Journal of Evolutionary Biology, 2025, **XX**, 1–9
https://doi.org/10.1093/jeb/voaf033
Advance access publication 28 March 2025
Research Article

JOURNAL OF
Evolutionary Biology
eseb
European Society for Evolutionary Biology

Inheritance of somatic mutations can affect fitness in monkeyflowers

Matthew A. Streisfeld[1,*], Jessie C. Crown[1], Jack J. McLean[1], Aidan W. Short[1], Mitchell B. Cruzan[2]

[1]Institute of Ecology and Evolution, University of Oregon, Eugene, OR, United States
[2]Department of Biology, Portland State University, Portland, OR, United States

Handling editor: Rebekah Rogers, Associate editor: Salvatore Cozzolino

*Corresponding author: Matthew A. Streisfeld, Institute of Ecology and Evolution, University of Oregon, Eugene, OR, United States. Email: mstreis@uoregon.edu

Abstract

Plants have the ability to transmit mutations to progeny that arise through both meiotic and mitotic (somatic) cell divisions. This is because the same meristem cells responsible for vegetative growth will also generate gametes for sexual reproduction. Despite the potential for somatic mutations to contribute to genetic variation and adaptation, their role in plant evolution remains largely unexplored. We conducted experiments with the bush monkeyflower (*Mimulus aurantiacus*) to assess the phenotypic effects of somatic mutations inherited across generations. By generating self-pollinations within a flower (autogamy) or between flowers on different stems of the same plant (geitonogamy), we tracked the effects of somatic mutations transmitted to progeny. Autogamy and geitonogamy lead to different segregation patterns of somatic mutations among stems, with only autogamy resulting in offspring that are homozygous for somatic mutations specific to that stem. This allowed us to compare average phenotypic differences between pollination treatments that could be attributed to the inheritance of somatic variants. While most experimental units showed no impacts on fitness, in some cases, we detected increased seed production, as well as significant increases in drought tolerance, even though *M. aurantiacus* is already well adapted to drought conditions. We also found increased variance in drought tolerance following autogamy, consistent with the hypothesis that somatic mutations transmitted between generations can impact fitness. These results highlight the potential role of inherited somatic mutations as a relevant source of genetic variation in plant evolution.

Keywords: acquired mutations, autogamy, cell lineage selection, *Mimulus aurantiacus*, geitonogamy, natural selection

Introduction

Mutations are the ultimate source of genetic variation. However, only mutations that are transmitted to subsequent generations will be relevant for evolution. Mutations are generated through both mitosis and meiosis, but for most animals, only variants that arise in the germline can be transmitted to progeny. This is because the germline is determined early in development and is separated from the somatic cell lineages that produce the rest of the organism's body. Therefore, somatic mutations that form outside of the germline are not heritable.

By contrast, plants undergo indeterminate growth, where shoot and root systems continually elongate and develop throughout a significant portion of their life cycle (Antolin & Strobeck, 1985; D'Amato, 1996). Growth of the shoot system in plants occurs at shoot apical meristems (SAMs), which contain a population of undifferentiated germ cells known as the central zone. In vascular plants, these cells divide to produce cells that differentiate into leaf and stem tissue necessary for growth and development, and they eventually produce the gametes required for sexual reproduction. This reservoir of pluripotent cells is replenished through mitotic division (Kwiatkowska, 2008), but as the shoot elongates, mutations may occur due to DNA replication errors. These somatic mutations can accumulate as the stem grows, resulting in distal areas of the shoot system possessing more somatic variants than their basal counterparts (Schultz & Scofield, 2009). In angiosperms, the gametes are not produced until later in development when the SAM is first converted to a floral meristem and then to a flower, indicating that somatic mutations that arise in the SAM may be transmitted to offspring. This leads to the possibility that somatic mutations are a source of genetic variation that can impact evolutionary processes.

Despite the potential for the inheritance of somatic variants that accumulated during vegetative growth, the role and relevance of somatic mutations within plants remain unsettled. Since plants possess the ability to pass on both meiotic and somatic mutations to progeny, one might expect the mutation rate per generation among plants would be noticeably higher than animals. However, mutation rates per generation appear to be similar between plants and animals (Gaut et al., 2011). Multiple explanations have been offered to explain this observation.

For example, germline segregation in plants may occur earlier in development than previously appreciated, with primordial germ cells physically separated from future somatic cells within the meristem (Lanfear et al., 2018). This suggests that somatic mutations arising during vegetative growth will only rarely be inherited by progeny, since future germ cells are found in isolated cell lineages (Cruzan, 2018). These segregated populations of germ cells could potentially have a slower rate of division than their somatic counterparts, and

Received October 16, 2024; revised February 21, 2025; accepted March 25, 2025

as a result, they would have a significantly lower mutation rate over time (Lanfear et al., 2018). Models based on quantitative cell lineage data from *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) were used to replicate patterns of cell division in SAMs and axillary meristems (Burian et al., 2016). These studies reported that cells were not constantly replaced within the central zone of the SAM, and instead persisted throughout vegetative growth, suggesting that plants possess mechanisms to prevent the fixation and eventual accumulation of deleterious genetic load (Burian, 2021).

Aside from the potential developmental modifications of germ cells, there are multiple forms of selection that can mitigate the effects of deleterious mutational load that accumulated during stem growth. For example, cell lineages in the SAM containing deleterious somatic mutations may be removed from the population of meristem cells due to cell lineage selection (CLS; Cruzan, 2018; Fagerstrom et al., 1998; Monro & Poore, 2009; Otto & Hastings, 1998). Since the size of the central zone is fixed and is replenished through mitotic division, cell lineages that replicate more slowly due to the expression of deleterious mutations will be replaced by cell lineages with accelerated division (Pineda-Krch & Lehtila, 2002). Models of stochastic growth have indicated that relatively minor differences in cell replication rates during development can result in significant changes in the proportion of mutant cells found within adults (Otto & Orive, 1995; Pineda-Krch & Lehtila, 2002). Other forms of selection that occur either when haploid pollen grains compete for access to the ovules (i.e., gametophytic selection) or during seed formation (i.e., selective embryo abortion) will further reduce the number of somatic variants that are transmitted to the offspring (Buchholz, 1922; Cruzan, 2018). These models are supported by Yu et al. (2020), who identified thousands of somatic variants among ramets (individual stems) of common eelgrass (*Zostera marina*) that showed evidence of natural selection. Furthermore, Cruzan et al. (2022) detected extensive variation in fitness among offspring of seep monkeyflower (*Mimulus guttatus*) due to the accumulation of somatic mutations during stem growth. Indeed, in some cases, mutations passed to offspring were deleterious, while in others, beneficial mutations led to improved fitness, implying a potentially important role of somatic mutations in plant evolution. However, it is important to note that most mutations are not expected to affect fitness, or if they do, they are more likely to be deleterious, suggesting that beneficial mutations will be rare (Charlesworth & Willis, 2009).

