EVALUATING A SIMPLE APPROXIMATION TO MODELING THE JOINT EVOLUTION OF SELF-FERTILIZATION AND INBREEDING DEPRESSION

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A comprehensive understanding of plant mating system evolution requires detailed genetic models for both the mating system and inbreeding depression, which are often intractable. A simple approximation assuming that the mating system evolves by small infrequent mutational steps has been proposed. We examine its accuracy by comparing the evolutionarily stable selfing rates it predicts to those obtained from an explicit genetic model of the selfing rate, when inbreeding depression is caused by partly recessive deleterious mutations at many loci. Both models also include pollen limitation and pollen discounting. The approximation produces reasonably accurate predictions with a low or moderate genomic mutation rate to deleterious alleles, on the order of $U = 0.02$–0.2. However, for high mutation rates, the predictions of the full genetic model differ substantially from those of the approximation, especially with nearly recessive lethal alleles. This occurs because when a modifier allele affecting the selfing rate is rare, homozygous modifiers are produced mainly by selfing, which enhances the opportunity for purging nearly recessive lethals and increases the marginal fitness of the allele modifying the selfing rate. Our results confirm that explicit genetic models of selfing rate and inbreeding depression are required to understand mating system evolution.

KEY WORDS: Mixed mating, plants, pollen limitation, pollen discounting, recessive lethal mutations, selfing.

Inbreeding depression, the relative decrease in fitness of inbred versus outbred individuals, is a critical force in the evolution of mating systems, with complex evolutionary dynamics. Numerous theoretical approaches have explored these dynamics, and sometimes its joint evolution with the mating system, when inbreeding depression is caused by overdominance (Uyenoyama and Waller 1991b), or by deleterious recessive alleles at one (e.g., Uyenoyama and Waller 1991a; Glémin 2003) or many loci with (Charlesworth et al. 1991) or without (Lande and Schemske 1985; Charlesworth et al. 1990; Lande et al. 1994) epistatic interactions or genetic linkage (Charlesworth et al. 1992). All these approaches stress the critical influence of the genetic basis of inbreeding depression and its evolutionary dynamics on mating system evolution (see, e.g., Porcher et al. 2009, for an example of how the dynamics of inbreeding depression substantially modify the predictions of an ecological model of mating system evolution). Yet, most models that address the effect of ecological forces on mating system evolution have overlooked the dynamics of inbreeding depression, which is often considered fixed (Goodwillie et al. 2005). Simplified models of mating system evolution abound because modeling the joint evolution of mating system and inbreeding depression requires detailed genetic models for both characters, which are often intractable.

One approximation to modeling the joint evolution of inbreeding depression and mating system was proposed by Lande and Schemske (1985) and later extended by Johnston (1998) to incorporate ecological mechanisms (seed and pollen discounting) in the evolution of plant mating systems. This approximation is used to find joint equilibria of the mating system and inbreeding depression, by examining the indirect selection gradient on small changes in the selfing rate, assuming the mating system evolves by infrequent small mutation steps. The mating system is assumed to undergo no direct selection, but evolves because of its influence on inbreeding depression. This approximation contains elements of evolutionarily stable strategies, as well as inclusive fitness by weighting selfed seed twice as much as outcrossed seed to account for the automatic advantage of selfing described by Fisher (1941). It can incorporate any genetic model of inbreeding depression, as well as ecological mechanisms influencing the selfing rate (Johnston 1998; Johnston et al. 2009; Devaux, Lande and Porcher in prep.).

This approximation ignores genotypic associations, due to both linkage (gametic) disequilibrium and identity (zygotic) disequilibrium (Haldane 1949; Crow and Kimura 1970, eq. 3.9.3), between the mating system locus and viability loci controlling inbreeding depression. Such genotypic associations have been observed in nature (Weber et al. 2012), and theory predicts that in some cases they can greatly influence the dynamics of invasion of a modifier of the selfing rate (Lande and Schemske 1985; Uyenoyama et al. 1993). Several models have quantified the impact of genotypic associations on the spread of selfing modifiers (Holsinger 1988; Charlesworth et al. 1990; Uyenoyama and Waller 1991a,b,c; Schultz and Willis 1995), but intermediate selfing rates were never evolutionarily stable in these models, unless they assumed inbreeding depression due to overdominance (Uyenoyama and Waller 1991b), which has little support from experimental data (Charlesworth and Willis 2009). As a result, no study has quantified the influence of genotypic associations between inbreeding depression and selfing modifiers on the evolutionarily stable selfing rates and the accuracy of an approximation assuming no genetic association. Although it has previously been thought that the approximation should be accurate for infrequent small mutations affecting the selfing rate (Lande and Schemske 1985; Johnston et al. 2009), this was never demonstrated.

