# The role of pseudo-overdominance in maintaining inbreeding depression

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**Abstract**

Classical models ignoring linkage predict that deleterious recessive mutations purge or fix within inbred populations, yet these often retain moderate to high segregating load. True overdominance generates balancing selection that sustains inbreeding depression even in inbred populations but is rare. In contrast, arrays of mildly deleterious recessives linked in repulsion may occur commonly enough to generate pseudo-overdominance and sustain segregating load. We used simulations to explore how long pseudo-overdominant regions (POD's) persist following their creation via hybridization between populations fixed for alternative mutations at linked loci. Balancing haplotype loads, tight linkage, and moderate to strong cumulative selective effects serve to maintain POD's, suggesting that POD's may most often arise and persist in low recombination regions (e.g., inversions). Selection and drift unbalance the load, eventually eliminating POD's, but

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this process is very slow when pseudo-overdominance is strong. Background selection across the genome accelerates the loss of weak POD's but reinforces strong POD's in inbred populations by disfavoring homozygotes. Further modeling and studies of POD dynamics within populations could help us understand how POD's affect persistence of the load and how inbred mating systems evolve.

Keywords: Inbreeding; purging; fixation; drift load; pseudo-overdominance; associative overdominance; POD; linkage; recombination.

# 1 Introduction

Mechanisms exist to ensure outcrossing in most species indicating that selection consistently acts against inbred progeny (Darwin, 1876). Nevertheless, numerous taxa inbreed either inadvertently (e.g., from local dispersal and/or mating) or via evolved adaptations, e.g., facultative self-fertilization in some snails and the self-fertilizing flowers found in many Angiosperms. Since Darwin's seminal work, inbreeding depression (ID) has been accepted as the primary selective force favoring outcrossing. Inbreeding greatly depresses fitness in most higher plants and animals (Keller and Waller, 2002) including many inbred populations (Byers and Waller, 1999). Although dominance, overdominance, and certain forms of epistasis can generate ID, evolutionists generally accept that true overdominance is rare and that most ID derives from dominance, i.e., the cumulative action of deleterious recessive mutations acting across many loci (Charlesworth and Charlesworth, 1987; Crow, 1993, 1999a).

By greatly increasing homozygosity, inbreeding exposes deleterious recessive mutations, reducing inbred fitness and selecting against those mutations. This purifying selection acts to purge unlinked, highly deleterious, recessive mutations, reducing the inbred load (Lande and Schemske, 1985a). Selection is far less effective against mildly deleterious mutations, allowing many to drift and fix whenever the product of effective population size ($N_e$) and their selective effect in homozygotes (s) approaches one or less (Crow and Kimura, 1970; Hedrick et al, 2016). This generates an appreciable fixed "drift load" in many small populations (Whitlock et al, 2000; Charlesworth, 2018). Strong selection acting against deleterious mutations at some loci also tends to fix some deleterious mutations segregating in repulsion at linked loci. This process is termed background selection (Charlesworth et al, 1997; Zhao and Charlesworth, 2016). Purifying selection, drift, and background selection all reduce heterozygosity and thus segregating variation and ID. It is thus puzzling why appreciable ID and outcrossing mechanisms persist in many inbred plant and animal populations (Waller, 2021, termed this "Darwin's paradox").

That heterozygosity, ID, and outcrossing mechanisms persist even in inbred popula-

tions suggests the existence of some force countering purging and fixation. Inbreeding reduces effective rates of recombination while increasing linkage and identity disequilibria, the associations occurring between alleles and genotypes at different loci (Sved, 1971; Lewontin, 1974). These associations generate selective interference among loci, reducing the efficiency of purifying selection (Roze, 2015). Some hypothesize that this selective interference might sustain enough segregating variation to account for persistent inbreeding depression and load (Winn et al, 2011). New mutations or the regular introduction of alternative fixed mutations in crosses between sub-populations provide other mechanisms to replenish variation and sustain ID (Charlesworth, 2018).

Overdominance generating balancing selection against both homozygotes provides another mechanism that could sustain heterozygosity and ID. Single-locus overdominance, however, is considered only a rare contributor to inbreeding depression relative to the deleterious recessive mutations that constitute most of the load segregating in natural populations (Charlesworth and Charlesworth, 1998; Crow, 1999a). Most apparent overdominance instead appears to reflect pseudo-overdominance (Charlesworth and Willis, 2009; Hedrick et al, 2016). According to expectations from previous theoretical works, whenever deleterious recessive mutations are linked in repulsion, pseudo-overdominance will emerge (Ohta, 1971). Because pseudo-overdominance is a consequence of commonly occurring minor deleterious loci, it should be more frequent than true overdominance. Extrapolating Kimura and Ohta's (1971) results on true and associative overdominance, when the cumulative overdominant effects are strong enough, pseudo-overdominant selection should act to sustain segregating genetic variation and inbreeding depression, even within small and inbred populations, making its consequences on selection highly relevant for understanding the evolution of inversions Kirkpatrick (2010), inbreeding depression (Waller, 2021; Glémin, 2021), and mutational dynamics within particular genomic regions (Zhao and Charlesworth, 2016; Becher et al, 2020). Waller (2021) reviewed these ideas and the evidence that localized zones of high heterozygosity exist generating balancing selection.

Here we evaluate whether the conditions necessary for maintaining pseudo-overdominance

are plausible and thus might help account for persistent genetic diversity and inbreeding depression particularly in partially self-fertilizing populations. We use theory and simulations to explore the evolutionary stability and dynamics of pseudo-overdominant blocks of load loci ("POD's" here following Waller, 2021). In particular, we first link existing theory on overdominant selection to evaluate the conditions necessary for the stability of POD's made up of varying numbers of segregating mutations. Unless they are associated with truly overdominant loci (i.e., sheltered load), it seems unlikely that POD's would arise in isolated populations. New mutations are more likely to go to fixation or be purged before enough are accumulated to generate a POD capable of affecting other genetic processes. It therefore seems more plausible that POD's would be observed in hybrid populations isolated long enough to allow for mutations in low recombination regions to fix. We therefore simulate admixture between two populations, each fixed for different sets of deleterious mutations within a specific genomic region. This allows us to estimate the parameter values needed to generate a POD with enough power to sustain heterozygosity and ID over many generations. We then explore how varying levels of linkage, dominance, selection, and self-fertilization affect POD persistence and erosion within populations. We also examine patterns of POD erosion in response to selection and recombination. These results lead to us to discuss when and how often POD's arise, how long they persist, and thus how evolutionarily significant they may be in sustaining heterozygosity, inbreeding depression, and segregating load.

