Phenotypic Effects of Somatic Mutations Accumulating during Vegetative Growth

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Abstract

The unique life form of plants promotes the accumulation of large numbers of somatic mutations that can be passed on to offspring in the next generation. However, rates of mutation accumulation remain similar in plants and animals. The solution to this paradox may be that plants have the potential to filter somatic mutations prior to seed dispersal via three forms of intra-generation selection. To provide an experimental test of this hypothesis, we used plants of *Mimulus guttatus* to compare the performance of offspring from self-pollinations made within the same flower (autogamy), to offspring from self-pollinations between stems on the same plant (geitonogamy). The fitness effects of somatic mutations are expected to differ between progeny from these crosses, as autogamy will result in homozygosity of a proportion of somatic mutations, but geitonogamous progeny will remain heterozygous. We developed a novel analytical approach to test whether the homozygous effects of somatic mutations were evident from differences in the means and variances of fitness between autogamous and geitonogamous progeny. Consistent with our predictions, the variance in progeny fitness among stems was significantly greater for autogamous compared to geitonogamous progeny from the same stem. Surprisingly, several autogamous progeny groups displayed increased fitness compared to geitonogamous controls, indicating that the effects of beneficial somatic mutations were prevalent in progeny. Phenotypic effects of somatic mutations were also evident from the positive association between mean fitness and the within-group variance in fitness for autogamous progeny. These results support the hypothesis that somatic mutations accumulate during vegetative growth, but they are filtered by the different forms of intra-generation selection, resulting in the culling of expressed deleterious mutations and the retention of beneficial mutations.

Impact Summary

Plants do not have a germline that is separate from their soma. Consequently, plants have the potential to generate large numbers of somatic mutations as they grow. However, rates of mutation accumulation in plants are generally similar to animals. We provide evidence that selection occurring on clonal cell lineages filters mutations and results in a disproportionately high frequency of beneficial mutations being passed on to the next generation. This is the first report indicating that somatic mutations accumulating during vegetative growth can affect the fitness of offspring in the next generation.
Introduction

Mutation is the source of variation for evolution and adaptation, but organisms differ in whether mutations originating during gamete formation (meiosis) or somatic growth (mitosis) contribute to heritable variation. For the vast the majority of organisms, including viruses, unicellular microbes, and some multicellular eukaryotes, mutations occur only during cell replication, so they primarily exhibit clonal evolution. By contrast, genetic modification occurring during somatic growth of animals is usually limited to the appearance of acquired mutations and the development of cancer (Nowell 1976; Beerenwinkel et al. 2015), neither of which contributes to heritable variation in populations. Plants differ from animals and microbes, because mutations contributing to heritable variation can arise during gamete formation and somatic growth. This is due to the fact that plants lack separate germ lines; the same germ cells in apical meristems are responsible for vegetative growth and sexual reproduction. Consequently, plants have the capacity for clonal evolution, as somatic mutations that occur in plant meristems can accumulate and be passed to the next generation (McKnight et al. 2002; Klekowski 2003; Schultz and Scofield 2009; Bobiwash et al. 2013; Dubrovina and Kiselev 2016; Watson et al. 2016; Schmid-Siegert et al. 2017). This aspect of plant biology is well known (Monro and Poore 2009; Ally et al. 2010; Reusch and Bostrom 2011), and somatic mutation accumulation has been important in agriculture, where the origin of many clonally-derived varieties of fruits, including citrus, apples, and wine grapes, have been cultivated by grafting from genetically differentiated bud tips (Aradhya et al. 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli et al. 2012; Jarni et al. 2015; Pelsy et al. 2015).

However, there is disagreement over the degree and biological importance of somatic mutation accumulation in plants (Schultz and Scofield 2009; Burian et al. 2016; Watson et al. 2016; Schmid-Siegert et al. 2017).

Clonal evolution occurs as individual cell lineages accumulate mutations during mitotic cell division (Elena and Lenski 2003; Greaves and Maley 2012; Long et al. 2015). Like mutations occurring during meiosis, somatic mutations are expected to be deleterious more often than beneficial, but the actual distribution of fitness effects of mutations (or their dominance levels) is generally unknown (Orr 2010). In diploids, new somatic mutations occur as a single copy within the genome, so their immediate fitness effects will depend on the combination of their selection coefficient \( s \) and their expression in the heterozygous state (dominance; \( h \)). It is difficult to assess the effects of somatic mutations, because clonal lineages lack recombination, and the phenotypic effects of beneficial mutations are confounded by the co-occurrence of detrimental genetic variants (clonal interference; Long et al. 2015). Similarly, although sexual reproduction does allow mutations to segregate among progeny, it is difficult to detect...
the phenotypic effects of novel mutations in eukaryotes with separate sexes, because the genomes of two individuals are combined in offspring (Long et al. 2015). In this study, we make crosses within clones of hermaphroditic plants to assess the phenotypic effects of novel mutations accumulating during somatic growth.

Plants have a number of advantages over other organisms for the study of clonal evolution. Separate stems on the same plant consist of multiple clonal cell lineages that are derived from the same zygote but may differ for mutations that have accumulated during stem elongation. To produce recombinant progeny segregating for somatic mutations unique to each stem, crosses can be made either within the same flower (autogamy) or between flowers on separate stems on the same plant (geitonogamy; Fig. 1). These crosses are both self-fertilizations, but they differ in the complement of somatic mutations that are unique to each stem. Progeny from autogamous crosses will segregate for the mutations that accumulated within a single stem, while the progeny from geitonogamous crosses will segregate for mutations from both stems. Thus, the average effects of mutations accumulating during stem growth can be evaluated by comparing the average fitness of progeny generated by autogamous and geitonogamous crosses. The potential for somatic mutations to accumulate during vegetative growth, and the ability to study their effects among recombinant progeny, make plants an attractive model for the study of fundamental processes of clonal evolution.

Plants grow from the division of a population of meristem cells within the stem tip that is known as the central zone. These cell lineages go on to produce future stem, leaf, and reproductive tissues (flowers). Competition for space and resources among cell lineages within the central zone can lead to clonal evolution during stem growth. Lineages that carry expressed deleterious mutations may have slower rates of division and can be eliminated as they are replaced by cell lineages displaying more rapid growth (Fig. 2). Similarly, expressed beneficial mutations will tend to be retained and accumulate during stem growth as these cell lineages outcompete others. In addition, plants may accumulate somatic mutations that are recessive and/or neutral with respect to fitness. Thus, as plant stems grow, they can accumulate unique complements of neutral, beneficial, and deleterious mutations, and they will become differentiated from other stems on the same plant.

