New Phytologist

Research

Reproductive compensation in the evolution of plant mating systems

Emmanuelle Porcher and Russell Lande

Department of Biology, 0116, University of California – San Diego, La Jolla, CA 92093, USA

Author for correspondence:
Russell Lande
Email: rlande@ucsd.edu

Received: *29 October 2004*
Accepted: *14 December 2004*

Summary

• Reproductive compensation, the replacement of dead embryos by potentially viable ones, is known to play a major role in the maintenance of deleterious mutations in mammalian populations. However, it has received little attention in plant evolution. Here we model the joint evolution of mating system and inbreeding depression with reproductive compensation.

• We used a dynamic model of inbreeding depression, allowing for partial purging of recessive lethal mutations by selfing.

• We showed that reproductive compensation tended to increase the mean number of lethals in a population, but favored self-fertilization by effectively decreasing early inbreeding depression. When compensation depended on the selfing rate, stable mixed mating systems can occur, with low to intermediate selfing rates.

• Experimental evidence of reproductive compensation is required to confirm its potential importance in the evolution of plant mating systems. We suggest experimental methods to detect reproductive compensation.

Key words: embryonic lethals, inbreeding depression, plant mating systems, reproductive compensation, seed : ovule ratio, self-fertilization.

New Phytologist (2005) **166**: 673–684

© *New Phytologist* (2005) **doi**: 10.1111/j.1469-8137.2005.01363.x

Introduction

In many plant species, the number of ovules produced by a plant exceeds the number of seeds eventually matured. The average percentage of ovules developed into seeds varies greatly among species, averaging 85% in annuals and 50% in perennial species (Wiens, 1984). The failure of ovules to develop into seeds can be attributed to unfertilized ovules, caused by pollen limitation and/or self-incompatibility reactions (Burd, 1994; Larson & Barrett, 2000). However, in an appreciable number of cases, successfully outcrossed ovules (zygotes and embryos) are aborted, due mainly, but not only, to resource limitation (Lloyd, 1980; Stephenson, 1981; Wiens, 1984; Lee, 1988). Several nonexclusive explanations for this apparently suboptimal resource allocation have been suggested. For example, excess flowers are likely to enhance both male reproductive success, via increased pollen production and export (Sutherland, 1987; Burd & Callahan, 2000), and female reproductive success, via increased pollinator attraction when pollen is limited (Burd, 1998 and references therein). Excess ovules can increase female fitness by allowing increased seed set during unpredictable periods of abundant pollinators or resources, or by providing reproductive assurance to cope with potential losses of ovules caused by herbivores, pathogens or weather (Lloyd, 1980; Stephenson, 1981; Kozlowski & Stearns, 1989). Finally, the production of supernumerary embryos provides an opportunity for plants to reduce the impact of genetic load on fertility by producing more, or better quality, offspring (see References below).

Two mechanisms can reduce the impact of the genetic load caused by deleterious mutations: (1) selective embryo abortion, an enhancement of offspring quality by differential abortion of embryos (Uma Shaanker *et al.*, 1988; Korbecka *et al.*, 2002); and (2) reproductive compensation, an increase in the number of viable offspring by replacement of dead embryos (Hastings, 2000). In selective embryo abortion, the number of aborted ovules depends solely on resources available for seed maturation, but abortion is not random. Embryos of

lesser quality are preferentially aborted, through either maternal control or, more likely, competition among embryos for resources (Korbecka *et al.*, 2002). Many theoretical and, to a lesser extent, empirical studies demonstrated that this nonrandom embryo abortion can increase progeny fitness, presumably via a positive relationship between competitive ability of embryos and seedling or adult fitness (Kozlowski & Stearns, 1989; Burd, 1998; Korbecka *et al.*, 2002 for a review of experimental evidence). Selective embryo abortion also decreases the impact of genetic load caused by deleterious mutations (Sorensen, 1982; Latta, 1995).

The concept of reproductive compensation is mainly used in human genetics (Hastings, 2000, 2001) and has been applied to some mammalian species (e.g. Charlesworth, 1994). It refers to the replacement of a dead embryo by a potentially viable one. Much embryo mortality is attributable to early acting, highly deleterious mutations (lethals and semilethals), whereas mildly deleterious mutations tend to act late in development during growth (Lande *et al.*, 1994; Husband & Schemske, 1996 in plants). In predominantly outbreeding species such as mammals, embryo mortality is primarily caused by dominant lethals (e.g. chromosome abnormalities in humans, Diamond, 1987 and references therein), although with biparental inbreeding recessive lethals may play a significant role (Cavalli-Sforza & Bodmer, 1999). In self-compatible hermaphroditic plants, a relatively high rate of embryonic mortality may occur in selfed embryos, in which individually rare, nearly recessive lethal alleles at many loci are more likely to become homozygous and hence exposed to selection (Charlesworth & Charlesworth, 1987; Lande *et al.*, 1994; Husband & Schemske, 1996; Charlesworth & Charlesworth, 1999). This inbreeding depression upon selfing tends to be greater in predominantly outcrossing populations with higher genetic loads (Lande & Schemske, 1985; Charlesworth *et al.*, 1990; Husband & Schemske, 1996; Byers & Waller, 1999).

