Phenotypic Effects of Somatic Mutations Accumulating during Vegetative Growth

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Submitted: _____; *Electronically published:* _____

Online enhancements: Three appendices. *Dryad Data:* _____

Abstract

The unique life form of plants promotes the accumulation of large numbers of somatic mutations that can be passed to offspring in the next generation. However, rates of mutation accumulation in plants remain similar to rates in animals. The explanation for this paradox may be that plants have the potential to filter somatic mutations by eliminating deleterious mutations prior to seed dispersal. We present experimental tests of this hypothesis using crosses in plants of *Mimulus guttatus*. The fitness effects of somatic mutations are expected to differ between progeny from self-pollinations made within the same flower (autogamy) relative to those made between stems on the same plant (geitonogamy), as autogamy leads to homozygosity of a proportion of somatic mutations, but progeny from geitonogamy remain heterozygous. Using two different approaches, we find that somatic mutations have consistent fitness effects on offspring. Surprisingly, several progeny groups from autogamous crosses displayed increases in fitness compared to progeny from geitonogamy controls, 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 natural selection, 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.

32 **Introduction**

33 Mutation is the source of variation for evolution and adaptation, but organisms differ in whether
34 mutations originating during gamete formation (meiosis) or somatic growth (mitosis) contribute to
35 heritable variation. For the vast majority of organisms, including viruses, unicellular microbes, and some
36 multicellular eukaryotes, sexual reproduction is rare or absent. In these organisms, mutations occur
37 during cell replication, and their primary process for adaptation and diversification is by clonal evolution.
38 By contrast, genetic modification occurring during somatic growth of animals is usually limited to the
39 appearance of acquired mutations and the development of cancer (Nowell 1976; Beerenwinkel et al.
40 2015), neither of which contributes to heritable variation in populations. This is due to the fact that in
41 metazoans, the germline is determined early in development and relatively few cell divisions occur
42 before the formation of gametes (the Weismann Barrier; Buss 1983). This may be true even for some
43 highly clonal animals, such as cnidarians, but it remains unclear whether clonal variation in corals is
44 transferred to the next generation remains (e.g., Schweinsberg et al. 2014), but not others (e.g., Barfield
45 et al. 2016).

46 Plants differ from animals and microbes, because mutations contributing to heritable variation
47 can arise both during gamete formation and somatic growth (Antolin and Strobeck 1985; Klekowski and
48 Godfrey 1989). This is due to the fact that plants lack separate germ lines; the same cells within apical
49 meristems subtend both vegetative growth and gamete development, leading to sexual reproduction.
50 Consequently, plants have the capacity for clonal evolution, as somatic mutations that occur in plant
51 meristems can accumulate and be passed to the next generation (McKnight et al. 2002; Klekowski 2003;
52 Schultz and Scofield 2009; Bobiwash et al. 2013; Dubrovina and Kiselev 2016; Watson et al. 2016;
53 Schmid-Siegert et al. 2017). This aspect of plant biology is well known (Monro and Poore 2009; Ally et al.
54 2010; Reusch and Bostrom 2011), and somatic mutation accumulation has been important in
55 agriculture, where the origin of many clonally-derived varieties of fruits, including citrus, apples, and
56 wine grapes, have been cultivated by grafting from genetically differentiated bud tips (Aradhya et al.
57 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli et al. 2012; Jarni et al. 2015; Pelsy et al. 2015).
58 However, there is disagreement over the degree and biological importance of somatic mutation
59 accumulation in plants (Schultz and Scofield 2009; Burian et al. 2016; Watson et al. 2016; Kuhlemeier
60 2017; Schmid-Siegert et al. 2017; Plomion et al. 2018; Hanlon et al. 2019).

61 Clonal evolution occurs as individual cell lineages accumulate mutations during mitotic division
62 (Otto and Orive 1995; Otto and Hastings 1998; Orive 2001; Elena and Lenski 2003; Greaves and Maley
63 2012; Long et al. 2015). Like mutations occurring during meiosis, somatic mutations are expected to be

64 deleterious more often than beneficial, but the actual distribution of fitness effects is generally unknown
65 (Orr 2010). In diploids, new somatic mutations occur as a single copy within the genome, so their
66 immediate fitness effects will depend on the combination of their selection coefficient (*s*) and their
67 expression in the heterozygous state (dominance; *h*). We expect expressed mutations that reduce rates
68 of cell division and growth will tend to be eliminated. Similarly, any mutations that elevate the rate of
69 cell division will tend to increase in frequency in the germ cell population. In general, it is difficult to
70 assess the effects of somatic mutations, because clonal lineages lack recombination, and the phenotypic
71 effects of beneficial mutations can be muted by the co-occurrence of detrimental genetic variants (Hill-
72 Robertson Effect; Selective Interference; Hill and Robertson 1966; Fogle et al. 2008). Although sexual
73 reproduction does allow mutations to segregate among progeny, it is difficult to detect the phenotypic
74 effects of novel mutations in eukaryotes with separate sexes, because the genomes of two individuals
75 are combined in offspring (Long et al. 2015). In this study, we make crosses within individual clones of
76 hermaphroditic plants to assess the phenotypic effects of novel mutations accumulating during somatic
77 growth.
78 Plants have several advantages over other organisms for the study of clonal evolution. Separate
79 stems on the same plant consist of multiple clonal cell lineages that are derived from the same zygote
80 but may differ for mutations that have accumulated during stem elongation. To produce recombinant
81 progeny segregating for somatic mutations unique to each stem, crosses can be made either within the
82 same flower (autogamy) or between flowers on separate stems of the same plant (geitonogamy; Fig. 1).
83 These crosses are both self-fertilizations, but they differ in the complement of somatic mutations that
84 are unique to each stem. Progeny from autogamous crosses will segregate for the mutations that
85 accumulated within a single stem, while the progeny from geitonogamous crosses will segregate for
86 mutations from both stems. After autogamy, somatic mutations unique to a stem will be homozygous in
87 approximately 25% of progeny, but they will be either heterozygous or absent in progeny from
88 geitonogamous crosses. Thus, the average effects of mutations accumulating during stem growth can be
89 evaluated by comparing the average fitness of progeny generated by autogamous and geitonogamous
90 crosses. The potential for somatic mutations to accumulate during vegetative growth, and the ability to
91 study their effects among recombinant progeny, make plants an attractive model for the study of
92 fundamental processes of clonal evolution.
93 Plants grow from the division of a population of meristem cells within the stem tip that is known
94 as the central zone. These germ cell lineages go on to produce future stem, leaf, and reproductive
95 tissues (flowers). As plants grow, individual ramets of the same genet (separate stems or vegetatively

