmosphere by diffusion from the epidermal cells of the petals. Other than petals stamens, pistils, pollen and even nectaries contribute to the floral bouquet (Flamini et al. 2003; Dotterl and Jurgens 2005; Farre-Armengol et al. 2013). FVOCs emitted from petals help in long-distance attraction of pollinators, while nectar and pollen odours could be indicators of reward quality. Samples of odours from pollen analysed with headspace techniques have been found to be chemically different from the scent of the whole flower (Flamini et al. 2003), and the diversity of compounds identified is often lower in pollen (Dobson et al. 1996). Odours from pollen are probably detected at short distances by insects, especially in cases where pollen emissions are quantitatively less abundant than those from the entire flower. However, many species may also present stronger pollen odours than others (Dobson and Bergstrom 2000). This helps pollen-foraging insects with a better chance to locate food and a more effective transport of pollen to stigmas (Dobson and Bergstrom 2000). In addition to these, trichomes and floral stomata are also considered to be a medium to release scent (Effmert et al. 2006). Molecular-level regulations such as scent biosynthetic gene expression and enzyme activity contribute to the tissue-specificity of scent emission (Muhlemann et al. 2014). Taken together, formation of FVOCs is often restricted to specific cell types or cell layers. For example, in snapdragon flowers the synthesis of methyl

benzoate is restricted to the inner epidermal layer of the upper and lower petal lobes (Muhlemann et al. 2014).

Temporal FVOC emission is tightly correlated with the activity of the pollinator species (Rodriguez-Saona et al. 2011). Diurnal and nocturnal volatile emissions are examples of temporal regulation. Flowers, which are pollinated by insects that are active during daytime produce volatiles diurnally, while plants that are visited by pollinators foraging at night emit volatiles nocturnally (Kolosova et al. 2001; Waelti et al. 2008). Whether the temporal change in FVOC emission is qualitative or quantitative really depends on the plant species under study. For instance, in the case of *Dianthus inoxianus*, the total amount FVOCs does not change over the day and night cycle, but the levels of compounds that aid in pollinator attraction varies according to visitor activity (Balao et al. 2011). The temporal emission of FVOCs is also regulated transcriptionally (Kolosova et al. 2001; Hendel-Rahmanim et al. 2007; Nagegowda et al. 2008; Nieuwenhuizen et al. 2009). Along with these, FVOCs emission is also regulated based on the developmental stage of the flower. When the flowers are ready for pollination, the levels of volatile emission are at maximum. The emission level drops after pollination and/or senescence (Muhlemann et al. 2006; Rodriguez-Saona et al. 2011). In summary, spatiotemporal regulation of FVOC emission can help in conserving metabolic costs, avoiding undesirable visitors and ensuring successful pollination by synchronizing with pollinator activity patterns.

5.4.2 Abiotic Factors

Several external factors are known to influence composition, quantity and timing of volatile emission. For example, increasing temperatures and CO₂ levels and altered precipitation patterns, can affect plant traits important for pollinator attraction (Jablonski et al. 2002; Scaven and Rafferty 2013). These changes are quite variable in intensity, timing and species but are generally likely to increase emissions of FVOCs (Penuelas and Staudt 2010). Higher emissions might increase the efficiency of reproduction by enhancing plant–pollinator interactions. Along with quantitative variations in emissions, abiotic factors can also cause qualitative variation in species-specific blends. In addition to this, other components of climate change such as air pollution and high concentrations of ozone, may induce a reduction in the efficiency of pollinators to forage for flowers by reducing the longevity of FVOCs once released and the distance at which they can be perceived by pollinators (McFrederick et al. 2008; Blande et al. 2010). Studies on the impact of abiotic factors are thus imperative to understand the future of pollinator-dependent crop plants in the growing crisis of climate change.

5.4.3 Biotic Factors

Along with abiotic factors, biotic interactions can also induce changes in the FVOC emission. Biotic factors include pollination as well as interactions with floral antagonists. It is known that flowers decrease or alter the emission of floral volatiles markedly after successful pollination (Muhlemann et al. 2006; Rodriguez-Saona et al. 2011). Such quantitative and/or qualitative post-pollination changes in FVOCs decrease the attractiveness of these flowers and increase the overall reproductive success of the plant by directing pollinators to flowers that are not yet pollinated (Schiestl et al. 1997). This is important especially for plants with low pollinator visitation rates whose reproductive success is largely pollinator limited (Neiland and Wilcock 1998). It also helps in reducing the carbon loss due to emission as well as to prevent additional visitation by flower antagonists (Muhlemann et al. 2006). The decrease in emission of FVOC is known to occur within 24–96 h of pollinator visitation after the pollen tubes reach the ovary (Negre et al. 2003).

Other than pollinator interactions, microbial interactions can also alter the FVOC emission. Floral microbiota can play a relevant role in the emission of floral scent, in *Sambucus nigra* flowers, where the removal of microorganisms caused a significant decrease in the amount of emitted FVOCs, without affecting floral physiology and VOC contents (Penuelas et al. 2014). In *Silene latifolia* flowers, infection by the anther smut fungus *Microbotryum violaceum* results in decreased total scent emission. This often caused discrimination against infected flowers by the pollinator (Dotterl et al. 2009). Below-ground association of arbuscular mycorrhizal fungi in *Polemonium viscosum* also reduces the intensity of FVOC emission. This shows that microbial interactions both below and above ground can modulate flower traits and thereby act as an additional layer of external regulatory mechanism (Becklin et al. 2011). Several other examples of microbial influence are discussed in the earlier section.

It is well known that upon herbivory, plants produce plant volatiles as an indirect defence mechanism (Dicke 2009). However, very few studies report induction of defence volatiles by flowers after florivory. For example, upon infestation of parsnip webworm, wild parsnip flowers are reported to emit higher amounts of octyl esters (Zangerl and Berenbaum 2009). Likewise, *Helicoverpa zea* larvae infested cotton flower buds induce emission of terpenes and fatty acid derivatives (Rose and Tumlinson 2004), which commonly deter florivores. Changes in FVOC emissions induced by leaf herbivory have been studied in several species (Effmert et al. 2008; Kessler et al. 2011; Pareja et al. 2012; Bruinsma et al. 2014). Such herbivore-induced changes can decrease or increase pollinator visitation and impact fruit and seed production by plants (Lucas-Barbosa et al. 2011; Bruinsma et al. 2014). Plants are known to emit volatiles upon herbivore damage to attract natural enemies of the herbivore such as parasitoid wasps. Although emission of herbivore-induced leaf volatiles is an excellent indirect defence strategy, this could have negative impact on overall reproductive fitness. For example, in *Solanum peruvianum* it was shown that herbivore-induced vegetative volatiles and FVOC emissions alter pollinator behaviour, making flowers of herbivore-attacked plants less attractive (Kessler

et al. 2011). In summary, herbivory can influence plant pollinators either by damaging the flowers directly, changing the composition of FVOC, causing metabolic changes or inducing vegetative volatile production that can in turn indirectly impact pollinator behaviour.

5.5 Collection and Analysis of FVOC

To date, several methods have been reported for efficient collection and analysis of VOCs (Tholl et al. 2006; Stashenko and Martínez 2008). They include extractive methods using harsh procedures like steam or water distillation, solvent extraction, supercritical fluid extraction and other traditional approaches that are still useful in large-scale preparations but suffer from disadvantages such as use of large amounts of harmful solvents and reagents, tedious time-consuming steps and loss of some components by decomposition. The increasing interest in VOC research has led to development of newer, milder and sensitive methods of extraction and detection. The rapid advancement in the analytical chemistry research has also improved the design of sophisticated instruments such as gas chromatography-mass spectrometry (GCMS) that has had enormous impact on floral volatile research. Here, we present known methods of collection and analysis of FVOCs.

Plant volatile collection usually involves collection of air in the headspace of the flowers. To do this, plants can either be enclosed inside jars in a laboratory set-up or in non-odour emitting wraps in field conditions. An adsorbent material is often used to trap the volatiles emitted from flowers that can be eluted into a suitable solvent or directly injected into a GCMS. The liquid sampling method usually requires a concentration step prior to GCMS analysis that can result in some sample loss and dilution. To avoid this, solvent-free methods such as thermal desorption (TD) method or solid-phase micro extraction (SPME) methods could be adopted. The collection of volatiles in this way is often referred to as “headspace” collection method. Headspace methods can be further classified as static and dynamic headspace. In static headspace method, volatiles from plant/flower are collected after encasing without any air circulation. In other words, the air is “static”. This is suitable for solvent-free micro extraction techniques such as SPME where a thin fibre of the adsorbent (usually 1 cm length) is inserted through an airtight septum into the volatile collection chamber. The fibre is left to adsorb till equilibration and then withdrawn. The fibre can then be directly desorbed onto the injection port in a GCMS for further analysis. There are several SPME adsorbents commercially available that usually consist of an inert material coated with different adsorbents such as polydimethylsiloxane (PDMS), divinylbenzene (DVB), carboxen, etc. Depending on the polarity and application, the adsorbent fibre needs to be chosen and optimized. Overall, HS-SPME-GCMS (Headspace SPME) is a method of choice for solvent-free collection of small sample sizes especially in field conditions. It also has the advantage of ease of automation as auto sampler for SPME is now available and the fibres are reusable after desorption. HS-SPME technique is widely used for volatile mapping studies where large numbers of plants/flowers have to be screened

(Vandendriessche et al. 2013). Some challenges associated with this sampling technique are high susceptibility to matrix effects, difficulty in identifying unknown compounds as the trapping efficiency is low, humidity issues and cumbersome quantification. To quantify using SPME technique, standards have to be adsorbed on the fibre at same equilibration time as the analyte and since this can differ depending on type of volatile mixture, sample volume, vapour pressure and temperature, usually the quantification in this method is cumbersome or impractical. Despite the challenges, there are many reports of FVOC collection using this method (Burzynski-Chang et al. 2018). A more recent static volatile collection approach has been to use sorbent PDMS tubes. These tubes are robust, cheap, can be cut to desired lengths simply with scissors and also used in natural field conditions. Earlier, such tubes were used for soil volatile collection where the inserted tubes can be easily removed and analysed by liquid chromatography (Mohney et al. 2009). Recently, similar method has been used with gas phase chromatography using thermal desorption (TD) method (Kallenbach et al. 2015). Taken together, the static headspace sampling technique has numerous applications but also suffers some disadvantages as mentioned. It is useful for real-time field volatile collection and quantification is possible under non-equilibrium conditions. It is imperative, therefore, to consider application and potential limitations carefully before choosing the right sampling method.

Dynamic headspace collection method involves continuous circulation of filtered air or any inert gas through the collection set-up. This technique is more often used in floral scent research than static headspace as it helps in collection of larger amounts of volatiles sufficient for detection as well as identification of unknowns. Since air or carrier gas is circulated continuously, the problem of humidity does not arise and this gives a clean adsorption process. The choice of adsorbent based on their binding affinity is crucial for the success of this method of sampling. However, this can be easily solved by mixed adsorbents where different material is packed in layers or beds (Ciccioli et al. 2002). Although headspace sampling involves constant flow of air, there are many ways to do this: (a) air is only pulled using a vacuum pump; (b) air is both “pushed” and “pulled” and (c) collection in a closed system where push and pull is performed inside the set-up (closed loop stripping method). All three procedures have their own advantages and problems and have been discussed in detail in Tholl et al. (2006). Since closed loop method minimizes contamination compared to open method, it is desirable for small samples as demonstrated with small, relatively less scented *Arabidopsis thaliana* flowers. In this report, it was shown that floral volatile composition obtained using closed loop stripping method has significantly higher signal-to-noise ratio compared to open push–pull method (Chen et al. 2003). Push–pull systems are easy to design and are useful for scented flowers. The only drawback of dynamic headspace method is that it needs to be performed in airtight conditions and care must be taken to seal any leakage. Nevertheless, this sampling method is superior, extremely useful and reproducible for volatile collection in greenhouse and climate-controlled chambers. Alternatively, static headspace is suitable for qualitative analyses and survey of VOC profiles.

Ideally, the two collection methods are complementary and can be used for collection of volatiles efficiently for different types of samples.

GC-MS is the best suited method for detection and analysis of volatiles and is often used in floral volatile studies (Knudsen et al. 2006). For this analysis, volatiles can be either eluted into suitable non-polar solvents and injected into GC or directly desorbed using thermal desorber unit. Automation in thermal desorption allows high-throughput injections. Alternatively, a micro vial kit can also be used. Here, small amount of liquid samples in disposable quartz micro vial is inserted at the tip of the probe. The probe is then guided into the injector port of GC using a special adapter. This method is particularly useful for very small samples such as pollen. In a study by Juergen and Dotterl (2004), pollen odours were measured from single anthers using this technique. The authors could identify 103 compounds from 12 Ranunculaceae species and report low intraspecific variation (Juergen and Dotterl 2004). From the GCMS run, total ion chromatograms are obtained, which provide information on the retention time of each compound and its mass spectrum consisting of characteristic ion fragmentation patterns. Then, using standard library databases such as Wiley and NIST, a suitable match can be obtained for known compounds. However, matching with library spectra and retention index can often be erroneous due to various complex ion dissociations and rearrangements. For proper reliable identification, Kovats indices have to be matched in at least two columns of different polarity as well as an authentic synthetic standard. Softer ionization techniques such as chemical ionization can be useful for obtaining stronger molecular ion peak that makes it sometimes easy to understand the fragmentation pattern of unknowns. Nevertheless, given the extensive literature on floral volatiles and the revival of mass spectral databases, identification and quantification of known volatiles can be performed with confidence if the sampling method is robust (Koo et al. 2013). Although MS spectra are sufficient to identify floral volatiles, sometimes to obtain more information on unknowns and to quantify them, tandem MS-MS techniques are employed. In MS-MS technique, parent ions are further fragmented and the resulting daughter ions are further analysed to gain more information on the original molecule. This method also gives more reliability to quantification of unknowns.

In addition to laboratory-based GCMS analysis, newer technologies such as portable GC are highly useful for field-based measurements. This miniaturized GC performs online GC analysis using surface acoustic wave (SAW) microbalance detector, also popularly known as Electronic zNose™ (Electronic Sensor Technology, Newbury Park, USA) (Kunert et al. 2002). Using this instrument, in a recent study, FVOCs of evening primrose were measured and the authors report the variation in emission with flower opening and closing (Oh 2017). Since this technique allows real-time monitoring of volatile emission with rapid detection, it is also known as “fast GC-SAW” technique. Another innovation in rapid volatile analysis is the use of toroidal ion trap mass spectrometer (TIT-MS). Wong et al. (2017) report analysis of essential oils from hybrid and commercial varieties of Hop (*Humulus lupulus*) using this technique. They used HS-SPME method for sample collection followed by separation on person-portable low thermal mass GC that utilizes low

power resistive heating of GC open tubular column. This GC coupled with ITMS (ppGC-ITMS; PerkinElmer Torion Technologies) was used to perform rapid, on-site measurement of leaf volatiles. The application of such portable advanced technologies is not only useful for research on plants, but also immensely beneficial to detect hazardous chemicals rapidly and accurately (Contreras et al. 2008). Despite these state-of-the-art technologies, traditional dynamic headspace sampling followed by offline GCMS remains the method of choice in laboratory studies on FVOCs.

5.6 Conclusion and Future Directions

This chapter outlines the various aspects of FVOCs ranging from biosynthesis, regulation, varied functions to collection and analysis. There has been tremendous progress in our understanding of floral scents in the past decade and this has stimulated considerable research in pollination, biochemistry and chemical ecology. We have explained in detail the diverse functions of FVOCs but still we need more studies to address the role of whole volatile blends on insect populations. Several groups report key floral compounds and their impact on pollinators and other insects; however, floral volatiles are usually released as a complex mixture of different chemicals whose role is still unclear. Also, whether the ecological effect of a particular chemical entity changes in the mixture or not remains unknown. Further, it also remains to be studied if the presence of one chemical compound can impact the attractiveness of others in the blend. With extensive studies on floral chemistry, it is clear that they shape beneficial as well as antagonistic ecological interactions in a community. This indicates that it is imperative to understand floral scents from whole-plant perspective and this would more likely shed light on the evolution of these signals.

A key question in biology is to understand variations in functional traits and in case of floral scents, the variations can arise from several factors. Abiotic factors play an important role in altering FVOC emission. For instance, it is well known that plants growing in different geographic gradients exhibit varying floral traits (Cosacov et al. 2014). With the increase threat of climate change and its influence on plant–pollinators interactions, quantitative approach is needed to evaluate their role in future climate-mediated changes in ecological networks. However, to date, very few studies are known to report such variation in FVOC with landscapes owing partly to the difficulty in collection of these scents. The advances made in analytical instrumentation with newer, faster, real-time measurements, it is now possible to ask exciting questions that address global changes in floral scents. The complexity of FVOCs highlights how plants change these signals according to local community dynamics and more functional studies are needed to understand such plasticity. These can then help us understand both their functions as well as selective forces that operate on these traits. Taken together, investigation of FVOCs in the context of environmental changes, community-level dynamics as well as integration with other sensory modalities should be an important focus of future research. Analysis of

whole plant “volat-ome” would allow phenotyping for efficient breeding programs tuned according to changing environmental conditions.

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A Snapshot of Evolutionary History of Floral Nectaries Across Angiosperm Lineages

6

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Abstract

Floral nectary (FN) represents an integral component of plant-pollinator interaction and thus its contribution to the evolutionary divergence of angiosperms cannot be ruled out. However, scattered information limits our present understanding of its direct role in driving floral evolution. We looked into the distribution pattern of the floral nectary to ascertain its evolutionary history across angiosperm phylogeny (APG IV). The analysis indicates that it is a highly homoplastic trait, i.e. it has been gained and lost many times independently in different lineages across angiosperms. Further, it appears that different forms of FN (sepal, petal, hypanthial and tepal) have evolved from the ancestral nectaryless flowers through separate transition events during the course of evolutionary history. The incidences of reversal to the nectaryless state are common. Interestingly, the inclusion of developmental genetic aspects in relation to angiosperm phylogeny has proved useful in refining the predicted scenarios in the evolution of various nectary types. The analysis also underlines that similar genes have been repeatedly recruited to specify floral nectary in different clades of angiosperms. These findings reiterate that selection works on a few genes of the Gene Regulatory Network of flower development and are thus more likely to underlie convergent evolution.

Keywords

Floral nectary · Pollination syndrome · Angiosperm phylogeny

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Glossary

Clade: A group of organisms that includes a common ancestor with all its descendants.

Synapomorphy: Derived characters that are shared between an ancestor and its descendants.

Symplesiomorphy: An ancestral trait or character shared by two or more taxa.

Pseudogenization: The process in which gene loses its function through disruption of its regulatory or coding sequence.

Subfunctionalization: The process by which each of the duplicated gene or parologue retains a subset of its original ancestral function.

Neofunctionalization: The process by which gene acquires a new function after a gene duplication event.

Parsimony: The best hypothesis of relationship that requires minimum number or fewest evolutionary changes.

6.1 Introduction

Flower is a key structural innovation, which has led to rapid diversification of angiosperms (Friis et al. 2006). There is a remarkable similarity in the structural organization of flowers among majority of the taxa, and a significant proportion (~90%) of them are pollinated by animals (Ollerton et al. 2011). It is well-known that floral traits (*floral suites*) exhibit convergence among unrelated taxa to interact with similar functional group of animals (Faegri and Pijl 1979; Fenster et al. 2004). The suites serve as filters and exert selection on the legitimacy of pollinators to bring about pollination (Shivanna and Tandon 2014). The interaction may result in establishing mutualism between the plant and pollinators to different degrees and promotes the fitness of plants through targeted pollen deposition on the stigma of the co-blooming conspecifics. Besides exhibiting diverse floral forms, colours and shapes to attract a wide spectrum of pollinators, flowers offer rewards to sustain their services, mostly in the form of nectar and pollen and less frequently by offering scented resins, oils and fragrance (Armburster 2012). Being the predominant floral reward, dynamics of nectar production and composition can regulate plant fitness and community organization (Kearns and Inouye 1993; Irwin et al. 2010; Singh et al. 2014). Thus owing to its direct influence on the reproductive success, nectar biology (development and function) has been a vital aspect of investigation in reproductive ecological studies since long (Baker and Baker 1975; Nicolson et al. 2007).

Nectar is synthesized and secreted by a localized gland, termed nectary (Vogel 1998c). Nectary may occur either on the vegetative (petioles, stipules, leaf blades, cotyledons) or the reproductive organs (receptacle, sepal, petal, stamen, filament, anther, ovary, style, stigma, fruits) (Bernardello 2007; Weber and Keeler 2013). Nectary morphology varies greatly from a well-structured (single or multicelled trichomes) to almost inconspicuous forms (Marazzi et al. 2013; Vogel 1998c). Further, nectaries among the plants show remarkable differences in histology and

composition of the nectar (Roguz et al. 2018; Tolke et al. 2018). A comprehensive classification to appreciate nectary diversity is not available so far. However, one weakly defined classification, based on its distribution and function, of nectaries into the floral and extrafloral types is still considered a standard. Whereas floral nectary (FN) is associated with the organs of floral whorls and is directly linked to pollination, the extra floral nectaries (EFN) are mainly present on the vegetative organs, and their secretions serve as food to ants and arthropods in lieu of protection from the predators. The EFNs are exclusively involved in plant defence mechanisms (Marazzi et al. 2013). However, there are exceptions that do not fall into this classification such as those of *Euphorbia* species, where the bract tips alternate with the nectary glands. They are essentially considered extrafloral, as they are positioned on the reduced inflorescence but certainly attract pollinators (Proctor et al. 1996).

The functional traits of the flower that promote timely and precise dispatch/receipt of pollen are linked to its developmental traits. Between the nectary and nectar, one may view the former as an integral component of flower development and the latter to be a functional component of the flower. Attempts are being made to explain as to how pollinators are able to induce changes in the developmental traits of the flower through the functional traits (Dellinger et al. 2019). The information may be strengthened by ascertaining the patterns that emerge from macroevolutionary processes of key floral developmental domains.

Floral nectary has profusely figured in numerous reproductive and ecological studies of angiosperms (Bernardello 2007). However, the scattered information limits our present understanding of its role played in driving floral evolution and consequent speciation and angiosperm diversification. In this chapter, we present a comprehensive assessment on the distribution pattern of FN's across angiosperm families, recognized in the APG IV system by Chase et al. (2016). For mapping the trait, published reports covering floral morphology, anatomy, ultra-structural and pollination data were used (Table 6.2). Then, a series of questions were asked with collated available evidences, regarding its origin and evolutionary patterns across angiosperm lineages. The specific issues addressed include (i) how frequently each FN type appeared across the entire phylogeny, (ii) what are the possible causes that might have favoured a particular type of FN and (iii) How many possible transitions between different types might have happened in the evolutionary history of angiosperms? We also looked into the clades/lineages, where origin, loss or evolutionary transitions in the trait have occurred. Finally, we discuss the role of underlying genes in the evolution of floral nectary across angiosperm phylogeny in the light of recent information from developmental genetics.

6.2 Phylogenetic Distribution of Floral Nectary

The distribution pattern of the FN across the angiosperm families shows that they are ubiquitous (Fig. 6.1). It is a phylogenetically widespread floral trait which was lost and gained on numerous occasions. Within the most basal ANA clade (Amborellales, Nymphaeales and Austrobaileyales), a sister group of

mesangiosperms (Chloranthales + Magnoliids) (Monocots (Ceratophyllales + Eudicots)), most of the families lack the floral nectary. This suggests that basal angiosperm flowers were nectaryless, and the nectaries present on the sepals in Cabombaceae+Nymphaeaceae (second node) and that on the tepals in Schisandraceae (third node) are most likely to be due to two independent transitions in these lineages.

At the branch below Austrobaileyales, the phylogenetic relationship amongst Chloranthales, Magnoliids, monocots and eudicots is not resolved (Chase et al. 2016), but the presence of similar nectaryless condition in the Chloranthaceae, as observed in the ancestral ANA members, supports its sister relationship with the ANA clade (Doyle et al. 2003; Tosaki et al. 2001; von Balthazar and Endress 1999). In magnoliids, the distribution of nectary shows remarkable differences in the developmental origins. Floral nectary originated on tepals (Calycanthaceae and Siparunaceae), petals (Magnoliaceae and Annonaceae), stamens (Winteraceae, Atherospermataceae, Gomortegaceae, Hernandiaceae, Monimiaceae, Lauraceae and Annonaceae), staminodes (Hernandiaceae and Lauraceae) and the stigma (Magnoliaceae and Annonaceae). In addition, few families also showed the absence of FN in the magnoliid clade (Vogel 1998a). Therefore, it is difficult to infer the ancestral state of magnoliid clade because of high variability of FN's in Canellales-Piperales and Laurales-Magnoliales. It is possible that the diverse floral nectaries originated via independent transitions in each family, most plausibly through parsimonious pathway or the shortest evolutionary route to support the present distribution pattern in magnoliids.

Monocots show an exceptional pattern of distribution of floral nectaries. Most of the families of its early branching (Acorales, Alismatales) and later-derived clades (Petrosaviales, Dioscoreales, Liliales, Asparagales, Arecales, Zingiberales and Commeliniales) share septal-floral nectaries. A few selected clades, namely, Poales, Pandanales, Triuridaceae (Dioscoreales), Orchidaceae and Hypoxidaceae (Asparagales), and Philydraceae and Commelinaceae (Commeliniales), show absence of septal nectaries. It is most likely that monocot flowers were ancestrally harbouring the septal nectary and lost it on several occasions in the abovementioned descendants, as evolutionarily, loss of a trait is much easier than to gain one (Glover et al. 2015). Further, other types of FN mapped on a few monocots are derived independently. Septal nectary appeared as synapomorphy, exclusively for the clades of monocots.

Ceratophyllales, which appeared as sister to the monocots and the eudicots in the tree, lacks floral nectaries. This state is also reflected at the nodes of an early branched Chloranthales and Magnoliids. This character state of nectary being ancestrally missing is possibly converged in the order. At the branch below Ceratophyllales, the distribution across the other early diverged eudicots demonstrate multiple origins of floral nectary. They occur on the following organs of flower: sepal (Ranunculaceae), petal (Lardizabalaceae, Menispermaceae, Berberidaceae), tepal (Ranunculaceae), staminal (Lardizabalaceae, Proteaceae, Sabiaceae, Ranunculaceae), staminode (Circaeasteraceae, Berberidaceae, Ranunculaceae), pistillode (Buxaceae), style (Buxaceae) and ovary

(Trochodendraceae). Such a huge diversity of FNs obscures the general pattern followed, in particular, at the nodes of the early eudicots (Ranunculales, Proteales, Buxales and Trochodendrales). However, two different research groups (Tamura et al. 1993; Erbar et al. 1999) on the basis of developmental studies gave evidence that diverse nectaries present in a majority of the families of Ranunculales are derived from stamen. Thus, FNs in all of them were interpreted to be staminally associated. If this is the case, it can be deduced that nectaries were ancestrally staminal for early eudicots as a whole, and repeated losses might have occurred in some of its descendants on independent occasions (Eupetalaceae, Papaveraceae, Nelumbonaceae, Plantanaceae). The development of nectaries on the petals (Lardizabalaceae, Menispermaceae, Berberidaceae), ovary (Trochodendraceae) and style (Buxaceae) appears to have been derived secondarily from the staminal nectary.

Further, in the late evolved eudicots (Gunnerales-Brassicales), i.e. up to Rosids, floral nectaries are confined only to the stamen or stamen-associated whorl. According to their position, they are categorised into (i) intrastaminal (nectaries borne between the staminal whorl and the ovary), (ii) extrastaminal (nectaries borne between the perianth and the androecium), (iii) interstaminal (nectaries located between the stamens), (iv) staminal, and (v) staminoidal types. These stamen-associated nectaries are found in almost all the lineages that group within Celastrales, Oxalidales, Sapindales, Huerteales, Crossomatales (except Stachyuraceae and Guamatlaceae), Geraniales, Malpighiales (most of its families), Vitales, Zygophyllales (except Krameriaeae) and Brassicales (except Gyrostemonaceae, Bataceae, Setchellanthaceae, Akaniaceae, Tropaeolaceae, Moringaceae, Caricaceae). The rest of the clades/lineages either completely lack FN or occasionally bear them on hypanthium or gynophore in specific lineages of Myrtales, Cucurbitales, Rosales and Brassicales. The recurring appearance of stamen-associated nectary across late evolved clades of eudicots points to the high possibility of a single-transition event occurred at the ancestral node of early eudicots (Ranunculaes). This condition of early eudicot flowers with ancestrally stamen-associated nectaries was also hypothesized by others (Erbar 2014), who investigated core eudicot nectaries. Thus, nectaries associated with stamen whorl can be considered as symplesiomorphies retained by the most clades within the Rosids. However, hypanthial or gynophore nectaries might have resulted from multiple transitions/shifts that occurred in the selected evolutionary lineages (Lythraceae, Onagraceae, Melastomataceae, Fabaceae, Rosaceae) and are thus derived. Asterids, which appear as sister to the Rosids, show different distribution patterns of FN particularly in their highly nested clades (Lamiids and Campanuliids). These clades have flowers with floral nectary positioned in the innermost whorl, i.e. gynoecial. It appears that the evolutionary shift possibly occurred at the node leading to Lamiids and Campanuliids. On the other hand, the early branching clades of Asterids, i.e. Berberidiopsales, Santalales and Caryophyllales, have the most pronounced incidence of stamen-associated nectaries.

The analysis revealed that FN is a highly homoplastic trait, i.e. it has been gained and lost many times, independently in different lineages across angiosperms. It is

further inferred that angiosperm flowers were ancestrally nectaryless and it has been acquired through many transitional events during the course of its evolution (Fig. 6.1). The study further indicates that FN evolved only twice in ANITA clade and restricted to nonreproductive whorls of the flower (sepals and petals). However, in the Magnoliids, that is, sister to (eudicots-Ceratophyllaceae)-monocots clade, there have been multiple transitions between the sepal, petal and staminal nectary states, although the exact pattern is not clear. After the divergence of monocots and eudicots, nectary associated with floral whorls became a prevalent state. In the monocots, a majority of the taxa show septal type of nectary and occasionally tepal nectary or sometimes both while in the eudicots stamen-associated nectaries (staminal, interstaminal, interstaminal extrastaminal, staminodal) are more predominant, especially among the Rosids and Asterids. In Asterids, there is a transition to ovarian nectaries, as observed in the highly nested clades Lamiids and Campanuliids, although reversals to sepal, petal and tepal nectary are not uncommon. These results further emphasize that nectary shift is more likely to have gradually occurred in one particular direction, i.e. centripetally from the outer whorls of the flower to inner most gynoecium.

Amongst the different forms of nectary, those associated with the non-reproductive whorls such as sepals (3.1%), petals (7.4%), tepals (4.5%) and hypanthium (4%) appeared less frequently in the families across phylogeny. Most of the transition events to nonreproductive whorl nectary amongst the angiosperm lineages occurred independently, i.e. sepals (11 transitions), tepal (10 transitions), hypanthial (13 transitions) and petals (26 transitions). However, those associated with the essential organs, i.e. the staminal and the gynoecial whorls (17.5%), septal nectary (12.7%), intrastaminal nectary (16.1%) and staminal nectary (11.0%), are found predominantly in a number of species-rich lineages (Table 6.1). Amongst different forms of nectary associated with essential organs, stylar, stigmatic and gynophore types have recruited independently via four, two and three transition events, respectively. Septal nectary is most likely originated once in the ancestral node of monocots followed by 19 independent losses in some of its descendants. Both stamen-associated nectary and ovarian type of nectary also appeared to be associated with one major transition event at the ancestral node of eudicots and asterids, respectively, followed by its frequent losses in some of its members (Fig. 6.1).

Such centripetal translocation of the floral nectaries, to the innermost whorl in the flower, appears to be linked with the evolution of selective accessibility of rewards to specialist pollinators. This is in agreement with the evidences from the studies performed on early paraphyletic clades of angiosperms (Nymphaeaceae, Aristolochiaceae, Piperaceae, Annonaceae) with radially symmetrical (*actinomorphic*) flowers (Citerne et al. 2010). Nectaries present either on the bracts or the petals of these open-type flowers offer an unrestricted access to a diverse range of generalist foragers. This may increase the visitation of different animals, thus limiting the pollination efficiency and consequently affecting the out-crossing rate of the species. In contrast, nectaries associated with the essential organs have higher incidences of occurrence across angiosperm families because the nectar offered at this position

Table 6.1 The frequency of appearance of FN and associated transition events across Angiosperm families. The types have been grouped based on the floral organ on which they occur

Total no. of families	416	Percentage	Transition events
Families with sepal nectary	13	3.12	11
Families with petal nectary	31	7.45	26
Families with tepal nectary	19	4.56	10
Families with hypanthial nectary	17	4.08	13
Families with extrastaminal nectary	28	6.73	1
Families with interstaminal nectary	10	2.40	
Families with intrastaminal nectary	67	16.10	
Families with staminal nectary	46	11.05	
Families with staminodal nectary	18	4.32	
Families with stigmatic nectary	3	0.72	2
Families with stylar nectary	5	1.20	
Families with septal nectary	53	12.74	19
Families with ovarian nectary	73	17.54	13
Families with pistillloid nectary	7	1.68	7
Families with gynophore nectary	3	0.72	3

ensures that pollinators would optimally contact the reproductive organs of the flower, thereby allowing the plant to maximise fitness through increase in the specificity of dispatch and receipt of pollen during pollinator's visits.

6.3 Genetic Switches Underlying Nectary Development

The multiple origins of floral nectaries among angiosperms highlight the question whether similar genetic mechanisms were involved or the existing gene of the developmental pathways has been reused, in the repeated evolution of floral nectary. A number of studies have demonstrated the involvement of key regulators in nectary formation across angiosperms (Lee et al. 2005; McKim et al. 2008; Min et al. 2019).

Preliminary studies indicate that the genetic pathway as laid by the canonical ABC model and its elaborations on the predominantly conserved flower architecture across diverged lineages, does not appear to be involved in floral nectary development (Bowman et al. 2012; Coen and Meyerowitz 1991; Krizek and Fletcher 2005). One reason may lie in the relative positioning of the floral whorls on the flower and the order in which they arise, which are fixed across the angiosperms. On the contrary, nectary is not present in the fixed position in the flower (Bernardello 2007). It arises in any position along the receptacle and is associated with any of the four floral whorls – sepal, petal, staminal and gynoecial in different clades of angiosperms. Thus, nectary is not constrained by the limitations of floral organ identity genes and probably represents one of the weak links between the floral developmental and functional modules. This is reflected at the molecular level which indicated that nectary is an ABC-independent floral structure (Baum et al. 2001).

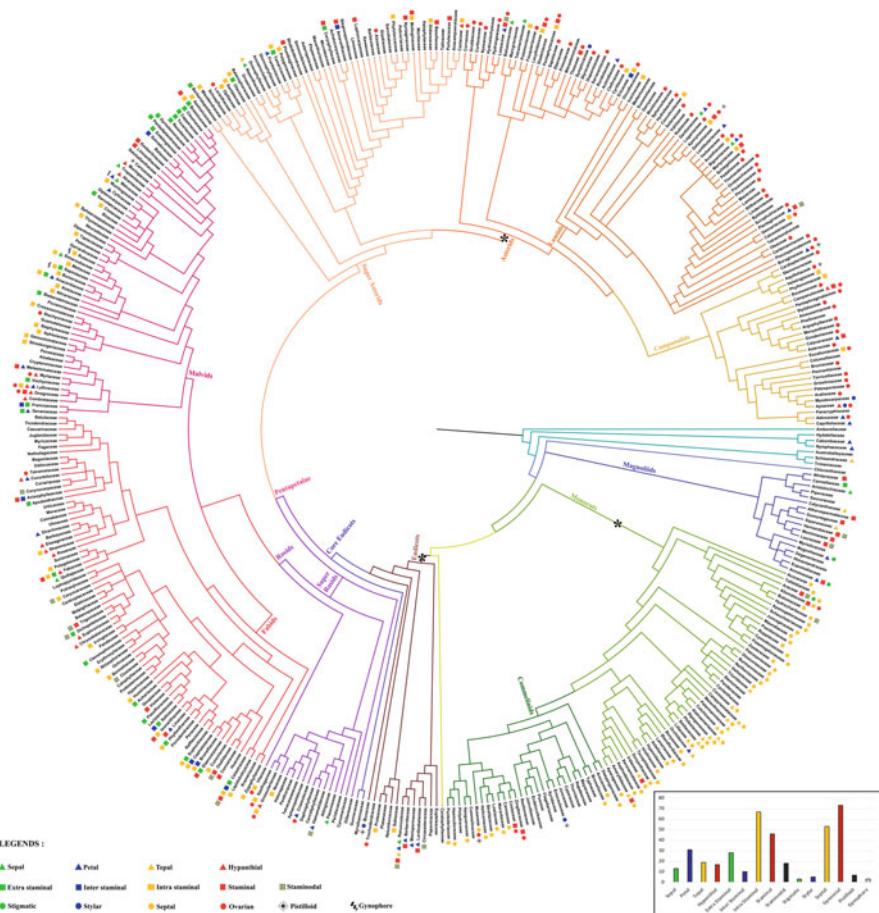


Fig. 6.1 Phylogenetic distribution of floral nectaries across angiosperms. The tree based on APG IV classification is constructed manually (through newick format) and visualized in Figtree Software (version 3.1). The relationships among orders and families shown are according to updated APweb (Stevens, 2017). Each nectary type is depicted by different shape colour (triangles – nonreproductive floral whorl nectary; circular – gynoecium-associated nectary; and square – androecium-associated nectary). Asterisk represents transition events that occurred at the ancestral nodes of monocots for septal, eudicots for stamen/stamen whorl associated and asterids for ovarian nectary. Inset depicts the frequency of each nectary type present across angiosperm phylogeny

The more convincing demonstrations of the involvement of only one or very few key genes in nectary development came from mutant and expression studies targeted downstream of the ABC genes (Bowman and Smyth 1999; Box et al. 2011; Lee et al. 2005; Min et al. 2019; Fourquin et al. 2014). In Orchidaceae, a KNOX gene ortholog from the MADS Box transcription factor family has been shown to play a key role in the development of nectary spur (Box et al. 2011). In Ranunculaceae, *STYLISH*

homologue isolated from a well-characterized mutant is associated with nectary development (Min et al. 2019). Another transcription factor characterized in *Arabidopsis thaliana*, known to be involved in nectary formation, is *CRABS CLAW (CRC)* (Bowman and Smyth 1999). A number of equally convincing studies from evolutionary distant families, i.e. Solanaceae (*Petunia hybrida*, *Nicotiana tabacum*, *Nicotiana benthamiana*), Brassicaceae (*Capparis flexuosa*, *Cleome sparsifolia*, *Lepidium africanum*), Malvaceae (*Gossypium hirsutum*), Asparagaceae (*Asparagus asparagoides*), Poaceae (*Oryza sativa*), Amborellaceae (*Amborella trichopoda*), Cabombaceae (*Cabomba caroliniana*), Papaveraceae (*Eschscholzia californica*) and Berberidaceae (*Epimedium sagittatum*), have shown that *CRC* is necessary for nectary development within two major clades of the eudicots: rosids and asterids (Fourquin et al. 2007; Lee et al. 2005; Morel et al. 2018; Nakayama et al. 2010; Orashakova et al. 2009; Sun et al. 2013). Another class of transcriptional factor found to be required for nectary development is *BLADE-ON-PETIOLE (BOP1 and BOP2)* (McKim et al. 2008).

It is important to note that the genes responsible for nectary development are already selected in the gene regulatory network (GRN), which governs different developmental processes in angiosperms. For instance, *KNOX* genes are implicated in leaf morphogenesis and shoot apical meristem (SAM) maintenance (Box et al. 2012; Jouannic et al. 2007). *STYLISH* gene is involved in the development of style and stigma, while *CRC* homologue aids in gynoecium morphogenesis (Pfannebecker et al. 2017). Thus, it is apparent from these evidences that *similar genes have been repeatedly recruited* to specify nectary in different clades of angiosperms. It further highlights that there are very few genes in GRN that are more suitable for evolutionary changes and thus more likely to underlie convergent evolution (Table 6.2).

The probable way by which the key genes shift their ancestral expression patterns to novel locations seems to be via changes in *cis*-regulatory sequences. These regulatory sequences lie in the proximity of coding sequences and are less constrained to maintain the triplet codon and hence emerged as potential regions of morphological evolution (Carroll 2008; Gompel et al. 2005; McGregor et al. 2007; Cretekos et al. 2008; Jeong et al. 2008; Frankel 2012). For example, the elaborated *KNOX* gene expression in the corolla nectar spur is likely to be a result of transposon insertions in putative *cis*-regulatory regions in the 5' untranslated region (5'-UTR) and first intron in the family Plantaginaceae (Golz et al. 2002). Furthermore, novel role for *CRC* orthologues in monocots (leaf and carpel development) and core eudicots (nectary development) is again a result of molecular changes in the *cis*-regulatory regions of the genes (Fourquin et al. 2007).

Gene duplication and subsequent functional divergence of paralogs have also been found integral to the repeated evolution of convergent traits (Averof and Akam 1995; Ohno 1970). The duplicated gene copy, which arose via tandem, proximal, transposed or whole genomic duplication (WGD) events, alleviates the selection constraints and thus resulted in pseudogenization, subfunctionalization or neofunctionalization (Lawton-Rauh 2003; Li 1983; Ohno 1970; Zhang et al. 2002). For example, *FRUITFULL* gene is primarily involved in cell proliferation

Table 6.2 List of published reports used for compiling data on floral nectaries across angiosperms

S. No.	Family	References
1	Amborellaceae	Thien et al. (2003)
2	Hydatellaceae	Rudall et al. (2007) and Taylor et al. (2010)
3	Cabombaceae	Erbar (2014) and Galati et al. (2019)
4	Nymphaeaceae	Brown (1938), Schneider and Jeter (1982), Vogel (1998a), Schneider et al. (2003) and Hiepko 1965
5	Austrobaileyaceae	Endress (1990)
6	Trimeniaceae	Bernhardt et al. (2003)
7	Schisandraceae	White and Thien (1985), Thien et al. (1983, 2009), Endress (1990) and Saunders (1998)
8	Canellaceae	Wilson (1986) and Erbar (2014)
9	Winteraceae	Gottsberger et al. (1980), Thien (1980) and Lloyd and Wells (1992)
10	Saururaceae	Thien et al. (1994) and Tanaka (1979)
11	Piperaceae	Vogel (1998a)
12	Aristolochiaceae	Meijer (1993), Bernardello et al. (1999), Daumann (1959), Faegri and van der Pijl (1979), Vogel (1998a) and Sakai (2002)
13	Myristicaceae	Bernardello (2007)
14	Magnoliaceae	Thien. (1974), Huang et al. (1999), Endress (1990), Allain et al. (1999), Daumann (1930) and Erbar (2014)
15	Degeneriaceae	Kubitzki (1993c)
16	Himantandraceae	Bernardello (2007) and Endress (1984a)
17	Eupomatiaceae	
18	Annonaceae	Kessler (1988), Endress (1990) and Silberbauer-Gottsberger et al. (2003)
19	Calycanthaceae	Vogel (1998a)
20	Siparunaceae	
21	Gomortegaceae	Fahn (1979), Cronquist (1981), Rohwer (1993, 2009), Smets (1986), Kubitzki (1993a, b), Endress and Lorence (2004) and Endress (1980)
22	Atherospermataceae	
23	Hernandiaceae	
24	Monimiaceae	
25	Lauraceae	
26	Chloranthaceae	von Balthazar and Endress (1999), Tosaki et al. (2001) and Doyle et al. (2003)
27	Acoraceae	Rudall and Furness (1997) and Buzgo and Endress 2000
28	Araceae	Cronquist (1981, 1988) and Vogel (1983, 1998d)
29	Tofieldiaceae	Daumann (1970), van Heel (1988), Smets et al. (2000) and Remizowa et al. (2006)
30	Alismataceae	
31	Butomaceae	
32	Hydrocharitaceae	Cronquist (1981, 1988), Vogel (1998d) and Bernardello (2007)
33	Scheuchzeriaceae	
34	Aponogetonaceae	Daumann (1970), van Heel (1988), Smets et al. (2000) and Remizova et al. (2006)

(continued)

Table 6.2 (continued)

S. No.	Family	References
35	Maundiaceae	Cronquist (1981, 1988) and Vogel (1998d)
36	Juncaginaceae	
37	Zosteraceae	
38	Potamogetonaceae	
39	Posidoniaceae	
40	Ruppiaceae	
41	Cymodoceaceae	
42	Petrosaviaceae	Rudall (2002) and Remizowa et al. (2006)
43	Nartheciaceae	Daumann (1970), Smets et al. (2000), Caddick et al. (2002), Rudall (2002), Remizowa et al. (2006) and Tobe et al. (2018)
44	Burmanniaceae	Daumann (1970), Smets et al. (2000), Caddick et al. (2002), Rudall (2002) and Remizowa et al. (2006)
45	Dioscoreaceae	Daumann (1970), Smets et al. (2000), Caddick et al. (2002), Rudall (2002) and Remizowa et al. (2006)
46	Triuridaceae	
47	Velloziaceae	Menezes (1973)
48	Stemonaceae	Caddick et al. (2002)
49	Cyclanthaceae	
50	Pandanaceae	
51	Campynemataceae	Rudall (2002) and Rudall and Eastman (2002)
52	Corsiaceae	
53	Melanthiaceae	Rudall (2002) and Tamura (1998)
54	Petermanniaceae	Conran and Clifford (1998)a
55	Alstroemeriaceae	Rudall (2002), Daumann (1970) and Conran and Clifford (1998b)
56	Colchicaceae	Rudall (2002), Daumann (1970) and Nordenstam (1998)
57	Philesiaceae	Rudall (2002) and Conran and Clifford (1998c)
58	Rhipogonaceae	Rudall (2002) and Conran (1998)
59	Smilacaceae	
60	Liliaceae	Rudall (2002), Daumann (1970), Nordenstam (1982), Kaniki and Persson (1997), Rudall et al. (2000) and Roguz et al. (2018)
61	Orchidaceae	Rudall (2002), Duamann (1970), Nilsson et al. (1987), Dressler (1990), Figueiredo and Pais (1992), Galetto et al. (1997), Bustos Singer and Cocucci (1999), Stpiczynska et al. (2005) and Hobbhahn et al. (2013)
62	Boryaceae	Rudall (2002) and Bernardello (2007)
63	Blandfordiaceae	
64	Asteliaceae	
65	Lanariaceae	
66	Hypoxidaceae	Vogel (1998d) and Kocyan and Endress (2001)
67	Doryanthaceae	Daumann (1970), Rudall (1998, 2002), Vogel (1998d), Smets et al. (2000), Rudall et al. (2003a), Bernardello (2007), Endress (1995)
68	Ixioliriaceae	
69	Tecophilaeaceae	
70	Iridaceae	
71	Xeronemataceae	

(continued)

Table 6.2 (continued)

S. No.	Family	References
72	Asphodelaceae	
73	Amaryllidaceae	
74	Asparagaceae	
75	Dasypogonaceae	Rudall (2002)
76	Arecaceae	Daumann (1970), Uhl and Moore (1971), Rudall et al. (2003b), Stauffer and Endress (2003) and Smets et al. (2000)
77	Hanguanaceae	Rudall et al. (1999)
78	Commelinaceae	Hrycan and Davis (2005)
79	Philydraceae	Hamann (1998)
80	Pontederiaceae	Daumann (1970) and Strange et al. (2004)
81	Haemodoraceae	Daumann (1970) and Simpson (1990, 1993, 1998)
82	Strelitziaceae	Daumann (1970) and Kronestedt and Walles (1986)
83	Lowiaceae	Larsen (1998) and Wen and Liao (1999)
84	Heliconiaceae	Kirchoff (1992)
85	Musaceae	Daumann (1970), Fahn and Benouaiche (1979), Kirchoff (1992) and Ren and Wang (2007)
86	Cannaceae	Daumann (1970)
87	Marantaceae	Daumann (1970) and Rao (1975)
88	Costaceae	Newman and Kirchoff (1992), Rao (1963), Smets et al. (2000), Smets and Cresens (1988) and Vogel (1998b)
89	Zingiberaceae	Newman and Kirchoff (1992), Rao (1963), Smets et al. (2000), Smets and Cresens (1988), Vogel (1998b), Box and Rudall (2006)
90	Typhaceae	Bernardello (2007)
91	Bromeliaceae	Bernardello (2007), Daumann (1970), Bohme (1988), Bernardello et al. (1991), Sajo et al. (2004) and Mosti et al. (2013)
92	Rapateaceae	Bernardello (2007)
93	Xyridaceae	
94	Eriocaulaceae	Bernardello (2007), Stutzel and Gansser (1995), Stutzel (1998), Smets et al. (2000) and Ramos et al. (2005)
95	Mayacaceae	Wolowski and Freitas (2015)
96	Thurniaceae	Rudall and Conran (2012)
97	Juncaceae	Bernardello (2007)
98	Cyperaceae	
99	Restionaceae	
100	Flagellariaceae	Rudall and Conran (2012)
101	Joinvilleaceae	
102	Ecdeiocoleaceae	
103	Poaceae	Bernardello (2007)
104	Ceratophyllaceae	Les (1993)
105	Eupteleaceae	Cronquist (1981) and Endress (1986)
106	Papaveraceae	Cronquist (1981), Liden (1993), Olesen (1996), Damerval et al. (2013) and Zhang and Zhao (2018)
107	Circaeasteraceae	Cronquist (1981), Tian et al. (2006) and Ren et al. (2004)

(continued)

Table 6.2 (continued)

S. No.	Family	References
108	Lardizabalaceae	Cronquist (1981), Kuwagoe and Suzuki (2003) and Endress (1995)
109	Menispermaceae	Cronquist (1981) and Endress (1986)
110	Berberidaceae	Fahn (1979), Cronquist (1981), Suzuki (1984), Smets (1986), Bernardello et al. (2000) and Endress (1989)
111	Ranunculaceae	Vogel (1993), Erbar et al. (1999), Smets and Cresens (1988), Kosuge (1994), Tamura (1993), Peterson et al. (1979), Fahn (1979), Cronquist (1981), Hodges (1997), Erbar et al. (1999) and Erbar (2014)
112	Sabiaceae	Douglas and Tucker (1996), Bernardello (2007), van de Water (1980)
113	Nelumbonaceae	Williamson and Schneider (1993)
114	Platanaceae	von Balthazar and Schonenberger (2009)
115	Proteaceae	Fahn (1979), Douglas and Tucker (1996) and Diaz-Forestier et al. (2016)
116	Trochodendraceae	Endress (1986)
117	Buxaceae	von Balthazar and Endress (2002), Daumann (1974), Smets (1988) and Vogel (1998c)
118	Myrothamnaceae	Wanntorp and Decraene (2005)
119	Gunneraceae	
120	Dilleniaceae	Tucker and Bernhardt (2000)
121	Peridiscaceae	Cronquist (1981)
122	Paeoniaceae	Zimmermann (1932), Elias (1983), Sanches-Lafuente (2002), Leins and Erbar (2010) and Erbar (2014)
123	Altingiaceae	Endress (1993b)
124	Hamamelidaceae	Endress (1993b), Mione and Bogle (1990) and Anderson and Hill (2002)
125	Cercidiphyllaceae	Endress (1993a)
126	Iteaceae	Hermsen et al. (2003)
127	Grossulariaceae	Cronquist (1981) and Erbar (2014)
128	Saxifragaceae	Bensel and Palser (1975a, b), Cronquist (1981), Smets (1986), Decraene et al. (1998b), Sandvik and Totland (2003) and Erbar (2014)
129	Crassulaceae	Cronquist (1981) and Said (1982)
130	Tetrapcarpaeaceae	Carlsward et al. (2011)
131	Penthoraceae	
132	Haloragaceae	Cronquist (1981) and Kubitzki (2007a)
133	Vitaceae	Cronquist (1981), Gerrath and Poslusny (1994), Zhang et al. (1999) and Erbar (2014)
134	Krameriaceae	Simpson (1982)
135	Zygophyllaceae	Cronquist (1981), Decraene and Smets (1991c), Wang et al. (2000) and Debandi et al. (2002)
136	Quillajaceae	Diaz-Forestier et al. (2016)

(continued)

Table 6.2 (continued)

S. No.	Family	References
137	Fabaceae	Bisby (1981), Vogel (1997), Ancibor (1969), Cocucci et al. (1992), Davis et al. (1998), Westerkamp and Weber (1999), Fahn (1979), Cronquist (1981), Tucker (2002) and Herendeen et al. (2003)
138	Surianaceae	Cronquist (1981)
139	Polygalaceae	Westerkamp and Weber (1999) and Cronquist (1981)
140	Rosaceae	Cronquist (1981), Smets (1986), Judd et al. (2002), Buban et al. (2003), Evans and Dickinson (2005), Weigend (2005) and Chwil et al. (2019)
141	Barbeyaceae	Dickinson and Sweitzer (1970), Cronquist (1981) and Judd et al. (2002)
142	Dirachmaceae	Link (1994a) and Decraene and Miller (2004)
143	Elaeagnaceae	Cronquist (1981) and Decraene and Miller (2004)
144	Rhamnaceae	Medan and Aegesen (1995)
145	Ulmaceae	Dickinson and Sweitzer (1970), Cronquist (1981) and, Judd et al. (2002)
146	Cannabaceae	
147	Moraceae	
148	Urticaceae	
149	Nothofagaceae	Cronquist (1981) and Judd et al. (2002)
150	Fagaceae	
151	Myricaceae	
152	Juglandaceae	
153	Casuarinaceae	
154	Ticodendraceae	
155	Betulaceae	
156	Apodanthaceae	Blarer et al. (2004)
157	Anisophylleaceae	Matthews and Endress (2004)
158	Coryncarpaceae	Narayana et al. (1986) and Matthews and Endress (2004)
159	Coriariaceae	Thompson and Gornall (1995)
160	Cucurbitaceae	Brown (1938), Vogel (1981, 1990, 1997), Nepi et al. (1996), Ashworth and Galetto (2002) and Fahn and Shimony (2001)
161	Tetramelaceae	Davidson (1973) and Matthews and Endress (2004)
162	Datiscaceae	Rieseberg et al. (1993)
163	Begoniaceae	Vogel (1998c)
164	Lepidobotryaceae	Link (1991), Tobe and Hammel (1993), Matthews and Endress (2005b)
165	Celastraceae	Sandvik and Totland (2003), Simmons (2004a b) and Matthews and Endress (2005b)
166	Huaceae	Simmons and Hedin (1999)
167	Connaraceae	Matthews and Endress (2002)
168	Oxalidaceae	Brown (1938), Matthews and Endress (2002)
169	Cunoniaceae	Vogel (1998a), Matthews and Endress (2002), Bradford et al. (2004), Humana and Valdivia (2004) and Diaz-Forestier et al. (2016)

(continued)

Table 6.2 (continued)

S. No.	Family	References
170	Elaeocarpaceae	Vogel (1998a), Matthews and Endress (2002), Bradford et al. (2004) and Humana and Valdivia (2004)
171	Cephalotaceae	
172	Brunelliaceae	
173	Pandaceae	Cronquist (1981)
174	Irvingiaceae	Link (1992b)
175	Ctenolophonaceae	Link (1992a)
176	Rhizophoraceae	Juncosa and Tomlinson (1987)
177	Ochnaceae	Cronquist (1981)
178	Bonnetiaceae	Dickinson and Weitzman (1998)
179	Clusiaceae	Robson (1961) and Decraene and Smets (1991b)
180	Podostemaceae	Cronquist (1981)
181	Hypericaceae	
182	Caryocaraceae	Dickinson (1990) and Prance and Freitas da Silva (1973)
183	Lophopyxidaceae	Cronquist (1981)
184	Putranjivaceae	
185	Elatinaceae	Cronquist (1981)
186	Malpighiaceae	Simpson and Neff (1983) and Sigrist and Sazima (2004)
187	Balanopaceae	Cronquist (1981)
188	Trigoniaceae	Matthews and Endress (2008)
189	Dichapetalaceae	
190	Euphroniaceae	
191	Chrysobalanaceae	Cronquist (1981) and Arista et al. (1997)
192	Humiriaceae	Link (1992a)
193	Achariaceae	Bernhard (1999a)
194	Violaceae	Smets (1986), Vogel (1998b) and Freitas and Sazima (2003)
195	Goupiaceae	Cronquist (1981)
196	Passifloraceae	Fahn (1979), Cronquist (1981) and Bernhard (1999b)
197	Lacistemataceae	Cronquist (1981)
198	Salicaceae	Cronquist (1981), Bullock (1994) and Machado and Oliveira (2000)
199	Rafflesiaceae	Beaman et al. (1988)
200	Euphorbiaceae	Cronquist (1981), Webster (1994), Freitas et al. (2001) and Michaelis (1924)
201	Linaceae	Brown (1938) and Cronquist (1981)
202	Ixonanthaceae	Link (1992d)
203	Picrodendraceae	Cronquist (1981)
204	Phyllanthaceae	Webster (1994)
205	Geraniaceae	Cronquist (1981), Link (1994b) and Vogel (1998c)
206	Francoaceae	Decraene and Smets (1999a), Decraene et al. (2001), Weigend (2005) and Jeiter et al. (2016)
207	Combretaceae	Cronquist (1981) and Bernardello et al. (1994)
208	Lythraceae	Schmid (1980), Cronquist (1981), Smets (1986) and Graham et al. (2005)

(continued)

Table 6.2 (continued)

S. No.	Family	References
209	Onagraceae	Cronquist (1981), Eyde (1981, 1982), Smets (1986), Anton et al. (2017)
210	Vochysiaceae	Oliveira (1996) and Hedges (1997)
211	Myrtaceae	Schmid (1980), Cronquist (1981), O'Brien et al. (1996) and Davis (1997)
212	Melastomataceae	Stein and Tobe (1989) and Vogel (1997)
213	Crypteroniaceae	Bernardello (2007)
214	Alzateaceae	
215	Penaeaceae	
216	Aphloioaceae	Mathews and Endress (2005a)
217	Geissolomataceae	
218	Strasburgeriaceae	
219	Staphyleaceae	
220	Stachyuraceae	Mathews and Endress (2005a)
221	Crossosomataceae	
222	Picramniaceae	Kubitzki (2007b)
223	Tapisciaceae	Dickinson (1986a)
224	Biebersteiniaceae	Link (1994b)
225	Nitrariaceae	Decraene and Smets (1991)c
226	Kirkiaeae	Cronquist (1981)
227	Burseraceae	Cronquist (1981) and Sunnichan et al. (2005)
228	Anacardiaceae	Cronquist (1981), Wannan and Quinn (1991), Gallant et al. (1998), Wunnachit et al. (1992) and Tolke et al. (2018)
229	Sapindaceae	Cronquist (1981), Decraene et al. (2000a), Cui et al. (2003), Leins and Erbar (2010), Solis et al. (2017) and Zini et al. (2014)
230	Rutaceae	Rachmilevitz and Fahn (1973), Fahn (1979), Cronquist (1981), Souza et al. (2003) and Caris et al. (2006)
231	Simaroubaceae	Cronquist (1981) and Bory and Clair-maczulajtys (1982)
232	Meliaceae	Brown (1938), Cronquist (1981), Lal (1994) and Moscheta et al. (2002)
233	Cytinaceae	Hobbhahn and Johnson (2015)
234	Muntingiaceae	Bawa and Webb (1983)
235	Neuradaceae	Decraene and Smets (1995)
236	Malvaceae	Vogel (2000), Donato (1991) and Leitao et al. (2005)
237	Sphaerosepalaceae	Horn (2004)
238	Thymelaeaceae	Cronquist (1981), Herber (2003), Cornara et al. (2005) and Bandera and Traveset (2006)
239	Bixaceae	Poppendieck (2003a, b) and Cronquist (1981)
240	Cistaceae	Fahn (1979), Cronquist (1981) and manetas and Petropoulou (2000)
241	Sarcolaenaceae	Cronquist (1981) and Bayer (2003)
242	Dipterocarpaceae	Ghazoul (1997), Harrison et al. (2005) and Ashton (2003)
243	Akaniaceae	Decraene et al. (2002)

(continued)

Table 6.2 (continued)

S. No.	Family	References
244	Tropaeolaceae	Rachmilevitz and Fahn (1975), Cronquist (1981) and Decraene and Smets (2001)
245	Moringaceae	Cronquist (1981) and Decraene et al. (1998a)
246	Caricaceae	Decraene and Smets (1999b)
247	Limnanthaceae	Link (1992)c
248	Setchellanthaceae	Tobe et al. (1999)
249	Koeberliniaceae	Mehta and Moseley (1981)
250	Bataceae	George (2003) and Decraene (2005)
251	Salvadoraceae	Cronquist (1981)
252	Emblingiaceae	
253	Tovariaceae	Fisel and Weberling (1990) and Decraene (2002)
254	Pentadiplandraceae	Decraene (2002)
255	Gyrostemonaceae	George (2003) and Decraene (2005)
256	Resedaceae	Cronquist (1981) and Kubitzki (2003)
257	Capparaceae	Fahn (1979), Cronquist (1981), Decraene et al. (2002) and Kers (2003)
258	Cleomaceae	Erbar and Leins (1997)
259	Brassicaceae	Norris (1941), Deng and Hu (1995), Davies et al. (1996, 1998), Fahn (1979), Cronquist (1981), Decraene et al. (2002) and Kers (2003)
260	Aextoxicaceae	Cronquist (1981) and Decraene (2004)
261	Berberidopsidaceae	Decraene (2004)
262	Olacaceae	Cronquist (1981)
263	Opiliaceae	Cronquist (1981) and Bullock (1994)
264	Santalaceae	Kjuit (1969), Cronquist (1981), Bhaskar (1992), Aronne et al. (1993) and Bernardello (2007)
265	Misodendraceae	Cronquist (1981)
266	Loranthaceae	Kjuit (1969) and Galetto et al. (1990)
267	Frankeniaceae	Brochmann et al. (1995)
268	Tamaricaceae	Brown (1938), Fahn (1979) and Cronquist (1981)
269	Plumbaginaceae	Galetto (1993) and De Laet et al. (1995)
270	Polygonaceae	Decraene and Smets (1991a) and De Melo et al. (2003)
271	Droseraceae	Murza and Davis (2003), Kerner von Marilaun and Oliver (1895) and Lowrie (2001)
272	Nepenthaceae	Kato (1993), Leins and Erbar (2010)
273	Drosophyllaceae	Ortega Olivencia et al. (1995)
274	Rhabdodendraceae	Nelson and Prance (1984)
275	Simmondsiaceae	Niklas and Buchmann (1985)
276	Physenaceae	Dickinson and Miller (1993)
277	Caryophyllaceae	Zandonella (1977)
278	Achatocarpaceae	Bullock (1994)
279	Amaranthaceae	Zandonella (1977)
280	Lophiocarpaceae	Hakki (2013)

(continued)

Table 6.2 (continued)

S. No.	Family	References
281	Aizoaceae	Zandonella (1977), Chesselet et al. (2002) and Ihlenfeldt (1960)
282	Phytolaccaceae	Zandonella (1977) and Bernardello et al. (1993)
283	Nyctaginaceae	Zandonella (1997), Vanvinckenroye et al. (1993) and Lopez and Galleto (2002)
284	Molluginaceae	Zandonella (1997)
285	Didiereaceae	Kubitzki (1993b)
286	Basellaceae	Zandonella (1997)
287	Portulacaceae	Zandonella (1997) and Vanvinckenroye and Smets (1996, 1999)
288	Cactaceae	Zandonella (1997), Barthlott and Hunt (1993), Nassar et al. (1997) and Aguero et al. (2018)
289	Nyssaceae	Eyde (1968, 1988), Cronquist (1981) and Batra (1999)
290	Hydrostachyaceae	Erbar and Leins (2004)
291	Hydrangeaceae	Bensel and Palser (1975c), Cronquist (1981) and Hufford (2001)
292	Loasaceae	Brown and Kaul (1981), Smets (1988), Hufford (1990), Weigend and Gottschling (2006), Weigend (2004) and Moody and Hufford (2000)
293	Curtisiaceae	Cronquist (1981)
294	Grubbiaceae	Kubitzki (2004)
295	Cornaceae	Eyde (1968, 1988), Cronquist (1981) and Batra (1999)
296	Balsaminaceae	Cronquist (1981), Smets (1986), Travers et al. (2003) and Fischer (2004a)
297	Marcgraviaceae	Tschapka and von Helversen (1999)
298	Tetrameristaceae	Balthazar and Schonenberger (2013)
299	Fouquieriaceae	Cronquist (1981) and Nabhan et al. (1999)
300	Polemoniaceae	Cronquist (1981), Smets (1986) and Wilken (2004)
301	Lecythidaceae	Mori et al. (1978), Cronquist (1981), Frame and Durou (2001), Prance (2004) and Prance and Mori (2004)
302	Sladeniaceae	Stevens and Weitzmann (2004)
303	Pentaphylacaceae	Weitzmann et al. (2004)
304	Sapotaceae	Pennington (2004)
305	Ebenaceae	Wallnöfer (2004)
306	Primulaceae	Vogel (1986, 1997), Caris et al. (2000) and Caris and Smets (2004)
307	Theaceae	Cronquist (1981)
308	Symplocaceae	Caris et al. (2002) and Nooteboom (2004)
309	Diapensiaceae	Scott (2004)
310	Styracaceae	Fahn (1979) and Saraiva et al. (1998)
311	Sarraceniaceae	Vogel (1998a)
312	Actinidiaceae	Schmid (1978) and Brown (1935)
313	Clethraceae	Brown (1938), Cronquist (1981) and Schneider and Bayer (2004)
314	Myrsinaceae	Vogel (1986, 1997), Otegui and Cocucci (1999), Caris et al. (2000) and Stahl and Anderberg (2004)
315	Cyrillaceae	Cronquist (1981) and Dute et al. (2004)

(continued)

Table 6.2 (continued)

S. No.	Family	References
316	Ericaceae	Brown (1938), Cronquist (1981), Palser (1961), Wallace (1977), Cronquist (1981), Palser et al. (1991), Anderson et al. (2000b) and Freitas et al. (2006)
317	Theophrastaceae	Cronquist (1981), Vogel (1986) and Caris and Smets (2004)
318	Oncotocetaceae	Dickinson (1986)
319	Icacinaceae	Cronquist (1981) and Karehed (2001)
320	Eucommiaceae	Cronquist (1981)
321	Garryaceae	
322	Rubiaceae	Brown (1938), Cronquist (1981), Smets (1986) and Galetto (1998)
323	Gentianaceae	Cronquist (1981), Chassot et al. (2001), von Hagen and Kadereit (2003), Vogel (1998a) and Wang et al. (2018)
324	Loganiaceae	Cronquist (1981)
325	Apocynaceae	Rao and Ganguli (1963), Boiteau and Allorge (1978), Galetto (1997), Venter et al. (2001), Christ and Schnepf (1988), Kevan et al. (1989), Kunze (1991, 1997), Woodson and Moore (1938), Haber (1984), Herrera (1991), Lin and Bernardello (1999), Monteiro and Demarco (2017)
326	Boraginaceae	Di Fulvio (1997, 1978), Fahn (1979), Cosa de Gastiazoro (1995), Di Fulvio et al. (1997) and Hofmann (1999)
327	Convolvulaceae	Fahn (1979), Cronquist (1981), Stucky and Beckmann (1982), Pinheiro and Schlindwein (1998), Galetto and Bernardello (2004), Keeler and Kaul (1984) and Said (1982)
328	Solanaceae	Bernardello (1987), Vogel (1991, 1998b), Cocucci and Galetto (1992), Rabinowitch et al. (1993) and Mione and Serazo (1999)
329	Montiniaceae	Decraene et al. (2000b)
330	Sphenocleaceae	Erbar (1995)
331	Hydroleaceae	Di Fulvio (1997) and Erbar et al. (2005)
332	Plocospermataceae	Struwe and Jensen (2004) and D'Arcy and Keating (1973)
333	Carlemanniaceae	Thiv (2004)
334	Oleaceae	Fahn (1979), Cronquist (1981) and Green (2004)
335	Calceolariaceae	Sersic (2004)
336	Gesneriaceae	Cronquist (1981), Maldonado and Otegui (1997), Perret et al. (2001) and Weber (2004)
337	Plantaginaceae	Philbrick and Anderson (1992), Leins and Erbar (2004), Primack (1978), Cronquist (1981), Nepi et al. (2003), Bello et al. (2004), Wagenitz (2004) and Fischer (2004b)
338	Scrophulariaceae	Galetto (1995b), Cronquist (1981), Gaffal et al. (1998), Vogel (1998b) and Nepi et al. (2003)
339	Stilbaceae	Linder (2004)
340	Byblidaceae	Conran and Carolin (2004)
341	Martyniaceae	Ihlenfeld (2004) and Thieret (1976)
342	Pedaliaceae	Cronquist (1981), Ihlenfeld (2004b) and Wortley et al. (2005)
343	Acanthaceae	Cosa (1975), Cronquist (1981), Piovano et al. (1999) and Schonenberger (1999)

(continued)

Table 6.2 (continued)

S. No.	Family	References
344	Bignoniaceae	Fahn (1979), Cronquist (1981), Galetto (1995a), Rivera (1996, 2000a, b), Gentry (1980, 1982) and Lopes et al. (2002)
345	Lentibulariaceae	Narayana and Satyavathi (1988), Vogel (1997) and Bernardello (2007)
346	Schlegeliaceae	Fischer (2004b)
347	Thomandersiaceae	Wortley et al. (2005)
348	Verbenaceae	Brown (1938), Fahn (1979), Cronquist (1981), Smets (1986) and Bernardello et al. (2000)
349	Lamiaceae	Cronquist (1981), Kumari (1986), Dafni et al. (1988), Zer and Fahn (1992), Petanidou et al. (2000) and Bernardello (2007)
350	Phrymaceae	Galetto (1995b) and Hazle and Canne-Hilliker (2005)
351	Paulowniaceae	Fischer (2004b)
352	Orobanchaceae	Fischer (2004b), Bekker and Kwak (2005) and Liu et al. (2015)
353	Stemonuraceae	Karehed (2001)
354	Cardiopteridaceae	Cronquist (1981), Karehed (2001) and Tobe (2012)
355	Phyllonomaceae	Karehed (2001) and Ao and Tobe (2015)
356	Helwingiaceae	Ao and Tobe (2015)
357	Aquifoliaceae	Loesener (1942) and Erbar and Leins (2010)
358	Rousseaceae	Lundberg and Bremer (2003)
359	Campanulaceae	Koopman and Ayers (2005), Fahn (1979), Cronquist (1981), Smets (1986), Erbar and Leins (1989), Galetto et al. (1993), Vogel (1998c) and Anderson et al. (2000a)
360	Pentaphragmataceae	Vogel (1998c)
361	Stylidiaceae	Cronquist (1981), Erbar (1992) and Laurent et al. (1998)
362	Alseuosmiaceae	Cronquist (1981) and Lundberg and Bremer (2003)
363	Phellinaceae	Lundberg and Bremer (2003)
364	Argophyllaceae	Cronquist (1981) and Lundberg and Bremer (2003)
365	Menyanthaceae	Cronquist (1981), Erbar 1997
366	Goodeniaceae	Cronquist (1981), Leins and Erbar (1989)
367	Calyceraceae	Erbar (1993)
368	Asteraceae	Brown (1938), Frey-Wyssling (1955), Galetto (1995c), Torres (1998), Vogel (1998), Mani and Saravanan (1999), Bernardello et al. (2000), Sancho and Otegui (2000), Ma et al. (2002), Visintin and Bernardello (2005), Wist and Davis (2006)
369	Escalloniaceae	Bensel and Palser (1975c), Bernardello et al. (2000), Diaz-Forestier et al. (2016)
370	Columelliaceae	Cronquist (1981), Chalcoff et al. (2006)
371	Bruniaceae	Quint and Classen-Bockhoff (2006)
372	Paracryphiaceae	Dickinson and Baas (1977)
373	Adoxaceae	Wagenitz and Laing (1984), Erbar (1994)
374	Caprifoliaceae	Brown (1938), Fahn and Rachmilevitz (1970), Weberling (1977), Wagenitz and Laing (1984), Davis (2003)

(continued)

Table 6.2 (continued)

S. No.	Family	References
375	Pennantiaceae	Erbar and Leins (2010)
376	Torricelliaceae	
377	Griselinaceae	
378	Pittosporaceae	Fahn (1979), Erbar and Leins (1995)
379	Araliaceae	Cronquist (1981), Erbar and Leins (1988) and Vezza et al. (2006)
380	Myodocarpaceae	Karehed (2003)
381	Apiaceae	Brown (1938), Cronquist (1981), Smets (1986) and Erbar (2014)

and limits the dehiscence zone of siliques in *A. thaliana*. However, its duplication in Solanaceae is associated with the acquisition of a novel function, i.e. fleshy-fruit formation, which highlights the importance of gene duplication events followed by neofunctionalization as a mechanism to allow the independent evolution of a distinct floral trait (Maheepala et al. 2019). The other instance of additional gene duplication and paralogs expression divergence is observed in species of Papaveraceae and Brassicaceae. Here, replum development in higher eudicot fruits is controlled by divergent expression of *REPLUMLESS* gene paralogs, thus adding to a novel feature of the fruits (Zumajo-Cardona et al. 2018). Further, repeated evolution of bilateral symmetry across angiosperms is associated with duplication and divergent expression patterns of *CYCLOIDEA* gene (Jabbour et al. 2014; Broholm et al. 2008; Chapman et al. 2008; Carlson et al. 2011).

Phylogenetic studies have provided significant insights into the evolutionary trends of different nectary types across angiosperms, and major transition events occurred at the ancestral nodes of selected clades. Integrating developmental aspect has also been proved useful in refining the presumed scenarios of changes in nectary types across angiosperm. For instance, it is assumed for core eudicots that stamen-associated nectary arose once in its ancestral flower followed by repeated losses in some of its descendants on independent occasions. All the developmental genetic evidences presented for core eudicots to date also pinpoint towards the existence of similar genetic regulatory module *CRC* or *CRC*-dependent pathway for nectary development (Fourquin et al. 2007; Lee et al. 2005; Morel et al. 2018; Nakayama et al. 2010; Orashakova et al. 2009; Sun et al. 2013). Thus, it reaffirms that pattern observed across core eudicots stems from a single transition event. Furthermore, the petal nectaries that appeared in the Ranunculales and Lamiales are assumed to be the result of two independent transition events which is also supported at the molecular level as two different developmental homologues *STYLISH* and *KNOX* have been found to be associated with each transition event (Box et al. 2011; Min et al. 2019). While most of the developmental genetic evidences are from the core eudicot lineages, one can assume that there is a striking parallelism (*trait that evolve through the same genetic modification*) in the independent evolution of floral nectary. While most of the comparative work of flower nectary developmental genetics has been undertaken in core eudicot lineages, whether the extensive parallel recruitment of a *CRC* dependent programme for floral nectary extends to non-core eudicot taxa is not yet known.

6.4 Conclusions

Floral nectary is a relatively common and phylogenetically distributed plant trait which has evolved and lost on numerous occasions amongst flowering plants. From nectaryless form of basal angiosperms, multiple transition events have contributed to instances of nonreproductive nectary types (sepal, petal, tepal, hypanthial) and reproductive types of nectary (staminal, interstaminal, intrastaminal, interstaminal, staminodal, stylar, stigmatic or ovarian). The present distribution sampling also suggests that nonreproductive whorl-associated nectaries appeared less frequently in the families across phylogeny when compared to those associated with reproductive whorl nectaries. The study further highlights the importance of developmental studies to resolve the hypothesized scenarios of changes in nectary types. The developmental aspect provides insights into the parallel recruitment of *CRC*-dependent developmental programme during independent transitions in core eudicots. However, the modifications to this programme in reverse transition to nectaryless state is still not known. Also it is yet to be determined if recruitment occurs via regulatory changes at *CRC* loci, or through evolutionary changes to one or multiple upstream regulators of *CRC*, or through a combination of these possibilities. Also, it is yet to be investigated whether the paralogues belonging to *CRC*-like gene lineages have any role in the evolution of nectary among the core eudicots, early diverging eudicots or monocots.

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Floral Symmetry – What It Is, How It Forms, and Why It Varies

7

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Abstract

Flowers are compressed shoots in which floral organs are arranged in whorls, generally in a symmetrical manner that may be an intrinsic feature of the flower. Floral symmetry varies across angiosperms; the states range from symmetrical (polysymmetrical, disymmetrical and monosymmetrical) to asymmetrical, with the polysymmetrical form perhaps being the most common state. The development of different states of symmetry has been widely studied at the morphological level, while the range of underlying molecular processes is not fully understood. Causes for this variation may be found in the ecological interactions of flowers, especially with pollinators, which may exert selective pressures that favour one or other symmetry state. However, the complexity of interactions among ecological, developmental and other intrinsic factors means that it is difficult to generalize. For instance, because pollinators such as bumblebees appear to prefer symmetry, it is possible that they and similar insect visitors exert selection pressure that favours symmetry over asymmetry; however, asymmetry has evolved several times. Phylogenetic analyses show that disymmetry, monosymmetry and asymmetry evolved multiple times across angiosperms, and that in most cases that have been studied, the same genetic pathway (involving the regulatory gene CYC2) has been involved in several independent origins of monosymmetry. It has also been shown that several lineages never alter their polysymmetrical state, which must have some underlying reasons. Finally, the question of how different symmetry states arise, and why they arise, must include the integration of a wide range of developmental, ecological and evolutionary explanations.

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Keywords

Asymmetry · Disymmetry · Floral morphospace · Molecular development · Monosymmetry · Phylogenetic patterns · Pollinator ecology · Polysymmetry · Symmetry correlates

Glossary

Disparity: Morphological disparity is the diversity of form found across different taxa, and is distinguished from phylogenetic or taxonomic diversity, which comprises the different types of species and other taxa.

Enabler, Enabling Trait: A newly evolved trait or variant that promotes the evolution of disparity by allowing increased exploration of a portion of the morphospace, perhaps within the limits set by evolutionary constraints in a specific lineage (Donoghue and Sanderson 2015).

