

THE EVOLUTION OF PLANT SEXUAL DIVERSITY

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Charles Darwin recognized that flowering plants have an unrivalled diversity of sexual systems. Determining the ecological and genetic factors that govern sexual diversification in plants is today a central problem in evolutionary biology. The integration of phylogenetic, ecological and population-genetic studies have provided new insights into the selective mechanisms that are responsible for major evolutionary transitions between reproductive modes.

EVOLUTION OF SEX



SEXUAL POLYMORPHISM

The co-occurrence within a single interbreeding population of morphologically distinct mating groups that are distinguished by differences in their sexual organs.

DICHOGAMY

Differences in the timing of pollen dispersal from anthers and stigma receptivity of flowers. In protandry, pollen is dispersed before stigmas are receptive, and in protogyny, stigmas are receptive before pollen is dispersed from anthers.

Flowers, the reproductive organs of angiosperms (flowering plants), are more varied than the equivalent structures of any other group of organisms. Because of this variation, interest in plant sexual diversity has a long and venerable history in biology. Linnaeus used variation in sexual structures of plants as the basis for his classification, Darwin wrote three volumes on plant reproductive biology and Fisher provided the population-genetic principles for understanding the evolution of plant mating systems. However, despite this attention, it is still not obvious why flowering plants have evolved such extraordinarily diverse means to achieve only one primary function — mating success. A central problem for evolutionary biologists is therefore to understand the selective forces that are responsible for the origins and maintenance of sexual diversity in flowering plants.

Here, I review recent advances in our understanding of the evolution and functional significance of sexual diversity in flowering plants. Several developments make it an opportune time to explore this topic. First, a rich theoretical framework and new advances in genetics and molecular biology have meant that sexual systems have become a unique testing ground for both phenotypic and genetic models of natural selection^{1–3}, as well as for phylogenetic analysis and comparative biology^{4–6}. Second, because plants are so amenable to experimental manipulation, they can be exploited as model systems for testing adaptive hypotheses on the function of sexual traits^{7–9}. Last, the recent unification of pollination biology and mating system studies¹⁰, two areas of

reproductive biology that remained separate for most of the past century, has led to new experimental findings on the mechanisms that drive sexual diversification in plants. Studies that integrate these theoretical, comparative and experimental approaches are providing novel insights into the complexities of plant sexual behaviour.

I begin this review by illustrating how a functional perspective is crucial for understanding the evolution of plant sexual diversity, using SEXUAL POLYMORPHISMS as exemplars for this approach. I then discuss new findings about the evolution of sexual dimorphism from hermaphroditism (cosexuality) and the evolution of self-fertilization from cross-fertilization, two major transitions among sexual systems in flowering plants. These transitions have arisen independently in numerous plant lineages, providing opportunities to search for general principles that can explain the origins of these two sexual systems. The key message of this review is that flowering plants show remarkable ecological and evolutionary lability in their sexual systems. The presence of this natural source of variation, combined with a knowledge of the genetic basis of sexual traits, provides biologists with outstanding opportunities to investigate general questions concerning the evolutionary biology of sex.

Strategies that promote cross-pollination

Flowering plants are predominantly hermaphroditic, with most species producing flowers that contain both female (pistils) and male (stamens) sexual organs. These sexual structures can be spatially separated in a

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INBREEDING DEPRESSION

The reduction in viability and fertility of inbred offspring compared with outbred offspring.

flower (herkogamy) or can function at different times (DICHOGAMY), so reducing the likelihood of self-pollination. Because of the modular growth of plants and the resulting production of multiple flowers and inflorescences (a group of flowers combined in a single reproductive unit), female and male gametes are deployed in diverse structural and temporal combinations at the flower, inflorescence, shoot and population levels. Determining the genetic and environmental

components of this sexual variation is an important challenge for plant biologists. Moreover, this variation has important functional consequences for mating behaviour and plant fitness.

First, if female and male gametes are produced simultaneously by a plant, opportunities for self-fertilization can occur. Numerous studies have shown that inbred offspring are less fit than outbred offspring and this INBREEDING DEPRESSION is generally recognized as one of the main selective forces that shape the evolution of plant mating strategies¹¹. Second, hermaphrodite plants acquire fitness through either of two sexual avenues — by being maternal or paternal parents to seeds produced in the next generation. The ‘gender’ of a plant therefore refers to the relative importance of these two avenues — the maleness or femaleness of the plant, measured as the proportion of a plant’s genes that are transmitted to offspring as a pollen or ovule parent. Plant gender¹² therefore provides a functional rather than a morphological view of plant sex, with the gender strategies of species being characterized by the relative contributions to fitness that result from maternal and paternal investment. Two dominant research themes have therefore arisen in contemporary studies of plant sexual diversity — the fitness consequences of outcrossing and selfing and the reproductive mechanisms that promote these alternatives¹³, and the evolution of gender strategies¹⁴. Both are a consequence of the fundamental hermaphroditic condition of flowering plants.

Since Darwin’s time, most adaptive interpretations of sexual diversity have focused on mechanisms that function to promote outcrossing and reduce the likelihood of inbreeding depression. However, this simple idea is more complicated than it can first seem. This is because fitness in hermaphrodites depends on the sum of both female and male fertility, and outcrossing can be promoted in diverse ways depending on whether we consider the interests of the maternal or paternal parent. Traditionally, floral traits have been interpreted as ‘anti-selfing’ mechanisms that passively encourage cross-pollination by preventing or discouraging self-pollination, thereby allowing more opportunities for ovules to be outcrossed. This involves the genetic contribution to the next generation as a maternal parent and focuses on the fate of ovules, as measured by seed set and the frequency of ovules that are outcrossed. However, an alternative perspective on the function of floral traits recognizes their paternal mating role in actively promoting pollen dispersal, thereby increasing fitness through male outcrossed siring success¹⁵. These viewpoints can be readily understood by considering functional interpretations of sexual polymorphisms that involve the spatial arrangement of female and male reproductive organs¹⁶.

