



HAL
open science

THE EVOLUTION OF SELF-FERTILIZATION AND INBREEDING DEPRESSION UNDER POLLEN DISCOUNTING AND POLLEN LIMITATION

Emmanuelle Porcher, Russell Lande

► **To cite this version:**

Emmanuelle Porcher, Russell Lande. THE EVOLUTION OF SELF-FERTILIZATION AND INBREEDING DEPRESSION UNDER POLLEN DISCOUNTING AND POLLEN LIMITATION. *Journal of Evolutionary Biology*, 2005, 18 (3), pp.497-508. 10.1111/j.1420-9101.2005.00905.x . mnhn-02265370

HAL Id: mnhn-02265370

<https://mnhn.hal.science/mnhn-02265370>

Submitted on 9 Aug 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

THE EVOLUTION OF SELF-FERTILIZATION AND INBREEDING DEPRESSION
UNDER POLLEN DISCOUNTING AND POLLEN LIMITATION

Emmanuelle Porcher and Russell Lande

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

RUNNING TITLE: Pollination biology and evolution of selfing

Corresponding author:

Emmanuelle Porcher
Department of Biology 0116,
9500 Gilman drive
University of California - San Diego,
La Jolla, CA, 92093

Phone: 1 858 822 2972

Fax: 1 858 534 7108

Email: eporcher@ucsd.edu

Send page proofs to:

Russell Lande
Department of Integrative Biology
University of California, Berkeley
Berkeley, CA, 94720-3140

ABSTRACT

1
2
3 We model the evolution of plant mating systems under the joint effects of pollen
4 discounting and pollen limitation, using a dynamic model of inbreeding depression, allowing
5 for partial purging of recessive lethal mutations by selfing. Stable mixed mating systems
6 occur for a wide range of parameter values with pollen discounting alone. However, when
7 typical levels of pollen limitation are combined with pollen discounting, stable selfing rates
8 are always high but less than 1 ($0.9 < s < 1$ in most cases); in this situation, complete selfing
9 does not evolve because pollen discounting becomes very large at high selfing rates, so that
10 the automatic advantage of selfing changes to a disadvantage. These results suggest that
11 mixed mating systems with high selfing rates can be maintained by selection, whereas mixed
12 mating systems with low to moderate selfing rates are more likely attributable to unavoidable
13 geitonogamous selfing.

14

15

16 Keywords: Geitonogamy / Inbreeding depression / Pollen discounting / Pollen limitation /
17 Self-fertilization.

18

19

20

INTRODUCTION

1
2 In self-compatible hermaphroditic plants, the distribution of mating systems is
3 generally considered bimodal, with a majority of populations exhibiting low ($0 < s < 0.2$) or
4 high ($0.8 < s < 1$) selfing rates (Schemske & Lande, 1985; Barrett & Eckert, 1990; Barrett *et*
5 *al.*, 1996). However, intermediate selfing rates occur in an appreciable fraction of natural
6 populations (Aide, 1986; Schemske & Lande, 1986; Vogler & Kalisz, 2001; Barrett, 2003),
7 and empirical evidence suggests that many might be stable (see e.g. Holsinger, 1991 and
8 references therein). During the past 20 years, plant evolutionary biologists have tried to
9 explain the maintenance of such stable mixed mating systems, which is not accounted for by
10 most theoretical genetic studies based on two major evolutionary forces: (*i*) the 50%
11 automatic advantage of selfing, due to the transmission, on average, of three copies of the
12 genome of selfing individuals (two as parents of selfed seeds and one a male parent of
13 outcrossed seeds on other plants) while outcrossing genotypes transmit two copies (Fisher,
14 1941) and (*ii*) inbreeding depression, the relative decrease in fitness of selfed vs. outcrossed
15 individuals due mainly to recessive deleterious mutations (Charlesworth & Charlesworth,
16 1987; Husband & Schemske, 1996). Explicit genetic models of inbreeding depression that
17 account for its joint evolution with the mating system (Lande & Schemske, 1985; Lande *et*
18 *al.*, 1994; Charlesworth *et al.*, 1990) predict a dichotomous outcome of evolution in a single
19 population: either complete selfing or complete outcrossing. In most of the theory developed
20 so far, the maintenance of stable mixed mating systems appeals either to genetic factors, with
21 different hypotheses on inbreeding depression, or to ecological factors, notably pollination
22 biology (but see Schoen & Lloyd, 1984; Holsinger, 1986).

23 A variety of genetic factors favoring the maintenance of intermediate selfing rates
24 have been proposed, including inbreeding depression due to overdominance (Campbell, 1986;
25 Charlesworth & Charlesworth, 1990), inbreeding depression due to partially recessive, very

1 mildly deleterious mutations (Latta & Ritland, 1994), biparental inbreeding in isolated
2 (Uyenoyama, 1986; Yahara, 1992) or structured (Ronfort & Couvet, 1995) populations,
3 negative relationship between inbreeding depression and number of generations of selfing in a
4 lineage, mimicking purging of genetic load (Maynard Smith, 1977; Damgaard *et al.*, 1992;
5 Latta & Ritland, 1993), and spatial or temporal environmentally-induced variation in
6 inbreeding depression (Cheptou & Mathias, 2001). However, many genetic models predict
7 mixed mating systems either for a narrow range of parameter values only, or under highly
8 specific assumptions that are probably rarely met in natural populations, e.g. inbreeding
9 depression due to overdominance or to very mildly deleterious mutations only.

10 Ecological models based on pollination biology consider the details of pollen transfer,
11 and allow variation of reproductive success components that are generally considered constant
12 in genetic models of plant mating system evolution. In particular, two main factors affecting
13 the evolution of selfing rate have been isolated in ecological models: pollen and seed
14 discounting. Pollen discounting (Nagylaki, 1976; Gregorius *et al.*, 1987; Holsinger, 1991;
15 Harder & Wilson, 1998) is the reduction of male reproductive success by outcrossing that
16 may accompany an increase in selfing rate, due to a decrease in amounts of exported pollen. It
17 has been observed in several natural populations (e.g. Chang & Rausher, 1998; Fishman,
18 2000 and references therein) and is identified as a major factor favoring the maintenance of
19 stable mixed mating systems under a wide range of conditions (Holsinger, 1991; Johnston,
20 1998) but is generally omitted in detailed genetic models of mating system evolution. Seed
21 discounting (Lloyd, 1992), the loss of outcrossed seeds due to selfing, is always complete in
22 genetic models: each selfing event occurs at the expense of one outcrossing event. However,
23 this needs not be always the case due to specific aspects of pollen transfer. In particular, if
24 seed set is limited by outcross pollen availability (due to low pollinator frequency or low plant
25 population density), selfing increases the proportion of ovules fertilized (reproductive

1 assurance, Lloyd, 1992; Holsinger, 1996) without an equal decrease in the number of
2 outcrossed seeds, and seed discounting is reduced. As with pollen discounting, seed
3 discounting or reproductive assurance may also be responsible for stable intermediate selfing
4 rates, but under more specific conditions (e.g. seed discounting larger than 1, Johnston, 1998,
5 or with a size-number trade-off of seeds, Sakai & Ishii, 1999).