To shed light on the evolutionary consequences of somatic mutations, we performed experiments to determine the phenotypic effects of inherited somatic mutations in the bush monkeyflower (*Mimulus aurantiacus* Curtis; Phrymaceae). *M. aurantiacus* is a woody, perennial shrub that is found throughout semi-arid regions of southwestern North America (McMinn, 1951). To track the effects of somatic mutations that accumulated within a stem, we took advantage of the fact that these plants are highly branching. Therefore, each stem can contain distinct germ cell lineages that are derived from the same zygote, implying that different sets of somatic mutations can accumulate across stems. By making crosses within the same flower (autogamy) or between flowers on different stems of the same plant (geitonogamy), we generated progeny that varied in their segregation of somatic mutations among stems. Both types of crosses are self-fertilizations, leading to high homozygosity of meiotic mutations. However, the offspring from each cross will differ in the complement of somatic mutations that they inherit. In a diploid plant, somatic mutations ($a \rightarrow a'$) will appear in the heterozygous state. Therefore, progeny generated via autogamy will produce 25% of their offspring that are homozygous ($a'a'$) for a somatic mutation that arose in that stem. By contrast, because mutations will differ between stems, the offspring of geitonogamous crosses will not be homozygous for somatic mutations that arose in either stem. Thus, by comparing phenotypic differences between progeny from autogamous and geitonogamous crosses, the average phenotypic effects of somatic mutations can be assessed (Bobiwash et al., 2013; Schultz & Scofield, 2009).

Cell lineage selection is expected to eliminate deleterious variants while favouring the retention of beneficial somatic mutations (Pineda-Krch & Lehtila, 2002). Comparing the average fitness of progeny produced via autogamy and geitonogamy can reveal the presence of these beneficial variants. If beneficial mutations are not completely recessive, homozygous ($a'a'$) and heterozygous offspring (aa') will have higher fitness than homozygous individuals without the mutation (aa). Thus, 75% of offspring produced by autogamy are expected to have higher fitness, but among the offspring from geitonogamy, 50% will be heterozygous and none will be homozygous for the mutation. This should lead to greater variance in fitness among autogamous progeny and a positive relationship between the mean and variance in the fitness of autogamous progeny (Cruzan et al., 2022).

We challenged progeny under drought conditions—a stress that *M. aurantiacus* routinely encounters in its native habitat (Sobel et al., 2019). By contrast, prior studies in *M. guttatus* grew plants in a highly distinctive environment that they had never encountered (salt stress), which may have contributed to the substantial fitness effects of somatic mutations they observed (Cruzan et al., 2022). Thus, we compared results between these two closely related plant species that differed not only in important life history characteristics, but also in the novelty of the selective pressures they experienced. Such a difference in selective environment is likely to impact the spectrum of mutations that affect fitness.

We followed fitness among progeny derived from autogamy and geitonogamy across multiple stages in the life cycle, including fecundity, germination, early seedling growth rates, survival under terminal drought conditions, and total biomass. Under a scenario where somatic mutations that arose in a parental stem are transmitted to offspring, we expect some autogamous pollinations to generate progeny with different average fitness than geitonogamous crosses involving the same pollen source (Cruzan et al., 2022). In addition, because of different segregation patterns of somatic mutations between the cross types, we expect progeny from autogamous pollination to display increased variation in average fitness compared to progeny from geitonogamous crosses. However, given that the selective environment was similar to their native habitat, the impact of somatic mutations on plant fitness may be diminished. Findings from this study contribute to our understanding of the relevance of somatic mutations in plant evolution.

Materials and methods

Experimental setup

To estimate the fitness effects of somatic mutations, we made autogamous and geitonogamous crosses in 26 *M. aurantiacus* genets that had been growing in an open plot in Eugene, Oregon, USA for 4 years. These genets were created through the crossbreeding of red- and yellow-flowered ecotypes of *M. aurantiacus* ssp. *puniceus* (Chase et al., 2017; Sobel & Streisfeld, 2015). We applied pollen from a single flower per stem to open stigmas on up to four different flowers on the same plant: one autogamous pollination on that same flower and up to three geitonogamous pollinations to flowers on different stems of the same genet. Similarly aged flowers were selected for pollination. *M. aurantiacus* flowers are slightly protogynous, with pollen becoming dehiscent 1-day post-anthesis. Thus, only flowers with recently dehiscent anthers were selected. Moreover, given that new flowers continue to open toward the distal ends of growing stems, all flowers used in the experiment were situated in similar spots along the stem. In addition, *M. aurantiacus* flowers contain a touch-sensitive stigma that closes when contacted with pollen and remains closed for the duration of the flower's lifespan (Fetscher & Kohn, 1999). Therefore, by requiring that stigmas were open prior to pollination, we could guarantee that no contaminating pollen was present on those flowers prior to (or following) our pollinations. Sufficient pollen was applied to saturate the surface of each stigma.

Hereafter, we refer to the offspring from a set of autogamous and geitonogamous crosses made from a single pollen donor as a 'unit'. Because somatic mutations can arise uniquely in any stem, we created multiple units from different stems on the same genet (mean: 1.8 per genet; range 1–4). Specifically, between 1 and 22 July 2021, we made 170 crosses, of which 163 developed into fruits. This included 42 individual units that successfully produced a fruit from the autogamous cross and at least two of the geitonogamous crosses. We note that this approach is an improvement over the method used by Cruzan et al. (2022), where pollen from two stems was reciprocally crossed to create autogamous and geitonogamous pollinations. In that case, distinct somatic mutations in each stem could not be controlled for, which may have impacted estimates of fitness. By using pollen from a single flower to produce multiple geitonogamous crosses on different stems, we were better able to control for different mutations among stems.

Fecundity

Fruits from each of the 42 units were collected when they turned brown and stored at room temperature for 2 months to allow them to mature fully. Each mature fruit was weighed to the nearest 0.1 mg. Seeds were carefully separated from their capsule, and all seeds from each fruit were weighed. Seeds were then photographed using a Sony Alpha 6000 digital camera and counted using ImageJ software.

