Reproductive compensation and embryo screening drive the evolution of polyembryony

September 1, 2021

Keywords

Self-Incompatibility

Polyembryony

Sibling Rivalry

Abstract

Simple polyembryony – where one gametophyte produces multiple embryos with different sires but the same maternal haplotype – is common in conifers, ferns, horsetails and other vascular plants. By providing a backup for inviable embryos, polyembryony may evolve as a mechanism of reproductive compensation. Alternatively, polyembryony may provide an opportunity for embryo screening and the preferential exclusion of low fitness embryos from the seed, perhaps acting as a mechanism of Self-Incompatibility (SI). To date, the evolution of polyembryony has not been modeled, and these verbal hypotheses have not been evaluated. We develop an infinite-site, forward population genetics model to test how these factors can favor the evolution of polyembryony, and how these underlying benefits of polyembryony shape the genetic load under a range of biological parameters. We find that reproductive compensation strongly favors the evolution of polyembryony, and embryo screening less strongly favors polyembryony. When embryo screening favors the evolution of polyembryony it increases embryo competitiveness, but does not act as an SI mechanism, as it does not trade low-fitness selfed offspring for high fitness outcrossed offspring. Remarkably we find nearly identical results in cases in which mutations impact either embryo or post-embryonic fitness (no pleiotropy), and in cases in which mutations have identical fitness effects embryo or post-embryonic fitness (extreme pleiotropy). In sum, the consequences of polyembryony depends on its function – decreasing the mean embryonic fitness when acting as a mechanism of embryo compensation and increasing mean embryonic fitness when acting as a mechanism of screening.

> Nature is, above all, profligate. Don't believe them when they tell you how economical and thrifty nature is.
>
> – Annie Dillard 1974.

Not only do most parents produce more offspring than will survive, but most organisms that provide parental care make more offspring than they will likely be able to nurture to independence. Frequent siblicide in the great egret, *Casmerodius albus*, provides a dramatic example of this – siblings kill one another, presumably over the ability to monopolize small food items (Mock 1984); why then do egret mothers continue laying eggs that will develop into offspring that will kill one another? Could such overproduction allow parents to screen for offspring quality (Forbes and Mock 1998), or does the "diverse portfolio" of offspring born over the breeding season allow parents to hedge their bets, ensuring survival of at least one chick (Forbes 2009)?

Simple polyembryony – in which a single maternal gametophyte is fertilized by multiple sperm cells to produce multiple embryos with genetically identical maternally derived genomes but distinct paternal genomes (Buchholz 1922; Schnarf 1937, cited in Dogra 1967) – provides an even more extreme, but perhaps less dramatic, example of this problem. Simple polyembryony is ubiquitous in gymnosperms (Willson and Burley 1983), and is found in many seedless vascular plants including ferns and horsetails (Buchholz 1922). The number of archegonia per seed typically varies from two to four in the genus *Pinus*, but can reach up to 200 (as reported in *Widdringtonia juniperoides* Saxton 1934), of which only a single single embryo typically survives in mature seed (Chamberlain 1966). We develop a population genomic model to both test the two major advantages of simple polyembryony described by Kärkkäinen and Savolainen (1993), which we dub *embryo screening* (akin to egret mothers screening for offspring quality) and *reproductive compensation* (akin to egret mothers hedging their bets) theories for the evolution of polyembryony, and to investigate how polyembryony changes embryonic and post-embryonic fitness and the genetic architecture of these complex traits. While these benefits of polyembryony have been acknowledged for some time, they have rarely been discussed together and their theoretical contributions to the evolution of polyembryony have not been disentangled (but see Ferreira et al. 2019, for an empirical study of the influences of these processes on seedling establishment in a population of *Carapa surinamensis* that produces mono- and polyembryonic seeds).

Reproductive compensation is an increase in seed set that occurs when embryo mortality is counteracted by an expanded supply of embryos (Sorensen 1982; Porcher and Lande 2005). Polyem-

bryony provides reproductive compensation if a lone embryo is less likely to develop into a successful seed than is a collection of sibling embryos. So, for example, if a proportion p of embryos are inviable, and the survival of each embryo is independent, a second embryo increases the probability that a seed contains a surviving embryo from $1 - p$ to $1 - p^2$ (Lindgren 1975).

Alternatively, polyembryony could be favored because, by allowing for "embryo screening," it results in the preferential exclusion (inclusion) of low (or high) fitness embryos from the seed. This would be particularly beneficial if embryonic and post-embryonic fitness are positively correlated, embryo screening (dubbed Developmental Selection by Buchholz (1922)) could favor the evolution of polyembryony. Such a correlation can arise either through pleiotropy (embryonic and post-embryonic fitness effects of the same mutation) across the life cycle, or if embryonic fitness determined by one set of loci predicted post-embryonic fitness produced by another set of loci. This later option seems particularly likely if inbred offspring are unfit across the life cycle, and as such, simple polyembryony is often interpreted as an inbreeding avoidance mechanism (e.g. Dogra 1967; Sorensen 1982) analogous to the self-incompatibility systems (hereafter SI) found in angiosperms. Koski (1971) and others contend that this gives way to evolution of the so-called "Embryo Lethal System" – an apparently coordinated self destruction mechanism revealed upon inbreeding (or the production of low-vigor embryos, more generally) in pines as a mechanism evolved to prevent selfing (Koski 1971; Sarvas 1962, e.g. page 162 onwards). Under this model, polyembryony does not prevent self-fertilization *per se*, but dampens self-fertilization's deleterious effects by allowing competition which would favor outcrossed over inbred progeny before major maternal resource allocation (Willson and Burley 1983; Sorensen 1982). As such, embryo screening could circumvent the constraint imposed by the unenclosed gymnosperm seed, which precludes prezygotic mate choice (e.g. SI systems Dogra 1967; Sorensen 1982; Willson and Burley 1983). We note that our use of the term "embryo screening" implies that the fitness of embryos can be effectively differentiated and reliably selected early in development; however, there is no guarantee that such screening will accurately predict fitness later in life rather than favoring dishonest signals that overwhelm a mother's ability to predict later fitness from early embryo vigor (McCoy and Haig 2020). Furthermore, the exact mechanism of such screening is not yet known, and here "embryo screening" applies to a situation where mother has an active role or where it is merely providing the venue for the life-or-death competition among siblings (Haig 1992).

Critically, the embryo screening model assumes that possibility of effective mechanism by which the fitness of embryos in a seed can be differentiated, a topic of much debate. Based on extensive

experimental work on *P. sylvestris*, Sarvas (1962) stated that embryo competition and "struggle for life" is quite apparent under microscopic observation, but unfortunately the details leading to this conclusion were not disclosed. Empirical studies evaluating these ideas are quite rare, and the evidence from these studies is mixed. Most such studies address the possibility of embryo screening by comparing the observed frequency of selfed embryos (which are likely to be low-fitness) to their expectation under a model without embryo screening. In a particularly careful study, O'Connell and Ritland (2005) conducted controlled pollination with varying levels of self-pollen with *Thuja plicata*, and found that the effect of embryo competition became apparent with a probability of selfing (0.75), that exceeds reasonable estimates of the frequency of self-pollination in most conifers. While subtle effects at lower selfing rates are plausible, other researchers argue that selfed embryo death primarily occurs after the dominant embryo is determined (Williams 2008), and embryo survival is determined by chance physical factors (Williams 2007; Mikkola 1969), undercutting the embryo screening model.

In addition to various selective forces favoring the evolution of polyembryony, polyembryony itself can have striking evolutionary consequences. Previous models have investigated how polyembryony (or less mechanistically explicit forms of reproductive compensation) could shape the genetic load (Latta 1995; Sakai 2019; Porcher and Lande 2005; Kärkkäinen et al. 1996) and the exposure of inbreeding depression (Kärkkäinen and Savolainen 1993; Hedrick et al. 1999). These models generally show that by removing selfed embryos early in development, polyembryony will prevent the effective purging of deleterious recessive mutations (Klekowski 1982; Haig 1992), and will therefore increase the number of deleterious mutations at equilibrium, and increase the extent of inbreeding depression. Despite its weakening the action of selection, in such models polyembryony also decreases the realized selfing rate, and ultimately increase population mean fitness. As such, polyembryony is often suggested as an explanation for the joint observation of high inbreeding depression (gymnosperms have an estimated 5-10 lethal equivalents per haploid genome (Lynch and Walsh 1998; Williams and Savolainen 1996)), the low realized selfing rates in gymnosperms (Kärkkäinen and Savolainen 1993; Hedrick et al. 1999), and the absence of a relationship between inbreeding depression and the primary selfing rate in gymnosperms (Husband and Schemske 1996). However other models of polyembryony make drastically different predictions — for example, Latta (1995) modelled the embryo screening component of polyembryony (which he called "embryo competition") and found that polyembryony increased the efficacy of selection and decreased the number of deleterious mutations per individual. Here, we find that much of these differences are attributable

to implicit, and often unacknowledged, modelling decisions that consider polyembryony as a mechanism of embryo compensation or embryo screening – that is, when polyembryony functions as a mechanism of embryo compensation we find an elevated embryo load because selection is relaxed (as found in e.g. Lande et al. 1994; Klekowski 1982; Haig 1992), while when functioning as a mechanism of embryo screening, polyembryony tends to more effectively remove the mutational load of embryos (as found in Latta 1995).

Previous research provides some insight into the evolutionary consequences of polyembryony, but contains numerous modelling assumptions that limit their applicability to major questions in the evolution of polyembryony and its consequences. For example, comparing cases with and without reproductive compensation, Porcher and Lande (2005) showed that reproductive compensation can favor the evolution of selfing and can allow for the maintenance of mixed mating systems, while Sakai (2019) showed that selective embryo abortion could allow for the maintenance of high levels of inbreeding depression in selfing species. But if the mating system of an initially monoembryonic population affects whether polyembryony evolves in the first place, this initial condition may affect subsequent mating system evolution after the transition to polyembryony. A second limitation with current theory of the evolutionary consequences of polyembryony is that each model has focused on a single dominance and selection coefficient. As such, while current theory predicts evolution of the number and frequency of deleterious mutations, the magnitude of genetic load, it cannot predict evolution of the distributions of dominance or selection coefficients, or the architecture of genetic load. This limitation has prevented theory from addressing Koski's (1971) hypothesis that the "Embryo Lethal System" evolved as an altruistic mechanism by which inbred embryos sacrifice themselves to prevent their mothers from selfing, as opposed to the parsimonious alternative that selfing simply exposes the elevated number of deleterious mutations that can accumulate under polyembryony.

Here we present a series of infinite-sites forward population genetic simulations of polyembryony. This model allows us to evaluate the relative importance of embryo screening and reproductive compensation to the evolution of polyembryony, the evolutionary consequences of alternative models of embryo selection, and to test the hypothesis that polyembryony can favor the evolution of an increased recessive load as a mechanism to prevent inbreeding. We limit our focus to "simple polyembryony" and do not consider cleavage polyembryony, in which a fertilized zygote can split into numerous genetically identical embryos (Agapito-Tenfen et al. 2012), or nucellar polyembryony, in which maternal tissue asexually develops into embryos (Lakshmanan and Ambegaokar

1984), sometimes competing with sexually derived embryos, as they are likely favored by distinct mechanisms (Ganeshaiah et al. 1991).

Methods

Overview

We present a series of models to disentangle the contribution of the potential evolutionary benefits of compensation and embryo screening to the evolution of polyembryony. To better understand how and when these factors favor the evolution of polyembryony, we vary the distribution of dominance and fitness effects and the probability of selfing. Importantly, by changing the underlying probability of selfing, we can address the possibility that polyembryony is analogous to self-incompatibility systems when embryo screening favors its evolution. Finally, when polyembryony does evolve, we ask how its evolution shapes the genetic load and its architecture.

The life cycle begins with $N = 1000$ diploid seeds, each of which has one or two embryos, depending on whether mothers are mono- or polyembryonic. Following embryo selection (which can include viability selection and/or embryo screening – see below), surviving seed parents for the next generation are chosen with replacement with a probability reflecting their post-embryonic fitness. Each time a seed parent is chosen, it generates one of the N seeds in the next generation, thus maintaining a constant population size of N viable plus inviable seeds every generation. Each embryo in the seed is fertilized independently. If directly selfed, which occurs with probability equal to the selfing rate, the seed parent of an embryo is also its pollen parent, otherwise the pollen parent is selected at random and in proportion to adult fitness. Next, gametes are formed by free recombination and each gamete acquires mutations. Finally, fusion between gametes generates the embryos for the next generation (Fig. 1).