Here we compare this approximation with a full genetic model for the joint evolution of both the selfing rate and inbreeding depression. Both models also include pollen limitation and pollen discounting, two ecological mechanisms that influence the evolution of plant mating systems and create the conditions for the maintenance of intermediate selfing rates (Holsinger 1991; Knight et al. 2005; Porcher and Lande 2005).

The Models
THE APPROXIMATION

We assume a large (effectively infinite) population with selfing rate $\bar{r}$ in which an initially rare modifier with selfing rate r appears. The resident and modifier genotypes may differ (1) in their total seed set T, due to pollen limitation, and (2) in the amount of pollen exported for outcrossing, P, due to pollen discounting (the decrease in pollen export caused by self-fertilization; Harder and Wilson 1998). The fitness of the modifier genotype affecting the selfing rate incorporates the automatic advantage of selfing (Fisher 1941), by weighting selfed seed twice as much as outcrossed seed

$$w = r\bar{w}_1 T(r) + (1-r)\frac{\bar{w}_0}{2}T(r) + (1-\bar{r})\frac{\bar{w}_0}{2}\frac{P(r)}{P(\bar{r})}T(\bar{r}),$$

where $\bar{w}_0$ and $\bar{w}_1$ are the mean fitnesses of outcrossed and selfed progeny, respectively (Lande and Schemske 1985; Johnston 1998, eq. 2a). The intensity of selection on the modifier with a small effect on the selfing rate is approximately proportional to the selection gradient:

$$\frac{1}{\bar{w}_0 T(\bar{r})}\frac{\partial w}{\partial r}\bigg|_{r=\bar{r}} = \frac{1}{2} - \delta + \left[\frac{r(1-\delta)}{T(\bar{r})}\frac{\partial T(r)}{\partial r} + \frac{(1-r)}{2T(\bar{r})}\frac{\partial T(r)}{\partial r} + \frac{1-\bar{r}}{2P(\bar{r})}\frac{\partial P(r)}{\partial r}\right]_{r=\bar{r}},$$

where $\delta = 1 - \bar{w}_1/\bar{w}_0$ is the inbreeding depression in the resident population assumed to be at mutation–selection equilibrium for the given selfing rate (Lande and Schemske 1985). Evolutionary equilibrium selfing rates occur when the selection gradient is 0, which yields the level of inbreeding depression that exactly counterbalances all other constraints on the evolution of selfing, that is the automatic advantage, reproductive assurance in the presence of pollen limitation, and pollen discounting

$$\delta(\bar{r}) = \frac{1}{2}\left[1 + \frac{\frac{\partial \ln T(r)}{\partial r} + (1-\bar{r})\frac{\partial \ln P(r)}{\partial r}}{1 + r\frac{\partial \ln T(r)}{\partial r}}\right]_{r=\bar{r}}. \quad (1)$$

The right-hand side of equation (1), hereafter referred to as the constraint function, can be compared to the inbreeding depression $\delta(\bar{r})$ expected at equilibrium in a population with selfing rate $\bar{r}$ under any explicit genetic model for inbreeding depression. Equilibrium selfing rates occur at the intersections of the constraint function and $\delta(\bar{r})$ (Fig. 1).

