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The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization

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• *Background* The field of plant mating-system evolution has long been interested in understanding why selfing evolves from outcrossing. Many possible mechanisms drive this evolutionary trend, but most research has focused upon the transmission advantage of selfing and its ability to provide reproductive assurance when cross-pollination is uncertain. We discuss the shared conceptual framework of these ideas and their empirical support that is emerging from tests of their predictions over the last 25 years.

• *Scope* These two hypotheses are derived from the same strategic framework. The transmission advantage hypothesis involves purely gene-level selection, with reproductive assurance involving an added component of individual-level selection. Support for both of these ideas has been garnered from population-genetic tests of their predictions. Studies in natural populations often show that selfing increases seed production, but it is not clear if this benefit is sufficient to favour the evolution of selfing, and the ecological agents limiting outcross pollen are often not identified. Pollen discounting appears to be highly variable and important in systems where selfing involves multiple floral adaptations, yet seed discounting has rarely been investigated. Although reproductive assurance appears likely as a leading factor facilitating the evolution of selfing, studies must account for both seed and pollen discounting to adequately test this hypothesis.

• *Conclusions* The transmission advantage and reproductive assurance ideas describe components of gene transmission that favour selfing. Future work should move beyond their dichotomous presentation and focus upon understanding whether selection through pollen, seed or both explains the spread of selfing-rate modifiers in plant populations.

Key words: Demography, inbreeding depression, mating systems, outcrossing, pollen discounting, pollination, seed discounting.

INTRODUCTION

Even a casual study of floral diversity reveals a bewildering array of variation that has long been considered to be a paragon of the adaptive evolutionary process (Darwin, 1859). In comparison to other organismal groups, flowering plants are particularly variable with respect to the placement of male and female gametes within and among individuals (Darwin, 1876; Barrett, 2002). The vast majority of angiosperm species have perfect flowers, where the pollen and ovules of a single individual are held in close spatio-temporal proximity. Given this arrangement, plants are faced with a strategic decision, on whether to reproduce through outcrossing, selfing, or some mixture of these possibilities (Barrett and Eckert, 1990). This simple question has stimulated a rich body of theoretical and empirical work in the field of plant mating-system evolution (Lande and Schemske, 1985; Goodwillie *et al.*, 2005). Addressing this idea is important because selfing appears to be driven by persistent natural selection in the wild, yet long periods of selfing have negative consequences on the genetic diversity, viability and diversification of plant lineages (Stebbins, 1957; Goldberg *et al.*, 2010). If it is possible to understand the factors driving the recurrent evolution of selfing in nature, then this field of research will explain the ultimate mechanisms underlying a major evolutionary trend in flowering plants (Eckert *et al.*, 2009a).

Theories on the evolution of self-fertilization have a deep history in plant evolutionary biology (Darwin, 1876; Fisher, 1941; Baker, 1955; Jain, 1976; Lloyd, 1979; Holsinger, 1996). In general, selfing is seen as a reproductive strategy that can replace outcrossing whenever the fitness of a selfing morph exceeds that of an outcrossing morph (Lloyd, 1979, 1992). Selection of selfing over outcrossing can theoretically occur for an extremely diverse array of reasons (Goodwillie *et al.*, 2005), ranging from its ability to shield individuals and populations from maladaptive gene flow (Antonovics, 1968; Grossenbacher and Whittall, 2011), competitive interactions (Cheptou and Dieckmann, 2002), and antagonists (Koslow and DeAngelis, 2006) or its ability to increase seed production (Darwin, 1876; Lloyd, 1980) or allele transmission in natural populations (Fisher, 1941). Each of the potential benefits of selfing may be countered by inbreeding depression, which has received extensive theoretical and empirical attention in studies of mating-system evolution (Lande and Schemske, 1985; Porcher and Lande, 2005; Byers and Waller, 1999; Keller and Waller, 2002). In terms of the factors that favour selfing, most effort has focused on two specific hypotheses for the evolution of this reproductive

strategy (Jain, 1976; Holsinger, 1996; Cheptou, 2004). The first and most long-standing hypothesis suggests that selfing evolves because it increases seed production when mates or pollinators are scarce, a phenomenon known as reproductive assurance (Darwin, 1876; Baker, 1955; Lloyd, 1965; Inouye *et al.*, 1996). The second hypothesis was derived relatively recently from population-genetic models showing that a gene for selfing has a $3:2$ transmission advantage over those causing outcrossing (Fisher, 1941; Holsinger, 1991). This potential gene-level advantage of selfing has been termed the automatic selection hypothesis.