When we consider the potential for meiotic and somatic mutations to contribute to the total mutational load of plant populations – particularly for long-lived plants – it becomes evident that not all of the mutations occurring during a plant’s lifespan are passed on to the next generation (Fig. 3). First, cell lineage selection will filter mutations displaying some degree of dominance, which will tend to increase the relative frequency of beneficial mutations in the next generation. In addition, some
proportion of recessive deleterious mutations will be eliminated during the haploid life stage due to pollen tube attrition and pollen competition (gametophytic selection; Mulcahy 1979; Cruzan 1989; Mable and Otto 1998; Armbruster and Rogers 2004; Arunkumar et al. 2013; Harder et al. 2016). A portion of deleterious mutations also will be homozygous in zygotes, which can lead to higher rates of seed and fruit abortion (Selective Embryo Abortion; Husband and Schemske 1995; Korbecka et al. 2002). Indeed, the effects of deleterious somatic mutations often are apparent as higher rates of embryo abortion after autogamous compared to geitonogamous pollinations, which is referred to as autogamy depression (Schultz and Scofield 2009; Bobiwash et al. 2013). In contrast, mutations that result in faster growth of stem cells will be favored by cell lineage selection and will have an elevated chance of being inherited. The sum effects of this intra-generation selection, which includes cell lineage selection, gametophytic selection, and selective embryo abortion, may result in non-random complements of mutations entering the next generation.

In this study, we use autogamous and geitonogamous self-pollinations to estimate the effects of somatic mutations segregating in offspring of Mimulus guttatus DC (Erythranthe guttata G.L. Nesom; Phrymaceae). Populations of M. guttatus display a wide range of life histories and mating systems – from highly selfing annuals to herbaceous perennials that outcross to varying degrees (Wu et al. 2008). In two separate experiments, we characterize the fitness effects of somatic mutations that accumulated during vegetative growth of perennial, self-compatible plants of M. guttatus that were subjected to novel environments. We made autogamous and geitonogamous self-pollinations across multiple stems and asked whether there was evidence for autogamy depression for seed set and embryo abortion. In addition, we estimated the fitness effects of somatic mutations segregating in progeny, which to our knowledge, has not been done before. We begin by describing our analytical approach in greater detail, followed by providing specifics of the two experiments. We show that somatic mutations accumulate during stem growth, as progeny from autogamous crosses have greater variance in mean fitness compared to progeny from geitonogamous crosses. We further reveal a disproportionate effect of beneficial mutations in some stems, which indicates that intra-generation selection may be filtering mutations to modify the distribution of fitness effects transmitted to progeny. The results from these experiments challenge widely accepted notions concerning mutation accumulation during vegetative growth and the distribution of fitness effects of mutations in plant populations.

**Approach**
While previous studies have detected autogamy depression expressed as seed and fruit abortion (reviewed in Bobiwash et al. 2013), no earlier work has evaluated the effects of somatic mutations on the fitness of progeny in the next generation. This is surprising, because somatic mutations affecting levels of embryo abortion also could have effects on the fitness of progeny. We use a comparative approach to evaluate the fitness consequences of somatic mutations that are unique to each stem.

For a diploid plant, we can assume that somatic mutations ($a \rightarrow a'$) will be in the heterozygous state when they first arise, so they will segregate in self-fertilized, autogamous progeny as 25% homozygous ($a'a'$), 50% heterozygous ($aa'$), and 25% the original homozygote ($aa$). By contrast, because progeny from geitonogamous crosses contain mutations that arose in separate stems, half of their progeny will be carrying unique mutations in the heterozygous state, and homozygotes for mutations that arose in a single stem will be absent. The difference in fitness of autogamous and geitonogamous progeny will depend on the selection coefficient ($s$) and the dominance ($h$) of somatic mutations unique to each stem and allows us to estimate the fitness effects of somatic mutations. For a single mutation, if we assume that the relative fitness ($w$) of the original homozygote ($aa$) is 1, then the heterozygote would have fitness $1+hs$, and individuals homozygous for the mutation would have fitness $1+s$ ($s$ ranges between -1 and 1, and $h$ ranges between 0 and 1).

If recessive or partially dominant somatic mutations (i.e., $h < 1.0$) accumulate during stem growth, we can use the contrast in fitness between autogamous and geitonogamous progeny to estimate the selection coefficient ($s$) associated with the mutation. Assuming a single mutation, and following the segregation ratios and fitnesses described above, the average fitness of autogamous progeny ($w_A$) due to a single mutation would be the weighted average of the three genotypes ($aa, aa', and a'a'$): $w_A = 0.25 + 0.5(1+hs) + 0.25(1+s)$. For geitonogamous progeny, the fitness effects of a single mutation in one stem would be $w_G = 0.5 + 0.5(1+hs)$. However, note that geitonogamy combines mutations that occur in two stems, so the fitness of progeny from geitonogamous crosses would be affected by the combination of mutations that have expression in the heterozygous state from both stems. Regardless, contrasting the fitness of progeny arising from these two cross types provides a close approximation of the fitness effects of somatic mutations that are unique to a stem, which we denote as $s \approx (w_A - w_G)$.

While the above calculations are for a single mutation, it is possible that multiple mutations are generated during mitosis in meristem cells during vegetative growth that are subsequently transmitted to progeny. Consequently, the fitness of offspring from autogamous crosses may be affected by the combination of mutations that arose within that stem, which would obscure the effects of individual
mutations. Nevertheless, we can assess the average effects of \( n \) somatic mutations unique to an individual stem. This is simply the difference in fitness between the autogamous and geitonogamous progeny from a single stem: \( \bar{s}_k = \bar{w}_k(A) - \bar{w}_k(G) \), where \( \bar{w}_k(A) \) and \( \bar{w}_k(G) \) are the average relative fitnesses of the autogamous and geitonogamous progeny from stem \( k \), respectively. Provided that environmental effects are controlled for with a common garden design, the parameter \( \bar{s}_k \) represents the combined effects of one or more mutations that have different magnitudes and/or directions of effects on fitness and serves as an indicator of the average fitness effects of all expressed somatic mutations that accumulated during vegetative growth.

In addition, even though autogamy and geitonogamy are both self-pollinations, as long as mutations do not have complete expression in heterozygotes (i.e. \( h < 1.0 \)), then the variance in fitness should be greater for autogamous progeny groups than for geitonogamous progeny groups from the same stem. Therefore, we can generate an independent estimate of \( s \) based on the variance in the fitness of autogamous offspring (Appendix 1). Somatic mutations that are unique to stems will have different effects on the variance in fitness among autogamous progeny depending on the strength of selection (\( s \)), their expression in the heterozygous state (\( h \)), and the number of mutations that accumulated (\( n \)). To evaluate these effects, we began by considering a single somatic mutation (\( n = 1 \)) occurring in a stem with genotype \( aa' \) that segregated in offspring in a 1:2:1 ratio. We simulated the fitness of autogamous progeny for different values of \( s \) and \( h \), such that fitness of the \( aa, aa' \), and \( a'a' \) genotypes was 1, 1+\( hs \), and 1+\( s \), respectively (\( s \) ranges from -1 to 1 and \( h \) ranges from 0 to 1). The outcome of a multiple regression analysis demonstrated that the large majority of variance in fitness (as measured by the standard deviation, SD) could be explained by the selection coefficient rather than dominance level (Appendix 1; Fig. S1). Moreover, the ratio between estimates of SD and \( s \) remained constant for different numbers of mutations (\( n \)) segregating among autogamous progeny (i.e. \( s \) scales linearly with SD), which allowed us to estimate the selective effects of mutations based on the SD as \( s_{SD} = c_{n,N}SD \), where \( c_{n,N} \) is a constant based on the number of independent mutations of equal effect (\( n \)) and the number of progeny sampled from a single autogamous fruit (\( N \)). For one to four mutations occurring in a stem, values of \( c \) range from 2.0 to \(~5.0\), based on sampling between 4 and 256 autogamous progeny (Appendix 1; Fig. S2).

