

Ecology and evolution of plant mating

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Sex in flowering plants is complicated by three distinctive features of their biology. First, being sessile, plants require vectors to transfer male gametes (pollen) between individuals. This reliance promotes the evolution of diverse floral adaptations associated with the particular agents responsible for pollen dispersal (animals, wind, water). Animals are most commonly employed as pollinators because their behavioural flexibility can be manipulated by plant traits. Second, most plants are hermaphrodite and so are capable of selfing, sometimes at the expense of outcrossing. Third, owing to the modular construction of plants, male and female gametes are packaged in a bewildering array of structural and temporal combinations at the flower, inflorescence, plant or population level, despite the basic hermaphroditic condition. Because of vector-mediated gamete transfer and elaborate sexual systems, plant mating can be highly promiscuous, with individuals mating with many sexual partners including themselves.

Pollination is fundamental among the complex ecological interactions that generate mating patterns because it determines mating opportunities by establishing the dispersion of pollen grains among flowers. Despite the obvious functional link between pollination and mating, research on these basic aspects of plant reproduction has followed separate paths during most of this century, with surprisingly little cross-fertilization. Most pollination studies have been ecological, with little consideration of how patterns of pollen transport might influence mating and plant fitness. In contrast, mating-system studies have been dominated by population genetic and theoretical approaches, paying scant attention to how the proximate ecological factors governing pollen dispersal influence mating. In recent years, the isolation of pollination and mating-system biology has begun to break down, with the publication of theoretical and empirical studies emphasizing both the ecological aspects of plant mating and the fitness consequences of different mating patterns. Here, we review some of these recent developments to illustrate why, following Darwin's early lead, plant mating continues to be one of the most active fields of enquiry in evolutionary biology. These developments involve advances in determining the proximate mechanisms governing mating, measuring mating complexity, and understanding the genetic and evolutionary consequences of different mating strategies.

The incidence of selfing and outcrossing and their measurement

In contrast to its zoological counterpart, the study of plant mating systems has been dominated by comparison of

Plants exhibit complex mating patterns because of their immobility, hermaphroditism and reliance on vectors for pollen transfer. Research on plant mating attempts to determine who mates with whom in plant populations and how and why mating patterns become evolutionarily modified. Most theoretical models of mating-system evolution have focused on the fitness consequences of selfing and outcrossing, stimulating considerable empirical work on the ecology and genetics of inbreeding depression. Less attention has been given to how the mechanics of pollen dispersal influence the transmission of self and outcross gametes. Recent work on the relation between pollen dispersal and mating suggests that many features of floral design traditionally interpreted as anti-selfing mechanisms may function to reduce the mating costs associated with large floral displays.

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the relative frequency of selfing and outcrossing¹. There are important biological reasons why the evolution of selfing, in particular, has attracted so much attention. First, the effects of selfing and outcrossing on fitness through inbreeding depression and heterosis are well established^{2,3}. Second, the frequency of outcrossing is the most important determinant of population genetic structure, affecting both genetic diversity within populations and genetic differentiation among them⁴. Finally, the acquisition of selfing profoundly influences floral evolution, affecting floral design and sexual resource allocation⁵. Each of these aspects of the biology of selfing and outcrossing has stimulated considerable theoretical and empirical work during the past decade.

Recognition of the significance of selfing for mating-system evolution prompted development of specific tools for measuring the relative frequency of selfing and outcrossing. Since Brown and Allard⁶ first demonstrated the utility of allozyme markers to estimate mating parameters 25 years

ago, estimation of the proportion of offspring produced by selfing, s , or its complement, the female outcrossing rate ($t = 1 - s$), has become routine for many botanists. It is important to recognize that s and t portray selfing and outcrossing through female function and only depict the proportions of successful gametes involved in selfing and outcrossing when referring to a population average of all phenotypes^{7,8}. This approach has few parallels with work on animal mating systems, probably because of the contrasting sexual systems (hermaphroditism versus dioecy) that predominate in the two groups. Notwithstanding this difference, many animals do not appear to maintain sufficient allozyme variation to enable quantitative analysis of mating parameters relevant to their reproductive biology (e.g. incidence of extra-pair copulations in 'monogamous' birds), hence the development of alternative genetic markers, such as DNA fingerprinting techniques in behavioural ecology. Although such techniques are also likely to provide powerful new insights into aspects of plant mating, particularly concerning male reproductive success, allozymes continue to be the main source of genetic markers for analysing selfing.

Two types of data are required to describe accurately the mating system of a plant population: (1) measures of fertility, and (2) estimates of the kinds of mating events that occur⁹. Fertility involves the relative contribution of individuals to the next generation through male and female gametes. Mating events are usually classified according to whether seeds originate from outcrossing, selfing or apomixis. Among outcrossed progeny, it is also possible to estimate the degree of biparental inbreeding, how often progeny are full

sibs, and the number of male parents represented in a seed crop⁹. Despite rapid progress in the use of genetic markers, it is worth noting that we are still some way from being able to determine for any plant population the basic mating information of who has mated with whom and how often. DNA markers with high allelic variation, such as microsatellite loci, seem to offer the best hope for achieving this goal¹⁰.

