Fitness Effects of Somatic Mutations Accumulating during Vegetative Growth

Mitchell B. Cruzan[1, 2], Matthew A. Streisfeld[3], and Jaime A. Schwoch[1]

[1]Department of Biology, Portland State University, Portland, OR 97207

[3]Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403

[2]Author for correspondence: cruzan@pdx.edu

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Abstract

The unique life form of plants promotes the accumulation of somatic mutations that can be passed to offspring in the next generation, because the same meristem cells responsible for vegetative growth also generate gametes for sexual reproduction. However, little is known about the consequences of somatic mutation accumulation for offspring fitness. We evaluate the fitness effects of somatic mutations in *Mimulus guttatus* by comparing progeny from self-pollinations made within the same flower (autogamy) to progeny from self-pollinations made between stems on the same plant (geitonogamy). The effects of somatic mutations are evident from this comparison, as autogamy leads to homozygosity of a proportion of somatic mutations, but progeny from geitonogamy remain heterozygous for mutations unique to each stem. In two different experiments, we find consistent fitness effects of somatic mutations from individual stems. Surprisingly, several progeny groups from autogamous crosses displayed increases in fitness compared to progeny from geitonogamy crosses, indicating that beneficial somatic mutations were prevalent in some stems. These results support the hypothesis that somatic mutations accumulate during vegetative growth, but they are filtered by different forms of selection that occur throughout development, resulting in the culling of expressed deleterious mutations and the retention of beneficial mutations.

Key words: Acquired mutations, autogamy, autogamy depression, cell lineage selection, dominance, *Erythranthe guttata*, geitonogamy, *Mimulus guttatus*, somatic mutations.

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 majority of organisms, including viruses, unicellular microbes, and some multicellular eukaryotes, sexual reproduction is rare or absent. In these organisms, mutations can occur during mitotic cell replication, and the primary mechanism for adaptation and diversification occurs via selection on cell lineages without recombination. By contrast, acquired mutations occurring during somatic growth of animals are not heritable. This is because in metazoans (but possibly excepting corals; Schweinsberg et al. 2014; Barfield et al. 2016), the germline is determined early in development and relatively few cell divisions occur before the formation of gametes (the Weismann Barrier; Buss 1983). Consequently, heritable mutations only occur in animals during the development of gonads and gametes.

Plants differ from animals and microbes, because mutations contributing to heritable variation can arise both during gamete formation and somatic growth (Antolin and Strobeck 1985; Klekowski and Godfrey 1989). This is due to the fact that plants lack a separate germline; the same germ cells within apical meristems subtend both vegetative growth and gamete development. Consequently, plants have the capacity to accumulate somatic mutations during vegetative growth that can 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; Yu et al. 2020). 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 extent and evolutionary importance of somatic mutation accumulation in plants (Schultz and Scofield 2009; Burian et al. 2016; Watson et al. 2016; Kuhlemeier 2017; Schmid-Siegert et al. 2017; Plomion et al. 2018; Hanlon et al. 2019).

In general, it is difficult to assess the effects of somatic mutations accumulating during growth because clonal cell lineages lack recombination, and the fitness effects of beneficial mutations can be muted by the co-occurrence of detrimental genetic variants (Hill-Robertson Effect; Selective Interference; Hill and Robertson 1966; Fogle et al. 2008). Although sexual reproduction does allow mutations to segregate among progeny, it is difficult to detect the fitness effects of somatic mutations in

64 eukaryotes with separate sexes. This is because the genomes of two individuals are combined, so
65 individual somatic mutations that occur in one individual will always be heterozygous in offspring (Long
66 et al. 2015). A more effective approach would be to make self-fertilizations in a hermaphroditic species,
67 so a proportion of offspring would be homozygous for novel somatic mutations. In this study, we make
68 crosses within individual clones of hermaphroditic plants to assess the fitness effects of inherited
69 mutations that accumulated during somatic growth.

70 Hermaphroditic plants have several advantages for the study of somatic mutations. Separate
71 stems on the same plant contain multiple germ cell lineages that are derived from the same zygote, but
72 they may differ for somatic mutations that have accumulated during stem elongation. To produce
73 recombinant progeny segregating for somatic mutations unique to each stem, crosses can be made
74 either within the same flower (autogamy), or between flowers on separate stems of the same plant
75 (inter-ramet geitonogamy – hereafter referred to simply as geitonogamy; Fig. 1). These crosses are both
76 self-fertilizations, but the offspring will differ in the complement of somatic mutations that they inherit.
77 Mutations unique to a stem will be homozygous in 25% of autogamous progeny, while none of the
78 progeny from geitonogamous crosses will be homozygous, and half will be heterozygous for unique
79 somatic mutations. Thus, the average fitness effects of somatic mutations unique to each stem can be
80 evaluated by comparing progeny generated by autogamous and geitonogamous crosses of the same
81 stem. The potential for somatic mutations to accumulate during vegetative growth, and the ability to
82 study their effects among recombinant progeny, make plants an attractive model for the study of
83 fundamental processes of clonal evolution.

84 Plants grow from the division of a population of undifferentiated meristem cells within the stem
85 tip that is known as the central zone. These germ cell lineages go on to produce future stem, leaf, and
86 reproductive tissues (flowers). As plants grow, individual ramets of the same genet (separate stems or
87 vegetatively propagated plants) can continue to become differentiated as they accumulate somatic
88 mutations (Fig. 2). Therefore, when we consider the potential for meiotic and somatic mutations to
89 contribute to the total mutational load of plant populations – particularly for long-lived plants – it
90 becomes evident that not all of the mutations occurring during a plant's lifespan are passed to the next
91 generation (Cruzan 2018 pages 86-98). Indeed, plants have mechanisms of "developmental selection"
92 (Buchholz 1922) that occur during vegetative growth and reproduction to filter the set of mutations that
93 are inherited by progeny. For example, because new somatic mutations occur as a single copy within the
94 diploid genome, their immediate fitness effects will depend on the combination of their fitness effects
95 and their expression in the heterozygous state. Mathematical models have demonstrated that multiple

96 unexpressed mutations (i.e. neutral and recessive deleterious mutations) are likely to accumulate as
97 germ cells divide, so any selective cell deaths that occur during somatic growth will reduce the total
98 mutational load and alter the composition of mutations carried by the germ cell population (Otto and
99 Orive 1995; Otto and Hastings 1998). Thus, lineages that carry expressed deleterious mutations causing
100 slower rates of division can be eliminated as they are replaced by cell lineages displaying more rapid
101 growth (Otto and Orive 1995; Otto and Hastings 1998; Orive 2001; Elena and Lenski 2003; Greaves and
102 Maley 2012; Long et al. 2015). (Fig. 2A). More rarely, expressed beneficial mutations will arise. Similar to
103 the process of clonal evolution in mammalian cancer, any mutations that elevate the rate of cell division
104 will tend to increase in frequency until they have replaced the entire germ cell population (clonal
105 selective sweep; Nowell 1976; Lang et al. 2013; Fig. 2B green arrows). This process – referred to as cell
106 lineage selection – will lead to some ramets containing a prevalence of deleterious somatic mutations,
107 while others could possess beneficial mutations. The potential for these clonal selective sweeps to occur
108 during vegetative growth has been confirmed by the observation of somatic mutations occurring at
109 frequencies near 50% (heterozygosity) within the stem tissue (Yu et al. 2020; Schwoch et al. unpublished
110 data). Hence, germ cell populations in plant apical meristems have the potential to undergo clonal
111 evolution during vegetative growth.
112 In addition to cell lineage selection, some proportion of recessive deleterious mutations may be
113 eliminated during the haploid life stage due to pollen tube attrition and pollen competition
114 (Gemetophytic Selection; Mulcahy 1979; Cruzan 1989; Mable and Otto 1998; Armbruster and Rogers
115 2004; Arunkumar et al. 2013; Harder et al. 2016). Finally, a portion of deleterious mutations will be
116 homozygous in zygotes, which can lead to higher rates of seed and fruit abortion (Selective Embryo
117 Abortion; Husband and Schemske 1995; Korbecka et al. 2002), thereby increasing the average fitness of
118 surviving offspring (Cruzan and Thomson 1997; Mena-Alí and Rocha 2005). The sum effects of these
119 processes of developmental selection, which includes cell lineage selection, gametophytic selection, and
120 selective embryo abortion, may filter the set of mutations that enters the next generation. Indeed, the
121 effects of deleterious somatic mutations often are apparent as higher rates of embryo abortion after
122 autogamous compared to geitonogamous pollinations, which is referred to as autogamy depression
123 (Schultz and Scofield 2009). While autogamy depression for seed and fruit abortion has been observed
124 in several species (reviewed in Bobiwash et al. 2013), no previous study has evaluated the fitness effects
125 of somatic mutations inherited by progeny. The question we address here is whether somatic mutations
126 that characterize individual ramets have measurable fitness effects in the offspring generation.

