Evolution of Plant Breeding Systems   Review

Deborah Charlesworth

Breeding systems are important, and often neglected, aspects of the natural biology of organisms, affecting homozygosity and thus many aspects of their biology, including levels and patterns of genetic diversity and genome evolution. Among the different plant mating systems, it is useful to distinguish two types of systems: 'sex systems', hermaphroditic versus male/female and other situations; and the 'mating systems' of hermaphroditic populations, inbreeding, outcrossing or intermediate. Evolutionary changes in breeding systems occur between closely related species, and some changes occur more often than others. Understanding why such changes occur requires combined genetical and ecological approaches. I review the ideas of some of the most important theoretical models, showing how these are based on individual selection using genetic principles to ask whether alleles affecting plants' outcrossing rates or sex morphs will spread in populations. After discussing how the conclusions are affected by some of the many relevant ecological factors, I relate these theoretical ideas to empirical data from some of the many recent breeding system studies in plant populations.

Introduction

Breeding systems have attracted interest for many reasons. Their evolution and change is particularly evident in plants. Flower size differences are easily visible, and it has long been noticed that small-flowered plants that quickly produce many seeds without pollinator visits are often closely related to species with more conspicuous flowers that either set seed only after pollinator visits or are self-incompatible. The change from outcrossing to inbreeding is a repeated evolutionary transition, occurring in many unrelated genera. Many self-incompatible species have self-fertile relatives (for example [1–4]). For instance, the closest relatives of the inbreeder *Arabidopsis thaliana* are self-incompatible (see Figure 1, top), and there are many similar examples in the Brassicaceae [5] and other plant families.

The directionality of such changes is often evident from the relationships of the species. Phylogenetic analyses often infer outcrossing systems in ancestral lineages [6,7], particularly when these take into consideration the low likelihood of *de novo* evolution of systems such as self-incompatibility or separate sexes (see below). Thus, the bottom part of Figure 1 shows frequent loss of outcrossing systems and rarer evolution of outbreeding from highly inbreeding populations.

However, adaptations promoting outbreeding, including the evolution of separate sexes, have also evolved many times, which is briefly discussed below.
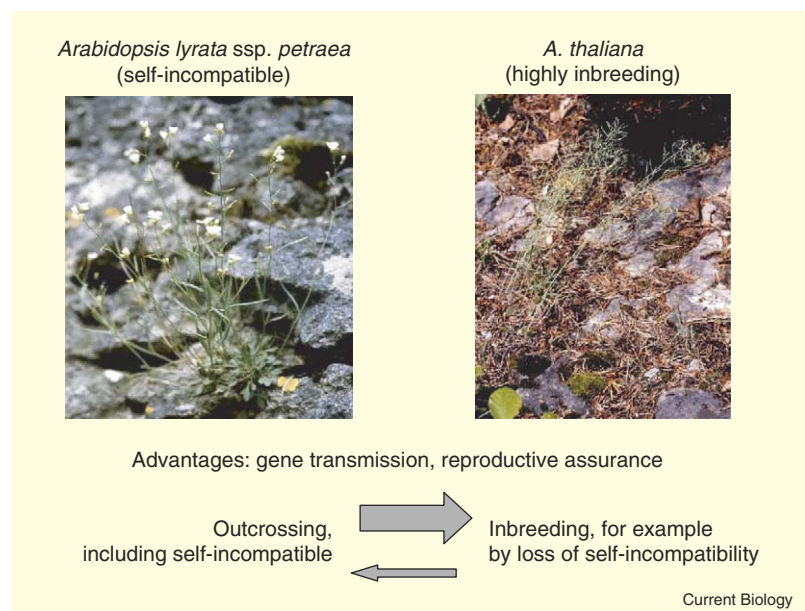
Breeding systems affect many important aspects of a population's ecology and evolution, including whether individuals are homozygous or heterozygous. A change to inbreeding rapidly increases homozygote frequencies, and thus individuals will express recessive or partially recessive deleterious mutations, and suffer lower survival probabilities and reduced fertility: 'inbreeding depression'. In the long term, these effects on survival and fertility lower mutation frequencies, and thus lead to reduced inbreeding depression (purging) [8]. Another effect of homozygosity is to allow expression of recessive advantages. A larger range of new mutations is therefore affected by selection, compared with outcrossing populations in which some degree of dominance is required [9]. However, inbreeding also lowers effective population size — a population of homozygotes has an effective size half that of an outcrossing diploid with the same number of individuals — implying greater effects of genetic drift, potentially reducing the ability of natural selection to eliminate disadvantageous mutations and increase the frequency of advantageous ones. Inbreeding populations are thus predicted slowly to deteriorate by fixing mildly deleterious mutations [10,11]. They will also tend to lose neutral genetic diversity.

Understanding mating system evolution is thus an important part of evolutionary biology. In addition to the ecological effects of breeding systems just mentioned, ecological factors must evidently be among the major influences on mating system evolution. For example population fragmentation might cause low availability of conspecific potential mates, or low availability of pollinators to ensure cross-fertilisation between individuals, and a similar situation may exist in species with a weedy or colonising lifestyle (which, like low density, may affect the effective availability of mates). Mating system evolution is also evidently likely to be affected by resource availability. Along with the supply of suitable pollinators, resources may limit plants' ability to produce fruits and seeds, with important effects on life history evolution and consequently on the mating system.

For instance, annual plants must produce seeds before dying, and even low quality offspring make some contribution to fitness, defined as the representation of an individual's genes in the progeny generation. It is thus not surprising that, even though self-fertilisation often leads to offspring of low quality because of inbreeding depression, many annuals have low

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Ashworth Lab. King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK.
Email: deborah.charlesworth@ed.ac.uk

Figure 1. Evolution of outcrossing and in-breeding.

Closely related outcrossing and inbreed-ing species in the genus *Arabidopsis* (upper part of figure). The lower part of the figure shows the different frequencies of transitions between outcrossing and inbreeding.

Arabidopsis lyrata ssp. *petraea*
(self-incompatible)

A. thaliana
(highly inbreeding)

Advantages: gene transmission, reproductive assurance

Outcrossing,
including self-incompatible

Inbreeding, for example
by loss of self-incompatibility

Current Biology

outcrossing frequencies in nature, especially as many are weeds, for which self-fertilisation provides the potential advantages of freedom from need for con-specifics, and from pollinating animals ('reproductive assurance').

In contrast, a perennial life history allows for the pos-sibility of producing offspring that will contribute to fit-ness in many years, and so evolution may favour a lower allocation of resources to reproduction, relative to an annual species [12]. In turn, this may lead to larger plant size, possibly affecting pollinator behaviour so that transfer of pollen between different individuals, and thus outcrossing, becomes less likely. The evolution of large plant size may thus be accompanied by other changes to restrict either the receipt of self pollen — in-volving the timing and structural separation of male and female stages of flowering, or even unisexuality of the whole plant — or self pollen acceptance — including chemical recognition systems ('self-incompatibility') that reject pollen of the same 'incompatibility type' as the maternal plant, and thus prevent self-fertilisation and also some matings between close relatives.

