Maintenance of High Inbreeding Depression in Selfing Populations: Effects of Coupling of Early- and Late-Acting Mutations

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15 late-acting locus

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ABSTRACT High estimates of inbreeding depression have been obtained in many plant populations with high selfing rates. However, deleterious mutations might be purged from such populations as a result of selfing. I developed a simulation model assuming the presence of mutations at two sets of loci, namely, early- and late-acting loci, and the selective abortion of embryos coupled with ovule overproduction. In the model, early-acting loci are expressed during embryo initiation, and less vigorous embryos are aborted. Late-acting loci are expressed after selective abortion ends; the surviving embryos (seeds) compete, and some of them form the next generation. If mutations are allowed to occur in both early- and late-acting loci, they increase in frequency in populations with high selfing rates in both sets of loci. However, this phenomenon does not occur if mutations occur in only the early- or late-acting loci. Consistent results are observed even if the total number of loci in which mutations are allowed to occur is the same among simulations with both early- and late-acting loci or only early- or late-acting loci, indicating that the presence of both sets of loci is the causal factor. Thus, the coupling effects of early- and late-acting mutations promote the maintenance of these mutations in populations with high selfing rates.

In organisms in which selfing is possible, such as hermaphroditic plants, the offspring produced by selfing are often less vigorous (as a result of inbreeding depression). Such inbreeding depression is caused by recessive or partially recessive deleterious mutations (CHARLESWORTH AND CHARLESWORTH 1999; CHARLESWORTH AND WILLIS 2009).

Selfing is expected to purge deleterious mutations from a population because embryos that are homozygous for deleterious mutations are produced, and they will disappear. However, substantial variation in inbreeding depression exists among plants with similar selfing rates, with estimates ranging from nearly 0 to approximately 0.9 in plants with selfing rates of 0 to 0.8 and from 0 to approximately 0.5 in plants with selfing rates greater

than 0.8 (WINN *et al.* 2011). Furthermore, BALDWIN AND SCHOEN (2019) experimentally forced selfing in self-incompatible populations and found that inbreeding depression was difficult to purge by selfing. Thus, many plants with high selfing rates maintain deleterious mutations. This discrepancy is a current problem in evolutionary biology. Furthermore, as explained below, the maintenance of deleterious mutations is strongly related to two other topics in evolutionary biology.

One of these topics is the evolution from outcrossing to selfing. This transition is a very common transition in flowering plants and has independently occurred in many plant taxa (STEBBINS 1974; BARRETT 2002). Selfing has two major short-term advantages. First, plants can produce seeds by selfing under pollinator- and/or mate-limitation conditions (DARWIN 1876; GOODWILLIE *et al.* 2005). Second, an allele promoting selfing has a transmission advantage over an allele promoting outcrossing because the former allele can be transmitted via the pollen parent of seeds produced by selfing (FISHER 1941; GOODWILLIE *et al.* 2005; ECKERT *et al.* 2006). However, if many deleterious mutations are maintained in selfing populations, these short-term advantages may be insufficient to outweigh the inbreeding depression in seeds produced by selfing. Furthermore, genetic load is a severe long-term disadvantage of selfing and can lead to the extinction of selfing populations (GOLDBERG *et al.* 2010; CHEPTOU 2019). An outcrossing population that maintains many deleterious mutations and begins to self may become extinct as a result of inbreeding depression. However, extinction may be avoided if deleterious mutations are rapidly purged. Therefore, understanding whether deleterious mutations are maintained in selfing populations is important.

The second topic is the evolution of selfing rates, which has been studied theoretically and empirically (LLOYD 1979; LANDE AND SCHEMSKE 1985; SCHEMSKE AND LANDE 1985; HOLSINGER 1991; UYENOYAMA AND WALLER 1991; LLOYD 1992; LATTA AND

RITLAND 1994; CHEPTOU AND MATHIAS 2001; GOODWILLIE *et al.* 2005; PORCHER AND LANDE 2005a; PORCHER AND LANDE 2005b; WINN *et al.* 2011; LANDE AND PORCHER 2015). In particular, the conditions that select for intermediate selfing rates have been extensively studied. Although intermediate selfing rates are commonly observed in plants (GOODWILLIE *et al.* 2005; WINN *et al.* 2011), general theory predicts that populations will evolve to complete outcrossing or selfing dependent on the degree of inbreeding depression (LLOYD 1979; LANDE AND SCHEMSKE 1985). In addition, if selfing purges deleterious mutations, then complete selfing should be evolutionarily stable (LANDE AND SCHEMSKE 1985). Hence, whether deleterious mutations are maintained or purged affects the evolution of selfing rate.

The selective interference hypothesis is a convincing hypothesis that has been proposed to explain the maintenance of deleterious mutations (GANDERS 1972; LANDE *et al.* 1994). This hypothesis states that if nearly all embryos produced by selfing are homozygous for highly deleterious mutations at certain loci and die without developing to the next generation, then no opportunity exists to select for heterozygous embryos or embryos without mutations. Although there is some evidence consistent with this hypothesis, whether selective interference alone can explain the maintenance of deleterious mutations is unclear (WINN *et al.* 2011). Additionally, for this mechanism to operate, most mutations must be recessive; hence, selective interference might be unlikely to maintain inbreeding depression (KELLY 2007; ROZE 2015).

Two previous studies examined the maintenance of deleterious mutations assuming early- and late-acting genes. The effect of the overproduction of ovules on the maintenance of deleterious mutations is analyzed in Porcher and Lande (2005b). They assumed that the same mutations become both early acting and late acting; the effects appear during embryo development (i.e., are early acting) if the mutations are homozygous but appear later (i.e., are late acting) if the mutations are heterozygous. Thus, early-acting and late-acting genes are

assumed not to differ. They showed that the overproduction of ovules (i.e., the production of more ovules than of seeds by the parent) promoted the maintenance of deleterious mutations. Lande and Porcher (2017) analyzed the effects of the interactions between early- and late-acting loci on the maintenance of deleterious mutations. For late-acting loci, they assumed quantitative genetic variance under stabilizing selection without considering the individual late-acting loci. They showed that there is a purging threshold rate of self-fertilization, before which the population is effectively outcrossing and deleterious mutations are maintained.

In the present paper, I propose a new mechanism by which deleterious mutations are maintained without being purged from populations with high selfing rates: the coupling effects of early-acting and late-acting loci. I also develop a model considering early-acting and late-acting loci. However, in my model, early- and late-acting loci are distinguished, and the effects of early- and late-acting mutations appear during their respective stages. (Whether they are homozygous or heterozygous does not affect the stage at which their effects appear.) In other words, early-acting loci are expressed during the initiation of embryos to the stage at which selective abortion of embryos ends (assuming overproduction of ovules), whereas late-acting loci are expressed only after selective abortion ends; these loci act during seed completion, seed germination, and seedling growth. Then, I consider inbreeding depression caused by early-acting and late-acting loci. Ovules are overproduced and therefore do not all develop into seeds mainly due to resource limitation, which is a very common phenomenon in plants (LEE 1988). I assume individual loci for both early- and late-acting loci and examine the effects of carrying both early- and late-acting mutations by the same embryos (coupling effects of mutations).

Model

125 ***Simulation process***

126 I carried out simulations to examine when deleterious mutations were maintained in
127 populations that underwent mutations at early- and late-acting loci. Each population consists
128 of n diploid hermaphroditic plants with a selfing rate s and random outcrossing among n
129 plants according to a prior selfing model (LLOYD AND SCHOEN 1992), as explained below.
130 There is no pollen limitation, and all ovules produced are successfully fertilized by self or
131 nonself pollen. I assumed nonoverlapping generations, with the parents in the new generation
132 selected from the seeds produced by the parents in the previous generation. After the initial
133 generation, mutations occur at rate m, which represents the number of mutations per gene per
134 generation. Inbreeding depression may occur if deleterious mutations become homozygous
135 through inbreeding. The simulations followed the dynamics of the deleterious mutations for
136 500 generations.

137 The symbols used in the model are defined in Table 1.

138

139 **Early- and late-acting loci:** Early- and late-acting loci are defined as described in the
140 introduction.

141

142 **Genomes:** Each haplotype genome consists of n_{link} chromosomes, each with the same
143 number of loci (n_{locus}). Of the n_{link} chromosomes, the first to the ith chromosomes carry both
144 early- and late-acting loci, where the first to the jth loci in each chromosome are early-acting
145 loci, and the others are late-acting loci. The other chromosomes carry only late-acting loci.

146

147 **Pollen and ovule production:** Each parent produces n_p pollen grains ($n_p = 4n_{pm}$, where n_{pm}
148 is the number of pollen mother cells) and n_o ovules. Deleterious mutations occur during
149 production of the megaspore and pollen mother cells, and the number of mutations in each

mother cell is sampled from a Poisson distribution with the parameter $4mn_{link}n_{locus}$. Recombination may occur during meiosis at rate r per chromosome per generation. If recombination occurs, a crossover between one pair of chromatids takes place at a randomly determined point (loci in the model are equidistant in cM). Furthermore, different chromosomes randomly segregate during meiosis. As a result, a pollen mother cell develops into 4 pollen grains, whereas one randomly selected megaspore, which develops from a megaspore mother cell, remains in the ovule.

Selfing and outcrossing: Within each parent, randomly selected sn_o ovules are fertilized by randomly selected sn_o self-pollen grains before outcrossing. The remaining $n_p - sn_o$ pollen grains contribute to the population's pollen pool. Outcrossing then occurs among all the n parents in the population. There is no pollen limitation, and the $(1 - s)n_o$ ovules remaining within each parent after selfing are fertilized by pollen that is randomly selected from the pollen pool.

Early selection: Deleterious effects are assumed to be the same for all mutations at any early-acting locus. The relative fitness values are $1 - d_e$ $(0 \leq d_e \leq 1)$ for homozygotes and $1 - h_e d_e$ $(0 \leq h_e \leq 1)$ for heterozygotes, where d_e is the selection coefficient against an individual mutation when homozygous, and h_e is the dominance coefficient. The fitness of an embryo is multiplicative and is determined by the product of the values of all early-acting loci:

$$(1 - d_e)^{hme} (1 - h_e d_e)^{hte},$$

where hm_e and ht_e are the numbers of homozygotes and heterozygotes, respectively.

Development into seeds: Because of resource limitation, each parent can produce at most n_s seeds, and the selective abortion of embryos may therefore occur. I assume that embryos with fitness values higher than a threshold of d_t can potentially develop into seeds. If the number of these embryos is greater than n_s, then n_s embryos are randomly selected by weighting their fitness values determined by the early-acting loci. Otherwise, all embryos develop into seeds (the number of seeds produced may be smaller than n_s).

Late selection: Deleterious effects are assumed to be equal for all mutations occurring at any late-acting locus. The relative fitness values of homozygotes and heterozygotes at a locus are $1 - d_l$ ($0 \leq d_l \leq 1$) and $1 - h_l d_l$ ($0 \leq h_l \leq 1$), where d_l is the selection coefficient, and h_l is the dominance coefficient. The fitness of an embryo (seed) during the late selection stage is multiplicative and is determined by the product of the fitness values of all late-acting loci:

$$(1 - d_l)^{hml} (1 - h_l d_l)^{htl},$$

where hm_l and ht_l are the numbers of homozygotes and heterozygotes, respectively.

Competition for the next generation: Among the seeds produced by the parents in each population, n seeds develop into the parents of the next generation. These seeds are randomly selected by weighting their fitness values due to selection caused by late-acting loci.