Evolutionary Constraint: Variation in species may arise within constraints of bauplan, physiology, biochemistry, or genetic developmental pathways that may be specific to specific lineages. While such constraints may be broken in diverse ways, they may also set the limits within which variation arises and diversification occurs, at least for some period of time. A consequence of constraints may be “enabling” variation.

Evolution and Development (Evo-Devo): The use of comparative data obtained from the study of developmental genetic processes to infer evolutionary patterns of trait development.

Floral Axis: A line that runs from the point of attachment of a flower to the flowering meristem, such that the four whorls are arranged around it, in a planar or spiral whorl.

Floral Whorl: A whorl in anatomy is an arrangement of similar organs in a circle around an axis that is perpendicular or near normal to the circle. In flowers, e.g., a corolla is a whorl of petals. In a flower there is a series of whorls of different organs, going from the outermost, calyx, corolla and androecium, to the innermost gynoecium. Geometrically, these form a set of coplanar concentric circles or a spiral of parallel rising circles.

Floral Orientation: In mature flowers, there are two types of orientation – *vertical*, when the receptacle or the area of attachment of the reproductive stems is in a horizontal plane such that the floral axis is nearly vertical, and *lateral*, when the receptacle is in a vertical plane and the axis nearly horizontal.

Floral Presentation: Different attributes of petal position may be important in floral ecology. With reference to the floral axis, the surface of petals may be distinguished as follows: the *adaxial* surface, adjacent to the floral axis is in most cases the surface that is presented to the pollinator; the *abaxial* surface, facing away from the axis is generally not visible to the pollinator. With respect to the shoot or inflorescence axis to which the flower is attached, petals are distinguished as follows: the *dorsal* petal is closest to the shoot axis, while the *ventral* petal is farthest from the axis. In monosymmetrical flowers, the dorsal petal may be modified to form a *flag* (banner), and the ventral petal may form a *landing platform*.

Modules: Modules are independent units that collectively define or construct a more complex structure. In flowering plants, the node, subtending internode, leaf and axillary bud make up the growth module that generates the plant body. The vegetative axillary or terminal meristem gets converted to a flowering meristem.

Synorganization: The appearance of new structures as a result of coordinated developmental re-organization, mainly due to *fusion* of identical, or two or more varied floral organs and *elaboration* (coordinated development); whorled phyllotaxis is a prerequisite for synorganization to occur.

7.1 Introduction

Land plants are sedentary organisms with indeterminate, modular growth, and their responses to biotic and abiotic environmental factors are shaped by these facts. Thus, if plants need to find a mate, or find new areas to colonize, they need agents – whether biotic or abiotic. In flowering plants, pollen and seeds are transported to variable distances by diverse means, thus “moving” an individual plant from one spot to another. Due to indeterminacy and modularity, the timing, and extent of flowering and other floral traits can be adjusted in response to prevailing conditions to a remarkable degree. Thus, floral and fruit biology are major aspects of the reproductive ecology of angiosperms.

The angiosperm flower is a sexual reproductive module. A typical flower is composed of four types of floral organs – sepals, petals, stamens and carpels – which are organized in four successive concentric or spiral whorls around the central axis of the flower, making up the perianth (calyx and corolla), androecium and gynoecium whorls. In some cases, an undifferentiated whorl of tepals makes up the perianth. The whorls have different functions – protection and attraction (calyx and corolla or perianth), male reproduction (androecium), and female reproduction (gynoecium). Variation in the floral form (morphological disparity) is governed by developmental and ecological factors acting under the forces of evolution within the constraints of phylogenetic history. The form of the flower may be understood in terms of *floral organization* (variation in number and arrangement of organs), *floral architecture* (variation in overall appearance, including symmetry, often due to synorganization), and *floral biology* (variation in presentation and dispersal of pollen and other rewards) (Endress 1996). In addition, there may be variation in attributes of floral organs, such as colour (especially in the corolla), as well as texture, structure and organization of component tissues.

In this chapter, we focus on a major aspect of floral architecture, *symmetry*. We discuss floral symmetry as related to floral reproductive biology, addressing questions of *what* it is, *how* variation in symmetry comes about, as learned through developmental studies, and *why* this variation comes about, as learned through ecological and evolutionary studies. We first discuss floral symmetry, the variants and traits correlated with it, then the development of types of floral symmetry including a brief sketch of what is known about the underlying molecular genetics

in model organisms. We then outline why changes in symmetry may occur, by discussing the present understanding of pollinator preferences and how this may exert selective pressures on symmetry traits and finally, examine macroevolutionary trends in symmetry traits, including a brief introduction to the notion of morphospace, and underlying genetic developmental processes that may enable repeated origins of monosymmetry. We will attempt to illustrate how insights from studies in diverse fields may contribute to a comprehensive understanding of traits, such as floral symmetry, that have long fascinated botanists.

7.2 Floral Symmetry

Flowers vary in symmetry – *what* is it? Symmetry is a geometric feature of all physical objects including organisms (Polak and Trivers 1994) and in this context, flowers. The simplest symmetry of an object is the similarity of two parts of the object with reference to a plane through an *axis of symmetry*, which in the case of floral symmetry is the (principal) axis of the flower (Giurfa et al. 1999). Is there “mirror” similarity of the obtained parts thus possible? If so, the object is symmetrical; otherwise, i.e., if no plane through the axis of symmetry permitting similarity of the two divided parts is available, it is asymmetrical. In biology, this is the sense in which the term symmetry is used. Thus in flowers the most common forms of symmetry are: *polysymmetry* (“radial symmetry” or “actinomorphy”) with multiple (more than two) axial planes, being such as each dividing the flower into two similar halves; *disymmetry*, having two, generally mutually perpendicular, axial planes of symmetry; *monosymmetry* (“bilateral symmetry” or “zygomorphy”), with just one axial plane dividing the flower into two exactly similar halves, and *asymmetry* (including enantiomorphy) with no axial plane of symmetry possible (Endress 1999; Neal et al. 1998; Damerval and Nadot 2007; Fig. 7.1). The degree of symmetry may differ between floral whorls, with multiple possible types of variations and combinations (Endress 2012). Here, we use the term symmetry to refer to the character, and the terms polysymmetry, disymmetry, monosymmetry, and asymmetry to refer to specific states of this character. Polysymmetry may be the most frequent state across angiosperms – based on family-level data, polysymmetry is the most frequent (82%), followed by monosymmetry (37%) and asymmetry (10%) (Neal et al. 1998). However, many monosymmetrical families are highly speciose so its overall prevalence at lower taxa ranks may be underestimated in the above data; yet a rough estimate suggests that polysymmetrical flowers may be more numerous (~61%) than other forms even when species-level data are used (estimated from Fig. S1 O’Meara et al. 2016).

Evolutionarily, flowers are compressed shoots, so floral parts emerge from the apical meristematic region of the floral shoots. The floral organs may be arranged spirally or in concentric circles along the central (often disciform) axis of the flower, determining phyllotaxy. However, the symmetry of the flower depends not on phyllotaxy, but instead on the number of units in each floral whorl (reviewed in Endress 1999). Spiral phyllotaxy in flowers can acquire all the three different

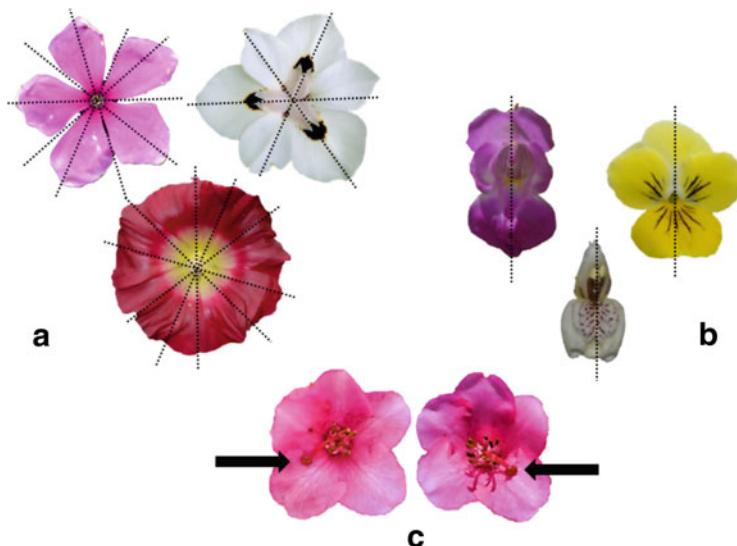


Fig. 7.1 Major symmetry types in flowers. (a) Polysymmetry in (clockwise) *Catharanthus roseus* (L.) G.Don [Apocynaceae], *Dites bicolor* (Steud.) Sweet ex Klatt [Iridaceae], *Alcea rosea* L. [Malvaceae]. (b) Monosymmetry in (clockwise) *Impatiens tripetala* Roxb. ex DC. [Balsaminaceae], *Viola pedunculata* Torr. & A. Gray [Violaceae], *Justicia gendarussa* Burm.f. (c) Asymmetry (enantiostyly) in *Rhododendron glaucophyllum* Rehder [Ericaceae]

symmetry outcomes as in whorled phyllotaxy: polysymmetry with a large number of units in floral components, monosymmetry with dorsiventral differentiation, or asymmetry with only few units per floral organ (Endress 1999). Floral arrangement in the inflorescence and presence of subtending bracts also affect symmetry (Endress 1999). In a compact, flattened inflorescence different degrees or types of monosymmetry may be present in the same inflorescence like central flowers being polysymmetrical and peripheral flowers abruptly or gradually monosymmetrical, e.g., in *Schizophragma* (Hydrangeaceae), *Iberis* (Brassicaceae) and several other taxa (Hufford 2004; Busch and Zachgo 2007; Endress 2012).

Overall floral monosymmetry is generally contributed by monosymmetrical traits in any one or more of the whorls, namely, corolla, stamens and style such that even if the corolla is polysymmetrical, the flower may be monosymmetrical due to monosymmetry traits of other whorls. For example, in flowers of *Rhododendron*, traits such as reflexed stamens, reflexed style and bundling of stamens tend to confer monosymmetry, while traits like straight stamens, straight style and free stamens tend to confer polysymmetry (Berry et al. 2018). In Apiales, overall monosymmetry or asymmetry is mostly due to a monosymmetrical or asymmetrical gynoecium (Nuraliev et al. 2019). Random developmental variation and stress may lead to minor quantitative deviations from monosymmetry in a population of predominantly symmetrical forms; this variation has been termed fluctuating asymmetry (Parsons 1992; Møller and Eriksson 1994).

Similar to monosymmetry, asymmetry in floral form may be due to all, some, or one, of the floral whorls. Many flowers are asymmetrical in highly organized ways, some may be asymmetrical because of simplification or reduction in the number of organs. One type of asymmetry is *enantiomorphy*, in which a polymorphic population contains mirror-image forms in which flowers are either left- or right-handed. In some species, only one form is present – either the left- or the right-oriented form. Enantiomorphs occur across angiosperms, but relatively infrequently (Endress 2012). In enantiomorphy, the two asymmetrical forms can be looked upon as a modification of monosymmetry, which results from dissimilarity between the two halves of the flower and that one form is a mirror form of the other. These asymmetrical forms may involve all or any floral whorls, even if enantiostyly (enantiomorphy involving the style) is the pattern most commonly discussed (Jesson and Barrett 2003; Marazzi et al. 2006). Species in one group of legumes (Subtribe Phaseolinae) are asymmetrical in that the keel is curved to one side (Endress 1999). Enantiostyly may be of two kinds – “monomorphic,” in which the two forms are found in the same plant (e.g., *Monochoria korsakowii* (Pontederiaceae)) and therefore not likely to have a genetic basis, and “dimorphic,” in which individual plants bear either left- or right-handed flowers (e.g., *Heteranthera multiflora*), which is a genetic trait (Jesson and Barrett 2003). Enantiostyly may be part of a syndrome of traits, termed reciprocal enantiostyly, in which there are two corresponding forms of anthers (heteranthery), the “feeding” and “pollinating” anthers and nectarless flowers (Dulberger 1981; Jesson and Barrett 2003). There are also rare cases of non-reciprocal enantiostyly where just the style is bent, e.g., *Exacum* (Gentianaceae; Lloyd and Webb 1992). The form variations may result from diverse types of bending of different parts of the flower – curvature, torsion, or resupination (Endress 2012).

Other types of asymmetry are more subtle; here, different whorls may display different symmetries in different planes of symmetry, and the asymmetry may also be due to aestivation (contort, imbricate, etc.) and minor variation in size or orientation of organs (Endress 2012).

7.3 Some Traits May Vary with Symmetry

7.3.1 Pigment Patterns

One trait which contributes to the attraction of the flower is the presence of pigment patterns on the corolla. Flowers exhibit a variety of patterns like spots, blotches and streaks on the corolla. In many plant groups, these patterns act as *nectar guides* (i.e., cues for the pollinator to find nectar at the base of the corolla tube) (see below). In *Rhododendron*, it was noted that pigment patterns tend to occur in monosymmetrical flowers (Leppik 1974; Berry et al. 2018). An angiosperm-wide survey revealed a statistically significant pattern – monosymmetrical flowers have monosymmetrical nectar guides and polysymmetrical flowers have polysymmetrical patterns of nectar guides (Dafni and Kevan 1996).

7.3.2 Surface Microstructure

The microstructure of the petal epidermal cells shows significant size and shape variation and elaborate cuticular ornamentation on various parts of the petal epidermis of the same flower (Kay et al. 1981; Ojeda et al. 2009; Guo 2015; Berry and Geeta 2019b). The microstructure of the corolla surface may or may not vary with symmetry of the flower – for instance, in the almost polysymmetrical corolla of *Ruellia simplex* the distribution of conical cells and other epidermal cell types is uniform on all petals, but in the strong monosymmetrical bilabiate corolla of *Justicia adhatoda* conical cells are present extensively on the lower lip, but are limited to the lateral margins of the upper lip (Berry and Geeta 2019b).

7.4 Development

Flowers vary in symmetry – *how* does this happen? One answer may lie in our understanding of the morphogenesis of symmetrical and asymmetrical flowers, another may lie in the underlying genetic developmental mechanisms.

Morphological developmental studies have revealed much about diversity in the patterns and processes of generation of symmetry in the floral form. Polysymmetrical flowers typically start development with similar organ primordia, starting with the appearance of sepals, then petals, stamens and carpels. In *Arabidopsis*, the sepals appear first, followed simultaneously by petals and stamens and finally, the carpels are formed; however, this sequence may vary to minor degrees within the family, Brassicaceae (Smyth et al. 1990). There are passing phases of asymmetry within the *Arabidopsis* flower primordium, for instance, in early stages the abaxial sepal is larger than others, but sepal sizes and appearance soon become similar and the flower is polysymmetrical at the adult stage (Smyth et al. 1990).

Floral monosymmetry may be categorized, based on the relative time of initiation of the trait, as *early* (mostly appearing in the perianth before the initiation of the reproductive organs) or *late* (appearing after all organs have been initiated). Early initiation of monosymmetry is common, but in some families (e.g., Lamiaceae) both types occur (Endress 2012; Reyes et al. 2016). Monosymmetrical flowers categorized as early are also likely to be *elaborate*. There is a great deal of variation in details of development in these flowers, but overall they follow the sequence of initiation of sepals, petals, stamens and carpels (Endress 2012). In *Antirrhinum majus*, sepals are initiated first, then petals and immediately after, the stamens (the primordium has a monosymmetric appearance at this stage) and finally, the carpels. Shortly after stamens are initiated, the petals fuse at the base to form a tube that grows rapidly after the initiation of the carpels, it is the abaxial petal that confers monosymmetry on the flower (Singh and Jain 1979; Vincent and Coen 2004). Generally, in Pentapetalae (a large clade of eudicots to which *A. majus* belongs), corolla monosymmetry may arise in one of the two similar ways – by modification of the adaxial petal or the abaxial petal. Initiation of the monosymmetry-conferring

petal (whether abaxial or adaxial petal) is generally in the median plane (Bukhari et al. 2017).

Asymmetrical forms such as in enantiostyly tend to appear relatively late in development at any time between the bud stage and anthesis (Jesson et al. 2003). In fluctuating asymmetry (FA), phenotypes are variable, usually, with different degrees of distortions of monosymmetry on one side of the axial plane of symmetry (Parsons 1992). FA occurs due to random events in development; its study going back to early genetic work. Due to developmental stability (canalization: Waddington 1942), most individuals develop into the “normal” symmetrical form that forms the bulk of the population but occasional random environmental effects may cause the form to deviate from the normal, generating right-handed and left-handed asymmetrical forms at random (e.g., Mather 1953). FA reflects developmental instability and its occurrence has been related to environmental stress (Parsons 1992; Markow 1995; Lewontin 2008).

7.4.1 Synorganization

Elaborate structures in floral architecture are possible due to synorganization (i.e., coordinated development including fusion) of the same type, or two or more varied floral organs and subsequent elaboration of the coordinated structure; whorled phyllotaxis is a prerequisite for synorganization to occur (Endress 2006). Tube flowers often have their petal primordia fused very early such that the developing corolla appears as a ring, with protuberances that develop into lobes (Kaplan 1967). Differential expansion of one of the lobes usually contributes to the development of monosymmetrical flowers (see above). Synorganization leads to the asymmetrical diadelphous androecium of Papilionaceae (Al-Nowaihi et al. 2001).

7.4.2 Developmental Transitions Between Symmetry States

Polysymmetry is often considered to be a “ground state” that are variously modified to generate monosymmetry, disymmetry, and asymmetry. However, developmental transitions may occur among different states in all forms of symmetry, due to factors that are not fully understood but that include effects of the inflorescence axis as well as the floral axis and subtending bracts, and the interactions among these effects (Endress 2012).

7.4.2.1 Inflorescence Effect

Flowers may undergo changes in symmetry during development such that polysymmetrical flowers, e.g., *Veratrum album*, pass through early monosymmetry, and monosymmetrical flowers, e.g., *Couroupita guianensis*, pass through early asymmetry. Such shifts in development are thought to be the result of the interplay of developmental gradients due to the main shoot (i.e., the inflorescence) and subtending bract, if present (Endress 1999). On the other hand, the type of

inflorescence may affect floral symmetry. A compact inflorescence can give the appearance of a flower-like superstructure, in which marginal flowers are modified (to attract and aid as landing platforms) as in *Lamium* (Lamiaceae), or *Gentiana* (Asteraceae). In these taxa and in families like Dipsacaceae, Apiaceae, Verbenaceae, and Caprifoliaceae, the peripheral flowers are monosymmetrical and the internal flowers are polysymmetrical. In these inflorescences, individual flowers are smaller in size than the pollinator, and the modification of marginal flowers into landing platforms is similar to the modification of central-ventral petals in monosymmetrical flowers (reviewed in Neal et al. 1998; Reyes et al. 2016).

Synorganization may occur between flowers to form a flower-like superstructure, e.g., in Asteraceae the outer monosymmetrical ray flowers give the impression of a corolla-like structure, and the central, small, polysymmetrical disc flowers give the impression of the reproductive whorls, where the overall structure appears polysymmetrical. Such organizational changes coupled with other elaborate architectural modifications generate the most complex forms of floral monosymmetry, as in Fabaceae, Lamiales, Orchidaceae, and Zingiberales (Endress 2012).

7.4.2.2 Flower Effect

Developmental transitions between different symmetry states may represent part of the underlying developmental process of the flower. For instance, early polysymmetry may pass into monosymmetry, e.g., *Antirrhinum majus*, where the early near-radial pentamerous flower becomes monosymmetrical in the androecial whorl due to reduction of the primordial adaxial stamen to a staminode (Endress 1999). An intensive study of Apiales reveals the types of factors in the flower that may be related to the evolution of monosymmetry, including reduction of floral organs and increased merosity of the gynoecium (Nuraliev et al. 2019).

7.5 Molecular Developmental Genetics

In their ABC model of flower development, Coen and Meyerowitz (1991) proposed a modular genetic program with three classes of homeotic genes (A, B, and C) that control the identity of the meristem and the identity of the different floral whorls. This genetic pathway is involved in floral development across angiosperms, while the specifics of the ABC model are modified in diverse ways that generate the diversity of floral forms seen beyond model organisms (Litt and Kramer 2010).

In setting up monosymmetry, the default state of multiple axial planes (five in pentamerous flowers) has to be changed to a single axial plane of symmetry. This usually is the dorsiventral plane, which is set up by regulatory genes of the *TCP* and *MYB* gene families. It was first shown in *Antirrhinum majus* that the *TCP* genes *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) expressed in the dorsal region determine dorsal identity; they activate the *MYB* gene *RADIALIS* (*RAD*) which restricts the expression of another *MYB* gene *DIVARICATA* (*DIV*) to the ventral and lateral regions (Galego and Almeida 2002; Corley et al. 2005; Green et al. 2010). The Orchidaceae, the supreme example of monosymmetry and asymmetry, differ

from the general case in that B-class MADS box genes appear to play key roles in the setting up of monosymmetry (Spencer and Kim 2018).

The developmental connection, noted above, between the flower and inflorescence in synorganized structures, is reflected at the molecular genetic level. Symmetry change is tightly linked to the expression of *CYC*-like genes in the compact inflorescence of *Gerbera hybrida* (Asteraceae), where *CYC*-like gene expression is found in the margins of the inflorescence, which bears monosymmetrical ray florets but not in the centre of the inflorescence, which bears polysymmetrical disc florets (Broholm et al. 2008).

7.6 Ecology – Natural Selection and Adaptive Variation

Flowers vary in symmetry – *why* does this happen? One answer may lie in selective forces that lead to the evolution of monosymmetry, another may lie in the history of specific lineages (see below). Given the need that plants have of agents for movement of reproductive units, the major selective factors are likely to be pollinators of flowers, but there may also be selection for fruit dispersal.

7.6.1 Pollinators

Pollinators of flowers include bees (Hymenoptera), butterflies (Lepidoptera), moths (Lepidoptera), hoverflies (Diptera), wasps (Hymenoptera), beetles (Coleoptera), ants (Hymenoptera), bats (Chiroptera) and birds like honeycreepers, sunbirds, parrots, honeyeaters and hummingbirds (Sprengel 1793; Frisch 1953; Faegri and van der Pijl 1979; Kevan and Baker 1983; Heinrich 1975; Barth 1991; Comba et al. 1999). Most pollinator-related studies have been done on bees, as they form a part of the major insect pollinator guild of Hymenopterans (Sprengel 1793; Knuth 1908; Proctor and Yeo 1973; Faegri and van der Pijl 1979; Kevan and Baker 1983; Barth 1991; Chittka and Menzel 1992). These and other studies have established the importance, for pollinators, of floral colour, odour and form. Bees can learn to distinguish monosymmetrical from asymmetrical forms (Giurfa et al. 1996; Plowright et al. 2017) but have an innate preference for symmetrical “flower-like” shapes, e.g., radial-polysymmetrical or dorsiventral-monosymmetrical (common floral shapes) over asymmetrical, random or transversal monosymmetrical shapes (Lehrer et al. 1995). However, across different plant and pollinator taxa, there are mixed reports in the field on whether symmetrical flowers are preferred over asymmetrical ones. In *Epilobium angustifolium*, bumblebees appear to prefer larger, more symmetrical flowers (Møller 1995), while there was no such preference in the case of bee flies visiting *Gorteria diffusa* (Midgley and Johnson 1998), and syrphid flies and butterflies visiting *Geranium robertianum* (Frey and Bukoski 2014).

Broad surveys reveal that monosymmetry is a specialization, in that a narrower range of pollinators visit monosymmetrical forms (Fenster et al. 2004; Gong and Huang 2009). Experiments using artificial flowers showed that bumblebees visit

disymmetrical flowers more than polysymmetrical flowers, suggesting that there is indeed a preference for a reduced number of planes of symmetry that may act as a selective force on flowers (Culbert and Forrest 2016). Field studies of the monosymmetrical flowers in *Commelina communis* show that both when approaching the flower and also while landing on the flower, pollinators, such as syrphid flies and bumblebees, preferred laterally oriented flowers compared to manipulated downward oriented flowers, on which they could not conveniently land, or vertically oriented flowers on which their visits were not effective in pollination; removing the landing platform by changing orientation has a greater effect (Ushimaru and Hyodo 2005).

Colour and other floral traits such as odour are major attractants to pollinators. The nature and role of colour vision in bees as pollinators were pioneered by Frisch (1914) and expanded by others such as Chittka (1992) and Chittka et al. (1994). In a study that removed a major attractant (colour), honey bees in Germany were exposed to grey-scale floral images of Australian flowers that had not been experienced by them before; they preferred images of “insect” flowers (known to be pollinated by insects) over “bird” flowers (Howard et al. 2019). It was shown that the honey bee’s preference was innate and an outcome of the co-evolution of the behaviour of insect pollinator and their preferred combination of flower shape attributes including symmetry (Howard et al. 2019).

7.6.1.1 Patterns

The idea that patterns on the corolla act as nectar guides to insect visitors initially was suggested by Sprengel (1793). This hypothesis has been supported in several laboratory and field studies for insects, especially bees, and birds (Manning 1956; Waser and Price 1985; Leonard and Papaj 2011; Hansen et al. 2012). This attraction of pollinators to patterned pigments can act as a selective pressure, as shown in studies where individuals with patterned flowers have a higher reproductive fitness than those that are unpatterned (Hansen et al. 2012; Ma et al. 2016; Zhang et al. 2017).

7.6.1.2 Surface Structures

Variation in petal microstructure may affect diverse traits such as optical properties of the flower and associated pollinator signalling (van der Kooi et al. 2019), wettability (Whitney et al. 2011), petal reflexion due to epidermal expansion (Baumann et al. 2007), tactile cues (Kevan and Lane 1985), and landing and locomotory grip for the pollinator (Whitney et al. 2009; Alcorn et al. 2012).

7.6.2 Flowers

Early thoughts on differences between monosymmetrical and polysymmetrical flowers are due to Berg (1960), who suggested that monosymmetrical flowers are more specialized than polysymmetrical flowers, and that laterally oriented flowers are more specialized than vertical ones. This hypothesis informed much of the

subsequent pollination studies, but with some modification (e.g., Armbruster et al. 1999). Monosymmetrical flowers are pollinated by fewer functional groups of pollinators, with narrower variation, compared to pollinators of polysymmetrical flowers (Fenster et al. 2004 [from Robertson 1929]; Gong and Huang 2009).

Asymmetrical flowers represent even greater specialization. In general, we might not expect to see asymmetry in flowers because of the apparent preference of pollinators for symmetrical flowers of all kinds. However, asymmetrical flowers do occur across angiosperms, albeit at lower frequencies (Endress 2012). Two species of *Vigna* in Brazil co-exist, even though sharing pollinators, due to differences in morphology and flowering time: asymmetrical flowers and early peak flowering time in one – *V. longifolia* – and the less restrictive monosymmetrical flowers and late peak flowering time in the other – *V. luteola* (de Souza et al. 2017). Such factors could conceivably lead to divergence of species, but may not have in this instance, as the two species appear to be widely separated in phylogenetic history (Delgao-Salinas et al. 2011). Enantiostyly, in which the style is held at a left- or right-oriented angle, is significantly associated with a corresponding differing curvature of the anthers (reciprocal enantiostyly); this apparently adaptive association is inferred to increase precision of pollen transfer and to reduce interference between anther and stigma – but this explanation may not hold in cases of non-reciprocal enantiostyly (Jesson and Barrett 2003).

Bumblebees prefer to visit larger, symmetrical flowers in *Epilobium angustifolium* (Onagraceae); larger flowers produce more nectar and fluctuating asymmetry is pronounced in smaller flowers (Møller 1995). In this instance, bumblebee preference for larger flowers appears to exert selection against those individuals that show developmental instability in the flower. In other cases, experiments suggest that pollinators may not distinguish among such variants in populations of *Gorteria diffusa* or *Geranium robertianum* (see above under “Pollinators”).

In Pentapetalae, corolla monosymmetry appears by modification of either the central-dorsal petal or the central-ventral petal – which either presents itself as a flag (“banner”) to the pollinator (modified dorsal petal) or acts as a landing platform (modified ventral petal) for the pollinator (Bukhari et al. 2017). Both patterns of corolla monosymmetry are present to similar extents across angiosperms; however, we do not know whether the banner is preferred over the landing platform (or vice versa) by particular types of pollinators that might exert selection pressure on the two modes of monosymmetrical flower presentation (Bukhari et al. 2017).

In pollination studies, floral architectural traits often are analysed for their association with other floral traits in their combined attraction for pollinator types, yielding the concept of the *pollination syndrome* (Faegri and van der Piejl 1979). The pollination syndrome, a two-way association between functional groups of pollinators and suites of floral traits, can generate selection pressures on floral traits due to pollinator preferences and traits (reviewed by Fenster et al. 2004). In *Rhododendron*, floral traits across different species were analysed in the context of known pollinators of these species, in order to identify suites of characters that might be termed pollinator syndromes. Signals of pollination syndromes involving

monosymmetry traits were obtained, for example, in flowers characterized by monosymmetrical corolla, reflexed stamen, loosely bundled stamens, reflexed style and presence of pigment patterns in species (*R. ponticum*, *R. reticulatum* and *R. simsii*) that are reported to be pollinated by insects, while flowers characterized by polysymmetrical corolla, hooked stamens, bundled stamens, hooked/straight style and absence of pigment pattern in species (*R. barbatum*, *R. macabeanum*, and *R. thomsonii*) that are reported to be pollinated by birds (Berry and Geeta 2019a).

7.6.3 Fruit Dispersal

A few cases of dispersal enabled by late-developing monosymmetry and asymmetry of fruit are discussed by Endress (2012). In Saxifragaceae, *Tiarella* with two unequal segments of the capsule, held horizontally such that the lower larger segment bends down backward when raindrops fall on the fruit, releasing the fruit in a “spring-board” mechanism (Savile 1975) is reported. Another studied genus is, *Chrysosplenium*, which has seed dispersal by raindrops splashing into an open vertical cup formed by the two symmetrical segments of the capsule (Savile 1975). Species with asymmetrical fruit are also reported, in which some are divaricate and others horizontal, suggesting variation in modes of dispersal (Pan and Ohba 2001). In these plants, the flower is either weakly monosymmetrical (e.g., in *Tiarella*), or symmetrical (e.g., in *Chrysosplenium*) but their fruit mature to develop strong monosymmetry (Endress 2012).

7.7 Evolution

7.7.1 Natural Selection of Symmetry States

Strong monosymmetrical flowers have bilabiate construction (also called “lip blossoms”) that help defend pollen-robbing and by the formation of a locked corolla throat where the upper-lip (or “roof”) completely covers and reaches close to the lower-lip (or “floor”), by developing a pollinator-specific alignment-channel (for extracting nectar), by increasing the reproductive surface (or “pollination chamber”) for contact with the pollinator, or by reduction in landing space of the lower-lip for bird pollination – which in turn assists precise pollen transfer and lower pollen-production per flower (Wester and Claßen-Bockhoff 2007). Based on increased reproductive efficiency for the plant and pollinator preference inferred from pollinator perception, learning ability and innate behaviour, it has been suggested that monosymmetry evolves from polysymmetry through directional selection (Neal et al. 1998; Reyes et al. 2016).

In a series of studies in a single species, *Erysimum mediohispanicum* Polatschek, Gómez et al. (2006, 2009a, b) and Gómez and Perfectti 2010) showed that populations are polymorphic in that flowers vary between poly- and monosymmetry

within and between populations. In some populations the insect visitors (honey bees, bee flies, beetles) prefer monosymmetrical flowers, exerting a selection pressure for monosymmetry. In this species monosymmetry is shown to be a heritable trait, and plants bearing monosymmetrical flowers have higher fitness in terms of number of seeds produced and number of seeds surviving to the juvenile stage in these populations, indicating that natural selection may effect change over time. However, populations differ in the degree and type of polymorphism and are subject to selection in different directions, depending upon the particular composition of pollinators (honey bees, bee flies, beetles). As pollinator assemblages vary geographically, phenotype fitness differs among populations, thus resulting in a selection mosaic across the different populations. Symmetry being a heritable trait, this results in a geographic mosaic of coevolutionary patterns.

In experimental studies that manipulated symmetry, results were variable – in *Hesperis matronalis* (Brassicaceae) there were no significant differences in either visitation rates or seed set (Weeks and Frey 2007); in *Impatiens pallida* (Balsaminaceae) manipulation of symmetry had no effect on female reproductive success measured as seed set (Frey et al. 2005), while in *Geranium robertianum* symmetry was associated with male, but not female, reproductive success (Frey and Bukoski 2014). A meta-analysis showed that selection is stronger when bees, long-tongued flies, or birds, rather than Lepidoptera or multiple animal taxa are the primary visitors; however, there were far too few studies of actinomorphic species to allow an evaluation of whether pollinators act as agents of selection on symmetry (Caruso et al. 2018). However, it has also been suggested that polysymmetry may have a selective advantage over monosymmetry under certain conditions, and this may explain why, while monosymmetry appears to be a key innovation for several species-rich clades, it is not so for other clades (Endress 2012).

7.7.2 Morphospace

A morphospace is the spectrum of morphological variation that theoretically is possible for a particular structure or organism (Fig. 7.2). The morphospace may be a linear interval for a single trait or a multidimensional geometric space that considers more than one trait. When such a morphospace is compared with variation that is actually observed, it may reveal that some regions of the morphospace are unoccupied, and these empty portions may point to selective forces or developmental constraints that might act on the trait group (Stebbins 1951; Richardson and Chipman 2003; Pigliucci 2007; Olson 2012; Salazar-Ciudad and Marín-Riera 2013; Chartier et al. 2014; Chipman 2017; Nuraliev et al. 2019).

Angiosperm-wide studies have pointed out that, of all possible combinations of floral character states that make up floral morphospaces, angiosperm species occupy just a small fraction of such morphospaces, so that the distribution is strongly skewed (Stebbins 1951; Chartier et al. 2014; O'Meara et al. 2016). These results have been generally interpreted to represent the effect of positive selection on these sets of traits. In 36 flowering plant species in Japan, monosymmetrical flowers

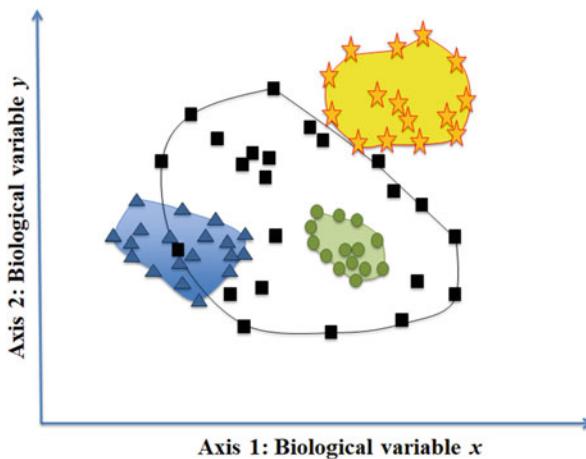


Fig. 7.2 The morphospace, a composite graphical representation of trait variation in a set of taxa (or other entities). The morphospace is delineated by the range of possible variation in multiple morphological traits (including shape and size) of an organism. This morphospace is a simple, 2-D representation of variation in two traits, measured by x and y. The occupied complex of morphospaces is shown by four different symbols representing four different species: blue triangles, yellow stars, green circles and black squares; each symbol places a single individual species in a corresponding morphospace. Each circumscribed area denotes the range of variation in these traits in a particular species group. Three species groups (blue, yellow, green) occupy non-overlapping morphospaces, while one (black) overlaps to different extents with two of the species groups. Several regions of the individual morphospaces are unoccupied. Occupancy may indicate selection for a particular combination of character states; non-occupancy may indicate selection *against* a different combination or, alternatively, a developmentally impossible combination

occupied a smaller part of the flower-size morphospace than did polysymmetrical flowers. In other words, flower size variation was significantly lower in monosymmetrical flowers compared to polysymmetrical flowers (Nikkeshi et al. 2015). The authors hypothesize that this pattern of size variation represents optimal size limits, which might have evolved under selection imposed by the size of pollinators and the need for precise deposition on and removal of pollen from the insect.

In a study on floral morphological disparity in order Ericales, the degrees of variation in floral organization and floral architecture were compared (Chartier et al. 2017). Each family occupied a small part of the floral morphospace occupied by the entire order. Variation in floral architecture (including symmetry) was higher than variation in floral organization; specifically, the androecium contributed the most in terms of morphological diversity compared to other floral whorls (Chartier et al. 2017). This suggests that floral architecture changes more readily than floral organization, perhaps because it is under greater selection pressure and also because underlying developmental processes are more readily modified. If floral organization is more subject to phylogenetic constraints, it may be less easily altered in evolution even if there were selection pressure on its features.

As selection can act in either direction (see above) but perhaps is biased toward monosymmetry, the absence of monosymmetry in some lineages could be due to lack of genetic variation perhaps due to development constraints, to selection *for* polysymmetry (or no selection *against* it), or it may be that, given enough time, monosymmetry eventually will evolve. The developmental genetic machinery appears to be labile enough in several lineages that monosymmetry evolved multiple times, involving the CYC2 pathway in the systems that have been studied (see below). It has been pointed out that other origins of monosymmetry from polysymmetry involve reduction in number of petals and other organs, and these evolutionary changes must use alternative developmental paths (Donoghue et al. 1998). The developmental evidence does not indicate constraints on the origin of the trait.

7.7.3 How Labile Is Floral Symmetry?

Floral symmetry changes come from either evolutionary elaboration or reduction (simplification) (Endress 2012). The development of symmetrical flowers may include monosymmetrical or asymmetrical stages, so evolutionary transformation may require only minor changes, for instance in timing (Busch et al. 2012).

Monosymmetry has evolved multiple times in several lineages across angiosperms, and has been proposed to be a key innovation that leads to high speciation. However, several lineages (e.g., Crossosomatales) never evolve monosymmetry, while others, such as Lamiales, are predominantly monosymmetrical (Endress 2012). Several studies have revealed repeated evolutionary origins of monosymmetry, as well as reversals to polysymmetry. Phylogenetic reconstruction of ancestral states reveals that in 761 species belonging to 110 families of angiosperms, from an ancestral state of polysymmetry, several origins of monosymmetry (about 130) and about half as many reversals to polysymmetry have occurred during evolution of the group (Reyes et al. 2016). Similar evolutionary patterns are detected also in particular clades of angiosperms. For instance, in Papaveraceae, evolutionary transitions may have gone from polysymmetry in the common ancestor with the closest group Ranunculaceae, to disymmetry in the ancestral Papaveraceae and in early Fumarioideae lineages such as *Hypecoum* and *Dicentra*, to monosymmetry in lineages containing taxa such as *Fumaria* and *Corydalis* (Sauquet et al. 2015). This stepped evolution is interesting in the context of experiments showing that bumblebees may prefer artificial disymmetrical flowers over polysymmetrical ones (see above). In analyses of subtle monosymmetry in different whorls of flowers in 96 species of *Rhododendron*, multiple gains and losses of monosymmetry features were detected to differing extents (Berry et al. 2018). These phylogenetic patterns reveal a great many changes, showing that symmetry is quite labile and prone to change in evolution. However, it is also true that some lineages are quite old, but remain polysymmetrical. For example, Vitaceae (~950 species: Wen et al. 2018), an early-branching lineage of rosids (~70,000 species: Wang et al. 2009), has been around for about 100 my (Wang et al. 2009). It is the

sister clade to the rest of rosids and has never evolved monosymmetry, even though the trait has arisen multiple times in the rest of rosids (Reyes et al. 2016). Are there developmental or other constraints in Vitaceae?

It has been suggested generally that variation in developmental processes brings about variation in floral architecture (including symmetry); that ecological factors acting at the community and population levels mainly influence floral biology; that phylogenetic constraints mainly act at the level of floral organization (Endress 1996). Given that there are several polysymmetrical lineages in which monosymmetry does not arise, are there constraints to evolution of monosymmetry in these lineages due to developmental genetic factors? Similarly, asymmetry is generally less common than monosymmetry. Is this because elaborate monosymmetry is itself less common, perhaps because of developmental constraints or perhaps because selection for asymmetry is less frequent?

Multiple, parallel recruitment of the *CYC2* gene developmental pathway have occurred in instances of different origins of floral monosymmetry (e.g., Dipsacales: Howarth and Donoghue 2005; Brassicaceae: Busch et al. 2012; Proteaceae: Citerne et al. 2017). Multiple reversals from monosymmetry to polysymmetry in the Malpighiaceae clade may have occurred due to at least two different developmental-genetic mechanisms that seems to be context-dependent and arise stochastically. Loss of *CYC*-like gene expression either due to some upstream regulation or loss of gene-function has been hypothesized for transitions that have occurred from monosymmetry to polysymmetry (Zhang et al. 2013; Hileman 2014). While different paralogues of *CYC*-like gene-lineages have been associated with the evolution of floral monosymmetry across many lineages of angiosperms, in others it may have involved changes in regulatory cis-elements, e.g., *Conandron ramondioides* (Gesneriaceae) (Hsin and Wang 2018). Overall, there do not appear to be developmental genetic constraints in generating the necessary variation to make monosymmetrical flowers, and other clade-specific causes must be sought. However, the developmental mechanism, involving B-class MADS box genes, that sets up monosymmetry/asymmetry in orchids (see above) has not been observed in other taxa – perhaps due to developmental constraints or lineage-specificity, or perhaps it has not been investigated more thoroughly.

Another aspect that has not been discussed much is the evolution of an *enabling* trait (Donoghue and Sanderson 2015), that having evolved, makes possible the evolution and elaboration of other traits. For instance, corolla monosymmetry may enable a certain set of morphologies that then are explored (developmentally and ecologically) within specific lineages, e.g., Lamiales. Such an enabling trait may be considered as the other, “positive” aspect of an apparent phylogenetic constraint.

7.7.4 Floral Symmetry and Pigment Patterns (Nectar Guides)

It has been pointed out that there is an association between floral symmetry and pigment patterns on the corolla (Leppik 1974; Dafni and Kevan 1996). This association may be expected because symmetry and nectar guides are both features that

help the pollinator navigate its way into the heart of the flower. That the two traits may evolve together was revealed by evolutionary correlation detected between floral symmetry traits (considered for the entire flower or whorl-wise) and pigment patterns on the corolla in *Rhododendron* (Berry et al. 2018; Box 7.1). Thus, monosymmetrical flowers with monosymmetrical corolla, reflexed and bundled stamens and reflexed styles have corolla pigment patterns (e.g., *R. lutescens*), while predominantly polysymmetrical flowers with polysymmetrical corolla, straight and loosely bundled stamens, and hooked styles do not have corolla pigment patterns (e.g., *R. cinnabarinum*). Multiple gains and losses of monosymmetry features or traits directly related to floral monosymmetry were tied to gains of pigment patterns; similarly, multiple gains and losses of polysymmetry were coupled with losses of pigment patterns. Thus, both traits (monosymmetry and pigment patterns) are evolutionarily labile and appear to evolve together. This repeated, correlated evolution indicates selection on these traits – a macroevolutionary pattern that is supported by known pollinator preferences (see above).

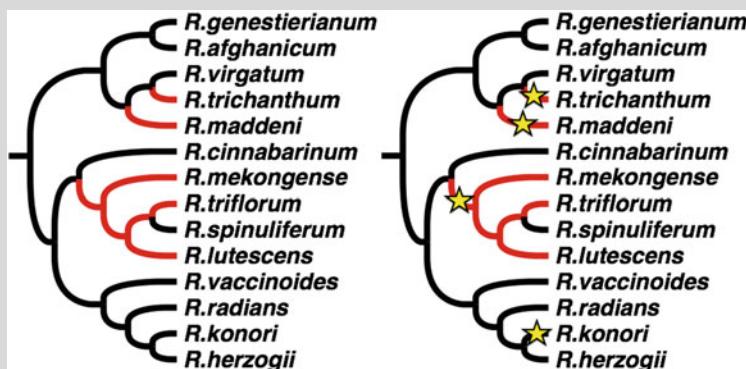
7.7.5 Does Monosymmetry Result in Higher Rates of Speciation?

If monosymmetry increases the specificity of pollination, then reproductive isolation is more likely and this should result in higher rates of speciation in monosymmetric lineages. Using diverse phylogenetic statistical approaches, this hypothesis had found support in some studies, but not in others (Sargent 2004; Reyes et al. 2015; Kay et al. 2006; O’Meara et al. 2016; Vamosi and Vamosi 2011; Vamosi et al. 2014). The methods of phylogenetic analyses may have much to do with the results obtained, but overall, it may be concluded that there does appear to be a relationship between monosymmetry and increased diversification rate, but that this may not hold in all lineages of angiosperms, and that more studies are needed of specific lineages. Other analyses point out the limitations of phylogenetic analyses of pollinator-driven evolution (Niet and Johnson 2012). Because the overall diversification of species is the complex outcome of a range of intrinsic and extrinsic factors, the question is a complex one that is still in the early stages of being tackled. For instance, investigators may need to look beyond a single character for a “synnovation,” an interacting combination of traits with a particular evolutionary consequence (Donoghue and Sanderson 2015). An example may be the combined effect of the presence of corolla, monosymmetry and reduced stamen number, which together appear to act as a “key synnovation” that promotes diversification (O’Meara et al. 2016). Furthermore, while monosymmetry has evolved multiple times across angiosperms and may be a key innovation for several species-rich clades, it may not be so for other clades, perhaps because the genetic switch for this change is simple and polysymmetry may have a selective advantage over monosymmetry under certain conditions (Endress 2012).

Box 7.1: Tree-Thinking

To detect macroevolutionary patterns that tell us what happened in history and to understand these patterns in terms of biological (including evolutionary) processes at diverse levels of organization (molecular, cellular, organismal, ecological), phylogenetic analysis is essential. The first step is to obtain a phylogenetic tree of relationships among taxa of interest, which involves acquiring data (usually molecular sequences) and analysing the data employing any or all of a range of methods (e.g., maximum parsimony – MP, maximum likelihood – ML, Bayesian inference – BI). The second step is to understand the evolution of trait/s of interest, given this phylogenetic history of the taxa. Such phylogenetic interpretation has been termed “tree-thinking.”

To understand when, in the history of a set of taxa, a trait arose, ancestral states are inferred from the character states of the taxa by mapping the states on a given phylogenetic tree. In this example (*below left*), MP analysis suggests a scenario in which polysymmetry characterized the most recent common ancestor of the *Rhododendron* species, and monosymmetry arose independently two times in two different lineages. Other scenarios take more steps and therefore, less preferred. Monosymmetry arose more than once in evolution, and therefore the trait in this set of taxa is not homologous (a single evolutionary origin in a recent common ancestor) but *homoplasious* (multiple independent origins due to parallelism, convergence or reversals).



Modified after Berry et al. (2018)

To determine whether variation in two traits, such as symmetry and pigment patterns, evolved together in a correlated manner, special statistical methods are used because, unlike data in standard statistical analyses, cross-species data may not represent independent data points (Felsenstein 1985). To show that two traits are associated due to independent events (and therefore correlated), relatedness due to common ancestry is taken into account by

(continued)

Box 7.1 (continued)

weighting the evolutionary replicates as the data points, using comparative statistical methods. In this ancestral state reconstruction of pigment patterns overlaid on evolution of monosymmetry (*above right*), independent origins of pigment patterns (stars) are associated with origins of monosymmetry. In a large enough tree with a large number of homoplasies, this association is shown to be statistically significant (e.g., Berry et al. 2018).

7.8 Conclusions

A great deal is understood about the morphological disparity in floral symmetry in terms of the development of form and major genes that control form; however, much remains to be understood. Future studies of floral architectural research could try to understand the origins and development of subtle variations of floral monosymmetry, some of which may have pollination functions. Why are some combinations of symmetry features more prevalent than others and still other forms completely absent (as in Ericales, see above), such that the morphospace is not fully occupied? Selection by pollinators is known to be effective in some, but not all, systems. What makes pollinator groups settle for particular combinations of symmetry and other features? Might it be useful, in this context, to consider the *enabling* aspects of phylogenetic constraints? Addressing these questions requires the combined study of the developmental organization and adaptive features to understand occupied and unoccupied regions of floral architecture morphospaces. Advances in answering these questions would necessarily come from the combined examination of studies in molecular developmental genetics, functional ecology and microevolution of floral symmetry analysed within a phylogenetic framework.

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Resource Allocation in Flowering Plants: Concept and Implications

8

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Abstract

The concept of resource allocation envisages that every organism partitions its resources to the competing activities of growth, maintenance, and reproduction. Studies conducted on flowering plants have revealed this partitioning to be plastic and regulated by life-history patterns and breeding system of the species. Plasticity in resource allocation can contribute to the outcomes of sex differentiation in higher plants. Besides, plants also compete for resources such as light and nutrients and competitive ability also has a bearing on resource allotment to different parts. There is a trade-off of resources in terms of investment in reproductive effort and fitness. Studies on resource mobilization in plants that are facing biotic as well as abiotic stress have revealed that photo-assimilatory pathways have an important role. Computed data have helped in developing predictive models for resource distribution that will help in the management of various crop species.

Keywords

Resource allocation · Breeding system · Reproductive effort · Trade-offs

8.1 Introduction

Theory of resource allocation, originally borrowed from microeconomics, advocates that every organism has a finite amount of resources that are partitioned for competing activities of growth, maintenance, and reproduction in a way that resources allocated to one function are therefore not available to the other (Cody 1966; Harper 1977; Harper and Ogden 1970; Sharma et al. 1999). Every organism thus optimizes

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on strategies to allocate resources according to its life-history pattern, and natural selection is assumed to prioritize some functions at the expense of others. Resource allocation maximizes the fitness of plants to the prevailing biotic and abiotic stresses. When the resources are limited, plants make a choice to limit the resources to a few functions rather than investing in many attributes. Diverse allocation patterns thus reflect survival strategies of plants resulting from different selection pressures (Jones and Little 1983).

Two important attributes of resource allocation strategy are *Reproductive Effort* (RE) and the concept of *Trade-offs* (Tuomi et al. 1983; Bazzaz and Reekie 1985). While the former refers to an individual's net investment of resources in reproduction, the latter advocates that there is a trade-off of resources between the competing activities; thus if one function is suppressed, the other may increase. An optimal pattern of allocation results in maximizing some output parameters. In evolutionary ecology, this output is primarily the measure of an individual's fitness and its contributions to future generations (Wilson 1983; Bazzaz et al. 2000). Such studies have been widely utilized in plants, as various functions can be approximately assigned to discrete structures. For example, carbon gain is primarily the function of leaves, nutrient uptake of roots, and reproduction of inflorescences, seeds, and ancillary structures (Bazzaz et al. 2000).

The concept of reproductive effort (RE), which is often equated with the reproductive allocation (RA), has received considerable attention primarily because of its economic significance and its direct relevance to the survival and evolution of a species. It is the net investment of resources in reproduction, which is diverted from vegetative activity (Tuomi et al. 1983; Bazzaz and Reekie 1985). The reproductive effort is the estimation of energy allocated to reproduction out of the total energy available to the individual (Primack and Antonovics 1982; Dunn and Sharitz 1991; Kaul 1998; Sharma et al. 1999; Kaul et al. 2002). This chapter highlights the resource allocation patterns in relation to various developmental events and trade-offs among flowering plants.

8.2 Resource Allocation Patterns: Static or Flexible

Primack (1979) opined that resource allocation pattern, in particular, the reproductive effort of a species or population, is a constant feature that does not alter even if the plants are subjected to various kinds of stresses (Harper and Ogden 1970; Van Andel and Vera 1977). Later studies on the contrary have shown that plants change their allocation pattern in response to several factors such as density, competition, availability of soil nutrients, microclimate of individual plants, etc. (Abrahamson 1979; Smith 1981; Waite and Hutchings 1982; Fahey 1983). When plants are not crowded, they behave more like r-selected species, allocating a larger proportion of their biomass to reproductive structure (high reproductive effort). When they are crowded, on the other hand, they behave more like k-selected species allocating lesser of their biomass to reproductive structures, and a greater proportion to the organs such as stems and leaves. Several studies have provided support for this

simple model of reproductive allocation (Thompson et al. 1991; Aarssen and Taylor 1992; Schmid and Weiner 1993).

The availability of soil nutrients is one of the factors that may influence resource allocation patterns (Brouwer 1962). One hypothesis is that plants growing in low nutrient environments allocate proportionally more resources to roots to increase their uptake capacity of limited soil resources (Bradshaw 1965; Chapin 1980; Wilson 1988; Gedroc et al. 1996). More “opportunistic” species (e.g., *Eleocharis palustris*, *Juncus tenuis*, *Lolium perenne*, *Oxalis corniculata*, *Poa compressa*, *Trisetum flavescens*, and *Veronica serpyllifolia*) are reported to show a significant response to nutrient availability in their allocation patterns than the non-opportunistic ones (e.g., *Carex flacca*, *Fragaria vesca*, *Luzula campestris*, or *Mentha arvensis*). Resource allocation pattern may also change with plant size (Pearsall 1927), i.e., they may be “allometric” in a broad sense, and it has been argued that some observed changes in allocation are primarily due to the size (Weiner 1988; Coleman et al. 1994; Coleman and McConaughay 1995). If the allocation to different structures changes with the size, any factor that influences plant size will thereby change the allocation pattern (Bloom et al. 1985).

According to *optimal allocation theory*, plants should allocate more resources to organs that capture the most limiting resources and less to those that are involved in obtaining non-limiting resources (Bloom et al. 1985). For example, under low nutrient conditions, plants should allocate relatively more of their biomass to roots while, under higher nutrient conditions, they should allocate more to shoots. While some studies provide support for the optimal allocation model (Shipley and Meziane 2002), others suggest that the differences in allocation patterns under varying nutrient conditions are largely due to allometric growth (Muller et al. 2000; Cahill 2003). Plants are generally more “rooty” early in development when they are young and small and become more “shooty” as they get larger. *Betula pendula* seedlings can alter their allocation to roots versus shoots in response to varying levels of some nutrients (Ericsson 1995).

In populations of *Murdannia keisak* inhabiting undisturbed sites, Dunn and Sharitz (1991) observed higher RE in comparison to the ones at thermally disturbed sites, indicating plasticity in the response of plants to the environment. Anronson and his coworker (1993) suggest that variability in resource allocation pattern holds adaptive significance in plants, particularly for those inhabiting fluctuating environment. They found that inter-population differences in reproductive effort in *Erucaria hispanica* and *Brachypodium distachyon* were greater under water stress, the main limiting factor in a desert. Their results also clearly indicate that variability in reproductive effort in response to drought is a critical and dynamic component of life-history strategy in annual species growing under variable xeric environment.

8.3 Resource Allocation in Relation to Breeding System and Life-History Patterns

Studies on related species following different breeding systems have demonstrated that species undergoing inbreeding allocate more resources to reproduction in comparison to the outbreeding counterparts (Sharma et al. 1999; Dhar 2004; Hamal 2010). Among the few species of *Plantago* (Sharma et al. 1999), predominantly inbreeding species namely *P. patagonica*, *P. drummondii*, and *P. ovata* exhibit 52%, 48%, and 56% reproductive effort, respectively, in comparison to the outbreeding species namely *P. lagopus* (32%) and *P. lanceolata* (27%). Obligate inbreeder *Trifolium dubium* invests 55% of its resources for reproduction in comparison to *T. pretense*, an obligate outbreeder, which spends only 13% of its resources for reproduction (Dhar 2004). Self-pollinating *Vicia hirsuta* spends 46.8% of its resources for reproduction while *V. sativa* with a mixed mating system uses only 17.5% of its resources for the same (Hamal 2010). Cleistogamous flowers invariably practice selfing and exhibit higher reproductive effort compared to their chasmogamous counterparts (Pitelka 1977; Primack 1979; Abrahamson 1979; Sharma and Koul 1995; Kaul 1998; Kaul et al. 2002). Such results are reflected through studies on the reproductive effort of cleistogamous and chasmogamous flowers on a single plant, for example, *Commelina benghalensis* (Kaul 1998; Kaul et al. 2002).

In most of the studies, annuals have been demonstrated to invest a large amount of resources to seed production which help these species to survive as also to invade and colonize new sites (Abrahamson 1975a, b; Pitelka 1977; Abrahamson 1979; Primack 1979; Sharma and Koul 1995; Sharma et al. 1999; Dhar 2004). Perennials, on the other hand, allocate a greater amount of their resources toward vegetative structures, which ensure long survival at a fixed site (Reekie and Bazzaz 1987; Anronson et al. 1993; Sharma and Koul 1995; Sharma et al. 1999; Dhar 2004). Annuals under optimal growth conditions divert almost all the resources produced during photosynthesis into the seeds whereas perennials commit only a limited proportion of resources to the reproductive phase of their life cycle (Archetti et al. 2009). In long-lived perennials with repeated flowering, investment in reproduction is typically not attempted until the requirements of survival have been met (Crawley 1996). Annual species of *Plantago* (*P. patagonica* 52% and *P. drummondii* 43%) invest an appreciable amount of resources for reproduction in comparison to their perennial counterpart namely *P. lagopus* (32%), *P. lanceolata* (27%), and *P. major* (21%) (Sharma et al. 1999). Similarly, *Trifolium dubium* and *T. fragiferum* invest 55% and 45% of their resources in reproduction while the perennials *T. pratense* and *T. repens* invest only 13% and 24% respectively. Sharma (2013) observed higher reproductive effort in polyploid *Artemisia nilagirica* as compared to the related diploid *A. scoparia*. The two species differ in sex expression, the former being a gynomonoecious and the latter a monoecious.

The cost of reproduction may also play a role in the evolution of plant reproductive systems by eliciting selection on life-history traits that are related to sexual dimorphisms, such as age and size at the time of first reproduction, frequency of

reproduction, and number of flowers. For example, in dioecious plants, females generally have higher reproductive allocation than males and thus the cost of reproduction is expected to be higher in females (Bawa 1980). Indeed, several studies on dioecious species suggest lower frequency of reproduction, higher mortality, and lower growth rates in females compared with males (Meagher and Antonovics 1982; Garcia and Antor 1995; Antos and Allen 1999; Nicotra 1999; Rocheleau and Houle 2001; Alvarez- Cansino et al. 2010; Obeso 2002). The higher seed production of female plants observed in several gynodioecious species, on the other hand, suggests that the overall resource allocation to reproduction is higher in females compared with hermaphrodites (Dawson and Geber 1999). To coexist with hermaphrodites, the females of gynodioecious species have to compensate for the lack of male function, and to outperform hermaphrodites in one or several aspects of female reproduction (Lewis 1941; Lloyd 1975; Charlesworth and Charlesworth 1978).

In the perennial gynodioecious *Geranium sylvaticum*, female plants generally produce more seeds than hermaphrodites (Asikainen and Mutikainen 2003). When combining the probability of flowering and seed production per plant to estimate the multiplicative change in fitness, female plants of *G. sylvaticum* showed a 56%, and the hermaphrodite showed a 39% decrease in fitness due to experimentally increased reproduction. Therefore, female plants seem to be more sensitive to the cost of reproduction in terms of seed fitness than hermaphrodites (Toivonen and Mutikainen 2012).

Methods for Estimating Reproductive Effort and Resource Allocation

Several methods have been used by diverse workers to quantify resource allocation patterns. The usage of each of these methods is related to the type of material and aim of the study. However, dry biomass allocation (b) and oxygen bomb calorimetry (a) are widely used in this order only.

- (a) Oxygen bomb calorimetry (Paine 1971): This method is the most direct method, wherein plant parts are separated and burned individually in an aerobic atmosphere to their original constituents that is carbon dioxide, water, and minerals. Energy released during burning is measured by the rise in temperature in the calorimeter. This energy is considered to be proportional to the energy used in producing the tissues. This method is considered to be reliable and relatively straightforward.
- (b) Dry biomass method: In this method used mostly for herbaceous annuals; mature plants; are taken and their different parts like root, stem, leaves, flowers, and fruits are separated, washed, and oven-dried for 24–48 h, at 55–80 °C. These dried plant parts are then weighed and compared to estimate relative energy allocation (Hickman and Pitelka 1975).

(continued)

- (c) Chemical component analysis: Different chemical components of different tissues of the plant are determined separately and compared to estimate energy allocation. The important chemical constituents are carbohydrate 4100 cal/ash-free gram, lipid 9450 cal/ ash-free gram, and protein 5650 cal/ ash-free gram (Paine 1971).
- (d) Crude estimates: Crude estimates of resource allocation involve counting and comparing attributes like number of flowers, number of seeds/fruits, number of pollen grains/ovule, leaf area, etc. (Harper 1977; Law 1979; Primack 1979; Lord 1980; Philipp 1980; Spira 1980). Leaf area/seed weight is one such estimate.

8.4 Sex Allocation

The investment made to the male and female reproductive functions in hermaphrodite individuals is referred to as sex allocation (Charnov 1982; Schoen 1982; Charlesworth and Charlesworth 1987; Mckone 1987; Sharma and Koul 1995; Sharma et al. 1999). This allocation has also been related to the breeding system practiced by a species. In outbreeding hermaphrodite angiosperms, the allocation to male and female functions is expected to be in the ratio of 1:1 (Charnov et al. 1976; Charnov 1979; Smith 1981; Ross and Gregorius 1983). A shift toward either sex is favored by natural selection only if it increases parental fitness for each unit of energy or resources spent (Goldman and Willson 1986; Lloyd 1988). The typical ratio of 1:1 deviates in individuals practicing predominant autogamy (Charnov 1982) with female allocation increasing with increase in the extent of self-pollination. In *Smyrnium olusatrum*, Lovett Doust and Harper (1980) observed that before fertilization plants distributed a greater (fivefold) proportion of phosphorus to male than to female organs. However, the cost incurred on the development of embryos reversed the ratio such that at seed maturity a greater proportion of phosphorus (twofold) was allocated to seeds than to stamens. Their observations also revealed that the distribution of resources, evaluated in terms of N, P and K content, to the male and bisexual flowers is in the ratio of 1:4.

In *Pseudocymopterus montanus*, Schlessman and Graceffa (2002) opined that the plants with higher reproductive effort had lower proportion of staminate flowers and were relatively more female than those with lower reproductive effort. Kour (2003) observed an insignificant difference in the relative allocation of resources to the pistil ($\bar{x} = 0.00011$ mg) and stamens ($\bar{x} = 0.00016$ mg) at the level of flower in *Scaligeria stewartiana*. However, the species spends more resources in the differentiation of male gametes ($\bar{x} = 0.408$ mg) as compared to females ($\bar{x} = 0.15$ mg) at the level of the plant.

Nectar represents a considerable investment of plants (Southwick 1984; Pyke 1991; Harder and Barret 1992; Ashman and Schoen 1997). By removing nectar from

flowers of *Blandfordia nobilis*, Pyke (1991) experimentally increased plant's net nectar production and reduced its ability to produce seeds. He concluded that there is a trade-off between pollinator-mediated fertilized seeds resulting from enhanced nectar production and decrease in seed number due to the costs of producing the nectar. Ashman and Schoen (1997) found that flowers of *Clarkia tembloriensis* experimentally maintained 35% longer than the average, invested proportionally more in nectar sugar (30%). These plants with long-lived flowers showed a 12% reduction in seed production, and this may be interpreted as the cost of flower maintenance. A similar trade-off between floral longevity and seed production was found in *Calochortus leichtlinii* (Holtsford 1985). Ashman and Schoen (1997) proposed a model that expresses the costs of maintenance as a trade-off between floral longevity and seed production and define an optimal floral longevity determined by both the rate of fitness gain and the cost of floral maintenance.

8.5 Trade-Offs

Investment in clonal growth is termed *vegetative reproductive effort* (VRE), while that in sexual reproduction is *sexual reproductive effort* (SRE). Several authors have attempted to assess the trade-offs between vegetative growth and reproduction and between clonal growth and sexual reproduction in plants practicing both. Several interesting results have emerged from such studies.

Liu et al. (2009) have elaborated this trade-off in *Sagittaria pygmaea* where plants could adjust their trade-off patterns to fit the nutrient variation. When the nutrient level is low, there is a weak trade-off between sexual reproduction and vegetative growth and between clonal propagation and vegetative growth; when the nutrient levels turn moderate, a strong trade-off occurs between sexual reproduction and clonal propagation; however, when the nutrient level is high, no trade-offs occur among these three different competing components. Odgen (1974) reported the reproductive strategy of *Tussilago farfara* under different levels of plant density and soil fertility by measuring both dry weights and caloric content using an oxygen bomb calorimeter. Most of the resources were found to be allocated to asexual reproduction in the case of plants growing in poor soils. The SRE, on the other hand, was not affected by soil fertility. Population density also influences vegetative reproduction which decreases with increase in density. Sexual reproduction, on the other hand, remained unaffected with respect to density. Abrahamson (1975a, b) estimated the RE of *Rubus hispida* in different habitats for two consecutive years. He found the VRE to be constant across the sites while SRE decreased with increased maturity of the sites. Also, while the VRE remained constant for both the years of the study, SRE was lower during the drier period of the 2 years. The density of vegetation was also found to affect the mode of reproduction. High density favored SRE, whereas low density favored VRE. Coelho and workers (2005) examined the effect of densely crowded conditions on the production of reproductive and vegetative structures in *Pistia stratiotes* (an aquatic macrophyte) to test whether there is a trade-off between clonal growth and investment in sexual

reproductive structures, and whether there is any shift in allocation pattern with plant size. The authors confirmed that individual plant biomass and the number of rosettes producing sexual reproductive structures and vegetative growth structures both increased with density. Increase in plant size resulted in increased proportional allocation to sexual reproductive structures and vegetative growth structures. However, the allocation of biomass to sexual reproduction did not occur at the expense of clonal growth. Bhuvi (2008) investigated the trade-offs between VRE and SRE by maintaining a set of plants in which sexual functions were curbed right from the inception and another set of plants without any manipulations. Comparisons made between the fresh biomass of open pollinated and the experimental vegetative plants revealed an increase in the VRE in the latter. For open pollinated plants, VRE as estimated with fresh biomass was 16% while for the experimental plants, the same averaged 22.2% for the 3 years of manipulations. However, curtailment of sexual functions led to the total loss of diosgenin, an important steroid obtained from the rhizome.

Several other studies have focused on the amount of resources diverted to sexual and asexual reproduction and their importance in the life-history pattern of a species. These include *Circae quadrifida* (Struik 1965), *Tussilago farfara* (Odgen 1974), *Solidago canadensis* (Bradbury and Hofstra 1976), *Zanthoxylum americanum*, (Popp and Reinartz 1988), *Trifolium repens* (Chapmann et al. 1992), *Helianthus tuberosus* (Westley 1993), *Pinguicula vulgaris* (Worley and Harder 1996), *Ulmus minor* (Lopez- Almansa et al. 2003), *Cryptotaenia canadensis* (Hawkins et al. 2005), and *Fallopia japonica* (Grimsby et al. 2007). Most of these studies confer evidence to a trade-off between sexual and asexual reproduction.

Biomass distribution between the competing reproductive functions (sexual and asexual reproduction) has been shown to vary with the genotype (Reekie 1991), nutrient availability (Biere 1995), and light and successional gradients (Ashmun et al. 1985; Jurik 1985). It has also been shown that allocation patterns can differ between rhizome and seed-derived plants of polycarpic species (Schmid and Bazzaz 1990; Schmid and Weiner 1993; Schmid et al. 1995) due to developmental effects (Schmid and Bazzaz 1990; Schmid and Weiner 1993) or due to selection pressures which result in favoring one mode of reproduction over the other (Schmid et al. 1995). Struik (1965) estimated the sexual and asexual efforts in *Circae quadrifida* with respect to different habitats. He reported that in mesic habitat, where flowering and fruit set was low, asexual RE was about four times the SRE. In dry mesic habitat where flowering and the fruit set was high, the asexual reproductive effort was about twice the sexual effort.

Studies on RE in *Trifolium repens* made by Turkington and Cavers (1978) suggested that greater resources are diverted to vegetative reproduction than sexual reproduction. Dhar (2004) reported that the roots and runners take the maximum share of resources (42%) in this species. Author suggested that the allocation of sufficient resources toward clonal propagation in *T. repens* may be because of the fact that the species experiences 50% ovule abortion. The decrease in resource utilization toward sexual reproduction may have increased the resource availability for clonal propagation. The same has been reported in *Ulmus minor*, which

propagates through root sprouting and experiences female sterility by pre-pollination and pre-zygotic ovule abortions and later seed abortion (Lopez-Almansa et al. 2003).

According to the *defense trade-off* hypothesis, plants may respond to the resource limitations by making choices, that is by investing more in anti-herbivore defense (Coley 1983; Koricheva et al. 2004; Dostalek et al. 2016). The *continuum of plant response to herbivory* hypothesis predicts the impact of herbivory may be detrimental, neutral, or beneficial for plant fitness depending on the plant's ability to replace tissue consumed by herbivores as influenced by the abiotic environment (Maschinski and Whitham 1989; Stowe et al. 2000). For example, plants may delay fruit production or produce fewer fruits following herbivore attack (Kersch-Becker and Lewinsoln 2012; Wang et al. 2016).

8.6 Resource Allocation Under Stress

Both the biotic as well as abiotic stresses are known to affect resource allocation. Eziz et al. (2017) collected data on resource allocation patterns in a sample of herbaceous and woody plants under drought conditions. Drought, one of the important abiotic stresses, significantly increased the fraction of root mass. Roots of herbaceous plants were found to be affected more sharply than the woody ones. In contrast, drought had a more negative impact on leaf mass fraction of woody plants as compared to herbaceous plants. Among the herbaceous plants, roots of the annuals respond more strongly to drought stress than perennial herbs, but the reproductive allocation showed a reverse pattern.

Li et al. (2019) determined sex-specific response to light in *Vallisneria spinulosa*. Authors found that both male and female plants differed in the adjustment of resource allocation. Under low light, females showed smaller reduction in the allocation of resources to vegetative tissues than males, suggesting female plasticity to increase carbon capture. On the contrary, males show a smaller reduction in reproductive allocation than females (flowers and inflorescences in males and fruits in females) suggesting that carbon limitation has greater impacts on sexual reproduction in females and less in males. Thus, sex-related resource currencies are important drivers for sex-specific variations in allocation patterns, with females safeguarding their vegetative carbon-rich biomass to satisfy future fruit and seed production.

Resources redistribute within inflorescences in response to different pollination intensities in *Sagittaria trifolia*. In a set of experiments, pollen supplementation of a single flower was found to significantly increase its fruiting probability; however, the same manipulation of an inflorescence did not increase its overall reproduction. In complex inflorescences, supplemental pollination had no effect on the reproductive success of flowers on the lateral or main branches. The resources were unlikely to be reallocated between main and lateral branches in the complex inflorescences, suggesting that flowering branches represent integrated physiological units (Dai et al. 2018).

Tonnabel et al. (2017) measured and analyzed relative allocation to reproduction vs. vegetative growth in *Mercurialis annua*—a dioecious taxon. Reproductively mature males and females differentially adjusted their resource allocation in response to varying plant densities. Males maintain the same relative allocation of resources to reproductive vs. vegetative tissues at all densities. In contrast, females reduced vegetative growth proportionally less than seed production at higher density. Resource currencies limiting male and female function differently were envisaged to have potentially led to the evolution of sex-specific strategies of resource acquisition and deployment; the females were found to be conserving resources for vegetative organs to ensure their future carbon-rich reproduction.

8.7 Conclusion

Studies on resource allocation have mostly been carried out in flowering plants. It has been understood that resources are allocated to plant organs that have more important functional roles such as ensuring reproductive success. Resource allocation patterns have been modeled for a few crop plants as well as some interesting plants growing in the wild. However, many aspects of patterns and processes involved in resource partitioning in plants that are growing in selected and specialized habitats have not been studied in detail. It would also be interesting to find out how plants are going to optimize their resources in response to changing climate. Besides, there is so much of plasticity in plants in terms of the photosynthetic pathways they adapt in response to the environment that no generalist models will be predictive enough. Therefore, experiments have to be carried out in controlled as well as field conditions on a variety of plant species in different habitats to assess the quantitative distribution and allocation. It is also surmised that plants will be subjected to more serious pests and pathogens in the changing climatic conditions, thus the patterns and allocation of resources to develop resistance and combat the stress will have to be more subsidized. There is also a need to develop more robust and novel tools and techniques to estimate the resources so that the data generated can help in a better understanding of the mechanisms involved in estimating resource distribution of perennial plants that represent a significant proportion of the phytodiversity.

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Dynamics of Eco-Evolutionary Forces in Shaping Dioecy

9

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Abstract

Evolution of dioecy among plants is a distinct phenomenon, debated extensively among biologists. It has now been realized that besides the underlying gender determination mechanisms, it is equally important to understand the contextual framework of eco-evolutionary forces that are instrumental in shaping dioecy in general. The theoretical framework of evolution of dioecy is well-argued in literature. Several empirical studies have indicated ecological factors like habitat, floral features, wind pollination, and clonality to be advantageous for establishing dioecy. Further, resource partitioning among genders is known to modulate the sex ratios, which is crucial for its evolutionary maintenance. How these factors influence evolutionary pathways and evolution of dioecy, has not been sufficiently investigated. Available phylogenetic analyses indicate that the factors are interlinked, and that they serve as usual correlates of dioecy. Although, such associations are not clearly elucidated in literature due to paucity of information about the prevailing sexual systems, further obscured by low species richness in existing dioecious clades. In this chapter, we present a conspectus of present understanding of ecological correlates of evolution and maintenance of dioecy, especially among the flowering plants. The information which has emerged so far indicates the involvement of multivariable eco-evolutionary suites. However, in order to appropriately characterize them, there is need to extend empirical studies on the complete range of sexual variation.

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Keywords

Dioecy · Wind pollination · Resource allocation · Sex ratio · Growth forms · Clonality

9.1 Introduction

Unlike animals, sexual systems among the flowering plants have a complex representation. While a significant proportion of the taxa bear bisexual flowers (hermaphrodite/co-sexual), nearly 6% among the angiosperms are known to be dioecious (Renner 2014). The combination of sexual conditions in plants is defined either at the individual or the population level (Sakai and Weller 1999). Dioecy, though not designed especially to promote outbreeding (*see* Darwin 1877), is characterized by the presence of separate plants of particular gender. Phylogenetic assessments reveal their random presence across many unrelated taxonomic groups, which are independently derived on multiple occasions (Renner 2014).

The underlying mechanisms (pathways) for the evolution of dioecy from hermaphroditic ancestral state are well-characterized. Theoretical models advocate that evolution of dioecy essentially requires invasion of sterility mutations of either of the sexual counterparts along with sex modifiers in a co-sexual population (Charlesworth 2016). It is also fervently believed that certain ecological parameters create salubrious conditions, enabling the evolutionary forces to take effect in manifesting dioecy (Charlesworth and Charlesworth 1978a, b; Lloyd 1982; Barrett 2002). These factors are also thought to be involved in the evolutionary maintenance of dioecy (Charlesworth and Charlesworth 1978a, b, 1981; Charlesworth 1989). The major factors include habitat, floral features, wind pollination and clonality. Besides the ecological factors, the prevailing sex ratio and resource allocation pattern may also influence its maintenance. However, some studies argue that these attributes become crucial once dioecy is established (Bawa 1980; Soza et al. 2012; Renner 2014). Phylogenetic studies also do not provide a general support for the hypothesis of correlated evolution in three eudicots clades, namely Rosids, Asterids and Eumagnoliids (Vamosi et al. 2003), yet it does not negate the association of dioecy with ecological correlates. The analysis also indicated several inter-correlations among the traits, but it is likely that clade-based analysis may be an underestimation, primarily for two reasons: First, dioecious clades show lower species richness (Heilbuth 2000); second sexual systems have not been comprehensively described for all the clades. Thus, it is crucial to examine detailed phylogenies of taxa (as complete as possible) to minimize the differences in the clade diversification (Käfer et al. 2016).

Studies that have highlighted the role of ecological suites in evolution of dioecy are in the contexts of (1) prerequisites; (2) maintenance and (3) co-evolution. Together these aspects constitute the ecological correlates of the dioecy (Table 9.1 and Fig. 9.1). It is important to point out here that in many taxa, the same plants

Table 9.1 Key reproductive and ecological correlates of dioecy

Correlate	Possible causal factor/hypothesis in support of correlation	Putative role in evolution/maintenance of dioecy
Small inconspicuous inflorescences with many flowers	Many-flowered inflorescences may lead to higher geitonogamous selfing, resulting in strong inbreeding depression in hermaphrodites, favouring selection of unisexuality. In unisexuals, many flowered inflorescences increase floral display and maximize pollination, hence assure reproduction.	Provide assistance in the evolution and maintenance of dioecy. This is also a prerequisite for wind as a pollination mode.
Fleshy fruit	Fleshy fruits, often dispersed by birds, may increase the chances of species' dispersal over long distances. Dioecious clades have been successful at long-distance colonization in tropical environments with fleshy fruits.	Association can be seen in lieu of maintenance and spread of dioecy.
Tropical distribution	Dioecious clades may be more vulnerable to extinction in temperate habitat due to susceptibility to fluctuations in availability of pollinators.	May be a co-evolved association.
Woody growth form (shrubs, lianas, trees)	Large plants increase geitonogamous selfing and strong inbreeding depression, thus favouring outcrossing and unisexuality.	Possible cause for evolution and maintenance of dioecy as well as for reproductive assurance.
Dry and open habitat	A shift to drier habitats with low insect pollinators may lead to wind-facilitated selfing and consequent inbreeding depression may favour dioecy. Moreover, the drier habitat facilitates pollen dispersal.	Serve as a maintenance factors for dioecy.
Abiotic pollination	Reduced flower and inflorescence size are conducive for wind pollination or generalist pollinators. Moreover, anemophily is believed to have appeared after the evolution of dioecy (Charlesworth 1993).	Key association for the maintenance of dioecy.
Resource allocation	Conflict between the paternal versus maternal functions for balanced resources, plays a significant role in the evolution of dioecy.	Acted as a casual factor as well as important for sexual dimorphism and thus maintenance of dioecy.
Clonality	Large clonal size may lead to evolution of dioecy in	A life-history trait, possibly contributed to evolution of dioecy.

(continued)

Table 9.1 (continued)

Correlate	Possible causal factor/hypothesis in support of correlation	Putative role in evolution/maintenance of dioecy
	hermaphrodites, to overcome inbreeding depression. However, extended period of clonal reproduction among dioecious species may also result in suppression of sexual reproduction and lower genetic diversity.	Clonality provides reproductive assurance, if sexual reproduction fails.
Sex ratio	Sex ratio is largely a consequence of dioecy, and its life-history traits including resource allocation.	Balanced sex ratio in a diclinous population is an indication of evolutionary, ecological and genetic stability.

