



INVITED REVIEW

Cross- and self-fertilization of plants – Darwin's experiments and what we know now

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The scientific work recorded by Darwin in his book on the cross- and self-fertilization of plants is an exemplar of interesting choices, careful technique, decades of work and methodical analyses that provided a plethora of robust results. The conclusions Darwin drew from his studies are, for the most part, valid and still relevant today. They focus primarily on providing data to support Darwin's hypothesis about the nature and importance of the benefits derived from cross-fertilization, although he conceded that a few plants seem to be invariably self-fertilized. The work is also a significant forerunner for the concepts of hybrid vigour and inbreeding depression. A wealth of information relating to flowers, flowering time, pollen efficacy, the results of cross- and self-pollination, the behaviour and impact of insects, seed and seed germination and plant breeding systems is scattered throughout the book. Darwin also related this book to several others he wrote in which some of these topics are included. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, **161**, 357–395.

ADDITIONAL KEYWORDS: cross-pollination – flowering time – inbreeding depression – plant height – seed set – self-pollination – self-sterility.

INTRODUCTION

It is 200 years since the birth of Charles Darwin and 133 years since the first edition of his book, *The Effects of Cross and Self-Fertilisation in the Vegetable Kingdom*, was published by John Murray in London. This paper is based on information largely found in an electronic copy of the first edition (Darwin, 1876; <http://www.gutenberg.org/etext/4346>) and a paper copy of the second edition (Darwin, 1888). For those less familiar with the book, Darwin divided the text into 12 chapters including an introduction and a final discussion of the general results. The first six chapters were devoted to the families and species examined and the results obtained. The next three chapters focused on the characters measured, and summarized the heights and weights (Chapter 7), constitutional vigour (Chapter 8), seed production

(Chapter 9) and the differences between cross- and self-fertilized plants. These were followed by chapters on the means of fertilization (Chapter 10) and the habits of insects with regard to pollination and fertilization (Chapter 11).

At the beginning of the introductory chapter, Darwin stated that there is 'abundant evidence that the flowers of most kinds of plants are constructed so as to be occasionally or habitually cross-fertilised'. He then set out the evidence that cross-fertilization 'is sometimes endured by the sexes being separated, and in a large number of cases . . . of plants [which] are called dichogamous, and have then been divided into two sub-classes: proterandrous [protandrous] species, in which the pollen is mature before the stigma, and proterogynous [protogynous] species, in which the reverse occurs.'. He added that cross-fertilization is 'ensured, in many cases, by mechanical contrivances . . .' and used examples such as heterostyly and the fact that some plants are self-sterile (self-incompatible) or partially so. His final category was flowers which 'present no apparent obstacle of any

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kind to self-fertilisation' but are 'frequently intercrossed, owing to the prepotency of pollen from another individual or variety over the plant's own pollen' (pp. B1 and B2).

Darwin believed 'that flowers are adapted for the production of seed and the propagation of the species' (p. B2), and he was concerned about both the means of cross-fertilization and, more particularly, the beneficial results of cross-fertilization to the succeeding progeny. He also believed that the construction of a flower provided ample opportunity for cross-pollination because self-fertilization was inevitably associated with inferior plants of often reduced fertility, which was detrimental to both short- and long-term survival. The extensive sets of experiments reported in the book were aimed at providing support for Darwin's hypotheses as set out in the text above, but it appears likely that his and other work on both animals and plants would have led him to this conclusion well before the completion of all reported pollinations and publication of the book. In carrying out such detailed work, Darwin provided some of the earliest and most convincing data for the phenomena now described as hybrid vigour and inbreeding depression.

The book contains a prodigious amount of data that took, for some species, 10 years to accumulate, and in total the studies covered 37 years of work according to Darwin himself (p. 4). Darwin was clearly a careful experimenter who made thorough analyses of the data he collected, sometimes aided by collaborators (e.g. Galton on statistics, p. 15), often pointing out any potential shortcomings, e.g. the effect of seasons and of years on the growth and flowering of his experimental plants. It is difficult to give Darwin's results full justice in this paper as there are so many interesting asides to the main themes. For example, he performed interesting pre-experiments and post-experiments on a few select species, and these provided further data for his main experimental work [for example, on the effects of pollen load and on the robustness of the self-incompatibility (sterility) systems of plants]. Darwin read the publications of, and corresponded with, a great number of scientists of the time, both from the UK and Europe, and the references scattered throughout the volume, primarily as footnotes, indicate this. He acknowledged that many relevant publications preceded his own (p. 4). On the other hand, Darwin wrote at a time when relatively few researchers were studying the reproductive biology of plants, and those that were often did so out of a commercial interest in the development of garden flowers (cultivars/varieties) and crop plants. The data he made reference to from the literature and his correspondence are often limited in scope and fragmentary, and occasionally it is difficult to deter-

mine whether or not the work is a personal communication. He often appears to be providing new data on plants previously not included, and this is borne out in the summary table (Table 1) in this article.

Today, the mode of presentation of scientific data is different and, at times, Darwin's presentation of information is laborious. As a result, searching for data in the book can be a tiresome process. However, as is shown below, the data are as relevant now as they were at the time of publication. This paper revisits that classic volume and reviews and discusses the plants Darwin chose for experimentation, the methods he employed in his experiments and his results. It also re-examines his conclusions in the light of what is known today.

THE PLANTS DARWIN USED IN THE EXPERIMENTS

Darwin had already begun experimenting on pollination before the Darwin family purchased Down House in the village of Downe in Kent in 1842, but he then added many plants he found growing wild in the fields surrounding the house and his garden. He had catalogued these within 2 years of moving in. He indicated that, for these plants, there were reasonably sized population numbers. As well as using his own garden plants, he purchased seed from nursery gardens (p. 321).

Darwin made clear in the first chapter (p. 26) that the species selected for experiments belonged to widely distinct families from a variety of countries. He did not indicate the thinking behind his choices of actual species or why particular countries might have been important, and he only occasionally referred to suppliers of seeds or plants. We can speculate that the choice of species must have been influenced by access to local living material, the species supplied as seed from collaborators, and those species he was able to germinate, grow and get to flower either in the garden or glasshouse under the conditions and with the materials and horticultural skills available in England at the time.

The list of plants that he cited in the book is extensive, and Table 1 includes 69 families, 158 genera and approximately 224 species. These numbers confirm that the representation of genera and species in any one family is generally poor, even though, occasionally, he tested several genera from the same family, e.g. Fabaceae, and grouped these family data together. He chose *Zea mays* L. because the plant is monoecious, and there were as yet no other data on a monoecious species (Chapter 6, p. 233). He presented the data 'not in any natural order but in that which was the most convenient for my

Table 1. The species Darwin used in his experiments or referred to in his book

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number
Acanthaceae	<i>Thunbergia alata</i> Bojer ex Sims	Few plants raised; problems with little pollen in anthers in early summer; no seed set early in the season but seeds set in autumn; self-fertile (pp. 96, 277, 331)	X					
Amaranthaceae	<i>Beta vulgaris</i> L.	Highly self-fertile (pp. 228, 230, 289, 367, 399)		X				
Amaryllidaceae	<i>Allium cepa</i> L.	Self-fertile; fewer and smaller capsules under a net (p. 369)						X
Amaryllidaceae	<i>Crinum</i> L.	Largely intercross across species (Lecoq, p. 396)						
Apiaceae	<i>Apium petroselinum</i> L.	Self-fertile (pp. 172, 277)		X				
Apocynaceae	<i>Cantharanthus roseus</i> (L.) G. Don (as <i>Vinca rosea</i> L.)	Excluding insects leads to no seeds set; will set if artificially cross-fertilized (p. 362)						
Apocynaceae	<i>Tabernaemontana echinata</i> Aubl.	Self-incompatible (Müller, pp. 331, 362)					X	
Apocynaceae	<i>Vinca major</i> L.	Excluding insects leads to no seeds set; will set if artificially cross-fertilized (p. 362)						
Araceae	<i>Arum maculatum</i> L.	Flies did escape and were covered with pollen (p. 420)						

Table 1. Continued

Family*	Taxon name†		Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number
Aristolochiaceae	<i>Aristolochia</i> L.	Some flies can escape flowers and travel to another (Hildebrand, p. 420)						
Asteraceae	<i>Carduus arctioides</i> Willd.	Leaves secrete honeydew in hot weather (Treviranus, p. 404)						
Asteraceae	<i>Lactuca sativa</i> L.	Self-fertile (pp. 173, 174, 369, 399)						X
Asteraceae	<i>Senecio cruentus</i> (L'Hér.) DC.	Self-incompatible; tendency to dioecy (pp. 335, 364); hybrid (Moore, p. 335)						
Asteraceae	<i>Senecio cruentus</i> (L'Hér.) DC. (as <i>Cineraria</i>)	Stigmas smeared with pollen from their own flower produced extremely poor seeds which proved incapable of germinating. Visited by bees and then produced plenty of seed. Utterly sterile with pollen from other flowers on the same plant (pp. 335, 364)						
Asteraceae	<i>Senecio heritieri</i> DC.	Hybrids (Moore, p. 335)						
Asteraceae	<i>Senecio maderensis</i> DC.	Hybrids (Moore, p. 335)						
Asteraceae	<i>Senecio populifolius</i> L.	Hybrids (Moore, p. 335)						

Asteraceae	<i>Senecio tussilaginis</i> Less.	Hybrids (Moore, p. 335)		
Balsaminaceae	<i>Impatiens</i> L.	Frequented by hummingbirds (Gould p. 371)		
Balsaminaceae	<i>Impatiens barbigera</i> (species not currently recognized)	Self-fertile (p. 366)		
Balsaminaceae	<i>Impatiens fulva</i> Nutt.	Sterile with its own pollen (Wilder, <i>Gardeners' Chronicle</i> , pp. 341, 367)		
Balsaminaceae	<i>Impatiens noli-me-tangere</i> L.	Self-fertile; some cleistogamous flowers (p. 367)		
Balsaminaceae	<i>Impatiens pallida</i> Nutt.	Sterile with its own pollen (Wilder, <i>Gardeners' Chronicle</i> , p. 341)		
Berberidaceae	<i>Mahonia aquifolium</i> Nutt.	Species intercross largely so cannot reach conclusions (p. 396)		
Berberidaceae	<i>Mahonia repens</i> (Lindl.) G. Don	Species intercross largely so cannot reach conclusions (p. 396)		
Betulaceae	<i>Corylus avellana</i> L.	Certain individuals are protandrous and others protogynous so can cross-fertilize (monoecious) (Müller, p. 390)		
Betulaceae	<i>Corylus avellana</i> L.	Certain individuals are protandrous and others protogynous so can cross-fertilize (monoecious)		
Bignoniaceae	<i>Bignonia</i> L.	Self-incompatible (p. 363)		
Boraginaceae	<i>Borago officinalis</i> L.	Self-fertile; reduced seed set (pp. 185, 186, 276, 293, 323, 362)	X	X