Ecology and evolution of selfing

The fundamental issues involved in the evolution of selfing were elegantly identified in Fisher's early treatment¹¹ of the transmission advantage of a selfing gene. His work provided the basic insight into why most later genetic models of mating-system evolution predict that selfing will be unconditionally favoured if selfed progeny have at least half the fitness of outcrossed progeny (Box 1). Several aspects of plant reproduction oppose this siring advantage of self-pollination, either by reducing it directly through pollen discounting or through inequalities in the fates of selfed and outcrossed progeny resulting from inbreeding depression (Box 1). The most thoroughly studied selective force maintaining outcrossing is substantial inbreeding depression in selfed progeny, which arises largely from the expression of recessive deleterious alleles in homozygous individuals^{2,3}.

The evolution of selfing should generally be accompanied by changes in the incidence and intensity of factors opposing it. In the most influential model considering the joint evolution of selfing and inbreeding depression, Lande and Schemske¹² predicted that predominant selfing and predominant outcrossing should be alternative stable outcomes of mating-system evolution in most plant populations. Despite valid criticism concerning sampling biases and the existence of some species with stable, mixed mating-systems, survey data that reveal bimodal distributions of selfing rates in natural plant populations tend to support this prediction (Refs 13–15 and Box 1). Models of mating-system evolution (reviewed in Ref. 3) have provided an enormous stimulus for experimentalists and recently have guided empirical work on the relation between selfing rates and inbreeding depression^{16,17}, the genetic architecture of inbreeding depression^{18,19}, and the fitness of selfed and outcrossed plants under contrasting environmental conditions^{20,21}.

Recent evidence confirms that the magnitude of inbreeding depression decreases with continued selfing as deleterious recessive alleles are expressed and purged through selection. Barrett and Charlesworth¹⁶ subjected plants from a selfing population and a predominantly outcrossing population of a water hyacinth (*Eichhornia paniculata*) to five generations of selfing followed by a generation of outcrossing. Fitness changed little during this experiment for the selfing population, whereas for the originally outcrossing population fitness declined during inbreeding and then recovered after outbreeding. In addition, a recent survey by Husband and Schemske¹⁷ revealed a significant negative correlation between cumulative inbreeding depression and the primary selfing rate of populations (Box 2). This study also identified differences between primarily selfing and outcrossing species in the timing of inbreeding depression during the life cycle: selfers typically express inbreeding depression late, whereas outcrossing species also commonly exhibit early-acting inbreeding depression. An increasing number of cases are known in which species with partial selfing maintain unexpectedly high levels of inbreeding depression (Refs 17 and 21). However, recent theoretical work²² has shown that selective interference among loci and high genomic mutation rates can prevent purging until a sharp threshold in the selfing rate is exceeded, thus providing an explanation for the

Box 1. The evolution of plant mating systems

Fisher¹¹ demonstrated that, all else being equal, a gene causing self-fertilization will increase in frequency each generation, because, on average, selfers contribute more gene copies to the next generation than outcrossers:

	Average gene contribution	
	Outcrosser	Selfer
Ovule parent	1	1
Pollen parent	1	2 ^a
Total	2	3 ^b

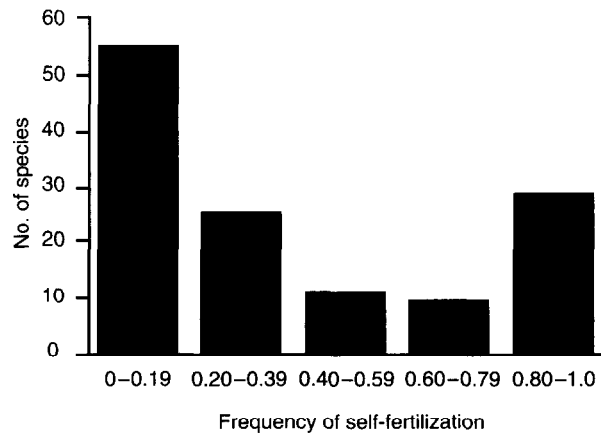
^acontribution diminished by pollen discounting.

^bcontribution diminished by inbreeding depression.

This three to two average bias in gamete transmission, which arises because a selfer sires its own seeds and seeds on outcrossers, has been termed the automatic selection advantage of self-fertilization.

Not all plants self, indicating that strong forces operate to prevent the spread of selfing genes. Fisher's argument requires that selfing and outcrossing involve distinctly separate pools of pollen. In contrast, when pollen involved in selfing diminishes the pool available for outcrossing (pollen discounting), a selfer's siring advantage is reduced proportionately. Holsinger²⁷ demonstrated theoretically that pollen discounting alone is sufficient to explain both why selfing does not always evolve, and the occurrence of mating systems that include a mixture of selfing and outcrossing. The few empirical studies of pollen discounting suggest that its incidence depends on how and when selfing occurs (see Box 4).

Even when pollen discounting does not reduce the pollination advantage of selfing, outcrossing may be favoured if selfed progeny have low fitness (w_s) relative to outcrossed individuals (w_o), so that they suffer inbreeding depression [$\delta = 1 - (w_s/w_o)$]. Modification of Fisher's model to incorporate inbreeding depression reveals that the advantage of selfing disappears if selfed progeny survive and reproduce only half as well as outcrossed progeny (that is, $\delta \geq 0.5$) (Ref. 2). Hence, if inbreeding depression alone counteracts the transmission advantage of selfing, mating-system evolution should be characterized by disruptive selection on mating patterns, with predominant selfing and predominant outcrossing as alternative stable states¹². This prediction can be assessed by testing whether the distribution of selfing rates for a random sample of plant species is bimodal. Unfortunately, the incidence of selfing has been estimated using allozyme markers for only a small, nonrandom fraction of seed plants and ferns that do not comprise phylogenetically independent samples. Notwithstanding these problems, the three surveys^{13–15} conducted to date all detected bimodal distributions. In the largest survey, involving 129 species of seed plants distributed among 67 genera and 33 families, a highly non-uniform distribution of selfing rates with a deficiency of partially selfing species was found¹⁴.