To model inbreeding depression, we use the Kondrashov model (1985), which describes the evolution of the distribution of number of partly recessive deleterious alleles per mature plant in the population when mutations occur at an infinite number of unlinked loci in an infinite population (Lande et al. 1994; Porcher and Lande 2005). We considered separately two contrasting classes of deleterious mutations that are believed to cause inbreeding
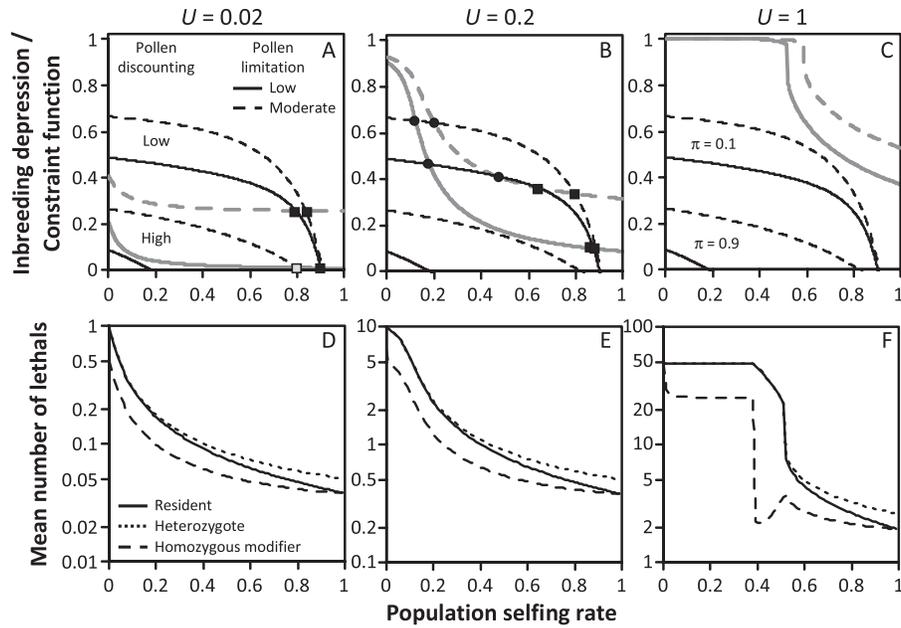
Figure 1. Inbreeding depression, constraint function, and genetic load (mean number of deleterious alleles) when inbreeding depression is caused by nearly recessive lethals. The three variables are plotted as a function of the selfing rate for different values of U, the genomic mutation rate. (A–C) Genetic inbreeding depression without (thick solid gray line) or with (thick dashed gray line) background inbreeding depression ($d = 0.25$) and constraint function (black lines) for different levels of pollen limitation and pollen discounting, with stable (squares) and unstable (circles) intermediate equilibria predicted at their intersections. Low and moderate pollen limitation (solid and dashed black lines, respectively), $\pi_o P_T = 4$ and 1.5, correspond to seed set of a completely outcrossing plant of 0.98 and 0.78. The upper pair of black solid and dashed lines correspond to low pollen discounting ($\pi = 0.1$), and the lower pair to high pollen discounting ($\pi = 0.9$). (D–F) Genetic load in genotypes at a modifier increasing the selfing rate, when the modifier allele is rare, but after associations with lethals have built up (resident, solid line; heterozygote, dotted line; homozygous modifier, dashed line). Figures D–F do not include background inbreeding depression ($d = 0$).

depression (Charlesworth and Willis 2009): nearly recessive lethal mutations versus partially recessive, mildly deleterious mutations. We also analyzed a model incorporating a constant "background" inbreeding depression into a Kondrashov model with nearly recessive lethals, because the equilibrium inbreeding depression due to stabilizing selection on quantitative characters, or to nearly additive, mildly deleterious mutations, undergoes relatively little purging in response to an increased selfing rate (Porcher and Lande 2005; and see Fig. S1).

MAINTENANCE OF MIXED MATING WITH POLLEN DISCOUNTING AND POLLEN LIMITATION

Pollen limitation and pollen discounting are described by the seed set $T(r)$ and pollen export $P(r)$ functions employing the mass-action model for pollination (Holsinger 1991; Porcher and Lande 2005). All genotypes are expected to produce the same number of flowers and the same amount of pollen P_T. A rare mating system modifier changes the selfing rate by altering the fraction α of pollen a genotype exports for outcrossing. Because the mating system modifier is rare, the amount of out-

cross pollen landing on the stigma of any plant is approximately $P_o = \bar{\alpha}\pi_o P_T$, where $\bar{\alpha}$ is the fraction of pollen exported by the resident genotype and π_o is the probability that pollen exported for outcrossing actually lands on a stigma. The amount of self-pollen landing on the stigmas of the resident and modifier genotypes are $\bar{P}_s = (1 - \bar{\alpha})\pi_s P_T$ and $P_s = (1 - \alpha)\pi_s P_T$, respectively, where π_s is the probability that pollen not exported actually lands on the stigma.

