Genome-doubling readily occurs in flowering plants (angiosperms) and is linked with speciation as well as major innovations (seeds and flowers). Beneficial traits (increased flower size and number) are often expressed by the resulting polyploid species, making them useful crops as well as increasing their invasive potential. However, after a century of research, the forces that drive polyploid evolution are still unclear. The primary mechanism of formation by gametic nonreduction creates an inherent negative selection pressure by the interaction of triploid sterility and pollen-swamping. I ask whether the combined cost of these emergent properties can be quantified by using an individual-based modelling approach. Specifically, I investigate whether increasing the rate of nonreduction is sufficient to give evolving polyploids enough of an advantage that they can reach stable fixation. Modelling this system revealed that in order to ultimately outcompete their diploid progenitors (as well as the sterile offspring that interploidy matings create), the rate of nonreduction had to exceed the natural rate 27-fold. Extinction probability above this rate was 0.686, and fixation of polyploidy was extremely unstable. The rate of nonreduction required to achieve stable fixation was 83-fold greater than the natural rate. Extinction probability reached 0.719, and the probability of attaining stable fixation was 0.070. These findings support the idea that polyploidy most often leads to evolutionary ‘dead-ends’. They also indicate

that the strength of triploid sterility must be drastically reduced, or some other beneficial adaptation would be required in order for polyploidy to evolve and persist.

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1 Introduction

Polyploidy arises via mechanisms of genome-doubling and describes organisms with more than two sets of chromosomes. No polyploid mammal or bird species have been found, as the condition appears to be fatal in these classes (Svartman et al. 2005; Wertheim et al. 2013). However, polyploidy has been observed in amphibians, fish, fungi, reptiles, and plants (Otto and Whitton 2000). Amongst these, genome-doubling is exceptionally well tolerated in plants; polyploidy is a ubiquitous feature in the evolutionary history of all flowering plants (angiosperms) (Jiao et al. 2011; Wood et al. 2009). Linked, both with speciation and major innovation, this extreme mutation that readily doubles the genomes of angiosperms has been the topic of much research as well as hot debate; why do polyploid lineages evolve and persist? A modelling approach will be used, here, to elucidate some of the main mechanisms at play in this system. In particular, there appears to be inherent selection against the evolution of polyploidy, due to the primary mechanism by which polyploids are formed: gametic nonreduction. During this process, unreduced gametes are produced, which leads to the creation of sterile offspring via pollen interactions known as 'pollen-swamping'. The resulting interplay between these mechanisms is thought to exert strong negative selection against the evolution of polyploidy, and yet it appears to be fundamental in all flowering plants.

1.1 The role of genome-doubling in plant diversification

The ubiquity of polyploidisation in plant lineages suggests that genome-doubling is a critical process in their evolution. Furthermore, polyploidisation often coincides with speciation; 15% of the time in angiosperms and 31% in ferns (Wood et al. 2009). Genome-doubling has even been linked with major biological innovations that led to the diversification of the seed plant (spermatophyte) and angiosperm phyla (Jiao et al. 2011). Seeds and flowers were both crucial in allowing plants to transition from an aquatic life-cycle to a terrestrial one by removing their dependence on water for reproduction. These adaptations are broadly considered as two of the most significant in the history of our planet because they enabled the 'greening' of earth. No wonder, then, that there has been a substantial effort made towards understanding the evolution of polyploidy and the mechanisms by which genome-doubling has been able to drive such adaptations. As a result, polyploidisation has been shown to drastically alter phenotypes, breeding system, and physiology within just a few generations due to the massive genomic alterations that newly created polyploids (neopolyploids) rapidly undergo (Adams and Wendel 2005; Levin 2002; Soltis et al. 2014b). Polyploids often also have larger flowers, seeds and stomata as well as being more robust all-round (Ramsey and Schemske 2002). Because of these attributes, genome-doubling has been crucial in the domestication of many crop plants, for instance;

wheat, maise, sugarcane, coffee, cotton and tobacco (Dubcovsky and Dvorak 2007; Otto and Whitton 2000). However, these same beneficial traits are also linked with the invasive potential of plants (te Beest et al. 2012).

Despite being broadly considered as one of the main driving forces in angiosperm evolution (Barker et al. 2016; Otto 2007; Soltis et al. 2014b), polyploids may actually contribute far less to diversification than their diploid counterparts (Mayrose et al. 2011). While genome-doubling significantly increases speciation rates of diploids, new polyploid lines do not appear to speciate further by that same mechanism and so speciate at a slower rate. Furthermore, polyploid extinction rates are far higher than those of diploids which further reduces their overall speciation rate relative to diploids (Arrigo and Barker 2012; Mayrose et al. 2011). While the validity of these findings has been the topic of some debate (Mayrose et al. 2015; Soltis et al. 2014a), they represent a current shift in opinion; away from the view of polyploidy driving speciation and back to the formerly popular idea that polyploidy most often leads to evolutionary 'dead ends' (Stebbins 1950).

1.2 Polyploid formation in angiosperms

There are multiple routes to producing polyploid offspring; genome-doubling occurs either intraspecifically (autopolyploidy) or via merging of genomes during hybridisation (allopolyploidy). The relative abundance and

rates of the appearance of each type have long been debated (Darlington 1937; Stebbins 1947). In the past, autopolyploids were not recognised as distinct species. They were categorised, instead, as cytotypes of their progenitors, leading to a gross underestimation of their prevalence (Soltis et al. 2007). Understanding of this topic in recent years has improved as new data have accumulated due to improved methods of determining ploidy levels: flow cytometry (Doležel et al. 2007). Both the rates of genome-doubling and the mechanisms by which it occurs are different in autopolyploids and allopolyploids (Ramsey and Schemske 1998). For quantifying the cost of pollen-swamping and triploid sterility, there is no need to include hybridisation in the model, so allopolyploid formation will not be explored here further. Autopolyploids, however, occur via the following routes:

1. Multiple pollen grains fertilising one ovule.
2. Production of entire polyploid shoots (and so also seeds) via somatic-doubling during tissue growth.
3. The production of unreduced gametes caused by aberrations in meiosis.

Of these mechanisms, the production of unreduced gametes is the most common pathway to polyploidy, and the most is known about the rates of polyploidisation via this route (Ramsey and Schemske 1998). With this in mind, gametic-nonreduction is the form of genome-doubling that

this model will include. It occurs because of problems with spindle function or cytokinesis which cause errors during meiosis. When meiosis goes awry in this way, the chromosomes are not reduced by half into two daughter cells. The resulting gametes are unreduced, i.e.:- diploid rather than haploid.

The nonreduction of gametes produces individuals of varying ploidy level with both odd and even-numbered sets of chromosomes. Consider a diploid population; under usual circumstances gametes produced would be haploid and F1 offspring, therefore, would be diploid just like their parents. However, when nonreduction occurs, and diploid gametes enter the system, there will also be fertilisation attempts between haploid and diploid gametes, or diploid and diploid gametes. As a result, offspring ploidy can range from two to four, and the population is now made up of parental diploids, as well as F1 diploids, triploids, and tetraploids. In the generation that follows (F2), ploidy level gets even more diverse due to further nonreduction and increased interploidy pairing of gametes (see figure 1).

1.3 Pollen-swamping and triploid sterility

Neopolyploids are thought to suffer rapid reproductive isolation via post-zygotic barriers because of the production of large numbers of sterile or inviable offspring. Triploids and other odd-numbered ploidy levelled individuals, are often

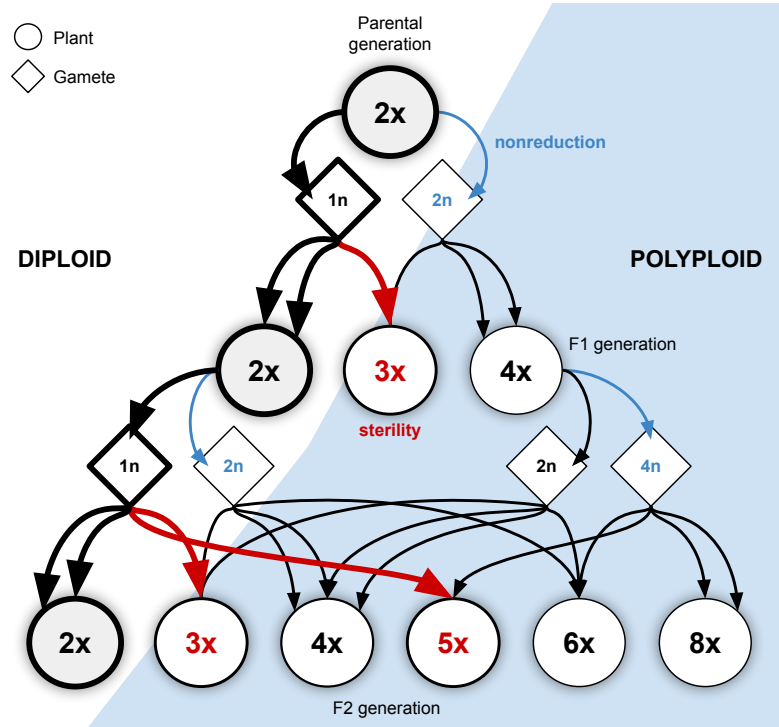

Figure 1. Polyploid formation via gametic nonreduction and pollen-swamping: Base ploidy level (number of sets of chromosomes) of individual plants (cirlces) is denoted 2x (diploid), 3x (tetraploid) and so on. Ploidy level of gametes (diamonds) is denoted with 1n (haploid), 2n (diploid) and so on. Blue arrows show genome-doubling occuring via nonreduction of gametes. Red arrows show the route by which haploid pollen swamps the gametes of polyploids to produce inviable offspring. Fat lines and borders indicate a higher frequency of occurance.

sterile due to developmental defects, genomic instability, a lack of endosperm that reduces seed viability, or because of problems with gametogenesis that produces inviable pollen (Costa et al. 2014; Sonnleitner et al. 2013). The following explanation of pollen-swamping is best digested alongside figure 1, which visualises the gametic interactions that lead to the production of both sterile and viable polyploids. Diploids

(or 2x individuals) produce mostly haploid gametes (or 1n gametes). Diploid gametes (2n) produced via nonreduction occur at a much lower frequency; less than six-in-a-thousand for non-hybrids (Ramsey and Schemske 1998). Because of this abundance of haploid gametes, the frequency of F1 diploid offspring (2x) is highest, followed by sterile triploids (3x), with viable tetraploids (4x) appearing at the lowest frequencies. Most unreduced female gametes that appear find themselves *swamped* by the pollen of diploids and only those that are found by similarly unreduced pollen can be successful in increasing the fitness of either diploid progenitor.