Table 1. Continued

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to greater than 50% seed production by weight or number
Brassicaceae	<i>Brassica campestris</i> L.	Heavy and fine seeds tend to yield the finest plants (Wilson, p. 353)						
Brassicaceae	<i>Brassica napus</i> L.	Pollen from a neighbouring variety of cabbage effaces the action of self-pollen. Pollen can fertilize <i>B. rapa</i> (writer in <i>Gardeners' Chronicle</i> , 1855, p. 395)						
Brassicaceae	<i>Brassica oleracea</i> L.	Self-fertile (pp. 98, 100, 101, 102, 262, 288, 292, 322, 365)	X		X		X	
Brassicaceae	<i>Brassica rapa</i> L.	When grown close to <i>B. napus</i> seedlings, resemble that species (a writer in <i>Gardeners' Chronicle</i> , 1855, p. 395)						
Brassicaceae	<i>Iberis amara</i> L.	Highly self-fertile (p. 365)						
Brassicaceae	<i>Iberis umbellata</i> L. (as var. <i>kermesiana</i>)	Highly self-fertile (pp. 103–106, 262, 289, 292, 315, 365, 394)	X		X			X
Brassicaceae	<i>Pringlea</i> W.Anderson ex Hook.f.	Wind-pollinated (p. 410)						
Brassicaceae	<i>Raphanus sativus</i> L.	Self-fertile; seeds per capsule less in self (1.85) than cross (3.5) – moderately self-fertile (pp. 365, 395)						X

Calceolariaceae	<i>Calceolaria</i> Heist.	Highly self-fertile (pp. 87, 369)	X		
Campanulaceae	<i>Campanula carpathica</i>	No seed set when insects excluded (p. 174)			
Campanulaceae	<i>Isotoma</i> (R.Br.) Lindl.	Self-fertile; reduced seed set (pp. 176, 364)			
Campanulaceae	<i>Lobelia fulgens</i> Humb. & Bonpl. ex Willd.	Self-incompatible (pp. 179–182, 274, 292, 294, 295, 323, 364)	X	X (2nd generation)	X
Campanulaceae	<i>Lobelia ramosa</i> Benth.	Self-incompatible (pp. 176–178, 293, 295, 325, 364)	X		
Campanulaceae	<i>Lobelia tenuior</i> R.Br.	(<i>L. ramosa</i> is probably a white variety of <i>L. tenuior</i> , p. 176)			
Campanulaceae	<i>Specularia perfoliata</i> A.DC.	Self-fertile; cleistogamic flowers (p. 174)			
Campanulaceae	<i>Specularia speculum</i> A.DC.	Self-fertile; Diptera pollinate (pp. 174–176, 276, 293, 369); petals close at night	X		X
Cannaceae	<i>Canna warszewiczii</i> A.Dietr.	Highly self-fertile (pp. 230, 278, 294, 323, 325, 369)		X	
Caryophyllaceae	<i>Dianthus caryophyllus</i> L.	Few capsules; few good seeds (pp. 132–139, 263, 274, 292, 309, 316, 319, 323, 325, 360)	X		X (changed over three generations)
Caryophyllaceae	<i>Lychnis dioica</i> L.	Rudimentary stamens and pistils are found (p. 413)			
Caryophyllaceae	<i>Stellaria media</i> (L.) Vill.	Self-fertile (p. 367)			X
Caryophyllaceae	<i>Viscaria oculata</i> Lindl.	Self-fertile (relative fertility measured as seeds per capsule low) (p. 323)	X	X	X

Table 1. Continued

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time			The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number
Cistaceae	<i>Cistus</i> L.	Hybrids which could be fertilized by pollen from any other but not by its own pollen. (from <i>Variation under Domestication</i> , p. 306)							
Convolvulaceae	<i>Convolvulus tricolor</i> L.	Cannot be kept pure unless kept at distance from other varieties (Verlot, p. 55)							
Convolvulaceae	<i>Ipomoea purpurea</i> (L.) Roth	Studied over 10 generations (pp. 28–62, 257–259, 289, 291, 297, 300, 308, 314, 322, 324, 368, 399)	X		X			X	
Convolvulaceae	<i>Ipomoea purpurea</i> (L.) Roth (as <i>Convolvulus major</i> Hort. ex Nichols)	Thrips seen to carry pollen to stigma. When growing outdoors habitually crossed by insects. Highly self-fertile. Ten generations studied of selfed & crossed plants – crossed plants larger & with greater vigour, but effects diminish over generations – impaired fertility of self-fertilized plants (p. 28)							

X

Dipsacaceae	<i>Scabiosa atropurpurea</i> L.	Protandrous, self-fertile; (very low self-seed set, p. 172)
Ericaceae	<i>Calluna vulgaris</i> (L.) Hull	Bees visit (p. 424)
Ericaceae	<i>Erica tetralix</i> L.	Perforated corolla (pp. 424, 429, 437)
Ericaceae	<i>Kalmia latifolia</i> L.	Self-incompatible (Beal, p. 359)
Ericaceae	<i>Rhododendron azaleoides</i> Desf.	Hybrid; bumble bees pollinate and rob flowers (p. 435)
Euphorbiaceae	<i>Mercurialis annua</i> L.	Moths eat pollen (p. 421)
Fabaceae	<i>Acacia magnifica</i> (species not currently recognized)	Fluid secreted by phyllodia which stand near the flowers is connected with their fertilization (Leighton, p. 407)
Fabaceae	<i>Acacia sphaerocephala</i> Cham. & Schtdl.	Secretions attract ants to defend plants (Delpino, Belt, p. 406)
Fabaceae	<i>Coronilla</i> L.	Bees may fertilize the flowers while sucking from holes on the side of the calyx (Farrar, p. 407)
Fabaceae	<i>Cytisus laburnum</i> L.	Self-fertile; reduced seed set (p. 362)
Fabaceae	<i>Erythrina</i> L.	Self-fertile (MacArthur); needs pollinator (p. 360)
Fabaceae	<i>Hedysarum onobrychis</i> L.	Visited by bees (p. 361)
Fabaceae	<i>Lathyrus grandiflorus</i> Sibth. & Sm.	Needs pollinator (pp. 155, 360)
Fabaceae	<i>Lathyrus nissolia</i> L.	Self-fertile (p. 367)

Table 1. Continued

Family*	Taxon name†		Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number
Fabaceae	<i>Lathyrus odoratus</i> L.	Darwin's notes on breeding systems				X		X
Fabaceae	<i>Lathyrus sylvestris</i> L.	Self-fertile; progeny from crossing varieties showed characteristics of hybrid vigour (pp. 153–160, 265, 295, 304, 316, 325, 367)						
Fabaceae	<i>Lotus corniculatus</i> L.	Corolla perforated (p. 432)						
Fabaceae	<i>Lotus</i>	Self-incompatible (p. 361)						
Fabaceae	<i>Lupinus luteus</i> L.	Self-fertile (pp. 147–148, 294, 296, 367, 399)	X				X	X
Fabaceae	<i>Lupinus pilosus</i> L.	Self-fertile (pp. 149, 367)	X					
Fabaceae	<i>Medicago lupulina</i> L.	Self-fertile; smaller seed by weight (means of fertilization described by Keimung <i>et al.</i> and Henson, p. 368)						
Fabaceae	<i>Melilotus officinalis</i> (L.) Lam.	Self-fertile; reduced seed set (p. 360)						
Fabaceae	<i>Ononis minutissima</i> L.	Self-fertile (pp. 167–68, 323, 367)	X					
Fabaceae	<i>Phaseolus multiflorus</i> Willd.	Self-fertile; reduced seed set; bumble bees can perforate flowers to collect nectar (pp. 433, 150–152, 168, 276, 293, 316, 360, 438)	X		X		X	
Fabaceae	<i>Phaseolus vulgaris</i> L.	Self-fertile but can be cross-pollinated (pp. 153, 168, 367)						X

Fabaceae	<i>Pisum sativum</i> L.	Self-fertile; insects rarely visit the flowers (p. 169); progeny from crossing varieties showed characteristics of hybrid vigour (pp. 160, 264, 367)	X	X
Fabaceae	<i>Sarothamnus scoparius</i> (L.) Wimm. ex W.D.J. Koch [= <i>Cytisus scoparius</i>]	Self-fertile but needs pollinator; low self-seed set (p. 163 ff.); plants from self-pollinations more susceptible to cold (pp. 166–167)	X	
Fabaceae	<i>Trifolium arvense</i> L.	Self-fertile; flowers are very small but visited by bees incessantly (pp. 367, 386)		X
Fabaceae	<i>Trifolium incarnatum</i> L.	Probably self-incompatible; very reduced seed set and poor quality seed when insects excluded (p. 361) (Bond, p. 368)	X	
Fabaceae	<i>Trifolium minus</i> Relhan	Pollinated by small moths (Bond, p. 368)		
Fabaceae	<i>Trifolium pratense</i> L.	Self-incompatible; bumble bee pollinated (pp. 361, 429, 438)	X	
Fabaceae	<i>Trifolium procumbens</i> L.	Self-fertile; suspected moth pollinated (p. 368)		
Fabaceae	<i>Trifolium repens</i> L.	Probably self-incompatible; very reduced seed set when insects excluded (p. 361)	X	
Fabaceae	<i>Vicia faba</i> L.	Self-fertile; reduced seed set when insects excluded (pp. 360, 405)	X	
Fabaceae	<i>Vicia hirsuta</i> (L.) Gray	Self-fertile; smallest flowers of any British leguminous plant (p. 367)		X

Table 1. *Continued*

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time			The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number
Fabaceae	<i>Vicia sativa</i> L.	Self-fertile (p. 367)							X
Fabaceae	<i>Wisteria sinensis</i> Sweet	7000 pollen grains for each ovule (Editor of <i>Botanical Register</i> , p. 378); perforation of corolla by bees (Gentry, p. 430)							
Gentianaceae	<i>Bartonia aurea</i> Lindl.	Self-fertilized progeny equal or superior to the crossed (pp. 170, 171, 277)	X						
Gentianaceae	<i>Chironia decussata</i> Vent.	Hypopetalous nectary (Link, p. 404)							
Geraniaceae	<i>Geranium phaeum</i> L.	Flowers continue to produce nectar (p. 423)							
Geraniaceae	<i>Pelargonium zonale</i> L'Hér.	Few capsules set when insects excluded (p. 359); some individuals feebly dichogamous, some plants strongly protandrous (pp. 142, 143, 301)	X						X
Gesneriaceae	<i>Gesneria</i> L.	Most or all the species are dichogamous (Ogle, p. 92)							
Gesneriaceae	<i>Gesneria pendulina</i> Lindl.	Crossed & selfed plants similar (pp. 92, 322)							X
Goodeniaceae	<i>Leschenaultia formosa</i> R.Br.	Self-incompatible (Darwin in <i>Gardeners' Chronicle</i> , p. 364)							