127 In this study, we use autogamous and geitonogamous self-pollinations to estimate the fitness
128 effects of somatic mutations segregating in offspring of *Mimulus guttatus* DC (*Erythranthe guttata* G.L.
129 Nesom; Phrymaceae). Populations of *M. guttatus* display a wide range of life histories – from annuals to
130 herbaceous perennials that outcross to varying degrees (Wu et al. 2008). We use perennial *M. guttatus*
131 plants that produce substantial vegetative growth prior to initiation of flowering. These plants are easily
132 propagated from rosettes, and selfing produces substantial numbers of seeds to allow for statistical
133 comparisons. In two separate experiments, we characterized the fitness effects of somatic mutations in
134 perennial *M. guttatus* plants in controlled environments.
135 We begin by describing the analytical approaches we use, followed by providing specifics of the
136 two experiments that we conducted. We show that somatic mutations accumulate during stem growth,
137 as progeny from autogamous crosses have greater variance in mean fitness compared to progeny from
138 geitonogamous crosses. We further reveal evidence for the presence of beneficial mutations in some
139 stems, which indicates that developmental selection may be responsible for filtering mutations and
140 modifying the distribution of fitness effects transmitted to progeny. The results from these experiments
141 provide the first evidence that inherited somatic mutations can have fitness consequences for plants in
142 the next generation, which suggests that somatic mutations may be an additional source of genetic
143 variation that can be used for adaptation.
144
145 **Approach**
146 We made autogamous and geitonogamous self-pollinations across multiple stems and estimated the
147 fitness effects of somatic mutations segregating in progeny. To increase the chances of detecting
148 somatic mutations that impacted fitness, we exposed plants to novel environments. According to
149 Fisher's geometric model, displacement from a fitness optimum increases the chances that any
150 mutation would move a genotype in a direction that increases fitness (Fisher 1930; Stearns and Fenster
151 2016). Furthermore, we evaluated fitness of seedlings under novel selection regimes in the greenhouse.
152 Exposing parental plants and seedlings to the same controlled environments is common in experimental
153 designs that test for the fitness effects of mutations accumulating across generations (Shaw et al. 2002;
154 Baer et al. 2007; Halligan and Keightley 2009), or in our case, for observing the fitness effects of somatic
155 mutations arising within a single generation.
156 For a diploid plant, we can assume that somatic mutations ($a \rightarrow a'$) will be in the heterozygous
157 state when they first appear. For progeny arising by autogamy, a mutation will segregate as 25%
158 homozygous ($a'a'$), 50% heterozygous (aa'), and 25% the original homozygote (aa). By contrast, because

159 progeny from geitonogamous crosses will be segregating for mutations that are unique to each stem,
160 half of them will be carrying mutations in the heterozygous state, and none of the progeny will be
161 homozygous for mutations that arose in a single stem. The fitness effects of somatic mutations
162 accumulating during vegetative growth have been quantified in terms of "autogamy depression" (δ_{AD})
163 using the formula; $\delta_{AD} = 1 - w_A/w_G$ (Schultz and Scofield 2009; Bobiwash et al. 2013), where w_A and w_G
164 are the relative fitnesses of progeny derived from autogamous and geitonogamous crosses, respectively.
165 Tests of autogamy depression have been applied previously only to estimates of fitness based
166 on fruit and seed set, and it generally assumes that fitness from autogamous crosses will be lower than
167 from geitonogamy. However, somatic mutations that are transmitted to progeny can affect fitness in the
168 offspring generation across different life stages, and beneficial mutations are possible. Thus, for each
169 stem, we examined the fitness effects of somatic mutations unique to stem k as: $\delta_{AD(k)} = \overline{w}_{k(A)} - \overline{w}_{k(G)}$,
170 where $\overline{w}_{k(A)}$ and $\overline{w}_{k(G)}$ are the average fitnesses of the progeny from autogamous and geitonogamous
171 crosses from stem k, respectively. We evaluate δ_{AD} for each stem separately, because we expect that
172 individual stems will have unique complements of somatic mutations and thus differ in their fitness
173 effects. By controlling for environmental effects with a common garden design, the parameter $\delta_{AD(k)}$ and
174 its sign describe the average magnitude and overall direction of the fitness effects of all expressed
175 somatic mutations that have been transmitted to the next generation. To evaluate the reliability of $\delta_{AD(k)}$
176 as an estimate of the fitness effects of somatic mutations, we simulated variation in the strength of
177 selection and dominance of somatic mutations across multiple pairs of stems (ramets). We determined
178 that values of $\delta_{AD(k)}$ were accurate across a range of values and assumptions for the effects of multiple
179 mutations (Appendix 1).
180 To provide additional confirmation of the $\delta_{AD(k)}$ estimates, we developed an alternative approach
181 for estimating the fitness effects of somatic mutations that was based entirely on the variance in mean
182 fitness among autogamous progeny. Individual germ cell lineages within the meristem may possess
183 unique sets of somatic mutations (Yu et al. 2020; Schwoch et al. in preparation), and flowers on the
184 same stem can develop from only a portion of the germ cells present in the meristem (McManus and
185 Veit 2002). Hence, no pair of flowers within a stem is likely to contain exactly the same complement of
186 mutations. Therefore, we generated an additional estimate of the fitness effects of somatic mutations
187 unique to a stem that is based on the variance in fitness of offspring from autogamous crosses. If
188 somatic mutations affect offspring fitness, the variance in fitness should be greater for progeny groups
189 from autogamy than from geitonogamy from the same stem, as long as mutations do not have complete
190 expression in heterozygotes (i.e. $h < 1.0$; Appendix 2). Somatic mutations that are unique to stems will

191 have different effects on the variance in fitness among progeny from autogamy depending on their
192 fitness (w) and their expression in the heterozygous state (h). Our analytical results demonstrated that
193 the large majority of variance in fitness among progeny from autogamy (as measured by the standard
194 deviation, SD) could be explained by the fitness effects of a mutation rather than the dominance level
195 (Appendix 2; Fig. S1). Moreover, the derived relationship allows us to estimate the fitness effects of
196 mutations based on the standard deviation in fitness according to the equation: $w_{SD} = cSD$, where c is
197 the slope of the linear relationship between w_{SD} and SD. For dominance levels of h = 0.0 and 1.0, c =
198 2.31, and the slope reaches a maximum of 3.01 when h = 0.5 (Appendix 2; Fig. S1). Thus, the variance in
199 fitness among progeny from autogamous crosses provides a good approximation of the magnitude of
200 the fitness effects (w) for somatic mutations accumulating in each stem. These two approaches are
201 independent of each other, and therefore provide an important means to confirm our estimates of the
202 average fitness effects of somatic mutations present in individual stems (Appendix 1 and 2).
203 From the considerations above, we can make three specific predictions that allow us to test for
204 the fitness effects of somatic mutations that occur during stem growth and are passed to offspring. First,
205 there should be greater variation among progeny groups from autogamy compared to those from
206 geitonogamy (i.e. as indicated by the stem x cross type interaction for progeny fitness). Second, the
207 average fitness of progeny from autogamy should be different from the progeny of geitonogamous
208 crosses from the same stem. The difference in the fitness of progeny from autogamous vs.
209 geitonogamous crosses provides an approximate estimate of the sign and magnitude of the average
210 fitness effects of somatic mutations for individual stems. In addition to influencing the mean fitness,
211 somatic mutations will increase the variance in fitness among progeny from autogamy. Consequently,
212 the third prediction we can make is that the mean fitness effects of somatic mutations will scale linearly
213 with the within-family standard deviation in fitness among progeny from autogamous crosses (Appendix
214 2). In general, we predict that if somatic mutations with fitness effects accumulate during vegetative
215 growth, then the mean progeny fitness should differ between autogamous and geitonogamous crosses,
216 and the variance in fitness of progeny from autogamous crosses would be greater than for progeny from
217 geitonogamous crosses. Below, we test these predictions using two different experiments with *Mimulus*
218 *guttatus*.
219
220 **First Experiment**
221 *Methods*–This experiment originally was designed to test the effects of somatic mutations on
222 seed set and ovule abortion (autogamy depression; Schultz and Scofield 2009; Bobiwash et al. 2013), so

223 we used multiple plants from several populations to obtain a large number of fruits. We grew plants of
224 *M. guttatus* in the Research Greenhouse facility at Portland State University from seed collected in July
225 2013 from three different populations in northern Oregon (Jackson Bottom Wetlands–JB: 45.501794 N, -
226 122.98776 W; and two from Saddle Mountain–SMB: 45.9861 N, -123.6859 W, and SMC: 45.9634 N, -
227 123.6837 W). We assumed that greenhouse conditions were different enough from field environments
228 to provide a novel selection regime. In August 2013, seeds were cold stratified on moist paper towels at
229 2°C for 30 days prior to being sown in soil. Seedlings were transplanted to pots (approximately 10 x 10 x
230 12 cm) and grown for seven months before the application of pollination treatments. Temperature was
231 maintained between 21–26°C during the day, and 15–21°C at night. Supplemental HID lights ran for 12
232 hours a day when the seedlings first emerged, and 14 hours a day during adult growth.
233 After plants became established and began producing multiple stems, we conducted
234 autogamous and geitonogamous self-pollinations using flowers on several stems (15 to 20 cm in length)
235 from two plants from each of four maternal families representing each of the three populations. Flowers
236 from pairs of stems on individual plants were reciprocally crossed (geitonogamy), or individual flowers
237 from these same stems were self-pollinated (autogamy). A total of 139 pollinations were conducted
238 across two treatments: limited (pollen was applied to stigmas with one touch from a plastic pipette tip)
239 or excess (where the stigma surface was coated with pollen). Pollinations were conducted on 12
240 different days (pollination date) over several weeks in July 2014. Mature fruits were collected and
241 placed individually into paper envelopes, and their contents were examined under a Leica MZ-16
242 stereoscope. The first 100 ovules from each fruit were categorized as filled seeds (brown, almond-
243 shaped), unfertilized ovules (small, flattened and light-colored), or aborted (larger than unfertilized,
244 dark-colored, shriveled). Ovules that were flattened and appeared to lack endosperm were assumed to
245 be unfertilized or aborted and were not used in germination tests. Seed set and ovule abortion were
246 analyzed using ANOVA models with the GLM procedure of SAS (SAS 2008), with population, maternal
247 plant nested within population, and pollination date as random effects, and cross type (autogamous or
248 geitonogamous) and pollination treatment as fixed effects. Seed set and ovule abortion data were
249 approximately normal so were not transformed prior to analysis.
250 We assessed the fitness of progeny arising by autogamy and geitonogamy in the same
251 greenhouse environment that was used to grow the parental plants. Seedlings from a subset of ten
252 maternal plants that had fruits from both cross types and at least 20 filled seeds were sown in soil and
253 transplanted to 36-cell trays (blocks) in September in a randomized incomplete block design. After three
254 months of growth, the progeny were scored for survival, and above ground biomass was measured after

255 drying at 60°C for at least 24 hours. Since all seedlings germinated within a few days of each other, the
256 final biomass is an estimate of growth rate. The fitness of each progeny was estimated as its final
257 biomass, which was log transformed and weighted by the survival frequency of progeny from the same
258 cross. Growth rate is considered to be an appropriate estimate of fitness for perennials (Younginger et
259 al. 2017). Furthermore, we evaluated fitness of seedlings under novel selection regimes in the
260 greenhouse rather than under field conditions, which allowed us to control for environmental
261 differences by having parental plants and seedlings exposed to the same conditions. These estimates
262 were rescaled relative to the maximum value from all crosses, so that $\overline{w}$ ranged from 0 to 1 across
263 progeny from all crosses. Data were analyzed to estimate the mean fitness for progeny from autogamy
264 ($\overline{w}_{k(A)}$) and geitonogamy ($\overline{w}_{k(G)}$) from each stem, and the variance in fitness (as measured by the
265 standard deviation; SD) for each group of progeny produced by autogamy and geitonogamy from single
266 stems.