Sticking with figure 1;

1. When reproduction of F1 individuals occurs the chances of producing viable offspring increases, but only minorly. Most of the gametes produced are still haploid (1n), with nonreduction creating 2n gametes like before.
2. There are also now some F1 tetraploids (4x) in the system; they produce 2n gametes under usual circumstances and unreduced 4n gametes at a lower rate.
3. The increase in 2n gametes produced by F1 individuals increases the probability that tetraploids will be produced in F2.
4. Additionally, 2n and 4n gametes produced can combine to create viable hexaploids (6x).
5. However, the majority of F1 gametes in the system are still haploid. Male 1n gametes, therefore, swamp female

gametes of all levels (1n through to 4n). The abundance of haploid gametes inevitably blocks access to other ploidy type pollen so that the majority of offspring produced are sterile triploids (3x) and pentaploids (5x).

Pollen-swamping, therefore, refers to the fitness deficit created by the overloading of female reproductive organs with incompatible pollen (typically haploid pollen created by diploids) which is usually most abundant in an angiosperm system. An example of this process in action has been observed in Spain, where the species distribution of a mixed diploid-hexaploid population of *Mercurialis annua* was disrupted. The diploids there gained such a competitive advantage by swamping neighbouring hexaploid populations with their pollen, that they displaced the hexaploids in multiple regions (Buggs and Pannell 2006). Exactly how strongly pollen-swamping selects against polyploidy, however, is not well known as pollination is difficult to measure in the wild. Finding out exactly how costly pollen-swamping is would help to disentangle how the adaptation of beneficial traits in polyploid lineages might allow them to overcome that cost in order to persist and become established. In other words, where is the bar that these traits need to reach in order to become beneficial?

1.4 The heart of the matter

Given the body of evidence that touts their rapid adaptability, why are so many neopolyploid lines doomed to extinction? Furthermore, under what conditions does polyploidy become advantageous and succeed so that these lineages become established and persist? A population is considered to have become established when the probability of extinction becomes unlikely. Therefore, the establishment phase is a critical period where any new plant population is at higher risk—as such, understanding the cost-benefit mechanisms for any neopolyploid is vital to predicting its chance of survival. The discussion surrounding the advantages and disadvantages of polyploidy is well-reviewed in Comai (2005), Ramsey and Ramsey (2014), and Otto (2007). However, even after a century of research, the factors that drive the success of polyploid establishment in the face of high extinction rates are still unclear.

Previous models offer contradictory predictions about how vital the rate of nonreduction is to polyploid establishment; mathematical models support the idea that it must be unrealistically high and adaptation is required (Felber 1991; Rausch and Morgan 2005), while a more recent, complex individual-based model showed that polyploids needed no adaptation and that natural rates of nonreduction were sufficient for establishment (Oswald and Nuismer 2011). The full picture is still not clear.

Theoretically, if the rate of nonreduction were increased so more 2n gametes were in the mating pool that creates F1 offspring (refer again to figure 1), then the strength of pollen-swamping should be reduced; more 2n gametes will mean more 2n + 2n fertilisation attempts and, therefore, more 4x offspring to balance out the sterile F1 triploids produced. Could modelling a system where the rate of nonreduction is artificially increased, show us how strong the adverse selection created by pollen-swamping really is? How high would the frequency of parental 2n gametes need to be in order for polyploids to gain a reproductive advantage over diploids in the system? Increased chance meetings of unreduced gametes should change the relative diploid-polyploid abundances of the population (see figure 2). If, for instance, there is a pool of 4 gametes where half of them are unreduced, and those unreduced gametes meet, then one viable polyploid and one diploid will be produced. However, if they do not meet then both the offspring produced will be sterile polyploids. This pattern would, of course, be less clear cut with larger pools of gametes, and possible outcomes would increase in diversity if some gametes could go unpaired. An individual-based model, where these interactions can be simulated *in-silico*, would tease out the most probable outcomes over many generations of evolution.

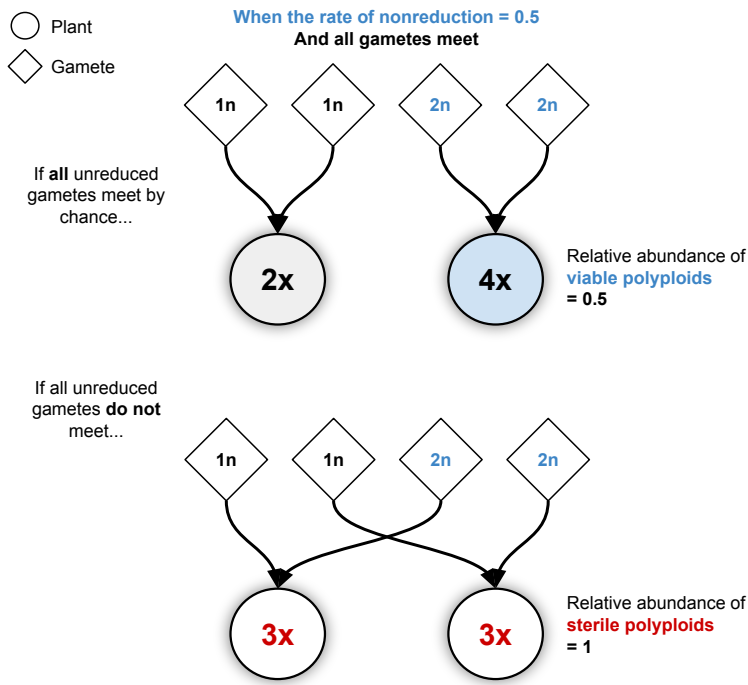

Figure 2. Relative abundance in response to chance and rate: a simplified example of how chance meetings of unreduced or reduced gametes might affect the relative abundance of offspring produced. In this scenario, a pool of half haploid (1n) gametes and half unreduced (2n) gametes produce either half diploid (2x), half viable polyploid (4x) offspring, or completely sterile polyploids (3x).

1.5 Aims

Using an individual-based modelling approach, I will investigate the response of relative polyploid and diploid abundances to changes in the rate of genome-doubling via nonreduction. The model will attempt to elucidate whether increasing the frequency of unreduced gametes in an angiosperm system will give the polyploids which evolve an advantage significant enough to overcome the cost of

pollen-swamping and triploid sterility. As such, the model will run under the assumption that all odd-numbered ploidy individuals (for example, triploids and pentaploids) are sterile. Neopolyploid fitness will, therefore, be negatively impacted due to the interplay between the abundance of haploid pollen and this 'triploid' sterility. I aim to quantify the theoretical cost of pollen-swamping by testing how high the rate of nonreduction would need to be in order to overcome the strength of negative selection which it creates. I ask:

1. **What relative abundance of sterile polyploids will arise with the rate of nonreduction?**
   For F1 offspring, while the frequency of unreduced gametes is far less than that of haploid gametes, the probability of producing sterile offspring should be equal to, or just below, the rate of nonreduction; because every unreduced gamete is most likely to be paired with a haploid one. However, as generations continue and viable polyploids appear, the number of unreduced gametes (2n and above) also increases. As shown in figure 2, this could potentially lead to situations where the relative abundance of sterile polyploid offspring is far greater. Will increasing the rate of nonreduction increase or decrease the relative abundance of sterile polyploids that appear in the system? Besides, what outcome will changes to the relative abundance of sterile individuals have on the system as a whole? Sterile

polyploids will still compete for resources, so booms in their abundance may cause instability in population dynamics. Whether this will be a cost or a boon to the evolution of polyploidy is unclear.

2. **When does the relative abundance of viable polyploid adults exceed half the rate of nonreduction?**
   For every unreduced gamete created, the probability of a viable polyploid being produced increases. The rate of nonreduction and the relative abundance of viable polyploids are, therefore, intrinsically linked. The maximum relative abundance of viable F1 polyploid offspring is half of the rate of nonreduction and can only occur if all unreduced male gametes are matched with unreduced female gametes. For polyploidy to evolve to fixation, the relative abundance must reach 1. Finding the rate of nonreduction at which the relative abundance of viable polyploids exceeds half the rate of nonreduction should show an improtant turning point. The unreduced gametes in the system should have begun to escape the adverse effects of pollen swamping, because when all unreduced gametes meet no sterile polyploids can be produced (see figure 2).
3. **Will increasing the rate of nonreduction lead to the fixation of polyploidy?**
   If the unreduced gametes can begin to escape the effects of pollen-swamping, it follows that the evolving

polyploid population should have a chance, then, to outcompete their diploid progenitors and reach a relative abundance of 1.