Heterostyly. In heterostylous species, populations are composed of two (distyly) or three (tristyly) floral morphs that differ reciprocally from one another in the positions in which (male) anthers and (female) stigmas (where pollen is deposited by pollinators) are located in flowers (FIG. 1c shows distyly). This sexual polymorphism

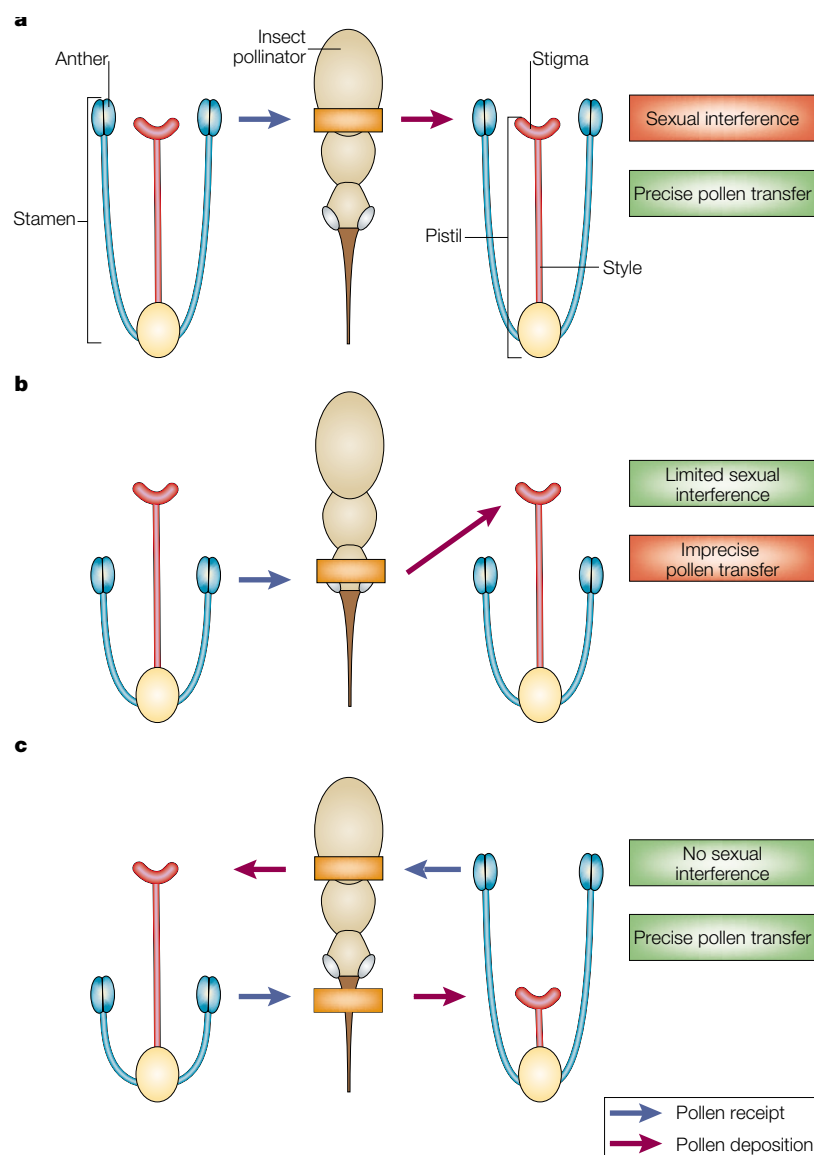


Figure 1 | Floral design and pollen transfer in animal-pollinated plants. The diagrams illustrate three floral designs that differ in the spatial arrangement of female (pistil) and male (stamen) sexual organs and in the location where pollen is deposited on visiting insect pollinators. The costs (red boxes) and benefits (green boxes) of these alternative arrangements are indicated with regard to the likelihood of sexual interference and self-pollination and to the precision of cross-pollen transfer. **a** | A population with a single floral phenotype in which anthers and stigmas are of equivalent height. **b** | A population with a single floral phenotype in which anthers and stigmas are spatially separated (herkogamy). **c** | A distylous population with two floral morphs that differ reciprocally in stigma and anther position. Distyly provides precise pollen transfer without the costs that are associated with sexual interference and self-pollination. Quantitative variation between plants in style and stamen length, as commonly occurs in conditions **a** and **b**, is usually governed by many genes of small effect. By contrast, the inheritance of distyly is governed by a major diallelic Mendelian locus.

has evolved independently in at least 28 animal-pollinated angiosperm families through the convergent selective pressures that are associated with cross-pollination. In addition to the sex-organ reciprocity that defines the polymorphism, heterostylous plants usually have a heteromorphic incompatibility system that prevents selfing and intramorph mating, although exceptions do occur^{17,18}. Heterostyly is simply inherited with a single diallelic Mendelian locus, with dominance controlling distyly, and two diallelic epistatically interacting loci, both with dominance governing tristyly¹⁹. In distylous species, there is evidence of SUPERGENE CONTROL of the morphological and physiological components of heterostyly, but virtually nothing is known about the molecular or developmental genetics of heterostyly, and even the loci that govern the polymorphisms have yet to be mapped.

Textbook interpretations of the adaptive significance of heterostyly usually describe the polymorphisms as simply 'outcrossing mechanisms', but this explanation is insufficient for two reasons. First, physiological self-incompatibility guarantees outcrossing, leaving unexplained the question of why the complex morphological components that characterize the polymorphism should have evolved. Second, by preventing intramorph mating, heteromorphic incompatibility actually restricts outcrossed mating opportunities, particularly in comparison with homomorphic incompatibility, in which there are numerous morphologically indistinguishable mating groups in a population (for a recent review of homomorphic self-incompatibility, see REF. 20).

A more complete interpretation of the adaptive significance of heterostyly recognizes different functional roles for the morphological and physiological components in promoting male and female components of fitness, respectively. So, reciprocal sex-organ positions in the style morphs function to increase male fertility by actively promoting more precise pollen dispersal among plants than would occur in populations with uniform sexual organs and herkogamy (FIG. 1). This is achieved by limiting functional interference between sexual organs, so reducing levels of male gamete wastage through self-pollination. By contrast, self-incompatibility in heterostylous plants passively safeguards against self-fertilization and inbreeding depression, thereby promoting the maternal component of fitness. Recognition of these dual functions resolves the apparent redundancy of two mechanisms that seem to have essentially the same role. In essence, the evolution of heterostyly reduces the conflict that many sexually uniform animal-pollinated plants face — achieving precise cross-pollen transfer between plants but at the same time avoiding sexual interference between female and male sexual organs and therefore self-pollination.

Enantiostyly. Heterostyly represents the best-studied simply inherited morphological polymorphism in plants, but recent studies have begun to focus on other examples of reciprocal sex-organ polymorphisms. Perhaps the most curious of these is ENANTIOSTYLY, a rare type of directional asymmetry in plants that involves mirror-image flowers. Although this sexual polymor-

phism has been known since the late nineteenth century, it is only recently that work aimed at understanding its evolution and functional significance has been conducted¹⁶. Interpretation of the functional significance of enantiostyly is complicated by the occurrence of several forms of this stilar polymorphism (FIG. 2). In monomorphic enantiostyly, left-styled and right-styled flowers occur on the same plant either mixed within inflorescences (by far the commonest form) or with individuals producing both left-styled and right-styled inflorescences. In dimorphic enantiostyly, individuals are genetically uniform for style direction, but populations contain left-styled and right-styled morphs. Monomorphic enantiostyly is widely distributed and occurs in at least ten angiosperm families, including both dicotyledons and MONOCOTYLEDONS. By contrast, dimorphic enantiostyly has arisen independently in only three monocotyledonous families. The contrasting phylogenetic distributions of the various forms of enantiostyly imply differing constraints on their evolutionary origins.