The reason why these two hypotheses have received the greatest attention is because they concern the direct reproductive advantages of selfing, and do not invoke other potentially less general ecological agencies that favour selfing over outcrossing (Lloyd, 1979; Uyenoyama *et al.*, 1993). In this paper, our goal is to discuss the current state of opinion on the relative importance of the reproductive assurance and transmission advantage hypotheses in explaining the repeated evolution of selfing in nature. We begin by revisiting theory concerning these components of natural selection so that the factors driving the evolution of selfing from outcrossing may be clearly understood (Lloyd, 1979, 1992). We follow this section by summarizing evidence gathered over the last 25 years toward addressing these hypotheses from observational, experimental and comparative approaches. Throughout this paper, we suggest that studies have too often treated these hypotheses as mutually exclusive alternatives, and have focused less often on their shared framework (Holsinger, 1996; Cheptou and Schoen, 2007). By discussing these hypotheses, their similarities, and their empirical support, we hope to focus future efforts toward best answering the question of why the evolution of selfing from outcrossing is the most commonly traversed axis in floral evolution in angiosperms.

THE BASIC MODEL FOR THE EVOLUTION OF SELFING

In considering these major hypotheses for the evolution of selfing, we revisit a phenotypic model developed by Lloyd (1992), as it describes gene transmission of plants in a stable population. In this example, one imagines the number of times gametes are passed on to offspring by competing plants, which are either entirely outcrossing or partially selfing. Outcrossing plants produce offspring through outcrossed ovules (x_x) and also through fertilizing other ovules that are available to be outcrossed in the population. Success as an outcross pollen donor depends on pollen fitness, or the number of ovules fertilized with outcross pollen (p_x). The total fitness gained by an outcrossing morph is then:

$$W_x = x_x + p_x$$

In contrast, selfing plants pass on two copies of gametes through ovules that are self-fertilized (y), and the fitness of these seeds may be reduced by inbreeding depression (δ, i.e. fitness is scaled by a factor of $1 - \delta$). Remaining ovules (x_s) are fertilized with outcross pollen, and this plant also achieves fitness through outcross pollen that fertilizes some

number of ovules (p_s):

$$W_s = 2(1 - \delta)y + x_s + p_s$$

Regardless of the actual shift in selfing rate, an increase in the amount of selfing will be selected whenever the gains from selfing exceed the losses, or when $W_s > W_x$:

$$2(1 - \delta)y > (x_x - x_s) + (p_x - p_s)$$

The gains from selfing arise through increased allele transmission through selfed seeds which are discounted by inbreeding depression. The losses in numbers of offspring arise from the loss of outcrossed seeds (the absolute seed discount, $x_x - x_s$) and the loss of outcrossed pollen fitness (the absolute pollen discount, $p_x - p_s$). This inequality is usually rearranged so that each of the discounts is expressed in terms of losses of outcrossed seeds and pollen fitness per selfed seed:

$$2(1 - \delta) > \left(\frac{x_x - x_s}{y}\right) + \left(\frac{p_x - p_s}{y}\right) \quad (1)$$

This entire inequality may be used in natural populations to determine whether selfing should evolve by natural selection. This perspective is useful because it identifies the components of fitness that are quantifiable and also potentially responsible for mating-system shifts. This perspective does not address the evolutionary stability of the selfing rate (Johnston, 1998; Goodwillie *et al.*, 2010), but nevertheless can be used to illuminate why the direction and magnitude of selection on mating-system modifiers arises (Table 1).

TESTS OF HYPOTHESES BASED UPON THE MODEL

Transmission advantage hypothesis

Now that we understand the pathways whereby outcrossing and selfing plants compete, in a Darwinian sense, we can

TABLE 1. *Empirical quantities required to test the transmission advantage and reproductive assurance hypotheses in nature*

Term	Component	Parameters required to estimate component
$\delta = 1 - (W_s/W_x)$	Inbreeding depression	W_s = lifetime fitness of selfed offspring
		W_x = lifetime fitness of outcross offspring
$(p_x - p_s)/y$	Pollen discounting rate	p_s = number of outcrossed seeds sired by selfing morphs
		p_x = number of outcrossed seeds sired by outcrossing morphs
$(x_x - x_s)/y$	Seed discounting rate	x_s = number of outcrossed seeds made by selfing morphs
		x_x = number of outcrossed seeds made by outcrossing morphs
		y = number of seeds produced by selfing

Both hypotheses require estimates of inbreeding depression and pollen discounting. The transmission advantage hypothesis assumes that seed discounting is complete, so this quantity is relevant only to the reproductive assurance hypothesis.

determine the necessary requirements for testing the transmission advantage hypothesis in nature. In a scenario where this mode of selection is operating, a mutation causing selfing spreads purely because it increases its transmission to offspring compared with a mutation that causes outcrossing, but there is no difference in the number of seeds produced by plants. In such a situation, selfed ovules (y) can be gained only by sacrificing ovules that are normally cross-fertilized in outcrossing plants (i.e. $y = x_x - x_s$). This hypothesis therefore describes selection operating at the level of genes causing outcrossing and selfing, since all else is equal at the individual level. Because of this perspective, selfing causes complete seed discounting, and the conditions for the evolution of selfing depend only upon the magnitude of inbreeding depression and the pollen-discounting rate:

$$\delta < \frac{1}{2} - \left(\frac{p_x - p_s}{2y} \right) \qquad (2)$$

If pollen discounting does not occur ($p_x - p_s = 0$), then selfing evolves whenever $\delta < 1/2$, as is expected under classic population-genetic theory (Fisher, 1941; Lande and Schemske, 1985). Pollen discounting is clearly the most important mechanism that can erase the transmission advantage of selfing, since selfing evolves under an increasingly narrower set of conditions as the magnitude of pollen discounting ($p_x - p_s$) increases (Nagylaki, 1976; Holsinger, 1991). Pollen discounting may occur for a variety of reasons, such as when selfing causes a reduction in the floral attractiveness to pollinators, the number of pollen grains per flower, or the quality of pollen grains that compete with those made by outcrossers (Holsinger, 1996).