4. **Do established and pioneering populations respond in the same way to increased rates of nonreduction?** Pioneering populations of diploids with only small numbers of individuals may be more susceptible to extinction than more established populations. It is possible, therefore, that there is a density-dependent response to increased nonreduction rates. Increased rates are likely to cause instability in the system as relative diploid-polyploid abundances struggle to stabilise. Therefore more extinction in pioneering populations is predicted, and fixation of polyploidy should be more likely when genome-doubling occurs in already established populations.

1.6 The study system

The model that follows was designed to simulate the life cycle of the yellow monkeyflower *Erythranthe gutatta* (formerly: *Mimulus guttatus*) as laid out by Peterson et al. (2016) and detailed in figure 3. *E. guttata* is a widespread, polymorphic herb that is found in a diverse range of habitats (Wu et al. 2008). Because ancient and recent genome-doubling is well documented in the species complex of this popular study organism (Beardsley et al. 2004; Buggs 2012; Simón-Porcar

et al. 2017), *E guttata* was a sensible choice for parameterising the model that follows. Yellow monkeyflowers can be annual or perennial, and figure 3 distinguishes between these modes by using grey arrows for the transitions that only perennials make, to form cloned rosettes (ros) via stolons. The project aims do not require the representation of rosettes in the model. The questions which it hopes to answer are specifically about how the patterns of polyploid evolution change in response to mechanisms of sexual reproduction. Therefore, only the seed (sd) and seedling (sdl) life stages from the life-cycle graph (figure 3) will be included. Peterson et al. (2016) have termed the reproductive life stage of the annuals as seedlings, but this seems confusing, so from here in they will be referred to as 'adults'.

2 Methods

An Individual-based model was created, from scratch, in *R v3.6.1* (R Core Team 2019), as an open-source R package *sploidy v.0.2.2* (McKeon 2020), and is available along with scripts and data on Github. In order to test the hypotheses presented, reproductive isolation of polyploids had to occur in a way that represented the production of sterile offspring via pollen swamping.

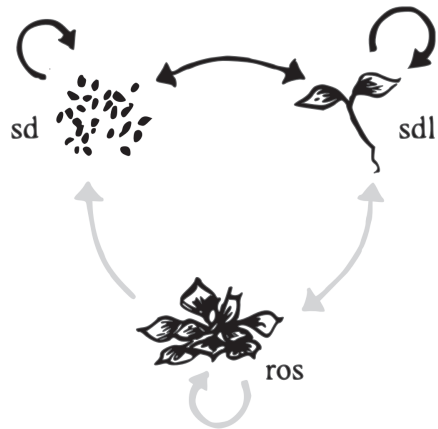

Figure 3. Life cycle of *Erythranthe guttata* (formerly: *Mimulus guttatus*) taken from Peterson et al. (2016). Seedlings (sdl) and rosettes (ros) are both sexually reproductive flowering stages which produce seeds (sd). Annual populations follow the black arrows, whereas perennials also follow grey arrows to form rosettes (asexually produced clones).

2.1 Model flow

From a starting population of seeds and adults, transition probabilities, seed survival probability, and germination rates were used to transition individuals through time, continuously from time t to time $t + 1$ (see figure 4 for a diagram of the model flow). Surviving seeds in time t either germinated to become adults in time $t + 1$ or they remained as seeds. Seed dormancy was limited by a longevity parameter that reduced the seed bank before survival probabilities were applied. Adults in time t became the mating pool for separate transitions involving reproduction, either to seed or straight to new adults in time $t + 1$.

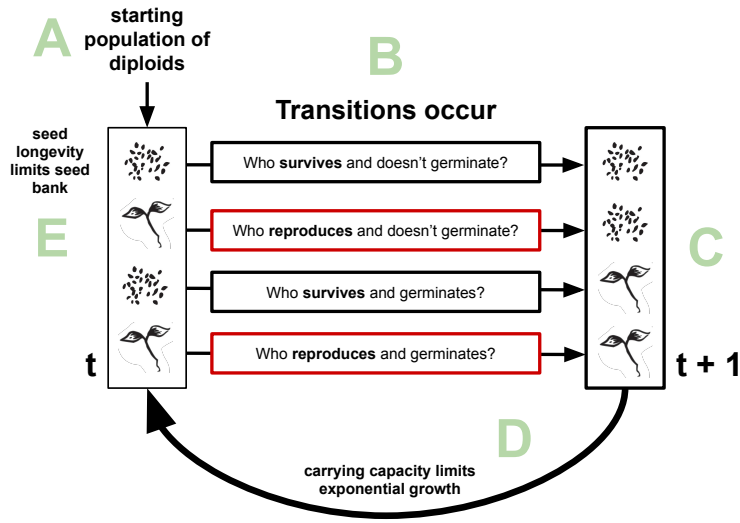

Figure 4. Model flow: From a starting population of diploid seeds and adults (A), transitions involving survival, reproduction and germination probabilities occur (B) to create the individuals present in the next generation (C). This process loops continuously from time *t* to time *t + 1* to simulate the life cycle of *E. gutta*. Carrying capacity limits any possible exponential growth by randomly selecting survivors across the entire landscape at the end of every generation (D). In addition, where seed survival probabilities would lead to generation for a seed bank, the size of this is limited with an enforced maximum longevity before seed survival is applied a the beginning of a new generation (E). Transitions (B) highlighted in red are where the mechanisms of genome-doubling and pollen-swamping occur (for more details see figure 5).

During reproduction (figure 5), sterile adults were ignored, and mothers were sampled first from the adult population. Pollen donors were paired with mothers so that every mother received pollen; sampling was done with replacement, so some plants pollinated multiple mothers while others did not pollinate any. Once all fertilisation attempts were decided, gametic nonreduction occurred at a specified rate, separately to both female and male gametes. The ploidy level of paired

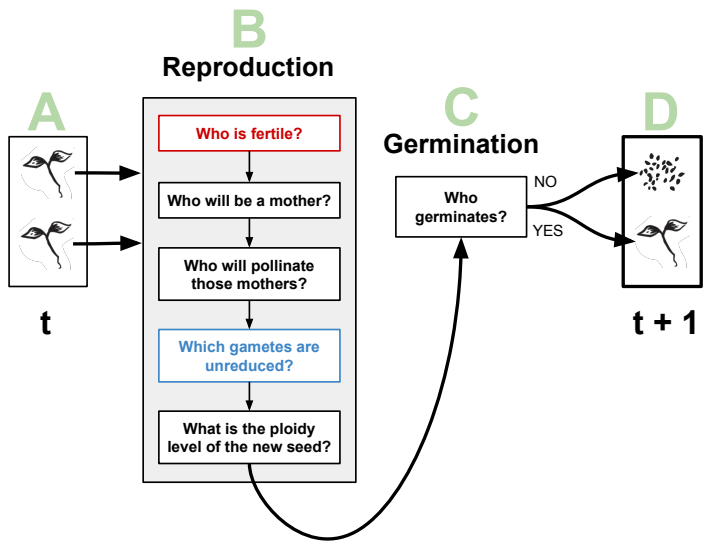

Figure 5. Reproduction and germination: For all transitions from adults in time *t* (A) to seeds or adults in time *t + 1* (D), individuals must go through reproduction (B) and germination (C). Mechanisms of reproduction which induce the effects of pollen-swamping (E) and triploid sterility (F) are highlighted in red, while the process that controls genome-doubling (G) is shown in blue.

gametes was then used to calculate the base ploidy level of the new seed. Those that germinated became adults in time *t + 1*, while those that did not remained as seeds. Once all the transitions were complete, carrying capacity limited any possible exponential growth by randomly selecting survivors from across the entire landscape, and the cycle repeated (figure 4).

2.2 Experiment design

The simulations began with a cohort of diploid individuals that underwent gametic nonreduction; a scenario which

loosely reflected the neo-autopolyploidisation recently discovered to be responsible for a mixed diploid-tetraploid population of *E. guttata* in the Shetland Isles (Simón-Porcar et al. 2017). Transition probabilities remained the same for all simulations (see below), but the rate of nonreduction and the starting population size was varied. Control simulations, without nonreduction, used 100 random starting population sizes between 1 and the carrying capacity of the landscape (K = 100000). Experimental simulations, with nonreduction, used another 1000 random starting population sizes in the same range (1:100000), as well as 1000 random rates of nonreduction between 0 and 0.5. This upper limit was chosen because once more than half of the gametes in the system are unreduced, they will become the most abundant type. Pollen-swamping, therefore, should no longer be in effect—it was not interesting to see if polyploids could evolve when the odds were stacked in their favour with a nonreduction rate above 0.5.

2.3 Transition data

Data for an *E. guttata* population that had stable properties and no clonal growth were used. The data (table 1) and formula (figure 6) used to calculate transitions (table 2) was taken from Peterson et al. (2016). The population chosen was a low-elevation perennial from a year when no rosettes were produced, so the life-stages match those modelled (seeds and

adults). Data existed for the same population in another year when rosettes did occur, so by choosing this population, there was room to build upon the results presented here at a later date.