Numerous theoretical and empirical studies demonstrate that reproductive compensation can play a major role in the maintenance of deleterious mutations in mammal populations (Charlesworth, 1994; Hastings, 2001; Overall *et al.*, 2002). Despite this potential role in mating system evolution, reproductive compensation has received little attention from plant evolutionary biologists. One noticeable exception, however, is the work conducted on polyembryonic gymnosperm species. Polyembryony refers to the existence of multiple embryos within an ovule. Among these, all but one are usually aborted (Haig, 1992), thus creating an opportunity for reproductive compensation within ovules (although this terminology is not generally used). A few studies have demonstrated that polyembryony generates decreased apparent (or secondary) selfing rates measured in seedlings (Kärkkäinen & Savolainen, 1993); favors increased frequencies of lethals (Klekowski, 1982; Hedrick *et al.*, 1999); but reduces the genetic load caused by deleterious mutations (Klekowski, 1982; Sorensen, 1982; Hedrick *et al.*, 1999).

Polyembryony is much less common in angiosperms than in gymnosperms, occurring in only 244 angiosperm species (Uma Shaanker & Ganeshaiah, 1996). However, reproductive compensation is also likely to occur because of competition for resources between developing embryos within an ovary (for multiovulated species), within a fruit, or even within a plant. Whether reproductive compensation is a cause or a consequence of low seed : ovule ratios, it can exert a major influence on the evolution of plant mating systems because, like selective abortion, it increases frequencies of deleterious mutations but decreases the realized inbreeding depression.

In this paper we model the influence of reproductive compensation on plant mating system evolution. We assume that embryo abortion is controlled by both resource availability and early inbreeding depression, determined by recurrent mutation to nearly recessive lethals at a very large number of loci (Kondrashov, 1985). We explore the impact of different levels of reproductive compensation on the mean number of lethals in a population with different selfing rates. We also investigate whether reproductive compensation can contribute to the maintenance of stable mixed mating systems composed of partial selfing and partial outcrossing.

The model

We analyze the joint evolution of selfing rate and inbreeding depression under reproductive compensation using an adaptive dynamics framework (Dieckmann, 1997) by evaluating the ability of a rare mutant with selfing rate s_2 to invade a resident population with selfing rate s_1. In contrast to adaptive dynamics, which often relies on a phenotypic approach, we assume that the selfing rate is controlled by a single diallelic locus (resident and mutant alleles) with additive effect. Hence the selfing rate of heterozygous genotypes at the locus controlling selfing is $s' = (s_1 + s_2)/2$. By using a purely genetic determination of selfing rate, we assume competing selfing, where self and outcross pollen land simultaneously on the stigma and compete for the same ovules (Lloyd, 1979). This mode of self-fertilization, which does not require special floral mechanisms, is thought to be the most common in natural populations (Holsinger, 1991).

Inbreeding depression

Inbreeding depression, the relative decrease in mean fitness of selfed vs outcrossed individuals, is classically divided into an early component affecting embryo development, and a late component affecting development of seedlings into adult plants (Husband & Schemske, 1996). Most early inbreeding depression is caused by nearly recessive, highly deleterious (lethal and semilethal) mutations that are nearly recessive [with dominance coefficient $h \approx 0.02$ (Simmons & Crow, 1977) where $h = 0$ represents complete recessivity]; late inbreeding depression is primarily caused by partially recessive, nearly additive, mildly

deleterious mutations [$h \approx 0.3$, where $h = 0.5$ represents perfect additivity; Simmons & Crow, 1977; Lande & Schemske, 1985; Husband & Schemske, 1996; Lynch & Walsh, 1998 (pp. 186–7); Charlesworth & Charlesworth, 1999]. Individually rare, nearly recessive lethals and semilethals are more likely to be exposed to selection as homozygotes in selfing populations than in randomly mating populations, so that early inbreeding depression can be partially purged by selfing (Lande & Schemske, 1985; Lande *et al.*, 1994). In contrast, the strength of selection on mildly deleterious mutations with nearly additive effects depends little on the mating system of the population, and this late component of inbreeding depression can be considered roughly constant throughout evolution (Lande & Schemske, 1985; Charlesworth *et al.*, 1990; Husband & Schemske, 1996).

Inbreeding depression caused by lethals is analyzed 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 appear only 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 an identical effect on fitness, being lethal when homozygous and with the same dominance coefficient, h, when heterozygous. We assume that death caused by homozygous lethals occurs during embryo development (early inbreeding depression), whereas the heterozygous effects of lethals are late-acting. The late component of inbreeding depression caused by nearly additive, mildly deleterious mutations is modeled via a constant 'background' inbreeding depression, d.

Reproductive compensation occurs whenever the number of viable embryos produced by an individual exceeds the maximum number of seeds that it can mature due to resource limitation. We assume that reproductive compensation occurs at the whole-plant level, so that dead embryos can be replaced by viable embryos from other flowers. Our results are likely to be essentially the same if reproductive compensation occurs at a lower level, e.g. within branches or within flowers, provided that many embryos compete within the compensated unit and that self and outcross pollen are randomly distributed across the plant. We also assume that all plants produce the same fixed number of ovules; excess resources cannot be diverted to additional flower production when embryo mortality is high.

In a population with a given mean number of heterozygous lethals, reproductive compensation has no effect on the early survival of selfed zygotes relative to outcrossed zygotes. However, the decrease in reproductive success of a partially selfing individual caused by early inbreeding depression is influenced by reproductive compensation, because dead embryos can be replaced by viable ones so that seed set may not be reduced. Complete reproductive compensation effectively cancels the early inbreeding depression, so that the reproductive success of an individual is controlled by resource limitation as well as late-acting effects of heterozygous lethals and mildly deleterious mutations. To account for this, we consider two definitions of inbreeding depression: (1) primary inbreeding depression, the relative decrease in fitness of selfed vs outcrossed zygotes; and (2) experimental inbreeding depression, the relative decrease in fitness of embryos obtained by experimental selfing vs outcrossing of parental plants.

