

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

E. PORCHER,\* J. K. KELLY,† P.-O. CHEPTOU,‡ C. G. ECKERT,§ M. O. JOHNSTON¶  
& S. KALISZ\*\*

\*UMR 5173 MNHN-CNRS-UPMC, Conservation des Espèces, Restauration et Suivi des Populations, Muséum national d'Histoire naturelle, Paris, France

†Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS, USA

‡UMR 5175 CEFE, Centre d'Ecologie Fonctionnelle et Evolutive (CNRS), Montpellier Cedex 05, France

§Department of Biology, Queen's University, Kingston, ON, Canada

¶Department of Biology, Dalhousie University, Halifax, NS, Canada

\*\*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA

## Keywords:

environmental variation;  
genetic load;  
mixed mating;  
purging.

## Abstract

The magnitude of inbreeding depression, a central parameter in the evolution of plant mating systems, can vary depending on environmental conditions. However, the underlying genetic mechanisms causing environmental fluctuations in inbreeding depression, and the consequences of this variation for the evolution of self-fertilization, have been little studied. Here, we consider temporal fluctuations of the selection coefficient in an explicit genetic model of inbreeding depression. We show that substantial variance in inbreeding depression can be generated at equilibrium by fluctuating selection, although the simulated variance tends to be lower than has been measured in experimental studies. Our simulations also reveal that purging of deleterious mutations does not depend on the variance in their selection coefficient. Finally, an evolutionary analysis shows that, in contrast to previous theoretical approaches, intermediate selfing rates are never evolutionarily stable when the variation in inbreeding depression is due to fluctuations in the selection coefficient on deleterious mutations.

## Introduction

Over the last 30 years, theoretical studies of plant mating systems have considered the role of inbreeding depression as central for the evolution of self-fertilization (Lloyd, 1979; Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990). Inbreeding depression is the main evolutionary force that opposes the automatic 50% advantage of selfing (Fisher, 1941); it dictates the cost of seed discounting and determines the extent to which selfing causes reproductive assurance (Lloyd, 1992), so that the predicted outcome of mating system evolution critically depends on inbreeding depression values. Simple evolutionary models with a constant inbreeding

depression,  $\delta$ , predict a dichotomous outcome of evolution: complete selfing when  $\delta < 0.5$  and complete outcrossing when  $\delta > 0.5$ . Importantly, incorporating purging, i.e. a decrease in inbreeding depression with increasing population selfing rate due to the elimination of recessive deleterious mutations by selection, does not change these conclusions (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1990; but see Uyenoyama & Waller, 1991a). Yet, the existence of natural populations with intermediate stable selfing rates is now widely acknowledged and a variety of theoretical models have been proposed to account for the maintenance of such mixed mating systems (reviewed in Goodwillie *et al.*, 2005). Although several models rely on ecology (e.g. pollination biology, Holsinger, 1991; Johnston, 1998; Vallejo-Marin & Uyenoyama, 2004; Porcher & Lande, 2005; Johnston *et al.*, 2009; or population dynamics, Morgan *et al.*, 2005) to explain mixed mating systems, numerous studies have elaborated on the prominent

*Correspondence:* Emmanuelle Porcher, UMR 5173 CERSP, Muséum national d'Histoire naturelle, 61 rue Buffon, F-75005 Paris, France.  
Tel.: 00 1 40 79 53 61; fax: 00 1 40 79 38 35; e-mail: porcher@mnhn.fr  
The first three authors contributed equally to this work. Other authors are listed alphabetically.

role of inbreeding depression by examining various genetic mechanisms (including overdominance, biparental inbreeding in isolated or structured populations, or decline in fitness with generations of selfing, Lloyd, 1979; Uyenoyama, 1986; Holsinger, 1988; Uyenoyama & Waller, 1991b; Latta & Ritland, 1993; Ronfort & Couvet, 1995; Rausher & Chang, 1999).

Recently studies have demonstrated that the magnitude of inbreeding depression is sensitive to environmental factors (reviewed in Armbruster & Reed, 2005; Cheptou, 2006; Willi *et al.*, 2007). Some theoretical models have shown that environmentally induced variation in inbreeding depression is likely to influence the evolution of mating systems, particularly to favour mixed mating systems (Cheptou & Mathias, 2001; Cheptou & Schoen, 2002). However, these models do not incorporate a genetic basis of inbreeding depression and hence do not include the purging process that can destabilize mixed mating systems in explicitly genetic models (e.g. Lande *et al.*, 1994; Husband & Schemske, 1996; Kelly, 2007). In addition, the effectiveness of purging itself may depend on environmental fluctuations, as a result of environment-dependent inbreeding depression (e.g. Bijlsma *et al.*, 1999), a phenomenon that has received little theoretical attention so far. To properly assess the actual role of environmental fluctuations in inbreeding depression on the evolution of plant mating systems, we therefore need to (1) determine the genetic mechanisms underlying fluctuations in inbreeding depression and (2) examine the combined effects of fluctuating inbreeding depression and purging on the evolution of selfing.

