

The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation

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Abstract

We model the evolution of plant mating systems under the joint effects of pollen discounting and pollen limitation, using a dynamic model of inbreeding depression, allowing for partial purging of recessive lethal mutations by selfing. Stable mixed mating systems occur for a wide range of parameter values with pollen discounting alone. However, when typical levels of pollen limitation are combined with pollen discounting, stable selfing rates are always high but less than 1 ($0.9 < s < 1$ in most cases); in this situation, complete selfing does not evolve because pollen discounting becomes very large at high selfing rates, so that the automatic advantage of selfing changes to a disadvantage. These results suggest that mixed mating systems with high selfing rates can be maintained by selection, whereas mixed mating systems with low to moderate selfing rates are more likely attributable to unavoidable geitonogamous selfing.

Introduction

In self-compatible hermaphroditic plants, the distribution of mating systems is generally considered bimodal, with a majority of populations exhibiting low ($0 < s < 0.2$) or high ($0.8 < s < 1$) selfing rates (Schemske & Lande, 1985; Barrett & Eckert, 1990; Barrett *et al.*, 1996). However, intermediate selfing rates occur in an appreciable fraction of natural populations (Aide, 1986; Schemske & Lande, 1986; Vogler & Kalisz, 2001; Barrett, 2003), and empirical evidence suggests that many might be stable (see e.g. Holsinger, 1991 and references therein). During the past 20 years, plant evolutionary biologists have tried to explain the maintenance of such stable mixed mating systems, which is not accounted for by most theoretical genetic studies based on two major evolutionary forces: (i) the 50% automatic advantage of selfing, due to the transmission, on average, of three copies of the genome of selfing individuals (two as parents of selfed seeds and one a male parent of outcrossed seeds on other plants) while outcrossing genotypes transmit two copies (Fisher, 1941) and (ii) inbreeding depression, the relative decrease in fitness of selfed vs. outcrossed individuals due

mainly to recessive deleterious mutations (Charlesworth & Charlesworth, 1987; Husband & Schemske, 1996). Explicit genetic models of inbreeding depression that account for its joint evolution with the mating system (Lande & Schemske, 1985; Charlesworth *et al.*, 1990; Lande *et al.*, 1994) predict a dichotomous outcome of evolution in a single population: either complete selfing or complete outcrossing. In most of the theory developed so far, the maintenance of stable mixed mating systems appeals either to genetic factors, with different hypotheses on inbreeding depression, or to ecological factors, notably pollination biology (but see Schoen & Lloyd, 1984; Holsinger, 1986).

A variety of genetic factors favouring the maintenance of intermediate selfing rates have been proposed, including inbreeding depression due to overdominance (Campbell, 1986; Charlesworth & Charlesworth, 1990), inbreeding depression due to partially recessive, very mildly deleterious mutations (Latta & Ritland, 1994), biparental inbreeding in isolated (Uyenoyama, 1986; Yahara, 1992) or structured (Ronfort & Couvet, 1995) populations, negative relationship between inbreeding depression and number of generations of selfing in a lineage, mimicking purging of genetic load (Maynard Smith, 1977; Damgaard *et al.*, 1992; Latta & Ritland, 1993), and spatial or temporal environmentally induced variation in inbreeding depression (Cheptou & Mathias, 2001). However, many genetic models predict mixed

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mating systems either for a narrow range of parameter values only, or under highly specific assumptions that are probably rarely met in natural populations, e.g. inbreeding depression due to overdominance or to very mildly deleterious mutations only.

Ecological models based on pollination biology consider the details of pollen transfer, and allow variation of reproductive success components that are generally considered constant in genetic models of plant mating system evolution. In particular, two main factors affecting the evolution of selfing rate have been isolated in ecological models: pollen and seed discounting. Pollen discounting (Nagyaki, 1976; Gregorius *et al.*, 1987; Holsinger, 1991; Harder & Wilson, 1998) is the reduction of male reproductive success by outcrossing that may accompany an increase in selfing rate, due to a decrease in amounts of exported pollen. It has been observed in several natural populations (e.g. Chang & Rausher, 1998; Fishman, 2000 and references therein) and is identified as a major factor favouring the maintenance of stable mixed mating systems under a wide range of conditions (Holsinger, 1991; Johnston, 1998) but is generally omitted in detailed genetic models of mating system evolution. Seed discounting (Lloyd, 1992), the loss of outcrossed seeds due to selfing, is always complete in genetic models: each selfing event occurs at the expense of one outcrossing event. However, this needs not be always the case due to specific aspects of pollen transfer. In particular, if seed set is limited by outcross pollen availability (due to low pollinator frequency or low plant population density), selfing increases the proportion of ovules fertilized (reproductive assurance, Lloyd, 1992; Holsinger, 1996) without an equal decrease in the number of outcrossed seeds, and seed discounting is reduced. As with pollen discounting, seed discounting or reproductive assurance may also be responsible for stable intermediate selfing rates, but under more specific conditions (e.g. seed discounting larger than 1, Johnston, 1998, or with a size-number trade-off of seeds, Sakai & Ishii, 1999).

Although both theoretical and empirical studies have emphasized the role of pollen discounting and pollen limitation in evolution of plant mating systems, no theoretical approach has combined them. These two factors have opposing effects on mating system evolution and are likely to co-occur in natural populations; their interaction may determine the expected outcome of evolution. More specifically, pollen discounting can be decreased under pollen limitation, as already stressed by Lloyd (1992): for example, if the selfing rate depends on the relative amount of self vs. outcross pollen landing on a stigma (competing selfing, Lloyd & Schoen, 1992), high selfing rates can be achieved with a smaller amount of self-pollen when outcross pollen is limited. Together with the larger fertilization success by highly selfing genotypes, this effect of pollen limitation may neutralize the effects of pollen discounting and strongly narrow the

range of conditions where stable mixed mating systems can exist. As outlined by Holsinger (1991) and Johnston (1998), there is thus a need for ecological models exploring this interaction.