Grossulariaceae	<i>Ribes aureum</i> Pursh	Nectar producing (Beal, p. 435)			
Hydrophyllaceae	<i>Nemophila insignis</i> Benth.	Self-fertile (pp. 182–185, 293, 303, 316, 323)	X		X
Iridaceae	<i>Gladiolus</i> L.	Hybrids which could be fertilized by pollen from any other but not by its own pollen. (from <i>Variation under Domestication</i> , p. 306)			
Iridaceae	<i>Iris</i> L.	Secretions from calyx (p. 404)			
Juglandaceae	<i>Juglans regia</i> L.	Some individuals proterandrous (Delpino, p. 391)			
Lamiaceae	<i>Ajuga reptans</i> L.	Self-fertile but more seeds in plants not netted (p. 368)			X
Lamiaceae	<i>Lamium album</i> L.	Visited by insects (Muller, p. 391; Bennett, p. 419)			
Lamiaceae	<i>Lamium purpureum</i> L.	Visited by insects (Bennett, p. 419)			
Lamiaceae	<i>Nepeta glechoma</i> Benth.	Visited by bees (Bennett, p. 419)			
Lamiaceae	<i>Origanum vulgare</i> L.	Crossed plants superior (pp. 94, 95, 292, 301)	X		
Lamiaceae	<i>Salvia coccinea</i> Juss. ex Murr.	Self-fertile; low seed production when not artificially self-pollinated (p. 363), but variable (p. 322)	X		X
Lamiaceae	<i>Salvia glutinosa</i> L.	Corollas can be perforated by bees (Ogle, p. 430)			
Lamiaceae	<i>Salvia grahamii</i> Benth.	Many perforated flowers; also visited by bumblebees (pp. 429, 431, 436)			
Lamiaceae	<i>Salvia tenorii</i> Spreng.	Self-fertile; very reduced seed set; pollinated by bees (p. 362)			X
Lamiaceae	<i>Stachys coccinea</i> Hook. & Arn.	Bees pollinate but some perforate flowers (pp. 431, 436)			

Table 1. *Continued*

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number
Liliaceae	<i>Lilium auratum</i> Lindl.	Sterile with its own pollen (Wilder, <i>Gardeners' Chronicle</i> , pp. 341, 367)						
Limnanthaceae	<i>Limnanthes douglasii</i> R.Br.	Highly self-fertile (pp. 145, 146, 293, 316, 323, 357, 399)	X		X			X
Linaceae	<i>Linum grandiflorum</i> Desf.	Self-sterile (p. 343)						
Linaceae	<i>Linum usitatissimum</i> L.	Self-fertile (Hoffman, p. 366)						
Linderniaceae	<i>Lindernia nummulariifolia</i> (D.Don) Wettstein [as <i>Vandellia nummularifolia</i> D.Don]	Self-fertile; bears perfect flowers; self-fertilization produces more seed (pp. 90, 278, 315, 322, 369)	X					X
Lythraceae	<i>Cuphea purpurea</i> Hort. ex Lem.	Self-fertile (pp. 323, 362)						
Malvaceae	<i>Abutilon darwinii</i> Hook.f.	Self-sterile in Brazil (Müller); self-sterile in the early part of the season, self-fertile later in the season; some plants moderately self-fertile in a single generation in England; pollinated by hummingbirds (pp. 333–335, 344, 358)						

Malvaceae	<i>Hibiscus africanus</i> Mill.	Self-fertile (pp. 140, 277, 292, 296; Kölreuter, p. 378)	X	X
Malvaceae	<i>Hibiscus vesicarius</i> Cav.	More pollen produced than necessary (Kölreuter, p. 378)		
Melastomataceae	<i>Centradenia floribunda</i> Planch.	Self-fertile; needs pollinator (p. 364)		
Melastomataceae	<i>Heterocentron mexicanum</i> Hook. & Arn.	Self-fertile; need pollinator (p. 364)		
Melastomataceae	<i>Monochaetum ensiferum</i> Hook.	Self-fertile; needs pollinator (p. 364)		
Melastomataceae	<i>Pleroma</i> D.Don	Self-fertile; few capsules set when insects excluded (p. 364)		
Melastomataceae	<i>Rhexia glandulosa</i> Bonpl.	Self-fertile; few capsules set when insects excluded (p. 364)		X
Menyanthaceae	<i>Villarsia parnassifolia</i> R.Br.	Produces a single flower daily (Kerner, p. 392)		
Nyctaginaceae	<i>Mirabilis</i> L.	Number of grains necessary for fertilization (p. 378)		
Nymphaeaceae	<i>Euryale amazonica</i> Poepp.	Lower seed set in capsules; self-fertile (Smith, p. 358)		
Nymphaeaceae	<i>Euryale ferox</i> Salisb.	Highly self-fertile (Caspary, p. 365)		
Nymphaeaceae	<i>Nymphaea</i> L.	Some species self-incompatible; some species self-fertile (Caspary, pp. 358, 365)		
Nymphaeaceae	<i>Victoria regia</i> Lindl.	Caspary, p. 365		
Oleaceae	<i>Forsythia viridissima</i> Lindl.	Not self-fertile (Wilder, p. 341, but eliminated in 1888 version)		

Table 1. *Continued*

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number
Oleaceae	<i>Fraxinus ornus</i> L.	Leaves as well as stems secrete (<i>Gardeners' Chronicle</i> , 1855, p. 404)						
Onagraceae	<i>Clarkia elegans</i> Dougl.	Self-fertile – crossed & self-fertilized plants produced capsules (pp. 169, 170, 294, 296, 316)	X				X	X
Orchidaceae	<i>Epipactis latifolia</i> All.	Pollinated by wasps (p. 426)						
Orchidaceae	<i>Maxillaria</i> Ruiz & Pav.	Sterile with own pollen (Munro, p. 331)						
Orchidaceae	<i>Oncidium</i> Sw.	Sterile with own pollen (Munro, p. 331)						
Orchidaceae	<i>Ophrys apifera</i> Huds.	Self-fertile; cleistogamous flowers (pp. 350, 369, 408, 442)						
Orchidaceae	<i>Ophrys insectifera</i> L. [as <i>O. muscifera</i> Huds.]	Flowers are rarely visited by insects, plants not self-fertile (pp. 385, 408)						
Orchidaceae	<i>Spiranthes autumnalis</i> (Balb.) Rich.	Pollen masses cannot be applied to the stigma until the labellum and rostellum have moved apart (pp. 393, 423, 424)						
Orobanchaceae	<i>Agalinis pedicularia</i> (L.) S.F.Blake [as <i>Gerardia pedicularia</i> L.]	Perforated corolla (Bailey, pp. 430, 437)						

Orobanchaceae	<i>Bartsia odontites</i> Huds.	Self-fertile but some seed shrivelled (p. 369)	
Orobanchaceae	<i>Euphrasia officinalis</i> L.	Self-fertile, flies get covered in pollen (p. 368)	
Orobanchaceae	<i>Rhinanthus alectorolophus</i> (Scop.) Pollich	Bees perforate (Müller, p. 435)	
Papaveraceae	<i>Adlumia cirrhosa</i> Raf.	Highly self-fertile (p. 366)	X
Papaveraceae	<i>Argemone ochroleuca</i> Sweet	Self-fertile (Hildebrand, p. 366)	
Papaveraceae	<i>Corydalis cava</i> Schweigg. & Kort.	Pollen from other flowers on same plant is more effective than self. Self-incompatible; self-fertile. (Hildebrand, Caspary, pp. 331, 358)	
Papaveraceae	<i>Corydalis halleri</i> Willd.	Slightly self-incompatible (Caspary, p. 331)	
Papaveraceae	<i>Corydalis intermedia</i> Mérat	Self-fertile (Caspary, p. 331)	X
Papaveraceae	<i>Corydalis lutea</i> (L.) DC.	Self-fertile but covered plant produced half as many capsules as uncovered – inbreeding depression (p. 359)	
Papaveraceae	<i>Corydalis ochroleuca</i> W.D.J.Koch	Flower mechanism like <i>C. lutea</i> – spontaneously fertilized flowers are by no means sterile (Hildebrandt, p. 359)	
Papaveraceae	<i>Corydalis solida</i> Sw.	Self-incompatible	
Papaveraceae	<i>Corydalis solida</i> Sw.	Self-incompatible (p. 358)	

Table 1. Continued

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to greater than 50% seed production by weight or number
Papaveraceae	<i>Eschscholzia californica</i> Cham.	Self-incompatible in Brazil; few capsules in UK (pp. 109–116, 263, 275, 290, 292, 294, 315, 319, 322, 332, 340, 343, 358, 444, 449)		X	X (5 out of 9 plants)		X (in 2nd year)	X
Papaveraceae	<i>Fumaria capreolata</i> L.	Self-fertile (p. 366)						
Papaveraceae	<i>Fumaria officinalis</i> L.	Self-fertile (Darwin & Müller, p. 366)						
Papaveraceae	<i>Glaucium luteum</i> Scop.	Self-fertile (Hildebrand, p. 366)						
Papaveraceae	<i>Hypecoum grandiflorum</i> Benth.	Highly self-sterile (Hildebrand, pp. 331, 359)						
Papaveraceae	<i>Hypecoum procumbens</i> L.	Self-fertile (Hildebrand, pp. 331, 366)						
Papaveraceae	<i>Papaver alpinum</i> L.	Few germinable seeds. Quite self-sterile (Hoffman, pp. 331, 358)						
Papaveraceae	<i>Papaver argemonoides</i> Ces.	Spontaneously self-fertilized (Hildebrandt, p. 366)						
Papaveraceae	<i>Papaver dubium</i> L.	Not so well fitted for self-fertilization as other poppies (Müller, p. 107)						
Papaveraceae	<i>Papaver orientale</i> L.	Crosses freely with other poppy species (p. 108)						
Papaveraceae	<i>Papaver rhoeas</i> L.	Often self-fertilized (p. 107)						

Papaveraceae	<i>Papaver somniferum</i> L.	Self-fertile – produces an abundance of capsules (pp. 108, 331, 365)		
Papaveraceae	<i>Papaver vagum</i> Jord.	Few capsules, few seeds; self-fertile (pp. 107, 109, 315, 358, 398)	X	X
Passifloraceae	<i>Passiflora alata</i> Curtis	Self-impotent but could be fertilized by pollen from its seedlings. No capsules set when insects excluded (Scott, p. 330; Darwin, <i>Variation of animals and plants under domestication</i> , p. 357)		
Passifloraceae	<i>Passiflora caerulea</i> L.	No capsules set when insects excluded (Darwin, <i>Variation of animals and plants under domestication</i> , p. 357)		
Passifloraceae	<i>Passiflora edulis</i> Sims	No capsules set when insects excluded (Darwin, <i>Variation of animals and plants under domestication</i> , p. 357)		
Passifloraceae	<i>Passiflora gracilis</i> J.Jacq.	Self-fertile; annual; fruits from self-pollination contain fewer seeds than those from cross-pollination (pp. 171, 276, 323, 365)	X	X
Passifloraceae	<i>Passiflora laurifolia</i> L.	No capsules set when insects excluded (Darwin, <i>Variation of animals and plants under domestication</i> , p. 357)		