The first genetic analysis of dimorphic enantiostyly has recently been conducted in the aquatic herb *Heteranthera multiflora* (Pontederiaceae)²¹. The polymorphism is controlled by a single Mendelian locus, with right-deflected styles dominant to left-deflected styles. The molecular and developmental mechanisms of left–right determination are unknown, but the rarity of dimorphic enantiostyly indicates that there might be strong constraints on its origin. These could be related to the difficulty of establishing positional information for the left–right axis that is required for style bending. Because a left–right asymmetry has to be aligned to both dorsal–ventral and apical–basal axes there might be limits to obtaining the appropriate positional information in many plant lineages. It is noteworthy that whereas monomorphically enantiostylous species are often radially symmetrical, the monocotyledonous families in which dimorphic enantiostyly has arisen have flowers with moderate bilateral symmetry. It would be interesting to investigate the expression of genes such as *cycloidea* and *radialis* in enantiostylous species. These genes convey positional information during floral development in flowers with bilateral symmetry²², and it is conceivable that similar types of gene could have a role in influencing 'left from right' in dimorphically enantiostylous species.

The simple Mendelian control of dimorphic enantiostyly results in an equilibrium expectation of 1:1 style-morph ratios in outcrossing populations. Equality of style-morph ratios is also a common feature of heterostylous populations. A recent study²³ of *Wachendorfia* (Haemodoraceae), a small genus of South African herbs, shows how the maintenance of enantiostyly is strongly influenced by the reproductive systems of populations. Equal frequencies of left-styled and right-styled plants are a general feature of *W. paniculata* populations (shown in FIG. 2c), and estimates of mating patterns with the use of marker genes confirm high outcrossing rates. By contrast, in the clonal *W. thrysiflora* and the selfer *W. parviflora*, morph ratios in populations are often strongly skewed, with some containing only a

SUPERGENE CONTROL

A cluster of tightly linked co-adapted genes that are inherited as a single unit and carry out related functions.

ENANTIOSTYLY

Mirror-image flowers in which the style bends either to the left side or the right side of the floral axis. The phenomenon can exist as a sexual polymorphism with left-styled and right-styled plants.

MONOCOTYLEDON

(Monocot). One of the two classes of flowering plants, monocots are characterized by one embryonic leaf (cotyledon). Maize, rice and other grasses are common monocots.