The evolution of mating systems raises some partic-ularly intriguing questions. It is clear that both genetic and ecological factors are important, but there has been disagreement about their relative importance. The issues are greatly clarified by understanding that selection acts on individuals in the short term, causing breeding system changes, probably often in relation to environmental changes — for example, reproductive assurance is likely to be important in colonising plants — and also has long-term consequences of such changes affecting population survival. Fitness differences between genotypes with different breed-ing systems, which depend on their ecological circum-stances, lead to evolutionary changes (short-term, or individual selection, effects). Because a population's breeding system influences its evolution and genetic diversity, the long-term consequences outlined above may also be important. Mating system patterns in

present day populations must therefore reflect the combined effects of the kinds of changes that evolve in different ecological situations — the input of breed-ing system changes — together with the survival or loss of populations and species with different breeding systems, again strongly influenced by their ecological circumstances.

Theories for Breeding System Changes
From the outline of some of the factors affecting mat-ing systems given above, it is clear that, even ignoring long-term consequences, a complete theory of breed-ing system changes will be very difficult, because it must include the selective forces that affect mating systems themselves, along with those affecting other aspects of plant fitness. How resources devoted to flowers affect pollination success — with, in many spe-cies, conspecifics and other species competing for an-imal pollinators — will interact with how outcrossing versus self-fertilisation affect offspring quality (which also depends on resources affecting fruit and seed output and quality). At the same time, resource alloca-tion to reproduction evolves in competition with de-mands for allocation to functions increasing survival and growth, in a life-history evolution context, and this may also involve aspects of competition between conspecific seedlings and further inter-species inter-actions in the process of seed dispersal. No theory combining life-history and resource allocation is avail-able, and a complete evolutionary model would prob-ably be too complex to be useful. Thus a general pre-dictive theory may be an unrealistic aim.

Instead, simplifying approaches have given us a fairly good understanding of several major factors in mating system evolution, despite this complexity. The advantage of this approach is that it generates testable predictions about single observable factors, for example, that low pollination levels tend to select for an ability to self-fertilise. Such predictions can be tested in the field and experimentally. A particularly
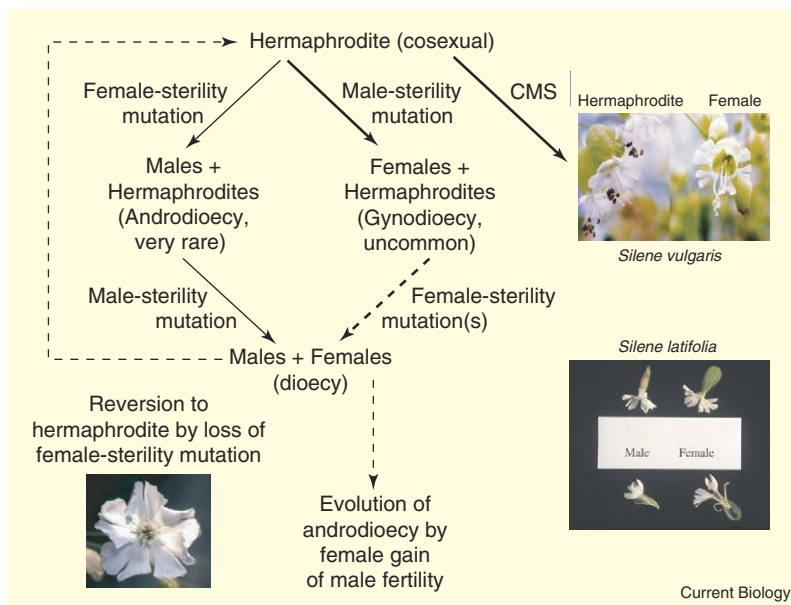
Figure 2. Evolution of two separate sexes from hermaphrodites, showing that at least two evolutionary steps are required.

Wider lines indicate commoner transitions, and dotted lines show changes that can occur once dioecy has evolved (reversion to hermaphroditism, and evolution of androdioecy). The figure illustrates the sex systems known within the genus *Silene*. The bladder campion (*S. vulgaris*) is an example of cytoplasmic male sterility (CMS) while the white campion, *S. latifolia*, is dioecious. Males with loss of female sterility (hermaphrodites carrying a Y chromosome) occasionally arise in *S. latifolia*; an example is shown at the bottom left-hand side of the figure. Androdioecy is not known in the genus *Silene*.

favourable circumstance for testing for selection (fitness differences) occurs when a population is polymorphic, and, remarkably, breeding systems are often polymorphic. Comparative tests, based on correlations between breeding systems and particular ecological situations, are also possible, because similar breeding system changes have occurred repeatedly.

As will be seen below, the evolution of inbreeding versus outcrossing, and even the initially puzzling evolution of separate sexes (dioecy) from hermaphroditism or monoecy (with separate male and female flowers on the same plant) are both simpler to understand than the evolution or maintenance of sexuality. A first, historically very helpful, insight was that mating systems are properties of individuals, not of populations. Thus an individual selection approach can be used to think about mating system evolution. It is particularly helpful to concentrate on the situations where a mutant is rare, as homozygotes can then be ignored, greatly simplifying the genetics, yet dealing with parts of the allele frequency range that tell us whether a population will change (whether the mutation will spread in the population). The mutation's fate is often determined by whether the phenotype of the rare heterozygotes gives higher fitness than the type initially present in the population; whether it will become fixed can similarly be studied by focusing on populations in which it is so common that homozygotes for the initial allele can be neglected [13]. These 'phenotypic selection models' are based on genetical principles, and are correct — they give identical results to those obtained by the more laborious calculations of genotype frequency changes — only under certain restrictions [14–16].

When changes evolve, the effects on the population outlined above are slow, because they rely on disadvantageous mutations and on genetic drift. In contrast, as will be seen, the individual selection increased fitness through reproduction of a selfing mutation can be very large, so that the long-term effects cannot prevent its spread throughout the population ('fixation'). We can thus study separately the input of breeding system changes, using an individual selection approach, and consider effects on the population's long-term survival and measures of 'population fitness' (such as whether it can produce enough seeds to persist) only as potentially affecting the maintenance of established breeding systems.

The Major Categories of Mating Systems

Mating systems descriptions involve three important aspects: first, whether sexual reproduction occurs at all; second, whether individuals have both sex functions ('cosexual', including hermaphroditic and monoecious plants) or whether some or all are unisexual males or females (dioecy) (reviewed in [17]); and third, whether cosexual individuals are self-compatible or not and, if compatible, what natural outcrossing rates are inferred using genetic markers or other approaches [18,19]. Hermaphrodite and monoecious species are 'sexually monomorphic', while dioecious populations are 'sexually polymorphic', having separate males and females (or other situations mentioned below, see Figure 2) determined by a genetic sex-determination system [20], or sometimes environmentally [21,22], or partially so (for example [23,24]).