2.4 Assumptions

- All individuals began as diploid.
- Polyploids arose by chance depending on the rate of nonreduction during all sexually reproductive transitions. The frequency of polyploids that appeared was an emergent property of the interactions between adult gametes which were first sampled randomly, before being paired, and then reduced or left unreduced (see figure 5).
- Even-numbered ploidy individuals were viable and could outcross or self-fertilise.
- Odd-numbered ploidy individuals were sterile and did not contribute to transitions.
- Pollen range spanned the entire landscape.
- All plants were hermaphrodite (in possession of perfect flowers that provided both male and female function), as is most common for *E. guttata* (Wise et al. 2011) and, indeed, all angiosperms; mothers and fathers were chosen via random sampling from the same pool of individuals.
- Seed dispersal spanned the entire landscape.

$$
\begin{array}{c}
 \\
\text{Seed}_{t+1} \\
\text{Seedling}_{t+1} \\
\text{Rosette}_{t+1}
\end{array}
\begin{array}{c}
\begin{array}{ccc}
\text{Seed}_t & \text{Seedling}_t & \text{Rosette}_t
\end{array} \\
\begin{pmatrix}
\text{D}(1-\text{G}) & \text{FOA}(1-\text{G}) & \text{FOA}(1-\text{G}) \\
\text{DG} & \text{FOAG} & \text{FOAG} \\
0 & \text{SR} & \text{SR}
\end{pmatrix}
\end{array}
$$

Figure 6. How the transition matrix (table 2, seedlings renamed as adults) was calculated from the data provided in table 1, according to this formula taken from Peterson et al. (2016).

Table 1. Components of transition: values used to calculate the transition matrix of a low-elevation perennial population of *Erythranthe guttata* (formerly: *Mimulus guttatus*) for a year in which no rosettes were produced. Seed survival (D) is not population specific; taken from Elderd and Doak (2006). All other components taken from Peterson et al. (2016) who recorded values specific to that *E. guttata* population in the year 2013 (N = 77). G = germination rate, O = ovule number per flower, F = flower production, S = winter survival, R = rosette production, A = proportional recruitment success of ovules relative to rosettes.

D	G	O	F	S	R	A
0.534	0.652	494	5.34	0	0	0.00067

Table 2. Transition matrix used for the model, based on the formula shown in figure 6 (adults were renamed from seedling) and the data from figure 1.

	Seed (t)	Adult (t)	Rosette (t)
Seed (t+1)	0.186	0.615	0.615
Adult (t+1)	0.348	1.152	1.152
Rosette (t+1)	0.000	0.000	0.000

- Seed dormancy was disabled to remove any lag in the effects of changing the rate of nonreduction; this fit with the lack of dormancy described for *E. guttata* (Willis 1993).
- Carrying capacity limited any exponential population growth that resulted in adult poulation sizes over 100000. This number was large enough to limit the effects of drift and small enough for processing power to remain efficient.

3 Results

Sterile polyploids were expected to become established at a relative abundance which roughly equalled the rate of nonreduction while this rate was low. However, as the rate of nonreduction was increased up to 0.5 the predictability of this relative abundance was expected to vary, either exceeding the rate of nonreduction or decreasing below it. A relative abundance of viable polyploids which exceeded half the rate of nonreduction was expected to signal a disappearance of sterile polyploids. Therefore, allowing fixation of polyploidy to occur and persist. A density-dependent effect was also expected to reduce chances of polyploidy fixation for pioneering populations.

The data output by the model was in the form of counts, with each row representing one generation of one simulation. Two datasets are analysed here, for; 1) 100 control simulations, and 2) 1000 experimental simulations. For each dataset, columns recorded the counts of individuals by the following subtypes: seeds, adults, diploids, polyploids, sterile polyploids, and viable polyploids. Sterile and viable polyploids were a subdivision of polyploids, and both diploids and polyploids were a subdivision of adults. Counts recorded were the number of individuals at the end of a generation, after all, transitions were completed. These were used to calculate relative abundance for each subtype. In addition, the experimental variables (rate of nonreduction and starting

population size) used for each simulation were recorded in every row, and all simulation instances were separated by identification code.

3.1 General patterns

The pattern of population growth over time when no genome-doubling occurred was consistent and predictable (figure 7). Stability was defined as having a total population that reached carrying capacity (K) and persisted around that point for the entire simulation. Fixation was defined as either diploids or polyploids being the only ploidy type persisting on the landscape. Without nonreduction, whatever the starting population size, the number of adults quickly stabilised in under 15 generations (mean = 3.07, sd = 2.52, N = 100) and persisted in a stable manner for the entire 1000 generations. When nonreduction was included in the experimental simulations, this stability gradually became disrupted as polyploids appeared in the system and began to spread. Broad patterns observed in the results could be split into four categories based on their outcome (figure 8):

1. Stable coexistence of a mixed diploid-polyploid population.
2. Complete extinction of both diploids and polyploids.
3. Unstable fixation of polyploidy.
4. Stable fixation of polyploidy.

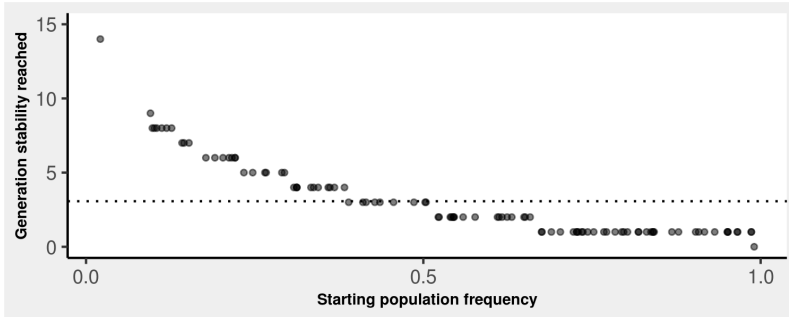

Figure 7. Speed of diploid stabilisation for 100 control simulations: stability was defined as having a total population that reached carrying capacity (K) and persisted around that point for the entire simulation. Each point represents one simulation. Starting population frequency is the number of adults at the beginning of the simulaiton relative to K, making the scale pioneering (close to zero) to established (close to 1).

Diploids never became fixated when nonreduction was enabled, as new polyploids arising from diploid progenitors would always keep appearing in the system. This process did not work the other way around; polyploids did not give rise to diploids.

Below nonreduction at a rate of 0.152, sterile polyploids coexisted with diploids and viable polyploids. All ploidy types became established at varying relative abundances so that the total population stabilised around K. In figure 9 this is visualised over time. Above nonreduction rates of 0.152, the only simulations in which polyploids were able to persist were those where only viable polyploids remained (figures 11 and 12). Increasing nonreduction rate caused booming sterile and viable polyploid populations to monopolise the landscape. Sterile polyploid populations peaked first, quickly followed

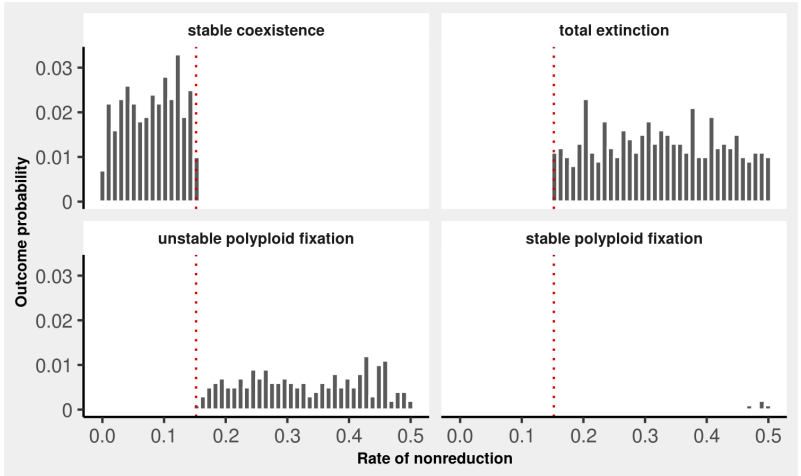

Figure 8. Outcome probability dependent on nonreduction rate: Each bin shows the probability of simulations within that group of nonreduction rates which ended in a particular outcome. Simulations ending with stable coexistence (top left) had a total adult population around the carrying capacity (K) with both diploids and polyploids surviving throughout all 1000 generations. Simulations with total extinction (top right) had neither diploid or polyploids surviving to 1000 generations. Those where the relative abundance of polyploids persisting through to 1000 generations was 1, were considered fixated. Simulations ending in unstable polyploid fixation (bottom left) had polyploid populations of only 1 or 2 individuals. Simulations ending with stable polyploid fixation (bottom right) had an established population around K which survived through to 1000 generations. The dotted red line marks the threshold rate of nonreduction (0.152) which divided outcome probability.

by viable polyploids just a few generations later. Viable polyploids tended to become more abundant than sterile polyploids with their relative abundances being over 0.5. The booming rise of polyploidy (in both sterile and viable forms) disrupted the production and survival of diploids in the system, causing population instability that most frequently led to total extinction (figure 10).