Inbreeding depression values

I calculated the inbreeding depression values for each of the final 10 generations of the simulations as follows: I calculated the numbers of seeds produced and the fitness values due to late-acting loci of each seed for each parent, for seeds produced by complete selfing, and

for seeds produced by complete outcrossing. Then, I calculated the three means across all parents and obtained three inbreeding depression estimates: 1) one for the number of seeds produced (reflecting selection on early-acting loci), 2) one for embryo survival ability (also reflecting selection on early-acting loci), and 3) one for seed competitive ability (reflecting selection on late-acting loci). These values were averaged over the last 10 generations.

Simulations carried out

I carried out three kinds of simulations: simulation 1, which includes simulation 1_E, simulation 1_L, and simulation 1_{EL}; simulation 2, which includes simulation 2_E, simulation 2_L, and simulation 2_{EL}; and simulation 3, which includes simulation 3_E, simulation 3_L, and simulation 3_{EL} (Table 2). Simulation 1 distinguishes the effects caused by the coupling of early- and late-acting mutations from those caused by selective interference. Simulation 2 examines the effects of coupling of early- and late-acting mutations by equalizing the potential total effects of early-acting mutations and those of late-acting mutations among the simulation runs (i.e., the numbers of early-acting loci and late-acting loci are the same among the simulation runs). Simulation 3 examines the effects of the relative numbers of early- and late-acting loci.

Simulation 1: comparisons between the coupling effects and selective interference: For this purpose, comparisons were performed between those simulations in which both coupling effects and selective interference can occur and those in which only selective interference can occur (it is difficult to conduct simulations in which only coupling effects occur). Here, the number of loci in which mutations are allowed to occur and the effects of individual mutations were equalized among the simulations because a higher number of mutations and/or larger effects of individual mutations lead to greater effects of selective interference.

Hence, I carried out simulations assuming that only early-acting loci existed (simulation 1_E), only late-acting loci existed (simulation 1_L), or both early- and late-acting loci existed (simulation 1_{EL}). I made the following assumptions for these simulations:

1) In all of these simulations, the total number of loci is 10,000; the numbers of early- and late-acting loci are, respectively, 10,000 and 0 in simulation 1_E, 0 and 10,000 in simulation 1_L, and 1,000 and 9,000 in simulation 1_{EL}.
2) The selection coefficient is the same for all mutations ($d_e = d_l = 0.05$, 0.2, or 0.5).
3) The dominance coefficient is also the same for all mutations ($h_e = h_l = 0.02$).
4) There is no threshold for the development into seeds (i.e., $d_t = 0$), and hence, the form of selection is the same for embryo competition, where early-acting genes express, and for seed competition for the next generation, where late-acting genes express.
5) The strength of selection is the same for embryo competition and seed competition; the numbers of embryos and seeds produced by a parent, n_o and n_p, are 36 and 6, respectively. Thus, 1/6 of embryos or seeds are selected in both the embryo and seed competitions.

The potential effects of mutations are the same in all of these simulations, and selective interference can take place, but the coupling of early- and late-acting mutations does not occur in simulations 1_E and 1_L.

Embryos developing into seeds are randomly selected in simulation 1_L, and seeds developing into the next generation are randomly selected in simulation 1_E. In all of simulation 1, $n_{link} = 5$ and $n_{locus} = 2{,}000$, and in simulation 1_{EL}, each chromosome contains 200 early-acting loci and 1,800 late-acting loci. The other parameter values used were s = 0.3–0.8, $m = 0.00005$, $r = 1$, and $n = 300$. The population size remained constant.

Simulation 2: comparisons when the numbers of early-acting loci and late-acting loci are the same among the simulation runs: In these simulations, the numbers of early-acting loci and late-acting loci are the same among the simulation runs, in contrast to simulation 1. This equalization removes the possible effects of having different numbers of respective loci on the maintenance of mutations. For example, the greater number of early-acting loci (or late-acting ones) will result in the higher degree of inbreeding depression caused by early-acting loci (or late-acting loci). I hence assumed that, in total, 1,000 early- and 9,000 late-acting loci exist in simulation 2. Then, I carried out simulations in which mutations were allowed only in early-acting loci (simulation 2_E), only in late-acting loci (simulation 2_L), or in both the early- and late-acting loci (simulation 2_{EL}). The potential effects of mutations caused by early-acting loci are the same between simulations 2_E and 2_{EL}, and those caused by late-acting loci are also the same between simulations 2_L and 2_{EL}, where the coupling of early- and late-acting mutations occurs only in simulation 2_{EL}.

For the main runs of simulation 2, I assumed $n_{link} = 5$ and $n_{locus} = 2{,}000$, and for each haplotype genome, one chromosome contains 1,000 early- and 1,000 late-acting loci, and the other chromosomes contain only 2,000 late-acting loci. Other assumptions were also made for the other runs (see below). For the other parameters, I used the following values: $s = 0.3–0.8$; $m = 0.00005$; $d_e = 1$; $d_l = 0.05$, 0.2, or 0.5; $h_e = h_l = 0.02$; $d_t = 0.2$; $r = 1$; $n = 300$; $n_s = 10$; and $n_o = n_p = 20$. The population size remained constant.

Effects of linkage: In addition, to examine the effects of linkage on the results, I carried out the following two kind of runs for simulation 2: 1) $n_{link} = 5$ and $n_{locus} = 2{,}000$; for each haplotype genome, each chromosome contains 200 early- and 1,800 late-acting loci (1,000 early- and 9,000 late-acting loci in total, as in the main runs of simulation 2) and 2) all loci are unlinked, all loci segregate independently, and there are 1,000 early- and 9,000

late-acting loci. Simulation 2 was used because if the numbers of early-acting loci and late-acting loci differ among the simulation runs, factors other than the linkage between early- and late-acting loci might affect the results, in which case the effects of the linkage might not be detected. The other parameter values used were the same as those in the main runs of simulation 2, except that r was not assumed for the runs without the linkage of chromosomes.

Effects of the dominance coefficient: Using simulation 2, I also examined the effects of the dominance coefficient in the late-acting loci, h_l. The mean dominance coefficient is 0.1 for *Caenorhabditis elegans* (PETERS *et al.* 2003), 0.17 for *Drosophila melanogaster* (FRY AND NUZHDIN 2003), and 0.197 for nonlethal mutations in yeasts (SZAFRANIEC *et al.* 2003), with large variances. Hence, I assumed h_l = 0.02, 0.1, 0.2, and 0.3 for the late-acting loci. More deleterious mutations are more likely to be recessive than less deleterious mutations (HUBER *et al.* 2018), and early-acting mutations are highly deleterious and nearly recessive (see Introduction of PORCHER AND LANDE 2005b). Hence, I assumed h_e = 0.02 for the early-acting loci for all simulations. Simulation 2 was used because if the numbers of early-acting loci and late-acting loci differ among the simulation runs, factors other than the dominance coefficient might affect the results. The other parameter values used were the same as those in the main runs of simulation 2.

Simulation 3: comparisons when the number of early-acting loci is greater than that of late-acting loci: I also examine the effects of the relative numbers of early- and late-acting loci by exchanging the numbers of respective loci used in simulation 2. In this simulation, n_{link} = 5 and n_{locus} = 2,000, as in simulation 2, but for each haplotype genome, each chromosome contained 1,800 early-acting loci and 200 late-acting loci. Hence, the effects of

300 selective interference could be strong in the early-acting loci in this simulation, whereas
301 those effects could be strong in the late-acting loci in simulation 2. The other parameter
302 values used were the same as those in the main runs of simulation 2.
303
304 ## Results
305 Figure 1 shows examples of the dynamics of the deleterious, recessive mutations that occur
306 in the early- and late-acting loci (simulation 2; 1,000 and 9,000 early- and late-acting loci,
307 respectively). The simulations start with no mutations at either the early- or late-acting loci.
308 If mutations are allowed to occur in both sets of loci, then their numbers increase over the
309 generations in both loci (solid lines). However, if mutations are allowed to occur in only the
310 early- or late-acting loci (1,000 and 9,000 locus mutations, respectively), then they remain
311 rare in both sets of loci (dotted lines).
312
313 ***Simulation 1***
314 Under most parameter values, the numbers of early- and late-acting mutations (left and right
315 panels, respectively, in Fig. 2) that are maintained are greater if both early- and late-acting
316 loci exist (red circles; simulation 1_{EL}) than if only early-acting loci (gray circles; simulation
317 1_E) or only late-acting loci exist (black circles; simulation 1_L). Thus, the presence of both sets
318 of loci promotes the maintenance of mutations under the same effects of selective
319 interference. Here, the differences are smaller for late-acting mutations. This trend may be
320 because the numbers of late-acting loci are similar between simulations 1_{EL} and 1_L (9,000
321 and 10,000, respectively) and, hence, the effects of selective interference should be similar
322 for late-acting loci (also see the explanation for the maintenance of mutations in simulations
323 2 and 3). On the other hand, if s = 0.7 - 0.8 and $d_e = d_l$ = 0.2 or = 0.5, the numbers of
324 early-acting mutations that are maintained are lower if both sets of loci exist than if only

early-acting loci exist. Additionally, if *s* is low or high, the numbers of late-acting mutations that are maintained are similar between the simulations when both sets of loci exist and when only late-acting loci exist.

Simulations 2 and 3

Maintenance of mutations: The numbers of early-acting mutations (left panels in Fig. 3) that are maintained are greater if mutations occur in both the early- and late-acting loci (red circles; simulation 2_{EL}). Early-acting mutations are not maintained only if *s* is very high. In contrast, if mutations do not occur in the late-acting loci, then the numbers of early-acting mutations that are maintained are very low, even if mutations occur in the early-acting loci (gray circles in Fig. 3; simulation 2_{E}).

The number of late-acting mutations (right panels in Fig. 3) is higher when mutations occur in both the early- and late-acting loci (red circles; simulation 2_{EL}), particularly if *s* is moderate. Late-acting mutations are rare only if *s* is very high. However, even if mutations do not occur in the early-acting loci, the numbers of late-acting mutations that are maintained tend to be high (black circles; simulation 2_{L}). This trend may be due to selective interference, which promotes their maintenance under the assumption that the number of late-acting loci (9,000) is greater than that of early-acting loci (1,000). In fact, if the numbers of early-and late-acting loci are 9,000 and 1,000, respectively, then the opposite results are observed (Fig. S1; simulation 3). The numbers of late-acting mutations that are maintained are very small if the mutations do not occur in the early-acting loci, whereas the numbers of early-acting mutations that are maintained are high even if mutations do not occur in the late-acting loci.

The mechanism modeled here increases the frequency of late-acting mutations that are present as heterozygotes; more late-acting mutations are present as heterozygotes if

mutations occur in both the early- and late-acting loci than if they occur only in the late-acting loci (Fig. S2). This case obtains particularly if s is not low and d_l is low.

Inbreeding depression: The inbreeding depression values related to the number of seeds produced (left panels in Fig. 4) and embryo survival ability (Fig. S3) were also greater if mutations occurred in both the early- and late-acting loci, except for very high values of s. The inbreeding depression values related to seed competitive ability (right panels in Fig. 4) also show similar patterns to the number of mutations maintained (Fig. 3).