Parameters and model details

Genome structure and mutation rate: Every generation, each haploid genome expects a Poisson distributed number (mean U) of *de novo* deleterious mutations at any one of an infinite number of unlinked sites (i.e. an infinite sites model). We investigate cases with $U = 0.5$ mutations per haploid chromosome per generation. This value is in the middle of estimates of the per generation haploid mutation rates in small statured plants (with ranges of 0.05 to 2.0 as reviewed by Schultz and Scofield (2009) where U is presented as the diploid rate, such that values are twice

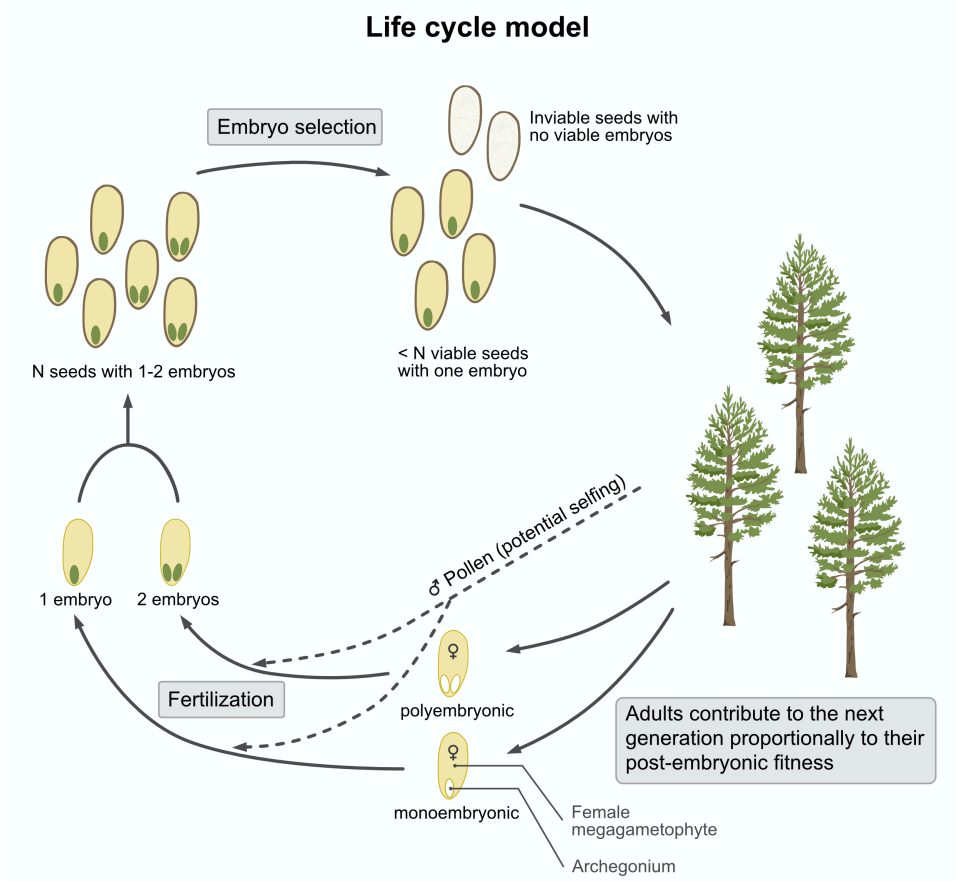

Figure 1: **Overview of the life cycle model:** The life cycle starts with N seeds, each with one or two embryos, followed by embryo and seed selection. Because of seed inviability, the number of plants will be less than N. Seed parents are chosen with replacement in proportion to their post-embryonic fitness, and directly self with probability p_{self}. Both embryos of polyembryonic mothers are fertilized independently, and pollen parents of non-selfed seed are sampled with from the population with replacement in proportion to each genotypes post-embryonic fitness. Seed parents carrying the dominant polyembryony allele, produce two archegonia per seed, while those without this allele produce only one.

ours). While this value could differ in large-statured long-lived plants (Schultz and Scofield 2009), initial explorations of numerous values of U led to qualitatively similar results (so long as populations didn't crash under their mutational load), suggesting that he precise value of U will not reflect our major conclusions.

The timing of mutational effects: We focus on the case in which half of *de novo* deleterious mutations impact embryonic fitness and the other half impact post-embryonic fitness. Our code also allows mutations to influence both embryonic and post-embryonic fitness pleiotropically. While our main results ignore the possibility that mutations may impact embryonic and post-embryonic

fitness, we have also considered a special case of "complete pleiotropy" which we present in the discussion.

The distribution of fitness and dominance effects of new mutations: For all parameter values, fitness effects, s, across loci are multiplicative and independent such that the fitness of the ith individual, w_i, equals the product of one minus the deleterious effect of their genotype at the kth locus, taken across all loci (i.e. $w_i = \Pi_k(1 - s_{ik})$). To investigate the impact of mutational architecture on the evolution of polyembryony, we compare models with a different value of fitness and dominance effects of new mutations. For s we present cases with $s = 0.1$, $s = 0.5$, $s = 1$, and $s = \text{Uniform}(20/N, 1)$. Dominance, h, can take any value between 0 and 1, but we present cases with full recessivity ($h = 0$) and full additivity ($h = 0.5$), as well as a case where the dominance of each mutation takes a random value between zero and one from the uniform distribution ($h = \text{Uniform}(0, 1)$). Thus, mutational effects span the range from quite deleterious to lethal, but will not reach fixation by random genetic drift. Practically, this means that we save considerable computational resources, and that we do not consider weakly deleterious mutations whose fixation is not effectively prevented by selection. In all simulations, we assumed that the distribution of fitness and dominance effects did not differ for mutations impacting the embryo and adult.

Selfing: With a probability equal to the p_{self} (which we systematically varied from zero to one in increments of 0.2) the seed parent was also chosen to be the pollen parent. Otherwise, mating was random, with pollen parents chosen and with replacement in proportion to adult fitness, using the `sample()` function in R (R Core Team 2020). We note that this random mating does not preclude selfing. Therefore, even with $p_{\text{self}} = 0$, one of every N_e embryos (approximately 0.001 when N = 1000, depending on seed survival rates and the variance in post-embryo fitness) is expected to have identical pollen and seed parents.

Evolution

Burn-in: For all parameter combinations, we forward simulated ten replicate processes for 2000 generations, ensuring that populations achieved mutation-selection-drift balance by visually examining the variability in the number of deleterious mutations over time and among replicates (Figure S1). For most parameter values, equilibrium was reached within this time frame (Figure S1). How-

ever, for recessive mutations in predominantly outcrossing populations (with selfing rates of 0, 0.2, or 0.4) this was not enough time to reach equilibrium. For these slowly equilibrating cases, we increased the burn-in period until 3000 generations, at which point equilibrium was largely achieved. Finally, with complete selfing and a non-recessive load with $s = 0.1$, the number of deleterious mutations seems somewhat unstable (that is, with this population size, mutation rate, and obligate selfing, additive mutations with $s = 0.1$ eventually reach fixation, and therefore the number of mutations per individual eventually increases – Figure S1). To ensure that our simulations performed sensibly, we compared results from these burn-ins into analytical expectations when known, specifically focusing on the expected number of recessive alleles per individual (Gao et al. 2015).

Invasion of polyembryony: For each burn-in replicate, we ran many introductions of a dominant acting polyembryony allele, introduced at a frequency of $1/2N$, and kept track of the fate of this allele (loss or fixation) for each introduction. Due to computational considerations, we varied the number of introductions from 500 to 1000 for each model of polyembryony (below) for each burn-in replicate. That is, when polyembryony was strongly favored, a given simulation took longer to complete (because fixation from $1/2N$ takes more time than loss from $1/2N$). By contrast, when polyembryony is not strongly favored, individual simulations are faster (because loss occurs more quickly than fixation) and more precision was needed to distinguish fixation rates from neutrality. The R (R Core Team 2020) code for these forward simulations is available as a supplementary document.

Models of polyembryony

We aim to dissect the contribution of reproductive compensation and embryo screening to the evolution of polyembryony.

The reproductive compensation benefit of polyembryony is that having two potential embryos in a seed increases the probability that a seed will contain a viable embryo (Top panel of Figure 2A). Thus, the benefit of reproductive compensation occurs if the survival of at least one embryo ensures a viable seed. We can effectively remove this benefit of polyembryony by allowing seed viability to be determined by survival of an arbitrarily chosen embryo among that seed's two embryos.

The embryo screening benefit of polyembryony is that, mothers can increase their expected inclusive fitness by screening for vigorous embryos, which could result in higher fitness seeds (Bottom panel of Figure 2A). We allow for the benefit of embryo screening by selecting the embryo that

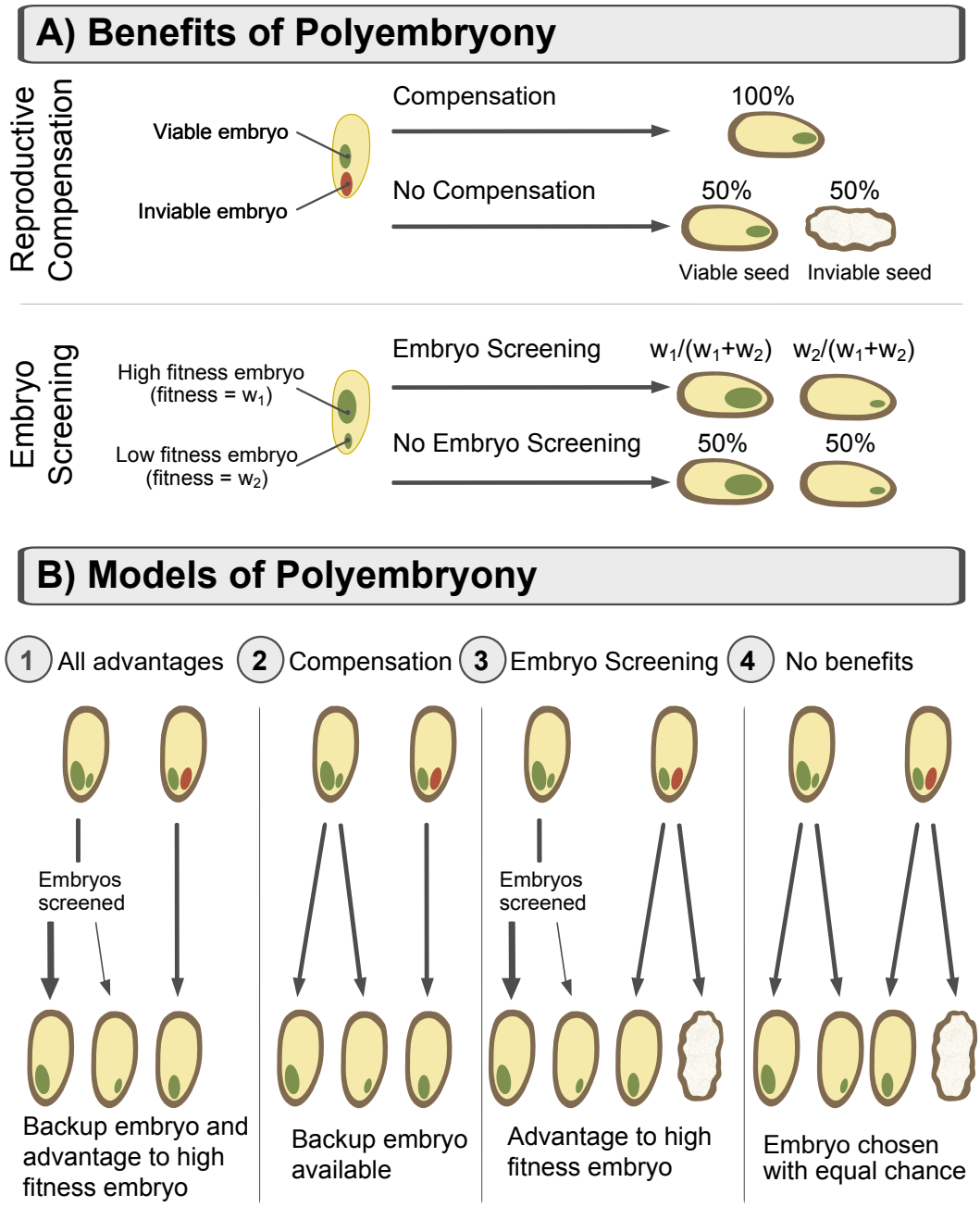

Figure 2: **Benefits ad models of polyembryony:** (**A**) Polyembryony offers two potential benefits – increasing the chances having a viable seed (Reproductive compensation, top panel), and increasing the chances that a high fitness embryo is in the seed (Embryo screening, bottom panel). (**B**) Factorially combining these benefits generates our four models for the evolution of polyembryony (see text for details).

makes it to the seed among surviving embryos with probabilities in proportion to their embryonic fitness. We can effectively remove this benefit of polyembryony by randomly choosing one among

viable embryos in a seed to become the dominant embryo and continue development.

Factorially combining these options results in four models for the evolution of polyembryony (Figure 2B). As we describe these models, we show how each model isolates the potential outcomes of polyembryony by considering the survival of focal embryo one, p_1, in a seed with a second embryo. We assume embryos one and two have survival probabilities w_1 and w_2, respectively, and we note the probability that embryo one is chosen by embryo screening as $\tilde{w_1} = \frac{w_1}{w_1+w_2}$.

1. All advantages: In this model, each embryo in the seed of a polyembryonic mother survives independently with a probability determined by its embryonic fitness. If only one embryo survives, this embryo develops into the seed, and if both embryos survive, the embryo that develops in the seed is chosen in proportion to the relative embryonic fitness of each embryo (i.e. if both embryos survive, the probability an embryo develops into a seed equals its fitness divided by the sum of the fitness of both surviving embryos). Thus, this model includes both potential benefits of polyembryony.