96 propagated plants) can continue to become differentiated as they accumulate somatic mutations (Fig.
97 2). Mathematical models have demonstrated that multiple unexpressed mutations (i.e. neutral and
98 recessive deleterious mutations) are likely to accumulate as germ cells divide, so selective cell deaths
99 during somatic growth will reduce the total mutational load and alter the composition of mutations
100 carried by the germ cell population (Otto and Orive 1995; Otto and Hastings 1998). Lineages that carry
101 expressed deleterious mutations may have slower rates of division and can be eliminated as they are
102 replaced by cell lineages displaying more rapid growth (Fig. 2A). Moreover, theoretical treatments
103 predict constant turnover, as expressed deleterious mutations cause the loss of a cell lineage along with
104 all of its somatic mutations (Otto and Orive 1995; Otto and Hastings 1998; Orive 2001). This process –
105 referred to as cell lineage selection – has the potential to maintain moderate numbers of somatic
106 mutations within each meristem over the life of each ramet. More rarely, expressed beneficial
107 mutations will arise. These variants will be retained and accumulate during stem growth as these cell
108 lineages outcompete others, which leads to a process analogous to a selective sweep (Otto and Orive
109 1995; Fig. 2B green arrows; Otto and Hastings 1998; Orive 2001). The end result is that some ramets will
110 be characterized by a prevalence of deleterious somatic mutations, while others could possess beneficial
111 mutations. The question we address here is whether somatic mutations that characterize individual
112 ramets have measurable effects on fitness in the offspring generation.
113 When we consider the potential for meiotic and somatic mutations to contribute to the total
114 mutational load of plant populations–particularly for long-lived plants–it becomes evident that not all of
115 the mutations occurring during a plant's lifespan are passed on to the next generation (Cruzan 2018
116 pages 86-98). As described above, the process of cell lineage selection will tend to filter deleterious
117 mutations and favor the retention of beneficial mutations. In addition, some proportion of recessive
118 deleterious mutations may be eliminated during the haploid life stage due to pollen tube attrition and
119 pollen competition (Gemetophytic Selection; Mulcahy 1979; Cruzan 1989; Mable and Otto 1998;
120 Armbruster and Rogers 2004; Arunkumar et al. 2013; Harder et al. 2016). A portion of deleterious
121 mutations also will be homozygous in zygotes, which can lead to higher rates of seed and fruit abortion
122 (Selective Embryo Abortion; Husband and Schemske 1995; Korbecka et al. 2002), thereby increasing the
123 average fitness of surviving offspring (Cruzan and Thomson 1997; Mena-Alí and Rocha 2005). Indeed,
124 the effects of deleterious somatic mutations often are apparent as higher rates of embryo abortion after
125 autogamous compared to geitonogamous pollinations, which is referred to as autogamy depression
126 (Schultz and Scofield 2009; Bobiwash et al. 2013). In contrast, mutations that result in faster growth of
127 stem cells will be favored by cell lineage selection and will have an elevated chance of being inherited.

128 The sum effects of this intra-generation selection, which includes cell lineage selection, gametophytic
129 selection, and selective embryo abortion, may result in non-random complements of mutations entering
130 the next generation.

131 In this study, we use autogamous and geitonogamous self-pollinations to estimate the effects
132 of somatic mutations segregating in offspring of *Mimulus guttatus* DC (*Erythranthe guttata* G.L. Nesom;
133 Phrymaceae). Populations of *M. guttatus* display a wide range of life histories – from annuals to
134 herbaceous perennials that outcross to varying degrees (Wu et al. 2008). We use perennial *M. guttatus*
135 plants that produce substantial vegetative growth prior to initiation of flowering. These plants are easily
136 propagated from rosettes, and selfing produces substantial numbers of seeds to allow for statistical
137 comparisons. In two separate experiments, we characterized the fitness effects of somatic mutations
138 that accumulated during vegetative growth of perennial *M. guttatus* plants that were subjected to novel
139 environments. We made autogamous and geitonogamous self-pollinations across multiple stems and
140 asked whether there was evidence for autogamy depression for seed set and embryo abortion. In
141 addition, we estimated the fitness effects of somatic mutations segregating in progeny. We begin by
142 describing our analytical approach in greater detail, followed by providing specifics of the two
143 experiments. We show that somatic mutations accumulate during stem growth, as progeny from
144 autogamous crosses have greater variance in mean fitness compared to progeny from geitonogamous
145 crosses. We further reveal evidence for the presence of beneficial mutations in some stems, which
146 indicates that intra-generation selection may be filtering mutations to modify the distribution of fitness
147 effects transmitted to progeny. The results from these experiments challenge widely accepted notions
148 concerning mutation accumulation during vegetative growth and the distribution of fitness effects of
149 mutations in plant populations.
150

151 **Approach**

152 While previous studies have detected autogamy depression expressed as seed and fruit abortion
153 (reviewed in Bobiwash et al. 2013), no earlier work has evaluated the effects of somatic mutations on
154 the fitness of seedlings in the next generation. We use a comparative approach to evaluate the fitness
155 consequences of somatic mutations that are unique to each stem.

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, so in progeny arising by autogamy, they will segregate as approximately
158 25% homozygous ($a'a'$), 50% heterozygous (aa'), and 25% the original homozygote (aa). By contrast,
159 because progeny from geitonogamous crosses will be segregating for mutations that are unique to each

160 stem, about half of them will be carrying mutations in the heterozygous state, and none of the progeny
161 will be 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 This equation is analogous to the measure of inbreeding depression based on differences in the relative
166 fitness of offspring derived from selfing vs. outcrossing.

167 Autogamy depression has been applied previously only to estimates of fitness based on fruit and
168 seed set, and it generally assumes that the fitness of progeny from autogamous crosses will be lower
169 than from geitonogamy. Thus, for each stem, we estimated the fitness effects of somatic mutations
170 unique to that stem ($\bar{s}_k$) as the difference in mean fitness between the progeny from autogamous and
171 geitonogamous crosses from a single stem: $\bar{s}_k = \bar{w}_{k(A)} - \bar{w}_{k(G)}$, where $\bar{w}_{k(A)}$ and $\bar{w}_{k(G)}$ are the average
172 fitnesses of the progeny from autogamous and geitonogamous crosses from stem *k*, respectively.
173 Provided that environmental effects are controlled for with a common garden design, the parameter $\bar{s}_k$
174 represents the combined effects of one or more mutations that have different magnitudes and/or
175 directions of effects on fitness, which provides an estimate of the average fitness effects of all expressed
176 somatic mutations that have accumulated during vegetative growth. To evaluate the reliability of $\bar{s}_k$ as
177 an estimate of *s* we conducted simulations by randomizing the selective effects and dominance of
178 somatic mutations in pairs of stems. We determined that values of $\bar{s}_k$ were accurate estimates of *s*
179 across a range of values and assumptions for the effects of multiple mutations (Appendix 1).

180 While simulations indicate that our estimates of $\bar{s}_k$should provide reliable information on the
181 sign and magnitude of the average fitness effects of somatic mutations unique to a stem, we also
182 developed an alternative approach for further validation. Individual germ cell lineages within the
183 meristem may possess unique sets of somatic mutations (Schwoch et al. in preparation), and flowers on
184 the same stem can develop from only a portion of the germ cells present in the meristem (McManus
185 and Veit 2002). Hence, no pair of flowers within a stem are likely to contain exactly the same
186 complement of mutations. Therefore, we generated an additional estimate of *s* that is based only on the
187 variance in fitness of offspring from autogamous crosses. If somatic mutations affect offspring fitness,
188 the variance in fitness should be greater for progeny groups from autogamy than from geitonogamy
189 from the same stem, as long as mutations do not have complete expression in heterozygotes (i.e. $h <$
190 1.0; Appendix 2). Somatic mutations that are unique to stems will have different effects on the variance
191 in fitness among progeny from autogamy depending on the strength of selection (*s*) and their expression

