JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12343

Selfing, adaptation and background selection in finite populations

A. KAMRAN-DISFANI & A. F. AGRAWAL

Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada

Keywords:

adaptation;
beneficial and deleterious mutations;
Hill–Robertson effects;
recombination;
selfing.

Abstract

Classic deterministic genetic models of the evolution of selfing predict species should be either completely outcrossing or completely selfing. However, even species considered high selfers outcross to a small degree (e.g. *Arabidopsis thaliana and Caenorhabditis elegans*). This discrepancy between theory and data may exist because the classic models ignore the effects of drift interacting with selection, that is, Hill–Robertson effects. High selfing rates make the effective rate of recombination near zero, which is expected to cause the build-up of negative disequilibria in finite populations. Despite the transmission advantage associated with complete selfing, low levels of outcrossing may be favoured because of the benefits of increasing the effective rate of recombination to dissipate negative disequilibria. Using multilocus simulations, we confirm that selfing reduces effective population size through background selection and causes negative disequilibria between selected sites. Consequently, the rate of adaptation is substantially reduced in strong selfers. When selfing rate is allowed to evolve, populations evolve to be either strong outcrossers or strong selfers, depending on the parameter values. Amongst selfers, low, but nonzero, levels of outcrossing can be maintained by selection even when all mutations are deleterious; more outcrossing is maintained with higher rates of deleterious mutation. The addition of beneficial mutations can (i) lead to a quantitative increase in the degree of outcrossing amongst stronger selfers but (ii) may cause outcrossing species to evolve into stronger selfers.

Introduction

Many plants and animals reproduce by self-fertilization and much effort has been put into understanding why this is so (Goodwillie *et al.*, 2005; Jarne & Auld, 2006). Nonetheless, some gaps remain and our goal here is to build upon existing population genetic results from a related problem – the evolution of recombination – to extend the models of selfing to incorporate the effects of drift. Before doing so, it is first useful to briefly review some of the classic theory on selfing.

A hermaphrodite that efficiently self-fertilizes its own eggs as well as competes to fertilize the eggs of others will transmit more copies of its genes to the next generation than a pure outcrosser. Consequently, an allele for selfing experiences a transmission advantage that can drive the evolution of selfing, all else being equal (Fisher, 1941). However, the transmission advantage is weakened if the act of selfing reduces the pollen available to outcross (i.e. pollen discounting, Nagylaki, 1976; Charlesworth, 1980; Holsinger *et al.*, 1984). However, selfed offspring are often less fit than outcrossed offspring, and inbreeding depression plays a major role in determining whether selfing is favoured over outcrossing (Lloyd, 1979; Charlesworth, 1980). A new selfing mutant is expected to have higher fitness than outcrossing residents if $\delta < \frac{1}{2}(1 - \kappa)$, where δ is the level inbreeding depression and κ is the level of pollen discounting (Charlesworth, 1980).

Deleterious recessive alleles segregating at mutation–selection balance are thought to be the major cause of inbreeding depression (Charlesworth & Charlesworth, 1999; but see Long *et al.*, 2013). In lineages that self, deleterious alleles are purged, reducing inbreeding depression, strengthening the advantage of selfing. Because of this purging, Lande & Schemske (1985)

Correspondence: Aneil F. Agrawal, Department of Ecology & Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada.
Tel.: +1-416-946-5563; fax: +1-416-978-5878;
e-mail: a.agrawal@utoronto.ca

predicted that species should be either completely outcrossing (with high inbreeding depression) or completely selfing (with low inbreeding depression). These predictions were confirmed in deterministic modifier simulations (Charlesworth *et al.*, 1990).

However, surveys of selfing rates across both plants (Goodwillie *et al.*, 2005) and animals (Jarne & Auld, 2006) indicate that a fairly high fraction of species have intermediate levels of selfing ($20 < S < 80\%$). There are a number of ecological factors missing from the classic 'genetic' models that provide plausible potential explanations for 'intermediate' levels of selfing, including reproductive assurance (Lloyd, 1979, 1992), escalating levels of pollen discounting at higher selfing levels (Johnston, 1998), frequency-dependent pollination dynamics (Holsinger, 1991; Porcher & Lande, 2005) and host–parasite coevolution (Agrawal & Lively, 2001). We will not further consider such ecological factors or the problem of intermediate selfing rates but a good review can be found in Goodwillie *et al.* (2005).

The survey data also show that species considered strong selfers typically outcross at a low level (e.g. $95\% < S < 99.99\%$). For example, two model selfers have low but nonzero rates of outcrossing: *Arabidopsis thaliana*: $97.5\% < S < 99.7\%$ (Abbott & Gomes, 1989; Bomblies *et al.*, 2010) and *Caenorhabditis elegans*: $99\% < S < 99.99\%$ (Barriere & Felix, 2005; Cutter, 2006). Are the ecological factors listed above needed to account for the low but nonzero levels of outcrossing in high selfers, or can an a purely genetic model explain these observations?

The classic models of selfing ignore the role of drift. In finite populations, an interaction between drift and selection, known as the Hill–Robertson effect (Hill & Robertson, 1966), causes the build-up of negative disequilibrium. As a consequence, modifiers increasing recombination can be favoured because recombination dissipates negative disequilibrium, allowing faster response to selection (Felsenstein, 1974; Barton & Otto, 2005). When recombination rates are initially low, the negative disequilibrium can be large, creating strong selection for recombination. Using a simulation model with fitness variation arising from recurrent deleterious mutation in a finite population, Keightley & Otto (2006) showed that selection for nonzero recombination can overcome large costs. In highly selfing populations, the effective recombination rate is near zero (Nordborg, 1997), so we expect that negative disequilibria will accumulate, possibly creating selection for nonzero outcrossing to increase the effective recombination rate.

Deterministic models have previously shown that selfing rates can evolve through the effects of recombination (Uyenoyama & Waller, 1991a). However, it has been argued that selection on selfing rates arising from the effects of recombination breaking down disequilibria amongst fitness-affecting loci will be weak relative to the consequences of selfing enhancing segregation (Uyenoyama & Waller, 1991b). This argument may not hold if there are many polymorphic sites in the genome, as occurs with high mutation and weak selection, because there can be a substantial build-up of negative disequilibria if the actual or effective recombination rate is very low.

If low levels of outcrossing are maintained to ensure that the effective recombination rate is not too low, then we may expect that more outcrossing will be needed in cases where the actual recombination rate is low compared to where it is high. However, Uyenoyama & Waller (1991a) analysed a model with a single fitness locus that led to the opposite prediction. In their model, selfing increased homozygosity, allowing for a faster response to selection amongst selfed offspring compared to outcrossed offspring, thereby providing a benefit to the allele that increased selfing. This benefit only persists as long as the selfing allele avoids recombining away from the good homozygous genotypes it created. This led to the conclusion that '…tighter linkage uniformly promotes the evolution of selfing' (p. 22). This prediction was supported in multilocus simulations by Charlesworth *et al.* (1992), who observed that 'modifiers increasing outcrossing were more likely to be eliminated when linkage was assumed than when we assumed free recombination'.

On the other hand, it has been suggested by numerous authors that the benefits of recombination may select for some outcrossing (Stebbins, 1957; Maynard Smith, 1978; Kondrashov, 1985; Charlesworth *et al.*, 1991). In one test of this idea, David *et al.* (1993) used multilocus stochastic simulations to examine the evolution of alleles that caused low levels of outcrossing in obligately selfing populations of small size ($N = 100$ or 1000) and without inbreeding depression. These authors found that alleles causing low levels of outcrossing fixed more often when there was directional selection on 80 highly polymorphic loci than when there was no such selection. However, under no conditions did the outcrossing allele fix more often than the neutral expectation (50% in this case), presumably because of the transmission disadvantage associated with outcrossing. Furthermore, differences in fixation rates between simulations with and without directional selection may have been due to concomitant differences in N_e rather than the influence of recombination benefits mitigating the transmission disadvantage.

