The genetic consequences of fluctuating inbreeding depression and the evolution of plant selfing rates

Emmanuelle Porcher, John K Kelly, Pierre-Olivier Cheptou, Christopher G Eckert, Mark Johnston, Susan Kalisz

The genetic consequences of fluctuating inbreeding depression and the evolution of plant selfing rates

Emmanuelle Porcher[1*], John K. Kelly[2*], Pierre-Olivier Cheptou [3*], Christopher G. Eckert[4], Mark O. Johnston[5], and Susan Kalisz[6]

[1]UMR 5173 MNHN-CNRS-UPMC, Conservation des Espèces, Restauration et Suivi des Populations, Muséum national d'Histoire naturelle, 61 rue Buffon, F-75005 Paris, France.

[2]Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Ave., Lawrence, Kansas 66045-7534 USA

[3]UMR 5175 CEFE, Centre d'Ecologie Fonctionnelle et Evolutive (CNRS), 1919 Route de Mende, F-34293 Montpellier Cedex 05, France.

[4]Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6 Canada

[5]Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1 Canada

[6]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA

[*]Authors contributed equally to this work. Other authors are listed alphabetically.

Running title: Genetics of fluctuating inbreeding depression

Corresponding author: Emmanuelle Porcher

UMR 5173 CERSP, Muséum national d'Histoire naturelle, 61 rue Buffon, F-75005 Paris, France.

Email: porcher@mnhn.fr

Phone: 00 33 1 40 79 53 61

Fax: 00 33 1 40 79 38 35

1   **Abstract**

2   The magnitude of inbreeding depression, a central parameter in the evolution of plant

3   mating systems, can vary depending on environmental conditions. However, the underlying

4   genetic mechanisms causing environmental fluctuations in inbreeding depression, and the

5   consequences of this variation for the evolution of self-fertilization, have been little studied.

6   Here, we consider temporal fluctuations of the selection coefficient in an explicit genetic

7   model of inbreeding depression. We show that substantial variance in inbreeding depression

8   can be generated at equilibrium by fluctuating selection, although the simulated variance

9   tends to be lower than has been measured in experimental studies. Our simulations also reveal

10   that purging of deleterious mutations does not depend on the variance in their selection

11   coefficient. Finally, an evolutionary analysis shows that, in contrast to previous theoretical

12   approaches, intermediate selfing rates are never evolutionarily stable when the variation in

13   inbreeding depression is due to fluctuations in the selection coefficient on deleterious

14   mutations.

15

16   **Keywords**: Mixed mating / genetic load / purging / environmental variation.

17

18

1   **Introduction**

2   Over the last thirty years, theoretical studies of plant mating systems have considered

3   the role of inbreeding depression as central for the evolution of self-fertilization (Lloyd, 1979;

4   Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990). Inbreeding depression is the

5   main evolutionary force that opposes the automatic 50% advantage of selfing (Fisher, 1941),

6   it dictates the cost of seed discounting and determines the extent to which selfing causes

7   reproductive assurance (Lloyd, 1992), so that the predicted outcome of mating system

8   evolution critically depends on inbreeding depression values. Simple evolutionary models

9   with a constant inbreeding depression, δ, predict a dichotomous outcome of evolution:

10   complete selfing when $\delta < 0.5$ and complete outcrossing when $\delta > 0.5$. Importantly,

11   incorporating purging, *i.e.* a decrease in inbreeding depression with increasing population

12   selfing rate due to the elimination of recessive deleterious mutations by selection, does not

13   change these conclusions (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990;

14   but see Uyenoyama & Waller, 1991a). Yet, the existence of natural populations with

15   intermediate stable selfing rates is now widely acknowledged and a variety of theoretical

16   models have been proposed to account for the maintenance of such mixed mating systems

17   (reviewed in Goodwillie et al., 2005). Although several models rely on ecology (e.g.

18   pollination biology, Holsinger, 1991; Johnston, 1998; Vallejo-Marin & Uyenoyama, 2004;

19   Porcher & Lande, 2005; Johnston et al., 2009 or population dynamics, Morgan et al., 2005) to

20   explain mixed mating systems, numerous studies have elaborated on the prominent role of

21   inbreeding depression by examining various genetic mechanisms (including overdominance,

22   biparental inbreeding in isolated or structured populations, or decline in fitness with

23   generations of selfing, Lloyd, 1979; Uyenoyama, 1986; Holsinger, 1988; Uyenoyama &

24   Waller, 1991b; Latta & Ritland, 1993; Ronfort & Couvet, 1995; Rausher & Chang, 1999).

1   Recently studies have demonstrated that the magnitude of inbreeding depression is

2   sensitive to environmental factors (reviewed in Armbruster & Reed, 2005; Cheptou, 2006;

3   Willi et al., 2007). Some theoretical models have shown that environmentally-induced

4   variation in inbreeding depression is likely to influence the evolution of mating systems,

5   particularly to favour mixed mating systems (Cheptou & Mathias, 2001; Cheptou & Schoen,

6   2002). However, these models do not incorporate a genetic basis of inbreeding depression and

7   hence do not include the purging process that destabilizes mixed mating systems in most

8   explicitly genetic models (e.g. Lande et al., 1994; Husband & Schemske, 1996; Kelly, 2007).

9   In addition, the effectiveness of purging itself may depend on environmental fluctuations, as a

10   result of environment-dependent inbreeding depression (e.g. Bijlsma et al., 1999), a

11   phenomenon that has received little theoretical attention so far. To properly assess the actual

12   role of environmental fluctuations in inbreeding depression on the evolution of plant mating

13   systems, we therefore need to (1) determine the genetic mechanisms underlying fluctuations

14   in inbreeding depression and (2) examine the combined effects of fluctuating inbreeding

15   depression and purging on the evolution of selfing.

16   Here, we use a common genetic model of inbreeding depression to examine one

17   possible genetic mechanism of fluctuating inbreeding depression and to test whether such

18   fluctuations can maintain mixed mating systems when purging occurs. Consistent with the

19   bulk of experimental data (Charlesworth & Charlesworth, 1999), we assume that inbreeding

20   depression is caused by partly recessive, deleterious mutations. Such a mutation can be

21   characterized by its selection coefficient (S) and its dominance coefficient (h). From this

22   genetic perspective, environmental variation in inbreeding depression could result from

23   fluctuations in either S or h or both (Bijlsma et al., 1999; Armbruster & Reed, 2005), but little

24   is known so far regarding the most likely source(s) of variation in inbreeding depression

25   (Cheptou, 2006). Numerous experimental observations of larger inbreeding depression in

1 stressful environments (e.g. Bijlsma et al., 1999; and reviews in Armbruster & Reed, 2005;

2 Willi et al., 2007) suggest that environmental conditions may influence the selection

3 coefficient of deleterious alleles, although other experiments comparing the mean fitness of

4 *Arabidopsis thaliana* mutation accumulation lines across light and nutrient gradients failed to

5 demonstrate any environment-specific effects of spontaneous mutations (Chang & Shaw,

6 2003; Kavanaugh & Shaw, 2005). In the present study, we assume that fluctuations in

7 inbreeding depression are caused by temporal changes in the selection coefficient of

8 deleterious mutations. We apply an analytical approach assuming an infinite population size.

9 We address (1) the effect of temporal fluctuations of selection coefficients on the temporal

10 variation in inbreeding depression, and compare this predicted variation to that observed in

11 natural populations, (2) the effectiveness of purging under fluctuating selection, and (3) the

12 evolution of mating systems. We specifically investigate whether intermediate selfing rates

13 are evolutionarily stable when inbreeding depression is subjected to fluctuating selection and

14 is allowed to co-evolve with the mating system.

15

1 **The model**

2   In the following theoretical approach, we define mixed mating systems as mating

3 systems with a selfing rate $r > 0$ and $r < 1$, which is broader than the definition used in

4 empirical studies (generally $0.2 < r < 0.8$; reviewed in Goodwillie et al., 2005), because we

5 focus on genetically determined selfing rates, with no environmental effects and no

6 measurement errors.