Here, we use a common genetic model of inbreeding depression to examine one possible genetic mechanism of fluctuating inbreeding depression and to test whether such fluctuations can maintain mixed mating systems when purging occurs. Consistent with the bulk of experimental data (Charlesworth & Charlesworth, 1999), we assume that inbreeding depression is caused by partly recessive, deleterious mutations. Such a mutation can be characterized by its selection coefficient ( $S$ ) and its dominance coefficient ( $h$ ). From this genetic perspective, environmental variation in inbreeding depression could result from fluctuations in either  $S$  or  $h$  or both (Bijlsma *et al.*, 1999; Armbruster & Reed, 2005), but little is known so far regarding the most likely source(s) of variation in inbreeding depression (Cheptou, 2006). Numerous experimental observations of larger inbreeding depression in stressful environments (e.g. Bijlsma *et al.*, 1999; and reviews in Armbruster & Reed, 2005; Willi *et al.*, 2007) suggest that environmental conditions may influence the selection coefficient of deleterious alleles, although other experiments comparing the mean fitness of *Arabidopsis thaliana* mutation accumulation lines across light and nutrient gradients failed to demonstrate any environment-specific effects of spontaneous mutations (Chang & Shaw, 2003; Kavanagh & Shaw, 2005). In the present study, we assume

that fluctuations in inbreeding depression are caused by temporal changes in the selection coefficient of deleterious mutations. We apply an analytical approach assuming an infinite population size. We address (1) the effect of temporal fluctuations of selection coefficients on the temporal variation in inbreeding depression, and compare this predicted variation to that observed in natural populations, (2) the effectiveness of purging under fluctuating selection and (3) the evolution of mating systems. We specifically investigate whether intermediate selfing rates are evolutionarily stable when inbreeding depression is subjected to fluctuating selection and is allowed to co-evolve with the mating system.

## The model

In the following theoretical approach, we define mixed mating systems as mating systems with a selfing rate  $r > 0$  and  $r < 1$ , which is broader than the definition used in empirical studies (generally  $0.2 < r < 0.8$ ; reviewed in Goodwillie *et al.*, 2005), because we focus on genetically determined selfing rates, with no environmental effects and no measurement errors.

### Inbreeding depression with a fluctuating selection environment

Inbreeding depression, the relative decrease in mean fitness of selfed vs. outcrossed individuals, is described by the model of Kondrashov (1985), which models the evolution of the distribution of the number of homozygous and heterozygous deleterious mutations per individual in an infinite population with selfing rate  $r$ . Mutation occurs at an infinite number of loci, following a Poisson process with rate  $U$  per diploid zygote per generation. Each generation, the selection coefficient of mutations,  $S$ , is randomly sampled from a normal distribution truncated to the interval  $[0,1]$ , with mean  $\mu_S$  and variance  $\sigma_S^2$  before truncation. This selection coefficient is applied to all mutations, which also have the same dominance coefficient,  $h$ . Mutations have multiplicative effects on fitness, so that the fitness of an individual carrying  $x$  homozygous mutations and  $y$  heterozygous mutations is given by  $(1 - S)^x(1 - hS)^y$ .

Each generation, the modelled population undergoes mating, followed by mutation to deleterious alleles and selection. Recursion equations describing the distribution of number of homozygous and heterozygous mutations per individual are detailed in an online appendix and can also be found in Kondrashov (1985) or Charlesworth *et al.* (1990). These equations were iterated for 1000 generations (C++ code for numerical iterations available upon request from E.P.), by which time the population had reached steady state for all parameter combinations. To identify steady state, we considered the growth rate in the number of mutations across generations,  $N_{t+1}/N_t$ ; the population was considered at steady state when the

average growth rate over 50 generations did not differ significantly from 1 (one sample Student's *t*-test). Equations were subsequently iterated for another 1000 generations to record fluctuations in inbreeding depression at (statistical) steady state. For each set of parameter values, we recorded (1) the mean and variance of the number of homozygous and heterozygous deleterious mutations per individual and (2) the mean and variance of inbreeding depression, measured over the last 1000 generations. Because inbreeding depression is constrained between 0 (in the present study) and 1, the variance in inbreeding depression depends on the mean (e.g. the variance must be 0 if the mean is 0 or 1). To account for this dependence on the mean, we compared the simulated variance in inbreeding depression to the maximum possible variance:

$$\sigma_{\max}^2 = \mu_{\text{ID}}(1 - \mu_{\text{ID}}) \quad (1)$$

where  $\mu_{\text{ID}}$  is the mean inbreeding depression.