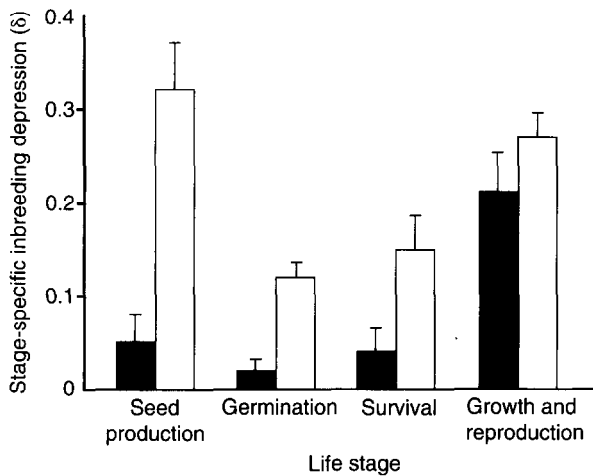


In addition to pollen discounting and inbreeding depression, other genetic³ and reproductive factors²⁶ not considered in the simple fisherian argument outlined above can also modify the evolutionary dynamics of selfing and outcrossing. In particular, biparental inbreeding, reproductive assurance and the mode of self-pollination can all play important roles^{3,26,28,39}. Figure redrawn, with permission, from Ref. 14.

Box 2. Inbreeding depression: relation to mating system and life cycle stage

How does the magnitude of inbreeding depression vary with the selfing rate and does the timing of inbreeding depression in the life cycle differ between species, depending on their mating systems? Husband and Schemske¹⁷ recently addressed these questions with an analysis of published data on inbreeding depression and selfing rates of seed plants. They examined the relation between cumulative inbreeding depression, calculated as the product of the mean relative fitness of selfed and outcrossed progeny at four life history stages, and the primary selfing rate (the proportion of selfed progeny at fertilization) for a sample of angiosperm and gymnosperm populations. For the combined sample, the magnitude of inbreeding depression varied negatively with the frequency of selfing ($r_s = -0.42, p < 0.01, n = 44$ populations). Predominantly selfing species exhibited 43% less average inbreeding depression than predominantly outcrossing species. These results support the view that inbreeding depression evolves in conjunction with the mating system and that prolonged selfing decreases inbreeding depression by reducing genetic load.

Inbreeding depression appears to involve many genes, some of which are expressed at specific stages in the life cycle². If such genes have different fitness consequences then the timing as well as the magnitude of inbreeding depression could evolve with changes in selfing. Husband and Schemske compared the magnitude of inbreeding depression at four stages in the life cycle of predominantly selfing (shaded bars) and predominantly outcrossing (open bars) species.



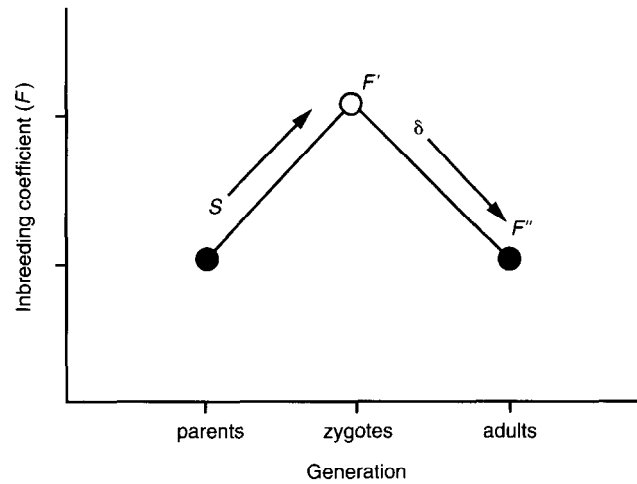
Most selfers expressed the majority of inbreeding depression late in their life cycles, whereas outcrossing species commonly exhibited substantial inbreeding depression throughout their life cycles. These results suggest that most early-acting inbreeding depression is associated with relatively few recessive lethals that can be purged easily through inbreeding. In contrast, inbreeding depression later in the life cycle seems to result from many weakly deleterious mutations, which are more difficult to purge. Figure redrawn, with permission, from Ref. 17.

maintenance of high inbreeding depression in species with a moderate degree of selfing.

Fitness comparisons of selfed versus outcrossed progeny grown under field conditions often detect more-severe inbreeding depression than when plants experience the less stressful conditions of a glasshouse or garden^{2,20}. To avoid the complications associated with environment-dependent aspects of inbreeding depression, Ritland²³ developed a marker-based method for measuring inbreeding depression in the field, based on changes in the inbreeding coefficient between life history stages. Recent applications of this method revealed different intensities of inbreeding depression in annual species of monkeyflower (*Mimulus*) with contrasting selfing rates (Ref. 24 and Box 3), and enabled field estimates of inbreeding depression in swamp loosestrife (*Decodon verticillatus*), a long-lived clonal species in which experimental estimates of lifetime inbreeding depression are nearly impossible to obtain²¹.