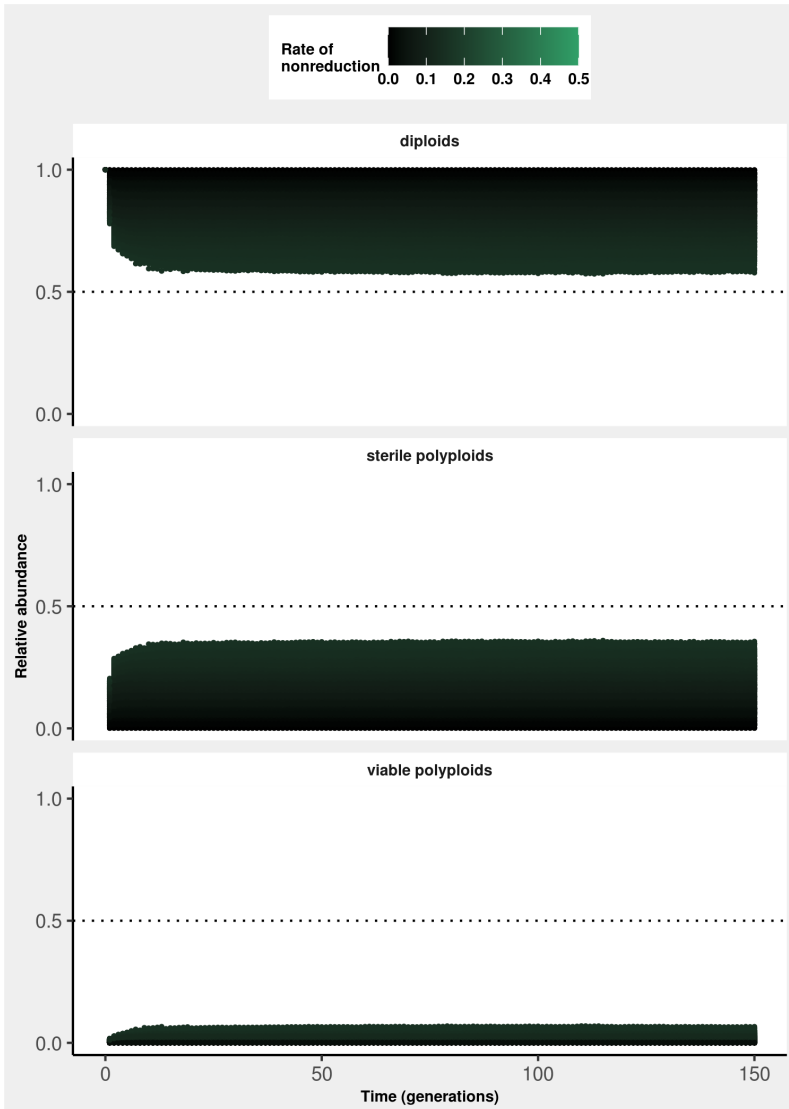

Figure 9. Coexistence: changes in relative diploid-polyploid abundances that culminate in coexistence. Each point represents 1 of 1000 simulations where coexistence occured (N = 337). Points are coloured from black to green based on the rate of nonreduction; simulations that ran with higher rates are brighter green. The dotted black line highlights an abundance of half the population (0.5).

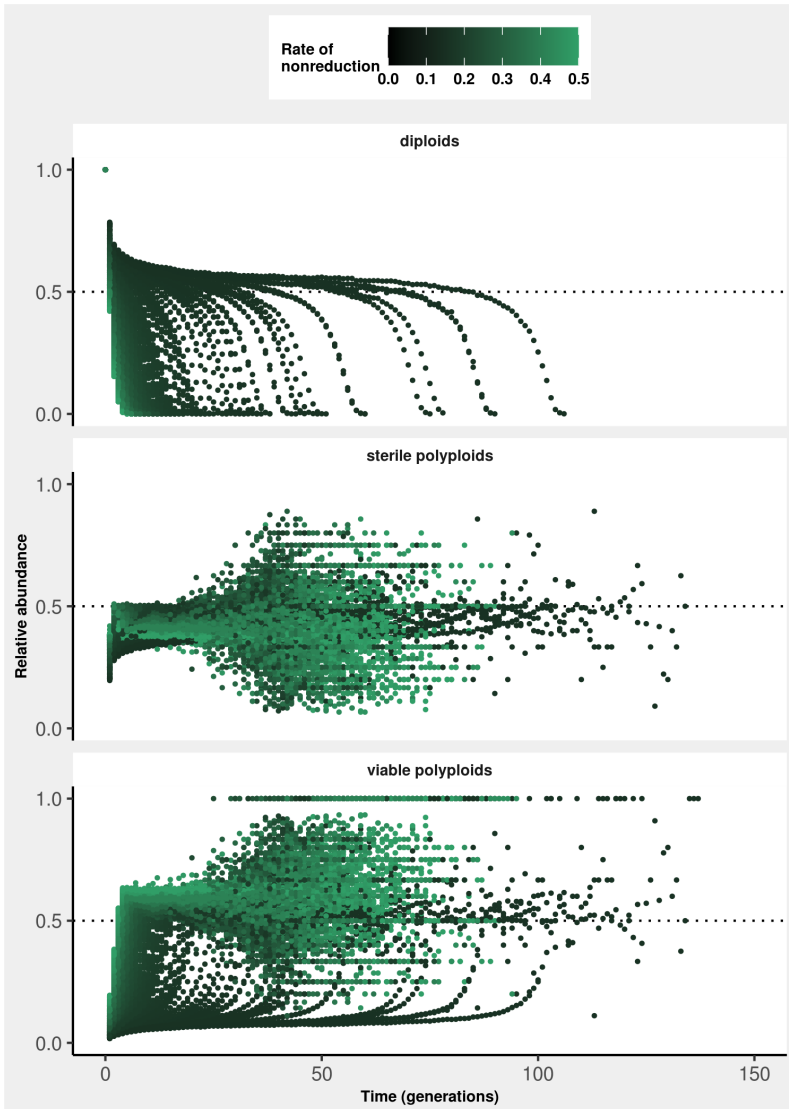

Figure 10. Total extinction: changes in relative diploid-polyploid abundances that culminate in extinction. Each point represents 1 of 1000 simulations where total extinction occured (N = 455). Points are coloured from black to green based on the rate of nonreduction; simulations that ran with higher rates are brighter green. The dotted black line highlights an abundance of half the population (0.5).

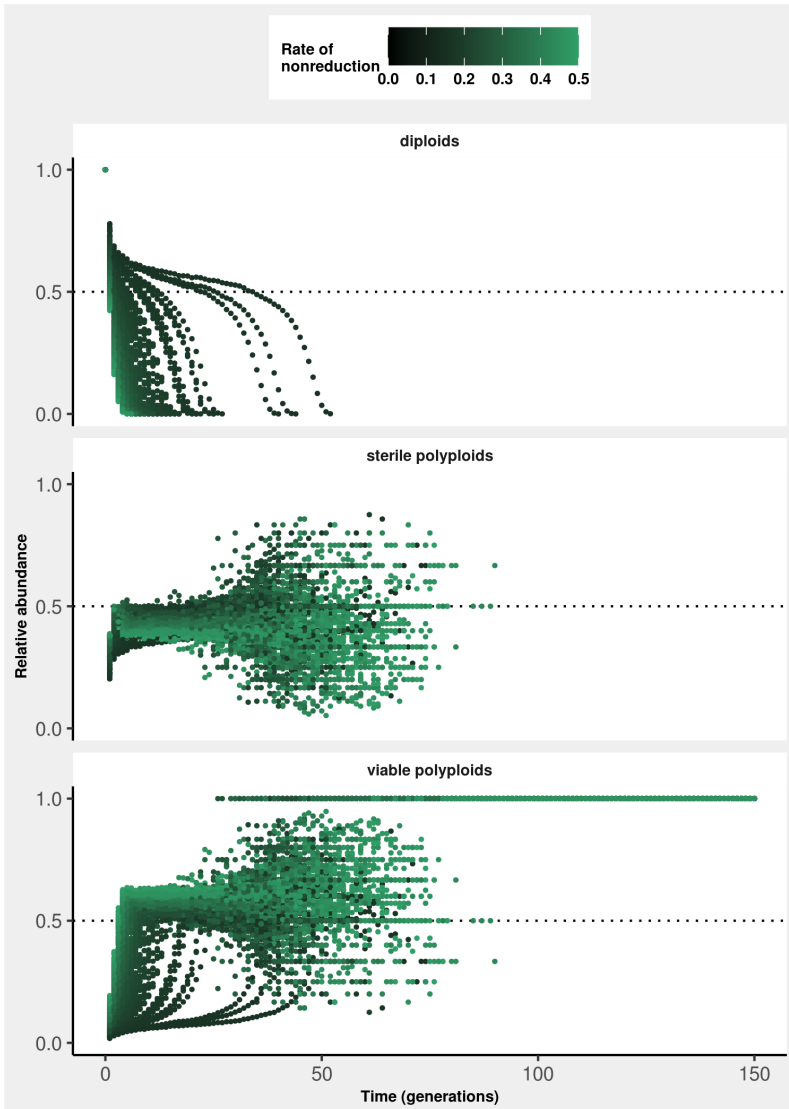

Figure 11. Unstable polyploid fixation: changes in relative diploid-polyploid abundances that culminate in unstable polyploid fixation. Each point represents 1 of 1000 simulations where unstable polyploid fixation occured (N = 204). Points are coloured from black to green based on the rate of nonreduction; simulations that ran with higher rates are brighter green. The dotted black line highlights an abundance of half the population (0.5).