192 in the heterozygous state (*h*). To evaluate these effects, we considered a single somatic mutation
193 occurring in a stem with genotype *aa'* that segregated in offspring in a 1:2:1 ratio following autogamy.
194 We assumed that the fitness of the *aa*, *aa'*, and *a'a'* genotypes was 1, 1+*hs*, and 1+*s*, respectively (where
195 *s* ranges from -1 to 1, and *h* ranges from 0 to 1). Our analytical results demonstrated that the large
196 majority of variance in fitness among progeny from autogamy (as measured by the standard deviation,
197 SD) could be explained by the selection coefficient rather than the dominance level (Appendix 2; Fig. S1).
198 Moreover, the derived relationship allows us to estimate the selective effects of mutations based on the
199 standard deviation in fitness according to the equation: $s_{SD} = cSD$, where *c* is the slope of the linear
200 relationship between s_{SD} and *SD*. For dominance levels of *h* = 0.0 and 1.0, *c* = 2.31, and the slope reaches
201 a maximum of 3.01 when *h* = 0.5 (Appendix 2; Fig. S1). Thus, the variance in fitness among progeny from
202 autogamous crosses provides a good approximation of the magnitude of the selection coefficient (*s*) for
203 somatic mutations accumulating in each stem. However, because *s* is estimated from the standard
204 deviation, only the magnitude of selection but not its direction can be inferred. Thus, although each
205 approach has potential shortcomings (but for different reasons), identifying consistent results using
206 both approaches would provide a robust estimate of the average selective effects of somatic mutations
207 present in individual stems (Appendix 1 and 2).
208 From the considerations above, we can make three specific predictions that allow us to test for
209 the fitness effects of somatic mutations that occur during stem growth and are passed on to offspring.
210 First, there should be greater variation among progeny groups from autogamy compared to those from
211 geitonogamy (i.e. as indicated by the stem x cross type interaction for progeny fitness). Second, the
212 average fitness of progeny from autogamy should be different from the progeny of geitonogamous
213 crosses from the same stem. The difference in the fitness of progeny from autogamous vs.
214 geitonogamous crosses allows us to make an approximate estimate of the sign and magnitude of
215 selection coefficients for individual stems. In addition to influencing the mean fitness, somatic mutations
216 will increase the variance in fitness among progeny from autogamy. Consequently, the third prediction
217 we can make is that the selective effects of somatic mutations will scale linearly with the within-family
218 standard deviation in fitness among progeny from autogamous crosses (Appendix 2). In general, we
219 predict that if somatic mutations with fitness effects accumulate during vegetative growth, then the
220 mean fitness should differ between progeny from autogamy and geitonogamy, and the variance in
221 fitness of progeny from autogamous crosses would be greater than for progeny from geitonogamous
222 crosses. Below, we test these predictions using two different experiments with *Mimulus guttatus*.
223

224 **First Experiment**

225 *Methods*–This experiment originally was designed to test the effects of somatic mutations on
226 seed set and ovule abortion (autogamy depression; Schultz and Scofield 2009; Bobiwash et al. 2013), so
227 we used multiple plants from several populations to obtain a large number of fruits. We grew plants of
228 *M. guttatus* in the Research Greenhouse facility at Portland State University from seed collected in July
229 2013 from three different populations in northern Oregon (Jackson Bottom Wetlands–JB: 45.501794 N, -
230 122.98776 W; and two from Saddle Mountain–SMB: 45.9861 N, -123.6859 W, and SMC: 45.9634 N, -
231 123.6837 W). We assumed that greenhouse conditions were different enough from field environments
232 to provide a novel selection regime that would increase the chances mutations would enhance fitness
233 because genotypes were further from the new fitness optimum (Fisher 1930; Bull and Otto 2005;
234 Stearns and Fenster 2016). In August 2013, seeds were cold stratified on moist paper towels at 2°C for
235 30 days prior to being sown in soil. Seedlings were transplanted to pots (approximately 10 x 10 x 12 cm)
236 and grown for seven months before the application of pollination treatments. Temperature was
237 maintained between 21–26°C during the day, and 15–21°C at night. Supplemental HID lights ran for 12
238 hours a day when the seedlings first emerged, and 14 hours a day during adult growth.

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

256 We assessed the fitness of progeny arising by autogamy and geitonogamy in the same
257 greenhouse environment that was used to grow the parental plants. Seedlings from a subset of ten
258 maternal plants that had fruits from both cross types and at least 20 filled seeds were sown in soil and
259 transplanted to 36-cell trays (blocks) in September in a randomized incomplete block design. After three
260 months of growth, the progeny were scored for survival, and above ground biomass was measured after
261 drying at 60°C for at least 24 hours. The fitness of each progeny was estimated as its final biomass,
262 which was log transformed and weighted by the survival frequency of progeny from the same cross.
263 Biomass is considered to be an appropriate estimate of fitness for perennials (Younginger et al. 2017).
264 These estimates were rescaled relative to the maximum value from all crosses, so that $\overline{w}$ ranged from 0
265 to 1 across progeny from all crosses. Data were analyzed to estimate the mean fitness for progeny from
266 autogamy ($\overline{w}_{k(A)}$) and geitonogamy ($\overline{w}_{k(G)}$) progeny from each stem, and the variance in fitness (as
267 measured by the standard deviation; SD) for each group of progeny produced by autogamy and
268 geitonogamy from a single stem.
269 *Results*–Levels of seed set and germination were similar between cross types, but embryo
270 abortion was greater in autogamous crosses. The mean number of seeds produced after autogamous
271 (56.72 ±2.24, N = 87) and geitonogamous (57.52 ±3.42, N = 52) pollinations was similar (Cross in Table
272 S2), and there was little variation among populations (Pop in Table S2), maternal plants nested within
273 population (Mat(Pop) in Table S2), and pollination dates (Date in Table S2). Limited pollinations
274 produced fewer seeds than excess pollinations (Pollen in Table S2), but the effect of pollen dosage in the
275 overall model was not significant. However, the mean number of aborted ovules was greater for
276 autogamous (17.36 ±1.27, N = 87) than for geitonogamous (13.90 ±1.70, N = 52) pollinations (Cross in
277 Table S3), which is consistent with the predicted effects of autogamy depression (Schultz and Scofield
278 2009; Bobiwash et al. 2013). There also were significant differences among populations in the level of
279 ovule abortion (Pop in Table S3), which perhaps reflects differences in historical inbreeding and levels of
280 genetic load. There were significant differences in ovule abortion among maternal plants nested within
281 population (Mat (Pop) in Table S3), but not for pollination treatments (Pollen in Table S3) or pollination
282 dates (Date in Table S3). The overall model for ovule abortion was significant (Table S3). Seed
283 germination was similar between autogamous (mean = 13.03 out of 20 planted per fruit) and
284 geitonogamous crosses (mean = 13.28; $F_{1,101}$ = 0.16, P = 0.694). Of the 354 seedlings that germinated,
285 202 survived, and survival was higher for progeny from autogamy (67%) compared to geitonogamy
286 (50%; chi-square = 12.03, P = 0.0005). Nearly all surviving plants flowered by the end of the experiment,
287 and flower production was correlated with above ground biomass (r = 0.58, P < 0.001, N = 282).