Because both genetic and ecological factors are potentially important determinants of mating system evolution, a complete understanding of the maintenance of stable mixed mating systems requires theoretical approaches accounting for both genetics and ecology in a realistic manner. However, genetic models usually omit pollination ecology, and most theory on the role of pollination biology in mating system evolution neglects critical genetic components. In most pollination-based models generating stable mixed mating systems, inbreeding depression is either omitted (Holsinger, 1991), or considered constant throughout evolution (Lloyd, 1979; Sakai, 1995; Sakai & Ishii, 1999). Yet, theoretical (Lande & Schemske, 1985; Charlesworth *et al.*, 1990) and empirical (Husband & Schemske, 1996) studies suggest that the component of inbreeding depression due to recessive lethal (or semi-lethal) mutations can be purged by selfing, and tends to be smaller in highly selfing than in highly outcrossing populations. In what represents the only theoretical approach so far combining pollination biology and the joint evolution of selfing rate and inbreeding depression, Johnston (1998) confirmed that pollination processes, and notably pollen discounting, play a major role in maintaining mixed mating systems, but he also demonstrated that the conditions for stability and the values of stable selfing rates strongly depend on inbreeding depression. However, Johnston (1998) neglected associations between deleterious mutations and genes influencing the selfing rate, and his approach accurately describes the dynamics only for mutations with small effects on the selfing rate. Accounting for linkage and zygotic disequilibria during the joint evolution of inbreeding depression and mating system can have a major influence. For example, mutations generating large increases in selfing rate can invade a population experiencing high levels of initial inbreeding depression, due to purging of deleterious mutations in the selfing genotypes (Lande & Schemske, 1985; Charlesworth *et al.*, 1990); this can eventually lead to complete selfing even when it could not evolve by small steps.

Here, we combine for the first time in a mathematical model the effects of pollen discounting and pollen limitation, together with a genetic model allowing a joint evolution of plant mating system and inbreeding depression. We use a mass-action model of pollination processes (Holsinger, 1991), wherein selfing rate is determined by the relative amounts of self vs. outcross pollen landing on the stigma. We refine Johnston's approach (1998) by using a dynamic model for the component of inbreeding depression due to nearly recessive lethal mutations at a very large number of loci (Kondrashov, 1985), with a constant background component of inbreeding depression attributable to mildly

deleterious mutations (Lande & Schemske, 1985; Husband & Schemske, 1996). Using an adaptive dynamic framework, we examine the stable selfing rates under various conditions of pollen discounting, pollen limitation and mutation rates to lethals.

The model

We model a mutant allele at a modifier locus affecting selfing, arising at low frequency in an initially monomorphic population, referred to as the resident population. Notations are summarized in Table 1.

Self-fertilization

The mass-action model (Holsinger, 1991) assumes competing self-fertilization (Lloyd & Schoen, 1992), such that self-pollen and outcross pollen arrive simultaneously on the stigma and compete for the fertilization of ovules; the selfing rate is then determined by the relative amounts of self and outcross pollen landing on stigmas. In comparison to prior or delayed selfing, which often requires specific floral mechanisms (Holsinger, 1991), competing self-fertilization is thought to be more common and is unavoidably involved in geitonogamous selfing, the pollination of flowers by flowers from the same plant, due to pollinator behaviour. Under competing self-fertilization, substantial amounts of self-pollen may be required to achieve high selfing rates, which likely reduces male reproductive success by diminishing exported pollen (pollen discounting, Harder & Wilson, 1998) and is modelled as follows.

We assume that all plants produce the same number of ovules and have the same pollen/ovule ratio. In the following, the amount of pollen produced by all flowers,

P , corresponds to number of pollen grains per ovule. Of pollen produced by genotype G , a fraction α_G is exported for outcrossing and $1 - \alpha_G$ remains for selfing. For all genotypes, the probabilities that outcross pollen and self-pollen actually land on a stigma are π_o and π_s , respectively. Hence, the amount of self-pollen falling on stigmas of genotype G is $P_{sG} = (1 - \alpha_G)\pi_s P$. The amount of outcross pollen P_o received by all genotypes depends on the average rate of pollen export in the population $\bar{\alpha} = \sum_G f_G \alpha_G$ (where f_G is the frequency of genotype G): $P_o = \bar{\alpha}\pi_o P$. Under competing selfing, the primary selfing rate s_G of genotype G , after fertilization, is the ratio of self-pollen to total pollen landing on the stigma,

$$s_G = P_{sG}/(P_{sG} + P_o) = (1 - \alpha_G)/(1 - \alpha_G + \bar{\alpha}\pi), \quad (1)$$

where $\pi = \pi_o/\pi_s$ is the relative success of outcross vs. self-pollen. In this model, variation in selfing rate among genotypes is due to differences in the rate of pollen export α_G , rather than differences in the probabilities that outcross or self-pollen reaches a stigma, π_o and π_s , which are assumed identical for all genotypes.

Pollen limitation and fertilization success

In addition to the automatic advantage, self-fertilization also provides 'reproductive assurance' when outcross pollen is limited by lack of pollinators or low population density of plants (Lloyd, 1992; Holsinger, 1996). Natural populations frequently experience pollen limitation, resulting in decreased seed set by individuals with larger outcrossing rates (e.g. Larson & Barrett, 2000). Pollen limitation is modelled by varying the total amount of outcross pollen $\pi_o P$ that would land on stigmas in a monomorphic, completely outcrossing ($\bar{\alpha} = 1$) population, a quantity likely influenced by pollinator

Table 1 Summary of notation.

Symbol	Meaning	Value	Reference
1. Genetic model of inbreeding depression			
U	Genomic mutation rate to lethals	0; 0.02; 0.2; 1	Simmons & Crow (1977); Klekowski & Godfrey (1989); Lande <i>et al.</i> (1994)
h	Dominance coefficient of lethals	0.02	Simmons & Crow (1977)
D	Inbreeding depression due to lethals	[0, 0.999992]	
d	Background inbreeding depression	0.25	Husband & Schemske (1996)
δ	Total inbreeding depression ($\delta = D + d - Dd$)	[0, 0.999994]	
2. Mass action model of selfing			
P	Total amount of pollen produced by a plant	–	
α_G	Fraction of pollen exported by genotype G	[0, 1]	
π_o, π_s	Probability that outcross (self-) pollen lands on a stigma	–	
π	Relative success of outcross vs. self-pollen (π_o/π_s)	$[10^{-6}, 1]$	
P_{sG}	Amount of self-pollen landing on stigmas	[0, ∞]	
P_o	Amount of outcross pollen landing on stigmas	[0, ∞]	
s, s_G	Selfing rate (of genotype G)	[0, 1]	
3. Pollen limitation			
T_G	Fraction of ovules fertilized on genotype G (seed set)	–	
T	Relative seed set of selfing vs. outcrossing plants	0.4; 1	Burd (1994)

availability. We assume that the fraction of ovules fertilized on genotype G is an increasing function of total pollen landing on the stigma (Kohn & Waser, 1985; Waser & Price, 1991; Mitchell, 1997):

$$T_G = 1 - \exp[-P_o - P_{sG}] = 1 - \exp[-\pi_o P(\bar{x} + (1 - \alpha_G)/\pi)]. \quad (2)$$

Thus, if the total amount of pollen $P_{sG} + P_o$ is larger than about 4, pollen limitation is negligible. Since amounts of pollen represent here number of pollen grains per ovule, this implies that full fertilization requires more than one pollen grain per ovule, as commonly observed in experimental studies of the relationship between pollen load and seed set (Kohn & Waser, 1985; Waser & Price, 1991; Mitchell, 1997).