The predicted mean and variance in inbreeding depression were compared to empirical means and variances in inbreeding depression, estimated in contrasting environments (compiled by Armbruster & Reed, 2005). Most of these studies measured inbreeding depression in only two environments, which were obviously not chosen at random, but instead likely represent extremes of a distribution (e.g. stressful vs. benign environments). Therefore, it was not appropriate to estimate variance using the classical estimator,

$$s^2 = \frac{1}{n-1} \sum (x_i - \bar{x})^2.$$

We thus chose to calculate the sample variance,

$$\sigma_{\text{ID}}^2 = \frac{1}{n} \sum (x_i - \bar{x}_{\text{ID}})^2.$$

Due to small sample sizes and nonrandom sampling, this crude procedure provides an order of magnitude estimate for the actual variance in inbreeding depression.

### Evolutionarily stable mating systems

We analysed the joint evolution of selfing rate and inbreeding depression using an adaptive dynamics framework (Dieckmann, 1997). The fitness of a rare mutant with selfing rate  $r'$  is compared to that of the resident genotype, with selfing rate  $r$ . We assumed that the selfing rate is controlled by a single locus, with two alleles. Once the population with selfing rate  $r$  reached steady state (after 1000 generations), a mutant allele with a selfing rate  $r'$  different from the resident was introduced at a low frequency in linkage and identity equilibrium with deleterious mutations, and the recursion equations were numerically iterated for 1000 generations to detect invasion (or not) by the mutant.

We deduce stable selfing rates using pairwise invasibility plots (PIP), in which regions of invasion (in grey)

and noninvasion (in white) of a rare mutant are plotted against the selfing rate of the resident genotype,  $r$  and the selfing rate of the mutant,  $r'$  (see Fig. 3). Hence, regions located below the  $r = r'$  line correspond to emergence of a mutant with a smaller selfing rate than the resident, and vice versa. If a mutant with selfing rate  $r' > r$  can invade (i.e. the point with coordinates  $(r, r')$  is located in a region of invasion), then evolution favours increased selfing rates and vice versa. Evolutionary equilibria occur at the intersection of the  $r = r'$  line and a line separating regions of invasion and noninvasion. Details regarding the criteria to infer stability of equilibria can be found in Dieckmann (1997). The equilibrium selfing rates discussed here, denoted by white circles on the figures, are evolutionarily stable (they cannot be invaded by neighbouring mutant selfing rates) and are evolutionary attractors or convergence stable (evolution by a series of small steps proceeds towards the equilibrium). We refer to these equilibria as stable selfing rates.

### Parameter values

We either chose parameter values according to experimental values or we used a wide range of biologically plausible values. The genomic mutation rate to deleterious mutations was  $U = 0.02, 0.2$  or  $1$ , which encompasses the range of experimental estimates among multicellular organisms (reviewed in García-Dorado *et al.*, 2004). The dominance coefficient of mutations was either  $h = 0.02$ , as in the only available experimental data for highly deleterious mutations (Simmons & Crow, 1977) or  $h = 0.3$ , a more reasonable value for mildly deleterious mutations (García-Dorado *et al.*, 2004). Exploratory analyses showed that the mean selection coefficient of deleterious mutations had little effect on the results presented here, which is consistent with previous theory showing that equilibrium inbreeding depression in a population depends little on the selection coefficient of deleterious mutations, and is much more influenced by their dominance coefficient (e.g. Charlesworth *et al.*, 1990). Hence, we considered a single, intermediate value for the average selection coefficient of deleterious mutations,  $\mu_S = 0.5$ , so that this coefficient, which is constrained between 0 and 1, could be submitted to significant environmental variation. The initial variances were set to  $\sigma_S^2 = 0.01$  and  $0.09$ , but the latter was actually smaller ( $\sigma_S^2 = 0.06$ ) due to truncation of  $S$  values below 0 or above 1. Note that truncation did not bias the mean selection coefficient. For comparative purposes, we also considered constant selection ( $\sigma_S^2 = 0$ ), with  $S = 0.5$ . Finally, we considered different values of the selfing rate, across the range of  $r = 0-1$ .

### Analytical approximation

To characterize the effect of purging on the selection of self-fertilization in a fluctuating environment, we

compared the results of the Kondrashov model with stochastic variation in selection coefficients to Cheptou & Schoen's (2002) analytical phenotypic approach. Following Lande & Schemske (1985), an approximate measure of the strength of selection on a rare genotype with selfing rate  $r'$  is given by the expected fitness of such genotypes:

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

where  $r$  is the mean selfing rate in the population and  $\delta$  is the inbreeding depression.