Table 1. *Continued*

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number
Passifloraceae	<i>Passiflora quadrangularis</i> L.	Some individuals set no capsules when insects excluded (Darwin, <i>Variation of animals and plant under domestication</i> , p. 357)						
Passifloraceae	<i>Passiflora racemosa</i> Brot.	No capsules set when insects excluded (Darwin, <i>Variation of animals and plant under domestication</i> , p. 357)						
Phrymaceae	<i>Mimulus luteus</i> L.	Highly self-fertile (pp. 10, 63–81, 259–61, 286, 296, 301, 307, 315, 319, 322, 324, 348, 369, 393, 399)	X		X (half)		X (half)	X
Phrymaceae	<i>Mimulus roseus</i> Dougl. ex Lindl.	Bees enter flowers and get coated with pollen, self-fertilization prevented (p. 63)						
Plantaginaceae	<i>Antirrhinum majus</i> L.	Self-fertile; reduced seed set, few perforated corollas (pp. 363, 432)						
Plantaginaceae	<i>Digitalis purpurea</i> L.	Self-incompatible; few poor capsules (pp. 81, 84–87, 288, 299, 363, 452)		X				
Plantaginaceae	<i>Linaria cymbalaria</i> (L.) Mill.	Freely visited by bees (pp. 386, 428)						

Plantaginaceae	<i>Linaria vulgaris</i> (L.) Mill.	Self-incompatible (pp. 9, 88, 322, 363)	X		
Plantaginaceae	<i>Penstemon argutus</i> Paxton	Perforated flowers (p. 429)			
Plantaginaceae	<i>Plantago</i> L.	Pollen weighed (Blackley, p. 378); changing from wind to insect pollination (Delpino & Müller, p. 403)			
Plantaginaceae	<i>Veronica agrestis</i> L.	Self-fertile (p. 369)			X
Plantaginaceae	<i>Veronica chamaedrys</i> L.	Insects visit these flowers (p. 369)			
Plantaginaceae	<i>Veronica hederifolia</i> L.	Insects visit these flowers (p. 369)			
Poaceae	<i>Leersia oryzoides</i> (L.) Sw.	Produces an abundance of cleistogamous flowers, but rarely capable of cross-fertilization (Darwin, <i>Different forms of flowers</i> , p. 350)			
Poaceae	<i>Phalaris canariensis</i> L.	Hermaphrodite grass better suited for cross-fertilization (Hildebrand, p. 235). Different results in open ground (pp. 236, 237, 293)	X	X (in open ground)	X (in pots)
Poaceae	<i>Zea mays</i> L.	Monoecious; self-fertile (pp. 16–18, 233–4, 288, 293, 369)	X	X	X
Polemoniaceae	<i>Leptosiphon</i> Benth.				
Polemoniaceae	<i>Leptosiphon androsaceus</i> Benth.	Self-fertile (p. 368)			
Polygonaceae	<i>Fagopyrum esculentum</i> Moench	Early flowering of crossed plants, heterostyled (pp. 228, 293)	X	X	
Polygonaceae	<i>Rheum rhaponticum</i> L.	Part wind, part insect pollinated (Diptera) (p. 403)			

Table 1. *Continued*

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time			The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number
Primulaceae	<i>Anagallis collina</i> Schousb.	Self-fertile; extraordinary difference in fertility between the crossed and self-fertilized plants (pp. 217, 218, 267, 317, 323, 325)	X					X	
Primulaceae	<i>Cortusa matthioli</i> L.	Self-fertile (Scott, p. 362)							
Primulaceae	<i>Cyclamen persicum</i> Mill.	Self-incompatible (pp. 215, 216, 293, 317, 323, 362, 448)	X		X			X	
Primulaceae	<i>Cyclamen repandum</i> Sm.	Self-fertile but inbreeding depression (Lecoq, p. 215)							
Primulaceae	<i>Primula</i> L. [as <i>Polyanthus</i> , a cultivated form or hybrid of <i>P. veris</i> L.]	Prepotency of pollen – used as source of pollen (pp. 397, 398)							
Primulaceae	<i>Primula elatior</i> Hill	Bees collect from short styled variety (Müller, p. 425). Bumblebees try to suck at mouths of flowers, but fail, so make holes (Müller, p. 430)							
Primulaceae	<i>Primula grandiflora</i> Bastard	One of parents of hybrid (with <i>P. officinalis</i>) (Gordon, p. 380)							
Primulaceae	<i>Primula mollis</i> Nutt. ex Hook.	Self-fertile; homomorphic (not heterostylous) (Scott, p. 368)							X

Primulaceae	<i>Primula officinalis</i> Hill	One of parents of hybrid (with <i>P. grandiflora</i>) (Gordon, p. 380)			
Primulaceae	<i>Primula scotica</i> Hook.	Self-fertile; not heterostyled; no capsules set if insects excluded (Scott, p. 362)	X	X	
Primulaceae	<i>Primula sinensis</i> Lour.	Heterostyled; self-fertile (pp. 225, 227, 279, 293, 296)			
Primulaceae	<i>Primula veris</i> L. (variety sent by Scott)	Equal styled and red-flowered variety; self-fertile (p. 222)	X	(X, p. 223)	
Primulaceae	<i>Primula veris</i> (as var. <i>officinalis</i>)	Heterostyled (pp. 219 ff.); insect pollination effects correct pollen transfer; pollination by the same form gives either no seed or reduced seed set; seedlings from the latter pollinations are often dwarfed and more or less sterile (pp. 219, 221, 267, 268, 293, 317, 351, 397–398)	X		X
Proteaceae	<i>Synaphea</i> R.Br.	Stigma is screened from the pollen in the same flower by being held by a stamen (Bentham, p. 415)			
Ranunculaceae	<i>Aconitum napellus</i> L.	Strongly protandrous; it would therefore be more or less sterile unless bees carried pollen from the younger to the older flowers. White but not blue flowers are perforated by bees – these not pollinated and are rarer (Ogle, p. 431)			

Table 1. Continued

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time		The effect of exclusion of insects§			
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross flowers earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number	
Ranunculaceae	<i>Adonis aestivalis</i> L.	Self-fertile (Hoffmann claimed it is proterandrous) (pp. 128, 277, 365)		X						
Ranunculaceae	<i>Anemone</i> L.	Self-fertile (p. 396)								
Ranunculaceae	<i>Delphinium consolida</i> L.	Many capsules; c. 50% seed set; self-fertile (pp. 129, 130, 322, 358, 423)	X							X
Ranunculaceae	<i>Ranunculus acris</i> L.	Self-fertile (p. 365)								X
Resedaceae	<i>Reseda lutea</i> L.	Some individuals few and poor capsules; some individuals self-fertile (pp. 117–119, 339, 365)	X						X	
Resedaceae	<i>Reseda odorata</i> L.	Some individuals self-incompatible; some individuals self-fertile (pp. 119–123, 289, 316, 328, 336, 358, 365)	X	X						X
Rosaceae	<i>Geum urbanum</i> L.	Pollen produced only 10× too much (Gartner, p. 378)								
Rosaceae	<i>Poterium sanguisorba</i> L.	Has become anemophilous whilst most Rosaceae remain entomophilous (p. 410)								
Rosaceae	<i>Prunus avium</i> (L.) L.	Nectar-secreting glands on petioles wither away on old leaves (Reinke, p. 405)								

Rosaceae	<i>Prunus laurocerasus</i> L.	Secreting nectar does not attract ants or wasps (p. 405)		
Rubiaceae	<i>Galium aparine</i> L.	Self-fertile (p. 369)		
Rubiaceae	<i>Posoqueria fragrans</i> Darwin	Movement of stamen on pollination contrivance (Müller, pp. 5, 393)		
Rutaceae	<i>Dictamnus fraxinella</i> Pers.	Bees visit flowers (p. 419)		
Scrophulariaceae	<i>Verbascum lychinitis</i> L.	Self-fertile (pp. 89, 341, 369)		X
Scrophulariaceae	<i>Verbascum nigrum</i> L.	Self-incompatible (pp. 89, 330, 341)		
Scrophulariaceae	<i>Verbascum phoeniceum</i> L.	Self-incompatible but can become self-fertile (pp. 330, 341, 364)		
Scrophulariaceae	<i>Verbascum thapsus</i> L.	Highly self-fertile; frequently visited by pollen-collecting bees (pp. 89–90, 341, 369)	X	
Solanaceae	<i>Brugmansia</i> Pers.	Long-beaked hummingbirds fertilize but short-beaked ones bore holes in flowers (Müller, p. 371)		
Solanaceae	<i>Nicotiana</i> L.	Can be used for crosses (Kölreuter, p. 210)		
Solanaceae	<i>Nicotiana glutinosa</i> L.	Highly self-fertile (pp. 203, 205–208, 210, 212–215, 266–7, 279, 288–290, 293–295, 323, 325, 349, 368)	X	X (in 3rd year)
Solanaceae	<i>Nicotiana tabacum</i> L.			
Solanaceae	<i>Nolana prostrata</i> L.f.	Self-fertile (pp. 186, 187, 277, 321, 323)	X	

Table 1. Continued

Family*	Taxon name†	Darwin's notes on breeding systems	Plant height‡		Flowering time			The effect of exclusion of insects§	
			Progeny of cross taller than selfed	Progeny of cross not taller than selfed	Progeny of cross earlier than selfed	Progeny of cross and self flower at same time	Progeny of self flowers earlier than cross	Leads to less than 50% seed production by weight or number	Leads to greater than 50% seed production by weight or number
Solanaceae	<i>Petunia violacea</i> Lindl.	No capsules set when insects excluded; fewer capsules set from self-pollination than from cross-pollination (pp. 188 ff., 265, 274, 289, 293, 294, 309, 316, 319, 323, 325, 362)	X		X		X (3 out of 5 in 4th generation)	X (changed over 2 generations)	
Solanaceae	<i>Solanum tuberosum</i> L.	For some varieties, there was seed set only when varieties were crossed (Tinzmann, 1846, p. 362); visited by Diptera (Müller) and bumblebees (p. 369)							X
Strelitziaceae	<i>Strelitzia</i> Ait.	Fertilized by Nectarinidae (p. 371)							
Tropaeolaceae	<i>Tropaeolum minus</i> L.	Self-fertile, protandrous; flowers adapted for cross-fertilization by insects (pp. 144, 145, 293, 316, 323)	X		X				X

Tropaeolaceae	<i>Tropaeolum tricolor</i> Lindl. (named as <i>tricolorum</i> in volume)	Self-fertile (table F, p. 323); nectar producer (p. 430)			
Typhaceae	<i>Typha</i> L.	Wind-pollinated (p. 377)			
Urticaceae	<i>Cecropia</i> Loefl.	Small food bodies produced to attract ants (Müller, p. 406)			
Violaceae	<i>Viola canina</i> L.	Excluding insects leads to no seeds set; will set if artificially fertilized (p. 357)			
Violaceae	<i>Viola tricolor</i> L.	Self-fertile but excluding insects leads to poor capsules with few seeds; partially sterile; inbreeding depression is evidenced (pp. 123, 126, 127, 286, 289, 292, 296, 304, 316, 325, 358, 423 ff.)	X		X
Zingiberaceae	<i>Hedychium</i> J.Koenig	Self-fertile; needs pollinator (p. 364)			

Totals: families, 69; genera, 158; species, 224 (an approximate number as a result of some plants only being named to genus by Darwin).