7

8 *Inbreeding depression with a fluctuating selection environment*

9   Inbreeding depression, the relative decrease in mean fitness of selfed vs. outcrossed

10 individuals, is described by the model of Kondrashov (1985), which models the evolution of

11 the distribution of the number of homozygous and heterozygous deleterious mutations per

12 individual in an infinite population with selfing rate r. Mutation occurs at an infinite number

13 of loci, following a Poisson process with rate U per diploid zygote per generation. Each

14 generation, the selection coefficient of mutations, S, is randomly sampled from a normal

15 distribution truncated to the interval [0,1], with mean μ_S and variance σ_S^2 before truncation.

16 This selection coefficient is applied to all mutations, which also have the same dominance

17 coefficient, h. Mutations have multiplicative effects on fitness, so that the fitness of an

18 individual carrying x homozygous mutations and y heterozygous mutations is given by $(1 -$

19 $S)^x(1 - hS)^y$.

20   Each generation, the modelled population undergoes mating, followed by mutation to

21 deleterious alleles and selection. Recursion equations describing the distribution of number of

22 homozygous and heterozygous mutations per individual are detailed in an online appendix

23 and can also be found in Kondrashov (1985) or Charlesworth et al. (1990). These equations

24 were iterated for 1000 generations (C++ code for numerical iterations available upon request

25 from E.P.), by which time the population had reached steady-state for all parameter

1   combinations. To identify steady state, we considered the growth rate in the number of

2   mutations across generations, N_{t+1}/N_t: the population was considered at steady state when the

3   average growth rate over 50 generations did not differ significantly from 1 (one sample

4   Student's t-test). Equations were subsequently iterated for another 1000 generations, to record

5   fluctuations in inbreeding depression at (statistical) steady state. For each set of parameter

6   values, we recorded (1) the mean and variance of the number of homozygous and

7   heterozygous deleterious mutations per individual and (2) the mean and variance of

8   inbreeding depression, measured over the last 1000 generations. Because inbreeding

9   depression is constrained between 0 (in the present study) and 1, the variance in inbreeding

10   depression depends on the mean (e.g. the variance must be 0 if the mean is 0 or 1). To account

11   for this dependence on the mean, we compared the simulated variance in inbreeding

12   depression to the maximum possible variance:

13   $\sigma_{max}^2 = \mu_{ID}(1 - \mu_{ID})$ [1]

14   where μ_{ID} is the mean inbreeding depression.

15   The predicted mean and variance in inbreeding depression were compared to empirical

16   means and variances in inbreeding depression, estimated in contrasting environments

17   (compiled by Armbruster & Reed, 2005). Most of these studies measured inbreeding

18   depression in only two environments, which were obviously not chosen at random, but

19   instead likely represent extremes of a distribution (e.g. stressful vs. benign environments).

20   Therefore, it was not appropriate to estimate variance using the classical estimator

21   $s^2 = \frac{1}{n-1}\sum(x_i - \bar{x})^2$. We thus chose to calculate the sample variance, $\sigma_{ID}^2 = \frac{1}{n}\sum(x_i - \bar{x}_{ID})^2$:

22   due to small sample sizes and non-random sampling, this crude procedure provides an order

23   of magnitude estimate for the actual variance in inbreeding depression.

24

1 *Evolutionarily stable mating systems*

2   We analyzed the joint evolution of selfing rate and inbreeding depression using an

3 adaptive dynamics framework (Dieckmann, 1997). The fitness of a rare mutant with selfing

4 rate r' is compared to that of the resident genotype, with selfing rate r. We assumed that the

5 selfing rate is controlled by a single locus, with two alleles. Once the population with selfing

6 rate r reached steady-state (after 1000 generations), a mutant allele with a selfing rate r'

7 different from the resident was introduced at a low frequency in linkage and identity

8 equilibrium with deleterious mutations, and the recursion equations were numerically iterated

9 for 1000 generations to detect invasion (or not) by the mutant.

10   We deduce stable selfing rates using Pairwise Invasibility Plots (PIP), in which regions

11 of invasion (in grey) and non-invasion (in white) of a rare mutant are plotted against the

12 selfing rate of the resident genotype, r and the selfing rate of the mutant, r' (see Fig. 3).

13 Hence, regions located below the $r = r'$ line correspond to emergence of a mutant with a

14 smaller selfing rate than the resident, and vice versa. If a mutant with selfing rate $r' > r$ can

15 invade (i.e. the point with coordinates (r, r') is located in a region of invasion), then evolution

16 favours increased selfing rates and vice versa. Evolutionary equilibria occur at the intersection

17 of the $r = r'$ line and a line separating regions of invasion and non-invasion. Details regarding

18 the criteria to infer stability of equilibria can be found in Dieckmann (1997). The equilibrium

19 selfing rates discussed here, denoted by white circles on the figures, are evolutionarily stable

20 (they cannot be invaded by neighbouring mutant selfing rates) and are evolutionary attractors

21 or convergence stable (evolution by a series of small steps proceeds towards the equilibrium).

22 We refer to these equilibria as stable selfing rates.

23

24 *Parameter values*

1 　　　We either chose parameter values according to experimental values or we used a wide

2 range of biologically plausible values. The genomic mutation rate to deleterious mutations

3 was $U = 0.02$, 0.2 or 1, which encompasses the range of experimental estimates among

4 multicellular organisms (reviewed in García-Dorado et al., 2004). The dominance coefficient

5 of mutations was either $h = 0.02$, as in the only available experimental data for highly

6 deleterious mutations (Simmons & Crow, 1977) or $h = 0.3$, a more reasonable value for

7 mildly deleterious mutations (García-Dorado et al., 2004). Exploratory analyses showed that

8 the mean selection coefficient of deleterious mutations had little effect on the results

9 presented here, which is consistent with previous theory showing that equilibrium inbreeding

10 depression in a population depends little on the selection coefficient of deleterious mutations,

11 and is much more influenced by their dominance coefficient (e.g. Charlesworth et al., 1990).

12 Hence, we considered a single, intermediate value for the average selection coefficient of

13 deleterious mutations, $\mu_S = 0.5$, so that this coefficient, which is constrained between 0 and 1,

14 could be submitted to significant environmental variation. The initial variances were set to

15 $\sigma_S^2 = 0.01$ and 0.09, but the latter was actually smaller ($\sigma_S^2 = 0.06$) due to truncation of S

16 values below 0 or above 1. Note that truncation did not bias the mean selection coefficient.

17 For comparative purposes, we also considered constant selection ($\sigma_S^2 = 0$), with $S = 0.5$.

18 Finally, we considered different values of the selfing rate, across the range of $r = 0$ to 1.

19

20 *Analytical approximation*

21 　　　To characterize the effect of purging on the selection of self-fertilization in a fluctuating

22 environment, we compared the results of the Kondrashov model with stochastic variation in

23 selection coefficients to Cheptou and Schoen's (2002) analytical phenotypic approach.

24 Following Lande and Schemske (1985), an approximate measure of the strength of selection

25 on a rare genotype with selfing rate r' is given by the expected fitness of such genotypes:

1 $$w = r'(1-\delta) + \frac{1}{2}(1-r') + \frac{1}{2}(1-r) \qquad\qquad [2]$$

2 where r is the mean selfing rate in the population and δ is the inbreeding depression.

3 If δ varies in time and if evolution of the selfing rate occurs much slower than the fluctuations

4 in inbreeding depression, the conditions for invasion of a rare genotype with selfing rate r' are

5 given by its long-run growth rate (Lande, 2007, 2008), i.e. the geometric mean of fitness over

6 time or similarly by the expectation of the logarithm of the annual fitness, E[Log(w)] (Kisdi &

7 Meszena, 1995). Without any specific information on the distribution of δ, this quantity can

8 be approximated using a second order approximation (Jensen approximation: Bulmer, 1994)

9 by:

10 $$s = E[\log(w)] \approx \log[E(w)] - \frac{1}{2\,E(w)^2}\,\mathrm{var}(w) \qquad\qquad [3]$$

11 Assuming small effects of mutation on the selfing rate, the evolution of self-fertilization can

12 be analysed by modelling the invasion of a rare genotype with selfing r' in a population with

13 selfing rate r. The success of invasion can be evaluated via $D(r)$, the selection gradient

14 $\partial s / \partial r'$ at r: if the latter is positive (negative) mutants with slightly higher (lower) selfing rate

15 may successfully invade. The selection gradient $D(r)$ for an inbreeding depression

16 distribution with mean μ_δ and variance σ_δ^2 is given by:

17 $$D(r) = \frac{0.5 - \mu_\delta}{1 - \mu_\delta r} - \frac{\left(1 - \dfrac{r}{2}\right)r}{(1-\mu r)^3}\sigma^2 \qquad\qquad [4]$$

18 (see Cheptou & Schoen, 2002 for details). A necessary condition for mixed selfing rates to be

19 stable is thus that $D(r) = 0$ (no mutant can invade) for intermediate values of the selfing rate

20 ($0 < r < 1$). Moreover, a sufficient and more stringent condition for mixed selfing rates to

21 evolve is that boundaries ($r = 0$ and $r = 1$) are unstable, i.e.:

22   (1) $D(0) > 0$ (Increased selfing is favoured in a fully outcrossing population)

1   (2) $D(1) < 0$ (Increased outcrossing is favoured in a fully selfing population)

2   We incorporated purging in this analytical approach by making both μ_δ and σ_δ^2

3   functions of the population selfing rate, r. This and equation [4] yield the following conditions

4   for maintenance of mixed mating:

5   (1) $\mu_\delta(0) < 0.5$ [5]

6   (2) $\sigma_\delta^2(1) > 2[1 - \mu_\delta(1)]^2[0.5 - \mu_\delta(1)]$ [6]

7   We tested whether the values generated by the Kondrashov model with fluctuating selection

8   (mean and variance of inbreeding depression at $r = 0$ and $r = 1$) met these conditions for the

9   maintenance of mixed mating.

10
11

1 **Results**

2 *Effectiveness of purging under fluctuating inbreeding depression*

3   Temporal variation in the selection coefficient of deleterious mutations appeared to

4 have little effect on the extent of purging (Fig. 1). Regardless of the mean (not shown) and

5 variance of selection coefficients, the relationship between the average inbreeding depression

6 at equilibrium and the population selfing rate differed little between variable vs. constant

7 selection, i.e. the occurrence of purging was unchanged as the variance in selection

8 coefficients was increased. As expected, inbreeding depression was large in predominantly

9 outcrossing populations, and decreased with increasing selfing rate. The decrease was

10 progressive under moderate mutation rate (U = 0.02 or 0.2, Fig. 1) or large dominance

11 coefficient (h = 0.3, Fig. 1), but sharper under higher mutation rates and with nearly recessive

12 mutations (U = 1 and h = 0.02, Fig. 1). This pattern was unaffected by fluctuations in the

13 selection coefficient of deleterious mutations, although the average inbreeding depression in a

14 predominantly outcrossing population tended to be slightly lower under large fluctuations of

15 selection than in a constant environment (Fig. 1, σ_S = 0.3 and h = 0.02).

16

17 *Magnitude of variation in inbreeding depression*

18   As expected, temporal fluctuations in the selection coefficient of deleterious mutations

19 generated variation in inbreeding depression across generations. Variation in inbreeding

20 depression increased with the selection coefficient, σ_S^2 up to ca. 0.05. It was also affected by

21 the population selfing rate and the purging process, with a peak in variance when the average

22 inbreeding depression was closest to 0.5. Because mean and variance of inbreeding

23 depression are not independent (equation [1]), the changes in variance under different

24 population selfing rates were in great part attributable to changes in mean inbreeding

25 depression. Depending on the mutation rate and dominance coefficient of deleterious

1   mutations, variation in mean inbreeding depression explained 51-97% of the variance of

2   inbreeding depression (these values were obtained by examining the correlation between the

3   variance in the simulations and the maximum variance in inbreeding depression calculated

4   from equation [1]).

5

6   *Comparison of simulated vs. observed variation in inbreeding depression*

7   Overall, the empirical data generally exhibited more variation in inbreeding depression

8   than simulations (Fig. 2, σ^2_{ID} up to 0.18 and 0.046, respectively). The parameter space of μ_δ

9   (mean inbreeding depression) and σ^2_δ (variance in inbreeding depression) where the

10   analytical model of Cheptou and Schoen (2002) predicts stable mixed mating systems

11   (equation [4], $D(r) = 0$) are plotted on Figure 2 (shaded area). Interestingly, none of the

12   empirical points fall within this parameter space. Despite relatively low variance in

13   inbreeding depression generated by our theoretical approach, the simulations nonetheless

14   generated some situations for which Cheptou and Schoen's (2002) model predicts stable

15   mixed mating: this was the case with $U = 0.2$, $h = 0.02$, $\sigma_S = 0.3$ and $U = 1$, $h = 0.02$, $\sigma_S = 0.3$

16   (combination of μ_δ and σ_δ^2 within the shaded area, Fig. 2).

17

18   *Evolution of self-fertilization under fluctuating inbreeding depression.*

19   Evolutionarily stable selfing rates can be deduced from Pairwise Invasibility Plots

20   (PIP, Fig. 3), where regions of invasion (in grey) and non-invasion (in white) of a rare mutant

21   are plotted against the selfing rate of the resident genotype, r and that of the mutant, r'.

22   Evolutionarily stable selfing rates are located at the intersection of the $r = r'$ line and a line

23   separating regions of invasion and non-invasion, and are characterized by the fact that no

24   mutant can invade (i.e. the areas just above and below are white on the PIP). The main result

25   of our study is that intermediate selfing rates were never evolutionarily stable (Fig. 3), even

though large variances in the selection coefficient of deleterious mutations generated

fluctuations in the magnitude of inbreeding depression. This was true regardless of the

mutation rate, the dominance coefficient of deleterious mutation, as well as the mean and

variance of the selection coefficient of mutations (and hence variance in inbreeding

depression). The only stable mating systems were complete outcrossing with nearly

codominant deleterious mutations ($h = 0.3$, Fig. 3) and complete outcrossing or complete

selfing (depending on the initial conditions) with nearly recessive mutations ($h = 0.02$, Fig. 3).

In addition, the threshold for the selection of pure selfing or pure outcrossing did not depend

on the variance in inbreeding depression. Thus, in our genetic model, fluctuations in

inbreeding depression do not favour the maintenance of mixed mating.

This was confirmed by considering the modified version of Cheptou and Schoen

(2002) analytical approach, accounting for purging via selfing rate dependent inbreeding

depression. Under this model, condition (2) for the maintenance of stable mixed mating

(namely $D(1) < 0$, invasion of a mutant with selfing rate < 1 in a completely selfing

population) requires substantial variance in inbreeding depression in a completely selfing

population where average inbreeding depression is generally low. Under the Kondrashov

model, high variance is generated by a high mutation rate (e.g. $U = 1$, Fig. 1). However, this

set of parameters also generates high average inbreeding depression (μ_δ close to 1) at $r = 0$,

which invalidates condition (1) for the maintenance of mixed mating (namely $D(0) > 0$,

invasion of a mutant with the selfing rate > 0 in a completely outcrossing population, $\Leftrightarrow$

$\mu_\delta < 0.5$). Hence, although some combinations of μ_δ and σ_δ fell within the 'mixed mating'

window of Fig. 2, sufficient conditions for stable mixed mating (1) and (2) were never met.

Mixed mating can therefore never be maintained when inbreeding depression is modelled

with partly recessive deleterious mutations in an infinite population (as in Kondrashov, 1985).

1 **Discussion**

2   In this study, we generated fluctuating inbreeding depression using a genetic model

3 where environmental variation affects the selection coefficient. This allowed us to

4 characterize purging under a fluctuating environment and analyse the evolution of plant

5 mating systems in this context, specifically testing for conditions that allow the maintenance

6 of mixed mating.

7

8 *Fluctuations in selection coefficients have little effect on purging*

9   Temporal variation in the selection coefficient of the deleterious mutations has very

10 little effect on the average equilibrium inbreeding depression. This is consistent with single

11 locus theory in randomly mating populations: Crow and Kimura (1970) showed that with

12 incompletely recessive ($h > 0$), mildly deleterious mutations ($S \gg u$) mutant homozygotes are

13 kept sufficiently rare by selection so that most selection occurs against mutant heterozyogtes.