If  $\delta$  varies with time and if evolution of the selfing rate occurs much slower than the fluctuations in inbreeding depression, the conditions for invasion of a rare genotype with selfing rate  $r'$  are given by its long-run growth rate (Lande, 2007, 2008), i.e. the geometric mean of fitness over time or similarly by the expectation of the logarithm of the annual fitness,  $E[\log(w)]$  (Kisdi & Meszina, 1995). Without any specific information on the distribution of  $\delta$ , this quantity can be approximated using a second-order approximation (Jensen approximation: Bulmer, 1994) by:

$$s = E[\log(w)] \approx \log[E(w)] - \frac{1}{2E(w)^2} \text{var}(w). \quad (3)$$

Assuming small effects of mutation on the selfing rate, the evolution of self-fertilization can be analysed by modelling the invasion of a rare genotype with selfing  $r'$  in a population with selfing rate  $r$ . The success of invasion can be evaluated via  $D(r)$ , the selection gradient  $\partial s / \partial r'$  at  $r$ : if the latter is positive (negative) mutants with slightly higher (lower) selfing rate may successfully invade. The selection gradient  $D(r)$  for an inbreeding depression distribution with mean  $\mu_\delta$  and variance  $\sigma_\delta^2$  is given by (see Cheptou & Schoen, 2002 for details):

$$D(r) = \frac{0.5 - \mu_\delta}{1 - \mu_\delta r} - \frac{(1 - \frac{r}{2})r}{(1 - \mu r)^3} \sigma_\delta^2 \quad (4)$$

A necessary condition for mixed selfing rates to be stable is thus that  $D(r) = 0$  (no mutant can invade) for intermediate values of the selfing rate ( $0 < r < 1$ ). Moreover, a sufficient and more stringent condition for mixed selfing rates to evolve is that boundaries ( $r = 0$  and  $1$ ) are unstable, i.e.:

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

We incorporated purging in this analytical approach by making both  $\mu_\delta$  and  $\sigma_\delta^2$  functions of the population selfing rate,  $r$ . This and eqn (4) yield the following conditions for maintenance of mixed mating:

$$\mu_\delta(0) < 0.5, \quad (5)$$

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

We tested whether the values generated by the Kondrashov model with fluctuating selection (mean and

variance of inbreeding depression at  $r = 0$  and  $1$ ) met these conditions for the maintenance of mixed mating.

## Results

### Effectiveness of purging under fluctuating inbreeding depression

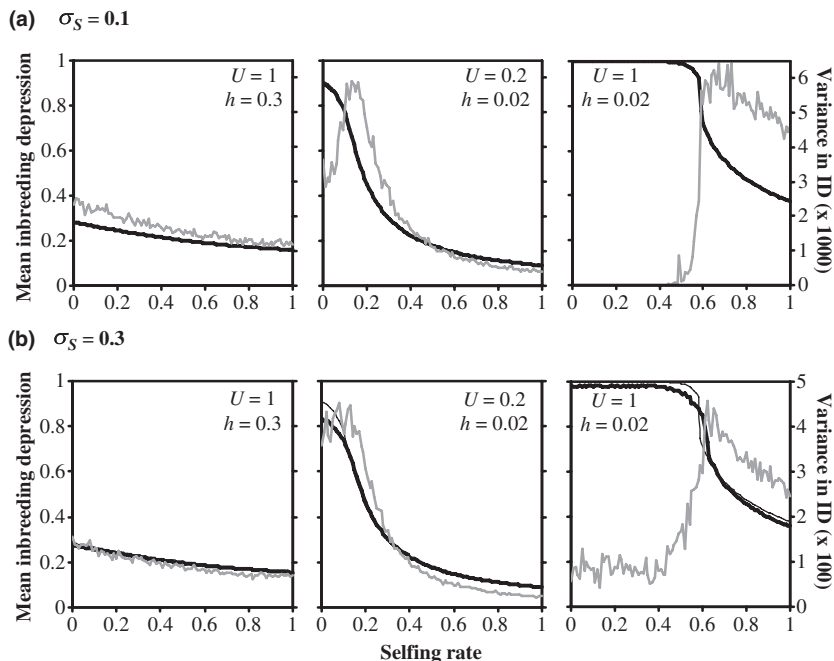
Temporal variation in the selection coefficient of deleterious mutations appeared to have little effect on the extent of purging (Fig. 1). Regardless of the mean (data not shown) and variance of selection coefficients, the relationship between the average inbreeding depression at equilibrium and the population selfing rate differed little between variable vs. constant selection, i.e. the occurrence of purging was unchanged as the variance in selection coefficients was increased. As expected, inbreeding depression was large in predominantly outcrossing populations, and decreased with increasing selfing rate. The decrease was progressive under moderate mutation rate ( $U = 0.02$  or  $0.2$ , Fig. 1) or large dominance coefficient ( $h = 0.3$ , Fig. 1), but sharper under higher mutation rates and with nearly recessive mutations ( $U = 1$  and  $h = 0.02$ , Fig. 1). This pattern was unaffected by fluctuations in the selection coefficient of deleterious mutations, although the average inbreeding depression in a predominantly outcrossing population tended to be slightly lower under large fluctuations of selection than in a constant environment (Fig. 1,  $\sigma_S = 0.3$  and  $h = 0.02$ ).