Under competing selfing, the primary selfing rate r of the modifier genotype, after fertilization, is the ratio of self-pollen to total pollen landing on the stigma $r = P_s/(P_s + P_o)$, which can also be written

$$r(\alpha) = \frac{(1 - \alpha)\pi_s P_T}{(1 - \alpha)\pi_s P_T + \bar{\alpha}\pi_o P_T} = \frac{1 - \alpha}{1 - \alpha + \bar{\alpha}\pi}, \quad (2)$$

where $\pi = \pi_o/\pi_s$, the relative success of outcross versus self-pollen, quantifies the strength of pollen discounting (Porcher and Lande 2005).

The amount of pollen the modifier genotype exports for outcrossing is $P = \alpha\pi_o P_T$, which can be written as a function of the

selfing rate using equation (2), $\alpha = 1 - r\bar{\alpha}\pi/(1 - r)$, so that

$$P(r) = \left(1 - \frac{r\bar{\alpha}\pi}{1 - r}\right)\pi_{o}P_{T}. \tag{3}$$

Under pollen limitation, the total seed set T of the modifier genotype depends on the amounts of self and outcross pollen landing on the stigma,

$$T = 1 - \exp[-P_{o} - P_{s}] = 1 - \exp[-\pi_{o}P_{T}(\bar{\alpha} + (1 - \alpha)/\pi)],$$

which can also be written as a function of the selfing rate r using equation (2),

$$T(r) = 1 - \exp[-\pi_{o}P_{T}\bar{\alpha}/(1 - r)]. \tag{4}$$

From equation (4), one can see that $\pi_{o}P_{T}$, the amount of pollen exported by a completely outcrossing individual that reaches a stigma, can be used to quantify pollen limitation independently of the population mating system.

Noting that $\bar{\alpha} = (1 - \bar{r})/[1 - \bar{r}(1 - \pi)]$ and differentiating equations (3) and (4), we find

$$\left.\frac{\partial\ln P(r)}{\partial r}\right|_{r=\bar{r}} = -\frac{\pi}{(1 - \bar{r})^2} \text{ and}$$

$$\left.\frac{\partial\ln T(r)}{\partial r}\right|_{r=\bar{r}} = \frac{[1 - T(\bar{r})]\pi_{o}P_{T}}{T(\bar{r})(1 - \bar{r})[1 - \bar{r}(1 - \pi)]}.$$

These are used to obtain the constraint function from equation (1). The intersection between the constraint function and inbreeding depression $\delta(\bar{r})$ is then explored numerically to obtain the equilibrium selfing rates expected under the approximation (Fig. 1A–C). The stability of an equilibrium is found by comparing the values of the constraint function and $\delta(\bar{r})$ around the equilibrium: equilibrium selfing rates are evolutionarily stable if an increase in $\bar{r}$ results in the inbreeding depression being larger than the constraint function (greater costs than benefits of selfing) and vice versa.

THE FULL GENETIC MODEL

The equilibrium selfing rates predicted by the approximation are compared to those obtained using the full genetic model of Porcher and Lande (2005) describing joint evolution of both mating system and inbreeding depression in an infinite population. This uses the same pollination model, where the selfing rate, seed set, and pollen export are controlled by the fraction α of pollen exported for outcrossing. The pollination model is coupled with the Kondrashov (1985) model, in which inbreeding depression is caused either by nearly recessive lethals (with or without an additional constant background inbreeding depression) or by nearly additive, mildly deleterious mutations. To model the evolution of the selfing rate, we added an unlinked locus modifying the mating

system to the genetic model of inbreeding depression, which accounts for associations between deleterious mutations and alleles influencing the selfing rate. In a resident population at mutation–selection equilibrium, a modifier allele with a pollen export fraction α slightly different from the resident is introduced at a low frequency in linkage and identity equilibrium with deleterious mutations. We examine the fate (invasion or not) of this initially rare modifier allele after 2000 generations to find the evolutionarily stable selfing rates, that is resident selfing rates that cannot be invaded.