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

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

352 high salt in the hydroponics system, as described above. A total of 239 seedlings from 11 fruits (five
353 autogamous and six geitonogamous) were randomly and evenly distributed among 12 hydroponic tubs
354 to ensure equal representation across blocks (tubs). Plant size was measured as the product of the
355 length and width of vegetative spread after two months of growth and was used as a proxy for biomass.
356 Salt concentration increased from 10 mM - 37.5 mM over the course of the experiment to induce
357 mortality (~57% across all progeny groups). Fitness was estimated as plant size (log transformed to
358 improve normality) weighted by the survival frequency for progeny from the same 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 **Selection Coefficient 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. Since the results from these two experiments are qualitatively

384 similar, we combined them to estimate the selection coefficients of somatic mutations in each stem (ten
385 comparisons of autogamy and geitonogamy from Experiment 1 and four from Experiment 2, for a total
386 of 14 comparisons). Specifically, we tested the second and third predictions described above. We expect
387 that the effect of somatic mutations will be greater for progeny from autogamy, so their mean fitness
388 should be different from progeny of geitonogamous crosses on the same stem (providing an estimate of
389 $\bar{s}_k$). Moreover, the within-family variance in fitness among progeny should be greater after autogamy
390 compared to geitonogamy (providing an estimate of s_{SD}). These estimates of the selective effects of
391 somatic mutations should be similar, so we assessed whether there was a positive relationship between
392 the variance in fitness among progeny from single fruits produced by autogamous pollination (s_{SD}) and
393 the selection coefficient estimated from the average fitness of progeny from autogamous and
394 geitonogamous crosses to the same stem ($\bar{s}_k$).

395 Estimates of the average selective effects of somatic mutations unique to each stem were made
396 based on the fitness of progeny from autogamous ($\overline{w}_{k(A)}$) and geitonogamous ($\overline{w}_{k(G)}$) crosses (Table S1
397 in Appendix 3). In both experiments, fitness was estimated as the above ground biomass weighted by
398 the survival of seedlings from the same fruit and scaled to a maximum value of 1.0. The average
399 selective value of mutations ($\bar{s}_k$) for each stem (first experiment) or stem/node combination (second
400 experiment) was calculated as the difference in fitness between progeny from autogamous and
401 geitonogamous crosses, as described above ($\bar{s}_k$ = $\overline{w}_{k(A)}$ - $\overline{w}_{k(G)}$). We observed extensive variation in $\bar{s}_k$
402 among stems, with nine stems that were significantly different from zero (Fig. 3). Four of the stems had
403 average selective effects of somatic mutations that were positive, suggesting a net beneficial effect of
404 somatic mutations transmitted to offspring. In addition, the average fitness of progeny from
405 autogamous and geitonogamous crosses on the same stems or nodes was positively correlated (Fig. S2;
406 Table S6). Note also that the $\bar{s}_k$ estimates that deviated the most from zero generally had the highest (or
407 lowest) $\overline{w}_{\mathrm{A}}$ values relative to all of the stems (Fig. S3). However, this was not always the case, as one
408 stem (stem 9) with a value of $\bar{s}_k$ close to zero produced progeny with relatively high fitness after both
409 autogamous and geitonogamous pollination.

410 To make additional estimates of the average selection coefficients for mutations unique to each
411 stem or node, by using the standard deviation in progeny fitness from autogamous crosses based on the
412 relationship $s_{SD} = cSD$, where c = 3, which assumes dominance close to h = 0.5 (qualitatively similar
413 results are obtained if we choose other values of c between 3 and 2.31; Appendix 2). Because the sign of
414 s_{SD} could not be inferred directly from this approach, we used the sign estimated from the $\bar{s}_k$method
415 (Fig. 4; Appendix 2). Our simulations show that the sign of $\bar{s}_k$is almost always estimated correctly

416 (Appendix 1). For stems with values of $\bar{s}_k$ greater than zero, there was a strong positive relationship
417 between $\bar{s}_k$ and estimates of s_{SD} made from the within-family variation among progeny from autogamy
418 (Fig. 4). In contrast, the relationship for negative values of $\bar{s}_k$ appeared to be driven largely by a single
419 observation. Note that this observation was not supported by a similarly high value of s_{SD}, and the
420 remaining negative selection coefficients were more modest based on both estimates. This one highly
421 negative, and apparently inaccurate estimate of $\bar{s}_k$, may be due to a large influence of mutations from
422 the second stem on the fitness of geitonogamous progeny (note that this stem had the highest estimate
423 of $\overline{w}_{k(G)}$; Fig. S2). It is also notable that the variance in fitness for progeny from autogamy did not
424 decline to zero for values of $\bar{s}_k$ close to zero, which could be due to the presence of both beneficial and
425 detrimental mutations, and possibly genetic background effects (i.e. epistasis), but it may also reflect
426 uncontrolled environmental variation. Overall, the results from both approaches reveal consistent
427 estimates of the average fitness consequences associated with the accumulation of somatic mutations
428 in individual stems.
429
430 **Discussion**
431 The observation of higher variation in fitness among stems for progeny from autogamous compared to
432 geitonogamous self-pollinations provides strong evidence for the accumulation of somatic mutations
433 during vegetative growth in *Mimulus guttatus*. Two estimates of the effects of selection due to somatic
434 mutations provided consistent results on offspring fitness: 1) Differences in the mean fitness between
435 progeny from autogamy and geitonogamy ($\bar{s}_k$), and 2) variation in fitness of autogamous progeny (s_{SD}).
436 We found evidence for the prevalent effects of beneficial mutations in progeny, with estimates of $\bar{s}_k$
437 and s_{SD} exceeding 0.1 in four cases, while estimates for negative selection coefficients were more
438 modest (mostly > -0.15). These results are consistent with the hypothesis that somatic mutation
439 accumulation during vegetative growth is substantial enough to have demonstrable effects on progeny
440 fitness and to contribute to standing genetic variation. Furthermore, the observation that progeny from
441 autogamous crosses occasionally had higher fitness than progeny from geitonogamous crosses argues
442 for the accumulation of beneficial mutations and implies that many deleterious mutations are culled by
443 the various types of intra-generation selection prior to seed dispersal. These results suggest that somatic
444 mutations are generated during vegetative growth, but many of them are filtered due to intra-
445 generation selection, which results in a shift in the distribution of fitness effects for the mutations that
446 are passed on to the next generation.

447 Somatic mutations accumulating during vegetative growth had an overall positive effect for four
448 of the stems tested. This result is contrary to widely held views that the accumulation of beneficial
449 somatic mutations should be exceedingly rare (Crow 1993; Charlesworth and Willis 2009). However, a
450 potential explanation for these findings is afforded by the unique biology of plants; somatic mutations in
451 meristem cells could contribute to higher rates of division for some cell lineages over others (Otto and
452 Orive 1995; Otto and Hastings 1998). Thus, cell lineage selection has the potential to retain beneficial
453 somatic mutations, as individual cell lineages divide at faster rates. Although there may be few
454 opportunities for beneficial changes to alter basic cellular metabolism, it is becoming apparent from
455 experimental evolution studies with microbes that even basic aspects of cellular metabolism can be
456 sensitive to environmental conditions, which can increase the chances that mutations in clonal
457 populations are beneficial (e.g., Lee and Marx 2013; Maharjan et al. 2015). In this regard, cell lineage
458 selection in a plant meristem is analogous to clonal evolution in microbial populations, and it represents
459 a potentially powerful forum for the removal of deleterious somatic mutations while favoring the
460 retention of beneficial ones. In addition, gametophytic selection and selective embryo abortion can act
461 as prominent filters (Mulcahy 1979; Cruzan 1989; Mable and Otto 1998; Armbruster and Rogers 2004;
462 Arunkumar et al. 2013; Harder et al. 2016), but they are most likely to have effects by culling deleterious
463 mutations. Regardless, the combined effects of these different forms of intra-generation selection
464 appear to have had a considerable effect on filtering of somatic mutations, such that the distribution of
465 fitness effects among stems has shifted to include more beneficial mutations than expected.
466 An alternative explanation for the observed phenotypic changes is that exposure to
467 environmental stress has induced heritable epigenetic modifications (Quadrana and Colot 2016).
468 However, epigenetic modifications are often considered to be consistent in direction and predictable, as
469 they are hypothesized to represent an adaptive response to historic exposure to similar stressors
470 (Baulcombe and Dean 2014; Itabashi et al. 2018). The results of the experiments described here are
471 unlikely to support a role for epigenetics, because phenotypic responses in the next generation were
472 inconsistent in direction and magnitude, and they were not predictable based on environmental
473 exposure of the parent stem. Indeed, among stems and nodes, the mean fitness of progeny from
474 autogamy displayed both increases and decreases compared to the geitonogamy controls, which is
475 consistent with the hypothesis that individual ramets are accumulating unique complements of somatic
476 mutations. Furthermore, these conclusions are supported by the observation of numerous somatic
477 variants in the transcriptomes of meristem tissue from multiple ramets derived from a single genet