*Families follow APG III (2009).

†Species names are given unless this is unclear; then only the genus is given.

‡Plant height and seed weight data are from Chapter 7; plant height comparisons were sometimes based on only four plants.

§Species were interpreted as primarily cross-fertilized (< 50% seed set) or often self-fertilized (> 50% seed set) when insects were excluded.

purpose'. The purpose of his efforts was to show that nature abhors selfing and its products.

Table 1 has been populated with data from Darwin and references of others cited in the book, in an attempt to bring disparate data into a single, easily accessible listing.

DICHOGAMY

Darwin did not discover dichogamy, but he did see it as playing an important role in effecting cross-pollination and thus supporting his hypothesis. He cited Hildebrand (1867: 383) that dichogamy is found in the greater number of species. In several cases, at the start of sections on particular species, there is a description of the plants being 'proterandrous' or 'strongly proterandrous' (for example, pp. 129, 142 or 185) and adapted for cross-fertilization by insects (Table 1).

FLORAL MORPHOLOGY

Darwin often described the structure of flowers, visits of insects and contrivances that enabled or facilitated cross-pollination (for example, p. 123, *Viola tricolor* L.; p. 383, papilionaceous species. Some of these data may have been obtained from other work to which he often referred, but he used the information both to explain how he would pollinate flowers and why specific results were obtained.

Darwin's most surprising omission is his lack of voucher specimens for the species he used, but apparently this was known to his peers (Professor John Parker, Director, University of Cambridge Botanic Garden, personal communication, 2009). In Table 1, names Darwin and others used are listed, but the absence of vouchers makes further identification of exactly what he used impossible. Data for some genera are therefore recorded without a species name.

EXPERIMENTAL TECHNIQUES

Most experiments were carried out in the glasshouses and garden of Down House in Kent, largely in a potting shed behind the house, from 1842 until publication of the book in 1876. He was supported by at least one gardener and sometimes helped in the experimentation by his children (Boulter, 2008).

In the introductory remarks to Chapter 1, Darwin provided interesting insights into the various methods he used, such as isolating plants for pollination under nets, germinating seed 'on the chimney piece in a warm room' and the selection of germinated seed, growing plants in pairs and in competition in pots or rows, germinating light-sensitive seeds in pots, the uniform composition of soil in pots, water-

ing, separation by a superficial partition and illumination of self and crossed progeny (pp. 10–13).

CONTROLLED POLLINATIONS

The reasons for, and the thinking behind, the pollination techniques used are not entirely clear and often are not recorded. Darwin may simply have used those suggested by correspondents or adapted methods he had read about. He could certainly have gained technical knowledge from published work, through correspondence and perhaps through specific visits to sites at which people were undertaking pollination and propagation work. Pollination techniques of the sort he employed would have been routinely used by nursery breeders and growers. Undoubtedly, Darwin would have been eager to gain such skills. In 1838, there is a record of Darwin in Loddiges garden in Hackney where he remarked upon the 1279 varieties of roses, but there is no record that he obtained any pollination or horticultural skills from here. Darwin may also have gained some technical pollination data from his voyage on the *Beagle*, but he did not record this. Some of the techniques he employed had often been used before [e.g. Thomas Fairchild's work with pinks (species and hybrids of *Dianthus* L.) in 1717], and Darwin mentions many papers from the 1840s and 1850s which he read in the *Gardeners' Chronicle* [for example, Anderson (1853) on the need for excluding insects from experiments], but he might have developed or modified some techniques himself. Isaac Anderson, who was involved in hybridization, was also a correspondent (see <http://www.darwinproject.ac.uk/darwinletters/calendar/entry-3948.html>). Many of the techniques Darwin used are those which could be employed today.

Darwin transferred pollen from anther to stigma using a paintbrush, but he did not tell us how he cleaned the pollinating brush to ensure no cross-contamination. He ensured that he placed sufficient pollen on the stigma to cover the stigmatic surface (p. 24), although he did not attempt to monitor whether exactly the same amounts were put on each stigma when making cross- and self-pollinations. For him cross-fertilization 'always means a cross between distinct plants which were raised from seeds and not from cuttings or buds' (p. 10). Self-fertilization 'always implied that the flowers in question were impregnated with their own pollen' and the seed collected was the product of self-fertilized flowers. In his cross- and self-pollination experiments, Darwin used muslin netting to prevent the entry of insects to flowers, and he thus prevented inadvertent contamination of stigmas by pollen from flowers other than those which he had himself deposited. However, when performing crosses, Darwin did not emasculate ('castrate') the

flowers by removing the stamens, and thus a degree of self-pollination would have been inevitable (p. 23). As a comparison with the selfing and crossing in the experimental plants, he allowed others to open-pollinate to ensure that the plants were capable of setting fruit and seed when untreated by him (he appears to have used little of this open-pollinated seed in further experimentation).

In fact, he carried out a host of carefully controlled self- and cross-pollinations which are all set out in the volume. He collected the resulting seed, examined its colour and seed size, and measured seed weight. He then germinated seeds and grew on the plants, often in strong competition situations, and, by this means, he was able to provide considerable evidence to support his hypothesis that outcrossing (outbreeding) was beneficial, whereas selfing (inbreeding) was detrimental.

In some experiments, he attempted to pollinate immature stigmas because he believed that cross-pollen might become effective when the stigma became 'matured' (receptive). Little is said in the book about how he or others determined that stigmas had developed to a state of receptivity to pollen (today, we can view amounts of secretions or use histochemical techniques) or that he used bud pollination techniques. He also experimented with mixed pollinations, such as self-pollination followed 23 h later by cross-pollination. From this, he derived his hypothesis about the prepotency of cross-pollen (p. 393 onwards; see below for a discussion on this topic). He was also interested in the effect of pollen load: the potential effects caused by large rather than small amounts of pollen application on subsequent seed set. Information and a discussion about this are described below (section on the effect of pollen load).

Occasionally, Darwin recorded different characters in different experiments to reduce what he perceived as a greater chance of error. For example, in the case of the relative fertility of flowers crossed with either pollen from a distinct plant and with their own pollen, he attempted to remove the error of pollen being applied to the stigma at the wrong time being the cause of failure to produce effects, rather than the greater or lesser potency of the pollen. This is demonstrated by work recorded in table F (p. 322/3) which shows degrees of fertility from cross- and self-pollination based on the average number of seeds per capsule. He pointed out, citing *Nolana prostrata* Hort. Monsp. ex Dunal, that had he recorded the number of capsules, the difference between the results of self- and cross-pollination would have been more marked. In *N. prostrata*, 30 flowers on a number of plants were cross-fertilized and produced 27 capsules in total, each with five seeds. Thirty-two flowers on the same plants were self-fertilized and produced six capsules

with five seeds in each. As Darwin, in this case, used only the result of the number of seeds per capsule in table F, this meant that the results for crossing and selfing were the same, namely five seeds per capsule.

Darwin also carried out pre-experimentation, with *Linaria* Mill. and *Dianthus*, in relation to the use of plant height as a measure of the differences in plants derived from cross- and self-fertilization (p. 9).

COMPETITIVE GROWTH CONDITIONS

Seeds were germinated and grown in large pots or outside in rows in the cultivated part of the Down House garden. However, Darwin used at least two different cultivation methods. In one, he germinated seeds in sand and then transplanted pairs of plants into soil in pots, one the product of self-fertilization and the other the product of cross-fertilization, germinated at the same time. In the other method, he germinated the seed in soil in pots, because he stated that these seeds were inhibited in their germination by the light (p. 12). For those grown in pots, the seed progeny from self-pollinations were sown on one side and the seed from the same species from cross-pollinations on the other. The seeds were allowed to germinate and the plants to grow to flowering and fruiting. Darwin attempted to use the latter method of planting to provide similar and uniform conditions for all plants and from year to year, although he acknowledged that, in successive generations, seasonal variations did have an effect (p. 21). For some species, successful seed germination produced too many plants (Darwin did not attempt to remove any or thin the growth) and, in this case, he could observe whether some were outcompeted (p. 239).

MEASUREMENTS OF PLANT HEIGHT, WEIGHT AND FLOWERING TIME

Plant height was measured using a ruler. Darwin provided over several pages details of what he did and why (p. 13 and onwards). For some species, Darwin also measured plant weight using a simple balance. He often recorded time from planting to seed germination and time to first flowering for progeny derived from cross-fertilizations and self-fertilizations (for example, p. 146).

FRUIT AND SEED SET, SEED WEIGHT AND MORPHOLOGY

Estimates of fertility (based on fruit and seed set) were made. Counts of seed set were based on: (1) the number of capsules; (2) the number of mature seeds in the mature ovaries or fruits; and (3) seed weight. With regard to seed morphology, he used his eyes and,

possibly, a light microscope (not recorded) to determine the quality of seed produced. Light coloured, small and/or shrivelled seeds were considered to be non-germinable and infertile.

An interesting question is whether the characters he measured would be used today. Some undoubtedly would, but plant height probably would not be deemed to be a reliable measure.

WHAT DID DARWIN DISCOVER? A SUMMARY OF THE RESULTS AND DISCUSSION IN THE BOOK

PREPOTENCY OF CROSS-POLLEN

Darwin believed that cross-pollen was 'prepotent', i.e. heteromorphic pollen was more potent than the homomorphic, selfed kind. He strengthened the case for his hypothesis concerning the benefits of cross-fertilization by providing evidence of the 'fertilising power of pollen from another variety or individual being greater than that of the plant's own pollen' (p. 393 ff.). He performed preliminary work on both *Mimulus luteus* L. and *Iberis umbellata* L., in which self-pollination is automatic. He allowed self-pollination to take place, but he then applied cross-pollen using pollen from varieties with differing flower colour. The result clearly showed that the progeny were derived from the cross-pollination and fertilization, as all offspring had the flower colour to be expected from a cross-fertilization. Darwin carried his experiment further by attempting to discover the period of time needed to elapse before cross-pollen would no longer be as effective or more effective than self-pollen. He self-pollinated ragged jack [Darwin does not give the Latin name, but from our research there are three candidates; the most likely is *Lychnis flos-cuculi* L. and this is probably a locally used name (Britten & Holland, 1886); less likely are *Senecio jacobaea* L. (http://www.cabicompendium.org/NamesLists/CPC/Full/SEN_JA.htm) and *Brassica oleracea* cv. 'Ragged Jack' (<http://www.pfaf.org/database/plants.php?Brassica+oleracea+ramosa>)], and then added cross-pollen 23 h later. He also self-pollinated *Primula veris* L. and added cross-pollen 24 h later. The results for both experiments indicated that the cross-pollen continued to be more effective in fertilization even after these time periods. For *P. veris*, Darwin knew that pollination with the self-pollen was illegitimate, but he had already determined that illegitimate pollination could produce a moderate seed set (p. 398). Prepotency of pollen later became the subject of a paper in *Nature* by Ward (1900), who was aware at the time of double fertilization in the embryo sacs of plants. However, it was not until Bateman (1956) coined the term 'cryptic

self-incompatibility' that a potential link was made between the incompatibility system and the prepotency of pollen, indicating that there is a genetic cause for the increased potency of cross-pollen. Barrett (1988) suggested that prepotency might be widespread in the flowering plants (angiosperms) and that, even for self-compatible species, in those plant families in which homomorphic and heteromorphic incompatibility is found, pollen tube growth is often significantly faster in cross-pollen than in self-pollen. He also indicated that self-pollen may be at a further disadvantage because embryo abortion may be involved in some instances, and embryo abortion may be a feature of inbreeding depression.