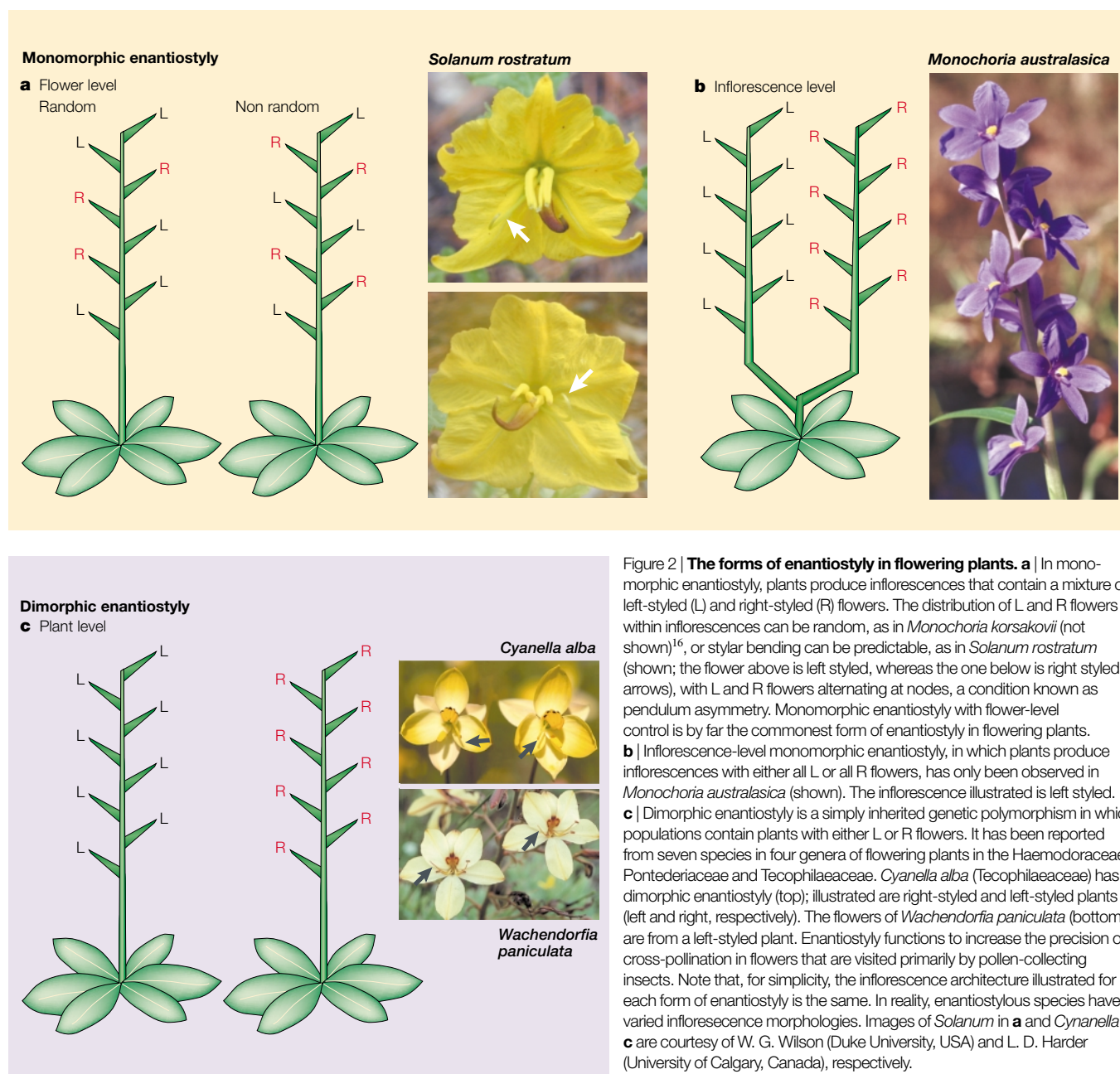


Figure 2 | The forms of enantiostyly in flowering plants. a | In monomorphic enantiostyly, plants produce inflorescences that contain a mixture of left-styled (L) and right-styled (R) flowers. The distribution of L and R flowers within inflorescences can be random, as in *Monochoria korsakowii* (not shown)¹⁶, or stylar bending can be predictable, as in *Solanum rostratum* (shown; the flower above is left styled, whereas the one below is right styled; arrows), with L and R flowers alternating at nodes, a condition known as pendulum asymmetry. Monomorphic enantiostyly with flower-level control is by far the commonest form of enantiostyly in flowering plants. **b** | Inflorescence-level monomorphic enantiostyly, in which plants produce inflorescences with either all L or all R flowers, has only been observed in *Monochoria australasica* (shown). The inflorescence illustrated is left styled. **c** | Dimorphic enantiostyly is a simply inherited genetic polymorphism in which populations contain plants with either L or R flowers. It has been reported from seven species in four genera of flowering plants in the Haemodioraceae, Pontederiaceae and Tecophilaeaceae. *Cyanella alba* (Tecophilaeaceae) has dimorphic enantiostyly (top); illustrated are right-styled and left-styled plants (left and right, respectively). The flowers of *Wachendorfia paniculata* (bottom) are from a left-styled plant. Enantiostyly functions to increase the precision of cross-pollination in flowers that are visited primarily by pollen-collecting insects. Note that, for simplicity, the inflorescence architecture illustrated for each form of enantiostyly is the same. In reality, enantiostylous species have varied inflorescence morphologies. Images of *Solanum* in **a** and *Cyanella* in **c** are courtesy of W. G. Wilson (Duke University, USA) and L. D. Harder (University of Calgary, Canada), respectively.

RECIPROCAL HERKOGAMY
Sexual polymorphisms in which floral morphs differ reciprocally in the locations of female and male sex organs within flowers (for example, heterostyly and enantiostyly).

FLEXISTYLY
A sexual polymorphism in which populations contain two floral morphs that differ in the temporal patterns of style growth and orientation.

single style morph. These deviations from the equilibrium expectation result from stochastic forces, such as founder events, with clonal propagation or selfing reducing the rate at which populations can progress to 1:1 morph ratios. The equal style-morph ratios in *W. paniculata* are significant because, unlike in heterostyly, there is no evidence of incompatibility systems that prevent intramorph mating in enantiostylous species. This implies that the reciprocal floral morphologies alone promote intermorph mating and equal morph ratios, through segregated pollen deposition on alternate sides of the bodies of insect pollinators. Segregated pollen deposition, but in a vertical plane, has been shown for pollinators that visit heterostylous

flowers (FIG. 1c). Observations of bees that visit enantiostylous flowers, and experimental studies using pollen dyes and marker genes, have confirmed these expectations²¹ and support the interpretation that enantiostyly functions in a manner that is analogous to heterostyly.

Flexistyl. Both of the preceding sexual contrivances that promote more proficient cross-pollination in animal-pollinated hermaphroditic flowers involve RECIPROCAL HERKOGAMY. A new floral strategy has recently been discovered that combines both reciprocal herkogamy and dichogamy in a single stylar polymorphism^{24,25}. The condition, termed FLEXISTYLY, is reported from a genus of tropical gingers — *Alpinia*

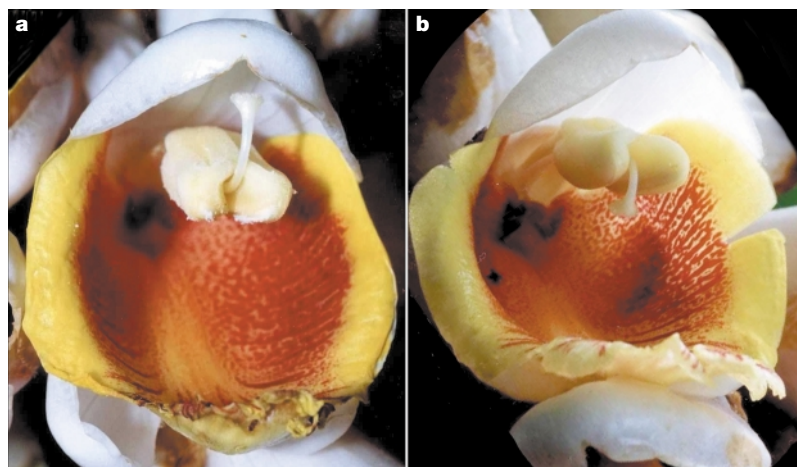


Figure 3 | Flexistylly in *Alpinia* (Zingiberaceae). Populations of *Alpinia* (tropical ginger from China) contain equal frequencies of two morphs that differ in style growth and the timing of female and male function. **a** | The protandrous morph of *Alpinia zerumbet*, which is male in the morning and female in the afternoon, and **b** | the protogynous morph, in which the reverse sequence of sex expression occurs. This new sexual polymorphism was recently discovered in Yunnan Province in South-West China by Qing-Jun Li and colleagues. Photographs courtesy of Q.-L. Li, Xishuangbanna Tropical Botanical Garden, Mengla, China.

spp. (Zingiberaceae) from China. Populations are composed of equal frequencies of two style morphs, one that functions as female in the morning and male in the afternoon, and the other in which the sex roles are temporally reversed (FIG. 3). Styles of flowers that disperse pollen in the morning are curved upwards, so that stigmas are spatially separated from anthers and cannot touch pollinating bees. At noon, after male function is complete, styles grow downwards into a position where stigmas contact pollinators. Patterns of style growth in the reciprocal morph are reversed, with stigmas receiving pollen in the morning and upward style curvature occurring in the afternoon, which reduces interference with pollen donation.

Flexistylly is an example of heterodichogamy²⁶, a poorly understood sex-phase polymorphism that involves protandrous (male function precedes female function) and protogynous (female function precedes male function) floral morphs, that has been reported from 11 families of flowering plants. Nothing is known about the genetics of flexistylly in *Alpinia* but, as for heterostyly and enantiostyly, it seems likely that the polymorphism is simply inherited. Indeed, studies of the genetics of heterodichogamy in walnuts²⁷ and pecans²⁸ have shown that a single diallelic locus governs the two sexual phases, with the protogynous condition dominant to protandry. In common with heterostyly and enantiostyly, negative frequency-dependent selection is the most likely mechanism that maintains 1:1 morph ratios in populations of heterodichogamous species.

These examples of stylar polymorphisms illustrate how simply inherited developmental changes in the positions of sex organs can have profound implications for the mating biology of populations. Spatial separation of stigmas and anthers is widespread in flowering plants, preventing interference between female and male sex functions and reducing the

intensity of self-pollination (FIG. 1b). However, if these sexual organs become too widely separated, pollinators might contact only one set of organs or touch them with different body parts while visiting flowers. This can reduce the precision of cross-pollination and decrease both female and male components of fitness. When this occurs, populations become vulnerable to invasion by developmental variants with altered sex-organ position that benefit by having their stigmas and anthers in matching reciprocal positions to the resident phenotype. Negative frequency-dependent mating guarantees that neither phenotype will gain ascendancy, resulting in a balanced polymorphism that maintains sexual diversity.

Unlike homomorphic self-incompatibility, in which dozens of physiologically distinct mating types with different pollen and stigma specificities coexist in populations, only a very small number of mating groups can be maintained with stylar polymorphisms. This is undoubtedly because of the difficulties in achieving multiply segregated sites for pollen deposition on animal pollinators, owing to the vagaries of the pollination process. The three mating groups that occur in tristylous populations probably represent the upper limit that can be achieved for morphological polymorphisms that function to promote the precise transfer of pollen through the geometry of pollinator contacts.