Box 3. Measuring inbreeding depression in the field

Inbreeding affects the population structure of partially selfing plants and influences the evolution of mating systems^{2,3}, so that measures of inbreeding depression are highly desirable. Unfortunately, the extent of inbreeding depression depends on the environment in which a plant develops^{2,20}, which requires that it be measured under field conditions. Measurements can be difficult to obtain, particularly for early stages in the life cycle. To overcome these problems, Ritland²³ developed a non-experimental population genetic technique for estimating inbreeding depression that takes advantage of changes in the inbreeding coefficient (F) between life history stages, which can be obtained from electrophoretic data. In a partial selfer, the inbreeding coefficient increases from adult to seed through selfing, but subsequently declines throughout the life cycle owing to selection against selfed offspring. The magnitude of this decline reflects the intensity of inbreeding depression.



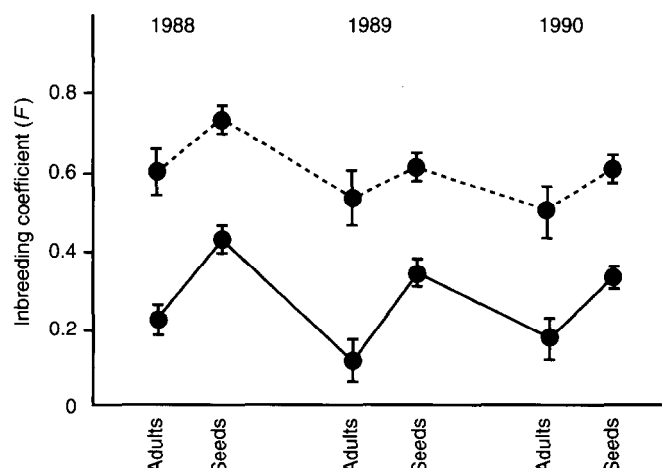
For annual species, inbreeding depression can be estimated indirectly by:

$$\hat{\delta} = 1 - \frac{(1-s)F''}{F' - F'' + (1-s)F''}$$

where F' and F'' are the inbreeding coefficients of the progeny generation before and after selection, respectively, and s is the selfing rate of the parental generation. For long-lived plants, inbreeding depression can be inferred from estimates of the parental F and s , by assuming that the population under study maintains an equilibrium F in each adult generation, so that:

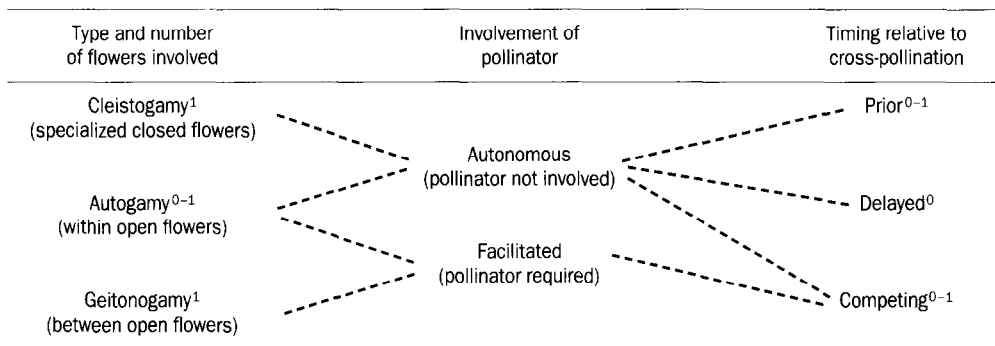
$$\hat{\delta} = 1 - \frac{2(1-s)F}{s(1-F)}$$

Dole and Ritland²⁴ employed this marker-based approach to compare the magnitude of inbreeding depression in two sympatric annual *Mimulus* species with contrasting mating systems. By estimating F and s for adults over three consecutive generations they found that the relative fitness of selfed progeny averaged only 0.19 in the more outcrossing *M. guttatus* ($s = 0.63$) (solid line) and 0.32 in the largely selfing *M. platycalyx* ($s = 0.84$) (dashed line). This result is in accord with the hypothesis that selfing reduces genetic load through purging. However, their data also indicate that partially selfing species can harbour substantial genetic load. Figure below was redrawn, with permission, from Ref. 24.



Box 4. Functional classification of the modes of self-pollination in flowering plants

Self-pollination is the outcome of various processes that differ in reproductive expenditure, pollinator involvement and timing relative to cross-pollination. Lloyd and Schoen¹ recognized eight modes of self-pollination, which are not mutually exclusive:



The dashed lines connect modes that can occur concurrently. The superscripts indicate the associated intensity of pollen discounting, which can range from none (0) to complete (1). The expected intensity of pollen discounting varies among selfing modes. This variation is evident in the five published studies of pollen discounting³², as significant discounting was only found in experiments with multi-flowered plants, in which geitonogamy was possible.

Two examples, from Lloyd²⁶, illustrate that it is important to distinguish between the different modes of self-pollination because they differ in the amount of reproductive assurance that they provide and in their influence on the fitness obtained through pollen and ovules. Delayed selfing is always favoured because it involves no pollen discounting and increases fertilization whenever a flower has been insufficiently cross-pollinated. In contrast, selection promoting selfing should never increase geitonogamy because the features necessary for a pollinator to move pollen between flowers on the same plant are the same as those needed for cross-pollination. Consequently, geitonogamy probably involves complete pollen discounting (Box 5), which eliminates the pollination advantage of selfing (Box 1). Hence, mode of self-pollination significantly influences whether and under what circumstances natural selection favours selfing²⁶.