From the considerations above, we can make specific predictions about the fitness effects of somatic mutations that occur during stem growth and are passed on to offspring. If individual stems are characterized by unique mutations, autogamous progeny groups should have greater variance in fitness among stems compared to geitonogamous progeny from the same stems, because autogamous progeny...
will be homozygous for each of these mutations 25% of the time. Consequently, we can make an
approximate estimate of the selection coefficient based on the average effect of mutations by
comparing the fitness of autogamous and geitonogamous progeny from the same stem. Moreover, if
intra-generation selection has the potential to filter mutations, we expect that a non-random subset of
the accumulated mutations will be passed on to progeny. For example, cell lineage selection may
eliminate deleterious mutations that have effects on cell growth rates, but only if they are expressed in
the heterozygous state. In contrast, beneficial mutations would be more likely to be retained if they are
expressed in the heterozygous state. While beneficial mutations are generally considered to be rare,
they may be detectable in autogamous progeny if mutation rates are high and if cell lineage selection is
effective.

In addition to having an effect on the mean fitness, the presence of somatic mutations also will
increase the variance in fitness of autogamous progeny. Consequently, the second prediction we can
make is that the variance in fitness among progeny from single fruits produced by autogamous
pollination should be positively associated with the average fitness effects of somatic mutations unique
to each stem (Appendix 1). In general, we predict that if somatic mutations with fitness effects are
accumulating during vegetative growth, then the variance in fitness of autogamous progeny from
different stems would be greater than for geitonogamous progeny. Below, we test these predictions
using two different experiments in *Mimulus guttatus*.

First Experiment

**Methods** – This experiment originally was designed to test the effects of somatic mutations on
seed set and ovule abortion (autogamy depression; Schultz and Scofield 2009; Bobiwash et al. 2013), so
we used multiple plants from several populations to obtain a large number of fruits. We grew plants of
*M. guttatus* in the Research Greenhouse facility at Portland State University from seed collected in July
2013 from three different populations in northern Oregon (Jackson Bottom Wetlands – JB: 45.501794 N,
-122.98776 W; and two from Saddle Mountain – SMB: 45.9861 N, -123.6859 W, and SMC: 45.9634 N, -
123.6837 W). We assumed that greenhouse conditions were different enough from field environments
to provide a novel selection regime for clonal evolution during vegetative growth. In August 2013, seeds
were cold stratified on moist paper towels at 2°C for 30 days prior to being sown in soil. Seedlings were
transplanted to pots (approximately 10 x 10 x 12 cm) and grown for seven months before the
application of pollination treatments. Temperature was maintained between 21–26°C during the day,
and 15–21°C at night. Supplemental HID lights ran for 12 hours a day when the seedlings first emerged, and 14 hours a day during adult growth.

After plants became established and began producing multiple stems, we conducted autogamous and geitonogamous self-pollinations using flowers on several stems (15 to 20 cm in length) from two plants from each of four maternal families representing each of the three populations. Flowers from pairs of stems on individual plants were reciprocally crossed (geitonogamy), or individual flowers from these same stems were self-pollinated (autogamy). A total of 139 pollinations were conducted across two treatments: limited (pollen was applied to stigmas with one touch from a plastic pipette tip) or excess (where the stigma surface was coated with pollen). Pollinations were conducted on 12 different days (pollination date) over several weeks in July 2014. Mature fruits were collected and placed individually into paper envelopes, and their contents were examined under a Leica MZ-16 stereoscope. The first 100 ovules from each fruit were categorized as filled seeds (brown, almond-shaped), unfertilized ovules (small, flattened and light-colored), or aborted (larger than unfertilized, dark-colored, shriveled). Unfertilized and aborted ovules were flattened and appeared to lack endosperm and were not used in germination tests. Differences in seed set and ovule abortion were analyzed using ANOVA models with the GLM procedure of SAS (SAS 2008), with population, maternal plant nested within population, and pollination date as random effects, and cross type (autogamous or geitonogamous) and pollination treatment as fixed effects. Data were approximately normal so were not transformed prior to analysis.

We assessed the fitness of autogamous and geitonogamous progeny in the same greenhouse environment that was used to grow the parental plants. Seedlings from a subset of ten maternal plants that had fruits from both cross types and at least 20 filled seeds were sown in soil and transplanted to 36-cell trays (blocks) in September in a randomized incomplete block design. After three months of growth, the progeny were scored for survival, and above ground biomass was measured after drying at 60°C for at least 24 hours. The fitness of progeny was estimated as its final biomass (log transformed to improve normality), weighted by the survival frequency of progeny from the same cross. Biomass is considered to be an appropriate estimate of fitness for perennials (Younginger et al. 2017). These estimates were rescaled relative to the maximum value from all crosses, so that \( \bar{w} \) ranged from 0 to 1. Data were analyzed to estimate the mean fitness for autogamous (\( \bar{w}_{k(A)} \)) and geitonogamous (\( \bar{w}_{k(G)} \)) progeny from each stem, and the variance in fitness (as measured by the standard deviation; SD) for each group of autogamous and geitonogamous progeny from a single stem.
Results – Levels of seed set and germination were similar between cross types, but embryo abortion was greater in autogamous crosses. The mean number of seeds produced after autogamous (56.72 ±2.24, N = 87) and geitonogamous (57.52 ±3.42, N = 52) pollinations was similar (F_{1,101} = 0.22, P = 0.640), and there was little variation among populations (F_{2,86.5} = 0.66, P = 0.517), maternal plants (F_{18,101} = 0.79, P = 0.709), and pollination dates (F_{11,101} = 1.59, P = 0.113). Limited pollinations produced fewer seeds than excess pollinations (F_{1,101} = 4.78, P = 0.031), but the overall model was not significant (F_{33,101} = 1.43, P = 0.089; Table S1). However, the mean number of aborted ovules was greater for autogamous (17.36 ±1.27, N = 87) than for geitonogamous (13.90 ±1.70, N = 52) pollinations (F_{1,101} = 7.52, P = 0.007), and there also were significant differences among populations in the level of ovule abortion (F_{2,66} = 9.76, P < 0.001; Table S2). There were significant differences in ovule abortion among maternal plants (F_{2,101} = 10.60, P < 0.001), but not for pollination treatments (F_{1,101} = 0.01, P = 0.998) or pollination dates (F_{11,101} = 1.77, P = 0.070; model F_{33,101} = 2.76, P < 0.001). Seed germination was similar between autogamous (mean = 13.03 out of 20 planted per fruit) and geitonogamous crosses (mean = 13.28; F_{1,101} = 0.16, P = 0.694). Of the 354 seedlings that germinated, 202 survived, and survival was higher for autogamous (67%) compared to geitonogamous progeny (50%; chi-square = 12.03, P = 0.0005). Nearly all surviving plants flowered by the end of the experiment, and flower production was correlated with above ground biomass (r = 0.58, P < 0.001, N = 282).