In the all benefits model, the probability that embryo makes it to the seed is the probability it survives and embryo two dies ($w_1(1-w_2)$) plus the probability that both survive, and embryo one ends up in the seed ($\tilde{w_1}(w_1 w_2)$).

$$p_{1|\text{all benefits}} = w_1(1-w_2) + \tilde{w_1}(w_1 w_2) \tag{1}$$

2. Compensation only: In this model, each ovule in the seed of a polyembryonic genotype survives independently with a probability determined by its embryonic fitness. If only one embryo survives, this embryo develops into the seed, and if both embryos survive, the embryo that develops in the seed is chosen at random. As such, polyembryony provides the benefit of increasing the probability that a seed survives, but does not provide the added benefit of embryo screening. This model resembles the case of reproductive compensation (Porcher and Lande 2005), as inviable genotypes can be replaced. With this type of selection, embryo viability is not dependent on the fitness of the other embryo, however embryos with inviable siblings are more likely to become seed than are embryos with viable siblings.

Thus, in the compensation model, the probability that embryo makes it to the seed is the probability it survives and embryo two dies ($w_1(1-w_2)$) plus the probability that both survived and that this embryo was randomly chosen to end up in the seed ($w_1 w_2/2$).

$$p_{1|\text{compensation}} = w_1(1 - w_2) + 0.5(w_1 w_2) \tag{2}$$

This equation is similar to the all benefits model (Eq. 1), with the difference that $\tilde{w}_1$ is replaced by 0.5 highlighting the absence of embryo screening in the compensation only model.

3. Embryo Screening only: We can indirectly infer the advantages and consequences of embryo screening by comparing the all benefits and compensation models above. However, directly modelling the benefits and consequences of screening alone from the compensation is somewhat complex because it is difficult to model the evolution of relative embryo quality (i.e. the basis for screening), when there is only one embryo in a seed (i.e. before the introduction of polyembryony). We therefore developed the following approach to model allow for selection on embryo quality before and after polyembryony evolved while removing the natural advantage of reproductive compensation associated with polyembryony. In this model, a seed in a polyembryonic genotype survives with a probability equal to the embryonic fitness of an arbitrarily chosen embryo (embryo 1 in our simulation). Thus, if this embryo dies but the other lives, the seed still dies. As such, monoembryonic and polyembryonic genotypes will have the same probability of developing a viable seed (i.e. in both cases seed survival is determined by the fitness of a single random embryo). However, if both embryos survive, the embryo that develops in seed is chosen in proportion to the relative embryonic fitness of each embryo.

Thus, in the embryo screening model, the probability that embryo makes it to the seed is the probability it survives and embryo two dies divide by two $(0.5(w_1(1 - w_2)))$ plus the probability that both survive, and embryo one ends up in the seed $(\tilde{w}_1(w_1 w_2))$.

$$p_{1|\text{all benefits}} = 0.5(w_1(1 - w_2)) + \tilde{w}_1 w_1 w_2 \tag{3}$$

We note that this outcome is similar to the all benefits model (Eq. 1), with the difference that $w_1(1-w_2)$ is weighted by 0.5, so as to effectively removes the benefit of reproductive compensation from this model, while maintaining embryo screening.

However, while our model of embryo screening compensation without embryo screening seems biologically plausible, our model of embryo screening without reproductive compensation does not – it is unclear how survival of a seed could be determined by the fitness of a random embryonic genotype it carries that may or may not end up in the seed. As such, this scenario is introduced,

not as an attempt to model biological reality, but rather to provide a theoretical control to allow us to better understand the biological world. That is, while biology may not allow for embryo screening without an incidental benefit of enhanced embryo survival our modeling approach can, which allows us to isolate the evolutionary consequences of each.

4. No benefit: In this model, one of the two embryos in the seeds is randomly chosen to make it into the seed, and the seed is inviable if this embryo is inviable. As such, there should be no advantage of polyembryony. This model acts as a control to ensure that our simulation scheme meets neutral expectations and that our control for each potential benefit of polyembryony is properly implemented.

Results

.

Burn-in simulations

We discuss the results from our burn-in simulations, as they set the scene for the evolution of polyembryony. Throughout the discussion of burn-in results, we focus on mutations impacting embryo fitness, as results for post-embryonic fitness follow similar qualitative and quantitative patterns (Figure S1). Genomes saved at the end of the burn-in are available for download here.

Comparison to published analytical results: Before discussing specific results, we evaluate whether our simulations behave sensibly by comparing model output to known analytical results – namely, the expected number of recessive lethal mutations per diploid genome in a panmictic population. Based on classic results of Li and Nei (1972), Gao et al. (2015) show that the expected number of recessive lethals per (diploid) individual in a finite, panmictic population equals $U\sqrt{2\pi N_e}$, where U is the mutation rate per haploid genome, and N_e is the effective population size. For the case of recessive embryo lethals in outcrossers, we find a mean of 18.6 mutations per diploid genome, a value remarkably consistent with the predicted value of 18.4 (Compare dashed white line to simulation results in Figure 3A). That is, if $U = 0.25$, as we are only concerned with mutations impacting embryo fitness (half of total mutations), and $2N_e \approx 1745$, the mean number of surviving embryos across replicates in the final generation. Additional exploratory simulations (not shown) found a consistent agreement with theory across a range of mutation rates.

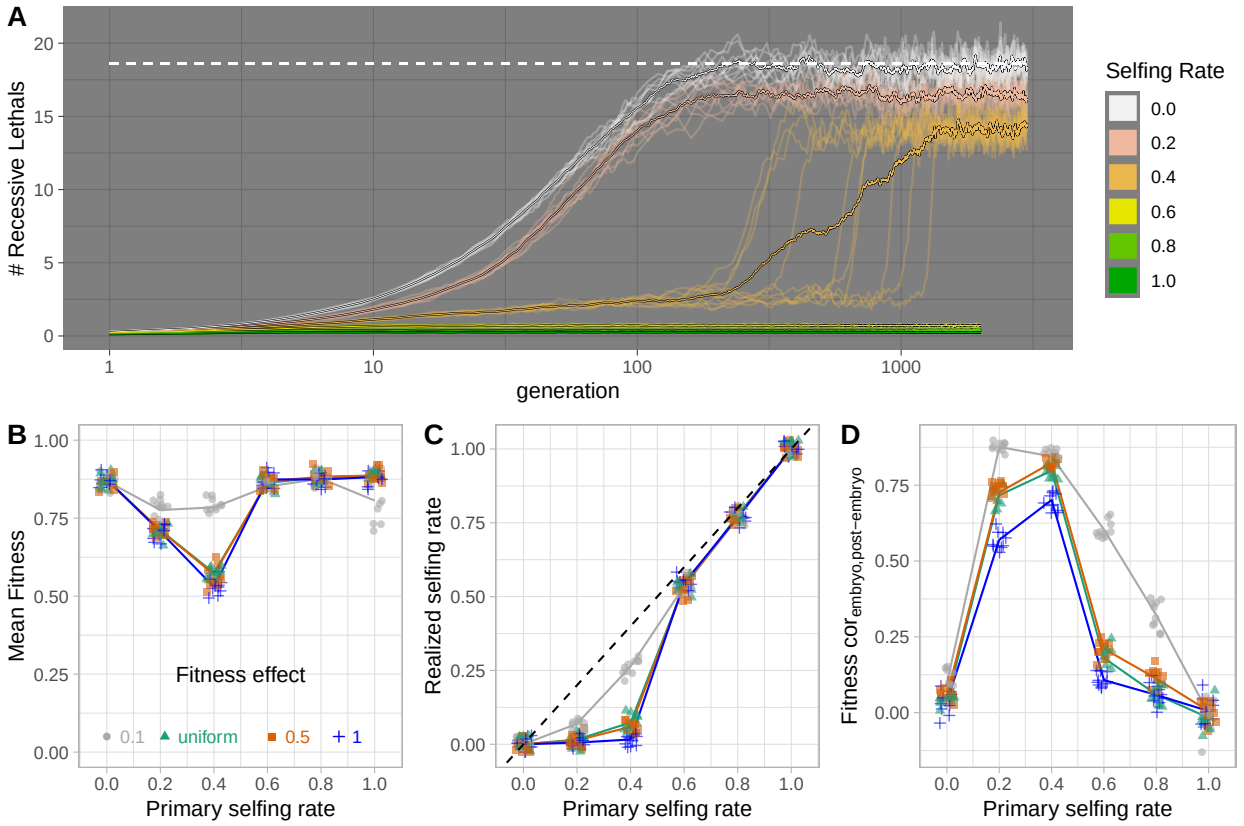

Figure 3: **Results from burn-in: (A)** The mean number of recessive lethal alleles per haploid genome over time. Each line is one of ten replicates for each selfing rate, designated by color. The dashed white line shows the theoretical expectation for a randomly mating population in our simulation, while the larger colored line shows the mean across replicates. Results with different dominance and selection coefficients are presented in Figure S1. Figures **(B-D)** show features of the population 'burn-in' populations after the load equilibrates. Points are slightly jittered to show the data - with one value for each replicate simulation for a given combination of selfing rates on the x, and fitness effects of new mutations in color, lines connect means. In **C**, the one to one line is shown by the dashed black line. All mutations are fully recessive. Results with different dominance coefficients are presented in Figure S2.

Novel Burn-in Results: Recessive lethal mutations are effectively purged with predominant selfing (selfing rate > 0.5), while a large number of deleterious mutations accumulate with predominant outcrossing (Figure 3A). With an intermediate selfing rate of 0.4, the population appears to reach an equilibrium, relatively modest number of recessive mutations, until this rapidly and dramatically increases, presumably reflecting a transition from effective purging to interference among deleterious mutations (Lande et al. 1994; Porcher and Lande 2016). Across all parameter combinations, the number of deleterious mutations at equilibrium decreased as mutations became more deleterious and more additive (Figure S1A). Additionally, across all dominance and selection

coefficients, the number of deleterious alleles in a population decreased with the selfing rate. However, the results for obligate selfers were somewhat unstable with weak selection and non-recessive dominance coefficients, when mutations can occasionally fix, and therefore continue increasing in number (Figure S1).

When mutations are recessive ($h = 0$), mean fitness is lowest with intermediate selfing rates, and is generally highest with high levels of selfing or outcrossing (Figure 3B). This pattern is most pronounced when recessive mutations are lethal ($s = 1$), exceptionally deleterious ($s = 0.5$), or where selection coefficients were drawn from a uniform distribution, and more subtle with a selection coefficient of $s = 0.1$ (Figure 3B). By contrast, when mutational effects are additive ($h = 0.5$) or are drawn from a uniform distribution, mean fitness increases with the selfing rate, with significantly positive slopes ranging between 0.045, and 0.082 (Figure S2A, Table S1), presumably because selfing increases the variance in fitness, allowing for more effective selection. In these non-recessive cases, mean embryo fitness is roughly similar, regardless of the fitness effects of individual mutations (Figure S2, modelling mean fitness = f(selfing, s), the p-value for the effect of s is 0.059 and 0.25, for cases with a uniform and additive load, respectively, Table S2). Reassuringly, this grand mean fitness under obligate outcrossing for non-recessive alleles of 0.78 is in line with its predicted value of 0.78 from Haldane's 1937 classic result that mean fitness equals e^{-U} (where U is the mutation rate per haploid genome, which equals 0.5 divided by two, as half of mutations will impact embryo fitness). Somewhat surprisingly, mean post-embryonic fitness does depend on the selection coefficient (Fig. S2D), suggesting that selection at one life stage impacts outcomes at another as suggested by Sakai (2019).

With intermediate selfing rates and recessive gene action we observe a much higher primary than realized selfing rate, suggesting that inbreeding depression underlies much of the embryo death in these cases (Fig. 3C). By contrast, we observe a nearly perfect relationship between primary and realized selfing rates under non-recessivity (Fig. S2B). We observe a strong positive correlation between embryo and post-embryo fitness for recessive gene action and intermediate selfing rates, but no relationship otherwise (Fig. 3D, and Fig. S2C). Together these results support the intuition that if competition acts to remove selfed embryos, this benefit of polyembryony will be most relevant when mutations are recessive.

Invasion of polyembryony

We compare the fixation probability of a new mutant that confers polyembryony, across all models described above. We find that, when the polyembryony allele fixes, it tends to fix more quickly when polyembryony provides reproductive compensation than when it does not (Fig. 4A, Fig. S4, Table S3). Similarly, polyembryony is most likely to fix when it provides reproductive compensation – in some cases, single mutations have up to a fifteen percent chance of reaching fixation, a 300-fold increase in the probability, relative to neutral expectations (Fig. 4B & 4C). The benefits of embryo screening alone also favored the evolution of polyembryony, but had a more modest effect – in some cases, single mutations have up to a one percent chance of reaching fixation, a 20-fold increase in the probability, relative to neutral expectations. Reassuringly, fixation proportions from the no benefits model matched neutral expectations, with approximately $1/2N = 0.0005$ introductions resulting in fixation (See Table S3, and compare the solid lines to the dashed line in Figure 4B).

Other biological parameters such as the selfing rate, and the dominance and selective coefficients of deleterious mutations also impact on the evolution of polyembryony, often depending on their interaction. Below we discuss the effects of selfing rate and additive vs. recessive modes of gene action, noting that results from the uniform mode of gene action are qualitatively similar to the additive model (Fig. S3, Table S3).

The benefit of reproductive compensation strongly favored the evolution of polyembryony for all biological parameters investigated (Fig. 4). Figure 4C displays the fixation proportions for the compensation models (row two in Fig. 4B) on a linear scale to reveal the effect of selfing rate and selective effects of new mutations.