# 2 Approaches

## 2.1 Load needed to generate a POD

Kimura and Ohta (1971) demonstrated that when the selective effects generating true overdominance are strong enough, a stable equilibrium can exist that perpetuates the two overdominant alleles indefinitely even within a fully self-fertilizing population. Consider a scenario in which two haplotypes, noted H1 and H2, occur within a diploid population self-fertilizing at rate $\sigma$. Each homozygote suffers a fitness reduction ($s_1$ or $s_2$) compared

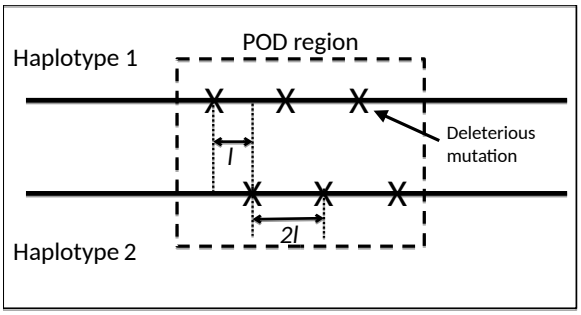


Figure 1: Genetic structure of the POD region (delimited by the dashed box). Deleterious mutations (represented by crosses) linked in cis occur at a distance $2\ell$ cM from each other along the same chromosome, alternating (at a distance $\ell$ cM) with trans mutations on the opposite chromosome. Close, regular, and alternating spacing of recessive deleterious mutations along both haplotypes ensure linkage and pseudo-overdominance.

to the heterozygote fitness. In the case of true overdominance, they showed that a stable polymorphism will persist at an over-dominant locus (with both alleles at a frequency of 0.5) when:

$$\sigma < \frac{2s_2(1-s_2)}{s_1+s_2-2s_1s_2}. \tag{1}$$

This equilibrium always exists when both segregating homozygotes reduce fitness by at least half ($s1$, $s2 > 0.5$). Beyond this point, selection acts to maintain overdominance even as the selfing rate approaches one as selection removes homozygotes faster than they are created by selfing (Rocheleau and Lessard, 2000). Inequality (1) will always be true when $s1 = s2$ except in completely selfing populations. The higher the self-fertilization, the more balanced the load must be (see Supp. Fig. A2).

We use this threshold under true overdominance to estimate the number of fully linked load loci within a block of pseudo-overdominant alleles (POD) required to generate the necessary level of overdominance needed to maintain a stable equilibrium (see Eq. 1). This much balancing selection ensures that the POD will persist. To make the models equivalent, we assume complete linkage among matched sets of mildly deleterious mutations, all with the same coefficient of selection $s = 0.01$ and plausible levels of

dominance $h = 0.2$ matching empirical observations in yeast and *Drosophila* – Agrawal and Whitlock (2011). We further assume that each haplotype carries the same number $n$ of deleterious mutations and that mutations within the POD are evenly spaced, occurring at intervals of $\ell$ cM between alternating trans-mutations on opposing haplotypes (Fig. 1). As fitness effects are considered multiplicative across loci, an individual's fitness is:

$$W = (1 - hs)^{he}(1 - s)^{ho} \tag{2}$$

where $he$ and $ho$ are the number of heterozygous and homozygous mutations, respectively, carried by the individual. Assuming no cross-overs occur within the POD, homozygosity only occurs in individuals that carry two copies of the same haplotype (genotype $H_1H_1$ or $H_2H_2$). In this case, the coefficient of selection acting against either homozygote ($s_H = s_1, s_2$), relative to the fitness of the heterozygote $H_1H_2$ ($W_{AA}/W_{Aa}$) is:

$$s_H = 1 - \frac{(1 - s)^n}{(1 - hs)^{2n}}. \tag{3}$$

This expression allows us to determine the number of deleterious alleles per haplotype necessary to sustain enough overdominance to preserve both haplotypes via stable balancing selection (see Supplementary Material):

$$n = \frac{\log(1 - s_H)}{\log(1 - s) - 2\log(1 - hs)} \tag{4}$$

Given the $s$ and $h$ values assumed above and the need for $s_H$ to be at least 0.5, 115 or more load loci per haplotype are needed to sustain the POD indefinitely (Supp. File 1, Fig. S1). As with true overdominance, strong and symmetrical selection create the most favorable conditions for sustaining both haplotypes.

## 2.2 Inbreeding depression

Inbreeding depression is a local variable, reflecting the number of heterozygotes maintained in a population. The general equation used to estimate inbreeding depression

is:

$$\delta = 1 - \frac{W_s}{W_o} \tag{5}$$

where $W_s$ is the fitness of selfed offspring and $W_o$ that of outcrossed offspring (Charlesworth and Charlesworth, 1987). When mutations are deleterious, and accounting for drift, this variable depends on the haploid mutation rate $U$, the coefficient of selection $s$ and the dominance of mutations $h$ (see equation 3 from Bataillon and Kirkpatrick 2000):

$$\delta_s = 1 - \exp\left[-U\left(\frac{(1-2h)(1+F)}{2(h+F-hF)} - \frac{(1-2h)(1+F)(1-2hs)}{8(h+F-hF)^2 sN}\right)\right], \tag{6}$$

where $F = \sigma/(2-\sigma)$ is the equilibrium inbreeding coefficient (expected deviation from Hardy-Weinberg equilibrium of genotype frequencies). Though this expression for $F$ remains true for weak overdominance (Glémin, 2021), when there is strong overdominance, the inbreeding coefficient depends on the coefficients of selection and allelic frequencies (Appendix A4 from Kimura and Ohta, 1971). In our case with symmetrical selection against homozygotes, this term is given as:

$$\hat{F} = \frac{2 - s_H - \sigma + s_H\sigma - \sqrt{(2-s_H)^2 - 2\left(2 - s_H - s_H^2\right)\sigma + (1-s_H)^2\sigma^2}}{2s_H}. \tag{7}$$

$\hat{F}$ will tend to zero with increasing $s_H$ (see Fig. A1 in Supp. File 1), eventually strengthening selection enough to remove homozygotes from the population faster than they are generated. Selfing populations subject to strong overdominant selection thus tend to behave like outcrossing ones as low fitness homozygotes are eliminated. At equilibrium, this inbreeding load $\delta_{od}$ for symmetrical overdominance can be written as:

$$\delta_{od} = \frac{(1+\hat{F})i}{2 - s_H} \tag{8}$$

where, when $s_1 = s_2 = s_H$, $i = \frac{s_H^2}{2s_H}$ - see Eq. A2from Supp. File 1 and Kimura and Ohta (1971). We provide the general expressions for $\hat{F}$ and $\delta_{od}$ in Supp. File 1 (see Eq. A3).

If we assume that selection against deleterious mutations elsewhere in the genome and overdominant selection do not interfere with one another and fitness effects remain multiplicative (see for example Kirkpatrick and Jarne 2000), the upper limit of the expected level of inbreeding depression will be:

$$\delta = 1 - (1-\delta_{od})(1-\delta_s), \tag{9}$$

setting $F$ in Eq. 6 to $\hat{F}$.

Depending on the strength of overdominant selection, $\delta_{od}$ can increase with the selfing rate $\sigma$ (Charlesworth and Charlesworth, 1987, 1990). The opposite tendency is observed for $\delta_s$, which decreases with $\sigma$ as purging becomes more efficient. It is therefore possible to have $\delta$ maintained at similar levels in outcrossers and selfers, depending on the rates of background mutation $U$ and the strength of the POD.