Here, we use stochastic multilocus simulations to examine the causes and consequences of selfing. We first examine how selfing (i) reduces effective population size through background selection, (ii) affects disequilibrium amongst deleterious mutations and (iii) impedes the fixation of beneficial mutations. We then consider the evolution of selfing rates in populations with only deleterious mutations and those adapting to new environments where the beneficial rate may be reasonably high. Charlesworth *et al.* (1992) also used

multilocus simulations to examine the evolution of selfing. They measured fixation probabilities of modifiers of reasonably large phenotypic effect. In contrast, our focus is on determining equilibrium selfing rates using a continuum of alleles. Moreover, we examine larger population sizes ($N = 10^4$ rather than 400) and investigate the effects of beneficial mutations in addition to deleterious mutations acting alone.

Model description

Simulations were written in C++. Each diploid individual carries two copies of a single chromosome with 5000 uniformly spaced loci. Most of these loci affect fitness, with multiplicative fitness effects across loci. In simulations with only deleterious mutations, fitness is given by $W_{Del} = (1 - s_{Del})^{n_{HomDel}}(1 - h_{Del}s_{Del})^{n_{HetDel}}$ where n_{HomDel} and n_{HetDel} are the numbers of loci at which an individual is homozygous and heterozygous for a deleterious mutation. In models that include beneficial mutations, total fitness is given by $W = w_{Del}{*}w_{Ben}$ where $W_{Ben} = (1 + \alpha)^{2n_{HomBen}+\beta n_{HetBen}}$, which for $\alpha \ll 1$ is nearly equivalent to the more standard parameterization $W_{Ben} = (1 + s_{Ben})^{n_{HomBen}}(1 + h_{Ben}s_{Ben})^{n_{HetBen}}$ with $s_{Ben} = 2\alpha$ and $h_{Ben} = \beta/2$. We report parameter values in terms of s_{Ben} and h_{Ben} using the relationships above.

One locus determines the selfing rate, S, but does not affect fitness; we arbitrarily decided to situate this locus one-third of the way along the chromosome. Each allele has a real value between 0 and 1. An individual's selfing rate is given by the average value of its two alleles at the selfing locus.

Each generation, N offspring are produced from the previous generation. Each offspring is created as follows. A potential mother is chosen at random. If the individual's fitness (scaled relative to the maximum fitness in the population) is greater than a random deviate ~Uniform[0, 1], then the individual will be the mother of the next offspring. The process is repeated until a suitable mother is found. If the mother's selfing rate is greater than another random deviate (Uniform [0, 1]), then the offspring will be produced by selfing. If not, then a father is chosen by selecting a random individual i and testing whether a random deviate (Uniform[0, 1]) is less $(W_i/W_{max})(1 - S_i\kappa)$, where κ is the pollen discounting parameter, S_i is the selfing rate of individual i and W_{max} is the maximum fitness in the population. The process is repeated until a suitable father is found. The inclusion of the pollen-discounting parameter allows the inherent transmission advantage associated with selfing to be controlled such that there is a 1.5-fold transmission advantage when $\kappa = 0$ but no advantage when $\kappa = 1$ (Charlesworth, 1980). Having identified a mother and father, a gamete is created from each parent. For a given parent, the gamete is created from the two homeologous parental chromosomes with c crossover events, where $c \sim$ Poisson[mean = M], with M representing the genome map length. The c crossover events are assigned to random positions along the chromosome. (Because there is typically 1–2 chiasmata per chromosome in many species (Bell, 1982), the use of larger values of M can be thought of as approximating a genome with multiple chromosomes.) After creating the two gametes and combining them to make the new zygote, then x deleterious mutations are added, where $x \sim$ Poisson[mean = $U(1 - p_{Ben})$] and U is the diploid mutation rate. The x mutations are added to randomly chosen fitness loci, changing the existing alleles to deleterious ones (if an allele is already deleterious, it remains so). Similarly, y beneficial mutations are added to the genome, where $y \sim$ Poisson[mean = Up_{Ben}]. Mutation occurs at the selfing locus with probability μ_{Self}. When a mutation occurs, a random number z is added to the existing value of the affected allele, where $z \sim$ Normal[mean = 0, variance = σ^2_{Self}]. If the new allelic value exceeds 1 or is below 0, it is changed to the appropriate boundary value.

We tested the simulation to ensure it behaved properly with respect to mutation load and inbreeding depression compared to previous studies (e.g. Charlesworth *et al.*, 1990).

Simulations to measure linkage disequilibrium

For each set of parameters, we initiated populations with, on average, $U/h_{Del}s_{Del}$ deleterious alleles per individual and then did a burn-in period of 2000 generations. Every 20 generations for the next 3000 generations, we measured the variance in the number of deleterious alleles per (haploid) chromosome. We also measured the frequency of deleterious alleles at each fitness-affecting locus.

The observed variance in the number of deleterious alleles per chromosome can be decomposed as $v_o = \sum_i q_i(1 - q_i) + 2\sum_i \sum_{j>i} D_{ij}$ where q_i is the frequency of the deleterious allele at locus i and D_{ij} is the linkage disequilibrium between loci i and j. The first summation in the preceding equation is the expected variance, v_e, in the absence linkage disequilibrium. Computationally, it is much easier to calculate allele frequencies for each locus and compute v_e than it is to directly calculate all of the D_{ij} terms. We can quantify the contribution of disequilibrium to the variance in deleterious alleles per chromosome as $v_o - v_e$. In these simulations, selfing rates did not evolve.

Simulations in which selfing evolves

Each simulation began with a burn-in period of 2000 generations during which there was complete outcrossing. Following this, a single pulse of variation was added to the selfing locus by mutating each allele using $\sigma^2_{Self} = 0.05$. The probability of mutation at this locus

was then set to a relatively high rate of $\mu_{Self} = 0.05$ to speed the rate of evolution. After generation 10 000, the mutation rate and effect size were reduced, $\mu_{Self} = 0.001$ and $\sigma^2_{Self} = 0.025$, and the simulation continued for at least another 5000 generations. During the last 3000 generations, the mean selfing rate was calculated over nonoverlapping 500-generation intervals. If the difference between the minimum and maximum selfing rates over these six 500-generation intervals was < 0.02, then the simulation was terminated. Otherwise, the simulation was continued for another 5000 generations and again average selfing rates were examined over the final six 500-generation intervals. This process continued until the difference between the minimum and maximum selfing rates over the six 500-generation intervals was < 0.02.

Results

Background selection and selfing

From the perspective of a focal site, the presence of deleterious alleles elsewhere in the genome causes variance in fitness, reducing N_e. If these deleterious sites are linked to the focal site, then the variation in fitness experienced by the focal site will be correlated across generations, causing a greater reduction in N_e. This phenomenon is known as background selection and its effects on genome evolution can be substantial (Charlesworth, 2012). The strength of the background selection was quantified by Hudson & Kaplan (1995) and Nordborg *et al.* (1996) for outcrossing populations. Following Nordborg *et al.* (1996), the effect of background selection on a neutral locus from a selected site separated by a physical distance x is $B = N_e/N = \exp[-\mu hs/2(hs + R[x])^2]$, where μ is the mutation rate at the selected site, hs is the strength of selection on rare deleterious alleles, and $R[x]$ is the probability that two sites that are physical distance x apart will be separated by recombination. The effect of background selection due to deleterious mutations occurring across the genome can be calculated as

$$B = exp\left[-\int_0^{zG} \frac{\mu hs}{2(hs + R[x])^2}\,dx - \int_{zG}^{G} \frac{\mu hs}{2(hs + R[x])^2}\,dx\right] \quad [1]$$

where G is the physical length of the genome and z is the relative position of the focal neutral site along this length.