The average fitness of autogamous and geitonogamous progeny varied widely among individual stems, which was evident as a significant interaction between cross type and stem identity (F_{8,201} = 5.52, P < 0.0001; Table S3). Little of the variation in progeny fitness was explained by differences among stems (F_{8,201} = 0.74, P = 0.676), but larger amounts were explained by cross type (F_{1,201} = 3.09, P < 0.0805) and among blocks (F_{14,201} = 3.11, P = 0.0002). When data were analyzed separately for each cross type, we found that mean autogamous progeny fitness varied among stems to a much greater degree (F_{8,130} = 4.44, P < 0.0001) than geitonogamous progeny fitness (F_{8,70} = 1.47, P = 0.1866). For four of the ten stems, \( \bar{s}_k \) was significantly less than zero, which indicates that mean fitness of autogamous progeny was less than geitonogamous progeny. However, average fitness of autogamous progeny was significantly greater than geitonogamous progeny for two of the stems, which suggests that somatic mutations with beneficial phenotypic effects were transmitted to offspring (Fig. 4). These data are analyzed further along with data from the second experiment described below.
Second Experiment

Results of the first experiment are consistent with the hypothesis that somatic mutations arising during vegetative growth are inherited and can have substantial phenotypic effects in the next generation. However, the first experiment was not designed specifically to compare the effects of autogamous and geitonogamous pollination on offspring fitness. Furthermore, the results from the first experiment are inconsistent with widely held views of the process of mutation accumulation in plants – i.e., that somatic mutation rates are repressed and beneficial mutations are extremely rare (Groot and Laux 2016; Watson et al. 2016; Sarkar et al. 2017). Therefore, to confirm these results, we performed a second experiment to provide a more robust assessment of the effects of autogamous and geitonogamous self-pollination on the fitness of offspring. The second experiment used a single clone of *M. guttatus* to make comparisons between autogamous and geitonogamous pollinations paired at the same node. The plant chosen was a self-compatible perennial that displayed vigorous vegetative growth. We attempted to induce somatic mutations by exposing plants to stressful conditions conferred by high salinity under hydroponics cultivation. A control hydroponics treatment without added salt was included with an expectation that fewer mutations would appear under low stress. This design provided a more refined test of the hypothesis that the fitness effects of somatic mutations differed in the offspring of autogamous and geitonogamous crosses.

Methods – To assess the fitness effects of mutations that accumulated during vegetative growth, a single plant (genet BV, obtained from Willamette Gardens native plant nursery, Corvallis, OR) was vegetatively propagated to generate 12 plants (ramets) that were exposed to high salinity and control conditions. Plants were grown in pea gravel (4 – 8 mm) in pots placed in four 53 L tubs using a flood and drain hydroponics system (flooding at 15 min intervals). Two tubs had no added salt and were used as controls, and two tubs had high salinity. The initial salt concentration in the high salinity treatment was 5 mM but increased weekly to 25 mM after plants became established. Salt concentrations were monitored using a conductivity meter to ensure stable concentrations. To provide nutrients, 30 ml of hydroponics fertilizer (FloraGrow, Planet Natural, Bozeman, MT) was added per tub. During the course of the experiment, some plants grew substantially. The fastest growing ramets were transplanted three to four times over the next three months by removing a single rosette and transplanting it back into the hydroponics system.

To promote stress recovery, plants were transplanted to soil for six months, which included a two month vernalization period in a growth chamber (4°C and 8 h light; Conviron E8, Controlled Environments Ltd., Winnipeg, Manitoba, Canada). After vernalization, plants were returned to the
Autogamous and geitonogamous pollinations were made to pairs of flowers at single nodes or consecutive nodes (seven nodes and 14 pollinations total) on the largest ramets in each of the control and high salt treatments. To account for somatic mutation turnover that may occur due to the effects of cell lineage selection during stem growth, we compared progeny from autogamous and geitonogamous pollinations at pairs of flowers from the same node. Without a priori knowledge of the expression of somatic mutations in heterozygotes, it is difficult to determine the best pollen donor for geitonogamous crosses. Consequently, we opted to generate the most diverse geitonogamous progeny possible by pollinating flowers with pollen from a ramet from the other treatment (i.e. salt pollinated with control pollen, and control pollinated with salt pollen). The fruits were collected, and the total number of fertilized, unfertilized, and aborted seeds were counted under a dissecting microscope. Seeds were planted in soil in trays with three seeds per cell. Seeds were cold stratified in moist soil for three weeks before they germinated in the greenhouse.

To determine whether autogamous seedlings from ramets exposed to salt stress showed improved performance under the same conditions, all progeny were exposed to high salinity. After germination and establishment in soil, seedlings from autogamous and geitonogamous crosses to control and salt stress ramets were transplanted into pots filled with pea gravel and subjected to high salt in the hydroponics system, as described above. A total of 239 seedlings from 11 fruits (five autogamous and six geitonogamous) were randomly and evenly distributed among 12 hydroponic tubs to ensure equal representation across blocks (tubs). Plant size was measured as the product of the length and width of vegetative spread after two months of growth and was used as a proxy for biomass. Salt concentration increased from 10 mM - 37.5 mM over the course of the experiment to induce mortality (~57% across all progeny groups). Fitness was estimated as plant size (log transformed to improve normality) weighted by the survival frequency for progeny from the same cross.

Results – Similar to experiment 1, there was greater variation in the fitness of autogamous compared to geitonogamous progeny, as indicted by a significant interaction between cross type and stem/node identity (F_{4,226} = 3.44, P = 0.0095). There was no consistent difference between autogamous and geitonogamous progeny fitness when averaged across stems (F_{1,226} = 0.59, P = 0.444), but there were larger differences among individual stems/nodes (F_{6,226} = 3.44, P = 0.051; Table S4). For autogamous progeny, there was significant variation among stems (F_{2,89} = 3.76, P = 0.028) and nodes (nested within stems; F_{3,89} = 2.59, P = 0.0596), but not for geitonogamous progeny (F_{1,136} = 0.63, P = 0.431 and F_{4,136} = 1.19, P = 0.317 for stems and nodes, respectively). There were significant differences in average size among the four tubs (F_{11,226} = 4.51, P < 0.0001), but there was no consistent difference in
the performance of progeny based on the treatment history of their maternal ramets ($F_{1,126} = 0.02, P = 0.888$), and there was no interaction between cross type and historical treatment ($F_{1,136} = 0.64, P = 0.423$). Three nodes were dropped from further analysis, because one of the paired fruits aborted or produced fewer than five seedlings. However, similar to experiment 1, and consistent with the hypothesis that somatic mutations accumulate during stem growth, the variance in fitness among autogamous progeny was greater than for geitonogamous progeny. Also similar to experiment 1, the fitness of autogamous progeny was greater than geitonogamous progeny for half of the stems (Fig. 4).

Since results from the two experiments were similar, the data for four nodes from experiment 2 were analyzed together with the first experiment, as described in the next section.