## 2.3 Recombination and POD's

Thus far, we have assumed complete linkage in order to apply one-locus overdominance theory to infer the strength of selection against homozygotes necessary to sustain a stable equilibrium. However, some recombination will occur, allowing the strong linkage disequilibrium among loci within a POD to erode over time. If recombination within the POD block is rare (reflecting tight linkage), any new haplotype $H_c$ will be generated by a single recombination event. As the positions of deleterious alleles among different haplotypes alternate in trans relative to flanking mutations on the same chromosome in $H_1H_2$ heterozygotes (Fig. 1), the recombinant haplotype $H_c$, and its complement will also carry $n$ deleterious homozygous mutations, except when recombination occurs at a tip of the POD. A single mutation is then cleaved, trimming the load to $n-1$ deleterious alleles. Using a system of Ordinary Differential Equations, the change in frequencies $P_1$, $P_2$ and $P_c$ of the three respective haplotypes $H_1$, $H_2$ and $H_c$ can be expressed:

$$\begin{aligned}
\Delta_{P_1} &= \frac{P_1((1-\hat{F})(1-s_{c,1})P_c + (1-s_H)((1-\hat{F})P_1 + \hat{F}) + (1-\hat{F})P_2) - P_1\overline{W}}{\overline{W}} \\
\Delta_{P_2} &= \frac{P_2((1-\hat{F})(1-s_{c,2})P_c + (1-s_H)((1-\hat{F})P_2 + \hat{F}) + (1-\hat{F})P_1) - P_2\overline{W}}{\overline{W}} \\
\Delta_{P_c} &= \frac{P_c((1-\hat{F})(1-s_{c,1})P_1 + (1-s_c)((1-\hat{F})P_c + \hat{F}) + (1-\hat{F})(1-s_{c,2})P_2) - P_c\overline{W}}{\overline{W}}.
\end{aligned} \tag{10}$$

The mean fitness of the population $\overline{W}$ is the sum of the expected genotypic frequencies after selection (see Supplementary Material), and $s_c$, $s_{c,1}$ and $s_{c,2}$ are the coefficients of selection associated respectively with haplotypes $H_cH_c$, $H_cH_1$ and $H_cH_2$. Resolving this system for arbitrary values of $s_c$, $s_{c,1}$ and $s_{c,2}$, one finds that the only possible equilibria

are those involving the fixation of one of the three haplotypes, or maintaining only two of them. Hence a rare haplotype $H_c$ should either be lost, go to fixation or replace one of the initial haplotypes and co-exist with the remaining haplotype. In order for $H_c$ to increase in frequency, $\Delta_{P_c}$ must be positive at the moment it enters the population, otherwise it will be eliminated immediately. Assuming $P_c$ is of order $\epsilon$ ($\epsilon$ being very small), the expression for $\Delta_{P_c}$ for the leading order of $P_c$ (noted $\bar{\Delta}_{P_c}$) can be derived. In a population at equilibrium with $P_1 = P_2 = (1-\epsilon)/2$:

$$\bar{\Delta}_{P_c} = \frac{2((1+\hat{F})s_H - s_{c,1} - s_{c,2} - \hat{F}(2s_c - s_{c,1} - s_{c,2}))}{2 - s_H - \hat{F}s_H}. \tag{11}$$

The denominator of this expression is always greater than 0 for $s_H < 1$. To understand the behavior of $\bar{\Delta}_{P_c}$, we simplify the above equation by setting $\hat{F}$ to 0 (no self-fertilisation or very strong overdominant selection with $s_H \approx 1$, see Supp Fig. A1). In this case Eq. 11 is $2(s_H - s_{c,1} - s_{c,2})/(2 - s_H)$. If no mutations have been cleaved through recombination (*i.e* $H_c$ carries $n$ mutations), the numerator $2(s_H - s_{c,1} - s_{c,2}) \leq 0$ (see Eq. B1 in Supp. File 2 for expressions of $s_{c,1}$ and $s_{c,2}$). In this case $\bar{\Delta}_{P_c}$ is always negative (see Fig. B2 in Supp. File 2) and $H_c$ haplotypes will be selected against. If however $H_c$ carries $n-1$ mutations, the resulting coefficients of selection (Eq. B2 in Supp. File 2) lead to a positive $\bar{\Delta}_{P_c}$ (as the numerator can in this case be positive). The larger $\hat{F}$ (or the selfing rate $\sigma$) the more positive the resulting $\bar{\Delta}_{P_c}$ (Fig. B2 in Supp. File 2). $\bar{\Delta}_{P_c}$ also becomes more positive as $n$ decreases. This is true for different values of $s_H$ (*i.e.* only $n$ changes), but also for $s$, $h$ and $n$ chosen for a constant $s_H$ (Eq. 3 and see Fig. B2 in Supp File 2).

Combined with the previous expectations detailed above, we predict that if a POD is initially stable, its eventual loss will usually occur gradually as recombination at the distal ends of the POD cleaves off mutations. This creates haplotypes with improved relative fitnesses. The reduced zones of stable equilibria for $s_c = s_H$ in selfing populations (Fig. A2, in Supp. File 1) means that POD's will destabilise, erode, and more easily be lost in such populations, resulting in the rapid fixation of one of the original haplotypes or a recombinant. Again, this process will occur faster when fewer load loci are involved

as the loss of one mutation has a greater effect on the relative fitness. Persistent POD's only occur if mutations have small deleterious effects. In outcrossers, POD's can persist for longer as new haplotypes are not strongly counter selected when $s_c = s_H$, and when $s_c < s_H$ (*i.e.* a mutation is cleaved) selection is not as positive as in selfing populations (see Fig. B2 in Supp. File 2). Drift thus plays an important role in how recombinants increase in frequency in small populations.

# 3 Simulations

We use an individual-based simulation program in C++ to evaluate whether and for how long POD's can be maintained under various conditions. Populations consist of $N$ sexual diploid individuals, self-fertilizing at a fixed rate, $\sigma$. Each individual is represented by two vectors carrying the positions of deleterious mutations along a single chromosome with map length $R$ Morgans. Recombination occurs uniformly through the genome. Mutations in and outside the POD have a fixed effect, with respective coefficients of selection, $s$ and $s_d$, and dominances, $h$ and $h_d$. Individual fitness is calculated as shown in Eq. 2. To assess the effects of background selection we introduce new mutations sampled from a Poisson distribution with parameter $U$, the haploid mutation rate. The positions of these mutations are uniformly sampled, and they can be introduced anywhere in the genome (infinite-alleles model). Generations are discrete (no overlap) and consist of three phases: *i*) introducing new mutations, *ii*) selection, and *iii*) recombination and gamete production.

## 3.1 Introducing pseudo-overdominance

To assess how well POD's persist in populations, we introduce an arbitrary ideal haplotype structure expected to favour POD persistence. This consists of two complementary haplotypes, $H_1$ and $H_2$, each carrying $n$ mutations. As before, cis-mutations occur at regular intervals (every $2\ell$ cM) along each haplotype and mutations are staggered, spreading the load evenly through the POD and ensuring pseudo-overdominance (Fig. 1). The probability that a recombination event occurs between two trans-mutations is

then $\ell$. The total length of the POD zone is then the product of the number of loci involved and the map distances between them, or 2 $\ell$ $n$ cM.

## 3.2 Initial conditions and variables measured

Each simulation begins with two populations of size $N$. Each population is fixed for either $H_1$ or $H_2$. After a burn-in period of 4 000 generations, a new population of size $N$ is created by randomly sampling individuals from the two source populations, with each population contributing 50% of individuals to the new population. Simulations then run for another 4000 generations. Samples of 100 individuals are taken every 10 generations to estimate inbreeding depression, which we compare to the theoretical expectations presented above (Eqs. 6, 8 and 9). We also use these samples to estimate heterozygosity within and outside the POD zone (POD $H_e$ and genome $H_e$, respectively) as:

$$H_e = \sum_{j=1}^{100} \frac{he_j}{L}. \tag{12}$$

where $he_i$ is the number of heterozygous mutations carried by individual $j$ and $L$ is the total number of segregating sites in the genomic region of interest. At higher mutation rates, singletons will be frequent. This will reduce $H_e$ by inflating $L$. We thus also measure heteroyzgosity within the POD block keeping $L$ fixed at $2n$, the number of initial segregating sites, giving us a measure of "absolute" heterozygosity $\hat{H}_e$, to accurately represent how POD's erode. As long as a POD is stable, $\hat{H}_e$ should be equivalent to the frequency of heterozygotes expected at equilibrium in a true overdominant system, given by (Kimura and Ohta, 1971, see Supp. File 1):

$$P_{12} = 1 - \frac{(1+\hat{F})i(s_1(1-x)+s_2x)}{s_1 s_2}. \tag{13}$$

Decreases in this frequency indicate erosion of the POD.