6 Although both theoretical and empirical studies have emphasized the role of pollen
7 discounting and pollen limitation in evolution of plant mating systems, no theoretical
8 approach has combined them. These two factors have opposing effects on mating system
9 evolution and are likely to co-occur in natural populations; their interaction may determine the
10 expected outcome of evolution. More specifically, pollen discounting can be decreased under
11 pollen limitation, as already stressed by Lloyd (1992): for example, if the selfing rate depends
12 on the relative amount of self vs. outcross pollen landing on a stigma (competing selfing,
13 Lloyd & Schoen, 1992), high selfing rates can be achieved with a smaller amount of self
14 pollen when outcross pollen is limited. Together with the larger fertilization success by highly
15 selfing genotypes, this effect of pollen limitation may neutralize the effects of pollen
16 discounting and strongly narrow the range of conditions where stable mixed mating systems
17 can exist. As outlined by Holsinger (1991) and Johnston (1998), there is thus a need for
18 ecological models exploring this interaction.

19 Because both genetic and ecological factors are potentially important determinants of
20 mating system evolution, a complete understanding of the maintenance of stable mixed
21 mating systems requires theoretical approaches accounting for both genetics and ecology in a
22 realistic manner. However, genetic models usually omit pollination ecology, and most theory
23 on the role of pollination biology in mating system evolution neglects critical genetic
24 components. In most pollination-based models generating stable mixed mating systems,
25 inbreeding depression is either omitted (Holsinger, 1991), or considered constant throughout

1 evolution (Lloyd, 1979; Sakai, 1995; Sakai & Ishii, 1999). Yet, theoretical (Lande &
2 Schemske, 1985; Charlesworth *et al.*, 1990) and empirical (Husband & Schemske, 1996)
3 studies suggest that the component of inbreeding depression due to recessive lethal (or semi-
4 lethal) mutations can be purged by selfing, and tends to be smaller in highly selfing than in
5 highly outcrossing populations. In what represents the only theoretical approach so far
6 combining pollination biology and the joint evolution of selfing rate and inbreeding
7 depression, Johnston (1998) confirmed that pollination processes, and notably pollen
8 discounting, play a major role in maintaining mixed mating systems, but he also demonstrated
9 that the conditions for stability and the values of stable selfing rates strongly depend on
10 inbreeding depression. However, Johnston (1998) neglected associations between deleterious
11 mutations and genes influencing the selfing rate, and his approach accurately describes the
12 dynamics only for mutations with small effects on the selfing rate. Accounting for linkage and
13 zygotic disequilibria during the joint evolution of inbreeding depression and mating system
14 can have a major influence. For example, mutations generating large increases in selfing rate
15 can invade a population experiencing high levels of initial inbreeding depression, due to
16 purging of deleterious mutations in the selfing genotypes (Lande & Schemske, 1985;
17 Charlesworth *et al.*, 1990); this can eventually lead to complete selfing even when it could not
18 evolve by small steps.

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

1 component of inbreeding depression attributable to mildly deleterious mutations (Lande &
2 Schemske, 1985; Husband & Schemske, 1996). Using an adaptive dynamic framework, we
3 examine the stable selfing rates under various conditions of pollen discounting, pollen
4 limitation and mutation rates to lethals.

5

6 THE MODEL

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

10 *Self fertilization*

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

22 We assume that all plants produce the same number of ovules and have the same
23 pollen/ovule ratio. In the following, the amount of pollen produced by all flowers, P ,
24 corresponds to number of pollen grains per ovule. Of pollen produced by genotype G , a
25 fraction α_G is exported for outcrossing and $1 - \alpha_G$ remains for selfing. For all genotypes, the

1 probabilities that outcross pollen and self pollen actually land on a stigma are π_o and π_s ,
 2 respectively. Hence, the amount of self pollen falling on stigmas of genotype G is $P_{sG} = (1 -$
 3 $\alpha_G)\pi_s P$. The amount of outcross pollen P_o received by all genotypes depends on the average
 4 rate of pollen export in the population $\bar{\alpha} = \sum_G f_G \alpha_G$ (where f_G is the frequency of genotype
 5 G): $P_o = \bar{\alpha} \pi_o P$. Under competing selfing, the primary selfing rate s_G of genotype G, after
 6 fertilization, is the ratio of self pollen to total pollen landing on the stigma,

$$7 \quad s_G = P_{sG} / (P_{sG} + P_o) = (1 - \alpha_G) / (1 - \alpha_G + \bar{\alpha} \pi) \quad (1)$$

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

12

13 *Pollen limitation and fertilization success*

14 In addition to the automatic advantage, self-fertilization also provides “reproductive
 15 assurance” when outcross pollen is limited by lack of pollinators or low population density of
 16 plants (Lloyd, 1992; Holsinger, 1996). Natural populations frequently experience pollen
 17 limitation, resulting in decreased seed set by individuals with larger outcrossing rates (e.g.
 18 Larson & Barrett, 2000). Pollen limitation is modeled by varying the total amount of outcross
 19 pollen $\pi_o P$ that would land on stigmas in a monomorphic, completely outcrossing ($\bar{\alpha} = 1$)
 20 population, a quantity likely influenced by pollinator availability. We assume that the fraction
 21 of ovules fertilized on genotype G is an increasing function of total pollen landing on the
 22 stigma (Kohn & Waser, 1985; Waser & Price, 1991; Mitchell, 1997):

$$23 \quad T_G = 1 - \exp[-P_o - P_{sG}] = 1 - \exp[-\pi_o P (\bar{\alpha} + (1 - \alpha_G) / \pi)]. \quad (2)$$

24 Thus, if the total amount of pollen $P_{sG} + P_o$ is larger than about 4, pollen limitation is

1 negligible. Since amounts of pollen represent here number of pollen grains per ovule, this
2 implies that full fertilization requires more than one pollen grain per ovule, as commonly
3 observed in experimental studies of the relationship between pollen load and seed set (Kohn
4 & Waser, 1985; Waser & Price, 1991; Mitchell, 1997).

5

6 *Inbreeding depression*

7 Inbreeding depression is attributable to nearly recessive, highly deleterious (lethal and
8 semi-lethal) mutations and to partially recessive (nearly additive), mildly deleterious
9 mutations (Simmons & Crow, 1977; Lande & Schemske, 1985; Husband & Schemske, 1996;
10 Charlesworth & Charlesworth, 1999). Individually rare, nearly recessive, lethals and semi-
11 lethals are much more likely to be exposed to selection as homozygotes in selfing populations
12 than in randomly mating populations, and this component of inbreeding depression can be
13 partially purged by selfing (Lande & Schemske, 1985; Lande *et al.*, 1994). In contrast, the
14 strength of selection acting on mildly deleterious mutations with nearly additive effects
15 depends little on the mating system of the population and this component of inbreeding
16 depression can be considered roughly constant throughout evolution (Lande & Schemske,
17 1985; Husband & Schemske, 1996).