14 As a result, the dynamics of the mutant allele in a constant environment are approximately a

15 linear function of the selection coefficient, S, and can be written $dq/dt \approx u - hSq$ (Crow &

16 Kimura, 1970; Lande & Schemske, 1985), where q is the mutant allele frequency and u is the

17 per locus mutation rate. With fluctuating selection, this yields $E(dq/dt) = u - E(hS)q$. At

18 equilibrium ($dq/dt = 0$), $q = u/E(hS)$. Hence, in randomly mating populations under fluctuating

19 selection, inbreeding depression at a single locus depends solely on the mean selection

20 coefficient (or dominance coefficient) of the deleterious mutation, not on its variance. Our

21 results extend these predictions with a single locus to many loci, as well as non-random

22 mating. Similarly, when selfing is enforced in a previously completely outcrossing

23 population, fluctuations do not affect the time required for the population to reach the new

24 equilibrium inbreeding depression (not shown), which is due to the fact that, under complete

25 selfing, highly recessive deleterious mutations are quickly exposed to selection and a few

1 generations of strong selection are enough to purge most of the load (e.g. Lande & Schemske,

2 1985).

3   A number of experimental approaches have demonstrated that inbreeding depression

4 varies across environments and generally appears to be stronger in more stressful

5 environments, generally defined as environments reducing mean fitness (Armbruster & Reed,

6 2005). As a consequence, purging might be inefficient in relatively benign environments,

7 where inbreeding depression is weak (e.g. Bijlsma et al., 1999; Keller & Waller, 2002;

8 Armbruster & Reed, 2005). Such faster reduction in inbreeding depression was observed by

9 Kristensen et al. (2003) and Swindell and Bouzat (2006) in inbred laboratory populations of

10 *Drosophila* species submitted to stressful environments (high temperature, competition,

11 chemical stress) vs. benign environments. Their *Drosophila* data are however not directly

12 comparable to our theoretical results suggesting that variable selection has little effect on

13 purging because in these experimental studies, environment-dependent purging was examined

14 in two or more contrasting, but constant environments. In the variable environment simulated

15 here, our results suggest that only five to ten generations of stress can increase inbreeding

16 depression and are enough to purge most of the load (not shown), regardless of the average

17 effects of mutations/stress in the environment, because the rate of purging is much faster than

18 mutation accumulation (Lande & Schemske, 1985).

19

20 *Fluctuations in selection coefficients can generate substantial variation in inbreeding*

21 *depression*

22   Although the time-averaged inbreeding depression is little affected by fluctuating

23 selection, the *current* inbreeding depression can exhibit substantial variation across

24 environments. In the present study, we considered mutations that were moderately deleterious

25 on average ($\mu_S = 0.5$), but this intermediate selection coefficient was allowed to vary between

17

1 0 (neutral mutations) and 1 (lethal mutations) following a truncated normal distribution with

2 variance between 0.01 and 0.06. As a result, the observed variance in inbreeding depression

3 ranged between 5 x 10^{-4} and 0.05, and was highest when the mean inbreeding depression was

4 close to 0.5, which was generally observed at intermediate selfing rates (with nearly recessive

5 deleterious mutations, $h = 0.02$, Fig. 1). Although empirical data on variation in inbreeding

6 depression magnitude are scarce, several studies (e.g. Jiménez et al., 1994; Koelewijn, 1998;

7 Cheptou et al., 2001; Haag et al., 2002) find that their study organisms exhibit more variation

8 in inbreeding depression than any of our simulations (regardless of parameter values). This

9 would suggest that varying S in time, as simulated here, might not be the only mechanism

10 generating the observed variation in the experimental data. It is however important to keep in

11 mind that experiments on inbreeding depression do not necessarily mimic natural conditions,

12 and that the variance in inbreeding depression was generally estimated from two data points

13 (i.e. two experimental conditions). Obviously there is a need for experimental studies that

14 estimate inbreeding depression over wider ranges of environmental conditions. In addition,

15 the magnitude of inbreeding depression in natural populations depends on the history of

16 populations (Kristensen et al., 2003; Leimu et al., 2008), so that it could be less sensitive to

17 environmental variation than suggested by greenhouse experiments (e.g. Galloway &

18 Etterson, 2007). Comparison of inbreeding depression variation in the wild (see for instance

19 Dole & Ritland, 1993) would be more relevant.

20

21 *Purging cancels out the role of fluctuating inbreeding depression in the maintenance of mixed*

22 *mating systems*

23 Based on a phenotypic model with no purging of deleterious mutations, Cheptou and

24 Schoen (2002) predicted that intermediate selfing rates can be stable with fluctuating

25 inbreeding depression under some combinations of mean and variance in inbreeding

depression (see Fig. 2). Our results with an explicit genetic model of inbreeding depression contrast with their predictions: mixed mating systems were never evolutionarily stable, although some parameter combinations appear to generate a mean and variance of inbreeding depression consistent with stable mixed mating systems under Cheptou and Schoen's approach (Fig 2, e.g. a high mutation rate to deleterious alleles ($U = 1$), nearly recessive mutations ($h = 0.02$) and substantial fluctuation in the selection coefficient of deleterious mutations). Evidently, it is purging in our model that makes mixed mating unstable. While the mean and variance are independent parameters in Cheptou and Schoen's model, these quantities intrinsically covary in an explicit genetic model. Purging creates a strong positive feedback that selects for selfing (Lande & Schemske, 1985) and that overwhelms the negative feedback produced by fluctuating inbreeding depression (Cheptou & Schoen, 2002).

A heuristic understanding of why the genetic architecture of inbreeding depression can disrupt the maintenance of intermediate selfing rates was seen using an analytical approach. By incorporating selfing rate-dependent mean and variance of inbreeding depression into Cheptou and Schoen's (2002) analytical approach, we derived sufficient conditions for mixed mating: moderate mean inbreeding depression under complete outcrossing ($\mu_\delta < 0.5$) together with appreciable variance in inbreeding depression under complete selfing. In our simulations, these conditions were never met because the genetic architecture of inbreeding depression generates two contrasting situations that depend on the characteristics of the deleterious mutation. First, a low mutation rate (not shown) or nearly codominant deleterious mutations that can be eliminated by selection even in a heterozygous state ($h = 0.3$, Fig. 1) result in moderate inbreeding depression in a completely outcrossing population (condition (1)). However, this also generates a low inbreeding depression (and hence a low variance in inbreeding depression) under complete selfing, due to extensive purging, so that condition (2) is not met. Alternatively, a high mutation rate to nearly recessive mutations ($U = 1$ and $h = $

1 0.02, Fig. 1) creates situations in which the mean inbreeding depression under complete

2 selfing is close to 0.5, so that the variance in inbreeding depression can be large (condition

3 (2)). However, with this characteristic of mutations, condition (1) is not met due to the

4 absence of purging in a completely outcrossing population, generating large mean inbreeding

5 depression.

6

7 *Some limits of the model*

8   Although fluctuating selection coefficients can generate substantial inbreeding

9 depression, our approach suggests that when purging occurs, fluctuating inbreeding

10 depression is very unlikely to maintain intermediate selfing rates. This probably depends

11 strongly on several assumptions of our model. First, Kondrashov's (1985) approach assumes

12 infinite population size, whereas in natural populations purging is a stochastic process (Byers

13 & Waller, 1999) that can be slowed down in small populations (Glémin, 2003), but should

14 also be reinforced by the possibility of biparental inbreeding in finite populations. The role of

15 demographic stochasticity in the evolution of genetic load and mating systems has been

16 addressed in a limited number of studies (e.g. Charlesworth et al., 1992; Wang et al., 1999;

17 Bataillon & Kirkpatrick, 2000; Theodorou & Couvet, 2002; Glémin, 2003; Guillaume &

18 Perrin, 2006) but the interaction between demography, genetics and environmental variation

19 remains to be explored. Second, we assumed that environmental variation affected the

20 selection coefficient of a large number of strictly identical, unlinked deleterious mutations.

21 Although little is known regarding the actual mechanisms for environment-dependent

22 inbreeding depression (Armbruster & Reed, 2005), recent studies suggest the existence of

23 environment-specific deleterious alleles (e.g. Vermeulen & Bijlsma, 2004), i.e. alleles that are

24 usually neutral but become deleterious in specific (e.g. stressful) environments. Such

25 environment specific alleles, which are rarely submitted to selection, may accumulate in the

genome and eventually add up to environment-independent inbreeding depression in stressful

environments, which can affect the evolution of mating systems. However, our model

suggests that their effects are likely to remain minor if these alleles are expressed frequently

enough, because purging occurs within a couple of generations under selection. Lastly, other

genetic mechanisms such as variation in dominance or even purely additive gene action when

the strength of stabilizing selection varies may create inbreeding depression variance under

heterogeneous environments (Lande & Schemske, 1985).

