Combining population genetics and demographical approaches in evolutionary studies of plant mating systems

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The striking amount of variation in the mating systems of higher plants has stimulated fruitful research by both ecologists and population geneticists. Historically, these two schools of thought have developed independent theoretical treatments and empirical approaches to account for the evolution of such diversity. We highlight the approach adopted by each field. Population geneticists have developed an approach centred on gene properties of individuals and their role on the evolution of self-fertilisation (transmission rules and the deleterious role of mutations), while ecologists have mostly focused on demographic properties of self-fertilisation (seed production, colonisation ability of selfers). As a result, the two approaches sometimes use conflicting notions of fitness. The recent empirical advances on inbreeding depression, a topic typically motivated by population genetic questions, have emphasized the need to adopt a demographical perspective for fitness. In this paper, we suggest generalizing this approach in mating system evolution and we expect further improvements by integrating demographic and genetics perspectives.

For more than two centuries, the diversity of plant mating systems has captured the curiosity and imagination of biologists. Changes in the mating system are among the most frequent evolutionary transitions in plants. Not only is the mating system a central focus for the study of adaptation (e.g. adaptive changes in flower morphology related to plant–pollinator interactions), but it also plays an important role in determining the distribution of genetic variation in populations (Baker 1955, Stebbins 1957, Holsinger 2000).

The adaptive nature of plant mating systems has been studied intensively, especially in hermaphroditic species, which represent more than 70% of the plant kingdom (Yampolsky and Yampolsky 1922). One focus has been on the evolution of self-fertilisation. Interestingly and though their scientific approach differ, both ecologists and population geneticists have contributed to our current understanding of the evolution of plant mating systems (Barrett and Harder 1996). Their studies have led to extensive theoretical developments (especially a large body of population genetics theory for mating system evolution) along with many experimental tests of theoretical hypotheses. Over the years, an important body of data on variation in selfing rates in the Angiosperms has also been collected (Vogler and Kalisz 2001).

As noted by several authors (Uyenoyama et al. 1993, Barrett and Harder 1996, Holsinger 1996), these two traditions have been developed along independent paths, and while a number of researchers have combined tools from ecology and population genetics in studies of mating system evolution. It would be interesting to go one step further, since to date, despite numerous models and hypotheses, neither ecological nor genetic theory have provided a completely adequate explanation for the selection and maintenance of the enormous range of selfing rates found in hermaphroditic plants (Barrett and Harder 1996).

In this paper, our aim is to focus on the logic of the arguments developed in mating system theory and to clarify inconsistencies. We propose that adopting a demographical perspective for fitness components may be a way to unify the different factors affecting
self-fertilisation and to consider its evolution under general ecological scenarios.

**Reviewing self-fertilisation theory**

As reviewed by Holsinger (1996), the selective advantage of selfing can be viewed as arising in one of two ways (Fig. 1):

1. selfing increases ovule success when seed production is limited by pollen transfer (reproductive assurance hypothesis or fertility advantage)
2. selfing exhibits an intrinsic advantage in pollen transmission (automatic selection or cost of outcrossing).

The first principle was proposed by Darwin (1876) who focused on self-pollination as the means of ensuring seed set either when outcrossing partners are absent or when pollinators are scarce. This has been referred to as the “reproductive assurance hypothesis” (Jain 1976). The second principle, based on gene transmission mechanisms, was proposed by Ronald Fisher (Fisher 1941), who argued that genes favoring selfing (mating system modifiers) are automatically selected because they benefit from a 50% transmission advantage compared to “outcrossing” genes. On the other hand, the deleterious effect of inbreeding on progeny performance (or inbreeding depression) (Knight 1799), is considered to be the main factor selecting against selfing. These three general components: pollen limitation, the cost of outcrossing and inbreeding depression are the cornerstones of most theories for the evolution of self-fertilisation (Lloyd 1979, 1980).