Inbreeding depression

Inbreeding depression is attributable to nearly recessive, highly deleterious (lethal and semi-lethal) mutations and to partially recessive (nearly additive), mildly deleterious mutations (Simmons & Crow, 1977; Lande & Schemske, 1985; Husband & Schemske, 1996; Charlesworth & Charlesworth, 1999). Individually rare, nearly recessive, lethals and semi-lethals are much more likely to be exposed to selection as homozygotes in selfing populations than in randomly mating populations, and this component of inbreeding depression can be partially purged by selfing (Lande & Schemske, 1985; Lande *et al.*, 1994). In contrast, the strength of selection acting on mildly deleterious mutations with nearly additive effects depends little on the mating system of the population and this component of inbreeding depression can be considered roughly constant throughout evolution (Lande & Schemske, 1985; Husband & Schemske, 1996).

Inbreeding depression due to lethals, D , is analysed using a modified version of Kondrashov's (1985) model, to describe evolution of the distribution of number of heterozygous lethal alleles per individual in an infinite population. This model assumes a very large (effectively infinite) number of unlinked loci mutating to nearly recessive lethals, with genomic mutation rate per generation U . Each mutation occurs at a new locus (or one not currently segregating in the population) and is therefore unique. Consequently, in an infinite population where outcrosses occur at random between unrelated individuals, homozygous lethals only appear by selfing. A multilocus genotype can be described by the number of heterozygous lethals, because (i) recessive lethal alleles segregate independently and are never homozygous in mature plants and (ii) all lethal mutations have identical effect on fitness, being lethal when homozygous and with the same dominance coefficient, h , when heterozygous. Inbreeding depression due to nearly additive, mildly deleterious mutations are modelled via a constant 'background' inbreeding depression, d .

Parameter values and evolutionary dynamics

Parameters were either assigned values according to experimental data (as indicated below) or were varied to cover the whole range of possible values (see Table 1 for a summary of parameter values). We allow the genomic mutation rate to lethals to be $U = 0$ (no inbreeding depression), 0.02, 0.2 or 1, which embraces the range of experimental estimates, from 0.02 (*Drosophila melanogaster*, Simmons & Crow, 1977) to 0.2 (red mangroves, Klekowski & Godfrey, 1989, extrapolated by Lande *et al.*, 1994). The dominance coefficient of lethals is $h = 0.02$, as in the only available experimental data, which are from *Drosophila* (Simmons & Crow, 1977). In a completely outcrossing population, the range $U = 0-1$ with $h = 0.02$ generates inbreeding depression due to lethals between $D = 0$ and 0.99992 (Porcher & Lande, 2005). We assume that the constant background inbreeding depression due to nearly additive, mildly deleterious mutations is $d = 0.25$ (as estimated by Husband & Schemske, 1996), except when $U = 0$, in which case $d = 0$, to model a situation with no inbreeding depression at all. The relative success of outcross vs. self-pollen, $\pi (= \pi_o/\pi_s)$, affects the strength of pollen discounting (see Results) and ranges between 10^{-6} (no pollen discounting) and 1 (strong pollen discounting). We consider two levels of pollen limitation by allowing the total amount of successful exported pollen to be $\pi_o P = 1$ or ∞ , which generates relative seed set of completely selfing vs. completely outcrossing individuals $t = 0.6$ (as in Burd, 1994) or 1 (no pollen limitation). Let A be the resident allele and B the mutant allele at the modifier locus. Within each level of pollen discounting, mutation rate and pollen limitation, we consider resident selfing rates from $s_{AA} = 0$ to 1 by setting the fraction of exported pollen between $\alpha_{AA} = 1$ and 0. The fraction of pollen exported from the homozygous mutant is also varied from $\alpha_{BB} = 1$ to 0, which generates an initial mutant selfing rate from $s_{BB} = 0$ to $1/(1 + \alpha_{AA}\pi)$ when the mutant is rare in the population. Thus we consider mutations with a wide range of possible effects on the selfing rate. Alleles at the modifier locus have additive effects on pollen export, so that $\alpha_{AB} = (\alpha_{AA} + \alpha_{BB})/2$.

Each generation, a population undergoes mating, mutation to lethals, and selection. Recursion equations, including the genetic basis of inbreeding depression, are given in the Appendix. The model fully accounts for linkage disequilibria and identity disequilibria (associations of genotypic states) between loci producing recessive lethals and the locus controlling the selfing rate. Assuming that the mutation rate at the locus controlling selfing is small enough, each successful mutant becomes fixed in the population before the next mutation appears; we therefore consider the invasibility of a mutant in a population at equilibrium. From a resident population monomorphic at the modifier locus, and initially containing no lethals, for each set of parameter values the

recursion equations were numerically iterated until closely approaching mutation-selection equilibrium, which occurs after a few dozen generations under large selfing rate or after several thousand generations under small selfing rate. A mutant allele with a selfing rate different from the resident was then introduced at a low frequency in linkage and identity equilibrium with lethals, and the recursion equations were numerically iterated for a hundred generations to detect invasion (or not) by the mutant.

Results

Analysis of invasion of an outcrossing population by a selfing mutant

Invasion of a mutant genotype with modified selfing rate depends on the relative values of inbreeding depression and automatic advantage of selfing. With no pollen limitation and no pollen discounting, the automatic advantage of a rare genotype with selfing rate s relative to a common outcrossing genotype is $s/2$, which for $s = 1$ becomes 50%, the commonly cited automatic advantage of selfing. Similarly, the decrease in reproductive success due to total inbreeding depression is $s\delta$ for a genotype with selfing rate s , which becomes δ for $s = 1$. The influence of pollen limitation and pollen discounting on the automatic advantage can be inferred using a phenotypic approach, ignoring Mendelian genetics but assuming sufficient genetic variation for the selfing rate to evolve (see below). By definition, this approach does not accurately describe the full genetic model, which allows the locus controlling the mating system to have various dominance effects for selfing rate and rate of pollen export, and also permits inbreeding depression to evolve jointly with the mating system. Nevertheless, the phenotypic approach provides simple analytical results regarding the main factors influencing the evolution of mating systems and can prove useful to examine results from the full genetic model.