18 Inbreeding depression due to lethals, D , is analyzed using a modified version of
19 Kondrashov's (1985) model, to describe evolution of the distribution of number of
20 heterozygous lethal alleles per individual in an infinite population. This model assumes a very
21 large (effectively infinite) number of unlinked loci mutating to nearly recessive lethals, with
22 genomic mutation rate per generation U . Each mutation occurs at a new locus (or one not
23 currently segregating in the population) and is therefore unique. Consequently, in an infinite
24 population where outcrosses occur at random between unrelated individuals, homozygous
25 lethals only appear by selfing. A multilocus genotype can be described by the number of

1 heterozygous lethals, because (i) recessive lethal alleles segregate independently and are
 2 never homozygous in mature plants, and (ii) all lethal mutations have identical effect on
 3 fitness, being lethal when homozygous and with the same dominance coefficient, h , when
 4 heterozygous. Inbreeding depression due to nearly additive, mildly deleterious mutations is
 5 modeled via a constant “background” inbreeding depression, d .

6

7

Parameter values and evolutionary dynamics

8 Parameters were either assigned values according to experimental data (as indicated
 9 below) or were varied to cover the whole range of possible values (see Table 1 for a summary
 10 of parameter values). We allow the genomic mutation rate to lethals to be $U = 0$ (no
 11 inbreeding depression), 0.02, 0.2, or 1, which embraces the range of experimental estimates,
 12 from 0.02 (*Drosophila melanogaster*, Simmons & Crow, 1977) to 0.2 (red mangroves,
 13 Klekowski & Godfrey, 1989, extrapolated by Lande *et al.*, 1994). The dominance coefficient
 14 of lethals is $h = 0.02$, as in the only available experimental data, which are from *Drosophila*
 15 (Simmons & Crow, 1977). In a completely outcrossing population, the range $U = 0$ to 1 with
 16 $h = 0.02$ generates inbreeding depression due to lethals between $D = 0$ and 0.99992 (Porcher
 17 & Lande, 2005). We assume that the constant background inbreeding depression due to nearly
 18 additive, mildly deleterious mutations is $d = 0.25$ (as estimated by Husband & Schemske,
 19 1996), except when $U = 0$, in which case $d = 0$, to model a situation with no inbreeding
 20 depression at all. The relative success of outcross vs. self pollen, $\pi (= \pi_o/\pi_s)$, affects the
 21 strength of pollen discounting (see Results) and ranges between 10^{-6} (no pollen discounting)
 22 and 1 (strong pollen discounting). We consider two levels of pollen limitation by allowing the
 23 total amount of successful exported pollen to be $\pi_o P = 1$ or ∞ , which generates relative seed
 24 set of completely selfing vs. completely outcrossing individuals $t = 0.6$ (as in Burd, 1994) or 1
 25 (no pollen limitation). Let A be the resident allele and B the mutant allele at the modifier

1 locus. Within each level of pollen discounting, mutation rate and pollen limitation, we
 2 consider resident selfing rates from $s_{AA} = 0$ to 1 by setting the fraction of exported pollen
 3 between $\alpha_{AA} = 1$ and 0. The fraction of pollen exported from the homozygous mutant is also
 4 varied from $\alpha_{BB} = 1$ to 0, which generates an initial mutant selfing rate from $s_{BB} = 0$ to $1/(1 +$
 5 $\alpha_{AA}\pi)$ when the mutant is rare in the population. Thus we consider mutations with a wide
 6 range of possible effects on the selfing rate. Alleles at the modifier locus have additive effects
 7 on pollen export, so that $\alpha_{AB} = (\alpha_{AA} + \alpha_{BB})/2$.

8 Each generation, a population undergoes mating, mutation to lethals, and selection.
 9 Recursion equations, including the genetic basis of inbreeding depression, are given in the
 10 Appendix. The model fully accounts for linkage disequilibria and identity disequilibria
 11 (associations of genotypic states) between loci producing recessive lethals and the locus
 12 controlling the selfing rate. Assuming that the mutation rate at the locus controlling selfing is
 13 small enough, each successful mutant becomes fixed in the population before the next
 14 mutation appears; we therefore consider the invasibility of a mutant in a population at
 15 equilibrium. From a resident population monomorphic at the modifier locus, and initially
 16 containing no lethals, for each set of parameter values the recursion equations were
 17 numerically iterated until closely approaching mutation-selection equilibrium, which occurs
 18 after a few dozen generations under large selfing rate or after several thousand generations
 19 under small selfing rate. A mutant allele with a selfing rate different from the resident was
 20 then introduced at a low frequency in linkage and identity equilibrium with lethals, and the
 21 recursion equations were numerically iterated for a hundred generations to detect invasion (or
 22 not) by the mutant.

23

24

RESULTS

25

Analysis of invasion of an outcrossing population by a selfing mutant

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

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

1 selfing rate s relative to a common outcrossing genotype is therefore $(1 + s + \alpha t - 2t)/(2t)$.

2 Using equation (1) with $\alpha_G = \alpha$ and $\bar{\alpha} \sim 1$, because the mutant is initially rare, yields $\alpha = 1 -$
 3 $s\pi/(1 - s)$. Following Lloyd (1992), the automatic advantage of selfing can thus be rewritten
 4 as $[1 + s - t - s\pi t/(1 - s)]/(2t)$ or

$$5 \quad \frac{s}{2} \left(\frac{2 - D_s - D_p}{t} \right) \quad (3),$$

6 where

$$7 \quad D_s = [t - (1 - s)]/s \quad (4)$$

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

$$9 \quad D_p = \pi t / (1 - s) \quad (5)$$

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

11 One important result obtained from the phenotypic approach is that the automatic
 12 advantage of selfing can actually turn into a disadvantage. As already noted by Lloyd (1992),
 13 seed discounting varies between 0 (when t approaches zero, i.e. with strong pollen limitation,
 14 or when $s = 0$) and 1 (with no pollen limitation). Pollen discounting approaches zero when the
 15 success of outcross vs. self pollen, π , approaches zero, or with strong pollen limitation (t
 16 approaches zero), because in both cases relatively small amounts of outcross pollen eventually
 17 land on stigmas, so that a high selfing rate can be achieved by using a small amount of self
 18 pollen. In contrast to Lloyd's (1992) model, pollen discounting can be larger than 1 with the
 19 mass-action model of fertilization, whenever $\pi t / (1 - s) > 1$. As a result, with no pollen
 20 limitation ($t = 1$ and $D_s = 1$), the automatic advantage of selfing becomes negative when $s > 1$
 21 $- \pi$. With pollen limitation, this condition is more restrictive. Because pollen discounting D_p
 22 is directly proportional to the relative success of outcross vs. self pollen, π , we vary π in the
 23 following to generate different levels of pollen discounting, from no pollen discounting ($\pi =$
 24 10^{-6}) to strong pollen discounting ($\pi = 1$)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