267 *Results*–Levels of seed set and germination were similar between cross types, but ovule abortion
268 was greater in autogamous crosses. The mean number of seeds produced after autogamous (56.72
269 ±2.24, N = 87) and geitonogamous (57.52 ±3.42, N = 52) pollinations was similar (Cross in Table S2), and
270 there was little variation among populations (Pop in Table S2), maternal plants nested within population
271 (Mat(Pop) in Table S2), and pollination dates (Date in Table S2). Limited pollinations produced fewer
272 seeds than excess pollinations (Pollen in Table S2), but the effect of pollen dosage in the overall model
273 was not significant. However, the mean number of aborted ovules was greater for autogamous (17.36
274 ±1.27, N = 87) than for geitonogamous (13.90 ±1.70, N = 52) pollinations (Cross in Table S3), which is
275 consistent with the predicted effects of autogamy depression (Schultz and Scofield 2009; Bobiwash et al.
276 2013). There also were significant differences among populations in the level of ovule abortion (Pop in
277 Table S3), which perhaps reflects differences in historical inbreeding and levels of genetic load. There
278 were significant differences in ovule abortion among maternal plants nested within population (Mat
279 (Pop) in Table S3), but not for pollination treatments (Pollen in Table S3) or pollination dates (Date in
280 Table S3). The overall model for ovule abortion was significant (Table S3). Seed germination was similar
281 between autogamous (mean = 13.03 out of 20 planted per fruit) and geitonogamous crosses (mean =
282 13.28; $F_{1,101}$ = 0.16, P = 0.694). Of the 354 seedlings that germinated, 202 survived, and interestingly,
283 survival was higher for progeny from autogamy (67%) compared to geitonogamy (50%; chi-square =
284 12.03, P = 0.0005). For the surviving plants that flowered by the end of the experiment, flower
285 production was correlated with growth rate (r = 0.58, P < 0.001, N = 282).

286 The average fitness of progeny from autogamy and geitonogamy varied widely among individual
287 stems. Consistent with our first prediction for the effects of somatic mutations on offspring fitness,
288 there was a significant interaction between cross type and stem identity (Table S4). Little of the variation
289 in progeny fitness was explained by differences among stems (Mat in Table S4), but larger amounts were
290 explained by cross type (Cross in Table S4) and among blocks (Tray in Table S4). When data were
291 analyzed separately for each cross type, we found that mean fitness of progeny from autogamy varied
292 among stems to a much greater degree ($F_{8,130} = 4.44$, $P < 0.0001$) than progeny from geitonogamous
293 crosses ($F_{8,70} = 1.47$, $P = 0.1866$); a result that supports our first prediction for the effects of somatic
294 mutations on progeny fitness. These data are analyzed further along with data from the second
295 experiment described below.
296
297 **Second Experiment**
298 The observation of greater variation in fitness among stems for progeny from autogamous
299 crosses in the first experiment is consistent with the hypothesis that somatic mutations arising during
300 vegetative growth are transmitted and can have substantial fitness effects in the next generation.
301 Furthermore, the results from the first experiment do not support the view that somatic mutations
302 during vegetative growth are rare (Burian et al. 2016; Groot and Laux 2016; Watson et al. 2016;
303 Kuhlemeier 2017; Schmid-Siegert et al. 2017), but they are consistent with several studies
304 demonstrating that somatic mutations can be inherited by offspring in the next generation (Klekowski
305 and Godfrey 1989; Schultz and Scofield 2009; Bobiwash et al. 2013). However, the first experiment was
306 not designed specifically to compare the effects of autogamous and geitonogamous pollinations on
307 offspring fitness. Thus, to confirm results from the first experiment, we performed a second experiment
308 to provide a more robust assessment of the effects of autogamous and geitonogamous self-pollination
309 on the fitness of offspring. The second experiment used a single clone of *M. guttatus* to make
310 comparisons between autogamous and geitonogamous pollinations paired at the same node. The plant
311 chosen was a self-compatible perennial that displayed vigorous vegetative growth. We exposed plants
312 to novel conditions using high salinity under hydroponics cultivation, which increased the potential that
313 mutations would have fitness consequences. Moreover, our recent genomic results confirm more low-
314 frequency somatic mutation among plants exposed to high salt stress compared to the control
315 hydroponics treatment without added salt (Schwoch et al. in preparation). This experimental design
316 provided a more refined test of the hypothesis that the fitness effects of somatic mutations differed in
317 the offspring of autogamous and geitonogamous crosses.

318 *Methods*–To assess the fitness effects of mutations that accumulated during vegetative growth,
319 a single plant (genet BV, obtained from Willamette Gardens native plant nursery, Corvallis, OR) was
320 vegetatively propagated to generate 12 plants (ramets) that were exposed to high salinity and control
321 conditions. Plants were grown in pea gravel (4–8 mm) in pots placed in four 53 L tubs using a flood and
322 drain hydroponics system (flooding at 15 min intervals). Two tubs had no added salt and were used as
323 controls, and two tubs had high salinity. The initial salt concentration in the high salinity treatment was
324 5 mM but increased weekly to 25 mM after plants became established. Salt concentrations were
325 monitored using a conductivity meter to ensure stable concentrations. To provide nutrients, 30 ml of
326 hydroponics fertilizer (FloraGrow, Planet Natural, Bozeman, MT) was added per tub. During the course
327 of the experiment, some plants grew substantially. We imposed selection to favor the fastest growing
328 ramets over the next three months by repeatedly removing single rosettes and transplanting them back
329 into the hydroponics system up to four times.
330 To promote stress recovery, plants were transplanted to soil for six months, which included a
331 two-month vernalization period in a growth chamber (4°C and 8 h light; Conviron E8, Controlled
332 Environments Ltd., Winnipeg, Manitoba, Canada). After vernalization, plants were returned to the
333 greenhouse to induce flowering. Autogamous and geitonogamous pollinations were made to pairs of
334 flowers at single nodes or consecutive nodes (seven nodes and 14 pollinations total) on the largest
335 ramets in each of the control and high salt treatments. To account for somatic mutation turnover that
336 may occur due to the effects of cell lineage selection during stem growth, we compared progeny from
337 autogamous and geitonogamous pollinations at pairs of flowers from the same node. Without *a priori*
338 knowledge of the expression of somatic mutations in heterozygotes, it is difficult to determine the best
339 pollen donor for geitonogamous crosses. Consequently, we opted to generate progeny from
340 geitonogamy by pollinating flowers with pollen from a potentially more genetically divergent ramet
341 from the other treatment (i.e. salt pollinated with control pollen, and control pollinated with salt
342 pollen). The fruits were collected, and the total number of aborted and mature seeds and unfertilized
343 ovules were counted under a dissecting microscope. Seeds were planted in soil in trays with three seeds
344 per cell. Seeds were cold stratified in moist soil for three weeks before they germinated in the
345 greenhouse.
346 To determine whether seedlings from autogamous crosses from ramets exposed to salt stress
347 showed improved performance under the same conditions, all progeny were exposed to high salinity.
348 After germination and establishment in soil, seedlings from autogamous and geitonogamous crosses
349 from control and salt stress ramets were transplanted into pots filled with pea gravel and subjected to

350 high salt in the hydroponics system, as described above. A total of 239 seedlings from 11 fruits (five
351 autogamous and six geitonogamous) were randomly and evenly distributed among 12 hydroponic tubs
352 to ensure equal representation across blocks (tubs). Plant size was measured as the product of the
353 length and width of vegetative spread after two months of growth and was used as a proxy for overall
354 plant performance. Since plants germinated within a few days of each other, plant size represents a
355 good estimate of growth rate. Salt concentration increased from 10 mM - 37.5 mM over the course of
356 the experiment to induce mortality (~57% across all progeny groups). Fitness was estimated as plant size
357 (log transformed to improve normality) weighted by the survival frequency for progeny from the same
358 cross.
359 *Results*–Similar to experiment 1 and consistent with our first prediction for the effects of
360 somatic mutations, there was greater variation in the fitness of progeny from autogamous compared to
361 geitonogamous crosses, as indicated by a significant interaction between cross type and stem/node
362 identity (Stem/Node*Cross in Table S5). There was no consistent difference in progeny fitness between
363 autogamous and geitonogamous pollinations when averaged across stems (Cross in Table S5), but there
364 were larger differences among individual stems/nodes (Stem/Node in Table S5). Also consistent with our
365 first prediction, there was significant variation in progeny fitness among stems ($F_{2,89}$ = 3.76, P = 0.028)
366 and nodes (nested within stems; $F_{3,89}$ = 2.59, P = 0.0596) for autogamous, but not geitonogamous
367 crosses ($F_{1,136}$ = 0.63, P = 0.431 and $F_{4,136}$ = 1.19, P = 0.317 for stems and nodes, respectively). There were
368 significant differences in average size among the blocks (Tray in Table S5), but there was no consistent
369 difference in the performance of progeny based on the treatment history of their maternal ramets ($F_{1,126}$
370 = 0.02, P = 0.888), and there was no interaction between cross type and historical treatment ($F_{1,136}$ =
371 0.64, P = 0.423). Three nodes were dropped from further analysis, because one of the paired fruits
372 aborted or produced fewer than five seedlings. However, similar to experiment 1, and consistent with
373 the hypothesis that somatic mutations accumulate during stem growth, the variance in fitness among
374 progeny from autogamy among stems was greater than for geitonogamy. Since results from the two
375 experiments were similar, the data for four nodes from experiment 2 were analyzed together with the
376 first experiment, as described in the next section.
377
378 **Fitness Estimates**
379 Results from the two experiments described above are consistent with the hypothesis that somatic
380 mutations unique to individual stems can have demonstrable effects on the fitness of progeny when a
381 proportion is made homozygous by autogamous self-pollination. In both experiments, the variance in