Although most attention during the past decade has focused on the fitness consequences of selfing and outcrossing, several workers have begun investigating the ecology of gene transmission and the relative success of self and outcross gametes²⁵. Here, the approach is to understand the mechanics of the pollination process by determining how, when and how much self and outcross pollen are transported to stigmas. Two conceptual advances have assisted this work. First, Lloyd and Schoen's¹ classification of modes of self-pollination (Box 4) has highlighted the fact that the selective forces responsible for the evolution of selfing depend critically on the particular mode of self-pollination²⁶. Second, recent models of mating-system evolution^{8,27,28} explicitly incorporate the relation of the incidence of selfing to a plant's pollination environment and indicate that outcrossing may be favoured even in the absence of inbreeding depression and that mixed mating can be evolutionarily stable whenever selfing can evolve^{27,28}. These predictions do not depend on the fitness of outcrossed progeny exceeding that of selfed progeny, only that a trade-off exists between success as a self and outcross pollen parent. Whether this trade-off occurs commonly, and to what extent it is contingent upon a species' pollination biology, requires empirical work.

Pollen dispersal, mating patterns and the evolution of floral design and display

How do pollen dispersal and mating interact to influence the evolution of floral traits? In addressing this issue, it is convenient to distinguish two concepts commonly used in floral biology. Floral design refers to characteristics of individual flowers including their structure, colour, scent and nectar production, whereas floral display describes the number of flowers open at one time and their arrangement in inflorescences. The primary function of both is to promote mating between plants; however, precisely how this is achieved has been the source of some confusion. Since Darwin, most features of floral design and display have been interpreted as mechanisms that passively encourage cross-

pollination by preventing or discouraging self-pollination, thereby allowing more opportunities for ovules to be outcrossed. Such 'anti-selfing hypotheses' have been invoked widely for the past century to explain the evolution of various 'breeding systems', such as heterostyly²⁹ and floral strategies^{30,31}. This perspective focuses on the fate of ovules, as measured by seed production and population-level outcrossing rates, and therefore emphasizes maternal contributions to the next generation.

During the past two decades, appreciation of the seemingly obvious fact that every seed has a mother *and* a father has increased attention on the relation between pollen dispersal and male fertility, particularly in animal-pollinated plants^{28,32}. The recognition of a plant's paternal role in mating leads to an

alternative interpretation that particular floral mechanisms actively promote more effective pollen dispersal, thereby augmenting fitness through outcrossed siring success³²⁻³⁴. This more recent perspective is particularly important for explaining the occurrence of floral traits such as herkogamy and dichogamy (spatial and temporal separation of male and female function, respectively) and sexual systems such as andromonoecy (plants with male and hermaphrodite flowers), which frequently occur in species with physiological self-incompatibility³². The apparent redundancy of several floral mechanisms that prevent selfing is resolved by recognizing that these floral traits may promote outcrossed siring success by limiting pollen discounting, a role that self-incompatibility can never serve³⁵.

The linkage between pollen dispersal, mating and the evolution of floral design and display can be illustrated by considering several aspects of a plant's attractiveness to pollinators. First, consider the relatively common case of plants with elaborate floral signals, but only a few ovules per flower, such as those in the Lamiaceae or Boraginaceae. Why should such flowers expend so much effort attracting pollinators when fertilizing all ovules requires only a few pollen grains, which could be delivered by a single pollinator? The answer probably lies more with siring success, than with seed production. In particular, pollinator attraction always benefits male function if decreased pollen removal increases the proportion of removed grains that fertilize ovules and if floral mechanisms restrict removal by individual pollinators^{34,36}. Attraction of many pollinators and restricted pollen removal also increase potential mate diversity because, with many pollinators following different foraging paths, an individual plant imports pollen from and exports pollen to a larger sample of the population.

Although plant attractiveness to pollinators often increases with the number of flowers open at one time, display size also bears mating costs, so that many species do not produce large displays even though they eventually produce many flowers during their flowering period. When a

plant displays many flowers simultaneously, pollinators that move within the display can transfer pollen among flowers on the same plant³⁷. Such geitonogamy bears two potential mating costs. Most obviously, in self-compatible species geitonogamy can lead to self-fertilization and inbreeding depression. Less appreciated until recently is the possibility that pollen used in self-pollination may not be available for outcrossing, potentially reducing the plant's success as a pollen parent. A recent marker-gene study³⁵, involving experimental manipulation of inflorescence size in bee-pollinated *E. paniculata*, provided the first experimental evidence for the predicted negative relation between selfing rate and outcrossed siring success as a result of geitonogamous pollen discounting (Box 5).

Aspects of floral design and display that mitigate the mating costs of geitonogamy but that promote the benefits of enhanced pollinator attraction that accompany mass flowering may be widespread, given that most animal-pollinated plants expose several to many flowers to the pollination process each day. Because of the importance of geitonogamy in governing the incidence of self-pollination and pollen discounting (see Box 5), the individual flower cannot be considered the operational unit of either male or female function in animal-pollinated plants. Rather, this role belongs to the entire floral display. This conclusion affects functional interpretation of the design of individual flowers, because the mating consequences of traits that affect pollen dispersal depend on how many flowers a pollinator visits on the same plant. Appreciation of the functional significance of floral architecture, the placement of sexual organs within flowers, and the schedules of male and female function therefore requires an understanding of their influences

Box 5. Pollen carryover, geitonogamy and outcrossed siring success

Pollen transfer between flowers on an individual plant (geitonogamous self-pollination) is possible whenever plants simultaneously display several functionally male and female flowers. Lloyd²⁶ pointed out that geitonogamy probably causes complete pollen discounting (see Box 4), so that plants with large floral displays may pay a large mating cost in terms of lost outcrossing opportunities. Three roles of floral design and display in self-pollination and outcrossed siring success can be illustrated with a simple model (see Ref. 32 for details) and the results of an experiment involving *Eichhornia paniculata* inflorescences manipulated to contain 3, 6, 9 or 12 flowers³⁵. For each mating parameter, we present the theoretical prediction followed by a related empirical result. In the experiment, plants of two sizes competed for siring opportunities within individual arrays, with plants with small displays outnumbering those with large displays so that both treatments were represented by the same total number of flowers. Allozyme markers were used to estimate seed paternity and the relation of selfing rate to flower position within an inflorescence and to inflorescence size.