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 non-invasion (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 favors 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 non-invasion. Details regarding the criteria to infer stability of an 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 non-invasion, so that it cannot be invaded by neighboring 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 equation (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

1 genotype, equation (1) with $\alpha_G = \bar{\alpha}$ leads to $\bar{\alpha} = (1 - s^*)/[1 - s^*(1 - \pi)]$, where s^* is the
 2 selfing rate of the resident genotype. Hence, the maximum selfing rate of a rare mutant
 3 depends on the selfing rate of the resident genotype as follows:

$$4 \quad s_{\max} = [1 - s^*(1 - \pi)]/(1 - s^* + \pi). \quad (6)$$

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

7

8 *Stable selfing rates without pollen limitation*

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

16 With no pollen discounting ($\pi = 10^{-6}$ and D_p close to zero), the automatic advantage of
 17 selfing is $s/2$ and the effects of inbreeding depression on equilibrium selfing rates depend on
 18 the mutation rate to lethals and on initial conditions. A small mutation rate to lethals ($U =$
 19 0.02) does not affect the outcome of evolution: complete selfing is always selected, because
 20 total inbreeding depression remains small compared to the automatic advantage of selfing (δ
 21 $< 1/2$), regardless of the resident selfing rate (Fig. 1E). With larger mutation rates to lethals (U
 22 ≥ 0.2), the outcome of evolution depends on the initial selfing rate, as also observed by
 23 Johnston (1998) with no pollen discounting. With $U = 0.2$, total initial inbreeding depression
 24 in a population with a small selfing rate is large enough to overcome the automatic advantage
 25 of selfing, and evolution proceeds towards complete outcrossing. In contrast, above a

1 threshold selfing rate of the initial population, purging of lethals reduces inbreeding
 2 depression below the automatic advantage and favors mutants increasing the selfing rate (Fig.
 3 1I). With a high mutation rate to lethals ($U = 1$), the pattern is similar, but mutants with large
 4 selfing rates can invade a population with small selfing rates, because of a dramatic purging
 5 of recessive lethals in the progeny of highly selfing mutants (Fig. 1M). As a result, the
 6 ultimate outcome of evolution is complete selfing if the selfing rate can evolve by large steps.

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

19

20 *Stable selfing rates with pollen limitation*

21 Pollen limitation alone does not maintain mixed mating systems. Under pollen
 22 limitation, seed discounting, D_s , becomes smaller than 1, because some of the selfed ovules
 23 could not have been outcrossed anyway due to limited amounts of outcross pollen, $\pi_o P$,
 24 landing on stigmas. Without pollen discounting, this effect of pollen limitation has little
 25 influence on stable selfing rates (Fig. 2, first column): complete selfing is favored, because the

1 advantage of selfing becomes larger than $\frac{1}{2}$. With an intermediate mutation rate to lethals (U
 2 = 0.2), pollen limitation confers a larger automatic advantage to mutants with large selfing
 3 rates, so that they can invade a population with a small selfing rate (Fig. 2I), which favors an
 4 evolution towards complete selfing by mutations of large effect on the selfing rate.

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

19 With pollen limitation and pollen discounting, inbreeding depression has little effect on
 20 the value of intermediate stable selfing rates, due to purging of most lethals at high selfing
 21 rates, but it strongly influences the existence of additional equilibrium selfing rates and the
 22 dynamics of evolution. Under a small mutation rate to lethals ($U = 0.02$), a large intermediate
 23 selfing rate is the only outcome of evolution (Fig. 2F-H). Under larger mutation rates to
 24 lethals ($U \geq 0.2$), complete outcrossing can be invaded by mutants with a high selfing rate, so
 25 that it can persist only if selfing evolves by small steps, as explained above (Fig. 2 J, K and N-

1 P). With $U = 0.2$ and $\pi = 0.5$, complete outcrossing is impervious to invasion and mixed
2 mating systems cannot be achieved if the initial selfing rate is low (Fig. 2L).

3

4

DISCUSSION

5 Our model accounting for pollination biology and the genetics of inbreeding
6 depression confirms the major role of pollination biology in the maintenance of stable mixed
7 mating systems. Stable intermediate selfing rates due to pollen discounting were discovered
8 previously by Holsinger (1991) and Johnston (1998), but these authors did not consider the
9 joint effect of pollen limitation, a ubiquitous factor which likely affects the evolution of
10 mating systems, especially under mechanistic models of self-fertilization. We show that when
11 pollen discounting and pollen limitation are combined, pollination biology alone cannot
12 explain stable low selfing rates ($s < 0.5$). We argue below that in self-compatible species
13 stable low to intermediate selfing rates might often be caused by unavoidable geitonogamy,
14 especially in large perennial plants with many flowers (Barrett, 2003; Porcher & Lande,
15 2005). In contrast, stable high selfing rates are likely attributable to a balance of genetic
16 factors and pollination mechanisms.

17

18 *Conditions for maintenance of stable mixed mating systems.*

19 Without pollen discounting, our genetic model of inbreeding depression, accounting
20 for the joint evolution of mating system and inbreeding depression, does not predict stable
21 mixed mating systems (Fig. 1 and 2, first column). The joint evolution of selfing and
22 inbreeding depression has previously been analyzed by Charlesworth *et al.* (1990). The
23 present approach assumes that inbreeding depression is due to a combination of nearly
24 recessive lethal mutations and mildly deleterious mutations with nearly additive effects, while
25 the model by Charlesworth *et al.* (1990) considered only one type of mutation in a given

1 population. We find that even with a more realistic genetic basis for inbreeding depression
2 and with pollen limitation, mixed mating systems cannot be explained by the opposing effects
3 of inbreeding depression and the automatic advantage of selfing without additional features of
4 pollination biology.

5 Our model confirms that pollen discounting is a major factor favoring the
6 maintenance of stable mixed mating systems, as previously demonstrated by Holsinger
7 (1991). We describe here that this is due to the automatic advantage of selfing, which can
8 actually turn into a disadvantage at high selfing rates (equation 3). Holsinger (1991), however,
9 did not consider the influence of inbreeding depression, assuming that it would not affect the
10 existence of stable intermediate selfing rates, but only lower them. As a result, the conditions
11 for existence of stable mixed mating systems obtained by Holsinger (1991) were broader than
12 what we find: he observed stable intermediate selfing rates whenever the relative success of
13 self vs. outcross pollen, π , was smaller than 1, predicting that mixed mating systems should be
14 very common, because it is unlikely that outcross pollen is more successful than self pollen.