Effects of the overproduction of ovules: The extent to which ovules are overproduced affects the preservation of deleterious mutations and thus the genetic load maintained in the population (Fig. 5). In this figure, the number of ovules produced by a plant, n_o, is equal to 20 in all panels; therefore, a greater value of n_s indicates a lower degree of ovule overproduction. If the selfing rate is high (s = 0.6, 0.65, and 0.7 in Fig. 5), a small or moderate degree of overproduction is sufficient for maintenance, and if the selfing rate is not very high (s = 0.5 in Fig. 7), deleterious mutations are maintained without overproduction if both early- and late-acting mutations occur (red circles; simulation 2_{EL}). However, if mutations do not occur either in the late- or early-acting loci (gray or black circles; simulation 2_E or 2_L), the number of mutations maintained is low and is almost independent of the maximum number of seeds produced.

Effects of linkage: Linkage does not qualitatively affect the results. The results are very similar to those presented in Figs. 3 and 4, even if each chromosome contains 200 early-acting loci and 1,800 late-acting loci (n_{link} = 5 and n_{locus} = 2000; Fig. S4) or all loci are unlinked and segregate independently (Fig. S5). However, the degree of inbreeding

depression in seed production becomes high (relative to that presented in Fig. 4) when both early- and late-acting mutations occur.

Effects of the dominance coefficient: Irrespective of the value of the dominance coefficient in the late-acting loci, h_l, the numbers of early-acting mutations that are maintained (Fig. S6) and the inbreeding depression values (Figs. S7 and S8) are higher if mutations occur in both the early- and late-acting loci than if mutations do not occur in the late-acting loci, although the differences between these two conditions become small with increases in h_l. On the other hand, the numbers of late-acting mutations that are maintained (Fig. S9) and the inbreeding depression values (Figs. S10) tend to be slightly higher if mutations occur in both the early- and late-acting loci than if mutations do not occur in the early-acting loci, but the differences are very small. This situation may also occur because selective interference promotes their maintenance under the assumption that the number of late-acting loci (9,000) is great.

Discussion

Coupling effects of early- and late-acting mutations

Early- and late-acting mutations are maintained if mutations occur in both sets of loci but are not maintained if they occur only in either the early- or late-acting loci (Figs. 1-4). Thus, for maintenance of mutations to occur, mutations must take place in both loci, which implies that the occurrence of early-acting mutations promotes the spread of late-acting mutations and vice versa.

These results can be understood intuitively. Let *E* and *L* be early-acting and late-acting deleterious alleles, respectively, and *e* and *l* be early- and late-acting wild-type alleles, respectively. Consider embryo production by *EeLl* parents, and assume that an *EeLl*

parent happened to produce *EELL* embryos at a higher frequency and *EELl* and *EeLL* embryos at low frequencies. For example,

parent A; *EELL*, *EeLl*, *Eell*, *eeLl*, *eell*

parent B; *EELl*, *EeLL*, *Eell*, *eeLl*, *eell*

The embryos of parent A are more successful than those of parent B because only *EELL* embryos are unable to survive to the next generation in parent A, whereas both *EELl* and *EeLL* embryos are unable to survive in parent B. In addition, because the frequencies of *E* and *L* alleles in the other embryos are higher in parent A than in parent B, more *E* and *L* alleles are passed to the next generation by parent A. Thus, for given frequencies of *EE* and *LL* in the embryos produced by a parent, more *E* and *L* alleles are passed to the next generation if *EELL* embryos happened to be produced more frequently, such that *EELl* and *EeLL* embryos are produced at lower frequencies. On the other hand, if embryos are overproduced, parent A is not disadvantageous compared to the parent with genotypes such as *eell* because a similar number of seeds develop in both parents. Thus, in comparison to other parents, parent A produces successful seeds bearing heterozygous early- and late-acting mutations at higher frequencies. Mutations spread through these heterozygotes.

Differences from selective interference

The coupling effect of early- and late-acting mutations differs from selective interference and, compared to selective interference, more strongly promotes the maintenance of mutations (Fig. 2). In these simulations, the total number of loci in which mutations are allowed to occur and the form and strength of selection are the same. However, the numbers of mutations that are maintained are much greater when coupling occurs than when selective

424 interference alone occurs for most of the examined parameters. Embryo competition, where
425 early-acting genes express, is a form of within-plant competition, whereas seed competition,
426 where late-acting genes express, is a form of among-plant competition. Thus, the effects of
427 selective interference can be expected to differ between embryo competition and seed
428 competition. Accordingly, selective interference should lead to the maintenance of the
429 greatest number of mutations when only early- or late-acting genes exist. However, the
430 number of mutations maintained is greatest when both genes exist, indicating that the
431 presence of both mutations is important.

432 The difference in the number of mutations maintained between the coupling effect
433 and selective interference may be due to differences between these phenomena in the
434 mechanisms by which mutations are maintained. In selective interference, mutations spread
435 through outcrossed embryos, and hence, strong selection against selfed embryos is necessary;
436 a lower survival rate of selfed embryos leads to a greater number of maintained mutations.
437 On the other hand, in the coupling effect, as explained above, mutations spread through
438 selfed embryos as well as through outcrossed ones through the coupling of early- and
439 late-acting mutations. Because the contribution of outcrossed embryos should be the same,
440 the coupling effect promotes the maintenance of mutations more strongly than does selective
441 interference.

442 The exception is the maintenance of early-acting loci when selfing rates and
443 selection coefficients are very high; more early-acting mutations are maintained when there
444 are 10,000 early-acting loci (Fig. 2). In this case, very strong inbreeding depression occurs,
445 and almost all selfed zygotes die, resulting in very strong selective interference. However, it
446 seems unlikely that mutations are maintained by selective interference when selfing rates and
447 selection coefficients are very high. In the simulations, 10,000 early-acting loci exist. (This
448 parameter value was used for comparisons between the coupling effect and selective

449 interference rather than to derive realistic predictions.) Given that the total numbers of loci
450 are 25,498 in *Arabidopsis thaliana* (KAUL *et al.* 2000) and approximately 35,000 in tomato
451 (SATO *et al.* 2012), it is doubtful that 10,000 early-acting loci actually exist. Thus, very
452 strong mutation effects caused by a very large number of early-acting loci and very
453 deleterious mutations might have promoted the strong purging of selfed embryos to an
454 unrealistic extent.
455
456 ***Effects of the overproduction of ovules***
457 An increase in the overproduction of ovules results in a higher number of deleterious
458 mutations that are maintained (Fig. 5). Porcher and Lande (2005b) similarly showed that a
459 higher degree of overproduction leads to the maintenance of a greater number of mutations.
460 Several adaptive reasons may explain the overproduction of ovules (summarized by
461 PORCHER AND LANDE 2005b) [e.g., enhanced male reproductive success via increased pollen
462 production and export generated from excess flowers (SUTHERLAND 1987; BURD AND
463 CALLAHAN 2000) and enhanced female reproductive success via pollinator attraction (BURD
464 1998)]. In any case, the present model is applicable to most plant species because the
465 selective abortion of embryos may occur, as not all embryos develop into seeds. Hence,
466 given that overproduction of ovules is very common (LEE 1988), the present model may
467 apply to most plant species.
468 However, the overproduction of ovules alone cannot lead to the maintenance of
469 deleterious mutations; the coupling of early- and late-acting mutations is necessary. This is
470 because if mutations do not occur either in the late- or early-acting loci, the number of
471 mutations maintained is low and is almost independent of the degree of overproduction (gray
472 and black circles in Fig. 5; simulations 2_E and 2_L). In contrast to the model of Porcher and
473 Lande (2005b), in which the same mutations become both early acting and late acting, the

474 present model distinguishes early- and late-acting loci, and the effects of early- and
475 late-acting mutations appear during their respective stages. Then, if mutations do not occur in
476 the early-acting loci, embryos developing into seeds are randomly selected, and the
477 overproduction of ovules does not affect the relative frequencies of homozygous and
478 heterozygous late-acting mutations and hence does not affect the maintenance of those
479 mutations. Furthermore, if mutations do not occur in the late-acting loci, the function of
480 overproduction—embryos that will not become vigorous seeds are removed—does not exist.
481 A high degree of overproduction rather enhances the removal of early-acting mutations
482 because of the severe competition among embryos and hence does not contribute to the
483 maintenance of early-acting mutations.
484
485 ***Effects of linkage***
486 Linkage is not an important factor in maintenance of mutations, as evidenced by the similar
487 results among the simulations in which linkage exists (Figs. 3 and 4), all loci are unlinked, or
488 all loci segregate independently (Fig. S5). Thus, the present results were not caused by
489 linkage disequilibrium.
490
491 ***Effects of the dominance coefficient***
492 The coupling effect occurs even if the dominance coefficient in the late-acting loci, h_l, is
493 higher than 0.02, although the effect diminishes with increasing dominance coefficient (Figs.
494 S6-S10). In organisms, mean values of the dominance coefficient range between
495 approximately 0.1 and 0.2, but the values are highly variable among mutations (FRY AND
496 NUZHDIN 2003; PETERS *et al.* 2003; SZAFRANIEC *et al.* 2003; HUBER *et al.* 2018). Hence,
497 many mutations with dominance coefficients much smaller than those means are included,
498 and such mutations should be more effectively maintained by the coupling effect than

mutations with high dominance coefficients. Thus, mutations with small dominance coefficients may contribute more strongly than those with large dominance coefficients to the high inbreeding depression in selfing populations.

Implications for studies of selfing and inbreeding depression

The present results can aid in the understanding of empirical studies of selfing and inbreeding depression. Here, I discuss several topics.

The mechanism modeled here increases the frequency of late-acting mutations present as heterozygotes (Fig. S2). If late-acting mutations are present as heterozygotes, seeds are more vigorous than they are if the same number of mutations are maintained at higher homozygote frequencies. Hence, the risk of extinction of populations caused by the accumulation of deleterious mutations may be lower if mutations are present as heterozygotes in the seeds. Thus, the probability that populations with high selfing rates can persist is higher under the present mechanism than under selective interference alone.

The present study has implications for the study of reproductive allocation in flowers, i.e., the allocation to pollinator attraction and male and female organs, as it shows that the overproduction of ovules affects the extent of inbreeding depression and, hence, the vigor of selfed seeds. Thus, ovule production and outcrossing strategy may be related to each other in a previously unconsidered way. For example, a change in the number of ovules produced results in a change in the vigor of selfed seeds, leading to changes in the evolutionarily stable selfing rate and resource allocation to enhance outcrossing. The adaptive significance of ovule overproduction, such as through bet-hedging and selective abortion (KOZLOWSKI AND STEARNS 1989), may also change under the present model. Thus, the present study may contribute to future studies on these and other topics.