For a given mean number of lethals, reproductive compensation does not affect the primary inbreeding depression, which always includes both early and late components of inbreeding depression. However, with reproductive compensation, primary inbreeding depression is not readily measurable, because parental seed set is controlled in part by resource availability, which can mask embryo mortality. Experimental inbreeding depression always includes the late component of inbreeding depression, and may also include the early component if resources are abundant or with a large number of lethals (no compensation; see Appendix). For a given mean number of lethals, reproductive compensation decreases experimental inbreeding depression. In the absence of reproductive compensation, primary inbreeding depression and experimental inbreeding depression are identical.

We also monitor the mean fitness in a partially selfing population relative to that in a completely outcrossing population. The mean fitness of a population with selfing rate s, $\bar{W}(s)$, is defined here as the mean fraction of zygotes reaching the adult stage, including effects of resource limitation and reproductive compensation. In a completely outcrossing population with no reproductive compensation, in the Kondrashov model at mutation–selection equilibrium the mean number of heterozygous lethals in zygotes is U/h and the mean fitness is e^{-U} (Kondrashov, 1985; Porcher & Lande, 2005a). We describe resource limitation by the parameter c, the maximum fraction of ovules that can be matured. Therefore with resource limitation the equilibrium mean fitness in a completely outcrossing population is ce^{-U}, so that the relative mean fitness of a population with selfing rate s is $\bar{w}(s) = \bar{W}(s)/(ce^{-U})$.

Details of the model appear in the Appendix. A population undergoes mating, mutation and selection each generation. The seed production of a genotype depends on the selfing rate and on the number of homozygous lethals contributing to death of embryos, as well as on resource availability limiting the maximum fraction of ovules that can be matured (c). The opportunity for reproductive compensation arises when the number of viable embryos produced by a plant exceeds the number of seeds that can be matured. For various selfing rates s_1 of the resident population, we monitor the average number

of heterozygous lethals, the relative mean fitness, and the primary and experimental inbreeding depression at equilibrium. We then evaluate the ability of a rare mutant with selfing rate s_2 to invade the resident population.

Parameter values

We allow the genomic mutation rate to lethals to be $U = 0.02$, 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). We assume that the constant background inbreeding depression caused by nearly additive, mildly deleterious mutations is $d = 0.25$ (Husband & Schemske, 1996). We allow the proportion of ovules that can be matured to seeds to be $c = 1$ (no opportunity for reproductive compensation), 0.9, 0.5 or 0.1. Within each level of resource availability and mutation rate, we consider resident selfing rates s_1 ranging from 0 to 1 and, for each resident selfing rate, the mutant selfing rate s_2 is also varied from 0 to 1. Hence we consider all possible mutations with small, intermediate or large effects on the selfing rate.

Results

Mean number of lethals and inbreeding depression with no reproductive compensation

Our dynamic model allows purging of nearly recessive lethal mutations by selfing so that, with no reproductive compensation, the average number of heterozygous lethals and the (primary and experimental) inbreeding depression decrease when selfing rate increases (Fig. 1, $c = 1$), as demonstrated by Lande & Schemske (1985). Under a very high mutation rate to lethals ($U = 1$), this decrease is discontinuous: virtually no purging occurs under small selfing rates, and there is a severe drop in the number of heterozygous lethals above a threshold selfing rate (Fig. 1c, Lande *et al.*, 1994). This inefficiency of purging of nearly recessive lethals at low selfing rates is attributable to high inbreeding depression and survival rates close to zero for selfed offspring. The selfed progeny of an individual carrying x heterozygous lethals survive with probability $(^3/_4)^x$. Therefore, with a primary selfing rate s at fertilization, the secondary selfing rate or the proportion of observed selfed seedlings in the progeny of an individual carrying x heterozygous lethals is $s^*(x) = s(^3/_4)^x/[1 - s + s(^3/_4)^x]$. This is a decreasing function of the number of heterozygous lethals, so that with a large number of lethals, the secondary selfing rate can be close to zero even with intermediate values of the primary selfing rate; the population is then effectively close to random mating, and purging is inefficient. Note that the relationship between primary and secondary selfing rate is not affected by reproductive compensation in this model, because dead embryos are replaced by viable selfed and outcrossed embryos in proportions identical to those before compensation.

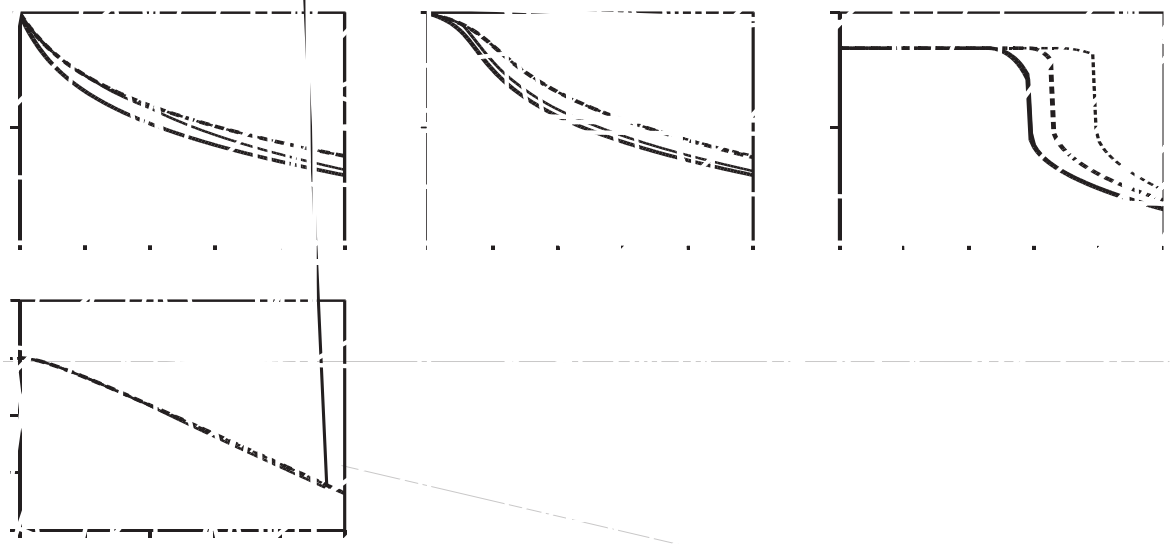
With no compensation, the mean fitness either decreases monotonically with increased selfing rates, primarily because of late-acting background inbreeding depression ($U = 0.02$, Fig. 1d); or increases at intermediate selfing rates because of the severe drop in the mean number of lethals ($c = 1$, $U = 0.2$ and 1; Fig. 1e,f).