### Magnitude of variation in inbreeding depression

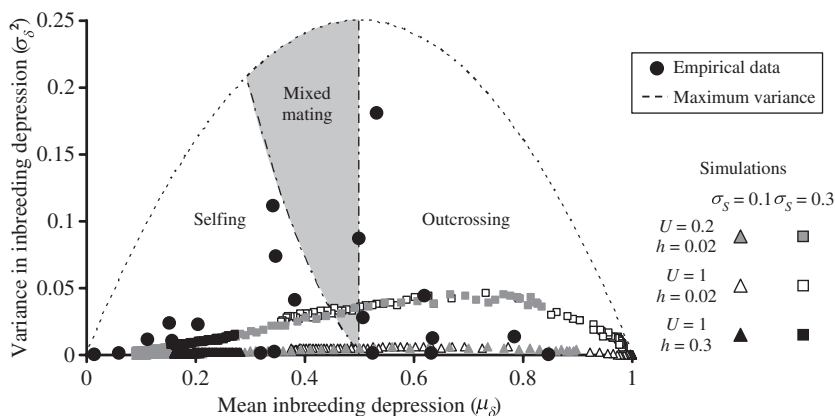
As expected, temporal fluctuations in the selection coefficient of deleterious mutations generated variation in inbreeding depression across generations. Variation in inbreeding depression increased with the selection coefficient,  $\sigma_S^2$  up to ca. 0.05. It was also affected by the population selfing rate and the purging process, with a peak in variance when the average inbreeding depression was closest to 0.5. Because mean and variance of inbreeding depression are not independent (eqn 1), the changes in variance under different population selfing rates were in great part attributable to changes in mean inbreeding depression. Depending on the mutation rate and dominance coefficient of deleterious mutations, variation in mean inbreeding depression explained 51–97% of the variance of inbreeding depression (these values were obtained by examining the correlation between the variance in the simulations and the maximum variance in inbreeding depression calculated from eqn 1).

### Comparison of simulated vs. observed variation in inbreeding depression

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



**Fig. 1** Equilibrium inbreeding depression under fluctuating selection. Mean inbreeding depression (thick black lines) and variance in inbreeding depression (grey lines, measured over generations) are calculated over 1000 generations at steady state. The average selection coefficient of deleterious mutations is  $\mu_S = 0.5$ . Expected inbreeding depression in a constant environment ( $\sigma_S = 0$ , thin black lines) is sometimes exactly superimposed on the curves of mean inbreeding depression.



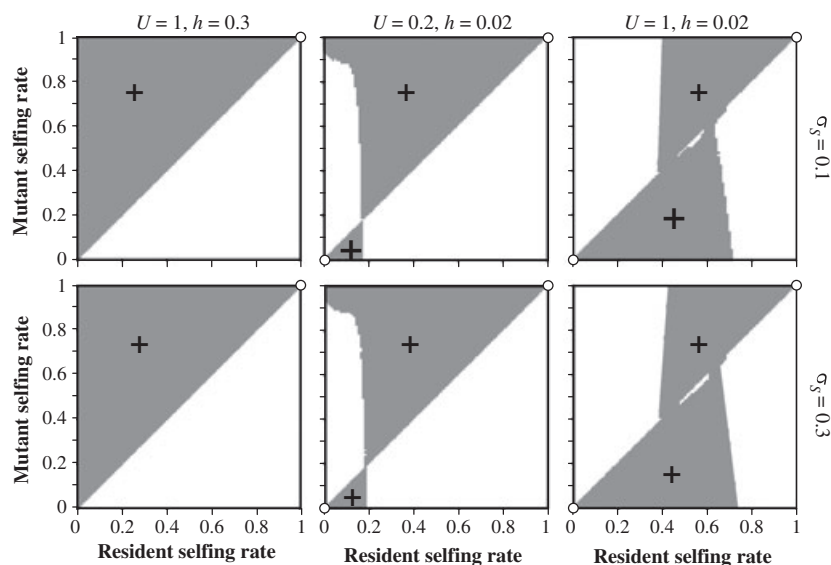
**Fig. 2** Predicted and empirically derived estimates of the mean and variance in inbreeding depression. Empirical data (black circles) are from Ambruster & Reed (2005). Predicted values (squares and triangles) are from our simulation approach and are the same as in Fig. 1. The shaded area corresponds to combinations of mean and variance in inbreeding depression for which mixed mating systems are expected to be stable under Cheptou & Schoen’s (2002) model, based on Jensen approximation (see Cheptou & Schoen, 2002 for a numerical example using a truncated Gaussian distribution). The dotted black envelope line represents the maximum variance in inbreeding depression  $\mu_{\delta}(1 - \mu_{\delta})$ , as a function of the mean inbreeding depression,  $\mu_{\delta}$  ( $0 < \mu_{\delta} < 1$ ).