Drought tolerance

To determine if somatic mutations can affect fitness in an environment that closely mimics natural conditions, we used a terminal drought experiment (as in Sobel et al., 2019). The ecotypes of *M. aurantiacus* ssp. *puniceus* are desiccation-tolerant shrubs that have adapted to endure seasonal droughts in southern California (Sobel et al., 2019). Because of these seasonal droughts, drought sensitivity likely serves as a principal agent of selection for these ecotypes in the wild.

Our primary goal was to determine if there were phenotypic differences between offspring generated from autogamy and geitonogamy within the same unit. This is because distinct somatic mutations are expected to arise and segregate in each unit, and thus, there is no a priori expectation that pollination treatment will affect fitness in the same way across units. Indeed, in some units, we might expect deleterious variants to be transmitted to offspring, thereby reducing fitness among autogamous offspring (Cruzan et al., 2022). Alternatively, given that offspring were tested in an environment that they already are likely adapted (i.e., drought), most somatic variants will probably have few measurable effects on fitness. Finally, on rare occasions, it is possible that the transmission of a somatic variant (or variants) will improve offspring fitness, resulting in a significant increase in net fitness for that unit following autogamy. As a consequence, the relevant comparisons for fitness are between the offspring of autogamous and geitonogamous crosses within a unit, rather than between them.

We conducted the drought experiment across a random sample of 10 units. Due to the segregation of somatic variants in progeny, we focussed on maximizing the sample size of offspring segregating for potential somatic mutations within these 10 units, rather than testing all possible units. The only two requirements for selecting these units were: (1) that they were complete (i.e., that fruits were produced from all three of the geitonogamous crosses as well as the autogamous pollination), and (2) that there were at least two genets that each contained two units. This latter requirement was included to explore whether variation in somatic mutations among stems of the same genet led to differences in offspring fitness. After randomly selecting 10 units that met these criteria, eight genets were represented, such that two of the genets each consisted of two units.

Seeds were spread on moist potting soil and placed in a grow room at 22 °C, with fluorescent lighting set to a 16-hr photoperiod. Trays were misted and bottom watered as needed to keep the soil moist. From each of the 10 units, we selected 96 seedlings for the drought experiment (48 from the autogamous cross and 48 seedlings from the geitonogamous crosses), resulting in a total of 960 seedlings. These seedlings should segregate for somatic mutations that arose in the stems that subtended the donor or recipient flowers. Seedlings were transplanted into individual cone-shaped pots (21 cm deep) filled with potting soil, which were placed into random positions within separate 98-cell racks for each unit. Racks were placed in the University of Oregon greenhouse and bottom watered as needed for two weeks to allow seedlings to recover and establish their roots in the deep cones. After this, no water was added. From this point forward, a single researcher scored plant health every other day using a scale between 0 and 4 (as described in Sobel et al., 2019). A score of 0 indicated no sign of drought stress. A score of 1 indicated initial signs of drought stress, including the adaxial side of the leaves curling under. A score of 2 indicated the first sign of true wilting. A score of 3 indicated systemic and severe wilting. A score of 4 indicated the death of the plant. The experiment ended once all plants were assigned a score of 4. Plants were measured at the same time each day throughout the experiment, and the identity of the pollination treatment was kept blind to the evaluator until the end of the experiment. At the end of the experiment, the above-ground plant material was harvested, dried, and weighed to provide a final estimate of biomass at the time the plant died.

To provide an estimate of drought tolerance from these time-series data, we fit a three-parameter logistic curve to the drought scores estimated from each plant on each day of the experiment. Then, we estimated the parameter '*b*', which occurs at the time (in days) when the drought score reaches 50% of its maximum. This corresponds to the rate at which each plant begins showing obvious signs of drought stress, such that a larger value of '*b*' indicates a more drought-tolerant plant. This was repeated separately for each plant within each of the 10 units used in the drought experiments. We also estimated the time (in days) for plants to reach a drought score of 4 (i.e., the survival time). Twelve plants out of the 960 tested across all 10 units were removed from further analysis because they died too quickly to obtain estimates of drought tolerance.

Seed germination and growth rate

In addition to survival due to drought, we also examined the effects of pollination treatment on early-life stages. From a subset of four of the units described above (two units each from genets A and C), we measured germination timing and early seedling growth rates. We began by filling eight 96-cell plug trays with moist potting soil, and for each unit, we randomly sowed 192 seeds derived from autogamy and 192 seeds from geitonogamy (64 seeds from each of the three geitonogamous crosses), with two seeds of the same cross-type per cell. The eight trays that contained the 1,536 seeds were placed in a grow room equipped with fluorescent lights and maintained at 22 °C with a 16-hr photoperiod. Trays were bottom watered and overhead misted as needed. Seedling emergence was recorded at the same time each day for 16 days after the first seedling emerged. Each day, seedlings were digitally photographed from above with a ruler in the frame, and we estimated the total leaf area using Adobe Photoshop. To estimate early seedling growth rates, we subtracted the total leaf area on the first day a seedling emerged from the total leaf area on the final day of the experiment and divided this by the number of days since the seeding emerged. Seedlings that emerged from these four units were selected randomly at the end of this experiment to be used in the drought experiment described above.

Data analysis

To compare these experiments with previous work in other herbaceous and woody plant systems that focussed on the effects of seed production following autogamous and geitonogamous pollinations (Bobiwash et al., 2013; Cruzan et al., 2022), we first explored the effects of pollination treatment on seed production prior to offspring being exposed to drought conditions. We began by averaging the seed counts and seed and fruit weights from the multiple geitonogamous crosses per unit. Because different somatic variants are expected to arise in each stem, we grouped seed and fruit data by unit, as they are all derived from the same pollen source. We then performed paired *t*-tests among units to determine if fruit weight, seed weight, and seed count differed between autogamous and geitonogamous pollinations. Due to an expected relationship among these metrics of fecundity, we estimated the correlation coefficient between all three pairs of values.

We then examined the overall effects of pollination treatment and unit on drought tolerance, survival time, and biomass across the 10 units. In these analyses, we tested for an effect of unit, pollination treatment, and their interaction on variation in the different components of fitness. An interaction between pollination treatment and unit would indicate variable effects of pollination treatment among units, likely due to different sets of somatic mutations segregating among units. Moreover, from a subset of 4 of the 10 units, we estimated whether germination and/or early seedling growth rates varied between pollination treatments.

For the drought tolerance data, we tested for an effect of pollination treatment, unit, and their interaction using the *aov* function in R. We then used the *emmeans* package (Lenth, 2019) to perform pairwise comparisons of the estimated marginal mean values of the response variable from the model, adjusting *p*-values for multiple testing. These analyses were repeated separately for the survival and biomass data, which each were square root transformed to improve normality. For the four units that also included germination and seedling growth rate data, we performed a MANOVA to test if the combined effects of the five fitness components (germination, growth, drought tolerance, survival, and biomass) estimated from each seedling differed across pollination treatments, units, as well as their interaction. The effects of individual response variables were further tested using separate two-way ANOVAs.