Glémin & Ronfort (2013) extended [1] to allow for selfing by replacing hs with $(h + F - hF)s$ (Caballero & Hill, 1992) and replacing $R[x]$ with $(1 - F)R[x]$ (Nordborg, 1997). Glémin & Ronfort (2013) used the linear function $R[x] = rx$, which works well if the total map length is small enough that double recombination events can be ignored but will underestimate the effects of background selection otherwise. For genomes in which more than one recombination event is likely, an alternative function should be used. Assuming no crossover interference, an appropriate function is Haldane's mapping function $R[x] = (1 - \exp[-2rx])/2$ (Nordborg *et al.*, 1996). With this function, it is possible to obtain an analytical solution for B, although the formula is complicated (see Data S1). In outcrossers, most of the background selection effect comes from sites that are closely physically linked to the focal site. The reduction in effective recombination in strong selfers means that sites across the genome, including other chromosomes, can contribute substantially to background selection, making the total effect much greater even when genome recombination maps are long (Fig. 1).

The analyses of Hudson & Kaplan (1995) and Nordborg *et al.* (1996) that lead to eq. [1] assume that the

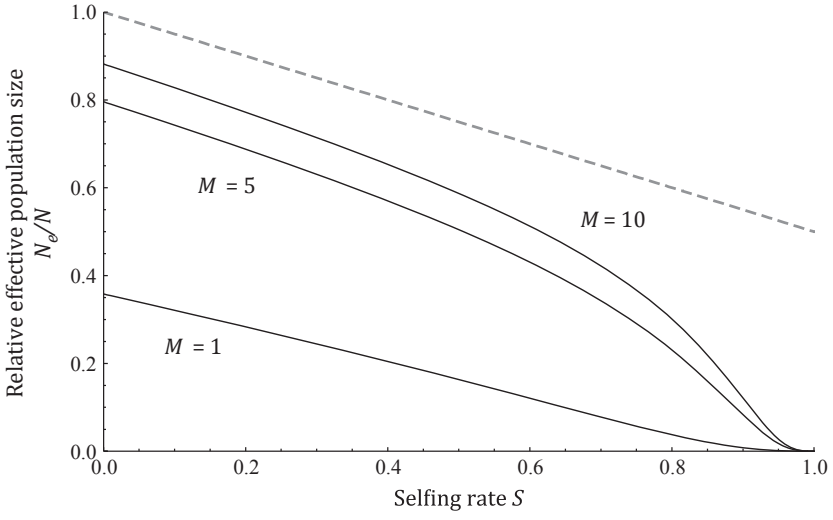

Fig. 1 Reduction in N_e with background selection and selfing. The dashed line shows the decline in population size due to selfing alone, $N_e/N = 1/(1 + F)$ where $F = S/(2 - S)$. The solid lines show the reduction in N_e when the effects of background selection are included, $N_e/N = B/(1 + F)$ where B represents the effect of background selection. Background selection causes a greater reduction in N_e when recombination is low (e.g. $M = 1$ vs. $M = 10$) and selfing rates are high. Parameters: $U = 1$, $h_{Del} = ¼$, $s_{Del} = 0.05$.

deleterious allele at each locus is at its deterministic equilibrium frequency and that there are no disequilibria amongst selected loci. These assumptions are more likely to be violated with selfing because selfing directly generates associations amongst loci and increases the effects of drift. The approximation works reasonably well when used to adjust N_e for predicting beneficial fixation rates for the conditions shown in Table 1. However, we find evidence (Table S1) that the approximation may severely underestimate N_e at high very high selfing rates (e.g. $S = 0.99$) and possibly overestimating N_e at high but less extreme selfing rates (e.g. $S = 0.9$).

Effects of selfing on variance and linkage disequilibrium

Figure 2 shows the observed variance in the number of deleterious alleles per haploid chromosome. The variance is lower when selfing is high. This effect is largely due to the fact that deleterious alleles are rarer in selfing populations because of more efficient purging under selfing (Crow & Kimura, 1970). From the observed allele frequencies, we can calculate the expected variance in the absence of linkage disequilibria, $\Sigma p_i q_i$. The difference between the expected and observed variances is due to linkage disequilibria. In some simulations (Fig. 2a), we observe positive disequilibria for intermediate levels of selfing. With intermediate levels of selfing, effective population sizes are sufficiently large that disequilibria are shaped by deterministic forces. Roze & Lenormand (2005) showed that positive disequilibria are expected in deterministic models with multiplicative selection when there is selfing. This is because selfing causes correlations in homozygosity across the genome (i.e. individuals homozygous at one locus tend to be homozygous at a second locus). Because selection is more efficient in homozygotes, the better allele at one locus becomes associated with homozygosity at a second locus. The same logic can be applied to both loci, explaining why good alleles at the two loci become associated with one another (D. Roze, pers. comm.).

With very high levels of selfing ($S > 0.95$), we observe negative linkage disequilibria. This likely reflects the fact that with high selfing, N_e is greatly reduced, allowing Hill–Robertson effects to become the dominant force shaping disequilibria. High selfing results in a considerable suppression of variance by negative disequilibria with $U = 1$ and $s = 0.05$ (Fig. 2a). With a lower mutation rate, there is little effect of disequilibria on the variance ($U = 0.1$, Fig. 2b). In this case, there are too few segregating alleles to reduce N_e through background selection and cause Hill–Robertson effects. The level of polymorphism can be increased at low mutation rates by making selection weaker because polymorphism should be proportional to U/s. When we do so ($U = 0.1$, $s = 0.005$; Fig. 2c), we again observe negative disequilibria at high selfing levels. Even though polymorphism levels are similar in Fig. 2c as in 2a, the disequilibria are not as strongly negative in Fig. 2c, presumably because the strength of Hill–Robertson effects depends not only on the level of polymorphism but also the strength of selection (Hill & Robertson, 1966; Barton, 1995; Barton & Otto, 2005).

Analytical results based on quasi-linkage equilibrium (QLE) assumptions predict that pairwise disequilibria should be proportional to q^2s^2 (Roze & Lenormand, 2005; Barton and Otto). Because q is proportional to μ/s, the sum of pairwise disequilibria across the genome is expected to depend on U^2 and be independent of s. This helps to explain the differences in disequilibria of Fig. 2a with Figs 2b and c as well as the similarity between Figs 2b and c for low and intermediate selfing

Table 1 Observed (± SE) and predicted values for fixation probabilities ($\times 10^{-3}$) of beneficial mutations as function of selfing rate S and map length M.

	$M = 1$		$M = 5$		$M = 10$	
S	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
0	8.11 ± 0.68	7.14	17.25 ± 1.20	15.79	16.33 ± 0.64	17.48
0.1	10.06 ± 0.80	6.74	14.75 ± 1.03	15.51	16.92 ± 1.95	17.27
0.5	4.83 ± 19	4.34	13.17 ± 0.74	13.36	15.92 ± 1.30	15.54
0.8	1.47 ± 0.10	1.26	5.20 ± 0.60	7.65	7.10 ± 0.63	9.98
0.9	0.33 ± 0.12	0.29	1.27 ± 0.10	3.02	1.57 ± 0.27	4.40
0.99	0.10 ± 0.04	0.05	0.13 ± 0.07	0.06	0.03 ± 0.03	0.06

In these simulations, a single beneficial allele was introduced to randomly chosen individual in the middle of the chromosome at generation 2000. The simulation was continued until fixation or loss occured (T generations). The simulation then continued for $max[T, 1000]$ generations (as a waiting period) before another beneficial allele was introduced. The process was repeated until 2000–5000 beneficial mutations had been introduced. This entire procedure was repeated six times for each parameter combination. SE was calculated based on the fixation rates in these six independent runs. Analytical predictions are based on the single locus model of Caballero & Hill (1992) but adjusting N_e to include the effects of background selection (as described in text). Note that at the highest selfing level, the fixation rate approaches the neutral expectation, $1/2N$. Parameters: $N = 10^4$, $U = 1$, $h_{Del} = ¼$, $s_{Del} = 0.05$, $h_{Ben} = ½$, $s_{Ben} = 0.02$.