Consider a plant population in which each pollinator visits V flowers on a plant. While visiting each flower a pollinator moves I grains from anthers to stigma (facilitated intrafloral self-pollination – see Box 4) and removes D pollen grains that are then transported to other flowers on the same or other plants. A proportion, κ (carryover fraction), of the pollen on a pollinator's body remains there during each visit to a flower, so that each flower's stigma receives the complementary proportion, $1 - \kappa$, from other flowers. D , I and κ all depend on floral design.

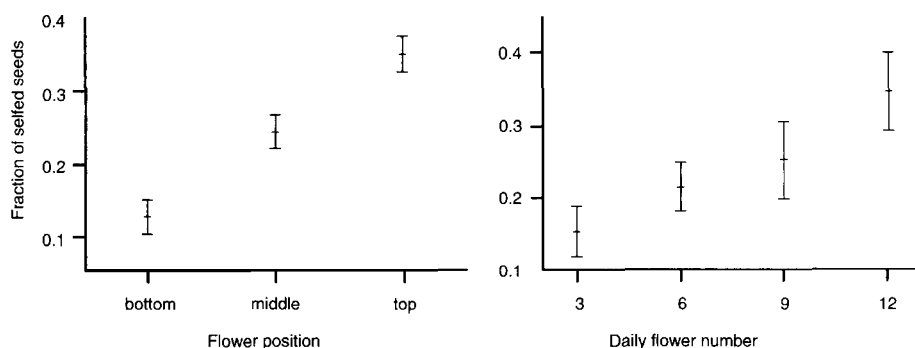
These features determine two aspects of the incidence of facilitated self-pollination. First, the fraction of self-pollen grains received by the j th flower visited on a plant:

$$\psi_j = 1 - \frac{D\kappa^{j-1}}{I + D}$$

increases asymptotically as a pollinator visits successive flowers and causes increased geitonogamous pollination. The increased self-pollination from bottom to top flowers within inflorescences observed during the *E. paniculata* experiment is consistent with such geitonogamy, because bumble bees (*Bombus* spp.) habitually visited flowers that were low on an inflorescence and then moved upwards. Second, the total incidence of selfing by the entire plant:

$$\Psi = 1 - \frac{D[1 - \kappa^V]}{V(I + D)(1 - \kappa)}$$

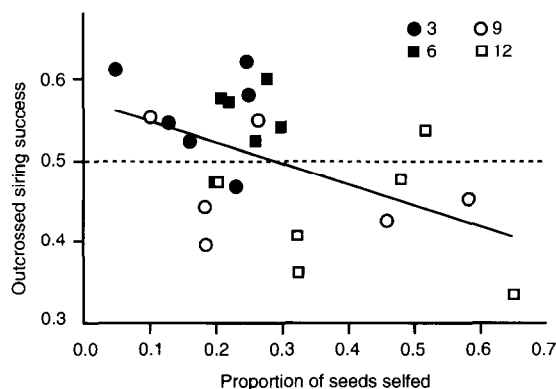
increases for large floral displays if pollinators visit more flowers per plant (i.e. larger V) and cause more geitonogamy. This relation was also evident in the *E. paniculata* experiment.



Finally, because pollen involved in geitonogamy discounts the number of grains destined for other plants, the proportion of outcrossed siring opportunities lost to geitonogamous pollen discounting is:

$$\lambda = 1 - \frac{1 - \kappa^V}{V(1 - \kappa)}$$

The expected negative relation between outcrossed siring success (proportion of all outcrossed seeds sired by plants of a particular inflorescence size, represented by the different symbols in the figure below) and the incidence of selfing was also evident in the *E. paniculata* experiment.



Because λ varies positively with V and negatively with κ , this form of pollen discounting could be reduced through smaller floral displays (with a corresponding increase in flowering period) and/or changes in floral design that enhance pollen carryover (see Ref. 32). Figures redrawn, with permission, from Ref. 35.

on a plant's aggregate mating success. Studies are needed to determine to what extent functional correlations between floral and inflorescence characters (e.g. Ref. 38) are the result of evolutionary interactions between floral design and display. Consideration of these interactions would particularly benefit from comparative analyses in a phylogenetic context.

Even if floral mechanisms limit the occurrence of selfing, plants can still experience appreciable inbreeding, depending on the extent of pollen dispersal relative to the genetic structure of the population. Plant populations frequently comprise overlapping patches of related individuals so that biparental inbreeding is probably common³⁹ and offspring resulting from crosses between near neighbours are often less fit than those from more distant crosses⁴⁰. Because pollinators mediate pollen transport within and among these patches, their foraging behaviour and the amount of pollen from a specific flower that remains on a pollinator's body during subsequent visits to recipient flowers (pollen carry-over) determine the number and diversity of mating opportunities and their consequences³². For example, C.M. Herrera (unpublished) recently found that lavender (*Lavendula latifolia*) flowers exposed to butterfly and to bee pollination produced more seeds than flowers visited solely by bees. More importantly, offspring from butterfly-pollinated seeds were considerably fitter when sown under the severe conditions of the parental population. Because butterflies fly farther between flower visits than bees, it seems likely that these differences resulted from greater opportunities for outcrossing among unrelated plants in flowers exposed to butterfly pollination. Hence, in addition to affecting reproductive success, the characteristics of pollination can bear direct demographic consequences, with obvious implications for the evolution of floral designs that promote extensive pollen dispersal.