Given the potential importance of pollen discounting in counteracting selection of selfing, it is of great interest to know whether it is commonly observed in plant populations. The measurement of pollen discounting has been conducted in a number of scenarios where the outcross seed paternity of outcrossing and selfing plants has been measured in competition (Table 2). Over a broad number of studies in several years and populations of the species *Ipomoea purpurea*, pollen discounting has rarely been implicated for mutations that alter floral colour and increase selfing rates (Rausher and Fry, 1993; Fehr and Rausher, 2004; Fry and Rausher, 1997; Coberly and Rausher, 2008), although it has been observed in populations where selfing phenotypes are common (Chang and Rausher, 1998). In *Eichhornia paniculata*, a selfing morph experiences no pollen discounting in diverse populations, yet actually has an advantage (negative pollen discounting) over a similar outcrossing morph when morph diversity is reduced in populations (Kohn and Barrett, 1994). Pollen discounting in *E. paniculata* has also been shown to be more important for plants with large displays, as pollinators can transport pollen between flowers on the same plant, thereby reducing outcross siring success (Harder and Barrett, 1995; Eckert, 2000). These results collectively suggest that pollen discounting may not always operate in natural populations and is highly dependent upon plant life history, yet is critical for understanding the magnitude and direction of selection on the mating system.

TABLE 2. *Studies where direct competition of outcrossing and selfing morphs permitted estimates of pollen discounting*

Species	Trait(s) associated with selfing	Pollen discounting? ($p_x - p_s$)	References
Ipomoea purpurea	Flower colour (A-locus)	~ 0	Fehr and Rausher, 2004; Coberly and Rausher, 2008
Ipomoea purpurea	Flower colour (W-locus)	~ 0	Rausher and Fry, 1993; Fry and Rausher, 1997
Ipomoea purpurea	Anther-stigma distance	~ 0: when selfing rare > 0: when selfing common	Chang and Rausher, 1998
Eichhornia paniculata	Modified stamen in M morph	~ 0: trimorphic populations < 0: mono-, di-morphic populations	Kohn and Barrett, 1994
Mimulus guttatus and *M. micranthus*	Multiple morphological traits	> 0	Ritland, 1991
Senecio squalidus	Radiate vs. non-radiate morph	> 0	Holsinger, 1992
Arenaria uniflora	Multiple morphological traits	> 0	Fishman, 2000

In each study, the average outcross paternity of outcrossing (p_x) and selfing (p_s) plants was inferred with polymorphic molecular markers.

In species where selfing morphs harbour many floral adaptations associated with the selfing syndrome, there is a trend for higher levels of pollen discounting (Ritland, 1991; Holsinger, 1992; Fishman, 2000). Pollen discounting appears in these species because selfing morphs have reduced floral attractiveness (Holsinger, 1992) or reduced pollen quality (Ritland, 1991; Fishman, 2000). In two of these cases, experiments have utilized quite divergent outcrossing and selfing morphs, so some of the pollen discounting in these experiments may in fact be caused by pre-zygotic or early post-zygotic incompatibilities between lineages (Ritland, 1991; Fishman, 2000). Although there are relatively few estimates of pollen discounting in these types of systems, these results imply that pollen discounting can range from being negligible to important depending upon the context, and that morphological adaptations for selfing are likely to cause pollen discounting (Holsinger, 1996; Takebayashi and Delph, 2000). Since many of the floral traits that accompany selfing in these lineages may have arisen long after the initial transition from outcrossing to partial selfing, these cases may not adequately determine whether pollen discounting hindered the first stages of the transition to selfing.

Reproductive assurance hypothesis

Reproductive assurance is distinct from automatic selection because it invokes elevated seed production in selfing plants compared with outcrossers (i.e. $x_s + y > x_x$). This hypothesis therefore describes selection operating at the individual level, because it invokes variation in the number of offspring produced by plants. The idea that selfing is an efficient means

of seed production has a long and storied history in plant evolutionary biology. This idea was originally cited in the works of Darwin (1859, 1876), and was reified by the observations of Baker (1955, 1965), Stebbins (1957) and Lloyd (1965, 1980), who examined bio-geographical and demographic correlates of selfing that arise in nature. Specifically, these important, early evolutionary botanists suggested that selfing evolves in isolated or marginal populations or those occurring on islands, as these habitats are often typified by chronically small populations or reductions in the efficiency of cross-pollination as a mode of reproduction. Currently, the specific agents limiting seed production through cross-pollination, such as the ephemeral habit, histories of invasion, and the reliance upon specialized pollinators, have been largely supported (Runions and Geber, 2000; Fenster *et al.*, 2005; Fenster and Rodriguez, 2007; van Kleunen *et al.*, 2008), although there are exceptions to this broad rule (Sutherland, 2004; Herlihy and Eckert, 2005; Cheptou and Massol, 2009). Given the broad interest in identifying the ecological factors that may cause the evolution of selfing because of its ability to provide reproductive assurance, we discuss important experimental issues and support for the hypothesis that have been identified in individual studies in recent history.