Acknowledgements

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622

623 Table 1. Symbols used in the present model and their definitions.

Symbol	Definition
d_e	Selection coefficient against an individual mutation when homozygous for early-acting mutations
d_l	Selection coefficient against an individual mutation when homozygous for late-acting mutations
d_t	Threshold for the fitness values of embryos above which embryos can potentially develop to seeds
h_e	Dominance coefficient for the early-acting loci
h_l	Dominance coefficient for the late-acting loci
hm_e	Numbers of homozygotes in the early-acting mutations
ht_e	Numbers of heterozygotes in the early-acting mutations.
hm_l	Numbers of homozygotes in the late-acting mutations
ht_l	Numbers of heterozygotes in the late-acting mutations
i	Number of chromosomes carrying both early- and late-acting loci in each haplotype genome (carried by the first to ith chromosomes)
j	Number of early-acting loci in the chromosomes carrying those loci (with the first to jth loci in each chromosome being the early-acting ones)
m	Mutation rate per gene per generation
n	Number of plants in the population
n_{link}	Number of chromosomes in each haplotype genome
n_{locus}	Number of loci in each chromosome
n_p	Number of pollen grains produced by a parent
n_{pm}	Number of pollen mother cells ($n_p = 4n_{pm}$)

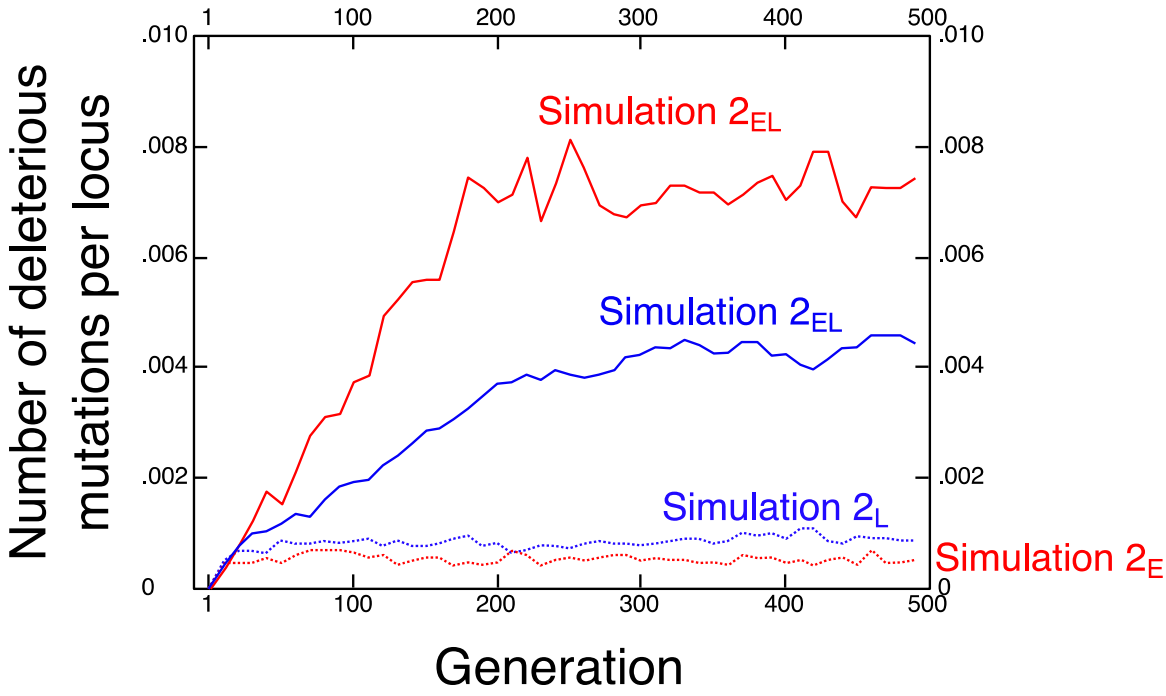
n_o	Number of ovules produced by a parent
n_s	Number of seeds a parent can potentially produce
s	Selfing rate

624

625 Table 2 Simulations carried out.

	Early-acting loci		Late-acting loci		Note	Purpose
	Number	Occurrence of mutations (indicated by "yes")	Number	Occurrence of mutations (indicated by "yes")		
Simulation 1					Total number of loci in which mutations may occur is the same.	Comparisons between the coupling effects and selective interference.
Simulation 1_{EL}	1,000	Yes	9,000	Yes		
Simulation 1_{E}	10,000	Yes	0			
Simulation 1_{L}	0		10,000	Yes		
Simulation 2					Numbers of early-acting loci and late-acting loci are the same among the simulation runs.	Removal of the possible effects of having different numbers of respective loci.
Simulation 2_{EL}	1,000	Yes	9,000	Yes		
Simulation 2_{E}	1,000	Yes	9,000			
Simulation 2_{L}	1,000		9,000	Yes		
Simulation 3					Numbers of early- and late-acting loci are reversed from those in simulation 2.	Examination of the effects of the relative numbers of early- and late-acting loci.
Simulation 3_{EL}	9,000	Yes	1,000	Yes		
Simulation 3_{E}	9,000	Yes	1,000			
Simulation 3_{L}	9,000		1,000	Yes		

626



627

628 **Figure 1** Examples of the dynamics of deleterious, recessive mutations occurring in early-
629 and late-acting loci (simulation 2). Red lines; early-acting mutations, blue lines; late-acting
630 mutations, solid lines; mutations occur in both sets of loci (simulation 2_{EL}), dotted lines;
631 mutations occur in the loci of the corresponding color but not in the other set of loci
632 (simulations 2_E and 2_L).

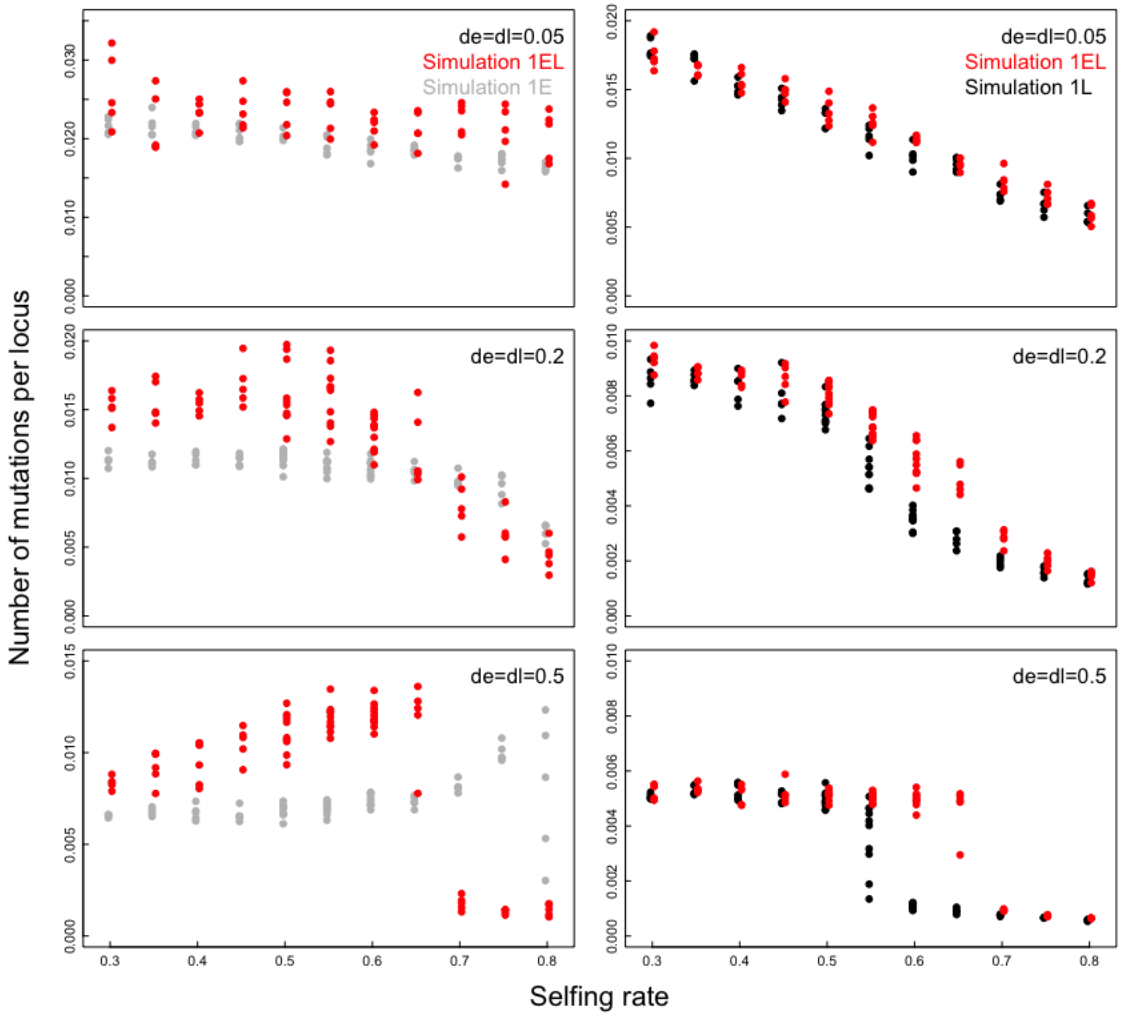


Figure 2 Comparisons of the numbers of mutations maintained between the coupling effects and selective interference (simulation 1). The dependences of the number of early-acting mutations (left panels) and late-acting mutations (right panels) per locus after 500 generations on the selfing rate, *s*, with the selection coefficients against an individual early- or late-acting mutation, d_e or d_l, shown. The total number of loci in which mutations are allowed to occur is 10,000 (n_{link} = 5 and n_{locus} = 2,000) in all simulations. Red circles; simulation 1_{EL}, in which the numbers of early-acting and late-acting loci are 1,000 and 9,000, respectively (with each chromosome containing 200 early-acting loci and 1,800 late-acting loci), and mutations occur in both the early- and late-acting loci. Gray circles; simulation 1_E, in which the numbers of early-acting and late-acting loci are 10,000 and 0, respectively, and mutations occur only in the early-acting loci. Black circles; simulation 1_L, in which the

645 numbers of early-acting and late-acting loci are 0 and 10,000, respectively, and mutations
646 occur only in the late-acting loci. The simulation results of 5 or 10 runs are shown for each
647 combination of parameter values.
648