THE EFFECT OF POLLEN LOAD (AMOUNT OF POLLEN EMPLOYED)

Darwin noted (p. 377) that flowers produce far greater amounts of pollen than is required for the fertilization of one flower and that this, in part, may be in response to effects of loss as a result of inclement weather (principally rain), pollinator inefficiencies and pollen-eating insects, all arguments which hold up well today. He described in his book the work of others who either counted or weighed pollen to demonstrate the variation in number from 4863 pollen grains in anthers of *Hibiscus* L. to 27 302 050 pollen grains in anthers of *Scirpus lacustris* L. [= *Schoenoplectus lacustris* (L.) Palla]. For *Hibiscus*, Kölreuter (1761) found that, of the 4863 pollen grains, only '60 grains were necessary to fertilize all the ovules of a flower' (p. 378). He also noted early on in the book (p. 24), citing the work of others, that, although as few as 50–60 pollen grains can be enough to fertilize all the ovules in an ovary, too few, perhaps only one or two, can result in poorly formed plants in the progeny. Darwin was also aware of the potential effects of large amounts of pollen on seed, as he had read that too much pollen could be detrimental. He thus undertook an experiment with *Ipomoea purpurea* (L.) Roth to test whether too little or an excess had any demonstrable effect. Although there were small differences in the number of capsules and seed set, and in seed weight or seed germination, for both cross- and self-pollinations, Darwin concluded that these differences were not sufficient, and thus amounts of pollen on the stigma, as long as there were sufficient pollen grains, would not affect his experiments.

EXPERIMENTS ON THE IMPACT OF CROSS- AND SELF-FERTILIZATION

Self-pollination and fertilization using flowers on the same plant

Using *Digitalis purpurea* L., *I. purpurea*, *M. luteus*, *Pelargonium zonale* L'Hér., and *Origanum vulgare* L.,

Darwin tested the hypothesis that crossing with pollen from different flowers on the same plant gives results no different from those when pollinating a flower with its own pollen (p. 297 ff.). He stated (p. 302) that 'the advantages of a cross depend on the progenitors of the crossed plants possessing somewhat different constitutions'. These plants possessing different constitutions would now be called different genotypes.

The effects of self-fertilization on flower colour, morphology and fertility

Although he admitted that he did not consider at first describing flower colour over some generations of self-fertilization, Darwin did note (p. 299 ff.) that flower colour became over time 'absolutely uniform in tint, or very nearly so' for *I. purpurea*, *M. luteus*, *Dianthus caryophyllus* L. and *Petunia violacea* Lindl. derived from self-pollination. He noted that, for example, in *I. purpurea*, self-fertilized plants produced a uniform petal colour after selfing for seven generations (although he suggested that this was apparent one or two generations previously, p. 59). For all these species, raised from purchased seed, the original plants 'varied greatly in colour'. What Darwin was seeing was the change from heterozygosity to homozygosity. He followed on with this work, cross-pollinating the plants of the later generations of self-fertilized plants with what he termed 'fresh' stock, and the progeny returned flowers of 'diversified tints'. This is the situation expected from the Mendelian segregation of genes.

Darwin noted in Chapter 9 (p. 320) that, in *I. purpurea*, the characteristics of self-fertilized plants after seven or eight generations included anthers of reduced size, flowers dropping after pollination and abnormal ('monstrous') flowers. In *P. violacea*, there were far greater numbers of shrivelled pollen grains in plants after four generations of self-fertilization.

Cross-pollination with plants from the same progeny

When intercrossing plants from the same stock, but not from the same individual, the progeny from the crosses, not surprisingly, showed distinct differences in weight from their self-pollinated relatives in the majority of instances (see table B; p. 245 ff.).

Cross-pollinations of selfed plants with fresh stock of the same species

In several of his experiments (see table C; p. 245 ff.), Darwin investigated the result of taking plants derived from self-fertilization, sometimes for several generations, and then crossing them with completely new plants of the same species derived from other stock. The progeny from the crosses showed a significant increase in height, weight and fertility

over those that were derived from self-fertilization, although this result was not universal.

The effect of self- or cross-fertilization on vigour and fecundity (p. 285 and ff.)

Much of the book focuses on measurements made on plants of the same species, raised either from cross-fertilization or self-fertilization often over several generations. Measurements included plant height (in inches), plant weight (in ounces), number of capsules set per plant, the number of seed set per capsule and the weight of a given number of seeds.

Plant height

It appears that Darwin's early work on cross- and self-fertilization in *Linaria vulgaris* (L.) Mill. may have been a reason for using plant height as a character to demonstrate differences created by the impact of inbreeding or outbreeding (p. 88). Darwin often measured only a sample of the tallest plants from each type of pollination (see p. 144). The final plant height, in many cases, was more often greatest in plants derived from cross-fertilization, and there are many tables in the book showing the numerous results that Darwin collected which demonstrated this. Under the competitive growing conditions Darwin often used, he measured the height of young plants derived by selfing and crossing, and, in many cases, the measurements of plant height demonstrated that plants derived from crossing grew taller more quickly than those from selfing.

He also produced, in a sixth generation of selfing in *I. purpurea*, a plant that Darwin named 'the Hero'. This plant arose spontaneously in the self-fertilized population and was taller than the plants from both cross- and self-fertilizations. Darwin continued to self it and compared it with its progeny from cross- and self-pollination in succeeding generations; it consistently bred true, being taller than all the other progeny. This may have been the result of a mutation, or possibly Darwin had, unsuspectingly, perhaps because of the growing conditions he used, selected a trait which resulted in such a tall plant (p. 47 onwards). Darwin considered that 'the Hero' was adapted for self-fertilization, having become more fertile than usual for the species when plants were self-pollinated. He noted that the anthers and pollen of progeny from 'the Hero' were well developed and compared favourably with those of progeny from cross-pollinated rather than selfed plants, and that seed set was higher, at an average of 5.2 seeds per capsule (p. 50). Interestingly, he also noted that 'some few of the flowers produced by the grandchildren of Hero were slightly monstrous' and that these characteristics were 'like those of the ordinary self-fertilised plants of the later generations'. Darwin's experiment

for the ninth generation was affected by poor growth conditions and he states that the results could not be fully 'trusted'. There were 26 exceptional cases in which the cross-fertilized plants did not exceed greatly the self-fertilized plants in height (p. 238). With perhaps the exception of 'the Hero', Darwin did not appear to deduce that some individual plants might have become adapted to a change in mating behaviour and that these would then fail to display disadvantages when compared with their cross-fertilized relatives. His methods and the means of evaluating the results almost certainly precluded the collection of this type of datum, and with even his careful observation he rarely identified the anomalies. In addition, his strong belief in his hypothesis appeared to make it difficult for him to be impartial. Darwin knew nothing of genotypic variation, although undoubtedly he saw some of its phenotypic results.

The final plant height might not now be considered as a character by which the vigour of a plant would best be measured, even when most measurements were made under competitive conditions. However, it is becoming clear that genes controlling plant height may be linked to those of flowering time, and that variation in these genes may have effects on seed production (Wang & Li, 2006; Blake *et al.*, 2009).

Flowering time

Darwin noted that many plants derived from cross-fertilization flowered earlier than those from self-fertilization, and that this may be related to plant height. Again, this was never universally so [for example, in *Tropaeolum minus* L. (p. 144), for three pots, the cross-pollinated progeny flowered first but, in the fourth pot, the cross- and self-pollinated progeny flowered simultaneously; in *M. luteus*, cross-pollinated progeny flowered first in nine of ten pots]. These data are summarized on pages 291–295.

Genes have now been identified that regulate seasonal flowering responses (*FT* and *FLC*; Greenup *et al.*, 2009) in *Arabidopsis* Heynh. in Holl & Heynh. (Brassicaceae) and cereals (Poaceae). The gene *FLC* has been recorded as a floral repressor regulated through the vernalization pathway (Locascio, Lucchin & Varotto, 2009). However, there may be epigenetic mechanisms at work, as the *AtMBD9* gene controls flowering time and axillary branching by modulating the gene expression through DNA methylation and histone acetylation (Yaish *et al.*, 2009). This recent work could explain some of Darwin's observed effects of both flowering time and plant height.

Seed set, number of seeds and seed weight

The number of capsules set, the number of seeds per capsule and the weight of seeds are reported in the

book, and these generally provide support to the hypothesis that cross-pollination provides a significantly better result than self-pollination. Results are variable for different species (table D) but, for example, in *T. minus* (p. 144), when 12 flowers were cross-pollinated, the yield was 11 capsules and 24 seeds against 18 self-pollinated flowers yielding 11 capsules and 22 seeds, a result expected under Darwin's hypothesis. The seeds from the self-pollination were heavier than those from the cross-pollination, and this was found to be true in 10 of the 16 species examined. Darwin, although surprised at the result, noted that, in general, the capsules from self-pollinations possessed fewer seeds per capsule, which may have contributed to enabling a larger seed size. The plants from the selfed seed were, in other respects, inferior to the plants from crosses (pp. 352–353).

Recent work on *Macadamia tetraphylla* L.A.S.-Johnson (Pisanu, Gross & Flood, 2009), which is weakly self-compatible, has indicated that the situation is perhaps even more complex than Darwin could have imagined. In experiments using trees from highly disturbed to intact habitats, and using pollen from donors located at distances from less than 10 m to up to 100 km distance, plants artificially pollinated using outcrossed pollen produced more seed per raceme than did open-pollinated or self-pollinated trees. Trees from moderately disturbed habitats provided the highest fruit production. Seed set and seed weight were affected by pollen source, with local- and distant-source pollen producing heavier seed.

Plant vigour and viability

Darwin examined the fertility (better defined as the productiveness according to Darwin) of plants in 23 species following self- and cross-pollination, using fruit and seed set as the primary characters, but also by looking at the same characters in the progeny. According to Darwin, this was a common feature of those attempting hybridization at the time (p. 312), as there was widespread interest in the fertility of the hybrid offspring obtained. Darwin recorded that Gartner (p. 313) showed that some plants could be crossed easily, but that the hybrids formed were sterile, whereas others could be crossed with difficulty, but the hybrids were fertile.