Under recessivity, the probability of fixation is maximized (approximately 15%) at intermediate selfing rates, suggesting that polyembryony can evolve to make up for offspring lost to early acting-inbreeding depression. Again assuming recessivity, obligate outcrossing more strongly favors the evolution of polyembryony than does obligate selfing (compare an approximately 10% fixation probability under random mating to a 2.5% fixation probability under obligate selfing, Fig. 4C), presumably reflecting the higher within-seed variance in fitness under obligate outcrossing leading to higher impact of polyembryony. The fitness effect of recessive deleterious mutations have only a modest effect on fixation proportion, varying slightly across selfing rate.

However, the compensation model also strongly favors the evolution of polyembryony with an additive load, suggesting that overcoming inbreeding depression is not the only driver of the

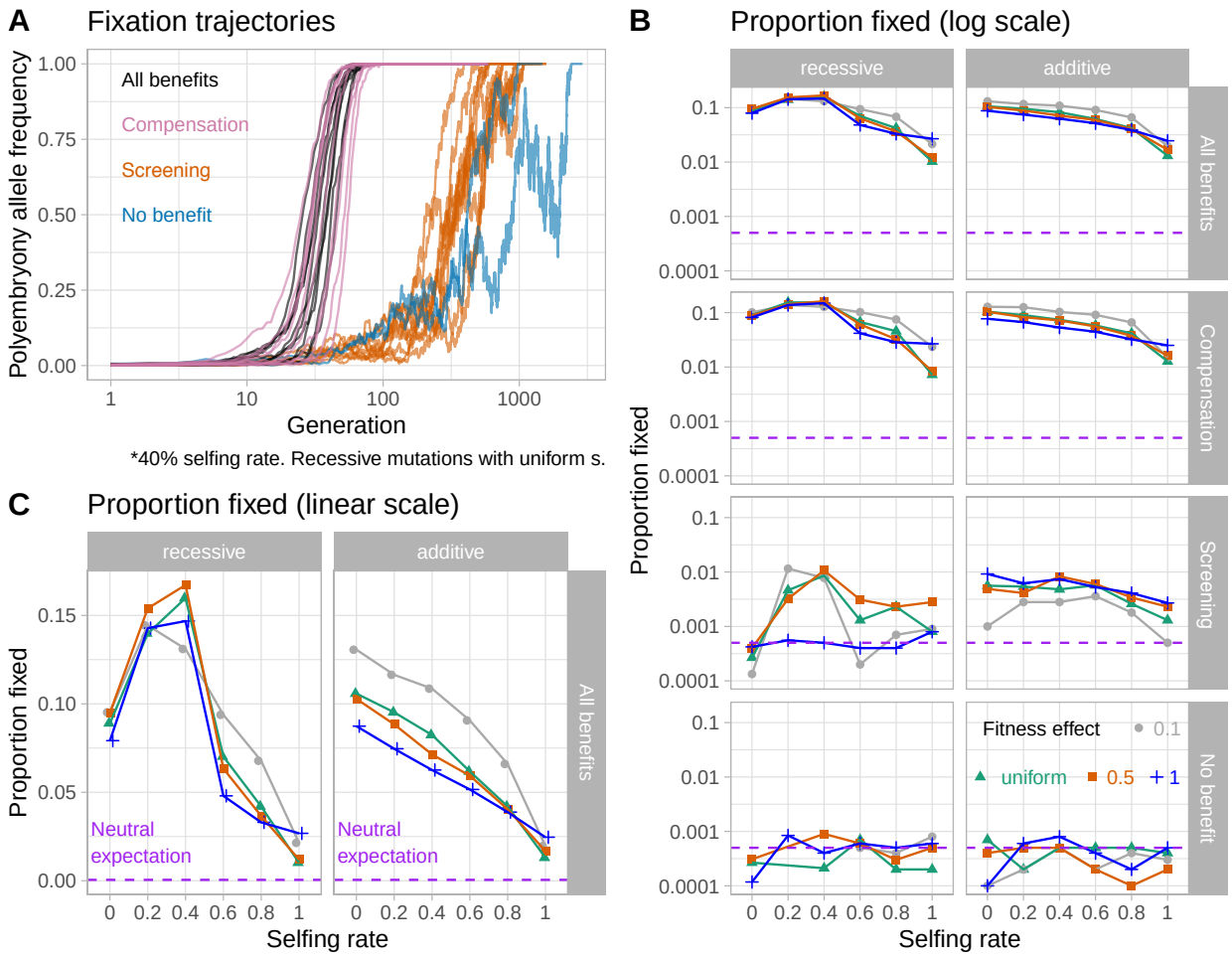

Figure 4: **The fixation of an allele conferring polyembryony: A)** Example trajectories of the fixation of the polyembryony allele with all benefits (black), the benefit of compensation (pink), the benefit of embryo screening (orange), and no benefit (blue). Note that generation on x increases on the log10 scale, but specific values are noted with their linear value. **B)** The proportion of introductions resulting in fixation of the polyembryony allele as a function of the selfing rate (x), the fitness effect of new mutations (color), the mode of gene action (columns), and the benefit of polyembryony (rows). The dashed pink line displays the expectation under neutrality. Note that fixation proportion on y increases on the log_{10} scale, but specific values are noted with their linear value. **C)** The proportion of introductions resulting in fixation of the polyembryony allele as a function of the selfing rate (x), the fitness effect of new mutations (color), the mode of gene action (columns), with all the benefits of polyembryony. The values are identical to those in the first row of **B**, but are presented on a linear scale to highlight the effect of selfing rate on fixation probability.

evolution of polyembryony. (Second row, second column, Figure 4B). In cases with additive gene action, the fixation probability of a polyembryony allele decreases with the selfing rate, again reflecting the lack of within-seed variance in fitness (i.e. the benefits of replacing an inviable embryo decrease as the probability that its replacement will also die increases). Additionally, under

additivity (or if mutations take their dominance coefficients from a uniform distribution, Table S3, Figure S3) a load composed of highly deleterious mutations is less likely to foster the evolution of polyembryony than a load composed of a larger number of mild mutations (compare $s = 1$ (blue) to $s = 0.5$ (orange) or s=uniform (teal) to $s = 0.1$ (grey), Fig. 4). This surprising result might reflect the fact that while mean fitness does not depend on fitness effects of new mutations, the survival of maternal sibling-embryos becomes more dependent on one another as mutational effects get larger (Figure S5). As such, with large effect mutations, a backup embryo is less useful as if one dies the other is likely to die as well.

The benefit of embryo screening also favors the evolution of polyembryony. However, fixation probabilities are approximately five- to ten-fold lower for this model than for the reproductive compensation model. With a recessive load and intermediate selfing rates (0.20 or 0.40), embryo screening results in the fixation of the polyembryony allele in approximately one percent of introductions, a twenty-fold increase relative to the neutral expectation of 0.05%. Somewhat surprisingly, embryo screening favors polyembryony for a non-recessive load (Third row, second column of Fig. 4B), even though embryo fitness was uncorrelated with post-embryo fitness in these models (Fig. S2). This likely reflects the benefit of producing grand-children with higher embryonic fitness who will out-compete their siblings (analogous to models of "runaway sexual selection" Kirkpatrick 1982). Under both additivity and a uniform distribution of dominance effects, the probability of fixation of an allele conferring competitive polyembryony is greatest in predominantly outcrossing populations (selfing rates of 0.40 or less), decreasing as the selfing rate increases. Here, the probability of fixation is greatest when the load is composed of alleles of large effect, a result that runs counter to that found in the compensation model with an additive load.

All benefits results in fixation probabilities qualitatively similar to the reproductive compensation model (Fig. S3, Table S3) – reflecting the importance of the benefits of reproductive compensation, to the evolution of polyembryony.

No benefits results in fixation probabilities consistent with neutral expectations (Fig. S3, Table S3).

Evolutionary consequences of polyembryony

We compare how different models of the evolution of polyembryony shape key features of a population, including the proportion of surviving seeds, the realized selfing rate and the architecture of genetic load. Although a strict version of the embryo screening only model is unlikely to occur in nature, its inclusion allows us to distinguish the individual effects of competition and compensation when both would be operating in nature (i.e. the all benefits model). Because results were qualitatively similar across all selection coefficients (save the decrease in fitness with recessive mutations, $s = 0.1$ and high selfing rates, which did not always converge Fig. S1), and because results from the additive model and the uniformly distributed dominance coefficient model did not differ qualitatively, we focus on results from the cases in which the selection coefficients of new mutations are selected at random from a uniform distribution, exploring cases in which mutations are recessive or additive (Figure 5).

Curiously, the benefit of embryo screening alone did not impact the realized selfing rate (Fig. 5), even with recessive mutations and intermediate selfing rates. This result is a consequence of features of both our model and biological reality. Specifically, there are limited opportunities for competition between selfed and outcrossed embryos in a seed (Williams 2007), as this only occurs with a probability equal to two times the variance in the selfing rate (the probability that exactly one of two embryos is from a self-fertilization event) times the probability that both are destined to survive. By contrast, with a recessive load a benefit of compensation decreases the realized selfing rate, and increases the number of mutations impacting embryo fitness in partially selfing populations (Fig. 5).

The benefit of compensation (in both the compensation and all benefits model) resulted in a strong increase in seed survival. Under a recessive load, this effect was maximized with intermediate selfing rates, while it decreased steadily with selfing rate under a (partially) additive load. By selecting for higher fitness embryos, the benefit of embryo screening alone subtly increased seed survival for all models of dominance investigated so long as the selfing rate was not too large (Fig. 5). Consequently, the expected embryo fitness of the surviving seeds subtly increases with the benefit of embryo screening, but decreases with compensation. These benefits appear to act additively, such that the expected embryo fitness decreases in the all benefits model but does so less severely than in the compensation model.

Regardless of the mode of gene action, the screening model does not increase the expected post-

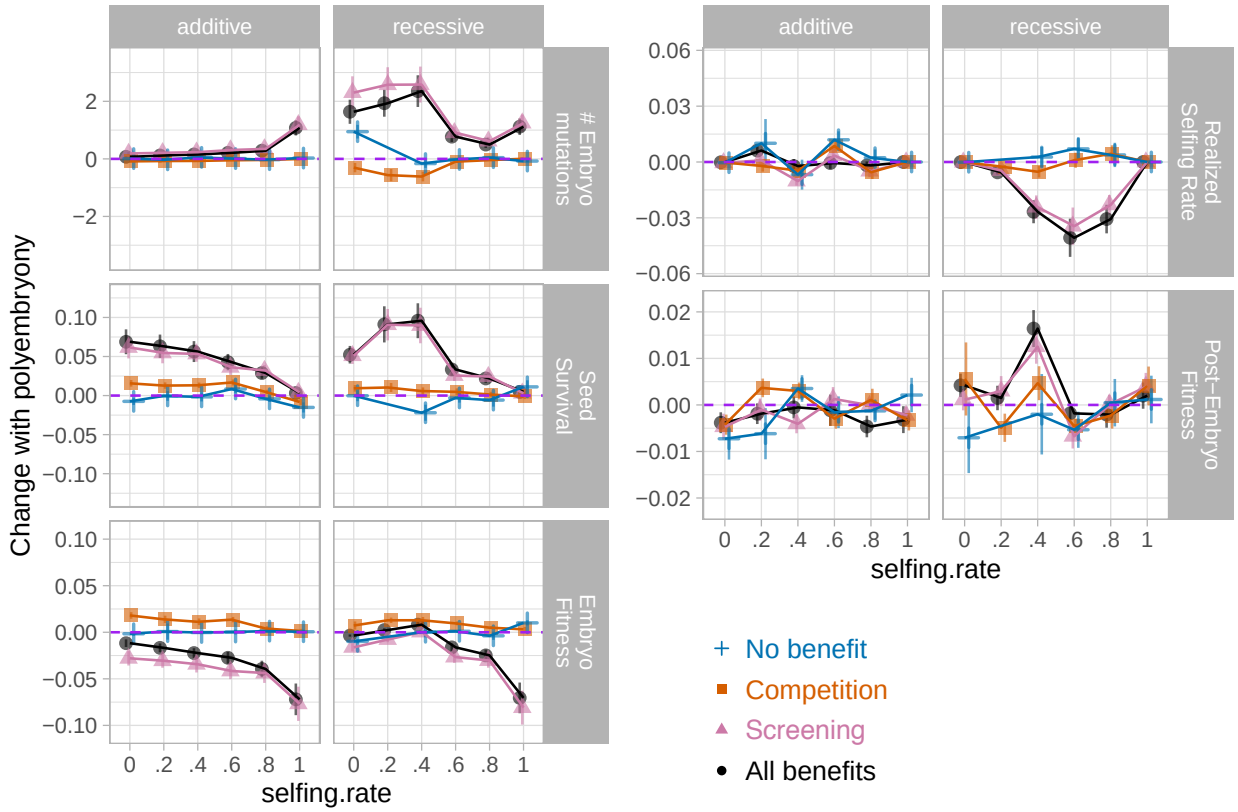

Figure 5: **The evolutionary impact of polyembryony.** How the evolution of polyembryony impacts the per individual number of mutations impacting embryo fitness, expected seed survival, mean embryo fitness, the realized selfing rate, and mean post-embryo fitness (rows), when mutations are additive or recessive (columns), across selfing rates (x-axis), for each model of polyembryony (color).

embryo fitness of surviving seeds (Fig. 5). While post-embryo fitness modestly increases with the evolution of polyembryony under a model of screening with intermediate selfing rates and a recessive load, this increase pales in comparison to the benefits of higher seed viability which accompanies reproductive compensation. Together, these lines of evidence suggest that polyembryony does not evolve as a mechanism to prevent self-fertilization, and is not analogous to the system of self-incompatibility observed in angiosperms.