For the reproductive assurance hypothesis to explain the evolution of selfing, plants must be able to self-fertilize in the absence of a pollinator (Lloyd, 1992). Direct tests of the reproductive assurance hypothesis therefore require at minimum that the effect of the autonomous mode of selfing on seed production be quantified (Eckert and Schaefer, 1998). This component of selfing may be estimated by comparing the seed production of intact flowers, which have the capacity to autonomously self, with those that have been emasculated (Schoen and Lloyd, 1992). These manipulative experiments have been conducted in a wide diversity of species (>50 species), and there is broad support for autonomous selfing providing reproductive assurance (Eckert *et al.*, 2006). That being said, the ability of selfing to provide reproductive assurance will also depend strongly on the timing and mode of self-pollen deposition, because it can lead to substantial seed discounting (Lloyd, 1992). Early self-pollen deposition (i.e. prior or competing selfing) should lead to large amounts of seed discounting, which can actually erase the reproductive assurance benefits of selfing (Herlihy and Eckert, 2002), while delayed selfing only boosts seed production once opportunities for outcrossing have passed. Seeing how seed discounting has been estimated much less often than pollen discounting (Vaughton and Ramsey, 2010; Vaughton *et al.*, 2010), more work is needed to determine its magnitude in nature.

Even if emasculation experiments demonstrate that autonomous selfing provides reproductive assurance, elevated seed production in selfing plants is not enough to determine whether selfing should evolve by reproductive assurance. This problem arises because simple comparisons of seed production ignore seed discounting and the cost of meiosis in outcrossed seeds (Holsinger, 1996; Herlihy and Eckert, 2002; Cheptou and Schoen, 2007). If the cost of meiosis is accounted for, the evolution of selfing occurs when:

$$\delta < \frac{(2y + x_s + p_s) - (x_x + p_x)}{2y} \qquad (3)$$

The numerator of this expression contains the number of gametes passed on to offspring by selfing plants minus the number of gametes passed on to offspring by outcrossing plants. As the number of ovules fertilized by self pollen (y) increases, conditions for the evolution of selfing become more favourable. In contrast, if either the seed discounts ($x_x - x_s$) or pollen discounts ($p_x - p_s$) become increasingly large, selfing will be only be selected when values of inbreeding depression are increasingly small. This approach clarifies that both seed *and* pollen discounting contribute equally to cancel the advantage of selfing when it boosts seed production.

Outlining the logic behind the transmission advantage and reproductive assurance hypotheses is important because it clarifies the shared framework underlying these ideas (Fig. 1). Most importantly, both hypotheses describe selection of selfing because of the cost of meiosis incurred by outcross seeds. As such, these ideas are not independent of each other, even though they are often presented as such in the mating-system literature (Cheptou, 2007). The transmission advantage hypothesis is applicable only when the total seed production of plants must be equal, such that elevated selfing rates necessarily result in fewer outcross seeds (i.e. the seed discounting rate equals 1). If, however, autonomous selfing is associated with elevated seed production, it is still necessary to evaluate the magnitude of pollen and seed discounting, as each of these factors can counteract selection for selfing, even if it provides reproductive assurance (Fig. 1). Both of these discounting rates have rarely been measured in natural populations and, to our knowledge, never been estimated jointly in a single species to evaluate whether autonomous selfing should be selected (Herlihy and Eckert, 2002; Eckert *et al.*, 2006).