478 (Schwoch et al. in preparation). Thus, the results of the current study appear to indicate that somatic
479 mutations accumulating during stem growth are responsible for the observed phenotypic effects.
480 The potential for the acquisition of mutations during vegetative growth is a well-known aspect
481 of plant biology (Klekowski 2003; Schultz and Scofield 2009; Bobiwash et al. 2013), but no previous study
482 has demonstrated the effects of somatic mutations on the fitness of progeny in the next generation.
483 Most studies have focused on the detrimental effects of somatic mutations; chloroplast mutants have
484 been observed in a number of species (Klekowski 2003), declines in pollen fertility were found in older
485 clones of quaking aspen (Ally et al. 2010), and higher rates of seed and fruit abortion after autogamous
486 pollinations were found in several studies (reviewed in Bobiwash et al. 2013). In contrast, agriculturalists
487 have taken advantage of beneficial somatic mutations by conducting clonal selection to improve
488 economically important plants (Aradhya et al. 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli
489 et al. 2012; Jarni et al. 2015; Pelsy et al. 2015), and a handful of studies report phenotypic responses to
490 selection in asexual lineages. For example, Breese et al. (1965) succeeded in selecting for increased
491 tillering ability (production of new grass stems) within genets of perennial ryegrass (*Lolium perenne*).
492 Similarly, artificial selection on clonal lineages effectively improved branching in the red seaweed,
493 *Asparagopsis armata* (Monro and Poore 2009). The current study on *M. guttatus* contributes to this
494 literature by highlighting the potential for plants to exhibit significant levels of clonal variation within a
495 single generation.
496 The transmission of beneficial mutations in autogamous crosses may explain some heretofore
497 difficult to understand results from mutation accumulation studies. Our results suggest that beneficial
498 somatic mutations are likely to be partially dominant (i.e. not completely recessive), because they would
499 have to be expressed in the heterozygous state to be favored by cell lineage selection. Mutations
500 accumulating during vegetative growth could contribute to standing genetic variation, but autogamy
501 may be more effective for the accumulation of beneficial somatic mutations in populations than
502 geitonogamy or outcrossing, because beneficial mutations could be made homozygous in progeny in a
503 single generation. Depending on the crossing design, outcrossing would take at least two generations for
504 beneficial somatic mutations to be made homozygous, and under geitonogamy they would remain
505 heterozygous. It is striking that high rates of beneficial mutation accumulation have been observed in at
506 least some mutation accumulation studies in the autogamous plant *Arabidopsis thaliana* (Shaw et al.
507 2002; Rutter et al. 2010; Rutter et al. 2012), but not in outcrossing and partially-selfing species of
508 *Amsinckia* (Schoen 2005). With a few exceptions (e.g., Baer et al. 2005; Denver et al. 2010), nearly all
509 mutation accumulation studies on animals consistently show a prevalence of deleterious mutations

510 (Baer et al. 2007; Halligan and Keightley 2009). Similarly, our results suggest that the adaptive potential
511 of autogamous plants may be greater than previously thought, which may help explain the wider
512 geographic ranges of selfing compared to closely-related outcrossing species (Grossenbacher et al.
513 2015). Although intra-generation selection has the potential to contribute to adaptation in all plants, its
514 effects may be enhanced in autogamous lineages, because beneficial mutations arising during
515 vegetative growth have a greater chance of becoming homozygous in offspring and being retained
516 across generations.

517 As stems grow, mutations are generated during every mitotic cell division, so the potential for
518 somatic mutation accumulation in plants appears substantial. Thus, understanding how long-lived
519 plants, such as trees, avoid mutational meltdown from the accumulation of deleterious somatic
520 mutations remains a longstanding question. Paradoxically, however, the rate of mutation accumulation
521 observed across generations in plant and animal genomes is similar (Gaut et al. 2011). One explanation
522 for this pattern is that somatic mutations in plants are repressed during vegetative growth, similar to
523 animal germlines, which would protect lineages from the negative effects of mutation accumulation
524 during development of the soma (Plant Germline Hypothesis; Burian et al. 2016; Cruzan 2018; page 90).
525 An alternative hypothesis posits that somatic mutations are generated in apical meristems during plant
526 growth, but these mutations are filtered by intra-generation selection occurring prior to the
527 establishment of offspring (Somatic Mutation Accumulation Hypothesis). Because plants have retained
528 the capacity to undergo clonal evolution from their algal ancestors, the ability to filter mutations during
529 growth and reproduction has existed for some time, and thus intra-generation selection has the
530 potential to skew the distribution of fitness effects of transmitted mutations to include a larger
531 proportion of beneficial mutations than would be expected through random processes. In addition, this
532 provides a reasonable explanation for why longer-lived plants appear to have slower rates of mutation
533 accumulation across generations (Yue et al. 2010; Gaut et al. 2011). This could be due to the fact that
534 longer generation time leads to more time between recombination events, which can lead to more
535 background selection in non-recombining cell lineages during vegetative growth (Cruzan 2018; pages 94-
536 95). Recent studies in oaks and spruce (Plomion et al. 2018; Hanlon et al. 2019), as well as our
537 unpublished data from *M. guttatus*, confirm that multiple somatic variants are likely, even in plants of
538 very different size. Moreover, results from the current study identify the presence of beneficial
539 mutations that may be passed on to offspring in the next generation, likely a consequence of intra-
540 generation selection. Future work that combines information from experiments evaluating the genomic
541 consequences of somatic variation with anatomical estimates of stem cell population dynamics will

allow for the development of new models that provide insights into the extent and limitations of somatic evolution in plants.

In conclusion, despite the potential for somatic mutation accumulation to generate novel genetic variation in plant populations, its role in evolution remains almost entirely unexplored. Our estimates of the selective effects of somatic mutations were consistent across several methods and indicate that some stems possessed a prevalence of deleterious mutations while others produced autogamous progeny with high fitness, which indicates a prevalence of beneficial mutations. Even though high levels of mutation accumulation are often believed to be detrimental, the basic biology of plants suggests that the role of somatic mutations in plant evolution should be considered carefully in the future. Moving forward, our results indicate that we must keep in mind that all eukaryotes are not necessarily equivalent, and features of the organism's biology may have unexpected consequences for the evolutionary phenomena that we observe. Future lines of investigation will improve our understanding of these fundamental aspects of plant development and evolution that may have contributed to the remarkable diversification of plants, and may help to account for some of the extensive variation in mutation rates detected among lineages.