Darwin's results were based on allowing plants which were the progeny of previously cross-fertilized or self-fertilized parents to set seed under open pollination (insect-aided) conditions, or to allow these plants to self-pollinate spontaneously under nets which excluded insects (p. 313). The numbers of capsules set were recorded, with the results for a few species of greater interest to Darwin being based on both capsule and seed set, as seed set was considered to be a more accurate reflection of fertility. Darwin

considered these results to be less than fully trustworthy because of his belief that the fertility of a plant was influenced by its age, health, soil and water conditions and by the temperature to which it was exposed.

One of Darwin's first studies was on the primroses (*Primula vulgaris* Huds.) in his garden, some of which he covered with muslin (so that insects could not get through to fertilize the flowers). Thus, he could compare self-pollinated seed production with that normally achieved through cross-pollination. He artificially pollinated flowers using a paintbrush, transferring pollen between flowers on the same plant as well as between plants. He noted that primroses have two different flower forms (thrum and pin) and observed that 'good fertilisation' (production of good numbers of seed) would only occur when a thrum plant fertilized a pin and vice versa (i.e. cross-fertilization). [Darwin also studied and wrote about heterostyly in *Primula veris* in his book *The Different Forms of Flowers on Plants of the Same Species* (Darwin, 1877); see Weller (2009) for a review and update on this work].

Darwin showed that the progeny of those species which normally outcross exhibit a greater degree of reduction in vigour and fertility if they are derived from self-pollination than those species which normally or habitually self. Most probably, those which self have eliminated factors which cause inbreeding depression during their evolution, and have retained those that are essential for their survival. Lande & Schemske (1985) developed genetic models which could account for the extremely high levels of inbreeding depression that can be observed in some outcrossing species which are forced to self (> 50%). They described two stable states, namely predominant selfing and predominant outcrossing. Each species has its own mechanism which depends on its history and its genes, such as those affecting inbreeding depression. Those species which are able to persist with self-fertilization are those with fewer deleterious recessive mutations. As noted by Leach & Mayo (2005), in inbreeding of normally outcrossing species, partly and completely recessive deleterious mutations will contribute far more to variance. Recovery of vigour following crossing would be a result of covering up, as heterozygotes, of rare recessive defects (heterozygote advantage).

Darwin spent time looking at how species in his garden altered. This seemed to be especially so when species found themselves at the edge of a population e.g. hedge parsley (probably *Anthriscus sylvestris* (L.) Hoffm.), with an altered flower colour (Boulter, 2008). On the other hand, he did not spend much time looking for direct evidence for transitions from outcrossing to self-fertilization, perhaps because of his

belief that selfing was an abomination. It is now believed (Mable, 2008) that transitions to selfing are common across flowering plants. Selfing does have its advantages, especially when a species finds itself in isolation; however, its main disadvantage is inbreeding depression, which means that deleterious recessive mutations are more clearly exhibited. Another disadvantage of inbreeding is the loss of genetic diversity for the isolated plants. One might expect, as indicated above, that the transition to self-fertilization, although perhaps necessary for survival in the short term, could lead to inbreeding depression, which might ultimately mean extinction.

An interesting comparison of the mating behaviours of many related species within one genus has recently been published by Miller, Levin & Feliciano (2008). *Lycium* L. is a genus which is thought to have arisen in South America, but spread from there throughout the world. There are now about 80 different species. When the authors first looked at North American species, they found a perfect correlation between self-compatibility, the presence of polyploidy and the separation of male and female flowers (versus self-incompatibility, diploidy and cosexuality). Such a system would keep the possibility of inbreeding depression low. African *Lycium* shows a similar link between ploidy and cosexuality, and, at least for two species, self-incompatibility as well. Thus, evidence shows that self-incompatibility persisted after the spread into Africa, even though the plants would have been isolated.

Polyploid species are thought not to exhibit so much inbreeding depression. Slotte *et al.* (2008) studied polyploidy in species of *Capsella* Medik. across Europe, North Africa and Asia. It is known that both diploid and polyploid species in this genus can self. However, the authors noted that *C. rubella* Reut. and *C. bursa-pastoris* (L.) Medik., although predominantly selfing, can outcross up to 12%, depending on the environment. In Europe at least, genes have been seen to move between these species, showing that even polyploid species may acquire genes from other species, and thus may not be completely isolated, nor indeed subjected to inbreeding depression.

Barringer & Geber (2008) recently published conflicting evidence in species of *Clarkia* Pursh for the relationship between polyploidy and inbreeding depression. Diploid and (allo)tetraploid species were compared, with a selfing and an outcrossing species of each being used for the comparisons. Outcrossers with both diploid and allotetraploid chromosome complements showed more inbreeding depression when selfed than did the comparable selfing species. Polyploid species were only slightly less fit (i.e. they showed slightly more inbreeding depression) than did the diploid outcrossers. The authors stated that they

could detect no really significant interaction between mating system or ploidy with inbreeding depression, but mating systems seemed to be more important overall.

Self-sterile (self-incompatible) and self-fertile plants

Darwin had previously reported on the subject of self-sterility (self-incompatibility; Darwin, 1868, Chapter 17; p. 330) and was aware of previous reports, including those of Kölreuter (1761–66) and Scott (personal communication) on *Verbascum phoeniceum* L. (p. 330), Gartner (personal communication) on *Verbascum nigrum* L., *Lobelia fulgens* Willd. and five species of *Passiflora* L., and Scott (personal communication), Munro (personal communication) and Müller (1868a) on South American orchids. Müller (1868b, 1870), in addition, was cited for *Bignonia* L. (an unknown species), *Tabernaemontana echinata* Aubl. and several Amaryllidaceae and Liliaceae, Hildebrand (1866, 1873) was cited for *Corydalis cava* Schweigg. & Kort. and *Hypocoum grandiflorum* Benth., and Hoffmann (1875) for *Papaver alpinum* L. Darwin noted that there were differences between species within genera, again citing Hildebrand (1873); *Hypocoum grandiflorum* is self-sterile, whereas *H. procumbens* is fairly self-fertile. However, Darwin was also aware that the expression of self-sterility within a species was unstable and that, in *Verbascum phoeniceum*, ‘these plants afterwards became more or less self-fertile in a strangely fluctuating manner’ and, in the case of *Passiflora* spp., ‘slight changes in their conditions, such as being grafted onto another stock or a change of temperature, rendered them self-fertile’. Recent data also support the potential for a temperature impact on the expression of self-incompatibility in a species. Using maize, Lin *et al.* (2009) identified a gene they called *tsg1 1* (thermo-sensitive genic self-incompatibility), with a single allele, governing the expression of self-incompatibility at low or high temperatures.

In his book, Darwin recorded data from *Eschscholtzia californica* Cham., *Abutilon darwinii* Hook.f., *Senecio cruentus* DC., *Reseda odorata* L., *Reseda lutea* L. and *Thunbergia alata* Bojer ex Sims. All species were considered and recorded by Darwin as self-sterile (self-incompatible) from previous observations but, on closer and more meticulous examination, Darwin found that the results were not as clear cut for every species (Table 1). In the case of *Eschscholtzia californica* (p. 331 ff.), Müller, working in Brazil, found a self-sterile plant and Hildebrand, in Germany, found that plants he grew were largely self-sterile. Darwin was already aware that garden plants grown in the UK were self-fertile, and he thus requested seed from Müller. Plants from the seed Müller sent were grown on to flowering, and there

was capsule and seed set from artificial self-pollinations. However, Darwin showed that the numbers of seed produced from artificial cross-pollination of these plants were significantly higher than those from self-pollination: an average of 12 seeds per capsule from self-fertilization and 80 seeds per capsule from cross-fertilization. It appears that the species provides an example of a ‘leaky’ self-incompatibility system and Darwin invoked low temperature as a factor in the result. Darwin subsequently grew on progeny from self-fertilized parentage which gave further progeny with an increased self-fertility, indicating that he may have been inadvertently selecting self-fertile progeny, perhaps as a result of the growing conditions.

Early in the spring, *Abutilon darwinii* was recorded as self-sterile, and this was a rare case in which Darwin, presumably using a microscope, noted that pollen tubes following self-pollination do actually penetrate the stigma. In the latter part of May and June, these same plants produced capsules spontaneously under a net used to exclude insects and also set capsules when artificially self-pollinated, albeit at a relatively low success rate and with few seeds per capsule. Darwin considered the increase in self-fertility to be linked to the UK hothouse conditions and the effect of growing plants from the Southern Hemisphere in the Northern Hemisphere. *Thunbergia alata* appeared to be similar and was recorded as ‘self-sterile early in the season, but at a later period produced many spontaneously self-fertilised fruits’, once again demonstrating the instability of the expression of self-incompatibility. Darwin also considered the situation in *Reseda odorata* and *R. lutea* to be similar (p. 336 ff). He carried out selfing and crossing experiments with these plants over 3 years from 1868. In the first year, his seven plants produced seed from crossing, but not from selfing, indicating that *R. odorata* was self-sterile. Using ‘fresh’ seed, the origin of which was not identified, Darwin then self-pollinated a further four plants in 1869, and found that three were self-fertile, especially in early summer, and only one was self-sterile. (This latter plant was still more self-fertile than those used in the first year of the experiments in 1868). In the final year of the experiment, Darwin, once again, used ‘fresh’ plants and, of the six that were self-fertilized, two were more or less self-sterile and four were self-fertile. Darwin again considered temperature to be a factor in this result, although the variation suggests that genotype is a more likely factor. Three self-fertilized capsules were produced from the two more or less self-sterile plants and five seeds were germinated and grown on in 1871. Around 30 capsules were subsequently found on the plants which had been kept under nets, but the majority were either empty

or contained only one seed, indicating that they had still inherited the self-sterility of the parent plants. As empty capsules were set, this indicates that some pollen tubes may have fertilized ovules, but that the development of seed failed at a later stage and sufficiently late to allow the capsule to develop to more or less maturity.

Remarkably, although Darwin outlined the variation in the expression of self-incompatibility in several species, his work appears to have been forgotten by others when the genetics of self-incompatibility became established for gametophytic systems, starting with *Nicotiana* L. (East & Mangelsdorf, 1925), followed by sporophytic systems (Gerstel, 1950). The expectation appears to have been that self-incompatibility systems controlled by genes would be highly efficient, and this led to the rigid interpretation of results with low levels of seed set from self-fertilization being ignored as experimental error. This unfavourably influenced our understanding of a less than fully stable mechanism in many species. There were exceptions to this, such as the results reported from variable conditions of temperature (Hopper, Ascher & Peloquin, 1967) in *Lilium longiflorum* Thunb.