With pollen limitation and pollen discounting, the automatic advantage of a rare, partially selfing genotype relative to a common outcrossing genotype depends on the relative fertilization success of outcrossing vs. selfing genotypes, t ($0 < t < 1$) and on the relative rate of pollen export of the partially selfing genotype, α . In this calculation we omit the effects of inbreeding depression. On average, each generation, completely outcrossing individuals transmit t copies of their genome as female parents of their own seeds, and t copies as male parents of outcrossed seeds on other plants. Rare, partially selfing genotypes transmit $2s$ copies of their genome by selfing their own seeds, $1 - s$ copies as female parents of their own outcrossed seeds, and αt copies as male parents of outcrossed seeds on other plants. With pollen limitation and pollen discounting, the automatic advantage of a rare genotype with selfing rate s relative to a common

outcrossing genotype is therefore $(1 + s + \alpha t - 2t)/2t$. Using eqn 1 with $\alpha_G = \alpha$ and $\bar{\alpha} \sim 1$, because the mutant is initially rare, yields $\alpha = 1 - s\pi/(1 - s)$. Following Lloyd (1992), the automatic advantage of selfing can thus be rewritten as $[1 + s - t - s\pi t/(1 - s)]/2t$ or

$$\frac{s}{2} \left(\frac{2 - D_s - D_p}{t} \right), \quad (3)$$

where

$$D_s = [t - (1 - s)]/s, \quad (4)$$

quantifies seed discounting, the loss of outcrossed seeds due to selfing, and

$$D_p = \pi t/(1 - s) \quad (5)$$

quantifies pollen discounting, the decrease in male reproductive success due to selfing.

One important result obtained from the phenotypic approach is that the automatic advantage of selfing can actually turn into a disadvantage. As already noted by Lloyd (1992), seed discounting varies between zero (when t approaches zero, i.e. with strong pollen limitation, or when $s = 0$) and 1 (with no pollen limitation). Pollen discounting approaches zero when the success of outcross vs. self-pollen, π , approaches zero, or with strong pollen limitation (t approaches zero), because in both cases relatively small amounts of outcross pollen eventually land on stigmas, so that a high selfing rate can be achieved by using a small amount of self-pollen. In contrast to Lloyd's (1992) model, pollen discounting can be larger than 1 with the mass-action model of fertilization, whenever $\pi t/(1 - s) > 1$. As a result, with no pollen limitation ($t = 1$ and $D_s = 1$), the automatic advantage of selfing becomes negative when $s > 1 - \pi$. With pollen limitation, this condition is more restrictive. Because pollen discounting D_p is directly proportional to the relative success of outcross vs. self-pollen, π , we vary π in the following to generate different levels of pollen discounting, from no pollen discounting ($\pi = 10^{-6}$) to strong pollen discounting ($\pi = 1$).

Numerical analysis of the full genetic model

Graphic presentation of results

In the following, we use an adaptive dynamics framework (Dieckmann, 1997; Geritz *et al.*, 1998) to present the numerical results. Stable selfing rates are deduced from pairwise invasibility plots, in which regions of invasion (in grey) and noninvasion (in white) of a rare mutant are plotted against the selfing rate of the mutant, s , and the selfing rate of the resident genotype, s^* (see figures). Hence, regions located below the $s = s^*$ line correspond to emergence of a mutant with a smaller selfing rate than the resident, and *vice versa*. A mutant with selfing rate s can invade a resident population with selfing rate s^* if the point with coordinates (s^*, s) is located in a region of invasion. If the mutant can invade

and $s > s^*$, then evolution favours increased selfing rates and *vice versa*. Evolutionary equilibria occur at the intersection of the $s = s^*$ line and a line separating regions of invasion and noninvasion. Details regarding the criteria to infer stability of equilibrium can be found in Dieckmann (1997). The equilibrium selfing rates discussed here, denoted by white circles on the figures, are evolutionarily stable (the vertical line through this equilibrium lies within a region of noninvasion, so that it cannot be invaded by neighbouring mutant selfing rates) and are evolutionary attractors or convergence stable (evolution by a series of small steps proceeds towards the equilibrium). We refer to these equilibria as stable selfing rates. An example of evolutionary trajectories leading to a stable selfing rate is given on Fig. 1d.

On the pairwise invasibility plots, a striped region indicates selfing rates that cannot be achieved by a rare mutant. Under competing selfing, the fraction of outcross pollen $\bar{\alpha}$ exported by the resident genotype constrains the selfing rate of an initially rare mutant. From eqn 1, the maximum selfing rate of a rare mutant, when all its pollen is used for selfing ($\alpha = 0$), is $s_{\max} = 1/(1 + \bar{\alpha}\pi)$. Assuming that the population is monomorphic for the resident genotype, eqn 1 with $\alpha_G = \bar{\alpha}$ leads to $\bar{\alpha} = (1 - s^*)/[1 - s^*(1 - \pi)]$, where s^* is the selfing rate of the resident genotype. Hence, the maximum selfing rate of a rare mutant depends on the selfing rate of the resident genotype as follows:

$$s_{\max} = [1 - s^*(1 - \pi)] / (1 - s^* + \pi). \tag{6}$$

This equation bounds the striped region of selfing rates that cannot be achieved by an initially rare mutant (see figures).

Stable selfing rates without pollen limitation

With no inbreeding depression ($U = 0$) and no pollen limitation ($\pi_0 P = \infty$), seed discounting is complete ($D_s = 1$) and pollen discounting for a rare mutant $D_p = \pi / (1 - s)$ is an increasing function of the mutant selfing rate. Genotype-dependent pollen discounting and automatic advantage produce stable intermediate selfing rates under a wide range of conditions ($\pi > 0$, Fig. 1a–d). The stable selfing rate is $s = 1 - \pi$, as previously found by Holsinger (1991); whenever the probability that outcross pollen falls on the stigma is smaller than that for self-pollen ($\pi < 1$), mixed mating systems are maintained by selection.