Acknowledgements

We thank J. Thompson, E. Perez, and our research greenhouse manager Linda Taylor for plant maintenance and assistance with this research in the greenhouse and in the lab. Several people made helpful suggestions on this manuscript including N. Diaz, M. Grasty, and J. Persinger. This research was supported by an NIH NIGMS BUILD EXITO grant (5TL4GM118965-03, 5UL1GM118964-03, and 5RL5GM118963-03) to Portland State University.

Data Archiving

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

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764 Figure Captions

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

767 Fig. 2. An illustration of cell lineage selection as it acts on a number of cell lines that differ in growth rate
768 within the apical meristem of a plant. A) Expressed deleterious mutations are predicted to lead to the
769 demise of a cell line (brown cells) that is replaced by neighboring cell lines. The appearance of a
770 mutation that increases cell growth rate in the current environment results in a selective sweep of the
771 meristem (light green cells). B) Multiple ramets of the same genet become differentiated by unique
772 somatic mutations during vegetative growth. Most ramets carry deleterious mutations (orange, red,
773 purple, and pink arrows), but expressed beneficial mutations cause selective sweeps of the meristem in
774 some ramets (green arrows).

775 Fig. 3. Estimates of $\bar{s}_k$ for fourteen different stems (ramets) from two separate experiments (Experiment
776 1–blue bars; Experiment 2–orange bars) based on mean progeny fitness after autogamous $\overline{w}_{k(A)}$ and
777 geitonogamous $\overline{w}_{k(G)}$ self-pollinations ($\bar{s}_k$ = $\overline{w}_{k(A)}$ - $\overline{w}_{k(G)}$). Horizontal lines represent standard errors.
778 Asterisks indicate values of $\bar{s}_k$ that are significantly different from zero based on the *t* value, calculated
779 as $t = \bar{s}_k/\sqrt{SE}$ with *n-1* df, where *n* is the mean of sample sizes for progeny from autogamy and
780 geitonogamy. The relationship between $\overline{w}_{k(A)}$ and $\overline{w}_{k(G)}$ across stems is shown in Fig. S2. Means and
781 sample sizes for progeny groups are available in Table S6 and Fig. S3.

782 Fig. 4. Relationships between estimates of selection coefficients, based either on the difference in
783 fitness of progeny from autogamy and geitonogamy ($\bar{s}_k$), or the standard deviation in fitness within
784 progeny groups from autogamy for each stem (s_{SD}). Estimates of s_{SD} corresponding to negative values of
785 $\bar{s}_k$ were transformed to negative values. Estimates from Experiment 1 are indicated by blue circles and
786 from Experiment 2 are orange squares. Dashed lines indicate the separate relationships for positive and
787 negative values of the selection coefficients.

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789

Fig. 1

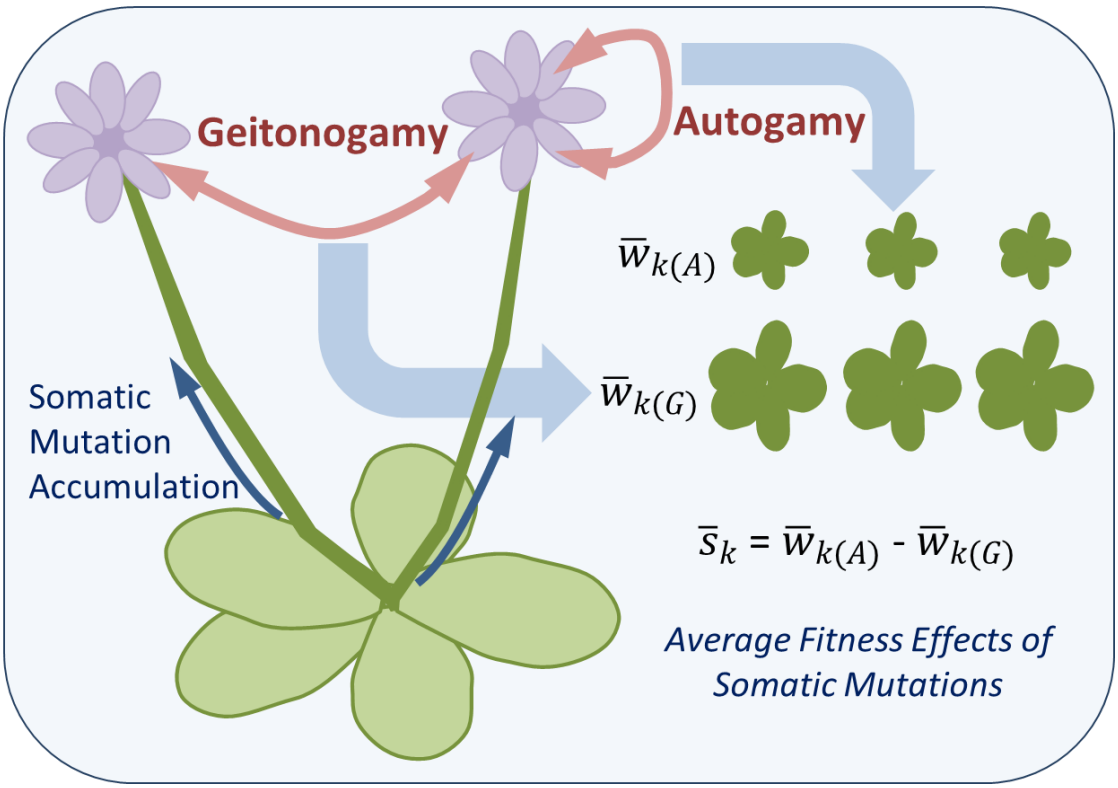

Geitonogamy
Autogamy
$\overline{w}_{k(A)}$
$\overline{w}_{k(G)}$
Somatic
Mutation
Accumulation
$\overline{s}_k = \overline{w}_{k(A)} - \overline{w}_{k(G)}$
Average Fitness Effects of
Somatic Mutations