(genets) may shift gender within the same or alternate season(s) in response to a variety of reasons, including nutrient availability, temperature, precipitation and even aging (Mahmud and Struwe 2019). The effects of gender lability are similar to those that arise from dioecious condition, as they face similar constraints of pollination requirements. However, we have excluded this aspect for brevity, and in this chapter we have primarily focused on the eco-evolutionary dynamics of dioecy.

9.2 Gender Configuration and Floral Features

During sexual differentiation, populations may exhibit several combinations of genders, resulting in a mixture of natural populations of imperfectly differentiated individuals. Such populations are comprised of plants with separate or combined sexes or polymorphic gender. The presence of unisexual and bisexual flowers may also occur in different combinations in the same plant. Thus, the terminology to define the gender and sexual system (on the basis of their structure and function) is intricate; the major ones are presented in Table 9.2 (*also see* Westergaard 1958; Ross 1982; Sakai and Weller 1999).

In order to elucidate the extent of variation in gender combinations besides the functional assessment of flowers, the role of other accessory floral organs (such as calyx and corolla) vis-a-vis fitness consequences is also important (Charlesworth and Charlesworth 1981; Barrett 2002). Several studies have indicated that unisexual flowers tend to possess small perianth lobes (without any differentiation into calyx and corolla); large, condensed and exposed inflorescences; absence of nectar and fragrance, dry pollen grains, high pollen production, and uniovulate pistils. However, most of these features do not appear to have evolved independently and are rather derived in order to support each other (correlated evolution), and to provide reproductive assurance (Vamosi et al. 2003). The clades harbouring such a suite appear to have adopted wind as a suitable pollination mechanism during their shift to pollinator-limited habitats (*see* Sect. 9.6), as it imparts better capabilities for capture

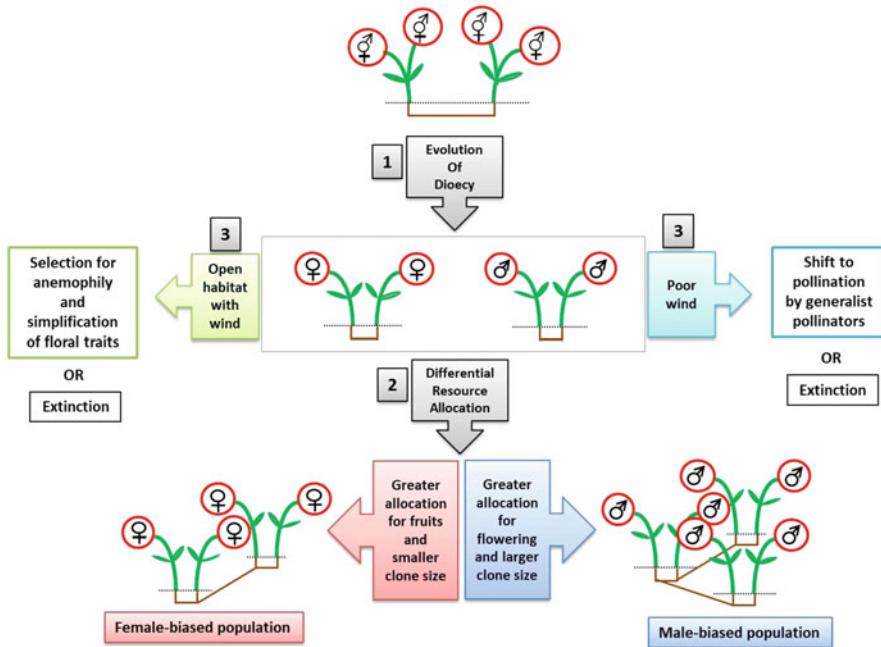


Fig. 9.1 Eco-evolutionary pathways associated with dioecious taxa. **1.** Evolution of dioecy from the co-sexual ancestors. The prevailing clonality (brown lines) in co-sexual ancestors may aggravate the consequence, and set a stage for the evolution of dioecy through geitonogamy and consequent inbreeding depression (*based on* Barrett 2002; Charlesworth 1989). **2.** There may be differential resource investment among the genders between the sexual and vegetative reproduction. Females invest lower amount of resources to clonal growth than the males. Thus, male-biased sex ratio among dioecious taxa is more common (*based on* Barrett 2015). The patterns of sex ratios among dioecious taxa mostly appeared as the consequence of life-history traits. **3.** Selection for wind pollination in dioecious clades with reduced floral suites is easier in windy or pollinator-limited environment. On the other hand, shift to closed habitats with pollinator availability may permit selection for generalist pollinators. Failure to adapt to a suitable mating strategy in the new environment may lead to local extinction (*based on* Culley et al. 2002). In this association of eco-evolutionary pathways, if clonality is considered to be absent in the co-sexual ancestors, the causes and consequences of dioecy largely remains the same

of airborne pollen amidst the possible incidence of pollination by insects as well (Freeman et al. 1981; Niklas 1985; e.g. *Siparuna*, Renner and Won 2001; *Schiedea*, Golonka et al. 2005). Another reason for the condensed inflorescences to become a strong correlate of dioecy could be in the context of inbreeding depression caused by greater possibility of geitonogamous selfing within the inflorescence in a bisexual system (Vallejo-Marín et al. 2010).

Dioecious taxa usually produce dry-type and small pollen which keeps the pollen airborne due to low settling velocity (Whitehead 1969; Niklas 1985). Because of high pollen and low ovule production, these taxa usually have high pollen-to-ovule ratio. According to Charnov (1982), high pollen production ensures ovule

Table 9.2 List of terms to define gender and sexuality. (Adapted and modified from Sakai and Weller 1999)

Term for gender	Definition	Examples
<i>Monomorphic populations</i>		
Hermaphrodite	Flowers with both male and female organs.	90% of flowering plants
monoecious	Flowers with separate sexes on the same individuals.	<i>Zea mays, Humulus, Ecballium, Myrica, Mercurialis</i> , etc.
Gynomonoecious	Individuals have female and hermaphrodite flowers.	<i>Daisies, Cirsium (Asteraceae)</i> , etc.
Andromonoecious	Individuals have male and hermaphrodite flowers.	<i>Aralia, Gingidia, Lignocarpa, Olea europaea, Passiflora incarnata</i> , etc.
Dioecious	Individuals with separate sex (i.e. male and females)	<i>Silene latifolia</i> , etc.
Gynodioecious	Individuals either female or hermaphrodite	<i>Plantago lanceolata, Daphne laureola, Leucopogon melaleuroides</i> , etc.
Andro dioecious	Individuals either male or hermaphrodite.	<i>Datisca glomerata, Ulmus minor</i> , etc.
<i>Polymorphic populations</i>		
Polygamomonoecious	Populations of plants with staminate, pistillate and perfect flowers on the same plant.	<i>Hippophae rhamnoides</i>
Trioecious/ subdioecious	Hermaphrodite, staminate and pistillate plants.	<i>Wurmbea dioica, Schleichera oleosa, Consolea spinosissima</i> , etc.

fertilization and maximizes reproductive success. Generally, the pistillate flowers of dioecious taxa are uniovulate, which also can be viewed as a mechanism to enhance reproductive success. In multi-ovulate systems, many ovules might remain unfertilized due to low/insufficient pollen load resulting in low reproductive success. Thus, the production of numerous uniovulate flowers, distributed over an inflorescence, would enhance pollen capture and lower the overall cost (Friedman and Barrett 2011).

Floral features of genders may also vary depending on the overall combination in a sexual system; for example in *Sagittaria latifolia*, monoecious populations have significantly smaller flowers than the dioecious populations (Yakimowski et al. 2011). In certain species, sexual dimorphism can be observed between the male and female flowers, which can have fitness consequences. For example, large and bright yellow perianth with orange anthers in males of subdioecious *Thymelaea hirsute* promotes pollinator attraction for outcrossing (Caporali et al. 2006). However, evolution of sexual dimorphism may put dioecious plants (especially exhibiting insect pollination) at higher risk of extinction under pollinator-limited environment (Vamosi and Otto 2002). This in turn explains the existence of inconspicuous flowers and wind pollination in most of the dioecious species.

9.3 Dioecy: Distribution, Habitat and Growth Form

Ecological suites are habitat dependent. In the context of evolution and maintenance of dioecy; habitat (dry/moist) or geography (tropical/temperate/island) plays an important role. The essential processes, for example, wind pollination, rely on ecological factors like low humidity, open area and sparse vegetation. In several studies, a positive correlation has been noted between dioecy and habitat or the geography, primarily in terms of growth form (habit), some life-history traits and resource availability (Vamosi et al. 2003; Renner 2014). Among certain dioecious lineages, dioecy have shown a significant correlation with woodiness (84%); tropical distribution (69%) and fleshy fruits (56%) (Vamosi and Vamosi 2004). However, counter-evidences are also beginning to appear against some of the correlates from recent studies.

Geographically, dioecious flora is predominant in the tropics (Bawa 1980; Renner and Ricklefs 1995; Vamosi et al. 2003). It has been shown that tropical dioecious clades are species rich in comparison to the temperate ones. One likely cause for such a distribution could be the gradually changing climatic conditions over the geological time scale (tropical climate, during Eocene era). Available fossil evidences and geological studies indicate that change in climatic conditions led to the formation of temperate zones and retraction of tropical parts. Consequently, the dioecious plants became less prevalent from the temperate regions due to lower survivorship and vulnerability in the depauperate pollinator environment (Vamosi and Otto 2002). The other reasons for the association were highlighted in the light of life-history traits such as fleshy fruits, and insect-pollination. Bird-assisted long-distance fruits dispersal enabled the migration of dioecious floras towards tropical climes (more favourable regimes). Such migrations are thought to be successful in long-distance colonization in the tropical conditions of Hawaiian flora (Sakai et al. 1995; Vamosi et al. 2003). Similarly, pollination by generalized pollinators is frequent among the tropical dioecious flora rather than wind, which may not be a favourable trait under moist evergreen forests (Bawa 1980).

Although there are convincing evidences in favour of tropical preponderance of dioecy, their occurrence in temperate regions cannot be underestimated. The reasons for miscalculation could be due to bias of sampling from one type of geographical region (e.g. Renner and Ricklefs 1995) or clade based analysis (e.g. Vamosi et al. 2003), and also due to limited number of pertinent studies. Several examples of successful dioecious genera such as *Rumex*, *Hippophae*, *Wurmbea*, *Myrica* are available from the temperate regions and these are successful colonizers. Temperate regions provide better prerequisites of aerodynamics for anemophily than the tropical ones, and also low availability of pollinators makes wind pollination a crucial process for reproductive success. In contrast, thicker vegetation in the tropics hinders wind currents and consequently effective pollen dispersal. Moreover, temperate regions are somewhat drier than the tropical forests; drier habitats facilitate pollen dispersal and the evolution of dioecy (reviewed in Culley et al. 2002). Thus, wind pollination is likely a better alternative in such environments. Additional examples of wind-pollinated dioecious taxa growing in temperate regions, such as sea

buckthorn (Mangla and Tandon 2014), *Ulmus* (Roy et al. personal communication), etc. also refute the notion of long-distance migration of dioecious flora towards tropical habitats. In sea buckthorn, although the fruits are fleshy, birds are not involved in seed dispersal (Mangla et al. 2015).

A distributional bias of dioecious is also seen in tropical islands. Among the islands, those with moist forests bear greater proportion of dioecious taxa than the drier ones (Baker and Cox 1984). Several studies over the years, such as those carried out in Hawaii, New Zealand and New Caledonia, have demonstrated a higher incidence of dioecy in the tropical islands (Bawa 1980; Sakai et al. 1995; Schlessman et al. 2014). The findings indicate that dimorphic colonizers and in situ evolution of dioecy might be the causes for such a distribution.

Dioecy in tropical flora also correlates with life-history traits like large size, woodiness, perennial growth form, fleshy fruits, and wind pollination/generalized pollinators (Sakai et al. 1995; Fox 1985). Such a predilection can be explained on the basis that annuals are more susceptible to annual environmental and pollination fluctuations. Among the growth forms, trees exhibit higher frequency of dioecy than the herbaceous plants (Bawa 1980). Long-lived species have better chances to survive in poor seasons of pollination (Lloyd 1982; Freeman et al. 1979). Certain dioecious species tend to acquire larger size due to clonal reproduction, which in turn provides better chances of reproduction or pollination. Climbing (woody as well as herbaceous) habit has also been found to be strongly correlated with dioecy (Renner and Ricklefs 1995), but only in tropical flora. This association of dioecy with woodiness and large plant size has also been hypothesized as an obligatory need to promote outcrossing in long-lived trees, possibly with excess geitonogamous selfing.

Life-history traits can also account for varied distribution of dioecious taxa among the monocots and dicots. Dicots with more varied range of growth form exhibit higher frequency of dioecious taxa than the monocots, as the latter are mostly herbaceous. Among the dicots also, primitive species are rich in dioecious taxa as compared to advanced ones (Renner and Ricklefs 1995). This unequal distribution between dicot lineages can be explained by the union between female and male floral organs basal angiosperms (Endress 1990), while the complex floral structure in the relatively advanced taxa makes it difficult for the ontogenetic suppression of one sex.

It may be inferred that differences in habitats offer different constraints for the plants for adaptation. These constraints often lead to optimization of sexual system for the successful completion of life cycle in a particular habitat.

9.4 Dioecy and Fleshy Fruits

The common occurrence of fleshy fruits in dioecious taxa is often associated with the habitat conditions, and their successful colonization in the tropics (Bawa 1980; Givnish 1980; Vamosi et al. 2003; Vamosi and Vamosi 2004). Long-distance colonization requires efficient dispersal mechanisms to facilitate population expansion. Incidentally, seed dispersal in dioecious clades is often facilitated by animals

(Vamosi et al. 2003; Vamosi and Vamosi 2004). Fleshy fruit is one such adaptation that favours interaction of animals, particularly birds, with plants. The woody habit, tropical distribution and inconspicuous flowers also correlate with fleshy fruits. High investment in the formation of fleshy fruits is balanced by forming non-showy and reduced flowers. However, some evidences gathered show a weak association between dioecy and fleshy fruits. For example, in *Silene*, dioecy is not associated with fleshy fruits (Muyle et al. 2018). Similarly, Matallana et al. (2005) and Ohya et al. (2017) could not find significant association, as fleshy fruits were associated in the same proportion among the hermaphrodites and dioecious plants. Numerous studies have suggested that dioecious plants are at higher risk of extinction than the hermaphrodites. Although, they can escape this fate by association with various dispersal modes or life-history traits (e.g. clonality) that they possess (Käfer et al. 2016). Thus, to draw any conclusion, it may be useful to look into the diversification rates and their consequences on life-history traits of dioecious species.

9.5 Resource Allocation

Plants partition available resources between vegetative growth and reproduction to improve fitness-related components (Agren 1988; Harris and Pannell 2008). The relative and optimal allocation of resources for the formation of essential floral organs gives an idea about the trade-off between male and female functions (Charnov 1982; Delph et al. 1993). The basic conflict of balanced resource partitioning for paternal versus maternal functions seems to have played a significant role in the evolution of dioecy (Lloyd 1979; Bawa 1980; Charlesworth and Charlesworth 1981). Success of sterility mutations in hermaphrodite populations depends on appropriation of resources in favour of prevailing sexual function. Complete allocation of resources to one gender may result in their better performance, for example, higher pollen production in *male-like forms* will result in greater success of seed-siring in *female-like forms* (Charlesworth and Charlesworth 1981; Rottenberg 2000; Spigler and Ashman 2011).

Gynodioecy, monoecy and androdioecy are the three prominent pathways of evolution of dioecy (Charlesworth and Charlesworth 1979; Barrett 2002). In these pathways, the transitory stages and the combinations thereof reflect the manifestations of trade-off between sexual functions. In gynodioecious species, the two genders (females and hermaphrodites) are expected to be more similar in their reproductive allocation, as both have to produce seeds (Charlesworth and Charlesworth 1981; Schultz 2009). However, in gynodioecious *Daphne laureola* (Alonso et al. 2007), *Schiedea* sp. (Weller et al. 2007), and *Leucopogon melaleuroides* (Vaughton and Ramsey 2011), the females are reported to allocate ~1.5-fold more biomass, resulting in more fruits than the hermaphrodites. In androdioecious taxa, greater amount of resources are allocated among the hermaphrodites (functionally female, male sterile) than that in the males (source of pollen grains, high pollen fertility), as seen in *Ulmus minor* (López-Almansa et al. 2003). In monoecious pathway, the intermediate stages possess considerable amount

of quantitative variation in the allocation, and also show a range of variation in the female and male fertility (Charlesworth and Charlesworth 1978b; Spigler and Ashman 2011). For example, in *Hippophae rhamnoides*, which is shown to exhibit subdioecy, assessment of resource allocation pattern in terms of production of pollen, their fertility, viability and fruit-set, among the three gender morphs, helped in corroborating the pathway (Mangla et al. 2019). In general, these studies advocate for separate assessment of resource allocation among all the transitory phenotypes, because the cost of reproduction and trade-offs are often fixed among the pathways (Charlesworth and Charlesworth 1981).

The differential investment patterns of resources among the dioecious plants may result in dimorphism in life-history traits (like vegetative growth, plant or clone size, phenology, floral traits, sex ratio, etc.). There are several studies which have also indicated the potential role of age, biotic and abiotic factors (e.g. carbon and nitrogen, water availability, light) in sexual dimorphism (Dawson and Geber 1999; Obeso 2002; Barrett and Hough 2012). Typically, the males invest more resources for flowering (male floral display), while the females relatively do so more for fruits and seeds (Delph 2009). In animal-pollinated unisexual flowers, larger male floral displays are the result of pollinator-mediated selection and ensure pollinators visitation for better mating opportunities (Eckhart 1999). On the other hand, among the anemophilous unisexuals, greater investment in male function (profuse pollen production) increases the chances of mating. In contrast, female function is mostly limited by resources than mating opportunities, because of greater allocation to fruits and seeds during the latter part of the season (Bateman's principle; Bateman 1948; Yakimowski et al. 2011). Male plants are known to exhibit anthesis earlier than females as well as limit their daily floral display or the flowering schedules. This is a probable compromise to overcome the cost of daily and continuous flowering along with inefficient pollen dispersal/low pollinator availability (Harder and Thomson 1989; Mangla and Tandon 2014). Similarly, dimorphism among the unisexuals commonly exists for flower size; staminate flowers are usually larger than pistillate, for example, *Sagittaria latifolia*, *Ecballium elaterium*, etc. and animal-pollinated unisexuals from the temperate regions (Eckhart 1999; Yakimowski et al. 2011).

Unequal trade-off of resources among the unisexuals may also lead to clonal propagation. Typically in females, greater allocation of resources favours sexual reproduction (Lloyd and Webb 1977; Willson 1979; Agren 1988). In general, the males invest more in clonal propagation while females do so more for sexual reproduction. This allocation pattern influences the clone size, comprising bigger male ramets than those in the females, and male biased sex ratio (see Sect. 9.7). The females also allocate more biomass to photosynthetic tissue and exhibit higher photosynthesis rate (Dawson and Geber 1999; Nicotra 1999; Álvarez-Cansino et al. 2010; Vaughton and Ramsey 2011). However, females have lower growth rates and higher mortality. Greater trade-off between growth and reproduction could be expected (especially in females, for example, *Baccharis dracunculifolia*; Espírito-Santo et al. 2003), which will be more pronounced in stressful or resource-limited environment, often resulting in higher levels of sexual dimorphism (e.g. *Mercurialis annua*; Hesse and Pannell 2011).

9.6 Wind Pollination

Wind pollination is considered to be a derived condition, and a strong correlate of dioecy (Culley et al. 2002). A recent study suggests that at least 31.6% of the dioecious taxa are anemophilous (Renner 2014). This is due to certain floral suites which have evolved along with the dioecy and facilitated anemophily (Friedman and Barrett 2009). It appears that anemophily in dioecious species appeared as a consequence rather than being a cause. It is important to question why such a pollination system appeared as the preferred strategy among a high proportion of the dioecious taxa. Two hypotheses have been posed to explain this outcome – *pollinator limitation hypothesis* and *pollinator discrimination hypothesis*.

Wind pollination is believed to have evolved on several occasions in entomophilous lineages (Friedman and Barrett 2008; Culley et al. 2002). However, it is doubtful whether the transition is driven by pollinator limitation. The shift in pollination mode is more advantageous only when ecological suites adversely affect or limit pollinator availability (Whitehead 1969; Culley et al. 2002). As per the *pollinator limitation hypothesis*, it could be presumed that the pollinator limitation in a species might arise due to habitat shift or when plants colonize new areas with limited or no pollinators (Weller et al. 1998; Goodwillie 1999; Culley et al. 2002). The decline in pollinator services (number/frequency) may impose selection pressure for the alternative pollination mechanism. The decline may also occur due to transfer of low or inferior quality of pollen load. Then, the prevalence of optimum wind currents in the open habitat conditions provides a chance for adopting wind pollination. Depending on the floral traits of the species, two routes may be followed. If the population has hermaphrodite flowers, autonomous selfing would provide higher chances of reproductive assurance. Alternatively, if the population has diclinous flowers, wind pollination would enhance the chances of pollination (though limited). Thus, similar ecological conditions and selective agents could result in two contrasting evolutionary outcomes (Friedman and Barrett 2008; Culley et al. 2002).

In contrast to the above conditions, in non-windy environments the species might switch to a new biotic pollinator or selfing mechanisms (autogamy, cleistogamy, etc.); otherwise, it would become extinct (Fig. 9.1). Consequently, combination of biotic and wind pollination (*ambophily*) may appear as an alternate or intermediate transitional state (towards wind pollination), with low pollinator abundance. However, the pollinators would be of generalist category. This has been observed in several dioecious taxa which are generally pollinated by wind and unspecialized biotic pollinators (e.g. *Salix*). The considerations for ambophily as a correlate are doubtful (Culley et al. 2002), especially in the case of diclinous species, due to limited number of studies.

A closer look into *pollinator limitation hypothesis* highlights its reliance on the floral traits. So, it can be presumed that evolution of dioecy precedes anemophily. A recent analysis also established that anemophily evolved more frequently in lineages that were pre-adapted with unisexual flowers and/or unisexual plants (Friedman and Barrett 2008). Though, the sequence of events through which the correlation has

been achieved has yet not been established. A few antagonistic explanations are also apparent in literature. Based on *pollinator discrimination hypothesis*, pollinator could discriminate between the male and females based on floral rewards (Kaplan and Mulcahy 1971). The pollinator's visit to the female flower would be disadvantageous, if the rewards come only from the males in the form of pollen grains. In such cases species may show transition to anemophily, supporting precedence of dioecy over wind pollination. Similarly, evolution of anemophily has been reported in *Leucadendron* (Midgley 1987), and *Thalictrum* (Kaplan and Mulcahy 1971; Soza et al. 2012). Charlesworth (1993), however, argued this hypothesis on the basis that dioecy is mostly accomplished via monoecy or gynodioecy. Therefore, evolution of dioecy followed by anemophily may not rely on just pollinator discrimination. Reduction in the fixed costs of floral display in both genders could be another factor that permitted wind pollination followed by evolution of dioecy (Lloyd 1982; Charlesworth 1993). The argument by Charlesworth (1993) appears to be acceptable because dioecy often evolved in response to inbreeding depression emerging from wind-mediated selfing or shift to habitats with limited pollinator availability. However, pollinator limitation and recurrent wind in a habitat are not the only selective pressures involved in frequent appearance of anemophily. Ecological suites required for successful evolution of wind pollination also includes habitat condition, floristic composition, seasonal variation, low species diversity, open vegetation, high number of conspecifics, low humidity, gregarious plant growth, and dry conditions (see Sect. 9.3).

The floral traits of most of the dioecious taxa have modified along with the evolution of dioecy, and are to some extent gender dependent due to differential resource allocation pattern (see Sect. 9.5). The anemophilous syndrome of dioecious taxa includes small non-showy flowers, reduced perianth, condensed inflorescence and exerted position, enhanced and exposed stigma site and size, copious production of small and dry-type pollen grains with smooth exine surface, etc. These features are prerequisites for fulfilling the aerodynamics of pollen dispersal and subsequent capture by the stigma (Niklas 1987; Paw and Hotton 1989; Golonka et al. 2005). Some of these traits might not be crucial but undeniably have facilitated evolution of anemophily in dioecious taxa. Assessment based on phylogenetic analysis has revealed that anemophilous species are not found in lineages with large or complex flowers (Linder 1998). Another similar analysis has suggested that anemophily is mostly evolved in the clades with inconspicuous and non-showy flowers (Friedman and Barrett 2009). However, certain ambophilous or entomophilous diclinous taxa (e.g. *Salix*, *Piper*, *Tamus communis*) possess pale yellow-white flowers and are also pollinated by small generalist pollinators (e.g. bees, flies).

Many studies have highlighted the probable role of inflorescence architecture in the evolution of anemophily. The structural organization of inflorescence and flower position, directly or indirectly, influences pollen capture and stigmatic load in them (Freeman et al. 1981). Niklas (1985) has also suggested that condensation of inflorescences may promote pollen capture. For example, in *Schiedea*, more condensed inflorescences enhance pollen dispersal and pollen capture (Weller et al. 1998; Golonka et al. 2005). The exposed inflorescence and high pollen

production is advantageous for dioecious taxa. In *Salix*, *Morus* and grasses like *Carex*, inflorescence is a catkin or is pendulous where even a low wind current disperses pollen in high density and overcomes the loss of untargeted deposition. On the other hand, exposed feathery stigmas in female flowers efficiently capture the airborne pollen grains. These examples indicate that most of the floral traits of wind-pollinated dioecious taxa are needed for (pollen release and capture) aerodynamics (Whitehead 1969; Regal 1982; Ackerman 2000) and facilitate the evolution of wind pollination in dioecious taxa.

9.7 Clonality

Clonality is of frequent occurrence among the angiosperm lineages, and is believed to have resulted from several ecological and evolutionary advantages (Vallejo-Marín et al. 2010). However, sometimes clonality can also be detrimental to plant fitness (Barrett 2015). The consequences of vegetative reproduction vary among hermaphrodites and unisexuals (*reviewed* in Vallejo-Marín et al. 2010). The long-term consequences of clonality include change in mating strategies, inbreeding depression, pollen discounting, limited resource availability and, eventually, loss of sexual reproduction. Types of vegetative propagules (bulbils, suckers, tubers, rhizomes, etc.) and their dissemination may influence the dynamics of sex ratio and spatial genetic structure of populations. In this section, we discuss the possible consequences of clonality among unisexuals with special emphasis on *clonality* as an ecological factor driving and maintaining dioecy and *consequences of clonality* on dioecious populations.

With the evolution of dioecy, shortcomings of self-pollination and inbreeding depression are prevented (Charlesworth 1993). It can be hypothesized that clonality among the hermaphrodites may also set the stage for the evolution of dioecy (Fig. 9.1; Barrett 1984). Clonal propagation usually leads to aggregation of ramets, or the expansion of genets to larger size, and enhance the chances of selfing through geitonogamy. Darwin predicted this association between dioecy and large plant size after studying trees (Darwin 1877). A later study on seed plants found positive correlation between large plant size and dioecy (Bawa 1980). Studies among aquatic plant groups (marine and fresh) with pronounced clonal propagation have also shown prevalence of dioecy among them. Similarly, strong association between separate sexes and the ability to produce specialized asexual propagules have been revealed in mosses (Longton and Schuster 1983; During 2007). Relation between large clone size and evolution of dioecy has long been studied in many families like Apiaceae and Araliaceae (Webb 1999). Dioecious *Aralia nudicaulis* covers large areas due to extensive clonal growth and forms large patches. It had been demonstrated that due to high inbreeding, evolution of dioecy was favoured (Barrett 1984). In the aquatic plant *Sagittaria latifolia*, both the monoecious and the dioecious populations exhibit extensive clonality. It is believed that geitonogamous selfing is promoted by clonality, and consequent inbreeding depression led to the evolution of dioecy in the species (Dorken et al. 2002). Several other evidences

suggest that dioecy is the derived condition in *S. latifolia* (Dorken et al. 2002; Dorken and Barrett 2004). However, in heterostylous *Eichhornia* spp., extensive clonal propagation seems to have disruptive influence on tristyly. There are evidences which highlight the breakdown of tristyly and promotion of self-fertilization in the introduced as well as the marginal populations (Barrett 2015). However, phylogenetic studies to favour association between dioecy and clonality among angiosperms are obscure in literature.

Dioecy brings benefits of outcrossing and sire reproductively fit progeny, but at the same time uncertainties of sexual reproduction are very high in populations with separate sexes. The species have to rely on generalist pollinators or abiotic pollination and will also have to thrive under harsh ecological conditions. Events amounting to pollination failure under such conditions may also exert selection on plants to exploit other mechanisms of perpetuation, besides sexual reproduction (Eckert 2002; Allem 2003; Vallejo-Marín et al. 2010; Wilson and Harder 2003), the most common being the clonal mode. Clonal reproduction allows a genotype to persist and increase in situations where mates are scarce (see Vallejo-Marín and O'Brien 2007), making it an ecologically stable strategy. Here, *Hippophae rhamnooides* can be cited as a suitable example. This species grows in the cold, desert region of India. The species, besides producing seeds, also reproduces extensively through root suckers, forming gregarious patches of the same gender. There is agamospermy as well in the species. The three modes of reproduction together provide reproductive assurance to the species under extreme environment (Mangla et al. 2015). The vegetative mode generates the clones; the sexual mode engenders new combinations (heterogeneity) and ensures dispersal of diaspores (Allem 2003; Vallejo-Marín et al. 2010; Hörandl and Hojsgaard 2012).

The differential resource investment among the genders (Lloyd and Webb 1977; Willson 1979) led to theories proposing differences in investments towards sexual and clonal reproduction. Numerous studies have reported lower investment in clonal growth by females than that by males (Putwain and Harper 1972; Popp and Reinartz 1988; Houssard et al. 1994). For example, in *Dioscorea japonica* (Mizuki et al. 2010), *Rumex acetosella* (Putwain and Harper 1972), *Xanthoxylum americanum* (Popp and Reinartz 1988), *Tamus communis*, *Mercurialis perennis* (Munguia-Rosas et al. 2011) and *Lindera melissifolia* (Gustafson et al. 2013), female plants produce fewer propagules (ramets) than males, and the female clones are smaller than the male. The reason for lower investment in clonal growth by the females may be attributed to preferential allocation of resources to the development of showy and fleshy fruits (Vamosi et al. 2003). However, there are several factors which can lead to larger female clones (see Sect. 9.8). In *Silene latifolia*, molecular analysis established that the difference in clone size was not significant either at the genet or the ramet level (Yakimowski and Barrett 2014b).

Extended period of clonal reproduction results in expansion of more competitive genotypes (e.g. *Solidago* sp.) and suppression of sexual reproduction, thereby resulting in lower genetic diversity (Hartnett and Bazzaz 1985; Honnay and Bossuyt 2005; Barrett 2015). This pattern of fine-scale spatial genetic structuring has been observed in perennial dioecious clonal forest herbs (Vandepitte et al. 2009, 2010) as

well as woody species (Douhovnikoff et al. 2005; Premoli and Steinke 2008). Such spatial separation between sexes may also result in extinction of species or alternatively if species rely on agamospermy; dioecious plants may completely eliminate the necessity of males and eventually may result in loss of sex. Marginal populations of clonal dioecious species may show pronounced effect of spatial separation. For example, populations of *Elodea canadensis* in Europe are represented by males only (Sculthorpe 1967), and similarly *Fallopia japonica* var. *japonica* populations in the United Kingdom are represented by only females (Hollingsworth and Bailey 2000).

Another outcome of clonality in dioecious species is “sterility”, which appears to be largely influenced by ecological and genetic factors. For example, in clonal, tristylous aquatic plant *Decodon verticillatus*, the population at the northern range limits (Eastern North America) has mostly lost the ability to reproduce by sexual means, and clones are represented only by single genotype. On the other hand, populations in the southern range are maintaining tristly, produce enormous number of seeds and are genetically diverse. Glasshouse studies have shown more winter survival of sterile clones than fertile clones from the northern range (Eckert 2002). This study suggests temperature as a limiting factor for sexual reproduction in northern range limits of the species helped by clonal propagation. Similarly, in a dioecious tree species *Populus tremuloides*, high level of male sterility was observed in older clones than younger clones (Namroud et al. 2005).

It is well known that the dioecy is an outcome of selection and fixation of favourable alleles in hermaphrodite populations (Charlesworth 2016). Prevalence of clonality among dioecious taxa may be regarded as an avoidance of the breakup of favourable gene combinations (Hojsgaard et al. 2013). However, other genetic factors such as accumulation of deleterious mutations are equally important for limiting sexual reproduction in clonal populations (Eckert 2002; Vallejo-Marín et al. 2010). In this context, clonal dioecious populations can reach an evolutionary dead end, with limited possibility of diversification.

9.8 Sex Ratio: A Correlate for Maintenance of Dioecy

Once established in the population, maintenance of the gender dimorphism is evolutionarily crucial for effective gene dispersal and fixation (Charlesworth and Charlesworth 1979; Geber et al. 1999). Thus in this context, the relative proportion of genders (secondary sex ratio) is an indication of the evolutionary, ecological and genetic stability for diclinous species (Barrett et al. 2010; Sinclair et al. 2012). Dioecious species with genetic determination mechanism produce two types of seeds, that is, males and females, and their relative number represents the primary sex ratio. However, only a few seeds of the lot produced are able to germinate and establish as a mature individual, due to mortality at different stages of growth. Such a situation leads to variation from the hypothetical 1:1 for male and female in nature (Barrett et al. 2010; Field et al. 2012; Pickup and Barrett 2013).

There are numerous studies which have reported sex ratio in various dioecious species along with variations thereof. It has been proposed that if the sex ratio

deviates from 1:1, there are chances of breakdown of dioecy (Crossman and Charlesworth 2013) or it may lead to local extinction of the species (Heilbuth 2000). These studies have highlighted that balanced or skewed sex ratio could be associated with abiotic and biotic factors such as pollination, growth form, clonality, cost of reproduction, resource availability, etc. Sex ratio could also be influenced by various other factors like difference in the competitive ability of the genders, mortality, spatial segregation of sexes (resource rich or poor areas) and age/size (Field et al. 2013). Thus, sex ratio is an important consequence of dioecy and the underlying ecological factors that govern it. In the evolutionary context, sex ratio appears as an essential correlate of dioecy and seems crucial for its maintenance. There are several ecological factors which are obligatory to maintain a population with a viable sex ratio.

In the clonal dioecious species, it is difficult to identify and segregate and identify individual plants due to gregarious growth. Therefore, mostly, the data on sex ratio of clonal dioecious species is generated by computing the flowering stems, rather than the frequency of the plants. Moreover, difference in age of reproductive maturity of the genders also influences the ratio. Such estimates may give an over-representation of one gender and suggest a bias in populations/species. For example, in one of the phylogenetic analyses, female-biased sex ratio was also found associated with clonality, especially in herbaceous species. However, in the same study, estimation of sex ratio of 243 angiosperm species belonging to 61 families revealed that male-biased sex ratios were twice as common as female-biased sex ratios. This analysis suggests that male-biased populations are frequent among dioecious taxa and favoured due to genetic and ecological factors (Field et al. 2013).

Many a times, differential clonal growth among genders can also generate a bias, for example, *Aralia nudicaulis* forms extensive clones by rhizome system and exhibit male-biased sex ratio (Barrett and Hellenurum 1981). Similarly, in *Dioscorea japonica* (Mizuki et al. 2010); *Rumex acetosella* (Putwain and Harper 1972), *Xanthoxylum americanum* (Popp and Reinartz 1988) and *Mercurialis perennis* (Vandepitte et al. 2009), female plants produce fewer daughter propagules (ramets) than males. Such condition leads to male-biased sex ratio at certain stages of life-history.

The association between clonality and sex-biasness may be resource or habitat dependent, as observed in *Hippophae rhamnoides*. This species showed female-biased sex ratio among the natural populations located in western Himalayas of India (*personal observation*), while those growing at the coastal sand dunes of Meijendal (The Netherlands) are reported to be male-biased (de Jong and Van der Meijden 2004). It is reasoned that because the females allocate more resources to reproduction, their survival rates become lower than the males under stressful xeric habitats (Waser 1984; Geber et al. 1999). According to Field et al. (2013), male-biased ratio tends to increase with latitude and altitude. *Sagittaria latifolia*, a clonal dioecious species occurs in a variety of habitats in the northern part of America, and the sex ratio ranges from male to female biased (Yakimowski and Barrett 2014a, b). In *Lindera melissifolia*, of the 11 populations studied in total, nine had biased sex ratios, with six male and three female-biased sites (Gustafson et al. 2013). In

Vallisineria americana, a strong male-biased sex ratio was observed in shallow water, while sex ratios gradually became female-biased, at greater water depths (Lokker et al. 1994).

Evolutionary status of dioecious species may influence, and often lead to male-biased sex ratio. *Fuchsia mirophylla* and *F. thymifolia* exhibit two sexual systems, gynodioecy and dioecy, respectively. Almost 90% of the hermaphrodites in gynodioecious populations are functionally males, and the ratio of females to functional males is nearly 1:1. However, some dioecious populations are male-biased (Arroyo and Raven 1975). For example *Mussaenda pubescens* (Li et al. 2010) and *Nothapodytes nimmoniana* (Sharma Manju et al. 2010) show cryptic dioecy (morphologically hermaphrodite but functionally dioecious) and heterostyly. The long-styled morphs are functionally females and the short-styled are functionally males. In *M. pubescens*, a male-biased sex ratio is evident with a greater number of small morph being present in the populations investigated (Li et al. 2010).

The reasons for male-biased sex ratio can be explained in terms of low resource allocation and biomass invested towards the reproduction, early reproductive maturity (flowering), increased growth, high survival ability and longevity in males than in females (Fig. 9.1; Lloyd and Webb 1977; Charnov 1982; Armstrong and Irvine 1989). In *Oemleria cerasiformis*, male-biased sex ratio was observed due to differential mortality rate (Allen and Antos 1993). High mortality in females at different development stages suggests that it could be because of more investment in reproduction by females and thus less survivorship. Additionally, females grow slower than the males, and thereby get overtapped by other woody species that decrease its probability to survive. *Myrica esculenta*, which is also known to drift from 1:1 (equilibrium) ratio, supports the occurrence of skewed and male-biased sex ratio (Khanduri et al. 2019).

Freeman et al. (1976) reported the presence of more males in five dioecious species from northern Utah, growing on xeric microsites, while the moist microsites represented more females. Similarly, in *Aralia nudicaulis* forest population's male-biased sex ratio is attributed to limited resources (generally light) causing difference in survivorship and growth of sexes. The requisite high resource investment in reproduction by females leads to decreased surviving ability and limited growth as compared to males (Barrett and Hellenurum 1981). Vandepitte et al. (2010) found that large populations of the dioecious perennial *Mercurialis perennis* in shaded forest sites were more likely to maintain balanced sex ratios while in the high light environment, male-dominated populations were found. In a study involving dioecious perennial shrub *Pistacia chinensis* (Yu and Lu 2011), there was an increase in male-biased populations on nutrient-poor soils. There was also a decrease in effective population size, and reduced connectivity among populations.

Female-biased sex ratios are found to be less common among dioecious angiosperms (Delph 1999; Barrett et al. 2010), and limited mechanisms have been proposed to account for this pattern. One plausible explanation can be derived in the context of sex chromosomes. For the species with sex chromosomes, primary population must be at equilibrium with a ratio of 1:1, but this may not be always true. According to Lloyd (1974) stronger female bias sex ratio are more likely to be

expected in species with heteromorphic sex chromosomes. This relation between gender bias and chromosome differentiation can also be explained on the basis of differential performance of pollen grains (microgametophytes) determining 'male' and 'female' (*the certation hypothesis*; Correns 1922). In XY system, Y chromosome (male determining) shows accumulation of deleterious mutation followed by degeneration (Ming et al. 2011). As a consequence of Y chromosome degeneration, Y bearing pollen showed poorer competitive ability than X bearing pollen. X bearing pollen are able to outcompete those with Y during pollen tube growth, thereby eventually leading to a female biased progeny ratio (Smith 1963; Lloyd 1974; Charlesworth 2002; Stehlík and Barrett 2005; Delph 2019). Studies on different species of *Silene*, *Rumex hastatulus*, *R. nivalis* etc. with heteromorphic sex chromosomes demonstrated that large pollen load on stigma, leads to more pollen competition and thus results in female biased sex ratio (Conn and Blum 1981; Stehlík and Barrett 2005; Delph 2019).

Sex-specific differences in mortality may also contribute to female biasness (Lloyd 1974; Field et al. 2012). *Salix sachalinensis* exhibits varying female-biased ratio at different developmental stages. Population with plants at younger stage exhibits male-biased ratio and become female-biased at the older stages. This change in sex ratio with age is because of difference in timing of maturation of both the sexes. The males often mature early (precocious reproduction) thus exhibiting male-biased ratio at younger age while the number of female plants increases at later stages (Ueno et al. 2007). *Rumex acetosella* is another example which shows a marked age-dependent variation in population sex ratio. Agamospermy is also a mechanism observed in certain dioecious plants and proposed to cause female biasness, as in *H. rhamnoides* (Mangla et al. 2015). Such examples are intriguing and in turn highlight the importance of knowledge of the age and past history of population for correctly interpreting the sex ratios of a species and its populations.

The female sex-ratio biasness may also vary with changing ecological conditions. For example, in *Rumex nivalis*, females attain their highest frequencies at lower elevation sites while males are more frequent at higher elevations. Another reason implicated was higher mortality of the male than the female plants, particularly under low nutrient conditions. Apart from that, demographic aspects of the local mating environment may influence progeny sex ratios, for example, in *Rumex* sp. females closer to males captured more pollen (high pollination intensity) and produced more female-biased sex ratios than more isolated females (Stehlík et al. 2008). *Acer negundo* is a common dioecious tree species of mid-elevation riparian zones throughout the western United States. As with many other dioecious species, the ratio of male to female individuals in the species, along stream margins in the Intermountain West, where soil water and other resources are abundant, is about 0.67, while the male-to-female ratio on the drier margins of riparian ecosystems (in this case, >1 m from stream channel) is about 1.61 (Ward et al. 2002).

There is no single factor that solely affects sex ratio in dioecious plants. The ecological, environmental, genetic factors more or less equally contribute to sexual biasness. However, differential cost of reproduction in both sexes seems to be the major cause of unequal sex ratio prevailing in dioecious species. One important

condition that draws attention is the cases of male-biased populations/species. It is yet to be shown whether such populations/species are moving towards extinction due to limited availability of mates.

9.9 Conclusion

Dioecious systems are better explored in the context of prevailing genetic mechanisms. However, little has been done to combine the information with ecological aspects and their role in evolution of dioecy. Though various theories have attempted to explain the associations between habitat conditions and dioecy, these theories need to be rigorously tested with suitable taxa and plant systems. With the above account, it may be surmised that various ecological factors are working in unison for shaping dioecy in plants. There are several theories which have attempted to define the association and their possible evolutionary relationships. However, these theories need more investigations on dioecious taxa distributed in different parts of the world occupying different ecological niches. Hypotheses based on just a few representatives might fail to test the correlation (e.g. fleshy fruits), and to provide conclusive evidences. It would also be interesting to look at dioecy in relation to the changing climatic regimes and understand the response of the species at regional and macro levels.

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Secondary Pollen Presentation in Flowering Plants 10

Chandan Barman, Vineet Kumar Singh, and Mrinalini Kakkar

Abstract

Successful pollen transfer among the compatible conspecifics is an essential attribute of sexual reproduction among flowering plants. The plants maximize their male fitness by improving the efficiency of pollen dispersal to as many conspecifics as possible. The precision with which pollen is carried by biotic vectors is also influenced by the manner in which the pollen is presented by the flowers. The method of presenting the pollen to the vectors can be either from the anthers directly (primary presentation) or that from the other floral organs (secondary presentation). The significance of these methods mainly lies in the targeted deposition of pollen for successful mating. Here, we focus on the structural and mechanistic diversity of secondary pollen presentation among angiosperms. The knowledge of these floral attributes is important to understand the intricacy of reproductive mechanisms that are integral to the selection for establishing successful plant-pollinator interaction and maximizing plant fitness.

Keywords

Floral morphology · Floral rewards · Floral specialization · Pollination · Pollen dispersion · Pollen presenter · Non-sexual organ presenter

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10.1 Introduction

Systematic studies on the functional morphology of flowers and pollination were initiated largely with the pioneering work by Sprengel and Darwin (Ladd 1994). These studies have continued to fascinate biologists for long because they have been fundamental to our present understanding of sexual reproduction and evolution in flowering plants (Tandon and Mohan Ram 2010). One of the significant aspects of sexual reproduction among flowering plants is the successful transfer of the pollen grains from anthers to the receptive stigma of the same or preferably the other flowers (Shivanna and Tandon 2014). Factors influencing the efficiency of pollen transfer largely act as selective forces in the evolution of floral structures in angiosperms. It is widely concluded that this selectively decreases the contact of stigma with that of plant's own pollen (Richardson and Stephenson 1991). A number of structural and mechanistic adaptations are known to minimize this inter-sexual conflict caused due to reduced herkogamy, and to ensure outcrossing. These adaptations include physical separation of male and female flowers within the inflorescence, temporal separation of sexual phases during anthesis, physical guides exercising passive control over the movement of a pollinator, parts exercising active control over the orientation of a pollinator towards the pollen and the stigma, and incompatibility barriers. Failure of such strategies, if happens, may result in the elimination of unwanted crosses by abortion of ovules or juvenile seeds through postzygotic selection (Howell et al. 1993).

Almost, in all angiosperms, pollen grains are produced in the anthers, and subsequently release them through dehiscence of anthers. Pacini (2000) described various possibilities that may occur during the release of pollen grains from the anther, which are summarized below:

- (a) As in gymnosperms and plants with male inflorescence in aments such as Betulaceae, the anther opens completely and the pollen, not held back by pollenkitt, is released at once.
- (b) The pollen grains are released by the movement of the anther (*Ricinus communis*) or filament (*Parietaria judaica*) (Bianchini and Pacini 1996).
- (c) Pollen grains are held in the anther by pollenkitt, tryphine, viscin threads, or other mechanisms or the anthers open slowly and gradually exposing the pollen grains to the insects; this is known as *primary pollen presentation* and of common occurrence in angiosperms (Hesse et al. 2000).
- (d) The pollen grains are presented in other parts of the flowers from anthers, for their transport through various dispersing agents. This is known as *secondary pollen presentation* (SPP) and the part of the flower onto which the pollen is loaded is known as *pollen presenter*. Thus, SPP is the developmental relocating of the dispersed pollen grains from the anthers onto another floral organ such as non-fertile components of androecium or gynoecium or onto the perianth/floral bracts, which then act as the pollen-presenting organ to the pollinators.

Guthrie and Salter (1950) coined the term *pollen presenter* with respect to the family Proteaceae. Pollen presenters can be divided into two functional groups based on the loading of pollen grains onto the presenter: *Active pollen presenter* and *Passive pollen presenter*. In the active types, the presenter is a modification of the style. Growth of the style pushes the presenter to collect the pollen grains from the anthers by piston mechanism and later the pollen grains are extruded for presentation. The active presenters are frequent among the species of Lobeliaceae, Goodeniaceae, Brunoniaceae, Calyceraceae, and Asteraceae.

In passive types, on the other hand, pollen is deposited onto the modified part of the style by anthers in a floral bud. Pollen adhesion to the presenter can be aided by sticky material of pollen grains, lipidic secretion from the anthers, or by the presence of hair on the presenter. Members of Ericaceae, Fabaceae, Proteaceae, Myrtaceae, Polygalaceae, Campanulaceae, Rubiaceae, Cannaceae, Marantaceae and *Cyphia* of Lobeliaceae have passive type of presenter.

10.2 Mechanisms of Secondary Pollen Presentation (SPP)

Secondary pollen presentation was first described by Sprengel in 1793. SPP, according to Faegri and van der Pijl (1979), is defined as “the location of pollen on floral organs other than anthers, for biotic vectors”. Thus, a mandatory intervention of biotic vector becomes a prerequisite to effect successful pollination among species with SPP system. So far, there is no report where vegetative part of a plant acts as a presenter. However, pollen grains of wind-pollinated taxa, where incidental loading of pollen grains onto the vegetative parts may occur and remain ineffective in reproduction, have been reported in some taxa. Twelve different types of SPP systems have been identified in angiosperm families. These can be briefly explained under two broad categories namely, *Non-sexual Organ Presentation* and *Sexual Organ Presentation*. This classification is based on floral parts that play a role in the pollen presentation.

10.2.1 Non-sexual Organ Presentation

It may occur via enveloped perianth or exposed bracts in three different ways.

(a) *Type 1: Pollen presentation inside a bloom*

In this type, pollen grains are presented inside a modified extrafloral structure, which provides protection from the external environment. The mechanism is commonly observed in Areceae and many aroids, where the pollen grains fall from the spadix and get deposited over the base of the spathe. Galil and Meiri (1992) described a unique phenomenon in *Arisarum vulgare*, where the fully hydrated pollen grains are presented at the base of the spathe closer to the female receptive part. However, the hydrated pollen grains within the chamber cannot germinate. Germination only happens when they are removed from the chamber

and dehydrated into the atmosphere. This hydrated state of the pollen grains prevents autonomous selfing in these species. *Arisarum vulgare* is a fly-pollinated species (Faegri and van der Pijl 1979), and it enhances the chance of cross-pollination though it requires a vector for the removal of pollen grains to facilitate germination.

(b) *Type 2: Exposed pollen presentation on Perianth*

Here, the pollen grains are presented exposed (structural protection is not provided) onto the hairs present on the perianth lobes. It is observed in the case of *Acrotriche serrulata* (Ericaceae). Flowers are small, pale-green, emit strong odour and produce copious amount of nectar. The flowers are pollinated by diurnal lizards and nocturnal mammals (Johnson et al. 2011). The petal lobes are situated away from the anthers and the loading of pollen is achieved during anthesis. As the perianth lobes reflex out during anthesis, the hairs take away pollen from the anthers by forming a comb-like structure. Adhesion of pollen to the comb is achieved with the help of viscous pollen coat substances. In *A. serrulata*, female phase starts only after the removal of petal lobes by floral visitor (*Antechinus* sp., a small marsupial mammal) during its foraging (McConchie et al. 1986).

(c) *Type 3: Petal presenters with exposed pollen presentation*

This type of SPP has also been reported in *Sechium talamancensis* (Cucurbitaceae), where pollen grains get adhered to the glandular hairs on the distal end of the petals due to anther dehiscence prior to anthesis (Jadeja 2015). Also, the outward movement of petals during anthesis may facilitate attachment of pollen to the petals. SPP mechanism of this type is uncommon among angiosperms. Howell et al. (1993) placed this type under the category of *perianth presenters with exposed pollen presentation*. Direct exposure of pollen grains to the external environment may reduce pollen viability with the passage of time. However, even a single pollination event results in adequate seed-set in the species.

10.2.2 Sexual-Organ Presentation

10.2.2.1 Androecial Presenters

In this SSP system, pollen presentation takes place on the non-fertile part of androecium. The advantage of this mechanism is that the duration of pollen availability is increased, which would have otherwise been lost, as during normal anther dehiscence. This mechanism, however, is less beneficial for reproduction in shifting the pollen grains within the androecium. The following three different types of androecial presenters can be observed in different families.

(a) *Type 4: Pollen grains presented on hairs at the base of the anther filament*

It is observed in *Santalum acuminatum*, a member of Santalaceae (Sedgley 1982). The hairs present at the base of stamens capture the pollen grains from the dehisced anthers and present them. Flowers in this species are self-compatible and require a vector for successful pollination. Compared to normal anther

presentation, the presence of staminal hairs near the anthers, and away from stigma, results in a slight benefit to this species. These types of staminal hairs are also observed in other *Santalum* species. Other types of androecial presenters may exist in nature, where pollen is presented from the filament or staminodes.

- (b) *Type 5: Pollen grains directly deposited on the labellum-staminode for presentation*

Fan et al. (2015) reported such an SPP in *Zingiber densissimum* (Zingiberaceae), where the sole fertile anther is present close to the structure consisted of the labellum fused with the staminode. During anther dehiscence, pollen grains are directly deposited on the structure for presentation.

- (c) *Type 6: Pollen grains directly deposited on anther hairs arising from the staminal slit*

A novel type of androecial presentation is observed in *Brandisia hancei* (Lamiales), where the hairs on the entire abaxial surface of a dehisced anther (including the longitudinal furrow and aperture) serve as the presenter organ (Ren et al. 2018). Under natural conditions, the flowers of *B. hancei* are horizontally oriented, with four anthers facing downward and show approach herkogamy. The anthers dehisce longitudinally and pollen is passively shifted to the anther hairs from pollen sac, which ultimately serve as structures for secondary pollen presentation.

10.2.2.2 Gynoecial Presenters

In secondary pollen presentation system, gynoecial presenters are the most diverse and widespread. Gynoecial presenters have been sub-categorized into six different types, based on pollen location and the stigmatic position of pistil. All systems with gynoecial presenters either exhibit protandry or pollen dispersal takes place simultaneously with the onset of stigma receptivity in them.

- (a) *Type 7: Terminal stylar presenters with passive pollen placement and concealed stigmas*

In this type, the terminal part of a modified style acts as a presenter. The receptive stigmatic surface in the beginning remains concealed between appressed stigmatic lobes or alternatively in a distal depression (Collins and Rebelo 1987; Nilsson et al. 1990; Sedgley 1992). Loading of pollen onto the presenting organ takes place passively when the flower is unopened. Anther dehiscence leads to loading of pollen directly onto the terminal presenting region of the style (Carolin 1961; Johnson and Briggs 1983; Nilsson et al. 1990). It observed in Rubiaceae (Verdcourt 1958) and Proteaceae (Johnson and Briggs 1975; Collins and Rebelo 1987; Mabberley 1987).

In Rubiaceae, pollen grains are deposited and the outer surface of the appressed stigmatic lobes act as a presenter. The style is not distinguished into a terminal and sub-terminal region. The style may show no obvious modifications, or it can be of a more elaborate shape with increased surface to hold the pollen mass. SPP of this type has been termed as *Ixoroid type* (Nilsson et al. 1990). Ixoroid pollen presentation is similar to the terminal stylar

presentation as found in Asteraceae. However, loading mechanism is passive and it occurs before anthesis in Asteraceae.

Three different types of presenters have been reported in Rubiaceae (Ladd and Donaldson 1993). In the first case, the style becomes narrower towards the tip having no modification, such as hairs or swelling, where pollen will be loaded. After pollen loading, the presenter divides into two arms which later on recurve to display the receptive stigma (*Ixora*, *Alberta*). The second type is marked by the presence of smooth or short hairs with fusiform shape. The stigmatic tissue emerges out of the grooves along the margins of the presenter (e.g. *Gardenia*, *Pavetta*). However, in the third case, a barrel-shaped presenter is located at the top of the style with a terminal stigma. The stigma may emerge after sometime or may appear initially but it becomes receptive only after anthesis. The surface of the presenter may be ribbed or papillate (e.g. *Cephalanthus*, *Pachystigma*, *Fabogia*).

In Proteaceae, the upper part of the style is modified into pollen presenter and is either a swelling or provision of hairs, or an outgrowth of sterile tissue of stigma. The presenter encloses the stigma and is accessed through a small aperture, or it grows out after anthesis. The presenters are exposed only when the perianth collapses during anthesis. In a number of taxa (e.g. *Banksia*, *Dryandra*, *Leucospermum*), the extension growth of style creates a tension, which leads to the initial release from the lower part of the perianth, while the still fused perianth lobes enclose the presenter. The tepal lobes release the presenter by springing it away from the floral axis after anthesis. The simplest presenter forms comprise a slightly swollen style end with a small apical slit which gives access to the enclosed stigma. Some of the presenters in this family are sticky in nature (Yeo 1993). Proteaceae members are known to exhibit the greatest morphological diversity of pollen presenter in angiosperms.

(b) *Type 8: Terminal stylar presenters with passive pollen placement and sub-terminal stigmas*

In this SPP system, a spoon-like receptacle at the distal end of the style forms a presenter, with a sub-apical receptive part of stigma. Pollen loading on the presenter is achieved due to close proximity of the androecium with gynoecium, pollen gets loaded directly into the receptacle before anthesis. SPP of this type occurs frequently in Marantaceae and Polygalaceae (Hickey and King 1981; Mabberley 1987).

In Marantaceae, pollen presentation is mediated by an explosive stylar movement (Classen-Bockhoff 1991; Locatelli et al. 2004). The flowers are bisexual, asymmetric and showy, which usually attract pollinators through staminodes instead of petals. Prior to anthesis, the pollen is deposited on the pollen plate, present at the back of the style head (adjacent to stigma cavity). In a freshly opened flower, access to the nectar is restricted by style and the staminodes and the mouth parts of a pollinator are forced to touch the triggering appendage. This mechanical stimulus is then transferred to the style via a basal plate, which triggers the style to spring forward due to the mechanical tension in the hooded staminode. This way foreign pollen is scrapped from the mouth of the pollinator

to the stigmatic cavity, and self-pollen is loaded on the mouth parts of insects from the stylar head (Classen-Bockhoff and Heller 2008).

Floral structure of Polygalaceae indicates that the presenter is enclosed in a keeled corolla, a structure similar to that observed in a pea-flower (Hickey and King 1981; Toelken 1983, Mabberley 1987). Keeled flowers perform two functions – one is pollination, and the other is the extension of the male phase by providing protection to the pollen source.

In Apocynaceae, the stylar head is either divided into three regions (an upper, sterile region, a median lateral region, and a lower receptive region), as reported in *Rauvolfia* (Koch et al. 2002); or into two regions (a sterile region at the tip, and a subcapitellate stigma below it), as reported in *Wrightia tomentosa* (Barman et al. 2018). The anthers form a dome-shaped structure enclosing the pistil and dehisce introrsely, depositing the pollen on the sterile region of the stylar head. Thus, the presenter in this family remains unexposed within the anther dome. To access the concealed nectar, the pollinators insert its mouth part through the gap between two adjacent anthers and thus, pollen grains are transferred from one flower to another. SPP in Apocynaceae is associated with herkogamy, which indicates a specialized pollination mechanism in this group (Yeo 1993; Albers and van der Maesen 1994). Pollination in this group of plants, either self or cross, is completely impossible without the aid of a vector.

(c) *Type 9: Terminal stylar presenters with active pollen placement*

Here, the terminal part of a modified style forms a presenter. As the style emerges through a connate ring of anthers, pollen grains are actively loaded into the distal portion of the style. Auto-pollination in type is prevented by closely appressed stigmatic lobes. This SPP is a characteristic feature of Lobeliaceae, Asteraceae, and Calyceraceae (Wernham 1912, Hickey and King 1981; Toelken 1983; Richards 1986; Mabberley 1987).

In the entire Asteraceae, the style is involved in SPP. In a floral bud, the anthers dehisce introrsely to release the pollen grains into the anther tube. As the style elongates within this anther tube, it pushes (*pump mechanism*) or brushes (*brushing mechanism*) the pollen out of the anther tube, mostly by stylar hairs, also termed sweeping hairs. Ladd and Donaldson (1993) listed four forms of presenters in Asteraceae: (1) the presenter located at style apex, cone-shaped and thickened (with or without hairs), e.g. *Arctotis*, *Heterolepis*, etc.; (2) the presenter located at the style apex, cone-shaped but non-thickened (with or without hairs), e.g. *Felicia*; (3) the presenters located on the upper part of the style tapering at the tip, covered with antrorse hairs, e.g. *Corymbium*, *Lactuceae*; and (4) the presenter is truncate, composed of the tissue covering the stigma which is capped by a semicircle blunt hairs at the apex of each arm (e.g. *Senecio*, *Athanasia*). Most of the Asteraceae members are protandrous but some appear to be protogynous (Lactucoideae). In the latter type, the tip of the stigmas is exposed first and then the pollen is carried out of the anther tube by elongation of the style. In the self-compatible members of Asteraceae, outcrossing is promoted by spatial separation of the male and female flowers within the capitula. Moreover, the adaption conferred by the asymmetry among flowers is also

believed to contribute to the sophisticated pollination mechanism in this group of plants (Leppik 1977).

Pollen presenter in Lobeliaceae is characterized by the presence of a small tuft/ring of hairs near the tip of the style, above which a small amount of sterile tissue covers the receptive stigma. The hairs are much more elongated and robust as compared to hairs found in Campanulaceae. The tip of the hairs may be rounded or acute in some species, whereas in others, it may be corrugated. The function of presenter happens when elongation of style through the staminal tube drives the released pollen to come out of the tube. Flowers are protandrous in Lobeliaceae. The style pushes the presenter out of the staminal tube and the presenter lobes reflexed to expose the papillate stigma in this group. *Cyphia* is an exception where the anther tube is lacking. The presenter is a bulbous swelling covering the stigma with short hairs on it. Pollen grains are shed onto the presenter passively and remain within the throat of corolla. After anthesis, the stigma may protrude through the end of the presenter or to the sides below the tip. Thus, it forms a papillate mound or remains in the middle of the presenter as a sticky depression.

In Calyceraceae, the mechanism of the pollen presenter is quite similar to that of Lobeliaceae, and acts in the same way by the elongation of the style pushing the pollen grains out of the anther tube.

(d) *Type 10: Sub-terminal stylar presenters*

In this type of system, pollen grains are presented along the modified style at a distance from the terminal stigmatic zone. The pollen grains are passively deposited onto the presenter due to the close association of the anthers and the style at the bud stage. SPP of this type has been reported in Campanulaceae, Cannaceae, Fabaceae and Myrtaceae. However, they show differences in their presentation mechanism due to variability in floral morphology. Flowers of Campanulaceae and Myrtaceae are actinomorphic while those of Cannaceae and Fabaceae are zygomorphic.

In Campanulaceae, pollen is presented on the middle and upper part of the style, behind the stigma. The presenter is quite uniform in most of the genera of this family. Presence of unicellular, often retractile hairs, a unique feature of the Campanulaceae presenters, supports the pollen before it is removed. The hairs on the presenter in a flower bud are hyaline and turgid. The pollen is shed before anther dehiscence. In the buds of *Campanula*, the anthers are dehisced introrsely and the grains are deposited on the middle or upper part of the style, which is densely covered by pollen-collecting hairs. The style elongates only after anthesis and pollen is exposed to pollinators (D'Antraccoli et al. 2019). The same mechanism of SPP has also been reported in *Phyteuma*.

The Campanulaceae is dominated by entomophilous flowers (Faegri and van der pijl 1979; Lloyd and Yates 1982). Absence of floral visitors, spontaneous stylar hair retraction and backward curling of the stigmatic lobe as reported in *Campunla*, and could facilitate selfing due to overlap of sexual phases (Nyman 1993). This can be a strategy of reproductive assurance in self-compatible species (Lloyd 1979). Breeding system of the species can be correlated with

the retraction of the pollen-collecting hairs (Nyman 1992). In some species, the pollen-collecting hairs facilitate auto-pollination by retaining pollen until the reflexed stigmatic lobes touch the pollen, e.g. *Wahlenbergia* sp. (Smith 1992), whereas in some of the *Campanula* species pollen become nonviable by this time (Nyman 1992). The Campanulaceae-exploited SPP system is extremely flexible. With very few modifications, the system has been adapted by obligate xenogamous annuals, perennials, autogamous annuals and species which follow mixed-mating strategy (Nyman 1992).

Myrtaceae is characterized by its brush blossoms, here anthers act as the most attractive part of the flower. The five genera of *Chamelaucium* bear suballiance bracts or petals and/or sepals. These organs serve as cues for attraction and pollen is presented on a modified part of the style. The presenter consists of hairs or papillae surrounding the upper part of the style beneath the apical stigma. Papillae are found in the genus *Actinodium*, while other genera bear short and simple hairs or long and branched hairs. These presenters occur in all the species of *Homoranthus*, *Actinodium*, *Darwinia* and ~70% of *Verticordia* species. Styler elongation is considered to help maintain the pollinator fidelity, through the separation of reward (nectar) from the fertile parts and thus, encourages outcrossing.

In Cannaceae, there is no distinct demarcation of pollen presenting structure from the style except that the style is very broad to its length. Passive loading of pollen from anther takes place along the side of this broad part below the apical stigmatic region. Ladd (1994) has considered this mechanism as ‘micro-herkogamy’, as the pollen is presented just a fraction of millimetre from the stigma. As the pollinator lands on the labellum, it loads the stigma with outcross pollen grains at the time of entering the flower, and subsequently, it collects the pollen from the style while leaving.

In Fabaceae, two different modes of presentation have been reported. In the first case, pollen is deposited onto a region on the style below the stigma, either on the hairs (Lavin and Delgado 1990) or a region which is broadened section of the style (e.g. *Sphaerolobium macrantherum*). The anthers and style remain enclosed within the keel petal and are visited by suitable pollinators. Pollen is transferred from the presenter hairs to body surface the pollinator, simultaneously deposition of pollen from the animal to the stigma may also take place.

In the second type, pollen is deposited anteriorly inside the keel petal, while the upper edges of the keel are strongly appressed along the entire length, except at the apex of the acumen. When a pollinator forages the flower, then pollen is squeezed out of the acumen. There are two slightly different mechanisms by which this occurs. In some taxa (e.g. *Luflinus*) the anther filaments swell at anthesis forming the piston, which forces the pollen out of the acumen (Weberling 1989). In the other case, the stamens are arranged in two whorls with the shorter whorl fitting below the longer one as they lie in the keel. Anthers of the upper stamens dehisce first into the apex of the keel petal, while the undehisced second whorl acts as a piston to force the pollen out of the acumen, when a pollinator manipulates the keel (Kalin Arroyo 1981). The presence of

dimorphic stamen whorls is a common feature in family Fabaceae. The explosive pollination mechanism of some Fabaceae (e.g. *Medicago*) has been described as secondary pollen presentation (Brantjes and van der Pijl 1980). However, this process does not entail the placement of pollen on any secondary structure in the flower, and is best categorized as pollen release rather than secondary presentation (also supported by Yeo 1993).

(e) *Type 11: Exposed stigmatic presenters*

In this type, pollen grains are either presented directly on the receptive stigmatic surface, where they are available for cross-pollination, or it is presented onto the extension or swelling at the top of the style. This mechanism has been observed in Rubiaceae. In *Cephalanthus*, the pollen is presented on the soon-to-be receptive part of the stigma (at least 12 h before the anthesis). Individual flowers of *Cephalanthus* are arranged into compact spherical capitulate inflorescences, forming a brush blossom. In Rubiaceae, the presenters can be of various types – (1) the style gradually tapers to the tip with virtually no modification, such as hairs to show where pollen will be deposited. The presenter splits into arms which recurve to display the stigma (e.g. *Ixora*, *Alberta*); (2) the presenter has an elongation fusiform shape, and may be smooth or have short hairs. The stigmatic tissue expands out of grooves along the sides of the presenter (e.g. *Gardenia*, *Pavetta*); (3) the presenter is a barrel-shaped structure at the top of the style with an apical stigma, which appears after a period of time, or is initially visible but becomes receptive sometime after anthesis. The surface may be ribbed or papillate (e.g. *Cefhalanthus*, *Pachystigma*, *Fadogia*).

(f) *Type 12: Indusial stigmatic presenters*

Pollen is presented within a cup called indusium, present at the terminal part of the style. This type of SPP has been reported only in Goodeniaceae and the closely related Brunoniaceae. All the 11 genera in Goodeniaceae possess a cup-like pollen presenter located at the top of the style. During the late bud stage, the tissue of the apical stigmatic area starts to expand from a cup-like structure, with the stigmatic region as the floor (except in *Lechenaultia* where stigmatic region is present to the external surface of the cup). Loading of pollen takes place actively, where the cup collects the pollen grains as the style elongates. The presenter then turns right angle to the elongating style, such that the mouth of the cup (presenter) faces the abaxial petals. Such an orientation facilitates contact pollinators with that of the presenter when the pollinators probe the flower from the front. Pollen is released either by knocking the presenter by the pollinator or by the development of the stigma out of the cup. The positioning of the presenter in Goodeniaceae can be compared to the positioning of the anther tube in Lobeliaceae.

In Brunoniaceae, the presenter is similar to that cup-shaped structure found in Goodeniaceae. However, it has hyaline and expanded crescentic lobes, which lack hairs forming the upper sides of the cup. The presenter is well-exerted from the flower and it does not turn right angle to that of the style. Thus, the presenter develops vertically, and the pollen grains are not tightly contained and are easily

dislodged from the cup. At maturity, a bilobed papillate stigma develops from the cup in a way as observed in Goodeniaceae.

10.3 Functional Significance

The secondary pollen presentation is a floral mechanism of pollination adaption, achieved by an individual flower. The insights into this mechanism help to understand the basic elements involved in pollination success. The pollination success is affected by both male function (dispersal of pollen) and female function (receipt of pollen). The ultimate aim of plant is to leave more progeny and that too of better quality. The functional significance of SPP can be appreciated in terms of (a) protection and delivery systems, and (b) pollen presentation on exposed sites of a flower.

- (a) *Protection and delivery systems*: Protection and deliver system of pollen evolves simultaneously. If the delivery of sufficient amount of pollen grains on a single visit is the primary requirement, then protection of pollen (awaiting delivery) would also have to evolve accordingly. ‘Explosive deposition’ or ‘explosive cloud’ types of pollen release becomes evident when pollen is delivered on a solitary visit (Yeo 1993). These types of explosive release may not always account for SPP but there is high probability that pollen is deposited on a particular region, from where the immediate release takes place on tripping. Protection and delivery system also increases the pollination efficiency by placing the pollen at the exact position from where it may be transferred to the pollinator. Some systems may guide or manipulate pollinators to place the pollen precisely on a specific body part. ‘Explosive deposition’ type is known in several families, whereas asymmetric ‘release’ types are found in Leguminosae and Polygalaceae.
- (b) *Exposed site pollen presentation*: The main character here is that pollen-presenters have different physical structures compared to that of the anthers. These structures may have different mechanical properties and their implementation may account for different spatial arrangements of pollen. Thus, pollen from a single anther-cylinder can be divided up by presentation on five corolla lobes (*Zinnia*) or pollen from several anthers may be deposited on the style (Proteaceae, Campanulaceae, Rubiaceae, Apocynaceae). Likewise in protection and delivery system, function of exposed site presentation is approximation of pollen deposition site and pollen reception site.

In Proteaceae, the pollen-presenting style regulates the distance between pollen and nectar in bird-pollinated species. Similar to the protective systems, the exposed site can play a crucial role in exact deposition of pollen onto the pollinator’s body parts. Again, far-exerted styles are common in many members of Proteaceae. Stiffening of styles in this group is probably an adaptation to ornithophily. Here, the pollen is transferred to the stiffening style and thus these

plants avoid stiffening of anthers, which may save some of the materials (although photosynthate is not likely to be in short supply in these plants).

In Asteraceae, there is a lack of space between the individual floret as well as in the capitulum. Combination of successive pollen presentation and secondary pollen presentation in this family may help floral functioning when space is limited. Yeo (1993) suggested pollen dosing as a frequent feature of release system in Asteraceous capitulum. However, quantitative information is lacking in this family and is more evident in Leguminosae (small amount of pollen is released during insect visit) and Campanulaceae (pollen is released progressively).

Yeo (1993) has listed some special cases where secondary pollen presentation has appeared, where the functions are as follows:

- In Myristicaceae and Rafflesiaceae, SPP results in a widespread pollen over the pollinator;
- In Loasaceae, adaptation to special bees;
- In Sterculiaceae, small flies use the hollow petals as pollination chambers and these flies are induced to enter into this chamber to collect the deposited pollen;
- In Hydrocharitaceae, SPP is a primary requirement to imitate the reward-providing stigmatic branches;
- In Epacridaceae, pollen grains placed on the corolla lobes to be removed from flooding by the nectar which fills the corolla tube;
- In Myrtaceae, pollen is mixed with an oily substance from an anther gland which is useful for sticking pollen to beaks of birds and beetles.

10.4 Evolutionary Perspective

The efficiency of sexual function is generally enhanced by the availability of gametes. Among the angiosperms, SPP is largely a contribution of a flower as a unit rather than an inflorescence, and its major consequence is to enhance male fitness by prolonging the availability of pollen and reaching out to conspecifics in greater proportion (Yeo 1993). Despite the variation in time of flower opening (anthesis) and pollen dispersion (anther dehiscence), SPP ensures that nothing goes in vain. As SPP exploits the components of floral structure for presentation, this process also adds to the cues for attraction. Together, these floral contrivances maximize the opportunities for timely deposition of pollen load on floral visitors/stigma, during their visits at the time of the receptive phase of flowers. However, this precision of lodging and dislodging the pollen would have not occurred without manipulating the pollinators foraging behaviour by the flower (Yeo 1993). In outcrossing plants, for effective pollination there is an intense rivalry between selection to place pollen grains and stigmas in the same position. A combination of dichogamy and self-incompatibility that avoids interference between male and

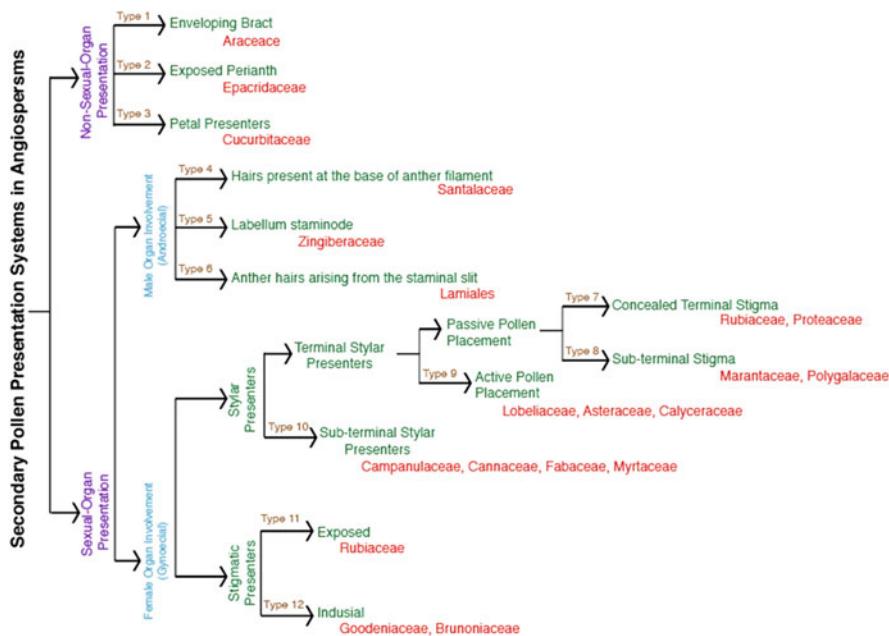


Fig. 10.1 Structural diversity of secondary pollen presentation systems (SPP) among angiosperms

female organs acts as a selective force in the evolution of flower as well act as a key factor in the evolution of presenters (Webb and Lloyd 1986).

The observed variability in SPP systems (Fig. 10.1) suggests that the mechanism might have evolved many times, possibly along with the manifestation of different anther dehiscence mechanisms (Howell et al. 1993). Within the monocots, the presenters found in Araceae and Zingiberaceae are not homologous suggesting evolution of SPP at least twice in this family. While in Cannaceae and Marantaceae, multiple origin of SPP in angiosperms indicates that SPP is adaptive in nature (Yeo 1993). In *Blyxa* (Hydrocharitaceae), the pollen and its presenter made to look like the styles, and this adoption of character is presumably be rooted in some aspect of the behaviour of the pollinators that the flower exploits. Thus mimicry (intraspecific) has also been documented to the evolution of secondary pollen presentation (Yeo 1993). The evolutionary aspects of adaptive values of SPP are primarily based on morphological descriptions, and very few studies have been experimentally performed to highlight how plants get benefitted through interaction of SPP and behaviour of pollinators during pollination (Fan et al. 2015; Castro et al. 2008).

In dicotyledonous families, like Rubiaceae, SPP must have evolved several times within each family (Imbert and Richards 1993). Within a flower, SPP is more linked with the gynoecium presentation than the other parts. This highlights that the SPP trait has evolved specifically, a number of times due to its presence in unrelated families. This further suggests that a common ancestor has diversified and has evolved independently. Many authors have suggested that mutations that favour

precise placement of pollen offer selective advantages, and this eventually lead to the development of gynoecial secondary pollen presentation (Carolin 1961). In Campanulaceae, the adoption of bell-shaped flower with a central style created the problem of pollen presentation. The visitor touches the stigmas with their ventral surface and if the anthers surround the style, then pollination will be ineffective because of introrse dehiscence. And if the anthers spread out inside the bell, they will touch the visitor dorsally. The introrse anthers create structural constraints. Thus, SPP is a way of overcoming these difficulties. Those genera which have departed from this flower-form have retained SPP and have taken further evolutionary steps once pollen presentation character was channelized. Similarly, the dense inflorescence had been a constraint towards SPP in Proteaceae and Asteraceae. In Proteaceae, however, the phylogeny implies that the origin of SPP has taken place in distinct lines, the more primitive character tend to have loose inflorescences. This indicates the occurrence of similar response in different lines to a particular selection pressure. Members of Leguminosae, Papilioideae, Papaveraceae and Polygalaceae are adapted to bee-pollination (Yeo 1993). Evolution of separate-petalled flowers pollinated by bees is the acquisition of zygomorphy and protective role of petals. Protection might have been called for either to save the pollen from the degradations of other insects or to control the proportion of the pollen that the bees take for their own needs. The first step towards zygomorphy and pollen protection by petals will culminate in the adoption of SPP. This implies that pollen presented secondarily can be better protected than primary presentation. The numerous parallel lines within Leguminosae indicate how strong are the selective pressures have been implemented once the initial steps come into play.