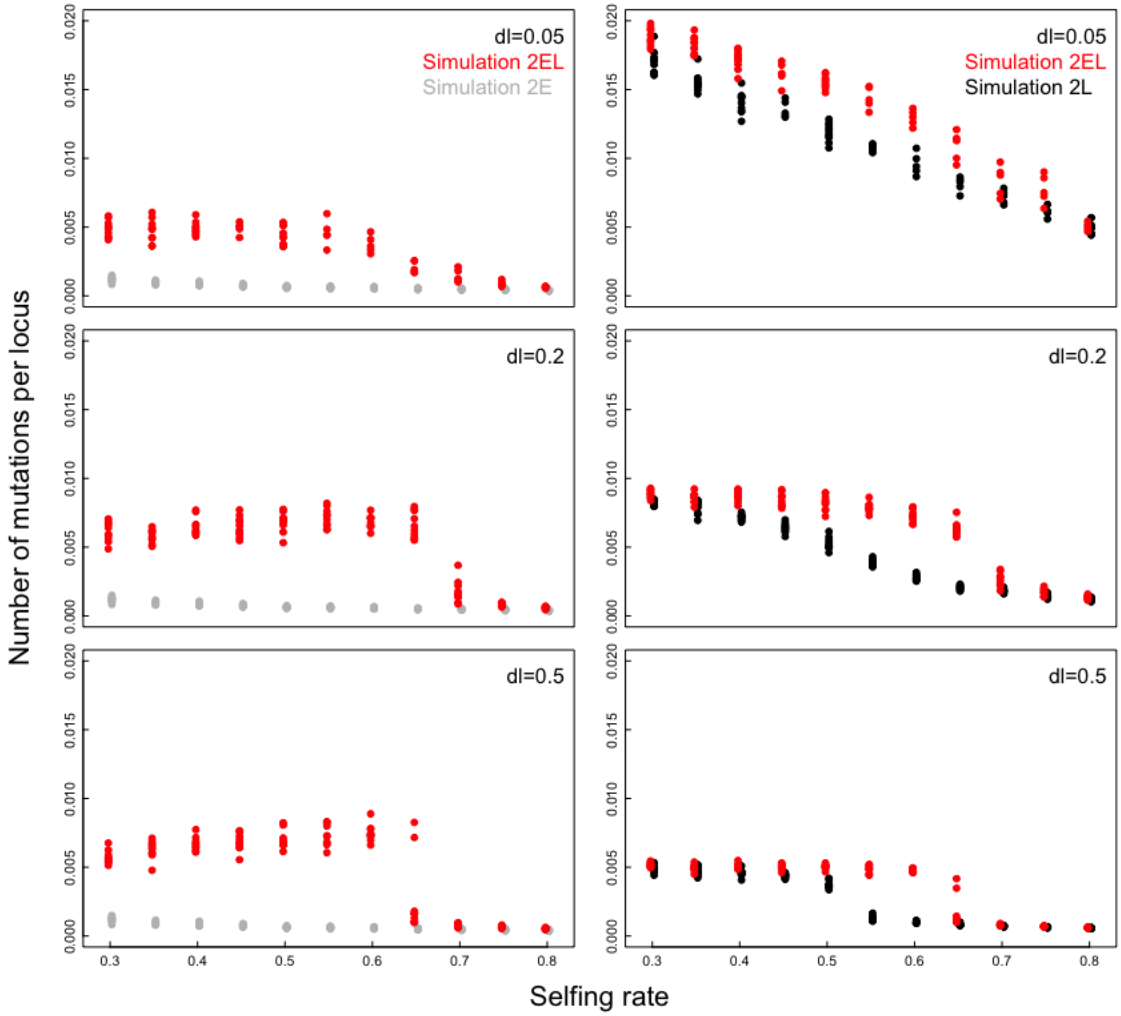


Figure 3 Comparisons of the numbers of mutations maintained among the simulation runs having the same number of early-acting loci and the same number of late-acting loci (simulation 2). The dependences of the number of mutations per locus after 500 generations on the selfing rate, s, with the selection coefficient against an individual late-acting mutation, d_l, shown. In all panels, the numbers of early-acting and late-acting loci are 1,000 and 9,000, respectively, and the early-acting mutations are lethal ($d_e = 1$) if they occur. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles; simulation 2_L, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 5 or 10 runs are shown for each combination of parameter values.

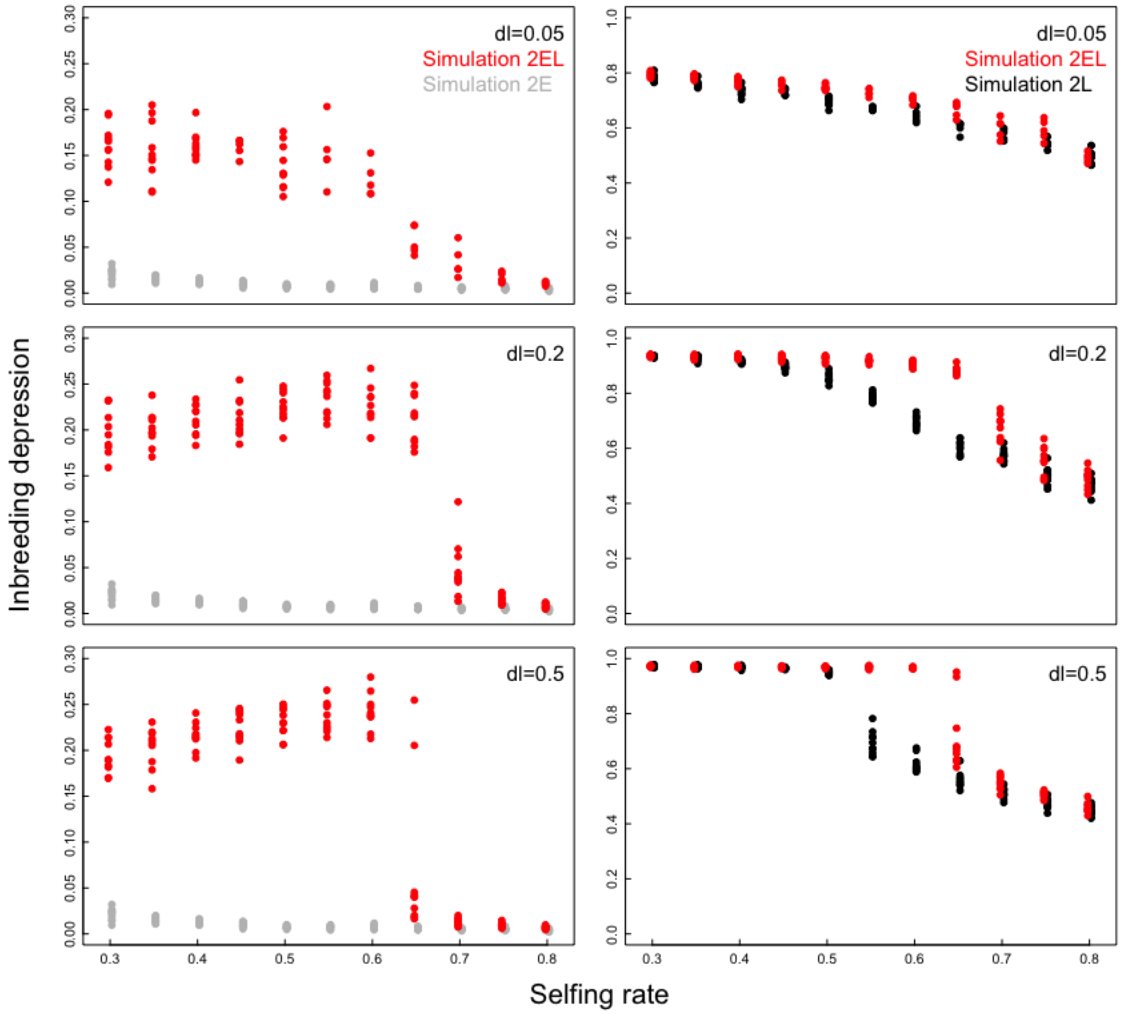


Figure 4 Comparisons of the degree of inbreeding depression among the simulation runs having the same number of early-acting loci and the same number of late-acting loci (simulation 2). The dependences of the effects of inbreeding depression on seed production (left panels) or seed competitive ability (right panels) after 500 generations on the selfing rate, s, with the selection coefficient against an individual late-acting mutation, d_l, shown. In all panels, the numbers of early-acting and late-acting loci are 1,000 and 9,000, respectively, and the early-acting mutations are lethal (d_e = 1) if they occur. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles;

672 simulation 2_L, in which mutations occur in the late-acting loci but not in the early-acting loci.

673 The simulation results of 10 runs are shown for each combination of parameter values.

674

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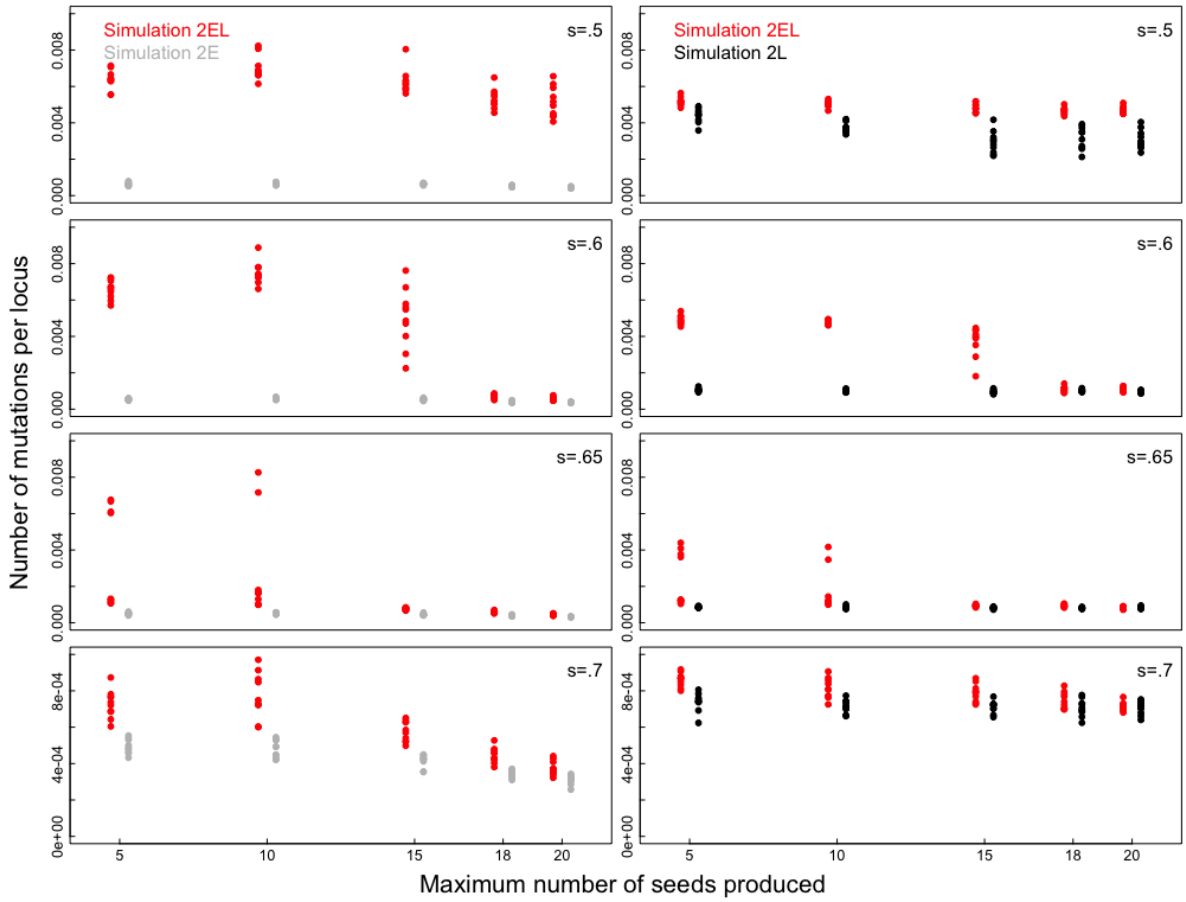

676

677 **Figure 5** The effects of the degree of overproduction of ovules (simulation 2). The

678 dependences of the number of early-acting mutations (left panels) and late-acting mutations

679 (right panels) per locus after 500 generations on the selfing rate, s, with the maximum

680 number of seeds produced by a plant, n_s, shown. The number of ovules produced by a plant,

681 n_o, is equal to 20 in all panels; therefore, a greater value of n_s indicates a lower degree of

682 ovule overproduction. The selection coefficients against an individual early-acting mutation

683 and an individual late-acting mutation are $d_e = 1$ and $d_l = 0.5$, respectively, in all panels. Red

684 circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray

circles; simulation 2_E, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles; simulation 2_L, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 10 runs are shown for each combination of parameter values.