(Fig. 2,  $\sigma_{ID}^2$  up to 0.18 and 0.046 respectively). The parameter space of  $\mu_{\delta}$  (mean inbreeding depression) and  $\sigma_{\delta}^2$  (variance in inbreeding depression) where the analytical model of Cheptou & Schoen (2002) predicts stable mixed mating systems (eqn 4,  $D(r) = 0$ ) are plotted on Fig. 2 (shaded area). Interestingly, none of the empirical points fall within this parameter space. Despite relatively low variance in inbreeding depression generated by our theoretical approach, the simulations nonetheless generated some situations for which Cheptou & Schoen’s

(2002) model predicts stable mixed mating: this was the case with  $U = 0.2, h = 0.02, \sigma_S = 0.3$  and  $U = 1, h = 0.02, \sigma_S = 0.3$  (combination of  $\mu_{\delta}$  and  $\sigma_{\delta}^2$  within the shaded area, Fig. 2).

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

Evolutionarily stable selfing rates can be deduced from PIP (Fig. 3), where regions of invasion (in grey) and



**Fig. 3** Evolutionarily stable selfing rates under fluctuating inbreeding depression. The average selection coefficient of deleterious mutations is  $\mu_S = 0.5$ . Regions where the mutant invades ('+') are in grey, regions where it does not invade are in white. White circles indicate evolutionarily stable selfing rates.

noninvasion (in white) of a rare mutant are plotted against the selfing rate of the resident genotype,  $r$  and that of the mutant,  $r'$ . Evolutionarily stable selfing rates are located at the intersection of the  $r = r'$  line and a line separating regions of invasion and noninvasion, and are characterized by the fact that no mutant can invade (i.e. the areas just above and below are white on the PIP). The main result 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 & 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 ( $\mu_\delta$  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  $\mu_\delta$  and  $\sigma_\delta$  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).

## Discussion

In this study, we generated fluctuating inbreeding depression using a genetic model where environmental variation affects the selection coefficient. This allowed us to characterize purging under a fluctuating environment and analyse the evolution of plant mating systems in this context, specifically testing for conditions that allow the maintenance of mixed mating.

### Fluctuations in selection coefficients have little effect on purging

Temporal variation in the selection coefficient of the deleterious mutations has very little effect on the average equilibrium inbreeding depression. This is consistent with single locus theory in randomly mating populations: Crow & Kimura (1970) showed that with incompletely recessive ( $h > 0$ ), mildly deleterious mutations ( $S \gg u$ ) mutant homozygotes are kept sufficiently rare by selection so that most selection occurs against mutant heterozygotes. As a result, the dynamics of the mutant allele in a constant environment are approximately a

linear function of the selection coefficient,  $S$ , and can be written  $dq/dt \approx u - hSq$  (Crow & Kimura, 1970; Lande & Schemske, 1985), where  $q$  is the mutant allele frequency and  $u$  is the per locus mutation rate. With fluctuating selection, this yields  $E(dq/dt) = u - E(hS)q$ . At equilibrium ( $dq/dt = 0$ ),  $q = u/E(hS)$ . Hence, in randomly mating populations under fluctuating selection, inbreeding depression at a single locus depends solely on the mean selection coefficient (or dominance coefficient) of the deleterious mutation, not on its variance. Our results extend these predictions with a single locus to many loci, as well as nonrandom mating. Similarly, when selfing is enforced in a previously completely outcrossing population, fluctuations do not affect the time required for the population to reach the new equilibrium inbreeding depression (data not shown), which is due to the fact that, under complete selfing, highly recessive deleterious mutations are quickly exposed to selection and a few generations of strong selection are enough to purge most of the load (e.g. Lande & Schemske, 1985).