Finally, we tested the hypothesis that the variance in drought tolerance following autogamy was higher for units that also had a higher mean fitness following autogamy. If somatic mutations improve offspring fitness, variation in fitness should be greater for progeny groups from autogamy than from geitonogamy. This is because somatic mutations will segregate as homozygotes and heterozygotes in autogamous progeny but will remain heterozygous in the progeny of geitonogamous crosses. For each of the 10 units, we calculated the percent difference in the standard deviation of drought tolerance between pollination treatments and regressed this against the percent deviation in mean drought tolerance between autogamous and geitonogamous treatments, as estimated from *emmeans*. The percent deviation in mean drought tolerance is similar to the parameter $\delta_{AD(k)}$ calculated in Cruzan et al. (2022), which represents the difference in mean fitness between autogamy ($w_{k(A)}$) and geitonogamy ($w_{k(G)}$) on each stem, k ($\delta_{AD(k)} = w_{k(A)} - w_{k(G)}$). Thus, this estimate reveals the extent that mean phenotypes differ due to pollination treatment.

Results

Seed production

We identified significant effects of cross type on seed production. Specifically, among the 42 units from 26 genets generated in this experiment, autogamous pollination consistently resulted in more seeds than geitonogamous pollination (mean autogamy = 260.7, 92 sd; mean geitonogamy = 234.5, 71.9 sd; paired *t*-test, $p = .014$; Figure 1, Supplementary Figure S1). Indeed, in 69% of the 42 units, the total seed count per fruit was higher following autogamous crosses. As expected, this pattern was similar for fruit weight and total seed weight (both $p = .004$), which were both strongly correlated with seed count and with each other (seed count vs seed weight: $r = 0.87$; seed count vs fruit weight: $r = 0.58$; seed weight vs fruit weight: $r = 0.79$).

Patterns of selection in offspring

Seedling drought tolerance varied between cross types and among units (Table 1). The interaction was also statistically significant, indicating that drought tolerance

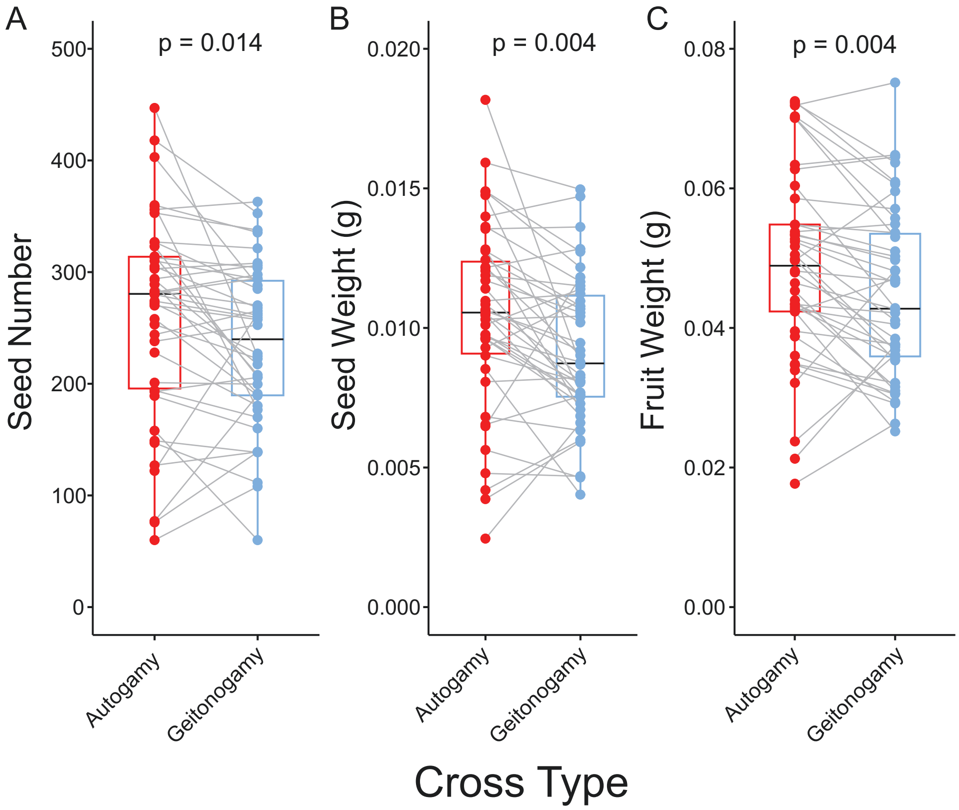

Figure 1. In each panel, grey lines connect the fecundity estimates from autogamous and geitonogamous pollinations from each of the 42 units in the experiment. Box plots show the median (in black), the bottom and top of the boxes correspond to the first and third quartile, respectively, and whiskers represent 1.5 times the interquartile range. *p*-values above each plot were estimated using paired *t*-tests. A) Seed number per fruit, B) total seed weight per fruit, and C) total fruit and seed weight. Values for geitonogamous crosses were averaged from the two or three crosses made within each unit.

varied depending on pollination treatment and unit, which is expected if somatic mutations arise independently in different units. Indeed, post hoc testing revealed that seedlings derived from autogamous pollination showed significantly higher drought tolerance relative to geitonogamy in units A1, C1, and C2 (Figure 2; Supplementary Figure S2). The other seven units showed no difference in drought tolerance between pollination treatments (Supplementary Table S1). Similarly, survival time varied among units and cross types (Table 1), with seedlings derived from autogamy in units A1, C1, and C2 surviving moderately longer than seedlings from geitonogamy (range of percent deviation in mean survival time between autogamy and geitonogamy: 2%–5%; Figure 2). By contrast, seedlings survived marginally longer following geitonogamy in unit R2 (adjusted $p = .084$, Supplementary Table S1), implying that somatic mutations may have resulted in the transmission of deleterious variation to offspring in that unit. In addition, drought tolerance and survival time were strongly positively correlated with each other ($r = 0.61$, $t = 23.6$, df = 946, $p < 2.2 \times 10^{-16}$). Consistent with previous work in this species that showed smaller plants were able to withstand drought better than larger seedlings (Sobel et al., 2019), drought tolerance was negatively correlated with biomass ($r = -0.57$, $t = -21.4$, df = 946, $p < 2.2 \times 10^{-16}$). However, despite this strong correlation, there was no effect of pollination treatment on biomass (Table 1). Only unit A1 showed a significant difference in biomass, with seedlings derived from geitonogamy having higher mean biomass than seedlings that were created from autogamy (Supplementary Table S1).