ECOLOGICAL CORRELATION AND HISTORICAL APPROACHES

Ecological correlates of selfing

The approach outlined above requires a great deal of knowledge that is often specific to a single species and an environment. Since plants species differ widely in floral morphology, mode of selfing, pollination biology and environmental circumstances, a broader perspective may be more widely successful in addressing the relative importance of the transmission advantage and reproductive assurance hypotheses. Since the transmission advantage hypothesis assumes that outcrossing and selfing plants produce the same number of seeds, this idea cannot be addressed through large-scale ecological studies in natural populations. The reproductive assurance hypothesis, however, has long been thought to explain shifts to selfing, because this strategy is thought to be associated with pollinator-poor environments or scenarios where mates are uncommon (Wyatt, 1986; Husband and Barrett, 1991; Kalisz *et al.*, 2004). Although there is interest in understanding the reasons why selfing evolves, most tests of the reproductive assurance hypothesis have focused on whether selfing boosts seed production [i.e. $R = 1 - ($seed set$_{emasculated}/$seed set$_{intact})$]. As a consequence, reproductive assurance is operationally defined, as the seed benefit of selfing can arise in response to any number of a
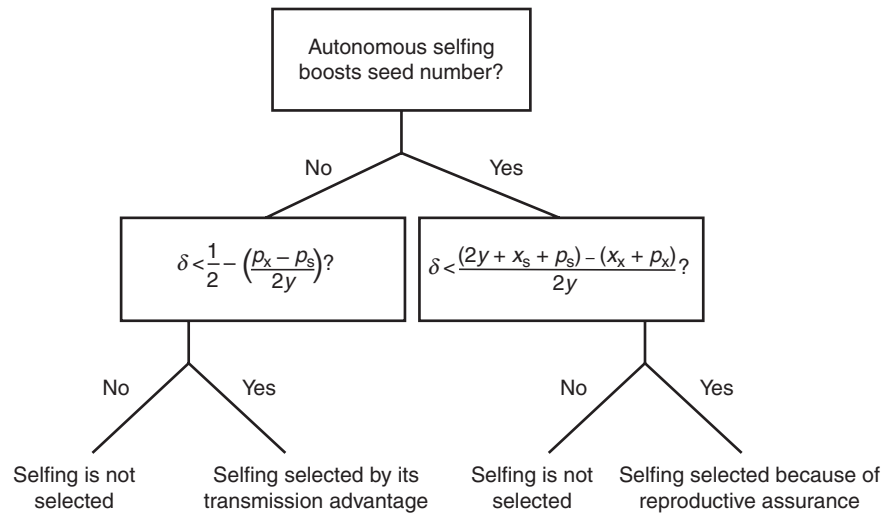
FIG. 1. The conceptual framework shared by two hypotheses for the evolution of selfing. Selfing will evolve by its gene-level transmission advantage if it is not erased by pollen discounting ($p_x - p_s$) or inbreeding depression (δ). If selfing increases seed production, selfing will be selected if the number of alleles passed on by plants capable of selfing ($2y + x_s + p_s$) is greater than the number transmitted by outcrossers ($x_x + p_x$), and exceeds a threshold inbreeding depression. Pollen and seed discounting ($x_x - x_s$) may both counter selection of selfing in this case.

diverse suite of mechanisms in nature (Eckert and Schaefer, 1998; Kalisz and Vogler, 2003; Kalisz *et al.*, 2004). Given this issue, more work is needed to test hypotheses about specific environmental or population-level characteristics that are responsible for selecting for selfing in nature.

Outcross pollen quantity may be constrained by any environmental or genetic factor that reduces the gross number of outcross pollen grains that are received at the stigma level by plants (Burd, 1994; Knight *et al.*, 2005; Aizen and Harder, 2007). This concept of quantity was invoked by Baker (1955), Stebbins (1957) and Lloyd (1980), and has most often been interpreted to occur in small populations where mates are uncommon or when reduced pollinator availability or activity occurs. To shed light on the agents of selection that generate natural selection for selfing, we compiled data from studies that examined variables associated with increased selfing in plants. Studies that tested the reproductive assurance component of selfing (i.e. an increase in seed production through autonomous selfing) and a specific environmental correlate were included (Table 3). In general, reduced pollen quantity (i.e. low plant density or reduced pollinator activity) is often associated with a reproductive assurance benefit of selfing, although studies have also commonly failed to identify ecological agents associated with elevated seed production in self-fertile plants in comparison to their outcrossing relatives. Perhaps most surprising is the fact that very few studies have connected the reproductive assurance benefit of selfing specifically to how effective pollinators are in their foraging behaviour in maintaining outcrossing, even though these vectors are often invoked as causing reproductive assurance (Rick *et al.*, 1979; Wyatt, 1986; Husband and Barrett, 1992; Karron *et al.*, 2004; Moeller, 2006; Fishman and Willis, 2008; Fenster and Rodriguez, 2007; Bodbyl Roels and Kelly, 2011).

Reductions in pollen quantity, although dominant in the literature concerning reproductive assurance, may not be entirely sufficient to explain selection of selfing in natural populations.

Outcross pollen quality may also generate selection for selfing when plants are unable to successfully use outcross pollen to produce viable offspring (Aizen and Harder, 2007). This constraint will be important when plants share S-alleles (Campbell and Husband, 2007), as these will result in self-incompatibility reactions that prevent pollen from fertilizing ovules (for a review, see Busch and Schoen, 2008). Mate-limitation of outcross seed production has commonly been observed to decline in the face of limited S-allele diversity (Young and Pickup, 2010; Campbell and Husband, 2007), although this is not always true for species with gametophytic pollen recognition (Holderegger *et al.*, 2008). Incompatibilities arising because of pollen–pistil incompatibilities between species will also be important in limiting outcross pollen success in plant communities (Fishman and Wyatt, 1999), as pollen from other species should usurp ovules. Surprisingly, the heterospecific component of pollen quality has rarely been considered as a factor contributing to reproductive assurance (Fishman and Wyatt, 1999; Table 3), and this factor should be considered in the future, especially given recent broad support for overlapping species ranges in explaining shifts to selfing in *Mimulus* (Grossenbacher and Whittall, 2011).