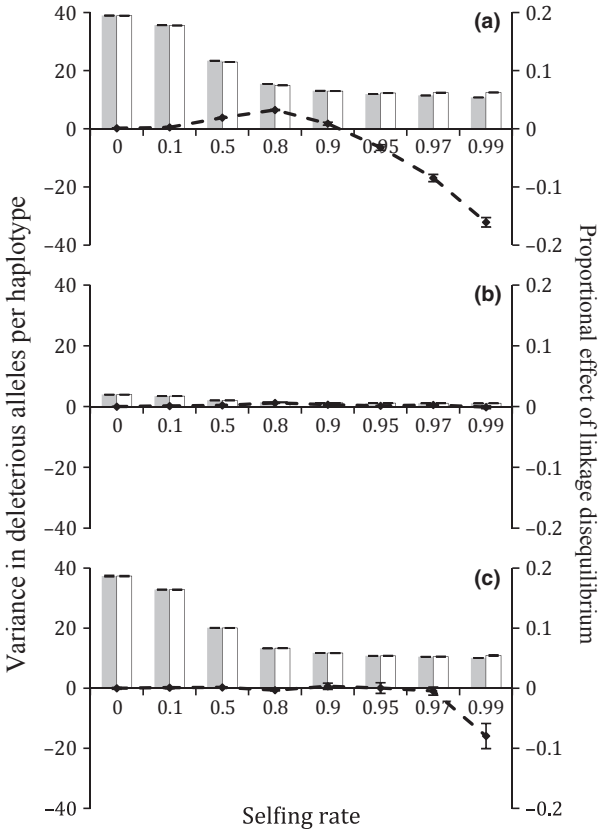

Fig. 2 Variance in haplotype quality and linkage disequilibrium at different levels of selfing. The left axis measures the variance in the number of deleterious alleles per haplotype (single chromosome) as observed in simulations (v_o, shaded bars) or as expected given the observed allele frequencies and assuming no linkage disequilibrium (v_e, open bars). The right axis shows the proportional effect of linkage disequilibrium on the variance, $(v_o - v_e)/v_o$. Estimates are averages from five independent simulations for each parameter combination. Error bars are standard errors measured from the variance amongst simulations. The value from each simulation is an average over the last 3000 generations of the 5000-generation-long simulation (taking measures every 20 generations). Parameter values: $N = 10^4$, $h_{Del} = 0.25$, $M = 5$ (a) $U = 1$, $s_{Del} = 0.05$; (b) $U = 0.1$, $s_{Del} = 0.05$; (c) $U = 0.1$, $s_{Del} = 0.005$.

rates. However, at high selfing rates, the QLE predictions are not expected to apply and it is clear from Fig. 2 that disequilibria depends on s as well as U.

Effects of selfing on rates of adaptation, accumulation of deleterious alleles and inbreeding depression

In extremely large but finite populations, selfing increases the fixation rate of recessive beneficial mutations and decreases the fixation of dominant beneficials, but should not alter the fixation rate of beneficial alleles with additive effects (Caballero & Hill, 1992; Charlesworth *et al.*, 1992). In more moderately sized populations, Glémin & Ronfort (2013) emphasized that fixation rates are reduced by selfing via the background selection effects on N_e. As expected, we find that fixation rates are dramatically reduced in highly selfing populations (Table 1). The analytical predictions are based on the study of Caballero & Hill (1992) but incorporating the effects of background selection via reductions in N_e using eq. [1]. The match between simulation results and analytical predictions is not perfect but is reasonably good. However, the fixation rate drops to the neutral rate ($1/2N = 5 \times 10^{-5}$) at the higher selfing levels, so there is little power to compare the analytical predictions to the simulations in this case.

Table 2 shows fixation of deleterious alleles from a separate set of simulations without beneficial mutations in populations with high selfing rates. Selection prevents the fixation of deleterious alleles provided there is a reasonable amount of outcrossing. However, deleterious alleles fix at an appreciable rate when selfing rates are very high. At very high selfing rates, the match between the predicted and observed number of deleterious alleles is poor and this is likely due to the underestimation of N_e by [1] at very high selfing rates (Table S1).

Table 2 also shows inbreeding depression. Even at very high selfing rates, inbreeding depression values are close to the deterministic predictions. Bataillon & Kirkpatrick (2000) predicted substantial reductions in inbreeding depression below the deterministic prediction in populations with small N_e but we found little evidence of this effect. However, we cannot quantitatively assess their predictions because our approximation for N_e based on [1] becomes unreliable when selfing rates are high (Tables 2, S1).

Evolution of selfing rates with deleterious mutation only

As predicted from the classic theory (Charlesworth, 1980), when pollen discounting κ was above some threshold, κ^*, high levels of outcrossing were maintained, otherwise high rates of selfing evolved. We did not find the critical κ^* precisely but rather found boundaries for it, that is, $\kappa' < \kappa^* < \kappa''$, where $\kappa'' - \kappa' = 0.05$. The equilibrium selfing rates observed above and below κ^* are shown in Table 3. (The corresponding inbreeding depression values at equilibrium are given in Table S2).

In the classic theory (Charlesworth, 1980), increased selfing is favoured in an outcrossing population when $\kappa < 1 - 2\delta$. Inbreeding depression in outcrossing populations is given by $\delta = 1 - \exp[-\frac{(1-2h)}{4h}U]$ (Charlesworth & Charlesworth, 1987), which gives a good match to our simulations (simulations: $\delta \approx 0.21$ for $U = 0.5$ and

Table 2 Inbreeding depression and fixation of deleterious alleles.

U	s_{Del}	N	S	Inbreeding depression	Number of deleterious alleles fixed
1	0.05	10^4	0.8	0.25 [0.24]	0.0 ± 0.0 [0.0]
			0.9	0.24 [0.23]	0.0 ± 0.0 [0.0]
			0.93	0.24 [0.23]	0.0 ± 0.0 [0.0]
			0.95	0.25 [0.23]	0.0 ± 0.0 [0.0]
			0.98	0.25 [0.22]	0.7 ± 0.3 [148]
			0.99	0.26 [0.22]	24.0 ± 1.5 [730]
			0.999	0.25 [0.22]	86.0 ± 3.1 [969]
1	0.01	10^4	0.8	0.25 [0.24]	0.0 ± 0.0 [0.0]
			0.9	0.24 [0.23]	0.0 ± 0.0 [0.0]
			0.93	0.24 [0.23]	0.0 ± 0.0 [0.0]
			0.95	0.24 [0.23]	0.3 ± 0.3 [0.1]
			0.98	0.26 [0.22]	12.7 ± 0.9 [985]
			0.99	0.24 [0.22]	163.3 ± 14.2 [1000]
			0.999	0.21 [0.22]	356.3 ± 25.7 [1000]
1	0.05	2000	0.8	0.26 [0.24]	0.0 ± 0.0 [0.0]
			0.9	0.25 [0.23]	0.0 ± 0.0 [0.0]
			0.93	0.25 [0.23]	2.0 ± 1.0 [0.0]
			0.95	0.26 [0.23]	5.0 ± 1.7 [4.0]
			0.98	0.26 [0.22]	18.3 ± 1.2 [726]
			0.99	0.25 [0.22]	75.0 ± 4.0 [941]
			0.999	0.25 [0.22]	143.0 ± 4.7 [994]
0.5	0.05	10^4	0.8	0.13 [0.13]	0.0 ± 0.0 [0.0]
			0.9	0.13 [0.12]	0.0 ± 0.0 [0.0]
			0.93	0.13 [0.12]	0.0 ± 0.0 [0.0]
			0.95	0.12 [0.12]	0.0 ± 0.0 [0.0]
			0.98	0.12 [0.12]	0.0 ± 0.0 [0.0]
			0.99	0.12 [0.12]	0.0 ± 0.0 [0.0]
			0.999	0.12 [0.12]	8.7 ± 0.3 [1.4]