Table 1. Results from two-way analysis of variance, testing the effects of unit, pollination treatment, and their interaction on three response variables (drought tolerance, survival time, and biomass) in 10 units. Survival time and biomass were square root transformed. Results of post hoc contrasts can be found in Supplementary Table S1. df = degrees of freedom.

Source	df	*F* value	*p*
Drought tolerance			
Unit	9	24.42	$<2 \times 10^{-16}$
Pollination treatment	1	4.44	.036
Unit × Pollination treatment	9	2.34	.013
Survival time			
Unit	9	15.46	$<2 \times 10^{-16}$
Pollination treatment	1	5.51	.019
Unit × Pollination treatment	9	3.13	.001
Biomass			
Unit	9	27.00	$<2 \times 10^{-16}$
Pollination treatment	1	2.42	.120
Unit × Pollination treatment	9	0.99	.451

From four of the above units, we also measured the number of days for seeds to germinate and the growth rates of young

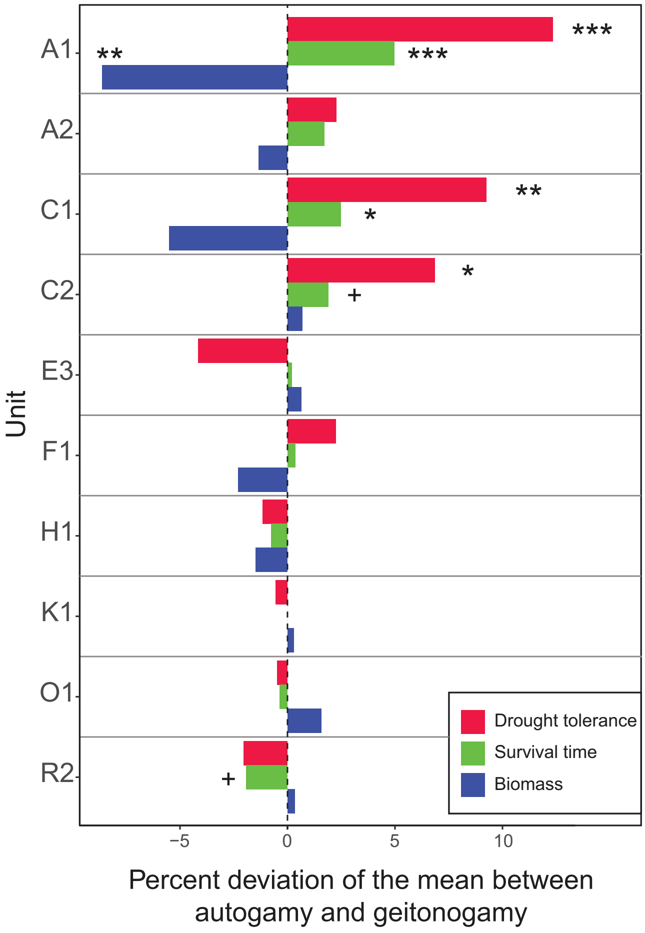

Figure 2. Shown is the percent deviation in the mean drought tolerance and the square root transformed survival time and biomass. Positive deviations indicate a higher mean value in seedlings derived from autogamy than geitonogamy, while a negative deviation indicates that the mean trait value is higher in plants derived from geitonogamy. Statistical significance is tested based on post hoc comparisons of the estimated marginal means following a complete two-way ANOVA model with predictor variables unit, cross type, and their interaction, and adjusting *p*-values for multiple testing. Distributions of the raw data for each pollination treatment and unit can be found as boxplots in Supplementary Figure S2. *** $p < .001$, ** $p < .01$, * $p < .05$, + $p < .1$.

Table 2. MANOVA results from the four units that were measured for all five aspects of fitness: germination timing, early seedling growth rate, drought tolerance, survival, and biomass. df = degrees of freedom, which are shown both for Pillai's statistic and the approximate *F* value. All variables were square root transformed prior to analysis. The effects of these predictors on individual response variables can be found in Supplementary Table S2.

Source	Pillai (df)	Approx. *F*	df	*p*
Unit	0.39 (3)	10.9	15, 1,089	$<2.2 \times 10^{-16}$
Pollination treatment	0.09 (1)	6.9	5, 361	3.7×10^{-6}
Unit × Pollination treatment	0.05 (3)	1.1	15, 1,089	.33

seedlings. We used MANOVA to test if there was a significant effect of unit, cross type, or their interaction among the combined set of five fitness estimates. We found an overall effect of pollination treatment and unit but no significant interaction (Table 2). These results imply that at least some of the fitness components contribute to differences between pollination treatment and among units, but the effect does not depend on their interaction. Post hoc testing of individual fitness measures revealed no differences in the timing of germination or early seedling growth rates based on pollination treatment, but differences in drought tolerance, survival, and biomass were consistent with the full analysis involving all 10 units (Supplementary Table S2).

The most striking differences in mean fitness between pollination treatments come from the offspring in unit A1. Indeed, we found significant differences in drought tolerance, survival, and biomass (Supplementary Table S1), with higher mean drought tolerance and survival and lower biomass for seedlings derived from autogamous crosses (Figure 2). Specifically, drought tolerance and survival were approximately 2 days longer, on average, among offspring produced by autogamy in this unit relative to geitonogamy (mean drought tolerance: autogamy = 17.3 days, 4.8 sd; geitonogamy = 15.4 days, 3.8 sd; mean survival: autogamy = 28.1, 4.5 sd; geitonogamy = 25.5, 4.4 sd). By contrast, mean biomass was lower for autogamy (0.09 g, 0.05 sd) than for geitonogamy (0.11 g, 0.05 sd). This difference resulted in a negative correlation between drought tolerance and both early seedling growth rates and biomass (Supplementary Figures S3 and S4), again consistent with previous findings in this species that revealed smaller plants were better able to withstand drought conditions (Sobel et al., 2019). Interestingly, pollination treatment led to consistent differences in drought tolerance and survival time for both units C1 and C2. By contrast, unit A1 showed an effect of pollination treatment on variation in these traits, but unit A2 did not. These units were created from different stems (and pollen sources) on genets A and C. Thus, even though units A1 and A2 were both produced using pollen derived from the same genet, they showed striking differences in their fitness effects due to the pollination treatments, implying that somatic mutations that accumulated in one stem may not be present elsewhere in the plant.