Basing one’s argument on the most influential papers (most highly cited) during the period 1985 to 2000, one of the most important theoretical developments in this field was the recognition of the importance of considering both the cost of outcrossing and inbreeding depression and of including aspects relevant to the genetic architecture of inbreeding depression (Lande and Schemske 1985, Holsinger 1988, Charlesworth and Charlesworth 1990, Charlesworth et al. 1990, 1991). This studies have led to the important notion that inbreeding depression is dynamically linked to selfing rate through purging of deleterious mutations (though see Byers and Waller 1999), as well as the potential importance of genetic associations between fitness loci and modifier loci (Holsinger 1991a). Also, there have been important theoretical insights about the cost of outcrossing. First, Uyenoyama (1986) pointed out that the cost of outcrossing is less than 50% when outcrossed partners are related (biparental inbreeding; Ronfort and Couvet 1995). When outcrossing occurs among relatives the automatic advantage of selfing is no longer 50% but less. Second, the cost of outcrossing may be reduced as a result of “pollen discounting”, i.e. when selfers contribute less to outcrossing as pollen donors than outcrosses do. Of interest, Holsinger (1991b) analysed the evolution of selfing when pollen export is dynamically linked to selfing rate (the “mass action model”) and showed that partial self-fertilisation is evolutionarily stable whenever selfing can evolve, thus demonstrating the fundamental importance of pollination biology for a

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**Fig. 1.** Two principles for the advantages of self-fertilization. (A) Mating system modifier genes are represented by dots; an allele coding for self-fertilization is shown as a black dot, while an allele coding for outcrossing is shown as an open dot. Transmission pathways from parent to offspring are shown as arrows; solid arrows represent gene transmission to progeny by the parent capable of self-fertilization, while dashed arrows represent transmission pathways for the outcrossing parent. The recognition that genes are transmitted by pollen and ovules leads to a definition of fitness expressed as the number of genes transmitted to the progeny (gene counting approach). Because the number of pollen grains produced is assumed to be large relative to the number of ovules (Bateman’s principle), a selfing genotype enjoy a 50% advantage in gene transmission relative to an outcrossing genotype. This results in the cost of outcrossing (Fisher 1941). (B) Reproductive assurance advantage of selfing. The large circles represent individual parents and their progeny. The arrows represent the production of discrete offspring by parents, with solid arrows representing pollinator-independent offspring production, and dashed arrows representing pollinator-dependent offspring production. Selfing provides an advantage by ensuring offspring production when lack of cross-pollinators limits seed set. This results in reproductive assurance.
proper understanding of the cost of outcrossing. All these developments share a common viewpoint, that is, fitness components are analysed as a genetic property of individuals (number of genes transmitted and deleterious effects of genes). At the same time, this approach has often been free of the demographical structure linked to mating system evolution and its effect on fitness components, which imposes a number of limitations on the scenarios considered (below).

An alternative tradition in mating system studies has focused on the importance of pollen limitation and, in particular, on the demographic impact of selfing. Specifically, Darwin’s ideas (below) were largely popularized by Baker (1955) and Baker (1967) in an island biology context. Baker proposed that: “With a self-compatible individual a single propagule is sufficient to start a sexually reproducing colony (after long distance dispersal), making its establishment much more likely than if the chance of the two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required”. Though Baker did not explicitly mention demography, he implicitly focused on the problems posed by low population densities for ensuring outcrossing during colonisation. More recently, the theoretical formalisation of Baker’s law by Pannell and Barrett (1998) explicitly analysed the demographic consequences of outcrossing and the importance of a minimum number of colonizers in a metapopulation to maintain outcrossing. Following this logic, the advantage of selfing in Pannell and Barrett’s model is measured in terms of seed production per individual because they focused on the role of selffertilization in colonisation ability. In contrast to the population genetic tradition which focuses on the number of genes transmitted, ecological approaches have emphasized on demographical factors such as population density and the colonisation process as important selection pressures driving mating system evolution.

If both fields have identified the major fitness components of selfing, it is fundamental to keep in mind that the automatic advantage of selfing and the fertility advantage do not apply to the same level of selection (see Lloyd 1977 and 1979 for a thoughtful discussion). On the one hand, automatic selection is linked to the number of genes transmitted by an individual (maternal and paternal contribution) and the evolutionary processes is considered as a change in gene frequency. This method is the relevant metrics for analysing evolutionary processes (below). In particular, by emphasizing the fact that the rate of colonisation is favoured by selfing, Baker’s law (Pannell and Barrett’s 1998 metapopulation model) considers that selection is governed by group selection (Lloyd 1979).

The way the fertility advantage of selfing is considered in ecological studies is particularly telling and may potentially lead to confusion. Indeed, it is often noted that an outcrosser will be at a disadvantage when pollinators are scarce because it produces few seeds (Klips and Snow 1997, Herrera et al. 2001, Anderson et al. 2003, Schueller 2004, Ward et al. 2005). This is true, but it must be reminded that when focusing on genes, each outcrossed seeds (maternal contribution) as well as each sired seeds in the population (paternal contributions) contain exactly one copy of the out-crossing individual. Yet ecological studies seldom note that the outcrosser will be at disadvantage because it also exports less pollen.