382 fitness among progeny groups from autogamy was significantly greater than among progeny from
383 geitonogamy from the same set of stems. As the results from these two experiments are qualitatively
384 similar, we estimated the fitness effects of somatic mutations in each stem (ten comparisons of
385 autogamy and geitonogamy from Experiment 1 and four from Experiment 2, for a total of 14
386 comparisons). Specifically, we tested the second and third predictions described above. Since 25% of
387 somatic mutations unique to a stem will be homozygous in autogamous progeny, we expect that their
388 fitness effects will be greater than for progeny from geitonogamous crosses on the same stem
389 (providing an estimate of $\delta_{AD(k)}$). In addition, the within-family variance in fitness among progeny should
390 be greater after autogamy compared to geitonogamy (providing an estimate of w_{SD}). These estimates
391 of the fitness effects of somatic mutations should be similar, so we assessed whether there was a
392 positive relationship between the variance in fitness among progeny from single fruits produced by
393 autogamous pollination (w_{SD}) and the fitness effects of somatic mutations unique to each stem
394 estimated from the average fitness of progeny from autogamous and geitonogamous crosses to the
395 same stem ($\delta_{AD(k)}$).
396 Estimates of the average fitness effects of somatic mutations unique to each stem were made
397 based on the fitness of progeny from autogamous ($\overline{w}_{k(A)}$) and geitonogamous ($\overline{w}_{k(G)}$) crosses (Table S1
398 in Appendix 3). In both experiments, fitness was estimated as growth rate weighted by the survival of
399 seedlings from the same fruit and scaled to a maximum value of 1.0. We have demonstrated that
400 growth rate is correlated with flower production, and it has been shown to be a good predictor of
401 fitness among perennial plants (Younginger et al. 2017). The average fitness effects of mutations ($\delta_{AD(k)}$)
402 for each stem (first experiment) or stem/node combination (second experiment) were calculated as the
403 difference in fitness between progeny from autogamous and geitonogamous crosses, as described
404 above ($\delta_{AD(k)} = \overline{w}_{k(A)} - \overline{w}_{k(G)}$). We observed extensive variation in $\delta_{AD(k)}$ among stems, with nine stems
405 that were significantly different from zero (Fig. 3). Four of the stems had average fitness effects of
406 somatic mutations that were positive, suggesting a net beneficial effect of somatic mutations
407 transmitted to offspring. In addition, the average fitness of progeny from autogamous and
408 geitonogamous crosses on the same stems or nodes was positively correlated (Fig. S2; Table S6). Note
409 also that the $\delta_{AD(k)}$ estimates that deviated the most from zero generally had the highest (or lowest)
410 $\overline{w}_{k(A)}$ values relative to all of the stems (Fig. S3). However, this was not always the case, as one stem
411 (stem 9) with a value of $\delta_{AD(k)}$ close to zero produced progeny with relatively high fitness after both
412 autogamous and geitonogamous pollination.

413 To make an independent estimate of the average fitness effects for mutations unique to each
414 stem or node, we used the standard deviation in progeny fitness from autogamous crosses based on the
415 relationship $w_{SD} = cSD$, where c = 3, which assumes dominance close to h = 0.5 (qualitatively similar
416 results are obtained if we choose other values of c between 3 and 2.31; Appendix 2). Because the sign of
417 w_{SD} could not be inferred directly from this approach, we used the sign estimated from the $\delta_{AD(k)}$ method
418 (Fig. 4; Appendix 2). Our simulations show that the sign of $\delta_{AD(k)}$ is almost always estimated correctly
419 (Appendix 1). For stems with values of $\delta_{AD(k)}$ greater than zero, there was a strong positive relationship
420 between $\delta_{AD(k)}$ and estimates of w_{SD} made from the within-family variation among progeny from
421 autogamy (Fig. 4). In contrast, the relationship for negative values of $\delta_{AD(k)}$ appeared to be driven largely
422 by a single observation. Note that this observation was not supported by a similarly high value of w_{SD},
423 and the remaining negative fitness effects were more modest based on both estimates. This one highly
424 negative estimate of $\delta_{AD(k)}$, may be due to a large influence of mutations from the second stem on the
425 fitness of geitonogamous progeny (note that this stem had the highest estimate of $\overline{w}_{k(G)}$; Fig. S2). It is
426 also notable that the variance in fitness for progeny from autogamy did not decline to zero for values of
427 $\delta_{AD(k)}$ close to zero, which could be due to the presence of both beneficial and detrimental mutations,
428 and possibly genetic background effects (i.e. epistasis), but it may also reflect uncontrolled
429 environmental variation. Overall, the results from both approaches reveal consistent estimates of the
430 average fitness consequences associated with the accumulation of somatic mutations in individual
431 stems.
432
433 **Discussion**
434 In this study, we have shown that somatic mutations can accumulate during vegetative growth and have
435 substantial fitness effects on plants in the next generation. Consistent with previous work (Bobiwash et
436 al. 2013), we observed significant autogamy depression in the form of greater ovule abortion in
437 autogamous relative to geitonogamous crosses. However, we also detected more variation in survival
438 and growth rate for progeny from autogamous compared to geitonogamous self-pollinations, which
439 provides the first evidence that somatic mutations that accumulated during vegetative growth can have
440 demonstrable effects on the fitness of plants in the next generation. Both the differences in the mean
441 fitness between progeny from autogamy and geitonogamy ($\delta_{AD(k)}$) and variation in fitness of autogamous
442 progeny (w_{SD}) provided consistent estimates of the average fitness effects of somatic mutations
443 segregating in progeny. We found evidence for the effects of beneficial mutations in progeny from some
444 stems, with estimates of $\delta_{AD(k)}$ and w_{SD} exceeding 0.1 in four cases, while estimates for negative fitness

445 effects were more modest (mostly > -0.15). Furthermore, the observation that progeny from
446 autogamous crosses occasionally had higher fitness than progeny from geitonogamous crosses argues
447 for the accumulation of beneficial mutations and implies that many deleterious mutations are culled by
448 the various types of developmental selection prior to seed dispersal. These results imply that even
449 though numerous somatic mutations are generated during vegetative growth, many of them are filtered
450 due to different forms of developmental selection, which results in a shift in the distribution of fitness
451 effects for the mutations that are passed to the next generation.

452 Somatic mutations accumulating during vegetative growth had an overall positive effect for four
453 of the stems tested. This result is contrary to widely held views that the accumulation of beneficial
454 somatic mutations should be exceedingly rare (Crow 1993; Charlesworth and Willis 2009). However, an
455 explanation for these findings is afforded by the unique biology of plants; somatic mutations in germ
456 cells in the central zone of the apical meristem could contribute to higher rates of division for some cell
457 lineages over others (Otto and Orive 1995; Otto and Hastings 1998). This hypothesis is supported by the
458 observation that a minority of somatic mutations occur at high frequencies in meristem tissue (Yu et al.
459 2020; Schwoch et al. unpublished data), suggesting that clonal selective sweeps have occurred during
460 stem elongation. Thus, cell lineage selection has the potential to retain beneficial somatic mutations, as
461 individual cell lineages divide at faster rates resulting in clonal evolution during vegetative growth.

462 Although there may be few opportunities for beneficial changes to alter basic cellular
463 metabolism, it is becoming apparent from experimental evolution studies with microbes that even basic
464 aspects of cellular metabolism can be sensitive to environmental conditions, which can increase the
465 chances that mutations in clonal populations are beneficial (e.g., Lang et al. 2013; Lee and Marx 2013;
466 Maharjan et al. 2015). In this regard, cell lineage selection in a plant meristem represents a potentially
467 powerful forum for the removal of deleterious somatic mutations while favoring the retention of
468 beneficial ones. In addition, gametophytic selection and selective embryo abortion can act as prominent
469 additional filters (Mulcahy 1979; Cruzan 1989; Mable and Otto 1998; Armbruster and Rogers 2004;
470 Arunkumar et al. 2013; Harder et al. 2016), but they are most likely to have effects by culling deleterious
471 mutations. Regardless, the combined effects of these different forms of developmental selection appear
472 to have had a considerable effect on filtering of somatic mutations under controlled conditions in the
473 greenhouse, such that the distribution of fitness effects among stems has shifted to include more
474 beneficial mutations than expected. Future studies should test whether similar findings are found under
475 field conditions, which could indicate a prominent role for somatic mutation in local adaptation.

476 An alternative explanation for the observed fitness differences is that exposure to
477 environmental stress has induced heritable epigenetic modifications (Quadrana and Colot 2016).
478 However, epigenetic modifications are considered to be consistent in direction and predictable, as they
479 are hypothesized to represent an adaptive response to historic exposure to similar stressors (Baulcombe
480 and Dean 2014; Itabashi et al. 2018). The results of the experiments described here are unlikely to
481 support a role for epigenetics, because fitness responses in the next generation were inconsistent in
482 direction and magnitude, and they were not predictable based on environmental exposure of the parent
483 stem. Indeed, among stems and nodes, the mean fitness of progeny from autogamy displayed both
484 increases and decreases compared to the geitonogamy controls, which is consistent with the hypothesis
485 that individual ramets are accumulating unique complements of somatic mutations. Furthermore, these
486 conclusions are supported by the observation of numerous somatic variants in the transcriptomes of
487 meristem tissue from multiple ramets derived from a single genet (Yu et al. 2020; Schwoch et al.
488 unpublished data). Thus, the results of the current study appear to indicate that somatic mutations
489 accumulating during stem growth are responsible for the observed fitness effects.
490 The potential for the acquisition of mutations during vegetative growth is a well-known aspect
491 of plant biology (Klekowski 2003; Schultz and Scofield 2009; Bobiwash et al. 2013), but no previous study
492 has demonstrated the effects of somatic mutations on the fitness of progeny in the next generation.
493 Moreover, most studies have focused on the detrimental effects of somatic mutations; chloroplast
494 mutants have been observed in a number of species (Klekowski 2003), declines in pollen fertility were
495 found in older clones of quaking aspen (Ally et al. 2010), and higher rates of seed and fruit abortion after
496 autogamous pollinations were found in several studies (reviewed in Bobiwash et al. 2013). In contrast,
497 agriculturalists have taken advantage of beneficial somatic mutations to improve economically
498 important plants (Aradhya et al. 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli et al. 2012;
499 Jarni et al. 2015; Pelsy et al. 2015), and a handful of studies report phenotypic responses to selection in
500 asexual lineages. For example, Breese et al. (1965) succeeded in selecting for increased tillering ability
501 (production of new grass stems) within genets of perennial ryegrass (*Lolium perenne*). Similarly, artificial
502 selection on clonal lineages effectively improved branching in the red seaweed, *Asparagopsis armata*
503 (Monro and Poore 2009). The current study on *M. guttatus* contributes to this literature by highlighting
504 the potential for plants to exhibit significant levels of clonal variation within a single generation.
505 The transmission of beneficial mutations in autogamous crosses may explain some heretofore
506 difficult to understand results from mutation accumulation studies. Our results suggest that beneficial
507 somatic mutations are likely to be partially dominant (i.e. not completely recessive), because they would