Since Darwin's work, and for many subsequent authors, there has been an actual or tacit acceptance that outbreeding is an ancient characteristic of plants and that inbreeding is derived. This may well be true for plants which have the self-incompatible breeding system (known to Darwin as self-sterility), for which, to date, there has been no substantive experiment showing that self-incompatibility can be created *de novo* from self-compatible plants (although this has been shown using genetic manipulation techniques; see, for example, Liu *et al.*, 2007). Progress is being made in our understanding of how flowering plants prevent selfing, how the transition to self-compatibility occurs, what are the causes of inbreeding depression and how it is avoided. Lloyd & Schoen (1992), noting Darwin's contributions, provided a review of the possible mechanisms or modes of self-pollination, how self-pollination could be facilitated or constrained, how differences in self- versus cross-pollen can determine the ability (or not) to self-pollinate, and how environmental factors can affect self-pollination. As a result of their review, one can only conclude that the ability to self is extremely variable, even within a single flower of a plant. On the other hand, much of the recent research has been carried out with only a few families, genera and species. Not only is it limited taxonomically, it offers few criteria for predicting the reproductive behaviour of any one species, perhaps new to science and poorly defined. For example, are most isolated species self-compatible, and are most species in cultivation also

self-compatible (Zohary & Hopf, 2000; Glémin & Bataillon, 2009)?

A great deal of recent research on the nature of self- and cross-fertilization in higher plant species has centred on self-compatibility and self-incompatibility systems in Brassicaceae and, more specifically, *Arabidopsis* spp. Liu *et al.* (2007) were able to move genes between the self-incompatible *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz and the self-compatible *Arabidopsis thaliana* (L.) Heynh., so that the latter species became self-incompatible. In reality, *A. thaliana* has what the authors (Liu *et al.*, 2007) suggested is transient self-incompatibility, or cryptic self-compatibility, which allows this species to self after opportunities for crosses may be exhausted. When genes were moved from *A. lyrata* to *A. thaliana*, the self-incompatible genes could be expressed. Mable (2008) investigated *A. lyrata* from natural populations in which previously it had been thought to be an obligate outcrosser. We now know that both inbreeding and outbreeding populations occur and, in Europe, in isolated areas, when selfing is the only means of reproduction, seeds are formed, but there is high inbreeding depression and high seed mortality. In North America, there is much variation in the level of self-incompatibility and the actual rates of outcrossing. The results of Mable (2008) indicate that there are modifiers of the self-incompatibility genes, which determine the strength of the self-incompatibility system. The loss of self-incompatibility appears to have occurred independently in many different areas, and fluctuations in inbreeding occur between years within the same populations, a situation mirrored by Darwin's results for self-sterile species. Mable (2008) described evidence for maternal effects, population history and environmental differences which can influence the level of inbreeding depression. One experiment, relevant to Darwin's data, looked at the differences in the percentage germination and length of germination period between selfed and outcrossed plants from three different mothers. Progeny from one mother, when outcrossed, showed significantly faster germination than progeny from the others.

In order to maintain self-incompatibility, species such as *A. lyrata* need to have many alleles at the self-incompatibility site of the genome, and these are under multi-allelic balancing selection (Schierup & Vekemans, 2008). These genes would definitely not be neutral with respect to selection. Self-compatible species, such as *A. thaliana*, would require less diversity, but there is no evidence of a species-wide selective sweep associated with the rise of selfing in *A. thaliana* (Shimizu *et al.*, 2008). In fact, *A. thaliana*, although self-compatible, still has considerable diversity around the self-incompatibility 'S' locus (Tang *et al.*, 2007) and has been shown to outcross, albeit at

a low level, with hoverflies identified as the likely pollinators (Lawrence, 1976). Although transitions to selfing are expected to cause reductions in effective population sizes, with increases in the expression of deleterious mutations and decreases in advantageous mutations (as Darwin might have predicted), there does not seem to be a reduction in the efficacy of selection associated with self-fertilization (Wright *et al.*, 2008). This may be a result of the production of large population sizes and the purging of deleterious mutations, together with increased recombination and mutation rates in selfing species. Genomic conflicts can also be reduced by reducing the number of transposable elements, having biased gene conversions and spreading cytoplasmic male sterility. Thus, selfing need not lead to inbreeding depression. Glémin, Bazin & Charlesworth (2006) showed that habitually self-pollinating plants have lower levels of polymorphism in a wide set of plant species.

Rea & Nasrallah (2008) reviewed the evidence for the many ways in which plants prevent selfing, and attributed the successes of flowering plants (over other plants) at least partially to their ability to cross-fertilize, to widen the gene pool and to prevent extinction. Darwin would have wholeheartedly approved of this conclusion! However, Wright *et al.* (2007) showed that the lower GC content of genes in self-compatible *A. thaliana*, when compared with self-incompatible *A. lyrata*, may have little to do with any reduction in the efficacy of natural selection in the selfing *A. thaliana*. Tang *et al.* (2007) showed that there is still a great deal of diversity at the self-incompatibility locus in the selfing *A. thaliana*, so that there could not have been a single, mutated, inactivated allele as the key step to becoming a selfing species. Haudry *et al.* (2008) looked at the rates of recombination in two outcrossing and two selfing grasses. They found that, although there were GC changes in genes between outcrossing and selfed species, selection efficiency was only weakly affected by the mating system. The authors suggest that, in outcrossers, there may in fact be even less selection efficiency as a result of increased base substitutions.

It is thought that self-incompatibility is ancestral to self-compatibility, and that many species, when isolated and mates are limiting, convert to self-compatibility. Busch & Schoen (2008) reviewed the evidence for why self-incompatibility systems might break down. Mate limitation and/or a lack of pollinator activity can be analysed using modern molecular tools. Simple gene changes may be the mechanism, either to the self-incompatibility genes themselves or to modifier genes. No self-incompatibility is 100% efficient, and there is considerable evidence that self-incompatibility systems are 'leaky', particularly

in flowers that are produced late in inflorescences, allowing self-fertilization (pseudo-self-compatibility). Natural selection may ensure that it is these self-compatible plants which survive. However, gene changes may be dangerous, as doubling of recessive harmful genes can lead to significant inbreeding depression. Isolated species can also become clonal or apomictic (egg cell is unreduced and unfertilized but develops into a new embryo), eliminating the need for any breeding at all and ensuring no changes to the genetic complement. Another mechanism found is doubling of the chromosome number in self-fertile species. The introduction of polyploidy can help to retain normal genes on at least one set of chromosomes, even if harmful genes arise on others. Inbreeding depression would be less apparent in these polyploid plants.

Self-seed set following the exclusion of insects

Darwin did not set out to identify breeding systems (mating systems) when he examined seed set following artificial, open and insect-excluded self-pollination, but, by using the species he did, he encompassed some of the plant breeding systems that are now known. However, he did this without knowing about chromosome number, polyploidy, DNA amount and the genetic control of characters that he was measuring. He did not know about deleterious recessive genes, epistasis, heterozygote advantage and overdominance (although he was aware of inter-specific hybridization). He also knew nothing about conditions such as outbreeding depression which would have run contrary to his hypothesis.

In the latter half of his book, Darwin brought together data from the scientific literature, from his own correspondence and from his own experimental work on breeding behaviour (cross- or self-pollination). By utilizing the method of enclosing plants in insect-proof nets, he also added the influence of an array of insect visitors on the production of seed for 172 genera and 240 plant species (Table 1). Data were recorded for dioecious, monoecious and hermaphrodite species, and for monomorphic and dimorphic species which held a special interest for him. Darwin established two categories (Chapter 10) into which he could place plants, based on seed production following the auto-self-pollination of plants. The first category included all plants that set no seed or that set less than half the number of seed produced by an open-pollinated plant of the same species, and the second category included all those plants that were 'quite' fertile or yielded more than half the number of seed that would be produced by an open-pollinated plant of the same species. Several characteristics of the flower could be presumed to contribute to his results, and it could be that more than one

character combined to play a role in the final result. These characteristics included the physical separation of stigma and pollen, the temporal separation of stigma and pollen (dichogamy), male sterility and self-sterility (self-incompatibility).

Darwin understood that, because many flowers were hermaphrodite, they could self-pollinate, and that the chances of cross-pollination had to be enhanced by a variety of means. He had already published a book in 1862 on the contrivances by which British and foreign orchids were fertilized by insects (Darwin, 1862; see also Micheneau, Johnson & Fay, 2009) and, from that and further work, he determined that conspicuous flowers, mechanical structures, dichogamy, pollen-ovule ratios and, when present, heterostyly, nectar and odour all influenced the effectiveness of crossing. He noted that inconspicuous flowers and, in particular, cleistogamous flowers were more prone to self-pollination, and as a comparison listed characteristics for cleistogamous flowers, such as flowers closed, petals more or less rudimentary, not brightly coloured, no nectar, no smell, small anthers, few pollen grains and stigmas little developed. He also noted that anemophilous (wind-pollinated) flowers resembled cleistogamous flowers in petal size, colour, absence of nectar and seed production, but they were not closed and often had many pollen grains and large or plumose stigmas. All these data are relevant today.

Darwin saw that a most significant influence was the pollinator, and thus devoted a whole chapter (Chapter 11) to the habits of insects, including those with habits such as perforating flowers for nectar and thus bypassing any role in effective pollination. It is hardly surprising that insects generally effectively enable pollination and that, although selfing has more disadvantages than crossing in some circumstances, there are occasions when bursts of genetic diversity are released or facilitated by occasional crossing. What Darwin was not aware of was that the slowly increasing pollution, the indiscriminate use of pesticides and the destruction of habitats would contribute to a reduction in pollinator numbers, and thereby impact greatly on the fecundity of flowering plant species, although he clearly identified that problems would arise if insects were to become locally or globally rare (p. 410).

CONCLUSIONS

Charles Darwin did not know whether his work would be new or outstanding, but he remarked that he found the projects enjoyable. Had he been living today, it appears highly likely that he would have found work equally if not more enjoyable. The results he presented are sound and they have mostly been

proven to be true. However, his data did not answer all his questions, they did not always give him what he expected and he had to rely on others for some supporting results. Darwin considered that the most important conclusion he made (p. 27) was that the 'mere act of crossing by itself does no good'. He realized that individuals that are crossed need to differ 'slightly in constitution' or demonstrate spontaneous variation. Darwin was describing differing genotypes.

Although Darwin studied and published his work on selfing and inbreeding depression well over 100 years ago, and much has been added since that time to our understanding of these phenomena, largely as a result of our knowledge of DNA and genes, there are still many questions to be answered. For example, why are so few species fixed at complete selfing or complete outcrossing (Schemske & Lande, 1985)? Is it because of inbreeding depression that barriers to self-fertilization are widespread and varied (Rea & Nasrallah, 2008)? If inbreeding depression is harmful, why is there no widespread reversion to self-incompatibility in nature? Why have we continued to breed and grow mainly self-compatible crop plants, and how have humans been able to select out genes which contribute to inbreeding depression in these (Pickersgill, 2009)? Will the changes to pollinators that we are now experiencing mean that it will be mainly self-compatible (or wind-pollinated) species that will survive? Will we have more apomictic and/or polyploid species of higher plants? Is there any way to help newly developed self-compatible species so that inbreeding depression might not occur?

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