Mean number of lethals and inbreeding depression with reproductive compensation

Resource limitation ($c < 1$) provides the opportunity for reproductive compensation. At any given selfing rate, reproductive compensation increases the mean number of lethals and the primary inbreeding depression at mutation–selection equilibrium (Fig. 1a–c,g–i). Nevertheless, reproductive compensation increases the relative mean fitness of the population (Fig. 1d–f) and tends to decrease the experimental inbreeding depression (Fig. 1j–l). This is because of the immediate effects of reproductive compensation, which effectively diminish the early inbreeding depression. For example, with $U = 1$, at low to intermediate selfing rates, the relative mean fitness does not decrease with increased selfing rates when resources are limited ($c < 1$, Fig. 1f).

The mean number of lethals increases at mutation–selection equilibrium because reproductive compensation acts more strongly for genotypes carrying more heterozygous lethals. The proportion of viable embryos in the progeny of an individual with primary selfing rate s and carrying x heterozygous lethals is $V(s,x) = 1 - s[1 - (^3/_4)^x]$, a decreasing function of the number of lethals. In the absence of compensation, genotypes with larger number of lethals have smaller seed set. If the conditions for reproductive compensation are met (see below), seed set is controlled by resource availability, and differences in seed set decrease among genotypes. Individuals carrying more heterozygous embryonic lethals have a reduced disadvantage compared with a situation without compensation. For this reason, reproductive compensation increases the mean number of heterozygous lethals maintained at mutation–selection equilibrium.

The opportunity for reproductive compensation increases under more stringent resource limitation and a smaller mutation rate to lethals. Under a high mutation rate to lethals ($U = 1$) and weak resource limitation ($c = 0.9$), with intermediate to high selfing rates, the vast majority of individuals carry a large number of lethals and produce fewer embryos than can be matured: reproductive compensation never occurs, which explains similar mean numbers of lethals for $c = 1$ and $c = 0.9$ (Fig. 1c). Alternatively, with a low mutation rate to lethals ($U = 0.02$) and strong resource limitation (e.g. $c = 0.5$), the vast majority of individuals carry few heterozygous lethals and their viable embryos always exceed what they can mature to seeds; complete reproductive compensation



therefore occurs regardless of the selfing rate, and genetic loads and mean fitness are identical for $c = 0.5$ and $c = 0.1$ (Fig. 3g,h). Note that with complete compensation (e.g. $d = 0.02$, $c = 0.1$) the experimental inbreeding depression is used to the background inbreeding depression ($d = 0.25$), plus an additional effect of selection on heterozygous lethals, which actually favors selfed individuals. This is caused by the purging of lethals by selfing, so that selfed seedlings are, on average, associated with fewer heterozygous lethals, and total inbreeding depression is slightly smaller than 0.25 (Fig. 1i, $c = 0.5$ or 0.1).

Stable selfing rates with reproductive compensation

The following results are presented as pairwise invasibility plots (Dieckmann, 1997), where the invasion of a resident population by a mutant genotype with a different selfing rate is plotted as a function of the resident selfing rate s_1 and the selfing rate of a rare mutant s_2 (Fig. 4). Regions where the mutant invades are indicated by dots; regions where it cannot invade are in white. Equilibria occur at the intersection of lines separating different regions of invasion. Details regarding the