508 have to be expressed in the heterozygous state to be favored by cell lineage selection. Mutations
509 accumulating during vegetative growth could contribute to standing genetic variation, but autogamy
510 may be more effective for the accumulation of beneficial somatic mutations in populations than
511 geitonogamy or outcrossing, because beneficial mutations could be made homozygous in progeny in a
512 single generation. Depending on the crossing design, outcrossing would take at least two generations for
513 beneficial somatic mutations to be made homozygous, and under geitonogamy they would remain
514 heterozygous. It is striking that high rates of beneficial mutation accumulation have been observed in at
515 least some mutation accumulation studies in the autogamous plant *Arabidopsis thaliana* (Shaw et al.
516 2002; Rutter et al. 2010; Rutter et al. 2012), but not in outcrossing and partially-selfing species of
517 *Amsinckia* (Schoen 2005). With a few exceptions (e.g., Baer et al. 2005; Denver et al. 2010), nearly all
518 mutation accumulation studies on animals consistently show a prevalence of deleterious mutations
519 (Baer et al. 2007; Halligan and Keightley 2009). Similarly, our results suggest that the adaptive potential
520 of autogamous plants may be greater than previously thought, which may help explain the wider
521 geographic ranges of selfing compared to closely-related outcrossing species (Grossenbacher et al. 2015;
522 Grant and Kalisz 2020). Although developmental selection has the potential to contribute to adaptation
523 in all plants, its effects may be enhanced in autogamous lineages, because beneficial mutations arising
524 during vegetative growth have a greater chance of becoming homozygous in offspring and being
525 retained across generations.

526 As stems grow, mutations can be generated during every mitotic cell division, so the potential
527 for somatic mutation accumulation in plants appears substantial. Thus, understanding how long-lived
528 plants, such as trees, avoid mutational meltdown from the accumulation of deleterious somatic
529 mutations remains a longstanding question. Paradoxically, however, the rate of mutation accumulation
530 observed across generations in plant and animal genomes is similar (Gaut et al. 2011). One hypothesis
531 for this pattern is that, similar to animal germlines, a subset of cell lines in the apical meristem
532 undergoes fewer mitotic divisions, which would protect lineages from the negative effects of mutation
533 accumulation during development of the soma (Plant Germline Hypothesis; Burian et al. 2016; Cruzan
534 2018; page 90). An alternative hypothesis posits that somatic mutations are generated in apical
535 meristems during plant growth, but these mutations are filtered by developmental selection prior to the
536 establishment of offspring (Somatic Mutation Accumulation Hypothesis). Because plants have retained
537 the capacity to undergo clonal evolution from their algal ancestors, the ability to filter mutations during
538 growth and reproduction has existed for some time, and thus developmental selection has the potential
539 to skew the distribution of fitness effects of transmitted mutations to include a larger proportion of

540 beneficial mutations than would be expected through random processes. In addition, this provides a
541 reasonable explanation for why longer-lived plants appear to have slower rates of mutation
542 accumulation across generations (Yue et al. 2010; Gaut et al. 2011). This could be due to the fact that
543 longer generation time leads to more time between recombination events, which can lead to more
544 background selection in non-recombining cell lineages during vegetative growth (Cruzan 2018; pages 94-
545 95). Recent studies in oaks, spruce, and eel grass (Plomion et al. 2018; Hanlon et al. 2019; Yu et al.
546 2020), as well as our unpublished data from *M. guttatus*, confirm that multiple somatic variants are
547 likely, even in plants of very different size. Moreover, results from the current study identify the
548 presence of beneficial mutations that were passed to offspring in the next generation, likely a
549 consequence of developmental selection. Future work that combines information from experiments
550 evaluating the genomic consequences of somatic variation with anatomical estimates of stem cell
551 population dynamics will allow for the development of new models that provide insights into the extent
552 and limitations of somatic evolution in plants.
553 In conclusion, despite the potential for somatic mutation accumulation to generate novel
554 genetic variation in plant populations, its role in evolution remains almost entirely unexplored. Our
555 estimates of the fitness effects of somatic mutations were consistent across two different methods and
556 indicate that some stems possessed a prevalence of deleterious mutations while others produced
557 autogamous progeny with high fitness, which indicates the presence of beneficial mutations. Even
558 though high levels of mutation accumulation are often believed to be detrimental, the basic biology of
559 plants suggests that the role of somatic mutations in plant evolution should be considered carefully in
560 the future. Moving forward, our results indicate that we must keep in mind that all eukaryotes are not
561 necessarily equivalent, and specific features of the organism's biology may have unexpected
562 consequences for the evolutionary phenomena that we observe. Future lines of investigation will
563 improve our understanding of these fundamental aspects of plant development and evolution that may
564 have contributed to the remarkable diversification of plants, and may help to account for some of the
565 extensive variation in mutation rates detected among lineages.

566

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Data Archiving

Data and all scripts for data analysis will be submitted to DataDryad upon publication.

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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 differ in growth rate within the apical meristem of a plant. A) Expressed deleterious mutations are predicted to lead to the demise of a cell line (brown cells) that is 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). B) Multiple ramets of the same genet become differentiated by unique somatic mutations during vegetative growth. Most ramets carry deleterious mutations (orange, red, purple, and pink arrows), but expressed beneficial mutations cause clonal selective sweeps of the meristem in some ramets (green arrows).

Fig. 3. Estimates of $\delta_{AD(k)}$ for fourteen different stems (ramets) from two separate experiments (Experiment 1–blue bars; Experiment 2–orange bars) based on mean progeny fitness after autogamous $\overline{w}_{k(A)}$ and geitonogamous $\overline{w}_{k(G)}$ self-pollinations ($\delta_{AD(k)} = \overline{w}_{k(A)} - \overline{w}_{k(G)}$). Horizontal lines represent standard errors. Asterisks indicate values of $\delta_{AD(k)}$ that are significantly different from zero based on the t value, calculated as $t = \delta_{AD(k)}/\sqrt{SE}$ with *n-1* df, where *n* is the mean of sample sizes for progeny from autogamy and geitonogamy. The relationship between $\overline{w}_{k(A)}$ and $\overline{w}_{k(G)}$ across stems is shown in Fig. S2. Means and sample sizes for progeny groups are available in Table S6 and Fig. S3.

Fig. 4. Relationships between estimates of fitness effects of somatic mutations, based either on the difference in fitness of progeny from autogamy and geitonogamy ($\delta_{AD(k)}$), or the standard deviation in fitness within progeny groups from autogamy for each stem (w_{SD}). Estimates of w_{SD} corresponding to negative values of $\delta_{AD(k)}$ 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 fitness estimates.

Fig. 1

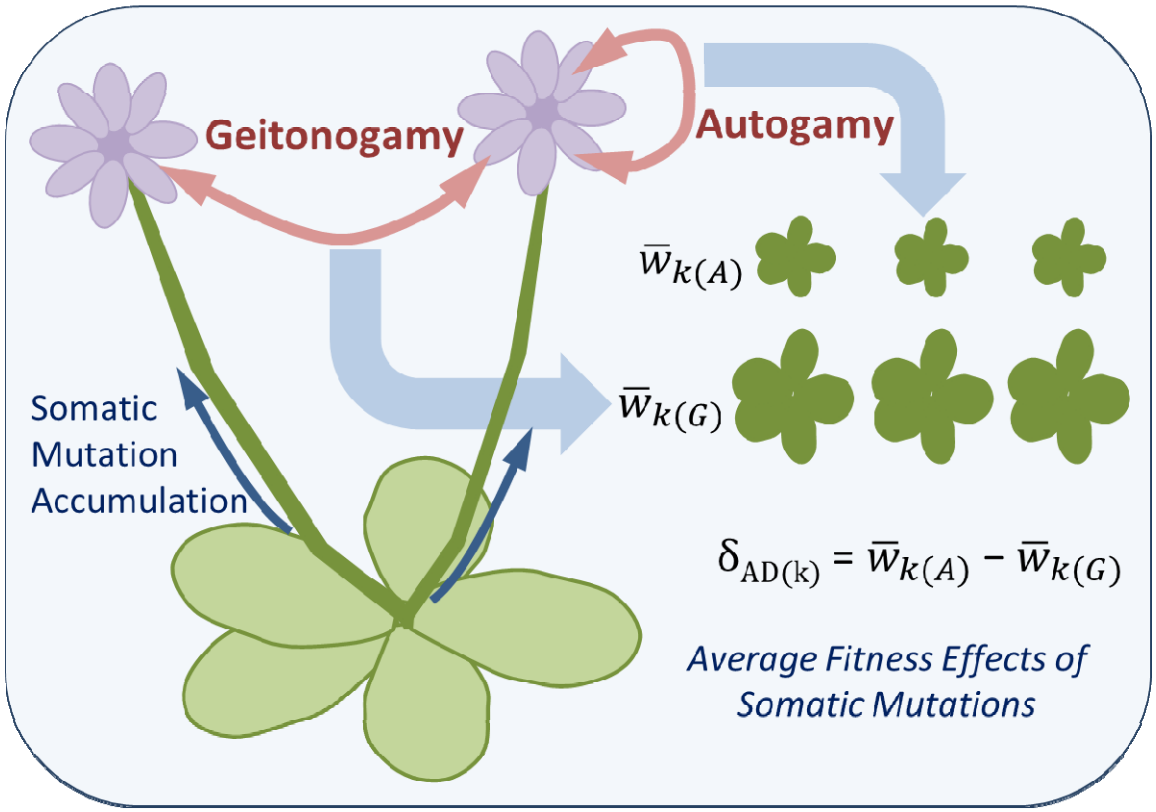
Geitonogamy
Autogamy
$\overline{w}_{k(A)}$
$\overline{w}_{k(G)}$
Somatic
Mutation
Accumulation
$\delta_{AD(k)} = \overline{w}_{k(A)} - \overline{w}_{k(G)}$
Average Fitness Effects of
Somatic Mutations