As shown in Figure 2, the genus *Silene* includes cosexuals and dioecious species, with many hermaphrodite species and also 'gynodioecious' species having unisexual females [25], while dioecious *Saggitaria* species have monoecious relatives [26], among many other examples [27–29]. There are also a few sexually polymorphic species with cosexuals and males ('androdioecious'), all with close dioecious relatives ([23,30], reviewed in [31]).

Sex systems are relatively easy to determine, though flower morphology is sometimes misleading, because flowers of plants that have recently evolved dioecy may appear cosexual [32]. Functional mating systems in cosexuals are much more difficult to determine in the wild, as matings cannot be directly observed when pollinators, or wind or water pollination, are involved, and often only female reproduction is

accessible. Even functional tests of male fertility can be misleading, because seed set does not guarantee that viable progeny will be produced in natural conditions [33]. Inbreeding can, however, be quantified using genetic markers. Markers can be used to estimate frequencies of homozygote and heterozygote genotypes (which, assuming a population at equilibrium under a defined mating system, such as a given frequency of ovule self-fertilisation, can yield an estimate of this 'selfing rate').

Recent changes in frequencies of inbreeding can also be detected [34]. Alternatively, sets of seed parents and their progeny can be genotyped, yielding selfing rate estimates without assuming that equilibrium has been reached [35]. From a large body of such estimates, as well as from earlier careful observations in the field, it has been established that many annual plants have low outcrossing rates (as predicted above), while longer-lived plants tend to be more outcrossing [18]. Different outcrossing frequencies among cosexuals can be caused by timing differences in the male and female phases of flowers (or of whole plants in monoecious species) or by presence or absence of self-incompatibility. It is also clear that a substantial minority of flowering plants have intermediate outcrossing rates [13,18,36].

Genetic Models of Mating System Evolution
Outcrossing rates
Models of mating system evolution have emphasised genetic effects, even though, as already explained, ecological circumstances, such as pollinator abundance or plant density, must often be important. Their complexity and variety, however, creates difficulties in developing any general theories. In contrast, there are some generally applicable strong genetic effects. Although many organisms are outcrossing, theory tells us that, like asexuality, inbreeding has a large advantage due to the increased transmission of gametes to the next generation — it gives an advantage to alleles that increase the rate of self-fertilisation or other inbreeding [37]. For selfing, the effect is smaller than the two-fold advantage of asexual reproduction, unless the allele causing increased selfing also causes reproductive resources to be re-allocated from pollen production to the extent that female fertility is increased. Even with no such effect, however, an allele causing complete selfing has an initial 50% advantage when introduced into an outcrossing population. This advantage arises because selfing individuals transmit two gametes to their own seeds, and may in addition contribute gametes if their pollen fertilises ovules of other plants. These simple models predict that complete selfing should evolve, unless some strong selective force acts against inbreeding.

Models involving individuals with different selfing rates must take into account this genetic force, alongside the influences of ecological circumstances that may affect the evolutionary outcome. If the density of conspecific plants, or of pollinators, is low, selfing has an additional potentially strong advantage, as a plant that is unable to self-fertilise has a lower chance of producing progeny either through its own seeds, or via its pollen generating outcrossed seeds on other maternal plants. This makes the maintenance of outcrossing even harder to explain.

Other circumstances can, however, reduce the advantage of mutations causing a change from outcrossing to inbreeding. A highly selfing individual may sire no outcrossed seeds on other plants (complete 'pollen discounting'). This complete loss of male fitness via outcrossing abolishes the advantage to selfing [38]; lesser discounting reduces the advantage [39]. If selfing occurs by pollinators moving from flower to flower on the same plant ('geitonogamous' pollination), each pollinator visit causing self-pollination of a flower is a missed potential outcross pollination event. Pollen discounting is likely when selfing is due to a mutation making the flowers small, so that anther–stigma separation is reduced and self pollen is deposited. Such flowers may produce less pollen, or pollen may be less available to pollinators (sometimes, flowers remain closed or only partially open). Smaller flowers will generally also attract fewer pollinator visits. The reduced outcrossing lowers contributions to the individuals' fitness through pollen; they may then have only a small fitness advantage relative to outcrossing individuals, or no advantage. Similarly, 'competing self-fertilisation', in which pollinators can bring either self pollen or pollen from other plants, is less advantageous than 'delayed self-fertilisation', in which ovules are fertilised by self pollen only after outcrossing opportunities have ended [13,39].

The general conclusion that selfing often has a large genetic advantage is important because it highlights the need for a strong disadvantage, to account for the common existence of outcrossing systems. Inbreeding depression is likely often to be the most important such disadvantage. Theoretical models show that strong inbreeding depression — with, in the simplest models, outbred offspring having more than twice the survival rate of inbred ones — can prevent the invasion of an outcrossing population by a selfing mutant [13,40,41]. These results have prompted many experiments that indeed have yielded empirical evidence, from a wide diversity of plants, that inbred progeny often have low survival and fertility, with a tendency for lesser effects in more inbred populations (reviewed in [19]). In some plant populations, inbreeding depression can be severe throughout life (for example [42–45]), as is notorious in some conifers [46]. Inbreeding depression is, however, effectively decreased if inviable seeds are replaced with others, making selfing more advantageous [47], while pollen discounting adds a disadvantage to selfing, effectively increasing inbreeding depression.

More Realistic Models and Intermediate Outcrossing
The simple phenotypic models for the evolution of outcrossing outlined above ignore many ecological details that may be important in particular situations. For instance, many different situations may lead to evolution of intermediate outcrossing rates (reviewed in [19,48]). These include situations with competing self-fertilisation when higher self-fertilisation lowers the fraction of outcrossed ovules [13] and the somewhat similar mass action model [49], seedling competition [50], biparental inbreeding [51], trade-offs
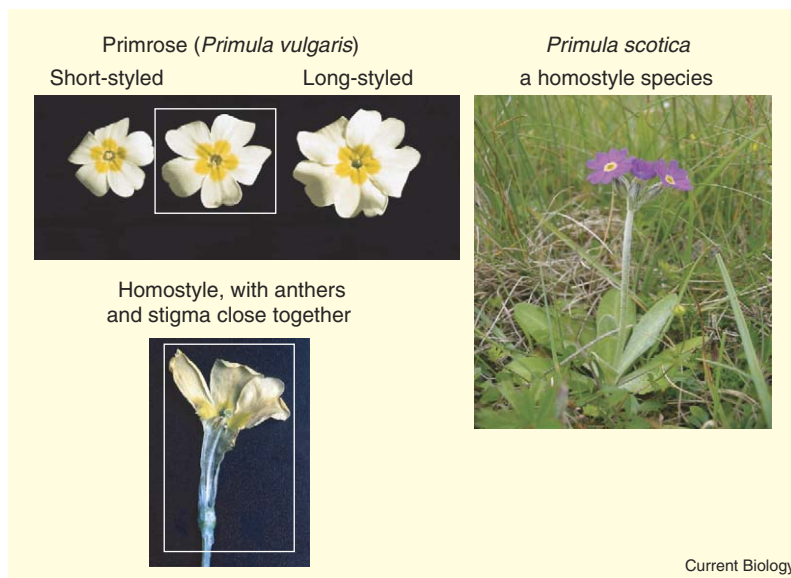
Figure 3. Heterostylous systems in the genus *Primula*, and the breakdown of heterostyle.