A number of experimental approaches have demonstrated that inbreeding depression varies across environments and generally appears to be stronger in more stressful environments, generally defined as environments reducing mean fitness (Armbruster & Reed, 2005). As a consequence, purging might be inefficient in relatively benign environments, where inbreeding depression is weak (e.g. Bijlsma *et al.*, 1999; Keller & Waller, 2002; Armbruster & Reed, 2005). Such faster reduction in inbreeding depression was observed by Kristensen *et al.* (2003) and Swindell & Bouzat (2006) in inbred laboratory populations of *Drosophila* species submitted to stressful environments (high temperature, competition, chemical stress) vs. benign environments. Their *Drosophila* data are however not directly comparable to our theoretical results suggesting that variable selection has little effect on purging because in these experimental studies, environment-dependent purging was examined in two or more contrasting, but constant environments. In the variable environment simulated here, our results suggest that only five to ten generations of stress can increase inbreeding depression and are enough to purge most of the load (data not shown), regardless of the average effects of mutations/stress in the environment, because the rate of purging is much faster than mutation accumulation (Lande & Schemske, 1985).

#### Fluctuations in selection coefficients can generate substantial variation in inbreeding depression

Although the time-averaged inbreeding depression is little affected by fluctuating selection, the *current* inbreeding depression can exhibit substantial variation across environments. In the present study, we considered mutations that were moderately deleterious on average ( $\mu_S = 0.5$ ), but this intermediate selection coefficient was

allowed to vary between 0 (neutral mutations) and 1 (lethal mutations) following a truncated normal distribution with variance between 0.01 and 0.06. As a result, the observed variance in inbreeding depression ranged between  $5 \times 10^{-4}$  and 0.05, and was highest when the mean inbreeding depression was close to 0.5, which was generally observed at intermediate selfing rates (with nearly recessive deleterious mutations,  $h = 0.02$ , Fig. 1). Although empirical data on variation in inbreeding depression magnitude are scarce, several studies (e.g. Jiménez *et al.*, 1994; Koelewijn, 1998; Cheptou *et al.*, 2001; Haag *et al.*, 2002) find that their study organisms exhibit more variation in inbreeding depression than any of our simulations (regardless of parameter values). This would suggest that varying  $S$  in time, as simulated here, might not be the only mechanism generating the observed variation in the experimental data. It is however important to keep in mind that experiments on inbreeding depression do not necessarily mimic natural conditions, and that the variance in inbreeding depression was generally estimated from two data points (i.e. two experimental conditions). Obviously there is a need for experimental studies that estimate inbreeding depression over a wider range of environmental conditions. In addition, the magnitude of inbreeding depression in natural populations depends on the history of populations (Kristensen *et al.*, 2003; Leimu *et al.*, 2008), so that it could be less sensitive to environmental variation than suggested by greenhouse experiments (e.g. Galloway & Etterson, 2007). Comparison of inbreeding depression variation in the wild (see for instance Dole & Ritland, 1993) would be more relevant.

#### Purging cancels out the role of fluctuating inbreeding depression in the maintenance of mixed mating systems

Based on a phenotypic model with no purging of deleterious mutations, Cheptou & Schoen (2002) predicted that intermediate selfing rates can be stable with fluctuating 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. Although 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 & 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 (data 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  $h = 0.02$ , Fig. 1) creates situations in which the mean inbreeding depression under complete selfing is close to 0.5, so that the variance in inbreeding depression can be large [condition (2)]. However, with this characteristic of mutations, condition (1) is not met due to the absence of purging in a completely outcrossing population, generating large mean inbreeding depression.

### Some limits of the model

Although fluctuating selection coefficients can generate substantial inbreeding depression, our approach suggests that when purging occurs, fluctuating inbreeding depression is very unlikely to maintain intermediate selfing rates. This probably depends strongly on several assumptions of our model. First, Kondrashov's (1985) approach assumes infinite population size, whereas in natural populations purging is a stochastic process (Byers & Waller, 1999) that can be slowed down in small populations (Glémin, 2003), but should also be reinforced by the possibility of biparental inbreeding in finite populations. The role of demographic stochasticity in the evolution of genetic load and mating systems has been addressed in a limited number of studies (e.g. Charlesworth *et al.*, 1992; Wang *et al.*, 1999; Bataillon & Kirkpatrick, 2000; Theodorou & Couvet, 2002; Glémin, 2003; Guillaume & Perrin, 2006) but the interaction between demography, genetics and environmental variation remains to be explored. Second, we assumed that environmental variation affected the selection coefficient

of a large number of strictly identical, unlinked deleterious mutations. Although little is known regarding the actual mechanisms for environment-dependent inbreeding depression (Armbruster & Reed, 2005), recent studies suggest the existence of environment-specific deleterious alleles (e.g. Vermeulen & Bijlsma, 2004), i.e. alleles that are usually neutral but become deleterious in specific (e.g. stressful) environments. Such 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.