Selection Coefficient Estimates

Results from the two experiments described above are consistent with the hypothesis that somatic mutations unique to individual stems can have demonstrable effects on the fitness of progeny when a proportion is made homozygous by autogamous self-pollination. In particular, the variance in fitness among autogamous progeny groups was significantly greater than among geitonogamous progeny from the same set of stems in both experiments. Both experiments also provide us with the unexpected result that the average effects of somatic mutations on fitness are significantly positive for four of the stems (Fig. 4). Since the results from these two experiments are qualitatively similar, we opted to combine them for additional analyses (ten comparisons of autogamy and geitonogamy from Experiment 1 and four from Experiment 2, for a total of 14 comparisons). Specifically, we test the prediction that somatic mutations accumulating in stems should lead to a positive relationship between the variance in fitness among progeny from single fruits produced by autogamous pollination ($s_{SP}$) and the selection coefficient estimated from the average fitness effects of autogamous and geitonogamous progeny ($s_k$).

Estimates of the selective effects of somatic mutations unique to each stem were made based on the fitness of autogamous ($\bar{w}_{k(A)}$) and geitonogamous ($\bar{w}_{k(G)}$) progeny. In both experiments, fitness was estimated as the above ground biomass weighted by the survival of seedlings from the same fruit and scaled to a maximum value of 1.0. The average selective value of mutations ($s_k$) for each stem (first experiment) or stem/node combination (second experiment) was calculated as the difference in fitness between autogamous and geitonogamous progeny, as described above ($s_k = \bar{w}_{k(A)} - \bar{w}_{k(G)}$). The average fitness of autogamous and geitonogamous progeny from the same stems or nodes was positively correlated (Fig. S3; Table S5). While stems displaying the highest positive selection coefficients tended to have higher fitness for autogamous progeny relative to geitonogamous progeny (Fig. S4), this was not
always the case, as one stem (stem 9) with a value of \( s_k \) close to zero produced progeny with relatively high fitness after both autogamous and geitonogamous pollination. Overall, comparisons between autogamous and geitonogamous progeny produced a range of estimates of \( \bar{s}_k \), eight of which were significantly different from zero (Fig. 4).

To make independent estimates of the average selection coefficients for mutations unique to each stem or node, we used the standard deviation in progeny fitness from autogamous crosses based on the relationship described in Appendix 1 \( (s_{SD} = c \sigma_{\bar{w}}) \), where \( c_{1,N} = 2 \), which assumes that variation is primarily due to a single locus and a small number of progeny). We assume a minimal value for \( c_{n,N} \) because the variance in progeny fitness is probably inflated by environmental variation. We changed the sign of \( s_{SD} \) to be negative or positive depending on the sign of \( \bar{s}_k \) (Fig. 5; Appendix 1). For values of \( \bar{s}_k \) greater than zero, there was a strong positive relationship between \( \bar{s}_k \) and estimates of \( s_{SD} \) made from the within-family variation among autogamous progeny (Fig. 5). In contrast, the relationship for negative values of \( \bar{s}_k \) appeared to be driven largely by a single observation. Even though this observation was supported by a similarly highly negative value of \( s_{SD} \), the remaining negative selection coefficients were more modest based on both estimates. It is also notable that the variance in fitness for autogamous progeny did not decline to zero for values of \( \bar{s}_k \) close to zero, which could be due to the presence of both beneficial and detrimental mutations, and possibly genetic background effects (i.e. epistasis), but it may also reflect environmental variation. Overall, the results from estimates of variance in fitness among autogamous progeny confirm the wide range of values of \( \bar{s}_k \) obtained by comparisons with geitonogamous progeny, and they suggest that most deleterious mutations in these crosses were relatively modest.

**Discussion**

The observation of higher variation in fitness among stems for progeny from autogamous compared to geitonogamous self-pollinations provides strong evidence for the accumulation of somatic mutations during vegetative growth in *Mimulus guttatus*. Our estimates of the effects of somatic mutations based on differences in the mean fitness between autogamous and geitonogamous progeny \( (\bar{s}_k) \), and from variation in fitness of autogamous progeny \( (s_{SD}) \), were consistent between the separate experiments. We found evidence for beneficial mutations being transmitted to progeny, with estimates of \( \bar{s}_k \) and \( s_{SD} \) exceeding 0.1 in four cases, while estimates for negative selection coefficients were more modest (mostly > -0.15). These results are consistent with the hypothesis that somatic mutation accumulation during vegetative growth is substantial enough to have demonstrable effects on progeny fitness.
Furthermore, the observation that autogamous progeny from four of the tested stems had higher fitness than progeny from geitonogamous crosses argues for the accumulation of beneficial mutations and implies that many deleterious mutations are culled by the various types of intra-generation selection prior to seed dispersal. These results suggest that somatic mutations are generated during vegetative growth, but many of them are filtered due to intra-generation selection, which results in a shift in the distribution of fitness effects for the mutations that are passed on to the next generation.

Somatic mutations accumulating during vegetative growth had an overall positive effect for four of the stems tested. This surprising result is contrary to widely held views that the accumulation of beneficial somatic mutations should be exceedingly rare (Crow 1993; Charlesworth and Willis 2009). However, a potential explanation for these findings is afforded by the unique biology of plants; somatic mutations in meristem cells could contribute to faster growth and division of cells during stem growth. Thus, cell lineage selection has the potential to retain beneficial somatic mutations, as individual cell lineages outcompete others for limited space and resources. Although there may be few opportunities for beneficial changes to alter basic cellular metabolism, it is becoming apparent from experimental evolution studies with microbes that even basic aspects of cellular metabolism can be sensitive to environmental conditions, which can lead to the appearance of large numbers of beneficial mutations in clonal populations (e.g., Lee and Marx 2013; Maharjan et al. 2015). In this regard, cell lineage selection in a plant meristem is analogous to clonal evolution in microbial populations, and it represents a potentially powerful forum for the filtering of somatic mutations. In addition, gametophytic selection and selective embryo abortion can act as prominent filters, but they are most likely to have effects on culling of deleterious mutations. Regardless, the combined effects of these different forms of intra-generation selection appear to have had a considerable effect on filtering of somatic mutations, such that the distribution of fitness effects among stems has shifted to include more beneficial mutations than expected (Fig. 3).

An alternative explanation for the observed phenotypic changes is that exposure to environmental stress has induced heritable epigenetic modifications (Quadrana and Colot 2016). However, epigenetic modifications are generally consistent in direction, are predictable, and are thought to represent an adaptive response to historic exposure to similar stressors (Baulcombe and Dean 2014; Crisp et al. 2016; Itabashi et al. 2018). The results of the experiments described here do not support a role for epigenetics, because phenotypic responses in the next generation were inconsistent in direction and magnitude, and they were not predictable based on environmental exposure of the parent stem. Indeed, among stems and nodes, the mean fitness of autogamous progeny displayed both
increases and decreases compared to the geitonogamy controls, which is consistent with the hypothesis that individual ramets are accumulating unique complements of somatic mutations. Furthermore, these conclusions are supported by the observation that many low-frequency but few high-frequency unique genetic variants have been found in the transcriptomes of ramets derived from a single genet of *M. guttatus* with a history of exposure to salt stress (Schwoch et al. unpublished data). Thus, the current study indicates that somatic mutations accumulating during stem growth can have phenotypic effects.