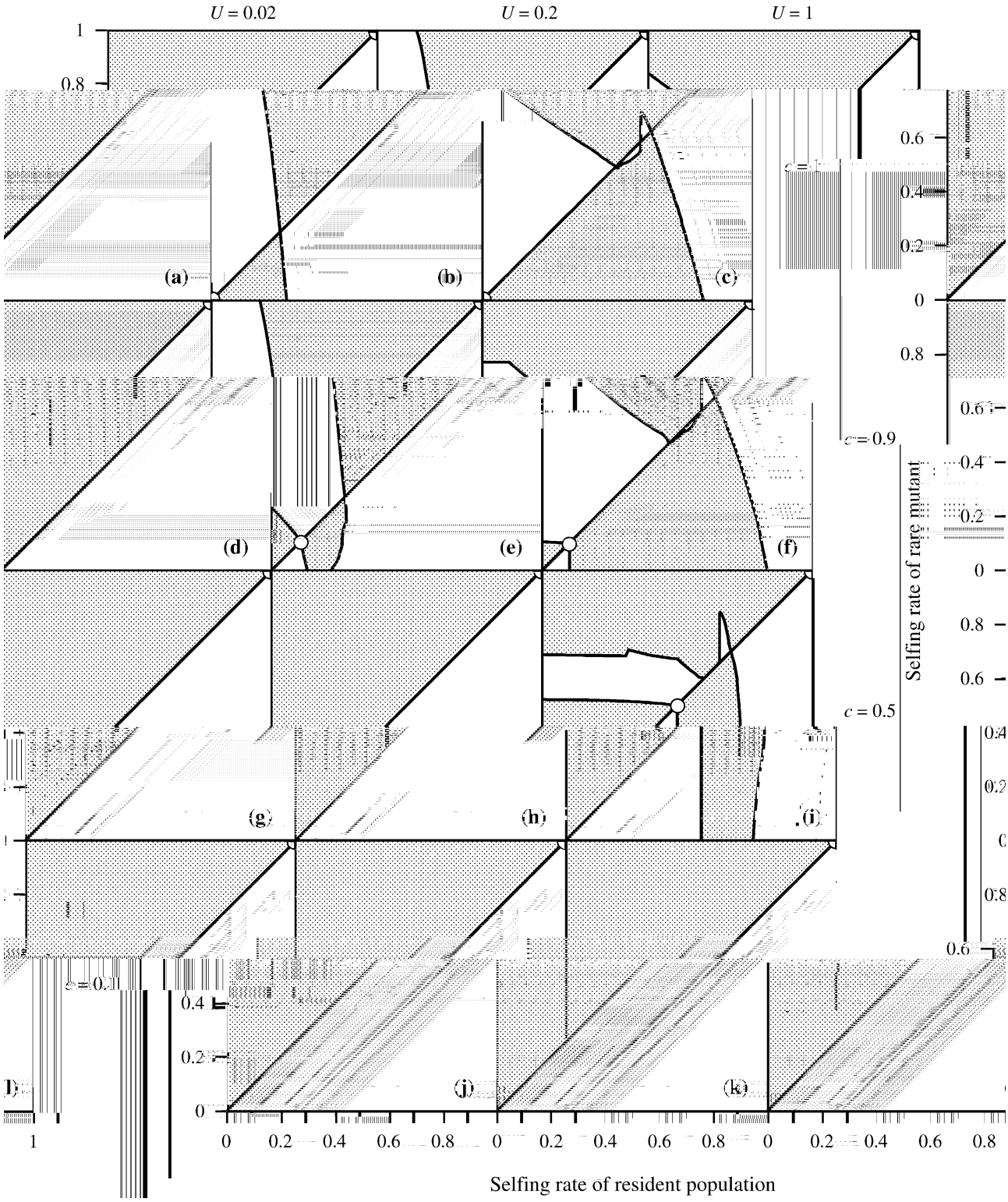


Fig. 2 Pairwise invasibility plots and stable selfing rates under different mutation rates to lethals, *U*, and levels of resource limitation, *c*. Invasibility of a rare mutant affecting the selfing rate depends on the selfing rate of the resident population and the selfing rate of the mutant, with regions of invasion indicated by dots and regions of noninvasion in white. Open circles represent stable selfing rates, except where these appear as a quarter circle in the upper right or lower left corner of panels.

criteria for inferring the stability of an equilibrium can be found in Dieckmann (1997). Here we are primarily interested in stable equilibrium selfing rates, which are both evolutionarily stable (cannot be invaded by neighboring mutant selfing rates) and convergence stable (evolution by a series of small steps proceeds towards the equilibrium).

Without reproductive compensation, the stable selfing rate depends on the mutation rate to lethals, and hence on primary inbreeding depression and, in some cases, on the initial selfing rate (Fig. 2a–c). Rare selfing genotypes in a completely outcrossing resident population benefit from a 50% automatic advantage because of the transmission, on average, of three copies of their genome (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). Under a low mutation rate to lethals ($U = 0.02$), the mean number of heterozygous lethals in a population remains small, so that the primary inbreeding depression never exceeds 0.5 (Fig. 1g) and cannot oppose the automatic advantage of selfing. Consequently, a mutant increasing the selfing rate can always invade, and complete selfing is the only stable mating system (Fig. 2a).

With an intermediate mutation rate to lethals ($U = 0.2$), the primary inbreeding depression in the resident population exceeds 0.5 under small selfing rates (Fig. 1h) and only mutants decreasing the selfing rate can invade, so that complete outcrossing is the stable mating system (Fig. 2b). However, above a threshold selfing rate ($s_1 > 0.28$), purging of lethals reduces the primary inbreeding depression below 0.5 in the resident population (Fig. 1h), and mutants increasing the selfing rate can invade, leading to complete selfing.

A high mutation rate to lethals ($U = 1$, Fig. 2c) creates a similar pattern, although a more complex one because of the precipitous drop in the mean number of lethals maintained by mutation at intermediate selfing rates (Fig. 1i). In addition, mutants with a high selfing rate can invade in a population with limited selfing, because of a dramatic purging of lethals in the progeny of highly selfing individuals (Fig. 2c).

Reproductive compensation increases the relative mean fitness of a partially selfing population by increasing the seed set of plants carrying homozygous lethals, and tends to favor evolution towards higher selfing rates. However, its evolutionary effect on stable selfing rates depends on inbreeding depression and opportunities for compensation. When the primary inbreeding depression is smaller than the automatic advantage of selfing, complete selfing is the only stable mating system, regardless of reproductive compensation ($U = 0.02$, Fig. 2, first column). Similarly, when the mean number of lethals is small relative to the maximum fraction of ovules that can be matured, c, compensation always occurs and inbreeding depression has no influence on seed set, so selection always favors increased selfing rates and the only stable mating system is complete selfing. This occurs under strong resource limitation ($c \leq 0.5$ when $U = 0.2$, Fig. 2h,k; $c \leq 0.1$ when $U = 1$, Fig. 2l).