Additional evidence against the hypothesis that the embryonic lethal system evolves as an SI-like mechanism comes from the allele frequency spectrum of deleterious mutations (Fig. 5). We reason that if polyembryony was favored as an inbreeding avoidance mechanism, we would expect an increase in very rare recessive lethal alleles in the embryo screening model to ensure the death of selfed embryos (i.e. effective avoidance of inbreeding). Contrary to this expectation, we see no such shift in the frequency spectrum. Figure 6 shows that the allele frequency spectrum is comparable

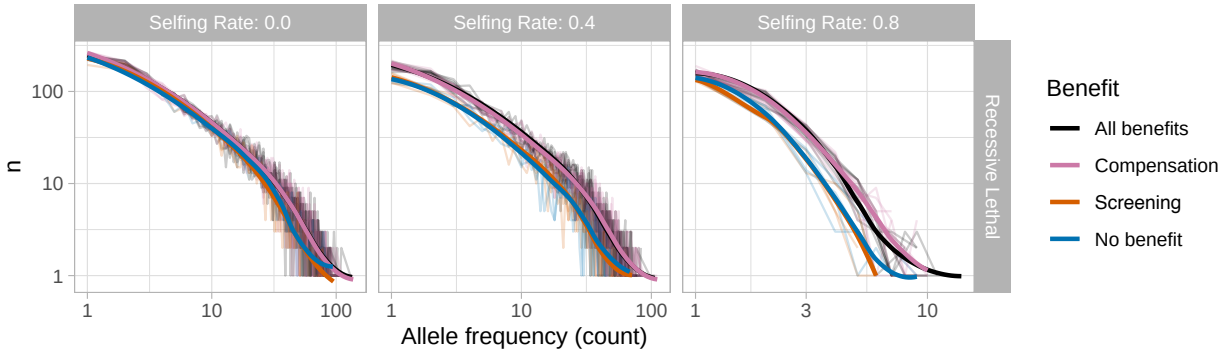

Figure 6: **The allele frequency spectrum** for embryo acting allele following the evolution of polyembryony under the recessive lethal model for a selfing rate of 0.00, 0.40, and 0.80 (left to right). Lines display averages of ten simulation replicates, and colors note the model of polyembryony. Note that the x axis with a selfing rate of 0.80 (right panel) is truncated relative to the other selfing rates, reflecting the effective purging of early acting recessive mutations with high selfing rates.

in the no benefit and embryo screening models, arguing against the idea that screening favored self-sacrifice in the form of an excess of rare recessive lethals. By contrast, there is a slight increase in the count of deleterious recessive mutations across all frequency classes in the compensation and all benefits models, reflecting the relaxation of embryo selection in these cases.

Discussion

We present four models to test the plausibility of the compensation and embryo screening theories for the evolution of polyembryony. We find that the evolutionary benefit of compensation – that is, the opportunity for a backup embryo to replace an inviable one – strongly favors the evolution of polyembryony. Relative to neutral expectations, the benefit of compensation results in between a twenty-fold increase in fixation probability above the neutral expectation with high selfing rates, and a two hundred-fold increase with intermediate to low selfing rates and a recessive load, all across a broad range of selection and dominance coefficients. By contrast, the benefit of embryo screening – that is, the opportunity of selection to prefer a higher fitness embryo – more weakly favored the evolution of polyembryony, resulting in between a zero-fold increase with high selfing rates, and a twenty-fold increase, with intermediate to low selfing rates and a recessive load, relative to neutral expectations. As such with both benefits, fixation probabilities closely follow expectations from the benefits of compensation alone.

Our work highlights the previously underappreciated result that the consequences of polyembryony depend on its function. When polyembryony functions as a compensation mechanism, mean

embryo fitness is reduced, but the probability of seed survival increases, consistent with previous work (e.g. Klekowski 1982; Porcher and Lande 2005). By contrast, when competition between embryos acts only to screen embryos, mean embryo fitness increases, seed survival does not change substantially, in line with models of competition alone (Latta 1995). With a recessive load, the benefit of compensation acts to decrease the effective selfing rate, while the embryo screening did not. With both the benefits of screening and compensation the evolutionary consequences of polyembryony is somewhere in between but is often closer to those expected from compensation.

The limited role of embryo screening in the evolution of polyembryony: It has long been assumed that a major benefit of polyembryony is that it provides an opportunity for embryos to compete (Sarvas 1962; Koski 1971), and therefore for mothers to screen for high fitness offspring. Not only was the benefit of screening itself – after removing the potential benefit of reproductive compensation – a comparatively weak force in the evolution of polyembryony, but it did not reliably increase seed fitness. In fact, under most selfing rates and dominance coefficients, screening more strongly favored the evolution of polyembryony with an additive load (in which there is no relationship between embryo and post embryo fitness) than a recessive load (in which there was such a relationship). This is surprising because there is a limited scope for selection on polyembryony when it cannot affect post-embryo fitness. In this case, selection on polyembryony only occurs within polyembryonic seeds themselves, and, since the embryos' maternal genomes are identical, only among the paternal genomes. Like runaway sexual selection (Fisher 1915), the automatic transmission advantage of selfing (Fisher 1941), or meiotic drive (Rhoades 1942), embryo screening is an example of a selective advantage that does not make a population necessarily more adapted to its environment.

Why doesn't embryo screening effectively remove selfed offspring and increase post-embryonic fitness (and could it ever)? In our model of embryo screening alone, screening is only relevant if both embryos were destined to live. As such, screening is most effective at removing selfed offspring when one seed is selfed and the other is not, and both seeds are destined to live (because if, for example, the selfed embryo was destined to die, reproductive compensation would allow for the replacement of this low-fitness, selfed embryo). Because predominant selfing (selfing rate > 0.5) purges the recessive load, and predominant outcrossing (selfing rate < 0.5) generates large inbreeding depression, in most cases in which selfed and outcrossed embryos could compete,

their fitness is either nearly equal, or the selfed embryo is destined to die. As such, embryo screening does not offer a more refined view into post-embryo fitness than is automatically accounted for by "hard selection" on seed viability. Our observation that embryo screening leads to more competitive embryos, rather than higher fitness plants is consistent with the claim of McCoy and Haig (2020) that Goodhart's law – 'When a measure becomes a target it ceases to be a good measure' – can undermine effective embryo selection. Despite our focus on the evolution of polyembryony, these results apply broadly and suggest that verbal models predicting that selective embryo abortion could limit the mating costs of selfing in plants with mixed mating systems (e.g. Huang et al. 2020), require more rigorous scrutiny.

Nonetheless, it is possible that pure "soft selection" (Wallace 1968, 1975) on embryos could reliably increase post-embryo fitness. That is, although we had trouble implementing this model computationally (e.g. we could not define the allele frequency spectrum of alleles determining success in soft selection before embryo screening evolved), nor could we map this onto a plausible biological mechanism, these challenges could reflect a shortcoming in our imagination, rather than a biological impossibility. Additionally, we note that even if the benefits of compensation initially favored the evolution of polyembryony, it is possible that the evolution of polyembryony was followed by novel recessive mutations experiencing soft selection and that therefore the benefits of embryo screening could maintain but not drive the evolution of polyembryony.

The embryo lethal system: Since Buchholz (1922), it has been argued that the embryo-lethal system, an apparently coordinated process of embryo death, could achieve a similar function to angiosperm self-incompatibility in the self-compatible gymnosperms. That is, coordinated death in the embryo stage would give way to highly outbred surviving adults (Sarvas 1962; Koski 1971). This would be an altruistic act in which an embryo sacrifices its predictably low fitness for a maternal half sibling. We did not observe the evolution of an embryo lethal system in response to the evolution of polyembryony, as would be expected if polyembryony favored altruistic self-destruction of more inbred embryos (e.g. we did not see a change in the allele frequency spectrum towards an excess of low-frequency recessive mutations). However, by relaxing selection on embryo viability, embryo compensation could indirectly result in an increase in the number of highly deleterious recessive mutations impacting embryo fitness. As such it appears that the embryo lethal system could reflect an elevated load tolerance rather than an exquisite adaptation (Gould and Lewontin 1979), as Williams (2007) argued forcefully based on developmental and genetic evidence.

Finally, we note that we cannot exclude possibilities which we did not model (e.g. pure soft selection). For example, in a single locus, 4 allele haploid system with pure soft selection, Archetti (2009) found that an "anti-robust" allele that amplified the deleterious effects of a mutation in juveniles so as to be replaced by a genotype that will result in a higher fitness adult will be favored. While one could interpret the "embryo lethal system" as an anti-robust system, it is unclear if such alleles could evolve in a complex diploid genome (e.g. pines).

Which has driven the evolution of polyembryony – Compensation or embryo screening? We find that the benefit of embryo compensation favors the evolution of polyembryony more strongly than does embryo embryo screening. However, we caution that whether compensation or embryo screening have actually favored the evolution of polyembryony depends on their biological plausibility and whether they reflect effective solutions to the problems they address. That is, we must consider biological processes outside of our model as we interpret our model results. For example, embryo screening could perhaps be most effectively achieved by placing more embryos in a seed, while compensation could be more effectively achieved by producing more seeds per plant.

Our models provide competing testable predictions to distinguish between predictions of the compensation and embryo screening model at within seed level, for simple polyembryony, assuming no pleiotropic effects. For example, we show that the evolution of polyembryony and its consequences depend on the selfing-rate and dominance coefficient. Specifically, with a recessive genetic load, embryo screening most strongly favors the evolution of polyembryony at intermediate selfing rates (Fig. 4B,C). The estimates of selfing rates for modern conifers can reach 0.30 - 0.60 (Sarvas 1962; Sorensen 1982), a range that favors polyembryony. We note, of course, that estimates of the primary selfing rate from extant conifers rate may differ substantially from the primary selfing rates of the population in which polyembryony arose.

Additionally, the two models make subtly different predictions about the difference between the realized and primary selfing rate. Relative to a monoembryonic ancestral population, polyembryony favored by embryo screening alone does not result in decrease in the difference between realized and primary selfing rates. By contrast, with a recessive load and intermediate selfing rates, polyembryony favored by compensation strongly amplified the difference between the realized and primary selfing rates. In nature, differences between primary and realized selfing rates are often observed in species with simple polyembryony (Lindgren 1975; Sorensen 1982; Kärkkäinen and Savolainen 1993; Lande et al. 1994), further emphasizing the probable role of compensation in the evolution

and maintenance of polyembryony.

The impact of pleiotropy across the life cycle: In the main results presented above, We assumed no pleiotropy across life stages – that is, mutations either impacted embryo or post embryo fitness. However, this is clearly untrue in nature. For example, severe loss of function mutations in key genes would likely decrease both embryo and seed fitness. We therefore wanted to evaluate the extent to which our results rested on the assumption of no pleiotropy. To do so, we re-ran all models under the assumption of complete pleiotropy – in which all mutations had identical fitness effects on embryonic and post-embryonic fitness.

Somewhat surprisingly, a model of exceptionally strong pleiotropy – in which each mutation had an identical effect on both embryonic and post-embryonic fitness – showed that reproductive compensation strongly favored the evolution of polyembryony, while embryo screening less reliably resulted in the fixation of polyembryony (Figure S6. Together, these results suggest that embryo compensation is likely the major selective force favoring the evolution of polyembryony,

We did not explore the case in which an allele antagonistically increased embryo fitness while decreasing post embryonic fitness. When such a mutation occurs with embryo screening, it could generate an ontogenic conflict. Empirical studies, e.g. mapping and measuring of inbreeding depression at different life stages (Koelewijn 1998), comparing gene expression across embryo development and later life stages (Raherison et al. 2012), and signatures of negative and positive selection in such genes would be valuable to further evaluate the potential importance of pleiotropy in the evolution of polyembryony. However, as our results suggest that reproductive compensation is the major driver of the evolution of polyembryony, it seems like this ontogenic conflict would play only a minor role in its evolution.

Finally, since polyembryony is primarily favored by the benefit of reproductive compensation, this benefit should be independent of the mechanism underlying embryo inviability. As such, polyembryony may be favored as a form of reproductive compensation for embryos whose viability is compromised by developmental hazards unrelated to genotype.

Screening, compensation and conflict in a pine nutshell: Gymnosperm seed with a maternal haploid megagametophyte, multiple genetically distinct embryos, genetically identical (cleavage) embryos, and strong inbreeding depression is a stage of evolutionary drama that deserves more attention, and we hope that the provided model will be used to broaden the investigations on the

evolutionary dynamics outside the angiosperm sphere. For example, in contrast to the opportunity for altruism to favor the embryo-lethal system, polyembryony also provides avenues for parental and embryonic conflict.

In simple polyembryony, embryos are potentially derived from different sires. A paternal genome carrying a mutation that sabotaged rival embryos carrying different paternal genomes could possess a net advantage even if doing so would reduce the probability that a viable seed is formed at all. Sabotage and anti-sabotage alleles would only be beneficial when expressed in a particular parental genome, so genomic imprinting that prevented expression in the wrong parental genome would also be advantageous.