Future research

Two avenues of future research on plant mating seem certain given recent developments in the field. First, the struggle to develop molecular markers for measuring male reproductive success will continue. Such information is critical for understanding fitness returns from investment in male function and the genetic and ecological factors determining male reproductive success. Second, investigations of both the fitness consequences of selfed and outcrossed progeny and the transmission dynamics of self and outcross gametes will be required to understand fully the evolution of plant mating systems. While more refined analyses of inbreeding depression will undoubtedly continue, this work is likely to be integrated with new research on the mechanics of the pollination process and how the transfer of self and outcross pollen within and between plants influences mating patterns. Finally, mating-system studies to date have adopted a largely ahistorical population-level perspective. This is likely to change as reconstruction of the phylogenetic history of reproductive traits provides a more comprehensive picture of the evolution of mating systems.

Acknowledgements

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References

- Lloyd, D.G. and Schoen, D.J. (1992) **Self- and cross-fertilization in plants. I. Functional dimensions**, *Int. J. Plant Sci.* 153, 358–369
- Charlesworth, D. and Charlesworth, B. (1987) **Inbreeding depression and its evolutionary consequences**, *Annu. Rev. Ecol. Syst.* 18, 237–268
- Uyenoyama, M.K., Holsinger, K.E. and Waller, D.M. (1993) **Ecological and genetic factors directing the evolution of self-fertilization**, *Oxf. Surv. Evol. Biol.* 9, 327–381
- Hamrick, J.L. and Godt, M.J. (1990) **Allozyme diversity in plant species**, in *Plant Population Genetics, Breeding and Genetic Resources* (Brown, A.H.D., Clegg, M.T., Kahler, A.L. and Weir, B.S., eds), pp. 43–63, Sinauer
- Brunet, J. (1992) **Sex allocation in hermaphroditic plants**, *Trends Ecol. Evol.* 7, 79–84
- Brown, A.H.D. and Allard, R.W. (1970) **Estimates of the mating system in open pollinated maize populations using isozyme polymorphisms**, *Genetics* 66, 133–145
- Horowitz, A. and Harding, J. (1972) **The concept of male outcrossing in hermaphrodite higher plants**, *Heredity* 29, 223–236
- Gregorius, H-R., Ziehe, M. and Ross, M.D. (1987) **Selection caused by self-fertilization I. Four measures of self-fertilization and their effects on fitness**, *Theor. Popul. Biol.* 31, 91–115
- Brown, A.H.D. (1990) **Genetic characterization of plant mating systems**, in *Plant Population Genetics, Breeding and Genetic Resources* (Brown, A.H.D., Clegg, M.T., Kahler, A.L. and Weir, B.S., eds), pp. 145–162, Sinauer
- Queller, D.C., Strassman, J.E. and Hughes, C.R. (1993) **Microsatellites and kinship**, *Trends Ecol. Evol.* 8, 285–288
- Fisher, R.A. (1941) **Average excess and average effect of a gene substitution**, *Ann. Eugen.* 11, 53–63
- Lande, R. and Schemske, D.W. (1985) **The evolution of self-fertilization and inbreeding depression. I. Genetic models**, *Evolution* 39, 24–40
- Schemske, D.W. and Lande, R. (1985) **The evolution of self-fertilization and inbreeding depression. II. Empirical observations**, *Evolution* 39, 41–52
- Barrett, S.C.H. and Eckert, C.G. (1990) **Variation and evolution of mating systems in seed plants**, in *Biological Approaches and Evolutionary Trends in Plants* (Kawano, S., ed.), pp. 229–254, Academic Press
- Soltis, D.E. and Soltis, P.S. (1992) **The distribution of selfing rates in homosporous ferns**, *Am. J. Bot.* 79, 97–100
- Barrett, S.C.H. and Charlesworth, D. (1991) **Effects of a change in the level of inbreeding on the genetic load**, *Nature* 352, 522–524
- Husband, B.C. and Schemske, D.W. **Evolution of the magnitude and timing of inbreeding depression in plants**, *Evolution* (in press)
- Fu, Y-B. and Ritland, K. (1994) **Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus***, *Genetics* 136, 323–331
- Johnston, M.O. and Schoen, D.J. (1995) **Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species**, *Science* 267, 226–229
- Dudash, M.R. (1990) **Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): A comparison in three environments**, *Evolution* 44, 1129–1139
- Eckert, C.G. and Barrett, S.C.H. (1994) **Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): Population genetic and experimental analyses**, *Evolution* 48, 952–964
- Lande, R., Schemske, D.W. and Schultz, S.T. (1994) **High inbreeding depression, selective interference among loci and the threshold selfing rate for purging recessive lethal mutations**, *Evolution* 48, 965–978
- Ritland, K. (1990) **Inferences about inbreeding depression based on changes of the inbreeding coefficient**, *Evolution* 44, 1230–1241
- Dole, J. and Ritland, K. (1993) **Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient**, *Evolution* 47, 361–373
- Kohn, J.R. and Barrett, S.C.H. (1994) **Pollen discounting and the spread of a selfing variant in tristylous *Eichhornia paniculata*: evidence from experimental populations**, *Evolution* 48, 1576–1594