Supplemental Material

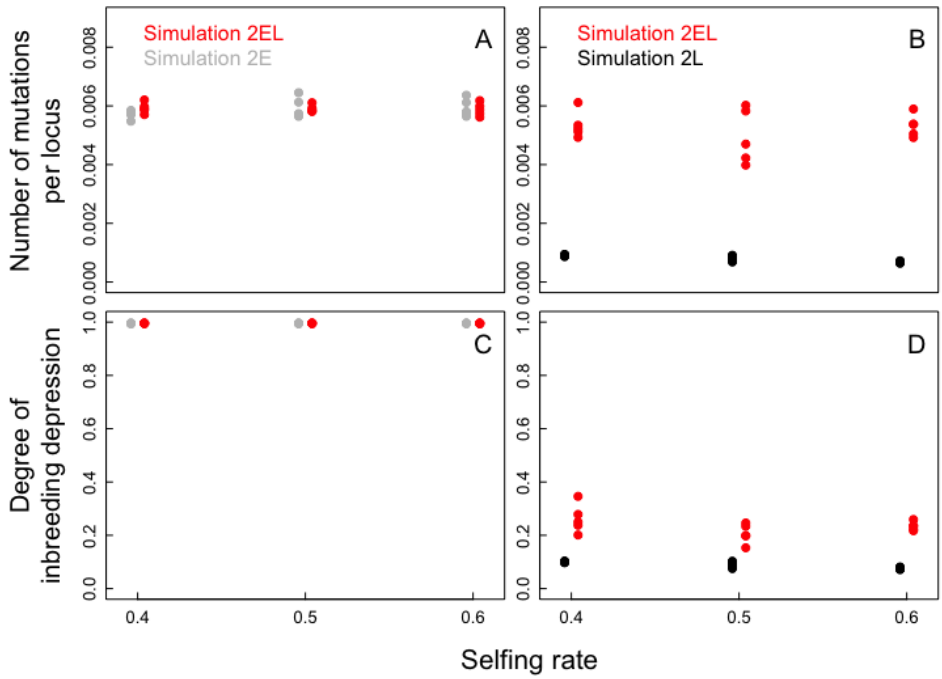

Figure S1 The effects of the relative numbers of early-acting and late-acting loci (simulation 3): comparison with the results in which each chromosome contains 1,800 early-acting loci and 200 late-acting loci (n_{link} = 5 and n_{locus} = 2000). The dependences of the numbers of early- (A) and late-acting (B) mutations per locus after 500 generations and the degree of inbreeding depression related to the number of seeds produced (C) and the seed competitive ability (D) on the selfing rate, s, are shown. In all panels, d_{e} = 1 and d_{l} = 0.5 if early- and late-acting mutations occur, respectively. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_{E}, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles; simulation 2_{L}, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 5 runs are shown for each combination of parameter values.

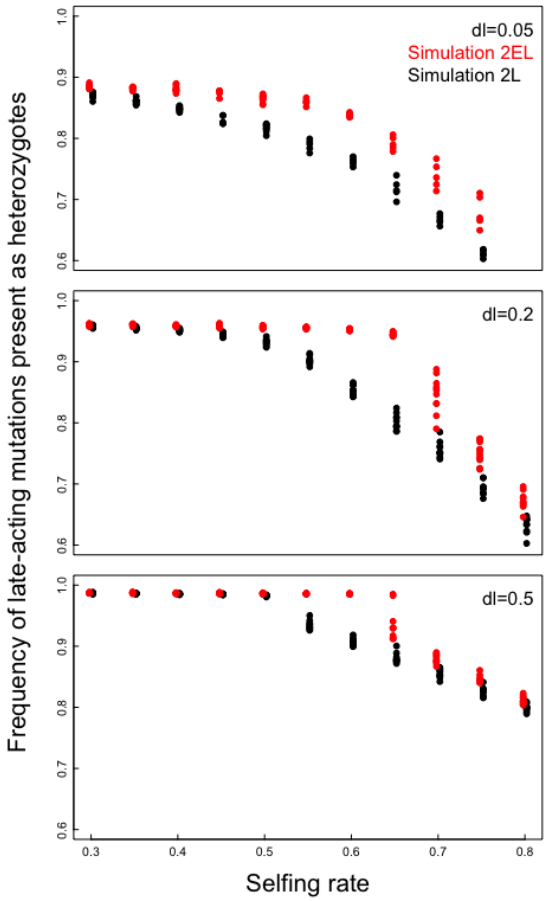

Figure S2 The dependences of the relative frequencies of late-acting mutations present as heterozygotes in the seeds produced after 500 generations on the selfing rate, s, and the selection coefficient against an individual late-acting mutation, d_l (simulation 2). In all panels, the early-acting mutations are lethal ($d_e = 1$) if they occur. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Black circles; simulation 2_L, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 10 runs are shown for each combination of parameter values.

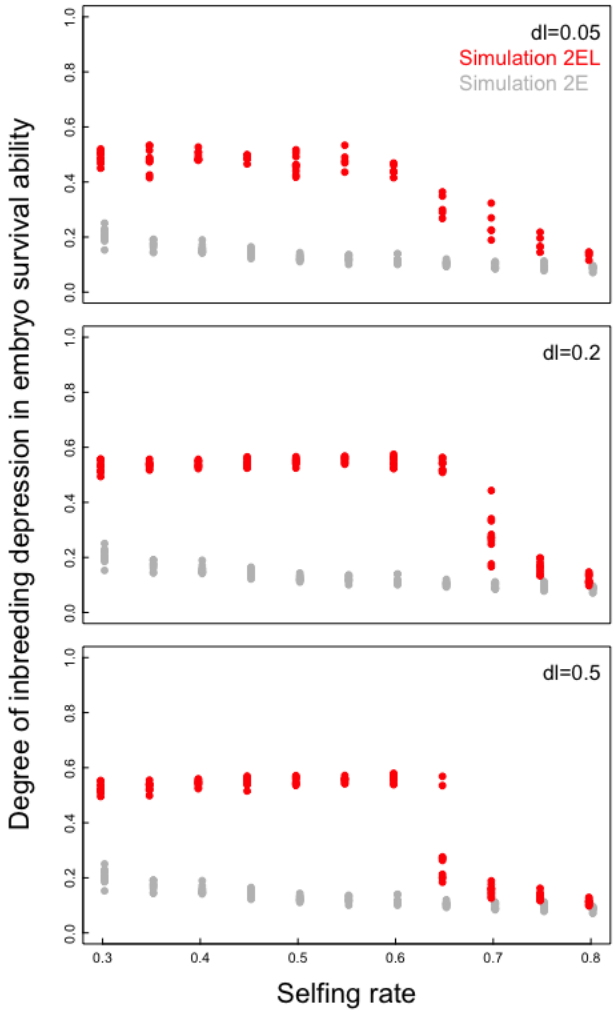

Figure S3 Comparisons of the degree of inbreeding depression among the simulation runs having the same number of early-acting loci and the same number of late-acting loci (simulation 2). The dependences of the effects of inbreeding depression on the embryo survival ability after 500 generations on the selfing rate, s, with the selection coefficient against an individual late-acting mutation, d_l, shown. The early-acting mutations are lethal (d_e = 1) in all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the early-acting loci but not in the late-acting loci. The simulation results of 10 runs are shown for each combination of parameter values.

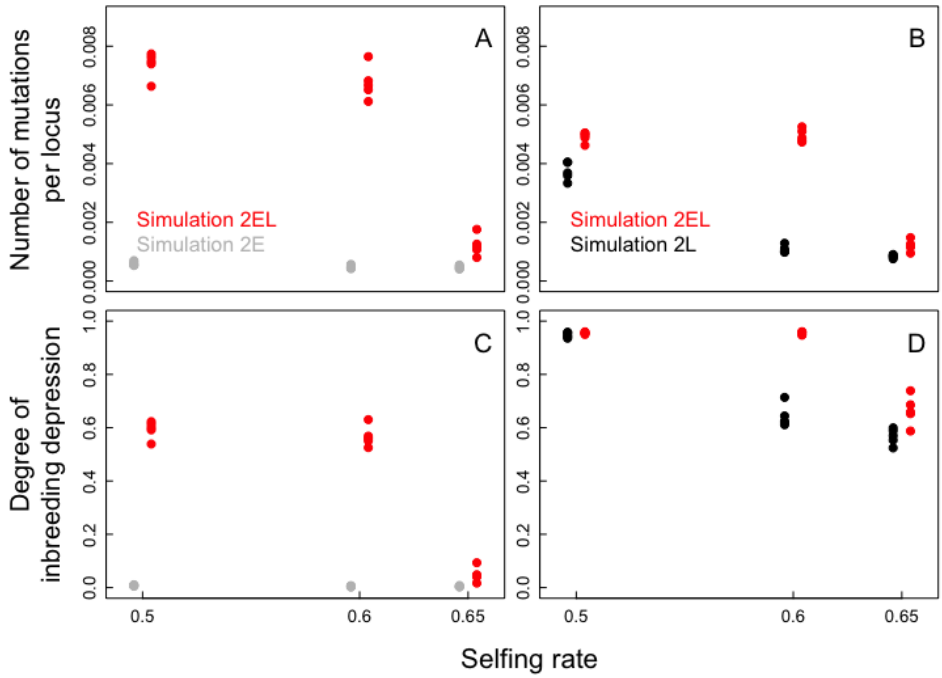

Figure S4 The results of the genetic architecture analysis in which each chromosome contains 200 early-acting loci and 1,800 late-acting loci (n_{link} = 5 and n_{locus} = 2000) (simulation 2). The dependences of the number of early- (A) and late-acting (B) mutations per locus after 500 generations and the degree of inbreeding depression related to the number of seeds produced (C) and the seed competitive ability (D) on the selfing rate, s, are shown. In all panels, d_{e} = 1 and d_{l} = 0.5 if early- and late-acting mutations occur, respectively. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_{E}, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles; simulation 2_{L}, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 5 runs are shown for each combination of parameter values.

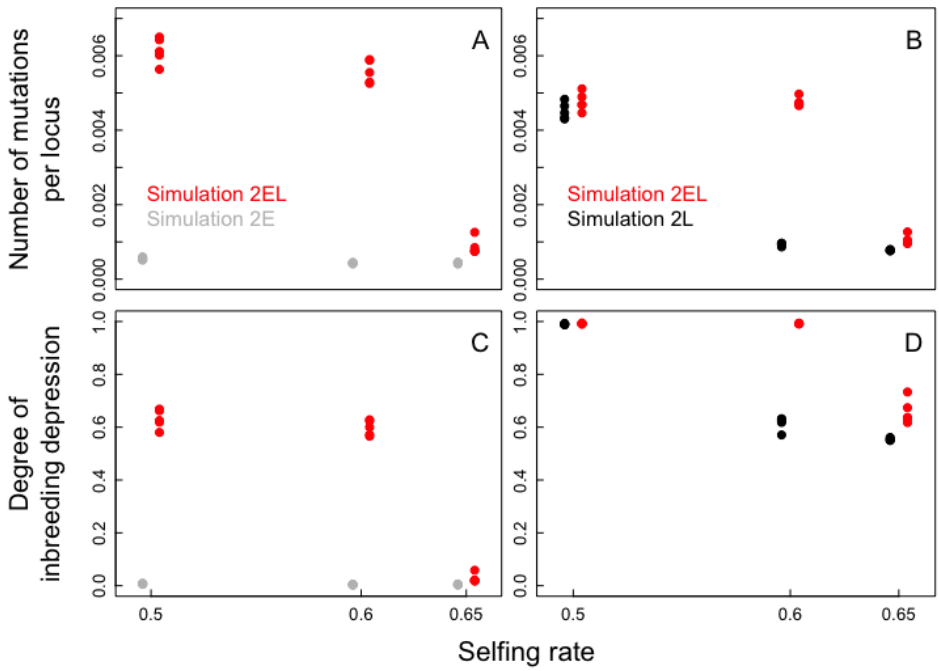

Figure S5 The results of the genetic architecture analysis in which all loci are unlinked, all genes segregate independently, and 1,000 early-acting loci and 9,000 late-acting loci are included (simulation 2). The dependences of the numbers of early- (A) and late-acting (B) mutations per locus after 500 generations and the degree of inbreeding depression related to the number of seeds produced (C) and the seed competitive ability (D) on the selfing rate, s, are shown. In all panels, $d_e = 1$ and $d_l = 0.5$ if early- and late-acting mutations occur, respectively. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the early-acting loci but not in the late-acting loci. Black circles; simulation 2_L, in which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 5 runs are shown for each combination of parameter values.