In addition to differences in fitness between pollination treatments, we also predicted that the transmission of beneficial somatic mutations should result in an increased variance in fitness in the offspring from autogamous pollinations compared to geitonogamous pollinations. This is because somatic mutations will segregate as both homozygotes and heterozygotes following autogamy, whereas they will remain in the heterozygous state in offspring after geitonogamy. Thus, offspring produced by autogamy will segregate a more diverse set of genotypes, resulting in higher variance in fitness. To investigate this, we explored the relationship between the percent deviation in mean drought tolerance between autogamy and geitonogamy and the percent deviation between these pollination treatments in the standard deviation for drought tolerance across each of the 10 units. We found a clear, positive relationship, such that units with higher mean drought tolerance following autogamy tended to also have a higher standard deviation in drought tolerance ($F = 9.03$; df = 1,8; $p = .017$; $R^2 = 0.53$; Figure 3). These results imply that there is a higher variance in drought tolerance following autogamy in the units that showed stronger fitness advantages, consistent with the effects of somatic mutations accumulating within stems and increasing drought tolerance as they segregate in offspring.

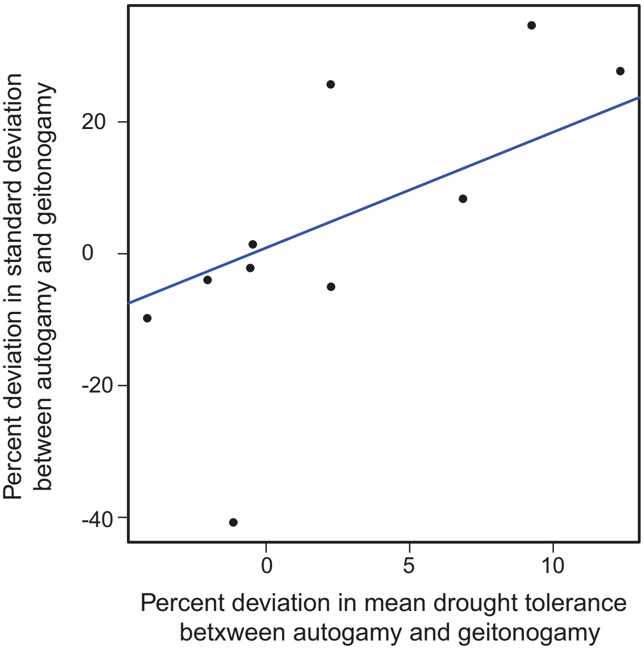

Figure 3. The difference in the standard deviation between autogamy and geitonogamy is higher in experimental units with a greater difference in mean drought tolerance following autogamy. Shown are the percent deviations in mean drought tolerance and standard deviation in drought tolerance between autogamy and geitonogamy among units. Positive deviations indicate that the mean (or standard deviation) is greater in seedlings derived from autogamy. The trendline from the linear regression between the two variables is shown.

Discussion

In this study, we demonstrated that the accumulation of somatic mutations in vegetative tissue can lead to variable impacts on the fitness of plants in the following generation. The general finding that fitness effects varied among units is consistent with prior experiments in *M. guttatus* (Cruzan et al., 2022), and with expectations from models of CLS (Fagerstrom et al., 1998; Monro & Poore, 2009; Otto & Hastings, 1998). Specifically, if differences in division rates are determined by somatic mutations, and cell lineages with faster growth can displace slower ones, we would expect mutations enhancing growth to be retained and those that grow slowly to be removed (Klekowski, 2003; Monro & Poore, 2009; Pineda-Krch & Fagerstrom, 1999; Poethig, 1987). Indeed, pollination treatment had no impact on fitness for most of the units, but in some cases, we detected modest beneficial or deleterious effects on seed production or drought tolerance, likely due to inherited somatic mutations. These results raise the potential that somatic mutations may be important for plant evolution.

In spite of slow division rates and possibly enhanced DNA repair capacity (Heyman et al., 2013; Yadav et al., 2009), plants often show reduced seed production (or increased embryo abortion) following autogamous compared to geitonogamous pollinations, which has been referred to as autogamy depression (Schultz & Scofield, 2009). This implies that meristem cells accumulate substantial levels of mutational load during stem elongation, likely due to the appearance of numerous somatic mutations. Autogamy depression for seed and fruit abortion has been observed in several species (reviewed in Bobiwash et al., 2013), including *M. guttatus* (Cruzan et al., 2022), and it has been argued to be stronger in longer-lived plants, as longer lifespan should correspond to more mitotic cell divisions and thus a greater opportunity for somatic mutation accumulation (Ally et al., 2010; Barrett, 2015; Schultz & Scofield, 2009).

Although *M. aurantiacus* is a long-lived perennial shrub, and some units did produce substantially fewer seeds following autogamy, there was an overall average increase in seed production following autogamy across all 42 units. Given that both cross types are self-fertilizations, these differences cannot be attributable to variation in the strength of inbreeding depression between treatments. Rather, these results suggest the potential that beneficial somatic variants whose fitness effects outweigh those of deleterious mutations were transmitted to progeny, resulting in a net increase in seed production. Because deleterious mutations can be filtered out prior to fertilization, there may have been a shift in the distribution of fitness effects that was skewed toward the transmission of more beneficial mutations rather than deleterious ones. Although these findings conflict with trends seen in other species that suggest autogamy depression is common (Bobiwash et al., 2013; Klekowski, 1998), they are consistent with a pattern seen in *Arabidopsis thaliana*, where there was unexpectedly high transmission of beneficial mutations during mutation accumulation studies (Rutter et al., 2010, 2012, 2018; Shaw et al., 2002). While these findings are intriguing, it is important to note that investigations into the fitness consequences of somatic mutations remain in their infancy, with much still to be learned about the segregation and development of the germline in plants (Burian, 2021). Although deep sequencing approaches have characterized somatic evolution in humans (Lawson et al., 2024; Martincorena et al., 2017), these studies are unable to directly estimate the fitness effects of the mutations. Combining these sequencing approaches in plant systems (e.g., *Mimulus*) where phenotypic selection experiments in offspring are possible will be critical to determine the consistency of these patterns.