Heterostyled flowers of the primrose *Primula vulgaris*, showing the self-compatible 'homostyle' flower type (left-hand side of the figure). On the right is shown a homostyled species whose distribution of which is confined to regions where pollination is often unreliable, due to bad weather.

between female and male functions [52,53], and pollen limitation, which can lead to a low, but non-zero, outcrossing, as commonly observed [54].

Since the development of these models, they have been successively improved by introducing greater genetic realism, including models of the genetic basis of inbreeding depression replacing the simple assumption of a fixed relative fitness of progeny produced by selfing [55–57]. For instance, intermediate outcrossing rates evolve if inbreeding depression either increases in successive generations, as a result of a contribution from overdominant loci [58], or varies temporally [59].

The genetic details of precisely how outcrossing systems can change are also important, because they determine whether the necessary genetic and phenotypic changes are likely to evolve, or difficult to evolve, thus affecting the rates of changes from outcrossing to inbreeding, and vice versa, which may be important in analysing rates of evolutionary transitions. A simple flower alteration could change an outcrossing flower to one with much higher self-fertilisation, such as the example of small flower size just mentioned, but even this case is not simple, as it must be remembered that there will also be other effects on fertilisations achieved; changes in anther–stigma separation can also probably be selected to best manage pollen removal by pollinators, and delivery when they visit the next flower, and to reduce interference between male and female functions [60]. This is an example of how selection probably very often acts on flower morphology — including the timing of the phases of male and female functions — to increase outcrossing success via pollen, by avoiding pollen discounting and minimising interference between male and female functions. Flowers represent the integrated outcome of selection of this kind, along with selection affecting the proportions of outcrossed and selfed seeds.

Self-Incompatibility Systems

Genetic details are evidently important in the evolution of self-incompatibility systems. An initial incompatibility allele, causing all pollen grains of its carrier plants to be recognised and rejected by its stigmas (as when the allele is expressed sporophytically in the plant producing the pollen), will have the simple effect of causing self-incompatibility, much as in the simplified models above that assume a mutation altering the outcrossing rate. However, an initial incompatibility allele expressed by the male gametophytes (pollen grains) will have different evolutionary dynamics. In a plant heterozygous for the mutant (incompatible) allele, half the pollen will carry the other allele. Thus the new allele does not render this plant self-incompatible, but, at best, increases its outcrossing rate. This advantage to the diploid parent is at the cost of a great disadvantage to the self-incompatible pollen genotype. Consequently, for such an allele to invade a population, inbreeding depression must be very large [55,61,62]. Interestingly, these models predict that non-functional alleles may persist alongside alleles causing incompatibility, another situation in which outcrossing rates may be intermediate, and indeed self-compatible individuals may coexist with incompatible ones in plant populations [1].

It is much harder to understand how outcrossing systems evolve when several different genetic changes are necessary. Despite their name, self-incompatibility systems do not involve a self-recognition system, but are based on genes encoding proteins with receptors and ligands that recognise one another (reviewed in [63]). The cross-incompatibility between some individuals is the basis for the advantage of rare alleles in plant incompatibility systems, which maintains many alleles in populations of plants with the homomorphic type of system, in which flower morphology is similar for all incompatibility types [64]. The evolution of such a system in a species that lacks incompatibility is likely to be a very rare event. Separate genes encode the pollen and pistil recognition proteins in both types of self-incompatibility system whose genes have been identified: the sporophytic system in Brassica and other related species [65–67], and the gametophytic systems with pistil S-RNases and pollen F-box proteins in the Solanaceae, Rosaceae and Antirrhinum (reviewed in
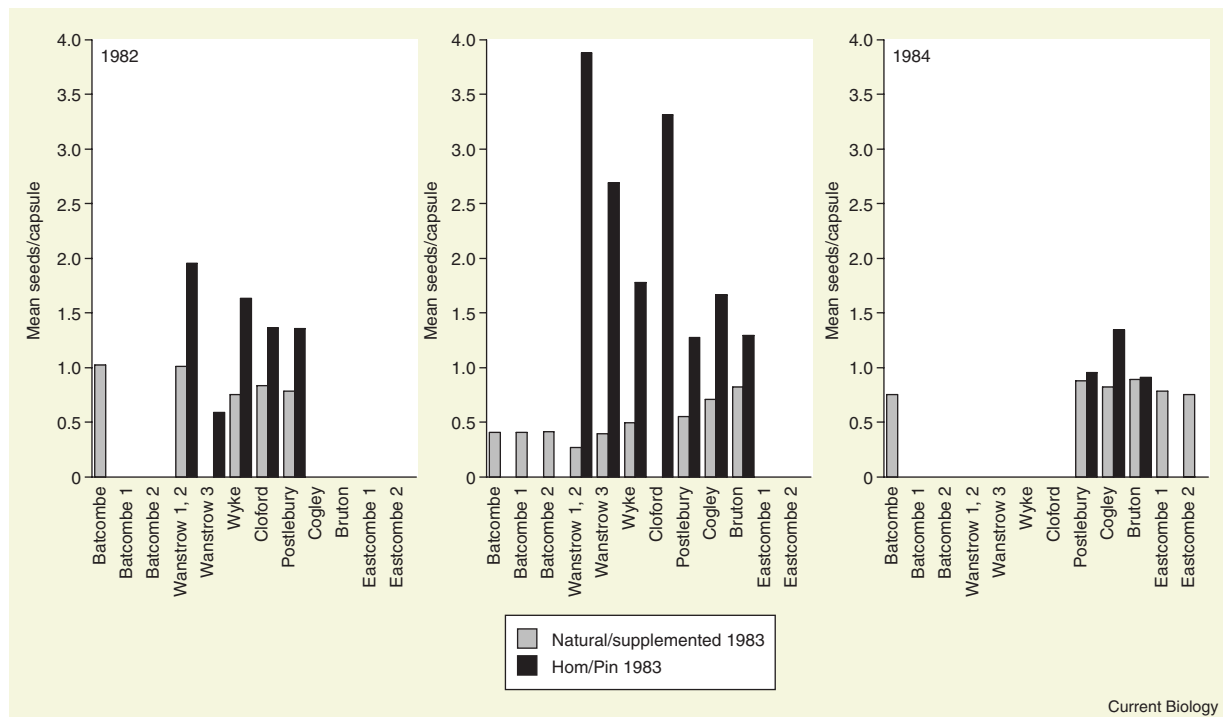
Figure 4. Reproductive assurance in several English natural populations of *Primula vulgaris* in which homostyle plants occur (data from [81]).