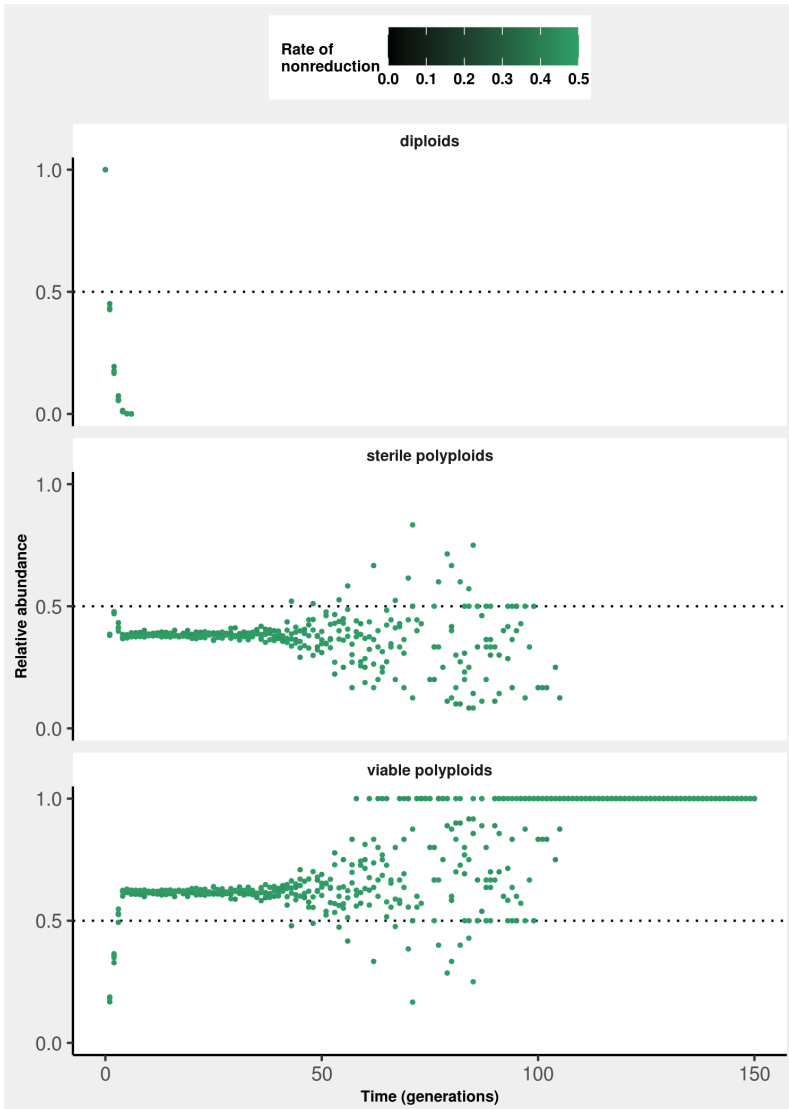

Figure 12. Stable polyploid fixation: changes in relative diploid-polyploid abundances that culminate in stable polyploid fixation. Each point represents 1 of 1000 simulations where stable polyploid fixation occured (N = 4). Points are coloured from black to green based on the rate of nonreduction; simulations that ran with higher rates are brighter green. The dotted black line highlights an abundance of half the population (0.5).

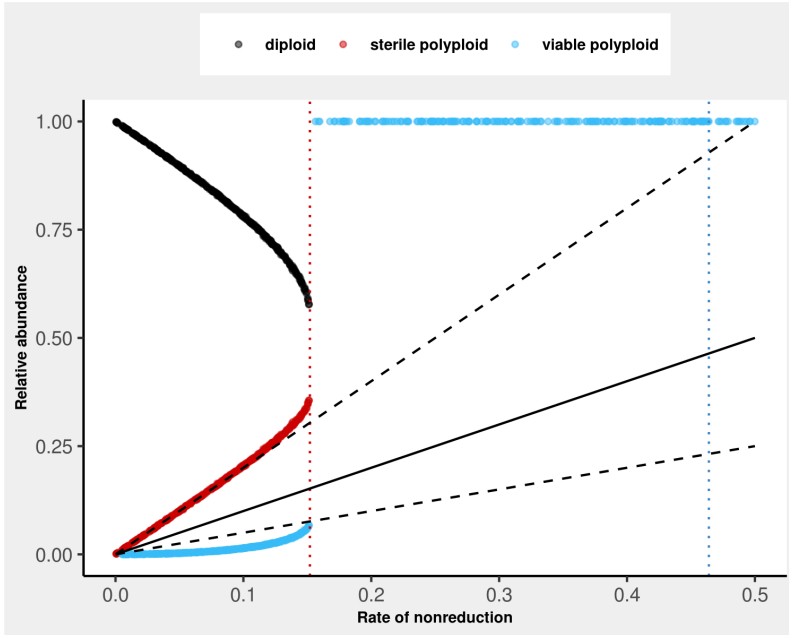

Figure 13. Relative abundance in repsonse to the rate of nonreduction: Points represent relative abundance of sterile polyploids (red) or viable polyploids (blue) persisting in the population at generation 1000. Each point represents one of the 545 simulations which avoided total extinction out of 1000 instances. The solid black line shows where relative abundance equals the rate of nonreduction, the black dashed line below marks an abundance equal to half the rate, and the black dashed line above marks one of double. The dotted red line marks the threshold rate of nonreduction (0.152) where the probable outcome of simulations changed. The dotted blue line marks the rate above which the fixated viable polyploids were established in a stable manner around the carrying capacity (0.464).

3.2 Sterile and viable polyploids

At low rates of nonreduction, sterile polyploids were expected to become established at a relative abundance which roughly equalled the rate of nonreduction. Their abundance was consistently double that. Figure 13 shows how the established relative abundances of diploids and polyploids (taken from populations which reached the end of the simulations) related

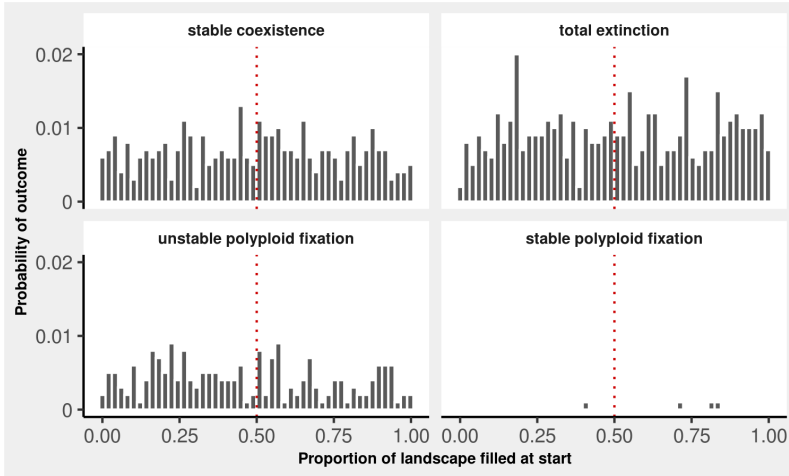

Figure 14. Outcome probability depending on starting population size: Each bin shows the probability of simulations within that group ending in a particular outcome. The dotted red line marks the halfway point between pioneering (close to 0) and established (close to 1) starting populations. Simulations ending with stable coexistence (top left) had a total adult population around the carrying capacity (100000) with both diploids and polyploids surviving throughout all 1000 generations. Simulations with total extinction (top right) had neither diploid or adults surviving to 1000 generations. Those where the relative abundance of polyploids persisting through to 1000 generations was 1, were considered fixated. Simulations ending in unstable polyploid fixation (bottom left) had polyploid populations of only 1 or 2 individuals. Simulations ending with stable polyploid fixation (bottom right) had a total adult population around the carrying capacity (100000) which survived through to 1000 generations.

to the rate of nonreduction. As the rate of nonreduction was increased, the relative abundance of sterile polyploids was expected to exceed the rate of nonreduction or decrease below it. As rate was increased, the relative abundance of established sterile polyploids began to climb steeply until the rate reached the threshold of 0.152. After this point, sterile polyploids no longer became established in any simulations which did not result in extinction. However, figures 10 and 11 show that

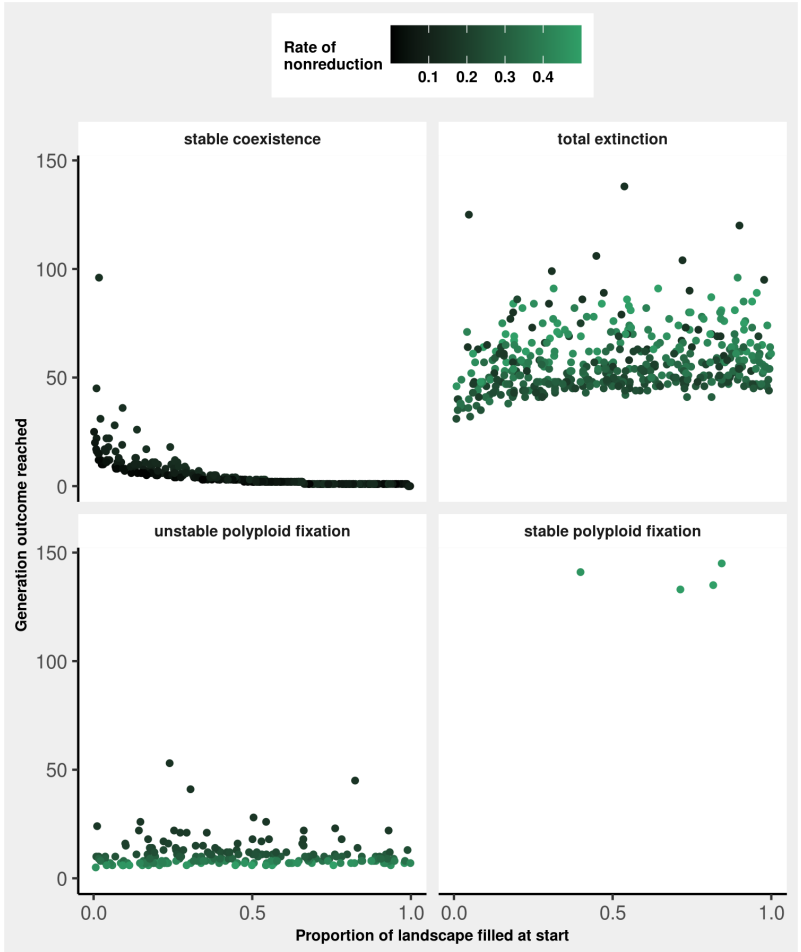

Figure 15. Speed of outcome depending on starting population size: each point represents one of 1000 simulations, divided into panels by the outcome observed; stable coexistence (top left), total extinction (top right), unstable polyploid fixation (bottom left), and stable polyploid fixation (bottom right). Stability was defined as having a total population frequency that reached 1 and persisted around that point for the entire simulation. Total extinction meant that neither diploids or polyploid survived. Unstable polyploid fixation describes simulations where diploids went extinct but the remaining polyploids persisted at very small population sizes (1 or 2 individuals). Points are coloured by the rate of nonreduction; low rates are black but as the rate increases the points become green.