With weak resource limitation, and intermediate to high lethal mutation rates, the magnitude of reproductive compensation depends on the selfing rate which, in some cases, creates conditions for the maintenance of stable mixed mating systems. In a population with a low selfing rate, all genotypes are fully compensated because the fraction of embryos dying due to homozygous lethals remains small (flat fitness curves at low selfing rates, e.g. Fig. 1f, $c = 0.9$ and $c = 0.5$). This favors the evolution of higher selfing rates, which produce, on average, fewer viable embryos because of inbreeding depression on selfing. During the evolution of a higher selfing rate, if resource limitation is not too strong, the average fraction of viable embryos eventually drops below c, the fraction of ovules that can be matured. Then reproductive compensation cannot occur, and selection favors a decreased selfing rate (Fig. 2e,f,i). This maintains a stable mixed mating system, with a low to intermediate selfing rate. At higher selfing rates ($s > 0.5$), purging of lethals reduces the inbreeding depression below 50%, which favors increased selfing. Thus when a stable mixed mating system exists, there also exists an unstable equilibrium selfing rate above which complete selfing is selected (Fig. 2e,f,i).

Discussion

Reproductive compensation and evolution of plant mating systems

Reproductive compensation tends to increase the equilibrium mean number of lethals in populations, as previously demonstrated in human populations (Hastings, 2000, 2001; Overall *et al.*, 2002) and for polyembryony in plants (Hedrick *et al.*, 1999). Nevertheless, its main effect on the evolution of plant mating systems is to favor increased selfing rates. This occurs because the usual effect of inbreeding depression in decreasing seed set is masked by reproductive compensation. In our model, early experimental inbreeding depression, measured by comparing relative seed sets of experimentally selfed vs outcrossed individuals, is smaller under more stringent resource limitation. With a large mean number of heterozygous lethals (producing a high abortion rate of selfed embryos and a high primary inbreeding depression), stringent resource limitation allows complete compensation, so that selfed and outcrossed individuals produce the same seed set, and there is no experimental inbreeding depression except that caused by the late-acting component. In contrast, with no resource limitation, selfed and outcrossed individuals differ in seed set if selfed individuals produce fewer viable embryos than can be matured. Thus reproductive compensation reverses the influence of environmental quality on inbreeding depression, which is often thought to be stronger in harsher environments (Ramsey & Vaughton, 1998; Cheptou & Mathias, 2001 and references therein). This arises from the role of reproductive compensation in the expression of early

inbreeding depression, a component that is seldom measured in studies of environment-dependent inbreeding depression.

A novel finding of our analysis is that reproductive compensation can promote the evolution of stable mixed mating systems. Although the distribution of selfing rates in natural plant populations tends to be bimodal, with most populations exhibiting predominant selfing or predominant outcrossing (Schemske & Lande, 1985; Barrett *et al.*, 1996), stable intermediate selfing rates are not uncommon in natural populations (Vogler & Kalisz, 2001; Barrett, 2003). Mixed mating systems are not explained by classical genetic models of mating system evolution, based only on automatic advantage of selfing and inbreeding depression (Lande & Schemske, 1985; Charlesworth *et al.*, 1990; Lande *et al.*, 1994). Several theoretical approaches have identified conditions favoring the maintenance of intermediate selfing rates. Most are based on genetic mechanisms (Campbell, 1986; Uyenoyama, 1986; Damgaard *et al.*, 1992; Latta & Ritland, 1994); pollination biology (Holsinger, 1991; Lloyd, 1992); or a combination of both (Johnston, 1998; Porcher & Lande, 2005b); other models also suggest that environmental variability (Cheptou & Mathias, 2001) or population dynamics (Holsinger, 1986; Cheptou & Dieckmann, 2002) might be involved. The role of resource limitation and reproductive compensation in the maintenance of mixed mating systems has not previously been investigated.

Here we demonstrate that resource limitation can be responsible for stable mixed mating systems with low to intermediate selfing rates, because reproductive compensation increases the mean fitness of a partially selfing population relative to a completely outcrossing population. With a dynamic model of inbreeding depression, mixed mating systems with high selfing rates ($0.5 < s < 1$) cannot be attributed to reproductive compensation, because high selfing rates purge most embryonic lethals so that complete selfing is the only stable mating system, regardless of the opportunity for compensation. We propose that stable mixed mating systems with intermediate to high selfing rates are generally maintained by the opposing effects of pollen limitation and pollen discounting (Porcher & Lande, 2005b).

Although only a few examples of stable intermediate selfing rates are shown here, they are likely to exist under a rather wide range of conditions. Stable mixed mating systems can be maintained by reproductive compensation if (i) resource limitation causes reproductive compensation to occur at low selfing rates, favoring increased selfing, until the mean fraction of viable embryos in the population drops below the fraction of ovules that can be matured; and (ii) primary inbreeding depression is sufficiently strong (>0.5) that, when the mean fraction of viable embryos is less than the fraction of ovules that can be matured, decreased selfing is selected. This never happens if the mutation rate to lethals is $U = 0.02$, but occurs whenever $c > 0.9$ for $U = 0.2$ and $c > 0.5$ for $U = 1$. Given the upper available estimates for the mutation rate to lethals in plants ($U = 0.2$, Klekowski & Godfrey, 1989; Lande *et al.*, 1994), this suggests that mixed mating systems could be maintained by reproductive compensation when the seed : ovule ratio is close to, but less than, 1.