We think that such an omission does not help to integrate the selective advantage of reproductive assurance in evolutionary models.

If we take a look at the bibliographic for the last 60 years (Source ISI Research), it shows that the seminal work of Fisher (1941) (335 citations) is mostly cited in genetically-oriented journals, whereas Baker (1955) (366 citations) or Baker (1967) (131 citations) are mostly cited in plant science journals. Moreover, papers citing both Baker (1955) and Fisher (1941) are few (54 citations) and none of the major theoretical contributions (cited below) cite both. For the sake of simplicity, many models have considered situations where demography can be neglected. However, recent empirical results have demonstrated that demography cannot be neglected since classical mating system parameters have to be considered in the light of demographical interactions among individuals. That is what we develop in the next part.

Inbreeding depression in wild hermaphroditic populations: the need for integrating population genetics and demography

Some light can be shed on the necessity to include demographical components of fitness by considering the way in which inbreeding depression has been treated in studies of mating system evolution. First described by Thomas Knight (1799) more than 200 years ago, inbreeding depression is expressed at the individual level, and is interpreted as arising from increased homozygosity (Charlesworth and Charlesworth 1987). In hermaphrodites, inbreeding depression is classically defined as \( \delta = 1 - \frac{W_i}{W_{out}} \), where \( W_i \) and \( W_{out} \) are the performances of selfed and outcrossed progeny respectively; e.g. biomass, number of flowers, survival, etc.)
Theory for the evolution of mating systems has stimulated many experimental measures of inbreeding depression (reviewed by Husband and Schemske 1996). The motivations for such studies include assessing how inbreeding depression acts to oppose the selection of selfing (Lloyd 1979) as well as understanding the genetic basis of inbreeding depression (Byers and Waller 1999). Unfortunately, other factors affecting inbreeding depression values (e.g. competition) have sometimes been considered as mere ‘complications’, things that should be avoided when studying inbreeding depression (reviewed by Barrett and Harder 1996).

Indeed, the experimental measure of inbreeding depression raises a number of challenges. For instance, consider one example of the analysis of inbreeding depression of survival to flowering in annual plants, where the environment could, in theory, play an extreme role. If growth conditions are very good for growth and survival, both inbred and outbred plants may survive until the end of the life cycle, and one would conclude that there is no inbreeding depression for survivorship. On the other hand, with intermediate mortality rates (e.g. due to competition among individuals or environmental stress), experimental results would likely suggest a positive inbreeding depression value. And lastly, if all plants die before reproduction, (e.g. because of stressful environmental conditions), the experiment would give no information about inbreeding depression. Though these simple examples may seem extreme, they demonstrate that in addition to genetic architecture, the estimated magnitude of inbreeding depression must depend on the ecological conditions acting on individuals (physical stress or competition). To our knowledge, no theoretical models have included environmental effects on inbreeding depression when studying the genetic architecture of ID, though empirical results on this point are now clearly established (Armbuster and Reed 2005). We believe that taking into account this phenomenon is crucial for understanding the selective effect of inbreeding depression in natural populations. Indeed, it can modify the purging process (Bijlsma et al. 1999) as well as evolutionary predictions concerning selfing. In our own experimental work we have illustrated the importance of demographical feedbacks on inbreeding depression in the case when the environmental conditions experienced by individuals in a population are linked to the evolution of the reproductive system itself (Cheptou and Schoen 2003). In that study, we measured inbreeding depression in competing stands and found that the relative composition of inbred and outbred plants (within natural populations, is determined directly by population selfing rate) substantially influences the magnitude of inbreeding depression (Fig. 2). In this context, the classical balance between the cost of outcrossing and inbreeding depression ought to be reinterpreted in the light of feedback from intraspecific competition, itself a function of the population’s rate of selfing (Cheptou and Dieckmann 2002). In other words, a gene favoring selfing may modify the competition environment in which its fitness is determined.

We would like to emphasize that including demographical components of fitness into the population genetics framework may help to generalise the selection on self-fertilisation under general ecological scenarios.

**An example: the evolution of self-fertilisation under stochastic pollination environment**

In the first part, we emphasized the fact that, though reproductive assurance and the cost of outcrossing select for selfing, they do not operate on the same level of selection. As a consequence, their joint effect is not necessarily trivial. Adopting a demographical view of fitness may help to clarify selection processes and avoid oversimplistic (and intuitive) arguments for the selection of selfing. We illustrate this point by analysing the selection of self-fertilisation under stochastic pollination environment.