Conclusion

Our model attempts to link theoretical genetic models of inbreeding depression with the

empirical observations that inbreeding depression is sensitive to environmental conditions.

We extend Kondrashov's framework to heterogeneous environments and show that

temporally varying selection on deleterious mutations maintains substantial variance in

inbreeding depression. However, this mechanism cannot in itself maintain stable mixed

selfing rates. Additional experimental and theoretical work is needed to elucidate the genetic

basis of environmental variation in inbreeding depression and its consequences for the

evolution of mating systems.

1   funded by Saint Gobain

1 **References**

2 Armbruster, P. & Reed, D.H. 2005. Inbreeding depression in benign and stressful

3   environments. *Heredity* **95**: 235-242.

4 Bataillon T. & Kirkpatrick, M. 2000. Inbreeding depression due to mildly deleterious

5   mutations in finite populations: size does matter. *Genet. Res.* **75**: 75-81.

6 Bijlsma, R., Bundgaard, J. & Van Putten, W.F. 1999. Environmental dependence of

7   inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* **12**: 1125-

8   1137.

9 Bulmer, M. 1994. *Theoretical evolutionary ecology*. Sinauer, Sunderland, MA.

10 Byers, D.L. & Waller, D.M. 1999. Do plant populations purge their genetic load? Effects of

11   population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Evol.*

12   *Syst.* **30**: 479-513.

13 Chang, S.M. & Shaw, R.G. 2003. The contribution of spontaneous mutation to variation in

14   environmental response in *Arabidopsis thaliana*: Responses to nutrients. *Evolution* **57**:

15   984-994.

16 Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression.

17   *Genet. Res.* **74**: 329-340.

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 Charlesworth, D., Morgan, M.T. & Charlesworth, B. 1992. The effect of linkage and

25   population-size on inbreeding depression due to mutational load. *Genet. Res.* **59**: 49-61.

Cheptou, P.O. 2006. Population biology - The ecology of inbreeding depression. *Heredity* **96**: 110-110.

Cheptou, P.O., Lepart, J. & Escarré, J. 2001. Inbreeding depression under intraspecific competition in a highly outcrossing population of *Crepis sancta* (Asteraceae): evidence for frequency-dependant variation. *Am. J. Bot.* **88**: 1424-1429.

Cheptou, P.O. & Mathias, A. 2001. Can varying inbreeding depression select for intermediary selfing rates? *Am. Nat.* **157**: 361-373.

Cheptou, P.O. & Schoen, D.J. 2002. The cost of fluctuating inbreeding depression. *Evolution* **56**: 1059-1062.

Crow, J.F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*. Harper and Row, New-York.

Dieckmann, U. 1997. Can adaptive dynamics invade? *Trends Ecol. Evol.* **12**: 128-131.

Dole, J. & Ritland, K. 1993. Inbreeding depression in two Mimulus taxa measured by multigenerational changes in the inbreeding coefficient. *Evolution* **47**: 361-373.

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

Galloway, L.F. & Etterson, J.R. 2007. Inbreeding depression in an autotetraploid herb: a three cohort field study. *New Phytol.* **173**: 383-392.

García-Dorado, A., López-Fanjul, C. & Caballero, A. 2004. Rates and effects of deleterious mutations and their evolutionary consequences. In: *Evolution of Molecules and Ecosystems* (A. Moya & E. Font, eds), pp. 20–32. Oxford University Press, NewYork.

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

Goodwillie, C., Kalisz, S. & Eckert, C.G. 2005. The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Ann.*

Rev. Ecol. Evol. Syst. **36**: 47-79.

Guillaume, F. & Perrin, N. 2006. Joint evolution of dispersal and inbreeding load. *Genetics* **173**: 497-509.

Haag, C.R., Hottinger, J.W., Riek, M. & Ebert, D. 2002. Strong inbreeding depression in a Daphnia metapopulation. *Evolution* **56**: 518–526.

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

Holsinger, K.E. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat*. **138**: 606-622.

Husband, B.C. & Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54-70.

Jiménez, J.A., Hughes, K.A., Alaks, G., Graham, L. & Lacy, R.C. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* **266**: 271–273.

Johnston, M.O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* **102/103**: 267-278.

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

Kavanaugh, C.M. & Shaw, R.G. 2005. The contribution of spontaneous mutation to variation in environmental responses of *Arabidopsis thaliana*: Responses to light. *Evolution* **59**: 266-275.

Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol*. **17**: 230-241.

Kelly, J.K. 2007. Mutation-selection balance in mixed mating populations. *J. Theor. Biol.* **246**: 355 -365.

1   Kisdi, V. & Meszena, G. 1995. Life-histories with lottery competition in a stochastic

2   environment - ESSs which do not prevail. *Theor. Popul. Biol.* **47**: 191-211.

3   Koelewijn, H.P. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago*

4   *coronopus*. *Evolution* **52**: 692–702.

5   Kondrashov, A.S. 1985. Deleterious mutations as an evolutionary factor. II. Facultative

6   apomixis and selfing. *Genetics* **111**: 635-653.

7   Kristensen, T.N., Dahlgaard, J. & Loeschcke, V. 2003. Effects of inbreeding and

8   environmental stress on fitness - using *Drosophila buzzatii* as a model organism.

9   *Conserv. Genet.* **4**: 453-465.

10   Lande, R. & Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding

11   depression in plants. 1. Genetics models. *Evolution* **39**: 24-40.

12   Lande, R., Schemske, D.W. & Schultz, S.T. 1994. High inbreeding depression, selective

13   interference among loci, and the threshold selfing rate for purging recessive lethal

14   mutations. *Evolution* **48**: 965-978.

15   Lande, R. 2007. Expected relative fitness and the adaptive topography of fluctuating

16   selection. *Evolution* **61**: 1835–1846.

17   Lande, R. 2008. Adaptive topography of fluctuating selection in a Mendelian population. *J.*

18   *Evol. Biol.* **21**: 1096-1105.

19   Latta, R.G. & Ritland, K.R. 1993. Models for the evolution of selfing under alternative modes

20   of inheritance. *Heredity* **71**: 1-10.

21   Leimu, R., Kloss, L. & Fischer, M. 2008. Effects of experimental inbreeding on herbivore

22   resistance and plant fitness: the role of history of inbreeding, herbivory and abiotic

23   factors. *Ecol. Lett.* **11**: 1101-1110.

24   Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in

25   plants. *Am. Nat.* **113**: 67-69.

Lloyd, D.G. 1992. Self and cross fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant. Sci.* **153**: 370-380.

Morgan, M.T., Wilson, W.G. & Knight, T.M. 2005. Plant population dynamics, pollinator foraging, and the selection of self-fertilization. *Am. Nat.* **166**: 169-183.

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

Rausher, M.D. & Chang, S.M. 1999. Stabilization of mixed-mating systems by differences in the magnitude of inbreeding depression for male and female fitness components. *Am. Nat.* **154**: 242-248.

Ronfort, J. & Couvet, D. 1995. A stochastic model of selection on selfing rates in structured populations. *Genet. Res*. **65**: 209-222.

Simmons, M.J. & Crow, J.F. 1977. Mutations affecting fitness in Drosophila populations. *Annu. Rev. Genet*. 11: 49-78.

Swindell, W.R. & Bouzat, J.L. 2006. Associations between environmental stress, selection history, and quantitative genetic variation in *Drosophila melanogaster*. *Genetica* **127**: 311-320.

Theodorou, K. & Couvet, D. 2002. Inbreeding depression and heterosis in a subdivided population: influence of the mating system. *Genet. Res.* **80**: 107-116.

Uyenoyama, M.K. 1986. Inbreeding depression and the cost of meiosis: the evolution of selfing in population practicing biparental inbreeding. *Evolution* **40**: 388-404.

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

Uyenoyama, M.K. & Waller, D.M. 1991b. Coevolution of self-fertilization and inbreeding depression. 2. Symmetrical overdominance in viability. *Theor. Popul. Biol.* **40**: 47-77.

1   Vallejo-Marin, M. & Uyenoyama, M.K. 2004. On the evolutionary costs of self-

2   incompatibility: Incomplete reproductive compensation due to pollen limitation.