Reproductive compensation in natural plant populations

Our model demonstrates that reproductive compensation may exert a significant influence on the evolution of plant mating systems, favoring increased selfing rates as well as maintaining stable mixed mating systems with low to intermediate selfing rates. However, reproductive compensation has rarely been observed in natural populations, and its actual role in the evolution of plant mating systems could be limited. Below, we suggest that reproductive compensation is seldom observed because it is not readily detectable in natural populations, and suggest some experiments by which it can be detected.

Reproductive compensation is unlikely to occur in pollen-limited populations because if the proportion of fertilized ovules is smaller than can be matured, there is no opportunity to replace dead embryos and seed set is not compensated. Pollen limitation is widespread in natural populations (Burd, 1994; Larson & Barrett, 2000), and many experimental studies have shown that seed set often can be increased by providing supplemental pollen to a flower. Yet no pollen limitation was found in 38% of study populations or species (Burd, 1994; but see Ashman *et al.*, 2004 for potential biases in detecting pollen limitation). Moreover, where pollen limitation has been observed, it varies greatly across years (Burd, 1994), creating the opportunity for occasional resource limitation and possible reproductive compensation.

A second reason why reproductive compensation is rarely detected in natural plant populations is that its demonstration is not straightforward. In species with a seed : ovule ratio smaller than 1 and no pollen limitation, demonstration of reproductive compensation requires observations of embryo abortion caused by deleterious mutations and no decrease in seed set resulting from this abortion. Early embryo death caused by deleterious mutations is commonly observed in experimental selfing of predominantly outcrossing species (Wiens *et al.*, 1987; Charlesworth, 1989; Wiens *et al.*, 1989; Krebs & Hancock, 1991; Husband & Schemske, 1996; see Johnston, 1992 for a review). However, because comparison of seed set by experimentally selfed vs outcrossed individuals is often used to demonstrate that genetic load causes abortion, such evidence is almost invariably associated with a decrease in selfed seed set, and therefore corresponds to examples where reproductive compensation does not occur (but see Wiens *et al.*, 1987, 1989).

Reproductive compensation and selective embryo abortion are likely to co-occur in natural populations, which further complicates the detection of reproductive compensation. For example, a difference between primary and secondary selfing rates (preferential abortion of selfed embryos) may indicate

reproductive compensation as well as selective embryo abortion. A few experimental studies have demonstrated that some, but not all, aborted embryos are otherwise viable (Casper, 1988; Melser & Klinkhamer, 2001). This, together with increased fitness of progeny, demonstrates that selective embryo abortion occurs, but does not rule out reproductive compensation. To our knowledge the only clear evidence of reproductive compensation in plant species comes from studies in polyembryonic species (Sorensen, 1982; Kärkkäinen & Savolainen, 1993), and relies on independent estimates of number of embryonic lethals and secondary selfing rates.

Despite the paucity of unambiguous evidence for reproductive compensation, the necessary conditions for it to occur are often met. First, some of the factors enabling reproductive compensation, e.g. resource-limited seed set or delayed distribution of resources after ovule fertilization, are also needed for selective embryo abortion, which has been demonstrated many times (Korbecka *et al.*, 2002). Second, the genetic load is often large enough in predominantly outcrossing populations to decrease significantly the proportion of viable embryos produced by selfing. Available experimental evidence suggests that the mutation rate to lethals can be as high as 0.2 (Klekowski & Godfrey, 1989; Lande *et al.*, 1994), especially in perennials, which exhibit lower seed : ovule ratios (Wiens, 1984). This is expected to maintain around 10 heterozygous lethals per individual on average in predominantly outcrossing populations. Such large numbers of lethals would cause high mortality rates of selfed embryos (≈95%) and a significant decrease in the fraction of viable embryos even for predominantly outcrossing species, which provides opportunity for compensation. In predominantly selfing populations, a mutation rate of $U = 0.2$ would maintain only about 0.4 heterozygous lethals per individual, causing low mortality rates of selfed embryos (≈10%). This ensures an appreciable production of viable embryos in predominantly selfing populations, so that reproductive compensation is likely to occur across all mating systems.

Detecting reproductive compensation requires observations that embryo mortality does not decrease seed set. Thus a measure of early primary inbreeding depression is needed in situations where comparison of seed sets by artificially selfed vs outcrossed individuals suggests little or no early experimental inbreeding depression. Procedures to estimate early primary inbreeding depression would involve manipulating the fraction of ovules that can be matured, or the fraction of viable embryos. This can be achieved by experimentally increasing resource availability or by limiting self or outcross pollen deposited on stigmas, thereby removing the opportunity for reproductive compensation. With compensation artificially prevented, a comparison of the mean seed set by experimentally selfed vs outcrossed plants directly reveals the early primary inbreeding depression.

Pollination experiments including pollen limitation may also reveal the level at which reproductive compensation occurs, for example by experimentally selfing or outcrossing single flowers or branches instead of whole plants. Some forms of density-dependent competition may resemble reproductive compensation at the plant level. In species with very limited seed dispersal, density-dependent competition among siblings would resemble compensation within plants. In species with long-distance seed dispersal, density dependence will tend to exert proportional effects on all families, with no compensatory effect among families that differ in number of lethals or selfing rate.

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Appendix: Recursion equations

Gamete production and mating

In the following, capital letters G and J refer to diploid genotypes and small letters g and j to haploid genotypes at the locus controlling the selfing rate, hereafter named the modifier locus.