PARAMETER VALUES

We varied genetic and ecological parameters to generate a wide range of intermediate stable selfing rates that can be compared between the two theoretical approaches. The genomic mutation rate to deleterious mutations was $U = 0.02$, 0.2, or 1; the dominance coefficient of lethals was set to $h = 0.02$ (references in Lande and Schemske 1985; Lande et al. 1994); mildly deleterious mutations were characterized by $s = 0.05$ and $h = 0.4$ (references in Halligan and Keightley 2009). We also analyzed a model with such nearly recessive mutation to lethals and a constant background inbreeding depression of $d = 0.25$ (Winn et al. 2011). The relative success of self versus outcross pollen π was varied between 10^{-4} (no pollen discounting) and 0.9999 (strong pollen discounting). We also considered a wide range for the strength of pollen limitation, from $\pi_{o}P_{T} = 0.5$ to $\pi_{o}P_{T} = 10^{10}$. These values correspond to seed sets of 0.4 and 1, respectively, for a completely outcrossing population. In the figures, we use a value of $\pi_{o}P_{T} = 1.5$ for moderate pollen limitation, corresponding to a seed set of 0.78 in a completely outcrossing population, which is representative of realistic values observed in natural populations (Knight et al. 2005).

In the full genetic model, we examined the spread of a rare modifier allele that increased or decreased the selfing rate compared to the resident population (see Porcher and Lande 2005 for details). Because we were interested in the accuracy of the approximation when the selfing rate evolves by small steps, we considered a rare modifier that increased or decreased selfing by 10^{-6}. The modifier allele was introduced at an initial frequency of 10^{-8} in a resident population at mutation–selection equilibrium for lethals. The modifier genotypes were initially at frequencies expected for a population with inbreeding coefficient $f = \bar{r}/(2 - \bar{r})$ (Wright 1921, 1969) and in linkage and identity equilibrium with lethal alleles. The recursion equations were numerically iterated for 2000 generations to detect successful invasion of the modifier of the selfing rate. We verified that if the resident population is initially not at a stable equilibrium selfing rate, a modifier allele causing a small change in the selfing rate toward the equilibrium eventually becomes fixed.
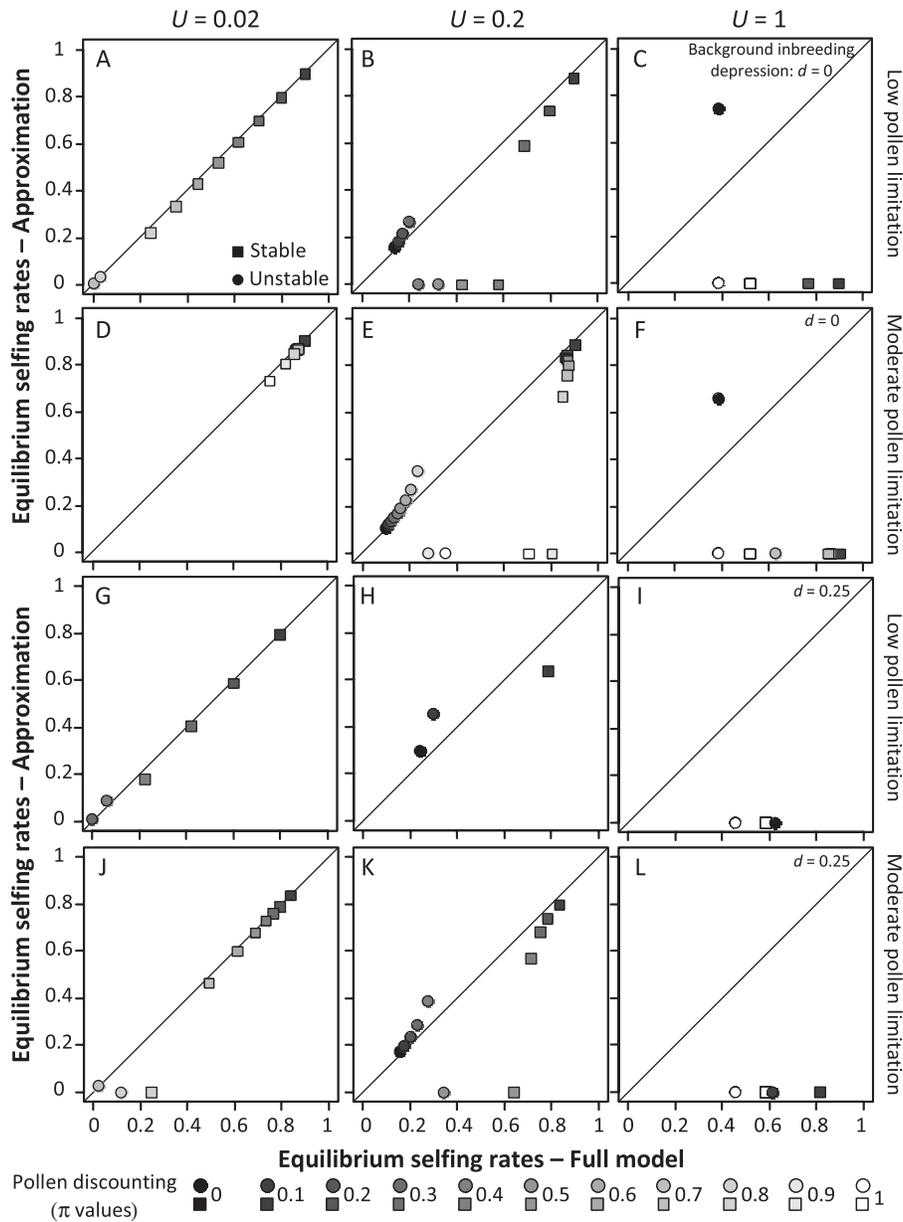
Figure 2. Equilibrium selfing rates predicted by the approximation versus the full genetic model, when inbreeding depression is caused by nearly recessive lethals, under different levels of pollen discounting, pollen limitation, and background inbreeding depression. Stable or unstable equilibria are indicated by squares or circles. Only intermediate equilibria are shown. Points on the *x*-axis correspond to intermediate equilibria predicted by the full genetic model that do not exist under the approximation. Complete outcrossing and complete selfing are always equilibria; their stability depends on the existence of intermediate equilibria. When there is a single stable intermediate selfing rate, *r* = 0 and 1 are unstable; when the stable intermediate selfing rate coexists with a lower unstable intermediate selfing rate, *r* = 0 is stable and *r* = 1 is unstable. Gray levels indicate pollen discounting values. Levels of pollen limitation as in Figure 1.