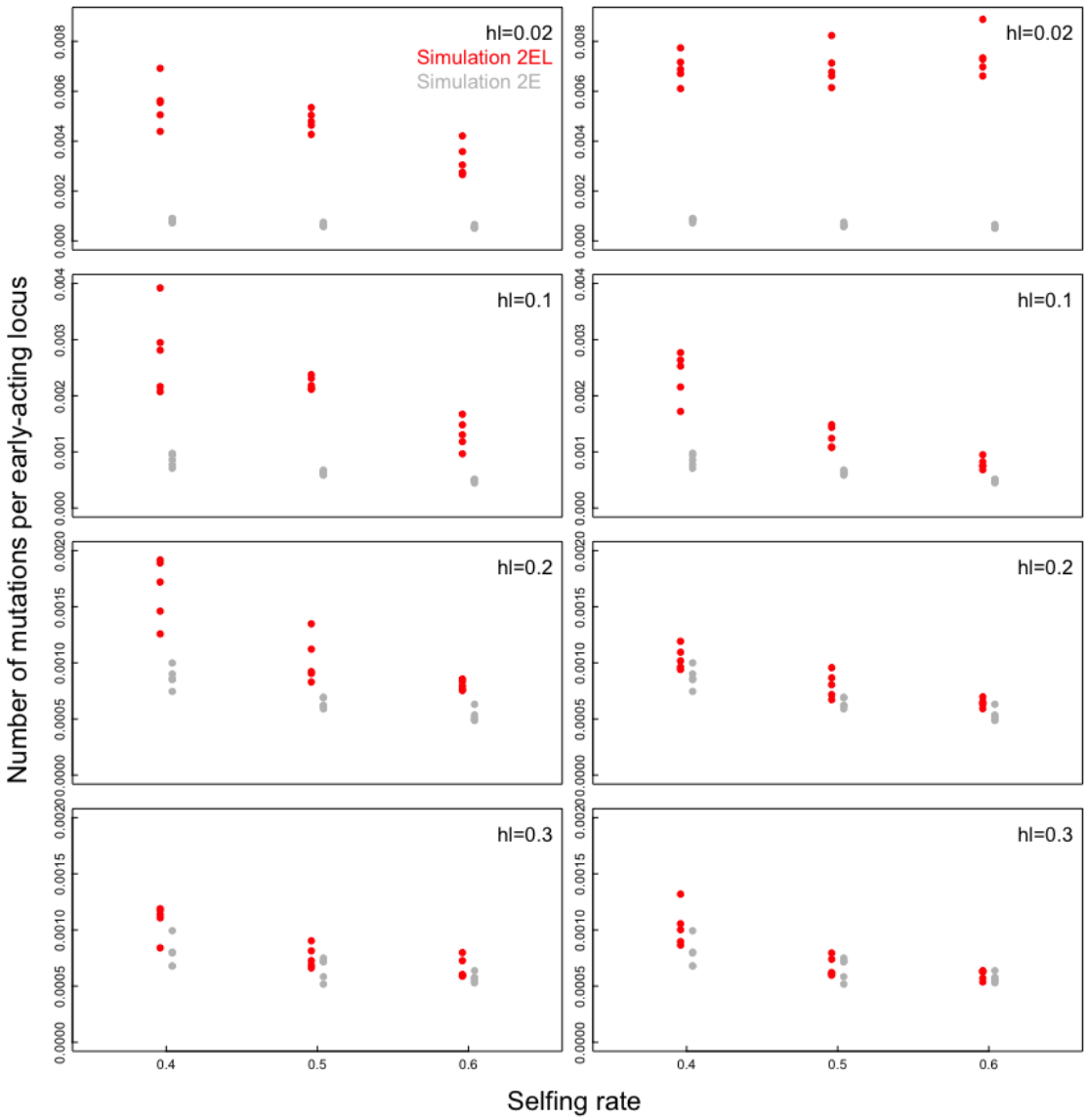

Figure S6 The effects of the dominance coefficient in the late-acting loci on the number of early-acting mutations maintained (simulation 2). The dependences of the number of early-acting mutations per locus after 500 generations on the selfing rate, s, the dominance coefficient in the late-acting loci, h_l, and the selection coefficient against an individual late-acting mutation, d_l (d_l = 0.05 and 0.5 in the left and right panels, respectively), are shown. The early-acting mutations are lethal (d_e = 1), and h_e = 0.02 in all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the

74 early-acting loci but not in the late-acting loci. The simulation results of 5 runs are
75 shown for each combination of parameter values.
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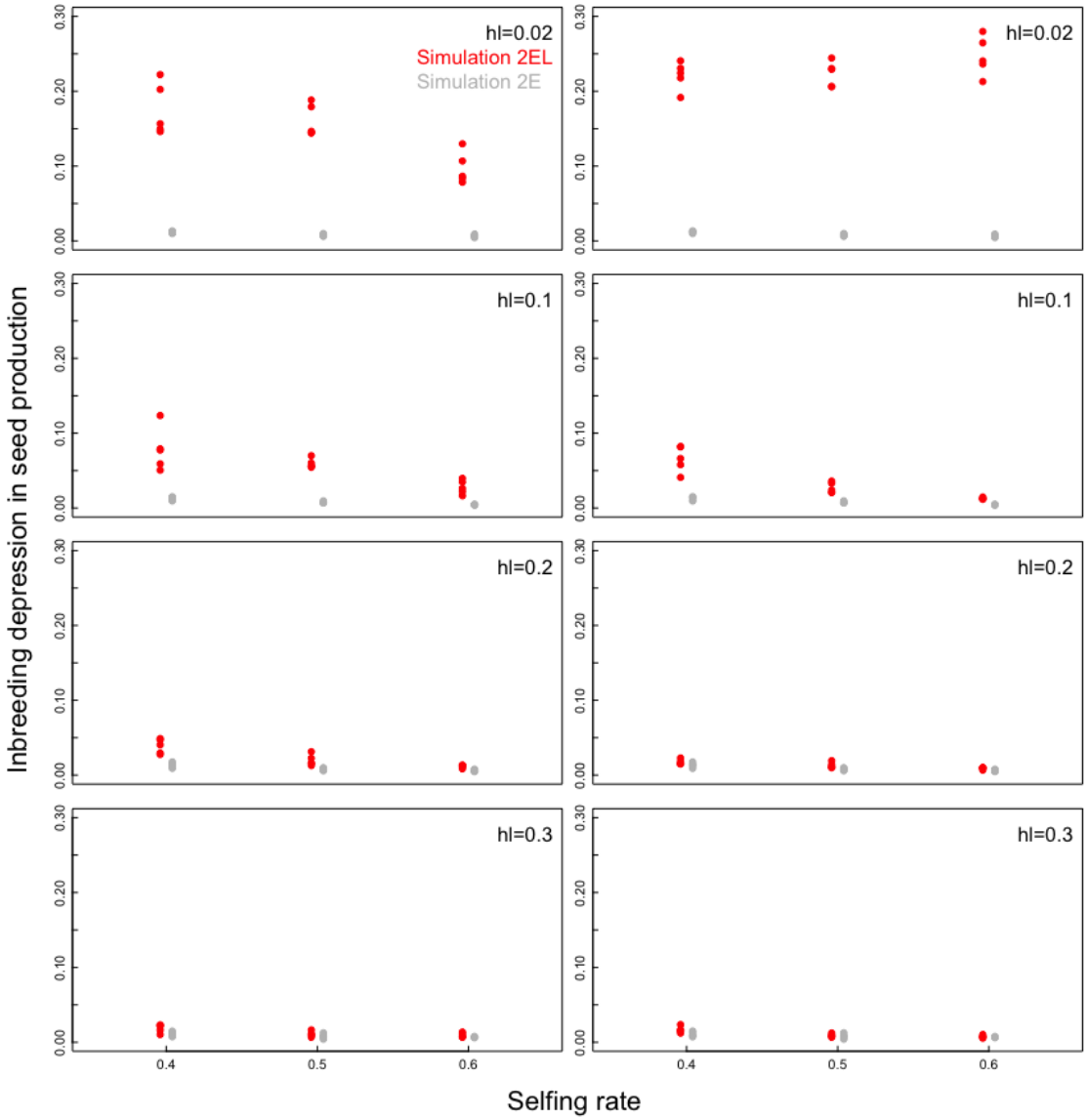

77

78 **Figure S7** The effects of the dominance coefficient in the late-acting loci on the
79 inbreeding depression in seed production (simulation 2). The dependences of the effects
80 of inbreeding depression on seed production after 500 generations on the selfing rate, s,
81 the dominance coefficient in the late-acting loci, h_l, and the selection coefficient against
82 an individual late-acting mutation, d_l (d_l = 0.05 and 0.5 in the left and right panels,
83 respectively), are shown. The early-acting mutations are lethal (d_e = 1), and h_e = 0.02 in
84 all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and
85 late-acting loci. Gray circles; simulation 2_E, in which mutations occur in the

86 early-acting loci but not in the late-acting loci. The simulation results of 5 runs are
87 shown for each combination of parameter values.
88