3   *Evolution* **58**: 1924-1935.

4   Vermeulen, C.J. & Bijlsma, R. 2004. Characterization of conditionally expressed mutants

5   affecting age-specific *Drosophila melanogaster*: Lethal conditions and temperature-

6   sensitive periods. *Genetics* **167**: 1241-1248.

7   Wang, J.L., Hill, W.G., Charlesworth, D. & Charlesworth, B. 1999. Dynamics of inbreeding

8   depression due to deleterious mutations in small populations: mutation parameters and

9   inbreeding rate. *Genet. Res.* **74**: 165-178.

10   Willi, Y., Dietrich, S. & van Kleunen, M. 2007. Inter-specific competitive stress does not

11   affect the magnitude of inbreeding depression. *Evol. Ecol. Res.* **9**: 959–974.

12

Figure Legends

2

3 **Figure 1**: Equilibrium inbreeding depression under fluctuating selection.

4 Mean inbreeding depression (thick black lines) and variance in inbreeding depression (grey

5 lines, measured over generations) are calculated over 1000 generations at steady state. The

6 average selection coefficient of deleterious mutations is $\mu_S = 0.5$. Expected inbreeding

7 depression in a constant environment ($\sigma_S = 0$, thin black lines) is sometimes exactly

8 superimposed on the curves of mean inbreeding depression.

9

10 **Figure 2**: Predicted and empirically derived estimates of the mean and variance in inbreeding

11 depression. Empirical data (black circles) are from Armbruster and Reed (2005). Predicted

12 values (squares and triangles) are from our simulation approach and are the same as in Figure

13 1. The shaded area corresponds to combinations of mean and variance in inbreeding

14 depression for which mixed mating systems are expected to be stable under Cheptou and

15 Schoen's (2002) model, based on Jensen approximation (see Cheptou & Schoen, 2002 for a

16 numerical example using a truncated Gaussian distribution). The dotted black envelope line

17 represents the maximum variance in inbreeding depression $\mu_\delta(1-\mu_\delta)$, as a function of the

18 mean inbreeding depression, μ_δ $(0 < \mu_\delta < 1)$.

19

20 **Figure 3**: Evolutionarily stable selfing rates under fluctuating inbreeding depression. The

21 average selection coefficient of deleterious mutations is $\mu_S = 0.5$. Regions where the mutant

22 invades ('+') are in grey, regions where it does not invade are in white. White circles indicate
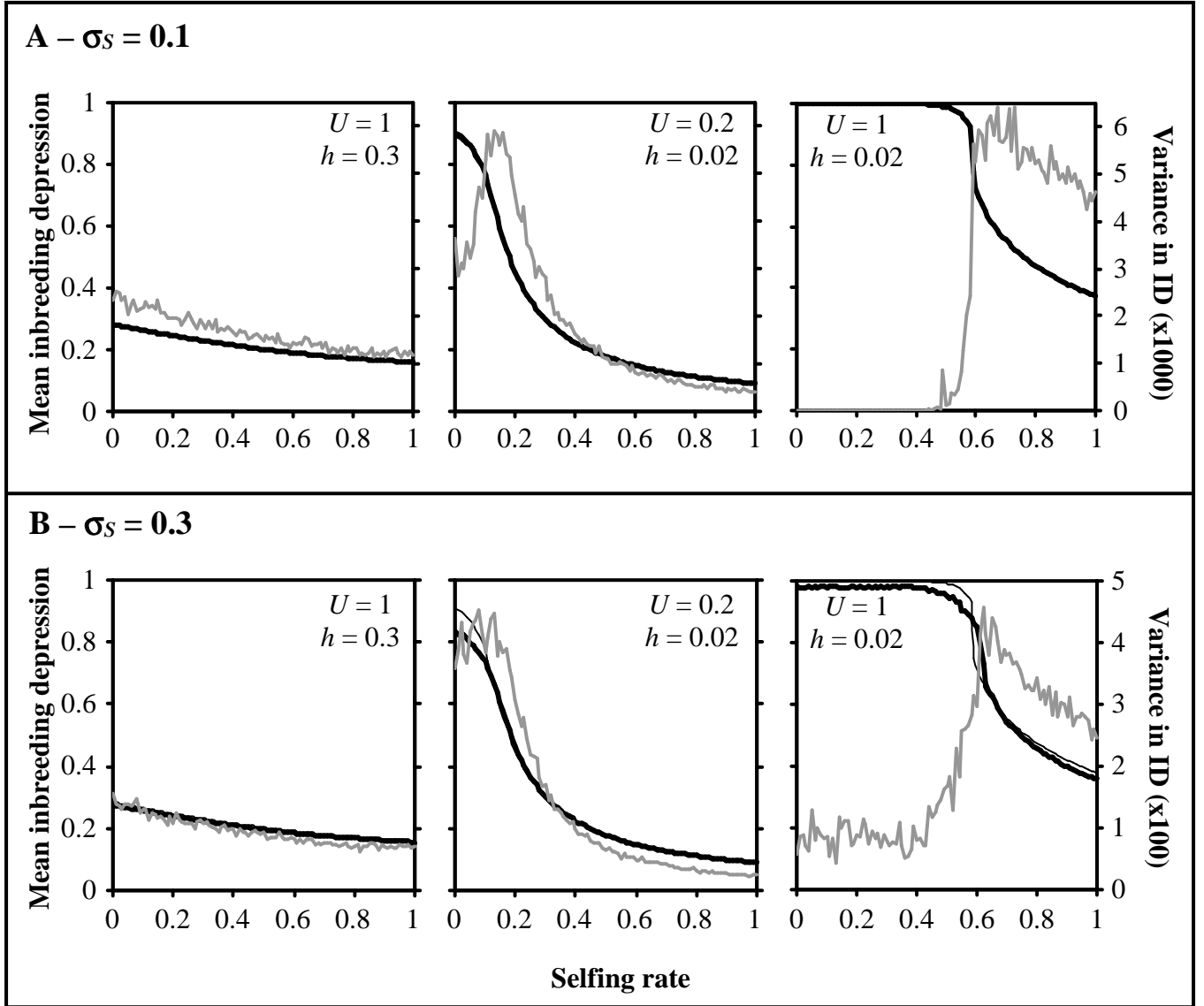
23 evolutionarily stable selfing rates.