## 3.3 Simulations run

We initially ran simulations for three population sizes ($N = 100$, 1000 and 5000) with no background selection ($U = 0$) and three fixed selfing rates $\sigma = 0$, 0.5 and 0.95. In these simulations we fix the number of mutations to be $n = 25$, 57 and 100, corresponding

to relative selection against homozygotes $s_H = 0.14$, 0.29 and 0.45. These result in overdominant selection sufficient to ensure stability when $\sigma = 0$, 0.5 or even (with a narrow range of stability) 0.95 (Fig. A2, dotted lines). We also compare three levels of linkage between cis-mutations (inter-mutaion lengths of $\ell = 5.10^{-3}$ cM, $1.10^{-3}$ cM and $5.10^{-4}$ cM). Finally, we explore the consequences of background selection on POD dynamics for $N = 1000$, $0 \leq \sigma \leq 0.95$, $n = 25$ and 100 and $\ell = 5.10^{-4}$ cM. In these simulations, we set the haploid mutation rate $U$ to either 0.1 or 0.5 and assume a total genomic map length of either $R = 1$ or 10 Morgans. To determine whether the heterozygosity maintained within the POD reflects selection rather than drift, we also ran control simulations where all alleles within the initial POD are neutral for all parameter sets mentioned above ($s$ and $h = 0$ within the POD). We ran 100 repetitions for each parameter set.

# 4 Results

## 4.1 POD dynamics

We first explore how various parameter values affect the stability of pseudo-overdominance, then determine the role of fixation and purging in destabilising and eroding POD's. We use heterozygosity within the POD as a measure of POD stability, with any decline from that expected under overdominant selection (Eq.13) implying erosion of the POD. Rates of decline in mean absolute heterozygosity $\hat{H}_e$ (see Eq.12 and accompanying text) strongly depend on how closely load loci are linked and levels of pseudo-overdominance (reflecting the coefficient of selection against homozygotes $s_H$). The number of load loci on a haplotype, $n$, has stronger effects as linkage increases. For tight linkage ($\ell = 5.10^{-4}$ cM) and a sufficiently large $n$, POD zones behave like true overdominant loci for 500+ generations (red lines in Fig. 2c and d). This reflects the strength of overdominant selection even under high rates of self-fertilisation, $\sigma$. When comparing POD dynamics to control simulations ($s = 0$, blue lines in Fig. 2), we confirm that POD selection greatly slows the decrease in $\hat{H}_e$ relative to the neutral case. However, once $\hat{H}_e$ drops to

a threshold reflecting the erosion of the structured haplotypes, selection quickly eliminates heterozygosity much faster than in the neutral case. This reflects POD instability occurring faster when linkage is weak ($i.e\ell = 5.10^{-3}$cM, red lines in Figs. 2a and b). As expected from the behavior of $\bar{\Delta}_{P_c}$ (Eq. 11), increased selfing tends to favor recombinants whether or not a mutation has been cleaved (Fig. B2 in Supp. File 2), destabilising POD's faster in selfing than in outcrossing populations.

Declines in $\hat{H}_e$ mainly reflect fixation at the load loci contributing to the POD (Fig. 3a for $\sigma = 0.95$ and Supp. Fig. S4a for $\sigma = 0$). Generally, when the POD is unstable (weak linkage and/or a small number of load loci $n$), fixation occurs throughout the POD. For more stable POD's, mutations are more commonly fixed on either end of the POD. Self-fertilising populations with low load (small n) quickly fix single recombinant haplotypes (Fig. 3b). For larger $n$, mutations fix more slowly, as recombination events cleave off single mutations at either end of the POD under tight linkage ($\ell = 5.10^{-4}$cM, Fig. 3c). In outcrossing populations, where fixation takes longer, we do not observe single recombinant haplotypes being fixed, though the pattern of fixation at the ends is maintained (Supp Fig. S4). This reflects the greater predicted stability of POD's in outcrossing populations despite weaker selection ( smaller $n$; Supp. File 1).

A surprising result emerges when we compare POD persistence $\hat{H}_e$ in populations of different size $N$ reflecting effects of genetic drift. Counter-intuitively, POD heterozygosity persists much longer (relative to neutral heterozygosity) in smaller populations than in larger populations (compare the red and blue lines in Fig. S3 where $N = 100$ to Fig. 2 and Fig. S2 where $N = 1000$ and 5000). While POD's are always strong and stable when many load loci are tightly linked (panels c and d in each figure), POD's that erode quickly after $\approx 1000$ generations in $N = 5000$ populations (Fig. S2, panels a, b) remain more heterozygous than expected out to $> 2500$ generations if N is 1000 (Fig. 2, a, b). At $N = 100$, POD heterozygosity lasts 1000 to 1500 generations longer than neutral heterozygosity (FIg. S3, a, b), though both decline greatly by 3000 generations unless many loci are strongly linked. In the presence of strong genetic drift, selection against or for new recombinant haplotypes is weakened (equivalent to bringing $\bar{\Delta}_{P_c}$ closer to 0).