As SPP evolved independently in many cases, some authors assume that it provides selective advantage by enhancing male and/or female fitness (Ren et al. 2018). For example, SPP promotes outcrossing by facilitating precise deposition of pollen onto the pollinators (Koch and von Helversen 2006; Fan et al. 2015). Moreover, SPP increases pollination efficiency through increased pollen deposition and competition on the stigmatic surface, which benefits female fitness in plants (Arathi and Kelly 2004). On the other hand, male fitness could be benefitted through the extension of male phase by portioning pollen release and preventing pollen loss (Hesse et al. 2000). One major disadvantage of SPP is that it could reduce female fitness in the gynoecial presenters. Due to the proximity of pollen on gynoecial presenters with that to the stigmatic surface, it may lead to self-interference, which can lead to competition with outcross pollen (Castro et al. 2008; Lin et al. 2012). Hence, the trade-off between the increase in fitness of male and female functions may occur and mechanisms like herkogamy, dichogamy and self-incompatibility may increase the magnitude of this problem (Castro et al. 2008; Lin et al. 2012).

High resources required for the cost of seed production confers advantage to the male function. Some of the features that SPP plants display favouring the availability of viable pollen are: (a) increased pollen load, through andromonoecy, (b) extended phase of pollen presentation, and (c) mechanisms and processes that ensure viability (Howell et al. 1993). SPP trait is highly diffuse, largely passive and does not necessarily appear to be a prerequisite to achieve pollination success. However, its

contribution to pollination success does allow one to speculate the mechanism to work as one of the components of evolutionary forces which lets flowers to manipulate the floral foragers to carry pollen from floral parts other than anthers. For SPP to be an evolutionary stable strategy (ESS), prolonging the dispatch of viable pollen in batches (pollen dosing) should also enhance the longevity of donating flowers in general and receptive phase of the flowers in particular. Further, the floral parts involved in pollen presentation, especially in exposed pollen presentation, ought to be structurally firmer as a consistent presenter. Such a protection is accompanied by concealment to prevent loss. Thus, in terms of SPP, floral design has a lot to do with the efficiency with which the presenter is consistently replenished with pollen from anthers. Protection and saving of unutilized pollen is one of the intrinsic issues in the maintenance and evolution of SPP systems (Yeo 1993). Whereas in the explosive flowers the entire pollen produce is dispensed, in many SPP systems pollen is dispersed in batches. The unutilized portion has to be protected from premature removal by biotic or abiotic means.

10.5 Conclusions

Secondary pollen presentation systems are distinct in nature and have been reported in diverse families across the flowering plants. Different methods have evolved where pollen is presented onto the style (Nyman 1993), in specialized regions of the style or stigma (Westerkamp and Weber 1999), or even over the stigmatic area (Imbert and Richards 1993), usually before anthesis. Evolutionary forces, perhaps those associate with facilitating outcrossing, have played a key role in altering/diversifying pollen presentation system in flowering plants as a unique trait. Among these, self-incompatibility and dichogamy appear to be crucial in the evolution of floral structure and pollen presenters among angiosperms. The advantage of secondary pollen presentation is the close presence of pollen donation site with that of the reception in a flower to save the resources devoted for the structural development of anthers in most groups with pollen presenters (except Lobeliaceae and some Fabaceae). Resources assigned for anther development can be utilized by other organs for attracting pollinators (except in Cannaceae, where anthers can act as an attractant for pollinators).

During pollen presentation, pollen grains are exposed to undesirable environmental conditions. Plants with SPP have evolved not only to protect the pollen grains but to monitor its removal and alter sexual-phase lengths accordingly. Strategies such as (a) anther dehiscence in a serial order, (b) delay in pollen presentation until arrival of vector, (c) anther dehiscence in a gradual manner, (d) reversible closure of the anther during harsh conditions, and (e) slow secondary presentation of the pollen from indusial cup to increase the male phase length during anthesis. The diversity of pollen presentation methods highlights that plants and the involved organisms are under continuous evolutionary pressure to improve male fitness for higher yield in plants.

In order to understand the functional role of SPP mechanism, studies on pollen deposition efficiency from the anther to the presenter, pollen transfer from secondary presenter to stigma by vectors, as well as pollination behaviour of most plants, should be linked properly. Moreover, investigations on pollen and stigma physiology, pollen-pistil interaction after self- and cross-pollination, and mating system analysis covering many populations at physiological and molecular level needs application of interdisciplinary approaches to understand the significance of SPP mechanism in flowering plants.

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Outbreeding in Angiosperms: Floral Architecture and Sexuality

11

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Abstract

Sexuality in flowering plants is distinctly correlated with mating systems. Maximum numbers of flowering plants are outbreeders, and cross-pollination is the predominant type of pollen transfer to increase genetic variation. Morphological traits that promote outbreeding include temporal (dichogamy) and spatial separation (herkogamy) of male and female functions of flowers, monoecy and dioecy. Monoecy permits both inbreeding and outbreeding, while outbreeding is the only means of reproduction in dioecious species. Outbreeding confers on plant an opportunity to ‘move’ its genes amongst conspecific populations and is largely influenced by the availability of pollinators. Though pollinator-driven mating is favoured by most plant species, certain populations may occasionally maximize their reproductive fitness under adverse conditions by adopting multiple strategies such as autonomous selfing, dichogamy, late and early selfing, prepotency and outbreeding. Sexual plasticity manifested through mixed-mating, multiple mating, biparental inbreeding and sex switching has helped plants not only reproduce sexually but also ‘move on’. In addition, pollination environment also alters the outcrossing rates in a population emphasizing the fact that mating systems are labile. How various populations maintain potential for such shifts needs to be investigated. This chapter focuses on the outbreeding strategies in plants and also discusses how mating systems may evolve over the next few decades in response to challenges presented by changing environment.

Keywords

Cosexuality · Dichogamy · Herkogamy · Inbreeding depression · Monoecy · Outbreeding · Pollination failure · Polycrossing · Reproductive assurance · Selfing

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11.1 Introduction

In flowering plants, sexual reproduction provides the only opportunity for genes to ‘move’ among conspecific populations through pollen and seeds. Sexual reproduction centres around flower, the reproductive unit. Flowers are primarily responsible for attracting pollinators and facilitating mating. They are able to acquire considerable morphological diversity with respect to the organization of the floral whorls. The vast majority of angiosperm flowers are perfect, bearing both the male (stamens) and female (pistils) parts. A number of species produce male and female flowers; both of them may be borne on the same plant (monoecy) or on different plants (dioecy). Flowering plants exhibit various mating possibilities – outbreeding, inbreeding or mixed breeding.

A population with bisexual flowers may perform both paternal function by contributing pollen and maternal function by receiving pollen from other plants or may even carry out selfing. Through these functions, the mating systems in a population greatly influence the extent of genetic diversity. Whereas outbreeding increases genetic diversity, inbreeding through selfing or frequent pollination within closely related individuals lowers the genetic diversity. However, continued outbreeding results in outbreeding depression, and selfing over several generations leads to inbreeding depression. Both systems over a period of time reduce reproductive success of the population. Also important is the manner in which outbreeding pollen is presented to the stigmatic surface. In nature, pollen presentation is largely governed by the plants growing in immediate vicinity. This pollen pool from the neighbouring plants is important because it provides the number of males competing for access to females. Furthermore, outbreeding is radically influenced by the type, amount and time at which pollen is deposited on the stigmatic surface. The floral traits mediate plant-pollinator interactions, and the role of floral features such as elongated pistils (Mulcahy 1979; Lankinen and Armbruster 2007) and delayed stigma receptivity (Lankinen and Madjidian 2011) in such interactions cannot be ignored. Further, floral traits can separate the male and female sex organs either in space (herkogamy), time (dichogamy) or both, thus encouraging outbreeding. As a result, outcrossing species generally possess floral mechanisms that actively promote pollen dispersal among plants and populations. The basis behind these variations is that hermaphrodite plants need to optimize both maternal and paternal fitness. Not forgetting that in most of these plants, the sexual organs are in close proximity to each other which increases the chances of self-interference (Barrett 2000; Webb and Lloyd 1986), which is detrimental to reproductive fitness (Castro et al. 2008; Waites and Agren 2006). The positioning of stigma and anther in the centre of the flower also has potential to interfere with pollen receipt and pollen removal. Several plants are known to avoid self-fertilization by protandry or protogyny, while many others have resolved this issue through sexual polymorphism which reduces self-interference making cross pollen dispersal effective. For example, species exhibit stylar polymorphism and at least four major classes – distyly and tristyly, stigma-height dimorphism and enantiostyly have been recognized. In such herkogamous plants, the reciprocal placement of stigmas (pollen receivers) and anthers (pollen

bearers) in the floral morphs promotes pollinator-mediated cross-pollination between morphs. The female and male functions in such cases are arranged in the same plant in a manner that there is less pollen wastage from self-pollination, making the entire process more economical. In some groups (e.g. *Fagopyrum esculentum*), effective pollen delivery gives recipient (female) a choice of mate from among many pollen grains that have been presented. In all cases, the thumb rule is precision of pollen transfer to stigma of another plant.

The major challenge outbreeding plant's face is from climate change and loss of pollinators. Since outbreeding mating systems are pollinator-driven mechanisms, any decline in pollinators would result in pollination failure.

11.2 What Is a Flower?

Flowers, the sexual reproductive structures, are usually comprised of four different whorls (as in perfect flowers) around the flanks of a condensed reproductive axis. The two inner whorls of male (staminate) and female (pistillate) reproductive structures perform the sexual function. In a large number of bisexual or hermaphrodite flowers, the female organs, the carpels occupy a central position in the flower and are surrounded by the male organs, the stamens. Importantly, the ovules of flowering plants are enclosed within ovary, and pollen tubes grow to reach the egg, aided during their journey by stigmatic and stylar tissues and their secretions (Endress and Igersheim 2000a, b). Carpels may arise separately or be fused together in a syncarpous gynoecium (Armbruster et al. 2002). The latter arrangement typically provides a larger landing platform for pollinating insects. The perfect or complete flowers also contain two accessory whorls of sterile organs (sepals and petals) surrounding their reproductive organs. These protect the delicate male and female tissues. They are generally showy and protective. In certain cases, the flowers (imperfect) may bear perianth represented as tepals, where the two accessory whorls (sepals and petals) remain undifferentiated. Furthermore, some imperfect flowers may be unisexual with only male or female whorl (Fig. 11.1). There are, however, some exceptions to the two basic flower types with *Lacandonia schismatica* and *Trithuria* known for ‘inside-out’ flowers, in which the carpels surround the central stamens (Rudall et al. 2007, 2009).

11.3 Floral Architecture

The term floral architecture though not commonly used in floral contexts has been described by Endress (1996), who differentiated between floral organization and floral architecture. According to him, floral organization describes the number and position of organs in a flower. Floral architecture has a wider description and takes into account the relative sizes of floral organs, their degree of fusion, besides formation of complex structures through synorganization. Such modifications increase the attractiveness of the flowers for pollinators. Formation of gynostegium

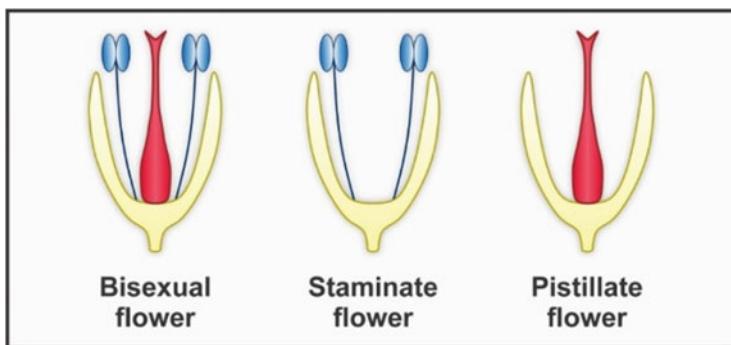


Fig. 11.1 Flower-level sexual expression of angiosperms showing bisexual and unisexual (staminate and pistillate) flowers. (Cardoso et al. 2018)

in Asclepiadoideae is an example of synorganization which defines the intimate structural connection of two or several neighbouring floral parts to form a functional system or apparatus (Endress 1990; Ronse De Craene 2010). While synorganization in Asclepiadoideae is due to fusion, in *Geranium robertianum* it is due to formation of ‘revolver’ architecture without fusion (Endress 2010). In this type, each compartment holds a part of the total nectar reward of the flower. As a consequence, potential pollinator has to probe each compartment to harvest all the nectar of the flower. Such a foraging mechanism increases the flower handling time, thereby increasing the likelihood of pollen transfer. Other examples of nectary chambers through synorganization are seen in *Codon* (Jeiter et al. 2016) with septa formed between the anther filament bases and the corolla tube and *Nasa* where five staminodal nectar scales are present (Weigend and Gottschling 2006).

The function of floral organs is somewhat defined. Calyx serves the function of protection, and corolla tends to be more elaborate to attract pollinators, while additional extra floral structures such as bracts and extrafloral nectaries become more conspicuous to facilitate protection and effective pollination.

11.4 Functions of Flower

Flower is considered a key evolutionary innovation, responsible for the evolutionary success of angiosperms amongst land plants (Specht and Bartlett 2009). It not only produces male (pollen) and female gametophytes (embryo sac) but also dispenses and receives pollen grains and facilitates fertilization and development of seeds and fruits. It screens the pollen grains and allows only the compatible one to function and, therefore, precisely bring about and control plant reproduction.

Flowers underpin mutualism between plants and pollinators, a key to pollination syndrome. Pollination, which drives outbreeding, is usually associated with more rapid speciation of plants, and it is through flowers that plant advertises the receptivity of floral organs. A myriad of floral features such as colour, shape, scent, reward

availability or time of flowering and, in several cases, also whole plants or groups of plants (such as floral density, flower number, flower height or spatial pattern) attract the pollinators/visitors. The more readily apparent floral presentation traits can be divided into attractants, the advertising signals and rewards, usually food stuffs.

Flowers in certain instances are also exploited as sheltered habitat for small visitors to rest in a place that is protected from bad weather, predators or parasites. In similar functions, flowers may also become reliable site for mates or hosts or preys and may offer a female oviposition site, providing shelters for eggs and larvae as in *Yucca-Tegeticula* mutualism. In rare cases, they are used as swarming up sites by insects in cold climates because of incoming solar radiation trapped by the flower.

11.5 Outbreeding Devices

Outbreeding is a mode of sexual reproduction where fusion of gametes from two different individuals results in an offspring with a new combination of genes. Thus, outbreeding increases the magnitude of genetic variation amongst individuals produced through sexual reproduction. There are several devices adopted by the plants to encourage outbreeding. Male cytoplasmic sterility and self-incompatibility are the two important genetically and physiologically controlled phenomena, respectively, that promote cross-pollination. However, sex separation can be achieved by simple presentation of male and female sexual organs at different time scale (dichogamy) or in different spatial orientation (herkogamy). The other common methods are dicliny and self-sterility.

11.6 Dichogamy: Sex Separation in Time

Dichogamy, a method to promote cross-pollination, is maturation of anthers and stigma of flowers at different times and has been invoked as a mating mechanism for avoiding inbreeding depression (IBD). It is of common occurrence among angiosperms (Sargent et al. 2006). Dichogamy reduces ‘selfing syndrome’ such as pollen discounting and ovule or seed discounting. According to Sargent et al. (2006), dichogamy is speculated to play a significant role as a mechanism to avoid selfing and promote outbreeding. In self-incompatible plants, it reduces sexual interference (Box 11.1) between pollen and stigma presentation within (intra) or among (inter) flowers. It works towards reducing intraflower interference between pollen export and receipt (Bertin and Newman 1993), making mating systems work as outbreeders. Sexual phases could be completely separated (complete dichogamy), completely overlapped (adichogamy), or partially overlapped also known as incomplete dichogamy (where male and female phases are partially overlapped) (Lloyd and Webb 1986; Stout 1928). In case of partial dichogamy as in sweet corn and squash, opportunities for autonomous selfing increase (Lloyd and Schoen 1992) because in such cases plant loses out opportunities for outcrossing.

Box 11.1 Protogynous diurnal synchronous dichogamy

1. Every flower e.g. Avocado (see Bergh 1975) opens twice where at first opening the pistil is mature, the stigma is receptive and the flower is functionally female. The pollen sacs remain closed. This is followed by second opening when the flower is functionally male. Dehiscence of the pollen sac occurs, and pollen is shed while the stigma shrivels and turns brown, losing receptivity.
2. All flowers of the tree or cultivar are functionally female before noon and functionally male in the afternoon (A group) or are functionally male before noon and functionally female in the afternoon (B group).
3. In group A, the elapsed time between the first and second opening is about 24 h and about 12 h for B group flowers.

Various types of dichogamy have been proposed:

1. **Based on order of presentation:** It can be protandry and protogyny governed by type of sexual phases expressed earlier during development. Protogyny relates to a flower in which the shedding of pollen occurs after the stigma has lost its receptivity, having the female sex organs maturing before the males, as in banana and cherimoya. On the other hand, when anthers mature before pistil, it is known as protandry, e.g. walnut. The dichogamous species may present the pollinating surfaces successively over time scale of hours as in avocado, days as in umbellifers, weeks as in most of the Aceraceae (de Jong 1976) or months apart as in figs (Weibes 1979).
2. **Based on degree of synchrony within a plant:** Several classes can be distinguished on the basis of degree and nature of synchrony among the blossoms on a single plant. It is synchronous if pollen and stigma presentation of all flowers at anthesis is simultaneous (and asynchronous dichogamy, which occurs when the pollen and stigma phases of different blossoms are not in phase with each other). During synchronous dichogamy, there may occur a switch from the male phase to the female phase or vice versa. In *Butomus umbellatus*, multiple consecutive cycles of synchronous dichogamy have been recorded. Each flower of the inflorescence is protandrous and spends 1 day in each of male and female phases, with an intervening neuter phase of 1 day. Thus, all open flowers have to be in the same phase (Bhardwaj and Eckert 2001). In carrot, a member of Umbelliferae, all flowers of the umbel go through successive male and female stages in synchrony before the next umbel order repeats the cycle (Cruden and Hermann-Parker 1977). In avocado, where flowers occur in panicles, an individual flower opens for 2 days, with distinct timings for male and female function. The first anthesis exhibits the female function lasting for 2–4 h after which the flower closes, only to open again on the second day. However, on second day, the male phase is functional in the same flower which had female phase functional and pollen is shed.
3. **The degree of separation:** Dichogamy is complete when there is no overlap in the presentation of the pollen and stigmas in a flower or the inflorescence. In such cases, the hermaphrodite flower initially enters male phase followed by female phase but only when the first phase is over. It may also enter female phase

followed by male. Dichogamy may be incomplete, when there is some overlap between the presentation of male and female phase, and this may affect outbreeding due to sex interference depending upon whether protandry or protogyny occurs. This is because at some stage, some of the pistils and some of the stamens in a flower mature at the same time. In Ranunculaceae and Rosaceae, where each flower has numerous pistils and stamens developing in succession over a period of time, incomplete dichogamy is observed. Complete dichogamy is, therefore, seen in flowers with single carpel and a few stamens. For example, *Polyalthia hypoleuca* exhibits complete protogyny (Rogstad 1994). In this plant, synchronized intrafloral protogyny is seen. Since flowers develop in serial cohorts, some of the flowers in successive cohorts exhibit complete intercohort dichogamy where stigma receptivity is separated by 2 days. This reduces chances of geitonogamy and also sex interference within an individual. A second mode is also observed where successive cohorts (serial) attain stigma receptivity day after day presenting staminate and carpellate phase flowers simultaneously in an individual flower. In such cases, chances of geitonogamy and sex interference exist.

4. **Based on floral units involved:** Dichogamy may be intra- or interfloral in plants with unisexual flowers and in monoecious or subdioecious populations with unisexual flowers. In monoecious condition, plant can both receive and disperse pollen and may ultimately function as maternal and paternal parents to the progeny. Such dual sex displays generally result in conflicts between the parental roles of plants during pollination and mating. Intrafloral and interfloral dichogamy is sometimes combined in an individual. For example, in many gynomonoecious species of Compositae, the marginal female florets open before the central hermaphrodite florets (interfloral protogyny), while the stamen reaches maturity before the pistil in each individual hermaphrodite flower of *Helianthus annuus* L. (Intra-floral protandry).

To ease out such conflicts of sexual interference, the pollen and stigmas of separate male and female flowers on one plant may be presented at different times; this is interfloral dichogamy. In light of this temporal selection, geitonogamous self-pollination can be viewed as a form of interfloral sexual interference. As in interfloral dichogamy, intrafloral sexual interference includes physical interference between sex functions, pollen clogging, and ovule discounting. When interference occurs between the male and female functions within the same flower of a plant, it is also referred to as pollen-stigma or pollen-pistil interference (see Bertin and Newman 1993). Two types of intrafloral interference have been studied. In the first type, structure and position of the female and male sexual organs within a flower may physically interfere with pollinator movement, making it ineffective. This happens when stamens obstruct or hamper positioning of the pollinator resulting in poor pollen deposition. As a result, several ovules are left unfertilized (ovule discounting). There may be cases where reduced pollen export may result when pistil prevents effective pollen pick up by the pollinator (Box 11.2). In the second type, ‘pollen clogging’ occurs in which the presence of self-pollen on the stigma of self-incompatible plants will reduce the surface area that could otherwise be available for outcross pollen, and this would directly affect the siring ability of outcross pollen (Herlihy and Eckert 2007). As this pollen is also not available for export to other plants, they may further reduce male siring success (Barrett 2002).

Box 11.2 Sexual interference

Pollen-stigma interference is a complex phenomenon wherein maternal or paternal fitness is obstructed when one sexual function (male or female) affects the other. ‘Stigma clogging’ is one such interference where self-pollen may occupy the stigma of self-incompatible plant, reducing its reproductive fitness. The pollen load restricts deposition of cross-pollen or displaces it, or damages the reactive surface of the stigma (van der Pijl 1978) besides reducing the growth of compatible tubes (Howlett et al. 1975). In several other cases, stamens may also reduce access to stigma or prevent incoming pollen from another plant to get deposited on a stigma. Similarly, the presence of carpels may reduce or obstruct pollinator’s access to pollen surfaces or redeposit pollen that has landed on the stigma before it can be disseminated to other flowers (Lloyd and Yates 1982). Sexual interference can potentially take several distinct forms with contrasting reproductive consequences, such as pollen discounting, ovule discounting and other related phenomenon resulting in decline of both, male and female reproductive fitness. Such sexual interference has been recognized to occur within flowers and between flowers.

11.7 Herkogamy: Sex Separation in Space

Herkogamy is a trait in which identical flowers are borne by the plant, but pollen release and receipt are separated in space within each flower of an individual plant. It also includes examples which may show protandry and protogyny. In self-compatible and pseudocompatible (plants with low level of self-fertility in self-incompatible species) species, herkogamy is an adaptive character that decreases chances of self-pollination. Generally, herkogamy, which promotes outbreeding, is also believed to reduce fully or partially self-interference between pollen receipt and pollen dispatch. In cases with large spatial separation between anther and stigma, herkogamy decreases efficiency of pollen transfer. Excessive herkogamy reduces the possible contact between pollinators and sexual organs. For example, in *Mimulus aurantiacus* with scanty herkogamy, Fetscher (2001) found that pollinators transferred twice as much pollen in flowers with closed stigma compared to those with open stigma. In this case, open stigmas physically interfere with pollen export. Another benefit of herkogamy over dichogamy is that floral visitor performs ‘double duty’ of depositing incoming pollen and removing pollen during a single visit (Baker and Hurd 1968). Further, herkogamy also reduces the investment on attractants and rewards unlike that in dichogamy where they have to be made available for an extended period.

Herkogamy can be further divided into several types:

- (i) *Homomorphic herkogamy* is seen in plants where all flowers are identical and may be further divided into ‘unordered’ or ‘ordered’ homomorphic herkogamy. In the former case, several contacts occur between the pollinator and stigma/pollen but in no particular sequence. The size of anther and stigma is small, and pollinator does not face any constraints; as a result of which, some self-pollination can occur, e.g. Myrtaceae, in which stigmas of individual flowers are placed beyond numerous stamens. In the ordered type, such contacts are fewer. With pollen and stigma positioned along the path of the pollinator and when the pollinator moves towards the flower, contact occurs. In this mechanism, precision of contact is important as chances of second contact are generally bleak. In some flowers (e.g. *Brasenia*), stigmatic area is unifacial and is placed outwards so that even if contacted by the pollinator, pollen deposition is discouraged by the stigma. There are three types under ordered herkogamy.
- (ii) Approach herkogamy involves specialized pollinators like bees and humming birds that perform with a lot of precision. As in *Yucca*, moths deliberately pollinate herkogamous flowers (Riley 1873). In this case, pollen and stigma are so placed that stigma is contacted first and pollen is picked later. Second contact may not occur after insect picks up the pollen. After collecting pollen balls, female moth flies to the other flower, oviposits in the ovary and, in this attempt, gives pollen to the stigma. As stigma is approached first, it is approach herkogamy.
- (iii) Reverse herkogamy is said to occur in flowers with tubular corolla, where anthers are placed near the throat and stigma is deep seated in the flower. As in butterfly-pollinated *Daphne*, the pollinator first contacts anthers and later the stigma which is the reverse route – normally stigmatic contact is followed by that with the anthers.
- (iv) Movement herkogamy wherein the floral parts are able to move in response to presence of the visitor. This involves sensitivity of floral parts or the architecture of the flower which is mobility based. This is exemplified by orchids where pollinia bend forward between flowers and get positioned in contact with stigma of next flower during insect’s flight.
- (v) Reciprocal herkogamy – individual blossoms are herkogamous and hermaphroditic, with two or three different forms of flowers, either on the same or separate plants. These classes combine some of the features of homomorphic and interfloral herkogamy; pollen and stigmas are separated in space within each blossom, but pollination precision is maintained between blossoms or plants by the reciprocal morphs. In the first type of heterostyly as in *Psychotria*, two morphs with reciprocal lengths of style and stigma occur on different or the same plants. The second class of reciprocal herkogamy is enantiomorphy where plants have two morphs, left- and right-handed flowers; anthers and stigmas are segregated to different sides. The two flower forms may occur within inflorescences either randomly located or in fixed position, e.g. in *Wachendorfia* and *Barberetta*, all flowers on a single plant are of one form, while in *Dilatris*, both floral forms occur on all individuals (Fig. 11.2a–c).

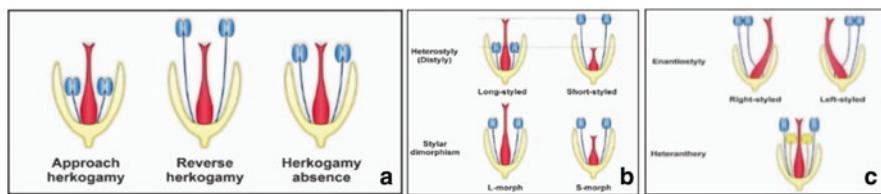


Fig. 11.2 a–c Outbreeding devices. **a** – The vertical herkogamy and its absence; **b** – Heterostyly and stayer dimorphism; **c** – Reciprocal enantiostyly and heteranthery with stamen differentiation. (Cardoso et al. 2018)

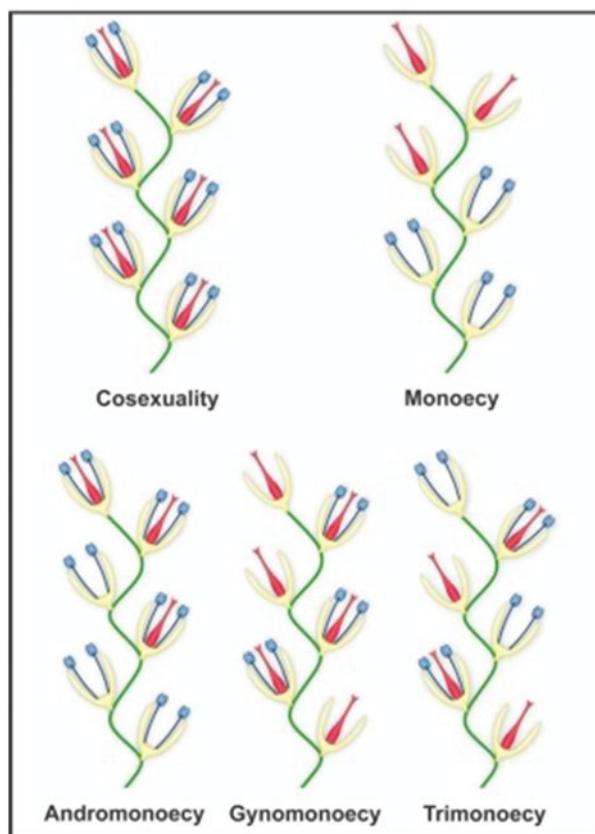
- (vi) Interfloral herkogamy – Many plants that produce both pollen and ovules in part or in a whole in unisexual blossoms can be regarded as exhibiting interfloral herkogamy. The two pollination surfaces are separated in space and presented simultaneously. This is most clearly seen in monoecious species where the distinct functions of pollen presentation and pollen receipt are performed simultaneously by male and female blossoms, respectively.

11.8 Choice of Sex

Studies on the breeding system also reveal the functional understanding of parental investments in producing offspring (Lloyd 1979; Lloyd and Bawa 1984; Sakai and Weller 1999). Based on the distribution of male and female parts of the flower, species have been variously termed as cosexual, hermaphrodites, monoecious, gynomonoecious, andromonoecious, polygamous or trimonoecious and dioecious (Fig. 11.3; also see Chap. 9). Cosexual in broad terms is a plant that produces both pollen and ovules but varies in their reproductive effort, e.g. *Arisaema*; hermaphrodite is a flower with both the male and female reproductive organs, e.g. tomato; monoecious plants have male and female reproductive organs on separate flowers but the same plant, like squash and pumpkins; gynomonoecious is an individual with both hermaphrodite and pistillate flowers as in *Silene vulgaris*; polygamous is mango with bisexual and unisexual flowers on the same plant; trimonoecious is with male, female and bisexual flowers on same plant; dioecious is mulberry with separate male and female plants, and there exist a few more situations (<https://www.cropsreview.com/types-of-sex.html>).

Further, all plant species can be accommodated within two types – ‘gender monomorphism’ and ‘gender dimorphism’. The former category of populations (hermaphrodite and monoecious conditions) is morphologically bisexual with both stamens and pistils in the flowers. Both sexes are expressed as in *Sonchus*. However, functionally they act either as a female or a male parent depending on the genetic contribution the plant makes to the next generation in terms of providing pollen or the ovule (or seed). By contrast, populations with ‘gender dimorphism’ show both, morphological and functional biomodality in gender. This is because the plants

Fig. 11.3 Population-level sexual expression of angiosperms showing the different types of individuals found on each system including cosexuality, monoecy, andromonoecy, gynomonoecy and trimonoecy. (Cardoso et al. 2018)



(e.g. papaya, *Asparagus* and Yam) in such cases are composed of two distinct sexual morphs (male and female plants) that function primarily as either female or male parents.

It is observed in several plants that gender adjustment can occur throughout the flowering season. Adjustment is expressed either as shifts in proportion of maleness and femaleness or there may be a switch over from all male to all female phases. In diphasy, the plant can remain all female or all male for the entire flowering season. Environmental cues and various other factors such as wounding and physical trauma usually result in shifts in sex. Cucumbers, for example, often produce male flowers under conditions of low soil moisture or low relative humidity, but physical trauma often results in a switch to femaleness as also observed in papaya.

Some maples (*Acer pennsylvanicum*) do not grow as canopy trees but come up quickly in temporary openings or gaps in the forest producing male flowers on the young trees. When more saplings start to grow up in the tree gap, the canopy starts closing over *Acer* and it gets shaded light which may lead to its death. In such situations as just before canopy closure, the *Acer* tree switches sex and becomes female and allocates all its resources to maternal function in one large reproductive burst (Borges 1998).

11.9 Dioecy – An Evolutionary Dead End?

Dioecy, characterized by the presence of distinct male and female plants, is rare in flowering plants with only about 6% of an estimated total of 240,000 species of angiosperms reported to be dioecious (Dufay et al. 2014; Muyle et al. 2018). Out of the various breeding systems (Fig. 11.4) represented by monoecy, androgynoecy, gynodioecy and distyly, the most common pathway for evolution of dioecy is through monoecy (Charlesworth and Charlesworth 1978; Lloyd 1980; Dorken et al. 2002; Renner 2014). In *Nothapodytes nimmoniana*, dioecy has been suggested to evolve from distyly by sterilization of anthers in female and stigma in male flowers (Sharma et al. 2010). Dioecy probably functions to avoid inbreeding (Barrett 2013).

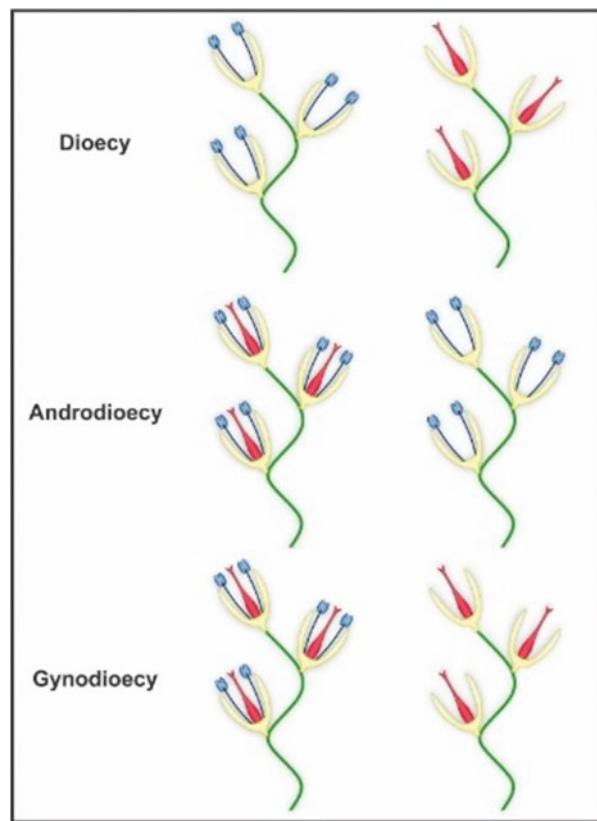
The widely accepted view for dioecy as an evolutionary dead end is now being looked from a different angle. The growing evidence from various plant systems indicates that several transitions in dioecy have given rise to other forms of mating systems (also see Chap. 9). The first documentation of reversion from dioecy to monoecy was documented in *Cotula* by Lloyd (1975a). In *Momordica*, Schaeffer & Renner (2010) indicated seven independent transitions from dioecy to monoecy. While gynodioecy-dioecy pathway (G-D pathway) has also been a common evolutionary process (Ross 1978; Barrett 1992; Weiblen et al. 2000; Ehlers & Bataillon, 2007; see Renner & Ricklefs, 1995), the androdioecious-dioecy occurs with low frequency.

Gynodioecy (wherein populations consist of separate female and hermaphrodite individuals) reported in about 1.4% of angiosperm genera (Richards, 1986; McCauley & Bailey, 2009) is considered to be an intermediate condition in the evolution from hermaphroditic to dioecious condition (Lloyd 1975b). Evolution in this direction is believed to have occurred repeatedly as a result of selection of self-fertile hermaphroditism (rather than hermaphroditism) for reproductive assurance during colonization. Male frequencies in these species are highly variable, while self-fertilization in hermaphroditism is delayed (see also Pannell 2002).

Three transitional phases are seen in G-D pathway. The first phase is characterized by the presence of mutations for male sterility in hermaphrodite population. Such male sterile individuals (now females) acquire seed-fertility advantage over the ‘resident’ hermaphrodites, and due to this advantage, they get established in the population. Such a population is gynodioecious in which the male function increases again to compensate for loss of female function (Fig. 11.5). Subsequently, full (or several partial) female-sterility mutation(s) invade the gynodioecious population. The ‘new males’ get established making the population subdioecious; this marks the second phase. The third phase marks the transition from subdioecy to dioecy during which the hermaphrodites gain fitness through male function achieved upon loss of female function (Charlesworth 1989a, b).

Not only a dioecious species can evolve from hermaphroditic, but dioecy can breakdown back into hermaphroditism as well (Fig. 11.6). Crossman and Charlesworth (2013) have suggested that pollen limitation may lead to extinction

Fig. 11.4 Population-level sexual expression of angiosperms showing the different types of individuals found on each system including dioecy, androdioecy and gynodioecy. (Cardoso et al. 2018)



of males in the population. The females become cosexual and self-fertile under such situations. Thus, species colonizing newly disturbed sites has a highly variable frequency of males. If there are a few males, any female in the population which is able to produce few pollen grains (female turned hermaphrodites) can fertilize its own ovules as well as those of other plants which lack sufficient pollen and, therefore, has low fertilization percentage. This is evolution away from dioecy, e.g. *Mercurialis annua*.

11.10 Limitations

Dioecy, to overcome pollination constraints, is believed to have given rise to sexual systems and polygamy through secondary processes. In dioecy, the seed production is contributed only by 50% of the individuals, the females, and pollinator movement from males to females is obligatory (Vamosi et al. 2006). Unisexual females are evidently exposed to a risk of failure to receive pollen, so that fruit set may fail leading to reproductive failure. With these limitations, dioecy has been explained in

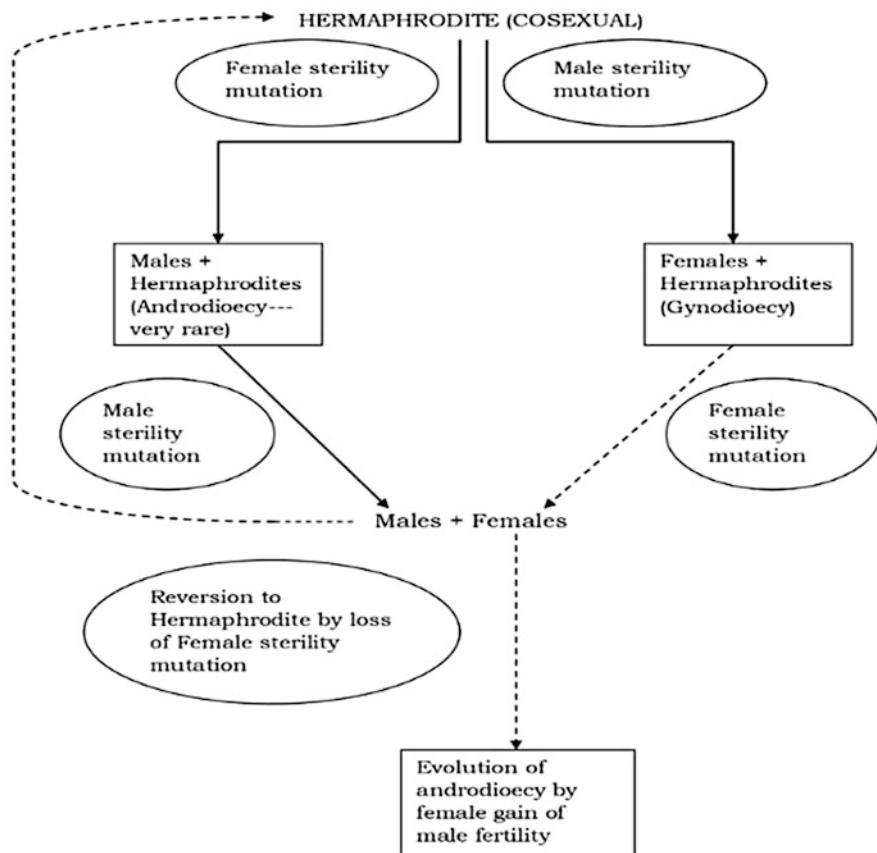
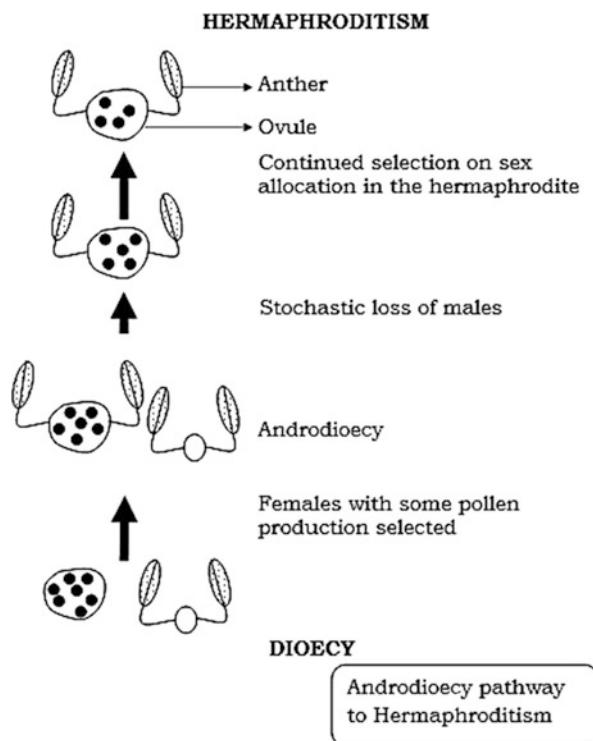


Fig. 11.5 Evolution of dioecy from hermaphrodites, showing that at least two steps are required via gynodioecy and androdioecy, latter being rather rare in occurrence. Broken lines depict possibility of going back to hermaphroditism via dioecy. Theories for the evolution of dioecy often focus on the conditions for invasion of populations by unisexual types. (Ross 1978)

terms of ‘dead-end hypothesis’ (Muyle et al. 2018). According to this hypothesis, the dioecious plant populations might experience evolutionary handicaps such as low seed dispersal, high genetic drift, low adaptation and ultimately species extinction. It may also lead to extinction of dioecious species, perhaps partially explaining why dioecious taxa include fewer species than sister taxa. It may be argued that even self-incompatible plants require another individual to mate with, but in such cases, interplant pollinator movement potentially contributes to seed production (Ohya et al. 2017). Despite the above said handicaps, dioecious plants maintain their populations in communities with co-occurring hermaphrodites, without any substantial difference in population growth rates between dioecious and cosexual plants (see Lloyd 1979). However, recent studies have indicated that dioecy may not be the dead end (Zhang et al. 2019). The dioecious plants are thought to mitigate these

Fig. 11.6 Evolution of hermaphroditism from dioecy



limitations by adopting ecological traits, such as insect pollination, animal-dispersed fleshy fruits, and precocious flowering.

11.11 Selfing Syndrome and Geitonogamy

Most of the plant species contain both female and male sexual organs. This state of bisexuality in the plants confers opportunities for self-fertilization, provided both male and female gametes are produced simultaneously. It possesses an advantage over outcrossing that is derived from cost of producing males (Box 11.3). Only about 10–15% of flowering plants are predominantly selfing (Wright et al. 2013). Selfing is expressed in several fundamentally different ways, described as modes of self-pollination (Lloyd and Schoen 1992). For the sake of convenience of understanding, self-pollination can be divided into cleistogamy, autonomous autogamy and geitonogamy. Occurring in closed flowers where outcrossing is impossible, cleistogamy, the most distinct mode of selfing, can be recognized immediately due to characteristic morphology (Lloyd 1982).

BOX 11.3: Cost of sex?

Consider two populations: one is asexual and one is fully sexual. Both have a stable sex ratio of 1:1. For simplicity, suppose that each female produces two offsprings. In the asexual case, the population will double every generation, since each member of the population produces two offsprings. In the sexual case, the population will remain constant, since half of all offsprings are males, and contribute to the next generation by fertilizing the females. The clone of offspring from an asexual female multiplies at twice the rate of the progeny descended from a sexual female, and a sexual female has only 50% of the fitness of an asexual female. This has been described by Maynard Smith as the twofold cost of sex (1978).

Autonomous selfing can occur before, during or after opportunities for outcrossing (Lloyd 1992). Depending upon time of self-pollination relative to cross-pollination, autonomous pollination can be divided into prior, competing and delayed self-pollination. Prior autogamy (occurring before outcrossing) operates when stigma is receptive and the anther dehisces; the pollinating stigmatic surfaces are oriented in a manner where they are in contact while flower is still closed (bud pollination). This is beneficial in poor weather. Competing selfing (concurrent) is similar to cross-pollination but is facilitated and occurs during early flowering along with cross-pollination as in *Roscoea schneideriana* and in Brassicaceae (anther rubbing in *Erysimum incanum*). Paired anthers surround the stigma, and due to close proximity of pollen and stigma, pollination occurs. Delayed selfing when the movements in flower parts at the end of anthesis bring some contact between pollen and stigma and unfertilized ovules is available. Among the various modes of autonomous selfing, only delayed selfing incurs no pollen and/or seed discounting costs at the flower level (Schoen and Brown 1991; Lloyd 1992). Delayed autonomous selfing is, therefore, generally regarded as adaptive since it allows seed production when pollinators are scarce or potential mates are unpredictable, allowing outcrossing to predominate when they are abundant (Wyatt 1983; Cruden and Lyon 1989; Goodwillie et al. 2005; Fenster and Martén-Rodríguez 2007; Chaudhary et al. 2018). It also occurs in plants with short life span (Barrett 2002). As delayed selfing combines the advantages of both selfing and outcrossing, it is often interpreted as a ‘best-of-both-worlds’ mating system. Hence, cross-pollination can occur early in floral life when pollinators are available, but delayed selfing guarantees successful pollination at the end of a flower’s lifespan if pollinator-mediated pollen deposition fails (Lloyd 1979; Kalisz and Vogler 2003; Jacquemyn and Brys 2008). For example, in *Incarvillea*, the adherent epipetalous stamens are also dragged along with strong winds, thus leading to contact of anthers with stigma in late anthesis (Qu et al. 2007).

Geitonogamy is the most distinct innovating pollen transfer between flowers of same plant and requires vectors as in cross-pollination (de Jong et al. 1993). It is a mix of properties with ecological benefits of cross-pollination and genetic properties of selfing. In the ecological benefits, may be included are reduced costs and the ovule and pollen discounting from participating in outcrossing. This is because pollen meant to be carried away by the pollinator, is captured by stigmas on the plant from which it originates. Such pollen is not made available for dispersal to other plants. Hence, geitonogamy removes pollen from the pool of male gametes which would otherwise be available for cross-pollination and thereby reduces paternal fitness.

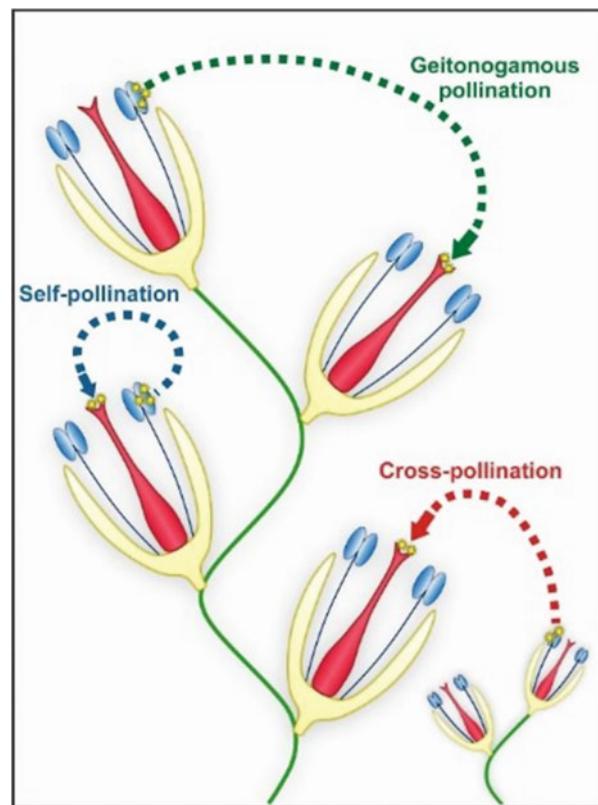
Geitonogamy may occur at least at three levels in a population, within branch, between branch and between ramet (an individual member of the clone). Geitonogamy is considered as one form of self-pollination in terms of genetic relations (Fig. 11.7). It is not only met within hermaphrodites but also seen in monoecious plants, e.g. as in plants because of the weight of the branches, pollen from upper inflorescence reaches lower flower stigma. In Ericaceae, at the end of flowering, stigma extends beyond the perianth whorls and gets pollinated by neighbouring pollen. It can also occur when visiting insects search nectar and move from flower to flower. In species with significant growth as in shrubs and trees, and those with many open flowers on a plant at the same time, a considerable amount of selfing can occur. In plants with large floral displays such as mass flowering trees and large clonal herbs, pollinators transfer pollen grains among flowers on a single plant, facilitating a considerable amount of geitonogamy (Lloyd and Schoen 1992).

Autogamous as well as geitonogamous selfing leads to high rates of self-fertilization in self-compatible species, but reduces reproductive success in self-incompatible species (de Jong et al. 1993; Klinkhamer and de Jong 1993). Further, selfing involves considerable mating costs because of inbreeding depression and pollen discounting associated with it. The latter phenomenon occurs when the plants while cross-pollinating encounter a loss of mating chances.

Facilitated self-pollination (Schneider and Buchanan 1980) may be considered as yet another mode of selfing. The visitors coming to flowers while collecting rewards may bring about some autogamy as well as geitonogamy, which depends on whether pollinator has visited other flowers of the same or other plants, before landing on this flower (Pazy 1984.). It is impossible to avoid such pollination when pollen and stigma are presented simultaneously to the visitor.

There is a twofold transmission advantage to the selfer. A selfing individual transmits two sets of genes to its offspring but the outbreeder only one set, leading to only a 50% advantage to the offspring (Fischer 1941; Maynard-Smith 1978). This is an intrinsic genetic advantage of self-fertilization (Box 11.2). Second advantage is termed as reproductive assurance (RA). While an obligate cross-fertilized plant faces the risk of not receiving enough pollen to set seeds independent of its resource status (Ashman et al. 2004), selfer is endowed with reproductive assurance, especially in

Fig. 11.7 Different types of pollination according to the source of pollen. (Cardoso et al. 2018)



cases where mates or pollinators are scarce (Mangla and Tandon 2011; see Shivanna 2015). This assurance of reproduction in hermaphroditic individuals is said to relieve plants of ‘Allee effect’, i.e. the difficulty of finding a sexual partner in low-density population (Kramer et al. 2008).

It is apparent that bisexuality in all the sexual systems, irrespective of their origin, confers species with RA through autogamy. However, it remains less investigated if autogamy occurs in hermaphroditic flowers produced in gyno- and androdioecious or polygamous (bisexual and unisexual) individuals, though a majority of gynodioecious species are reported to show this mode (see Shivanna 2015). In *Silene noctiflora*, a gynomonoecious species, bisexual flowers are known to ensure RA through autonomous selfing (Sharma et al. 2010).

Selfing, however, is believed to be limited by inbreeding depression (IBD) and pollen discounting. Plants have a reduced potential for adaptation. It also appears to have negative consequences on the genetic diversity, viability and diversification of plant lineages (Goldberg et al. 2010). Due to these reasons, selfing for long time was considered as ‘evolutionary dead end’ and inbreeding depression as one of the leading factors preventing the evolution of self-fertilization in plants. In face of these limitations, plants adapt themselves through ‘antidiscounting mechanisms’

also known as ‘anti-selfing mechanisms’ to achieve reproductive success. Floral designs such as herkogamy and dichogamy, which reduce chances of self-pollination, and male gamete wastage augment fitness through more proficient pollen dispersal mechanisms to other plants (cross-pollination).

11.12 Outbreeding vs Inbreeding

The evolutionary transitions have occurred in the direction from outcrossing to predominate selfing leading to the origin of dioecy. Selfing offers benefits to the plants as it releases them from requirements of mating partners. It also confers an inclusive fitness benefit outcome from increased relatedness between parents and inbred offspring and allows rapid colonization. The selfing individuals are known to have an innate transmission advantage over outbreeders because it leads to passing over both the copies of chromosomes through the progeny. Inbreeding thereby increases the expected proportion of alleles that any one parent shares with its offspring, including alleles favouring this mating system. However, selfing also results in diverse costs, inbreeding depression (IBD), pollen discounting and genetically uniform populations. The IBD defined as the reduction in progeny fitness from mating between related individuals is generally recognized as one of the main selective forces that shape evolution of mating strategies. While a hermaphrodite plant has a choice of selfing, it may also acquire fitness through either of two sexual functions – by being maternal (contributing ovule) or paternal (contributing pollen) parents to seeds produced in the next generation.

Whereas several studies have been carried out on reproductive decline and inclusive fitness (due to inbreeding), outbreeding appears to be overemphasized with respect to its advantages. Though outbreeding brings variations in the populations, it is marred by a less understood phenomenon, outbreeding depression. This results in decline of reproductive fitness and is related to dilution of genes associated with local adaptations and hybrid breakdown (Fenster and Galloway 2000). When the gene pools of ecologically and genetically distinct populations mix due to outbreeding, it results in reduced progeny fitness. The poor performance may either become expressed in F_1 progeny or may appear after several generations (Edmands 2007). Since outbreeding is a pollinator-driven mating strategy, it is evident that pollination failure may result in reproductive decline. Outbreeding has emerged as a complex phenomenon which remains less understood than selfing. For instance, when a pollinator visiting a hermaphrodite flower carries mixed pollen – pollen from several different sources – outbreeding performance is poor. This is because self-pollen, pollen from close relatives (biparental inbreeding) and pollen from one or more unrelated relatives, results in dichotomizing pollen pool into self versus outcross pollen (Karron et al. 2006). This effect becomes magnified when pollinators move only short distances and the pollen carryover is limited. Pollination in such cases with mixed pollen may affect non-self but inbred matings (see Mitchell

et al. 2009). How multiple paternity from pollen pool works in natural populations to maximize offspring fitness needs to be worked out. It will be equally rewarding to determine the ecological conditions that tend to promote biparental inbreeding. Such aspects of outbreeding need to be investigated.

11.13 Inbreeding and Reproductive Assurance (RA)

Though IBD impairs the benefits of selfing, RA is a benefit which plant derives upon self-fertilization. Reproductive assurance is an outcome of seed set even when pollinators and/or potential mates (pollen grains) are scarce. In plants, RA is likely the principal driver of the evolution of autonomous selfing, i.e. self-pollination without the assistance of pollinators (Baker 1955; Jain 1976; Lloyd 1980; Yahara 1992; Busch and Delph 2012; Shivanna 2015). According to Schoen and Brown (1991), Lloyd (1992) and Schoen et al. (1996) in cases where cross-pollination remained limiting due to scarcity of pollinators, selfing could be selected in spite of strong inbreeding depression. Whether RA is a major driver for the evolution of selfing, as first proposed by Darwin (1896), remains inconclusive. It is argued that selfing may not provide RA every year in every population. Also only autonomous selfing can provide these benefits and not geitonogamy. Whether or not the benefits of RA by selfing get diluted if the progeny is inferior to the outcrossed ones also needs to be studied. Further, selfing, which is considered a plant's way to avoid extinction in an impoverished pollinator environment, is somewhat inconsistent with perspective of Stebbins (1957), who considered selfing, an evolutionary dead end (Takebayashi and Morell 2001). Recent studies suggest that while evolution of selfing may optimize population persistence in the short term, it might be damaging to the lineage in the long term (Igic and Busch 2013).

On the other hand, outbreeding assures genetic variation. It has become an important tool with reproductive ecologists and plays a significant role in restoration of ecosystems and breeding of crop species. The 'seed mixes' required for restoration are obtained through polycross breeding. It is carried out under controlled conditions to produce synthetic varieties through random open pollination and is being adopted by breeders around the globe (Box 11.4).

BOX 11.4: Polycrossing for crop species

Year 1: The source nursery is raised with population of clones by planting several thousands of plants assembled from many sources. It provides a broad genetic base of the clonal lines for selection.

The germplasm in the nursery is screened and evaluated to identify superior individuals according to the breeding objectives.

Year 2: Clonal lines are raised with about 100 to 200 superior plants that are selected on phenotypic basis to multiply clonally. A clonal line nursery has about 20–25 plants derived from the same parental line.

The breeder may impose various biotic and abiotic selective pressure to aid in identifying about 25–50 most desirable clones.

Year 3: Polycross nursery has selected clonal lines which are maintained to generate seeds for progeny testing. Here each clone is pollinated by a random sample of pollen from all the other entries. A method of layout to achieve this objective is a square plot in which every clone occurs at least once in every row.

Seeds from each clone are harvested separately.

Year 4: Polycross progeny test is carried out in which seeds are harvested from the replicated clones and bulked for planting progeny rows for performance evaluation.

The top performing 5–10 clones are selected for inclusion in the synthetic cultivar.

Year 5: Syn-0 generation. The selected clones are vegetatively propagated and randomly transplanted into an isolated field for cross-fertilization to produce syn-0 seed.

Year 6: Syn-1 generation. The syn-0 seed is increased by planting in isolation. Equal amount of seed is obtained from each parent and mixed to ensure random mating in the field. Bulk seed is harvested from seed increased in syn-1 generation that may be released as a commercial cultivar provided sufficient seed is produced.

Year 7: Subsequent syn generations. Frequently, the syn-1 seed is not sufficient to release to farmers. Consequently, a more practical synthetic breeding is carried out to produce a commercial lot.

11.14 Cost of Sex

Plants are programmed to express their sexual preferences in several ways. Environmental cues, available energy resources and physiological status help plants to decide when to flower and undergo anthesis, how to orient their sex organs, how to advertise their assets to the pollinators, and much more. Plant also decides the organization and synorganization of flowers and pollinator visitation. The main aim

of all adjustments by the species is how to maximize their reproductive fitness and at what cost?

It is evident that there is an inherent resource cost of flowers. Floral organs draw carbon, nutrients and water from the vegetative portion of the plant not only during initial growth period and expansion but continuously over the flower's life span. In extremely resource-limited environments, resource allocation to floral display can be costly in terms of future growth and survival. Even under less extreme conditions, flowers vary in resource cost which affects plant reproductive success.

Sexual reproduction carries a cost which is evident from the fact that most plants need to achieve a minimum size and a specific number of leaves for reproduction. According to Lloyd (1984), three different types of costs of reproduction are incurred – (i) costs required for construction of structures related to reproduction or the acquisition and storage of energy for reproduction constitute the *production costs*, (ii) *fixed costs* that are incurred only once before embarking on reproduction and are not added subsequently (e.g. the production of peduncles and pedicels) and (iii) costs involved in offspring production. There appears to be a trade-off at the physiological level of resource allocation to either vegetative growth or reproduction.

11.15 Mating Choices: Challenges Ahead

As a result of anthropogenic activities, invasion by non-indigenous competitors, land abuse, habitat destruction and fragmentation and climate change, many plant and animal species have lost their natural habitats or have become restricted to small and isolated populations (Saunders et al. 1991; Jennersten et al. 1992; Aizen and Fein-Singer 1994). The plant populations face several challenges such as increased risk of pollinator limitation, increased inbreeding depression and the erosion of genetic diversity through genetic drift, all indicative of reduced reproductive fitness. They are also vulnerable to mutational meltdown which is accumulation of mildly detrimental mutations (Lande 1994). The plants have in general responded to these pressures by making several adaptations in all reproductive traits that are significant in perpetuation of the species. As outcross pollen limitations have arisen due to disruption of plant-pollinator interactions, there has been a shift towards self-fertilization especially autonomous type. Occasionally, the traits such as autonomous selfing initially appear to be adaptive, but studies prove that these are only short-term adjustments. On long-term basis, many shifts turn out to be maladaptive, decreasing fitness and consequently evolutionary suicide of fragmented population (Jacquemyn et al. 2012).

In face of various pressures from anthropogenic activities, the populations have to make a hard choice to either inbreed to face IBD or outbreed and become vulnerable to pollination failure and outbreeding depression. Since pollination is the first important stage through which plant genes 'move', it is important that plants outbreed. Outbreeding depends on pollinator visitation and may not operate if pollinators decline.

Pollination failure, a prominent consequence of disturbed and fragmented ecosystems, is manifested in various forms – pollen delivered to the stigma may be too little, too much, too late, too mixed in composition or too poor in quality. Though pollination mechanisms operating in disturbed ecosystems have been studied to a large extent, relatively little attention has been paid to the potential evolutionary consequences on plant mating systems in the populations (Eckert et al. 2010). Whether a particular species would adopt selfing or outbreeding or mixed mating in degraded habitat to achieve reproductive fitness cannot be generalized and has to be investigated in each population. Reproductive ecology focuses on the total reproductive fitness and natural selection of sexual traits in a population and measures reproductive success through male and female functions. The spatial and temporal parameters, in which plant reproductive diversity is being investigated, have broadened substantially through new techniques of genetics and molecular biology. As a result of advancements in field of reproductive ecology, evolutionary transitions are the focus of considerable research being carried out world over (Barrett et al. 2009). However, it still remains to be predicted, which evolutionary trajectory, a population or species will take when its pollination environment becomes critically disturbed (Hardr and Aizen 2010). After populations have become fragmented, particularly in long-lived species, changes in floral traits and flower's investments would also become significant as will the mating system. A relatively long period of time may be required for significant floral adaptations to become detectable. When pollinator abundance and pollinator limitation vary in space and time (Goodwillie et al. 2005, Eckert et al. 2010), plants may prefer a mixed mating system (Fig. 11.8). Under such scenario, establishment of a delayed-selfing mechanism may be most favourable, as it increases female fitness with little seed or pollen discounting (Kalisz et al. 2004). Concern has been raised that in extreme cases, plants may lose their capacity to reproduce sexually and only regenerate by vegetative offspring. Thus, a balance may shift in favour of clonal reproduction (Rossetto et al. 2004a, b), leading to homozygous population, ultimately resulting in a degeneration of life history traits associated with sexual reproduction (Eckert 2002).

Another important aspect of plant sexual systems is that plant-pollinator interactions do not occur in isolation, and if any change in pollination environment occurs, it affects the reproductive fitness of the plant. Restoring pollinator assemblages is essential for ecosystem restoration (Dixon 2009). In such degraded habitats if the pollinator nesting places are created, the decline in pollinator population may be checked. Besides, it is also important to consider that consistency of abiotic factors (wind) is greater than that of biotic pollination as the availability of animal pollinators can vary widely between sites and seasons.

In nature, each population exhibits depending upon time and space, one of the breeding systems only: self, mixed or cross. It may evolve into different system based on environmental constraints and genetic flexibility, and the entire process may take a long time through many generations. Since the plants expend a great proportion of their energy and resources towards sexual reproduction (cost of sex), they prefer free choice of mating under given environmental conditions. However,

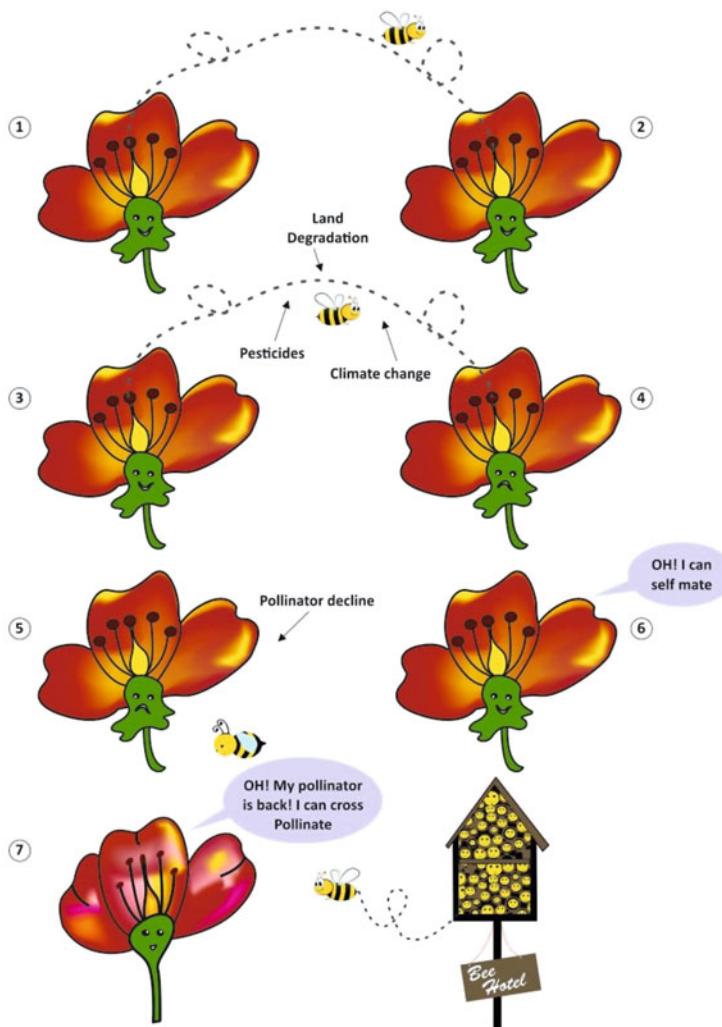


Fig. 11.8 Bisexual flower's thought process towards mode of pollination. I can cross pollinate and, in the absence of pollinators, can self-pollinate. However, man can make bee hotels to get back my pollinator and I revert to outbreeding!

the final decision of the plant on choice of mating is governed by the fitness consequences of outcrossing and selfing reproductive mechanisms.

As the anthropogenic activities continue to degrade ecosystems, it has become critical that alternatives are worked out to augment pollination services in both wild and captive populations. One possible solution to this problem is to infuse the collapsing population with individuals from a genetically and demographically fit population. Another solution which perhaps is time consuming is to carry out intentional hybridization. This is gradually becoming popular in fragmented

habitats. It would save a population from inbreeding depression, (conversely, it might also induce outbreeding depression!).

In nature, plants can increase or decrease their reliance to pollinator or adapt to autonomous selfing or reinforce interactions with pollinators. This can be accelerated if natural areas are restored and new breeding sites are created for pollinators and ‘pollination environment’ is improved. Places like highways and roadsides can be converted into microhabitats with framework species. To derive maximum benefit from such sites, it is important to keep the vegetation pesticide free. This is in light of the fact that second threat to pollinators is from pesticides, first being habitat destruction.

In most of the mating systems, plants spend a large fraction of their resources in producing offspring. They ‘advertise’ their reproductive maturity through floral traits, give discounts to pollinators by accepting specific and generalized pollinators and even extend ‘sale’ (end of season), i.e. delayed pollination of their products. All these efforts are channelized simply to perpetuate. In spite of these adaptations, if the plants are forced to abandon sexual reproduction due to anthropogenic damage, the plants will be left with no other choice but to ‘move’ on to asexual modes which would prove fatal in the long run.

11.16 Conclusion

Plants were for a long time classified as either inbreeders or outbreeders, but the sexual flexibility during evolution has given rise to systems which do not readily fit into either type. According to Eckert and Herlihy (2004), plants exhibit tremendous variation whether mating occurs via self-fertilization versus outcrossing, and many species practice a mixture of both. A shift from outbreeding to selfing and back has been frequently observed in several populations. Researches have indicated that in face of climate change, selfing would increase. A change in flower development has also been indicated in certain cases. One alteration is reduction in stigma-anther separation that reduces chances of herkogamy as in *Mimulus guttatus* (van Kleunen 2007). Decline in pollinator species and reduction in flower size are some of the common changes that would encourage selfing. In nature, there exists interplay of these mating systems, and the sexual preference in a population is governed by environmental pressures. However, outbreeding remains the significant mating system to permit introduction of genetic variations in a population. Outbreeding is desirable in degraded ecosystems, and the concept of seed mixes is picking up fast. Polycrossing has gained momentum in restoration programs, while small isolated populations can be saved through genetic rescue which again involves outbreeding. However, outbreeding is constrained by outbreeding depression or reduced offspring fitness from mating between two genetically dissimilar plants. Outbreeding has been adopted by populations ever since the origin of flowering plants. New vistas have opened up in our understanding of outbreeding packages in present-day context of mixed-mating systems, and it is pertinent that new tools are used to study the mating system. We also need to evaluate how the population demography in perturbed

habitats would change with and without pollinator-driven mating systems. This will help us understand the mechanisms that govern floral architecture, nutrient allocation and offspring fitness in plants with variable sex choice. We also need to augment performance of outbreeding system in rare and endangered species where the risk of pollination failure and outbreeding depression is high. For expanding our understanding on mating systems, we need to investigate the buffering action of multiple mating strategies in a given population. Moreover, the taxonomic diversity of pollinators in range of morphology, sensory, modality and behaviour displayed is to match the variation in flower form. While the advantages of selfing to a plant species cannot be ignored yet, the significance of outbreeding in bringing genetic variations cannot be undermined. It is only genetic variations that determine the evolutionary life span of a species.

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Sexual Selection in Angiosperms: Paradox Re-visited

12

Veenu Kaul and Madhu Raina

Abstract

The theory of sexual selection, proposed by Darwin, explains the evolution of sexually dimorphic characters in animals. The idea was and is still being used to elucidate the exaggerated sexual traits in male animals. He claimed females to be better in making the reproductive investments for offsprings than males. This made him suggest strategies wherein females choose the best fit males and males compete among themselves for mate acquisition. Today the concept has been developed extensively, and various other sexually related traits have been suggested to evolve in the same manner. By highlighting and merging the frameworks of sexual selection envisioned by various researchers from time to time, the present chapter discusses how sexual selection can occur in plants even though individuals do not make a direct interaction. Traits influencing export and reception of pollen in both hermaphrodites and dioecious taxa affect pollination which is likely to influence mate acquisition. In many instances phenotypes facilitating pollen export are in harmony with those that enhance pollen receipt. However, the reverse can also be true. This suggests that visitation rate of pollinator(s) limits both male and female functions in same or different directions. The present chapter summarizes the theory of sexual selection and attempts to review the conceptual developments in plants using evidences from different fields. The theory drives fundamental evolutionary processes such as trait elaboration and speciation including plant and floral morphologies. The empirical findings concerning potentially affected traits are discussed. At the same time, care has been taken to address the criticisms fairly.

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Keywords

Sexual selection · Mating success · Reproductive fitness · Mate acquisition · Export · Receipt · Competition · Strategy sexual conflict · Natural selection

12.1 Introduction: Historical Perspective in Brief

Charles Darwin has many firsts to his credit. A naturalist par excellence given the fact that the theories promulgated, observations made and evidences provided by him in the nineteenth century hold their ground firmly even today after more than 160 years. He was the first to conceptualize sexual selection in his classic book *On the Origin of Species by Means of Natural Selection* (1859), but the work along with extensive evidence provided by him materialized into another master piece entitled *The Descent of Man and Selection in Relation to Sex* (1871).

This theory was put forth to explain the possible significance and evolution of exaggerated, highly ornamented and/or flashy traits in males of several animal species. The theory was quite convincing in explaining the selection of these traits in animals, but Darwin did not explain its applicability to plants. The theory attempted to solve an apparent paradox – how characteristics that reduce survival can evolve. Although Darwin dedicated a chapter to secondary sexual characters in ‘lower classes of animals’, particularly the hermaphrodites, he did not apply sexual selection to them. He could not fathom how their ‘imperfect senses and low mental powers’ could make them appreciate each other’s beauty or even indulge in rivalry. It is, therefore, not surprising that he did not include any such chapter on plants.

12.1.1 Darwin on Sexual Selection

Darwin faced several challenges while forwarding the theory of evolution through natural selection. This theory operates by increasing the individuals carrying traits (genes) that bestow greater survival and reproductive advantage in relation to others of that population. Thus, the evolution of adaptive traits that confer greater survival to organisms is very well explained by this mechanism. In that case what would be the fate of those traits which are obviously detrimental to the very survival of the organisms? These pertained especially to the evolution of sexually dimorphic characters in animals often brilliantly colourful and conspicuous, such as bright plumage in birds, tusks of the male elephants, elaborate antlers in the male ungulates and so on. Together with elaborate and exaggerated ones like songs, odours, calls, ornaments, behaviours, etc. present in one sex only, these are likely to impose constraints on their survival ability and make them more vulnerable to the predators. As such natural selection should not favour the individuals bearing them and those would eventually get eliminated. Thus being costly in terms of resource and energy investments and with no contribution or apparent advantage to the bearer of these traits, Darwin’s theory could not easily explain their evolution as simple ecological

adaptations. Aware of this paradox and with deep insights into the problem, he approached it with a beautiful explanation christened as sexual selection theory. While some believe it to be a form of natural selection (Willson 1994) others consider it to be a distinct force (Wilkinson et al. 2015). It revolves around the principle of same-sex individuals competing for access to potential mates and opportunity to sire/fertilize the partner. Traits as per Darwin (1859, 1871) that could increase the number of mates would be favoured and selected as they would compensate for any negative effect on survival of offspring exhibiting these traits. He believed that the long colourful tails of peacocks were favoured and selected because males with longer and more colourful tails were preferred by the females. It, therefore, seemed a barter between the survival cost and increased mating success. Darwin (1871) considered male sex to be comparatively more affected by sexual selection, particularly in the light of evolution of display (Skogsmyr and Lankinen 2002). At the genetic level, gene(s) presumably responsible for such traits would have increased fitness on account of female preference. And notwithstanding the survival costs, it would spread in the population and get fixed over a period of time. It is, however, pertinent to mention that primary sexual characters like the differentiation and development of sex organs (ovaries, testes/androecia, gynoecia) were not included under the purview of sexual selection by Darwin. Clearly, therefore, ‘not all selection related to reproduction constitutes sexual selection’ (Jones and Ratterman 2009).

On the whole, the theory of sexual selection was not received as warmly as that of natural selection. The acceptance also came quite late for a number of reasons. First and foremost, among them was the idea of females exercising their choice in mate selection. The concept was not acceptable being in direct conflict with the male biased Victorian society at that time. Second was the premise of the choice being conscious. The deliberate choice or preference was not approved by Darwin himself since ‘conscious’ thinking and/or reasoning was unique to humans. Though he was convinced about the occurrence of female choice, but was not sure with the underlying mechanism. Third was his concept of competition taking place within species which usually was thought to occur only between species. It was even harder to expect it between sexes as well. Intra-sexual selection was still understandable because males as better competitors were evolving capacities to take on those of other species even predators, but inter-sexual selection was a counter-intuitive idea.

The underlying basis of making empirical studies on the essential features of sexual selection theory that could capture its complexity could not be clearly delineated until a century after Darwin gave this concept. And the reasons were lack of proper computational procedures. This need ultimately resulted in the development of various mathematical models. The proponents of these models described the mechanism responsible for sexual selection using leads from their own field of study and expertise. Difficulty(ies) arose on account of the sexual selection being a part of the natural selection on one hand and apparently contraindicative to its basic premise on the other. This confers paradoxical epithet to sexual selection theory and makes it more complicated.

Later, a progressive development of thought process on sexual selection led to the growth of an altogether different perspective on this premise. This formed a sound basis for studies of trait evolution in hermaphroditic animals. Subsequently, the thought of applying it to plants became prevalent in the late 1970s (Charnov 1979; Willson 1979; Queller 1983; Morgan 1992, 1994) but was met with diffidence and dissent. Acceptance of sexual selection being operative in plants has been rather too slow. Over time, the reproductive processes in animals have been increasingly recognized to show a parallel pattern in plants at least in some if not all traits and/or behavioural responses. And this was more obvious in outcrossing species. Considering that majority of the angiosperms are hermaphrodite (Yampolsky and Yampolsky 1922), this has taken a substantial time to achieve. In a symposium aimed to focus exclusively on comparisons of sexual selection in plants and animals, Stanton (1994) urged that ‘some colleagues and reviewers continue to view the application of sexual selection thinking to plant reproduction with deep suspicion’. It can be gauged from the rather miserly usage of the term ‘sexual selection’ in the published literature (see Willson 1994). According to Grant (1995), secondary sexual characters ‘are the defining features of sexual selection’, and since plants lack such characters, he questioned whether a similar argument could apply to both animals and plants. And this is despite the fact that a comprehensive review on secondary sex characters was made by Lloyd and Webb as early as 1977. Although an increasing number of studies governing these issues in plants have been published from time to time, the debate is yet to be settled (Lovett Doust 1990; Arnold 1994a, b; Willson 1994; Grant 1995; Richards 1997; Marshall 1998). Therefore, different ideas are under constant re-evaluation; no consensus seems to have emerged yet on how sexual selection works, what it should include and what are its effects and so on. As a result various concepts on sexual selection mirror this confusion (Skogsmyr and Lankinen 2002). Nonetheless, with huge amount of empirical work carried out in this direction coupled with the development of mathematical models, the theory of sexual selection has blossomed into an active field of evolutionary biology. Consequently, this process has been recognized as an evolutionary force powerful enough to drive trait elaboration and/or diversification within and among species (Andersson 1994; Wilkinson et al. 2015) that gears up the rates of molecular evolution (Swanson and Vacquier 1995, 2002; Aguade 1999) and thus promotes speciation (Kraaijeveld et al. 2011; Panhuis et al. 2001; Ritchie 2007; Servedio and Burger 2014).

12.2 Modern Perspectives

Sexual selection operates in two forms. Intra-sexual selection explains the evolution of traits responsible for generating male-male competition for gaining exclusive or preferential access to females, the latter remaining passive. Inter-sexual selection, on the other hand, is driven by females’ preference for the most colourful or conspicuous males.

That females exercised their choice or had preference with apparently no direct benefit was addressed to by Darwin as not conscious deliberation but an unconscious choice. This fuelled a difference of opinion between Darwin and Wallace because the latter believed it ‘implied conscious thought by females’ (Mota 2009/10).

Modern theory couples Darwin’s original ideas with recent insights that competition and choice can operate post-fertilization/mating (see Pischedda and Rice 2012). Sexual selection arises from the differences in reproductive success caused by competition for mates or their gametes when individuals with some trait variants outsmart members of the same sex, either directly by aggression or by virtue of being more attractive to the opposite sex (Darwin 1871; Parker 1970; Arnold 1994a, b). These processes may ultimately lead to the evolution of sexually selected traits, especially in the male, leading to increased attractiveness, such as vibrant colouration or dynamic courtship behaviours or exaggerated body modifications, or increased competitiveness through enlarged body size, weapons or armour (Andersson 1994). It is, therefore, gender biased and usually strongest in males (Janicke and Morrow 2019). While females committed for parental care lose mating opportunities, males tend to sire as many females as possible and, therefore, compromise on parental care. By doing so, they increase their respective fitnesses. Female reproductive success, therefore, is limited by the availability of nutrients. In males on the contrary, it primarily depends on the availability of productive females (Bateman 1948; Trivers 1972; Queller 1983; Skogsmyr and Lankinen 2002). These differences form the underlying basis of sexual selection and often end up in sexual conflict.