With no pollen discounting ($\pi = 10^{-6}$ and D_p close to zero), the automatic advantage of selfing is $s/2$ and the effects of inbreeding depression on equilibrium selfing rates depend on the mutation rate to lethals and on initial conditions. A small mutation rate to lethals ($U = 0.02$) does not affect the outcome of evolution: complete selfing is always selected, because total inbreeding depression remains small compared to the automatic

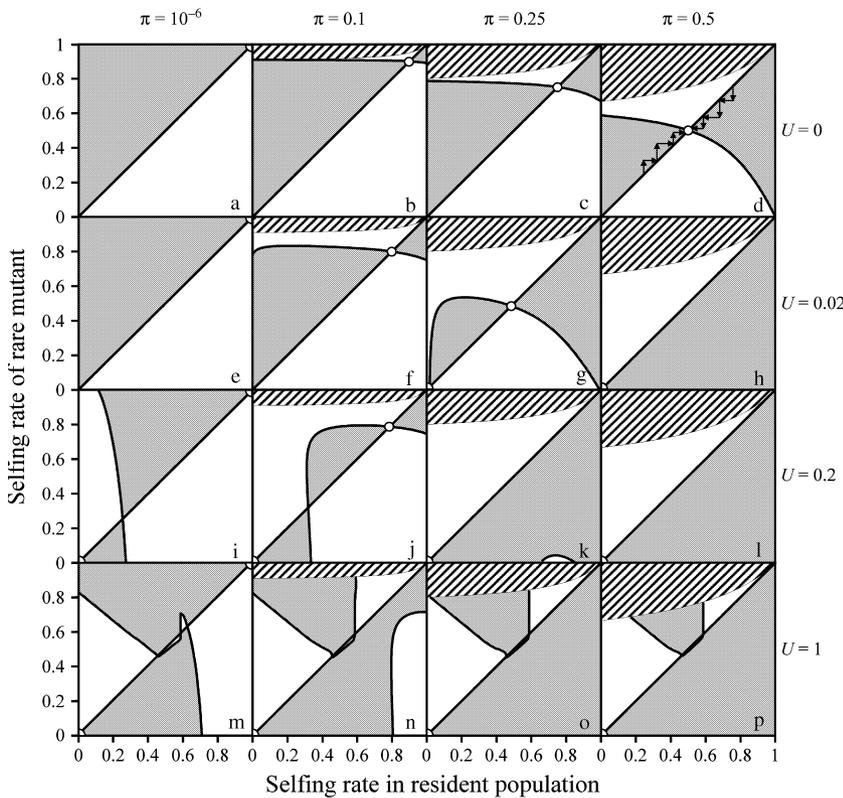


Fig. 1 Pairwise invasibility plots without pollen limitation, under various conditions of pollen discounting, π , and genomic mutation rate to lethals, U . Regions of invasion and noninvasion of a mutant affecting selfing rate are plotted against the selfing rate of the mutant when rare and the initial selfing rate in the resident population. Regions where the mutant invades are in grey, regions where it cannot invade in white. Striped regions correspond to high selfing rates that cannot be achieved by a mutant (too much outcross pollen available from the resident genotype, eqn 6). Stable equilibria are indicated by open circles except where the domain of attraction is very small, such that the equilibrium state is unlikely to persist. On panel d, two examples of evolutionary trajectories (series of invasions and fixations of mutants with a different selfing rate) are shown as arrows. Parameter values: background inbreeding depression $d = 0$ for $U = 0$ and $d = 0.25$ for $U \geq 0.02$; no pollen limitation ($\pi_0 P = \infty$).

advantage of selfing ($\delta < 1/2$), regardless of the resident selfing rate (Fig. 1e). With larger mutation rates to lethals ($U \geq 0.2$), the outcome of evolution depends on the initial selfing rate, as also observed by Johnston (1998) with no pollen discounting. With $U = 0.2$, total initial inbreeding depression in a population with a small selfing rate is large enough to overcome the automatic advantage of selfing, and evolution proceeds towards complete outcrossing. In contrast, above a threshold selfing rate of the initial population, purging of lethals reduces inbreeding depression below the automatic advantage and favours mutants increasing the selfing rate (Fig. 1i). With a high mutation rate to lethals ($U = 1$), the pattern is similar, but mutants with large selfing rates can invade a population with small selfing rates, because of a dramatic purging of recessive lethals in the progeny of highly selfing mutants (Fig. 1m). As a result, the ultimate outcome of evolution is complete selfing if the selfing rate can evolve by large steps.

With pollen discounting, accounting for inbreeding depression substantially alters the evolution of mating systems even for a moderate mutation rate to lethals, due to an interaction between the effects of pollen discounting and purging of inbreeding depression. Intermediate stable selfing rates still occur under moderate mutation rates to lethals and moderate levels of pollen discounting ($U \leq 0.2$ and $\pi \leq 0.25$, Fig. 1f, g and j), but are much lowered compared to equilibrium selfing rates without inbreeding depression. This drop in equilibrium selfing rates accelerates as the relative success of outcross vs. self-pollen π increases, because larger values of π favour smaller equilibrium selfing rates. Under smaller selfing rates, inbreeding depression is stronger, which reinforces the selection for smaller selfing rates. As a result, with pollen discounting and a dynamic model of inbreeding depression, complete outcrossing is the only outcome of evolution under a wide range of conditions (see Fig. 1; complete outcrossing is also the only stable selfing rate whenever $\pi > 0.5$ and $U > 0$, not shown).

Stable selfing rates with pollen limitation

Pollen limitation alone does not maintain mixed mating systems. Under pollen limitation, seed discounting, D_s , becomes smaller than 1, because some of the selfed ovules could not have been outcrossed anyway due to limited amounts of outcross pollen, $\pi_o P$, landing on stigmas. Without pollen discounting, this effect of pollen limitation has little influence on stable selfing rates (Fig. 2, first column): complete selfing is favoured, because the advantage of selfing becomes larger than $1/2$. With an intermediate mutation rate to lethals ($U = 0.2$), pollen limitation confers a larger automatic advantage to mutants with large selfing rates, so that they can invade a population with a small selfing rate (Fig. 2i), which favours an evolution towards complete selfing by mutations of large effect on the selfing rate.

With pollen discounting and pollen limitation, equilibrium selfing rates are generally close to but less than 1, although sometimes complete outcrossing is also stable (Fig. 2, three right columns). This remarkable influence of pollen limitation (in its absence, the only equilibrium selfing rate is zero under most conditions) is due to the decrease in seed discounting outlined above, but also to an interaction between the mating system and the effects of pollen discounting and pollen limitation. Pollen discounting is decreased under pollen limitation because (i) smaller amounts of self-pollen are required to achieve a given selfing rate (see eqn 5) and (ii) keeping pollen for selfing is not costly in terms of male reproductive success by outcrossing, because a lot of exported pollen is lost anyway due, e.g., to poor pollinator efficiency. Both effects are stronger under larger selfing rates of the resident population, because the total amount of outcross pollen landing on the stigma, $\bar{\alpha}\pi_o P$, is smaller and the number of ovules available for outcrossing is smaller, respectively. Thus, under pollen limitation, large selfing rates are selected as a result of decreased seed discounting and genotype-dependent pollen discounting.