Conifers and other gymnosperms provide unique opportunities to test key questions of plant mating system evolution and evolutionary conflict from a novel angle, especially now that their genomic resources are no longer seriously hindered by their large genome sizes. From the practical/empirical perspective, large seed size and gametophytic tissue allow easy identification of maternal haplotypes and alleles. Thus expression patterns and genetic diversity for example in potentially imprinted genes should be easy to quantify and identify in many conifer species.

Literature Cited

Agapito-Tenfen, S. Z., N. Steiner, M. P. Guerra, and R. O. Nodari. 2012. Patterns of polyembryony and frequency of surviving multiple embryos of the brazilian pine *Araucaria angustifolia*. Australian Journal of Botany 59:749–755.

Archetti, M. 2009. Survival of the steepest: hypersensitivity to mutations as an adaptation to soft selection. Journal of Evolutionary Biology 22:740–750.

Buchholz, J. T. 1922. Developmental selection in vascular plants. Botanical Gazzette 73:249–286.

Chamberlain, C. J. 1966. Gymnosperms: Structure and Evolution; With 397 Fig. Dover Publ.

Dillard, A. 1974. Pilgrim at Tinker Creek. Literature (Harper & Row). HarperPerennial.

Dogra, P. D. 1967. Seed sterility and disturbances in embryogeny in conifers with particular reference to seed testing and tree breeding in pinaceae. Studia forestalia Suecica pages 1–97.

Ferreira, D. N. d. S., J. L. C. Camargo, and I. D. K. Ferraz. 2019. Do polyembryonic seeds of carapa surinamensis (meliaceae) have advantages for seedling development? Acta Amazonica 49:97–104.

Fisher, R. A. 1915. The evolution of sexual preference. Eugenics Review 7:184–192.

———. 1941. Average excess and average effect of a gene substitution. Annals of Eugenics 11:53–63.

Forbes, L. S., and D. W. Mock. 1998. Parental optimism and progeny choice: When is screening for offspring quality affordable. Journal of Theoretical Biology 192:3–14.

Forbes, S. 2009. Portfolio theory and how parent birds manage investment risk. Oikos 118:1561–1569.

Ganeshaiah, K. N., R. Uma Shaanker, and N. V. Joshi. 1991. Evolution of polyembryony: Consequences to the fitness of mother and offspring. Journal of Genetics 70:103–127.

Gao, Z., D. Waggoner, M. Stephens, C. Ober, and M. Przeworski. 2015. An estimate of the average number of recessive lethal mutations carried by humans. Genetics 199:1243–1254.

Gould, S. J., and R. C. Lewontin. 1979. The spandrels of san marco and the panglossian paradigm: a critique of the adaptationist programme. Proceedings of the Royal Society B: Biological Sciences 205:581–598.

Haig, D. 1992. Brood reduction in gymnosperms. Pages 63–84 *in* M. Elgar and B. Crespi, eds. Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford.

Haldane, J. B. S. 1937. The effect of variation on fitness. American Naturalist 71:337–349.

Hedrick, P. W., O. Savolainen, and K. Karkkainen. 1999. Factors influencing the extent of inbreeding depression: an example from scots pine. Heredity 82 Pt 4:441–450.

Huang, Q., W. Wang, S. C. H. Barrett, and M. Ren. 2020. Plasticity in selective embryo abortion may limit the mating costs of geitonogamy in self-compatible plants: a hypothesis. American Journal of Botany 107:390–393.

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

Kärkkäinen, K., V. Koski, and O. Savolainen. 1996. Geographical variation in the inbreeding depression of scots pine. Evolution 50:111–119.

Kärkkäinen, K., and O. Savolainen. 1993. The degree of early inbreeding depression determines the selfing rate at the seed stage: model and results from *Pinus sylvestris* (scots pine). Heredity 71:160–166.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36:1–12.

Klekowski, E. J. 1982. Genetic load and soft selection in ferns. Heredity 49:191–197.

Koelewijn, H. P. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. Evolution 52:692–702.

Koski, V. 1971. Embryonic lethals of *Picea abies* and *Pinus sylvestris*. Metsäntutkimuslaitos.

Lakshmanan, K. K., and K. B. Ambegaokar. 1984. Polyembryony. Pages 445–474 *in* B. M. Johri, ed. Embryology of Angiosperms. Springer Berlin Heidelberg, Berlin, Heidelberg.

Lande, R., D. W. Schemske, and S. T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. Evolution 48:965–978.

Latta, R. G. 1995. The effects of embryo competition with mixed mating on the genetic load in plants. Heredity 75:637–643.

Li, W. H., and M. Nei. 1972. Total number of individuals affected by a single deleterious mutation in a finite population. American Journal of Human Genetics 24:667–679.

Lindgren, D. 1975. The relationship between self-fertilization, empty seeds and seeds originating from selfing as a consequence of polyembryony. Studia Forestalia Suecica 126:1–24.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits: Sinauer. Sunderland.

McCoy, D. E., and D. Haig. 2020. Embryo selection and mate choice: Can 'honest signals' be trusted? Trends in Ecology and Evolution 35:308–318.

Mikkola, L. 1969. Observations on interspecific sterility in picea. Annales Botanici Fennici 6:285–339.

Mock, D. W. 1984. Siblicidal aggression and resource monopolization in birds. Science 225:731–733.

O'Connell, L. M., and K. Ritland. 2005. Post-pollination mechanisms promoting outcrossing in a self-fertile conifer, *Thuja plicata* (cupressaceae). Canadian Journal of Botany 83:335–342.

Porcher, E., and R. Lande. 2005. Reproductive compensation in the evolution of plant mating systems. New Phytologist 166:673–684.

———. 2016. Inbreeding depression under mixed outcrossing, self-fertilization and sib-mating. BMC Evolutionary Biololgy 16:105.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raherison, E., P. Rigault, S. Caron, P.-L. Poulin, B. Boyle, J.-P. Verta, I. Giguère, C. Bomal, J. Bohlmann, and J. MacKay. 2012. Transcriptome profiling in conifers and the PiceaGenExpress database show patterns of diversification within gene families and interspecific conservation in vascular gene expression. BMC Genomics 13:434.

Rhoades, M. M. 1942. Preferential segregation in maize. Genetics 27:395–407.

Sakai, S. 2019. Maintenance of high inbreeding depression in selfing populations: Effects of coupling of early- and Late-Acting mutations. bioRxiv page 748699.

Sarvas, R. 1962. Investigations on the flowering and seed crop of *Pinus silvestris*. Metsatieteellisen tutkimuslaitoksen julkaisuja .

Saxton, W. T. 1934. Notes on conifers: IX. the ovule and embryogeny of widdringtonia. Annals of Botany 48:429–431.

Schnarf, K. 1937. Anatomie der Gymnospermen-Samen, vol. 10. Gebr. Borntraeger.

Schultz, S. T., and D. G. Scofield. 2009. Mutation accumulation in real branches: fitness assays for genomic deleterious mutation rate and effect in large-statured plants. Am Nat 174:163–75.

Sorensen, F. C. 1982. The roles of polyembryony and embryo viability in the genetic system of conifers.

Wallace, B. 1968. Polymorphism, population size and genetic load, in"population biology and Evolution"(RC lewontin, ed.) .

———. 1975. Hard and soft selection revisited. Evolution 29:465–473.

Williams, C. G. 2007. Re-thinking the embryo lethal system within the pinaceae. Canadian Journal

of Botany 85:667–677.

———. 2008. Selfed embryo death in *Pinus taeda*: a phenotypic profile. New Phytologist 178:210–222.

Williams, C. G., and O. Savolainen. 1996. Inbreeding depression in conifers: Implications for breeding strategy. Forest Science 42:102–117.

Willson, M. F., and N. Burley. 1983. Mate Choice in Plants: Tactics, Mechanisms, and Consequences. Princeton University Press.

Supplement

Table S1: Slope of the relationship between selfing rate and mean embryo fitness after burn-ins for non-recessive variants. All t values are associated with 59 degrees of freedom.

h	**s**	**estimate**	**lower 95% CI**	**upper 95% CI**	t	**p-value**
0.5	0.1	0.054	0.031	0.076	4.719	0.000015
0.5	0.5	0.085	0.076	0.094	19.114	<10-11
0.5	1	0.051	0.039	0.062	8.600	<10-11
0.5	uniform	0.082	0.072	0.091	16.413	<10-11
uniform	0.1	0.045	0.015	0.074	2.980	0.004203
uniform	0.5	0.078	0.068	0.088	15.313	<10-11
uniform	1	0.065	0.055	0.076	12.021	<10-11
uniform	uniform	0.083	0.074	0.091	19.445	<10-11

Table S2: Effect of selection coefficient on mean fitness following burn-in for non-recessive variants.

h	**estimate**	$F_{3,225}$	**p-value**
0.5	0.054	1.39	0.059
uniform	0.083	2.52	0.247

Table S3: The proportion of introductions of the polyembryony allele resulting in fixation.

$p_{\mathbf{self}}$	s	h	All benefits	Compensation	Competition	No benefit
0	uniform	uniform	0.1084 (n=5000)	0.1025 (n=10000)	0.0041 (n=10000)	0.0012 (n=10000)
0	uniform	recessive	0.089 (n=5000)	0.08937 (n=9500)	0.00027 (n=7500)	0.00027 (n=7500)
0	uniform	additive	0.1058 (n=5000)	0.1038 (n=10000)	0.0056 (n=10000)	7e-04 (n=10000)
0	0.1	uniform	0.129 (n=5000)	0.131 (n=10000)	0.0017 (n=10000)	2e-04 (n=10000)
0	0.1	recessive	0.09517 (n=6000)	0.10129 (n=7000)	0.00013 (n=7500)	0 (n=6500)
0	0.1	additive	0.1306 (n=5000)	0.128 (n=10000)	0.001 (n=10000)	1e-04 (n=10000)
0	0.5	uniform	0.1128 (n=5000)	0.0997 (n=10000)	0.0051 (n=10000)	4e-04 (n=10000)
0	0.5	recessive	0.095 (n=5000)	0.0885 (n=10000)	4e-04 (n=7500)	0.00031 (n=6500)
0	0.5	additive	0.1028 (n=5000)	0.1037 (n=10000)	0.0049 (n=10000)	4e-04 (n=10000)
0	1	uniform	0.0844 (n=5000)	0.0875 (n=10000)	0.0049 (n=10000)	0.001 (n=10000)
0	1	recessive	0.0792 (n=5000)	0.0817 (n=10000)	0.00042 (n=9500)	0.00012 (n=8500)
0	1	additive	0.0874 (n=5000)	0.077 (n=10000)	0.0092 (n=10000)	1e-04 (n=10000)
0.2	uniform	uniform	0.0946 (n=5000)	0.0885 (n=10000)	0.0033 (n=10000)	2e-04 (n=10000)
0.2	uniform	recessive	0.1398 (n=5000)	0.1539 (n=10000)	0.00462 (n=6500)	0 (n=9000)
0.2	uniform	additive	0.0952 (n=5000)	0.0901 (n=10000)	0.0054 (n=10000)	2e-04 (n=10000)
0.2	0.1	uniform	0.1178 (n=5000)	0.1211 (n=10000)	0.0015 (n=10000)	3e-04 (n=10000)
0.2	0.1	recessive	0.14436 (n=5500)	0.138 (n=6000)	0.01154 (n=6500)	0 (n=7000)
0.2	0.1	additive	0.1164 (n=5000)	0.1258 (n=10000)	0.0028 (n=10000)	2e-04 (n=10000)
0.2	0.5	uniform	0.0912 (n=5000)	0.089 (n=10000)	0.0048 (n=10000)	5e-04 (n=10000)
0.2	0.5	recessive	0.154 (n=5000)	0.1427 (n=10000)	0.00322 (n=9000)	0 (n=8500)