Historical approaches to testing hypotheses for the evolution of selfing

Each of the approaches outlined so far in this paper involves the study of intra-specific variation in the mating system, but often devotes little consideration to the histories of these populations (Barrett *et al.*, 1996). As is true for most major shifts in organismal structure and function, shifts in the mating system should produce population-genetic signatures that are readily discernible (Charlesworth, 2003). To understand why this may occur, we first consider the automatic selection hypothesis. For the innate transmission advantage of selfing to be realized, there must be sufficient vector-mediated transfer of pollen (Schoen *et al.*, 1996). In such a scenario, the effective

TABLE 3. *Studies examining associations between the reproductive assurance benefit of selfing and factors thought to limit outcross pollen in nature*

Species	Study[*]	Selfing = RA?[†]	Selfing variation[§]	Potential correlate of ↑ selfing?[¶]	References
Studies identifying an environmental correlate of RA					
Aquilegia canadensis	CP	Yes	*s*	↓ Plant density	Herlihy and Eckert, 2004
Arenaria uniflora	MP	Yes[‡]	Flower size	↑ Heterospecific pollen	Fishman and Wyatt, 1999
Bulbine vagans	MP	Yes	R	Inclement weather	Vaughton and Ramsey, 2010
Campanula spp.	CP	Yes	*s*	↓ Pollinator visitation	Inouye *et al.*, 1996
Clarkia xantiana	CP, CG	Yes	Herkogamy	↓ Pollinator abundance; ↓ plant density	Moeller and Geber, 2005; Moeller, 2006
Collinsia parviflora	CP, CG	Yes	R, flower size	↓ Pollinator visitation	Kennedy and Elle, 2008; Elle and Carney, 2003
Eichhornia paniculata	CP	Yes[‡]	*s*	↓ Plant density	Barrett *et al.*, 1989; Husband and Barrett, 1991
Linanthus spp.	CP	Yes[‡]	SC	↑ Variation in pollen limitation	Goodwillie, 2001
Phyllodoce aleutica	CP	Yes	*s*	↓ Pollinator activity	Kameyama and Kudo, 2009
Paris quadrifolia	CP	Yes	R	↓ Plant density	Jacquemyn and Brys, 2008
Primula vulgaris	CP, CG	Yes[‡]	Heterostyly	↓ Pollinator visitation	Piper *et al.*, 1986
Ranunculus reptans	CP	Unknown	SC	↓ Mate availability	Willi, 2009
Schizanthus spp.	MP	Yes	R	↑ Pollinator specialization	Perez *et al.*, 2009
Studies not identifying an environmental correlate of RA					
Aquilegia canadensis	CP	Yes	*s*	↓ Plant density range edges	Herlihy and Eckert, 2005; Eckert *et al.*, 2009*b*
Arenaria uniflora	CP	Unknown	Flower size	↓ Pollinator visitation	Wyatt, 1986
Crepis sancta	CP	Unknown	*s*	Earlier successional stages	Cheptou *et al.*, 2002
Datura stramonium	CP	Yes	R	↓ Plant density	van Kleunen *et al.*, 2007
Eichhornia paniculata	CP	Unknown	Heterostyly	↓ Pollinator visitation	Husband and Barrett, 1992
Eritrichium nanum	CP	Unknown	*s*	↑ Altitude	Wirth *et al.*, 2010
Gesnerieae spp.	CP	Yes	R	↑ Pollinator specialization	Marten-Rodriguez and Fenster, 2010
Helleborus foetidus	CP	Yes	R	↓ Pollinator visitation	Herrera *et al.*, 2001
Leavenworthia alabamica	CG	Yes[‡]	SC	Range edges	Busch, 2005
Nicotiana glauca	CP	Yes	R	↓ Pollinator visitation	Schueller, 2004

[*] Studies involve either correlations among populations (CP), manipulations of pollination environment (MP) or common garden experiments (CG).

[†] 'Yes' denotes that a study used floral emasculation in natural populations to test if selfing provides reproductive assurance.

[‡] In these cases, reproductive assurance is inferred because selfing plants produce more seed than closely related outcrossers

[§] Studies were included if they measured the selfing rate inferred with molecular markers (*s*), variation in self-compatibility (SC), key indicators of selfing, or the reproductive assurance benefit of selfing $R = 1 - $ (seed set$_{emasculated}$/seed set$_{intact}$).