The figure compares the results for 1982 to 1984 from naturally pollinated flowers of the 'pin' (long styled, self-incompatible) morph with flowers of the same morph experimentally pollinated to ensure excess pollen receipt (supplemented), and with naturally pollinated flowers of homostyle plants. Only the long-styled morph supplementation results are shown, because supplementation of flowers of the short-styled morph is difficult, but the few results were very similar to those shown. In 1982 and 1984, seed numbers per fruit under natural pollination were slightly below those achieved by supplemented flowers (the grey bars are only slightly below 1), indicating good pollination levels. Homostyles nevertheless often had somewhat higher seed set per fruit (and per plant) than the self-incompatible long-styled morph (black bars often higher than 1). In 1983, the flowering season was unusually rainy. Pollination was evidently poor, as pin plants' seed numbers under natural pollination were low, relative to flowers with pollen added (grey bars often less than 1). In that year, homostyle plants' seed output greatly exceeded that of pin plants (black bars much higher than 1). Thus the homostyle flowers benefited from reproductive assurance in 1983.

[68]). The problem of how new alleles can arise, given the requirement for coadapted alleles at the two incompatibility loci, is not yet solved [69,70].

The evolution of heterostylous systems with two or three floral morphs (Figure 3), often with cross-incompatibility among plants of the same morph [71,72] involves coordinated development of different flower parts. Again, successive changes at linked loci must have been involved, to avoid producing a high proportion of progeny with incorrect arrangements of the anthers and stigmas [73,74].

The trend to include greater realism has also led to study of relevant ecological factors. For example, population subdivision can lead to intermediate outcrossing rates [75]. Under certain conditions, intermediate outcrossing may also arise when low amounts of compatible pollen are received, which is likely to be important for the evolution and maintenance of self-incompatibility [76]. The importance of reproductive assurance has already been mentioned, and evidence for its action is accumulating. In populations of *Collinsia verna*, a self-compatible plant with generally low outcrossing rates and delayed selfing, outcrossing rates were lower in years when pollinator service was poor [77], and within-flower self-pollination was higher, leading to progeny suffering some

inbreeding depression, but not of high enough magnitude to negate the advantage of selfing.

Heterostylous systems have repeatedly broken down to yield more inbreeding populations [71,78]. Reproductive assurance is probably sometimes the selective reason for this. In a set of English populations of *Primula vulgaris*, pollinator service, reflected in numbers of seeds per fruit, and fruits per plant, varied from year to year (Figure 4). In these populations, as shown in Figure 3, some plants are self-compatible 'homostyles', which can self-fertilise within flowers, unlike the usual two incompatible Primrose 'morphs' [79,80]. In two good pollinator years, all plant morphs had fairly uniform, high seed output, but in one year, when the weather was unusually rainy at the flowering season, the self-fertile morph had much higher seed output than the incompatible morphs [81].

Evolution of Unisexuality
Like the maintenance of outcrossing, the evolution of unisexuality presents difficult puzzles. First, like the evolution of self-incompatibility systems, at least two successive stages are required, first evolving females or males — generating gynodioecious or androdioecious populations, respectively — and then changing the cosexuals into males or females, in one or more

steps [15] (illustrated in Figure 2). Second, both unisexual females and males in dioecious populations, with no male fertility, must have a strong disadvantage relative to cosexual plants, unless something compensates for the fertility loss.

The second difficulty is less severe for cytoplasmic male-sterility (CMS) than nuclear sterility factors. The spread of CMS mutations is not affected by the loss of male fertility, so these mutations can invade cosexual populations, given only a slight female fertility advantage; CMS factors are thus classic 'selfish' genetic elements. Higher female fertility of male sterile plants is likely if unisexuals reallocate resources to female functions, which is expected whenever developmental trade-offs occur between different functions that draw on the same pool of resources. Evidence for this has accumulated from many 'gynodioecious' populations, in which females and hermaphrodites are polymorphic and can be compared [82]. Populations with CMS polymorphisms may then be invaded by nuclear genes restoring male fertility, leading to complex genetic polymorphisms with female frequencies that vary greatly among populations [83], as theoretically predicted [84–88].

The chance that unisexual females receive pollen is evidently important for breeding system evolution, so population subdivision interacts with the genetic basis of male sterility. Recolonisation after extinction of local populations can either increase or decrease female frequencies in systems with mixed cytoplasmic/nuclear male-sterility, depending on the extinction rate and dominance of male fertility restorers; although females are more often absent by chance from small populations [83], restorer genes are also often absent, allowing the advantage of the sterility cytoplasm to operate [89,90]. Some populations are polymorphic for hermaphrodites and males (androdioecy), probably due to females of dioecious species evolving some ability to produce pollen in response to pollination limitation in colonising situations [91–93].

Two possible factors can compensate for loss of male functions caused by nuclear sterility genes: inbreeding depression favouring unisexuals, and trade-offs increasing the remaining sex function. Recent work is starting to uncover negative genetic correlations (trade-offs) in gynodioecious species between hermaphrodites' pollen and fruit production [94]. Realised seed or pollen production often depends on environmental quality. Many examples are known in which female fertility of cosexual plants appears more susceptible to limitation by low environmental quality than male fertility [33]. In many plants male reproduction begins at a younger age than seed production [95], or males grow larger [96], though differences are not invariably seen. In monoecious species, seed production may be more labile than pollen production [97], and this is also found in 'subdioecious' species, such as the spindle tree *Euonymus europeus* [32], in which females coexist with cosexuals that often have low fruiting ability ('inconstant males', see [98]), particularly in poor environments. In plants that change sex in response to environmental conditions, poor conditions often yield males while plants develop as females only in good conditions [21,22].

Animal pollinators are also likely to be a very important part of the environmental influences. If pollen is an important reward, females may be less attractive than males, and less re-visited. On the other hand, male fitness relies on pollinators visiting female plants, so that selection may favour lower rewards in the flowers of male plants. An evolutionary balance is to be expected between pressures increasing male attractiveness, increasing the fitness of males in competition with conspecific males, and decreasing it, inducing pollinators to leave a male plant, and, ideally next visit a female.

Conclusions

Given the complexity of factors that can influence the success of different mating systems, a general predictive theory seems unlikely. We can nevertheless identify the major variables, outcrossing rates and their consequences for the genetic phenomenon of inbreeding depression, and allocation of resources to pollination, including attracting and rewarding pollinating animals, and nourishing and protecting seeds. Ecological forces are clearly important, often determining whether a species' mating system is selected to change. Outcrossing rates depend on plant density, and on the density and nature of pollinators in animal pollinated populations, and seed production in many plants is limited by pollen supplies, since more seeds are often produced when natural pollination is supplemented experimentally, as in the two studies cited above [77,81]. The mode of pollination affects the selection on outcrossing versus selfing, and as explained above, many ecologically plausible situations can allow intermediate selfing rates.