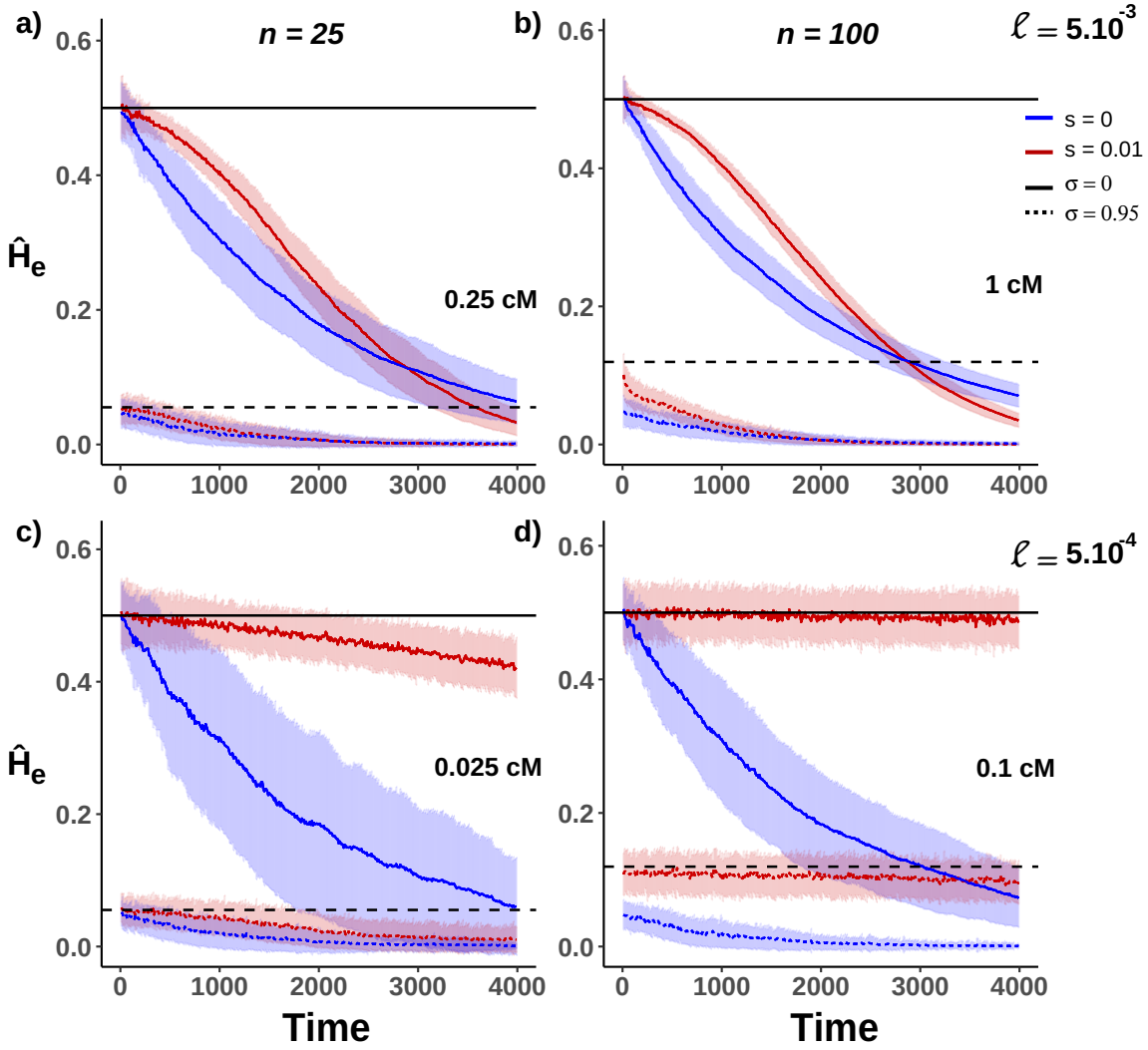


Figure 2: Declines in heterozygosity within the POD zone due to drift and selection. Graphs show declines in absolute heterozygosity $\hat{H}_e$ as a function of different levels of linkage ($\ell$) and differing numbers of load mutations within the POD zone ($n$), with resulting lengths of the POD zones given as bold numbers in each panel. Blue lines represent control cases where all POD mutations are neutral ($s = 0$). Red lines represent simulations run with deleterious mutations ($s = 0.01, h = 0.2$) within the POD zone. Full lines represent completely outcrossing populations ($\sigma = 0$) while lower dashed lines represent mostly self-fertilizing populations ($\sigma = 0.95$). Light shading around each line shows standard errors. Black lines represent heterozygosity expected with true overdominance (Eq. 13). Population size $N = 1000$ with no new mutations introduced anywhere in the genome ($U = 0$).

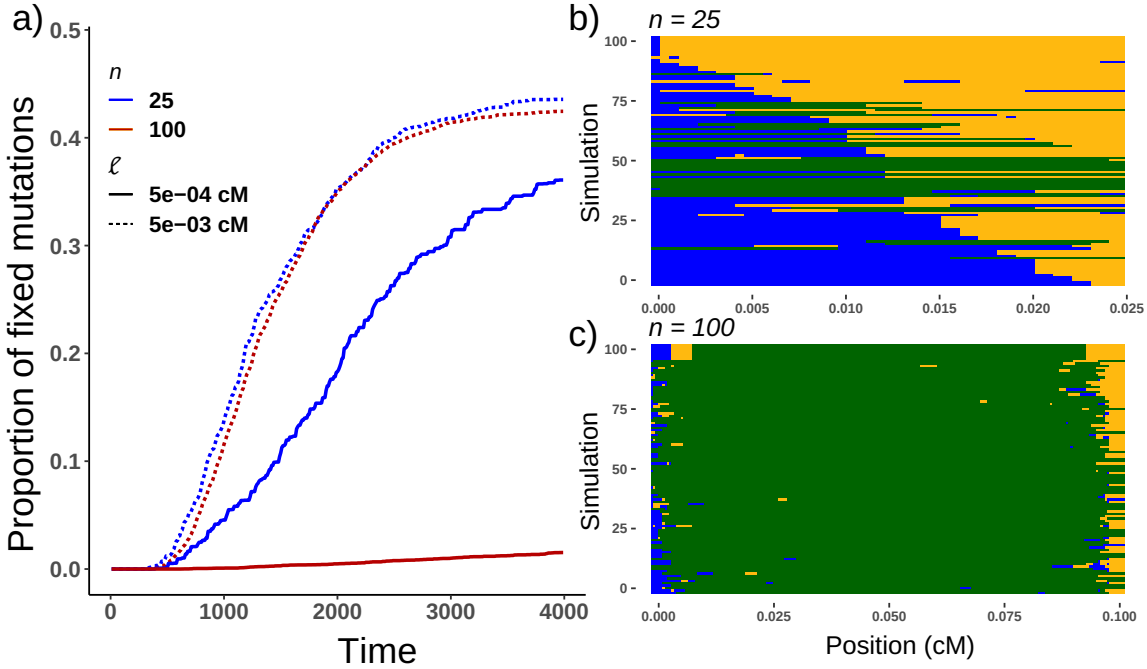


Figure 3: Fixation dynamics within the POD zone in highly selfing populations ($\sigma = 0.95$). a) Proportion of mutations fixed within the POD zone over time containing a POD with either loose or tight linkage (dotted or solid lines) reflecting longer ($\ell = 5.10^{-3}$ cM) or shorter ($\ell = 5.10^{-4}$ cM) POD regions. Weaker POD's ($n = 25$ load loci per haplotype) decay relatively rapidly losing most of their heterozygosity through fixation within 2000 generations (upper lines). Stronger POD's ($n = 100$) are robust persisting via strong balancing selection for many thousands of generations (lower coincident lines). b) and c) Maps showing distributions for 100 replicate simulations of where loci are fixed (blue and yellow pixels representing each haplotype) and still segregating (green pixels). Simulations ran for 4000 generations and outcomes are ranked along the length of the original POD. Weaker POD's (b) lost most of their heterozygosity to become fixed for either haplotype except for a few populations where both POD haplotypes still segregate at roughly even frequencies. Populations with stronger POD's (c) retained most of their heterozygosity except for fixing a few mutations at either end of the POD. Population size $N = 1000$ and background mutation rate $U = 0$.

Another factor contributing to this longer-lived stability is that recombinant haplotypes occur more rarely in smaller populations as there are fewer individuals (reducing the effective recombination rate). In selfing populations, increasing drift mostly increases the variance of $\hat{H}_e$ (shading around lines in Figs. 2, S2 and S3). Although mean $\hat{H}_e$ with POD selection initially remains higher than for control (neutral) simulations, it does so for a much shorter time-span. This increases the overlap between the control simulations and those with POD selection, making it difficult to distinguish between a decrease of heterozygosity due to drift and that due to selection against the POD.

The simulations also allow us to track the dynamics of mutation fixation and loss, illustrating further how stronger loads and tighter linkage promote POD stability (Fig. 3). With limited load ($n = 25$ load loci per haplotype), selection and drift act to dissolve the POD within a few thousand generations. The optimal structure of the POD, designed to maximize pseudo-overdominance and minimize effects of crossing over, may account for the initial $\approx 300$-generation stability even in these weak POD's (lower left, Fig. 3a). After that, however, mutations fix or purge quickly in the absence of strong balancing selection, resulting in a fairly even distribution of fixation across the length of the POD (Fig. 3b). When selection is strong ($n = 100$), however, the power of drift and selection to fix and purge these minor mutations is almost eliminated (lower curves, Fig. 3a). Nevertheless, some fixation of mutations occurs via crossing-over, mostly at the far ends of the POD (Fig. 3c). In this case, drift generally appears to be more potent than purifying selection particularly in selfing populations.