[¶] Factors that were investigated to see if they correlate positively (↑) or negatively (↓) with an increase in selfing.

population size (N_e) of this population will decline in response to increased levels of selfing because this shift alters the time it takes for alleles to coalesce in a common ancestor. If the mating-system transition is to complete selfing, all plants will be homozygous and the two alleles found within individuals will be identical by descent; the reduced time to coalescence therefore results in a 50 % reduction in the effective population size. More generally, the shift to a selfing rate *s* causes a relatively rapid departure from random mating equalling $F = s/(2 - s)$. This shift to greater amounts of selfing should therefore reduce the effective size of populations to a level equal to $N_e = N_e/(1 + F)$ (Pollak, 1987).

If selfing evolves because of reproductive assurance, expectations for reductions in the effective population size may be much larger. In particular, with the reproductive assurance hypothesis, declines in N_e should be greater than expected under the transmission advantage hypothesis, because the loss of vector-mediated pollination service causes some individuals to produce fewer seeds. This inflated variation in fitness will depress N_e and thereby cause reductions in genetic diversity that are greater than expected under the transmission advantage hypothesis (Schoen *et al.*, 1996). The transmission advantage hypothesis is therefore rejected whenever

N_e falls below neutral expectations (i.e. $N_e = N_e/(1 + F)$), since variation in seed production among individuals will depress N_e. Comparisons of allozyme and nucleotide diversity in closely lineages have repeatedly shown large losses of genetic diversity upon the adoption of selfing (Hamrick and Godt, 1996; Charlesworth, 2003), or high variance in diversity among populations, as would be expected in the face of demographic instability (Schoen and Brown, 1991). Reproductive assurance again serves as an umbrella term that encapsulates many possible sources of selection that limit outcross pollen quantity and quality, such as the reliance upon an unreliable or currently depauperate pollinator fauna, ephemeral flowering periods where cross-pollination is unlikely, reductions in population size and stability, or heterospecific pollen interference. Rejecting the transmission advantage hypothesis is therefore useful, but alternative methods may be needed to determine the specific reason(s) why outcross pollen may have been limited during the transition to selfing.

Several recent studies have examined the population-genetic history of closely related outcrossing and selfing populations in three species (North American *Arabidopsis lyrata*, *Eichhornia paniculata* and *Leavenworthia alabamica*). These studies have largely failed to reject the transmission advantage hypothesis

TABLE 4. *Species where selfing is thought to have evolved recently from outcrossing, and where population-genetic data have been used to evaluate the mechanism of natural selection triggering the shift in mating system*

Selfing taxon	Timing of event(s)	Decline in Θ $(4N_e\mu)$*	Reject automatic selection?	Ecological correlation with selfing	References
Arabidopsis lyrata	<10 ka	<50 %	No	None known	Hoebe *et al.*, 2009; Foxe *et al.*, 2010
Capsella rubella	<20 ka	>99 %	Yes	Floral adaptations, weedy habit	Foxe *et al.*, 2009; Guo *et al.*, 2009
Clarkia xantiana ssp. *parviflora*	<61 ka	~80 %	Yes	Floral adaptations, loss of pollinators	J. B. Pettengill and D. A. Moeller, University of Minnesota, USA, unpubl. res.
Eichhornia paniculata	<125 ka	~50 %	No	Floral adaptations, selfing in smaller populations	Ness *et al.*, 2010
Leavenworthia alabamica	<48 ka	~10 %	No	No known floral adaptations	Busch *et al.*, 2011
	<150 ka	100 %	Yes	Floral adaptations, selfing in smaller populations	Busch *et al.*, 2011

* Θ, scaled population parameter; μ, mutation rate per site per generation.

(Table 4). Interestingly, two of these comparisons (*A. lyrata* and *L. alabamica*) involve plant populations with little secondary adaptation to facilitate self-pollination, implying that these origins of selfing are recent, as is likely given recent glacial history and coalescent inferences (Foxe *et al.*, 2010; Ness *et al.*, 2010; Busch *et al.*, 2011). In each of these studies, however, there is ongoing gene flow between populations, which complicates tests of the transmission advantage hypothesis. For example, the automatic selection hypothesis is not rejected in *E. paniculata* using a population-genetic approach (Ness *et al.*, 2010), even though there are indications that reproductive assurance favours selfing from studies in natural populations (Barrett *et al.*, 1989; Husband and Barrett, 1991; Kohn and Barrett, 1994). The body of work conducted in *E. paniculata* demonstrates that there may often be conflicting evidence for and against the automatic selection hypothesis. In this case, the most likely reason for the discrepancy is that migration events after the evolution of selfing have restored genetic diversity to a level that is consistent with the expectations of the transmission advantage of selfing.