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

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

6

7 *Pollen limitation and stable high selfing rates*

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

23 Although pollen discounting and pollen limitation interact to favor high selfing rates,
 24 complete selfing is not stable. This was previously demonstrated by Holsinger (1991), in a
 25 model of plant mating system evolution based on pollen discounting only, and is due to a non-

1 linear increase in pollen discounting as the selfing rate increases. Our results confirm that,
2 even with strong pollen limitation favoring increased selfing via reproductive assurance,
3 complete selfing is not stable (except under the unrealistic conditions of large relative success
4 of outcross vs. self pollen, $\pi > 0.5$, and no inbreeding depression, Fig. 2D). This is attributable
5 to pollen discounting, which becomes larger than 1 at very high selfing rates, turning the
6 automatic advantage of selfing into a disadvantage (equation 3). This is consistent with
7 observations in natural populations. Although complete selfing is theoretically possible, e.g.
8 via cleistogamy or prior selfing (Lloyd & Schoen, 1992), most, if not all, highly selfing plant
9 species actually have $s < 1$ (Stebbins, 1957; Jain, 1976; Schemske & Lande, 1985). High
10 stable selfing rates close to but less than $s = 1$ could be attributable to the joint effect of
11 moderate pollen discounting and inbreeding depression without pollen limitation (e.g. Fig.
12 1F). However, in light of the frequent occurrence of pollen limitation (Burd, 1994; Larson &
13 Barrett 2000), we suggest that such mating systems could be maintained by the opposing
14 effects of strong pollen limitation and pollen discounting, favoring selfing rates close to but
15 less than 1, regardless of inbreeding depression and of the relative success of outcross vs. self
16 pollen.

17

18 *Conditions for maintenance of low selfing rates and complete outcrossing*

19 Our model shows that stable low to intermediate selfing rates, including $s = 0$, can be
20 maintained without pollen limitation (Fig. 1; Fig. 1D shows an intermediate selfing rate;
21 lower stable selfing rates are obtained with higher values of the relative success of outcross
22 vs. self pollen, $0.5 < \pi < 1$). Whether selection favors complete outcrossing or $s > 0$, and
23 whether mixed mating systems exhibit low or high selfing rates, depends on two key
24 parameters of the model: the genomic mutation rate to nearly recessive lethals U and the
25 relative success of outcross vs. self pollen π . The few available estimates suggest that U might

1 range between 0.02 (Simmons & Crow, 1977) and 0.2 (Klekowski & Godfrey, 1989; Lande *et*
2 *al.*, 1994). With $U = 0.02$ and no pollen limitation, complete outcrossing is stable whenever π
3 exceeds 0.1. Virtually nothing is known about the relative success of outcross vs. self pollen
4 in natural populations, because this quantity is not readily measurable. We expect it to be
5 smaller than 1, because large amounts of outcross pollen are lost during transport from one
6 plant to another, although self pollen might also be lost, e.g. by pollinator grooming during
7 geitonogamous selfing.

8 Our results show that (locally) stable outcrossing populations can be invaded by
9 mutants with a high selfing rate despite strong inbreeding depression (e.g. Fig. 1M-N or Fig.
10 2J-K, M-P). This is consistent with arguments of Lande & Schemske (1985) and simulations
11 of Charlesworth *et al.* (1990), but was not found by Johnston (1998), who neglected linkage
12 and identity disequilibria between lethals and genes influencing the selfing rate. However,
13 nearly complete selfing may be unlikely to evolve by a single mutation. Studies of the
14 genetics of plant mating systems suggest that the evolution of a high selfing rate may
15 sometimes be under control of major genes but does not evolve in a single step (McNair &
16 Cumbes, 1989; Fenster & Barrett, 1994; Fenster & Ritland, 1994; Fishman *et al.*, 2002;
17 Georgiady *et al.*, 2002). In addition, a single mutation producing a high selfing rate is likely to
18 have rather deleterious pleiotropic effects (Fisher, 1958; Wright, 1968).

19 In summary, the maintenance of low to moderate selfing rates requires an absence of
20 pollen limitation, and occurs under a narrow range of the mutation rate to lethals, U , and
21 relative success of outcross vs. self pollen, π . These conditions are somewhat restrictive and
22 might be met only rarely in natural populations, especially in light of widespread pollen
23 limitation (Burd, 1994; Larson & Barrett, 2000). We thus expect that many mixed mating
24 systems with low to moderate selfing rates are generally not maintained by selection, but are

1 most likely due to unavoidable geitonogamous selfing in populations where selection favors
2 complete outcrossing (de Jong *et al.*, 1993; Porcher & Lande, 2005).

3

4 *Concluding remarks*

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

17

18

ACKNOWLEDGEMENTS

19 This work was supported by grants from National Science Foundation, and the Miller
20 Institute for Basic Research in Science, University of California Berkeley, to R. Lande.

21

22

LITERATURE CITED

23 Aide, T.M. 1986. The influence of wind and animal pollination on variation in outcrossing
24 rates. *Evolution* 40: 434-435.

25 Barrett, S.C.H. 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm

- 1 and beyond. *Phil. Trans. R. Soc. Lond. B* 358: 991-1004.
- 2 Barrett, S.C.H. & Eckert, C.G. 1990. Variation and evolution of mating systems in seed
3 plants. Pp. 229-254 in S. Kawano, ed. *Biological approaches and evolutionary trends in*
4 *plants*. Academic press, London.
- 5 Barrett, S.C.H., Harder, L.D. & Worley, A.C. 1996. The comparative biology of pollination
6 and mating in flowering plants. *Phil. Trans. R. Soc. Lond. B* 352: 1271-1280.
- 7 Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in
8 fruit and seed set. *Bot. Rev.* 60: 83-139.
- 9 Campbell, R.B. 1986. The interdependence of mating structure and inbreeding depression.
10 *Theor. Pop. Biol.* 30: 232-244.
- 11 Chang, S-M. & Rausher, M.D. 1998. Frequency-dependent pollen discounting contributes to
12 maintenance of a mixed mating system in the common morning glory *Ipomoea*
13 *purpurea*. *Am. Nat.* 152: 671-683.
- 14 Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression.
15 *Genet. Res.* 74: 329-340.
- 16 Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary
17 consequences. *Ann. Rev. Ecol. Syst.* 18: 237-268.
- 18 Charlesworth, D. & Charlesworth, B. 1990. Inbreeding depression with heterozygote
19 advantage and its effect on selection for modifiers changing the outcrossing rate.
20 *Evolution* 44: 870-888.
- 21 Charlesworth, D., Morgan, M.T. & Charlesworth, B. 1990. Inbreeding depression, genetic
22 load, and the evolution of outcrossing rates in a multilocus system with no linkage.
23 *Evolution* 44: 1469-1489.
- 24 Cheptou, P-O. & Mathias, A. 2001. Can varying inbreeding depression select for intermediary
25 selfing rates? *Am. Nat.* 157: 361-373.