Results and Discussion

The approximation produces relatively accurate equilibrium selfing rates under low to moderate genomic rates of mutation to lethals (Fig. 2A, D and, to a lesser extent, B, E) or with nearly additive, mildly deleterious mutations (Fig. S2). We checked that with no inbreeding depression, $U = 0$, both models predict identical equilibrium selfing rates. For example, with no pollen limitation,

$T(r) = 1$, equation (1) simplifies to $\delta_e(\bar{r}) = 1/2[1 - \pi(1 - \bar{r})]$ and the evolutionarily stable selfing rate is $r* = 1 - \pi$ (Holsinger 1991). With low inbreeding depression, that is moderate genomic mutation rate to lethals ($U = 0.02$) or mildly deleterious mutations, and no pollen limitation, the stable selfing rates are also close to $1 - \pi$ for both models (Fig. 2A, S2A–C). As expected, higher pollen limitation generally favors larger stable selfing rates

but again the approximation is relatively accurate with moderate mutation rates (Fig. 2D, Fig. S2D–E).

With higher genomic mutation rates to lethals, $U = 0.2$ and 1, the equilibria predicted by the approximation can differ greatly from those of the full genetic model (Fig. 2) and the discrepancy between the two models increases as U increases. For $U = 0.2$, the predictions of the approximation agree reasonably well with those of the full model for limited pollen discounting, especially with pollen limitation (Fig. 2B, E), but the discrepancy between the models increases with more intermediate equilibrium selfing rates, which are obtained in this case with increasing pollen discounting. For low pollen limitation and high pollen discounting, the full model predicts mixed mating when the approximation predicts complete outcrossing (Fig. 2B, E). With a very high genomic mutation rate to lethals, $U = 1$, the approximation becomes completely inaccurate (Fig. 2C, F), predicting that the only evolutionarily stable mating system is complete outcrossing (and also complete selfing in the case of no pollen discounting), because the genetic inbreeding depression usually exceeds the constraint function (Fig. 1C). In contrast, the full genetic model predicts a number of evolutionarily stable intermediate selfing rates, most of which are close to 1 and depend little on pollen discounting and pollen limitation (see Porcher and Lande 2005).