Results of simulations in which selfing rate was set at a specified (nonevolving) level. Simulations were run for 5×10^4 generations with three replicate simulations per parameter combination. Inbreeding depression was measured over the final 2000 generations of the simulation. Standard errors for inbreeding depression values were < 0.01 (not shown). Expected levels of inbreeding depression based on $\delta = 1 - exp\left(-U\frac{(1+F)(1-2h)}{4(F+h-Fh)}\right)$ are given in brackets. Details of the inbreeding depression measures are as described in Table S3. The number of deleterious alleles fixed over the final 2000 generations (± SE) is shown. Analytical predictions, given in brackets, are based on the single locus model of Caballero & Hill (1992) but adjusting N_e to include the effects of background selection (as described in text). Other parameters: $M = 5$, $p_{Ben} = 0$, $h_{Del} = ¼$.

$\delta \approx 0.37$ for $U = 1$ with $h = ¼$). The observed critical value of pollen discounting required to prevent the evolution of selfing κ^* was higher than expected from classical theory ($\kappa^* = 1 - 2\delta$). This discrepancy likely arises because the classic prediction does not allow for the evolution of inbreeding depression or account for genetic associations that occur between loci that affect selfing rates and those that affect inbreeding depression (Campbell 1986; Holsinger 1988; Uyenoyama and Waller 1991).

For our baseline case, we used the following parameters: $U = 1$, $s_{Del} = 0.05$, $h_{Del} = ¼$, $M = 5$ and $N = 10^4$. With $\kappa < \kappa^*$, selfing evolved to ~97%. This equilibrium appeared independent of the level of pollen discounting, even down to $\kappa = 0$ (not shown), despite the fact that the transmission advantage of selfing increases as κ declines. To confirm that the nonzero level of outcrossing was not simply due to recurrent mutation at the modifier locus, we ran additional simulations. We first ran simulations as before to determine the equilibrium selfing rate, S_{eq}. We then turned off the mutation to the selfing locus ($\mu_{Self} = 0$) and set all individuals to be homozygous for complete selfing alleles ($S = 1$) and saved the state of the population. We then introduced an alternative selfing allele with a selfing rate equal to S_{eq}. The allele was introduced at a frequency of 10% (distributed in homozygous/heterozygous state at appropriate frequencies under S_{eq}). The introduction was repeated 100 times for each determination of S_{eq}, and S_{eq} was determined in five independent simulations. Under neutrality, the S_{eq} allele should fix 10% of the time but it fixed ~32% of the time (across the five independent determinations of S_{eq} combined P-value $< 10^{-16}$), indicating it is favoured by selection over a full selfing allele ($S = 1$). (In simulations where the two selfing alleles had nearly identical effects, fixation rate was at the expected neutral rate of 10%, SE = 0.1%.). With complete pollen discounting, $\kappa = 1$, equilibrium selfing rates were very low, $S_{eq} = 0.2\%$, and this nonzero selfing rate appeared to be maintained by mutation to the selfing locus. In simulations without mutation at the selfing locus but where an allele causing a selfing rate of S_{eq} was introduced at a frequency of 10% into a fully outcrossing population ($S = 0$), the S_{eq} allele was eliminated in all cases.

For the parameter values that we examined, the deleterious mutation rate U had the largest effect on the results. When the mutation rate was lowered from $U = 1$ to $U = 0.5$, the minimal level of pollen discounting required to maintained high outcrossing increased from $\kappa^* \approx 0.375$ to $\kappa^* \approx 0.675$, as expected because of the lower inbreeding depression with lower U (Charlesworth *et al.*, 1990). With $\kappa < \kappa^*$, populations evolved to have very high but not complete selfing: $0.99 < S_{eq} < 1$. We confirmed that even with $\kappa = 0$, alleles with $S_{eq} \approx 0.999$ fixed more often against a full selfing allele ($S = 1$) than expected under neutrality (i.e. fixation in ~12%, rather than 10%, of cases using 500 replicates for each of five separate determinations of S_{eq}; combined $P = 0.01$).

There was no strong effect of five-fold reductions in either N or s. For $U = 1$, reducing population size from $N = 10000$ to $N = 2000$ resulted in slightly more outcrossing when $\kappa < \kappa^*$ (i.e. $S_{eq} \approx 97\%$ vs. 95%). There was almost no difference in results between $s = 0.05$ and $s = 0.01$. With extremely strong selection, $s = 0.25$, the critical level of pollen discounting to maintain high levels of outcrossing was much higher ($\kappa^* \approx 0.725$ rather than $\kappa^* \approx 0.375$), as expected because purging is

Table 3 Equilibrium selfing rates with no beneficial mutations ($p_{Ben} = 0$).

							S_{eq}					
U	M	N	s_{Del}	h_{Del}	κ'	κ''	$\kappa' - 0.1$	$\kappa' - 0.05$	κ'	κ''	$\kappa'' + 0.05$	$\kappa'' + 0.1$
1	5	10^4	0.05	¼	0.35	0.4	0.964 ± 0.005	0.971 ± 0.003	0.966 ± 0.005	0.010 ± 0.001	0.007 ± 0.000	0.006 ± 0.001
1	1	10^4	0.05	¼	0.45	0.5	0.957 ± 0.008	0.940 ± 0.013	0.966 ± 0.005	0.010 ± 0.002	0.007 ± 0.000	0.006 ± 0.001
1	5	2000	0.05	¼	0.35	0.4	0.962 ± 0.007	0.944 ± 0.012	0.951 ± 0.011	0.016 ± 0.002	0.010 ± 0.002	0.007 ± 0.001
1	5	10^4	0.01	¼	0.35	0.4	0.970 ± 0.003	0.970 ± 0.004	0.969 ± 0.004	0.013 ± 0.002	0.009 ± 0.000	0.007 ± 0.001
1	5	10^4	0.25	¼	0.7	0.75	0.999 ± 0.000	0.999 ± 0.001	0.999 ± 0.002	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000
1	5	10^4	0.05	0.4	0.9	0.95	0.960 ± 0.004	0.962 ± 0.005	0.951 ± 0.013	0.028 ± 0.000	0.023 ± 0.000	NA
0.5	5	10^4	0.05	¼	0.65	0.7	0.991 ± 0.002	0.993 ± 0.002	0.994 ± 0.002	0.010 ± 0.001	0.008 ± 0.001	0.007 ± 0.000
0.5	1	10^4	0.05	¼	0.7	0.75	0.993 ± 0.002	0.996 ± 0.002	0.995 ± 0.002	0.013 ± 0.002	0.009 ± 0.001	0.007 ± 0.000
0.5	5	2000	0.05	¼	0.65	0.7	0.981 ± 0.007	0.992 ± 0.003	0.982 ± 0.006	0.013 ± 0.002	0.008 ± 0.001	0.007 ± 0.001
0.5	5	10^4	0.01	¼	0.65	0.7	0.989 ± 0.002	0.987 ± 0.002	0.982 ± 0.002	0.011 ± 0.000	0.010 ± 0.001	0.007 ± 0.000

Results are mean S_{eq} ± SE across replicate simulations (typically $n = 6$). Parameter combinations resulting in low selfing rates are shaded. One cell is marked 'NA' because $\kappa = \kappa'' + 0.1$ would be > 1 for this case.

much easier with strong selection (Charlesworth *et al.*, 1990). Moreover, the equilibrium level of selfing in the high selfing regime ($\kappa < \kappa^*$) was much higher (i.e. $S_{eq} \approx 99.9\%$ rather than 97%), presumably because with such strong selection, there are few segregating deleterious alleles, limiting the potential for negative disequilibria. This may be related to the result of Gordo and Campos (2008) that selection for recombination is reduced with strong selection.