12.3 Models

Sir Ronald Fisher (1915) promulgated the entire issue of sexual selection with mathematical modelling based on the genetics of the traits concerned. A classic contribution popular as the Fisher process or Fisherian runaway model, it considers that the ornament in males and preference for that ornament by females would evolve if it were genetically controlled and hence inherited. That means ‘females could carry the gene for preference and their sons the preferred trait’ (Mota 2009/10). Support in favour of this comes from the fact that such structures and behaviours often differ conspicuously among males within populations, and between closely related species, female preferences for these male characters vary only occasionally (Gray and Cade 2000; Brooks 2002; Grace and Shaw 2011; Oh et al. 2012). This could result in a runaway process since both the trait and the preference are subject to positive selection and would increase rapidly, concomitantly and in geometric fashion (Andersson 1994; Mota 2009/10). ‘That female choice was a very powerful selective force acting on male traits was demonstrated unquestionably by Andersson in 1982’ (Mota 2009/10). Nevertheless, female choice in various other instances cannot be per se a result of sexual selection. Instead, it is deemed to be a result of natural selection acting on offspring quantity and quality. If, however, the sexual selection involves competition over the quality as well as quantity of mates, then

female choice is an important component of sexual selection (Skogsmyr and Lankinen 2002). Several authors disagree with the term ‘quality’ since it might be misinterpreted with the effects of fecundity selection (Arnold 1994b).

Following Fisher’s model, scientific thought in this direction got serious and gained momentum only in the late 1970s. Till that time his ideas were received with a lot of scepticism and caution. Understanding of the Fisher process received an impetus when models based on population (Kirkpatrick 1982) and quantitative (Lande 1981) genetics were framed. Two more based on invasion analysis or adaptive dynamics (Pen and Weissing 2000) and individual-based simulations (Fawcett et al. 2007) were added later. Together these have revealed the efficacy of this process, but each is beset with advantages and limitations. These have been beautifully portrayed in Kuijper et al. (2012) wherein probable methods of overcoming the limitations have also been delineated.

While enlisting several reasons of the complexity of sexual selection models over the standard natural selection, these researchers have reviewed the various approaches put forth to fully conceptualize, capture and measure the relevant aspects of sexual selection. Many workers, however, do not agree to dichotomize the natural and the sexual selections (Willson 1979, 1994; Moore and Pannell 2011). According to Kuijper and his co-authors (2012), natural selection models are based on simplifying assumptions of asexual reproduction and random mating involving single fitness component, being equilibrium oriented and excluding multi-locus genetics, sex differences and associated intricacies. Sexual selection, on the other hand, is based on sexual reproduction and non-random mating, co-evolution of and associations between preferences and traits, several fitness components and their complex relationships, sex differences and their dynamics and many more.

12.4 Evolution of Female Choice; Costs and Benefits Thereof

To explain the mechanism by which female choices evolved, discussions on several related aspects have been consolidated into models. These are based on the costs involved and the benefits accrued thereupon. The following statements by Kuijper et al. (2012) summarize the cost and benefit surmise of the sexual selection process.

Females can benefit directly from expressing a preference, if the resulting choice of mates gives them a higher viability or fecundity than that without preference (Moller and Jennions 2001). Or, particular choice of mating partners may lead choosy females to have offspring with a higher reproductive value (Kokko et al. 2002), e.g. through attractive sons (Fisher 1915) or offspring of higher intrinsic quality (Zahavi 1975, 1977) in which case benefits are indirect.

In the opinion of some, female choice can evolve in the absence of any benefit (Kuijper et al. 2012 and references therein). Accordingly these are named so and are briefly described below:

- (A) *No benefits model*: This pertains to the evolution of female choice in the absence of any benefit to the choice maker. It invokes the sensory system like neural networks which impose female preferences.
- (B) *Direct benefits model*: The evolution of females' selectiveness is most easily explained by this model if females get directly and immediately benefitted from this feature. This can be in terms of 'parental care or nuptial gift or territory defense' (Jones and Ratterman 2009). Many arguments and counter-arguments have been provided in this direction (Price et al. 1993; Jones and Ratterman 2009; Ratikainen and Kokko 2010; Alonzo 2012; Kuijper et al. 2012; Tazzyman et al. 2012).
- (C) *Indirect benefits model*: In essence females are the recipients of only sperm from males. So how or what benefits accrue to them from this choice are not at all obvious. Whatever benefits if at all there are, some are neither immediate nor measurable. Instead it is the quality of her offspring that matters; this quality in terms of higher fitness is maintained if the female has paired with the preferred male. However, being selective or exercising choosiness is costly since the search for mates can expose the female to dangers or she may end up in remaining unfertilized (Kokko and Mappes 2005). That is why the female choice as the driver of sexual selection is considered to be relatively rare (Kirkpatrick and Barton 1997; Cameron et al. 2003). Jones and Ratterman (2009) further divided it into three broad categories (Fisherian, condition-dependent indicator and condition-independent indicator models).

Others like game theory (Maynard-Smith and Price 1973), handicap genes (Zahavi 1975, 1977), healthy males (Hamilton and Zuk 1982), good genes (Kodrick-Brown and Brown 1984) models, etc. are also there, but we would like to restrict till here only.

12.5 Sexual Selection in Plants: Interpretation, Understanding and Operational Features

Plants being sedentary are incapable of directly choosing their mate. Many constraints thereby make them less likely evolvable through these modes of selection (see Schaffner 1927). A few major constraints are sex-related features and predominant hermaphrodite sex expression. Secondary sex differences initially thought to be lacking are subdued and less conspicuous. Perhaps the selective forces producing such differences in animals are probably of less importance in plants. As already mentioned, majority of the plants are hermaphrodites with both the sexes present in the same individual, and traits are most often shared between the sexes. Therefore, their fitting into the conceptual framework of sexual selection seemed less likely. The application of sexual selection to plants gathered momentum in the late 1970s and thereafter (Charnov 1979; Willson 1979; Queller 1983; Stanton et al. 1986; Arnold 1994a, b; Charlesworth et al. 1987; Moore and Pannell 2011; Cocucci et al. 2014; Lankinen and Green 2015; Marshall and Evans 2016; Alonzo and Servedio

2019). Interpretations made on sexual selection in animals translate into the following on plants:

- (a) Female reproductive success is limited by the availability of resources (not pollen), while that of male is limited by pollinator visits.
- (b) Since pollen production (by males) is considerably higher than the ovule number (by females), competition ensues among males for success in siring.
- (c) The strongest sexual selection favours the sex which has the ‘strongest dependency of fecundity on mating success’ (Arnold 1994b). A complete understanding of this requires knowledge of the relationship between fecundity and mating success. Not one but many relationships exist; these are diverse, and assume many forms like linear, asymptotic, curvilinear and diminishing returns; and determine how the number of mates translates into number of offsprings. These help in measuring the intensity of selection on male and female mating successes and also the part of selection that operates on ‘any sexually selected trait’. Besides establishing the link from the trait to mating success to fitness, the total selection on a trait can be worked out by multiplying the selection coefficients that lie along this link.

Sexual selection in plants can be expected to operate when the asymmetry in resource investment to the offspring exists among the maternal and paternal counterparts (Tejaswini et al. 2001). While a major portion of resources is contributed by the female parent for the development of the seed, the male parent contributes limited energy present within the confines of a pollen grain. Primarily a female expends resources in the formation of a large number of showy flowers and other conspicuous features to lure legitimate pollinators. However, only a few of them ultimately turn into a fruit and contribute to the next generation (Willson 1979; Willson and Price 1977; Udovic 1981; Stephenson 1981; Bertin 1982; Mohan Raju et al. 2001). Thereafter, females spend energy on endosperm formation, the development of seed coat and/or even packaging seeds in the pod/fruit. Subsequent development inside the fertilized ovary is externally manifested in the fruit. Energy/resource expenditure by the maternal parent further extends to acquire various morphological adaptations in fruits and/or seeds to enhance dispersal thereof.

Thus, female expenditure is comparatively more which makes them become choosy over males, while males which spend less become competitive to produce as many males as possible. Females are also programmed to expend energy on maintaining various attributes of stigmatic structures to cleverly trap pollen in wind or water. These constraints are quite difficult to recognize and even escape attention in a bisexual flower. The unisexual ones in both monoecious and dioecious taxa display these differences relatively prominently (Sutherland 1986). A distinct asymmetry in the investment of resources emerges thereby.

12.6 Sexual Selection from the Perspective of Hermaphroditism

As mentioned before, majority of the flowering plants are hermaphrodites or bisexuals. A long-standing confusion prevails about the correct concept of secondary sexual traits in plants (Grant 1995; Richards 1997 but see also Lloyd and Webb 1977) even though flowering displays in many taxa are elaborate, ornamental as well as showy and often comparable to that in animals. Some believe that sexual selection is not important in hermaphrodites since traits are shared and investment in one reproductive function limits that in the other (Charlesworth et al. 1987; Grant 1995), while others consider it a major force in the evolution of floral diversity (Moore and Pannell 2011). For example, floral elaboration helps not only in receiving pollen but also in dispersing pollen through pollinators. Thus, floral traits and designs successful in luring as well as rewarding pollinators resulting in the accomplishment of pollination and subsequent fertilization must have been favoured and selected. Since both male and female functions are being influenced, the co-evolution can be the result of natural rather than sexual selection. However, it is not always the case since mating success can be regarded as a ‘direct function of the amount of pollen exported or received’ (Delph and Ashman 2006). Individuals with relatively elaborate floral displays are likely to acquire more pollinator visits and are better pollen dispersers and acquire higher fitness through mating success than those with limited displays. This could possibly be true for female function also when seed production is limited by pollinator abundance.

In general, the morphology of the flowers in a hermaphroditic plant is said to be the outcome of several selection pressures, and sexual selection is presumably one among them (Armbuster 1996). Although difficult to understand, this relationship between floral display/exaggeration and sexual selection does exist. Studies reflecting these are not many. Morgan (1992, 1994) used the quantitative genetic model to highlight that key characteristics are crucial for sexual selection in dioecious and hermaphrodite species alike. As opposed to dioecious taxa, plants bearing bisexual (hermaphrodites) or unisexual (monoecious taxa) flowers mate as both male and female parents. Therefore, grabbing opportunities to increase mating success through either of these functions would delineate the sexual selection process. And Bateman’s principles (1948) would hold true here as well; the only difference would be the usage of terms (sexual functions and/or gender in place of sexes).

Further, the evolution of exaggerated male ornamentation to have a selective advantage is constrained in terms of the runaway process (Fisher 1915, 1930). Runaway begins with viability selection (costs of traits, costs of preferences; see Kuijper et al. 2012) and leads to the evolution of variation in the reproductive structures/strategies of both the sexes (Morgan 1994). This covariance decreases as the cost of producing trait increases in hermaphrodites. Contrarily, in species with separate sexes, the females can be the silent carriers of the genes for a trait and will not be expressing them. In that situation, only the males carry the cost of the trait. However, the reverse can also be true. The optimal value, therefore, differs between the sexes; expression of traits by females is governed by natural selection alone, while the males will be affected by sexual selection (however, see Moore and

Pannell 2011). In hermaphrodites, males will carry genes with negative effects on survival. Expressed in all individuals, these, in turn, will lower selection on female preference. Consequently, covariance between the male traits and female preferences will occur occasionally. As a result, the runaway process itself will become less usual in hermaphrodites (Skogsmyr and Lankinen 2002).

It can, therefore, be concluded that the evolution of hermaphroditism is intensely affected by choice and competition for mates. Even if runaway processes are expected to be rare in these organisms, there are quite a few examples of inflated sexual traits. A striking example is the long pistils in *Hibiscus* spp. which comprise of five hairy red stigmas that spread in five directions in search of pollen to accomplish successful pollination and fertilization (Skogsmyr and Lankinen 2002). The unusually long nectar containing spurs of Darwin's orchid is the ultimate example.

Further, in nature differentiation of flowers with pollination specific characters can be considered examples of exaggerated traits selected sexually for increased mate acquisition by females. These include, for instance, flowers with plumose or feathery often early maturing stigmas in wind pollinated taxa or odour and nectar producing flowers with showy colourful designs and patterns and designs coupled with odour and nectar production in animal-pollinated ones can be considered examples of exaggerated traits selected sexually for increased mate acquisition by females. In addition to these, numerous adaptations recorded in flowers can be interpreted to be sexually selected. One such trait as per Arnold (1994b) could be a specific quantity of pollen deposited on the stigma; large quantity covers more surface area and does not let pollen from other nonspecific individuals to germinate. Thereby, it also ensures that all available ovules will get fertilized (also see the next section). Experimental evidence in the form of supplemental pollination treatments carried out in many bisexuals serves this purpose. However, recent work cautions against drawing any pre-emptive inferences. This is largely because trade-offs occur between several life history components. Finite resources available to every individual are to be partitioned between its several organs/functions/life cycle stages. So, if supplemental pollination does increase the seed set per flower substantially, it would be at the cost of the overall reduced seed set. Similarly, failure of seeds to mature would lead to decreased seed sets pointing towards pollen quality being the limiting factor. Thus, both quality and quantity of pollen would be subjected to selection. Parallel to this are contrivances that allow for staggered strategies of pollen and seed dispersals and/or their respective germinations or of anther maturation and pistil characteristics (e.g. enantiostyly, flexistyly, heterostyly) that reduce the level of interference between male and female presentations. The evolution of the stigma up and down the sides of style in *Dalechampia*, delayed (*Anemone canadensis*) or extended (*Cleome viscosa*) stigma receptivity, and several other features are sexually selected that maximize the number of pollen donors competing to fertilize their ovules. Coupled with these are many underlying chemical and molecular interactions among pollen grains/tubes on the stigma and/or style or plant-pollinator relationships. All these adaptations highlight the interplay of sexual and natural selections operating in hermaphrodite plants.

12.7 Promising Contenders for Sexual Selection in Plants

12.7.1 Floral Display

Among various traits, floral display is believed to be the first to evolve through sexual selection (Charnov 1979; Willson 1979; Queller 1983; Stanton et al. 1986). To accomplish successful pollination, nature facilitated the flower-pollinator co-evolution. A classic example of exaggeration in floral traits and also of flower-insect co-evolution is that of the orchid (*Angraecum sesquipedale* Thouars) and its moth pollinator predicted by Darwin although not yet identified at the time (Darwin 1862; Wasserthal 1997; Moore and Pannell 2011). Famous as Darwin's orchid, its flowers are spectacular in bearing a 27–43-cm-long spur with 40–300 µl nectar that fills up to 7–25 cm of its base (Arditti et al. 2012). The hawkmoth (*Xanthopan morgani praedicta*) pollinator discovered 41 years after Darwin matched perfectly to his description. Thus, the close association between the floral spur and the proboscis length of the moth is one of the most striking and beautiful examples of co-evolution and trait exaggeration. While flowers (or plants) achieve maximum reproduction through pollinator attraction, pollinators with longest proboscis get a haul of food. The evolution of spur length in species of *Aquilegia* was shown to be 'punctuated' and driven by pollinator shifts (Whittall and Hedges 2007). Since spurs evolved in response to plant's adaptations to unrelated pollinators varying in tongue lengths, the selection can be regarded as directional.

Mating success in such taxa is constrained by the availability of pollinators. At this level, while competition between pollen donors (and recipients) is operational, the question of female choice to select the most competent male takes a back seat (Stanton 1994). Nevertheless, the quantity of pollen deposited could influence female choice at a later stage. The evolution of traits that improve the ability to gain access to mates is possible irrespective of who makes the choice, for example, pollinators in animal-pollinated taxa or the traits themselves. Both male and female sexes may gain a benefit in function if investment in the floral traits is given a priority, which thus could be selected through natural selection (Charlesworth et al. 1987). Despite seed production and pollination/fertilization-based studies being many, enough information on the effect of inflorescence size on the total fitness of an individual and on the offspring quality is limited (Skogsmyr and Lankinen 2002). Since a healthy offspring is the most credible advantage to the female against an increase in the size of inflorescence, it still remains an unanswered question which function is more important in the selection of inflorescence size.

12.7.2 Inflorescence/Floral Traits and Pollinators

Pollinators are usually attracted towards flowers on account of several floristic features. These largely include bright flower colour, shape, odour, abundant pollen and copious nectar production and size of an individual flower or that of an inflorescence (Pleasants 1981; Pleasants and Stephen 1983; Stanton et al. 1986;

Eckhart 1993; Queller 1987; Philipp and Hansen 2000; Delph and Ashman 2006). Still, this is not unequivocally true; literature puts on record several studies that have failed to show this relationship (Meagher 1986, 1991; De Jong and Klinkhamer 1994; Jones and Reithel 2001). In *Aralia hispida*, pollinator visitation rates increased with umbel size and number (Thomson 1988). The rate, however, declined per flower as the size of inflorescence increased. Such a behaviour of pollinators vis-à-vis their visitation rates per flower has been found in several other species also (Delvin et al. 1992; Harder and Barrett 1995). Apparently, visitation rate as a function of inflorescence size depends on the availability of pollinators. Limited pollinators will accrue a benefit compared to circumstances of high pollinator availability (Thomson 1988). Notwithstanding pollinator abundance, the author found that individuals compete for pollinations, although not to the extent that would increase the visitation rates per flower.

When applying theoretical knowledge to nature, it is equally important to consider environmental variability, which in essence gives an indication towards selection to be less precise. Presumably, it does not act critically on a single flower within an inflorescence, but on the overall size of an inflorescence, i.e. it rather distinguishes between large or small inflorescences.

Ramesha et al. (2011) measured style lengths of flowers belonging to 31 wild outcrossed and 3 self-pollinated domesticated species growing in the botanical garden of their university campus. Results obtained were interesting. In outcrossed taxa where conspecific pollen deposited on the stigma is diverse, skewed distribution of lengths was found towards the higher side, whereas no such trend was observed in self-pollinated ones since the pollen is usually of uniform quality. These findings are in line with the premise of style length being a sexually selected character (Lankinen and Skogsmyr 2001a, b). In *Passiflora incarnata*, Dai and Galloway (2013) found that floral traits are influenced by sexual selection through female reproductive success.

Narrowing down further, there are certain issues of pollen dispersal and inflorescence features. For instance, pollen transfer among different flowers of a single inflorescence due to the frequent movement of visitors (Harder and Barrett 1995; Rademaker and De Jong 1998) results in intra-plant self-pollen deposition. And a substantial pollen mass goes waste if the plant turns out to be cryptic or partially or fully self-incompatible (Raina et al. 2017) leading to shared losses by males as well as females. Such conditions should trigger gradual pollen presentation (Harder and Thomson 1989) or non-interference between male and female phases, resulting in the evolution of traits promoting this behaviour. Together with this, traits that ensure low pollen uptake per visit would be selected if it balances pollen loss with increased visitation rates. The competition among pollen donors on the other hand would favour the production of pollen grains in large numbers to ensure adequate and successful fertilization of accessible females. This would cause an enormous increase in the number of male gametes per female (Bateman 1948; Charnov 1979; Willson 1979; Bawa 1980; Lloyd and Yates 1982). These workers urged that since female function insofar as pollen reception is concerned is short, it indicates that the correlation between female reproduction and floral traits is less

stochastic, allowing for a more continuous selection of these traits. However, in times of pollinator scarcity, female function is likely to get affected more initially on account of limited choice. When pollinators become abundant, sexual selection will operate on males to present their pollen gradually. Ultimately, the traits of floral advertisement will respond more towards the selection of male than female function when pollinators are few (Willson 1994).

Floral advertisement seems more understandable in terms of natural selection rather than sexual selection as it helps pollen receipt (benefiting female) as well as pollen dispersal (benefiting male). However, sexual selection arises from differences in mating success, while natural selection is due to the variance in all other fitness components. In hermaphrodites the mating success depends on the balance between the shared advertisement costs and benefits that accrue through the effectiveness of male and female functions. Further one cannot exclude the possibility of both the sexes becoming choosy. The conditions that make this happen must involve simultaneous action of sexual selection on both the sexes. Whether this is the norm or occurs under some specific set of conditions needs detailed empirical studies (Jones and Ratterman 2009).

Flower number, their longevity and seasonal distribution maintained in many taxa like asclepiads, orchids, aquilegias, etc. are primarily selected to enhance male competition (Queller 1983; Peter and Johnson 2006; Cocucci et al. 2014). The argument put forth is that females are often resource limited and, therefore, any feature increasing the pollination levels should more often be that of male and such traits should be common (Queller 1983). Female reproduction through seed formation is limited more by resources than by pollinators (Willson and Price 1980). But under conditions of ample resource and low or no pollinator availability, pollen limitation would still set in. This would, in all probability, trigger sexual selection through female function by favouring floral features that accelerate pollinator visits and thereby pollen receipt (Dai and Galloway 2013).

Further, on the basis of their studies on four asclepiads, Cocucci and co-workers (2014) have demonstrated the horns of pollinaria getting involved in confrontation with those of others and also in direct physical interaction with the pollinators. While considering these as ‘botanical equivalents of horns’ in many animals, these authors suggest that ‘neither self-pollination nor well defined sensory perception are required for sexual selection to take place through intra sexual struggles’. Intra-sexual competition is more intense for the sex that is over-represented in the population linking sexual selection with sex ratio (Pen and Weissing 2000). That is, attractive males are most likely to produce male-biased sex ratio.

12.7.2.1 Sexual Selection in Dioecious Taxa

While elaborating upon sexual selection in animals, Darwin (1871) suggested that investment made by females on the production and care of offsprings is substantially maximum than that contributed by males. For this, the female ensures that her offsprings are sired by the most competitive male. She exercises direct choice over males by allowing them to compete among themselves. Conversely, males invest heavily in traits required to attract the female or impart aggressiveness and/or

competitive ability for mate acquisition. In either case, the females or males have to pay a price; in the former increased fertility balances increased mortality and in the latter increased mortality compensates for mating success (Moore and Pannell 2011). However, the expectation of intra-sexual competition and female choice in being among the causal factors of sexual dimorphism is seemingly default. Whether, how and when these processes lead to sexual dimorphism are interesting questions that have been and are still being debated at length. Further, whether these are the causes and/or drivers of sexual dimorphism in plants need elaboration and clarity.

In dioecious plants even though dimorphism does not contrast strikingly with that in animals, differences subtle or conspicuous are found between males and females. These exist in size, display, design, packaging, etc. of flowers, e.g. hemp, hops, *Hippophae* and others. Besides, the sexes often differ in a number of secondary sexual traits not directly related to reproduction like morphology, architecture and physiology of leaf and shoot, bolting time, susceptibility to pests, pathogens and herbivores, etc. The following case study beautifully exemplifies this proposition.

Species of *Leucadendron* R. Br. (Proteaceae) are dioecious, some pollinated by insects and others by wind. An extremely interesting feature exhibited by these is the gender-linked leaf dimorphism. Generally leaves borne by the females are larger; in *Leucadendron xanthoconus*, for instance, involucral ones are pale green while in males these are yellow which completely obscure green ones below. After flowering all turn alike in colouration. This has been considered analogous to the displays by male animals (Bond and Maze 1999). Males of this species are prolific flower producers, receive more pollinator visits and have increased mortality rate. Thus, the cost of display is compensated by more pollinator visits. Female mating success, on the other hand, was not correlated to display. Therefore, selective pressures operating on male and female inflorescence displays vary leading to key differences in their corresponding vegetative structures. This made Bond and Maze (1999) to conclude that vegetative dimorphism evolved due to sexual selection on male display.

Earlier, leaf dimorphism was attributed to indirect selection on leaf area, i.e. as an allometric response to differences in inflorescence number driven by male-male competition for pollinators (Bond and Midgley 1988). However, Midgley (2010) raised many queries about the secondary sexual dimorphism in leaf size and found the explanations provided hitherto ‘unlikely and unsupported’. He generated data on a specific leaf area and water-use efficiency from leaves of both the sexes of many *Leucadendron* species and found ‘minimal gender differences’. In the re-analysis of the previous works together with these results, he narrowed down to this vegetative dimorphism being a result of ‘selection on flower size and architecture’.

However, it is not necessary that secondary sex differences are always related to reproductive features or behaviours. In many cases these may simply be due to selection resulting in differential capacities of resource investment and utilization. These trade-offs could possibly lead to gender specializations as well. In the opinion of Moore and Pannell (2011), viability selection operating here should not be confused with sexual selection. They consider viability and fecundity selections

parts of natural selection as opposed to that of sexual selection by others (Kuijper et al. 2012).

Meanwhile, sexes in several sexually dimorphic taxa differ in their micro-environmental requirements and hence do not occupy identical niches. Further, males are earlier in most of the phenological aspects/traits and sometimes continue later than females as well. According to Darwin (1871), females spend less on vegetative growth/reproduction and more on sexual reproduction because of the demands of fruit production. This results in males spreading more and surviving better than females. He exemplified the statement with his observations on strawberry. However, Janicke and Morrow (2019) are of the opinion that stronger sexual selection is operative in males in that they make more resource investment on mate acquisition even at the expense of health and survival compared to females. This is likely to manifest in a greater risk-taking behaviour and accidents in men, which in turn would increase susceptibility to diseases and immune response. Studies that actually demonstrate and quantify increased siring success using genetic/molecular markers are the pressing need of the hour.

Interestingly, lineages often differ in the several sexually dimorphic organisms; while some are strikingly colourful, others are not. Examples from animal kingdom are numerous; those from flowering plants are limited. Then why is there a disparity? Does sexual selection operate on some of these populations only or on all but with different intensities? Answers to these questions are still un- or under-explored except for the taxa where sex reversal is well known. In those cases, however, selection acts more strongly on females than males. This also means that sex ratios and mating systems are closely linked to the intensity of sexual selection (Jones and Ratterman 2009).

12.7.3 Male-Male (Intra-sexual) Competition

Plants, compared to animals, are inactive, less dynamic and incapable of exhibiting various behavioural features. These, therefore, lead to questions like do males compete in gaining access to the females? If yes, what strategy(ies) do they adopt? Of the different forms of competition among male gametes, two major strategies are discussed below.

12.7.3.1 Overwhelming of Gametes

Pollination in nature is random. A stigma receives a wide variety of genetically diverse pollen grains that far outnumber the ovules waiting to get sired in the ovary (Levin and Berube 1972; Stephenson and Bertin 1983). In this race, severe competition exists among male gametes to gain access to the ovary and fertilize ovules thereof, a behaviour analogous to male animals. That intra-sexual competition results in ovules being differentially sired by pollen from diverse genetic donors has been experimentally proved by the paternity data analysis of seeds of *Chamelirium luteum* (Meagher 1986, 1991) and *Asclepias* (Broyles and Wyatt 1990). This results in non-random fertilization and has repeatedly been observed

in cotton (Iyenger 1938), lima bean (Bemis 1958), onion (Currah 1981), Douglas fir (Apait et al. 1989), *Hibiscus moscheutos* (Snow and Spira 1991a, b, 1996), *Vigna unguiculata* (Radha et al. 1993), *Raphanus sativus* (Marshall and Ellstrand 1986; Marshall 1991) and so on.

Temporal variation or distribution is relatively more complex. In *Croton bonplandianum* the increased production of male flowers during early stages of the flowering increases the fitness gained by the pollen. Later, when female flowers outcompete male flowers, the fitness accrued through fertilized ovules increases and that by pollen declines; the mother plant diverts maximum resources towards the female flowers (Uma Shaanker and Ganeshiah 1984). This, however, does not mean that male contribution stops or ends, implying that males are not only early but with extended longevity as well. These traits in many taxa are maintained to enhance male competition assuming that measures utilized are unbiased. Apart from various attributes like number, shape and size, the internal environment or constitution of pollen grains in various species exercises sexual selection in overwhelming their gametes. Many bird- and insect-pollinated taxa are known to have starch-rich pollen grains (Baker and Baker 1983) which ensure the attraction of a wide range of pollinators and in turn assure their large-scale transfer (Mohan Raju et al. 2001). Packing pollen grains in different structures like pollinia, polyads, etc. (Kenrick and Knox 1982; Queller 1983; Nilsson et al. 1992) is also viewed as an evolutionary outcome of intra-sexual competition among plants to offer gamete pool and get it transferred to the stigmatic surface. Using *Asclepias exaltata*, Queller (1983) confirmed the sexual selection to be operative on the male function only. In his opinion, selection on males alone drives the timing of flowering in such a way that constancy in flower-fruit ratio is ensured (see also Cocucci et al. 2014).

12.7.3.2 Gamete Elimination and Obstruction Strategy

As mentioned earlier, swamping the area with more pollen grains is likely to increase not only the competitive rates but also the probability of occurrence of females. This, however, does not guarantee the reproductive success. The competition among males may still continue on the stigmatic surface once pollen grains get deposited over it. In this ‘gametic war’, a plant that produces vigorous pollen may acquire reproductive fitness by more competent gametes tactically outgrowing others through the tissues of the pistil. Some pollen grains, for example, with more vigorous tubes may pre-empt the fertilization of ovules (Stephenson et al. 1988a; Radha et al. 1993).

Following its deposition on the stigma, the pollen germinates, penetrates the cuticle and then produces pollen tubes that grow towards the ovules to accomplish fertilization (Herrero and Hormaza 1996). The female organs can exercise their choice or preference at all these stages. In case of conventional self-incompatibility (SI), the stigma does not allow pollen to germinate and thus follows sporophytic SI. In gametophytic SI, pollen germination is smooth but the growth of pollen tubes gets arrested inside the style. Interestingly, the choice can be exercised after sperm delivery or even syngamy, resulting in late-acting self-incompatibility (LSI) (Raina et al. 2017). Therefore, the acceptance of male by the female is challenged up till the

post-zygotic stage. The screening can be cryptic as well. And the success of male gamete encompasses the cumulative effect of pollen germination, rate of tube growth and fertilization (Bertin 1988). A compatible male gamete having superior attributes than its counterparts would succeed in siring more progeny. Therefore, ‘pollen competitive ability’ is another level of male competition, exemplified by various taxa like *Dianthus caryophyllus*, *D. chinensis* (Mulcahy and Mulcahy 1975; Tejaswini 1999), *Zea mays* (Sari-Gorla et al. 1983) and many others. Similarly, pollen from diverse genetic (but conspecific) sources exhibits different competitive potentials in the style. In this struggle the most competitive one accomplishes fertilization, e.g. *Hibiscus moscheutos* (Snow and Spira 1991a, b), *Kigelia pinnata* (Raina et al. 2017), *Commelina benghalensis* (Kaul and Koul 2009), and many others. In certain cases, the competition among gametes prevails even after fertilization. An extreme case of this competition is reported in *Kleinhowia hospita* (Uma Shaanker and Ganeshaiyah 1989). More competent pollen grains modify the stigma after germination in such a way that pollen tubes arriving late in the style are inhibited to grow further. Selection, thus, operates via the two sex tracts with both mutually exercising their preferences.

12.7.4 Female Choice

Sexual selection through female choice in plants is quite plausible. However, evidences in support of this are quite few. Examples of female components, stigma, style and ovarian tissue inhibiting pollen tube growth are many and diverse. While this ensures higher fitness gains by the female in terms of its offspring being genetically superior, there is an associated risk of some remaining unfertilized. In animals, this kind of ‘Swayamvara’ is exercised in a range of intriguing ways. It mainly includes a dance competition among males like in peacocks, tug of horns as seen in ungulates and even archery skills in human beings, claimed to have occurred as a part of Hindu mythology during ‘Swayamvara’ of Seeta and Draupadi. Likewise, females in plants are known to have choices over males in equally unique but distinct ways (see also Skogsmyr and Lankinen 2000).

12.7.4.1 Strategy to Invoke Pollen Competition

The presence of various stigmatic and stylar mechanisms or modifications is known to impose a severe competition among male partners so that the best of them could gain access to the ovary and fertilize the ovule (Ganeshaiyah et al. 1986; Ganeshaiyah and Uma Shaanker 1988a, b; Uma Shaanker and Ganeshaiyah 1988). In *Ipomopsis aggregata*, the stigma is quite long and provides a large surface area for the deposition of a huge quantity of pollen (Campbell 1989, 1992). A longer pistil may, in turn, increase competition among pollen grains and allows only the vigorous ones to effectively traverse the style and bring about fertilization (Mulcahy 1979). *Kigelia pinnata* also bears an 11.2-cm-long pistil with thigmotaxis stigma which undertakes a subtle screening of pollen tubes, rejecting selfed ones post fertilization probably after perceiving them to be of low quality (Raina et al. 2017). Inflorescence

(thyrs) in *K. pinnata* is flagelliflorous, that is, flowers are borne on long rope-like branches that dangle beneath the crown. Since flowers open at night, this condition provides ample opportunity for exposing the male and female sex organs sufficiently and making them conspicuous to pollinators. Similarly and as stated before, most species of *Dalechampia* have stigmatic surfaces extending from the stylar tip down its sides (Armbuster et al. 1995; Armbruster 1996) such that pollen landing on the edges of the stigma has to travel a long distance to reach the style and the ovary. Also, in different ornamental plants like *Hippeastrum* and *Hibiscus*, the pistil bears a long style with prolonged stigma receptivity (Mohan Raju et al. 2001) invoking intense and extended competition. These modifications or strategies of the female organ ensure that the best pollen fertilizes the ovules. In *Talinum mengessi* pollen germination is reportedly delayed up to 2 h possibly invoked by the stigma (female tissue) in an attempt to generate sufficiency in the variability of pollen genotypes received by it (Murdy and Carter 1987). In certain entomophilous and anemophilous species, stigma receptivity is delayed (Herrero 1983; Douglas and Cruden 1994; Dahl and Fredrikson 1996). This mechanism paves a way for the stigma to allow and receive pollen from different sources and in large numbers before the start of the race. In essence, this synchronizes the growth of the pollen tubes with the increased opportunity for the recipient plant to make a choice.

It is well known that pollen grains require water to hydrate them and become metabolically active to produce a pollen tube (Marshall and Folsom 1991; Raina and Kaul 2018). The germination process is also pH dependent. These pre-germination requirements are controlled and manipulated in a subtle way by the female sex organ. For instance, in *Leucaena leucocephala* a pouch-like stigma in an un-pollinated pistil contains viscous fluid of pH 5.0. Pollen germination is subject to the receipt of an optimum or critical pollen load. Amount equal to or more than this critical increases stigmatic pH to 6.0, which supports and increases the rate and extent of pollen germination (Ganeshiah and Uma Shaanker 1988a, b). In *Incarvillea emodi* and *Kigelia pinnata*, threshold pollen (cross) deposition is required for the permanent closure of stigmatic lobes (Verma et al. 2008, 2009; Raina et al. 2017). The same is true of *Moringa* (Uma Shaanker and Ganeshiah 1990). The production of fewer ovules per ovary is another attribute that can be potentially selected to increase the intensity of competition among pollen (Snow and Mazer 1988). Ganeshiah and his co-workers (1995) have shown that variability in the style lengths in monoecious figs tends to optimize the ovipositor length in the pollinating wasps. This not only ensures which flower types (short-styled or long-styled) are available to the wasp but also regulates seed production in the plants.

12.7.4.2 Strategy of Favoured Decision

The stigma, the primary centre for pollen receipt and recognition, acts as a gated structure to facilitate or inhibit its germination (Dumas and Gaude 1981; Raina et al. 2017). Style serves as a filter to select the superior male. Interestingly, therefore, the female exerts her secondary round of selection once males ensure early pollen germination. Manifested via self-incompatibility or other mechanisms, this strategy of female recognition causes pollen from different sources to grow at different rates

(Brannes and Cleveland 1963; Schemske and Fenster 1983). The growth inside the style is further regulated by the nutrients and/or signals supplied by the female tissue which may be chemical (Linskens and Pfahler 1977), electrical (Linskens and Spanjers 1973), hormonal (Jensen et al. 1983), genetic (Hinata and Okazaki 1986) or biochemical (Sharma and Shivanna 1986). Irrespective of their nature, these signals are known to trigger the interaction between female and male components assisting in the selection process of females. Thus the role of male (pollen) in these interactions cannot be ignored outrightly.

12.7.5 Pollen-Pistil Interaction

It is only in the angiosperms that the sporophytic tissue permits and also facilitates germination of the right kind of pollen followed by the growth and development of the pollen tube inside the pistil. Studies carried on *Petunia hybrida* and wild radish have established that a pistil can discriminate between a desired and an undesired gamete only after attaining maturity. This means that male mating success is certainly subject to the tests of the pistillate environment, another choice maker's strategy (Mulcahy et al. 1982; Cruzan 1993; Marshall 1998).

12.8 What Does Sexual Selection Confer?

Sexual selection is known to play an important role in the evolution especially of plants. Perhaps it is the sexual selection strategy of the females that has changed a carpel from being naked (lacking a pistil) to the one enclosed inside the pistil (Mulcahy 1979). It has been put forward that pistil development especially that of the stigma and style served the function of filters and contributed towards an intensified competition among gametes. This resulted in the placement of angiosperms at the highest rung of the evolutionary ladder (Tejaswini et al. 2001).

The increased load leading to competition among males has a direct bearing upon the progeny performance and has been established in natural populations of several taxa like *Petunia* (Mulcahy et al. 1975), *Turnera ulmifolia* (McKenna 1986), *Lotus* (Schlichting et al. 1987), maize (Frascaroli and Landi 1991), *Aureolaria flava* (Ramsetter and Mulcahy 1988), *Cassia fasciculata* (Lee and Bazzaz 1982a, b), *Cucurbita texana* (Johannsson and Stephansson 1997) and many more.

12.9 Sexual Selection Post Fertilization

As discussed earlier, male gametes compete for gaining access to the female gamete, and females, in turn, choose the most competent male. This tug of war is expected to end after fertilization. However, the process continues thereafter and selection operates to eliminate non-fit low-quality ones through selective seed or fruit abortion. Consistent with this is the fact that many plants differentiate ovules more than

they can afford to mature (Lee 1984; Stephenson et al. 1988b; Skogsmyr and Lankinen 2002), resulting in low seed to ovule ratios. The probability of fruits with less seeds undergoing abortion expectedly greater than the completely filled ones (Lee 1984; Stephenson and Winsor 1986) is believed to accrue benefit through severe pollen competition (Lee 1984; Mohan Raju et al. 2001). A plausible explanation is that fruits with less seeds result from no competition among pollen tubes, and abortion thereof paves a way to the selection of genetically superior genotype. It is equally likely that the partially filled or unfilled fruits may result from severe competition among pollen tubes such that none or few are allowed to fertilize. This would make seeds of less filled fruits genetically superior than those of completely filled ones. However, this is unlikely because the costs associated with the development of unfilled fruits would be quite high and the maternal parent (female) would not allow such fruits to develop. As per the published literature, abortion of fruits in plants is considered to be strictly governed by mate choice, and resource limitation apparently plays no role (Stephenson 1983; Stephenson and Winsor 1986). The issue is still debatable.

Sexual selection in multi-ovulated species may also manifest post-fertilization in terms of zygote competition and/or abortion of the seeds with malformed and defective embryos in the developing fruit (Raina and Kaul, unpublished). In actual practice, non-random abortion of seed has been assumed to be a clever strategy of the maternal tissue to eliminate the inferior offspring produced due to the siring by genetically poor paternal gamete. This has been seen in different taxa across the plant kingdom. For instance, in *Cassia fasciculata* (Lee and Bazzaz 1982a, b), *Phaseolus* spp. (Lee 1984; Weins 1984; Nakamura 1986), more than ten species of Fabaceae and several members of Pinaceae (Lloyd 1979), abortion is clearly related to the paternity of seeds and hence a process of sexual selection.

Contrary to the maternal strategy, the interaction can also be viewed as a sibling rivalry and parent-offspring conflict that can result in embryo abortion (Mazer 1987a, b; Mohan Raju et al. 2001; Skogsmyr and Lankinen 2002). Sibling rivalry becomes apparent when the developing embryos produce detrimental chemicals or hormones (Arathi et al. 1996) or acquire greater sink drawing ability (Weins et al. 1987). The former strategy inhibits the growth and development of weaker embryos while the latter allows diversion of higher amount of resources to some genetically superior fertilized ovules leaving others to starve. On account of various empirical evidences shown by Uma Shaanker and Ganeshiah (1997), the evolution of different reproductive features in plants is the manifestation of strategies and counter strategies of the mother plant and the offspring towards ensuring their respective interests.

Seed abortion has also been suggested to be a consequence of sibling rivalry for several other reasons like advantage over dispersal, competition post dispersal, predation escape or even to gain resources (Mohan Raju et al. 2001). From the offspring's point of view, to be a lone survivor is beneficial and, therefore, under such conditions developing embryos route to sibling rivalry and regicide (Ganeshiah and Uma Shaanker 1988a, b). Another interesting aspect of sibling rivalry could be viewed from the perspective of sexual selection or mate choice since it is established as a function of genetic relatedness among offsprings developing

inside a fruit. This argument is best exemplified by *Vigna unguiculata* (Radha et al. 1993) wherein the extent of resources allocated to developing seeds is greatly influenced by the genotypic composition of the competing siblings.

In *Lindera benzoin*, the number of pollen grains deposited on the stigma during open pollination has been directly related to the chances of fruit maturation (Niesenbaum and Casper 1994). Fruit abortion in this plant was not on account of ovule abortion or some incompatibility reactions, indicating the selective development of fruit under more intense pollen competition. Evidences have also been provided in favour of the diploid phase of the plants being affected by pollen competition, indicating the influence of gametic selection specifically on seed germination (Baskin and Baskin 2015). In *Sophora japonica* abortion of fruits was influenced by its position on the tree. Even the pattern of seed abortion chased the order of fertilization; ovules fertilized by fastest pollen tubes had the highest probability to reach maturity (O' Donnell and Bacesa 1993).

In the context of the aforementioned cases, post-fertilization selection raises some questions on sexual selection. Plants produce flowers abundantly but only few of them turn into fruits. Similarly, the production of ovules inside an ovary outnumbers the ovules getting transformed into seeds. Seed or fruit abortion being non-random depends on various factors like paternal genotype and the mate choice made at the early stage as the maternal selection turns to sibling rivalry at the later stages. Therefore, from the overwhelming of male gametes to female choice, sexual selection appears to ensure the selection of superior genotypes.

12.10 Sexual Selection and Natural Selection

We have, however, not broached one of the important parts in this chapter explicitly – the one concerning the fate of adaptations when sexual selection operates (see Hosken and House 2011). Recent studies along these lines focus on understanding the relationship between the sexual and natural selections although many workers either do not distinguish between the two (Moore and Pannell 2011) or consider sexual selection to be a form of natural selection (Willson 1994). However, it is increasingly becoming clear that the two processes are occurring and acting regularly. While the natural selection is responsible for adaptations, the effect of sexual selection on adaptations remains to be seen. Called ‘the lek paradox’, it concerns the possibility of genetic variation in sexually selected traits getting depleted under the influence of strong directional selection (Kirkpatrick and Ryan 1991; Pomiankowski and Moller 1995; Ritchie 1996; Rowe and Houle 1996). What factors then help in or contribute to the maintenance of this genetic variation under those conditions? What are the mechanisms underlying this paradox and how do these operate? Kuijper et al. (2012) believe that sexual selection would normally operate up to a naturally selected optimum but could go beyond if both the male competition and female choice are sufficiently weak (Hall et al. 2000). According to Nosil (2015), ‘this issue can be resolved if there is a trade-off between reproductive success and survival’ (see also Chenoweth et al. 2015; Johnston et al. 2013).

Nevertheless, concerted efforts are required to conduct empirical studies in this direction even though some have endeavoured to investigate these at the genomic level (Long et al. 2012; Chenoweth et al. 2015; Johnston et al. 2013; Nosil 2015).

12.11 Conclusions

This chapter reviews the theory of sexual selection and its impending role in angiosperms. Having a long history right from Darwin, research (both theoretical and empirical) today explores the possible paths leading to diversity and speciation on account of inflated male traits and female choice. It is suggested that sexual selection can be considered as a part of natural selection in the way that it concerns the interaction between two members of opposite sex. In this interaction all the traits evolved on account of competition over the quality and quantity of mates are included. And once the difference in the ability to attract or choose the mate comes into play, it will be selected. This difference will not restrict to natural and sexual selections but extends to that of sexual and parent-offspring conflicts.

Not all the authors referred to here have placed their work explicitly in the present context. When considering the previous review articles summarizing the research on sexual selection in plants, it is astonishing that the subject still remains controversial. Although a large amount of empirical studies favour sexual selection to act on plants, many scientists are against this interpretation. Nevertheless, we can think of two probable reasons for this. First, this study and various evidences would not have provided them with befitting insights into trait evolution in plants. Second, they felt that unambiguous mention of sexual selection would compromise the reliability of the study in the eyes of some readers. We hope that this chapter may help to clarify and illustrate why sexual selection in hermaphrodites is a convincing component of trait selection.

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Role of Apomixis in Perpetuation of Flowering Plants: Ecological Perspective

13

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Abstract

Apomixis or asexual formation of seeds involves three major features, absence of meiotic reduction, recombination and fertilisation, and thereby leads to formation of genetically uniform progeny. Though Winkler coined the term ‘apomixis’ in 1908 to describe ‘asexual reproductive process in place of sexual reproduction without nuclear or cell fusion’, the definition of apomixis is now restricted to the formation of seeds via an asexual process. Today apomictic forms are reported in several flowering plant taxa in species of both monocotyledonous and dicotyledonous genera. Although apomixis seems to be a simple phenomenon, several pathways are speculated to achieve it. Also this phenomenon has many subcomponents which should be achieved consistently and simultaneously to ensure formation of seeds (or next generation). There has been much focus on several facets of apomixis including cyto-embryological, molecular and biotechnological aspects. However, the origin of apomixis (independently in several plant taxa) and its ecological and evolutionary significance is still not completely deciphered. Nonetheless, we do understand that polyploidisation and/or hybridisation-associated shifts as well as environmental gradients play a role in establishing apomixis in most of the studied taxa. Further diversification occurs through several processes including mutation, chromosome rearrangements and aneuploidy, residual sexuality and backcrossing. While environmental changes bring about range shifts and secondary contact hybridisation of different ecotypes results in origin of apomictic lineages, apomixis is also an important factor in changing the ecological scenarios. Previously apomictic lineages were considered to be a dead end, but the current understanding treats them as a means of diversification of polyploid complexes and evolution in angiosperms.

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Keywords

Apomixis · Apospory · Diplospory · Parthenogenesis · Autonomous endosperm · Pseudogamous endosperm

Glossary

Amphimixis: or sexual reproduction Amphimixis is the process of reproduction where megasporangium mother cells undergo meiosis to form a reduced embryo sac and then form embryo after double fertilisation to give rise to seeds with both maternal and paternal contribution.

Apomixis: Apomixis (asexual seed formation) is a phenomenon in which a plant bypasses meiosis and fertilisation to form a viable seed.

Sporophytic apomixis or adventitious embryony: This variation of apomixis involves the formation of a maternal embryo from one or more somatic cells of the ovule.

Gametophytic apomixis: Those apomictic pathways where a diploid cell divides mitotically and differentiates to form an unreduced embryo sac which eventually gives rise to the maternal embryo.

Apospory: It is the type of gametophytic apomixis where the unreduced embryo sac arises from a somatic nucellar cell which acquires the developmental program of a functional megasporangium.

Diplospory: It is the type of gametophytic apomixis where the unreduced embryo sac arises from a megasporangium mother cell with suppressed or modified meiosis.

Parthenogenesis: It is the process of spontaneous fertilisation-independent development of the embryo from reduced or unreduced egg cell.

Polyphyletic origin: When a phenotype is derived independently from more than one common evolutionary ancestor.

Polyphenic origin: When multiple forms or phenotypes can arise from a single genotype by differing external conditions.

Geographic parthenogenesis: It is the term used for higher-latitude geographic distribution of asexuals compared to their sexual counterparts.

Sympatric: Two species are said to be sympatric if they occupy the same or overlapping geographic areas.

Syntopic: Two species are said to be syntopic if they can coexist without interference with each other.

Exaptive evolution: A character not selected by natural selection for its current utility.

Polyspory: Polysporic species include both bisporic (meiosis produces only two megasporangia, each with two haploid nuclei, and one of them forms functional megasporangium; therefore, two haploid nuclei are involved in the formation of embryo-sac) and tetrasporic species (meiosis produces one 4-nucleate megasporangium, and all haploid nuclei are involved in the formation of embryo-sac).

13.1 Introduction

Flowering plants have two modes of reproduction: amphimixis or sexual reproduction and asexual reproduction. Asexual reproduction can be achieved by means of vegetative propagation and agamospermy or asexual production of seeds; apomixis and agamospermy are usually used as synonyms (Bicknell and Koltunow 2004). Amphimixis involves fusion of male and female gametes and thus an alternation of sporophytic and gametophytic generations, while the absence of chromosome reduction in the pathway to embryo development is the defining feature of apomixis (Ozias-Akins 2006). Whereas sexual reproduction is a means to develop new genetic combinations, apomixis can help in the maintenance of desired plant phenotypes (Hojsgaard and Hörandl 2019).

All apomictic systems share at least three developmental components (Fig. 13.1): (i) apomeiosis, the generation of a cell capable of forming an embryo without prior (full reductional) meiosis; (ii) parthenogenesis, the spontaneous fertilisation-independent development of the embryo; and (iii) the capacity to either produce endosperm autonomously or to use an endosperm derived from fertilisation (Koltunow 1993). Based on apomeiosis, two main mechanisms for apomixis are recognised: sporophytic apomixis or adventitious embryony and gametophytic apomixis (Nogler 1984; Koltunow 1993; Crane 2001). The simplest pathway for apomixis is to avoid the production of a gametophyte and formation of a maternal embryo from one or more somatic cells of the ovule (sporophytic apomixis). Sporophytic apomixis differs from the gametophytic in that no alternation of generations intervenes prior to embryo development. Gametophytic apomixis encompasses those apomictic pathways where the maternal embryo originates from a diploid egg cell differentiated in an unreduced embryo sac. Gametophytic apomixis further shows classification into two main pathways: in the first case, the unreduced embryo sac arises from a somatic nucellar cell which acquires the developmental program of a functional megasporangium (apospory). Alternatively, the embryo sac arises from a megasporangium mother cell with suppressed or modified meiosis; here the pathway is termed diplospory. These pathways can be further subdivided into a variety of different developmental schemes described in several reviews (Nogler 1984; Asker and Jerling 1992; Crane 2001).

Parthenogenesis defined as spontaneous development of an embryo in the absence of fertilisation (diploid parthenogenesis, when the embryo develops from an unreduced egg cell) is the second component of gametophytic apomixis. The third (and last) component for the production of a functional apomictic seed is functional endosperm formation. Though a 2:3 embryo-to-endosperm ploidy ratio (related to the role of differential genomic imprinting on the maternal and paternal contributions) is of predominant occurrence among many species, in apomicts, the embryo-to-endosperm ploidy ratio can differ from normal 2:3 ratio depending on the mechanism of apomixis employed (Ozias-Akins 2006).

Apomixis can lead to the formation of variable progeny types. As discussed, combination of three steps, namely, non-reduction, loss of fertilisation, and parthenogenesis, results in apomixis and genetically identical progenies, but omission of

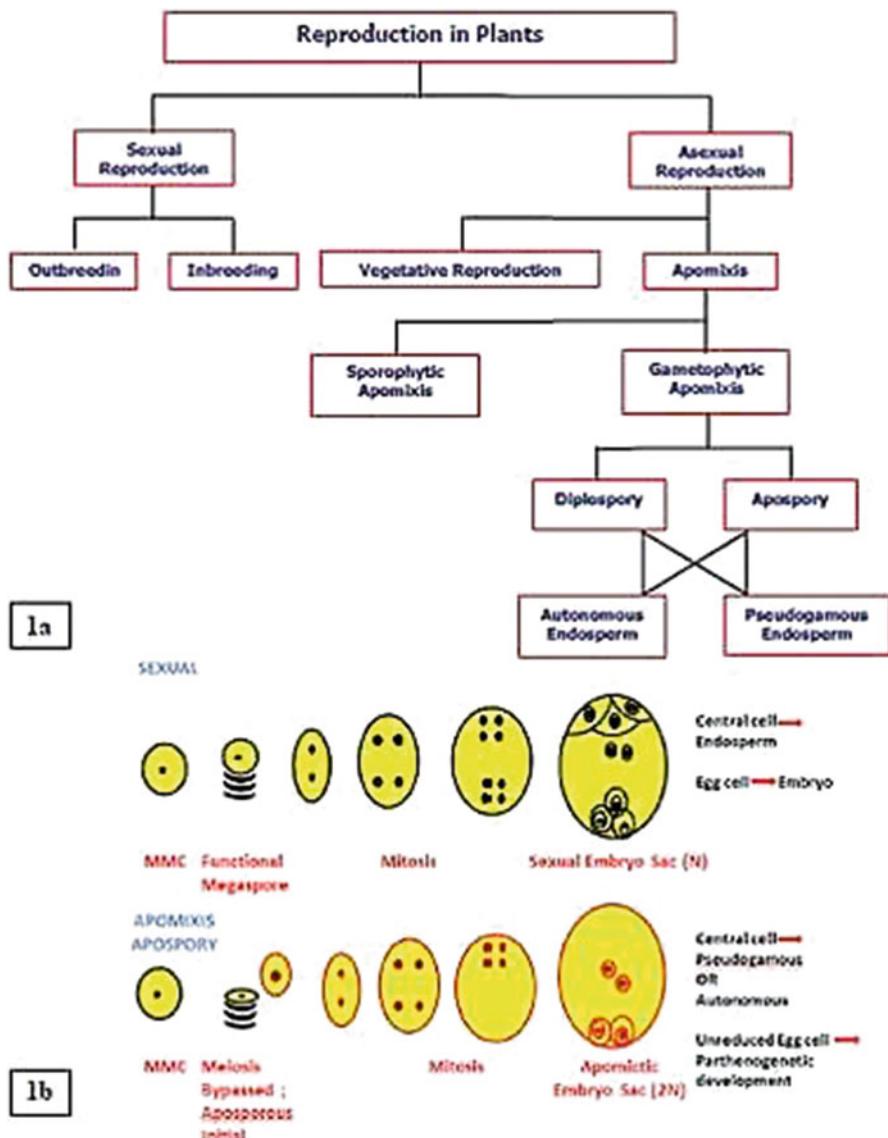


Fig. 13.1 (1a) Types of reproductive strategies in plants; (1b) components of apomixis as shown for apospory: (i) apomeiosis or by-pass of meiotic reduction, (ii) parthenogenetic development of egg cell to form the embryo, and (iii) endosperm development through autonomous or pseudogamous development

any one step is also a possibility in some cases, and therefore, in total four progeny types are possible:

- (a) $n + n$ or (BII) hybrids: normal sexual process; embryo development via fertilisation of two reduced gametes
- (b) $n + 0$: haploid parthenogenesis (gynogenesis or androgenesis); embryo development from one of the reduced gametes
- (c) $2n + n$ or (BIII) hybrids: embryo development from fertilisation of one reduced and one unreduced gamete
- (d) $2n + 0$: apomixis; embryo development from an unreduced gamete

The capacity to autonomously produce embryos from a reduced egg cell indicates that the locus controlling parthenogenesis is not as tightly linked to the genetic locus responsible for apomeiosis. The capacity to form BIII hybrids indicates that the unreduced embryo sac is functionally similar to embryo sacs derived from meiotic reduction in having a capacity to attract the pollen tube and that its egg cell can also successfully participate in the fertilisation process (Koltunow and Grossniklaus 2003).

Another important characteristic feature of apomixis to be considered for understanding the origin and evolution of apomorphic reproduction is its facultative nature with co-occurrence of both sexual and apomorphic embryo sacs. Rosenberg (1906, 1907) discussed it after observing the coexistence of meiotic and ameiotic structures in the ovules of *Hieracium*. It is essential that the sexual pathway of seed development remains intact in sporophytic apomicts as this is the sole source of endosperm tissue in such apomicts (Briggs and Walters 2016). In gametophytic apomixis also, many apomorphic taxa are facultative, meaning that a single individual can produce seeds through both sexual and apomorphic pathways. Furthermore, apomicts and their sexual relatives are often sympatric (but not necessarily syntopic) and morphologically difficult to differentiate (Albertini et al. 2010).

On one hand, where climatic fluctuations are thought to create range shifts and secondary contact hybridisation of different ecotypes results in apomorphic lineages; on the other hand, development of apomorphic genotypes also leads to new ecological scenarios (Hojsgaard and Hörandl 2019). In this chapter, we would be discussing this aspect of apomixis and its implication in propagation of flowering plants.

13.2 Distribution of Apomixis Across Taxa

13.2.1 History

Winkler coined the term ‘apomixis’ in 1908 to describe ‘asexual reproductive process in place of sexual reproduction without nucleus or cell fusion’ (Winkler 1908). This definition is very broad, and the definition of apomixis is now restricted to the formation of seeds via an asexual process (Richards 1997). It was Smith (1841) who gave the first description of apomixis in *Alchornea ilicifolia* (syn

Caelebogyne ilicifolia). Since then, apomictic forms have been reported in several hundred flowering plant species of both monocotyledonous and dicotyledonous genera with adventitious embryony, apospory, diplospory, and apospory and diplospory together occurring in 148, 110, 68, and 17 genera, respectively. This number may be an underestimate of the prevalence of apomixis in plants (Hojsgaard et al. 2014).

13.2.2 Distribution

The widespread distribution of apomixis amongst flowering plants and the occurrence of analogous phenomena in other organisms indicate that it has originated de novo several times (Albertini et al. 2010). Distributions of apomixis across angiosperms were recently reviewed by Hojsgaard et al. (2014), and an online database has been created for an easy access to updated lists (<http://www.apomixis.uni-goettingen.de>) (Fig. 13.2). According to this report, angiosperms have a general capacity to switch from sexual reproduction to apomixis, which mainly depends on diversity and genomic complexity of the clade (Hojsgaard et al. 2014). Both sporophytic and gametophytic types of apomixis and similarly both diplosporic and aposporic forms of gametophytic apomixis can be present in the same family. The occurrence of multiple types of apomixis in a family, genus, species, and sometimes individual genotype is interpreted as a result of overlapping or intersecting developmental pathways subjected to genotype and environment effects (Ozias-Akins 2006). Though in most cases apomicts within a single genus utilise a similar mechanism to produce asexual seed, members of closely related genera may differ widely in the apomictic mechanisms employed (Bicknell and Catanach 2006). Apomixis occurs sporadically among various clades of angiosperms, and several authors have noted a marked bias in the distribution of apomixis (Asker and Jerling 1992; Mogie 1992; Carman 1997). But in general, it has been found that apomixis biodiversity is paralleled by total biodiversity, i.e. more diverse orders, families (e.g. Asteraceae, Poaceae), and subfamilies contain more apomicts as well. Apomixis is possibly an ancient feature in some major clades particularly in commelinids, fabids, and lamiids (Hojsgaard et al. 2014). Carman (1997) listed 126 apomictic genera, 220 polysporic genera, and 255 polyembryonic genera. Sporophytic apomixis or adventitious embryony was supposed to occur more frequently in tropical than in temperate flora (Naumova 1992), but this view has been contested recently by Hojsgaard et al. (2014), who stated that both the forms (sporophytic and gametophytic) are more prevalent in the tropics in terms of total numbers. Adventitious embryony is more represented in diploid species, while in polyploids, other forms of apomixis are generally more prevalent. Adventitious embryony is found in several agriculturally important species like *Citrus* and mango (*Mangifera indica*) and in orchids. Naumova (1992) published the most comprehensive treatise on adventitious embryony.

As for the gametophytic apomixis, roughly three-fourths of gametophytic apomicts occur in just three families—the Rosaceae, Poaceae, and Asteraceae

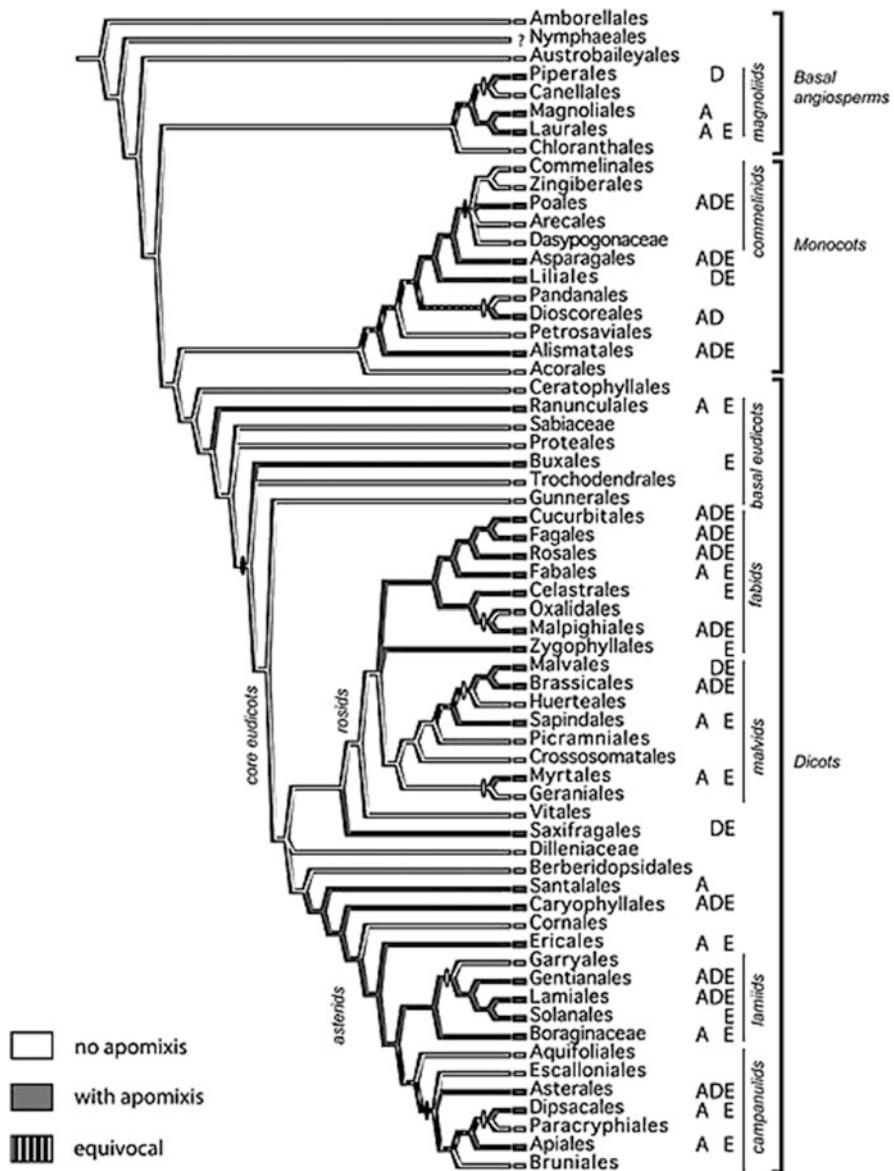


Fig. 13.2 Apomixis among orders of angiosperms. (From: Hojsgaard et al. 2014) (A) Apospory. (D) Diplospory. (E) Adventitious embryony

(Richards 1986; Asker and Jerling 1992). However, as discussed earlier, it might be because these are quite large and genetically complex families. Such patterns of distribution of apomicts may also reflect the presence of certain predisposing characteristics (cytological characteristic like massive nutritive nucellus in *Citrus*)

(Koltunow et al. 1995) or genetic characteristic like apparent linkage group in species like *Tripsacum dactyloides*, *Pennisetum squamulatum*, *Paspalum simplex*, and *Erigeron annuus* (Grimanelli et al. 2001) which make them amenable to the developmental and genetic changes characterising apomixis. With recorded taxa, apospory is found to be more frequent than diplospory (Hojsgaard et al. 2014). Since most gametophytic apomicts are polyploids, other factors like the higher frequencies of neopolyploids in temperate to arctic regions (Brochmann et al., 2004) may also make gametophytic apomicts more frequent in these regions.

Though parthenogenesis has been widely recorded in flowering plants (Asker and Jerling 1992; Hojsgaard et al. 2014), it occurs in most species at a low level. In comparison, the rates of parthenogenesis in the native apomicts like *Hieracium aurantiacum* and *H. piloselloides* are very high, 97.6% and 98.0%, respectively (Bicknell et al. 2003). Though as discussed before, there is a possibility of development of $n + 0$ progeny through haploid parthenogenesis (gynogenesis or androgensis), parthenogenesis has been more frequently studied as a component of apomictic development pathway. In fact, the geographical distribution of apomicts and sexual lineages has been labelled as *geographic parthenogenesis* (discussed later).

13.2.3 Variability in Endosperm Development

Autonomous endosperm development (endosperm – initiated autonomously without any contribution from male gamete) is of usual occurrence among the apomicts of Asteraceae and more common among species showing diplospory and adventitious embryony (Albertini et al. 2010). Pseudogamous (fusion of one of the sperms with the central cell to give rise to the endosperm and the degeneration of the other sperm without fusion with the egg cell) development of endosperm requires a pollination stimulus to occur and is more common in aposporous species, including the families Rosaceae and Poaceae but rare in the Asteraceae family. In endosperms for pseudogamous species, the expected level of the $4(n)$ maternal: $1(n)$ paternal ratio is often altered by the variable number of polar nuclei involved, the extent of their fusion, and the number and ploidy level of the male gamete(s) which operate in fertilisation. Thus, in many cases, the required 2:1 maternal-to-paternal ratio of the endosperm is maintained (Albertini et al. 2010). On the other hand, relaxation in the typical 2:3 embryo/endosperm ploidy balance requirement is also a norm in many apomictic plants (Matzk et al. 2000), for example, in the apomict *Poa pratensis* where two unreduced polar nuclei fuse with a sperm nucleus resulting in an embryo/endosperm ploidy ratio of 2:5. Other patterns of endosperm balance displayed and tolerated by apomictic species have been previously reviewed (Koltunow and Grossniklaus 2003). In *Tripsacum dactyloides*, a wide variety of maternal:paternal (m:p) ratios support functional endosperm development (Grimanelli et al. 1997). In *Paspalum notatum*, it was found that the crosses between sexual diploid and tetraploid plants result in seed abortion, whereas apomictic plants tolerate a wide range of m:p ratios (Quarin 1999). This demonstrates that a tolerance for unbalanced embryo:endosperm ratio is closely related to apomictic reproduction (Koltunow and

Grossniklaus 2003). In the case of sporophytic apomicts, usually an endosperm formed as part of the sexual process nourishes the somatic embryos. Such an endosperm is derived from fertilisation of two reduced polar nuclei with reduced male gamete, which results in the typical 2:3 embryo: endosperm ploidy ratio and normal development (Bicknell and Catanach 2006). It has been found that apomicts with autonomous endosperm formation tend to produce less viable pollen and, in some cases, are male sterile (Meirmans et al. 2006; Thompson et al. 2008). Exceptionally, *Commiphora wightii* is a sporophytic apomict, and most of the populations are made up of exclusively female plants (Gupta et al. 1996). Even when one or two male plants are present, in the population there is no pollination. Manual pollinations result in pollen germination, but the pollen tubes do not grow beyond the upper part of the style (Gupta et al. 1998). Thus, apomicts for this species do not require pollination stimulus for apomictic embryo formation and show autonomous endosperm development. In rare cases, even though neither the embryo nor the endosperm is fertilised, still apomicts require pollination to stimulate seed development (Bicknell et al. 2003). Reduced pollen acts as a means for gene flow from apomictic to sexual genotypes, for example, from apomictic tetraploids to sexual tetraploids in *Rubus* (Sochor et al. 2015; Šarhanová et al. 2017; Dickinson 2018). Therefore, its involvement in reproduction might be a way to maintain the apomictic genes in the long run.

13.2.4 Classification System

In apomictic plants, numerous morphologically or physiologically distinct yet interfertile varieties are found growing true to type from seed, for example, *Alchemilla*, *Hieracium*, *Pennisetum*, *Poa*, *Potentilla*, *Ranunculus*, *Rubus*, and *Taraxacum* (Bicknell and Catanach 2006). Apomixis forms a reproductive barrier at the level of genera or species. These separate apomictic genotypes are called as agamospecies, and their collection is referred to as an agamic species complex. Diverse pathways of reproduction in apomixis can give rise to varied levels of ploidy, leading to formation of an ‘agamic complex’ (Asker and Jerling 1992). Since there is no involvement of gametes in sporophytic apomixis, they do not form agamic complexes. Typically, apomictic lineages are confined to the terminal branches of phylogenetic trees (van Dijk and Vijverberg 2005).

13.3 Origin of Apomixis

13.3.1 Polyphyletic Origin

Since gametophytic apomixis has scattered distribution at the ordinal level (Fig. 13.2), apomixis is believed to have originated independently on several occasions (Hojsgaard et al. 2014). When occurrence of apomixis is superimposed on current phylogenetic tree, multiple independent origins as well as recurrent changes from facultative apomixis to obligate sexuality are observed (León-

Martínez and Vielle-Calzada 2019). Another fact supporting polyphyletic origin of apomixis is that the two main variants of apomixis, i.e. apospory and diplospory, do not appear to have a common ancestor (Briggs and Walters 2016; Albertini et al. 2019). The same genes might result in different forms of apomixis, and Bicknell et al. (2000) had also indicated allelic nature of two different forms of apospory in *Hieracium*. Nevertheless, apospory and diplospory are considered likely to be nonhomologous (van Dijk and Vijverberg 2005).

This repeated de novo evolution of apomixis is difficult to explain, as it would require highly specific changes in two conserved and molecularly complex processes, i.e. meiosis and syngamy. Also, to maintain viability, these mutations should occur simultaneously (Mogie 1992; Neiman et al. 2014). Apomeiosis without parthenogenesis would cause elevated ploidy in each generation which would be deleterious. On the other hand, mutants with haploid parthenogenesis would be weak and have recessive mutation load. Therefore, this phenomenon is easier to explain in species where apomixis is a single-locus trait. In species like *Poa pratensis*, a complex 5-locus genetic model has been postulated which includes single, unlinked genes for initiation of apospory, apospory prevention, parthenogenesis initiation, and parthenogenesis prevention, as well as a megasporule development gene (Matzk et al. 2005). In such cases, it becomes difficult to explain de novo origin of apomixis. Also, in *Potentilla puberula*, genetic contribution from at least one apomictic parent has been found to be necessary for the formation of new apomictic genotypes, and penta- and hexaploids derived from sexual backgrounds did not show apomixis and hence do not support de novo origin of apomixis (Nardi et al. 2018). Here, it has been suggested that perenniability and outcrossing may bring the required traits in the species, which then get clubbed together through chromosomal rearrangements (van Dijk and Vijverberg 2005).

13.3.2 Mutation Theory

Mutation theory considers that generation of new alleles through accumulation of mutations may lead to new genotypes via occasional sexual outcrossing (Muller's ratchet; Muller 1964; Asker and Jerling 1992; Majeský et al. 2012). The theory assumes that a combination of pre-existing tendencies for features like haploid parthenogenesis and relaxed 2 m:1p requirement for endosperm development along with dominant apomeiotic mutations would lead to apomixis (Mogie 1992; Carman 2001a). As discussed above, this theory would be easier to explain in species where apomixis is a single-locus trait, as it is difficult to assume that the alleged mutations required for all the three components of apomixis should occur simultaneously. Also, this theory falls short in explaining the variability in facultative nature of most apomicts (Carman 2001a). In some studies, it has been found that in the absence of some critical alleles at separate loci, major apomictic locus is also defective. Alternatively, certain genetic backgrounds have been found to induce apomixis even after lacking major apomixis-conferring linkage groups (Carman 2001a). These occurrences undermine mutation-based theories for apomixis.

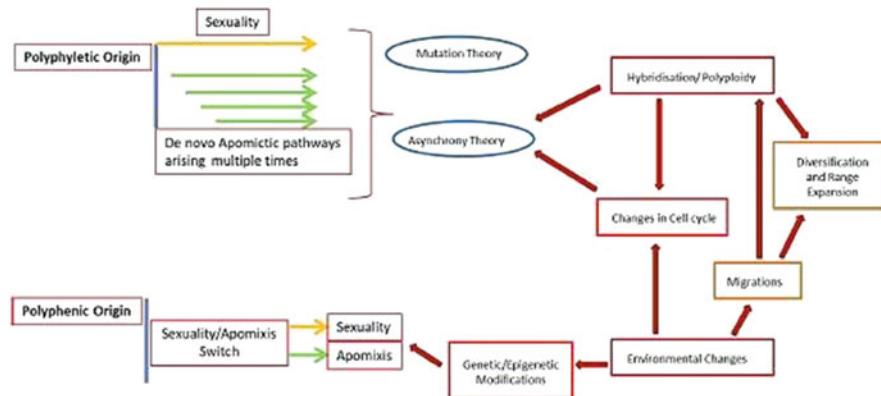


Fig. 13.3 Theories regarding origin of apomixis

13.3.3 Hybridisation-Derived Floral Asynchrony Theory

Carman (1997, 2001b, 2007) suggested that apomixis could result from temporal deregulation of alleles involved in demarcation of reproductive fate of cells. This deregulation may occur due to the hybridisation of divergent alleles. This is referred to as *hybridisation theory*. This has also been supported recently through transcriptomic profiling of apomixis in *Boechera*. This study showed that several genes conserved across both sexual and apomorphic systems are heterochronously expressed in apomorphic reproduction and could also be linked to parent of origin effects (Sharbel et al. 2009; Tucker and Koltunow 2009). According to this theory, asynchrony in megasporogenesis and embryo sac development in hybrids are the main cause of apomixis. It proposes that hybridisation of two such divergent ecotypes leads to heterozygosity which gets stabilised through polyploidisation and this multilocus heterozygosity leads to apomixis. Further, intergenic recombinations, mutations, and other chromosomal aberrations may lead to embryological events leading to reversion to sexuality or other anomalies. There are further controls in the form of rate of divergence among developmental programs, variability in expressivity of the genes involved, nutritional status, and epigenetic conditions (Carman 2001a).

Although it is difficult to provide a general theory for de novo origin of apomixis, it is clear that apomixis has evolved several times during evolution of angiosperms and several interrelated factors seem to play an important role (Fig. 13.3), including hybridisation, polyploidisation, and environmental factors (Hojsgaard and Hörlndl 2019).

13.3.4 Polyploidisation and Hybridisation

Apomicts differ from their sexual relatives not only in reproductive mode but also in ploidy (for most cases). Many wild apomictic species are characterised by hybridity and polyploidy (Richards 2003). Whereas odd ploidy levels (e.g. triploidy, pentaploidy) are often reliable predictors of the presence of apomixis, tetraploidy is the most common ploidy level among apomicts (Asker and Jerling 1992). An evolutionary correlation between vegetative reproduction and polyploidy has been reported in the central European flora (Herben et al. 2017). It is still unclear as to the relative contributions of hybridisation and polyploidy to asexual lineage origin and evolution, as both phenomena can have diverse regulatory consequences causing coordinated deregulation of the sexual pathway (Albertini et al. 2010). Two theories on the basis of cause and effect have been proposed; with one of them suggesting that polyploidy stimulates the development, penetrance, and maintenance of apomixis, and the other proposes that apomixis stimulates the formation and proliferation of polyploids (Bicknell and Catanach 2006). Functional roles for polyploidy in apomicts have been hypothesised to include epigenetic gene deregulation (Koltunow and Grossniklaus 2003), ploidy-dependent gene expression (Cervigni et al. 2008), ectopic gene expression resulting from genome asynchrony (Carman 1997), and restoration of hybrid fertility through allopolyploidy-induced sequence elimination (Rieseberg and Willis 2007). On the other hand, there are species which appear to express apomixis without any signs of hybridity or polyploidy. In *Paspalum*, the presence of apomixis in otherwise sexual diploid plant species has been reported (Ortiz et al. 2013; Hojsgaard and Hörandl 2019). Diploid apomicts are also reported following the experimental manipulation of several polyploid apomicts (Bicknell 1997; Kojima and Nagato 1997), but in almost all reported cases, the identified diploid apomict was weak and pollen sterile. These findings indicate that polyploidy is not an absolute requirement for the expression of apomixis, but in most systems, it may act to enhance the expression or promote the transmission of the trait (Bicknell and Catanach 2006). There is also a possibility that apomixis promotes polyploidy, and complexities associated with polyploidy were evolved following the establishment of apomictic reproduction and thus may be secondary with regard to its genetic control (Ozias-Akins and Van Dijk 2007). In any case, polyploidisation has often coincided with major evolutionary transitions and adaptive radiation of species and hence might play a role in spurts of adaptive speciation. It can provide new gene functions by supporting genetic redundancy and combining with other processes like recombination, gene mutation, transposon activity, and chromosomal rearrangement, which can act as drivers of diversification, speciation, and ultimately species evolution (Alix et al. 2017).

Apomicts in nature also often appear to have a hybrid origin (Šarhanová et al. 2017). As discussed before, Carman (1997, 2001b) proposed that hybridity itself is the cause of apomixis, and a combination of hybridity and polyploidy can lead to the disjunction of key regulatory events during critical stages of megasporogenesis, megagametogenesis, and fertilisation. Hybridisation among distantly related taxa is also an easier possibility with higher ploidy. A possible hybrid origin may also

explain the apparent puzzle of apomixis resulting from the summation of several components, each of which on its own would confer a selective disadvantage to the plant (Mogie 1992). Here, residual sexuality of apomicts is supposed to play a major role in reducing the mutation load of semi-lethal heterozygosity resulting from hybridity in asexual reproduction (Klekowsky Jr 1988; Niklas and Cobb 2016). Also, polyploidy may help to mitigate hybridisation-associated genome shock (Hegarty et al. 2006) and thus facilitate fixation of the hybrid (Soltis et al. 2010; Mason and Pires 2015).

13.3.5 Epigenesis

Epigenetic effects linked to hybridisation and/or polyploidisation can also affect expressivity of the different reproductive pathways (Grimanelli 2012) as reported in *Hieracium* (Koltunow et al. 2000; Krahulcová et al. 2011). It was found that DNA methylation in reproductive cells induces apomeiosis-like phenotypes (Garcia-Aguilar et al. 2010). As discussed below, environmental changes also affect the frequency of apomixis and point to the role of epigenetic factors in apomixis. This epigenetic model unites the mutation and hybridisation theories because epialleles can behave genetically like mutations, and epigenetic changes in gene expression have been documented after hybridisation (Lee and Chen 2001). The epigenetic control model also provides answer to the very improbable simultaneous origin of apomeiosis, parthenogenesis, and functional endosperm formation in apomictic plants (Mogie 1992).