A discrepancy between the full genetic model and the approximation is also observed, although to a lesser extent, with high mutation rates to mildly deleterious mutations and moderate pollen limitation ($U = 1$; Fig. S2F). Here, the full genetic model sometimes predict complete selfing when the approximation predicts mixed mating systems with a large selfing rate ($r > 0.6$). The combination of both types of mutations is therefore likely to exacerbate the difference between both models. This was not tested here, because the Kondrashov model with more than one mutation type is computationally demanding. Instead, we modeled the combined effects of nearly recessive, highly deleterious mutations and nearly additive, mildly deleterious mutations by adding a constant background inbreeding depression ($d = 0.25$) to the Kondrashov model with lethals. The results remain qualitatively the same, with larger discrepancies under higher mutation rates to lethals (Fig. 2G–L), but the inability of the approximation to predict mixed mating appears at lower genomic mutation rates, as expected (e.g., $U = 0.02$; Fig. 2J).

The discrepancy between the approximation and the full genetic model is caused by genotypic associations between alleles at the modifier locus and the genetic basis of inbreeding depression (Holsinger 1988; Charlesworth et al. 1990; Uyenoyama and Waller 1991a,c). In a partially selfing population, rare genotypes with a modified selfing rate may differ from the resident population in their average history of inbreeding, which impacts their associated genetic load (or mean number of re-

cessive deleterious alleles), whereas the approximation assumes identical genetic load regardless of the genotype at the modifier locus. For example, when the modifier allele is rare, plants homozygous for the modifier are initially produced mostly by selfing and therefore partially purged of recessive deleterious alleles (Fig. 1D–F). Extensive simulation showed that genetic associations between a modifier of selfing and recessive lethal mutations build up over a few dozen generations. As a result, a modifier allele that eventually invades and becomes fixed may initially decrease in frequency (Schultz and Willis 1995). Thus, the simulations were run for 2000 generations to detect successful invasion.

Differential purging of the load associated with modifier genotypes is transient; for a modifier of small effect destined for fixation the homozygous modifier gains genetic load as it becomes common (Schultz and Willis 1995). The initial differential purging for a rare modifier depends little on the magnitude of the modifier effect, and also occurs for (neutral) mutations with no impact on the selfing rate (Charlesworth 1991). However, it can strongly influence the fate of mating system modifiers even with very small effect, and alter the evolutionarily stable selfing rate and inbreeding depression by shifting the equilibrium selfing rate, especially when inbreeding depression is caused by nearly recessive lethals. Overall, this promotes enhancers of selfing under broader conditions than expected with the approximation, as demonstrated in earlier studies (Lande and Schemske 1985; Charlesworth et al. 1990; Uyenoyama and Waller 1991c; Schultz and Willis 1995). We have shown here that the joint evolution of inbreeding depression and mating system, combined with common mechanisms of pollination ecology, also favors mixed mating over complete outcrossing more often than predicted by the approximation.

Conclusion

We show that the accuracy of the approximation depends primarily on the genomic mutation rate to lethals, for which few estimates are available. The best estimates, from *Drosophila*, indicate that $U = 0.01$–0.03 per genome per generation (Fry et al. 1999; Charlesworth et al. 2004). Evidence suggests that annual plants may have comparable values of U (e.g., Jürgens et al. 1991 in *Arabidopsis thaliana*). For such species, the approximation is accurate. However, mutation rates to lethals may be an order of magnitude higher ($U = 0.2$) in large perennial or partially asexual species due to mutation accumulation in somatic cell divisions, as plants lack a separate germ line (Lande et al. 1994 and references therein; Remington and O'Malley 2000). The approximation also tends to underestimate stable selfing rates under high mutation rates to mildly deleterious, nearly additive alleles, although less so than with lethals. High mutation rates to mildly deleterious

alleles, sometimes greater than 1, may also occur in multicellular organisms (Halligan and Keightley 2009). With high U, the approximation greatly underestimates the evolutionarily stable selfing rates, so that explicit genetic models of both selfing rate and inbreeding depression are required for a comprehensive understanding of mating system evolution.

LITERATURE CITED

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. Genet. Res. 57:177–194.

Charlesworth, B., H. Borthwick, C. Bartolome, and P. Pignatelli. 2004. Estimates of the genomic mutation rate for detrimental alleles in *Drosophila melanogaster*. Genetics 167:815–826.

Charlesworth, D. 1991. The apparent selection on neutral marker loci in partially inbreeding populations. Genet. Res. 57:159–175.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10:783–796.

Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. Evolution 44:1469–1489.

———. 1992. The effect of linkage and population-size on inbreeding depression due to mutational load. Genet. Res. 59:49–61.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. Ann. Eugenics 11:53–63.