When the recombination map length was reduced from $M = 5$ to $M = 1$, more pollen discounting was required to maintain high levels of outcrossing (i.e. κ^* increased), consistent with the analytical work of Uyenoyama & Waller (1991a) and the simulations of Charlesworth *et al.* (1992). However, there was little, if any, quantitative effect on the equilibrium level of selfing when $\kappa < \kappa^*$. For example, holding $\kappa = 0$, we found very little effect over a 20-fold change in map length: S_{eq} ± SE = 96.6 ± 1.3%, 97.5 ± 0.3%, 96.6 ± 0.4% and 96.0 ± 0.5% for $M = 0.5$, 1, 5 and 10, respectively. This latter result is somewhat surprising given that outcrossing is presumed to be maintained to increase the effective rate of recombination. This result may indicate that there is selection against very tight linkage genome wide as occurs with extreme selfing but that a bit of outcrossing increases effective recombination between most sites to a sufficient level.

Evolution of selfing rates in adapting populations

Table 4 shows results of simulations in which some mutations are beneficial. Beneficial mutations affect the evolution of selfing in two major ways. In populations that would have high selfing rates without beneficial mutations, the addition of beneficial mutations can cause a substantial reduction in selfing rates (e.g. from

Table 4 Effect of beneficial mutations on equilibrium selfing rate.

		S_{eq}					
s_{Ben}	p_{Ben}	$\kappa = 0.25$	$\kappa = 0.3$	$\kappa = 0.35$	$\kappa = 0.4$	$\kappa = 0.45$	$\kappa = 0.5$
0	0	0.964 ± 0.005	0.971 ± 0.003	0.966 ± 0.005	0.010 ± 0.001	0.007 ± 0.000	0.006 ± 0.001
0.002	0.0001	0.963 ± 0.005	0.970 ± 0.008	0.957 ± 0.009	0.011 ± 0.001	0.008 ± 0.000	0.007 ± 0.001
	0.001	0.959 ± 0.005	0.969 ± 0.006	0.969 ± 0.008	0.014 ± 0.001	0.007 ± 0.000	0.007 ± 0.001
	0.01	0.967 ± 0.007	0.968 ± 0.004	0.967 ± 0.006	0.958 ± 0.006	0.012 ± 0.001	0.008 ± 0.000
0.02	0.0001	0.978 ± 0.004	0.959 ± 0.004	0.966 ± 0.004	0.012 ± 0.001	0.007 ± 0.001	0.006 ± 0.000
	0.001	0.970 ± 0.005	0.961 ± 0.005	0.959 ± 0.007	0.01*	0.010 ± 0.001	0.007 ± 0.001
	0.01	0.964 ± 0.007	0.967 ± 0.003	0.969 ± 0.004	0.970 ± 0.003	0.959 ± 0.004	0.01**
0.2	0.0001	0.943 ± 0.010	0.951 ± 0.011	0.935 ± 0.008	0.936 ± 0.015	0.01***	0.008 ± 0.002
	0.001	0.899 ± 0.017	0.862 ± 0.030	0.856 ± 0.018	0.882 ± 0.015	0.866 ± 0.024	0.818 ± 0.008
	0.01	0.781 ± 0.028	0.819 ± 0.037	0.802 ± 0.026	0.825 ± 0.020	0.817 ± 0.021	0.794 ± 0.025

Results are mean S_{eq} ± SE across replicate simulations (typically $n = 6$). Parameter combinations resulting in low selfing rates are shaded. Other parameters: $N = 10^4$, $U = 1$, $M = 5$, $s_{Del} = 0.05$, $h_{Del} = ¼$. *mean S_{eq} based on 4/6 replicates with low selfing rates; the other two had high selfing rates, ~0.96. **mean S_{eq} based on 5/6 replicates with low selfing rates; the other one had a high selfing rate, 0.97. ***mean S_{eq} based on 3/5 replicates with low selfing rates; the other two had high selfing rates, ~0.94.

S_{eq} = 0.97 to 0.90) but only when beneficial mutations have very large effects and occur frequently. More interestingly, the addition of beneficial mutations sometimes caused populations that would otherwise maintain high levels of outcrossing to evolve into strong selfers. This can be thought of as beneficial mutations causing a shift in the level of pollen discounting required to maintain high outcrossing. If beneficial mutations are recessive (dominant), κ^* is higher (lower) than when there are no beneficial mutations (Fig. 3). The sensitivity of κ^* to beneficial mutations is reduced with low recombination (Fig. 3, top vs. bottom).

Discussion

Selfing reduces the effective rate of recombination. This causes background selection to become much stronger, resulting in massive reductions in N_e for highly selfing species with high mutation rates (Fig. 1). As a consequence of reduced N_e, rates of adaptation are much lower in selfing populations (Table 1) and deleterious alleles fix more readily (Table 2). Indeed, selfing species often have lower levels of SNP diversity and less efficient purifying selection than their outcrossing relatives (Wright *et al.*, 2013), and in at least one case, there is evidence that selfers fix fewer beneficial mutations (Slotte *et al.*, 2010).

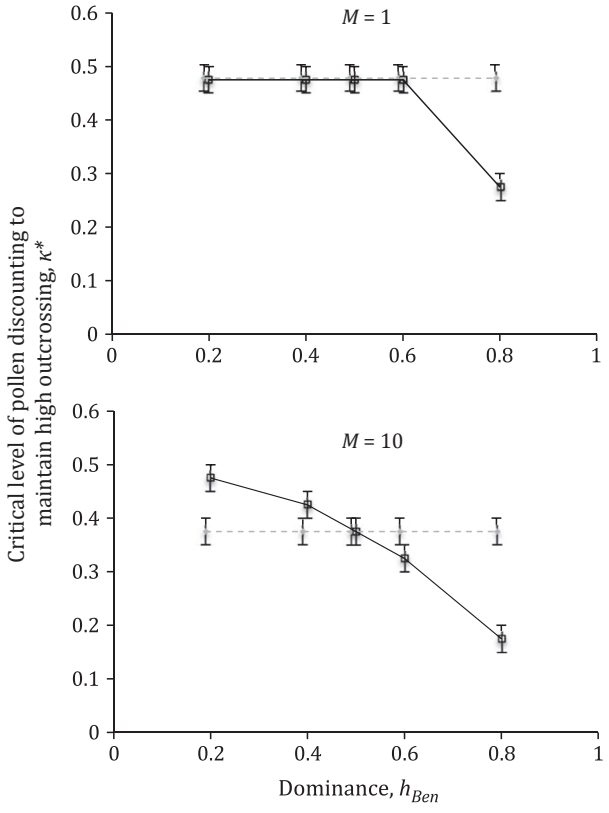

Fig. 3 Effect of the dominance of beneficial mutations, h_{Ben}, on the critical level of pollen discounting required to maintain high outcrossing rates, κ^*. Open symbols connected by solid black line show κ^* when beneficial mutations occur, p_{Ben} = 0.001. Grey points and dashed line give κ^* when all mutations are deleterious, that is, p_{Ben} = 0. Error bars represent lower (κ') and upper (κ'') bounds for κ^*. Parameters: $N = 10^4$, U = 1, s_{Ben} = 0.02, h_{Del} = ¼, s_{Del} = 0.05.