13.3.6 Environmental Changes

In *Ranunculus kuepferi*, spontaneous formation of apomictic seeds was found albeit at low frequencies in otherwise sexual, diploid wild populations. It was shown that these diploid populations are not hybrids, as they are geographically distant and isolated from each other as well as apomictic tetraploids (Cosendai et al. 2013; Schinkel et al. 2016). Here the important factor was suggested to be environmental effects like cold shocks and frost treatments during development which increased frequencies of apomictic seeds in warm-adapted, diploid *R. kuepferi* (Klatt et al. 2018). Even a small increase in the frequency of apomictic seeds is strong enough to make an impact through evolutionary time periods (Hojsgaard and Hörandl 2019). At the same time, whether stress will increase or decrease the frequency of sexual reproduction depends on the involved genotype and the type and severity of stress itself. *Boechera* pistils exposed to short-term stress (H_2O_2) as well as long-term stress (drought, sugar starvation, and BRZ) bring shifts from apomeiosis to meiosis. On the other hand, pharmacological treatments of glucose, epiBL, DTBA, and 5-azaC were able to induce apomictic reproduction in the sexual plants of *Boechera stricta* (Gao 2018). Other examples where environmental cues have been able to switch on the residual sexuality include *Boechera* under drought or heat stress

(Mateo de Arias 2015), *Ranunculus* under extended photoperiods (Klatt et al. 2016), *Eragrostis curvula* under drought or culture stress (Rodrigo et al. 2017). Thus, the frequency of apomeiosis in facultative gametophytic apomicts is affected by several environmental factors, such as temperature, photoperiod, soil salinity, and drought stress (Albertini et al. 2019). Environmental changes are likely to confuse cell cycle in plants; for example, nonconducive photoperiods have been known to cause abortion of meiosis (Moss and Heslop-Harrison 1968; Carman 2001a). This indicates that metabolic status of ovules is an important factor for sex/apomixis switch (Albertini et al. 2019). At the metabolic level, apomeiosis is postulated to occur in response to shifts in redox homeostasis (Gao 2018; Sherwood 2018). Such shifts could be a result of hybridisation and polyploidisation (Kirk et al. 2005) and might result in induced epigenetic changes to permit apomictic development in some cases (Albertini et al. 2019).

The trait of apomixis has also been described as a threshold trait. This feature along with asynchrony and environmental variation might explain the facultative nature of apomixis (Carman 2001a). It also explains multiple phenotypes with each phenotype defined by different threshold.

In some genera, apomicts show a tendency to inhabit regions at higher latitudes affected by the last glacial period (Bierzychudek 1985; Hörandl et al. 2008). Changing environmental conditions require rapid adaptation and hence show selection for recombination-based reproduction (Peck 1993, 1994; Lively and Morrani 2014). The “Fisher-Muller” population-genetic theory and, subsequently, several theories put forward the notion that sex/recombination though expensive helps in rapid adaptation to changing environment (Muller 1932; Fisher 1999; Brukhin and Baskar 2019). Once the adaptation to altered environment is achieved, selection works against sexual reproduction. It has been found in *Eragrostis curvula* (cultivar Tanganyika) that different stress conditions increased the expression of sexual reproduction and when the stress stops the reproductive mode shifts back to the apomixis original level (Rodrigo et al. 2017). Large-scale migrations of plants during periods of climatic oscillation may also result in widespread secondary contact hybridisation and resulting hybrids would show better fertility after polyploidisation (Stebbins 1985; Hewitt 2004; Hojsgaard et al. 2014). Thus, new genotypes might develop through hybridity and polyploidisation, which might lead to apomictic genotypes that are better adapted to the changed climate.

13.3.7 Polyphenic Origin

Some authors assume asexual reproduction to be an ancestral condition, as prokaryotes lack the meiotic ability (Niklas and Cobb 2016). Sexual reproduction probably evolved shortly after eukaryotic origin and, therefore, preceded the origin of the angiosperms (Cavalier-Smith 2001; van Dijk and Vijverberg 2005). While considering all kingdoms of living organisms, ancestral forms were supposed to largely maintain adapted gene complexes through asexual reproduction while retaining a capacity for producing genetic variants through sexual reproduction. It

is considered that over the course of evolution of land plants, asexual reproduction decreased due to evolution of seed habit and woody, self-supporting stems (Niklas and Cobb 2016).

As discussed before, it is commonly postulated that apomixis across the eukaryote kingdoms evolved from sexual reproduction by genetic or epigenetic modifications causing deregulation of events associated with sexual reproduction (Carman 1997; Grimanelli 2012; Neiman et al. 2014), but there is another point of view where apomixis is considered to be anciently polyphenic with sexual development. According to this view, apomixis and sexuality are determined through genetic or epigenetic modifications controlling the molecular capacities for apomixis or the apomixis switch. The view of polyphenic development is supported by several angiosperms showing inclinations towards cyclical apomixis. Other indications come through observations like origins of apomixis among related genera, facultative nature of most angiosperm apomicts, and environmental role in determining the frequency of apomixis (Albertini et al. 2019).

Due to multiple independent origins of apomixis across various plant taxa and varied pathways known for apomorphic reproduction, it is difficult to provide a single theory delivering conclusive evidence for its origin and maintenance within angiosperms. Nevertheless, we can conclude that hybridity, ploidy, and environmental adaptation are some of the factors affecting the process. At the level of cell, the actual switch to sexuality/apomixis might be a sum total of how these factors affect the cell cycle.

13.4 Ecological and Evolutionary Significance of Apomixis

13.4.1 Evolutionary History and Adaptive Value

It has been concluded that apomorphic, polysporic (species showing bisporic or tetrasporic), and polyembryonic species have a similar phylogenetic distribution, which shows their evolutionary relationship (Carman 1997; Carman et al. 2011; León-Martínez and Vielle-Calzada 2019). Though various reproductive developmental mechanisms share a close evolutionary history, the evolutionary relationship between asexuality and their component reproductive processes like unreduced gamete formation have not been dealt in detail. It might also be because individual components of apomixis are represented in much lower frequencies due to their deleterious effect in standalone capacity, but when clubbed together, they provide adaptive potential to taxa. Hence, it is generally not possible to study those separately. The formation of unreduced gamete is considered the most important part of apomorphic development. Though it is still unclear if unreduced gametes can be considered as means of exaptive evolution (i.e. having a use not selected by evolution) and evolvability (the capacity to generate adaptive genetic variation), there are several factors like occurrence of unreduced gamete formation across several lineages of organisms, presence of heritable genetic variation within and between species for unreduced gamete production, the involvement of unreduced

gametes in hybridisation events, and the production of unreduced gametes in response to stress which suggest that they could be a part of mechanism for evolutionary speciation (Mason and Pires 2015).

Adaptive value of sexuality and apomixis is still a much-discussed issue (Mirzaghdari and Hörandl 2018). Meiosis is mainly thought to provide benefit of DNA repair (Hörandl 2009; Hörandl and Hadacek 2013; Mirzaghdari and Hörandl 2018). Recombination can have positive effects by combining desirable characters but at the same time can also have negative effects on the fitness of populations by altering desired characteristics and requirement of a sexual counterpart to complete the reproductive life cycle (Niklas and Cobb 2016; Brukhin and Baskar 2019). Large population sizes and frequent mutations are required to make sexual reproduction profitable (Crow and Kimura 1965; Brukhin and Baskar 2019). Therefore, it is strange that so many plants still reproduce through sexual means. On the other hand, apomixis traditionally leads to limited gene flow but has been recently associated with increased diversity and diversification of angiosperms (Hojsgaard et al. 2014). Though theoretically unreduced gamete formation should be detrimental, reproductive stability through gametophytic apomixis can provide necessary selective force under stressful environments (Kreiner et al. 2017a, b; León-Martínez and Vielle-Calzada 2019). Factors like fixed heterozygosity, potential hybrid vigour, buffering of inbreeding depression, uniparental reproduction, benefits of reproduction through seeds like seed dormancy, and better dispersal are supposed to be responsible for the ecological and evolutionary success of apomicts (Hörandl 2006). Yet the apomicts are quite rarer than sexuals, and it might be due to low probability of getting together all necessary components to avoid meiosis and create a functional apomict (Brukhin and Baskar 2019).

13.4.2 Geographic Parthenogenesis

It is a term used to refer to different geographic distributions of sexuals and apomicts. Apomicts and their sexual relatives can be sympatric, but apomicts usually have larger geographic ranges due to species-specific factors (Hörandl 2006; Kirchheimer et al. 2018; Albertini et al. 2019). Thus, apomicts provide effective spread for their genotypes through space and time. Also, apomicts usually occupy higher altitudes and latitudes compared to their sexual relatives, but this distribution might be more related to glaciation events (Sochor 2016). Due to reasons like residual sexuality and increased heterozygosity (due to role of hybridisation and polyploidy), apomictic populations can exhibit as high genotypic diversity as well as higher allelic diversity which might provide expansion of ecological niche and increased flexibility to environmental changes. Evidence based upon genetic markers also supports this view. This may be the major reason why apomictic polyploids like *Taraxacum* spp. (Van Dijk 2003), *Hieracium pilosella* (Houliston and Chapman 2004), and *Cortaderia jubata* (Okada et al. 2009) serve as successful colonisers of new habitats especially of disturbed habitats. This has also been hypothesised to be the case for putative woody apomicts from New Zealand as

well (Molloy 2019). Apomixis itself leads to wider range expansions, and coupled with sexuality, it seems to be a source for novel geographically isolated lineages that have a capacity to further diversify by ecological and allopatric speciation (Hörandl and Hojsgaard 2012).

But like origin of apomixis, geographic parthenogenesis also cannot be explained fully through ploidies and differential hybrid origin, as seen for single ploidy level in *Rubus ser. Glandulosi* (Šarhanová et al. 2012; Sochor 2016). Another hypothesis for specific geographical distribution of apomict may be related to their inability to evolve in response to pathogen evolution, which might be higher at lower altitudes or latitudes, “Red Queen” hypothesis (Bell 1982; Verhoeven and Biere 2013; Lively and Morrani 2014). During host–parasite coevolution, cross-fertilisation is favoured over uniparental forms of reproduction. A continual environmental change seems to be the requirement for the long-term maintenance of sexual reproduction, and antagonistic coevolution with parasites seems to provide that constant source of environmental change (Lively and Morrani 2014). Another important point to consider here is that the study of distribution of geographical parthenogenesis is also not complete enough to draw any strong conclusions.

13.4.3 Role in Speciation

Despite having advantages of reproductive assurance, apomixis is much less common than sexual reproduction. Apomixis requires combination of several developmental processes and is often associated with infertility (Lloyd 1988). Though apomixis provides short-term advantage, extinction rates of apomicts are expected to be higher (van Dijk and Vijverberg 2005). It was used to be considered as an evolutionary dead end due to absence of genetic variation and adaptability to changing environments especially evolving pathogens (Levin 1975; Bell 1982) and accumulation of deleterious mutations (Muller 1964; Kondrashov 1982; van Dijk and Vijverberg 2005). However, this perception has now changed based on observations that there is much genetic variability in agamic complexes (Carman 1997; Whitton et al. 2008; Hörandl and Hojsgaard 2012). For example, a recent study with agamic complex of *Rubus* showed that this complex dates to last interglacial period and the apomicts helped to combine gene pools of the preglacial diploid ancestors and of recent sexuals from their region. Therefore, apomicts serve to preserve and spread genetic diversity (Sochor 2016). It might be possible because “apomixis genes” can escape extinction through transfer to new clones via hybridisation and can be maintained through sexual gene pool which might predate the splits of related genera. This may be responsible for the common origin of apomixis among related genera. Other factors like facultative sexuality and allelic divergence can also help reduce mutation accumulation of apomictic lineages (Hojsgaard and Hörandl 2015a). Through NGS sequencing, Lovell et al. (2017) found increased sequence diversity in apomictic populations. A positive correlation of number of genera per subfamily, family, and order with number of apomicts shows that apomixis might be working as a springboard for diversification

(Hojsgaard et al. 2014; Hojsgaard and Hörandl 2015b). Moreover, it might be a combined effect of apomict-sexuality ability of agamic complexes working in creating a higher diversity and removing harmful alleles at the same time (Hojsgaard and Hörandl 2015b; Brukhin and Baskar 2019). Apomixis causes rapid ecological differentiation through limited genetic cohesion and rapid range expansion (Brukhin and Baskar 2019).

An *Expanded Transition Theory* has been given by Hörandl and Hojsgaard (2012) to summarise the evolution of apomixis and its role in speciation, which supports Carman's (1997) hypothesis. The process is supposed to start with polyploidisation and/or hybridisation-associated shifts to facultative apomixis which further get diversified through mutation, chromosome rearrangements and aneuploidy, residual sexuality, hybridisation, and backcrossing. Reduced gene flow and mutation accumulation creates locally adapted genotypes. This diversification results in the range expansions of agamic complexes. These may get further complexed through reversal to sexuality which leads to allopatric speciation and eventual evolution of new genera. Thus, apomixis may facilitate diversification of polyploid complexes and evolution in angiosperms (Hörandl and Hojsgaard 2012).

13.5 Conclusion

It is clear that apomixis along with polyploidy provides effective avenues for range expansion and ecological plasticity, but further studies are required to discern the role of apomixis in the reticulate evolution and diversification of angiosperm genera. Embryological studies may help in better assessment of distribution of apomixis at the species level, while genome sequencing studies might help to dissect the role of hybridisation and polyploidy in perpetuation among apomicts. And further population-level assessments are needed to understand the distribution patterns of apomicts.

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Biotic Seed Dispersal Mechanisms of Tropical Rain Forests – Bats, Fishes, and Migratory Birds

14

Palatty Allesh Sinu, Anjana P. Unni, and Thomas Jose

Abstract

Seed dispersal is crucial for plants to maintain structure, diversity, population, and community. Although wind and water can disperse seeds, 75% of total terrestrial plants require animals to move their seeds to microsites that favor recruitment. Animals move seeds externally (epizoochory) and internally (endozoochory). While epizoochory is promising for plants, not many plants have evolved fruit/seed traits to favor it or evolved as a major dispersal mechanism in grasslands, scrub forests, and dry deciduous forests. Frugivorous animals perform endozoochory and long-distance dispersal of seeds, particularly in tropical rainforests. The quantity and quality of endozoochory are predicted by the abundance and fruit-handling behavior of animals. Birds, mammals, fishes, and ants are major biotic dispersal agents of tropics; but, crabs, wasps, and dung beetles are also often reported as seed dispersal agents of tropical plants.

India is a home for three tropical biodiversity hotspots. India is rich in terms of both the fleshy fruited plants and frugivorous birds and mammals. But, India has generated no commendable data on plant-animal interactions in general and plant-frugivore interaction in particular. In this chapter, we present three forms of seed dispersal that are relevant, but neglected for India – chiropterochory, ichthyochory, and long-distance seed dispersal by migrant birds. Although the proportion of fruit bat species in paleotropics is only about 25% of the total bat species, they are abundant, environment- and climate-resilient, resurgent, and local migrants according to the fruiting phenology of plants. Their fruits do not overlap with the fruits of birds – the major dispersal agents of day. Fishes in Cyprinidae are proposed as frugivores and seed dispersal agents of small-seeded riparian and swamp species for old-world tropics. But, India has so far not

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reported any case of fish-facilitated seed dispersal. India also hosts several transcontinental migratory water birds that can facilitate both epizoochory and endozoochory of water plants. India also facilitates regional and local migrations of many frugivorous and insectivorous birds across latitudes (north and northeast India to south India) and altitudes. But, we have no information on their contribution in shaping the plant community and structure in any Indian habitats.

Keywords

Frugivory · Seed dispersal · Chiropterochory · Ornithochory · Ichthyochory · Long-distance seed dispersal · Transcontinental dispersal · India · Fruit bats

14.1 Introduction

Seed dispersal is a critical step in the life history of plants, affecting their ecology, structure, diversity, biology, population structure and genetics, composition, spatial arrangement, and evolution (Cain et al. 2000; Terborgh et al. 2002; Bacles et al. 2006). Technically, the dispersal of seeds occurs when they are moved from the origin and deposited elsewhere (Schupp et al. 2010). But, an efficient seed dispersal occurs essentially when the seeds are moved toward the favorable microclimates where they can germinate and grow into adults (Howe and Smallwood 1982; Wenny and Levey 1998). The dispersed seeds among tropical trees may experience less exposure to competition, predation, and parasitism (Janzen 1970; Connell 1971; Howe and Miriti 2004). If the dispersal is to be successful, the fitness of dispersed seedlings should be more than undispersed seedlings. The resultant spatial distribution of seeds relative to their parent tree is referred to as the seed shadow (Janzen 1970; Janzen et al. 1976). It could be a few feet away from the parent plant or a continent away (Nogales et al. 2012; Viana et al. 2016; Kleyheeg et al. 2019). Through dispersal, plants may also colonize disturbed open habitats (Wunderle 1997; Puerta-Piñero et al. 2012), formed in response to climate and environmental changes (Corlett and Westcott 2013).

Although plants rely on different vectors – animals, wind, and water – for the dispersal of seeds, estimates suggest that 75% of all plants in tropical rainforests depend on frugivorous animals to disperse their seeds (Howe and Smallwood 1982). Nevertheless, seed dispersal is a complex process affected by several factors related to pre-dispersal, mid-dispersal, and post-dispersal stages (Corlett 2017; Aslan et al. 2019; Fig. 1). The complexity and strength of biotic interactions are key drivers of the evolution of reproductive strategies in plants (Aslan et al. 2019). Although abiotic means of transfer is quite common, the maximum dispersion is achieved through zoochory. Such transfers are often facilitated by special adaptations of plants to favor the dispersal agents. Zoochory, the animal-mediated seed dispersal, is considered the most successful method of seed dispersal in the tropics and includes epizoochory and endozoochory. Epizoochory is essentially the transfer of seeds externally, either through attachment to the fur of the animal or being intentionally carried away to store as a future food source. The seeds dispersed by this mechanism

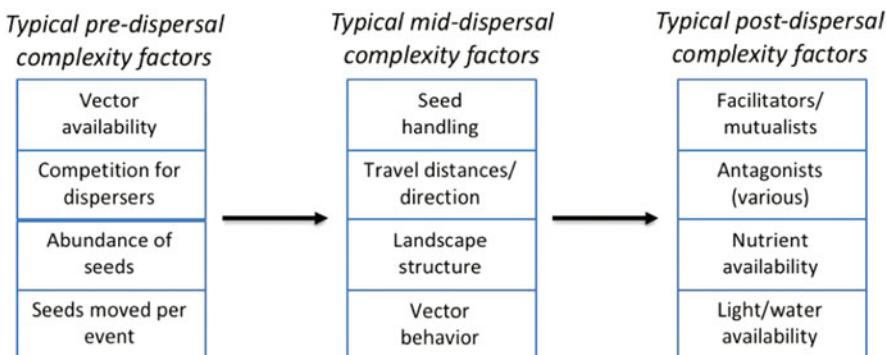


Fig. 14.1 Ecological complexity factors of seed dispersal. Adapted from Aslan et al. (2019)

are sticky, barbed, or with spines that facilitate attachment. In endozoochory, the seed enters the digestive system of the disperser. Seeds dispersed by endozoochory often have nutritive value.

For animal-dispersed fleshy fruits, seed shadows primarily resulted from the foraging behavior and movement of the frugivorous animals (Howe 1993; Sinu et al. 2012; Corlett 2017). The foraging behavior of the frugivores refers to the fruit-handling behavior (ingestion and subsequent defecation) and fruit-processing behavior (method of flesh removal, gut retention time, chemicals in digestive tracts, and defecation of seeds) (Traveset et al. 2008). The movement of frugivores refers to the time the animal spent on the parent tree after consuming fruits, intra-tree movement, inter-tree movement, and movement between tree and feeding or nesting roosts (Wenny 2001). These parameters of the frugivores along with their population size determine the quantity and quality – the distance and direction the seeds are dispersed and the condition of the seeds moved – of seed dispersal (Schupp 1993; Wang and Smith 2002; Levin et al. 2003). Therefore, every minute aspect of the foraging behavior and movement of frugivores seem to have a tremendous impact on the dispersal of seeds. It determines the fate of individual seed's survival probability (Condit et al. 2002; Terborgh et al. 2002), fate of the genetic structure of a population (Hamrick et al. 1993), and fate of a community and an ecosystem (Terborgh et al. 2002; Schupp et al. 2002; Levin et al. 2003).

Apart from the complex factors that Aslan et al. (2019) suggested (Fig. 14.1), Corlett (1998, 2017), while synthesizing the available literature on plants dispersed by frugivorous vertebrates of the Oriental region, came up with the following findings. First, small fruits are well dispersed as their potential seed dispersal agents include small vertebrates that thrive in fragmented and disturbed forests. Second, larger-seeded fruits are poorly dispersed as they rely on large mammals and birds, which are highly vulnerable to fragmentation, habitat loss, and hunting.

The evidences from Oriental tropics suggest that hornbills, elephants, gibbons, bulbuls, civets, and fruit bats are the major primary seed dispersal agents (Corlett 2017; Naniwadekar et al. 2019). Green pigeons, macaques, rodents, bears, and deer,

which were previously underestimated as seed dispersal agents, are proved to be efficient dispersal agents (Sengupta et al. 2015; Corlett 2017; McConkey 2018). Corlett (1998, 2017) is also of the opinion that fishes, rodents, and fruit bats could be major primary dispersal agents of the fragmented and secondary forests of old-world tropics, but need more studies. Secondary seed dispersal agents include rodents, dung beetles, ants, and crabs. While the former two are generalists, ant- and crab-dispersed seeds have elaiosomes or arils, the nutritive part they are interested in (Krishna and Somanathan 2014; Lengyel et al. 2009).

We have information on frugivores of some wild plants of south and north-east India. We have summarized this information in Table 14.1, which however may be incomplete. For instance, Tadwalkar et al. (2012) have suggested dispersal modes for 185 plant species of northern Western Ghats of India, but not identified the species or species group of dispersal agents. So, we have not included them in the list. Nevertheless, only very few have studied dispersal efficiency of the frugivores in any of these plant species.

Here, we review some of the less studied seed dispersal mechanisms relevant for India. We focus on long-distance seed dispersal by migratory birds, chiropterochory (seed dispersal by fruit bats), and ichthyochory (seed dispersal by fishes). We analyze the status of knowledge on these three dispersal modes and the threat they face.

14.2 Chiropterochory

Chiropterochory – seed dispersal by fruit bats – is considered a major mode of seed dispersal of tropical forests (Fujita and Tuttle 1991; Shilton et al. 1999; Stoner et al. 2007). But, it has received very little attention from the old-world tropics, especially India. It is estimated that globally over 300 plant species are receiving the service of fruit bats for dispersing their seeds, which include both the early succession and mature forest species (Fujita and Tuttle 1991; Shilton et al. 1999; Stoner et al. 2007; Kelm et al. 2008; Melo et al. 2009). Apart from the dispersal service the fruit bats render to angiosperms, bats also render pollination services to 528 plant species (Marshall 1983; Fleming et al. 2009) of which 360 are pollinated by phyllostomatid bats and 168 are pollinated by pteropodid bats.

The world's bat fauna consists of about 1100 species belonging to 201 genera and 18 families (Simmons 2005). Fruit bats are common in different parts of the world, but with great richness and abundance in paleotropics and neotropics. Paleotropics has 166 frugivorous and nectarivorous fruit bat species in the family Pteropodidae (Mickleburgh et al. 1992; Koopman 1993). India has 117 species of bats, but only less than a quarter of them are obligate fruit bats (Talmale and Pradhan 2009; Bates and Harrison 1997). Unlike neotropics, where the number of fruit bat and non-fruit bat species is more or less the same, in old-world tropics non-fruit bats dominate any given habitat studied (Bates and Harrison 1997; Talmale and Pradhan 2009; Raghuram et al. 2014; Debata et al. 2019). However, in neotropics, the fruit bats are dominated by small species belonging to Microchiroptera (Farnedo et al. 2015).

Table 14.1 Frugivores/potential dispersal agents of seeds of plants reported from India

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Goniothalamus wightii</i>	Annonaceae	Birds	Ganesh and Davidar (2001)
<i>Meiogyne panosa</i>	Annonaceae	Birds	
<i>Miliusa wightiana</i>	Annonaceae	Birds	
<i>Viburnum punctatum</i>	Caprifoliaceae	Birds	
<i>Daphniphyllum</i> sp.	Daphniphyllaceae	Birds	
<i>Antidesma menasu</i>	Euphorbiaceae	Birds	
<i>Macaranga peltata</i>	Euphorbiaceae	Birds	
<i>Mallotus tetracoccus</i>	Euphorbiaceae	Birds	
<i>Casearia ovata</i>	Flacourtiaceae	Birds	
<i>Gomphandra coriacea</i>	Icacinaceae	Birds	
<i>Nothopodytes nimmoniana</i>	Icacinaceae	Birds	
<i>Actinodaphne bourdillonii</i>	Lauraceae	Birds	
<i>Actinodaphne</i> sp.	Lauraceae	Birds	
<i>Alseodaphne semicarpifolia</i>	Lauraceae	Birds	
<i>Cinnamomum sulphuratum</i>	Lauraceae	Birds	
<i>Cinnamomum filipedicellatum</i>	Lauraceae	Birds	
<i>Cinnamomum travancoricum</i>	Lauraceae	Birds	
<i>Cryptocarya lawsonii</i>	Lauraceae	Birds	
<i>Litsea wightiana</i>	Lauraceae	Birds	
<i>Litsea mysorensis</i>	Lauraceae	Birds	
<i>Neolitsea cassia</i>	Lauraceae	Birds	
<i>Neolitsea fisheri</i>	Lauraceae	Birds	
<i>Persea macrantha</i>	Lauraceae	Birds	
<i>Phoebe lanceolatum</i>	Lauraceae	Birds	
<i>Michelia nilagirica</i>	Magnoliaceae	Birds	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Memecylon malabaricum</i>	Melastomataceae	Birds	
<i>Trichilia connaroides</i>	Meliaceae	Birds	
<i>Ficus micrococa</i>	Moraceae	Birds	
<i>Rapanea wightiana</i>	Myrsinaceae	Birds	
<i>Maesa indica</i>	Myrsinaceae	Birds	
<i>Eugenia thwaitesii</i>	Myrtaceae	Birds	
<i>Syzygium gardneri</i>	Myrtaceae	Birds	
<i>Syzygium travancoricum</i>	Myrtaceae	Birds	
<i>Gomphia serrata</i>	Ochnaceae	Birds	
<i>Helicia nilagirica</i>	Proteaceae	Birds	
<i>Ixora nigricans</i>	Rubiaceae	Birds	
<i>Octotropis travancorica</i>	Rubiaceae	Birds	
<i>Pavetta thomsonii</i>	Rubiaceae	Birds	
<i>Tricalysia apiocarpa</i>	Rubiaceae	Birds	
<i>Isonandra lanceolata</i>	Sapotaceae	Birds	
<i>Ternstroemia japonica</i>	Theaceae	Birds	
<i>Clerodendrum viscosum</i>	Verbenaceae	Birds	
<i>Canarium strictum</i>	Burseraceae	Birds large	
<i>Mastixia arborea</i>	Cornaceae	Birds large	
<i>Sclopia crenata</i>	Flacourtiaceae	Birds large	
<i>Beilschmiedia wightii</i>	Lauraceae	Birds large	
<i>Litsea glabrata</i>	Lauraceae	Birds large	
<i>Litsea insignis</i>	Lauraceae	Birds large	
<i>Dysoxylum malabaricum</i>	Meliaceae	Birds large	
<i>Holigarna nigra</i>	Anacardiaceae	Mammals	
<i>Diospyros malabarica</i>	Ebenaceae	Mammals	
<i>Diospyros sylvatica</i>	Ebenaceae	Mammals	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Elaeocarpus venustus</i>	Elaeocarpaceae	Mammals	
<i>Elaeocarpus munronii</i>	Elaeocarpaceae	Mammals	
<i>Elaeocarpus serratus</i>	Elaeocarpaceae	Mammals	
<i>Elaeocarpus tuberculatus</i>	Elaeocarpaceae	Mammals	
<i>Calophyllum austroindicum</i>	Guttiferae	Mammals	
<i>Garcinia travancoricum</i>	Guttiferae	Mammals	
<i>Aglaia elaeagnoidea</i>	Meliaceae	Mammals	
<i>Aglaia tamiyahdensis</i>	Meliaceae	Mammals	
<i>Ficus virens</i>	Moraceae	Mammals	
<i>Artocarpus heterophyllus</i>	Moraceae	Mammals	
<i>Myristica dactyloides</i>	Myristicaceae	Mammals	
<i>Eugenia floccosa</i>	Myrtaceae	Mammals	
<i>Syzygium mundagam</i>	Myrtaceae	Mammals	
<i>Prunus ceylanica</i>	Rosaceae	Mammals	
<i>Canthium travancoricum</i>	Rubiaceae	Mammals	
<i>Acronychia penduliflora</i>	Rutaceae	Mammals	
<i>Vepris bilocularis</i>	Rutaceae	Mammals	
<i>Palaquium ellipticum</i>	Sapotaceae	Mammals	
<i>Cullenia exarillata</i>	Bombacaceae	Mechanical	
<i>Vernonia travancorica</i>	Compositae	Mechanical	
<i>Agrostistachys borneensis</i>	Euphorbiaceae	Mechanical	
<i>Drypetes longifolia</i>	Euphorbiaceae	Mechanical	
<i>Euphorbia antiquorum</i>	Euphorbiaceae	Mechanical	
<i>Mallotus resinosus</i>	Euphorbiaceae	Mechanical	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Epiprinus mallowiformis</i>	Euphorbiaceae	Mechanical	
<i>Heritiera papilio</i>	Fabaceae	Mechanical	
<i>Ormosia travancorica</i>	Fabaceae	Mechanical	
<i>Hydnocarpus alpina</i>	Flacourtiaceae	Mechanical	
<i>Dimocarpus longon</i>	Sapindaceae	Mechanical	
<i>Gordonia obtusa</i>	Theaceae	Mechanical	
<i>Phyllanthus emblica</i>	Euphorbiaceae	Deer, chital, barking deer, langur, Indian gerbil	Prasad et al. (2004)
<i>Acacia intsia</i>	Leguminosae	<i>Elephas maximus</i>	Baskaran and Desai (2013)
<i>Artocarpus heterophyllus</i>	Moraceae	<i>Elephas maximus</i>	
<i>Bauhinia racemosa</i>	Caesalpiniaceae	<i>Elephas maximus</i>	
<i>Grewia hirsuta</i>	Tiliaceae	<i>Elephas maximus</i>	
<i>Grewia tiliifolia</i>	Tiliaceae	<i>Elephas maximus</i>	
<i>Mangifera indica</i>	Anacardiaceae	<i>Elephas maximus</i>	
<i>Tamarindus indica</i>	Fabaceae	<i>Elephas maximus</i>	
<i>Ziziphus mauritiana</i>	Rhamnaceae	<i>Elephas maximus</i>	
<i>Vitex glabrata</i>	Lamiaceae	<i>Paradoxurus hermaphroditus</i>	Chakravarthy and Ratnam (2015)
<i>Vitex glabrata</i>	Lamiaceae	<i>Megalaima lineata</i>	
<i>Vitex glabrata</i>	Lamiaceae	<i>Megalaima asiatica</i>	
<i>Vitex glabrata</i>	Lamiaceae	<i>Pycnonotus melanicterus</i>	
<i>Vitex glabrata</i>	Lamiaceae	<i>Treron apicauda</i>	
<i>Vitex glabrata</i>	Lamiaceae	<i>Treron sphenurus</i>	
<i>Prunus ceylanica</i>	Rosaceae	<i>Viverra zibetha</i>	
<i>Prunus ceylanica</i>	Rosaceae	<i>Tupaia glis</i>	
<i>Prunus ceylanica</i>	Rosaceae	<i>Rhyticeros undulatus</i>	
<i>Dillenia pentagyna</i>	Dilleniaceae	<i>Treron phoenicopterus</i>	Chatterjee and Basu (2015)
<i>Dillenia pentagyna</i>	Dilleniaceae	<i>Treron bicinctus</i>	
<i>Amoora rohituka</i>	Meliaceae	<i>Macaca mulatta</i>	
<i>Anogeissus latifolia</i>	Combretaceae	<i>Macaca mulatta</i>	Sengupta et al. (2015)

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Anthocephalus chinensis</i>	Rubiaceae	<i>Macaca mulatta</i>	
<i>Antidesma diandrum</i>	Euphorbiaceae	<i>Macaca mulatta</i>	
<i>Artocarpus chaplasha</i>	Moraceae	<i>Macaca mulatta</i>	
<i>Baccauria sapida</i>	Euphorbiaceae	<i>Macaca mulatta</i>	
<i>Beilschmedia gammieana</i>	Lauraceae	<i>Macaca mulatta</i>	
<i>Bridelia retusa</i>	Euphorbiaceae	<i>Macaca mulatta</i>	
<i>Careya arborea</i>	Lecythidaceae	<i>Macaca mulatta</i>	
<i>Casearia spp.</i>	Flacourtiaceae	<i>Macaca mulatta</i>	
<i>Chisocheton paniculatus</i>	Meliaceae	<i>Macaca mulatta</i>	
<i>Cissus elongata</i>	Vitaceae	<i>Macaca mulatta</i>	
<i>Crataeva unilocularis</i>	Capparaceae	<i>Macaca mulatta</i>	
<i>Elaeocarpus aristatus</i>	Elaeocarpaceae	<i>Macaca mulatta</i>	
<i>Elaeocarpus floribundus</i>	Elaeocarpaceae	<i>Macaca mulatta</i>	
<i>Elaeocarpus varuna</i>	Elaeocarpaceae	<i>Macaca mulatta</i>	
<i>Emblica officinalis</i>	Phyllanthaceae	<i>Macaca mulatta</i>	
<i>Eurya acuminata</i>	Theaceae	<i>Macaca mulatta</i>	
<i>Ficus benghalensis</i>	Moraceae	<i>Macaca mulatta</i>	
<i>Ficus benjamina</i>	Moraceae	<i>Macaca mulatta</i>	
<i>Ficus racemosa</i>	Moraceae	<i>Macaca mulatta</i>	
<i>Ficus spp.</i>	Moraceae	<i>Macaca mulatta</i>	
<i>Gmelina arborea</i>	Verbenaceae	<i>Macaca mulatta</i>	
<i>Leea spp.</i>	Leeaceae	<i>Macaca mulatta</i>	
<i>Mangifera indica</i>	Anacardiaceae	<i>Macaca mulatta</i>	
<i>Mangifera sylvatica</i>	Anacardiaceae	<i>Macaca mulatta</i>	
<i>Populus gamblei</i>	Salicaceae	<i>Macaca mulatta</i>	
<i>Polyalthia simiarum</i>	Annonaceae	<i>Macaca mulatta</i>	
<i>Premna benghalensis</i>	Verbenaceae	<i>Macaca mulatta</i>	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Spondias mangifera</i>	Anacardiaceae	<i>Macaca mulatta</i>	
<i>Syzygium cumini</i>	Myrtaceae	<i>Macaca mulatta</i>	
<i>Syzygium formosa</i>	Myrtaceae	<i>Macaca mulatta</i>	
<i>Talauma hodgsonii</i>	Magnoliaceae	<i>Macaca mulatta</i>	
<i>Zanthoxylum budrunga</i>	Rutaceae	<i>Macaca mulatta</i>	
<i>Zizyphus mauritiana</i>	Rhamnaceae	<i>Macaca mulatta</i>	
<i>Syzygium cumini</i>	Myrtaceae	Red-whiskered bulbul, white-cheaked barbet, oriental white-eye, crimson-backed sunbird, blossom-headed parakeet	Sinu et al. (2012)
<i>cassia fistula</i>	Fabaceae	Sloth bear	Sreekumar and Balakrishnan (2002)
<i>Artocarpus integerifolia</i>	Moraceae	Sloth bear	
<i>Artocarpus hirusta</i>	Moraceae	Sloth bear	
<i>Ficus</i> spp.	Moraceae	Sloth bear	
<i>Mangifera indica</i>	Anacardiaceae	Sloth bear	
<i>Ziziphus rugosa</i>	Rhamnaceae	Sloth bear	
<i>Z. oenopina</i>	Rhamnaceae	Sloth bear	
<i>Baccaurea courtallensis</i>	Euphorbiaceae	Sloth bear	
<i>Bridelia retusa</i>	Euphorbiaceae	Sloth bear	
<i>Aporosa lindeleyana</i>	Euphorbiaceae	Sloth bear	
<i>Elaeocarpus tuberculatus</i>	Elaeocarpaceae	Sloth bear	
<i>Phoenix humilis</i>	Palmaceae	Sloth bear	
<i>Ixora coccinea</i>	Rubiaceae	Sloth bear	
<i>Syzygium cumini</i>	Myrtaceae	Sloth bear	
<i>Lantana camara</i>	Verbenaceae	Sloth bear	
<i>Dillenia indica</i>	Dilleniaceae	<i>Elephas maximus</i>	Sekar and Sukumar (2013) and Sekar et al. (2015)
<i>Artocarpus chaplasha</i>	Moraceae	<i>Elephas maximus</i>	
<i>Careya arborea</i>	Lecythidaceae	<i>Elephas maximus</i>	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Saurauia nepalensis</i>	Actinidiaceae	Bird, mammals	Datta and Rawat (2008)
<i>Choerospondias axillaris</i>	Anacardiaceae	Mammals	
<i>Drimycarpus racemosus</i>	Anacardiaceae	Mammals	
<i>Lannea grandis</i>	Anacardiaceae	Bird, mammals	
<i>Mangifera sylvatica</i>	Anacardiaceae	Mammals	
<i>Spondias pinnata</i>	Anacardiaceae	Mammals	
<i>Miliusa roxburghiana</i>	Annonaceae	Birds	
<i>Polyalthia simiarum</i>	Annonaceae	Bird, mammals	
<i>Polyalthia</i> sp. 2	Annonaceae	Bird, mammals	
<i>Alstonia scholaris</i>	Apocynaceae	Wind	
<i>Wrightia tomentosa</i>	Apocynaceae	Wind	
<i>Oroxylum indicum</i>	Bignoniaceae	Wind	
<i>Radermachera sinica</i>	Bignoniaceae	Birds	
<i>Stereospermum chelonoides</i>	Bignoniaceae	Wind	
<i>Ehretia acuminata</i>	Boraginaceae	Bird, mammals	
<i>Ehretia laevis</i>	Boraginaceae	Birds	
<i>Canarium resiniferum</i>	Burseraceae	Bird, mammals	
<i>Canarium resiniferum</i>	Burseraceae	Birds	
<i>Garuga pinnata</i>	Burseraceae	Bird, mammals	
<i>Bauhinia purpurea</i>	Caesalpiniaceae	Wind	
<i>Crataeva religiosa</i>	Capparidaceae	Birds	
<i>Bhesa robusta</i>	Celastraceae	Birds	
<i>Calophyllum polyanthum</i>	Clusiaceae	Mammals	
<i>Garcinia xanthochymus</i>	Clusiaceae	Mammals	
<i>Kayea assamica</i>	Clusiaceae	Gravity, water	
<i>Mesua ferrea</i>	Clusiaceae	Birds?	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Terminalia bellerica</i>	Combretaceae	Mammals	
<i>Terminalia chebula</i>	Combretaceae	Mammals	
<i>Tetrameles nudiflora</i>	Datiscaceae	Wind	
<i>Dillenia indica</i>	Dilleniaceae	Mammals	
<i>Dillenia pentagyna</i>	Dilleniaceae	Bird, mammals	
<i>Diospyros toposia</i>	Ebenaceae	Mammals	
<i>Elaeocarpus aristatus</i>	Elaeocarpaceae	Mammals	
<i>Elaeocarpus ganitrus</i>	Elaeocarpaceae	Mammals	
<i>Aporusa octandra</i>	Euphorbiaceae	Bird, mammals	
<i>Baccaurea ramiflora</i>	Euphorbiaceae	Mammals	
<i>Bischofia javanica</i>	Euphorbiaceae	Birds, mammals	
<i>Bridelia retusa</i>	Euphorbiaceae	Birds	
<i>Croton roxburghii</i>	Euphorbiaceae	Wind, ballistic	
<i>Croton sp.1</i>	Euphorbiaceae	Wind, ballistic	
<i>Croton sp.2</i>	Euphorbiaceae	Wind, ballistic	
<i>Endospermum chinense</i>	Euphorbiaceae	Mammals	
<i>Glochidion assamicum</i>	Euphorbiaceae	Birds	
<i>Macaranga denticulata</i>	Euphorbiaceae	Birds	
<i>Mallotus philippensis</i>	Euphorbiaceae	Bird, mammals	
<i>Ostodes paniculata</i>	Euphorbiaceae	Wind	
<i>Sapium baccatum</i>	Euphorbiaceae	Birds	
<i>Sapium eugeniaefolium</i>	Euphorbiaceae	Birds?	
<i>Castanopsis hystrix</i>	Fagaceae	Gravity	
<i>Castanopsis indica</i>	Fagaceae	Gravity	
<i>Lithocarpus macrophylla</i>	Fagaceae	Gravity, wind	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Flacourтиa indica</i>	Flacourtiaceae	Birds	
<i>Gynocardia odorata</i>	Flacourtiaceae	Mammals	
<i>Altingia excelsa</i>	Hamamelidaceae	Gravity	
<i>Aesculus assamicus</i>	Hamamelidaceae	Gravity	
<i>Platea latifolia</i>	Icacinaceae	Birds	
<i>Engelhardtia spicata</i>	Juglandaceae	Wind	
<i>Actinodaphne angustifolia</i>	Lauraceae	Birds	
<i>Actinodaphne obovata</i>	Lauraceae	Birds	
<i>Alseodaphne peduncularis</i>	Lauraceae	Birds	
<i>Beilshmedia roxburghiana</i>	Lauraceae	Birds	
<i>Beilshmedia</i> sp. 2	Lauraceae	Birds	
<i>Beilshmedia</i> sp. 3	Lauraceae	Birds	
<i>Cinnamomum tamala</i>	Lauraceae	Birds	
<i>Cinnamomum cecicodaphne</i>	Lauraceae	Bird, mammals	
<i>Cinnamomum obtusifolium</i>	Lauraceae	Birds	
<i>Cryptocarya amygdalina</i>	Lauraceae	Birds	
<i>Cryptocarya</i> sp. 2	Lauraceae	Birds	
<i>Dodecadenia grandiflora</i>	Lauraceae	Birds	
<i>Litsea chinensis</i>	Lauraceae	Birds	
<i>Litsea monopetala</i>	Lauraceae	Birds	
<i>Litsea panamonja</i>	Lauraceae	Birds	
<i>Litsea</i> sp.	Lauraceae	Birds	
<i>Neolitsea umbrosa</i>	Lauraceae	Birds	
<i>Persea/Phoebe</i> sp.	Lauraceae	Birds	
<i>Phoebe attenuata</i>	Lauraceae	Birds	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Phoebe cooperiana</i>	Lauraceae	Birds	
<i>Phoebe lanceolata</i>	Lauraceae	Birds	
<i>Phoebe</i> sp.	Lauraceae	Birds	
<i>Leea indica</i>	Leeaceae	Birds	
<i>Duabanga grandiflora</i>	Lythraceae	Bird, mammals	
<i>Lagerstroemia parviflora</i>	Lythraceae	Gravity, wind	
<i>Lagerstroemia speciosa</i>	Lythraceae	Gravity, wind	
<i>Michelia champaca</i>	Magnoliaceae	Birds?	
<i>Talauma hodgsonii</i>	Magnoliaceae	Mammals	
<i>Talauma</i> sp. 2	Magnoliaceae	Mammals	
<i>Kydia calycina</i>	Malvaceae	Wind	
<i>Aglaia</i> sp. 1	Meliaceae	Birds	
<i>Aglaia</i> sp. 2	Meliaceae	Mammals	
<i>Aglaia spectabilis</i>	Meliaceae	Birds	
<i>Aphanamixis polystachya</i>	Meliaceae	Birds	
<i>Chisocheton paniculatus</i>	Meliaceae	Birds	
<i>Chukrasia tabularis</i>	Meliaceae	Wind	
<i>Dysoxylum binectariferum</i>	Meliaceae	Birds	
<i>Dysoxylum hamiltonii</i>	Meliaceae	Birds	
<i>Toona ciliata</i>	Meliaceae	Wind	
<i>Toona febrifuga</i>	Meliaceae	Wind	
<i>Albizia lucida</i>	Mimosaceae	Wind	
<i>Albizia procera</i>	Mimosaceae	Wind	
<i>Artocarpus chaplasha</i>	Moraceae	Bird, mammals	
<i>Ficus maclellandii</i>	Moraceae	Bird, mammals	
<i>Ficus altissima</i>	Moraceae	Bird, mammals	
<i>Ficus cyrtophylla/ clavata</i>	Moraceae	Bird, mammals	
<i>Ficus elastica</i>	Moraceae	Bird, mammals	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Ficus hookeri</i>	Moraceae	Bird, mammals	
<i>Ficus lamponga</i>	Moraceae	Mammals	
<i>Ficus mysorensis</i>	Moraceae	Bird, mammals	
<i>Ficus nervosa</i>	Moraceae	Bird, Mammals	
<i>Ficus pomifera</i>	Moraceae	Mammals	
<i>Ficus scandens</i>	Moraceae	Birds	
<i>Ficus</i> sp. 1	Moraceae	Birds	
<i>Ficus</i> sp. 2	Moraceae	Birds	
<i>Morus laevigata</i>	Moraceae	Birds	
<i>Horsfieldia kingii</i>	Myristicaceae	Birds	
<i>Knema angustifolia</i>	Myristicaceae	Birds	
<i>Syzygium formosum</i>	Myrtaceae	Birds	
<i>Syzygium megacarpum</i>	Myrtaceae	Bird, mammals	
<i>Syzygium</i> sp.	Myrtaceae	Birds	
<i>Syzygium syzygioides</i>	Myrtaceae	Birds	
<i>Linoceira macrophylla</i>	Oleaceae	Bird, mammals	
<i>Livistona jenkinsiana</i>	Palmae	Birds	
<i>Dalbergia paniculata</i>	Papilionaceae	Wind	
<i>Erythrina stricta</i>	Papilionaceae	Birds?	
<i>Helicia nilagirica</i>	Proteaceae	Mammals?	
<i>Hovenia acerba</i>	Rhamnaceae	Birds	
<i>Zizyphus</i> sp.	Rhamnaceae	Bird, mammals	
<i>Carallia brachiata</i>	Rhizophoraceae	Birds	
<i>Pygeum acuminatum</i>	Rosaceae	Birds	
<i>Pygeum</i> sp. 2	Rosaceae	Birds	
<i>Anthocephalus cadamba</i>	Rubiaceae	Mammals	
<i>Hyptianthera stricta</i>	Rubiaceae	Birds	
<i>Tricalysia</i> sp.	Rubiaceae	Bird, mammals	
<i>Evodia roxburghiana</i>	Rutaceae	Birds	
<i>Micromelum integrerrimum</i>	Rutaceae	Birds	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Zanthoxylum oxyphyllum</i>	Rutaceae	Birds	
<i>Zanthoxylum rhetsa</i>	Rutaceae	Birds	
<i>Meliosma dilleniaeefolia</i>	Sabiaceae	Birds?	
<i>Meliosma simplicifolia</i>	Sabiaceae	Birds?	
<i>Casearia graveolens</i>	Samydaceae	Birds	
<i>Xerospermum glabratum</i>	Sapindaceae	Mammals	
<i>Ailanthus grandis</i>	Simaroubaceae	Wind	
<i>Turpinia pomifera</i>	Staphylaceae	Mammals	
<i>Echinocarpus assamicus</i>	Sterculiaceae	Gravity, rodents	
<i>Pterospermum acerifolium</i>	Sterculiaceae	Wind	
<i>Pterospermum Lanceifolium</i>	Sterculiaceae	Wind	
<i>Pterygota alata</i>	Sterculiaceae	Wind	
<i>Sterculia hamiltonii</i>	Sterculiaceae	Birds	
<i>Sterculia villosa</i>	Sterculiaceae	Bird, mammals	
<i>Stryrax serrulatum</i>	Styracaceae	Birds	
<i>Schima wallichii</i>	Theaceae	Wind	
<i>Grewia microcos</i>	Tiliaceae	Mammals	
<i>Trema orientalis</i>	Ulmaceae	Birds	
<i>Laportea crenulata</i>	Urticaceae	Birds	
<i>Callicarpa macrophylla</i>	Verbenaceae	Birds	
<i>Gmelina arborea</i>	Verbenaceae	Mammals	
<i>Prema benghalensis</i>	Verbenaceae	Mammals	
<i>Vitex peduncularis</i>	Verbenaceae	Bird, mammals	
<i>Vitex pentaphylla</i>	Verbenaceae	Bird, mammals	
<i>Atlantia monophylla</i>	Rutaceae	Fruit bats	David et al. (2015)

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Azadirachta indica</i>	Meliaceae	Birds and bonnet macaque	
<i>Cansjeera rheedei</i>	Opiliaceae	Birds	
<i>Cissus vitiginea</i>	Vitaceae	Birds	
<i>Cordia dichotoma</i>	Boraginaceae	Bird, mammals	
<i>Ehretia pubecens</i>	Boraginaceae	Birds	
<i>Eugenia bracteata</i>	Myrtaceae	Birds	
<i>Ficus amplissima</i>	Moraceae	Bird, mammals	
<i>Ficus benghalensis</i>	Moraceae	Bird, mammals	
<i>Ficus microcarpa</i>	Moraceae	Bird, mammals	
<i>Ficus racemosa</i>	Moraceae	Mammals	
<i>Hugonia mystax</i>	Linaceae	Birds	
<i>Garcinia spicata</i>	Clusiaceae	Mammals	
<i>Glycosmis pentaphylla</i>	Rutaceae	Bird, mammals	
<i>Lannea coromandelica</i>	Anacardiaceae	Birds, bonnet macaque and three-striped palm squirrel	
<i>Manilkara hexandra</i>	Sapotaceae	Bird, mammals	
<i>Memecylon umbellatum</i>	Melastomataceae	Mammals	
<i>Ochna obtusata</i>	Ochnaceae	Birds	
<i>Olax scandens</i>	Olaraceae	Birds	
<i>Opilia amantacea</i>	Opiliaceae	Fruit bats	
<i>Pachygone ovata</i>	Menispermaceae	Mammals	
<i>Pamburus missionis</i>	Rutaceae	Mammals	
<i>Phoenix farinifera</i>	Arecaceae	Mammals	
<i>Salvadora persica</i>	Salvadoraceae	Birds	
<i>Securinega leucopyrus</i>	Euphorbiaceae	Birds	
<i>Syzygium cumini</i>	Myrtaceae	Mammals	
<i>Walsura trifolia</i>	Meliaceae	Birds	
<i>Zizyphus mauritiana</i>	Rhamnaceae	Mammals	

(continued)

Table 14.1 (continued)

Plant species	Plant family	Frugivore/dispersal agent	References
<i>Zizyphus oenoplia</i>	Rhamnaceae	Bird, mammals	
<i>Ptychosperma macarthurii</i>	Palmae	<i>Ocypterus griseus</i> , <i>Psilopogon viridis</i> , <i>Dendrocitta vagabunda</i> , <i>Eudynamys scolopaceus</i> , <i>Copsychus saularis</i> , <i>Spilopelia chinensis</i> , <i>Oriolus xanthomatus</i> , <i>Turdus simillimus</i> , <i>Myophonus horsfieldii</i> , <i>Pycnonotus cafer</i> , <i>P. jacous</i> , <i>Acridotheres javanicus</i> , <i>A. tristis</i>	Elsamol et al. (2019)
<i>Streblus asper</i>	Moraceae	Bulbuls (3 spp); myna (2 spp)	Aruna and Balasubramanian (2014)
<i>Santalum album</i>	Santalaceae	Asian koel, Indian grey hornbill, common myna, brahminy starling, brown-headed barbet, white-headed babbler, Indian grey hornbill, three-striped palm squirrel	Balasubramanian et al. (2011)
<i>Aquilaria malaccensis</i>	Thymelaeaceae	Wasp	Manohara (2013)
<i>Myristica fatua</i>	Myristicaceae	Crabs	Krishna and Somanathan (2014)
<i>Lantana camara</i>	Verbenaceae	Bulbuls (3 spp), myna (2 spp), babbler (2 spp), Asian koel, small green-billed malkoha, Blyth's reed warbler, common iora	Aruna and Balasubramanian (2017)
<i>Dysoxylum malabaricum</i>	Meliaceae	Malabar grey hornbill	Shivanna et al. (2003)
<i>Madhuca latifolia</i>	Sapotaceae	<i>Pteropus giganteus</i> , <i>Cynopterus sphinx</i> , <i>Rousettus leschenaultii</i>	Mahandran et al. (2018)
<i>Prosopis juliflora</i>	Fabaceae	Common palm civet	Khan et al. (2019)
<i>Ficus religiosa</i>	Moraceae	Common palm civet	
<i>Date palm</i>	Palmae	Common palm civet	

Although the population of fruit bats is not estimated in any Indian habitats, it is considered that they are abundant, environment and climate resilient, and resurgent. Among the fruit bat species in the old-world tropics, species of the spectacled Indian flying fox, *Pteropus*, have received disproportionately high focus. However, small fruit bats are also common in the paleotropics. Fruit bats are major dispersal agents of mainland and island flora (Utzurrum and Heideman 1991; Shilton et al. 1999; Hall

and Richards 2000; Mildenstein et al. 2005; Heer et al. 2010), including the defaunated newly created volcanic islands (Cox et al. 1991; Rainey 1995). Although fruit bats are seen along the elevational gradients in tropics, the lowland forests supply majority of their food resources (Stier 2003; Stier and Mildenstein 2005); and their services are considered important for lowland tropical forests also.

In paleotropics, the fruit bats (Chiroptera: Pteropodidae) help in dispersing and pollinating quite a good number of fruit crops, such as figs, rambutan, litchi, cashew, breadfruit, durian, and banana (Fujita and Tuttle 1991; Boyles et al. 2011; Kasso and Balakrishnan 2013). However, since monitoring the fruit bats at night is difficult, the importance of chiropterochory in wild species has received very little attention.

The diet of nectarivorous and frugivorous bats consists of both pollen and nectar (during flowering season) and fruits (fruiting season) (Bumrungsri et al. 2013). The fruit bat consumes both floral and fruit resources from same plant species during flowering and fruiting seasons, respectively (Hodgkison et al. 2003; Nathan et al. 2009; Bumrungsri et al. 2013; Mahandran et al. 2018). For instance, Bumrungsri et al. (2013) captured 607 nectarivorous bats of *Eonycteris spelaea* Dobson (Pteropodidae) and collected 1155 fecal samples of 506 bats and 1598 fur samples from 607 bats to study the diet of the species in Thailand. Pollen grains of *Durio* spp. were highly represented in the fecal samples (up to 42%) during the fruiting period, confirming that the bat mixes diets of both pollen and fruits (Bumrungsri et al. 2013). They also found that the fecal samples and pollen samples from bat furs had pollen remains of *Parkia* spp. (34%), *Musa* spp. (28%), and *Cocos nucifera* (<2.5%). There is no significant variation in the diet composition of adult males and females of *E. spelaea*.

Bats of different species often co-roost in the same sites. Stier and Mildenstein (2005) examined 771 fecal samples of the two co-roosting world's largest flying foxes (forearm length > 110 mm) – *Acerodon jubatus* and *Pteropus vampyrus lanensis* – for 2 years in the Philippines. They found that fig seeds were predominant in the fecal samples of both the species. Although they co-roost, *A. jubatus* is a natural forest obligate and specialist on fruits (particularly the fruits of hemiepiphytic figs) and leaves of mature lowland forests. But, *P. v. lanensis* is a generalist and feeds on leaves, flowers, and fruits from both natural forests and agroforests, such as mango, guava, star apple (*Chrysophyllum cainito*) and *Erythrina fusca*.

14.3 Bat- and Bird-Dispersed Fruits

The fruits that have bat dispersal syndrome are in general different from that dispersed by the birds, the major seed dispersal counterpart of bats in the day (Fleming and Kress 2011). But, some fruits like figs are eaten in good numbers by both the birds and bats. Jacomassa and Pizo (2010) in their studies on the effectiveness of birds and bats in dispersing the seeds of a pioneer species found that the bats are efficient in transporting considerably more number of seeds when compared to the birds. However, the germination rate and the time taken for germination were noticeably shorter for bird-dispersed seeds. So, the bats contribute to the quantitative

aspect of the seed dispersal, and the birds contribute to the qualitative aspect of the seed dispersal (Jacomassa and Pizo 2010). The fruit bat-dispersed fruits are normally drab green, pulpy, and positioned away from branches (Pijl 1957; Hodgkison et al. 2003; Mahandran et al. 2018). They mostly have hard rind and essential oils and are fibrous in nature (Galindo-González et al. 2000; Hodgkison et al. 2003; Bianconi et al. 2012). The bat-dispersed fruits also ripen more or less synchronously and emit a faint but distinct nocturnal odor (Corlett 1998; Shanahan et al. 2001; Hodgkison et al. 2003). The bird-dispersed fruits, however, are small, purplish, fleshy, soft skinny, and succulent. The bird-dispersed fruits remain attached to the branches at maturity, while the mammal-dispersed fruits fall at maturity (Vander Wall and Beck 2012). Unlike the bat-dispersed fruits, bird-dispersed fruits emit no fragrance at ripening and contain toxins (Levey et al. 2006). But, the birds' digestive enzymes can detoxify such fruit toxins (Struempf et al. 1999; Levey et al. 2006).

14.4 What Makes Fruit Bats Good Dispersal Agents?

The abundance during fruiting season, ability to travel long distance, and gut passage time predict the seed dispersal efficiency of a frugivore. Fruit bats meet all of these primary requirements to make them excellent dispersal agents in tropics (Morrison 1978a, 1980). Both the fruit bats and nectarivorous bats are efficient to read climate signals and travel several kilometers in search of food (Taylor 2006; Sherwin et al. 2013) and to travel between day roosts and feeding roosts and among fruiting trees (Start and Marshall 1976; Morrison 1978b). They perform local and regional migration based on food availability (Sherwin et al. 2013; Manandhar et al. 2018). They are environment and disturbance resilient and live in roosts on large common trees. For instance, the Indian flying fox (*Pteropus giganteus*) – a large fruit species – in Nepal valleys roosts on trees of *Ficus* spp., *Grevillea robusta*, *Elaeocarpus ganitrus*, *Dalbergia sissoo*, *Celtis australis*, *Populus* sp., *Persea* sp., *Linder* sp., and *Pinus roxburghii* in human-influenced habitats (Manandhar et al. 2018). Although they have limitations to disperse large-seeded fruits, they are excellent dispersal agents of small-seeded (<2–4 mm) fruits (Corlett 1998). Also, they defecate during flight, which allow random distribution of seeds. The median gut passage time for small seeds for large fruit bat species (*Eidolon helvum*) in West Africa was 72 min, but can go up to a maximum of 19 h (Abedi-Lartey et al. 2016). Considering the longer seed retention time in the gut, defecation in flight, and faraway nesting roosts (Shilton et al. 1999; Epstein et al. 2009; Oleksy et al. 2015; Weber et al. 2015; Abedi-Lartey et al. 2016), the fruit bats are likely to perform a very long-distance (>10–100 km) dispersal of seeds (Corlett 2017). Further, the fruit bats render dispersal service to plants in all types of habitats, from isolated single plant to an extremely small fragment to a contiguous mature forest, and have no reluctance to cross cleared areas (Griscom et al. 2007; Deshpande et al. 2016).

Ingestion and fruit handling may decapacitate the viability of seeds when it is passed through a mixture of digestive enzymes in the animals' digestive tracts (Traveset et al. 2008). Except the fruit bats belong to *Chiroderma* – which chew

seeds – all other bats swallow small seeds and are likely to disperse intact seeds (Nogueira and Peracchi 2003). Studies also suggest that gut passage of seeds of bats has no negative effect on the seeds (Fujita and Tuttle 1991; Galindo-González et al. 2000; Lopez and Vaughan 2004; Muscarella and Fleming 2007; Djossa et al. 2008; Marques and Fischer 2009; Heer et al. 2010). For instance, Heer et al. (2010), by seeding seeds of figs collected manually and collected from the fecal matter of fruit bats, showed that the gut passage positively influenced the germination of seeds.

The quality of seed shadow determines the dispersal efficiency of a frugivore. Some dispersal agents allow scattering of seeds, but some create clumping of seeds in one location. Depending on the nature of plants (climax or pioneer), this behavior is assessed positive or negative to the plants' reproduction. Most fruit bats have a nonrandom feeding roost away from the fruiting tree, normally within 20–300 m from the parent plant (Morrison 1978a; Tang et al. 2007, 2008, 2012; Corlett 2014; Weber et al. 2015). It depends on the size of both the fruit and the bat species (Mahandran et al. 2018). If fruits are large, such as a ripened mango, the bats eat pulp by sitting on the fruits and leave the seed on the tree itself or drop the seed under the tree. In that case, the dispersal ability of bats is assessed poor. But, large fruit bats belonging to *Pteropus* sp., which typically prefer large fruits for feeding, move the seeds to a smaller distance. But, some prefer to eat from the feeding spot on the parent tree (see Mahandran et al. 2018). But, the small fruit bats belonging to the genus *Cynopterus* do piecemeal on large fruits or gulp or carry small fruits to the foraging spots in some distance away or even to the roosting sites, which is normally several kilometers away.

The bats usually harvest fruits from trees and move to such spots for foraging, where they eat the flesh of fruits or bite off pieces of fruits and drink the fruit juice along with the small seeds. The dry pellet of the fruits will be spat out in the foraging spots. Since they use the same spots as feeding roosts, there is a high probability that the large seeds are deposited in clumps (Tang et al. 2008, 2012). But Shilton et al. (1999) suggest that fruit bats create a regular seed shadow for several meters to several kilometers (Shilton et al. 1999). In most cases, the seed shadows generated by the fruit bats are suitable for the germination of seeds (Fleming and Heithaus 1981; Shilton et al. 1999; Muscarella and Fleming 2007; Stoner et al. 2007; Mahandran et al. 2018).

The nature of roosting site and roosting behavior also might predict the fate of long-distance dispersal by fruit bats. Some roosts are on large trees (Manandhar et al. 2018). Some go to caves or to sites that are not suitable for seed germination. However, this has not been studied critically. *Cynopterus sphinx* (short-nosed fruit bat) after every foraging bout roosts for 5–30 min (Elangovan et al. 2001). The gut retention time also predicts the dispersal distance of fruit bats (Griscom et al. 2007; Muscarella and Fleming 2007; Tang et al. 2008). Shilton et al. (1999) suggest that the large fruit bats have an extended retention time of viable seeds in their gut. So, the seeds they eat often migrate to several kilometers.

Studies indicate that fruit bats, rather than consuming a random array of fruits, show preferences for some particular fruits over others. The diet contains fruits of different hardness and size (Marshall 1983; Dumont 2003). The force bats apply, for

Fig. 14.2 A mother and a pup of short-nosed fruit bat, *Cynopterus sphinx*, carry a fruit of *Madhuca latifolia* (Sapotaceae) to feeding roost (fruit weight, ~26 g; mother bat weight, ~50 g). Adapted from Mahandran et al. (2018)



biting fruits, is positively correlated to the body size of the bats. Although exceptions are likely, Dumont and O'Neal (2004) suggest that small fruit bats (~16 g) consume soft fruits, medium bats (31 g) eat soft and slightly harder fruits, and large bats (>800 g) eat extremely soft to extremely hard fruits.

Although studies on bat dispersal of seeds in Indian subcontinent are rare, it is generally perceived that fruit bats have an irreplaceable role in distributing seeds of several rare forest trees in tropics (Deshpande and Kelkar 2015). Quite a good number of studies reported diet of the fruit bats in India, *P. giganteus* (Elangovan et al. 2001; Karuppudurai and Sripathi 2018; Aung and Htay 2019). However, only very few studies critically studied seed dispersal efficiency of fruit bats in Indian subcontinent. Mahandran et al. (2018) studied the efficiency of three species of fruit bats, two small (*Cynopterus sphinx* and *Rousettus leschenaultia*) and one large (*P. giganteus*), in dispersing the seeds of *Madhuca latifolia* (Sapotaceae) – a tropical deciduous forest tree (Fig. 14.2). They found that the smallest bat (*C. sphinx*) visited the fruiting trees first and the largest bat (*P. giganteus*) reached last on the fruiting trees. While the smaller bat species spent time between 19:00 h and 22:00 h for foraging the fruits, the larger bat had two foraging peaks between 21:00 h and 22:00 h and at 03:00 h. There was also a spatial partitioning in the foraging layer of the canopy on fruiting trees for the larger and smaller bats. While the larger bats foraged in the upper canopy, the small bats foraged in the middle to lower canopy. *C. sphinx* moved fruits to about 32 m on average from the parent tree to the feeding roost. For *R. leschenaultia*, the feeding roost on average was about 45 m from the parent trees. Unlike these two, *P. giganteus* fed on fruits mostly on the parent tree itself; but they retrieved seeds of *M. latifolia* in the day roosts of *P. giganteus* at a

distance of about 7 km from the nearest tree. All three bats mostly spat the fruit remains and seeds at feeding roosts, and the germination rate of dispersed seeds was not different from that of undispersed seeds.

A recent unpublished study (Murugavel, unpublished) found that the subadults make longer foraging bouts (over 18 km one way) than the aged adults in Madurai district of Tamil Nadu state in south India. But, the aged adults may travel over hundred kilometers during migration. No other workers in India have studied the dispersal efficiency of fruit bats or analyzed the seedling community in foraging spots of fruit bats. In a preliminary observation, Sinu (unpublished) found seedlings of four species – *Artocarpus hirsutus*, *Leea indica*, *Caryota urens*, and *Annona* sp. – dominating in four foraging spots of an unknown fruit bat in a rural village of Ernakulam district in south India. Such studies might help us to know whether the seeds arriving to foraging spots of bats make a synergistic or a competing plant community. The seedling community that Sinu observed had a large tree (*A. hirsutus*), a small tree (*Annona* sp.), a shrub (*L. indica*), and a palm (*C. urens*).

14.5 Threats to Chiropterochory

Habitat loss is considered as a major threat to fruit bats (Mickleburgh et al. 1992, 2002). As mentioned before, lowland forests are benefited by chiropterochory. Incidentally, the lowland forests are highly affected by the major anthropogenic disturbances, such as commercial timber logging, land usage, and other developmental activities. Since biotic seed dispersal is a fine case of mutualism, the loss of lowland forest would lead to the parallel decline of both the bats and the bat-dispersed plants. But, fruit bats are known to be less vulnerable to fragmentation. Figs produce fruits almost all months in tropical climates. There is no difference of opinion that the figs are an important keystone resource for conserving fruit bats (Janzen 1979; Gautier-Hion and Michaloud 1989; Herre 1996; Shanahan et al. 2001; Bleher et al. 2003). Most fruit bats rely on figs for their major food supplements. Figs are an important source of a macronutrient, calcium, for the fruit bats (Stier and Mildenstein 2005). Maintaining figs in all possible places might help in conserving fruit bats and the service they provide to lowland forests.

Hunting large flying fox fruit bats belonging to *Pteropus* spp. (Chiroptera: Pteropodidae) for bushmeat and taboo is a common practice in paleotropics (Fujita and Tuttle 1991; Craig et al. 1994; Wiles et al. 1997; Brooke and Tschapka 2002; Sheherazade and Tsang 2015; Corlett 2017; Openshaw et al. 2017). For instance, in Niue Island in the South Pacific Ocean, the locals perform an annual 2-month hunting of fruit bats immediately after the hurricane season to ward off succeeding famine or drought. It is claimed that they capture over 1500 flying foxes per year. It has an irreversible impact on the seed dispersal service in the island (Brooke and Tschapka 2002). In some parts of Bangladesh, about 50% of villagers hunt bats (Openshaw et al. 2017). But, it is a tested fact that the populations of large frugivorous and small insectivorous bats are environment resilient and resurgent (Threlfall et al. 2011; Hanspach et al. 2012) and can rapidly increase their numbers if sufficient

measures are taken to protect small populations (Jones 1998; McConkey and Drake 2006; Farneda et al. 2015; Weber et al. 2015). Recent studies showed that while large fruit bats are most vulnerable to intensive agriculture practices, such as tea plantation (Wordley et al. 2017), the small fruit bats are not (Farneda et al. 2015). In Brazil, the frugivorous bats' abundance increases with the disturbance (Meyer et al. 2008; Farneda et al. 2015).

In a questionnaire survey in south India, Deshpande and Kelkar (2015) found that farmers are collecting about 100 cashew or areca nuts per day from a flying fox's roosts of 300–400 bats. Some locals appreciate the direct economic benefits they get from the fruit bats. However, a minority of the general public believes that bats are costly "pests" for their crops in production landscapes and orchards, such as sapota, rambutan, litchi, papaya, guava, pineapples, citrus, loquats, and mango (Chakravarthy and Girish 2003; Mahmood-ul-Hassan and Salim 2011; Vincenot et al. 2015a, b). Nowadays, fruit bats in India are in limelight due to their role in zoonotic diseases (Sharma et al. 2019). They are the carriers of Nipah virus, a symbiont of bats, but a deadly pathogen for human beings (Olival et al. 2019; Sharma et al. 2019). Although Nipah-related casualties in human beings occurred in 2018 and 2019 in the state of Kerala, no culling of animals has occurred so far. But, people are vigilant on visiting roosts of fruit bats and collecting fruits having bite marks of fruit bats.

14.6 Ichthyochory

Darwin (1859) recognized the dispersal of fruits of aquatic and riparian plants a long time ago. In a review, Barrat-Segretain (1996) has dealt with the reproductive and dispersal strategies of aquatic macrophytic plants. Seeds of aquatic plants are either entirely or partly from the food of many aquatic animals, including the water birds. Apart from endozoochory, dispersal by ingestion of seeds, ectozoochory – dispersal by external attachment of seeds on fins, feathers, and webs of aquatic animals – is also very common. Like terrestrial plants, aquatic plants also depend on wind, water, and animals for the dispersal of their seeds (Barrat-Segretain 1996). Here, we deal with the endozoochory by fishes.

It is possible that the early fishes might be the first vertebrate frugivores and animal seed dispersal agents ca. 300 mya (Tiffney 2004; Correa et al. 2015). In order to strengthen this belief, it has been found that earlier angiosperms had lived in freshwater systems, such as swamps (Eriksson et al. 2000), and produced fleshy fruits with small seeds (Friis et al. 2010). Further, the small vertebrates had an evolutionary radiation about 80 mya, after the evolution of the first angiosperm (Eriksson 2008). Many present-day fishes are in existence for 15 million years (Lundberg et al. 1986; Anderson et al. 2011; Correa et al. 2015). Field studies and stomach content analysis of fishes show that fruits and seeds are consumed and carried in the stomach of fishes (Goulding 1980; Banack et al. 2002; Mannheimer et al. 2003; Correa et al. 2007; Piedade et al. 2007; Galetti et al. 2008; Reys et al.

2009). Since mid-2000, researchers brought strong evidences for seed dispersal by fishes (see citations in Pollux et al. 2006).

At present, plants that live in swamps, lakes, river banks, and seasonally flooded forest plains are likely to be dispersed by the fishes (Horn 1997; Corlett 1998, 2017; Anderson et al. 2011; Pollux 2011). Plant species growing in several riparian and swampy habitats, such as *Aglaia*, *Artobotrys*, *Diospyros*, *Gonystylus*, *Hydnocarpus*, *Pandanus*, *Quassia*, *Sandoricum*, and *Syzygium*, are likely to be dispersed by the fishes. However, data, such as seed retention time in the fish gut, seed survival, and germinability after transport, are lacking for endozoochory by fishes (Pollux 2011).

The fish-dispersed fruits are buoyant and pulpy; the seeds vary in size from small to large; the defecated viable seeds are deposited in river/floodplain beds, which are not buoyant. There are evidences that the fishes in floodplains move seeds more toward favorable microsites, such as other patches of floodplain forests, rather than to permanent deep waters of rivers, which are unfavorable microsites for seed germination (Anderson et al. 2011). Sometimes the seed dispersal efficiency of fishes (in terms of the distance and quality of the habitats) is compared to that predicted for other large-bodied terrestrial dispersal agents, such as hornbills, elephants, and deers (Anderson et al. 2011). The size of fish-dispersed seeds varies between 2 and 4 cm in diameter. The gut retention time of seeds, an important attribute of long-distance dispersal agent, ranges between 34 h and 147 h in fishes, depending on the seed size (Anderson et al. 2011). Despite the growing evidences, only a few studies have experimentally assessed the seed dispersal quality of the fishes (Pollux 2011). Fruit/seed intake by the fishes can be intentional or unintentional. Since the tropical fruits are pulpy, the seeds might have remnants of flesh. Therefore, the frugivorous fishes in tropical parts intentionally feed the seeds (Kubitzki and Ziburski 1994). In temperate regions, the seeds are less or not pulpy, so the ingestion of seeds is mostly unintentional (Pollux et al. 2006).

Horn et al. (2011) suggest that 275 species of the fishes globally consume fruits and may perform seed dispersal. The Indomalayan (55 species, 25 genera, and 9 families of plants) and Australasian (6 species, 5 genera, and 4 families of plants) regions have documented less cases of ichthyochory when compared to those from the Neotropical region (149–151 species, 75 genera, and 17 families of plants) (Horn et al. 2011). Correa et al. (2015) gave a fine-scale analysis of the fish-fruit interaction in the neotropics and found that 69 Neotropical fish species have 566 species of fruits and seeds from 82 plant families in their diet. In monsoonal Australia, large grunters (Terapontidae) consume fig fruits (Davis et al. 2010).

Neotropics has maximum seasonally flooded forest due to the presence of the mighty Amazon River system. There, many species of fishes are frugivorous, and they seasonally migrate upstream during annual flooding. Coincidentally, maximum seeds are made available during this season. Horn (1997) pioneered the experimental study of seed dispersal of Neotropical fishes. He fed seeds of fig tree, *Ficus glabrata*, to a characid fish, *Brycon guatemalensis*. He collected seeds from fecal samples and found that the seeds retained the ability to germinate after gut passage. Through another experiment using radio telemetry, he got evidences that this fish migrated upstream during flooding. Seasonal migration of fishes may help the seeds to travel

longitudinally along the river and to maintain genetic and genotypic diversity in riparian plant populations and communities (Pollux et al. 2009; Pollux 2016). Gottsberger (1978) had attributed the homogeneous nature of the riparian plant community along the Amazonian river system to the greater abundance of frugivorous fishes. Characid fishes (e.g., *Colossoma* spp., *Brycon* spp.) are the major frugivorous species in neotropics (Anderson et al. 2011). There, even small fishes are crucial dispersal agents of herbaceous plants in seasonally flooded wetlands (Silveira and Weiss 2014).

Corlett reviewed the literature of ichthyochory for the oriental region first in 1998 (Corlett 1998) and gave an update in 2017 (Corlett 2017). Surprisingly, we have very less number of anecdotal observations on ichthyochory from old-world tropics although we have plenty of riparian forests and freshwater swamps. Euryphagous fishes (gulp anything that falls on water) are presumed major frugivores and dispersal agents of riverine plants. Cyprinid fishes (e.g., *Puntius* spp., *Leptobarbus* spp., *Tor* spp.) and clariid fishes (catfishes belonging to *Clarias* spp. and *Pangasius* spp.) are the likely frugivorous species in paleotropics (Corlett 1998). Both are capable of crushing hardest seeds. But, they are not present in neotropics.

14.7 Long-Distance Seed Dispersal

Usually, seed dispersal is not for that long distance. Only 1% of all seed dispersal accounts for long-distance dispersal (LDD) (Cain et al. 2000). An acceptable definition of long-distance dispersal seems to be any kind of seed dispersal which goes over 100 m (Cain et al. 2000). Autochory does not contribute to LDD because gravity-assisted dispersal and ballistic techniques would take the seeds only up to a short distance. However, if the ballistically dispersed seeds are aided by another medium, the chances of its travelling long distance will increase. This process of dispersal with the help of two or more agents one after the other is called *diplochory* (Vander Wall and Longland 2004).

The distance up to which the seed is dispersed will be dependent on a variety of factors such as the type of plant, the medium of dispersal, or even the weather. In some cases, it is observed that often the agents of long-distance dispersal are different than that of short-distance dispersal. For example, dandelion seeds which are generally considered to be dispersed with the help of wind have excellent potential for water-mediated dispersal due to its structure (Nathan et al. 2008).

Anemochory, hydrochory, and zoochory are the main mechanisms by which long-distance seed dispersal is done (Cain et al. 2000; Nathan et al. 2008). In the Anthropocene, humans with the aid of mechanized travelling methods act as seed dispersal agents knowingly or unknowingly (Taylor et al. 2012).

14.7.1 Long-Distance Dispersal by Migratory Birds

Animals – from very small butterflies to large whales – migrate across the globe in immense numbers for improved foraging materials, survival, and reproduction

(Alerstam et al. 2003; Dingle 2014). They transport nutrients, propagules, and other organisms that they forage or carry on their body from their native places or during their journeys. Since animal migration is seasonal, directional, and in a predictable route, they are likely to have a great impact in shaping the biodiversity on earth (Bauer and Høye 2014). However, animal movements and migrations have been incorporated only to a limited extent in ecological networks to understand ecological communities.

Long-distance dispersal is what enables colonization in isolated and remote habitats. Birds are the main vectors for long-distance dispersal. The new GPS tracking systems have somewhat helped overcome the challenges offered by this complex long-distance dispersal. Migratory birds carry seeds across long ranges and often in large numbers. It is estimated that 1.2% of the birds that migrate from Europe to Africa deposit seeds in Oceanic islands like the Canary Archipelago (Viana et al. 2016). Based on tracking data, these mallards can disperse seeds to over 290 and 410 km for short and long retention period assuming that they foraged just before migration. However, the pre-migratory fasting is a huge challenge in terms of dispersion as only 1% of the seeds ingested by mallards were found to be retained in mallards after a 12-h pre-migratory fasting (Kleyheeg et al. 2019). Characters like strong flight power and long retention time are characters that facilitate birds like ravens and gulls in Canary Islands in long-distance dispersal. Predatory migratory birds are also contributors to long-distance secondary dispersal (Nogales et al. 2012). Migratory birds also play a major role in determining the plant diversity in volcanic islands. While studying the angiosperm diversity in Surtsey and Anak Krakatau island, 68% of the angiosperms were brought in by the migratory birds, while in Anak Krakatau only about 23% was accounted by zochory. This was mainly because Surtsey island supported migratory seagulls, while Anak Krakatau supported largely the resident bird population (Nogales et al. 2012).