With pollen limitation and pollen discounting, inbreeding depression has little effect on the value of intermediate stable selfing rates, due to purging of most lethals at high selfing rates, but it strongly influences the existence of additional equilibrium selfing rates and the dynamics of evolution. Under a small mutation rate to lethals ($U = 0.02$), a large intermediate selfing rate is the only outcome of evolution (Fig. 2f–h). Under larger mutation rates to lethals ($U \geq 0.2$), complete outcrossing can be invaded by mutants with a high selfing rate, so that it can persist only if selfing evolves by small steps, as explained above (Fig. 2j, k and n,p). With $U = 0.2$ and $\pi = 0.5$, complete outcrossing is impervious to invasion and mixed mating systems cannot be achieved if the initial selfing rate is low (Fig. 2l).

Discussion

Our model accounting for pollination biology and the genetics of inbreeding depression confirms the major role of pollination biology in the maintenance of stable mixed mating systems. Stable intermediate selfing rates due to pollen discounting were discovered previously by Holsinger (1991) and Johnston (1998), but these authors did not consider the joint effect of pollen limitation, a ubiquitous factor which likely affects the evolution of mating systems, especially under mechanistic models of self-fertilization. We show that when pollen discounting and pollen limitation are combined, pollination biology alone cannot explain stable low selfing rates ($s < 0.5$). We argue below that in self-compatible species stable low to intermediate selfing rates might often be caused by unavoidable geitonogamy, especially in large perennials plants with many flowers (Barrett, 2003; Porcher & Lande, 2005). In contrast, stable high selfing rates are

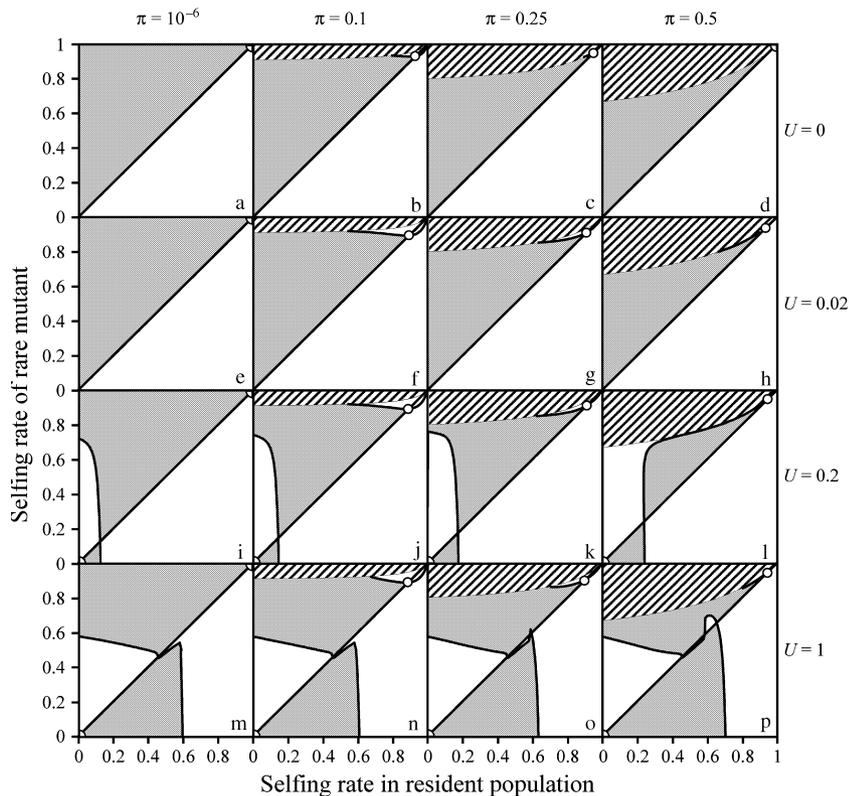


Fig. 2 Pairwise invasibility plots with pollen limitation ($\pi_o P = 1$), under various conditions of pollen discounting, π , and genomic mutation rate to lethals, U . Parameter values: background inbreeding depression $d = 0$ for $U = 0$ and $d = 0.25$ for $U \geq 0.02$.

likely attributable to a balance of genetic factors and pollination mechanisms.

Conditions for maintenance of stable mixed mating systems

Without pollen discounting, our genetic model of inbreeding depression, accounting for the joint evolution of mating system and inbreeding depression, does not predict stable mixed mating systems (Figs 1 and 2, first column). The joint evolution of selfing and inbreeding depression has previously been analysed by Charlesworth *et al.* (1990). The present approach assumes that inbreeding depression is due to a combination of nearly recessive lethal mutations and mildly deleterious mutations with nearly additive effects, while the model by Charlesworth *et al.* (1990) considered only one type of mutation in a given population. We find that even with a more realistic genetic basis for inbreeding depression and with pollen limitation, mixed mating systems cannot be explained by the opposing effects of inbreeding depression and the automatic advantage of selfing without additional features of pollination biology.

Our model confirms that pollen discounting is a major factor favouring the maintenance of stable mixed mating systems, as previously demonstrated by Holsinger (1991). We describe here that this is due to the automatic advantage of selfing, which can actually turn into a

disadvantage at high selfing rates (eqn 3). Holsinger (1991), however, did not consider the influence of inbreeding depression, assuming that it would not affect the existence of stable intermediate selfing rates, but only lower them. As a result, the conditions for existence of stable mixed mating systems obtained by Holsinger (1991) were broader than what we find: he observed stable intermediate selfing rates whenever the relative success of self vs. outcross pollen, π , was smaller than 1, predicting that mixed mating systems should be very common, because it is unlikely that outcross pollen is more successful than self-pollen.