p_{self}	s	h	All benefits	Compensation	Screening	No benefit
0.2	0.5	additive	0.0884 (n=5000)	0.0826 (n=10000)	0.0041 (n=10000)	5e-04 (n=10000)
0.2	1	uniform	0.0808 (n=5000)	0.0672 (n=10000)	0.0055 (n=10000)	4e-04 (n=10000)
0.2	1	recessive	0.1428 (n=5000)	0.1378 (n=10000)	0.00056 (n=9000)	0.00084 (n=9500)
0.2	1	additive	0.0746 (n=5000)	0.0669 (n=10000)	0.0062 (n=10000)	6e-04 (n=10000)
0.4	uniform	uniform	0.0762 (n=5000)	0.0736 (n=10000)	0.004 (n=10000)	2e-04 (n=10000)
0.4	uniform	recessive	0.1598 (n=5000)	0.1534 (n=10000)	0.0086 (n=10000)	0.00021 (n=9500)
0.4	uniform	additive	0.0824 (n=5000)	0.0738 (n=10000)	0.0048 (n=10000)	5e-04 (n=10000)
0.4	0.1	uniform	0.105 (n=5000)	0.109 (n=10000)	0.0017 (n=10000)	1e-04 (n=10000)
0.4	0.1	recessive	0.131 (n=5000)	0.1275 (n=10000)	0.00778 (n=9000)	0 (n=8500)
0.4	0.1	additive	0.1086 (n=5000)	0.103 (n=10000)	0.0028 (n=10000)	5e-04 (n=10000)
0.4	0.5	uniform	0.071 (n=5000)	0.0681 (n=10000)	0.0053 (n=10000)	3e-04 (n=10000)
0.4	0.5	recessive	0.1672 (n=5000)	0.1617 (n=10000)	0.0107 (n=10000)	9e-04 (n=10000)
0.4	0.5	additive	0.0714 (n=5000)	0.0718 (n=10000)	0.0083 (n=10000)	5e-04 (n=10000)
0.4	1	uniform	0.0542 (n=5000)	0.0547 (n=10000)	0.0066 (n=10000)	0 (n=10000)
0.4	1	recessive	0.1468 (n=5000)	0.149 (n=10000)	5e-04 (n=10000)	4e-04 (n=10000)
0.4	1	additive	0.0626 (n=5000)	0.0532 (n=10000)	0.0074 (n=10000)	8e-04 (n=10000)
0.6	uniform	uniform	0.0576 (n=5000)	0.0578 (n=10000)	0.0043 (n=10000)	7e-04 (n=10000)
0.6	uniform	recessive	0.0702 (n=5000)	0.0672 (n=10000)	0.0013 (n=10000)	7e-04 (n=10000)
0.6	uniform	additive	0.0618 (n=5000)	0.058 (n=10000)	0.0057 (n=10000)	5e-04 (n=10000)
0.6	0.1	uniform	0.0886 (n=5000)	0.0917 (n=10000)	0.0021 (n=10000)	3e-04 (n=10000)
0.6	0.1	recessive	0.0938 (n=5000)	0.1021 (n=10000)	2e-04 (n=10000)	5e-04 (n=10000)
0.6	0.1	additive	0.0906 (n=5000)	0.091 (n=10000)	0.0036 (n=10000)	2e-04 (n=10000)

p_{self}	s	h	All benefits	Compensation	Screening	No benefit
0.6	0.5	uniform	0.0546 (n=5000)	0.0512 (n=10000)	0.006 (n=10000)	7e-04 (n=10000)
0.6	0.5	recessive	0.0634 (n=5000)	0.0607 (n=10000)	0.0031 (n=10000)	6e-04 (n=10000)
0.6	0.5	additive	0.0594 (n=5000)	0.0557 (n=10000)	0.006 (n=10000)	2e-04 (n=10000)
0.6	1	uniform	0.0488 (n=5000)	0.0399 (n=10000)	0.0035 (n=10000)	8e-04 (n=10000)
0.6	1	recessive	0.048 (n=5000)	0.0417 (n=10000)	4e-04 (n=10000)	6e-04 (n=10000)
0.6	1	additive	0.0516 (n=5000)	0.0445 (n=10000)	0.0053 (n=10000)	4e-04 (n=10000)
0.8	uniform	uniform	0.0374 (n=5000)	0.0433 (n=10000)	0.0035 (n=10000)	4e-04 (n=10000)
0.8	uniform	recessive	0.042 (n=5000)	0.0455 (n=10000)	0.0023 (n=10000)	2e-04 (n=10000)
0.8	uniform	additive	0.042 (n=5000)	0.0416 (n=10000)	0.0026 (n=10000)	5e-04 (n=10000)
0.8	0.1	uniform	0.0658 (n=5000)	0.0665 (n=10000)	0.0022 (n=10000)	5e-04 (n=10000)
0.8	0.1	recessive	0.0678 (n=5000)	0.0747 (n=10000)	7e-04 (n=10000)	4e-04 (n=10000)
0.8	0.1	additive	0.066 (n=5000)	0.066 (n=10000)	0.0018 (n=10000)	4e-04 (n=10000)
0.8	0.5	uniform	0.0386 (n=5000)	0.0354 (n=10000)	0.0042 (n=10000)	0.0012 (n=10000)
0.8	0.5	recessive	0.0366 (n=5000)	0.0323 (n=10000)	0.0023 (n=10000)	3e-04 (n=10000)
0.8	0.5	additive	0.0404 (n=5000)	0.0375 (n=10000)	0.0034 (n=10000)	1e-04 (n=10000)
0.8	1	uniform	0.0308 (n=5000)	0.0301 (n=10000)	0.0032 (n=10000)	3e-04 (n=10000)
0.8	1	recessive	0.033 (n=5000)	0.0285 (n=10000)	4e-04 (n=10000)	5e-04 (n=10000)
0.8	1	additive	0.0388 (n=5000)	0.0322 (n=10000)	0.0041 (n=10000)	2e-04 (n=10000)
1	uniform	uniform	0.0132 (n=5000)	0.0138 (n=10000)	0.0011 (n=10000)	6e-04 (n=10000)
1	uniform	recessive	0.0102 (n=5000)	0.0072 (n=10000)	8e-04 (n=10000)	2e-04 (n=10000)
1	uniform	additive	0.013 (n=5000)	0.0127 (n=10000)	0.0013 (n=10000)	4e-04 (n=10000)
1	0.1	uniform	0.0242 (n=5000)	0.0223 (n=10000)	4e-04 (n=10000)	2e-04 (n=10000)

$p_{\mathbf{self}}$	s	h	All benefits	Compensation	Screening	No benefit
1	0.1	recessive	0.0214 (n=5000)	0.0235 (n=10000)	9e-04 (n=10000)	8e-04 (n=10000)
1	0.1	additive	0.0196 (n=5000)	0.0151 (n=10000)	5e-04 (n=10000)	3e-04 (n=10000)
1	0.5	uniform	0.0148 (n=5000)	0.0154 (n=10000)	0.0021 (n=10000)	6e-04 (n=10000)
1	0.5	recessive	0.012 (n=5000)	0.0084 (n=10000)	0.0028 (n=10000)	5e-04 (n=10000)
1	0.5	additive	0.0168 (n=5000)	0.0161 (n=10000)	0.0023 (n=10000)	2e-04 (n=10000)
1	1	uniform	0.023 (n=5000)	0.0228 (n=10000)	0.0023 (n=10000)	6e-04 (n=10000)
1	1	recessive	0.0268 (n=5000)	0.0267 (n=10000)	8e-04 (n=10000)	6e-04 (n=10000)
1	1	additive	0.0246 (n=5000)	0.025 (n=10000)	0.0027 (n=10000)	5e-04 (n=10000)

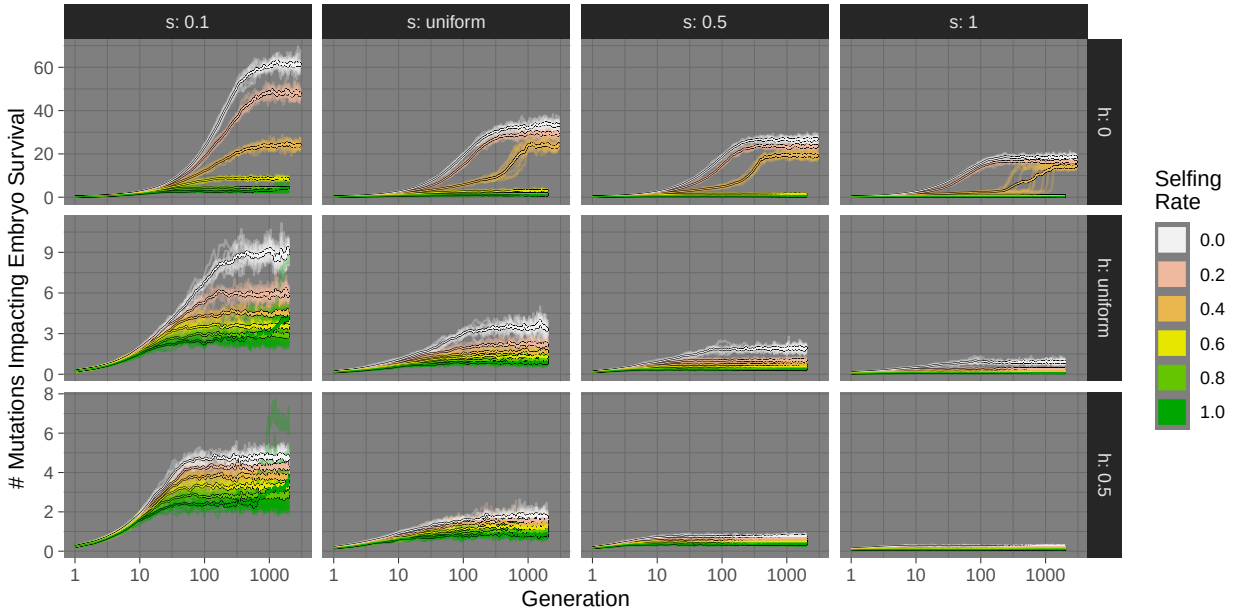

Figure S1: **The accumulation of mutations:** The number of deleterious mutations impacting embryo fitness over time in burn-in simulations, across selective (s) and dominance (h) coefficients, and selfing rates (on the x-axis).

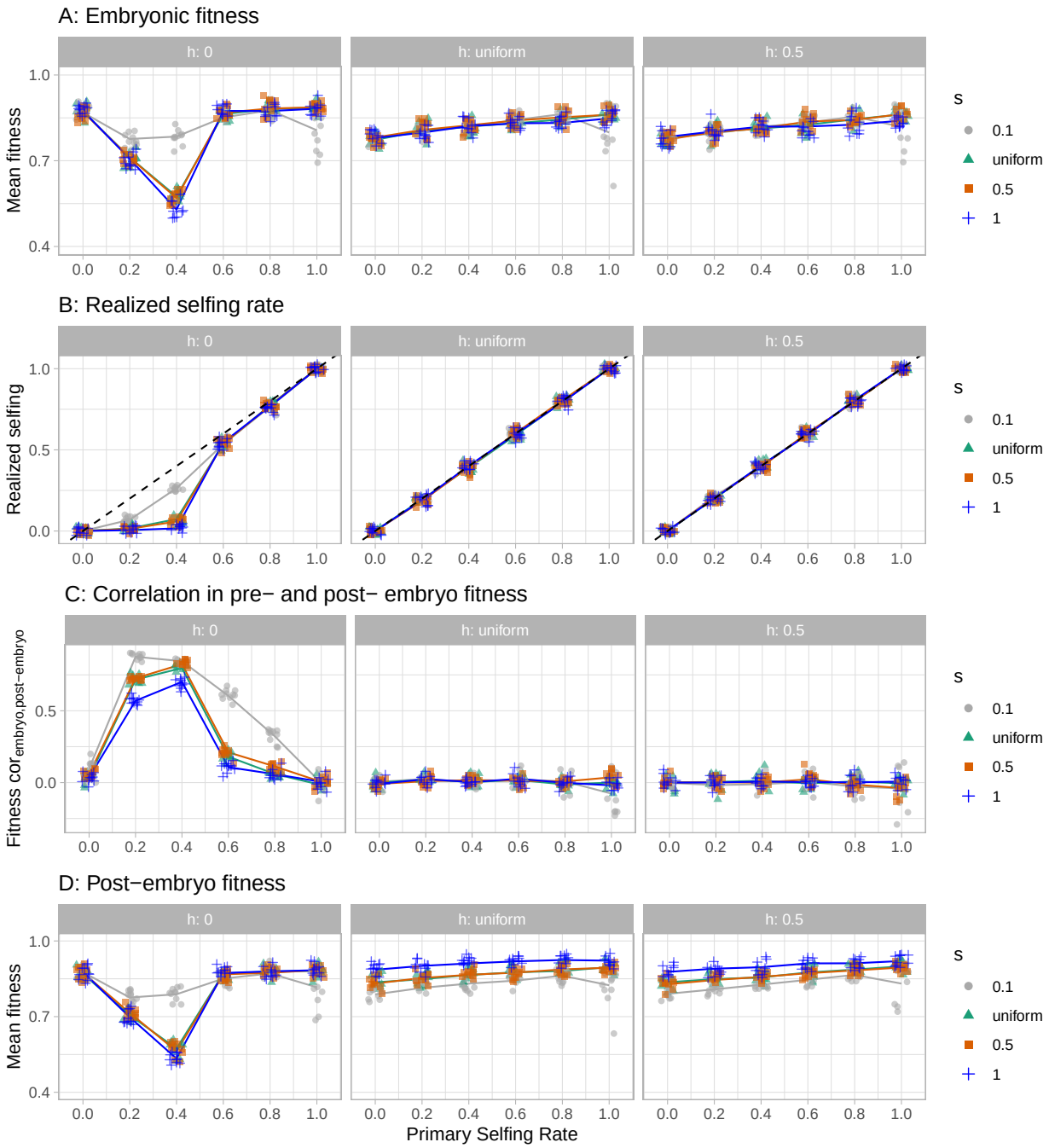

Figure S2: **Summaries of our simulated populations at equilibrium: (A)** The mean embryo fitness, **(B)** Realized selfing rate, **(C)** Correlation between embryo and postembryonic fitness, **(D)** Post-embryo fitness, across selective (s, colors and shapes) and dominance (h, facets) coefficients, and selfing rates (on the x-axis). The number of deleterious mutations impacting embryo fitness over time in burn-in simulations, across selective (s) and dominance (h) coefficients, and selfing rates (on the x-axis).