24

25

26

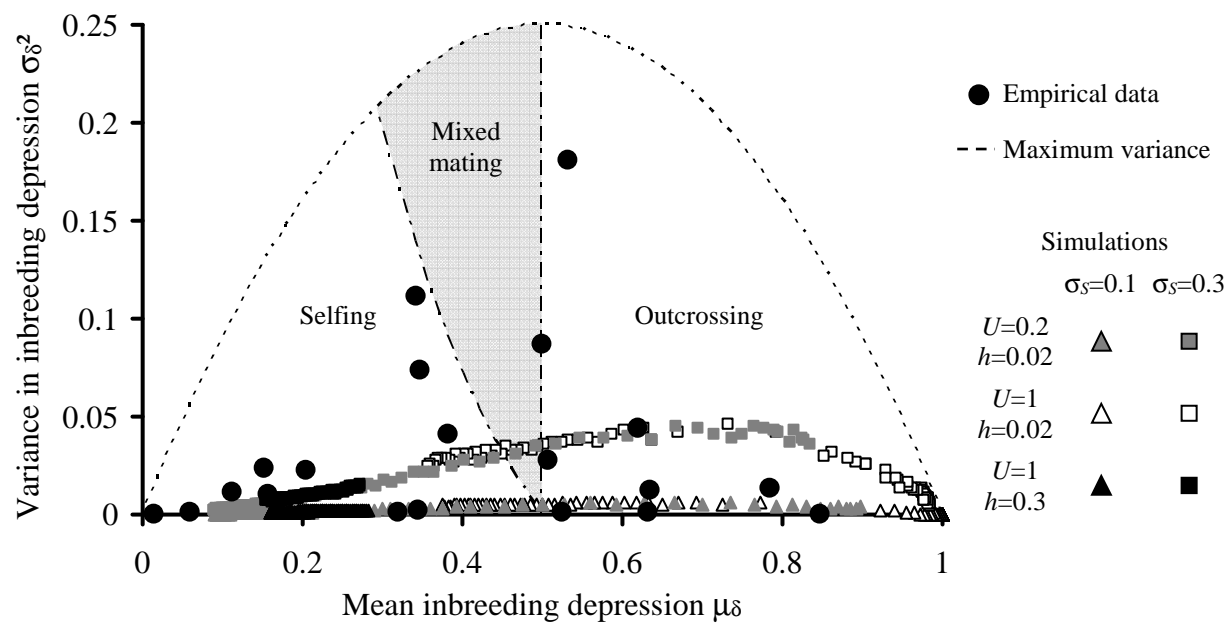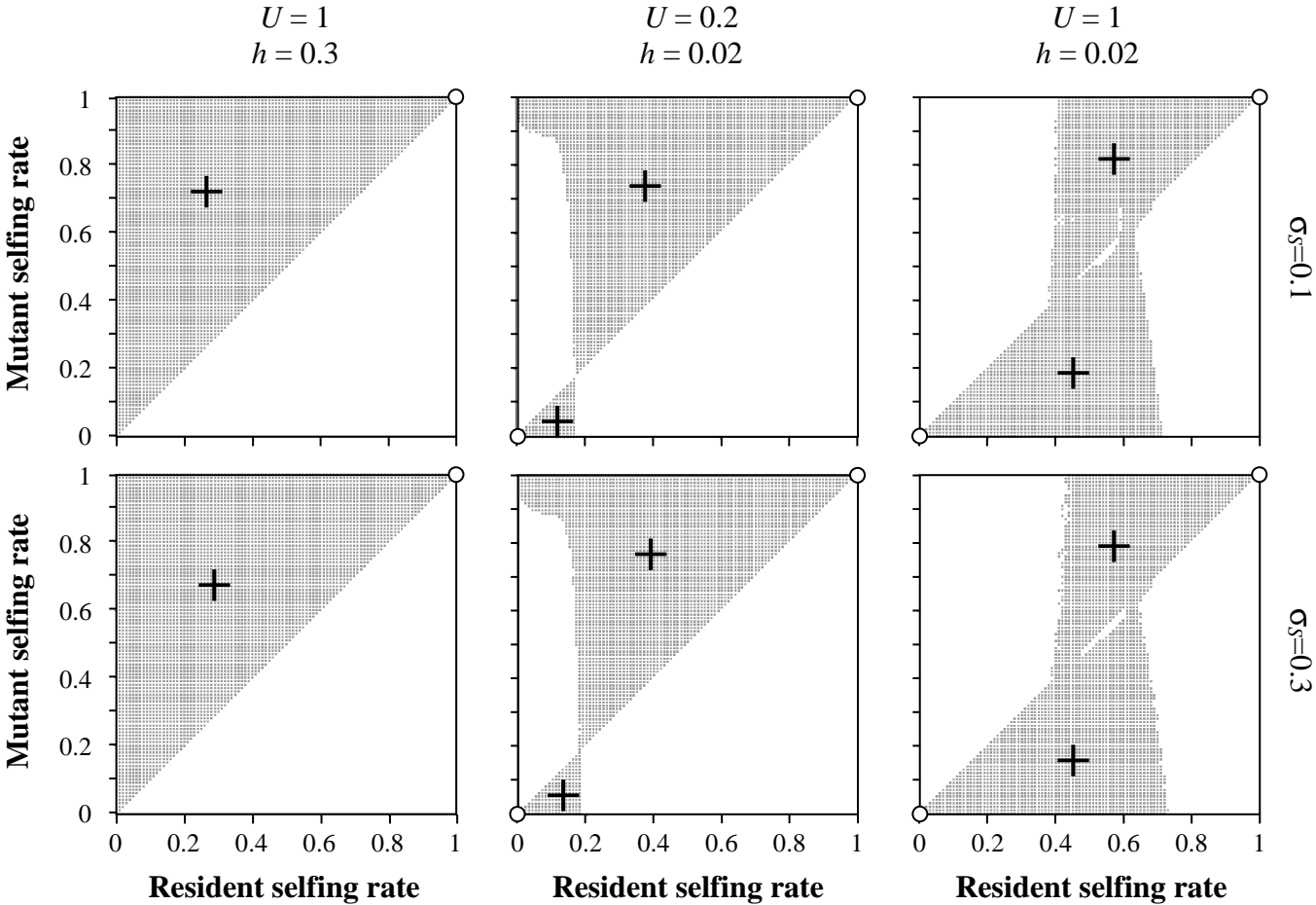**Figure 1**

Figure 2

Figure 3

Supporting information

Recursion equations for Kondrashov's model with fluctuating selection.

I. General equations

Here we derive recursion equations for the model describing the dynamics of inbreeding depression due partly recessive deleterious mutations in an infinite population with selfing rate r undergoing fluctuating selection. The dominance coefficient of mutations is h and their selection coefficient, S, is sampled at random each generation in a truncated Gaussian distribution with mean μ_S and variance σ_S^2. Mutation occurs at an infinite number of loci, and affects the number of heterozygous loci only, i.e. it occurs exclusively at loci that do not already carry mutations.

Let $q(ij)$ be the frequency of individuals carrying i mutations in the heterozygous sate and j mutations in the homozygous state. Similarly, $f(i)$ is the frequency of gametes carrying i deleterious mutations.

a. Selfing

The probability that a plant carrying n_1 heterozygous loci and n_2 homozygous loci produces by selfing an offspring carrying i ($0 \leq i \leq n_1$) heterozygous loci and j ($n_2 \leq j \leq n_1 + n_2$) homozygous loci is:

$$\binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\frac{1}{2}\right)^{2n_1-i}$$

Hence, the frequency of plants with i heterozygous loci and j homozygous loci obtained by selfing is:

$$q^{'}(ij) = r\sum_{n_1=i}^{\infty}\sum_{n_2=0}^{j}\binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\frac{1}{2}\right)^{2n_1-i} q(n_1n_2) \qquad (A1)$$

b. Outcrossing

The probability that a (n_1, n_2) plant produces a gamete with i mutations ($n_2 \leq i \leq n_1 + n_2$) is:

$$\binom{n_1}{i-n_2}\left(\frac{1}{2}\right)^{i-n_2}\left(\frac{1}{2}\right)^{n_1-i+n_2}$$

Hence, the frequency of gametes carrying i mutations in the population is:

$$f(i) = \sum_{n_2=0}^{i}\sum_{n_1=i-n_2}^{\infty}\binom{n_1}{i-n_2}\left(\frac{1}{2}\right)^{n_1} q(n_1n_2) \qquad (A2)$$

Because each mutation is unique, random mating in an infinite population never generates homozygous mutations. Therefore, the probability that an individual carrying n_1 heterozygous

and n_2 homozygous mutations produces by outcrossing a zygote with i heterozygous mutations (and no mutation in the homozygous state) is:

$$q(i0) = (1-r)\sum_{k=0}^{i} f_k f_{i-k} \qquad (A3)$$

c. Mutation and selection

Mutation follows a Poisson process, with mean number of new heterozygous deleterious mutations per genome of U per generation. The frequencies of zygotes after mutation are therefore:

$$q^{''}(ij) = \sum_{k=0}^{i} \frac{U^{i-k}e^{-U}}{(i-k)!} q^{'}(kj) \qquad (A4)$$

The probability that a zygote with x homozygous mutations and y heterozygous mutations survives to maturity is $(1 - S)^x(1 - hS)^y$. The frequency of mature plants with i heterozygous loci and j homozygous loci in the next generation is then:

$$q^{*}(ij) = \frac{(1-S)^j (1-hS)^i q^{''}(ij)}{\overline{w}} \qquad (A5)$$

where $\overline{w}$ is the mean fitness of the population:

$$\overline{w} = \sum_{i=0}^{\infty} \sum_{j=0}^{\infty} \left[(1-S)^j (1-hS)^i q^{''}(ij) \right] \qquad (A6)$$

Equations (A1–A6) completely define the recursion system. For each run, the initial population contained no deleterious mutation. Fluctuating selection was modeled by sampling each generation the selection coefficient of deleterious mutations, S, in a Gaussian distribution with mean μ_S and variance σ_S^2 (equations A5 and A6). Simulations were run until the system closely approached mutation-selection balance.

II. Addition of a locus controlling the selfing rate

We assume that selfing rate is controlled by a single locus (hereafter named 'modifier locus'), with two alleles: A is the resident and B is the mutant allele.