Accounting for the evolution of inbreeding depression and pollination biology in the evolution of plant mating systems strongly affects the stable equilibria, as Johnston (1998) previously demonstrated by combining a description of inbreeding depression (based on the results of Charlesworth *et al.*, 1990) with a simple model of pollen discounting. Because a larger inbreeding depression is maintained by mutation under smaller selfing rates, inbreeding depression and pollen discounting interact to produce a major decrease in the stable selfing rate as these two factors become stronger. Our results show that with inbreeding depression, a much larger success of self vs. outcross pollen ($\pi < 0.4$), together with small rates of mutation to lethals ($U < 0.2$), are required to observe stable mixed mating systems in the absence of pollen limitation. We did not explore the influence of

background inbreeding depression here, but we expect that increasing the value of background inbreeding depression would lower the stable selfing rate, as predicted by Holsinger (1991) for constant inbreeding depression. Johnston (1998) also concluded that stable intermediate selfing rates occur only if pollen discounting is an increasing function of selfing rate. His results are consistent with the mass-action model of selfing used here, where pollen discounting, $D_p = \pi t / (1 - s)$, is an increasing function of selfing rate.

Pollen limitation and stable high selfing rates

In our model, pollen limitation is described by the amount of outcross pollen available per ovule in a completely outcrossing population, $\pi_o P$. Larson & Barrett (2000) found that the seed set of open-pollinated, self-incompatible plants was, on average, 41% smaller than that of pollen-supplemented plants. The value of pollen limitation in Fig. 2 ($\pi_o P = 1$) produces a maximum 37% decrease in the fraction of fertilized ovules of completely outcrossing vs. completely selfing genotypes, which represents a reasonable estimate of pollen limitation in natural population. In Fig. 2, selection favours high selfing rates ($0.9 < s < 1$), regardless of the inbreeding depression or the success of self vs. outcross pollen. This occurs because pollen limitation decreases both seed and pollen discounting, and under high resident selfing rates reproductive assurance can overcome inbreeding depression as well as pollen and seed discounting. With less stringent pollen limitation (e.g. $\pi_o P = 2$, which produces a maximum 14% decrease in the seed set of completely outcrossing vs. completely selfing genotypes), equilibrium selfing rates are still large ($s > 0.5$, results not shown) even under strong pollen discounting ($\pi \leq 0.75$). Pollen limitation is therefore likely to be responsible for many observed stable mixed mating systems with high selfing rates.

Although pollen discounting and pollen limitation interact to favour high selfing rates, complete selfing is not stable. This was previously demonstrated by Holsinger (1991), in a model of plant mating system evolution based on pollen discounting only, and is due to a nonlinear increase in pollen discounting as the selfing rate increases. Our results confirm that, even with strong pollen limitation favouring increased selfing via reproductive assurance, complete selfing is not stable (except under the unrealistic conditions of large relative success of outcross vs. self-pollen, $\pi > 0.5$, and no inbreeding depression, Fig. 2d). This is attributable to pollen discounting, which becomes larger than 1 at very high selfing rates, turning the automatic advantage of selfing into a disadvantage (eqn 3). This is consistent with observations in natural populations. Although complete selfing is theoretically possible, e.g. via cleistogamy or prior selfing (Lloyd & Schoen, 1992), most, if not all, highly selfing plant species actually have $s < 1$ (Stebbins, 1957; Jain, 1976; Schemske

& Lande, 1985). High stable selfing rates close to but less than $s = 1$ could be attributable to the joint effect of moderate pollen discounting and inbreeding depression without pollen limitation (e.g. Fig. 1f). However, in light of the frequent occurrence of pollen limitation (Burd, 1994; Larson & Barrett, 2000), we suggest that such mating systems could be maintained by the opposing effects of strong pollen limitation and pollen discounting, favouring selfing rates close to but less than 1, regardless of inbreeding depression and of the relative success of outcross vs. self-pollen.

Conditions for maintenance of low selfing rates and complete outcrossing

Our model shows that stable low to intermediate selfing rates, including $s = 0$, can be maintained without pollen limitation (Fig. 1; Fig. 1d shows an intermediate selfing rate; lower stable selfing rates are obtained with higher values of the relative success of outcross vs. self-pollen, $0.5 < \pi < 1$). Whether selection favours complete outcrossing or $s > 0$, and whether mixed mating systems exhibit low or high selfing rates, depends on two key parameters of the model: the genomic mutation rate to nearly recessive lethals U and the relative success of outcross vs. self pollen π . The few available estimates suggest that U might range between 0.02 (Simmons & Crow, 1977) and 0.2 (Klekowski & Godfrey, 1989; Lande *et al.*, 1994). With $U = 0.02$ and no pollen limitation, complete outcrossing is stable whenever π exceeds 0.1. Virtually nothing is known about the relative success of outcross vs. self-pollen in natural populations, because this quantity is not readily measurable. We expect it to be smaller than 1, because large amounts of outcross pollen are lost during transport from one plant to another, although self-pollen might also be lost, e.g. by pollinator grooming during geitonogamous selfing.

Our results show that (locally) stable outcrossing populations can be invaded by mutants with a high selfing rate despite strong inbreeding depression (e.g. Fig. 1m and n or Fig. 2j, k and m–p). This is consistent with arguments of Lande & Schemske (1985) and simulations of Charlesworth *et al.* (1990), but was not found by Johnston (1998), who neglected linkage and identity disequilibria between lethals and genes influencing the selfing rate. However, nearly complete selfing may be unlikely to evolve by a single mutation. Studies of the genetics of plant mating systems suggest that the evolution of a high selfing rate may sometimes be under control of major genes but does not evolve in a single step (Macnair & Cumbes, 1989; Fenster & Barrett, 1994; Fenster & Ritland, 1994; Fishman *et al.*, 2002; Georgiadi *et al.*, 2002). In addition, a single mutation producing a high selfing rate is likely to have rather deleterious pleiotropic effects (Fisher, 1958; Wright, 1968).

In summary, the maintenance of low to moderate selfing rates requires an absence of pollen limitation, and

occurs under a narrow range of the mutation rate to lethals, U , and relative success of outcross vs. self-pollen, π . These conditions are somewhat restrictive and might be met only rarely in natural populations, especially in light of widespread pollen limitation (Burd, 1994; Larson & Barrett, 2000). We thus expect that many mixed mating systems with low to moderate selfing rates are generally not maintained by selection, but are most likely due to unavoidable geitonogamous selfing in populations where selection favours complete outcrossing (de Jong *et al.*, 1993; Porcher & Lande, 2005).