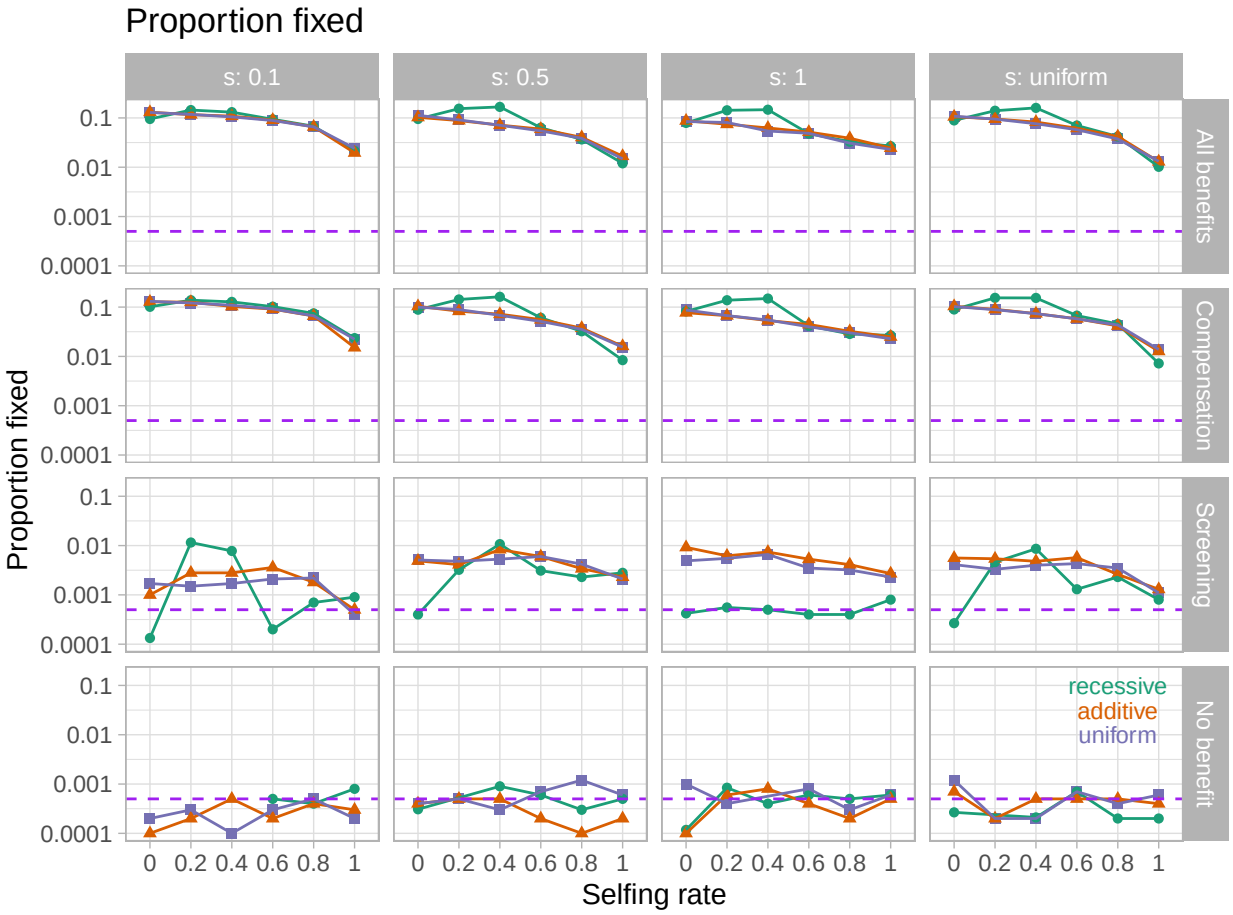

Figure S3: **Proportion of introductions resulting in fixation** as a function of selfing rate (x-axis), the benefit of polyembryony (rows), selection against new mutations (columns), and the dominance of new mutations (color). The purple line denotes neutral expectations. Note that fixation probabilities for additive mutations and those taking their value from a uniform distribution are very similar.

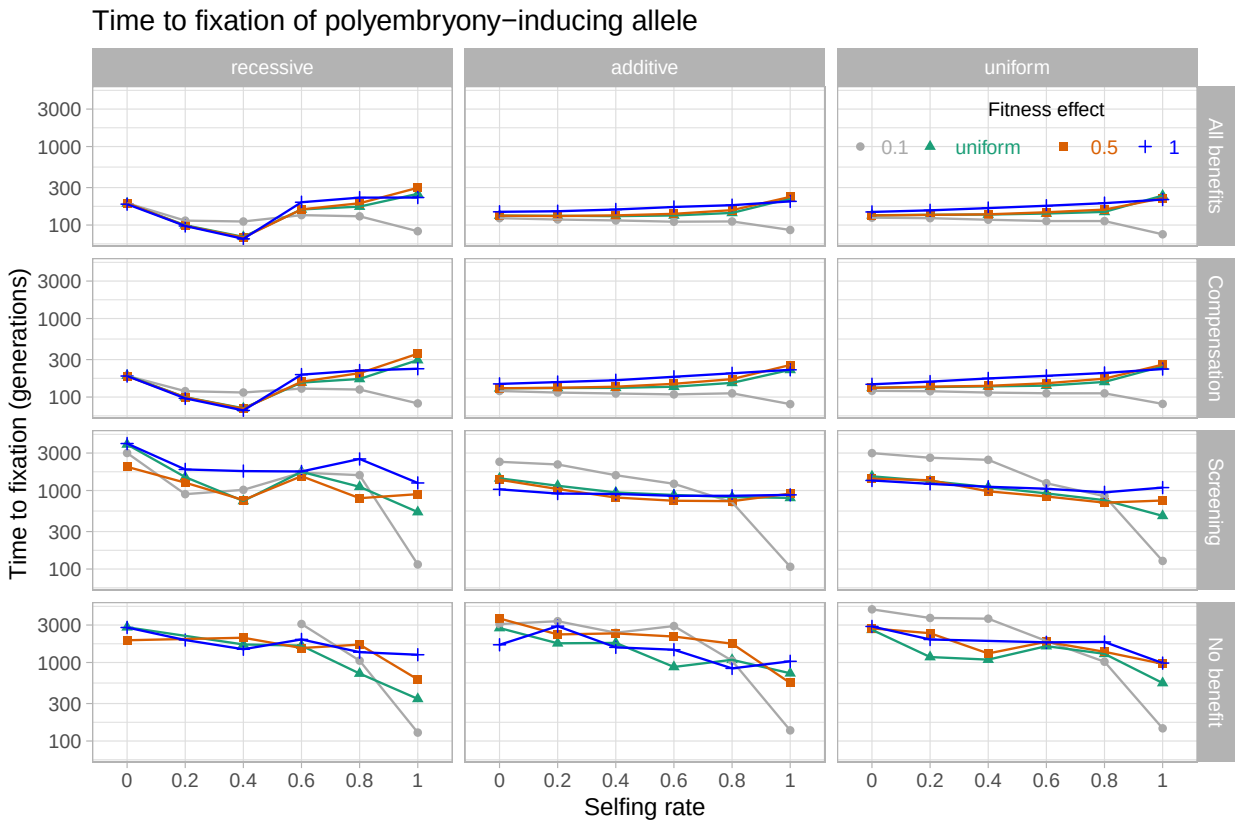

Figure S4: **Mean time to fixation** of the polyembryony allele across selective (s, colors and shapes) and dominance (h, faceted columns) coefficients, and selfing rates (on the x-axis), for each model (faceted rows).

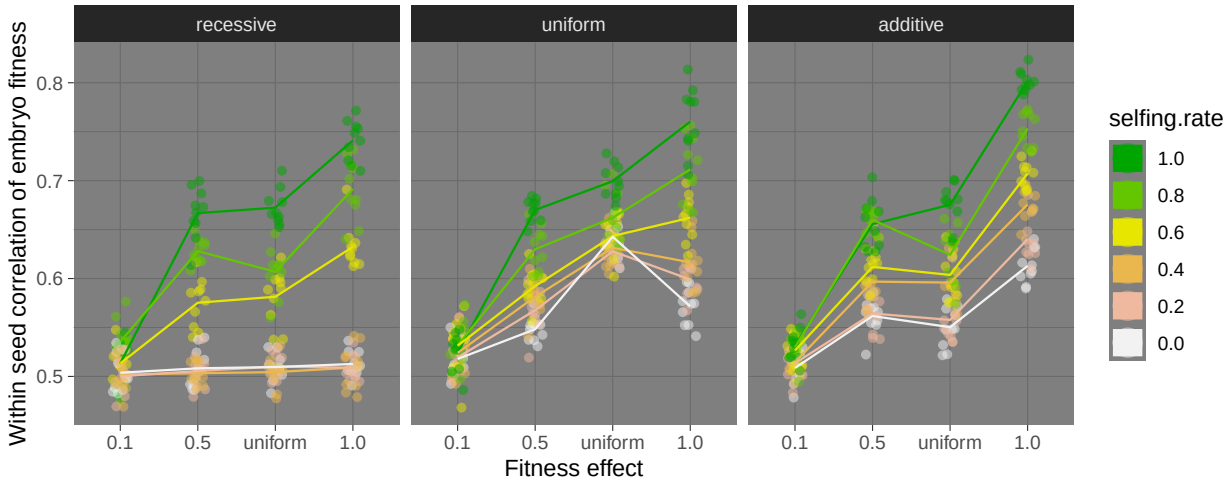

Figure S5: **Correlation in fitness** of the hypothetical two embryos in a seed, before polyembryony evolves as a function of the fitness effect of new mutations (on the x-axis), the selfing rate (color), and the dominance effect of new mutations (columns).

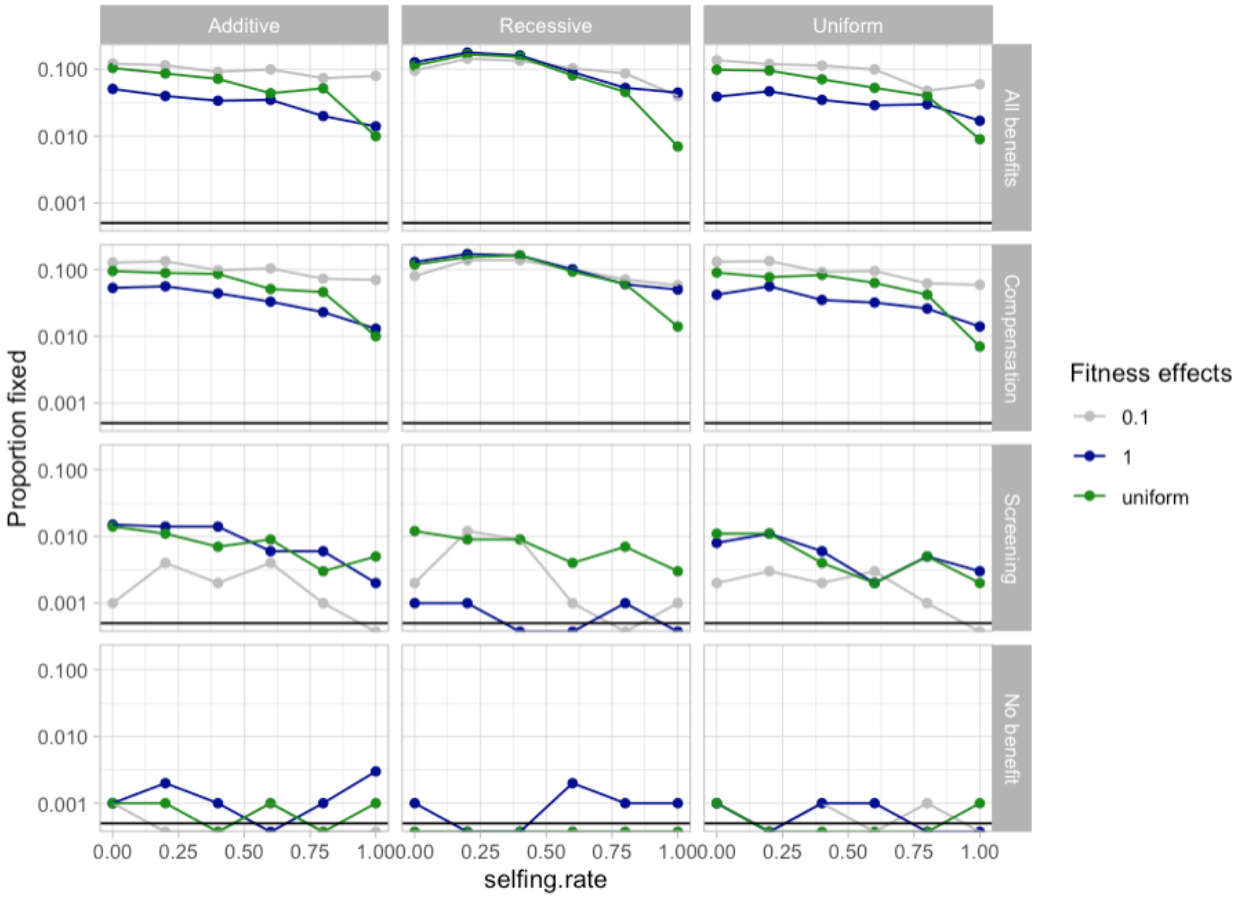

Figure S6: **Proportion of introductions resulting in fixation with complete pleitropy** as a function of selfing rate (x-axis), the benefit of polyembryony (rows), selection against new mutations (columns), and the dominance of new mutations (color). The black line denotes neutral expectations. In this model every mutations impacts both embryonic and post-embryonic fitness in the same way.