Although not explored well, epizoochory in the case of LDD by migratory birds cannot be ruled out. Carlquist (1967) estimated that 21% of the plants inhabiting the Juan Fernández archipelago was transported through the mud on migratory birds' feet. Understanding epizoochory in such cases is crucial as the passage of seeds through the guts of an active bird is found to decrease the seed viability. The viability of seeds decreased with the increasing body activity of the mallard, *Anas platyrhynchos* (Kleyheeg 2015). As migratory birds are highly active during their migratory activity, the seed viability in such cases should be further explored. Often the method of long-distance transport and the morphological syndrome of its dispersal are quite different. This is because of the strikingly unusual methods by which LDD occurs (Higgins et al. 2003). Realizing realistic dispersal patterns in LDD is quite challenging due to the opportunistic nature, limited sample size, and accuracy (Viana et al. 2016).

14.8 Conclusion

Considering the fruit traits of bat-dispersed fruits different from that of birds, the major counterparts of bats in the day, the seed dispersal service provided by fruit bats for tropical plants is important and irreplaceable. Unlike the speciose small fruit bats seen in neotropics, the fruit bats of old-world tropics are less speciose, but large. Although global literature suggests that fruit bats are environment, climate, and disturbance resilient, the old-world fruit bats face threat from agricultural intensification of certain monoculture crops, like tea and hunting, owing to their large size. However, it is a known fact that they can recover the population size if enough conservation measures are taken. Tropical India has many fleshy fruit crops and has fruit traits suitable for chiropterochory. Simultaneously, the lowland forests of old-world tropics that have maximum fleshy fruited plants and chiropterochory are facing threat from fragmentation and land-use change. The fruit bats' ability to forage in disturbed and fragmented forests makes them an important functional group of fragmented and secondary forests. India has generated sound knowledge on the distribution, biology, and diet of fruit bats, but most of them were animal focused, limiting the scope of the data from plants' recruitment perspective. Considering the paucity of literature related to chiropterochory in paleotropics, future studies may be directed to study chiropterochory from a plant community perspective. We also discussed ichthyochory and dispersal by migrant birds in this chapter. India, despite lacking a major river basin like the Amazon or Congo, has several major tropical rivers, rivulets, streams, wetlands, and even freshwater swamps. Ichthyochory is supposed to be an ancestral form of seed dispersal. But, a considerable progress on ichthyochory is made in neotropics, particularly in seasonally flooded Amazon. India is also a home for several winter migrants, such as wild ducks and other mallards from far northern latitudes. Because they are wetland specific, they might perform epizoochory of wetland plants. India also experiences local and regional migration of frugivorous birds across its latitudes, longitudes, and altitudes. Their contribution in local and regional plant movement also should be addressed in future studies.

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(Trans)Gene Flow: Mechanisms, Biosafety Concerns and Mitigation for Containment

15

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Abstract

Rapid progress in genetic engineering of plants has opened vistas for manipulation of traits to not only meet the ever-increasing need of food, feed, and fuel but also produce novel compounds in transgenic plants (genetically modified crops or GM crops). The widespread global adoption of transgenic plants has raised doubts and fears about its ill-impact on the health of intended consumers of end-products (humans and animals), on non-target organisms (pollinators, microbes, other biotic factors, etc.), and, environmental concerns (impact on biodiversity through escape of transgene and hybridization causing weediness, erosion of biodiversity). Transgene flow is a multistep process, influenced by several biotic and abiotic factors and pollen-mediated gene flow (PMGF) or gametic transmission is the most common mode. Some of these factors that influence (trans)gene flow include population structure and reproductive strategies of source/donor and sink/recipient species, overlap in geographical distribution, flowering phenology, genome compatibility, pollen-transfer mechanism (pollinators, wind direction and speed, pollen characteristics), pre- and post-fertilization barriers, survival of progeny as feral and volunteer population, etc. Several of these aspects have been investigated using both transgenic and non-transgenic plants and which revealed, for instance, that pollen-mediated flow can occur over large distances. Similarly, seed-mediated gene flow (SMGF) and vegetative propagule-mediated gene flow (VPMGF) of germplasm has also been established as a significant contributor to gene flow. Gene flow events are considered a threat if the resultant hybrids have equal or higher vigour,

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and are viable and fertile, as has been found in several cases. Indeed, increased vigour is a potential contributor to evolution of aggressive weediness and extinction of wild relatives through processes of genetic swamping, selective sweep, and genetic assimilation, an ecological consequence of (trans)gene escape. Horizontal gene flow, or gene flow between unrelated organisms (e.g. plants to microbes, humans, pollinators, etc.), is the major biosafety concern, although investigations have found such fears largely unfounded. Several containment and mitigation strategies including spatial and temporal barriers, biological barriers grouped under Genetic Use Restriction Technologies (GURTs) such as male sterility, bisexual/total sterility, cleistogamy, apomixis, maternal inheritance (transplastomics), preventing seed dispersal, secondary dormancy, competitive self-thinning, interfering with floral induction or bolting, are widely used or recommended to minimize or prevent (trans)gene escape. Transgenic technology is still a developing area, and next generation of plants generated through Genome Editing while addressing existing biosafety and environmental concerns are also likely to throw up newer challenges, including formulation of appropriate robust regulatory framework for which adequate data based on research needs to be collated and analysed.

Keywords

Transgenic · Gene flow · Biosafety

Abbreviations

CWR	Crop wild relatives
GURT	Genetic Use Restriction Technologies
HGT	Horizontal gene transfer
PGF	Pollen-mediated gene flow

Glossary Terms

Feral population: Plant population present in wild, non-agricultural fields and does not require human intervention for propagation (see Ellstrand et al. 2010).

Volunteer plants: Cultivated plants present in farmers' field that have not been deliberately planted or sown; often derived from over-wintering seeds, seeds that remain from previous seasons or seeds brought through agents of dispersal such as birds, other animals or wind.

Crop wild relatives: Wild relatives of domesticated crops, sharing common ancestry; usually harbour traits that are targets of introgression for crop improvement programmes.

15.1 Introduction

Gene flow, defined as *the incorporation of genes into gene pool of one population from one or more populations* by Futuyama (1998), has been shown to have profound evolutionary consequences including generation of aggressive weeds that are able to withstand herbicide tolerance (crop-to-weed gene flow; Barrett 1983), and extinction of wild relatives of crop species (Small 1984; Ellstrand and Elam 1993). Ellstrand et al. (1999) postulated that gene flow from crops to wild relatives is widespread, and may have several important consequences; and while analysing gene flow from crops to wild relatives evolutionary processes on a micro-scale can be understood (Ellstrand et al. 1999; Ellstrand 2014; Ellstrand and Rieseberg 2016).

In transgenic plants, gene flow refers to “transfer” of foreign gene (either conferring a novel or improved trait, or gene conferring selection/resistance against antibiotic for selection of transgenic event) into non-transgenic plants (vertical gene flow), or wild crop relatives (vertical/diagonal gene flow; Gressel 2015), or other organisms such as microbiota, consumer of the end-product (horizontal gene flow). Successful gene flow resulting in vertical or horizontal gene transfer is dependent on several factors including reproductive strategies of the “donor/source” and “recipient/sink” species, population structures, flowering phenologies, geographical barriers, pollinator availability and range, reproductive barriers, formation of viable seeds, and establishment of next generation capable of sexual reproduction (i.e. fertile offspring), uptake and transformation efficiency, and barriers to transfers of genetic material across species and taxonomic boundaries (in case of horizontal gene transfer). The probability of vertical gene flow is therefore higher than that of horizontal gene flow. The probability of vertical gene flow from GMO (source) to non-GMO (sink) is highest, followed by that from GM to wild crop relatives within the gene pool.

Various biological and ecological attributes of gene flow should be investigated before its implications on biosafety as a consequence of transgene flow from transgenic crop to wild relatives can be evaluated. It is imperative to note that biosafety risk assessment and evaluation as a result of transgene flow must be trait-specific. For example, as transgenic maize have been generated for insecticide resistance or herbicide tolerance or both, biosafety risk associated with each trait must be evaluated separately because a key outcome of gene flow is altered fitness that the “foreign gene/trait” confers upon the sink species, and whether the altered fitness is linked to survival advantage or disadvantage.

This chapter attempts to provide an overview of various dimensions of gene flow in context to transgenic plants, and biosafety concerns and strategies to minimize and contain transgene flow.

15.2 Gene Flow

Ecologically, gene flow is the *migration* or *movement* of genetic material from one *population* to another; biologically, gene flow refers to *transmission* of genetic material from one generation to the next (Slarkin 1985). The concept of gene flow has been intrinsically linked with the concept of biological species as proposed by Mayr (1942, 1963), and subsequently debated by Ehrlich and Raven (1969), Endler (1977), Jackson and Pounds (1979) and Stanley (1979). Vertical gene flow has been considered as a key driver of species existence and diversification. It is now widely accepted that there are no universal rules that govern gene flow across all species; gene flow is highly variable not only amongst various species, but even within a species and has multiple evolutionary outcomes (Slarkin 1985).

Transmission of gametes (pollen flow; pollen-mediated gene flow – PGF), and migration or movement of individuals (seed dispersal in plants) are the most common modes of gene flow within a species; gametic transmission (PGF) being a one-step process (upon successful fertilization leading to a viable and fertile offspring) to introduce an alternate allele or a novel gene from the donor (source) to the recipient (sink). Gressel (2015) refers to flow of genetic material/genes from crops and distantly related species with interbreeding barriers as diagonal gene flow (Gressel 2015). In rare cases, gene flow also occurs between species separated by extremely large evolutionary distances such as from animals/plants to microbes, and vice versa, outside the reproductive boundaries, and is referred to as horizontal gene flow (HGT) (Quispe-Huamanquispe et al. 2019; Pontiroli et al. 2009; Richardson and Palmer 2006; Broothaerts et al. 2005; Nielsen et al. 1998). With the widespread development, acceptance and cultivation of transgenic plants, gene flow (transgene flow/escape) as a biosafety issue has assumed significance. All the forms of gene flow – vertical, diagonal and horizontal are of concern when dealing with transgene escape, and its impact on biodiversity and biosafety. For the purpose of the current discussion, diagonal gene flow has been considered as part of vertical gene flow.

Gene flow or transfer of genetic material between crops and their wild relatives including weedy taxa is a two-way process and occurs naturally because of reproductive compatibility between crops and their progenitor species or crop wild relatives (CWRs) (Ellstrand et al. 1999; Lu 2008). Transgenic technology has made it easy to introduce any gene (cigene – from same species; transgene – from unrelated species) in the genome for production of genetically modified organism (Telem et al. 2013; Holme et al. 2013). The widespread introduction and cultivation of GM crops raises issues of biosafety and biodiversity as a consequence of absence or minimal reproductive barrier between crops (including genetically modified version of the crops) and CWRs.

Vertical gene flow can be classified into the following types (Fig. 15.1a; Lu 2008):

- (i) Crop-to-crop gene flow: flow of transgene from GM crop to non-GM crop (intraspecific)

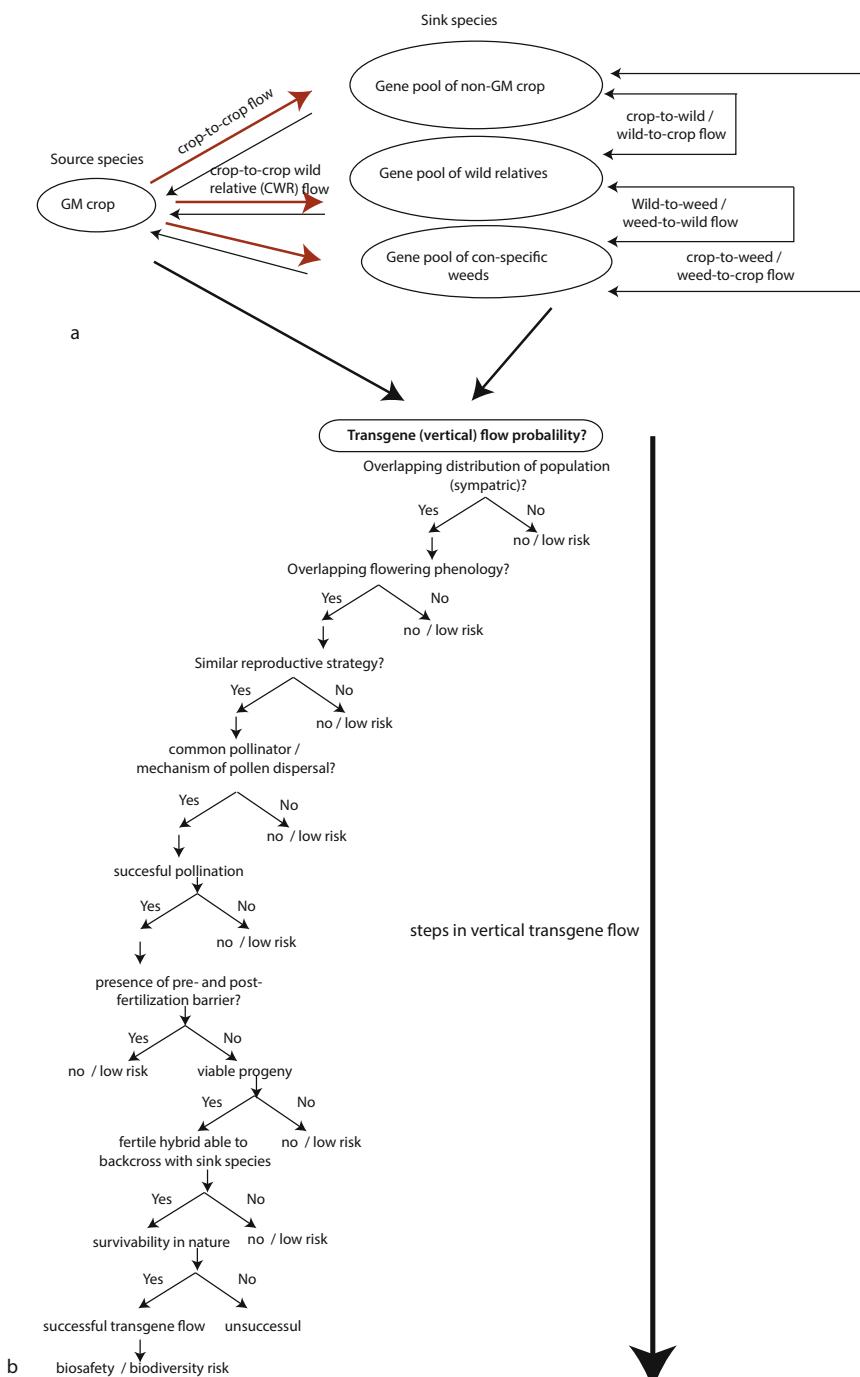


Fig. 15.1 (a). Gene flow between crops (GM and non-GM), wild relatives and conspecific weed: Red arrows indicate direction of transgene flow. (b). Vertical transgene flow is a multistep process and success at each step is required for introgression, establishment of and colonization by hybrids

- (ii) Crop-to-weed gene flow: flow of transgene from GM crop to conspecific weed (intraspecific)
- (iii) Crop-to-wild gene flow: flow of transgene from GM crop to its wild relative (interspecific; intergeneric)

The process of gene flow consists of several sub-steps, and success at each sub-step is essential for introgression of gene/transgene and establishment of hybrids. Factors that influence gene flow and transgene escape from GM crops (source species), introgression into non-GM crops and CWR (sink species), generation of and colonization by hybrid include (Fig. 15.1b; Gepts and Papa 2003; Ellstrand 2003, 2014; Campbell et al. 2006, 2009):

- (i) Geographical location of source and sink species (sympatric populations or minimal geographical distance and barriers)
- (ii) Reproductive strategies of the source and sink species (pollinators; pollination mechanism; pre-fertilization compatibility barrier; phenology)
- (iii) Post-fertilization barriers (viability of offspring)
- (iv) Cytotype and genome composition of hybrids permitting meiosis and gametogenesis (fertility of offspring)

15.2.1 Mechanisms of Gene Flow

Genetic engineering has been applied to modify or enhance useful traits or to eliminate harmful traits, and successful generation of genetically modified organisms (GMOs) has greatly enhanced production and cultivation of vital crops like maize, oilseed rape and soybean (Briefs, I.S.A.A.A. 2017). Subsequently, release of GM crops for cultivation raises biosafety issues as a number of transgenes have been found outside the GM crops in other organisms, in the gene pool of wild relatives, or in finished product. Therefore, major concerns related to release of GM crops are: food, feed and health safety, detection and regulation of transgenes outside the GM crop, and environmental safety.

The following section summarizes the various mechanisms of gene flow.

15.2.1.1 Pollen-Mediated Gene Flow (PGF/PMGF)

Movement/transfer of genes among individuals of different population through pollen grains that can fertilize the individuals of geographically isolated but reproductively compatible population is the most common way of gene flow (Ellstrand and Rieseberg 2016; Ellstrand et al. 2013). Factors that influence pollen-mediated gene flow are: distance between donor and recipient population, outcrossing rate of recipient population, morphological characters of pollen grains (weight, size, shape; monad, dyad, triad, tetrad, pollinia), pollen viability, climate conditions (wind direction and speed; rain), pollen loads of donor and pollinating agent

(e.g. anemophilous [wind], hydrophilous [water], entomophilous [insects], chiropterophilous [bats], orinthonophilous [birds]) (Lu 2008).

Several studies have investigated the effectiveness of PGF with respect to various factors such as pollinator species and range. Bees are one of the most important groups of pollinators, and gene flow is dependent not only on pollinator availability but also their flight range (Ribbands 1951). Cotton is an insect-pollinated crop with species of *Apis* (*Apis mellifera* L. *A. dorsata*, *A. florea*, *A. indica*) being the main pollinators; bumble bees (*Bombus* sp.) and leaf cutting bees (*Hymenoptera megachilidae*) are also involved in pollination (GEAC -Biology of Cotton 2011). Pollen-mediated gene flow has been shown to decrease exponentially as dispersal distance increases and ranges between 8% and 10% at a distance of 1 m, 0.08% at a distance of 20 m and 0.04% at a distance of 50 m under natural field conditions, in the presence of natural pollinator populations (Zhang et al. 2005). Another independent study established that pollen flow from cotton declines exponentially with increasing distance, and was found to be 7.65% at 0.3 m to <1% beyond 9 m with *A. mellifera* as the major pollinator; the authors however detected 0.04% pollen-mediated gene flow over 1.6 km (= 1 mile) distance when hybrids were detected (Van Deynze et al. 2005). Generation of hybrids have been studied by allowing hybridization between cultivated radish and individuals of naturally occurring or experimental wild populations, and recovering crop-weed hybrids (Klinger et al. 1991). In radish, an insect-pollinated crop, pollen grains from cultivated radish (*Raphanus sativus*) were able to cross-fertilize wild radish separated by up to 1000 m distance, demonstrating that hybrids can be obtained even when parental populations are separated over long distances (Klinger et al. 1991). The dependence of PMGF on pollinator species, as well as distance between source and sink has been shown when gene flow from transgenic cotton was evaluated in the presence of different pollinators – *Bombus ignitus*, *Apis mellifera* and *Pieris rapae*, and sink species (non-GM varieties of cotton) under greenhouse conditions (Yan et al. 2015). It was established that pollen-mediated gene flow was dependent on the species of pollinator present with highest gene flow levels with *Bombus ignites* as pollinator, followed by *Apis mellifera*, and least with *Pieris rapae* (Yan et al. 2015). Physical distance is another factor that influences pollen-mediated gene flow with non-GM cotton variety planted closer to GM-cotton yielding higher proportion of hybrid seeds (Yan et al. 2015). Physical barriers such as nets and separation crops are another determinant in regulating PMGF. Pollen grains of cotton have an average diameter of 120–150 µm and nets with different pore sizes ranging from 40 holes/cm², 60 holes/cm², 80 holes/cm² and 90 holes/cm² (all with pore sizes larger than the size of pollen grains) were used as physical barriers. Nets with 90 holes/cm² along with *Sorghum bicolor* as separation crop (average plant height of 3.2 m) proved most effective in preventing pollen-mediated gene flow as compared to nets with 40 hole/cm² with *Lycopersicon esculentum* (average plant height 1.5 m) as separation crop, which was least effective, as well as when no physical barriers were used (Yan et al. 2018).

Pollen-mediated gene flow of wind-pollinated or anemophilous crops is independent of biotic factors. Millwood et al. (2017) found that pollen-mediated gene flow in

wind-pollinated crops such as switchgrass (*Panicum virgatum*) is likely to occur over distances as far as 100 m with nearly 10% progeny of hybrid origin; the proportion of hybrid progenies resulting as an outcome of pollen-mediated gene flow was ca. 80% at a 10 m distance in the direction of wind flow (Millwood et al. 2017). PGF flow in rice was also found to be dependent on the direction of wind flow and 0.36–0.86% of hybrid progenies were a result of cross-fertilization by pollen from transgenic plant (source) to non-GM rice cultivars (sink) grown at a distance of 3–10 m (Messeguer et al. 2004). Other species in which PGF has been studied include oilseed rape (herbicide tolerance to non-GM plants; Hüsken and Dietz-Pfeilstetter, 2007), maize (introgression of phytase from GM to non-GM maize; Liu et al. 2015), rice (herbicide Basta tolerance from GM japonica rice to non-GM indica rice; Jia et al. 2007), creeping bentgrass (*Agrostis stolonifera*; herbicide glyphosate tolerance from creeping bentgrass to *Agrostis gigantean*, *Polypogon monspeliensis*; Zapiola and Mallory-Smith 2012, 2017), wheat (herbicide chlortoluron tolerance from GM to non-GM wheat; Loureiro et al. 2007, 2012) and *Populus trichocarpa* (Slavov et al. 2009), to name a few. Table 15.1 provides a representative list of various plants where PGF has been investigated.

15.2.1.2 Seed-Mediated Gene Flow (SMGF)

Natural dispersal of seed by wind, water, long-range migration of animals can cause this type of gene flow. Human activities like intentional seed exchange and trading of seed stock also cause gene flow in long-distance geographical areas (Lu 2008; Bohn et al. 2016). Gene flow through seed dispersal is considered an important factor in determining genetic structure of populations, colonization in fragmented habitats, and maintenance of connectivity in highly dispersed populations (Bacles et al. 2006). In severely deforested habitat, seed dispersal was found to be six times more effective than pollen dispersal in maintaining connectivity between populations of *Fraxinus excelsior* (Bacles and Ennos 2008).

The potential for seed-mediated gene flow was examined in transgenic safflower (*Carthamus tinctorius*), and various aspects of seed biology were examined (McPherson et al. 2009). The authors investigated the seed loss, viability and longevity (on soil surface and buried at different depths) of non-GM and GM (glufosinolate tolerant) safflower, density and growth parameters of safflower volunteer populations including seed production. Significantly, the short viability of safflower seeds did not allow viable seeds to be recovered from soil seed bank, implying that chances of seed-mediated gene flow in safflower is minimal (McPherson et al. 2009). Seed-mediated gene flow and emergence of volunteer populations across 51 fields, with commercial potato cultivation along with cereal as rotation crop, was studied over a period of time. Although the fecundity of volunteer potato crop and subsequent emergence of volunteer population declined over a period of time, presence of second-generation volunteer tubers and their ability to sprout post-dormancy to produce third-generation volunteer population revealed the chances of seed-mediated gene flow. Based on the data obtained, the researchers recommend the minimum rotation duration for GM-potato to be 1:4 (1 year GM-potato, 4 years other crops) with effective management practices to

Table 15.1 Representative examples of pollen-mediated gene flow, and generation of hybrids

S. no	Plant	Comments	References
1	Cotton	8–10% at a distance of 1 m, 0.08% at a distance of 20 m and 0.04% at a distance of 50 m.	Zhang et al. (2005)
		7.65% at 0.3 m to <1% beyond 9 m with <i>A. mellifera</i> ; 0.04% pollen-mediated gene flow over 1.6 km (=1 mile) distance.	Van Deynze et al. (2005)
		Pollen grains of cotton have an average diameter of 120–150 µm and nets with different pore sizes ranging from 40 holes/cm ² , 60 holes/cm ² , 80 holes/cm ² to 90 holes/cm ² were used as physical barriers. Nets with 90 holes/cm ² along with <i>Sorghum bicolor</i> as separation crop (average plant height of 3.2 m) proved most effective in preventing pollen-mediated gene flow as compared to nets with 40 hole/cm ² with <i>Lycopersicon esculentum</i> (average plant height 1.5 m) as separation crop, which was least effective, as well as when no physical barriers were used.	Yan et al. (2015, 2018)
2	Radish	Pollen flow detected upto 1000 m distance; hybrids exhibit higher vigour, produced more seeds, higher rate of hybrid seedling emergence, and the rate of emergence was earlier than parental seedlings. This trait correlated with higher seed output in hybrid seedlings. Higher number of hybrid seedlings survived until flower and increased colonization by hybrids was observed.	Klinger et al. (1991), Snow et al. (2001), and Hovick et al. (2011)
3	Switch grass (<i>Panicum virgatum</i>)	Ca. 80% at 10 m, and 10% at 100 m distance of wind flow.	Millwood et al. (2017)
4	Rice	Between 0.036% and 0.086% at 3–10 m distance.	Messeguer et al. (2004)
5	Foxtail millet (<i>Setaria italica</i>)	Gene flow was observed from <i>Setaria italica</i> to <i>S. viridis</i> ; weed hybrids were obtained displaying chloroplast-inherited and nuclear-inherited (dominant and recessive) transgene character.	Shi et al. (2008)
6	Maize (<i>Zea mays</i> ssp. <i>mays</i>)	Hybrids obtained between <i>Z. mays</i> ssp. <i>mays</i> and <i>Z. mays</i> ssp. <i>mexicana</i> (Chalco teosinte) displayed greater vigour than the two parents. Cobs of hybrids require lesser force to be detached and seeds can be easily dispersed.	Guadagnuolo et al. (2006) and Chavez et al. (2012)

(continued)

Table 15.1 (continued)

S. no	Plant	Comments	References
7	<i>Brassica</i> species	Hybrids seeds are produced at rates ranging from 9% to as high as 93% depending on the planting design and set-up using <i>Brassica rapa</i> (weed) and <i>Brassica napus</i> (crop).	Jorgensen and Andersen (1994)
		B. rapa (weed) x B. napus (crop) hybrids performed differentially in the absence (when hybrids are grown alone) or presence (as volunteer population) of competition, indicating that crop or weed alleles are likely to be favoured under different environmental conditions. This has direct implication on colonization by crop-weed hybrids.	Rose et al. (2009)
		Hybrids between B. napus and B. juncea resulted in an altered pollen/ovule ratio, indicating a shift in sex allocation towards more female investment, higher pollen transfer efficiency. Therefore, presence of transgenic plants among the wild population has been proposed to increase transgene escape by altering the sex allocation ratio of the wild weedy relatives.	Liu et al. (2018)
8	<i>Cucurbita pepo</i>	F1 hybrids between wild weedy <i>Cucurbita pepo</i> and cultivated transgenic <i>Cucurbita pepo</i> produce 41% more male flowers, 21% more female flowers and exhibit higher fecundity (producing an average of 28% more seeds as compared to wild type).	Spencer and Snow (2001)
9	Sunflower (<i>Helianthus annuus</i>)	Gene flow was observed from cultivated sunflower to wild weedy population; highest frequency of 23% was observed in wild populations at a distance of 3 m from crop; hybrids were obtained at a maximum distance of 1000 m. Seeds of hybrids shown to have lower dormancy and higher germination but overall lower fitness; some of the hybrids are tolerant to rust disease while wild parent is not; the flowering phenology between F1 hybrid and parents have extensive overlap with a possibility of backcross.	Arias and Rieseberg (1994), Snow et al. (1998), and Burke et al. (2002)

prevent tuber loss in field, and volunteer population to minimize seed-mediated gene flow (Phelan et al. 2015).

Several estimator models have been developed that allow comparison of rate of pollen or seed-mediated gene flow in angiosperms such as the r_{IBD} model (isolation by distance – IBD; Barrandeguy and García 2018), or Ennos index (island model; Ennos 1994). Use of these estimator models are recommended in order to accurately predict the chances of transgene flow and escape.

15.2.1.3 Vegetative Propagule-Mediated Gene Flow (VPMGF)

It occurs by dispersal of vegetative structures of plants such as tubers, tillers, rhizome, roots, etc., which helps in propagation and colonization of habitat. Dispersal of vegetative propagules occurs by water, wind or through anthropogenic activities. Gene flow by vegetative propagules is effective between populations separated by short distances. Vegetative structures such as tubers of transgenic crops (such as potato) can remain in field and lead to volunteer/feral population (Lu 2008; Phelan et al. 2015). These are, however, not the major factors of gene flow and transgene escape.

15.2.2 Fate of Hybrids

Gene flow outside of species boundaries can become a biosafety challenge if resultant hybrids are viable and fertile; not all hybridizations will lead to successful establishment of hybrids and therefore not all gene flow are biosafety risks. Spontaneous hybridization, fitness of hybrids and consequences for ecosystem has always been of concern among researchers, and it has been hypothesized that the fecundity of hybrids is likely to reach levels equivalent to those of the wild-type parents because of reduced differences in life-history traits that are necessary for successful survival, colonization and spread (Campbell and Snow 2007). Investigating the fate of hybrids under natural conditions to understand viability, fecundity, performance and colonization ability has therefore been accorded high priority. Intra-species transgene flow from transgenic varieties to non-GM varieties as a result of hybridization leads to successful establishment of hybrids (Ellstrand and Hoffman 1990). Indeed, F1 hybrids between transgenic *Cucurbita pepo* (ssp. *ovifera*, var. *ovifera*) and non-GM *Cucurbita pepo* (ssp. *ovifera*, var. *ozarkana* and var. *texana*) led to development of hybrids that have higher fecundity than the parents (Spencer and Snow, 2001). Hybrids between wild radish (*R. raphanistrum*) and cultivated radish (*R. sativus*; pollen source) were obtained by crossing and the fecundity of F1 hybrids was tested. In spite of lower fitness of hybrids due to delayed flowering (although hybrids produced more number of flowers than wild-type), low pollen fertility, and reduced seed set, frequency of alleles from cultivated radish in the population of wild radish ranged between 8% and 22% after three generation (Snow et al. 2001). The flowering period of the hybrids was extended even after it had ceased in wild-type parents; the continued flowering in hybrids was also shown to influence pollinator composition (Snow et al. 2001). Fecundity of the hybrids

reached similar levels, equivalent to that of wild types after 10 years; the difference in reproductive traits (flower number, low pollen fertility, and reduced seed set) observed in F1–F3 generation was lost, and F10 plants had nearly similar trait values, and differences were not statistically significant (Snow et al. 2010; Campbell et al. 2006). Similar results of fecundity of hybrid between cultivated *Sorghum bicolor* and wild weed *Sorghum halepense* were observed (Arriola and Ellstrand 1997). Several traits such as flowering time, pollen viability, panicle production, seed yield, tiller production and overall biomass of the hybrids and the weed Johnson grass did not show any significant difference, implying that a transgene that is beneficial or neutral for the sink species is likely to persist as crop–weed hybrid in nature (Arriola and Ellstrand 1997). Intraspecific hybrids obtained between both transgenic and non-transgenic plants of *Z. mays ssp. mays* and *Z. mays ssp. mexicana* (chalco teosinte) exhibited hybrid vigour for both vegetative (plant height, biomass) and reproductive (flowering time, seed production, dispersal) traits (Guadagnuolo et al. 2006). The authors observed that the growth performance of hybrids was better than the two parents used in the study, and thus recommended that transgenic maize can hybridize freely with wild maize (*Z. mays ssp. mexicana*; chalco teosinte), and that allele introgression into crop–weed hybrids is possible (Guadagnuolo et al. 2006). In fact, in a follow-up study, the cobs of the crop–weed hybrids were found to be requiring much less force to be detached from the hybrid plant indicating that both seed and cob can be easily detached and dispersed (Guadagnuolo et al. 2006; Chavez et al. 2012). Other plants where performance of crop–weed hybrids has been evaluated include *Brassica rapa* × *B. napus* (transgenic) hybrids (Rose et al. 2009); *Brassica juncea* × *B. napus* hybrids (Liu et al. 2018); wild radish (*Raphanus raphanistrum*) × cultivated radish (*Raphanus sativus*) (Hovick et al. 2011; Snow et al. 2001); *Cucurbita pepo* hybrids between wild weedy *Cucurbita pepo* and cultivated transgenic *Cucurbita pepo* (Spencer and Snow 2001); Sunflower (cultivated and wild weedy population) (Snow et al. 1998; Burke et al. 2002).

15.3 Why Gene Flow Matters? Ecological and Biosafety Issues

Gene flow, referred to as “evolutionary glue” (Mayr 1942; cited in Ellstrand 2014) is a major unavoidable driving force of evolutionary process. However, “alien genetic elements” introgressed into the genome of weedy and wild species through transgene flow from a crop species may confer selective advantage (increased colonization) or disadvantage (causing genetic erosion, extinction). What impact do the transgenes have on the recipient genome and, as a consequence what is the nature of selection pressure operating on the transgene in the recipient population has been subjected to wider discussion and deliberation (Ellstrand 2018; Gompert and Buerkle 2016; Ridley and Alexander 2016; Ellstrand et al. 2010).

Genetically, transgenes behave as dominant genes, and depending on the nature of transgene (herbicide resistance, insect resistance, floral traits such as altered flowering time, nutritional traits, root architecture), gene flow from crops to wild

varieties (through introgression) has two important ecological and evolutionary consequences for the wild relatives:

1. *Evolution of aggressive weeds*: Transgene conferring herbicide tolerance when incorporated into genome of wild crop relatives through gene flow reduces chances of weed control, increases in weediness, survival and colonization ability and population of weeds as they mimic the phenotype of crop plant and escape the process of eradication (Barrett 1983).
2. *Extinction of wild relatives*: Recurrent hybridization of wild, weedy species with cultivated species leads to introgression, and admixture of genomic component contaminates the wild genotype leading to genetic erosion. A major outcome is genomic incompatibility, meiotic defects, reduced fertility and thus fecundity of wild species eventually causing extinction (Small 1984). Constant introgression of genetic component from cultivated varieties through repeated outcrossing to crop wild relative (CWR) is considered a significant threat to biodiversity. Introgression increases the frequency of alleles from the cultivated species in CWR which decreases their fitness in wild habitat, leading to undesired consequences in terms of evolution and conservation of biodiversity (Lu 2013).

The problem of genetic erosion and generation of aggressive weeds are severe when introgressed genes are from ‘Genetically Modified’ crops containing either a cisgene or a transgene, and is considered a major ecological risk (Telem et al. 2013; Holme et al. 2013; Dale et al. 2002). Introgression of transgene into wild population depends on various factors such as genetic, environmental, ecological and human management variables. Recent studies suggest that introgression significantly affects genetic diversity and/or genetic homogeneity among demes in a meta-population (Ellstrand and Schierenbeck 2000; Stewart et al. 2003). Unintentional gene introgression can cause genetic erosion and decrease the fitness of wild varieties via three major processes: genetic swamping, selective sweep and genetic assimilation (Lu 2013).

1. *Genetic swamping or genetic pollution*: In this process, gene flow occurs between two genetically isolated populations, one large and the other small. Introgression of genes (or transgene)/alleles from large, source population causes reduction in the genetic diversity of smaller, sink population (Rhymer and Simberloff 1996). Genetic swamping involving allele-donating large population of cultivated crop, and allele-receiving small population of its wild relative (crop wild relative; CWR) may cause drastic reduction in population of CWR, even leading to its extinction in a certain geographical area (demographic swarming; Lu 2013). There are many instances where genetic swamping or demographic swarming has resulted in extinction of CWR. For instance, recurrent hybridization of cultivated rice with endemic variety has been proposed to be a major cause of near extinction of *O. perennis* ssp. *formosana* in Taiwan (Kiang 1979). Genetic swamping is a major concern when dealing with in situ conservation efforts of rare and threatened species where multiple causes (habitat fragmentation, cross-

- fertilization with related and commonly occurring species) threaten existence of rare and valuable wild species (Rutherford et al. 2019).
2. *Selective sweep*: It refers to a process when genetic variation surrounding a locus is lost because of strong positive selection. It normally occurs when a novel gene which confers a higher fitness probability is introduced into an individual of a population. Owing to higher fitness probability, the novel gene increases the chances of survival of the host over the other individuals and is under strong positive selection. Under strong selection pressure (positive or negative), genomic region of the population apparently becomes monomorphic due to loss of less viable trait. Both positive and negative selection lead to loss of genetic diversity (Charlesworth et al. 1993). Selective sweep has been observed in several crop species including *Zea mays* (Palaisa et al. 2004), *Sorghum* (Hamblin et al. 2004), and *Hordeum* (Morrell et al. 2003). An outcome of selective sweep in Maize is reduction in genetic variation surrounding the Y1 locus for phytoene synthase in yellow-maize varieties due to domestication, as compared to Y1 locus in white maize varieties (Palaisa et al. 2004). Similarly, directional selection in the wild maize ancestor at loci associated with agronomic traits is also under selective sweep, causing loss of genetic diversity (Tenaillon et al. 2004).
 3. *Genetic assimilation*: A process where allele of one population replaces the allele of another through introgression (Gepts and Papa 2003). Environmentally induced phenotypes become genetically fixed and no longer require environmental stimulus (Pigliucci and Murren 2003; Pigliucci et al. 2006). Genetic assimilation has severe impact on conservation of wild relatives and it may eventually lead to extinction of wild relatives (Ellstrand and Elam 1993).

15.3.1 Transgene Flow, Genetic Swamping, Selective Sweep and Genetic Assimilation

Transgenes are often associated with traits that confer selective advantage, and thus are dominant in nature. It has been postulated that selective advantage conferred by introgression of transgene may result in formation of “aggressive” genotypes, reduction in population size of wild-type non-GM or of CWR (without introgressed transgene) and eventual reduction in allelic diversity due to loss of members of gene pool. Genetic swamping may be of concern when the allele-donating large population is a cultivated GM crop, and small populations of crop wild relative (CWR) are allele receiving; hybridization and introgression of transgene from a crop species into a population of non-GM crop or of CWR will increase the frequency of the transgene in the population of CWR, a phenomena referred to as “genetic pollution” (Ellstrand 1992). Gene flow involving transgene will lead to introgression of not only the transgene but also of the surrounding locus (site of transgene integration from the host genome). As the frequency of the transgene increases in the population in a few generations due to positive selection, selective sweep will lead to monomorphism in the CWR sink population. Finally, genetic assimilation will

cause gradual replacement of wild-type genotype (in CWR) with the genotype of genetically modified crop.

15.3.2 (Trans)Gene Flow: Representative Case Studies

Globally, approximately 190 million hectares were planted with biotech crops or GM crops in 2017 of which nearly 100 million hectares are located in the developing world, and 80 million hectares in developed countries (Briefs, I.S.A.A.A. 2017). Across the globe, soybean (50%; 94.1 million hectares), maize (31%; 59.7 million hectares), cotton (13%; 24.1 million hectares) followed by canola (5%; 10.2 million hectares) (Briefs, I.S.A.A.A. 2017) are the predominantly cultivated transgenic crops. The area under cultivation of GM-crop (only cotton is allowed at present) in India is close to 11.5 million hectares. Transgene flow from crop to CWR is a major concern in habitats and geographical areas that are centres of origin and diversity of the GM-crop, and has a high diversity of members of primary gene pool, progenitor species, weedy wild relatives and land races that permit exchange of genetic information. A few representative examples of transgene flow are discussed below (see also Table 15.1) and a comprehensive review on gene flow from cultivated crop species to their wild relatives is presented elsewhere (Ellstrand et al. 1999).

Maize

Maize is the second most widely cultivated genetically modified crop and mostly modified for herbicide resistance and/or to enhance insect resistance traits. Maize pollen is dispersed by wind, and can travel long distances (Palaudelma's et al. 2009; Devos et al. 2005). Crop-to-crop gene flow has been estimated to be as high as 30% in farm scale evaluations (FSE) in UK in samples collected from 150 m distance, and even at 200 m distance (Weekes et al. 2007). Crop-to-landrace introgression and its subsequent effect have been evaluated in Mexico, which is considered as the centre of origin and diversity of maize, and where GM-maize is also grown. In Mexico, several landraces of maize are cultivated for local consumption (Duncan et al. 2019). The authors crossed landraces of maize with GM maize, and obtained introgressed lines (with ca. 94% landrace genome) through recurrent backcross with phenotypic selection. Comparison of field trial data for landraces with and without GM trait did not show any difference in cob and kernel characteristics, and the transgene had little or almost no effect on the target trait in the maize landraces (Duncan et al. 2019). Hybrids between maize and Mexican teosintes are known to have normal levels of fertility, and thus chances of transgene escape and spread are high (Rogers, 1950).

Concern about transgene escape from maize into its landraces was evaluated by sampling cobs and kernels of landraces grown in the centre of origin and diversity of maize in Mexico (Quist and Chapela 2001; Piñeyro-Nelson et al. 2009). The presence of transgenic DNA in kernel samples was demonstrated and subsequently verified by others (Quist and Chapela 2001; Serratos-Hernández et al. 2007).

However, several other studies have either failed to detect evidence of transgene in DNA samples of maize landraces collected from the same geographical area (Quist and Chapela 2001; Ortiz-García et al. 2005), or have questioned the experimental design, strategy and the methodology employed (Christou 2002).

Spontaneous hybrids between *Zea mays* ssp. *mays* with ssp. *mexicana* and ssp. *parviglumis* have been estimated to form at <1% and >50% rates, respectively (Ellstrand et al. 2007). Researchers suggest that frequency of introgression of maize transgene with its feral recipient crop teosinte (*Zea luxurians*) is very high and is independent of the distance of two grown crops (Doebley, 1990; Castillo-Gonzalez and Goodman 1997). In contrast, naturally formed hybrids have not been found, or attempt to obtain hybrids through manual pollination between transgenic maize and Eastern gamagrass (*Tripsacum dactyloides* L.), a member of the sub-tribe Tripsacinae, to which maize (*Zea mays* L.) also belongs, have not been successful, inspite of populations of the two species growing in close proximity in the USA where transgenic maize is widely cultivated. The data obtained suggests that probability of transgene flow and escape from maize to *Tripsacum dactyloides* L. is non-existent and therefore no environment safety risk exists (Lee et al. 2017).

Rice

Rice is an autogamous, self-pollinating crop. Gene flow from rice has been known to cause extinction of wild rice variety *O. perrenis* ssp. *formosana*, a CWR through genetic swamping or demographic swarming, in Taiwan (Kiang 1979). Messeguer et al. (2001) estimated the rate of transgene (*pat* gene conferring herbicide resistance) flow from *O. sativa* var. *japonica* to non-GM *O. sativa* var. *japonica* at around 0.09% (1 m distance) to 0.01% (5 m distance). Several subsequent investigations have confirmed extensive gene flow events from *O. sativa* to *O. rufipogon*; nearly 1.2% seedlings (296 out of 23,776) were identified as natural hybrids between *O. sativa* (pollen donor) and *O. rufipogon* at a distance of up to 42 metres under field conditions (Song et al. 2003). Rate of transgene flow (through detection of basta resistance in progenies, and molecular phenotyping using PCR for *bar* gene) from cultivated rice (*O. sativa*) has been estimated to be between 0.01% and 0.04% to weedy rice (*O. spontanea*/*O. sativa* f. *spontanea*), and 1.2%–2.2% to wild rice (*O. rufipogon*; Song et al. 2002; Chen et al. 2004; Jia et al. 2014). In another study, the frequency of transgene (*bar*) flow from *O. sativa* to *O. rufipogon* was estimated to be as high as 11–18% at a distance of 0–1 m, and 0.01% at 250 m; in contrast, no event of transgene flow and introgresion was observed from *O. sativa* to *Echinochloa crusgalli*, barnyard grass, even after long duration of co-existence (Wang et al. 2006).

Even though rice is an autogamous, self-pollinating crop, significant levels of gene flow have been recorded amongst varieties of *O. sativa*, *O. sativa* var. *japonica* and *O. sativa* var. *indica* (male sterile lines). Pollen from transgenic *O. sativa* var. *japonica* were found to sire progenies with *O. sativa* var. *indica* at a frequency ranging from 3.5–36% (at 0 m distance; hybrids could still be obtained at 0.007%

frequency at 150 m distance). It may, however, be noted that the sowing dates in the experiment were adjusted to obtain overlap in flowering time (Jia et al. 2007, 2014).

Wheat

Wheat, *Triticum aestivum* L., is an anemophilous, self-pollinated plant where pollen-mediated gene flow (PMGF) has been estimated to be ca. 1% (Poehlman and Sleper 1995- cited in Willenborg et al. 2009). In agreement with the natural rate of pollen-mediated gene flow, transgene flow and introgression from transgenic wheat lines into non-transgenic lines was estimated to be 0.6% (Willenborg et al. 2009). In contrast to this, potential outcrossing rates leading to formation of hybrids between cultivars of *T. aestivum* was estimated to be between 37% and 56%, and that between *T. aestivum* L. and *Triticum turgidum* L. var. *durum* (durum wheat) was between 5% and 30% (average 18%; Loureiro et al. 2007). The frequency of PMGF from two transgenic wheat cultivars (source) to three non-transgenic wheat cultivars (sink) was estimated to be between 0.029% and 0.034% at 0 m distance in two different sink varieties; in one plot, transgene flow frequency of 3.5% at 0 m distance was also detected. The authors also detected hybrids at a distance of up to 100 m from the source plant (Loureiro et al. 2012).

A major concern is transgene escape from cultivated GM crop to wild species, and therefore events of gene flow from *T. aestivum* (cultivated wheat) to its wild relatives, *Aegilops geniculata*, *A. neglecta* and *A. triuncialis*, were measured. Analysis of population structure using AFLP allowed researchers to estimate transgene among the F1 progenies; 2.8% were admixture of *A. geniculata* and *T. aestivum* (pollen donor) and the proportion of admixture in F1 progenies rose to 24% between wheat and *A. neglecta*, and 16% between wheat and *A. triuncialis* (Arrigo et al. 2011).

Threat of gene flow from bread wheat into jointed goatgrass (*Aegilops cylindrica*) is a major concern as *Aegilops cylindrica* grows as weed in areas of wheat cultivation, shares the D-genome of hexaploid wheat, and interspecific hybrids with high female fertility (37% at BC2 with *Aegilops cylindrica* as recurrent parent) can be obtained (Zemetra et al. 1998; Guadagnuolo et al. 2001; Gandhi et al. 2006). Transgene flow thus from GM wheat into *Aegilops cylindrica* can occur within a short period of time. Indeed, viable herbicide-resistant hybrids between wheat and goatgrass were recovered from field where imazamox-resistant wheat (cv. FS-4 IR wheat) variety was being grown, raising the concern that traits such as herbicide-tolerance can be introgressed from cultivated wheat into weeds; the fecundity of the hybrids can be restored in the backcrossed progenies (Seefeldt et al. 1998; Wang et al. 2001).

***Brassica* and Related Species**

Several species of *Brassica* are used as major sources for production of oilseed, vegetable and condiments, and a variety of traits are being modified through genetic modification (Briefs, I.S.A.A.A. 2017). Species of *Brassica* are entomophilous, with pollen transfer mediated through bee visits (although wind as a dispersal agent has

also been recorded; McCartney and Lacey 1991); species and varieties of each species are both self- and cross-pollinated.

Pollen grains from feral populations of rapeseed have been documented in commercial fields located as far as 1.5 km, and the density of 0–22 pollen grains/m³ at that distance was sufficient to cause fertilization of emasculated bait plants (Timmons et al. 1995). Experimental evidence suggests that 50% of the pollen is dispersed within a distance of 3 m and the amount of pollen available for long-distance dispersal reduces in an exponential manner (Lavigne et al. 1998). The effectiveness of cross-pollination has been suggested to be because of the dynamics of bee visits (frequency, distance travelled), and wind-pollination is not considered a major mechanism of cross-pollination (Eckert 1933; Ribbands 1951).

Three allopolyploid species of *Brassica*, *B. juncea* (AABB genome), *B. napus* (AACC) and *B. carinata* (BBCC) are known to have originated as a result of natural hybridization in pairwise combination between three diploid progenitors – *B. rapa* (AA), *B. nigra* (BB) and *B. oleracea* (CC), implying genesis of natural hybrid and gene flow (Nagahara 1935). The gene pool of *Brassicaceae* is quite broad, and intergeneric hybrids with other members of *Brassicaceae* such as *Eruca*, *Erucastrum*, *Diplotaxis*, etc. have been obtained largely under laboratory conditions requiring ovule-embryo-rescue because of strong incompatibility barriers (Warwick et al. 2009; Bing et al. 1995, Shivanna and Bahadur 2015). Natural, spontaneous cross-fertilization leading to formation of interspecific and intergeneric hybrids between a male sterile *B. napus* with *Brassica adpressa*, and, *Raphanus raphanistrum* have however been observed (Eber et al. 1994). Owing to the presence of shared genome between the diploid progenitor species and the resultant allopolyploid species, interspecific hybrids in *Brassica* can be obtained raising the possibility of transgene escape and introgression. When *B. rapa* (syn. *campestris*) and *B. napus* were planted in equal ratio under field condition, 13% and 9% of the seeds produced by *B. campestris*, and *B. napus*, respectively were found to be of hybrid origin (Jorgensen and Andersen 1994). A survey of a mixed weedy population of *B. napus* and *B. rapa*, approximately half of the plants (44 out of 102) were found to be admixtures containing genomes of both *B. napus* and *B. rapa* indicating extensive gene flow between the two species under natural conditions (Hansen et al. 2001). In another similar study in Russia, the rate of transgene flow from *B. napus*, detected as per cent of hybrid transgenic seeds was 4.1 in the progeny of *Brassica rapa* and 0.6 in the progeny of *Brassica juncea*. Both *B. rapa* and *B. juncea* are found as weeds in the rapeseed (*B. napus*) growing area in Russia (Mikhaylova and Kuluev 2018). Transgene escape (glyphosate, herbicide resistance) from *B. napus* and introgression into *B. juncea* and *B. carinata* grown in adjoining fields was estimated. Frequency of transgene escape measured as detection of interspecific hybrids was 0.024% in the case of *B. juncea*, and were found at a distance of up to 400 m; and that of *B. carinata* was 0.005% at a distance of 150 m (Séguin-Swartz et al. 2013). Seedlings of intergeneric hybrids such as that between *B. napus* and *Raphanus raphanistrum* have been shown to have much lower fitness than either of the parents, and are able to set a few seeds through selfing. Fitness of such hybrids

can be restored through backcross with either parent, thus leading to gene flow and introgression (Guéritaine et al. 2003).

Persistence of seeds of crop either in the field or under storage conditions for long duration because of dormancy is a significant contributor to seed-mediated gene flow. D'Hertefeldt et al. (2008) have detected herbicide-resistant (glufosinolate) GM-seedlings of rapeseed that emerged in fields where GM-trials were held 10 years earlier. After the conclusion of the trials, the site was being used for cultivation of sugar beet and barley following standard agricultural practices including harrowing, fertilizer and herbicide application. In spite of volunteer control measures, presence of volunteer rapeseed plants in the population demonstrated long-term persistence of GM seeds under natural conditions (D'Hertefeldt et al. 2008). The fact that 0.25–0.3% seeds of transgenic (kanamycin resistance; glufosinolate tolerance) rapeseed (*B. napus*) survive when buried at depths of up to 15 cm for 2-year period is a measure of fitness of over-wintering (Hails et al. 1997). The survivability and rate of emergence of seedlings of rapeseed (*B. napus*) and *Hirschfeldia incana* (hoary mustard), *Raphanus raphanistrum* (radish) and two intergeneric hybrids (rape × mustard) (rape × radish) was estimated at two sites in France (Dijon and Montpellier) over a 3-year period; seedling emergence (measured as a percentage of initial viable seeds) representing survivability of hybrid seeds obtained between *B. napus* × *Hirschfeldia incana* ranged from 0.12% (first year), 0.77% (second year) and 0.02% (third year), and that between *B. napus* × *Raphanus raphanistrum* L. was 0.04 (first year), 0.51% (second year) and 0.01% (third year) at Dijon. Similar trends in seedling emergence rates were observed for both the intergeneric hybrids at the Montpellier site (Chadoeuf et al. 1998). Crawley et al. (1993), however, did not detect any significant difference in growth performance, fitness (seed burial; dormancy; field persistence; overwintering) of transgenic and non-transgenic plants of *Brassica napus* subsp. *oleifera* under various ecological conditions and concluded that the transgene does not pose any risk of increased invasiveness and weediness (Crawley et al. 1993).

Sorghum

Most of the *Sorghum* species are diploid ($2n = 20$), but some such as *Sorghum halapense* are tetraploid ($2n = 4x = 40$) (Gómez et al. 1998). *Sorghum bicolor* can hybridize with its wild populations from the same species or with the other species such as Johnson grass (*Sorghum halepense*; a noxious weed), *Sorghum propinquum* and *Sorghum alnum* (Renganayaki et al. 2000; Paterson et al. 1995; Arriola and Ellstrand 1996, 1997). The extensive gene flow recorded between *S. bicolor*, *S. halepense*, *S. bicolor* ssp. *drummondii* (shattercane) is because of substantial overlap in flowering duration (Vanderlip and Reeves 1972; McWhorter 1961; Knight and Bennett 1953). Gene flow from *S. bicolor* to *S. halepense* was reported to occur over a distance of 200 m with the rate of gene flow being 0.09%, and is considered significant for the spread of crop alleles into CWR (Schmidt et al. 2013). In fact, *Sorghum* improvement programs have relied on cross-compatibility between various *Sorghum* species (Dillon et al. 2007). Globally, Johnson grass is known as a noxious weed; therefore, genetic modification of *Sorghum* for herbicide tolerance or

other improved traits and cultivation of GM-sorghum in close proximity to Johnson grass is not advised. *Sorghum bicolor* has been genetically altered to raise GM crops for various traits such as altered lignin content (Basu et al. 2011), digestibility (Henley et al. 2010, Che et al. 2016), water deficit and salinity (Maheswari et al. 2010) and disease resistance (resistance to stem borer; and, *Colletotrichum*; Girijashankar et al. 2005; Akosambo-Ayoo et al. 2011). Introduction or modification of such traits followed by introgression into CWR through transgene escape are likely to affect wild weedy relatives or CWRs of *Sorghum* by making them more sensitive to insect pests (modification of lignin), expand the range of CWR and increase competitive ability leading to increased dominance (abiotic and biotic stress tolerance) (Ohadi et al. 2017).

Other Crops

Gene flow analysis between cultivated and transgenic sugar beet (*Beta vulgaris* ssp. *vulgaris*) and its wild relatives have been performed and low levels of gene flow were observed from *Beta vulgaris* L. ssp. *vulgaris* (cultivated beet) to *Beta vulgaris* L. ssp. *maritima* (sea beet; Andersen et al. 2005; Arnaud et al. 2003). In one study, the authors concluded that gene flow from crop to CWR does not necessarily alter or reduce the genetic diversity of the weedy wild relative (Bartsch et al. 1999). In contrast to low levels of crop-to-weed gene flow, pollen-mediated weed-to-weed (subspecies within the *Beta vulgaris* complex) was observed to occur at extremely long distances (up to 9.6 km apart) with up to 11–17% progenies being sired by pollen grains from population located several kilometres away (Fenart et al. 2007).

Several authors have investigated gene flow in potato (*Solanum tuberosum*) and found a very low rate (Conner 1992, 1994; Conner and Dale 1996; Tynan et al. 1990). However, hybrid seedlings between transgenic and non-transgenic potato were obtained at frequency of 24% (when parents were grown in adjacent rows), 2% (at 3 m distance), 0.017% (at 10 m distance) and no hybrids were obtained when the parental lines were separated by 20 m distance (McPartlan and Dale 1994). The potential of pollen-mediated gene flow in potato is estimated to be low because of asexual mode of propagation (Conner and Dale 1996). Even if pollen dispersal does occur, a distance of 20 m has been suggested to be adequate to prevent cross-fertilization with other species.

Among crops, gene flow from cultivated sunflower (*H. annuus*) to CWR has been estimated to be high because of extensive sexual compatibility. The rate of gene flow was estimated to be nearly 27% between cultivated and wild sunflowers planted at a distance of 3 m; gene flow was detected even when the source and the sink populations were separated by a distance of 1000 m (Arias and Rieseberg 1994). In subsequent studies, 42% of the progenies present at the margin of the sunflower crops were found to be hybrids between the cultivated and CWR and that the crop alleles persisted for five generations in the hybrids (Whitton et al. 1997; Linder et al. 1998). Introgression of Bt-transgene (*Cry1AC*) from cultivated into wild weedy sunflower (both *H. annuus*) through backcrossing confers a selection advantage; weedy plants with the Bt-transgene suffered lower levels of insect pest damage, and

produced nearly 55% more seeds. The results suggest that Bt-introgression into weedy wild sunflower population will lead to survival advantage through higher biotic tolerance and increased hybrid fecundity (Snow et al. 2003). AFLP marker-based analysis showed that allele introgression between *H. annuus* (cultivated sunflower) and *H. petiolaris* (a closely related species) in sympatric populations occurs at a low frequency, is sporadic in nature, and chromosomal rearrangements act as barrier to introgression (Rieseberg et al. 1999a, b).

15.4 (Trans)Gene Flow as a Biosafety Issue

Research and development involving biological agents has unintended and unforeseen risks and hazards that may endanger the public health and environment. Biosafety is primarily a framework to manage and prevent such risks to public health and environment that could arise due to unintentional interactions between potentially dangerous biological agents and other susceptible agents or the environment (Kumar 2014). The primary objectives of biosafety program include the identification of biohazard, evaluation of associated risks and the containment of potentially harmful biological and chemical agents (Keese 2008; Gómez-Tatay and Hernández-Andreu 2019). Advancements in genetic engineering (GE) and molecular tools have enabled specific modification of quantitative and qualitative traits of important crops by introduction of foreign DNA and associated regulatory elements into an alien genetic background to obtain transgenics, and into the same genetic background to obtain cisgenics (Briefs, I.S.A.A.A. 2017; Telem et al. 2013). Release and widespread adoption of GM crops raises several biosafety concerns in relation to public health, economic, social, political, ethical issues, environmental risks such as biodiversity loss, superweed generation, HGT to microbiota, and several non-target effects including issues related to pollinator health (Table 15.2; Fig. 15.2a; Keese 2008; Prakash et al. 2011; Kumar 2014).

Gene flow, a natural and continuous process of the transfer of genetic material among populations, is one of the key mechanisms for evolution of allo-ploids; it has however never been documented to have caused a widespread danger to the public health and environment (Ellstrand 2003; Lu 2008; Ryffel 2014). Transgene escape (vertical or horizontal) from GM crop may have unpredictable and unintended effects because of introduction of the transgene into other organisms such as bacteria, viruses, other crops and even animals (Thomson 2001; Lu 2008; Quist 2010).

The issue of gene flow from transgenic to plants such as wild species, related species or other cultivated non-GM species, to pollinators, and to consumer as a biosafety issue must be looked at from various perspectives such as:

- (i) Relationship between the source and sink of gene flow
- (ii) End-use of the GM-product in question, for example, to be used as food (for human), feed (cattle, livestock), production of industrial product (GM for

Table 15.2 Fate and effects of transgene or transgene-expressed protein

Experiment type	Genes involved	Effects/toxicity/gene flow to microbiota	Conclusion
<i>Pollinator Health</i>			
Larval development/survival.	Bt toxins (lepidopteran-active: Cry1Ab, Cry1Ac, cry 9C); Bt toxins (coleopteran-active: Cry3B); Cry3A; Bt-corn (lepidopteran-active: Cry1Ab); Bt-corn pollen (lepidopteran-active: Cry1Ab, Cry1F); SBTI.	No effects or toxicity.	When these proteins were fed to the growing colony, it resulted in normal development and survival. Only SBTI resulted in reduced larval growth at extremely high concentration, which is unlikely to occur in natural conditions (Malone 2004).
Adult development/survival.	Cry1Ba; Cry1Ac; Bt-corn (lepidopteran-active: Cry1Ab); Bt-corn pollen (lepidopteran-active: Cry1Ab, Cry1F); serine protease inhibitors (Aprotinin, BBIa, CpTIa, POT-1a, POT-2a, SBTIa); OCI (chicken egg white cystatin); Chitinase; β -1,3 glucanase.	No effects or toxicity.	Serine protease inhibitors (Aprotinin, BBIa, CpTIa, POT-1a, POT-2a, SBTIa) result in reduced survival by a few days at extremely high concentration; unlikely to occur in natural conditions (Malone 2004).
Adult food consumption.	Cry1Ba.	No effects or toxicity.	Even higher doses did not affect the amount of food consumed by adults (Malone 2004).
Adult flight activity.	Cry1Ba; Aprotinin.	Cry1Ba did not have any effects while Aprotinin resulted in early flight activity, beginning a few days earlier.	Flight activity begins a few days earlier in case where proteins were fed at extremely high concentration; unlikely to occur in natural conditions (Malone 2004).
Adult digestive proteases	Aprotinin, POT-1, POT-2, SBTI	Inhibition of some proteases.	Inhibition of some proteases (Malone 2004).
Olfactory learning responses.	BBI; CpTI; SBTI; cysteine protease inhibitor (OC1b);	One inhibitor offered in sugar reward	CpTI offered in sugar reward reduced ability to learn;

(continued)

Table 15.2 (continued)

Experiment type	Genes involved	Effects/toxicity/gene flow to microbiota	Conclusion
	Chitinase; β -1,3 glucanase.	reduced ability to learn; others did not.	others did not (Malone 2004).
Foraging behaviour.	Bt-corn (lepidopteran-active: Cry1Ab); OCI and Chitinase (expressing in oilseed rape); Chitinase; β -1,3 glucanase.	No effects or toxicity.	Foraging behaviour was unchanged even at higher concentrations (Malone 2004).
<i>Fate of Transgene in Gastrointestinal Tract</i>			
Detection of transgene or its part.	M13 DNA (7250 bp) fragments in mice.	Part of transgene present.	Fragments of up to 1692 bp survived passage through the GIT (Schubbert et al. 1994, 1997).
	CP4-EPSPS transgene soybean.	Negligible amount of transgene fragment.	Small proportion (max 3.7%) of the CP4-EPSPS transgene survives passage through the stomach and the upper GIT; fully degraded in large intestine (Netherwood et al. 2004).
	Bt176 corn hybrid and chicken.	Transgene fragment absent.	No recombinant plant DNA fragments such as recombinant bla or cry1A(b) fragments could be found (Aeschbacher et al. 2005).
	GM corn, plasmid DNA encoding an Escherichia coli glutamate dehydrogenase (gdhA); weanling swine.	Transgene fragment absent.	gdhA transgene began degradation in the stomach and was nondetectable in the large intestine (Beagle et al. 2006).
Detection of transgene or its part in human intestinal epithelial cell line CaCo-2.	CP4 variant EPSPS transgene.	Negligible amount of transgene fragment.	0.06% of 633 bp long DNA fragments were found to be transported across polarized CaCo-2 cells (Sharma et al. 2007).

(continued)

Table 15.2 (continued)

Experiment type	Genes involved	Effects/toxicity/gene flow to microbiota	Conclusion
<i>Gene transfer to microbiota</i>			
Incorporation of plasmid DNA encoding for ampicillin resistance.	DNA persistence and integrity in various ex vivo and in vivo systems using gnotobiotic rats (either with <i>E. coli</i> or <i>B. subtilis</i>).	Small fragments were detected in stomach samples but no bacterial uptake (in vivo) was observed.	The transgene did not survive passage through the intact gastrointestinal tract. A few experiments show survival of transgene up to a level where gut microflora get transformed by transgenes. Most of the literature shows that transgenes do not survive harsh condition of GI tract. Thus, it may be concluded that gene transfer did not occur during the feeding experiments (Wilcks et al. 2004; Netherwood et al. 2004; Zuo et al. 2018).
Transgene in gut microbiota.	Survival of the transgene CP4-EPSPS from GM soya in the small intestine of human ileostomists.	3 out of 7 ileostomists were found to have the CP4-EPSPS transgene within their gut microbiota.	
Assessment of soil microbes and arthropod community.	Explore the ecological safety of perennial transgenic poplars in arthropod community regarding gene flow and other factors.	No effect on the microbial community structure or functional diversity.	
<i>Gene transfer to other crops</i>			
Evidence for gene flow between maize and its nearest wild relatives, the teosinte.	Enp1-8; Glu1-7; Pgd1-3.8; Pgm2-7.2; Enp1-14; Glu1-8; Glu1-11; Pgd1-1.8; Pgd2-8; Pgm2-7.2	Allozyme analysis and chloroplast DNA analysis reveals gene flow between maize and teosinte.	Genes placed in maize by genetic engineering could be transferred to teosinte and, under some circumstances, spread throughout teosinte populations (Doebley 1990).
Investigation of gene flow from transgenic to non-transgenic rice.	Pat gene.	<0.01% to 0.53% escape was detected in a normal side-by-side and circular plot design in varying conditions.	Based on the analyses of phenotypic, molecular and segregation data, it was observed that pollination of recipient plants with pollen of the transgenic source did occur at a significant frequency (Messeguer et al. 2001).

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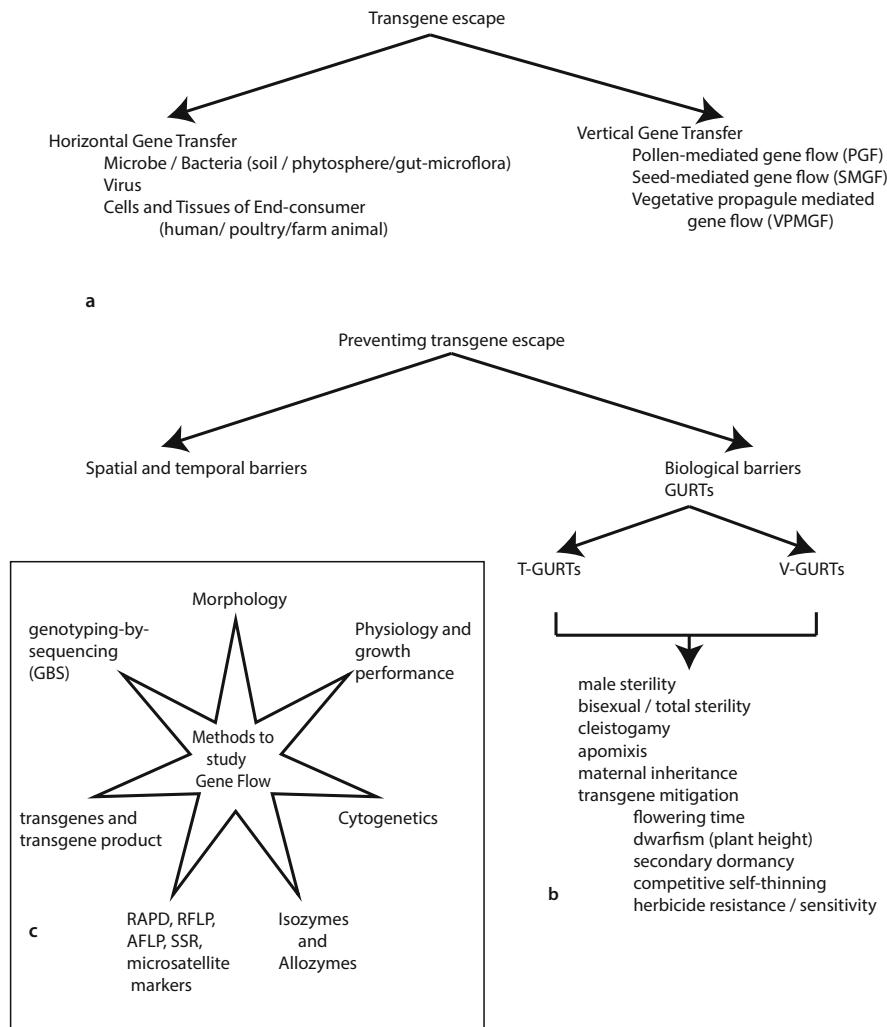
Table 15.2 (continued)

Experiment type	Genes involved	Effects/toxicity/gene flow to microbiota	Conclusion
Genetically characterize and determine the paternal origin of weeds and discuss the risks associated with the use of transgenic sugar beet cultivars.	Beta microsatellite Bvm3 locus (a total of 26 alleles present) alongwith 5 RFLP loci and nuclear DNA in Beta vulgaris.	A high degree of polymorphism was observed within all the groups, except for cultivated beets.	Parapatry sugar beet fields of northern France and coastal beets show no evidence for gene exchange between them (Desplanque et al. 1999).
Study how herbicidal resistant genes could flow from wheat to jointed goatgrass through crossing and backcrossing.	Imazamox herbicide – an imidazolinone herbicide.	The gene for resistance to imazamox was in the hybrids, and the majority of the seed produced by the hybrids possessed the resistance gene.	The rapid transfer of a herbicide-resistance trait from wheat to jointed goatgrass is a problem with serious implications for those who would use herbicide-resistant wheat in areas infested with jointed goatgrass (Seefeldt et al. 1998).
Study crop-to-wild transgene flow frequency and its persistence from GM <i>Brassica napus</i> to wild <i>Brassica juncea</i> .	Cry1Ac.	The frequency of gene flow from transgenic donor <i>B. napus</i> to wild brown mustard <i>B. juncea</i> was 0.66%.	In addition, although sympatric presence increased female investment in ovule production (lower P/O ratio), reduction in reproductive fitness in wild brown mustard as a result of competitive inferiority relative to Bt OSR was observed (Hansen et al. 2001).
Differentiation between natural and cultivated populations of <i>Medicago sativa</i> .	25 RAPD loci and allozyme assay.	RAPD markers revealed less within-population genetic diversity than allozymes, the quantitative and qualitative patterns of population structure were in full agreement with allozymes.	Crop-to-wild gene flow occurred in many locations, but some other mechanisms opposed cultivated traits to be maintained into natural populations (Jenczewski et al. 1999a, b).
Compare fitness components of F1 wild <i>Cucurbita pepo</i>	Zucchini Yellow Mosaic Virus and Watermelon Mosaic	The average fecundity of hybrids ranged from 453 to	F1 generation does not constitute a strong barrier to

(continued)

Table 15.2 (continued)

Experiment type	Genes involved	Effects/toxicity/gene flow to microbiota	Conclusion
with wild–crop hybrids within their native range.	Virus 2 resistance genes.	4497 seeds per plant and represented 15–53% of the numbers of seeds produced by wild plants.	introgression, despite the fact that some crop genes may be disadvantageous to wild squash.

**Fig. 15.2** (a) Modes of transgene escape; (b) strategies to prevent transgene escape; and (c) methods to study gene flow

increased bio-diesel; longer/stronger cotton fibre), edible vaccine (that will be purified).

15.4.1 Biosafety Concerns of Horizontal (Trans)Gene Transfer

In a Horizontal (trans)Gene Transfer (HGT), there is stable transfer of transgene from GM crops to unrelated organisms living in the vicinity or in close association (sharing overlapping ecological niches) via non-reproductive means such as phagotrophic uptake mechanism, endosymbiotic gene transfer, transduction, transformation (Dröge et al. 1998). Some of the genes involved in introducing or modifying traits such as herbicide resistance, insecticide resistance, antibiotic resistance, disease resistance and stress tolerance in GM crops involved genes that are of microbial origin (Kleter et al. 2005; Briefs, I.S.A.A.A. 2017). Some of the genes of bacterial origin that are approved to be used during generation of GM crops are herbicidal resistance genes such as 3-enolpyruvylshikimate-5-phosphate synthase (cp4 EPSPS), Glyphosate oxidoreductase (GOX), Phosphinothricin acetyltransferase (PAT); male sterility and fertility genes such as Barnase and Barstar, DNA adenine methylase (DAM); plant hormone metabolism genes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase and S-adenosylmethionine hydrolase (SAMase); transformation markers such as β -glucuronidase (GUS) and Nopaline synthase (NOS); insecticidal genes such as CRY etc. (Kleter et al. 2005).

Biosafety concerns about HGT from GM crops to microorganisms such as bacteria have been summarized as *horizontal transfer of transgenes is the elephant in room, and regulatory agencies such as European Food Safety Authority (EFSA) should no longer turn a blind eye to this potential menace* (Ho 2014).

The frequency and chances of success of HGT from GM crops depends on (Keese 2008):

- (i) Type of sink organism (eukaryote, prokaryote and virus)
- (ii) Genetic and ecological relationship between donor and recipient
- (iii) Function of the transferred gene
- (iv) Genetic and environmental niche similarity

Though every gene has equal chances of transfer, the level of persistence of transgene in the recipient sink species is dependent on the gene function and its downstream effects. For instance, genes with lethal effect may have zero persistence in the recipient organism, whereas genes most likely to be introgressed into bacterial genome are the ones of bacterial origin including antibiotic resistance genes that are routinely used during cloning, transgenic selection and subsequent generation of GM plants.

In case of HGT to microbes, recipient sink species may be soil bacteria, endosymbionts, or even bacteria in gastrointestinal microflora of animals (Dunfield and Germida 2004; Netherwood et al. 2004; Nawaz et al. 2018). Horizontal gene

transfer from GM crops to the microorganisms (living in soil, rumen or intestine) can occur only through rare “natural transformation” with the free DNA of bacterial origin (Kleter et al. 2005; Bertolla and Simonet 1999). However, there are barriers that restrict the chances of transfer such as persistence of intact DNA fragment, its integration in the bacterial genome and selective advantages of the transferred gene (Ceccherini et al. 2003; Keese 2008). An old but excellent article reviews the various dimensions of HGT from GM plants into bacteria (Nielsen et al. 1998).

Bacteria may be exposed to the transgenic DNA near decaying GM plant part or in the digestive tract of animals feeding on GM plant products. Transgenes gets degraded during the process of digestion and enzymatic degradation, leaving behind fragmented DNA (Table 15.2), which is recognized as nutrient source but not as potential substrate for bacterial evolution. While evidence for the persistence of transgenic DNA in phytosphere is present, no report shows the transformation of plant DNA to native soil microorganisms (Dunfield and Germida 2004). A few reports of bacterial transformation using DNA fragments from GM plants exist but these were conducted under optimized laboratory conditions which are unlikely to be present in the natural conditions. Comparative analysis of soil microflora from fields used for growing GM-maize (with *nptII* gene for resistance against kanamycin and neomycin) or non-GM maize showed equal prevalence of natural resistance against kanamycin and neomycin. Molecular analysis of 3000 independent bacterial isolates from the soil failed to detect *nptII* and thus any event of HGT from GM maize to soil bacteria was ruled out (Ma et al. 2011). Indeed, Schlueter et al. (1995), based on their experimental data obtained from artificially optimized “ideal” and “naturalized” conditions, concluded that the frequency of HGT from GM plants (potato, in this case) to bacteria (*Erwinia chrysanthemi*, a natural pathogen of potato) is extremely rare, to the point of being irrelevant and no biosafety risks are evident (Schlueter et al. 1995). In another study conducted over a 10-year period in field where GM-cotton with *bla* gene conferring resistance against lactam antibiotics were grown, no significant differences were detected in antibiotic resistance in soil bacterial population from field with GM-cotton or non-GM cotton (Demanèche et al. 2008). In contrast to previous reports, homologous recombination-mediated HGT from Transplastomic Tobacco into *Acinetobacter baylyi* strain BD413 present in the phytosphere has been observed. HGT was confirmed by detection of green fluorescence when promoterless aaD::GFP translational fusion construct present in the tobacco was introgressed into *Acinetobacter baylyi* upon homologous recombination which restored GFP activity (Pontiroli et al. 2009).

The results obtained so far clearly indicate that soil bacteria can uptake exogenous DNA at very low frequency under laboratory conditions (Ceccherini et al. 2003; Nicolia et al. 2014) whereas experiments in the field did not show any evidence of HGT (Ma et al. 2011). Transgene products have also been shown to be released by roots but do not contain the transgene per se and therefore are not a serious threat for HGT; rather such exudates may influence microbial population and act as a selection pressure and alter microbial community composition. HGT from GM crops to bacteria may impose strong selection pressure for bacterial resistance and may result in emergence of a new pathogen, or increase in pathogenicity of existing ones. These

bacteria may also transfer the transgene to other related/unrelated pathogens, thus altering ecological potential of the recipient bacteria (Dröge et al. 1998; Keese 2008). The evaluation of natural transformation events in soil or the phytosphere is challenging. Regardless of this, various studies have been carried out to observe the effects of the interaction of GM plants and bacteria, and most of the studies demonstrate it as a harmless phenomenon (Table 15.2).

The possibility of plant genes or transgenes being transferred from GM-food into human cells and tissues is directly dependent on their ability to survive harsh human gut conditions. Transgene (epsps; 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, conferring tolerance against the herbicide- glyphosate) from GM-soybean has been shown not to survive the gastrointestinal tract of humans; although when the digesta that has bypassed the gastrointestinal tract is collected and analysed, traces of epsps genes were detected from only one human subject out of 12 human subjects fed with meal of GM soya. Analysis of the gut microflora also ruled out the possibility of HGT from GM-soybean (Netherwood et al. 2004).

Several researchers rightly argued that an extremely small fraction of the plant DNA ingested by humans is likely to be of transgene origin; for example, only 0.00006% of about 70 µg DNA from 170 g of GM plant is likely to be of transgenic nature and the fear of transgene flow into human is unfounded. In contrast, the quantity of plant-based feed for cattle is much higher and so is the amount of plant DNA including transgene being ingested. It is highly unlikely that the DNA from GM crop will survive the environment in the gut (Nawaz et al. 2018).