In strong selfers, a substantial fraction of the genic variance in fitness arising from recurrent deleterious mutation is suppressed by negative disequilibria (Fig. 2), which arises because reduced recombination and low N_e increase the strength of Hill–Robertson effects. In recombination modifier models, negative disequilibria due to Hill–Robertson effects can select for nonzero recombination, even when recombination is intrinsically costly (Keightley & Otto, 2006; Roze, 2009; Hartfield *et al.*, 2010). In such models, recombination is favoured because genotypes generated via recombination are more variable in fitness; a modifier that creates highly variable offspring gains a long-term advantage by being associated with the most fit genotypes that contribute disproportionately to future generations. Our results are consistent with the same mechanism favouring the evolution of nonzero rates of outcrossing because with complete selfing, recombination is effectively zero.

In the recombination models, there is strong selection for some nonzero level of genetic mixing through this mechanism. As genetic mixing increases above zero, disequilibria weakens considerably removing selection for further modifier evolution and resulting in low but nonzero levels of recombination. Similarly, we see that negative disequilibria decline rapidly as selfing levels are reduced below 1 (Fig. 2). Consistent with this observation, our results indicate that the 'outcrossing for recombination' mechanism can select for low levels of outcrossing but not intermediate levels of outcrossing (e.g. $0.2 < S_{eq} < 0.8$).

Gordo and Campos (2008) suggested that there is little selection for recombination except when Muller's ratchet is operating. They found that fixation probability of alleles causing recombination (in a population of nonrecombiners) was only substantially higher than the neutral expectation under those conditions where the ratchet turned (i.e. deleterious alleles fixed). Moreover, they showed a reasonable correspondence between the rate of fitness decline caused by Muller's ratchet in the absence of recombination and fixation probability of alleles allowing recombination relative to the neutral expectation.

Our results are not directly comparable to those of Gordo and Campos (2008) because we examined the evolution of equilibrium selfing with a continuum of alleles' model rather than fixation probability of recombination modifiers in otherwise nonrecombining populations. Nonetheless, there is an interesting correspondence between S_{eq} and the selfing rate at which

the ratchet halts. Table 2 shows the number of deleterious alleles fixed in a 2000-generation period in populations at a specified (nonevolving) selfing rate. When the selfing rate is allowed to evolve, it seems to reach an equilibrium value slightly above the rate that would prevent the loss of fitness through fixation of deleterious alleles (see Table 3 for S_{eq} values and Table S3 for corresponding rates of deleterious fixations).

Deterministic simulation models with negative (synergistic) epistasis between deleterious mutations have also shown that high but not complete selfing can be evolutionarily stable (Charlesworth *et al.*, 1991; see also Kondrashov, 1985). Like Hill–Robertson effects, negative epistasis in deterministic models generates negative disequilibrium, creating the opportunity for outcrossing to be favoured through the benefits of enhanced recombination. However, the empirical data indicate that epistasis is not consistently negative; rather negative and positive epistases are both common (de Visser & Elena, 2007; Agrawal & Whitlock, 2010), and this variation in epistasis can result in net selection against recombination (Otto & Feldman, 1997).

As beneficial mutations spread from rare to common in finite populations, strong negative disequilibria can develop through Hill–Robertson effects, creating substantial selection for recombination (Felsenstein, 1974; Iles *et al.*, 2003; Barton & Otto, 2005; Hartfield *et al.*, 2010). Thus, we might have expected higher levels of outcrossing when beneficial mutations were included in the model. Under conditions when selfing rates were high in the absence of beneficial mutations, we found that the addition of beneficial mutations resulted in more outcrossing (Table 4). However, this effect only occurred when beneficials had large effects and occurred frequently, an unlikely situation.

More surprisingly, the inclusion of beneficial mutations caused some populations that were otherwise outcrossing to evolve into high selfers. This is likely due to the segregation effect described by Uyenoyama & Waller (1991a). An allele that increases selfing can be thought of as enhancing segregation, becoming associated with homozygotes of both the beneficial and resident alleles, whereas an outcrossing modifier is associated with heterozygotes. By being associated with homozygotes, the selfing subpopulation responds faster to selection. In this way, selfing modifiers become associated with a higher frequency of the beneficial mutation, thereby allowing higher selfing rates to evolve.

Table 4 shows that beneficial mutations can drive a shift to high selfing when additive beneficial mutations have moderate-to-large fitness effects. Although only a moderate rate of beneficials was required, this rate may be higher than what would be sustained over long evolutionary periods. However, such rates may not be unrealistic for short periods, as when populations experience novel environments and the opportunity for adaptation is high. As illustrated in Fig. 4, outcrossing populations can evolve into strong selfers during evolutionarily brief periods when the rate of beneficials is temporarily elevated. Because of the purging of inbreeding depression that occurs in this process, the population remains highly selfing even after the beneficial rate drops to zero.

Shifts to selfing are more likely when beneficial alleles are recessive as expected because increased selfing enhances beneficial fixation rate (Caballero & Hill,

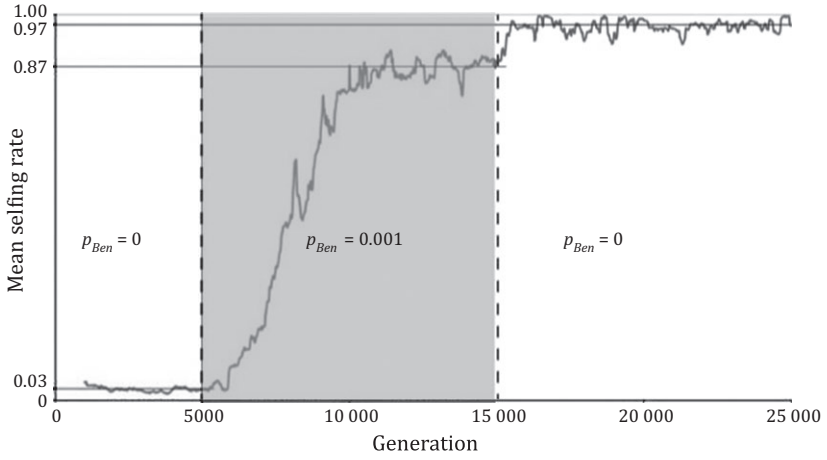

Fig. 4 Permanent shift to selfing driven by temporary increase in adaptation. In this example simulation, the population is initially completely outcrossing; all fitness-affecting mutations are deleterious (p_{Ben} = 0). Following a burn-in period, mutations begin to occur at the selfing locus but selfing rates remain low. At generation 5000, beneficial mutations of large effect begin to occur (p_{Ben} = 0.001, s_{Ben} = 0.2) and selfing rates increase. At generation 15000, no new beneficial mutations occur and the population evolves to an even higher rate of selfing. Parameters: $N = 10^4$, U = 1, M = 5, κ = 0.45, h_{Del} = ¼, s_{Del} = 0.05.