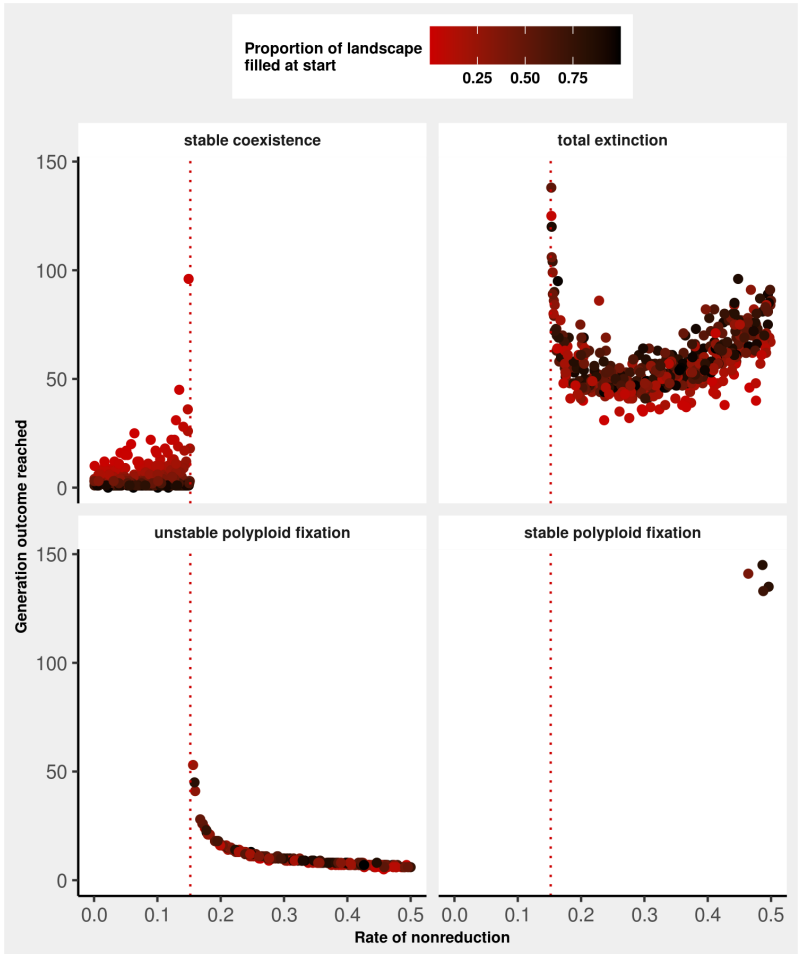

Figure 16. Speed of outcome depending on rate of nonreduction: each point represents one of 1000 simulations, divided into panels by the outcome observed; stable coexistence (top left), total extinction (top right), unstable polyploid fixation (bottom left), and stable polyploid fixation (bottom right). Stability was defined as having a total population frequency that reached 1 and persisted around that point for the entire simulation. Total extinction meant that neither diploids or polyploid survived. Unstable polyploid fixation describes simulations where diploids went extinct but the remaining polyploids persisted at very small population sizes (1 or 2 individuals). Points are coloured by starting population frequency; well established starting populations are black but as the frequency of individuals within them reduces the points become red so that the smallest pioneering populations are the brightest. The dotted red line marks the threshold rate of nonreduction (0.152) which divided outcome probability.

there were points during population instability where sterile polyploids frequently accounted for around three-quarters of the population.

A relative abundance of viable polyploids exceeding half the rate of nonreduction was expected to signal a disappearance of sterile polyploids and allow fixation of polyploidy to persist. Figure 13 shows that this was true. Under a rate of 0.152 very low relative abundances of viable polyploids become established in the system. As the rate of nonreduction increased, however, the shape of this relationship began to look exponential. Viable polyploids became established at relative abundances increasingly closer to half the rate of nonreduction. As the shape of this relationship reached a steep curve and looked set to hit the mark of half the rate, instability ensued: coexistence was no longer achieved (sterile polyploids and diploids no longer became established). Instead, there was only extinction or polyploid fixation.

3.3 Fixation of polyploidy

Fixation of polyploidy took one of two routes; 1) stable, or; 2) unstable. The pattern of demography change over time for these simulations was very similar (see figures 11 and 12). There was a period of polyploid growth where the relative abundance of viable polyploids exceeded that of the sterile polyploids, alongside a decline in diploids. Then a period where sterile and viable polyploids compete as viable

polyploids increase in abundance until they account for 100% of the population. Fixation never occurred below a nonreduction rate of 0.152 (figure 13). Over that rate, there was a 31.4% chance of polyploid fixation and a 0.6% per cent chance that this would be stable. At a rate of 0.464, the chance of achieving stable fixation increased to 7.0%, but the chance of unstable fixation dropped to 21.1%.

3.4 Response of pioneering versus established diploid populations

None of the outcomes (coexistence, extinction, or fixation) was more probable for either pioneering or established starting populations. Figure 14 shows that roughly even numbers of simulations fall on either side of this scale for all outcomes. A minor density-dependent effect was seen in the speed with which simulations reached their fate; small starting populations took longer to stabilise or went extinct more quickly. Figures 15 and 16 incorporate a time component; generation at which the outcome (coexistence, extinction, or fixation) was reached. Together, they show that the speed of achieving coexistence was influenced more by starting population size than by nonreduction rate; pioneering populations took longer to stabilise as the populations were further away from K. The converse was true for unstable fixation which was influenced by nonreduction rate rather than starting population size; the speed of reaching fixation

above rates of 0.152 increased with rate no matter whether starting populations were pioneering or established. Total extinction was affected by both; rate influenced the speed of extinction most, but established populations buffered against this somewhat and delayed the effect.

4 Discussion

The model results have shown that stable coexistence between diploids and polyploids is easily achievable despite the reproductive barriers which triploid sterility and pollen-swamping create. The estimated rate of actual nonreduction in non-hybrid angiosperms is 0.0056 (Ramsey and Schemske 1998), well below the threshold rate where coexistence in this model became impossible. The flip side of this is that, based on these results, the natural rate is also far too low for polyploidy to reach fixation without some other force coming into play. The model showed how critical the relative abundance of viable polyploids was to achieving fixation of polyploidy, and how the booming populations of sterile polyploids interfered with the diploids in the population even more than the polyploids; the relative abundance of diploids declined in response to the competition from booms of sterile polyploids.

In effect, the production of sterile offspring appeared to mediate the transition from a state of coexistence to one where polyploid fixation was possible. By competing with the

abundant diploids for resources and reducing their numbers, the sterile population made the chance meeting of rare (unreduced) gametes more probable, by making them less rare. The mechanism by which polyploids are formed (nonreduction) both creates the effect of pollen-swamping and then also removes it. The rates at which this happened here were much higher than the nonreduction estimated for autopolyploids. However, the frequency of unreduced gametes in allopolyploids is thought to be around 50-fold greater (Ramsey and Schemske 1998). This rate of nonreduction (0.275) falls quickly with the range where we saw a reasonable chance of polyploid fixation. Even so, the type of fixation most often observed within that range was always unstable; only minimal numbers of polyploids persisted despite achieving fixation. In reality, populations of this size would be incredibly sensitive to environmental stochasticity; extinction would be likely.

If polyploids tend to become extinct at the establishment phase, as suggested by Arrigo and Barker (2012), then it follows that traits which increase reproductive assurance should lead to mitigation of extinction risk. Perhaps this is why the polyploids we see established in nature exhibit traits like increased body size, flower size, and number. As well as increasing potential fecundity, these traits should increase the probability of self-pollination between flowers on the same individual (de Jong et al. 1993; Karron et al. 2004); further

assuring reproductive output. In a system where there is a method of fragmenting the mating pool, polyploids could avoid swamping from incompatible pollen via self-fertilisation. In the model presented here, under the assumption that pollen range encompasses the entire landscape, the ability to self over not-self would not increase the chance of self gametes meeting. Pollen range would need to be reduced, or some compatibility assortment (such as flower timing or pollinator specificity) would need to evolve in order for selfing to be advantageous. In natural populations of *E. guttata*, flowering time variation is broad and has a genetic basis (Monnahan and Kelly 2017). Could this be a mechanism of fragmenting the fertile population? Furthermore, can variation in flowering time, or other compatibility assorting traits, cause an increased chance of polyploidy fixation? Or, will these mechanisms be equally likely to evolve in diploids too?

4.1 Evolution of compatibility assortment

The increased size and stature of autopolyploids prolongs their development and leads to delayed flowering. This delay could cause reproductive isolation by altering pollinator specificity or increasing selfing probability (Ramsey and Ramsey 2014). While these effects may result in inbreeding and a loss of mates, rather than a cost, delayed flowering could also be thought of as a strategy to escape pollen swamping.