The evolution of separate sexes

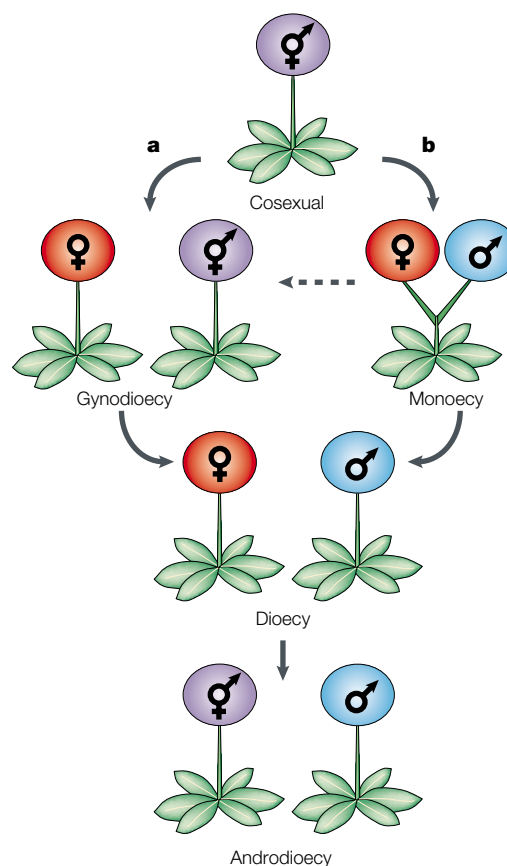
Few flowering plants (~10%) have unisexual flowers. This condition, known as *dicliny*, is associated with a wide spectrum of gender strategies that involve various combinations of female, male and hermaphrodite flowers at the plant and population levels. Despite the seemingly complex patterns of sexual diversity in flowering plants, two fundamentally distinct patterns of gender variation can generally be recognized, and virtually all species can be accommodated within these categories²⁹. Populations with 'gender monomorphism' show continuous variation in the relative genetic contributions that plants make to the next generation as female and male parents. For example, in species with *monoecy*, plants produce female and male flowers but usually vary in the relative numbers that they produce. By contrast, populations with 'gender dimorphism' show bimodality in gender because they are composed of two distinct sexual morphs that function primarily as either female or male parents. The morphs can be strictly unisexual, in which case the sexual system is known as *DIOECY*, or one morph is hermaphroditic and the other is female (*gynodioecy*³⁰) or male (*androdioecy*³¹). The bimodality in gender in gynodioecious and androdioecious populations arises because the 'hermaphrodite morph' functions largely as a male or female parent, respectively, owing to the presence of unisexual plants of the alternate sex in populations. Confusion often occurs in interpreting plant sexual systems³² because of the failure to understand the quantitative nature of gender and the need to adopt functional rather than morphological criteria when interpreting plant sexuality.

DIOECY

A sexual polymorphism in which populations contain female and male plants.

Box 1 | Pathways for the evolution of gender dimorphism in flowering plants

The accompanying figure illustrates the two main pathways from cosexuality to dioecism. In the gynodioecy pathway (a), male-sterility genes spread in cosexual populations, resulting in an intermediate stage that involves females and hermaphrodites. Genetic modifiers of female fertility gradually convert hermaphrodites to males, resulting in dioecy. There is considerable evidence for this pathway (see the main text). The monoecy pathway (b) is less well studied but is assumed to involve disruptive selection on female and male sex allocation in monoecious populations, which gradually increases gender specialization until unisexual plants originate. Monoecy has evolved numerous times from the dominant cosexual condition in angiosperm populations that have exclusively hermaphroditic flowers. This occurs through sterility mutations that produce unisexual flowers. A secondary pathway to dioecy from monoecy might involve a gynodioecious intermediate stage (dashed line)⁴⁶. A final pathway involves the evolution of the very rare sexual system androdioecy from dioecy. Genetic studies in *Datisca*³⁵ spp. (Datisceae) show that a single nuclear locus controls sex phenotype; at this locus, maleness is dominant to hermaphroditism in androdioecious *D. glomerata*. Female plants in dioecious populations are apparently converted into the hermaphrodite form by a recessive mutation that allows females to produce pollen. A single autosomal locus at which maleness is dominant also seems to govern sex expression in *Mercurialis annua*⁹⁹ (Euphorbiaceae), the only other intensively studied androdioecious species. There are no well-authenticated cases of androdioecy as an intermediate stage in the evolution of dioecy, and very limited evidence for the evolution of androdioecy from cosexuality.



Evolutionary pathways. Sterility mutations that give rise to unisexual flowers are a necessary starting point for the evolution of separate sexes from combined sexes. Two principal evolutionary pathways for the origin of dioecy are generally distinguished, both involving the transition from gender monomorphism to dimorphism (BOX 1). In the gynodioecy pathway, female plants invade cosexual populations through the spread of male-sterility mutations. Selection then favours male function in hermaphrodites through genetic modifiers of female fertility. Alternatively, in the monoecy pathway, DISRUPTIVE SELECTION on quantitative genetic variation in floral sex ratios within monoecious populations gradually increases gender specialization, culminating in the origin of female and male plants. These pathways therefore differ with regard to the types of gender variation on which selection acts and also in the relative importance of major versus minor genes that govern patterns of sexuality. A third pathway to dioecy from distyly has been documented in three plant families: Boraginaceae, Menyanthaceae and Rubiaceae³³. This transition is thought to involve increasing gender specialization of the long-styled and short-styled morphs, in most cases converting them into female and male plants, respectively. Little is known of the genetic basis of this transition or the selective mechanisms that are involved.

DISRUPTIVE SELECTION
Natural selection against the mean value of a quantitative trait, therefore favouring individuals at the two tails of the phenotypic distribution.

Comparisons of the genetic systems that govern the inheritance of heterostyly and dioecy reveal a striking difference between these two classes of sexual polymorphism. Both polymorphisms are polyphyletic and have arisen on numerous occasions in different lineages. However, although the genetics of heterostyly is remarkably uniform, involving the same one-locus (distyly) or two-locus (tristyly) control in unrelated groups¹⁹, analysis of the inheritance of sex determination in species with gender dimorphism illustrates significant variation^{34,35}, presumably reflecting the diverse pathways by which unisexuality can evolve. Genetic systems range from single locus to multiple loci, or involve morphologically distinct sex chromosomes³⁶. Biochemical and molecular analyses indicate that hormone levels have a central role in regulating sex expression in species with gender dimorphism^{37,38}, regardless of the type of inheritance. There have been no detailed studies on the role of hormones in controlling sex-organ growth in heterostylous species, although it seems very likely that they are also involved.