It is also firmly established that the evolutionary effects that drive mating system evolution are short-term, and that characteristics that lower population survival chances often cannot be opposed. The study of mating system evolution has uncovered several examples. Self-fertilisation has often evolved, despite the inbreeding depression it causes. It is not yet clear whether this leads to extinction of selfing species or populations. Although some evidence suggests a deficit of old selfing lineages, it is not conclusive about this question [99]. It is difficult to determine how long selfing lineages can persist, because phylogenetic analyses that compare numbers of transitions from outcrossing to selfing, and *vice versa*, mostly ignore the evidence that the former is a simple change that may readily occur (see above), whereas the evolution of an outcrossing mechanism where none previously existed must often be extremely unlikely, particularly when several successive changes are required (for instance, evolution of self-incompatibility or of dioecy, discussed above).

The evidence that self-incompatibility systems are maintained over long evolutionary times implies that changes to selfing rarely reverse [100]. This is partly because of the difficulty of evolving incompatibility systems, but also because inbred populations may often evolve low inbreeding depression (if they persist long enough). It is difficult to determine how long ago present-day selfers changed from outcrossing to selfing. This could be much more recent than the time of separation from the closest extant relative species,

as is thought to be true for *A. thaliana*'s change to selfing [101].

The evolution of dioecy also involves the evolution of two kinds of disadvantage. Unisexual females are evidently exposed to a risk of failure to receive pollen, so that fruit-set may fail. This can explain the many examples of reversion to cosexuality, including the androdioecious species mentioned earlier. It may also lead to extinction of dioecious species, perhaps partially explaining why dioecious taxa include fewer species than sister taxa [29]. A longer-term problem may come from the evolution of sex chromosomes. As selection eliminates X–Y recombination, Y-linked genes may accumulate deleterious mutations, and eventually be deleted [102], leading to reduced male survival and lower success of pollen carrying Y chromosomes. These examples show very clearly that it is simplistic to conclude that competition leads to beneficial outcomes, and that the theory of evolution makes no such prediction.

We now have a clear body of individual selection based theories and models, well integrated with approaches and techniques developed for testing ideas in experiments, field studies, and comparative analyses. It is to be hoped that more of the most important assumptions and predictions will be tested in the next few years. The many plants with intermediate outcrossing rates offer excellent opportunities for comparing the selective advantages and disadvantages of outcrossing. Studies of pollination, including comparisons of different floras along gradients of pollinator abundance, should help to clarify cases of thresholds when outcrossing cannot be maintained, and show how competition for pollinators, or the presence of other species flowering at the same time, may influence selection for inbreeding versus outcrossing, in turn affecting flower attractiveness and the allocation to rewards versus other plant functions. More tests of whether inbreeding depression is stonger under competition are needed, as this is assumed in some models.

The puzzle of how new self-incompatibility alleles arise remains to be solved, as do the genetic details of loss of incompatibility, and whether (and how) it can be regained once lost by a species. More evidence is also needed on the extent to which increased male function in cosexuals comes at a cost of lowered female fertility (and how much allocation to pollinator attraction may reduce pollen and ovule production); this is likely to illuminate the evolution of pollen/ovule ratios (which may affect pollination), as well as the evolution of dioecy and of sex chromosomes.

References

1. Lloyd, D.G. (1965). Evolution of self-compatibility and racial differentiation in Leavenworthia (Cruciferae). Contrib. Gray Herbarium Harv. Univ. *195*, 3–134.

2. Brauner, S., and Gottlieb, L.D. (1987). A self-compatible plant of Stephanomeria exigua subsp. coronaria (Asteraceae) and its relevance to the origin of its self-pollinating derivative S. malheurensis. Syst. Bot. *12*, 299–304.

3. Macnair, M.R., Macnair, V.E., and Martin, B.E. (1989). Adaptive speciation in Mimulus: an ecological comparison of M. cupriphilus with its presumed progenitor M. guttatus. New Phytol. *112*, 269–279.

4. Wyatt, R., Evans, E.A., and Sorenson, J.C. (1992). The evolution of self-pollination in granite outcrop species of Arenaria (Caryophyllaceae). VI. Electrophoretically detectable genetic variation. Systematic Botany *17*, 201–209.

5. Bateman, A.J. (1955). Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity *9*, 52–68.

6. Goodwillie, C. (1999). Multiple origins of self-compatibility in Linanthus section Leptosiphon (Polemoniaceae): Phylogenetic evidence from internal-transcribed-spacer sequence data. Evolution *53*, 1387–1395.

7. Schoen, D.J., L'Heureux, A.-M., Marsolais, J., and Johnston, M.O. (1997). Evolutionary history of the mating system in Amsinckia (Boraginaceae). Evolution *51*, 1090–1099.

8. Byers, D.L., and Waller, D.M. (1999). Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu. Rev. Ecol. and Systematics *30*, 479–513.

9. Charlesworth, B. (1992). Evolutionary rates in partially self-fertilizing species. Amer. Nat. *140*, 126–148.

10. Whitlock, M.C. (2002). Selection, load and inbreeding depression in a large metapopulation. Genetics *160*, 1191–1202.

11. Glémin, S. (2003). How are deleterious mutations purged? Drift versus nonrandom mating. Evolution *57*, 2678–2687.

12. Morgan, M.T., Schoen, D.J., and Bataillon, T. (1997). The evolution of self-fertilization in perennials. Am. Nat. *150*, 618–638.

13. Lloyd, D.G. (1979). Some reproductive factors affecting the selection of self-fertilization in plants. Am. Nat. *113*, 67–79.

14. Lloyd, D.G. (1977). Genetic and phenotypic models of natural selection. J. Theoret. Biol. *69*, 543–560.

15. Charlesworth, B., and Charlesworth, D. (1978). A model for the evolution of dioecy and gynodioecy. Am. Nat. *112*, 975–997.

16. Maurice, S., Couvet, D., Charlesworth, D., and Gouyon, P.-H. (1993). The evolution of gender in hermaphrodites of gynodioecious populations: a case in which the successful gamete method fails. Proc. Royal Soc. Lond., B. *251*, 253–261.

17. Barrett, S.C.H. (2002). The evolution of plant sexual diversity. Nat. Rev. Genet. *3*, 274–284.

18. Vogler, D.W., and Kalisz, S. (2001). Sex among the flowers: the distribution of plant mating systems. Evolution *55*, 202–204.

19. Goodwillie, C., Kalisz, S., and Eckert, C.G. (2005). The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. Annu. Rev. Ecol. and Systematics *36*, 47–79.

20. Charlesworth, D., and Guttman, D.S. (1999). The evolution of dioecy and plant sex chromosome systems. In Sex Determination in Plants, C.C. Ainsworth, ed. (Oxford: BIOS), pp. 25–49.

21. Condon, M.A., and Gilbert, L.E. (1988). Sex expression of Gurania And Psiguria (Cucurbitaceae) - neotropical vines that change sex. Am. J. Bot. *75*, 875–884.

22. Zimmerman, J.K. (1991). Ecological correlates of labile sex expression in the orchid Catasetum viridiflavum. Ecology *72*, 597–608.

23. Pannell, J. (1997). Mixed genetic and environmental sex determination in an androdioecious population of Mercurialis annua. Heredity *78*, 50–56.