If the model presented here were expanded so that the parental pool–which is sampled randomly to create seeds–was further subdivided depending on ploidy level, then fertilisation attempts would only be carried out between compatible individuals. Furthermore, the production of sterile offspring should be significantly reduced in this scenario. Consider a neotetraploid with delayed flowering; this individuals 2n gametes would no longer meet with haploid ones from the diploid population it arose in. Even its unreduced 4n gametes would not result in wasted effort, because the possible pairings would produce even-numbered ploidy offspring: 2n + 4n = 6x (hexaploid), or 4n + 4n = 8x (octoploid). Individuals could be given an inheritable trait value that denotes flowering time instead, to create more natural variation. Under these conditions, simulations could be used to test whether delayed flowering was more likely to evolve in polyploid or diploid lineages.

4.2 Selfing

Where diploid *M. annua* displaced hexaploid relatives via pollen-swamping, selfing only minorly allowed the hexaploids to mitigate these effects. Escaping pollen-swamping in *M. annua*, via selfing, was density-dependent and slight (Buggs and Pannell 2006). Various mechanisms (of morphology, genetics or flower sex and timing) attempt to control self-incompatibility in angiosperms in order to limit the

amount of inbreeding that can occur via self-pollination (Silva and Goring 2001). If, or once, flowering time variation evolved in the model, to fragment the mating pool, then the ability to self may present polyploids with a further advantage.

According to Ramsey and Ramsey (2014), self-incompatibility has been broadly expected to occur more in polyploids, an idea first proposed by Stebbins (1950) as a route by which polyploids could alleviate issues of reproductive isolation. Additionally, the idea that multiple gene copies are thought to buffer against the deleterious effects of inbreeding depression has bolstered this theory (Husband and Schemske 1997) and increased its popularity. There is some empirical evidence for increased selfing in angiosperm tetraploids (Cook and Soltis 2000; Husband and Schemske 1997), but a general pattern of association between polyploidy and selfing has not been found across a wide range of taxa (Mable 2004). A possible explanation for the lack of a more general association could be down to the probability of selfing in polyploidy being inherently linked with flowers. Remember how autopolyploids produce more numerous and larger flowers? Moreover, how this increases the probability of selfing?

Variation in life-history strategy has been observed in *E. guttata*; two populations in Oregon were mostly outcrossing with fluctuating partial selfing (Willis 1993). Could there be an association between the ability to self and the probability

of stable fixation of polyploidy? Self-fertilisation could be restricted in the model to investigate this concept further. A trait which modifies the probability of selfing could be included to add the possibility of variation in selfing to evolve. The model could then be used to test whether selfing evolves in polyploids alongside flowering time variation, or whether it is dependent on that variation preceding it.

4.3 Triploid sterility

Because triploids and other odd-numbered ploidy levelled individuals, are *often* sterile, the model assumed complete triploid sterility for simplicity. The mechanisms by which triploid sterility appears are developmental defects that stop triploids even being produced, genomic instability between unevenly matched sets of chromosomes that cannot pair effectively, problems with gametogenesis that produces inviable pollen, or a lack of endosperm which is essential for seed development (Costa et al. 2014; Sonnleitner et al. 2013). Based on this, the model made two assumptions regarding triploid sterility that could be improved:

1. All fertilisation attempts had an equal probability of success in producing offspring.
   Gamete pairings that would produce triploids or other odd-numbered ploidy offspring would have the same chance of transitioning via germination as any other seed in the system, but this is not strictly true. Interploidy

seed development is often disrupted so that the probability of producing viable triploid seeds is reduced. This reduction in seed output is termed the 'triploid block' (Marks 1966; Ramsey and Schemske 1998).

2. All odd-numbered ploidy individuals were completely sterile.

   In reality, this is not always strictly the case either; the reproductive potential of triploids can vary between species, by ploidy level, or by parental genome contribution. For example; the hybrid offspring of *E. guttata* and *Erythranthe peregrina* (formerly: *Mimulus peregrinus*) are sterile triploids with little potential for gene-flow (Buggs 2012), as are hybrids between *E. guttata* and *Erythranthe lutea* (Vallejo-Marin 2012). In contrast, Costa et al. (2014) show that odd-numbered ploidy types with more set of chromosomes (greater than 3) have reduced sterility; the extra gene contribution to endosperm made the seed production of pentaploids more effective than that of triploids. Furthermore, if the unreduced (larger) gamete is the maternal one, so there is a ratio of 2:1 maternal:paternal contribution of endosperm genes, then viable offspring can also be readily produced by triploids (Lin 1984).

4.4 Diploidisation

All neopolyploids enter a period of diploidisation, by which duplicated genes are discriminantly lost. This process occurs via various mechanisms, over many generations, until a state of diploidy has returned (Comai 2005). The model presented here included no rate or mechanism for this process. However, the diploidisation of neopolyploids would alter the mating dynamics in the system; relative abundances of reduced and unreduced gametes would change over time as polyploid lineages experienced this genetically reductive phase. Understanding how diploidisation alters the patterns of viable polyploid fixation and sterile polyploid abundance could further elucidate the real cost of pollen-swamping. Reduction of ploidy type towards diploid should constrain the diversity of ploidy types and increase the number of reduced gametes in the system. Would this increase the cost of pollen-swamping, and make the probability that unreduced gametes meet even more unlikely?

4.5 Evolution of the rate itself

Mutation rates are known to evolve (**?**). The rates of nonreduction vary between species (Ramsey and Schemske 1998), and as the mechanisms of nonreduction are based on developmental defects in meiotic machinery, they are also likely to evolve. However, as the rate of nonreduction adds a negative pressure to the survival and reproduction of diploids,

not just arising polyploids, this would theoretically constrain the rate. Higher rates of nonreduction in diploids are unlikely to evolve if they also reduce diploid fitness. Nevertheless, any polyploids which persist–either by chance or adaptation–would likely express higher rates of nonreduction, because the chance of fixation increased with rate alone. Successfully passing this trait on to viable offspring would pull the rate antagonistically upwards while the success of diploids would constrain it.

4.6 Observations in the wild

Even in the best-studied angiosperm systems, integration between genomic data and its ecological context has been lacking; this has hindered our ability to fully understand the driving forces in the evolution polyploidy (Soltis et al. 2016). In order to elucidate the mechanisms that may be at play (such as evolution of the nonreduction rate, self-incompatibility, delayed flowering or increased germination probability), much work is being done to find and describe wild neopolyploid populations (Ferrero et al. 2015; Schlaepfer et al. 2008; Simón-Porcar et al. 2017). The processes of adaptation can be better examined now that recent polyploidisation has been found in the wild. To date, much of the data has had the accumulation of genetic change over large spans of evolutionary history clouding the picture. Wild study populations will better inform us of the ecological

factors (such as; climate or predation pressure) which may be integral to understanding the fate neopolyploid lineages.

5 Conclusion

Increasing the frequency of unreduced gametes in the system modelled did give evolving neopolyploids enough of an advantage to overcome the combined cost of triploid sterility and pollen-swamping. In order for them to fully overcome the strength of negative selection working against them, the rate of nonreduction needed to be 83-fold greater than what we see in nature. At this high rate of nonreduction, between 0.464 and 0.5 there was a 7.0% chance that polyploidy would evolve to stable fixation, a 21.1% chance of unstable fixation, and a 71.9% chance of total extinction, where both diploid progenitors and evolving polyploids were eradicated. Evolving neopolyploids could also become fixated at tiny population sizes where one or two individuals would persist. Unstable fixation only occurred when the rate was 27-fold greater than what we see in nature. At these nonreduction rates between 0.152 and 0.464, there was 31.7% chance of polyploidy becoming fixated and a 68.3% chance of total extinction. Established populations suffered no less extinction than pioneering ones, though pioneering populations did reach their fate more quickly when they were doomed to failure.

The relative abundances of diploids, sterile polyploids and viable polyploids followed distinct trends depending on the

rate. Between nonreduction rates of 0 and 0.152, stable coexistence between all ploidy types was achieved 100% of the time. Diploids predominated, followed by sterile polyploids, with viable polyploids persisting at the lowest relative abundances. As the rate was increased through that range, the relative abundances of sterile and viable polyploids increased until both diploid and sterile polyploid abundances were close to 0.5, with diploids just over and sterile polyploids just under. Viable polyploids, at this point, were on the cusp of reaching a relative abundance that matched half the rate of nonreduction (0.076). Once that cusp was reached, sterile polyploid and diploid relative abundances collided around 0.5 and 100% of the populations were sent into a period of instability. During this turbulent phase, diploid abundance declined 100% of the time, with greater speed as the rate of nonreduction was increased. High relative abundances of sterile polyploids persisted around and over 0.5 long after diploid decline. Therefore, there was substantial sterile polyploid generation from the viable polyploid population due to interploidy fertilisation attempts between incompatible gametes. However, in all instances, viable polyploid abundance tended to be just above that of the sterile polyploids. Whether or not they managed to escape the production of sterile polyploids in order to become fixated was entirely down to chance.

When rare gametes meet, evolving polyploids can readily establish and persist at low relative abundances alongside their diploid progenitors, without any need for adaptation. However, these results also indicate that if polyploids did evolve such beneficial traits in order that they could become more abundant, this scenario should most often lead to evolutionary 'dead-ends'. Viable polyploids attempting to reach establishment were almost always wiped out by chance because of the constant production of sterile offspring. In order for that not to be the case, the strength of triploid sterility in the system would need to be far less. If this was true, the struggle between viable and sterile polyploids on the road to fixation should be far less in the hands of chance. These results do not show that explicitly, but further work could be done to model variation in the strength of triploid sterility. The idea that this might improve the chances of polyploid fixation could then be tested in a system that reflects some of the ways that triploid sterility has been shown to vary in nature.

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