Animals feeding on plants, herbivores, and pollinators can acquire transgene. Though any foreign DNA gets extensively degraded in the gastrointestinal tract, it is the most reasonable route of transgene entry into animals. DNA obtained through food may survive harsh digestive conditions and fragments up to a few hundred base pairs are detectable in the gastrointestinal tract. But no transgene could be detected in the tissues, organs and germline of subjected animals (Keese 2008; Nawaz et al. 2018).

Viruses have potential of recombination with other sequences of viral origin. In contrast to animal viruses, plant viruses are small and rarely contain host sequence. GM plants carrying viral DNA sequences in order to provide resistance from viral infection are potential source to transfer transgene to viruses (Keese 2008). Co-infection by viruses is the main reason for the background level of HGT to plant viruses. If a homologous, heterologous or non-host virus infects GM plant, they have potential for homologous and heterologous recombination with the viral transgene and can develop a new host range (Turturo et al. 2008).

Other instances where HGT has been investigated are pigs fed on Bt MON10/Bt MON810 maize where no traces of cry1Ab gene or Bt toxin were detected in pig gut (Walsh et al. 2012; Mazza et al. 2005), and broiler chicken fed on transgenic Bt corn (no HGT detected; Rossi et al. 2005), to cite a few examples.

Potential Effects of GM-Crops on Pollinator (Honey Bee) Health

Pollinators, responsible for transporting pollen grains, are presumed to be at a serious risk from GM plants as the presence of transgene or proteins expressed by transgene

may potentially have harmful effects on pollinator health, microorganisms living in the guts of the pollinator and subsequently human health (Malone 2004). Bees foraging and actively pollinating GM plants may have direct effects on its survival and lead to changes in developmental and behavioural changes (Brunet et al. 2019). Nectar is an unlikely source of novel proteins because it contains plant secretion including only carbohydrates and free amino acids with a few exceptions where proteins are present in very low amount. Pollen is the most likely source of transgene or its protein product (Malone 2004; Brunet et al. 2019). Various studies have been done to demonstrate the effects of transgene or proteins expressed by transgene using various methods but most studies show that direct feeding on the plant or exposure to the transgene or its product do not have any significant effect on the health of pollinator (Malone 2004; Ricroch et al. 2018; Dai et al. 2019). In a study involving two fields, one with transgenic (herbicide tolerant; LibertyLink™) canola and the other adjacent field with pollinator habitat comprising of a mixture of native wildflower species, no difference was observed in pollinator diversity, visit and foraging behaviour. The authors also conclude that GM crops showing herbicide tolerance are not directly harmful for the pollinators, but indirectly affect pollinator population by eliminating wild native plant species that may act as refugia (O'Brien and Arathi 2018). GM crops that require fewer herbicide and insecticide application may actually be beneficial for pollinator population. Recent studies show that glyphosate, Cry2Ab and Cry1F may affect the development of the larva and subsequent colony growth of stingless bee *M. quadrifasciata* in dose-dependent manner causing reduction in the pollination ability (Table 15.2; Vázquez et al. 2018; Seide et al. 2018).

15.4.2 Biosafety Concerns of Vertical (Trans)Gene Transfer

Vertical gene transfer can be pollen-mediated, seed-mediated or vegetative-propagule-mediated. Pollen-mediated transgene flow (PMGF /PGF) is the major biosafety concern if the transgene confers a strong fitness advantage, and therefore is under positive natural selection, allowing the transgene to spread quickly through hybridization and introgression regardless of low frequency of gene flow (Lu 2008; Quist 2010).

Pollen-mediated transgene flow requires at least three conditions (Messeguer 2003; Quist 2010; Fig. 15.1b):

1. Compatible wild relatives need to be growing within the pollen/seed dispersal range of the GM crop. The pollen range is affected by environmental (wind, humidity, temperature, etc.) and biological factors (plant height, pollen size, natural outcrossing rates, pollen viability, etc.).
2. The phenology of GM crop and the crop wild relative/weed should be overlapping.

3. Hybridization should result in viable and fertile offspring because reproductive barriers to introgression are generally strong. The offspring of the hybrid should also be viable and fertile; otherwise, gene flow would be restricted at this point.

Pollen-mediated transgene escape from GM to non-GM crop may lead to contamination, change in genetic diversity of traditional crops and change in farming practices. Crop-to-wild and crop-to-weed transgene escape may cause spread of resistance genes and lead to swarm effects, creation of new weeds, loss of wild germplasm (Lu 2013). Besides pollen-mediated, transgene escape can be seed-mediated and vegetative propagule-mediated. Both seed-mediated and vegetative-propagule-mediated transgene flow do not have any serious effects because transgene does not get integrated into the recipient populations (Van de Wiel et al. 2003; Lu 2008). The risks associated with GM crops need a stepwise assessment system that can estimate the frequency of transgene flow, determine its expression pattern and level and measure fitness change due to transgene expression. It may help us to predict potential environmental consequences under different conditions (Chèvre et al. 1997).

15.4.3 Transgenic Plants and Gene Flow: Analysis of Biosafety Concerns

Gene flow as biosafety issue must be analysed in reference to various factors and criteria that are necessary for gene flow to occur. A comprehensive analysis of transgenic maize is presented below as an illustrative example. Although in India current regulations do not permit open cultivation of transgenic plants for food use, biosafety guidelines have been prepared keeping in mind future needs and requirements (<http://dbtindia.gov.in/regulations-guidelines/regulations/biosafety-programme>).

Evaluation of Biosafety Issue of Transgenic Maize Transgenic maize has been generated using *Cry3/Cya1b Bt* gene (insect resistance), *EPSPS* (glyphosate tolerance; herbicide), *PAT* gene (glufosinolate; herbicide tolerance), *PAT + EPSPS* (glyphosate + glufosinolate) and stacked transgenics for both insect resistance and herbicide tolerance (*Cry1A + PAT*), *cspB* from *Bacillus subtilis* gene (drought tolerance), *ms45* (male sterility), *zm-aa1* (male sterility) (<https://www.isaaa.org/gmapprovaldatabase/crop/default.asp?CropID=6andCrop=Maize>). *Zea mays* (maize; $2n = 2x = 20$) has its centre of origin in Mesoamerican region, now Mexico and Central America. In India, transgenic maize is not yet approved for cultivation; however, non-GM maize is widely cultivated in India across majority of states (https://farmer.gov.in/M_cropstaticsmaize.aspx). Maize is generally grown as rainfed Kharif crop; however, in several parts of India, maize is also grown as rabi and as spring crop. Maize is also cultivated with pea, cauliflower, cotton, carrot, etc. as inter-crop (<https://farmer.gov.in/imagedefault/pestanddiseasescrops/normalmaizeproductiontechnologies.pdf>).

Maize is a monoecious plant; male flowers (tassel) are borne at the terminal ends of the main shoot and female flowers (ear) are produced in axillary positions. Male flowers are protandrous, and copious numbers of pollens are shed (beginning 2–3 days prior to receptivity of female flowers and continuing for up to 5–6 days), which are trapped by the “silk” of the female flowers that are present in the lateral branches, positioned lower on the main stem. Pollens remain viable for a short period only (ranging from 30 min to a maximum of 24 h; Luna et al. 2001). Pollen transmission is aided by wind, and majority of pollen grains land on silks within a short distance (Luna et al. 2001; Raynor et al. 1972); protandry and anemophily are major causes for cross-pollination rates of more than 95% (Biology of *Zea mays*, MoEFCC and DBT 2011). In cultivated maize, kernel (seed) dispersal is completely dependent on human intervention. The success of kernel germination from soil seed bank is low because of extremely high competition for resources among germinating seedlings, thus effectively ruling out presence of large volunteer and feral populations, except when cobs disintegrate due to disease or other human intervention such as use of partly crushed cobs for cattle-feeding or as manure (Pascher 2016).

Gene flow as a biosafety issue should be analysed in relation to the gene pool where exchange of genetic material can occur naturally, unaided by human intervention. The success rate of vertical gene transfer is highest among members of primary gene pool, member of tribe Maydeae to which *Zea mays* belongs, in this case. Among the members of Maydeae, *Zea*, *Euchlaena* and *Tripsacum* are of New World origin, whereas *Sclerachne*, *Trilobachne*, *Chionachne*, *Polytoca* and *Coix* are proposed to have originated in the Old World (Kaul 1973, Bor 1960- cited in Hore and Rathi 2007; Vallaeys 1948). In India, several genera that are considered a part of primary gene pool of *Zea mays* are found and include *Tripsacum dactyloides* ($2n = 2x = 36$; de Wet et al. 1982, 1983; de Wet and Harlan 1972) and *T. laxum* ($2n = 70, 72$; Ting 1960), *Coix lacryma-jobi* ($2n = 2x = 20$), *Chionachne* species, *Polytoca* species, *Trilobachne* species, and *Sclerachne* species (Biology of *Zea mays*—Maize, MoEFCC and DBT 2011). *Coix lacryma jobi* (Job’s tear) is of pan-Indian occurrence, cultivated for several economic products, with Malay Archipelago considered as the Centre of Origin (Nirodi 1955) and North-Eastern Centre considered as the Centre of Variability (Hore and Rathi 2007). Extensive maize germplasm diversity exists in India such as landraces termed as “Sikkim primitives”, some of which have different traits than Mexican primitives (Biology of *Zea mays*, MoEFCC and DBT 2011).

C. lacryma jobi is cultivated mainly during kharif season in NE India. *Coix* is monoecious and outcrossed; Mello et al. (1995) have reported natural outcrossing rates ranging between 33% and 72% in different populations of *Coix lacryma jobi* implying a high rate of cross-fertilization. Sapre et al. (1985) have reported occurrence of several intraspecific (*C. lacryma jobi*) and interspecific hybrids (among *Coix* species) providing evidence of gene flow. Ramirez (1997) reported several interspecific and intergeneric crosses for gene introgression in maize from wild relative. Reciprocal crosses were attempted between *Z. mays* ssp. *mays* (cultivated maize) and *Z. mays* ssp. *mexicana*; with *Z. mays* ssp. *diploperennis*, with *Z. mays*

ssp. perennis, and with *Coix lacryma-jobi*. In various crosses performed, the success rate in obtaining hybrid seeds ranged from as high as 60% (*Z. mays* ssp. *mays* and *Z. mays* ssp. *Mexicana*) to nearly 30% with *Coix lacryma-jobi* (Ramirez 1997). However, the so-called hybrid seeds obtained between *Z. mays* ssp. *mays* and *Coix lacryma-jobi* were not analysed further as these were considered as “*maternal seeds*” by the author. Other authors have also reported generation of hybrids of variable fertility between maize varieties (Purseglove 1972; cited in Biology of *Zea mays*-Maize, MoEFCC and DBT 2011), maize and annual teosintes except *Z. perrennis* (Wilkes 1977; Ellstrand et al. 2007). Kernels on cobs of maize x teosinte hybrids require substantially less force to detach and can disperse easily and have not been considered a barrier to dispersal and spread (Chavez et al. 2012). Gene flow among maize relatives has been documented to be bi-directional, albeit at a low frequency, thus allowing maize and teosintes to retain distinct genetic makeup and identity (Doebley 1990). Infertile and partially fertile hybrids from intergeneric crosses between maize and *Tripsacum* species (Eubanks 1997), between *Z. mays*, *Z. perrennis* and *Tripsacum dactyloides* (Iqbal et al. 2019), and between maize and wheat (Laurie and Bennett 1986) to name a few, have been reported. These hybrids were obtained by hand-crossing and the resultant hybrids were sterile and unstable due to chromosomal incompatibilities, implying that even if natural hybrids are formed between wild-relatives under rarest of conditions, their survival is a major constraint. Analysis of various factors suggests that the biosafety and environmental risks of transgene escape from insect-resistant and herbicide-resistant maize to teosintes is low and the foreign traits are not likely to increase the abundance of maize x teosinte hybrids (Devos et al. 2018).

Based on various past reports and investigations on traits, and biological properties of maize and its relatives, biosafety concerns of transgenic maize have been addressed in India. Potential of gene flow from maize to *Coix*, *Sclerachne*, *Polytoca*, *Chinachne*, *Trilobachne* exists because of their similar chromosome number, but limited studies have been performed to investigate the natural potential for hybrid formation, its fertility and fecundity (Biology of *Zea mays* (maize)-MoEFCC and DBT 2011).

In order to evaluate and address biosafety concerns of transgene flow, compilation of information on biology of crops and crop wild relatives including reproductive biology, ecology, genome information (compatibility), hybrids (fertility and fecundity), possibility of soil-seed bank, volunteers and feral population has been prepared as crop-specific handbooks (brinjal, cotton, okra, mustard and rice; MoEFCC and DBT 2011).

15.5 (Trans)Gene Flow: Containment and Mitigation Strategies

Biosafety has remained a major concern with regard to widespread acceptance and cultivation of GM crops. Gene flow from transgenics to other related plants and crop wild relatives (CWRs) may be a major concern in transgenic rice, sunflower, oilseed rape, sorghum, barley which have con-specific and closely related interbreeding

weeds in areas where these are cultivated. Bauer-Panskus et al. (2013) reviewed the documented cases of transgene introgression into wild and compatible relatives in several areas of USA, China, Canada and Mexico; the potential of transgene escape from crops in the centre of origin and diversification is highly undesirable and poses several environmental risks. Based on the information available for growth pattern, life-history traits of the GM crops (source) and sink species, strategies based on biochemical, molecular and genetic pathways have been proposed and devised for controlling gene flow (Fig. 15.2b; Table 15.3).

15.5.1 Spatial and Temporal Barriers

Physical isolation and barriers, such as nylon-mesh, net-houses and filters, can be the easiest strategy for preventing gene flow; they have been useful only under laboratories conditions and green houses, but are impractical in large agriculture fields. Isolation without any artificial barriers such as maintaining sufficient inter-crop distance is another viable option. Transgene flow frequency was observed to be decreasing significantly when distance between two compatible rice species increases (Rong et al. 2007). A combination of isolation distances and introduction of separation crops between donor (GM) and recipient compatible species has been an effective strategy for pollen-mediated gene flow (PGF) from transgenic cotton (Yan et al. 2018). In maize, the pollen deposition rates were found to be between 10 and 100 pollen/m² at a distance of 10 m from the source plant in the direction of wind flow; the deposition rate was only 0.001–0.0002 pollen grains/m² at a distance of 1000 m; more than 99% pollen grains of maize is deposited within a distance of 30 m (Jarosz et al. 2003, 2004, 2005). Incorporating the knowledge of distances pollen grains can travel with longevity of pollen viability can prove to be helpful for physical confinement of transgenes as has been analysed for rice (Pu et al. 2014). The authors also suggest that isolation distances between the source and sink species can be less effective in entomophilous as compared to anemophilous plants because of the large diversity of insect pollinators involved (Pu et al. 2014).

The level of gene flow measured as cross-pollination has also been shown to be dependent on the size of the agricultural plot or the area of cultivation. For example, in a plot size of 0.25 ha, the cross-fertilization was 1.77%, which got reduced to 0.83% as the plot size increased to 1 ha; (Mele 2004; cited in Devos et al. 2005), similar results have also been independently obtained by Weber (2005; cited in Devos et al. 2005). The over-all level of cross-fertilization has been estimated to be ca. 0.9% if the two adjacent plots are of the same size (Devos et al. 2005).

Difference in flowering time, or non-overlapping floral phenology between the source and sink species can decrease, or entirely eliminate the chances of outcrossing between two compatible species present in agriculture field. A gap of only 1 week in sowing time leading to difference in flowering period led to a reduction in cross-fertilization in recipient species by 50%, and by 75% when the sowing time difference was 3 weeks. Thus, spacing sowing time can significantly decrease the

Table 15.3 Strategies for preventing gene flow and their recent status in terms of practical usage in fields

Strategy	Crop	Evaluation	Drawbacks	References
<i>Physical containments</i>				
Isolation distances and separation crops	GM cotton	Significant decrease in PGF when isolation distance was increased or <i>Sorghum bicolor</i> was introduced as separation crop	Leaky gene flow due to wind, isolation distances and separation crops will be different for each crop	Yan et al. (2018)
	GM rice	Significant decrease in PGF with distance		Rong et al. (2005, 2010)
Reproductive isolation	GM citrus	Pollen competition contributed to the transgene flow		Pons et al. (2011)
	GM cotton	PGF is dependent on pollinator spp.		Yan et al. (2015)
Physical and genomic landscape ^a		Studying physical landscape, demographic and evolutionary history along with speciation genomics can measure and reduce gene migration		Ravinet et al. (2017)
<i>Biological containments</i>				
Genome incompatibility and difference in ploidy level ^a	Prevents recombination after pollination.	For example, <i>Aegilops tauschii</i> (weed) is compatible with D genome of wheat, while it is incompatible with B genome (Durum wheat).	It has not yet been carried out in transgenic, it may not be possible for all the GM plants.	Daniell (2002)
Synthetic incompatibility ^a	<i>Saccharomyces cerevisiae</i>	Genetic barrier was introduced between two strains using CRISPR/Cas9.	Not yet applied to any crop yet.	Maselko (2017)
Male sterility	GM tobacco and <i>B. napus</i>	TA29 gene mutant produces male sterile lines.	Compatible plants can pollinate the male sterile transgenic.	Mariani et al. (1990)
	<i>Nicotiana tabacum</i> and	Expression cassette. PrMC2pro::		Zhang et al. (2012)

(continued)

Table 15.3 (continued)

Strategy	Crop	Evaluation	Drawbacks	References
	<i>Eucalyptus</i> (spp.)	barnaseH102E confers complete pollen ablation.		
Bisexual Sterility	<i>Arabidopsis</i>	Loss of function mutation in meiosis specific genes like SWI1 and RAD51C genes produce bisexual sterility.	Transgenic crop needs to be propagated vegetatively.	Wang et al. (2012)
	Tobacco	Expression of barnase using the AtDMC1 promoter and barstar using the ACT2 promoter can efficiently produce bisexual sterile lines.		Kobayashi et al. (2006)
	<i>Panicum virgatum</i>	Overexpress of maize Corngrass1 (Cg1) gene causes absence of flowering along with higher biomass yield.		Chuck et al. (2011)
	<i>Arabidopsis thaliana</i> and <i>Nicotiana tabacum</i>	Complete male sterility and partial female sterility, SDS and BARNASE genes.		Huang et al. (2016)
No flowering	GM poplars ^a	Suppression of flowering genes can act as transgene mitigator.	Transgenic crop needs to be propagated vegetatively.	Klocko et al. (2018)
Cleistogamy	<i>Brassica napus</i> ^a	Clg mutant	Only few genes available for limited number of crops.	Fargue et al. (2006) and Faisal et al. (2018)
	<i>Oryza sativa</i>	cl7(t)		Ni et al. (2014)
Apomixis	<i>Paspalum notatum</i> fluggé	Low PGF due to apomixis and difference in ploidy level.	Only few genes available for limited number of crops.	Sandhu et al. (2010)

(continued)

Table 15.3 (continued)

Strategy	Crop	Evaluation	Drawbacks	References
GM gene deleter/transgene excision	<i>N. tabacum</i>	Site-specific recombination system creates non-transgenic pollens and seed.	Might interfere with normal propagation.	Luo (2007)
Maternal inheritance	<i>N. tabacum</i>	Cytoplasmic male sterility (CMS92) was incorporated.	Practically possible only for plants with strict maternal inheritance;	Svab and Maliga (2007)
	<i>N. tabacum</i>	Glyphosphate resistance is obtained by genetically modifying the chloroplast genome.	transgenic plant can backcross with male parent plant, leaky paternal chloroplast leakage.	Daniell et al. (1998)

Transgene mitigation

Indehiscent fruits ^a	<i>A. thaliana</i>	<i>SHATTERPROOF</i> genes are required for fruit dehiscence in <i>Arabidopsis</i> .	Might cause extinction of wild relatives.	Liljegren (2000)
	Brassicaceae	<i>INDEHISCENT</i> controls fruit dehiscence in <i>Brassica</i> fruits.		Girin et al. (2010)
	Brassicaceae	<i>FRUITFULL</i> gene produces pod shatter-resistant <i>Brassica</i> fruit.		Ostergaard et al. (2006)
Secondary dormancy ^a	<i>A. thaliana</i>	Several potential regulators have been identified for seed dormancy.		Finkelstein et al. (2008)
No flowering	GM poplars ^a	Suppression of flowering genes can act as transgene mitigator.		Klocko et al. (2018)
Dwarfing genes	<i>B. napus</i>	Δgai dwarfing gene.		Al-Ahmad et al. (2006)

^aStrategies which have not yet been tested for preventing gene flow but hold potential for future applications

chances of gene flow (Brookes et al. 2004, and Ortega Molina 2004 – cited in Devos et al. 2005).

Flowering similarity index compares synchrony in extent of flowering between species that are likely to cross-hybridize to predict efficiency of temporal isolation.

Flowering patterns of different species are transformed into a probability-density distribution (i.e. distribution of flowering density) which is then evaluated as a function of temporal distance, using a proxy of pollen density. This has been proposed to be a better indicator of probability of hybridization between crop relatives (Ohigashi et al. 2014). This index should therefore be used to predict the likelihood of gene flow than using simple measurements of overlap of flowering days. Ohigashi et al. (2014) have shown temporal barriers of flowering can be effective in preventing undesirable hybridization of transgenics and wild relatives of soybean. However, this strategy will require proper assessment and collection of quantitative data on floral characteristics including pollen density, pollen shedding of not only the source species, but all compatible weeds and wild varieties growing in the vicinity.

Since none of these physical measures are able to completely abolish the chance of gene flow, biological barriers and gene mitigation can play a better role in preventing transgene flow.

15.5.2 Biological Barriers

Biological barriers utilize biology of the crop for confining the transgene to only the desirable crop, thus preventing gene flow. Pollen grains are the major biological route of transfer of genetic material from one plant to the other. Genetic engineering tools can be used to modify the biological character such as flower development, male and female fertility, cleistogamy and apomixis, to prevent escape of transgenes. A range of molecular tools to prevent transgene flow using biological barriers are grouped as Genetic Use Restriction Technologies (GURTs) (Hills et al. 2007; Lombardo 2014). GURTs have been further divided into trait-GURT or trait-specific GURT (T-GURT) and varietal-GURT (V-GURT); the former (T-GURT) limit the transgene expression to GM plant (Mascia and Flavell 2004; Daniell 2002), whereas V-GURT limit transgene flow by interfering with plant development and reproduction such as pollen or seed viability, inheritance and transmission (Lombardo 2014; Hills et al. 2007). V-GURT have therefore also been equated with suicidal or terminator technologies (Lombardo 2014). The following section deals with various mitigation strategies that are considered as type of GURT:

- *Male sterility* can play direct role in preventing PGF. This can be done by hindering pollen development, maturation or dispersal. Several genes and other regulatory elements have been identified in the pathway of pollen development and maturation (Gómez et al. 2015) which can be targeted to prevent PGF. One of the earliest works in achieving male sterility was mutation in tapetum-specific gene (TA29) of transgenic tobacco and oilseed rape plants (Mariani et al. 1990). To date, several other genes have been identified and used for creating male sterile lines such as RAD51Ci lines with non-viable pollen, and SWIi lines displayed a complete absence of pollen in *Arabidopsis thaliana* (Wang et al.

2012). A novel transgene excision strategy in which a site-specific recombination system of LoxP-FRT recombinase (derived from fusion of phage CRE/LoxP and yeast FLP/FRT; termed as “GM-gene-deleter” system) driven by pollen-specific promoters (*BGP1*, *LAT52*) and seed-specific promoter (*PAB5*) to control the expression of *FLP* gene has been used to delete functional transgene, leading to production of non-transgenic pollens and seeds from transgenic plants and therefore can be another molecular tool for gene containment through both pollen and seeds (Luo et al. 2007). Since only fitness of the male gamete is affected by this strategy, there are chances of the transgenic flower getting pollinated by weed or wild-type plants, which will eventually create an undesirable hybrid which still has the transgene. Also, chances of male sterile lines being perfect (100% sterile) are nearly impossible, so chances of transgene escape still remain.

- *Bisexual/total sterility* that prevents plants from setting seeds through both self- and cross-pollination can be useful in overcoming the chances of contamination of non-GM species with the transgenes, as well as of GM plants being pollinated by non-transgenics. Bisexual or total sterility has been achieved by differential expression of BARNASE and BARSTAR genes in *Nicotiana* (Kobayashi et al. 2006). BARNASE (encoding RNase) was expressed using gamete-specific promoter (AtDMC1), whereas BARSTAR (BARNASE inhibitor) was expressed under ACT2 promoter (regulates gene expression in all tissues except gametes). Transgenic plants with both Barnase and Barstar were found to be totally sterile and no seed set was obtained after both selfing (due to aberrant male and female fertility) and cross-pollination (due to aberrant female fertility) (Kobayashi et al. 2006). Recently Huang et al. (2016) created bisexual sterile line in *A. thaliana* and *N. tabacum* by targeting *SOLO DANCERS (SDS)* gene, which are meiotic-specific cyclins. The researchers created fusion of *SDS* with *BARNASE* and achieved completely male and female sterile plants in both *A. thaliana* and *N. tabacum* demonstrating its applicability in widely different systems (Huang et al. 2016). However, such steps of inducing complete sterility in any transgenic system are drastic as these will need to be vegetatively propagated.
- *Cleistogamy* is another trait that has the potential to minimize gene flow by completely preventing outcrossing, while maintaining the fertility of the transgenic plant. A mutant *Brassica napus* displaying cleistogamous flowers ('C' gene mutant) was shown to have more than 94% autogamy and tenfold less pollen release than an open flower *Brassica napus* under similar field conditions (Fargue et al. 2006). Another *Brassica napus* cleistogamous mutant Zhong9-Clg (mutation in *Clg* locus in Zhong9 variety; *CLG* locus encodes RINGv E3 ubiquitin ligase) has been developed and has the potential to minimize or prevent gene flow (Faisal et al. 2018; Qu et al. 2014). In rice, EMS mutagenesis screen allowed identification of mutants with cleistogamy, the locus was identified as a novel allele of *DEP2* (involved in panicle morphology) and termed as *cl7(t)*; genetic improvement of *cl7(t)* mutant allowed recovery of lines with improved agro-nomic traits with cleistogamous flowers and such lines hold promise of developing transgenic lines with reduced dangers of transgene escape (Ni et al. 2014). Similarly, in another mutant of *Oryza sativa*, *superwoman1-cleistogamy1 (spw1-clg1)*

cls1) line that displays cleistogamy, outcrossing with related varieties of *O. sativa* was reduced significantly (Lombardo et al. 2017).

Cleistogamy has been studied in several crops such as pigeon pea, wheat, rice, barley, sorghum and was found to have different genetic mechanism in each crop (Ni et al. 2014; Saxena et al. 1992; Chhabra and Sethi 1991; Maeng et al. 2006; Merwine et al. 1981; Turuspekov et al. 2004; Nair et al. 2010); indeed studies in *O. sativa* show that cleistogamy can also be induced by genetic manipulation of several genes (Faisal et al. 2018; Lombardo et al. 2017; Maeng et al. 2006). Existence of such divergent genetic mechanisms does not permit devising a common strategy to induce cleistogamy as a universal approach to prevent transgene escape. This approach is also impractical for crops like maize, cassava and cucurbits in which flowers are unisexual.

- *Apomixis* occurs naturally in many plant species, when embryo, seed and endosperm develop without fertilization. Along with absence of fertilization (neither self- nor cross-breeding), obligatory apomorphic plant must also be male sterile to completely prevent gene flow. Pseudogamous (fertilization of central cell by one of the male gametes to produce endosperm while the other male gamete degenerates without fertilizing the egg) apomorphic lines of transgenic forage grass *Paspalum notatum* Flüggé have been shown to lower PGF when apomixis along with ploidy barrier was used (Sandhu et al. 2010). PGF from glufosinate-resistant transgenic apomorphic *Paspalum notatum* Flüggé to non-transgenic, non-apomorphic (sexual) *Paspalum notatum* Flüggé even at a close distance of only 0.5–3.0 m was only 0.03% (Sandhu et al. 2010). The low frequency or rate of gene transfer was also on account of the tetraploid nature of the apomorphic and diploid nature of the sexual plants with the resultant hybrids being triploids or aneuploids with severely reduced vigour and survival rate. Extremely rare presence of obligate apomixis, moderate male sterility and chances of apomorphic GM being invasive in nature are some of the possible hurdles for commercial usage of this technique for transgene containment.
- *Maternal inheritance*: The phenomena of maternal inheritance of organelle and organellar genome offer another strategy for preventing pollen-mediated transgene escape or gene flow. Boynton et al. (1988) and Svab et al. (1990) pioneered the technique of chloroplast transformation in *Chlamydomonas* and *Nicotiana*, respectively. Since then, it has been extended to several crop plant species (tobacco, soybean, potato, tomato, cotton) for manipulating and modifying various traits (Bock 2015).

Daniell et al. (1998) used chloroplast transformation to obtain transplastomic plants which exhibited complete maternal inheritance pattern for the herbicide resistance gene *EPSPS*, thus demonstrating the utility of plastid transformation as a containment strategy of transgene escape (Daniell et al. 1998). Similarly, transplastomics has been achieved in sugar beet (De Marchis et al. 2009), *Brassica oleracea* (Liu et al. 2008), tobacco (Svab and Maliga 1993), potato (Valkov et al. 2011), tomato (Ruf et al. 2001) and soybean (Dufourmantel et al. 2007), to name a few crops with an objective to prevent PGF.

It is assumed that chloroplast shows maternal inheritance in most of the angiosperms, but paternal inheritance of chloroplasts has been observed in some species such as dicotyledonous kiwifruit (Chat et al. 1999), *Petunia hybrida* (Horn et al. 2017), and most of the gymnosperms shows paternal inheritance of plastids. Even if the species is known to be maternally transmitting chloroplast, some level of paternal chloroplast leakage has been observed between *Brassica* compatible varieties (Haider et al. 2009) and *Setaria italica* (Wang et al. 2004; Shi et al. 2008). As there are minimal chances of chloroplast being transferred to the progeny through pollen, transgenes can be targeted in chloroplast genome, thus preventing PGF. However, owing to the instances of chloroplast leakage, transplastomics strategy also cannot completely prevent transgene outflow.

15.5.3 Transgene Mitigation (TM)

None of the containment methods discussed above based on spatial, temporal separation or biological incompatibility are foolproof, and have probabilities of leaky gene flow, that is, there are chances of transgenics to hybridize with their closely related wild or weedy species. Transgene mitigation as an approach is based on the strategy of having primary transgene being closely flanked by some mitigating gene (Gressel and Valverde 2009). These flanking mitigating genes are either neutral or beneficial to the crop, and are either lethal or confer selective disadvantage for the recipient weeds. Thus, introgression of transgene into the weed genome renders competitive disadvantage to the sink or recipient species. Mitigation gene can be decided based on the difference in growth parameters or chemical susceptibility of weed and crop. Processes such as seed dormancy, seed ripening, fruit shattering, plant growth and flowering pattern can influence the fitness of the weeds. Several genes involved in these pathways have been deciphered, which can act as candidates for transgene mitigation for reducing transgene flow (Table 15.3).

- Weedy plants disperse their seeds over a period of time. Thus, genes that prevent fruit shattering and hinder seed dispersal can be considered harmful for weeds. Genes such as *SHATTERPROOF* (Liljegren et al. 2000), *INDEHISCENT* (Girin et al. 2010), *FRUITFULL* (Ostergaard et al. 2006) have been studied in *Arabidopsis* and *Brassica* which render the siliques indehiscent. These genes can prove to be advantageous for transgenic crops by preventing loss due to fruit shattering, and disadvantageous for weeds by eliminating hybrid weeds through preventing seed dispersal, and can thus be considered as candidate genes in transgene mitigation strategy.
- Secondary dormancy is an adaptation of weeds during the course of evolution which allows them to survive for long periods in soil seed bank. Several genes related to this pathway have been described (Finkelstein et al. 2008; Zhang et al. 2017). Careful selection of some of these candidate genes that can be used to remove seed dormancy in weeds, trigger germination even under suboptimal or

non-permissive conditions and thus affect survival of hybrid seeds obtained from gene flow between transgenic crop and weed species can be also considered as another transgene mitigation strategy (Pipatpongpinyo et al. 2019; Kena 2017; Baek and Chung 2012).

- It is known that seedlings of weeds exhibit competitive self-thinning, where thousands of progeny compete for survival and only few make up to the full grown weeds (Park et al. 2003; Freckleton and Watkinson 1998; Palmblad 1968). Self-thinning is largely a function of plant geometry and therefore genes that drastically affect plant architecture and growth such as dwarfing genes can act as transgene mitigators (Lonsdale and Watkinson 1983). These genes can shorten the height of hybrid weed, thus making them prone to elimination by their non-hybrid cohort. This has been carried out and established in green houses as well as fields for tobacco and oilseed rape using gibberelin-insensitive gene (*gai*) (Al-Ahmad et al. 2005, 2006).
- Other traits and genes that are potential candidates for transgene mitigation include suppression of bolting and flowering that can affect the fitness of weeds. Several genes such as *FLC* (Hong et al. 2011), *VRN1* (Fu et al. 2005) and *Anti-GIGANTEA* (Curtis et al. 2002) are known to alter flowering-related traits and have the potential to suppress bolting and flowering of weed species.
- Herbicide resistance is a naturally occurring phenomenon in crops. Genes that induce the suppression of naturally occurring herbicide resistance can also be candidates for gene mitigation. In this strategy, a transgenic crop is created which contains gene of interest as well as a mitigator gene, which suppress herbicide resistance. In the following generation, a crop which is naturally resistant to mitigator herbicide will be planted and application of mitigator herbicide will eliminate the undesired hybrid (weed x transgenic) as well as volunteer transgenic plants which were created in previous generation. Lin et al. (2008) used RNAi cassette (to suppress bentazon detoxification enzyme CYP81A6) as mitigating partner of transgene, rendering bentazon susceptibility to the transgenic plant and undesired hybrid (weed x transgenic). Rotation of several types of transgenics, which have different types of mitigators (genes for herbicide-sensitivity), has also been advised (Gressel 1999; Gressel and Valverde 2009).

15.6 Methods to Study Gene Flow

Gene flow between crops, and crop wild relatives produces hybrids that share morphological and physiological traits, and molecular, genetic and isozyme pattern with parents. Analysis of such shared traits have been used to detect instances of gene and transgene flow (Fig. 15.2c), study the viability and fecundity of hybrids, with genetic and molecular analysis providing the most unambiguous evidence. Hybrids contain alleles from both parents which can be detected by using isozymes, allozymes, RAPD, RFLP, AFLP markers and progeny segregation of morphological and physiological traits (Ellstrand et al. 1999). Majority of the studies rely on the

detection of the transgene, transgene product, or the trait conferred by the transgene as a confirmation of gene flow.

Morphological features include kernel pericarp colour and shape in maize (Nieh et al. 2014); leaf colour in maize (Luna et al. 2001); paired kernel rows, female spikelet, exposed kernels, non-shattering rachis, and polystichous ear in *Tripsacum dactyloides* X *Zea diploperennis* hybrid (Eubanks 1995, 1997); spikelet and panicle characteristics in *Sorghum bicolor* and *halepense* hybrid (Arriola and Ellstrand 1996); fitness of hybrid seedlings of crop and wild sunflower, *H. annuus* (Snow et al. 1998); a combination of leaf phenotype (petiole and blade shape, abaxial leaf colour, margin dentation) with RAPD and microsatellite markers in transgenic poplars (Di-Fazio et al. 2012).

Allozymes/isozymes that have been used to detect gene flow include Enp 1-8, Glu1-7, Pgd1-3.8, Pgm2-7.2 in hybrids between cultivated maize and *Zea diploperennis*, *Z. perennis* or *Zea luxurians* (Doebley 1990); 6Pgd-3-a in hybrids between cultivated and wild sunflower (Arias and Rieseberg 1994); adenylate kinase locus, ADK-1 in hybrids between cultivated crop, *Sorghum bicolor* and CWR, *S. halepense* (Johnson grass; Arriola and Ellstrand 1996). In common beans, allozyme analysis has been used to estimate genetic diversity, and hybridization events, and gene flow (Santalla et al. 2002).

Among genetic loci, chloroplast DNA-based markers in hybrids between maize and CWR (Doebley 1990); microsatellite loci and flow cytometry in sugar beet x sea beet hybrids (Andersen et al. 2005); introgression of RAPD markers in hybrids between *Helianthus petiolaris* and *H. annuus* (Rieseberg et al. 1999b), and cultivated sunflower populations (Linder et al. 1998); SSR markers in rice (Song et al. 2006); RAPD markers along with allozyme in *Medicago sativa* (Jenczewski et al. 1999a, b); detection of species/cultivar-specific AFLP markers to detect gene flow in hybrids between *Helianthus petiolaris* and *H. annuus* (Rieseberg et al. 1999a); AFLP markers specific to crop and weed Brassicas (*B. napus*, and *B. rapa*, respectively; Rose et al. 2009); resistance to the herbicide chlorsulfuron, conferred by dominant mutation in AcetoLactate Synthase (ALS) in *B. napus* x *B. juncea* hybrids (Liu et al. 2010).

Although cytology can be used to detect hybrids of species with variable ploidy levels such as *Brassica napus* ($2n = 4x = 38$), a tetraploid and *Brassica rapa* (syn. *Campestris*; $2n = 2x = 20$) (Jorgensen and Anderson 1994), it is a tedious and cumbersome strategy to estimate and detect gene (transgene) flow.

The detection of transgene to confirm gene transfer has emerged as the most reliable method and several researchers have relied on detection of transgene to confirm gene transfer such as detection of nptII and GUS in potato (Skogsmyr 1994); transplastomic UidA (encoding GUS enzyme) in *Petunia hybrida* (Horn et al. 2017); chitinase and glucanase transgene in wheat (Rieben et al. 2011); EPSPS (5-enol-pyruvylshikimate-3-phosphate synthase) transgene in creeping bentgrass (*Agrostis stolonifera* L.; Watrud et al. 2004); GFP and Bt in *Brassica* (Halfhill et al. 2001).

A recent development has been the use of genotype information based on whole genome sequence obtained through genotyping-by-sequencing (GBS) to detect gene flow as in sunflower population in Argentina (Mondon et al. 2018).

HGT from GM plants to sink species is based on detection of transgene, transgene product, or trait (Nielsen et al. 1998) such as *nptII* as marker for HGT from GM-maize to soil bacteria (Ma et al. 2011); antibiotic resistance and GFP fluorescence from transplastomic tobacco to bacteria (Pontiroli et al. 2009); PCR-based detection of Cry1A(b), Zein and Sh-2 gene derived from GM-corn in samples of gut contents of crop, gizzard, jejunum, cecum and blood from broiler chicken (Rossi et al. 2005), and piglets (Mazza et al. 2005; Walsh et al. 2012).

15.7 Next-Generation Genome-Edited Plants: Are These GM Crops with Similar Risks?

Traditional tool of genetic modification of crop includes “copying and pasting” a gene or genetic segment of interest from a heterologous source such as animals/bacteria/plant into crop plants, terming them as GMOs. In order to avoid deleterious and harmful effects of transgenes and cisgenes, newer tools are now being pursued that introduce new plant traits by making few changes in the plants genome itself through genome/gene editing (GE). Thus, next-generation transgenic plants developed based on GE tools are considered non-GMO because the bacterial gene-editing machinery used in this process does not become part of the plant genome and can be segregated based on Mendelian segregation pattern (Metje-Sprink et al. 2018; Chen et al. 2018; He et al. 2018; Zhang et al. 2018; Luo et al. 2015). Among the various genome-editing techniques (ZFNs, TALENs and CRISPR/Cas), CRISPR/Cas has emerged as the most prevalent technique of genome editing due to its high efficiency and accuracy. Although genome-editing techniques overcome some of the challenges faced by traditional GMOs such as incorporation of transgene and cisgene, it does not overcome the trouble of gene flow. Since, the native edited gene will follow Mendelian principle, it has equal chances of introgressing in other compatible genomes through cross-breeding, similar to the foreign transgene. For example, CRISPR-Cas9 based genome editing tool can be effectively used to create biological containments of transgene by targeting genes that are involved in plant reproduction, and to induce sterility. This has been achieved by targeting poplar orthologs of LEAFY and AGAMOUS and creating genetically edited sterile poplar (Elorriaga et al. 2015). CRISPR/Cas9 has been used to create knockout mutants of OsACOS12 (Zou et al. 2017), OsNP1 (Chang et al. 2016) in rice and Ms1 (Okada et al. 2019) and Ms45 gene in wheat (Singh et al. 2018) rendering the plants’ male sterile. Genome-editing techniques cannot directly control the gene flow, but can efficiently assist other strategies such as male and bisexual sterility, cleistogamy, apomixes as well as gene mitigation and play a significant role in minimizing gene flow. Owing to the lack of foreign DNA being introduced during genome/gene editing, such crops have been kept out of the purview of strict regulation as are GM crops in several countries. For example, the USDA-APHIS has decided not to regulate GE crops as it does for GM crops (Van Eenennaam et al. 2019; <https://www.the-scientist.com/the-nutshell/usda-will-not-regulate-crispr-edited-crops-29852>); in contrast, the European Union has decided to apply stringent regulatory norms as

applicable to GM crops also on GE crops (Callaway 2018; Eckerstorfer et al. 2019a, b; Ledford 2019). As the field of GE is still evolving, a robust regulatory framework to address biosafety and environmental concerns are still being worked out for widespread acceptance of GE crops in agriculture (Eriksson et al. 2018; Eckerstorfer et al. 2019a, b; Friedrichs et al. 2019).

15.8 Conclusions

Gene flow is a natural phenomenon and is the key to evolutionary process for speciation and species' existence; natural gene flow occurs through vertical gene transfer and is usually subjected to strict taxonomic and species boundaries. Gene flow is a multistep process and success at each step is essential for generation of hybrids, establishment and spread. In nature, gene flow consequences include genetic swamping, selective sweep, and genetic assimilation. The major agent of gene flow is mediated via pollen grains, whereas seed and vegetative propagules play smaller roles. The development of transgenic crops that is akin to horizontal gene transfer has raised several concerns about biosafety and environmental issues such as loss of biodiversity, loss of genetic purity and contamination of species, and species extinction. Transgenes behave as dominant genes, and depending on the traits they modify, may confer survival advantage to the progenies, raising concerns about evolution of aggressive weeds, and extinction of wild relatives; additionally, biosafety concerns have also been raised for transfer of genes across species boundaries through horizontal gene transfer into bacteria, viruses and other organisms, and detrimental effects on pollinator health. To address unforeseen risks, several strategies based on spatio- and temporal barriers, and GURTs have been devised towards minimizing pollen-mediated gene flow, and transgene mitigation. In spite of a mountain of available literature, transgene escape has not yet been proven to cause genetic swamping/genetic pollution, selective sweep and genetic assimilation in CWR. It is too early to answer the questions. Whether such concerns are valid we don't know yet. The key to minimizing or preventing admixture of genetic material has been, and will remain an exhaustive characterization of germplasm including that of crop relatives, pollinators, and other abiotic factors. Next-generation, genome-edited crops, hopefully, will go a long way towards allaying fears of biosafety and environment while addressing the needs of food, feed and nutritional security.

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'Global Pollinator Crisis' and Its Impact on Crop Productivity and Sustenance of Plant Diversity

16

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Abstract

Pollination is an essential requirement for fruit and seed set. It is, therefore, crucial for crop productivity and sustenance of flowering plant diversity in their natural habitats. Nearly 90% of flowering plants use a range of animals to achieve pollination. Human-induced environmental changes in recent decades have markedly reduced the diversity, density and distribution of pollinators around the world, resulting in *global pollinator crisis*. The crisis is also threatening the survival of managed pollinators that are being used routinely for decades for pollination services of a large number of crop species grown in monoculture cropping system. Thus, pollination constraints have raised serious concern on the sustenance of crop productivity and plant diversity in the coming decades. Concerted efforts are being made around the world to study pollinator and pollination both in natural and agricultural habitats to mitigate the crisis. Recent approaches have been to use integrated pollination services using the wild as well as managed pollinators for crop species and to make the agricultural and natural habitats favourable for the sustenance of pollinators. Unfortunately, biologists in the tropics in general and India in particular have remained indifferent about pollinators and pollination services of wild as well as pollinator-dependent crop species. Serious efforts are needed to initiate extensive studies on the pollination ecology of our crops and wild species and make all possible efforts to identify and alleviate the pollinator crisis.

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Keywords

Agricultural habitats · Climate change · Crop productivity · Environmental degradation · Flowering plant diversity · Managed pollinators · Natural habitats · Pollination constraint · Pollinator crisis · Pesticide effects

16.1 Introduction

Humanity has to face two major challenges in the coming decades:

- Substantial increase in crop productivity to feed the growing population
- Conservation of biodiversity

Earlier chapters have highlighted the importance of pollination in fruit and seed production. Fruits and seeds are the economic products of a majority of the crop species; therefore, sustenance of pollination services is the basic requirement to ensure and improve crop productivity. In the absence of adequate pollination, cultivation of the best available variety and application of the best agronomic practices would not improve the yield. Maintenance of biodiversity, in general, depends on the regular natural recruitment of plants to sustain populations. In a healthy population, the mortality of individuals is equal to or less than the number of births. When recruitment becomes a constraint, the number of deaths exceeds that of births. This gradually reduces the number of individuals in the population leading to its endangerment and eventually to local extinction. In flowering plants, adequate pollination is a prerequisite for the production of sufficient number of fruits and seeds needed for an effective recruitment. If pollination is inadequate, the number of seeds formed would be reduced which would lead to the recruitment constraint. Thus, pollination is one of the important eco-services, essential not only to meet the challenge of increased crop productivity but also for the conservation of flowering plant diversity.

Plants are sedentary and cannot achieve pollination on their own. They have effectively outsourced this function to other agencies. Nearly 90% of all wild species of flowering plants and 75% of crop species use a range of animals to achieve pollination, and the remaining use abiotic agents – wind or water – for this purpose (Klein et al. 2007; Ollerton et al. 2011). Cereals, which meet the bulk of our calorific needs, do not depend on biotic pollinators as they are self- and/or wind pollinated. However, cereals do not meet our requirements of proteins, vitamins particularly of vitamin A and micronutrients. Most of the other crops such as legumes and oil, fruit and vegetable crops depend on biotic pollinators partially or fully. They are the major sources of proteins, vitamin A and micronutrients (Eilers et al. 2011; Chaplin-Kramer et al. 2014). It has also been found that the loss of biotic pollinators especially insects has implications on the diversity of vegetables, fruits and oilseeds. The yield, productivity and quality of these important crops are decreasing across the

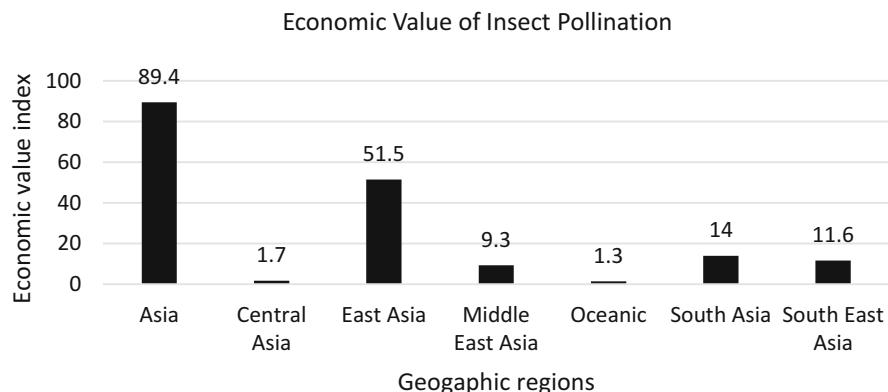


Fig. 16.1 Economic value index of pollination service in various regions of Asia. (Source: Gallai et al. 2009)

world, especially Southeast Asian regions. The spice-yielding plants, medicinal plants producing alkaloids and essential oils are particularly vulnerable.

Among the insects, bees are the most proficient and skilled pollinators of wild as well as the crop plants and are known to contribute 73% of the global pollination service. The remaining plants are pollinated by flies, bats, wasps, beetles, birds and butterflies. It is estimated that biotic pollinators together perform pollinator service worth US \$215 billion a year (Gallai et al. 2009, Fig. 16.1). According to Regan et al. (2015), birds carry out pollination when there is limited insect density and activity. Prominent birds that carry out pollination services include hummingbirds, honeyeaters and sunbirds. It is shown that birds have taken over pollination service in areas where temperature is low and rainfall is high. Plants growing in isolated islands with poor colonization by insects also show ornithophily (Cronk and Ojeda 2008).

Historically, biotic pollination used to be adequate, as the pollinators were abundant on and around agricultural and natural habitats. Pollination was taken for granted in the absence of any constraints. However, following the onset of monoculture practice of cropping since the 1940s in the Western countries, in which cultivation of the same crop is extended to hundreds of hectares without any patches of natural/semi-natural vegetation to sustain pollinators, insufficient pollination became a major constraint in several crop species. This problem was overcome by the use of managed pollinators, such as honeybees and bumblebees (James and Pitts-Singer 2008). This industry has developed over the years into a well-organized multibillion dollar industry (Shivanna 2014). The farmer signs the contract with the bee keeper well before the flowering period of the crop in which all relevant details such as the number of colonies needed, the period for which the colonies need to be maintained in the field and the rental value the farmer has to pay to the bee keeper for the pollination services are specified. The bee keeper brings the required number of colonies at the right time and keeps them in the farmer's field for the required period.

The bees will take care of the pollination services. When the contract period is over, the bee keeper takes the colonies to other crop fields where the pollination services are needed. Thus, the bee keeper uses bee colonies as ‘mobile pollination units’. The main income of the bee keepers comes from renting their bee colonies for pollination services rather than by selling the honey and wax. This scenario continued and expanded over the years without much problem until recently. Pollination was not a constraint even in wild species; abundant seeds used to be produced for adequate recruitment of new individuals in the populations. Thus, sustenance of species in their natural habitat was not a concern.

The situation has changed dramatically during the last few decades as a result of human-induced environmental changes (Thakur and Bhatnagar 2013). These changes are responsible for reducing the density, diversity and distribution of not only natural pollinators around the world, but they have also threatened the survival of managed pollinators. These developments raised serious concern about the ‘global pollinator crisis’ and its impact on pollination services of the crops and wild species (Holden 2006; Murray et al. 2009). Many of the pollinators may become extinct in tropics before their role in pollination is documented (Koul et al. 2018).

In the light of mounting evidences on the decline in the diversity and density of pollinators in recent decades, many international initiatives were undertaken to study and remedy pollination services. IPBES (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services) was set up in 2012 (in collaboration with several UN agencies) to assess available information on pollination services. Its report, released in 2016 (IPBES 2016), confirmed the decline of over 16.5% of vertebrate and 40% of invertebrate pollinators, including bees and butterflies across the world. The report also highlighted the need for sustenance and enhancement of pollination services for food and nutritional security. Another important point emphasized in the report was that in many developing countries the data on pollinators is non-existent, and there is an urgent need for collecting such data to sustain pollination services. An attempt is made in this chapter to briefly discuss the drivers of human-induced environmental changes and their impact on pollinators and pollination services of pollinator-dependent crops in agricultural habitats and of wild species in natural habitats. It also highlights India’s scenario and points out the lack of even the baseline data needed to lessen the pollinator crisis in crop and wild species.

16.2 Human-Induced Environmental Changes

The main drivers of human-induced environmental changes are habitat loss, its degradation and climatic changes. Together, all three aggravate the impact on pollination services. Conversion of natural habitats for agriculture, urbanization, industrialization and mining and to raise plantation crops has been the major culprit of habitat loss and its fragmentation. Mounting levels of pollution, extensive use of pesticides and herbicides and spread of invasive alien species have been identified as the major causes of habitat degradation (Tilman et al. 2001; DeFries et al. 2004; see

Murray et al. 2009; Ghazoul and Sheil 2010). In many parts of the world, land degradation is worsening over the years and has reached a critical level particularly on the oceanic islands (Sax and Gaines 2008; IPBES 2018). Many native plants and invasive species show overlapping phenological calendars. The invaders have relatively stronger cues for pollinator attraction. They affect native plant-pollinator interactions and impede their reproductive success (Traveset and Richardson 2006; Bartomeus et al. 2008). For example, the invasive *Lythrum salicaria* is shown to alter the interaction between the dominant pollinator, the bumblebee and the native plant *Decodon verticillatus* (King and Sargent 2012). According to Ghazoul (2004), invasive species have an ecological consequence on the single plant-pollinator interaction as well as on the plant-pollinator networks. Invasive alien animal species acting as competitors or predators could set pollinators at risk.

16.3 Impact of Environmental Changes on Wild Species

16.3.1 Environmental Changes

Since the 1990s, there has been a surge in reports indicating the decline of pollinators, both in the natural and agricultural habitats, and its impact on pollination services (Buchmann and Nabhan 1996; Kearns et al. 1998; Kremen and Ricketts 2000; Klein et al. 2007; Wilcock and Neiland 2002; Samejima et al. 2004; Hegland et al. 2009; Murray et al. 2009; Potts et al. 2010; Obute 2010; Aslan et al. 2013, Tylianakis 2013, Dicks et al. 2016, IPBES 2016). Biesmeijer et al. (2006) analysed bee and hoverfly assemblages in Britain and the Netherlands based on about one million records from the National Entomological databases. They found significant decline in local bee diversity in both the countries since 1980; the trends were divergent for hoverflies. The decline in bees was more prevalent in specialist floral visitors (visits confined to one or a few plant species), particularly of non-migrant species with reproduction confined to one generation per annum (univoltine) when compared to generalist, migrant and multivoltine species. Interestingly, they reported a parallel decline in the distribution of outcrossing plant species pollinated by declining pollinators. On the other hand, abiotically pollinated (by wind or water) species showed an increase in their distribution, and autogamous species showed an intermediate response. Thus, the studies correlated the decline of plant species to their biotic pollinators. Similarly, Cameron et al. (2011) reported a decline in the relative abundance of four important bumblebee species across the USA; the decline was up to 96% in abundance, and their distributional range was reduced by 23–87%. The assessment of over 120 years of historical data from a temperate forest, Illinois, has demonstrated that the sharp decline of wild pollinators has significantly disrupted the quantity and quality of pollination services in the region (Burkle et al. 2013). Inbreeding depression, loss of variability, reduced genetic vigour and elimination of poor offspring in several plant species have also been reported to be the consequence of limited pollination. The biomass of flying insects has declined by 75% in protected areas of Germany (Hallmann et al. 2017) and by 97% in protected

Puerto Rico rainforest when compared to the last 3–4 decades (Lister and Garcia 2018). It is estimated that nearly 40% of the global insect species are threatened with the possibility of extinction over the next few decades (Sanchez-Bayo and Wyckhuys 2019). Lepidoptera, Hymenoptera and Coleoptera, which comprise a majority of insect pollinators, are believed to be the most affected groups of terrestrial insects.

Recently, Powney et al. (2019) carried out a more intensive analyses based on 715,392 biological records of 353 wild bee and hoverfly species of the United Kingdom, between 1980 and 2013. Their models estimated the proportion of occupied 1 km² grids. They estimated a net loss of over 2.7 million occupied 1 km² grid cells across all pollinator species. There was a widespread variation in the trends between species. The losses were concentrated among rare species, and a 55% decline was associated with species of upland habitats. These studies highlight the possible deterioration of pollination services of wild species. There are also many reports on the effects of habitat fragmentation on pollinator activity, as fruit set in the smaller fragments of forests tends to be lower than the larger ones (Aguilar et al. 2006, da Silva Melo et al. 2014).

A recent meta-analysis has shown that the exclusion of vertebrate pollinators can lead to nearly 60% loss of fruit or seed set among flowering plants (Ratto et al. 2018). The impact is greater in plants which have obligate dependence on a particular pollinator; for example, in a bat-pollinated species, fruit set is reduced by ~80% when bats are excluded. Urbanization and rapid industrialization have led to shifting of bat pollinators from their habitats. This has led to reduced number of bats in semi-urban and peri-urban areas, thus leading to a decline in the pollination service of bat-pollinated plant species. Many birds are on RED data list and land-use change is the major driver of bird population decline. Forest degradation and fragmentation affect the behaviour of avians and thus have potential implications for bird-pollinated species (Hadley and Betts 2009). Pollen limitation owing to lower pollinator visitation rates has been shown to result in reduced fruit set in the bird-pollinated mangrove *Bruguiera gymnorhiza* (Wee et al. 2014).

So far, the consequence of environmental changes has been largely investigated on diurnal pollinators. The impact of these factors on nocturnal pollinators has not been given much importance. The activity of nocturnal pollinators is shown to get affected with artificial illumination during night around the world (Callum et al. 2015). For example, in *Cirsium oleraceum*, visits of nocturnal pollinators to flowers were shown to reduce by 62% when compared to dark areas (Knop et al. 2017); it also reduced fruit set by 13% in illuminated areas even when diurnal pollinators made numerous visits to the flowers.

Crop species are pollinated not only by honeybees but also by a large number of wild pollinators, including both social and solitary bees. The residues of the pesticides applied to crop species get incorporated in the pollen and nectar rewards of both wild and managed pollinators. Many pesticides kill them at higher concentrations. At low levels, pesticides may not kill the bees but markedly affect their ability to learn, forage and reproduce. Neonicotinoid pesticides have been shown to negatively affect the density of wild bees, nesting of solitary bees and

colony growth and reproduction of bumblebees (Brittain et al. 2010; Rundolf et al. 2015; Baron et al. 2017a, b) and thus impose a substantial risk even to wild pollinators. A novel class of neonicotinoids that was seen as less potent pesticides having novel properties and low residence time needs to be re-examined from the pollinator's perspective (Bhandhari and Koul 2018).

The herbicide Roundup is the world's most widely used and effective herbicide. Until recently, it was considered as the safest herbicide to animals as it targets an enzyme found only in plants and microbes. Recent studies, however, have shown that glyphosate, the major component of the Roundup, impairs the navigational and learning abilities of bees. It has also been shown to disrupt the microbial community of the bee's digestive system, thereby making them more vulnerable to infection (Motta et al. 2018).

16.3.2 Climate Change

Climate change influences organisms in a complex way and is not easy to analyse. There are two important effects of climate change as far as the pollination services of the wild species are concerned. One is the migration of species to higher altitudes, and the other is shift in the timing of phenoevents associated with the species. As highlighted earlier (Chaps. 1 and 2), phenology refers to the timings of recurring seasonal events associated with the organisms; the timing, intensity and longevity of flowering and fruiting are important phenoevents which are very sensitive to climate change. Phenological stages in most of the insects include timings of egg laying and development of the larva, pupa and adult. The most effective way of studying these shifts in plant species is by comparing early records of species distribution and the timing of their phenological events over the last several decades with those of the present. In many developed countries, especially those located in Europe, dependable records of phenological details, often going back to >100 years, are available and have been used to study species migration and their phenological shifts. The flowering phenology of cherry blossoms (*Prunus* species) in Japan is the longest (over 1200 years) phenological record available (see Primack et al. 2009). Herbarium specimens deposited in standard herbaria provide the location of collection and the period of flowering and fruiting at the time of their collection. The comparison of older herbarium sheets with the present field studies has been another approach to study species migration and their phenological shifts.

Environmental features such as temperature, light, precipitation and melting of snow act as cues to initiate phenological events. Phenoevents, particularly the flowering and fruiting, are evolved over thousands of years based on the availability of pollinators and seed dispersers to achieve optimal reproduction and recruitment. The migration of one of the partners to higher altitude or a change in the phenology of plants or its pollinators is likely to uncouple these synchronized events and thus affect pollination efficacy.

A number of studies have established climate change-induced shifts in the distribution and phenology of plants as well as animals (Grabherr et al. 1994;

Parmesan et al. 1999; Cleland et al. 2007; Parmesan and Yohe 2003; Porter and Semenov 2005; Beckage et al. 2008; Pimm 2009; Miller-Reshing and Forrest 2010; Miller-Rushing et al. 2010; Lovejoy and Hannah 2005; Lobell et al. 2011). As the habitat becomes unsuitable for the species due to ambient warming, many plant and animal species extend their distributional range towards higher altitudes (in mountain areas) and towards poles (in plains) to find favourable conditions. Often, many species are unable to keep pace with the changing environment and eventually become extinct. Studies analysing the details of over 110 years on bumblebees of North America and Europe have reported that many species are disappearing from their earlier range due to their inability to keep pace with the climate change (Kerr et al. 2015).

There are a number of reports on the shift in flowering time induced by climate change. A comparison of the data available for species growing in the Red River Valley, North Dakota, USA, from 1910 to 1961 has revealed significant increase in spring temperatures and lengthening of the growing season over the years (Dunnell and Travers 2011). A considerable number of species have shifted their first flowering time earlier or later compared to the previous century. Advances in the days of celebration of the cherry blossom festival in Japan in recent decades are attributed to the climate-induced shift in flowering phenology. This festival is an important cultural event in Japan, celebrated during April, which coincides with the peak flowering period of cherry blossom trees (Primack et al. 2009). The analysis of the dates shows the trees flowered an average of 7 days earlier from 1971 to 2000 than the average of all earlier records for the species. This change happens to be in tune with the rise in the temperature. Projections for the coming years indicate that by 2100, the trees are likely to flower nearly 30 days earlier (Allen et al. 2013).

In spite of sufficient evidences for species migration and their shift in phenology in response to climate change, very few studies demonstrate the effects of such shifts on pollination efficacy. In temperate regions, melting of ice initiates leafing episodes in plants, followed by flowering in the spring. Warming of the weather also induces hatching of the hibernating insects which feed on newly developed foliage. By the time the plants bloom in the spring, the insects emerge and pollinate their flowers. Global warming may uncouple such synchronized events; pollinators may emerge before plants flower or plants may flower before the emergence of their pollinators. Such asynchrony would adversely affect both pollination efficacy and pollinator sustenance. Investigations on these aspects are limited. (Thomas et al. 2004; Hegland et al. 2009; Settele et al. 2008; Thomson 2010; Settele et al. 2016). Based on the available data on the flowering time of plants and their bee pollinators in the North-Eastern USA, Bartomeus et al. (2011) reported that there was no visible asynchrony between flowering and pollinators. A long-term study since the mid-1970s in the Mediterranean Basin has indicated that unlike the earlier decades, when the availability of pollinators and flowering were synchronized, during the last decade insect phenoevents showed a steeper advance than those of plants (Gordo and Sanz 2005). Similarly Forrest and Thomson (2011) reported a complete asynchrony between the flowering of *Lathyrus* and one of its pollinators *Hoplitis fulgida*.

Simulation studies by Memmott et al. (2007) predicted a decline in 17–50% of floral resources to pollinators, mainly because of phenological shifts; this may result

in the local disappearance of pollinators and plant species. Early spring due to climate change tends to induce early flowering in *Corydalis ambigua*, before the appearance of its pollinator (bumblebee), leading to lower pollination success and fruit set (Kudo and Ida 2013). In an alpine region of Japan, although there was a synchrony in normal year between the flowering of plant species and the abundance of generalist pollinators, like bumblebees (Kudo 2014), there was an asynchrony between the onset of flowering and bee emergence during the warmer years.

Asynchrony in phenoevents may not be a serious limitation to generalist pollination systems in which each plant species is pollinated by a number of pollinators and each animal species may pollinate a number of plant species. Both plant species and pollinators can switch to other species when one of the partners is missing. However, asynchrony may be of significant disadvantage to the specialized pollination systems in which each plant species is pollinated by one species-specific pollinator and each pollinator species pollinates one plant species. One such specialized pollination system is sexual deception found in several orchid species. The flower of such species resembles the female insect of the pollinator species and also emits species-specific pheromone to attract male pollinator (see Shivanna 2019). Male pollinators generally emerge from hibernation a few days before the emergence of females. The flowering of orchids synchronizes with the emergence of males; they readily visit flowers and pollinate them in the absence of females. When once the females emerge, males hardly visit the flowers, as females lure away the males from the flowers. Thus, the flowering of orchids before the females appear is critical for a successful pollination. Early spring may result in the emergence of females earlier than normal and affect pollination efficacy (Robbirt et al. 2014).

Climate change also affects the weather pattern. The floods, droughts and forest fires have become more intense and frequent in many parts of the world in recent years. Precipitation patterns, including rains, snow and hail, also have an implication on various attributes of pollination (Morton and Rafferty 2017). It is believed that the regions located at higher altitudes are likely to experience a heavier precipitation pattern with a global rise in temperature (IPCC 2018). Wet climates show a low pollinator diversity because high rainfall results in the disruption of foraging opportunities, possibly through failure of anthesis, pollen degradation and nectar dilution (Lawson and Rands 2019). On the other hand, fluctuations in the rainfall pattern can significantly influence the pollinator service due to altered physiology during reproductive stages, leading to low nectar production, gender expression, decline in floral resources and shift or replacement in communities of an effective pollinator (Phillips et al. 2018; Mukherjee et al. 2019).

16.4 Impact of Environmental Changes on Managed Pollinators and Pollination of Crop Species

In recent decades, habitat loss, use of pesticides, climate change and a range of ailments are reducing the managed honeybee colonies, thus creating problems in sustenance of pollination of crop species (Roubik 1995; James and Pitts-Singer

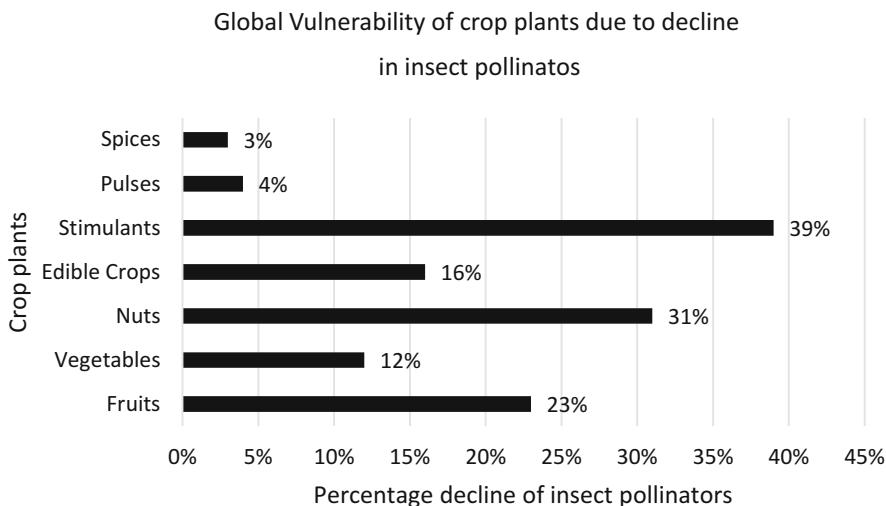


Fig. 16.2 The decline of production of selected insect-pollinated crops that are vulnerable to the decline of insect pollinators. (Source: Gallai et al. 2009)

2008; Fig. 16.2). Agrochemicals including pesticides pose a serious threat to pollinators and pollinator networks. Pollutants affect the cognitive ability of pollinators, impair their foraging behaviour, induce memory constraints, increase flower-handling time and reduce their pollen-collecting efficiency (Al Naggar, 2016). The consumption of contaminated and toxic pollen and nectar can have deleterious effects on their nesting behaviour, brood and cell production and often results in high rate of mortality among insects and insect larvae, and sometimes the collapse of colonies has been clearly pointed out by many scientists (Torchio 1983; Copping 2016). Even feral honeybee colonies are declining due to various diseases (Kraus and Page 1995). In a five-year survey (2013–2017) of honeybees, stingless bees and solitary bees in Brazil, 19,296 out of 37,453 colonies and nests were reported to be lost. The losses were highest for the Africanized honeybees (*Apis mellifera*), followed by stingless bee *Tetragonisca angustula* (Castilhos et al. 2019). In North America and Europe, the losses of honeybee colonies have increased dramatically since 2006 because of the prevalence of colony collapse disorder (CCD) in which the worker bees leave the colony for collecting pollen and honey but never return. The disorder seems to affect the navigational ability of the bees. In the USA, the average annual losses of bee colonies since 2010–2011 have been as high as 38% (Taylor 2019). Although a number of possible causes have been suggested for CCD disorder, none of them has been confirmed. Many studies have indicated that it may be due to the synergistic effects of a number of causes – *Varroa* mites, malnutrition, pathogens and pesticides (van Engelsdorp et al. 2009; Bosseva 2018).

Incidentally, CCD seems to affect only *Apis mellifera*, the major managed pollinator used for crop pollination. As honeybees are used to pollinate over 50 crop species in North America and Europe, CCD has significantly reduced the

number of bee colonies (Anonymous 2010). The dependence of agricultural crops on pollination by a single species (*A. mellifera*) has become risky (Winfree 2008). Further, the rental value of honeybee colonies has increased enormously in recent years because of the reduction in the number of available bee colonies. Almond is the main crop of California; 80% of the world's production of almonds comes from California. The acreage of almond cultivation has been steadily increasing. During its flowering season in February, it requires over two million bee colonies for pollination services. The cost of renting bee colonies has jumped from about \$ 45 in 2004 to over \$ 200 in 2018, reducing the profitability of orchard owners.

Pollination services through the managed pollinators are predicted to decline in the coming years due to global warming (Rader et al. 2013). Under high temperature (beyond threshold levels), bees spend lesser amount of time in foraging and more in cooling their bodies (Heinrich 1979). Honeybees are also sensitive to high winds and their foraging activity gets reduced. However, wild pollinators are less sensitive to wind speed and global warming and thus provide pollination services even under adverse conditions (Brittain et al. 2013a; Rader et al. 2013). Thus, the presence of several wild species in agricultural habitats would provide better pollination services because of their differential responses to global warming (Rader et al. 2013) and other environmental conditions.

In the light of the above problems faced by managed pollinators, attempts were initiated to domesticate wild pollinator species so that they would be available as additional pollinators for crop species at a lower cost. Success has been achieved in commercializing some of the solitary bee species (James and Pitts-Singer 2008; Ward et al. 2010; Koh et al. 2018). For example, an alkali bee (*Nomia melanderi*) builds millions of nests in the ground in one ha. They are sold as nest-filled earth blocks. Alfalfa leafcutting bees (*Megachile rotundata*) and mason bees (*Osmia* spp.) build nests in the above-ground tunnels in naturally occurring plant parts such as hollow twigs and also in artificial holes drilled in commercial boards. These bees are generally sold as in nest-filled hollow twigs/bee boards.

In recent years, numerous reports have highlighted the role of wild pollinators, even in the presence of managed pollinators (Greenleaf and Kremen 2006; Brittain et al. 2013b; Garibaldi et al. 2013). Studies on 600 field sites on 41 crops around the world have shown that wild pollinators enhance fruit set of crops even in the presence of managed honeybees. Managed honeybees thus supplement, rather than substitute, pollination services carried out by wild pollinators (Garibaldi et al. 2013). As mentioned above, wild bees appear to be more effective and withstand global warming better than commercial bees (Rader et al. 2013). The trend to safeguard pollination services of crop plants is to develop integrated pollination services by the judicious use of managed honeybee colonies along with other managed bees such as alkali bees, alfalfa leafcutting bees and blue orchard bees. For example, in almond orchards the number of honeybee colonies could be reduced by 50% by the use of the alternative pollinator, blue orchard bee (*Osmia lignaria*), thus reducing the rental value needed for sustaining pollination services (Ward et al. 2010; Koh et al. 2018). A parallel approach is to increase the density and diversity of wild pollinators by making agricultural habitats pollinator-friendly. Each species of social bees has a

microclimatic window, and their foraging activity is optimal within this temperature window; their efficiency goes down when the ambient temperature goes beyond this window (Corbet et al. 2008). The temperature window and foraging activity of wild bees are much wider. There are mounting evidences to indicate the importance of native biodiversity in buffering the impacts of global warming and other environmental changes in sustaining pollination services of crop species. Some of the approaches to make agricultural habitats pollinator-friendly include maintaining natural/semi-natural land strips amidst and around agricultural fields, providing artificial nesting sites and reducing the use of harmful agrochemicals (James and Pitts-Singer 2008; Rollin et al. 2019).

Additional methods that are being tested to safeguard pollination services in the coming decades include bee vaccination against various diseases and the development of pollinator drones. The queen bee is fed with sugar solution containing the vaccine, and the vaccinated queen bee is then introduced to the hive, resulting in the production of a new generation of bees with immunity to the disease (Salmela et al. 2017; Jacobs 2018). Experimental samples are being marketed in the name of Prime BEE. Many companies in the USA and Japan have already tested prototypes of pollinator drones and showed their ability to bring about pollination under laboratory conditions (Chechetka et al. 2017; Lallensack 2017). By employing machine language (GPS and AI), the robots are programmed to carry out the function of pollination services. Although many challenges remain, concepts from bio-inspired robotics may help scientists to engineer machines that possess at least some of the desirable properties of biological organisms, such as adaptivity, robustness, versatility and agility.

Managed beekeeping practice potentially affects the topology and functioning of pollination networks in natural ecosystems. The manual increment of managed honeybees in natural habitats can adversely affect the biodiversity of wild pollinators, ecosystem functioning and their resistance to global environmental change (Valido et al. 2019, Mallinger et al. 2017). Majority of studies have concluded that managed bees negatively influence or have the potential to adversely affect the populations of wild bees through competition, changes in plant communities or transmission of pathogens. However, studies are needed to establish whether competition between honeybees and native bees has a long-lasting survival threat and consequences.

16.5 Indian Scenario

In spite of the fact that the global pollinator crisis has become a reality, Indian biologists seem to have remained totally indifferent about the problems faced by the pollinators and pollination services. As human-induced environmental changes in India are likely to be more intense than those in the Western countries, pollination services of both wild and cultivated species of our country are likely to be seriously compromised. But we do not know much about what is happening to our pollinators and pollination services neither in our natural nor agricultural habitats. Studies on the

density, diversity and distribution of pollinators in the natural habitats and the impact of human-induced environmental changes on them are urgently needed. A comparison of pollination efficacy between self-incompatible and self-compatible species of ten plant species around Puducherry, South India, by Nayak and Davidar (2010) has shown higher levels of pollination limitation in self-incompatible species. This is because self-compatible species do not need the interplant movement of pollinators for effective pollination, but self-incompatible species are dependent on the inter-plant movement of pollinators and thus require more pollinator activity.

The pollination services of pollinator-dependent crops have also been ignored. Although the country is self-sufficient in cereals, the production of oils, pulses, fruits and vegetables is insufficient. This has created a 'hidden hunger' caused by deficiencies in proteins, micronutrients (particularly iron and zinc) and essential vitamins (especially vitamin A) in staple diet (Eilers et al. 2011). About 40% of the population in India is made up of vegetarians. They are dependent solely on vegetable sources for these essential requirements. India, Southeast Asia and central and southern Africa are hotspots for pollinator-dependent crops for the supply of micronutrients and show high nutritional vulnerability (Chaplin-Kramer et al. 2014). According to FAO (2015), 195 million undernourished people live in India. We have over 40% of undernourished children of the world (Global Hunger Index 2016). It is, therefore, inevitable to increase significantly the productivity of pulses, fruits and vegetables which depend on pollinators fully or partially.

Apple yield in Himachal Pradesh and Jammu and Kashmir used to suffer on account of pollination limitation (Partap 1998). Regular use of managed pollinators in apple orchards in India has overcome this limitation. However, this has not spread to other apple-growing countries in the Himalayas. Coffee growers in Coorg too are a worried lot as the main pollinator, *Apis dorsata*, nests on the strands of large trees which too are increasingly at risk (Krishnan et al. 2012). In any agricultural landscape, adequate pollinators are required for optimal crop yield. Inadequate pollinators in cash crops like cocoa and oil palm plantations have been identified as one of the major causes of their poor yields (Groeneveld et al. 2010; Rao and Law 1998).

For most of the other crops, we do not even have the information about their pollination status under natural conditions. Limited studies on large cardamom (*Amomum subulatum*), an important cash crop of the North-East (Sinu and Shivanna 2007), and a few vegetables (Bhattacharya and Basu 2016) have shown marked pollination deficiencies, and the yields can be substantially increased by enhancing pollinator density in *Amomum* and by the use of managed pollinators in vegetables. According to Basu et al. (2011), decreased crop productivity on account of the loss of pollinators has a long-term impact on the farming sector that accounts for almost a fifth of the nation's gross domestic product. The nodal agency for Indian agricultural crops, the Indian Council of Agricultural Research (ICAR), has initiated a number of research and extension programmes to make agriculture climate resilient, but there are hardly any programmes on pollination services of our crop species.

16.6 Concluding Remarks

Mounting evidences around the world have clearly shown that the ‘global pollinator crisis’ is a reality. The crisis is seriously threatening not only our crop productivity but also the sustainability of flowering plant diversity. This affects the functioning of natural ecosystems and thus the food and nutritional security. The world has realized fully the impact of this crisis on human welfare and has initiated a number of programmes to study and mitigate the pollinator crisis. According to a recent UN report on climate change (October 2018), overshooting 1.5 °C rise by the end of the century, as agreed at the Paris meeting, is going to have devastating effects on ecosystems, communities and global food security. The report warns that the world cannot afford even a 2 °C rise.

In spite of the serious concern of the world, tropical countries in general and India in particular have remained indifferent as far as pollinators and pollination services are concerned. A study conducted by the Pest Management Regulatory Agency (PMRA) identified 73 major pollen monitoring programmes across the world and only 2 exist in Asia. One of the reasons for this lack of interest appears to be that pollination is not a part of the curriculum of studies in any agricultural universities in the country. Thus, agricultural scientists are not fully aware of the importance of pollination or detailed methodologies to study pollination. We need to understand that the pollination is also crucial for many traditional medicines as modern pharmaceuticals, up to 25%, are derived from plant-based compounds; therefore, pollination constraints need to be investigated in medicinally important plants. Roy et al. (2016) pointed out that managing urban green spaces and connecting isolated vegetation patches by designing corridors may restore pollinator communities and pollination services delivered by them. It is important also to look at social and other tangible and non-tangible values of pollinators as pollination is important to maintain the values of many cultural and heritage sites and reserved areas. Thus, any convention on safeguarding these areas needs to recognize pollinators and pollinator-dependent plants as globally important. Unless immediate steps are initiated to initiate extensive studies to sustain pollination services of our pollinator-dependent crops and wild species, we may not be able to sustain even the existing level of crop productivity in the coming years or to sustain our vast biodiversity.

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