In addition to fecundity, we also measured variation in five aspects of fitness among the offspring of autogamous and geitonogamous pollinations. These components of selection acted at different stages of the plant life cycle, beginning with germination and continuing through early seedling growth rates, drought tolerance, survival, and total biomass. We did not find significant differences in fitness between pollination treatments for germination or early seedling growth rates in the four units tested, and many of the remaining units showed no measurable differences between pollination treatment for the other fitness estimates (Supplementary Table S2). However, we found three units (A1, C1, and C2) that showed increases in tolerance to drought in offspring derived from autogamy. We also found a clear relationship that offspring groups that had higher mean drought tolerance following autogamy also had higher variance in drought tolerance, which is in line with previous results demonstrating an increased variance in fitness in seedlings following autogamous pollination (Cruzan et al., 2022). In one case (unit R2), we found slightly higher survival in seedlings following geitonogamy, which suggests that the transmission of deleterious somatic mutations may have occurred in the autogamous line. Thus, these results reveal the variable effects of somatic mutations on fitness among units, with both beneficial and deleterious variation occasionally being transmitted to offspring, implying a potential role for somatic variation in local adaptation.

Despite detecting significant differences for fitness components in some of the units, the observed differences in mean phenotypes between pollination treatments were small (range of percent deviation in mean drought tolerance in units A1, C1, and C2 between autogamy and geitonogamy: 6.9%–12.3%). This result is expected because offspring were grown in an environment that closely matches their native habitat. The populations of *M. aurantiacus* used in this experiment occur naturally in chapparal communities of southern California, which are characterized by hot, dry summers, and short winters that are cool and moist (Beeks, 1962). Seedling recruitment tends to be very low due to the rapid drying of the soil in spring and summer. Thus, the terminal drought experiment we conducted closely mimics the conditions experienced by seedlings in the wild (Sobel et al., 2019). As a result, we would expect that plants were already near their adaptive peaks for drought tolerance, suggesting that most new mutations would not greatly improve fitness (Orr, 2005). By contrast, previous work in the herbaceous *M. guttatus* revealed that somatic mutations accumulating during vegetative growth had large, beneficial effects in offspring in 5 of the 14 stems tested (Cruzan et al., 2022). In that case, the parental plants and progeny were grown in a novel environment (hydroponic salt stress), implying that there was likely a broader spectrum of mutations that could have improved the phenotype in that new environment. As a consequence, our findings of statistically significant interactions between pollination treatment and unit for both drought tolerance and survival are consistent with our a priori predictions that few of the units should demonstrate phenotypic effects in this environment and that any fitness effects we did observe should be small.

Although the segregation of somatic mutations in offspring can obscure overall statistical patterns between pollination treatments, we can still see the net fitness effects of these variants in individual progeny. Specifically, we observed individual plants derived from autogamous pollination that have exceptional values of fitness, especially for drought tolerance, survival, and biomass. For example, in units A1 and C1 (the units that show the largest differences in mean drought tolerance between pollination treatments), we see that the plants with the highest drought tolerance are derived from autogamy. These plants have drought tolerance values that are more than three standard deviations above the mean (Supplementary Figure S5). This trend continues with the later-acting fitness components, such that these same plants also have consistently extreme values of survival and biomass. We also found a corresponding negative relationship between drought tolerance and early seedling growth rate, such that smaller plants tended to survive longer under drought conditions. These results are consistent with those of Sobel et al. (2019), who found that smaller *M. aurantiacus* plants tended to better withstand desiccation. They suggested that the reduced leaf area of smaller plants likely resulted in lower transpiration, leading to greater drought tolerance and thus longer survival under terminal drought conditions. Thus, the segregation of somatic variants appears to result in some progeny with extreme values of fitness. As noted above, future studies that take advantage of the power of deep sequencing will allow us to identify individual somatic variants that accumulated in parent stems. By following the segregation of these specific mutants in the next generation, we can track the fitness consequences of these variants in different environments.

In conclusion, we find evidence for highly variable fitness effects of somatic mutations being transmitted to progeny, with occasional beneficial effects, raising the possibility that somatic variation can be an important source of genetic variation affecting plant evolution. By comparing these results with those from the closely related *M. guttatus* with different life history characteristics (Cruzan et al., 2022), the current study found similar, though more subtle, fitness consequences following autogamy. Thus, despite differences in the species, environmental conditions, and life history strategies of the plants studied, the effects of somatic mutations on fitness appear to be consistent, further highlighting the potential relevance of somatic mutation for plant evolution.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

All analyses were performed in R. The data and code used for all analyses and to generate all figures are available here: https://doi.org/10.5061/dryad.bnzs7h4nf.

Author contributions

Matthew Streisfeld (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Funding acquisition [equal], Investigation [lead], Methodology [lead], Writing—original draft [lead], Writing—review & editing [lead]), Jessie Crown (Formal analysis [supporting], Methodology [supporting]), Jack McLean (Formal analysis [supporting], Methodology [supporting], Writing—original draft [supporting]), Aidan Short (Formal analysis [supporting], Methodology [supporting], Software [supporting]), and Mitch Cruzan (Conceptualization [equal], Investigation [equal], Methodology [equal], Writing—review & editing [supporting])

Acknowledgments

We would like to thank members of the Cruzan lab for their feedback on earlier drafts of this manuscript. We also thank S. Medbury for his assistance with plant care in the University of Oregon greenhouses. This work was supported by an award from the National Science Foundation (NSF-DEB 2051242) to MAS and MBC.

Conflicts of interest

None declared.

References

Ally, D., Ritland, K., & Otto, S. P. (2010). Aging in a long-lived clonal tree. *PLoS Biology*, *8*(8), e1000454. https://doi.org/10.1371/journal.pbio.1000454

Antolin, M. F., & Strobeck, C. (1985). The population genetics of somatic mutation in plants. *American Naturalist*, *126*, 52–62.

Barrett, S. C. H. (2015). Influences of clonality on plant sexual reproduction. *Proceedings of the National Academy of Sciences*, *112*(29), 8859–8866. https://doi.org/10.1073/pnas.1501712112

Beeks, R. M. (1962). variation and hybridization in southern California populations of *Diplacus* (Scrophulariaceae). *El Aliso*, *5*, 83–122.

Bobiwash, K., Schultz, S. T., & Schoen, D. J. (2013). Somatic deleterious mutation rate in a woody plant: Estimation from phenotypic data. *Heredity*, *111*(4), 338–344. https://doi.org/10.1038/hdy.2013.57

Buchholz, J. T. (1922). Developmental selection in vascular plants. *Botanical Gazette*, *73*(4), 249–286. https://doi.org/10.1086/332991

Burian, A. (2021). Does shoot apical meristem function as the germline in safeguarding against excess of mutations? *Frontiers in Plant Science*, *12*, 707740. https://doi.org/10.3389/fpls.2021.707740

Burian, A., & Barbier de Reuille, K. (2016). Patterns of stem cell divisions contribute to plant longevity. *Current Biology*, *26*(11), 1385–1394.