When focusing on the role of reproductive assurance, people have often focused on the uncertainty of the pollination environment (temporal heterogeneity) (Baker 1967, Herlihy and Eckert 2002, Kalisz and Vogler 2003) and have considered selfing as a way to maximise seed production (Burd 1995). In population genetics models (Lande 1982, Charlesworth 1994), the classical assumption is the stability of environment, which rules out stochastic variants and density dependent effects and so the exploration of evolutionary processes under those scenarios has been left out (Caswell 2001). The conclusions drawn from population genetics models built on stable environment assumptions are not valid under general demographic scenarios. Note that taking into account general ecological scenarios may be particularly relevant as regards the maintenance of mixed mating systems because it often leads to frequency- and density-dependent fitness (Heino et al. 1998), a necessary conditions for negative feedbacks to arise (Goodwillie et al. 2005).

Here we define fitness components in a demographical context, including for instance density-dependence of stochastic environments and integrate them into a population genetics framework in order to derive evolutionary predictions (Metz et al. 1992, Brommer 2000). Let us consider a simple model including pollen limitation (reproductive assurance) inspired from Lloyd’s (1979) work, and extending it to stochastic environments – a variant of Morgan and Wilson’s (2005) model. Thus, consider a hermaphroditic annual.
plant population experiencing inbreeding depression and pollinator limitation. According to the classical population genetics model, the fitness of a rare mutant with a selfing rate \( r \) in a resident population with selfing rate \( R \) can be measured as the number of genes transmitted to the next generation, that is:

\[
W(r,R,t) = r(1-\delta) + \left[ \frac{1-r}{2} \right] + \frac{1-R}{2} e(t)
\]

Where \( \delta \) is the inbreeding depression parameter and \( e \) characterizes pollinator efficiency, “the proportion of available ovules fertilized with the aid of an external agent” (Lloyd 1979). From the perspective of reproductive assurance, we consider that \( e \) may fluctuate in time as the result of among-year variation in pollinator

Fig. 2. Experimental evidence of ecological feedback on fitness driven by the mating system. Experiments on the partial selfer *Amsinckia douglasiana* \((s = 0.25)\). In partially selfing annual plant population, the population selfing rate in a large population is defined as the proportion of inbred and outbred individuals. Consider the focal individual shown in (A). Ecological interactions (competition) in a highly selfing population occur mainly among inbreds, whereas in a highly outcrossing population, they occur mainly among outbreds. Cheptou and Schoen (2003) measured the performances of focal plants (inbred and outbred) by varying the proportion of inbred and outbred plants (on the hexagon), thus simulating competition from complete outcrossing to complete selfing. (B) shows experimental results (fitted number of flowers produced by focal plants; mean, SE) as a function of the proportion of inbred neighbors in a competing stand (310 plants m\(^{-2}\)) in *Amsinckia douglasiana*. Absolute fitness for inbred (dotted line) and outbred (solid line) as well as inbreeding depression \((\delta = 1 - \frac{W_{\text{self}}}{W_{\text{out}}} \text{ thin line})\) changes with the frequency of inbred plants, showing that the population selfing rate feedbacks on individual performances and on inbreeding depression as a result of ecological interactions in the population. Note: the possibility that inbreeding depression can be influenced by the composition of the stand, which was rediscovered by Schmitt and Ehrhardt (1990) was anticipated by Darwin (1876) who wrote: “With *Nicotiana* the crossed were to the self-fertilized in height, when grown extremely crowded together in pots, as 100 to 54; when grown much less crowded in pots as 100 to 66, and when grown in the open ground, so as to be subjected to but little competition, as 100 to 72.”
activity (Kalisz and Vogler 2003). As a particular case in point, when environmental conditions are constant, the evolution of selfing is favored if \(1 - \delta - \frac{e}{2} > 0\) (Lloyd 1979). Next, let’s assume instead, that there is a stochastic pollination process resulting from among-year variation in the pollinator population. Specifically, each year, pollination efficiency, \(e\), is taken from a probability distribution (with mean \(\bar{e}\) and variance \(\sigma^2\)). If the population demography is assumed to be globally stable on an ecological timescale—neither goes extinct nor grows exponentially—then, the direction of selection for self-fertilisation is given by the sign of \((1 - \delta - \frac{e}{2}) - f(R)\sigma^2\) (see Box 1 for mathematical details). This characterizes the selection of selfing under stochastic pollination. Interestingly, \(f(R)\) embodies the feedback on selfing, modulating the spread of selfing genes in stochastic environments. \(f(R)\) is always positive and increases with \(R\). So contrary to the environmentally stable situation, selection in this case depends on the population selfing rate \(R\), and consequently the selection gradient may vanish for intermediate selfing rates— in other words, stochastic pollination may create negative feedback on selfing, which in turn allows for the maintenance of mixed mating (Fig. 3). This result may be likened to bet-hedging theory, which states that a good strategy is the one that maximises the long-term fitness (geometric mean). Note that a qualitatively similar result may sometimes be found when inbreeding depression fluctuates in time (see Cheptou and Schoen 2002 for a theoretical model, and Dole and Ritland 1993 for an empirical example).