- 1 Damgaard, D., Couvet, D. & Loeschke, V. 1992. Partial selfing as an optimal mating strategy.
2 *Heredity* 69: 289-295.
- 3 de Jong, T., Waser, N.M. & Klinkhamer, P.G.L. 1993. Geitonogamy: the neglected side of
4 selfing. *Trends Ecol. Evol.* 8: 321-325.
- 5 Dieckmann, U. 1997. Can adaptive dynamics invade? *Trends Ecol. Evol.* 12: 128-131.
- 6 Fenster, C.B. & Barrett, S.C.H. 1994. Inheritance of mating-system modifier genes in
7 *Eichhornia paniculata* (Pontederiaceae). *Heredity* 72: 433-445.
- 8 Fenster, C.B. & Ritland, K. 1994. Quantitative genetics of mating system divergence in the
9 yellow monkeyflower species complex. *Heredity* 73: 422-435.
- 10 Fisher, R.A. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.* 11:
11 53-63.
- 12 Fisher, R.A. 1958. *The genetical theory of natural selection*, Ed. 2. Dover, New York.
- 13 Fishman, L. 2000. Pollen discounting and the evolution of selfing in *Arenaria uniflora*
14 (Caryophyllaceae). *Evolution* 54: 1558-1565.
- 15 Fishman, L., Kelly, A.J. & Willis, J.H. 2002. Minor quantitative trait loci underlie floral traits
16 associated with mating system divergence in *Mimulus*. *Evolution* 56: 2138-2155.
- 17 Georgiady, M.S., Whitkus, R.W. & Lord, E. M. 2002. Genetic analysis of traits distinguishing
18 outcrossing and self-pollinating forms of currant tomato, *Lycopersicon pimpinellifolium*
19 (Jusl.) Mill. *Genetics* 161: 333-341.
- 20 Geritz, S.A.H., Kisdi, É., Meszéna, G. & Metz, J.A.J. 1998. Evolutionarily singular strategies
21 and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* 12: 35-57.
- 22 Gregorius, H-R., Ziehe, M. & Ross, M.D. 1987. Selection caused by self-fertilization. I. Four
23 measures of self-fertilization and their effects on fitness. *Theor. Pop. Biol.* 31: 91-115.
- 24 Harder, L.D. & Wilson, W.G. 1998. A clarification of pollen discounting and its joint effects
25 with inbreeding depression on mating system evolution. *Am. Nat.* 152: 684-695.

- 1 Holsinger, K.E. 1986. Dispersal and plant mating systems. The evolution of self-fertilization
2 in subdivided populations. *Evolution* 40: 405-413.
- 3 Holsinger, K.E. 1991. Mass-action models of plant mating systems: the evolutionary stability
4 of mixed mating systems. *Am. Nat.* 138: 606-622.
- 5 Holsinger, K.E. 1996. Pollination biology and the evolution of mating systems in flowering
6 plants. *Evol. Biol.* 29: 107-149.
- 7 Husband, B.C. & Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding
8 depression in plants. *Evolution* 50: 54-70.
- 9 Jain, S.K. 1976. Evolution of inbreeding in plants. *Ann. Rev. Ecol. Syst.* 7: 469-495.
- 10 Johnston, M.O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology
11 versus deleterious mutations. *Genetica* 102/103: 267-278.
- 12 Klekowski, E.J., Jr. & Godfrey, P.J. 1989. Ageing and mutation in plants. *Nature* 340: 389-
13 391.
- 14 Kohn, J.R. & Waser, N.M. 1985. The effect of *Delphinium nelsonii* pollen on seed set in
15 *Ipomopsis aggregata*, a competitor for hummingbird pollination. *Am. J. Bot.* 72: 1144-
16 1148.
- 17 Kondrashov, A.S. 1985. Deleterious mutations as an evolutionary factor. II. Facultative
18 apomixis and selfing. *Genetics* 111: 635-653.
- 19 Lande, R. & Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding
20 depression in plants. 1. Genetics models. *Evolution* 39: 24-40.
- 21 Lande, R., Schemske, D.W. & Schultz, S.T. 1994. High inbreeding depression, selective
22 interference among loci, and the threshold selfing rate for purging recessive lethal
23 mutations. *Evolution* 48: 965-978.
- 24 Larson, B.M.H. & Barrett, S.C.H. 2000. A comparative analysis of pollen limitation in
25 flowering plants. *Biol. J. Linn. Soc.* 69: 503-520.

- 1 Latta, R.G. & Ritland, K.R. 1993. Models for the evolution of selfing under alternative modes
2 of inheritance. *Heredity* 71: 1-10.
- 3 Latta, R.G. & Ritland, K.R. 1994. Conditions favoring stable mixed mating systems with
4 jointly evolving inbreeding depression. *J. Theor. Biol.* 170: 15-23.
- 5 Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in
6 plants. *Am. Nat.* 113: 67-69.
- 7 Lloyd, D.G. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization.
8 *Int. J. Plant Sci.* 153: 370-380.
- 9 Lloyd, D.G. & Schoen, D.J. 1992. Self- and cross-fertilization in plants. I. Functional
10 dimensions. *Int. J. Plant Sci.* 153: 358-369.
- 11 Maynard Smith, J. 1977. The sex habit in plants and animals. Pp. 315-331 in F.B.
12 Christiansen & T.M. Fenchel, eds. *Measuring selection in natural populations*.
13 Springer-Verlag, Berlin.
- 14 Macnair, M.R. & Cumbes, Q.J. 1989. The genetic architecture of interspecific variation in
15 *Mimulus*. *Genetics* 122: 211-222.
- 16 Mitchell, R.J. 1997. Effects of pollination intensity on *Lesquerella fendleri* seed set: variation
17 among plants. *Oecologia* 10: 382-388.
- 18 Nagylaki, T. 1976. A model for the evolution of self-fertilization and vegetative reproduction.
19 *J. Theor. Biol.* 58: 55-58.
- 20 Porcher, E. & Lande, R. 2005. Loss of gametophytic self-incompatibility with evolution of
21 inbreeding depression. *Evolution* 59: 30-44.
- 22 Ronfort, J. & Couvet, D. 1995. A stochastic model of selection on selfing rates in structured
23 populations. *Genet. Res.* 65: 209-222.
- 24 Sakai, S. 1995. Evolutionarily stable selfing rates of hermaphroditic plants in competing and
25 delayed selfing modes with allocation to attractive structures. *Evolution* 49: 557-564.

- 1 Sakai, S. & Ishii, H.S. 1999. Why be completely outcrossing? Evolutionarily stable
2 outcrossing strategies in an environment where outcross-pollen availability is
3 unpredictable. *Evol. Ecol. Res.* 1: 211-222.
- 4 Schemske, D.W. & Lande, R. 1985. The evolution of self-fertilization and inbreeding
5 depression in plants. 2. Empirical observations. *Evolution* 39: 41-52.
- 6 Schemske, D.W. & Lande, R. 1986. Mode of pollination and selection on mating system: a
7 comment on Aide's paper. *Evolution* 40: 436.
- 8 Schoen, D.J. & Lloyd, D.G. 1984. The selection of cleistogamy and heteromorphic diaspores.
9 *Biol. J. Linn. Soc.* 23: 303-322.
- 10 Simmons, M.J. & Crow, J.F. 1977. Mutations affecting fitness in *Drosophila* populations.
11 *Annu. Rev. Genet.* 11: 49-78.
- 12 Stebbins, G.L. 1957. Self fertilization and population variability in higher plants. *Am. Nat.* 91:
13 337-354.
- 14 Uyenoyama, M.K. 1986. Inbreeding depression and the cost of meiosis: the evolution of
15 selfing in population practicing biparental inbreeding. *Evolution* 40: 388-404.
- 16 Vogler, D.W. & Kalisz, S. 2001. Sex among the flowers: the distribution of plant mating
17 systems. *Evolution* 55: 202-204.
- 18 Waser, N.M. & Price, M.V. 1991. Outcrossing distance effect in *Delphinium nelsonii*: pollen
19 loads, pollen tubes, and seed set. *Ecology* 72: 171-179.
- 20 Wright, S. 1968. *Evolution and genetics of populations*. Vol. 1. University of Chicago Press,
21 Chicago.
- 22 Yahara, T. 1992. Graphical analysis of mating system evolution in plants. *Evolution* 46: 557-
23 561.
- 24