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Fig. 2

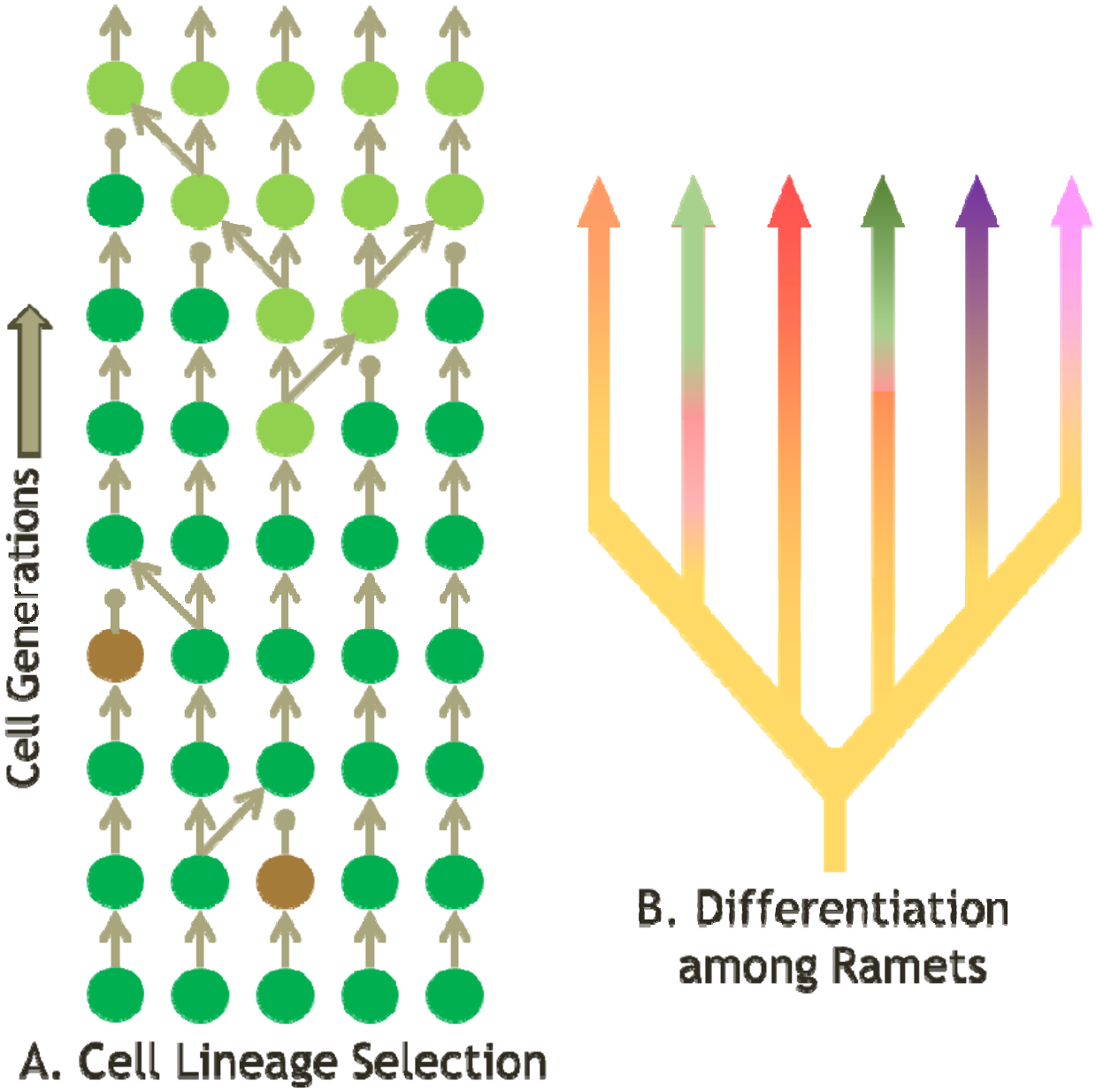

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Fig. 3

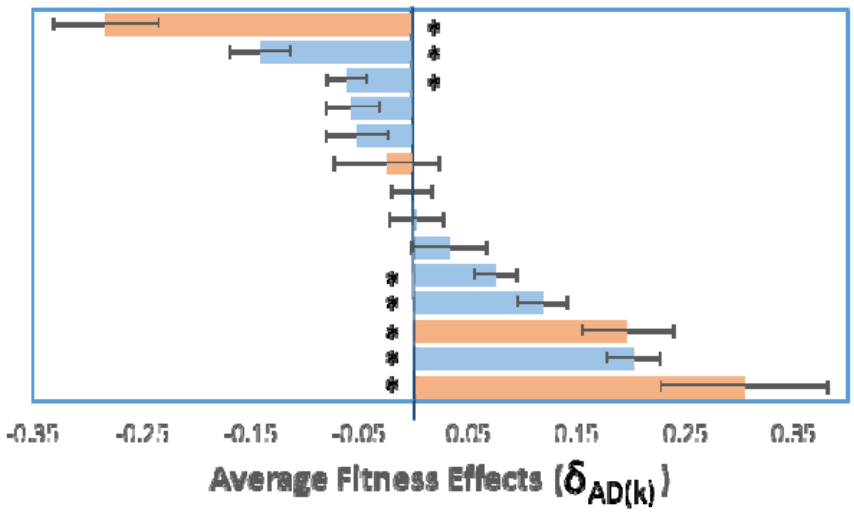

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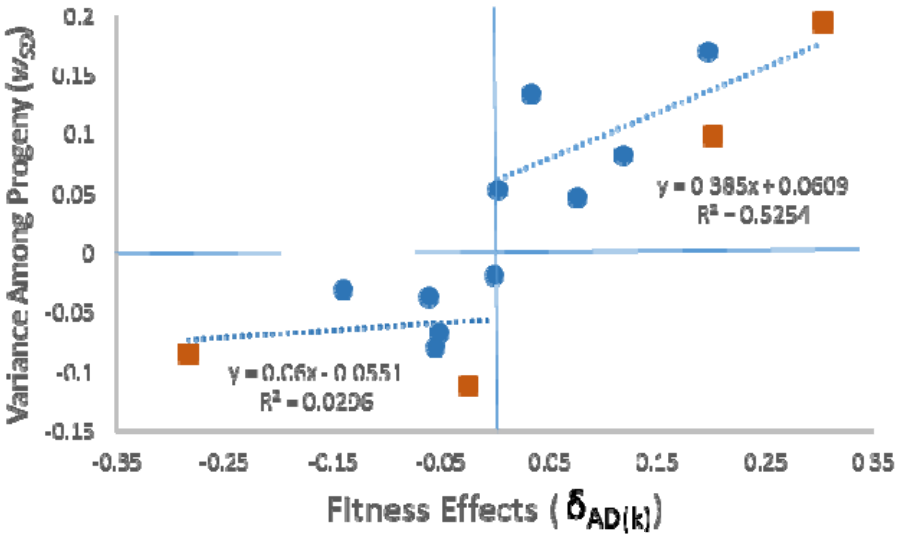
Fig. 4



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825 **Appendix 1. Validity of the $\delta_{AD(k)}$ Estimate**

826 For a pair of stems from the same genet, we assume that w_G represents the fitness of geitonogamous
827 progeny after a cross between the pair of stems, and that w_A represents the fitness of autogamous
828 progeny after selfing a single flower on one stem:

829 $w_G = 1 + 0.5(h_1s_1+h_2s_2)$

830 $w_A = 0.25(1) + 0.5(1+ h_1s_1) + 0.25(1+s_1)$

831 Where, s_1 and s_2 represent the selective effects of mutations, and h_1 and h_2 represent the dominance of
832 single somatic mutations in stem 1 and stem 2, respectively.

833 For simulations, we randomly chose values of s ranging from -0.2 to 0.2 for 200 pairs of stems. Values of
834 h for $s > 0$ were allowed to range from 0 to 0.7 for each stem. Because deleterious alleles tend to be
835 recessive, values of h were constrained to range between 0 and 0.1 for $s < 0$. Dominance values were
836 chosen assuming that deleterious somatic mutations with high levels of expression would be eliminated
837 by cell lineage selection during vegetative growth, and beneficial mutations with high dominance would
838 not display strong fitness effect differences between autogamous and geitonogamous progeny. To
839 match our experimental design in *Mimulus guttatus*, we calculated the fitness of autogamous progeny
840 (w_A) for one of the stems in each pair. We then calculated w_G using information on s and h for both
841 stems in each pair. Then we calculated the difference in fitness between each estimate as a measure of
842 autogamy depression ($\delta_{AD(k)} = w_A - w_G$). This simulation was repeated 20 times (4,000 pairs of stems
843 total) and average values were calculated.

844 <u>Results:</u>

845 1. Estimates of $\delta_{AD(k)}$ and s_1 are strongly correlated, with R^2 values ranging from 0.76 to 0.82 (one
846 example of 200 random estimates):
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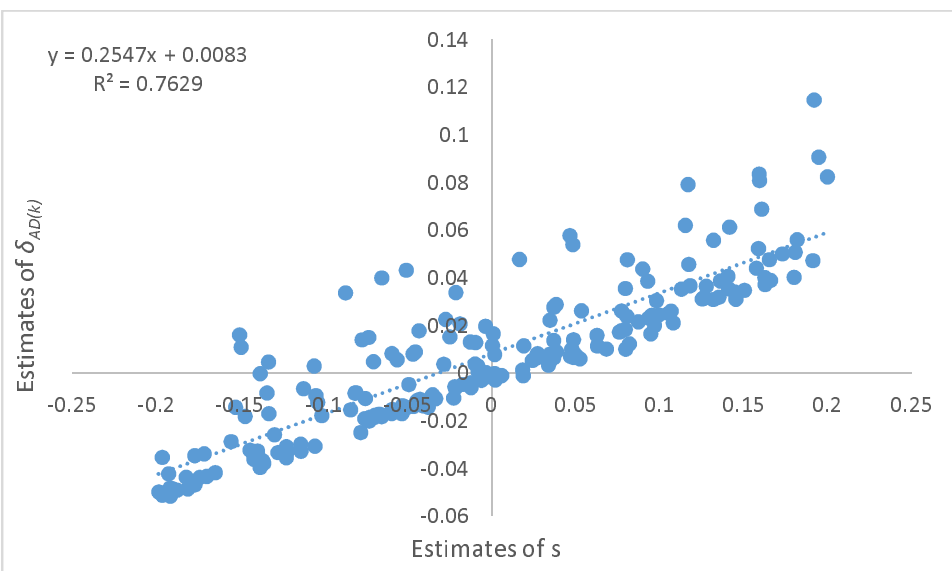

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2. The frequency of $\delta_{AD(k)}$ estimates having the opposite sign of s_1 was very low. Mean number of correct signs was 178.7 (+/- 1.04 standard error) and was 21.30 (+/-1.04; or 12%) for incorrect signs.
3. Estimates of $\delta_{AD(k)}$ with incorrect signs were closer to zero. Mean absolute values of s for $\delta_{AD(k)}$ estimates with incorrect signs was 0.054 (+/- 0.0021) and was 0.106 (+/-0.0011) for correct signs. Hence, estimates of $\delta_{AD(k)}$ that are further from zero are more reliable estimates of s_1.