The potential for the acquisition of mutations during vegetative growth is a well-known aspect of plant biology (Klekowski 2003; Schultz and Scofield 2009; Bobiwash et al. 2013), but no previous study has demonstrated the effects of somatic mutations on the fitness of progeny in the next generation. Most studies have focused on the detrimental effects of somatic mutations; chloroplast mutants have been observed in a number of species (Klekowski 2003), declines in pollen fertility were found in older clones of quaking aspen (Ally et al. 2010), and higher rates of seed and fruit abortion after autogamous pollinations were found in several studies (reviewed in Bobiwash et al. 2013). In contrast, agriculturalists have taken advantage of beneficial somatic mutations by conducting clonal selection to improve economically important plants (Aradhya et al. 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli et al. 2012; Jarni et al. 2015; Pelsy et al. 2015), and a handful of studies report phenotypic responses to selection in asexual lineages. For example, Breese et al. (1965) succeeded in selecting for increased tillering ability (production of new grass stems) within genets of perennial ryegrass (*Lolium perenne*). Similarly, artificial selection on clonal lineages effectively improved branching in the red seaweed, *Asparagopsis armata* (Monro and Poore 2009). The current study on *M. guttatus* contributes to this literature by highlighting the potential for plants to exhibit significant levels of clonal variation within a single generation.

Moreover, the unexpected transmission of beneficial mutations in autogamous crosses may explain some heretofore difficult to understand results from mutation accumulation studies. Our results suggest that beneficial somatic mutations are partially dominant, because they would have to be expressed in the heterozygous state to be favored by cell lineage selection. Consequently, we expect autogamy would be more effective for the accumulation of beneficial somatic mutations in populations than geitonogamy or outcrossing. It is striking that high rates of beneficial mutation accumulation have been observed in at least some mutation accumulation studies in the autogamous plant *Arabidopsis thaliana* (Shaw et al. 2002; Rutter et al. 2010; Rutter et al. 2012), but not in outcrossing and partially-selfing species of *Amsinckia* (Schoen 2005), or in any animal species (Halligan and Keightley 2009). Similarly, our results suggest that the adaptive potential of autogamous plants may be greater than
previously thought, which may help explain the wider geographic ranges of selfing compared to closely-related outcrossing species (Grossenbacher et al. 2015). Although intra-generation selection has the potential to contribute to adaptation in all plants, its effects may be enhanced in autogamous lineages, because beneficial mutations arising during vegetative growth have a greater chance of becoming homozygous in offspring and being retained across generations.

As stems elongate, mutations are generated during every mitotic cell division, so the potential for somatic mutation accumulation in plants appears substantial. Thus, understanding how long-lived plants, such as trees, avoid mutational meltdown from the accumulation of deleterious somatic mutations remains a longstanding question. Paradoxically, however, the rate of mutation accumulation observed across generations in plant and animal genomes is similar (Gaut et al. 2011). One explanation for this pattern is that somatic mutations in plants are repressed during vegetative growth, similar to animal germlines, which would protect lineages from the negative effects of mutation accumulation during development of the soma (Burian et al. 2016; Cruzan 2018; page 90). An alternative hypothesis posits that somatic mutations are generated in apical meristems during plant growth, but these mutations are filtered by intra-generation selection occurring prior to the establishment of offspring. Because plants have retained the capacity to undergo clonal evolution from their algal ancestors, the ability to filter mutations during growth and reproduction has existed for some time, and thus intra-generation selection has the potential to skew the distribution of fitness effects of transmitted mutations to include a larger proportion of beneficial mutations than would be expected through random processes. In addition, this provides a reasonable explanation for why longer-lived plants appear to have slower rates of mutation accumulation across generations (Yue et al. 2010; Gaut et al. 2011). This is due to the fact that longer generation time leads to more time between recombination events, which can lead to more background selection in non-recombining cell lineages during vegetative growth (Cruzan 2018; pages 94-95). From our results from crosses within plants of *M. guttatus*, it appears that a disproportionate number of beneficial mutations is passed on to offspring in the next generation, likely a consequence of intra-generation selection. Future work that combines information from experiments evaluating the genomic consequences of somatic variation with anatomical estimates of stem cell population dynamics will allow for the development of new models that provide insights into the extent and limitations of somatic evolution in plants.

In conclusion, despite the potential for somatic mutation accumulation to generate novel genetic variation in plant populations, its role in their evolution remains almost entirely unexplored. Even though high levels of mutation accumulation are often believed to be detrimental, the basic
biology of plants suggests that the role of somatic mutations in plant evolution should be considered carefully in the future. Moving forward, our results indicate that we must keep in mind that all eukaryotes are not necessarily equivalent, and unique features of the organism's biology may have unexpected consequences for the evolutionary phenomena that we observe. Future lines of investigation will improve our understanding of these fundamental aspects of plant development and evolution that may have contributed to the remarkable diversification of plants, and may help to account for some of the extensive variation in mutation rates detected among lineages.

Acknowledgements

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Data Archiving

Data will be submitted to DataDryad.

Author Contributions

MBC and JAS designed and conducted the experiments. MBC developed the analysis methods. MBC and MAS wrote the manuscript
Literature Cited


Data Archiving:
Figure Captions

**Fig. 1.** Experimental design to test for the average fitness effects of somatic mutations accumulating in stems during vegetative growth. See text for further explanation.

**Fig. 2.** An illustration of cell lineage selection as it acts on a number of cell lines that are competing for space and resources within the apical meristem of a plant. Expressed deleterious mutations are predicted to lead to the demise of a cell line (brown cells) that are replaced by neighboring cell lines. The appearance of a mutation that increases cell growth rate in the current environment results in a selective sweep of the meristem (light green cells).

**Fig. 3.** Predicted effects of cell lineage selection, gametophytic selection, and selective embryo abortion on the distribution of selective effects of mutations passed on to the next generation. Mutations accumulate during meiosis for the formation of haploid gametophytes and mitotic cell divisions during vegetative growth. The large majority of these mutations is deleterious (red region), but a small fraction is beneficial (green region). Episodes of intra-generation selection, including cell lineage selection, gametophytic selection, and selective embryo abortion, may reduce the load of deleterious mutations, while allowing the beneficial ones to be passed to the next generation.

**Fig. 4.** Estimates of $\bar{s}_k$ for fourteen different stems (ramets) from two separate experiments (Experiment 1 – blue bars; Experiment 2 – orange bars) based on mean progeny fitness after autogamous $\bar{w}_{k(A)}$ and geitonogamous $\bar{w}_{k(G)}$ self-pollinations ($\bar{s}_k = \bar{w}_{k(A)} - \bar{w}_{k(G)}$). Horizontal lines represent standard errors. Asterisks indicate values of $\bar{s}_k$ that are significantly different from zero based on the $t$ value, calculated as $t = \bar{s}_k / \sqrt{SE}$ with $n-1$ df, where $n$ is the mean of sample sizes for autogamous and geitonogamous progeny. The relationship between $\bar{w}_{k(A)}$ and $\bar{w}_{k(G)}$ across stems is shown in Fig. S3. Means and sample sizes for progeny groups are available in Table S5 and Fig. S4.