24. Yamashita, N., and Abe, T. (2002). Size distribution, growth and inter-year variation in sex expression of Bischofia javanica, an invasive tree. Ann. Bot. *90*, 599–605.

25. Desfeux, C., Maurice, S., Henry, J.P., Lejeune, B., and Gouyon, P.H. (1996). Evolution of reproductive systems in the genus Silene. Proc. Roy. Soc. Lond. B. *263*, 409–414.

26. Dorken, M.E., and Barrett, S.C.G. (2004). Sex determination and the evolution of dioecy from monoecy in Sagittaria latifolia (Alismataceae). Proc. Roy. Soc. Lond. B. *271*, 213–219.

27. Renner, S.S., and Ricklefs, R.E. (1995). Dioecy and its correlates in the flowering plants. Am. J. Bot. *82*, 596–606.

28. Renner, S.S., and Won, H. (2001). Repeated evolution of dioecy from monoecy in Siparunaceae (Laurales). Systematic Biol. *50*, 700–712.

29. Heilbuth, J.C. (2000). Lower species richness in dioecious clades. Am. Nat. *156*, 221–241.

30. Rieseberg, L.H., Philbrick, C.T., Pack, P.P., Hanson, M.A., and Fritsch, P. (1993). Inbreeding depression in androdioecious populations of Datisca glomerata (Datiscaceae). Am. J. Bot. *80*, 757–762.

31. Pannell, J.R. (2002). The evolution and maintenance of androdioecy. Annu. Rev. Ecol., Evol. Systematics *33*, 397–425.

32. Darwin, C.R. (1877). The Different Forms of Flowers on Plants of the Same Species (London: John Murray).

33. Verdu, M., Montilla, A.I., and Pannell, J.R. (2004). Paternal effects on functional gender account for cryptic dioecy in a perennial plant Proc. Roy. Soc. Lond. Series B: Biological Sciences *271*, 2017–2023.

34. Enjalbert, J., and David, J.L. (2000). Inferring recent outcrossing rates using multilocus individual heterozygosity: application to evolving wheat populations. Genetics *156*, 1973–1982.

35. Ritland, K. (1993). Estimation of mating systems. In Plant Genetics and Breeding, Vol. Part A, S.D. Tanksley and T.J. Orton, eds. (Amsterdam: Elsevier), pp. 289–301.

36. Baker, H.G. (1959). Reproductive methods as a factor in speciation in flowering plants. Cold Spring Harb. Symp. Quant. Biol. 24, 177–191.

37. Fisher, R.A. (1941). Average excess and average effect of a gene substitution. Ann. Eugen. 11, 53–63.

38. Nagylaki, T. (1976). A model for the evolution of self fertilization and vegetative reproduction. J. Theoret. Biol. 58, 55–58.

39. Lloyd, D.G. (1992). Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int. J. Plant Sci. 153, 370–380.

40. Lande, R., and Schemske, D.W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39, 24–40.

41. Charlesworth, B. (1980). The cost of sex in relation to mating system. J. Theoret. Biol. 84, 655–671.

42. Willis, J.H. (1993). Effects of different levels of inbreeding on fitness components in Mimulus guttatus. Evolution 47, 864–876.

43. Herlihy, C.R., and Eckert, C.G. (2002). Genetic cost of reproductive assurance in a self-fertilizing plant. Nature 416, 320–323.

44. Ramsey, M., Vaughton, G., and Peakall, R. (2006). Inbreeding avoidance and the evolution of gender dimorphism in Wurmbea biglandulosa (Colchicaceae). Evolution 60, 529–537.

45. Schneller, J.J., and Holderegger, R. (1997). Vigor and survival of inbred and outbred progeny of Athyrium filix-femina. Int. J. Plant Sci. 158, 79–82.

46. Savolainen, O., Kärkkäinen, K., and Kuitinien, H. (1992). Estimated numbers of embryonic lethals in conifers. Heredity 69, 308–314.

47. Porcher, E., and Lande, R. (2005). Reproductive compensation in the evolution of plant mating systems. New Phytologist 166, 673–684.

48. Johnston, M.O. (1998). Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. Genetica 102/103, 267–278.

49. Holsinger, K.E. (1991). Mass action models of plant mating systems: the evolutionary stability of mixed mating systems. Am. Nat. 138, 606–622.

50. Lloyd, D.G. (1980). Demographic factors and mating patterns in angiosperms. In Demography and Evolution in Plant Populations, O.T. Solbrig, ed. (Oxford: Blackwell), pp. 67–88.

51. Uyenoyama, M.K. (1986). Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution 40, 388–404.

52. Gregorius, H.-R. (1982). Selection in plant populations of effectively infinite size. II. Protectedness of a biallelic polymorphism. J. Theoret. Biol. 96, 689–705.

53. Charlesworth, D., and Charlesworth, B. (1978). Population genetics of partial male-sterility and the evolution of monoecy and dioecy. Heredity 41, 137–153.

54. Porcher, E., and Lande, R. (2005). The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. J. Evol. Biol. 18, 497–508.

55. Porcher, E., and Lande, R. (2005). Loss of gametophytic self-incompatibility with evolution of inbreeding depression. Evolution 59, 46–60.

56. Latta, R., and Ritland, K. (1994). Models for the evolution of selfing under alternative models of inheritance. Heredity 71, 1–10.

57. Charlesworth, D., Morgan, M.T., and Charlesworth, B. (1990). Inbreeding depression, genetic load and the evolution of outcrossing rates in a multi-locus system with no linkage. Evolution 44, 1469–1489.

58. Charlesworth, D., and Charlesworth, B. (1990). Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44, 870–888.

59. Cheptou, P.O., and Mathias, A. (2000). Can varying inbreeding depression select for intermediary selfing rates? Am. Nat. 157, 361–373.

60. Routley, M.B., Bertin, R.I., and Husband, B.C. (2004). Correlated evolution of dichogamy and self-incompatibility: A phylogenetic perspective. Int. J. Plant Sci. 165, 983–993.

61. Uyenoyama, M.K. (1988). On the evolution of genetic incompatibility systems. II. Initial increase of strong gametophytic self-incompatibility under partial selfing and half-sib mating. Am. Nat. 131, 700–722.

62. Charlesworth, D., and Charlesworth, B. (1979). The evolution and breakdown of S-allele systems. Heredity 43, 41–55.

63. Charlesworth, D., Vekemans, X., Castric, V., and Glémin, S. (2005). Plant self-incompatibility systems: a molecular evolutionary perspective. New Phytologist 168, 61–69.

64. Vekemans, X., and Slatkin, M. (1994). Gene and allelic genealogies at a gametophytic self-incompatibility locus. Genetics 137, 1157–1165.

65. Schopfer, C.R., Nasrallah, M.E., and Nasrallah, J.B. (1999). The male determinant of self-incompatibility in Brassica. Science 286, 1697–1700.

66. Takayama, S., Shiba, H., Iwano, M., Shimosato, H., Che, F.-S., Kai, N., Suzuki, G., Hinata, K., and Isogai, A. (2000). The pollen determinant of self-incompatibility in Brassica campestris. Proc. Natl. Acad. Sci. USA 97, 1920–1925.