Concluding remarks

Pollen discounting is likely to be a major factor explaining the maintenance of mixed mating systems in plants under a wide range of conditions. However, when pollen discounting is combined with pollen limitation in a model accounting for the joint evolution of inbreeding depression and plant mating system, the conditions for maintenance of stable intermediate selfing rate are reduced, and stable selfing rates maintained by selection are high ($0.5 < s < 1$ in general, but $0.9 < s < 1$ when realistic pollen limitation is considered). Hence, we propose that mating systems with low to moderate selfing rates are rarely maintained by selection and arise mainly as a consequence of unavoidable geitonogamy. Complete selfing is never stable because at high selfing rates pollen discounting turns the automatic advantage of selfing into a disadvantage. Although outcrossing populations can theoretically be invaded by mutants with a high selfing rate, despite a high inbreeding depression, this appears to happen rarely if ever in nature.

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Appendix – recursion equations

Let A be the resident allele and B the mutant allele at the modifier locus. In the following, capital letters G and J refers to diploid genotypes (AA, AB or BB) and small letters g and j to haploid genotypes (A or B) at the modifier locus.

Gamete production and mating

The probability that a plant with y heterozygous lethals produces, by selfing, a viable zygote with x ($\leq y$) heterozygous lethals is $C_y^x (1/2)^x (1/4)^{y-x} = C_y^x (1/2)^{2y-x}$ (Lande *et al.*, 1994), where $C_y^x = y!/[x!(y-x)!]$. Therefore, the relative frequency of zygotes with diploid genotype G at the modifier locus, carrying x heterozygous lethals, and originating from selfing of J genotypes is

$$q_J^G(x) = \sum_{y=x}^{\infty} k_1 T_J f_J(y) C_y^x (1/2)^{2y-x}, \quad (\text{A1})$$

where $f_J(y)$ is the frequency of plants with genotype J carrying y heterozygous lethals and k_1 is a constant accounting for Mendelian transmission of alleles at the modifier locus ($k_1 = 1, 0.5$ or 0.25 depending on heterozygosity of J). Equation A1 describing selfing also includes selection on homozygous lethals: for genotypes carrying x heterozygous lethals, a proportion $1 - (3/4)^x$ of their offspring carry at least one homozygous lethal; they are not viable and are not included in eqn A1. For

these parental genotypes, the seed set by selfing is reduced by a factor $(3/4)^x$, and the sum of eqn A1 over all parental genotypes, all offspring genotypes and all numbers of lethals is smaller than one.

The probability that a plant with y heterozygous lethals produces a gamete with x ($\leq y$) heterozygous lethals is $C_y^x(1/2)^y$. Hence, the probability that individuals with genotype G at the modifier locus produce gametes with haploid genotype g and carrying x lethals is

$$q_G^g(x) = \sum_{y=x}^{\infty} k_2 f_G(y) C_y^x (1/2)^y, \tag{A2}$$

where k_2 reflects Mendelian inheritance of the genotype at the modifier locus ($k_2 = 1$ or 0.5 depending on the heterozygosity of G).

Because each mutation is unique, random mating in an infinite population never generates homozygous lethals. The probability that a genotype G produces, by outcrossing, a zygote with genotype J and carrying x heterozygous lethals is $\sum_{y=x}^{\infty} G_G^g(y) p_j(x-y)$, where symbolically $J = gj$ and $p_j(x)$ is the frequency of pollen with genotype j and carrying x mutations.

Hence, the recursion equations for mating are:

$$\begin{aligned} f_{AA}^*(x) &= (1-d)s_{AA}T_{AA}q_{AA}^{AA}(x) + (1-d)s_{AB}T_{AB}q_{AB}^{AA}(x) \\ &+ (1-s_{AA})T_{AA} \sum_{y=0}^x q_{AA}^A(y)p_A(x-y) \\ &+ (1-s_{AB})T_{AB} \sum_{y=0}^x q_{AB}^A(y)p_A(x-y), \end{aligned} \tag{A3}$$

$$\begin{aligned} f_{AB}^*(x) &= (1-d)T_{AB}s_{AB}q_{AB}^{AB}(x) \\ &+ (1-s_{AA})T_{AA} \sum_{y=0}^x q_{AA}^A(y)p_B(x-y) \\ &+ (1-s_{BB})T_{BB} \sum_{y=0}^x q_{BB}^B(y)p_A(x-y) \\ &+ (1-s_{AB})T_{AB} \sum_{y=0}^x [q_{AB}^A(y)p_B(x-y) \\ &+ q_{AB}^B(y)p_A(x-y)], \end{aligned} \tag{A4}$$

$$\begin{aligned} f_{BB}^*(x) &= (1-d)s_{BB}T_{BB}q_{BB}^{BB}(x) + (1-d)s_{AB}T_{AB}q_{AB}^{BB}(x) \\ &+ (1-s_{BB})T_{BB} \sum_{y=0}^x q_{BB}^B(y)p_B(x-y) \\ &+ (1-s_{AB})T_{AB} \sum_{y=0}^x q_{AB}^B(y)p_B(x-y). \end{aligned} \tag{A5}$$

The genotypic frequencies in the pollen pool depend on the relative fractions of pollen exported by the different genotypes as follows:

$$\begin{aligned} p_A(x) &= \frac{\alpha_{AA}q_{AA}^A(x) + \alpha_{AB}q_{AB}^A(x)}{\alpha_{AA}f_{AA} + \alpha_{AB}f_{AB} + \alpha_{BB}f_{BB}}, \\ p_B(x) &= \frac{\alpha_{BB}q_{BB}^B(x) + \alpha_{AB}q_{AB}^B(x)}{\alpha_{AA}f_{AA} + \alpha_{AB}f_{AB} + \alpha_{BB}f_{BB}}. \end{aligned} \tag{A6}$$

Mutation and selection due to heterozygous lethals

Mutation to nearly recessive lethals follows a Poisson process, with a mean number of new heterozygous lethal mutations per genome of U per generation. The frequencies of zygotes after mutation are therefore, for any diploid genotype G:

$$f_G^{**}(x) = \sum_{y=0}^x f_G^*(x-y) \frac{e^{-U} U^y}{y!} \tag{A7}$$

The probability that a zygote with x heterozygous mutations survives to maturity is $(1-h)^x$, where h is the dominance coefficient of lethals. The frequency of mature plants with x heterozygous lethals in the next generation is then, for any genotype:

$$f_G'(x) = \frac{(1-h)^x}{\bar{W}} f_G^{**}(x), \tag{A8}$$

\bar{W} is the mean fitness of a population:

$$\bar{W} = \sum_{x=0}^{\infty} (1-h)^x [f_{AA}^{**}(x) + f_{AB}^{**}(x) + f_{BB}^{**}(x)]. \tag{A9}$$

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