Fry, J. D., P. D. Keightley, S. L. Heinsohn, and S. V. Nuzhdin. 1999. New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 96:574–579.

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

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

Haldane, J. B. S. 1949. The association of characters as a result of inbreeding and linkage. Ann. Eugenics 15:15–23.

Halligan, D. L., and P. D. Keightley. 2009. Spontaneous mutation accumulation studies in evolutionary genetics. Annu. Rev. Ecol. Evol. S. 40:151–172.

Harder, L. D., and W. G. Wilson. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. Am. Nat. 152:684–695.

Holsinger, K. E. 1988. Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. Evolution 42:1235–1244.

———. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. Am. Nat. 138:606–622.

Johnston, M. O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. Genetica 102-3:267–278.

Johnston, M. O., E. Porcher, P.-O. Cheptou, C. G. Eckert, E. Elle, M. A. Geber, S. Kalisz, J. K. Kelly, D. A. Moeller, M. Vallejo-Marin, et al. 2009. Correlations among fertility components can maintain mixed mating in plants. Am. Nat. 173:1–11.

Jürgens, G., U. Mayer, R. A. Torres Ruiz, T. Berleth, and S. Miséra. 1991. Genetic analysis of pattern formation in the *Arabidopsis* embryo. Develop. Suppl. 1:27–38.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T. L. Ashman. 2005. Pollen limitation of plant reproduction: pattern and process. Annu. Rev. Ecol. Evol. S. 36:467–497.

Kondrashov, A. S. 1985. Deleterious mutations as an evolutionary factor. 2. Facultative apomixis and selfing. Genetics 111:635–653.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. Evolution 39:24–40.

Lande, R., D. W. Schemske, and S. T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. Evolution 48:965–978.

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

Porcher, E., J. K. Kelly, P.-O. Cheptou, C. G. Eckert, M. O. Johnston, and S. Kalisz. 2009. The genetic consequences of fluctuating inbreeding depression and the evolution of plant selfing rates. J. Evol. Biol. 22:708–717.

Remington, D. L., and D. M. O'Malley. 2000. Whole-genome characterization of embryonic stage inbreeding depression in a selfed loblolly pine family. Genetics 155:337–348.

Schultz, S. T., and J. H. Willis. 1995. Individual variation in inbreeding depression: the roles of inbreeding history and mutation. Genetics 141:1209–1223.

Uyenoyama, M. K., and D. M. Waller. 1991a. Coevolution of self-fertilization and inbreeding depression. 1. Mutation-selection balance at one and two loci. Theor. Popul. Biol. 40:14–46.

———. 1991b. Coevolution of self-fertilization and inbreeding depression. 2. Symmetrical overdominance in viability. Theor. Popul. Biol. 40:47–77.

———. 1991c. Coevolution of self-fertilization and inbreeding depression. 3. Homozygous lethal mutations at multiple loci. Theor. Popul. Biol. 40:173–210.

Uyenoyama, M. K., K. E. Holsinger, and D. M. Waller. 1993. Ecological and genetic factors directing the evolution of self-fertilization. Ox. Surv. Evol. Biol. 9:327–381.

Weber, J. J., S. G. Weller, A. K. Sakai, A. Nguyen, N. D. Tai, C. A. Dominguez, and F. E. Molina-Freaner. 2012. Purging of inbreeding depression within a population of *Oxalis alpina* (Oxalidaceae). Am. J. Bot. 99:923–932.

Winn, A. A., E. Elle, S. Kalisz, P.-O. Cheptou, C. G. Eckert, C. Goodwillie, M. O. Johnston, D. A. Moeller, R. H. Ree, R. D. Sargent, et al. 2011. Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. Evolution 65:3339–3359.

Wright, S. 1969. Evolution and the genetics of populations. Theory of gene frequencies. Vol. 2. University of Chicago Press, Chicago.

———. 1921. Systems of mating. II. The effect of inbreeding on the genetic composition of a population. Genetics 6:124–143.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Inbreeding depression, constraint function and genetic load (mean number of deleterious alleles) when inbreeding depression is caused by nearly additive, mildly deleterious alleles ($s = 0.05$, $h = 0.4$).

Figure S2. Equilibrium selfing rates predicted by the approximation versus the full genetic model, when inbreeding depression is caused by nearly additive, mildly deleterious alleles ($s = 0.05$, $h = 0.4$), under different levels of pollen discounting and pollen limitation.