67. Kusaba, M., Dwyer, K., Hendershot, J., Vrebalov, J., Nasrallah, J.B., and Nasrallah, M.E. (2001). Self-incompatibility in the genus Arabidopsis: characterization of the S locus in the outcrossing A. lyrata and its autogamous relative, A. thaliana. Plant Cell 13, 627–643.

68. Kao, T.H., and Tsukamoto, T. (2004). The molecular and genetic bases of S-RNase-based self-incompatibility. Plant Cell 16, S72–S83.

69. Uyenoyama, M.K., Zhang, Y., and Newbigin, E. (2001). On the origin of self-incompatibility haplotypes: transition through self-compatible intermediates. Genetics 157, 1805–1817.

70. Chookajorn, T., Kachroo, A., Ripoll, D.R., Clark, A.G., and Nasrallah, J.B. (2003). Specificity determinants and diversification of the Brassica self-incompatibility pollen ligand. Proc. Natl. Acad. Sci. USA 101, 911–917.

71. Barrett, S.C.H. (1992). Heterostylous genetic polymorphisms: model systems for evolutionary analysis. In Evolution and Function of Heterostyly, A. Omary, ed. (Heidelberg: Springer-Verlag), pp. 1–29.

72. Barrett, S.C.H., and Harder, L.D. (1996). Ecology and evolution of plant mating. Trends Ecol. Evol. 11, 73–79.

73. Charlesworth, D., and Charlesworth, B. (1979). A model for the evolution of distyly. Amer. Nat. 114, 467–498.

74. Lloyd, D.G., and Webb, C.J. (1992). The evolution of heterostyly. In Evolution and function of heterostyly, S.C.H. Barrett, ed. (Heidelberg: Springer-Verlag), pp. 151–178.

75. Holsinger, K.E. (1986). Dispersal and plant mating systems: the evolution of self-fertilization in subdivided populations. Evolution 40, 405–413.

76. Vallejo-Marin, M., and Uyenoyama, M.K. (2004). On the evolutionary costs of self-incompatibility: incomplete reproductive compensation due to pollen limitation. Evolution 58, 1924–1935.

77. Kalisz, S., Vogler, D.W., and Hanley, K.M. (2004). Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430, 884–887.

78. Husband, B.C., and Barrett, S.C.H. (1993). Multiple origins of self-fertilization in tristylous Eichhornia paniculata (Pontederiaceae): inferences from style morph and isozyme variation. J. Evol. Biol. 6, 591–608.

79. Crosby, J.L. (1949). Selection of an unfavourable gene-complex. Evolution 3, 212–230.

80. Piper, J., Charlesworth, B., and Charlesworth, D. (1984). A high rate of self-fertilization and increased seed fertility of homostyle primroses. Nature 310, 50–51.

81. Piper, J., Charlesworth, B., and Charlesworth, D. (1986). Breeding system evolution in Primula vulgaris and the role of reproductive assurance. Heredity 56, 207–217.

82. Shykoff, J.A., Kolokotronis, S.O., Collin, C.L., and Lopez-Villavicencio, M. (2003). Effects of male sterility on reproductive traits in gynodioecious plants: a meta-analysis. Oecologia 135, 1–9.

83. Nilsson, E., and Agren, J. (2006). Population size, female fecundity, and sex ratio variation in gynodioecious Plantago maritima. J. Evol. Biol. 19, 825–833.

84. Delannay, X., Gouyon, P.-H., and Valdeyron, G. (1981). Mathematical study of the evolution of gynodioecy with cytoplasmic inheritance under the effect of a nuclear restorer gene. Genetics 99, 169–181.

85. Charlesworth, D. (1981). A further study of the problem of the maintenance of females in gynodioecious species. Heredity 46, 27–39.

86. Gouyon, P.H., Vichot, F., and Damme, J.v. (1991). Nuclear-cytoplasmic male sterility: single point equilibria versus limit cycles. Am. Nat. 137, 498–514.

87. Frank, S.A. (1989). The evolutionary dynamics of cytoplasmic male sterility. Am. Nat. 133, 345–576.

88. Jacobs, M.S., and Wade, M.J. (2003). A synthetic review of the theory of gynodioecy. Am. Nat. 161, 837–851.

89. Couvet, D., Ronce, O., and Gliddon, C. (1998). The maintenance of nucleocytoplasmic polymorphism in a metapopulation: the case of gynodioecy. Am. Nat. 152, 59–70.

90. Manicacci, D., Couvet, D., Belhassen, E., Gouyon, P.-H., and Atlan, A. (1996). Founder effects and sex-ratio in the gynodioecious Thymus vulgaris L. Mol. Ecol. 5, 63–72.

91. Pannell, J. (1997). Widespread functional androdioecy in Mercurialis annua L. (Euphorbiaceae). Biol. J. Linnean Soc. *61*, 95–116.

92. Pannell, J. (1997). The maintenance of gynodioecy and androdioecy in a metapopulation. Evolution *51*, 10–20.

93. Pannell, J.R., and Barrett, S.C.H. (1998). Baker's law revisited: Reproductive assurance in a metapopulation. Evolution *52*, 657–668.

94. Ashman, T.-L. (2003). Constraints on the evolution of males and sexual dimorphism: Field estimates of genetic architecture of reproductive traits in three populations of gynodioecious Fragaria virginiana. Evolution *57*, 2012–2025.

95. Allen, G.A., and Antos, J.A. (1993). Sex ratio variation in the dioecious shrub Oemleria cerasiformis. Am. Nat. *141*, 537–553.

96. Rocheleau, A.F., and Houle, G. (2001). Different cost of reproduction for the males and females of the rare dioecious shrub Corema conradii (Empetraceae). Am. J. Bot. *88*, 659–666.

97. Dorken, M.E., and Barrett, S.C.G. (2004). Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of Sagittaria latifolia (Alismataceae): a clonal aquatic plant. J. Ecol. *92*, 32–44.

98. Lloyd, D.G., and Bawa, K.S. (1984). Modification of the gender of seed plants in varying conditions. Evol. Biol. *17*, 255–338.

99. Takebayashi, N., and Morrell, P.P. (2001). Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. Am. J. Bot. *88*, 1143–1150.

100. Igic, B., Bohs, L., and Kohn, J.R. (2006). Ancient polymorphism reveals unidirectional breeding system shifts. Proc. Natl. Acad. Sci. USA *103*, 1359–1363.

101. Shimizu, K.K., Cork, J.M., Caicedo, A.L., Mays, C.A., Moore, R.C., Olsen, K.M., Ruzsa, S., Coop, G., Bustamante, C.D., Awadalla, P., *et al*. (2004). Darwinian selection on a selfing locus. Science *306*, 2081–2084.

102. Charlesworth, B., and Charlesworth, D. (2000). The degeneration of Y chromosomes. Phil. Trans. Roy. Soc. Lond. B. *355*, 1563–1572.