**Fig. 5.** Relationships between estimates of selection coefficients from the difference in fitness of autogamous and geitonogamous progeny ($\bar{s}_k$), and the standard deviation in fitness within autogamous progeny groups from each stem ($s_{SD}$). Estimates of $s_{SD}$ corresponding to negative values of $\bar{s}_k$ were transformed to negative values. Estimates from Experiment 1 are indicated by blue circles and from Experiment 2 are orange squares. Dashed lines indicate the separate relationships for positive and negative values of the selection coefficients.
Fig. 1

\[ \bar{S}_k = \bar{W}_{k(A)} - \bar{W}_{k(G)} \]

*Average Fitness Effects of Somatic Mutations*
Fig. 2
Fig. 3

![Diagram showing the relationship between mutation accumulation, total mutation load, and various stages of plant life, including meiosis, somatic growth, and selection processes like intra-generation selection, cell lineage selection, gametophytic selection, and selective embryo abortion. The diagram illustrates how mutations at different stages can be deleterious or beneficial.]
Fig. 4
Fig. 5

- Graph showing selection estimated from SD against selection ($S_k$).

- Equation: $y = 0.7923x + 0.0818$ with $R^2 = 0.3768$.
- Equation: $y = 0.7555x + 0.1686$ with $R^2 = 0.502$. 

Legend: Circles and squares represent different data sets.
Appendix 1. Estimating the selection coefficient due to somatic mutations from the variance in fitness among progeny.

In this Appendix, we will demonstrate that the standard deviation (SD) in autogamous progeny fitness can be used to estimate the selection coefficient for different numbers of somatic mutations occurring within a stem. The variance in fitness among autogamous progeny can be affected by the selection coefficient ($s$), dominance effects ($h$), and the number of somatic mutations that are unique to each stem ($n$). To evaluate these effects, we simulated the expected fitness of the $aa$, $aa'$, and $a'a'$ progeny genotypes for nine values of $s$ ranging from -0.01 to -0.3, and 10 values of $h$ in increments of 0.1 between 0 and 1, for a total of 90 combinations (assuming a single somatic mutation $aa'$ within the parental stem). For each combination, four individual offspring were generated to correspond to the expected segregation frequencies, such that there was one $aa$ genotype, two $aa'$, and one $a'a'$. The fitness of each genotype was calculated as $w_{aa} = 1.0$, $w_{aa'} = (1+hs)$, $w_{a'a'} = (1+s)$, and the standard deviation (SD) in fitness was calculated for each progeny group of four individuals. We estimated the independent effects of $s$ and $h$ on SD using a multiple regression model, with the SD in progeny fitness as the dependent variable. We found that most of the variance in fitness was explained by $s$ (slope=0.44, $P << 0.001$) but not $h$ (slope=0.004, $P=0.013$; $R^2=0.989$ for the model including both $s$ and $h$). The highest variance in fitness occurs at $h=0.0$ or $h=1.0$, but the SD scaled linearly with $s$. Because the fitness of the heterozygous genotype is always intermediate to the fitness of the homozygotes (i.e., unless $h = 0.0$ or $1.0$), the level of dominance makes only a minor contribution to the SD, and is thus not considered further (Fig. S1).

Next, to evaluate the effects of different numbers of somatic mutations ($n$) on variation in fitness of autogamous progeny, we used the same fitness and segregation values for each locus as above and averaged the fitness effects across loci. For simplicity, we considered the effects of completely dominant mutations and assumed equal and additive effects across independently segregating mutations. For example, for a single mutation ($n=1$), we obtain the following fitnesses when $s = 0.3$ and $h = 1.0$:
Genotype (n=1)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>aa</td>
<td>1.0000</td>
</tr>
<tr>
<td>aa'</td>
<td>1.3000</td>
</tr>
<tr>
<td>aa''</td>
<td>1.3000</td>
</tr>
<tr>
<td>aa''''</td>
<td>1.3000</td>
</tr>
<tr>
<td>aa''''''</td>
<td>0.1500</td>
</tr>
</tbody>
</table>

With the same values of \(s\) and \(h\), we obtain the following distribution of genotypes and fitnesses for two mutations (assuming one progeny per genotype to account for independent segregation at both loci):

Genotype (n=2)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>aa</td>
<td>1.00</td>
</tr>
<tr>
<td>aa'</td>
<td>1.00</td>
</tr>
<tr>
<td>bb</td>
<td>1.00</td>
</tr>
<tr>
<td>bb'</td>
<td>1.00</td>
</tr>
<tr>
<td>bb''</td>
<td>1.30</td>
</tr>
<tr>
<td>bb''''</td>
<td>1.30</td>
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<tr>
<td>bb''''''</td>
<td>1.30</td>
</tr>
<tr>
<td>aa</td>
<td>1.00</td>
</tr>
<tr>
<td>aa'</td>
<td>1.00</td>
</tr>
<tr>
<td>aa''</td>
<td>1.00</td>
</tr>
<tr>
<td>aa''''</td>
<td>1.00</td>
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<tr>
<td>aa''''''</td>
<td>1.00</td>
</tr>
<tr>
<td>aa''''''</td>
<td>0.1500</td>
</tr>
<tr>
<td>aa''''''</td>
<td>0.0949</td>
</tr>
</tbody>
</table>

To demonstrate that the selection coefficient can be estimated from these standard deviations in progeny fitness, we used the segregation ratios described above to generate fitness distributions for several values of \(s\) and \(n\) (with \(h = 1\); see examples for \(n = 1\) and \(n = 2\) above). To equalize the effects of population size across different numbers of loci, the number of individuals segregating for \(n = 1, 2, 3,\) and 4 mutations was replicated so all autogamous families were of the same size (\(N = 256\)). As we increased the number of independently segregating mutations, the SD among autogamous progeny declined rapidly and started to level off after three mutations (Fig. S2). Nevertheless, for each value of \(n\), the SD increased linearly with \(s\) (e.g., Fig. S1), and the ratio between the SD and the selection coefficient remained constant. This allowed us to define a new term \(c\) for each value of \(n\) that captures this strong relationship between \(s\) and SD, such that \(c_{n,N} = s/SD\), where \(c_{n,N}\) is a constant for each combination of \(n\) and \(N\). Therefore, \(s\) can be estimated from variation in fitness among autogamous progeny, according to the equation \(s_{SD} = c_{n,N}SD\). Since this estimate of the selection coefficient is based solely on variation among progeny, the sign of \(s_{SD}\) has to be determined by comparing the fitnesses of autogamous and geitonogamous progeny.