Outcrossing The probability that a plant with y heterozygous lethals produces a gamete with x ($\leq y$) lethals is $\binom{y}{x}\left(\frac{1}{2}\right)^y$ (Lande *et al.*, 1994). Hence the probability that individuals with genotype G at the modifier locus produce gametes with haploid genotype g and carrying x lethals is $q_G^g(x) = \sum_{y=x}^{\infty} k_1 f_G(y)\binom{y}{x}\left(\frac{1}{2}\right)^y$, where $f_G(y)$ is the frequency of plants with genotype G at the modifier locus carrying y heterozygous lethals, and k_1 reflects Mendelian inheritance of the genotype at the modifier locus (k_1 = 1 or $^1/_2$ depending on the heterozygosity of G). Assuming that all genotypes contribute equally to the pollen pool (so there is no pollen discounting associated with selfing), the frequencies of pollen grains carrying x lethals and the resident, R, or mutant, M, allele are:

$$p_R(x) = q_{RR}^R(x) + q_{RM}^R(x) \text{ and } p_M(x) = q_{MM}^M(x) + q_{RM}^M(x).$$

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

Selfing The probability that a plant with y heterozygous lethals produces, by selfing, a viable zygote with x ($\leq y$) heterozygous lethals is $\binom{y}{x}\left(\frac{1}{2}\right)^x\left(\frac{1}{4}\right)^{y-x} = \binom{y}{x}\left(\frac{1}{2}\right)^{2y-x}$ (Lande *et al.*, 1994). Therefore, the relative frequency of zygotes with diploid genotype J at the modifier locus, carrying x heterozygous lethals, and originating from selfing of G genotypes is $q_G^J(x) = \sum_{y=x}^{\infty} k_2 f_G(y)\binom{y}{x}\left(\frac{1}{2}\right)^{2y-x}$, where k_2 is a constant accounting for Mendelian transmission of alleles at the modifier locus (k_2 = 1, $^1/_2$ or $^3/_4$ depending on the heterozygosity of G and J).

Seed set and reproductive compensation The equation above describing selfing also includes selection on homozygous embryonic lethals: for genotypes carrying x heterozygous lethals, a proportion $1 - (^3/_4)^x$ of their offspring produced by selfing carry at least one homozygous lethal; they are not viable and are not included in the equation. Hence, assuming that (i) early inbreeding depression is due to homozygous lethals only; and (ii) seed set is not pollen-limited, the proportion of ovules producing viable embryos, for a genotype with selfing rate s and carrying x heterozygous lethals, is $V(s,x) = [(1-s) + s(^3/_4)^x] = 1 - s[1 - (^3/_4)^x]$.

We assume that seed production is limited by resource availability or genetic load, but not by pollen availability. Let c be the maximum fraction of ovules that can develop into seeds, due to resource limitation. If the fraction of viable embryos is smaller than the fraction of ovules that can develop into seeds, $V(s,x) < c$, no compensation occurs: embryos dying because of homozygous lethals are not replaced by viable embryos and the relative seed set by such individuals is $V(s,x)$. In contrast, if $V(s,x) > c$, dead embryos are replaced by viable ones; the relative seed set is c, and a fraction $1 - c/V(s,x)$ of viable embryos is aborted. We assume that abortion occurs at random among viable embryos, i.e. there is no selective abortion favoring embryos with fewer heterozygous lethals. Hence the overall frequency of viable embryos surviving the abortion process in the progeny of individuals with selfing rate s and carrying x heterozygous lethals is $a(s,x) = \min[1, c/V(s,x)]$; it is assumed to be identical for viable selfed embryos and outcrossed embryos. As a result, the equations for zygote production are modified as follows. The probability that individuals with diploid genotype G at the modifier locus produce gametes with haploid genotype g and carrying x lethals is $q_G^g(x) = \sum_{y=x}^{\infty} k_1 a(s_G,y) f_G(y)\binom{y}{x}\left(\frac{1}{2}\right)^y$, and the relative frequency of zygotes with diploid genotype J at the modifier locus, carrying x heterozygous lethals, and originating from selfing of G genotypes is $q_G^J(x) = \sum_{y=x}^{\infty} k_2 a(s_G,y) f_G(y)\binom{y}{x}\left(\frac{1}{2}\right)^{2y-x}$, where s_G is the selfing rate of genotype G.

Accounting for the additional effect of background inbreeding depression, d, on selfed progeny, the recursion equations are:

$$f_{RR}^*(x) = (1-d)(s_1 q_{RR}^{RR}(x) + s' q_{RM}^{RR}(x)) + (1-s_1) \sum_{y=0}^{x} q_{RR}^R(y)p_R(x-y) + (1-s') \sum_{y=0}^{x} q_{RM}^R(y)p_R(x-y) \quad \text{(Eqn A1)}$$

$$f_{RM}^*(x) = (1-d)s' q_{RM}^{RM}(x) + (1-s_1) \sum_{y=0}^{x} q_{RR}^R(y)p_M(x-y) + (1-s_2) \sum_{y=0}^{x} q_{MM}^M(y)p_R(x-y) + (1-s') \sum_{y=0}^{x} (q_{RM}^R(y)p_M(x-y) + q_{RM}^M(y)p_R(x-y)) \quad \text{(Eqn A2)}$$

$$f_{MM}^*(x) = (1-d)(s_2 q_{MM}^{MM}(x) + s' q_{RM}^{MM}(x)) + (1-s_2) \sum_{y=0}^{x} q_{MM}^M(y)p_M(x-y) + (1-s') \sum_{y=0}^{x} q_{RM}^M(y)p_M(x-y) \quad \text{(Eqn A3)}$$

Mutation and selection caused by recessive 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:

(Eqn A4)

The probability that a zygote with x heterozygous mutations survives to maturity is $(1 - h)^x$. The frequency of mature