## 4.2 Background selection and POD

Background selection acts in two opposing ways on the observable heterozygosity $H_e$, depending on the strength of POD. When fewer loci ($n$) contribute to a POD and linkage is loose (larger $\ell$), recurrent deleterious mutations speed the decay of heterozygosity. For more stable POD's, however, background selection has little effect. In fact, when selfing and mutation rates are high ($U = 0.5$), POD heterozygosity actually increases (Fig. 4). This effect is more pronounced in earlier generations following POD formation via

admixture (see results after 100 and 1000 generations in Supp. Fig. S5). Conversely, POD selection increases the heterozygosity of deleterious mutations elsewhere in the genome (see Supp. Fig. S6) suggesting these two forms of selection reinforce each other. That is, POD's appear to decrease the efficiency of purging deleterious mutations throughout the genome while selection against mutations segregating outside the POD zone appear to strengthen the balancing selection maintaining heterozygosity by further disfavoring homozygotes.

Background selection acting at other loci in the genome thus affect POD dynamics in two distinct ways. Weaker POD's (small $n$ and $s_H$) with loose linkage (larger $\ell$) are vulnerable to the effects of recurrent deleterious mutations elsewhere in the genome, speeding the decay of POD heterozygosity (Supp. Fig. S7). This ressembles classical effects of background selection on neutral variation (Fig. 4a). In contrast, stronger, more stable POD's in predominantly outcrossing populations are barely affected by strong background selection (*i.e.* for $U = 0.5$, Fig. 4b). Yet strong background selection in mostly selfing populations strengthens the POD by favoring heterozygotes. This effect is evident early on, after the admixture event (see Supp. Fig. S5 a and b at 100 generations, and c and d at 1000 generations). These two forms of selection mutually reinforce each other as POD selection increases the heterozygosity of deleterious mutations elsewhere in the genome (Supp Fig. S6). This reduces the efficiency with which selection acts against segregating deleterious mutations in other regions (by reducing the frequency of homozygotes) while selection acting at those load loci either barely affect the POD or strengthen the balancing selection maintaining the POD. Although background selection amplifies the selection maintaining the POD (especially in highly selfing populations), it has little effect on fixation dynamics within the POD (Supp. Fig. S8).

## 4.3 Inbreeding depression

As expected, the overdominance generated by a POD increases the amount of inbreeding depression, $\delta$, expressed in a population (compare lower lines in Fig. 5a and 5b). However, it also reduces the decline in $\delta$ with $\sigma$ relative to unlinked expectations. Back-

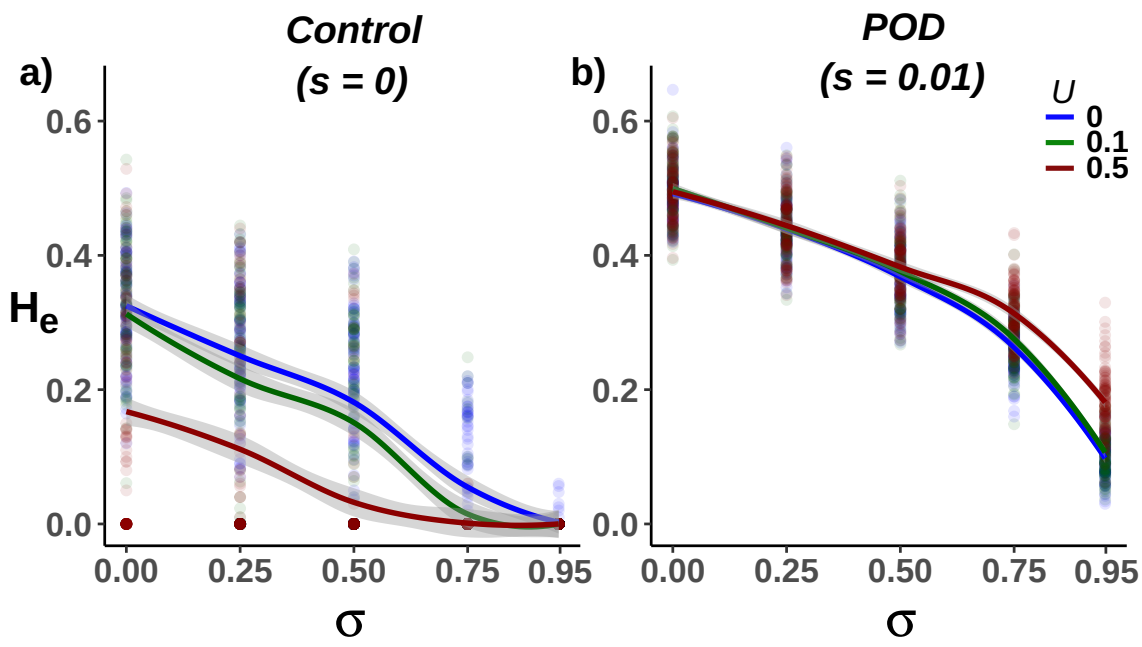


Figure 4: Opposing effects of background selection on heterozygosity dynamics in the presence and absence of a POD. Observed heterozygosity ($H_e$) after 4000 generation as a function of the rate of self-fertilization ($\sigma$) in the population. Total genome size R is 10 cM. The POD initially contains n = 100 hetrozygous mutations per haplotype. a) $H_e$ decay for loci lacking selection (control, $s = 0$). b) Results for loci subject to selection ($s = 0.01$, $h = 0.2$). Background mutation rates are $U = 0$, 0.1 and 0.5 mutations per haploid genome per generation, and all new mutations are under selection with $s_d = 0.01$ and $h_d = 0.2$. Linkage between trans-mutations $\ell = 5.10^{-4}$ cM and population size is $N = 1000$.

ground selection ($U > 0$), again as expected, accentuates inbreeding depression in highly selfing populations relative to classicexpectations especially soon after admixture, with or without a POD (Supp. Fig. S9). Fixation of the load via drift and purging of the load via purifying selection both reduce levels of inbreeding depression. In opposition to these, both background selection and the balancing selection associated with a POD instead sustain or increase inbreeding depression, even at high selfing rates (right side, Fig. 5 and Supp. Fig. S9). The marked departure of simulation results from theoretical expectations (Eq. 9) reflects how assuming independence among load loci actively misleads us especially at high selfing rates (where strong identity and linkage disequilibria occur). Distributions of observed values of $\delta$ in the simulations also become highly variable as background selection increases (Fig. 5c). This presumably reflects the cumulative effects of random mutations accruing over 4000 generations. Notably, the presence of a POD accentuates this variability (Fig. 5d). This underlines how variable POD erosion dynamics are, even to the point of generating bimodal distributions of $\delta$ (Fig. 5d).