Considerable theoretical^{39–41} and empirical work^{42–44} has been conducted on the gynodioecy pathway but, until recently, little attention has been paid to the monoecy pathway^{45,46}. Indeed, for this pathway it is still unclear whether the transition is determined solely by selection on quantitative genetic variation that governs

LIFE-HISTORY TRADE-OFF

When allocation of resources to one life-history trait reduces investment in another trait.

POLLEN DISCOUNTING

The loss of outcrossed siring success as a result of self-pollination.

sex allocation or whether major sterility genes are also involved. Our ignorance of the monoecy pathway is surprising because comparative evidence indicates that dioecy might often originate from this condition⁴⁷. Monoecious populations produce unisexual flowers and have many of the ecological and life-history traits that seem to be adaptive in dioecious species. A recent molecular phylogenetic study⁴⁸ provides convincing evidence of the multiple origins of dioecy from monoecy in the primarily neotropical shrub family Siparunaceae, although the selective mechanisms that are responsible are unclear.

Selective mechanisms. Theoretical models generally identify three key factors that govern the evolutionary transition from gender monomorphism to dimorphism — the fitness consequences of selfing and outcrossing, the optimal allocation of resources to female and male function, and the genetic control of sex expression¹. It is of crucial importance to determine the circumstances under which an increase in the selfing rate results in inbreeding depression in ancestral cosexual populations. Such conditions provide opportunities for the spread of unisexual variants that benefit through outcrossing advantage. The theory of nuclear inheritance of male sterility indicates that females can spread in ancestral cosexual populations if the product of the hermaphrodite selfing rate (r) and inbreeding depression (δ) exceeds 0.5. This criterion has been met in several recent experimental studies of gynodioecious species^{49–51}. For females to spread in cosexual populations they must produce more than twice as many successful seeds as hermaphrodites to overcome the inherent loss of male function that

arises from male sterility. More recent theoretical models on the evolution of gender dimorphism have focused on sex allocation and LIFE-HISTORY TRADE-OFFS⁵², and genetic associations between male-sterility genes and those that cause inbreeding depression⁵³. Such associations between genes that govern sexual traits and those at fitness-determining loci remained elusive until recently⁵⁴.

A link between the evolution of gender dimorphism and conditions that promote inbreeding depression might occur through the action of polyploidy. Chromosome doubling in plants can result in the breakdown of self-incompatibility to self-compatibility⁵⁵, providing opportunities for self-fertilization. Molecular phylogenetic evidence in North American *Lycium* (Solanaceae) indicates that gender dimorphism has evolved only in species that are polyploid and self-compatible but whose closest relatives are diploid and self-incompatible⁵⁶. A similar pattern also seems to have originated independently in South African species of *Lycium*, and additional evidence from 12 unrelated genera in other families indicates that polyploidy might have acted as a trigger for the evolution of gender dimorphism⁵⁶. In this model, polyploidy disrupts the functioning of self-incompatibility, resulting in selfing and inbreeding depression. These events are thought to provide the necessary conditions for the spread of male-sterile mutants and the evolution of dioecy through the gynodioecy pathway. Because the breakdown of self-incompatibility by polyploidy seems to occur commonly in flowering plants, it will be important to determine why the transition to gender dimorphism does not happen more often. Recessive male-sterility genes are a large component of the segregation load of many seed plants^{57,58} and hence the appropriate genetic variation seems unlikely to be limiting.

Another condition that can potentially lead to gender dimorphism involves large plant size⁴⁵. In species of large stature (for example, shrubs and trees) or with extensive clones (for example, aquatics), a considerable amount of selfing can occur because of the presence of many open flowers on a plant at the same time. Geitonogamy, the transfer of pollen between flowers on the same plant, is likely to be a principal source of selfing in such mass-flowering plants⁵⁹. For example, in the water-pollinated clonal seagrass *Zostera marina* a relation between clonal reproduction and rates of selfing was recently detected by using microsatellite markers⁶⁰. Geitonogamous selfing can involve considerable mating costs that result from both inbreeding depression and the loss of outcrossed mating opportunities through POLLEN DISCOUNTING⁶¹. This is because pollen captured by stigmas on the plant from which it originates is not available for dispersal to other plants. Experimental evidence indicates that this male mating cost has probably acted as an important selective force in promoting the evolution of diverse floral strategies⁶², including the segregation of female and male sex functions⁶³.

Recent marker gene investigations of the clonal aquatic plant *Sagittaria latifolia* provide evidence that geitonogamous selfing and strong inbreeding

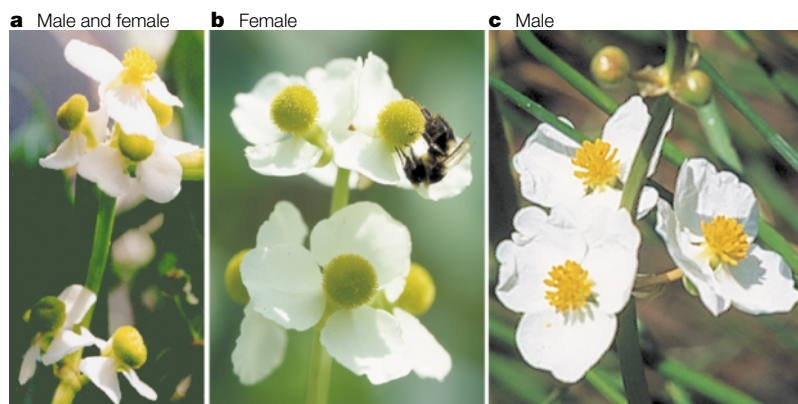


Figure 4 | Intraspecific variation as a tool for studying the evolution and maintenance of monoecy and dioecy. The clonal aquatic *Sagittaria latifolia* (Alismataceae) is unusual in having both monoecious (a) and dioecious (b, c) sexual systems⁴⁶. a | A monoecious inflorescence with mostly female flowers and a male flower at the top of the inflorescence. b, c | Inflorescences of female (b) and male (c) plants. Populations with combined versus separate sexes can be found growing in the same geographical area often in close proximity. Despite being fully interfertile, studies of genetic variation at allozyme loci indicate that there is little gene flow between monoecious and dioecious populations⁶⁴. Recent studies by Marcel Dorken and myself in Ontario, Canada, indicate that populations of the two sexual systems commonly occur in different wetland habitats. Monoecious populations inhabit ephemeral, disturbed habitats, whereas dioecious populations are more often restricted to large river systems and permanent wetlands. The influence of clone size on selfing rates is thought to be important in the evolution of dioecy from monoecy (see the text). Photographs in a and b courtesy of M. E. Dorken, University of Toronto, and L. D. Harder, University of Calgary, Canada, respectively.