From the point of view of reproductive assurance, it may be tempting to consider that a population adapted to a stochastic pollination environment is the one that maximises seed production over years; i.e. the best strategy is a mix of outcrossing for good years and selfing bad pollination years (bet hedging). As shown by Fig. 4, it is not the case. This model reveals that evolutionary stable strategies do not maximize seed production, as seen in other examples from evolutionary biology (Maynard Smith 1982). On this point, it is important to note that Darwin’s and Baker’s verbal interpretations are not accurate since they are implicitly based on the maximisation of seed production (Ashman et al. 2004, Burd 1995). Indeed, the selection of selfing is driven by gene transmission for which seed production is only one component of the fitness. The practical consequence is that mere observation of a population that appears to be employing selfing for optimising seed production in the face of an uncertain pollination does not constitute

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**Box 1. The evolution of self-fertilisation under stochastic pollination environments.**

The fitness at time \(t\) of a mutant, with selfing rate \(r\) in a resident population with selfing rate \(R\) in stochastic pollination environment, is given by the following equation:

\[
W_t(r, R) = r(1 - \delta) + \left[1 - r \frac{1}{2} + \frac{1 - R}{2}\right]e_t
\]

(1)

The long-term exponential growth rate is given by:

\[
s(r, R) = E[\text{Ln}w(r, R)]
\]

The population is demographically stable i.e. \(s(R, R) = E[\text{Ln}w(R, R)]\) and we approximate \(s(r, R)\) using Jensen approximation \(E[\text{Ln}(w(r, R))] = \text{Ln}[E(w(r, R))] - \frac{\text{Var}(w(r, R))}{2E(w(r, R))}\). Then, the direction of selection is given by the sign \(\frac{\partial s}{\partial r}\) evaluated at \(r = R\) i.e. the sign of the quantity (where \(\bar{e}\) and \(\sigma^2\) are mean and variance of the temporal pollination efficiency):

\[
D(R) = \frac{1 - \bar{e}/2 - \delta}{\sigma(1 - R) + R(1 - \delta)} - \frac{1 + R^2(1 - \delta)/2 - \delta + R(1.5 + 1.5\delta)}{[\sigma(1 - R) + R(1 - \delta)]^2}
\]

With \(A > 0\) and \(B > 0\)

The evolutionary stable strategy \(R^*\) is given by solving \(D(R) = 0\). The outcome of selection is summarised in Fig. 3.
a proof of adaptation to its environment. As we have shown (Fig. 4), a population producing fewer seeds may actually be closer to the evolutionary stable selfing rate.

In summary, this model reveals several interesting features. First, it combines a classical ecological scenario with basic population genetics models, which allows for the maintenance of mixed selfing rates. Second, it explicitly demonstrates that the criterion of seed maximisation under stochastic environment is not accurate from an evolutionary point of view.

Though it is true that: (1) selfing increases ovule success when seed production is pollen-limited; and (2) selfing exhibits an intrinsic advantage in pollen transmission, we can see that the outcome is not trivial under general ecological scenarios.

Conclusions and perspectives

Our aim has been to integrate concepts of population genetics and concepts of the ecological theory of mating system evolution. The demographical view of ecologists has often led to the use of the “optimization criteria” applied on seed production, whereas population geneticists have based their evolutionary analysis on the spread of a gene increasing the selfing rate, with the focus on conditions for its spread and invasion. Ecologists have explored various ecological scenarios with little theoretical formalisation, whereas population geneticists have mostly formalised gene transmission process within populations. On the one hand, population genetics have developed an important theoretical framework, but on the other hand the demographical theory allows the extension of evolutionary predictions to complex ecological scenarios. According to us, since models that do not integrate environmental components may not be relevant in the light of recent studies, future models in the field of mating systems ought to take these components into account.

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