We note that the formulation used above for w_G could result in estimates that are out of range when large numbers of mutations are involved (i.e., $w_G = 1 + 0.5 \sum h_i s_i$ for a large number of loci segregating for deleterious alleles). A better estimate may be the average effect of n loci segregating for deleterious alleles (i.e. $\sum h_i s_i / n$). If we apply this approach for two loci (one mutation per stem) we obtain,

$$w_G = 0.25 + 0.25(1 + h_1s_1) + 0.25(1 + h_2s_2) + 0.25(1 + (h_1s_1+h_2s_2)/2)$$

$$w_G = 1 + 0.375(h_1s_1+h_2s_2)$$

Using this formula, the relationship between $\delta_{AD(k)}$ and s_1 is stronger (R^2 ranging from 0.86 to 0.90), with only an average of 13 out of 200 estimates with opposite signs (7%). The average of correct signs was similar to the one above (0.105, +/- 0.0007), but the mean for incorrectly assigned values was much closer to zero (0.038, +/- 0.0022).

Another model for the effects of alleles at multiple loci on fitness assumes the interaction is multiplicative. In this case the estimate of w_G becomes,

$$w_G = 0.25 + 0.25(1 + h_1s_1) + 0.25(1 + h_2s_2) + 0.25(1 + (h_1s_1h_2s_2))$$

$$w_G = 1 + 0.25(h_1s_1+h_2s_2) + 0.25(h_1s_1h_2s_2)$$

Using this formula, the relationship between $\delta_{AD(k)}$ and s_1 is stronger (R^2 ranging from 0.93 to 0.95), with only an average of 7.1 out of 200 estimates with opposite signs (4%). The average of correct signs was similar to the ones above (0.103, +/- 0.0010), but the mean for incorrectly assigned values was closer to zero (0.023, +/- 0.0020).

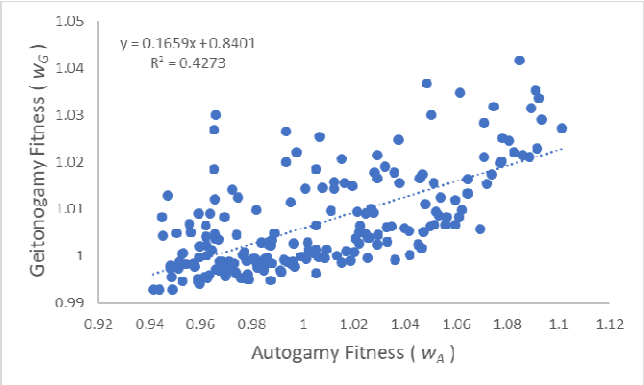
<u>Interpretation:</u>

These results indicate that our estimate of $\delta_{AD(k)}$ is a valid estimate of s for stem 1. However, it does appear that we are underestimating s (i.e., the slope of the line is less than 1), as these estimates do not consider the effects of multiple somatic mutations or possible genetic background effects (i.e. epistasis).

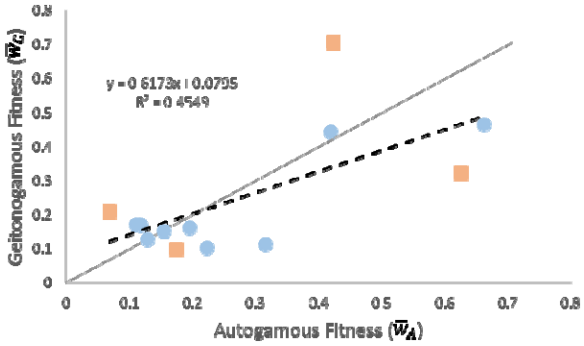
It is also notable that our simulation assumes a wider range of h values than are generally observed for deleterious mutations. Estimates indicate that the product of hs for deleterious alleles is generally around 0.02, and that the relationship between s and h is generally negative (hyperbolic; Lynch et al. 1999). Minor effects of deleterious mutations are expected for our data, because somatic mutations having strong effects in the heterozygous condition would be eliminated by cell lineage selection. Adding the constraint of a negative relationship between h and s to our simulation model would marginally improve the predictive power of $\delta_{AD(k)}$ for estimates of s.

These simulations also support our use of the correlation between w_A and w_G to confirm that our estimates are similar to the simulation estimates.

From the simulations:



From our data:



Appendix 2. Estimating the selection coefficient due to somatic mutations from the variance in fitness among progeny.

In this Appendix, we demonstrate that the standard deviation (SD) in fitness among progeny from autogamous crosses can be used to estimate the selection coefficient for the average effects of somatic mutations occurring within a stem. The variance in fitness among progeny from autogamy can be affected by the selection coefficient (*s*) and the dominance effects (*h*) of somatic mutations unique to a stem.

To evaluate the effects of a single mutation on progeny fitness, we assume fitnesses of;

$w_{aa} = 1$

$w_{aa'} = 1 + hs$

$w_{a'a'} = 1 + s$

The mean progeny fitness is then calculated as

$\bar{w} = ¼(1) + ½(1+hs) + ¼(1+s)$

Which simplifies to

$\bar{w} = (1 + ½\ hs + ¼\ s)$

The variance in progeny fitness can be calculated as

$V(w) = ¼(w_{aa} - \bar{w})^2 + ½(w_{aa'} - \bar{w})^2 + ¼(w_{a'a'} - \bar{w})^2$

After substituting, we get

$V(w) = [¼\ (- ½\ hs - ¼\ s)^2] + [½\ (½\ hs - ¼\ s)^2] + [¼\ (\ ¾\ s\ - ½\ hs\ )^2]$

For simplification, we separate the effects of homozygous and heterozygous genotypes (terms within the three sets of square brackets)

V(w) = [1] + [2] + [3]

and evaluate each one;

[1]: $¼\ (- ½\ hs - ¼\ s)^2 = ¼\ (- ½\ hs\ (- ½\ hs - ¼\ s)- ¼\ s(- ½\ hs - ¼\ s)) = (\ 1/16\ h^2s^2 + 1/16\ hs^2 + 1/64\ s^2\ )$

[2]: $½\ (½\ hs - ¼\ s)^2 = ½\ (½\ hs\ (½\ hs - ¼\ s) - ¼\ s(½\ hs - ¼\ s)) = ½\ (1/4\ h^2s^2 – 1/8\ hs^2 – 1/8\ hs^2 + 1/16\ s^2\ ) = (\ 1/8\ h^2s^2 - 1/8\ hs^2 + 1/32\ s^2\ )$

[3]: $¼\ (\ ¾\ s\ - ½\ hs\ )^2 = ¼\ (\ ¾\ s\ (\ ¾\ s\ - ½\ hs\ ) - ½\ hs\ (\ ¾\ s\ - ½\ hs\ )) = ¼\ (9/16\ s^2 – 3/8\ hs^2 – 3/8\ hs^2 + ¼\ h^2s^2\ ) = (9/64\ s^2 - 3/16\ hs^2 + 1/16\ h^2s^2\ )$

By combining and then simplifying these three results, we get

$V(w) = 3/16\ s^2 - ¼\ hs^2 + ¼\ h^2s^2$

And the standard deviation can be obtained by taking the square root of V(w)

$SD(w) = \sqrt{V(w)} = 0.433\ s - ½\ s\sqrt{h} + ½\ hs$

We note that this relationship holds regardless of the sign (positive or negative values of s), so for this relationship we can substitute *w* for *s*. The standard deviation in progeny fitness scales linearly with the selective effects of somatic mutations, and the effects of dominance on variation among progeny is minor (Fig. S1; which shows values of *s* or *w* predicted from values of SD for fitness ranging from 0 to 0.5). We note that the standard deviation is highest when mutations are completely recessive or dominant (h = 0 or 1) and SD(w) = 0.433s. When h = 0.5, the standard deviation is at its minimal value and SD(w) = 0.332s. By rearranging, we can obtain the regression equations to estimate the selective effects of somatic mutations from the standard deviation in progeny fitness as $s_{SD} = cSD$, where *c* is the slope of the regression line in Fig. S1. From this analysis, it is clear that the effects of *h* on the within-family SD in fitness is minor compared to the effects of *s*. We obtain estimates of the regression slopes from the standard deviation in progeny fitness to produce the relationships in Fig. S1;

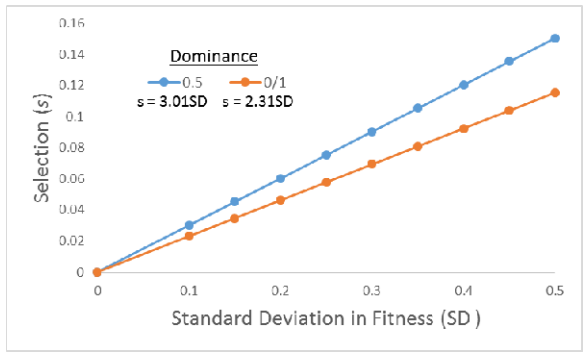


Fig. S1. The effects of selection (*s*) or fitness (*w*) and dominance (*h*) on the standard deviation (SD) in fitness among progeny. The effect of dominance on variation in fitness is minimized when *h = 0.5* and maximized when *h = 0.0* and *h = 1.0*.