$f_{AA}(i,j)$, $f_{AB}(i,j)$, and $f_{BB}(i,j)$ are the frequencies of the three diploid genotypes, carrying i and j mutation in the heterozygous and homozygous state, respectively.

$f_A(i)$ and $f_B(i)$ are the frequencies of gametes carrying allele A (respectively B) and i mutations.

a. Selfing

$q_J^G(ij)$ is the frequency of zygotes with genotype G at the modifier locus, carrying i heterozygous loci and j homozygous loci and originating from selfing of J genotypes. We have:

1    $$q_{\mathrm{AA}}^{\mathrm{AA}}(ij) = \sum_{n_1=i}^{\infty} \sum_{n_2=0}^{j} \binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\tfrac{1}{2}\right)^{2n_1-i} q_{\mathrm{AA}}(n_1 n_2)$$ (A7)

2    $$q_{\mathrm{AB}}^{\mathrm{AA}}(ij) = \tfrac{1}{4}\sum_{n_1=i}^{\infty} \sum_{n_2=0}^{j} \binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\tfrac{1}{2}\right)^{2n_1-i} q_{\mathrm{AB}}(n_1 n_2)$$ (A8)

3    $$q_{\mathrm{BB}}^{\mathrm{BB}}(ij) = \sum_{n_1=i}^{\infty} \sum_{n_2=0}^{j} \binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\tfrac{1}{2}\right)^{2n_1-i} q_{\mathrm{BB}}(n_1 n_2)$$ (A9)

4    $$q_{\mathrm{AB}}^{\mathrm{BB}}(ij) = \tfrac{1}{4}\sum_{n_1=i}^{\infty} \sum_{n_2=0}^{j} \binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\tfrac{1}{2}\right)^{2n_1-i} q_{\mathrm{AB}}(n_1 n_2)$$ (A10)

5    $$q_{\mathrm{AB}}^{\mathrm{AB}}(ij) = \tfrac{1}{2}\sum_{n_1=i}^{\infty} \sum_{n_2=0}^{j} \binom{n_1}{i}\binom{n_1-i}{j-n_2}\left(\tfrac{1}{2}\right)^{2n_1-i} q_{\mathrm{AB}}(n_1 n_2)$$ (A11)

6

7

8 *b. Outcrossing*

9

10 $f_G^g(i)$ is the frequency of gametes with haploid genotype g at the modifier locus, carrying i

11 mutations and originating from plants with diploid genotypes G.

12

13    $$f_{\mathrm{AA}}^{\mathrm{A}}(i) = \sum_{n_2=0}^{i} \sum_{n_1=i-n_2}^{\infty} \binom{n_1}{i-n_2}\left(\tfrac{1}{2}\right)^{n_1} q_{\mathrm{AA}}(n_1 n_2)$$ (A12)

14    $$f_{\mathrm{AB}}^{\mathrm{A}}(i) = \tfrac{1}{2}\sum_{n_2=0}^{i} \sum_{n_1=i-n_2}^{\infty} \binom{n_1}{i-n_2}\left(\tfrac{1}{2}\right)^{n_1} q_{\mathrm{AB}}(n_1 n_2)$$ (A13)

15    $$f_{\mathrm{BB}}^{\mathrm{B}}(i) = \sum_{n_2=0}^{i} \sum_{n_1=i-n_2}^{\infty} \binom{n_1}{i-n_2}\left(\tfrac{1}{2}\right)^{n_1} q_{\mathrm{BB}}(n_1 n_2)$$ (A14)

16    $$f_{\mathrm{AB}}^{\mathrm{B}}(i) = \tfrac{1}{2}\sum_{n_2=0}^{i} \sum_{n_1=i-n_2}^{\infty} \binom{n_1}{i-n_2}\left(\tfrac{1}{2}\right)^{n_1} q_{\mathrm{AB}}(n_1 n_2)$$ (A15)

17

18 $p_g(i)$ is the frequency of pollen grains with genotype g at the modifier locus and carrying i

19 mutations.

20

21    $$p_{\mathrm{A}}(i) = f_{\mathrm{AA}}^{\mathrm{A}}(i) + f_{\mathrm{AB}}^{\mathrm{A}}(i)$$ (A16) $$p_{\mathrm{B}}(i) = f_{\mathrm{AB}}^{\mathrm{B}}(i) + f_{\mathrm{BB}}^{\mathrm{B}}(i)$$ (A17)

22

23

24

25 *c. Recursion equations for mating*

26

27   Let r_{AA}, r_{AB}, and r_{BB} be the selfing rates of genotypes AA, AB and BB, respectively.

28 In the main text, $r_{\mathrm{AA}} = r$ and $r_{\mathrm{BB}} = r'$.

29

30    $$q_{\mathrm{AA}}^{'}(i0) = r_{\mathrm{AA}} q_{\mathrm{AA}}^{\mathrm{AA}}(i0) + r_{\mathrm{AB}} q_{\mathrm{AB}}^{\mathrm{AA}}(i0) + (1-r_{\mathrm{AA}})\left[\sum_{k=0}^{i} f_{\mathrm{AA}}^{\mathrm{A}}(k) p_{\mathrm{A}}(i-k)\right] + (1-r_{\mathrm{AB}})\left[\sum_{k=0}^{i} f_{\mathrm{AB}}^{\mathrm{A}}(k) p_{\mathrm{A}}(i-k)\right]$$ (A18)

31

32      $$q_{\mathrm{AA}}^{'}(ij) = r_{\mathrm{AA}} q_{\mathrm{AA}}^{\mathrm{AA}}(ij) + r_{\mathrm{AB}} q_{\mathrm{AB}}^{\mathrm{AA}}(ij),\, j>0$$ (A19)

33

$$q'_{BB}(i0) = r_{BB}q^{BB}_{BB}(i0) + r_{AB}q^{BB}_{AB}(i0) + (1-r_{BB})\left[\sum_{k=0}^{i} f^{B}_{BB}(k)p_{B}(i-k)\right] + (1-r_{AB})\left[\sum_{k=0}^{i} f^{B}_{AB}(k)p_{B}(i-k)\right] \quad (A20)$$

$$q'_{BB}(ij) = r_{BB}q^{BB}_{BB}(ij) + r_{AB}q^{BB}_{AB}(ij), j>0 \quad (A21)$$

$$q'_{BB}(i0) = r_{AB}q^{AB}_{AB}(i0) + (1-r_{AA})\left[\sum_{k=0}^{i} f^{A}_{AA}(k)p_{B}(i-k)\right] + (1-r_{BB})\left[\sum_{k=0}^{i} f^{B}_{BB}(k)p_{A}(i-k)\right]$$

$$+(1-r_{AB})\left[\sum_{k=0}^{i} \left(f^{A}_{AB}(k)p_{B}(i-k) + f^{B}_{AB}(k)p_{A}(i-k)\right)\right] \quad (A22)$$

$$q'_{AB}(ij) = r_{AB}q^{AB}_{AB}(ij), j>0 \quad (A23)$$

c. Mutation and selection

The frequencies of zygotes after mutation are, for any genotype G:

$$q''_{G}(ij) = \sum_{k=0}^{i} \frac{U^{i-k}e^{-U}}{(i-k)!}q'_{G}(kj) \quad (A24)$$

Selection acts both on mutations in heterozygous and homozygous state

$$q^{*}_{G}(ij) = \frac{(1-S)^{j}(1-hS)^{i}q''_{G}(ij)}{\bar{w}} \quad (A25)$$

where $\bar{w}$ is the mean fitness of the population:

$$\bar{w} = \sum_{i=0}^{\infty}\sum_{j=0}^{\infty}\left[(1-S)^{j}(1-hS)^{i}(q''_{AA}(ij) + q''_{AB}(ij) + q''_{BB}(ij))\right] \quad (A26)$$

Equations (A7–26) completely define the recursion system. As before, fluctuating selection was modeled by sampling each generation the selection coefficient of deleterious mutations, S, in a Gaussian distribution with mean μ_S and variance σ_S^2 (equations A5 and A6). For each run, a small frequency of a mutant allele, with selfing rate r_{BB}, was introduced in linkage and identity equilibrium with deleterious mutations, into a population with selfing rate r_{AA} at mutation-selection equilibrium. Simulations were run for 1000 generations to detect invasion (or not) by the mutant.