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

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

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 ($k_1 = 1, 1/2$ or $1/4$ 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 equation (A1). For these parental genotypes, the seed set by selfing is reduced by a factor $(3/4)^x$, and the sum of equations (A1) over all parental genotypes, all offspring genotypes and all numbers of lethals is smaller than 1.

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

$$q_G^g(x) = \sum_{y=x}^{\infty} k_2 f_G(y) C_y^x \left(\frac{1}{2}\right)^y \quad (\text{A2})$$

where k_2 reflects Mendelian inheritance of the genotype at the modifier locus ($k_2 = 1$ or $\frac{1}{2}$ 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:

$$f_{AA}^*(x) = (1-d)s_{AA}T_{AA}q_{AA}^{AA}(x) + (1-d)s_{AB}T_{AB}q_{AB}^{AA}(x) + (1-s_{AA})T_{AA}\sum_{y=0}^x q_{AA}^A(y)p_A(x-y) + (1-s_{AB})T_{AB}\sum_{y=0}^x q_{AB}^A(y)p_A(x-y) \quad (\text{A3})$$

$$f_{AB}^*(x) = (1-d)T_{AB}s_{AB}q_{AB}^{AB}(x) + (1-s_{AA})T_{AA}\sum_{y=0}^x q_{AA}^A(y)p_B(x-y) + (1-s_{BB})T_{BB}\sum_{y=0}^x q_{BB}^B(y)p_A(x-y) + (1-s_{AB})T_{AB}\sum_{y=0}^x [q_{AB}^A(y)p_B(x-y) + q_{AB}^B(y)p_A(x-y)] \quad (\text{A4})$$

$$f_{BB}^*(x) = (1-d)s_{BB}T_{BB}q_{BB}^{BB}(x) + (1-d)s_{AB}T_{AB}q_{AB}^{BB}(x) + (1-s_{BB})T_{BB}\sum_{y=0}^x q_{BB}^B(y)p_B(x-y) + (1-s_{AB})T_{AB}\sum_{y=0}^x q_{AB}^B(y)p_B(x-y) \quad (\text{A5})$$

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

$$p_A(x) = \frac{\alpha_{AA}q_{AA}^A(x) + \alpha_{AB}q_{AB}^A(x)}{\alpha_{AA}f_{AA} + \alpha_{AB}f_{AB} + \alpha_{BB}f_{BB}} \quad p_B(x) = \frac{\alpha_{BB}q_{BB}^B(x) + \alpha_{AB}q_{AB}^B(x)}{\alpha_{AA}f_{AA} + \alpha_{AB}f_{AB} + \alpha_{BB}f_{BB}} \quad (\text{A6})$$

19

1 *Mutation and selection due to heterozygous lethals*

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

5
$$f_G^{**}(x) = \sum_{y=0}^x f_G^*(x-y) \frac{e^{-U} U^y}{y!} \quad (\text{A7})$$

6 The probability that a zygote with x heterozygous mutations survives to maturity is
 7 $(1-h)^x$, where h is the dominance coefficient of lethals. The frequency of mature plants with x
 8 heterozygous lethals in the next generation is then, for any genotype:

9
$$f_G'(x) = \frac{(1-h)^x}{\bar{W}} f_G^{**}(x) \quad (\text{A8})$$

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

11
$$\bar{W} = \sum_{x=0}^{\infty} (1-h)^x \left[f_{AA}^{**}(x) + f_{AB}^{**}(x) + f_{BB}^{**}(x) \right] \quad (\text{A9})$$

12

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	[1]
h	Dominance coefficient of lethals	0.02	[2]
D	Inbreeding depression due to lethals	[0, 0.999992]	
d	Background inbreeding depression	0.25	[3]
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	$[0, \infty]$	
P_o	Amount of outcross pollen landing on stigmas	$[0, \infty]$	
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	[4]

[1] Simmons & Crow, 1977; Klekowski & Godfrey, 1989; Lande et al., 1994. [2] Simmons & Crow, 1977. [3] Husband & Schemske, 1996. [4] Burd, 1994.

FIGURES CAPTIONS

1
2
3 Figure 1. Pairwise invasibility plots without pollen limitation, under various conditions of
4 pollen discounting, π , and genomic mutation rate to lethals, U . Regions of invasion and non-
5 invasion of a mutant affecting selfing rate are plotted against the selfing rate of the mutant
6 when rare and the initial selfing rate in the resident population. Regions where the mutant
7 invades are in grey, regions where it cannot invade in white. Striped regions correspond to
8 high selfing rates that cannot be achieved by a mutant (too much outcross pollen available
9 from the resident genotype, equation 6). Stable equilibria are indicated by open circles except
10 where the domain of attraction is very small, such that the equilibrium state is unlikely to
11 persist. On panel D, two examples of evolutionary trajectories (series of invasions and
12 fixations of mutants with a different selfing rate) are shown as arrows. Parameter values:
13 background inbreeding depression $d = 0$ for $U = 0$ and $d = 0.25$ for $U \geq 0.02$; no pollen
14 limitation ($\pi_o P = \infty$).

15
16 Figure 2. Pairwise invasibility plots with pollen limitation ($\pi_o P = 1$), under various conditions
17 of pollen discounting, π , and genomic mutation rate to lethals, U . Parameter values:
18 background inbreeding depression $d = 0$ for $U = 0$ and $d = 0.25$ for $U \geq 0.02$.