### Acknowledgments

We thank other members of the 'Understanding the Paradox of Mixed Mating Systems' working group (Elizabeth Elle, Monica A. Geber, Carol Goodwillie, David A. Moeller, Richard H. Ree, Risa D. Sargent, Mario Vallejo-Marín and Alice A. Winn) for insightful discussions, and the National Evolutionary Synthesis Center (NESCent) for supporting this working group. Part of the simulations were run using resources of the Computational Biology Service Unit from the Muséum National d'Histoire Naturelle, which was partially funded by Saint Gobain.

### References

- Armbruster, P. & Reed, D.H. 2005. Inbreeding depression in benign and stressful environments. *Heredity* **95**: 235–242.
- Bataillon, T. & Kirkpatrick, M. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet. Res.* **75**: 75–81.



- Bijlsma, R., Bundgaard, J. & Van Putten, W.F. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* **12**: 1125–1137.
- Bulmer, M. 1994. *Theoretical Evolutionary Ecology*. Sinauer, Sunderland, MA.
- Byers, D.L. & Waller, D.M. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Evol. Syst.* **30**: 479–513.
- Chang, S.M. & Shaw, R.G. 2003. The contribution of spontaneous mutation to variation in environmental response in *Arabidopsis thaliana*: responses to nutrients. *Evolution* **57**: 984–994.
- Charlesworth, D. & Charlesworth, B. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* **44**: 870–888.
- Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res.* **74**: 329–340.
- Charlesworth, D., Morgan, M.T. & Charlesworth, B. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* **44**: 1469–1489.
- Charlesworth, D., Morgan, M.T. & Charlesworth, B. 1992. The effect of linkage and 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.
- 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.
- 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-dependent variation. *Am. J. Bot.* **88**: 1424–1429.
- 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, New York.
- 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. *Annu. 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.
- Kisdi, V. & Meszina, G. 1995. Life-histories with lottery competition in a stochastic environment – ESSs which do not prevail. *Theor. Popul. Biol.* **47**: 191–211.
- Koelewijn, H.P. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution* **52**: 692–702.
- Kondrashov, A.S. 1985. Deleterious mutations as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* **111**: 635–653.
- Kristensen, T.N., Dahlgaard, J. & Loeschcke, V. 2003. Effects of inbreeding and environmental stress on fitness – using *Drosophila buzzatii* as a model organism. *Conserv. Genet.* **4**: 453–465.
- Lande, R. 2007. Expected relative fitness and the adaptive topography of fluctuating selection. *Evolution* **61**: 1835–1846.
- Lande, R. 2008. Adaptive topography of fluctuating selection in a Mendelian population. *J. Evol. Biol.* **21**: 1096–1105.
- Lande, R. & Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding depression in plants. 1. Genetics models. *Evolution* **39**: 24–40.
- Lande, R., Schemske, D.W. & Schultz, S.T. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution* **48**: 965–978.
- Latta, R.G. & Ritland, K.R. 1993. Models for the evolution of selfing under alternative modes of inheritance. *Heredity* **71**: 1–10.
- Leimu, R., Kloss, L. & Fischer, M. 2008. Effects of experimental inbreeding on herbivore resistance and plant fitness: the role of history of inbreeding, herbivory and abiotic factors. *Ecol. Lett.* **11**: 1101–1110.

- Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in 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.
- Vallejo-Marin, M. & Uyenoyama, M.K. 2004. On the evolutionary costs of self-incompatibility: Incomplete reproductive compensation due to pollen limitation. *Evolution* **58**: 1924–1935.
- Vermeulen, C.J. & Bijlsma, R. 2004. Characterization of conditionally expressed mutants affecting age-specific *Drosophila melanogaster*: lethal conditions and temperature-sensitive periods. *Genetics* **167**: 1241–1248.
- Wang, J.L., Hill, W.G., Charlesworth, D. & Charlesworth, B. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* **74**: 165–178.
- Willi, Y., Dietrich, S. & van Kleunen, M. 2007. Inter-specific competitive stress does not affect the magnitude of inbreeding depression. *Evol. Ecol. Res.* **9**: 959–974.

## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Recursion equations for Kondrashov's model with fluctuating selection.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Received 30 September 2008; revised 7 January 2009; accepted 8 January 2009