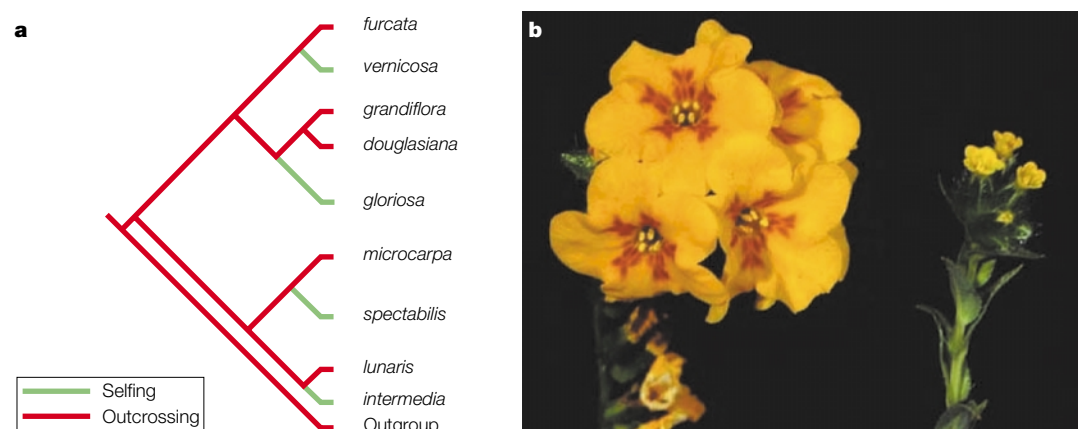


Figure 5 | Multiple origins of predominant self-fertilization in flowering plants. a | Phylogenetic reconstructions based on restriction-site variation in the chloroplast genome were used to investigate the evolutionary history of mating systems in the annual genus *Amsinckia* (Boraginaceae)⁷⁰. The results of these phylogenetic analyses indicate that predominant selfing might have evolved from outcrossing on at least four occasions in the genus. Outcrossing species are distylous and have large flowers, whereas selfing species are homostylous and have much smaller flowers. The repeated pattern of short branch lengths that separates selfers from their nearest outcrossing relatives in each of the four lineages in the phylogeny indicates that selfing in *Amsinckia* might be of recent origin. Current work on this system is aimed at determining the molecular genetic consequences of this change in mating systems. **b** | Difference in flower size between related outcrossers and selfers. The figure illustrates the large-flowered, outcrossing *A. furcata* (left) and its small-flowered, predominantly selfing, sister taxa *A. vernica* (right). Photograph courtesy of Daniel J. Schoen, McGill University, Canada.

depression have had a role in the evolutionary transition from monoecy to dioecy⁶⁴. Populations of both sexual systems occur together in this species, making it an excellent model organism for studying the evolution of combined versus separate sexes (FIG. 4). Many dioecious plants are large, indicating that geitonogamous selfing in ancestral cosexual populations might have been important in the evolutionary origin of dioecy, with unisexual plants benefiting from the genetic advantages of guaranteed outbreeding.

Comparative biology. Dioecy is widely distributed in flowering plants, occurring in nearly half of all families, including both basal and derived groups. However, the overall frequency of angiosperm species that are dioecious is only ~6% (REF. 47), implying that dioecy is rarely associated with successful evolutionary diversification. A recent comparative analysis⁶⁵, using sister-group comparisons of angiosperm taxa with contrasting sexual systems, showed that dioecious lineages have fewer species than their cosexual sister taxa at both the family and genus levels. This pattern might have resulted because dioecious species have a higher risk of extinction than non-dioecious relatives; indeed, a theoretical investigation⁶⁶ of the consequences of dioecy versus cosexuality for successful seed dispersal provides some support for this possibility.

Reduced speciation in dioecious clades, compared with their cosexual sister taxa, might also contribute to the lower species richness of dioecious lineages. Dioecy is commonly associated with unspecialized pollination systems that involve wind, water or generalist pollinators, rather than the more specialized pollinators that commonly drive floral diversification and reproductive isolation in many cosexual flowering plant groups^{67,68}.

A future challenge for comparative biologists will be to distinguish between the relative importance of low speciation versus high extinction in accounting for the reduced species richness of dioecious clades.

The evolution of self-fertilization

Despite the genetic benefits of outcrossing, significant numbers of angiosperm species (~20%) have evolved predominant selfing (autogamy) as a sexual strategy. Indeed, the multiple origin of small-flowered, predominantly autogamous species from outcrossing species is an almost universal feature of herbaceous plant families^{4,69–71} (FIG. 5). In contrast to the sexual systems that are discussed in the preceding sections, selfing is generally associated with reduced genetic variation in quantitative characters⁷², ALLOZYME LOCI⁷³ and neutral diversity at nucleotide polymorphisms^{74,75}; the origins of selfing might therefore be expected to retard evolutionary potential. Indeed, selfing, rather than dioecy, has most commonly been viewed in the literature as an ‘evolutionary dead-end’, although comparative evidence to support this assertion has been surprisingly hard to find⁷⁶ because of the paucity of well-resolved species-level phylogenies for most plant groups. Although selfing does release individuals from the requirement of having mating partners, allowing rapid colonization of unoccupied space⁷⁷, it is also associated with diverse costs, including inbreeding depression, pollen discounting and genetically uniform populations. Explanations of why selfing originates so often therefore require an understanding of both its consequences for fitness and the ecological context in which it evolves.

Theoretical models of the evolution of mating systems^{78,79} posit two main opposing genetic outcomes of selfing — the transmission advantage of alleles that

ALLOZYME LOCI
Loci that code for different electrophoretic forms of the same enzyme as a result of allelic differences.

SEED DISCOUNTING

The formation of self-fertilized seeds from ovules that, if they had not been self-fertilized, would have been cross-fertilized.

increase selfing rates, versus the reduced fitness of offspring that result from inbreeding. The models typically predict two alternative evolutionarily stable endpoints: predominant selfing or predominant outcrossing. However, empirical estimates of outcrossing rates by using genetic markers (FIG. 6) indicate that at least one-third of all species surveyed practise significant amounts of both selfing and outcrossing (mixed mating)⁸⁰. Indeed, for animal-pollinated species, the mating system is best considered a quantitative character rather than a discrete character that divides plant species. In some cases, this variation can even be found within species, with populations ranging from complete outcrossing to selfing⁸¹. Interestingly, survey data for wind-pollinated species show a bimodal distribution of outcrossing rates, with a strong deficiency of species with mixed mating⁸⁰. So, at least for this group, the existing data seem to be consistent with theoretical models.

Both adaptive and non-adaptive explanations can explain these contrasting patterns. Mixed mating in animal-pollinated species might arise to ensure reproduction ('reproductive assurance') when pollen vectors are scarce or unpredictable, a situation that might be less relevant in wind-pollinated plants. Alternatively, the selfing component to mixed mating might arise through geitonogamy and might be a non-adaptive cost that is associated with the large floral displays that are typically required to attract animal pollinators. If so, what accounts for the low frequency of mixed mating in wind-pollinated plants? Is this simply an issue of the limited taxonomic sampling of this group, or are there biological reasons why wind-pollinated plants might be less prone to mixed mating?