# 5 Discussion

We evaluated whether blocks of recessive mutations expressing pseudo-overdominance (POD's) can persist long enough to have significant evolutionary effects. If so, they might help to account for previously enigmatic results like the persistence of genetic diversity and inbreeding depression often observed in small or inbreeding populations. In our models, POD's did persist when linkage was tight and cumulative selective effects from both haplotypes were strong and evenly balanced. Tight linkage, in particular, creates genomic regions where cumulative pseudo-overdominance creates balancing selection strong enough to sustain POD's for long periods. When recombination occurs frequently, selection and drift become more effective, breaking up POD's by eroding mutations from their ends or by unbalancing the load. These forces then work as in classic models to purge and fix partial POD haplotypes and the mutations they carry. Whether POD's exist in sufficient number and power to affect evolutionary dynamics

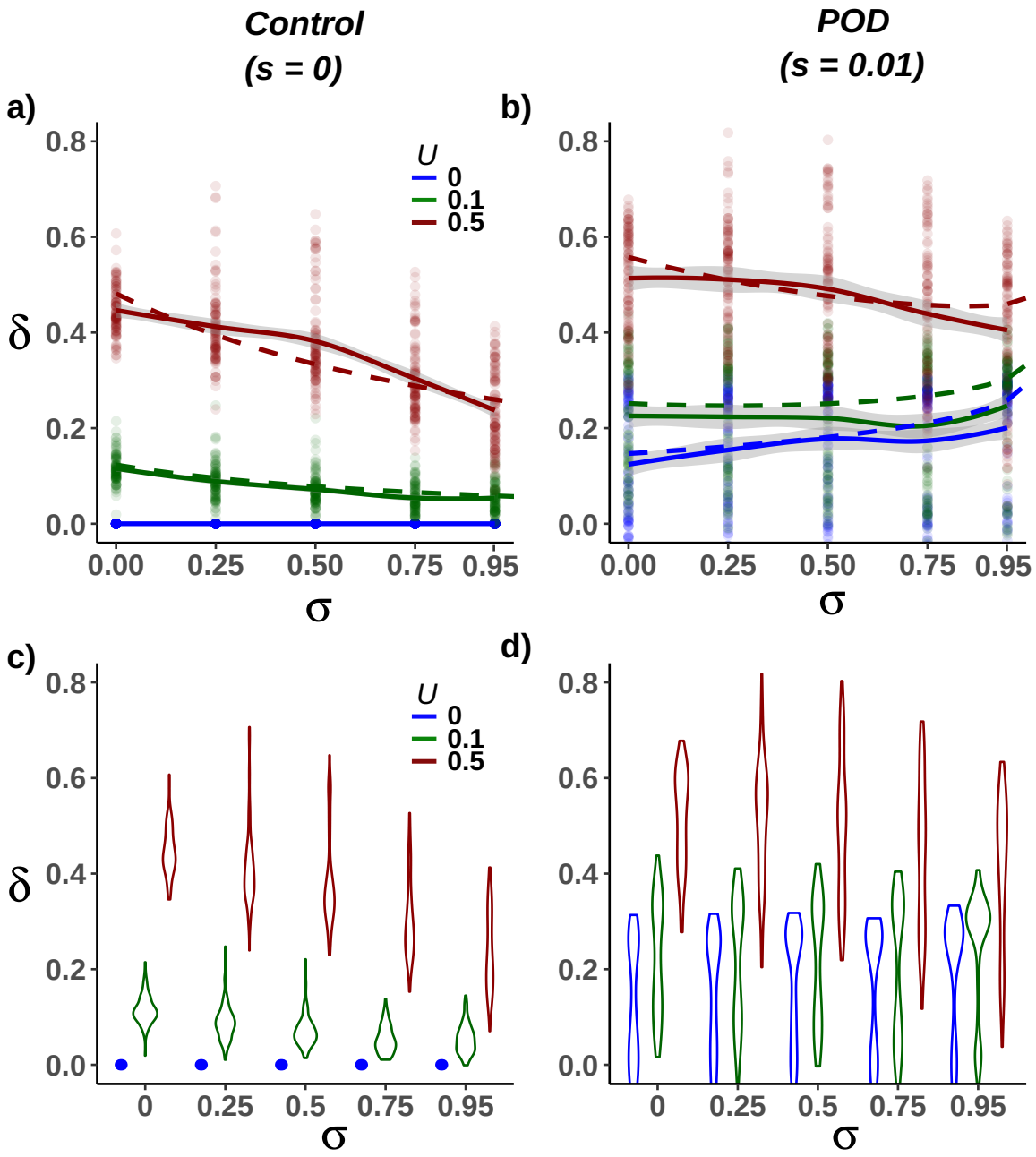


Figure 5: Inbreeding depression ($\delta$) as a function of the selfing rate ($\sigma$) 4000 generations after admixture for simulations without ($s = 0$, a and c) and with POD selection ($s = 0.01$, b and d) for different background haploid mutation rates $U$. Top panels: $\delta$ as a continuous function of $\sigma$. The dots represent $\delta$ for individual simulation runs while full lines show mean observed $\delta$ and the dashed lines show theoretical expectations for inbreeding depression calculated using equation 9. Bottom panels: Violin plots of the distributions of $\delta$ for different rates of $\sigma$. Other parameter values are as in Fig. 4.

thus hinges on the relative rates at which they are created and destroyed. Although we did not examine POD origins directly, our models demonstrated that POD's subject to strong and balanced selection can persist for long periods given tight linkage. This makes them a potentially important "player" in the evolutionary dynamics of the genetic load, both within the POD's and elsewhere in the genome (e.g., by affecting background selection and reducing homozygosity and purging, as shown in our results). Such effects are important as models that ignore linkage and genic associations predict the rapid loss of genetic variability and inbreeding depression via purging and fixation.

## 5.1 Assumptions and structure of the model

Our model envisioned that isolated populations, fixed for alternative sets of recessive deleterious mutations, fused to create hybrid populations expressing heterosis (from high levels of pseudo-overdominance). Populations regularly become isolated, diverge genetically, and then hybridize. If these populations were small or inbred, they will have fixed many mildly deleterious mutations as selective effects of order 1/2Ne or less are effectively neutral (Crow and Kimura, 1970). Inter-population crosses reveal that this fixed, or drift, load can be appreciable (Willi et al, 2013; Spigler et al, 2017). The same phenomena are observed in plant and animal breeding programs where inbred lines that may be low in fitness are hybridized to gain high fitness. This heterosis was once thought to reflect true overdominance but finer genetic characterizations reveal this to be mostly pseudo-overdominance (Crow, 1999b). Thus, our scenario for POD origins may be plausible.

The model developed by Harkness et al (2019) suggests that heterosis from between-line crosses may be short-lived. This could reflect how loads are often unbalanced, allowing rapid purging and fixation (and the former accelerate the latter via background selection). Nevertheless, particular chromosome segments in maize have unexpectedly high heterozygosity, suggesting the action of pseudo-overdominance (Brandenburg et al, 2017). The rapid and more complete decay of heterozygosity in Harkness et al's (2019) model may reflect their assumption of uniform rates of recombination along chromo-

somes. Our model shows this could limit POD emergence.

Strongly overdominant loci (Kimura and Ohta, 1971) persist for remarkably long periods (e.g., self-incompatibilty loci in plants and histocompatibility loci in animals) but are considered rare. For sets of recessive mutations to generate similarly strong and persistent pseudo-overdominance, they must be closely linked. Clusters of such mutations could occur by chance within small genomic regions (as assumed in our model) given mildly deleterious mutations are common and broadly dispersed through the genome. If their cumulative selective effects were strong and balanced across haplotypes, a POD would result. Further load mutations in or near these regions would be sheltered from selection, allowing them to accumulate and reinforce the overdominance as been observed for the *Arabidopsis halleri* S-locus (Llaurens et al, 2009).