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*(11), 783–796. https://doi.org/10.1038/nrg2664

Chase, M. A., Stankowski, S., & Streisfeld, M. A. (2017). Genomewide variation provides insight into evolutionary relationships in a monkeyflower species complex (Mimulus sect. Diplacus). *American Journal of Botany*, *104*(10), 1510–1521. https://doi.org/10.3732/ajb.1700234

Cruzan, M. B. 2018. *Evolutionary biology—A plant perspective*. Oxford University Press.

Cruzan, M. B., Streisfeld, M. A., & Schwoch, J. A. (2022). Fitness effects of somatic mutations accumulating during vegetative growth. *Evolutionary Ecology*, *36*(5), 767–785. https://doi.org/10.1007/s10682-022-10188-3

D’Amato, F. (1996). Role of somatic mutations in the evolution of higher plants. *Caryologia*, *50*(1), 1–15.

Fagerstrom, T., Briscoe, D. A., & Sunnucks, P. (1998). Evolution of mitotic cell-lineages in multicellular organisms. *Trends in Ecology and Evolution*, *13*(3), 117–120. https://doi.org/10.1016/S0169-5347(97)01314-1

Fetsscher, A. E., & Kohn, J. R. (1999). Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *American Journal of Botany*, *86*, 1130–1135.

Gaut, B., Yang, L., Takuno, S., & Eguiarte, L. E. (2011). The patterns and causes of variation in plant nucleotide substitution rates. *Annual Review of Ecology, Evolution, and Systematics*, *42*(1), 245–266. https://doi.org/10.1146/annurev-ecolsys-102710-145119

Heyman, J., Cools, T., Vandenbussche, F., … De Veylder, L. (2013). ERF115 controls root quiescent center cell division and stem cell replenishment. *Science*, *342*, 860–863.

Klekowski, E. J. (2003). Plant clonality, mutation, diplontic selection, and mutational meltdown. *Biological Journal of the Linnean Society*, *79*(1), 61–67. https://doi.org/10.1046/j.1095-8312.2003.00183.x

Kwiatkowska, D. (2008). Flowering and apical meristem growth dynamics. *Journal of Experimental Botany*, *59*(2), 187–201. https://doi.org/10.1093/jxb/erm290

Lanfear, R. (2018). Do plants have a segregated germline? *PLoS Biology*, *16*(5), e2005439. https://doi.org/10.1371/journal.pbio.2005439

Lawson, A. R., Abascal, F., Nicola, P. A., … Martincorena, I. (2024). Somatic mutation and selection at epidemiological scale. *medRxiv*, 2024–2010.

Lenth, R. (2019). *Emmeans: Estimated marginal means, aka least-squares means. R package version 1.4.2*. https://CRAN.R-project.org/package=emmeans

Martincorena, I., Raine, K. M., Gerstung, M., … Campbell, P. J. (2017). Universal patterns of selection in cancer and somatic tissues. *Cell*, *171*(5), 1029–1041.

McMinn, H. E. (1951). Studies in the genus *Diplacus*, scrophulariaceae. *Madrono*, *11*, 33–128.

Monro, K., & Poore, A. G. B. (2009). The potential for evolutionary responses to cell-lineage selection on growth form and its plasticity in a red seaweed. *American Naturalist*, *173*(2), 151–163. https://doi.org/10.1086/595758

Orr, H. A. (2005). The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*, *6*(2), 119–127. https://doi.org/10.1038/nrg1523

Otto, S. P., & Hastings, I. M. (1998). Mutation and selection within the individual. *Genetica*, *102-103*(1-6), 507–524.

Otto, S. P., & Orive, M. E. (1995). Evolutionary consequences of mutation and selection within an individual. *Genetics*, *141*(3), 1173–1187.

Pineda-Krch, M., & Fagerstrom, T. (1999). On the potential for evolutionary change in meristematic cell lineages through intraorganismal selection. *Journal of Evolutionary Biology*, *12*, 681–688.

Pineda-Krch, M. P., & Lehtila, K. (2002). Cell lineage dynamics in stratified shoot apical meristems. *Journal of Theoretical Botany*, *219*(4), 495–505.

Poethig, R. S. (1987). Clonal analysis of cell lineage patterns in plant development. *American Journal of Botany*, *74*(4), 581–594. https://doi.org/10.1002/j.1537-2197.1987.tb08679.x

Rutter, M. T., Roles, A., Conner, J. K., … Fenster, C. B. (2012). Fitness of *Arabidopsis thaliana* mutation accumulation lines whose spontaneous mutations are known. *Evolution*, *66*(7), 2335–2339. https://doi.org/10.1111/j.1558-5646.2012.01583.x

Rutter, M. T., Roles, A. J., & Fenster, C. B. (2018). Quantifying natural seasonal variation in mutation parameters with mutation accumulation lines. *Ecology and Evolution*, *8*(11), 5575–5585. https://doi.org/10.1002/ece3.4085

Rutter, M. T., Shaw, F. H., & Fenster, C. B. (2010). Spontaneous mutation parameters for *Arabidopsis thaliana* measured in the wild. *Evolution*, *64*(6), 1825–1835. https://doi.org/10.1111/j.1558-5646.2009.00928.x

Schultz, S. T., & Scofield, D. G. (2009). Mutation accumulation in real branches: Fitness assays for genomic deleterious mutation rate and effect in large-statured plants. *The American Naturalist*, *174*(2), 163–175. https://doi.org/10.1086/600100

Shaw, F. H., Geyer, C. J., & Shaw, R. G. (2002). A comprehensive model of mutations affecting fitness and inferences for *Arabidopsis thaliana*. *Evolution*, *56*(3), 453–463. https://doi.org/10.1111/j.0014-3820.2002.tb01358.x

Sobel, J. M., Stankowski, S., & Streisfeld, M. A. (2019). Variation in ecophysiological traits might contribute to ecogeographic isolation and divergence between parapatric ecotypes of *Mimulus aurantiacus*. *Journal of Evolutionary Biology*, *32*(6), 604–618. https://doi.org/10.1111/jeb.13442

Sobel, J. M., & Streisfeld, M. A. (2015). Strong premating reproductive isolation drives incipient speciation in *Mimulus aurantiacus*. *International Journal of Organic Evolution*, *69*(2), 447–461.

Yadav, R. K., Girke, T., Pasala, S., … Reddy, V. (2009). Gene expression map of the *Arabidopsis* shoot apical meristem stem cell niche. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 4941–4946.

Yu, L., Boström, C., Franzenburg, S., … Reusch, T. B. H. (2020). Somatic genetic drift and multilevel selection in a clonal seagrass. *Nature Ecology and Evolution*, *4*, 952–962. https://doi.org/10.1038/s41559-020-1196-4