For a single mutation, \(c_{1,4} = 2.0\) when there are four progeny (\(N = 4\)), and it increases to 2.31 when \(N = 256\). For two independently segregating mutations, \(c_{2,256} = 3.26\) when \(N = 256\), for three or more loci \(c = 4.55\), and for \(n = 4\), the value of \(c = 5.07\). For example, for a single mutation with \(s = -0.3\), \(h = 1.0\), and \(n = 1\), the fitness of each genotype category will be 1.0, 0.7, and 0.7 for the \(aa\), \(aa'\), and \(a'a'\) genotypes, respectively. The SD among four individuals representing the segregation ratio of 1:2:1 (i.e. one \(aa\), two \(aa'\), and one \(a'a'\)) is 0.15 (see above), so \(s_{SD} = 2SD = 2(0.15) = 0.3\) (the same value is obtained whether \(s\) positive or negative). For a single mutation with \(s = 0.1\), \(SD = 0.05\) so \(2(0.05) = 0.1\). Similarly, estimates of \(s\) can be obtained assuming multiple mutations. By inspection, we note that \(c\) ranges from

\[c_{n,N} = \frac{s}{SD}\]

\[s_{SD} = c_{n,N}SD\]

\[c = \frac{s}{SD}\]

\[s = cSD\]
2.0 to ~5.0 for 1 to 4 mutations for progeny groups between \( N = 4 \) and \( N = 256 \). While progeny sample sizes vary among fruits, this approach provides an approximate estimate of the average effects of mutations. Based on this range of values, we can infer that relatively few mutations were likely segregating in each stem. These estimates of the selection coefficient provide a set of values that scale with estimates based on comparing the fitnesses of autogamous and geitonogamous progeny (Fig. 5).

Fig. S2. The standard deviation (SD) in fitness among autogamous progeny from a single self-pollination declines rapidly with the number of independently segregating loci.
Appendix 2. Supplemental Tables and Figures

Table S1. Analysis of variance results for seed set after autogamous and geitonogamous pollinations (Cross) with limited and excess pollen (Pollen). Population (Pop), maternal plant nested within population (Mat(Pop)), and pollination date (Date) were declared as random effects in the model.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>33</td>
<td>21623.26022</td>
<td>655.25031</td>
<td>1.43</td>
<td>0.0888</td>
</tr>
<tr>
<td>Cross</td>
<td>1</td>
<td>100.647606</td>
<td>100.647606</td>
<td>0.22</td>
<td>0.6400</td>
</tr>
<tr>
<td>Pop</td>
<td>2</td>
<td>552.228601</td>
<td>276.114301</td>
<td>0.60</td>
<td>0.5487</td>
</tr>
<tr>
<td>Pollen</td>
<td>1</td>
<td>2183.436724</td>
<td>2183.436724</td>
<td>4.78</td>
<td>0.0312</td>
</tr>
<tr>
<td>Mat(Pop)</td>
<td>18</td>
<td>6490.364191</td>
<td>360.575788</td>
<td>0.79</td>
<td>0.7089</td>
</tr>
<tr>
<td>Date</td>
<td>11</td>
<td>8000.908005</td>
<td>727.355273</td>
<td>1.59</td>
<td>0.1127</td>
</tr>
<tr>
<td>Error</td>
<td>101</td>
<td>46182.07311</td>
<td>457.24825</td>
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<td></td>
</tr>
</tbody>
</table>

Table S2. Analysis of variance results for aborted ovules after autogamous and geitonogamous pollinations (Cross) with limited and excess pollen (Pollen). Population (Pop), maternal plant nested within population (Mat(Pop)), and pollination date (Date) were declared as random effects in the model.

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
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<td>9337.61468</td>
<td>282.95802</td>
<td>2.76</td>
<td>&lt;.0001</td>
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<tr>
<td>Cross</td>
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<td>449.176948</td>
<td>449.176948</td>
<td>4.38</td>
<td>0.0389</td>
</tr>
<tr>
<td>Pop</td>
<td>2</td>
<td>2174.689160</td>
<td>1087.344580</td>
<td>10.60</td>
<td>&lt;.0001</td>
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<tr>
<td>Pollen</td>
<td>1</td>
<td>0.597410</td>
<td>0.597410</td>
<td>0.01</td>
<td>0.9393</td>
</tr>
<tr>
<td>Mat(Pop)</td>
<td>18</td>
<td>2217.350757</td>
<td>123.186153</td>
<td>1.20</td>
<td>0.2751</td>
</tr>
<tr>
<td>Date</td>
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<td>1995.681210</td>
<td>181.425565</td>
<td>1.77</td>
<td>0.0695</td>
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<tr>
<td>Error</td>
<td>101</td>
<td>10363.24458</td>
<td>102.60638</td>
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</table>
Table S3. Analysis of variance for progeny fitness (biomass weighted by survival of seedlings) after autogamous and geitonogamous pollinations (Cross) for ten maternal plants (Mat) and their interaction (Mat*Cross). Planting block (Tray) was declared as a random effect in the model.

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<tbody>
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<td>1.42418594</td>
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</tr>
<tr>
<td>Tray</td>
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<td>0.45040769</td>
<td>0.03217198</td>
<td>3.11</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mat</td>
<td>9</td>
<td>0.06845581</td>
<td>0.00760620</td>
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<td>0.6756</td>
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<td>0.03196532</td>
<td>0.03196532</td>
<td>3.09</td>
<td>0.0805</td>
</tr>
<tr>
<td>Mat*Cross</td>
<td>9</td>
<td>0.51373985</td>
<td>0.05708221</td>
<td>5.52</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>168</td>
<td>1.73638043</td>
<td>0.01033560</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table S4. Analysis of variance for progeny fitness (biomass weighted by survival of seedlings) after autogamous and geitonogamous pollinations (Cross) for seven paired pollinations at individual nodes (Stem/Node) and their interaction (Stem/Node*Cross). Hydroponic planting block (Tub) was declared as a random effect in the model and initial size of seedlings (Initial Size) was entered as a covariate.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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<td>0.05712351</td>
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<td>0.04259691</td>
<td>4.51</td>
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<td>Stem/Node</td>
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<td>0.02011923</td>
<td>2.13</td>
<td>0.0514</td>
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<td>Cross</td>
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<td>0.00555294</td>
<td>0.59</td>
<td>0.4441</td>
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<tr>
<td>Stem/Node*Cross</td>
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<td>0.13005812</td>
<td>0.03251453</td>
<td>3.44</td>
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<tr>
<td>Error</td>
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<td>0.00944634</td>
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Table S5. Means and standard deviations (SD) for progeny groups from autogamous and geitonogamous pollinations in experiments 1 and 2. Experiment 1 included ten different genets. Experiment 2 was conducted with two ramets (C1 and D1) from the same genet.

<table>
<thead>
<tr>
<th>Exp</th>
<th>ID</th>
<th>N</th>
<th>Autogamy</th>
<th>SD</th>
<th>N</th>
<th>Geitonogamy</th>
<th>SD</th>
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<td>9</td>
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<td>0.195</td>
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<td>0.193</td>
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<td>0.114</td>
<td>0.041</td>
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<td>0.065</td>
<td>8</td>
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<td>0.195</td>
<td>0.158</td>
<td>9</td>
<td>0.162</td>
<td>0.071</td>
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<td>0.082</td>
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<tr>
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</table>

13.93  11.14
Fig. S3. The relationship between the autogamous and geitonogamous progeny from the 14 comparisons from experiments 1 (blue circles) and 2 (orange squares) as listed in Table S5. The solid line represents a 1:1 relationship and the dashed line represents the best-fit to the data.
Fig S4. Means and standard errors (lines) for fitness estimates of autogamous (upper panel) and geitonogamous (lower panel) progeny groups from experiment 1 (blue bars) and 2 (orange bars) listed in Table S5. Progeny groups are ordered as in Fig. 4.