Wind-pollinated plants commonly have sexual strategies, such as dichogamy, that might reduce the likelihood of geitonogamy. For example, grasses have ephemeral flowers that often have remarkable asynchrony of sex function, and it seems likely that these traits have been strongly selected to prevent the

inevitable selfing that could occur with wind as a pollen vector. Unfortunately, reproductive biologists have devoted most of their attention to showy, animal-pollinated plants; the sexual strategies of wind-pollinated plants have, by comparison, been seriously neglected. Investigations of the relations between floral display and sex function in wind-pollinated plants are needed to determine mechanisms that might limit mixed mating. In addition, microevolutionary studies that investigate why wind pollination has evolved from animal pollination are essential, given the importance of this transition in flowering plants.

Of particular importance in species with mixed mating is to determine the relative fitness of selfed versus outcrossed offspring by comparing the inbreeding coefficients of parents and progeny. Genetic markers have been used to investigate this problem^{64,82,83}. With strong inbreeding depression, the realized mating system of populations might be predominantly outcrossing, if offspring that arise from self-fertilization seldom reach reproductive maturity. Indeed, very early abortion of selfed embryos might bias estimates of outcrossing upwards, on the basis of mature seeds or seedlings. This effect might contribute to the uniformly high outcrossing estimates reported for most trees and other long-lived plants⁴ that commonly show considerable early-acting inbreeding depression⁸⁴.

An alternative to early embryo abortion is ovule discounting^{85,86}, whereby plants curtail ovule development shortly after self-pollination, thereby preventing self-fertilization from occurring. This has been interpreted as a strategy to limit investment in seeds with no genetic future and therefore to save resources for future reproductive episodes. Sexual strategies that reduce gamete loss and wasted reproductive investment might be commonly used by plants to ensure the production of optimal numbers of high-quality offspring.

The frequent association between predominant selfing and the annual life form⁴, and the finding that selfing commonly arises in geographically or ecologically marginal areas of species' ranges, implicates uncertain pollination conditions as the crucial ecological context for the evolution of selfing. Although other selective forces that are associated with pollination can promote the evolution of selfing⁸⁷, reproductive assurance seems likely to be the principal mechanism that drives the evolution of autonomous modes of selfing in plants⁸⁸. However, experimentally testing the reproductive assurance hypothesis can be complex, as a recent study of the spring-flowering herb *Aquilegia canadensis* (Ranunculaceae) illustrates⁸⁹.

A. canadensis occurs in small populations and pollinator service is often unreliable. These conditions favour the evolution of intraflower self-pollination as a means of ensuring seed set. Although in *A. canadensis* this mode of selfing increases seed production when opportunities for outcrossing are limited, floral manipulations and marker-gene studies showed that these benefits are often negated by severe SEED DISCOUNTING⁹⁰. In the populations investigated⁸⁹, ovules that would have been outcrossed were instead selfed and the resulting

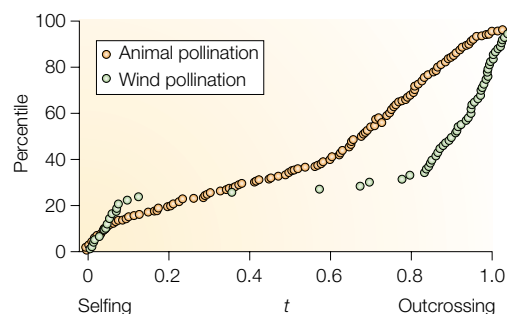


Figure 6 | The difference in the distribution of outcrossing rates in animal-pollinated and wind-pollinated plant species. The graph plots the percentile versus ranked estimates of t , the outcrossing rate, for 169 animal-pollinated species and 59 wind-pollinated species⁸⁰. The data are of significance for theoretical models of the evolution of mating systems that predict bimodality of outcrossing rates. Moreover, the difference between the two distributions raises important issues concerning the functional link between the pollination biology of species and their mating systems.

COALESCENT ANALYSIS

A means of investigating the shared genealogical history of genes. A genealogy is constructed backwards in time starting with the present-day sample. Lineages coalesce when they have a common ancestor.

QUANTITATIVE TRAIT LOCI

(QTL). Loci that control quantitative traits identified by showing a statistical association between genetic markers and phenotypes that can be measured.

offspring had very low fitness because of strong inbreeding depression. Hence, in *A. canadensis*, reproductive assurance bears a strong genetic cost. Why this species regularly selfs at ~75%, despite the harmful genetic consequences, is still a mystery, but the study nevertheless shows the importance of determining whether seed discounting occurs when testing the reproductive assurance hypothesis.

Future developments

Considerable progress has been made in our understanding of the evolutionary lability and function of plant sexual traits through imaginative experiments on floral characters and through the use of genetic markers. In addition, molecular studies have aided in the reconstruction of the phylogenetic history of sexual systems and have provided new information on the consequences of mating-system variation for genetic diversity. All three avenues of research seem likely to continue in earnest as researchers exploit the striking interspecific and intraspecific variation in sexual systems that characterize many flowering plant groups. Future work will undoubtedly benefit from the following recent technical and analytical innovations.

A wider array of genetic markers, particularly microsatellites, should provide more precise individual-based measurements of mating patterns, especially those involving male parentage⁹¹. This will enable investigators to assess more accurately the role of sexual traits in promoting mating success, particularly through effective pollen dispersal. Improved phylogenetic methods for inferring character evolution

and ancestral states⁹² should increase the understanding of the historical component in the evolution of sexual systems. Phylogeographic⁹³ and COALESCENT ANALYSIS of gene genealogies⁹⁴ offer exciting opportunities to distinguish between the roles of historical contingency and contemporary selective forces in structuring geographical patterns of intraspecific variation in sexual diversity. Last, molecular dissection of floral phenotypes by using QUANTITATIVE TRAIT LOCI-mapping techniques will give new insights into the genetic architecture of sexual traits and contribute crucial data to the debate on the relative importance of major versus minor genes to evolutionary change⁹⁵.

This review has focused primarily on floral design, particularly the strategic deployment of sex organs within and between flowers. But flowers are usually embedded within a larger reproductive milieu that is dictated, in part, by inflorescence characteristics and the architectural forms of plants. Placing plant sexuality in this broader structural framework requires functional analyses of the interactions between floral and inflorescence design, and general patterns of plant growth. Although several recent studies have investigated functional and genetic relations between flowers and inflorescences^{96,97}, we have only a limited understanding, at present, of the hierarchical control of sex allocation⁹⁸ that arises because of the modular growth of plants. Future work on plant sex should abandon the myopic view of flowers as the principal reproductive units and explore the functional interrelations between flowers, inflorescences and plant architecture, within the broader context of life-history evolution.

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