- 26 Lloyd, D.G. (1992) **Self- and cross-fertilization in plants. II. The selection of self-fertilization**, *Int. J. Plant Sci.* 153, 370–380
- 27 Holsinger, K.E. (1991) **Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems**, *Am. Nat.* 138, 606–622
- 28 Holsinger, K.E. **Pollination biology and the evolution of plant mating systems**, *Evol. Biol.* (in press)
- 29 Lloyd, D.G. and Webb, C.J. (1992) **The selection of heterostyly**, in *Evolution and Function of Heterostyly* (Barrett, S.C.H., ed.), pp. 179–207, Springer-Verlag
- 30 Lloyd, D.G. and Webb, C.J. (1986) **The avoidance of interference between the presentation of pollen and stigmas in angiosperms, I Dichogamy**, *N. Z. J. Bot.* 24, 135–162
- 31 Bertin, R.I. (1993) **Incidence of monoecy and dichogamy in relation to self-fertilization**, *Am. J. Bot.* 80, 557–560
- 32 Harder, L.D. and Barrett, S.C.H. (1996) **Pollen dispersal and mating patterns in animal-pollinated plants**, in *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants* (Lloyd, D.G. and Barrett, S.C.H., eds), pp. 140–190, Chapman & Hall
- 33 Bell, G. (1985) **On the function of flowers**, *Proc. R. Soc. London Ser. B* 224, 223–265
- 34 Harder, L.D. and Thomson, J.D. (1989) **Evolutionary options for maximizing pollen dispersal of animal-pollinated plants**, *Am. Nat.* 133, 323–344
- 35 Harder, L.D. and Barrett, S.C.H. (1995) **Mating cost of large floral displays in hermaphrodite plants**, *Nature* 373, 512–515
- 36 Harder, L.D. and Wilson, W.G. (1994) **Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants**, *Evol. Ecol.* 8, 542–559
- 37 de Jong, T.J., Waser, N.M. and Klinkhamer, P.G.L. (1993) **Geitonogamy: the neglected side of selfing**, *Trends Ecol. Evol.* 8, 321–325
- 38 Harder, L.D. and Cruzan, M.B. (1990) **An evaluation of the physiological and evolutionary influences of inflorescence size and flower depth on nectar production**, *Funct. Ecol.* 4, 559–572
- 39 Waller, D.M. (1993) **The statics and dynamics of mating system evolution**, in *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Thornhill, N.W., ed.), pp. 97–117, University of Chicago Press
- 40 Waser, N.M. (1993) **Population structure, optimal outbreeding and assortative mating in angiosperms**, in *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Thornhill, N.W., ed.), pp. 173–199, University of Chicago Press

Sex against virulence: the coevolution of parasitic diseases

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Parasites – here broadly defined as damage-producing organisms, including microbial pathogens, traditional parasites and small herbivores – are ubiquitous and influence either directly or indirectly almost every conceivable level of biological organization. The impact parasites have on the evolution and ecology of their hosts depends on their virulence, the driving force in host–parasite coevolution. Virulence, *per se* beneficial for neither parasite nor host, cannot be a property of a parasite alone; rather, it is a product of the host–parasite interaction. Different host genotypes from the same population do not suffer equally when infected with the same parasite strain, and different parasite strains cause variable levels of virulence in the same host genotype^{1–3}.

Most studies on the evolution of virulence have concentrated on parasite evolution, assuming that virulence is maintained by genetic trade-offs between virulence and other fitness components of the parasite. For example, parasite-induced host mortality was shown to be negatively correlated with host recovery rate (which contributes to parasite mortality) in Australian rabbits infected with the myxoma virus^{4,5} and positively correlated with the multiplication rate of a microsporidian parasite in *Daphnia* hosts³. Therefore, it has

Reciprocal selection is the underlying mechanism for host–parasite coevolutionary arms races. Its driving force is the reduction of host lifespan or fecundity that is caused by a parasite.

Parasites evolve to optimize host exploitation, while hosts evolve to minimize the ‘parasite-induced’ loss of fitness (virulence). Research on the evolution of virulence has mostly emphasized the role of parasite evolution in determining virulence. However, host evolution, accelerated by sexual recombination, contributes to the evolution and expression of virulence as well. The Red Queen hypothesis predicts that genetic variation among host offspring facilitates selection for reduced virulence. Here, we outline a synthesis between current thinking about the evolution of virulence and the evolution of sex.

been suggested that to maximize fitness a parasite should optimize the trade-off between virulence and other fitness components⁵. This optimality concept for the evolution of virulence, however, largely neglects genetic variation among hosts in their interaction with parasites. Such variation results in differential reproductive success among hosts and would, in the absence of parasite evolution, lead to reduced virulence. Given the high evolutionary rate of parasites^{4,6}, host evolution can often be ignored in a first approximation, but for a better understanding of the evolution of virulence it is essential to understand the host’s evolutionary response and in particular the role of genetic recombination in host evolution.

It has been suggested that sexual reproduction of hosts is a means to overcome the disadvantage of the low evolutionary rate that an asexual host would have in comparison with its rapidly evolving parasites^{7–9}. Combining current theory of the advantage of genetic recombination and outbreeding with the theory on the

evolution of virulence, one would predict that hosts continuously evolve to reduce virulence, while their parasites evolve to keep virulence as close as possible to an optimal level for their own life histories. In this arms race, a high

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