$w = 2.31*SD(w)$ for $h = 0$ or 1, and

$w = 3.01*SD(w)$ for $h = 0.5$

From these relationships we can estimate the selective effects of somatic mutations from the standard deviation in progeny fitness as $w_{SD} = cSD$, where *c* is the slope of the regression lines in Fig. S1.

Appendix 3. Supplemental Tables and Figures

Table S1.

	Autogamy			Geitonogamy				
Stem	$\bar{w}_A$	**N**	**Stdev**	$\bar{w}_G$	**N**	**Stdev**	$\delta_{AD(k)}$	$\bar{w}_A - \bar{\bar{w}}_G$
JB51	0.407	33	0.171	0.144	7	0.080	0.263	0.078
JB52	0.314	12	0.161	0.295	9	0.068	0.019	-0.015
JB54	0.258	8	0.066	0.298	11	0.103	-0.040	-0.071
SMB11	0.286	22	0.072	0.260	9	0.077	0.009	-0.043
SMB51	0.256	11	0.054	0.323	4	0.035	-0.067	-0.073
SMB52	0.273	7	0.072	0.280	6	0.051	-0.007	-0.056
SMB53	0.245	4	0.021	0.327	6	0.108	-0.081	-0.083
SMB54	0.289	10	0.023	0.245	5	0.056	0.044	-0.040
SMC11	0.303	16	0.071	0.238	8	0.085	0.065	-0.026
SMC12	0.356	8	0.071	0.239	6	0.059	0.117	0.027
C12	0.423	36	0.170	0.707	5	0.153	-0.283	0.094
C13	0.626	9	0.195	0.322	7	0.237	0.090	0.297
D17	0.692	5	0.170	0.485	4	0.314	0.207	0.363
D18	0.419	12	0.130	0.443	13	0.211	-0.025	0.090

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

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

Table S3. Analysis of variance results for aborted ovules after autogamous and geitonogamous pollinations (Cross) with limited and excess pollen (Pollen). Population (Pop), maternal stem nested within population (Mat(Pop)), and pollination date (Date) were declared as random effects in the model.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	33	9337.61468	282.95802	2.76	<.0001
Cross	1	449.176948	449.176948	4.38	0.0389
Pop	2	2174.689160	1087.344580	10.60	<.0001
Pollen	1	0.597410	0.597410	0.01	0.9393
Mat(Pop)	18	2217.350757	123.186153	1.20	0.2751
Date	11	1995.681210	181.425565	1.77	0.0695
Error	101	10363.24458	102.60638		

Table S4. Analysis of variance for progeny fitness (biomass weighted by survival of seedlings) after autogamous and geitonogamous pollinations (Cross) for ten maternal stems (Mat) and their interaction (Mat*Cross). Planting block (Tray) was declared as a random effect in the model.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	33	1.42418594	0.04315715	4.18	<.0001
Tray	14	0.45040769	0.03217198	3.11	0.0002
Mat	9	0.06845581	0.00760620	0.74	0.6756
Cross	1	0.03196532	0.03196532	3.09	0.0805
Mat*Cross	9	0.51373985	0.05708221	5.52	<.0001
Error	168	1.73638043	0.01033560		

Table S5. 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 (Tray) was declared as a random effect in the model and initial size of seedlings (Initial Size) was entered as a covariate.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	1.31384069	0.05712351	6.05	<.0001
Tray	11	0.46856600	0.04259691	4.51	<.0001
Initial Size	1	0.46861414	0.46861414	49.61	<.0001
Stem/Node	6	0.12071536	0.02011923	2.13	0.0514
Cross	1	0.00555294	0.00555294	0.59	0.4441
Stem/Node*Cross	4	0.13005812	0.03251453	3.44	0.0095
Error	203	1.91760656	0.00944634		

Table S6. 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. 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.

Exp	ID	N	Fitness Autogamy	SD	N	Fitness Geitonogamy	SD
2	C13	9	0.626	0.195	7	0.322	0.237
1	JB51	33	0.316	0.193	7	0.114	0.041
2	D17	7	0.662	0.170	60	0.465	0.314
1	SMC12	8	0.222	0.080	6	0.103	0.046
1	SMC11	16	0.175	0.065	8	0.099	0.071
1	JB52	12	0.195	0.158	9	0.162	0.071
1	SMB11	22	0.155	0.082	9	0.152	0.096
1	SMB54	10	0.128	0.020	5	0.129	0.072
2	D18	12	0.419	0.130	13	0.443	0.211
1	JB54	8	0.118	0.063	11	0.17	0.109
1	SMB52	7	0.117	0.069	6	0.173	0.057
1	SMB51	11	0.11	0.040	4	0.171	0.048
1	SMB53	4	0.069	0.021	6	0.21	0.108
2	C12	36	0.423	0.188	5	0.706	0.153
		13.93			11.14		

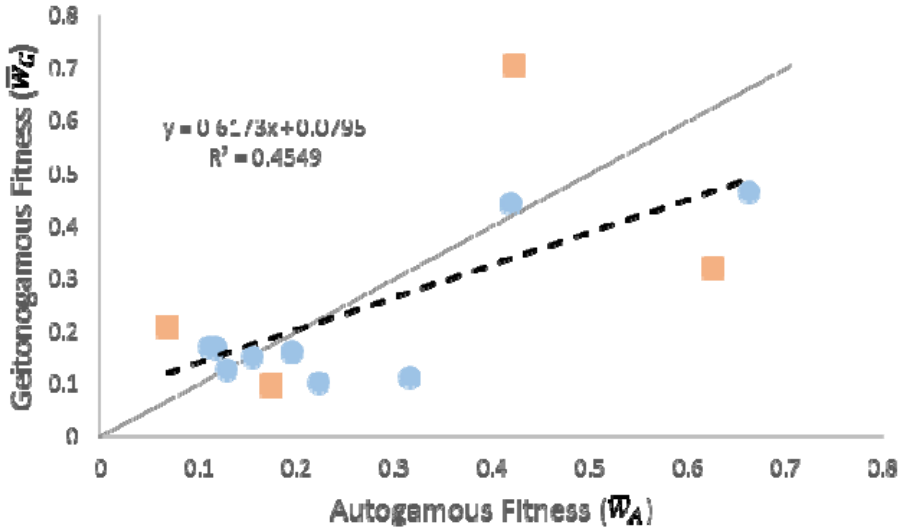


Fig. S2. The relationship between the autogamous and geitonogamous progeny from the 14 comparisons from experiments 1 (blue circles) and 2 (orange squares) as listed in Table S6. The solid line represents a 1:1 relationship and the dashed line represents the best-fit to the data. Stems falling above the solid line apparently had a prevalence of deleterious somatic mutations whereas those falling below the line displayed a prevalence of beneficial somatic mutations.

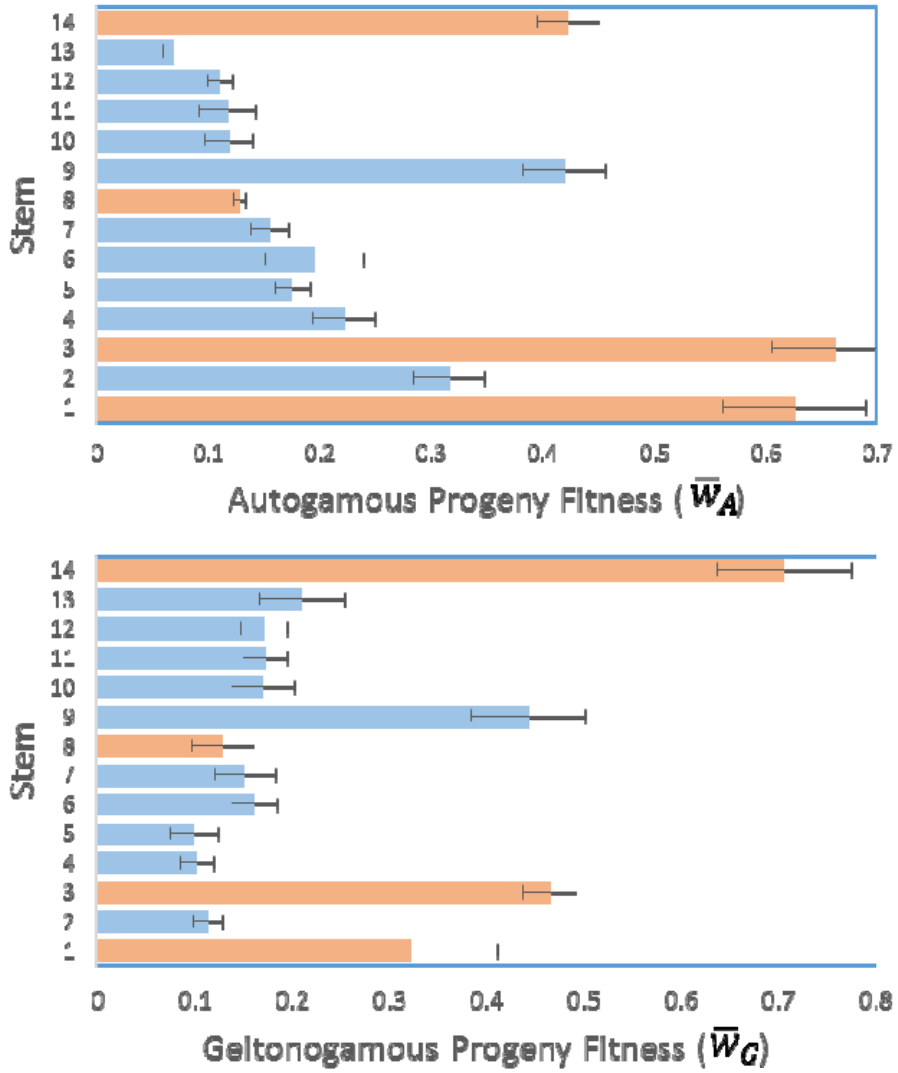


Fig S3. Means and standard errors (lines) for fitness estimates of autogamous (upper panel) and geitonogamous (lower panel) progeny groups from experiment 1 (blue bars) and 2 (orange bars) listed in Table S6. Progeny groups are ordered as in Fig. 4.