Fig. 2

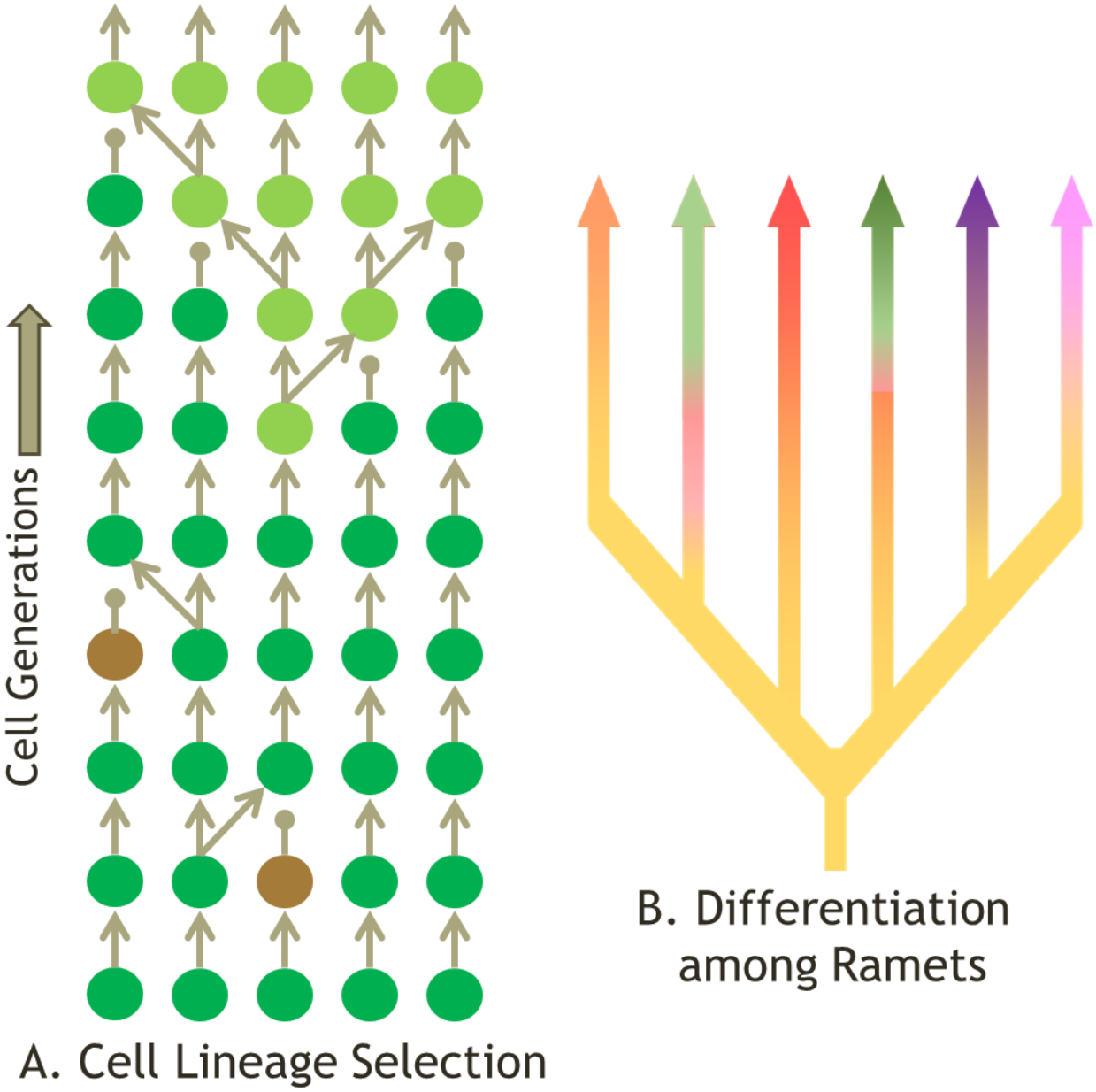
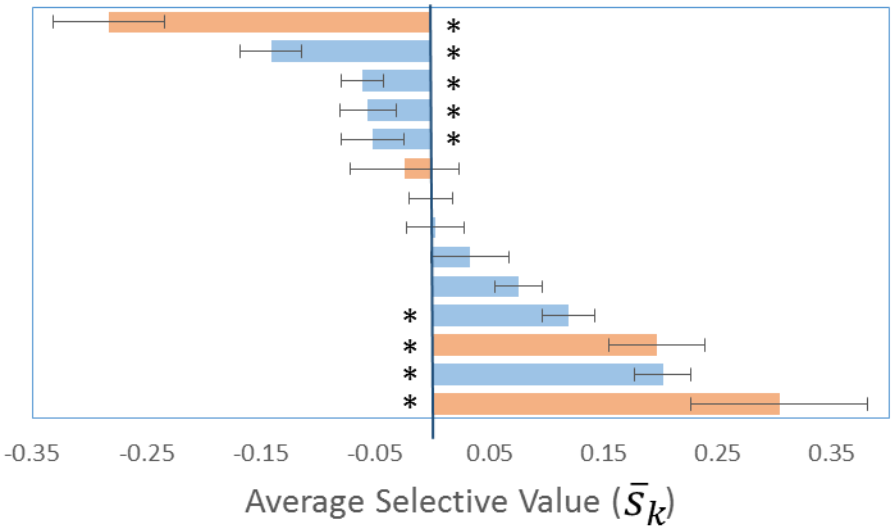

Cell Generations
A. Cell Lineage Selection
B. Differentiation among Ramets

Fig. 3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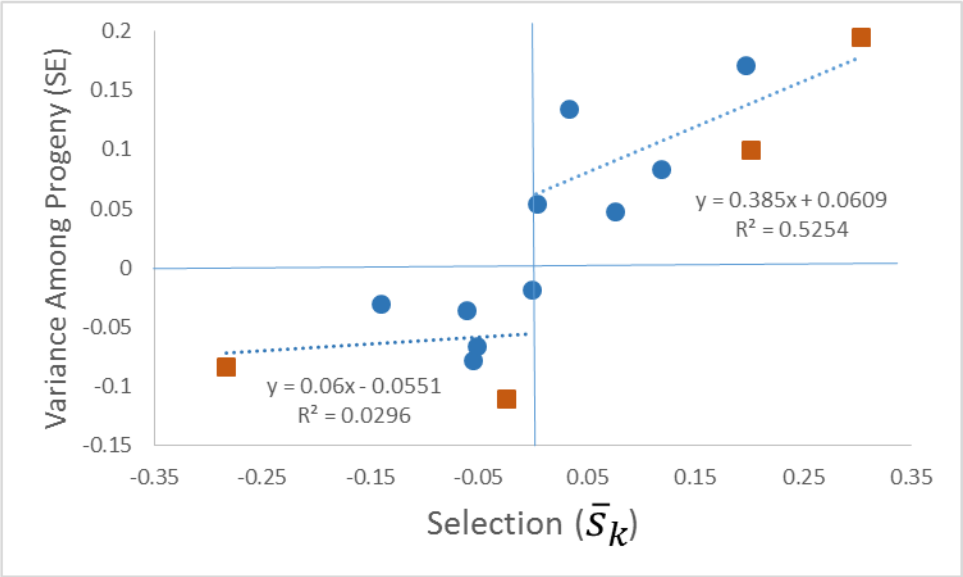
-0.35
-0.25
-0.15
-0.05
0.05
0.15
0.25
0.35
Average Selective Value ($\bar{S}_k$)

Fig. 4


Variance Among Progeny (SE)
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.35
-0.25
-0.15
-0.05
0.05
0.15
0.25
0.35
Selection ($\bar{s}_k$)
y = 0.385x + 0.0609
R² = 0.5254
y = 0.06x - 0.0551
R² = 0.0296