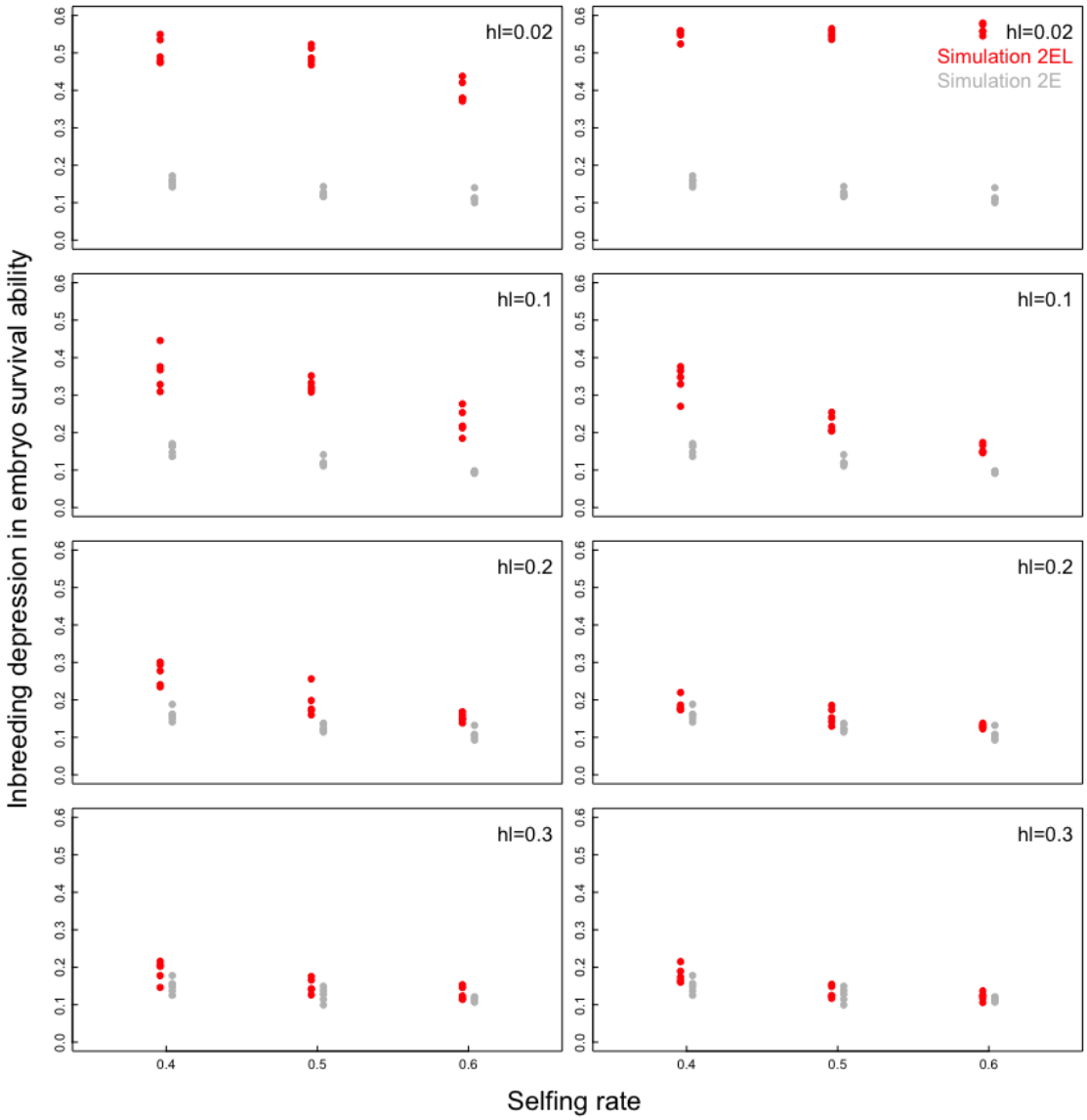

Figure S8 The effects of the dominance coefficient in the late-acting loci on the inbreeding depression in embryo survival ability (simulation 2). The dependences of the effects of inbreeding depression on the embryo survival ability after 500 generations on the selfing rate, s, the dominance coefficient in the late-acting loci, h_l, and the selection coefficient against an individual late-acting mutation, d_l (d_l = 0.05 and 0.5 in the left and right panels, respectively), are shown. The early-acting mutations are lethal (d_e = 1), and h_e = 0.02 in all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Gray circles; simulation 2_E, in which mutations

99 occur in the early-acting loci but not in the late-acting loci. The simulation results of 5
100 runs are shown for each combination of parameter values.
101

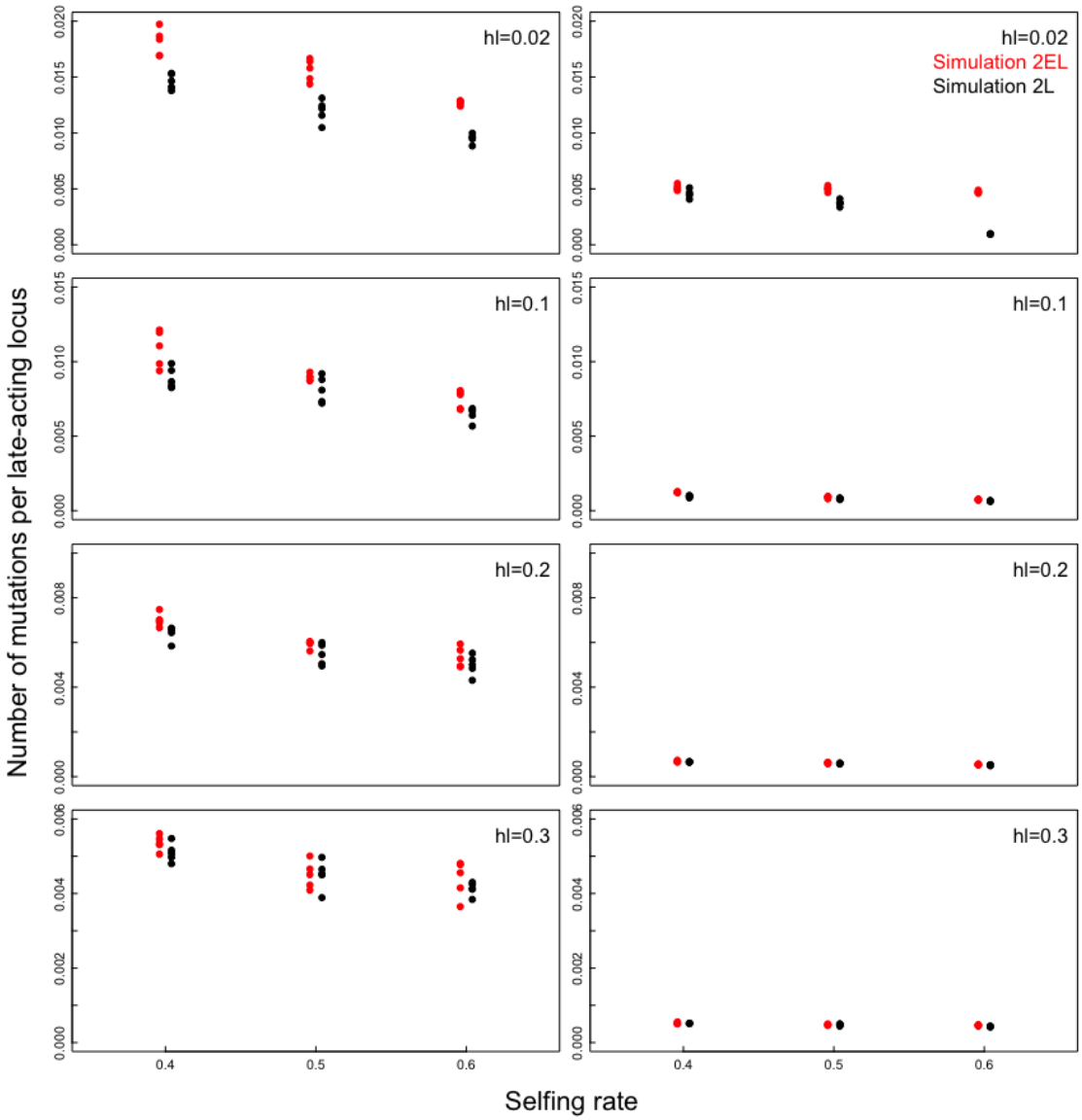

Figure S9 The effects of the dominance coefficient in the late-acting loci on the number of late-acting mutations maintained (simulation 2). The dependences of the number of late-acting mutations per locus after 500 generations on the selfing rate, s, the dominance coefficient in the late-acting loci, h_l, and the selection coefficient against an individual late-acting mutation, d_l (d_l = 0.05 and 0.5 in the left and right panels, respectively), are shown. The early-acting mutations are lethal (d_e = 1), and h_e = 0.02 in all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Black circles; simulation 2_L, in which mutations occur in the late-acting

loci but not in the early-acting loci. The simulation results of 5 runs are shown for each combination of parameter values.

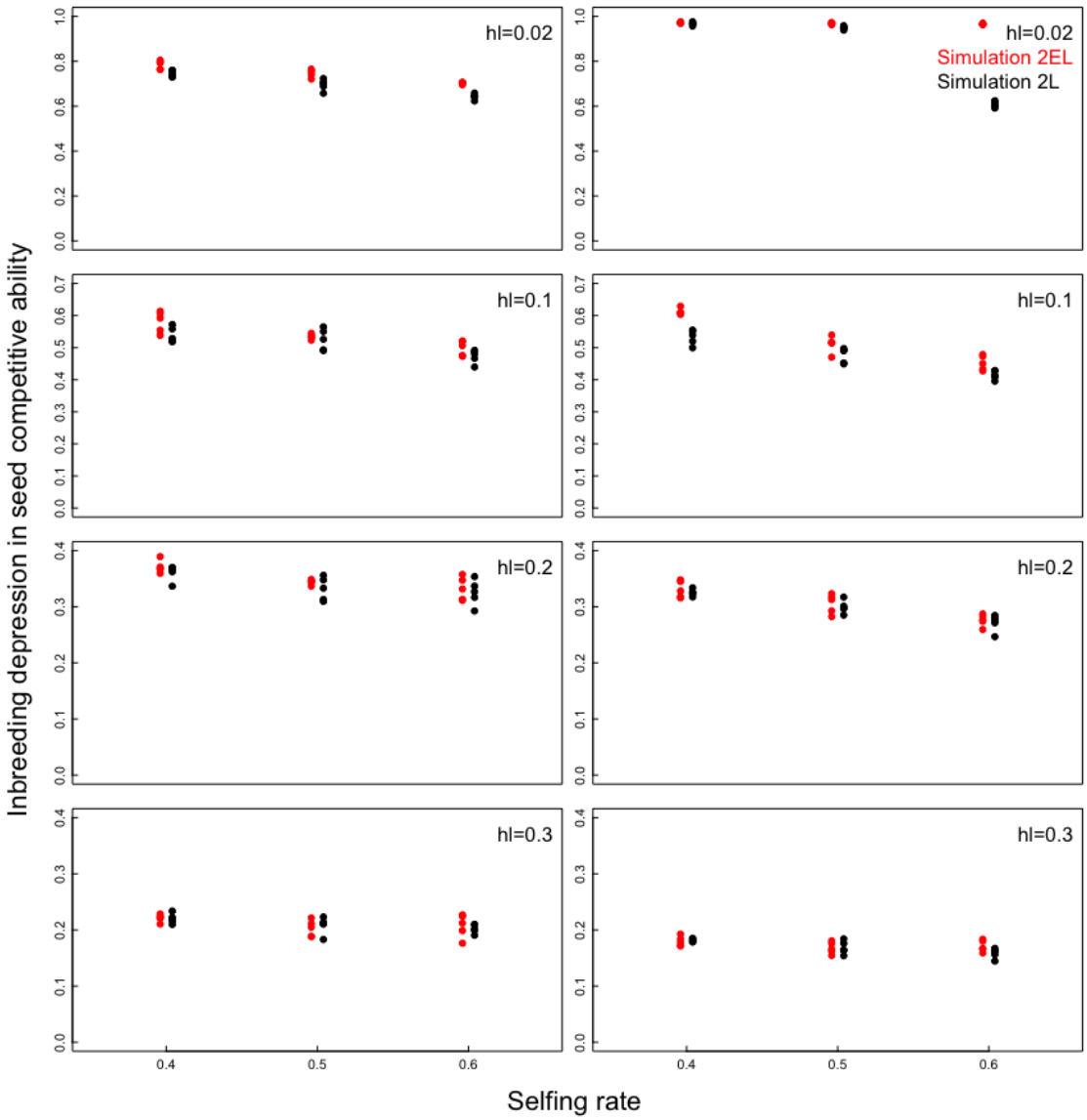

Figure S10 The effects of the dominance coefficient in the late-acting loci on the inbreeding depression in seed competitive ability (simulation 2). The dependences of the effects of inbreeding depression on the seed competitive ability after 500 generations on the selfing rate, s, the dominance coefficient in the late-acting loci, h_l, and the selection coefficient against an individual late-acting mutation, d_l (d_l = 0.05 and 0.5 in the left and right panels, respectively), are shown. The early-acting mutations are lethal (d_e = 1), and h_e = 0.02 in all panels. Red circles; simulation 2_{EL}, in which mutations occur in both the early- and late-acting loci. Black circles; simulation 2_L, in

which mutations occur in the late-acting loci but not in the early-acting loci. The simulation results of 5 runs are shown for each combination of parameter values.