19

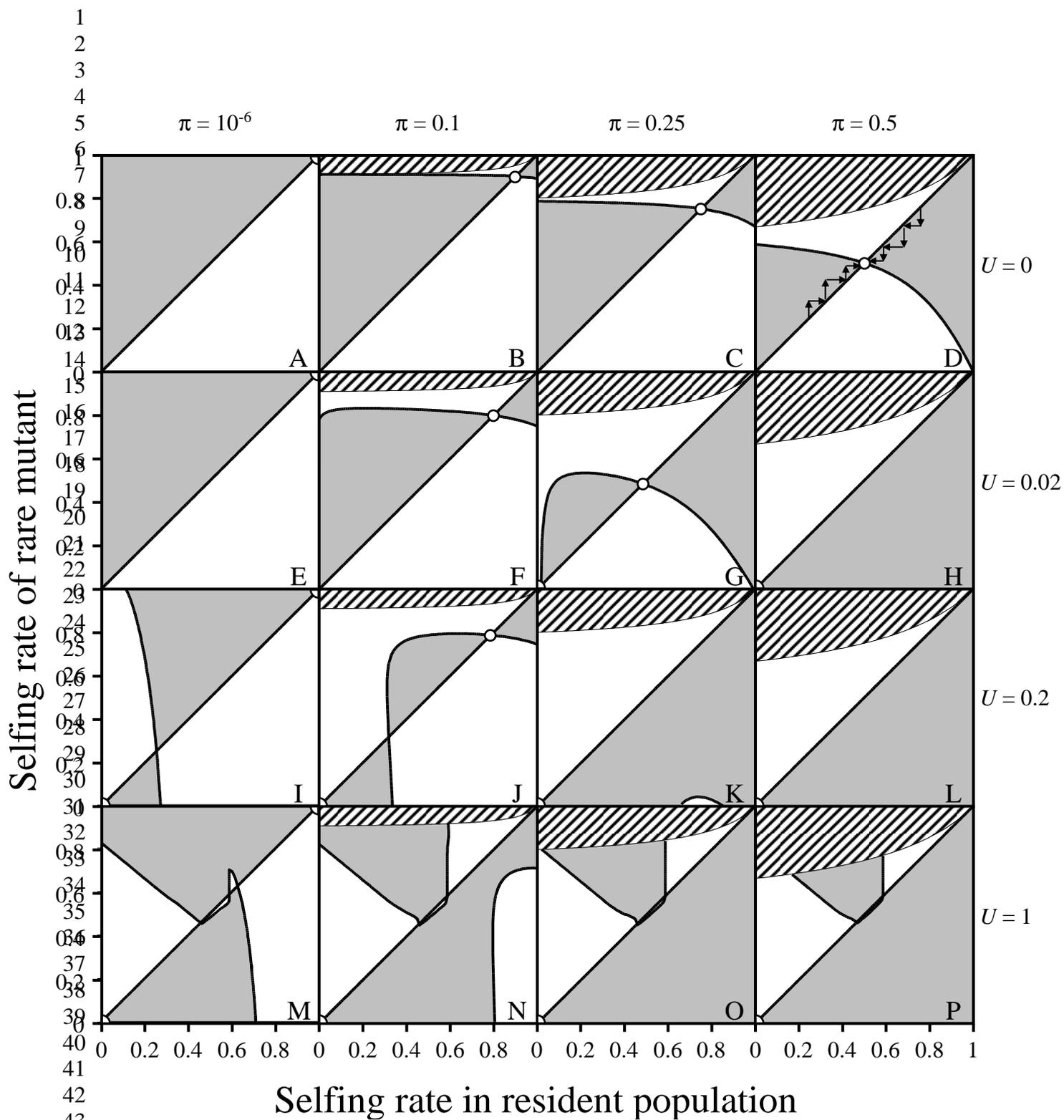


Figure 1

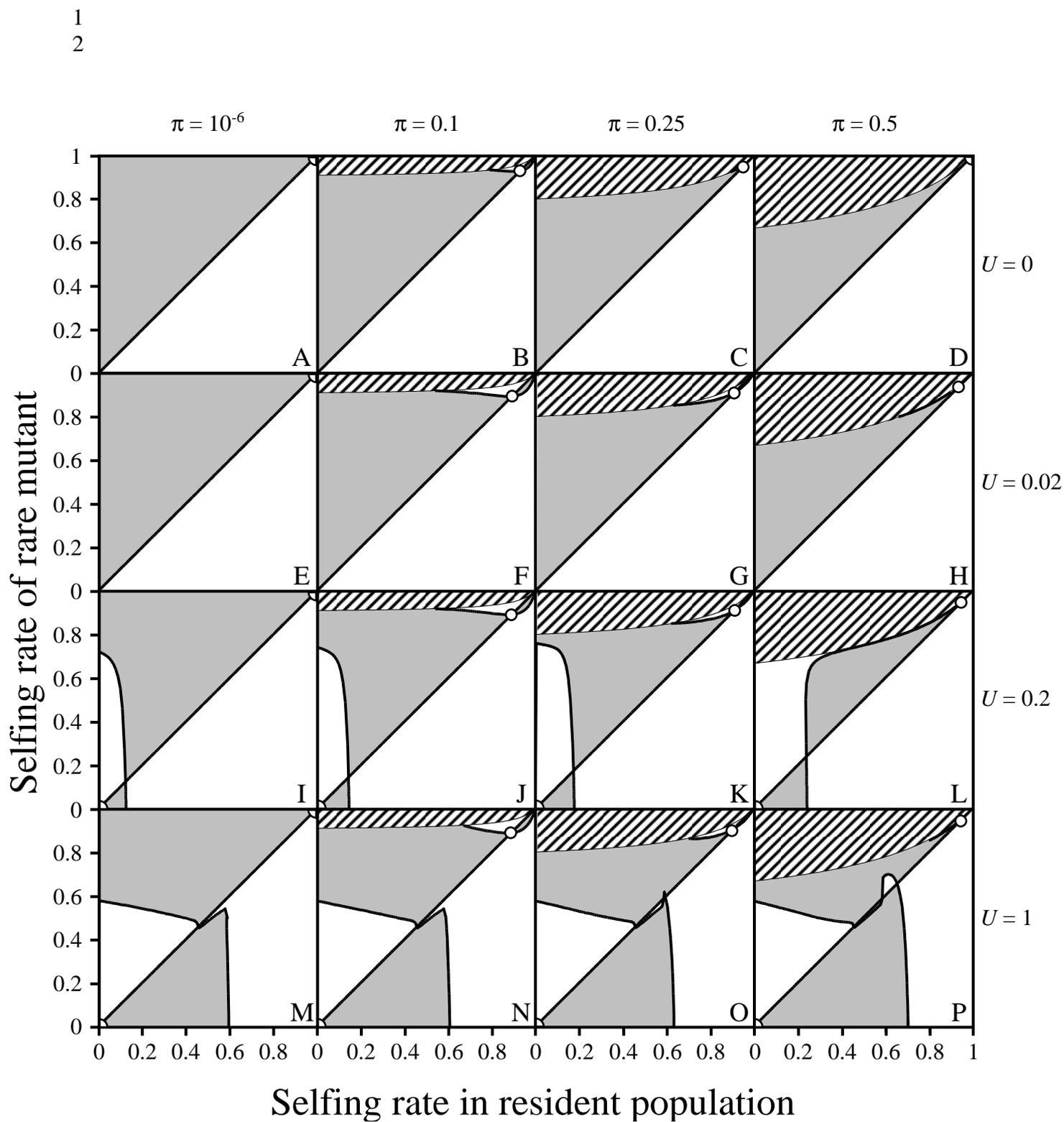


Figure 2