Our idealized model further assumed that mild mutations were evenly distributed along the incipient POD in repulsion to alternating mutations on the opposite chromosome. This maximized pseudo-overdominance and made it difficult to unbalance the load as internal recombinants expressed the same load (Fig. 1). Simulations that more realistically assumed randomly positioned load loci within the POD resulted in shorter-lived POD's (Supp. Figs. S10 and S11 vs. Fig. 2). This faster loss reflects how recombination and selection more easily unbalance the load between haplotypes when it is unevenly distributed. The linkage required for longer stability became an order of magnitude higher (*i.e.*, a map distance of $\ell = 10^{-5}$ cM) than when simulations assumed alternating load loci. However, this higher linkage resulted in more stability allowing fewer load loci (*Supp. Fig. S10c*). Recombination became so rare that linked load loci acted like a single overdominant locus. In our model, POD's needed more load loci to generate the strong balancing selection needed to stabilize the POD.

Additional modeling could add more realistic variation not only in the number and spacing of mutations but also their effects and dominance levels. More variable mutational effects and spacing would likely enhance the power of selection and drift to eliminate POD's by enhancing the power of recombination to disrupt the balanced selection maintaining POD's (rather than chewing them away at their ends). Our models

focused on POD erosion and loss, but understanding which mechanisms facilitate their formation would also help us understand how often POD's evolve. Notably, the presence of true (one locus) overdominance and inversions could favor POD's, extend their persistence, and thus enhance their evolutionary effects. It might also be worthwhile to explore whether modifier loci could evolve to reduce rates of recombination, particularly near the center of POD's, providing another POD-stabilizing mechanism.

## 5.2 Conditions favoring POD formation and persistence

The strong selection and tight linkage needed to sustain POD's in our models suggest that such conditions should enhance POD formation. Crosses between small or inbred populations with independent drift loads would generate highly heterozygous individuals and large segregating loads in the first generation. Subsequent recombination and selection, however, likely eliminates most proto-POD's. Any POD strong enough to persist, however, would reduce the effective rate of inbreeding ($\hat{F}$, Eq. 7) limiting the rate at which deleterious recessive mutations purge. Fixation at loci outside the POD would continue in small and inbred populations. We might therefore expect POD's to emerge recurrently in situations where populations regularly become small, isolated, and subject to fixation, then hybridize, regenerating strong heterosis. Truly overdominant loci (like the S- and HMC loci mentioned above) might also favor POD formation.

Conditions generating tight linkage should likewise favor POD formation as high linkage can compensate for less balanced and lower loads. Chromosomal inversions that block recombination, in particular, shelter deleterious recessive mutations from selection as heterozygotes, enhancing opportunities for pseudo-overdominant effects to evolve. Kirkpatrick (2010) observes that the genetic basis for inversion overdominance has not been determined but notes that pseudo-overdominance is plausible. Jay et al (2021) dissected the structure and genealogy of inversions contributing to heterosis in *Heliconius* butterflies. These date back to hybridization events as long as 3M years ago suggesting that high heterosis following hybridization may have spawned POD's. Interestingly, subsequent mutations (primarily transposable elements) increased the size

of these inversions and their load, reinforcing the pseudo-overdominance and inbreeding depression. This supports our conjecture as do recent simulations (Berdan et al, 2021) showing recurrent mutations can strengthen POD's.

## 5.3 Empirical evidence on POD's

As noted above, balanced polymorphisms expressing overdominance exist in many species and may stably persist for long periods. These are sometimes associated with local adaptation in plants like the five ancient polymorphisms found by Wu et al (2017) that predate the divergence of *Arabidopsis* from *Capsella*. Adaptive or inversion polymorphisms represent an alternative route to POD formation from the hybridization model outlined here. Inversions may not be associated with adaptive differences, however.

Trans-specific inversions, polymorphisms, and ancient S- and HMC loci all support the notion that overdominance can sustain polymorphisms for millions of years. Some researchers have found genomic regions supporting high heterozygosity and segregating variation suggestive of POD's. These exist in *Zea mays* (McMullen et al, 2009) and 22 low-recombination regions within the human genome near centromeres (Gilbert et al, 2020). Kremling et al (2018) confirmed that many rare variants in maize have deleterious effects confirming that "even intensive artificial selection is insufficient to purge genetic load." Brandenburg et al (2017) identified 6,978 genomic segments with unexpectedly high heterozygosity in land races of maize. Heterozygous segments were more likely to harbor deleterious mutations than the rest of the genome and several were deeply conserved across multiple land races. Brown and Kelly (2020) found an uneven distribution of load loci in *Mimulus guttatus* with deleterious alleles often localized to particular regions. These "Rare Allele Clusters" predicted fitness. Waller (2021) reviews further evidence of possible POD signatures.

## 5.4 Implications

POD's that arise and persist in natural populations would have several evolutionarily significant effects. Most immediately, POD's act to reinforce the persistence of inversions

by increasing selection against recombinant homozygotes and enhance levels of molecular variation by sustaining deleterious recessive mutations that would otherwise be lost to selection and/or drift. Higher levels of genetic variability are usually interpreted as favorable for adaptation but POD loads are maladaptive in increasing expression of the segregational load and reducing selection against newly arising deleterious recessive mutations. These effects could compromise both short- and longer-term population viability (particularly in small, isolated, and/or inbred populations that produce many homozygous progeny). They would also weaken the usually positive correlations between heterozygosity and fitness (expected when heterozygosity provides an inverse measure of individual inbreeding). The ability of POD's to shelter and potentially increase the load might lead to progressive increases in POD size and deleterious effects with outcomes similar to "mutational meltdown" models (Gabriel et al, 1993). Such effects might favor modifier loci that increased rates of recombination to prevent the genome from 'congealing' (Maynard Smith, 1977).

POD's would also affect the evolution of plant and animal mating systems by sustaining segregating deleterious mutations and the inbreeding depression they generate. Early models of mating system evolution sought to explain variable levels of self-fertilization as the outcome of selection acting on progeny expressing higher or lower levels of inbreeding depression. These predicted that increased rates of selfing should expose deleterious mutations to selection, reducing their frequency and thus inbreeding depression. As mutations were purged, ID would decline leading to run-away selection for complete selfing (Lande and Schemske, 1985b). Such models, however, neglected to account for linkage, drift dynamics, and the associations among loci that arise in small and inbred populations, all of which considerably modify classical predictions (Uyenoyama et al, 1993). More complete models display more complex dynamics and outcomes including the persistence of mixed mating systems in some cases. Nevertheless, we generally expect both purging and fixation due to drift to reduce segregating variation and thus inbreeding depression in smaller and more inbred populations. The abililty of many small inbred populations to retain genetic variation and inbreeding depression has puzzled many as

has the absense of purely inbreeding taxa (Byers and Waller, 1999; Winn et al, 2011). The chance presence and variable sizes and strengths of POD's among these populations might help to account for these paradoxes.

Just how POD's influence mating system evolution remains an open question. Harkness et al (2019) suggest that the short-lived heterosis observed in hybridizing populations should result in, at most, a temporary selective advantage to outcrossing but heterosis due to POD's could also favor outcrossing. If initial levels of pseudo-overdominance decline as recombination breaks up POD haplotypes, selfing could again be favored. Persistent POD's might provide more sustained selection favoring outcrossing. Our models suggest that mutations causing heterosis are often fixed in contrast to Harkness et al (2019). This might allow recurrent hybridization events to reinitiate POD formation, creating recurrent selection that could favor outcrossing in species with mixed mating systems.

How often POD's arise and persist, how commonly they occur within natural populations, and how restricted they are to genomic regions with tight linkage all deserve more attention. Do most POD's occur near centromeres or within inversions? Do POD's arise and dissipate regularly following recurrent hybridization? Further comparative genomic data should cast light on all these questions.

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