1992; Charlesworth *et al*., 1992), whereas the converse is true for dominant effects (Fig. 3). The model of Uyenoyama & Waller (1991a) would predict that shifts to selfing would be more likely to occur when recombination was low (because of the prolonged association between the selfing modifier and the beneficial), but we observed the opposite. This difference is likely explained by the smaller number of beneficials that fix in populations with low recombination rates (Fig. 3), limiting the opportunity for selfing modifiers to hitchhike to high frequencies. Consistent with Uyenoyama & Waller (1991a), shifts to selfing are more likely when modifier effect sizes are not too low (not shown).

Selfing species often live in different environments than their outcrossing progenitors. This observation is usually interpreted as being a function of invasion demography. Only a few individuals establish in the new habitat, so that selfing or biparental inbreeding is a necessity initially. Inbreeding depression is purged in the process and selfing evolves. Our work provides an alternative explanation for the evolution of high selfing during the invasion of new habitats or following environmental change in an existing habitat that does not require a demographic bottleneck.

Acknowledgments

We thank Tanja Schwander and Denis Roze for organizing the Jacques Monod Conference and the resulting collection of articles. We thank Stephen Wright for numerous insightful conversations about linkage and selfing. We also thank D. Roze and two anonymous reviewers for helpful comments on the manuscript. This work was supported by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to AFA and USRA to AKD).

References

Abbott, R.J. & Gomes, M.F. 1989. Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L) Heynh. *Heredity* **62**: 411–418.

Agrawal, A.F. & Lively, C.M. 2001. Parasites and the evolution of self-fertilization. *Evolution* **55**: 869–879.

Agrawal, A.F. & Whitlock, M.C. 2010. Environmental duress and epistasis: how does stress affect the strength of selection on new mutations? *Trends Ecol. Evol.* **25**: 450–458.

Barriere, A. & Felix, M.A. 2005. High local genetic diversity and low outcrossing rate in Caenorhabditis elegans natural populations. *Curr. Biol.* **15**: 1176–1184.

Barton, N.H. 1995. Linkage and the limits to natural selection. *Genetics* **140**: 821–841.

Barton, N.H. & Otto, S.P. 2005. Evolution of recombination due to random drift. *Genetics* **169**: 2353–2370.

Bataillon, T. & Kirkpatrick, M. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet. Res.* **75**: 75–81.

Bell, G. 1982. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press, Berkley.

Bomblies, K., Yant, L., Laitinen, R.A., Kim, S.T., Hollister, J.D., Warthmann, N. *et al.* 2010. Local-scale patterns of genetic variability, outcrossing, and spatial structure in natural stands of *Arabidopsis thaliana*. *PLoS Genet.* **6**: e1000890.

Caballero, A. & Hill, W.G. 1992. Effects of partial inbreeding on fixation rates and variation of mutant genes. *Genetics* **131**: 493–507.

Campbell, R.B. 1986. The interdependence of mating structure and inbreeding depression. *Theor. Popul. Biol.* **30**: 232–244.

Charlesworth, B. 1980. The cost of sex in relation to mating system. *J. Theor. Biol.* **84**: 655–671.

Charlesworth, B. 2012. The effects of deleterious mutations on evolution at linked sites. *Genetics* **190**: 5–22.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.

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, B., Morgan, M.T. & Charlesworth, D. 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genet. Res.* **57**: 177–194.

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.

Crow, J.F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*. Burgess Publishing Company, Minneapolis.

Cutter, A.D. 2006. Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer Caenorhabditis elegans. *Genetics* **172**: 171–184.

David, J.L., Savy, Y. & Brabant, P. 1993. Outcrossing and selfing evolution in populations under directional selection. *Heredity* **71**: 642–651.

Felsenstein, J. 1974. Evolutionary advantage of recombination. *Genetics* **78**: 737–756.

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

Glémin, S. & Ronfort, J. 2013. Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. *Evolution* **67**: 225–240.

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.

Gordo, I. & Campos, P.R.A. 2008. Sex and deleterious mutations. *Genetics* **179**: 621–626.

Hartfield, M., Otto, S.P. & Keightley, P.D. 2010. The role of advantageous mutations in enhancing the evolution of a recombination modifier. *Genetics* **184**: 1153–U464.

Hill, W.G. & Robertson A. 1966. Effect of linkage on limits to artificial selection. *Genet. Res.* **8**: 269–294.

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.
Holsinger, K.E., Feldman, M.W. & Christiansen, F.B. 1984. The evolution of self-fertilization in plants: a population genetic model. *Am. Nat.* **124**: 446–453.
Hudson, R.R. & Kaplan, N.L. 1995. Deleterious background selection with recombination. *Genetics* **141**: 1605–1617.
Iles, M.M., Walters, K. & Cannings, C. 2003. Recombination can evolve in large finite populations given selection on sufficient loci. *Genetics* **165**: 2249–2258.
Jarne, P. & Auld, J.R. 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. *Evolution* **60**: 1816–1824.
Johnston, M.O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* **102–3**: 267–278.
Keightley, P.D. & Otto, S.P. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* **443**: 89–92.
Kondrashov, A.S. 1985. Deleterious mutations as an evolutionary factor II. Facultative apomixis and selfing. *Genetics* **111**: 635–653.
Lande, R. & Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding depression in plants I. Genetic models. *Evolution* **39**: 24–40.
Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**: 67–79.
Lloyd, D.G. 1992. Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant Sci.* **153**: 370–380.
Long, T.A.F., Rowe, L. & Agrawal, A.F. 2013. The effects of selective history and environmental heterogeneity on inbreeding depression in experimental populations of *Drosophila melanogaster*. *Am. Nat.* **181**: 532–544.
Maynard Smith, J. 1978. *The Evolution of Sex*. Cambridge University Press, Cambridge.
Nagylaki, T. 1976. Model for evolution of self-fertilization and vegetative reproduction. *J. Theor. Biol.* **58**: 55–58.
Nordborg, M. 1997. Structured coalescent processes on different time scales. *Genetics* **146**: 1501–1514.
Nordborg, M., Charlesworth, B. & Charlesworth, D. 1996. The effect of recombination on background selection. *Genet. Res.* **67**: 159–174.
Otto, S.P. & Feldman, M.W. 1997. Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theor. Popul. Biol.* **51**: 134–147.
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.
Roze, D. 2009. Diploidy, population structure, and the evolution of recombination. *Am. Nat.* **174**: S79–S94.
Roze, D. & Lenormand, T. 2005. Self-fertilization and the evolution of recombination. *Genetics* **170**: 841–857.
Slotte, T., Foxe, J.P., Hazzouri, K.M. & Wright, S.I. 2010. Genome-wide evidence for efficient positive and purifying selection in *Capsella grandiflora*, a plant species with a large effective population size. *Mol. Biol. Evol.* **27**: 1813–1821.
Stebbins, G.L. 1957. Self fertilization and population variability in the higher plants. *Am. Nat.* **91**: 337–354.
Uyenoyama, M.K. & Waller, D.M. 1991a. Coevolution of self-fertilization and inbreeding depression. I. 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. III. Homozygous lethal mutations at multiple loci. *Theor. Popul. Biol.* **40**: 173–210.
de Visser, J.A.G.M. & Elena, S.F. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat. Rev. Genet.* **8**: 139–149.
Wright, S.I., Kalisz, S. & Slotte, T. 2013. Evolutionary consequences of self-fertilization in plants. *Proc. R Soc. B-Biol. Sci.* **280**: 20130133.

Supporting information

Additional Supporting Information may be found in the online version of this article:
Table S1 Estimates of *Ne/N* based on the simulations.
Table S2 Inbreeding depression at equilibrium.
Table S3 Fixation of deleterious alleles.
Data S1 Analytical approximation for effects of background selection.

Received 1 October 2013; revised 23 December 2013; accepted 11 January 2014