In contrast to cases where the shift to selfing did not involve a large loss of genetic diversity, there are three cases in which reproductive assurance has been implicated. Perhaps the strongest evidence comes from the mustard genus *Capsella*. In this genus, *Capsella rubella* is highly self-fertile and is thought to have evolved from a recent common ancestor with self-incompatible *C. grandiflora*. Interestingly, there has been a nearly complete loss of genetic diversity in *C. rubella* in comparison to its outcrossing sister species (Foxe *et al.*, 2009; Guo *et al.*, 2009). Selfing also appears to have evolved in response to reproductive assurance in a lineage of *Leavenworthia alabamica* and in *Clarkia xantiana* ssp. *parviflora*, as there have been large losses of genetic diversity in comparison to conspecific outcrossing populations (Table 4). Intriguingly, in each of these three recently derived selfing lineages (*C. rubella*, *L. alabamica* and *C. xantiana* ssp. *parviflora*) there are a large number of floral adaptations for self-pollination (e.g. shorter petals, alterations in anther position, and increased rates of spontaneous seed production). The joint observation of these derived floral syndromes, coupled with a genetic signature of a population bottleneck, provide perhaps the strongest support for the importance of reproductive assurance in driving shifts to self-fertilization.

SO WHICH MECHANISM IS MORE IMPORTANT IN EXPLAINING THE EVOLUTION OF SELFING?

The transmission advantage and reproductive assurance hypotheses have guided research in plant mating-system evolution for a very long time (Darwin, 1876; Fisher, 1941; Jain, 1976). It is important to remember that other hypotheses may explain shifts to selfing and have garnered empirical support (for a review, see Goodwillie *et al.*, 2005). Further, the simple equations presented here ignore associations and evolutionary feedbacks that have been shown to be important in determining the eventual outcome of mating-system evolution (Uyenoyama *et al.*, 1993; Cheptou and Schoen, 2007). Nevertheless, our simplified perspective is meant to focus upon the seed and pollen components of fitness that drive mating-system evolution. In this paper, we have endeavoured to show that the two major hypotheses for the evolution of selfing spring forth from the same framework, involving selection at the gene (transmission advantage) and both the gene and individual levels (reproductive assurance). The transmission advantage hypothesis is a special case, since it applies only when selfing does not boost seed production, whereas reproductive assurance applies more generally because it incorporates variation in seed production among outcrossing and selfing plants. In both of the conceptual approaches presented here (e.g. field experiments of competing plants or population genetic approaches), predictions of the reproductive assurance therefore include the cost of meiosis that forms the conceptual basis for the transmission advantage of selfing.

Since the transmission advantage hypothesis is a special case of the reproductive assurance hypothesis, it stands to reason that it will be applicable less often in natural populations. Interestingly, in two lineages where the evolution selfing does not involve floral adaptation for self-pollination, population-genetic analyses imply that this strategy may have evolved because of its transmission advantage (Table 4). It is more often the case, however, that selfing appears to boost seed production or to be associated with demographic instability (Eckert *et al.*, 2006; Table 3), although this is not always true. More work must focus on recently derived selfing lineages to

test accurately the predictions of the transmission advantage and reproductive assurance hypotheses with a population-genetic approach, since each of these mechanisms produces a unique historical signature on genetic diversity, but only over a relatively short time scale (Schoen *et al.*, 1996). In direct studies of mating-system polymorphisms, observations of a seed benefit associated with selfing cannot be assumed to generate selection for selfing in nature, since the seed and pollen discounting rates may erase this selective advantage (Lloyd, 1992). Whether selfing evolves because it provides reproductive assurance must therefore be tested by explicitly measuring the pollen and seed discounting rates. Interestingly, no known study has jointly estimated these rates (Eckert *et al.*, 2006). Both of these factors are sensitive to the timing of self-pollination and the pollination environment (Chang and Rausher, 1998; Vaughton *et al.*, 2010), so future work must examine the spatial and temporal variability expected in the environment to understand fully the selection of mating-system modifiers. One very interesting conclusion taken from these studies is that the degree of pollen discounting is likely to be strongly dependent upon whether traits causing selfing have morphological impacts on flowers or pollen presentation, as predicted (Holsinger, 1996). No generality has yet emerged on the magnitude of seed discounting since it has rarely been estimated (Schoen and Lloyd, 1992), but this factor likely plays a major role in maintaining outcrossing, especially if selfing is not delayed (Lloyd and Schoen, 1992; Herlihy and Eckert, 2002).

The reproductive assurance hypothesis appears to have been supported by a diverse array of empirical tests, and is emerging as one of the most generally accepted reasons for the evolution of selfing in angiosperms. Indeed, direct studies have repeatedly shown that selfing boosts seed production, and population-genetic studies often find large losses of genetic diversity or high variance in diversity among populations, as would be expected in species where demographic instability triggers the evolution of selfing. Unfortunately, reproductive assurance is implicated by rejecting rather simplistic null hypotheses which may not adequately describe the expected complexity of mating-system evolution. In particular, it may often be the case that the process of mating-system evolution is aided by each of the direct advantages of selfing sequentially. This added layer of complexity has not yet been incorporated into population-genetic models, nor can it be addressed in field experiments lacking a historical component. Indeed, the initial spread of a mutation causing selfing might often be favoured because of reproductive assurance. Following the purging of some of the segregating mutational load, the equilibrium selfing rate might largely depend upon the tug-of-war between the transmission advantage of selfing and inbreeding depression. Making progress in understanding the relative importance of these mechanisms may be particularly difficult if this is often the case.

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