Appendix 1. Validity of the $\bar{s}_k$ Estimate

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

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

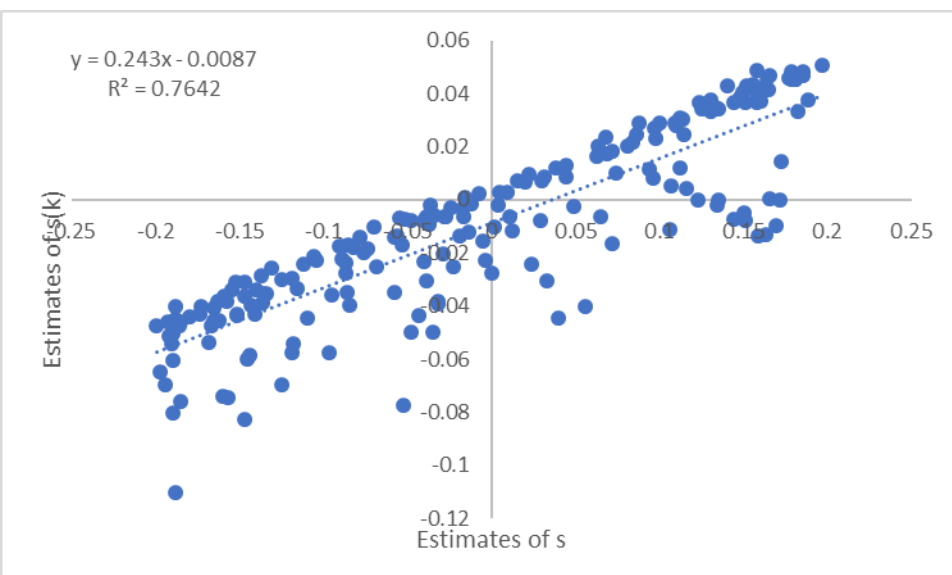
$$w_A = 0.25(1) + 0.5(1+ h_1s_1) + 0.25(1+s_1)$$

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

For simulations, we randomly chose values of s ranging from -0.2 to 0.2 for 200 pairs of stems. Values of h for $s > 0$ were allowed to range from 0 to 0.7 for each stem. Because deleterious alleles tend to be recessive, values of h were constrained to range between 0 and 0.1 for $s < 0$. To match our experimental design in *Mimulus guttatus*, we calculated the fitness of autogamous progeny (w_A) for one of the stems in each pair. We then calculated w_G using information on s and h for both stems in each pair. Then we calculated the difference in fitness between each estimate as a measure of autogamy depression ($s_k = w_A - w_G$). This simulation was repeated 20 times (4,000 pairs of stems total) and average values were calculated.

<u>Results:</u>

1. Estimates of s_k and s_1 are strongly correlated, with R^2 values ranging from 0.76 to 0.82 (one example of 200 random estimates):



2. The frequency of s_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 s_k with incorrect signs were closer to zero. Mean absolute values of s for s_k estimates with incorrect signs was 0.054 (+/- 0.0021) and was 0.106 (+/-0.0011) for correct signs. Hence, estimates of s_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 s_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 s_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).

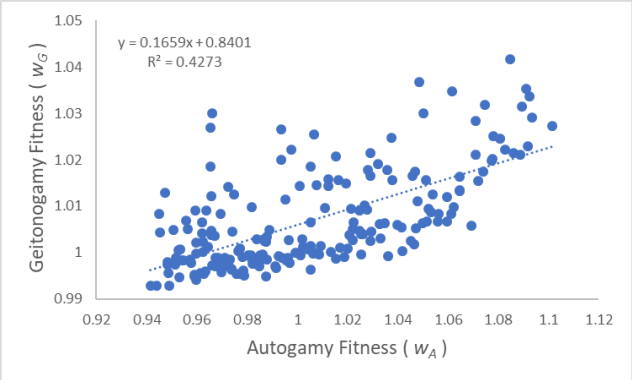
<u>Interpretation:</u>

These results indicate that our estimate of s_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).

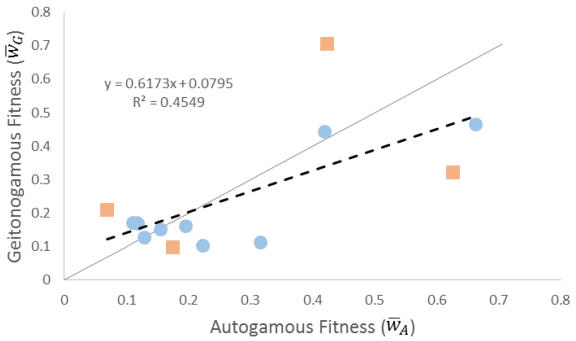
It is also notable that our simulation assumes a wider range of h values than are generally observed for deleterious mutations. Estimates indicate that the product of hs for deleterious alleles is generally around 0.02, and that the relationship between s and h is generally negative (hyperbolic). Minor 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 s_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

$\overline{w} = ¼(1) + ½(1+hs) + ¼(1+s)$

Which simplifies to

$\overline{w} = (1 + ½\ hs + ¼\ s)$

The variance in progeny fitness can be calculated as

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

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)

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

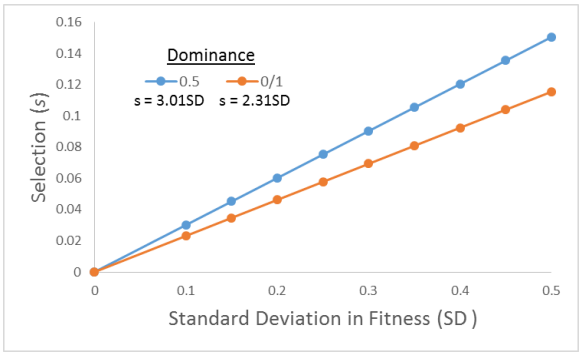


Fig. S1. The effects of selection (*s*) and dominance (*h*) on the standard deviation (SD) in fitness among progeny. The effect of dominance on variation in fitness is minimized when *h = 0.5* and maximized when *h = 0.0* and *h = 1.0*.

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* 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;

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

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

From these relationships we can estimate the selective effects of somatic mutations from the standard deviation in progeny fitness as $s_{SD} = cSD$, 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**	$\bar{s}_k$	$\bar{w}_A - \bar{\bar{w}}_G$
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		

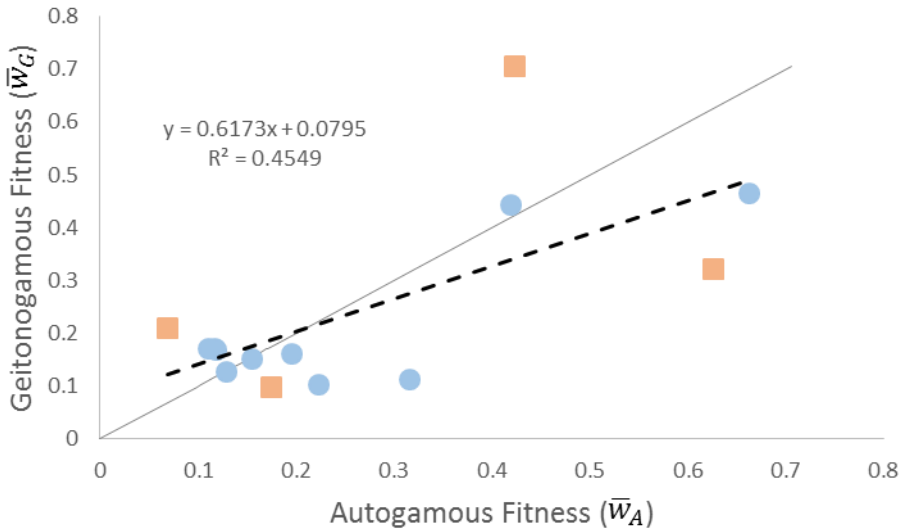


Fig. S2. The relationship between the autogamous and geitonogamous progeny from the 14 comparisons from experiments 1 (blue circles) and 2 (orange squares) as listed in Table S6. The solid line represents a 1:1 relationship and the dashed line represents the best-fit to the data. Stems falling above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

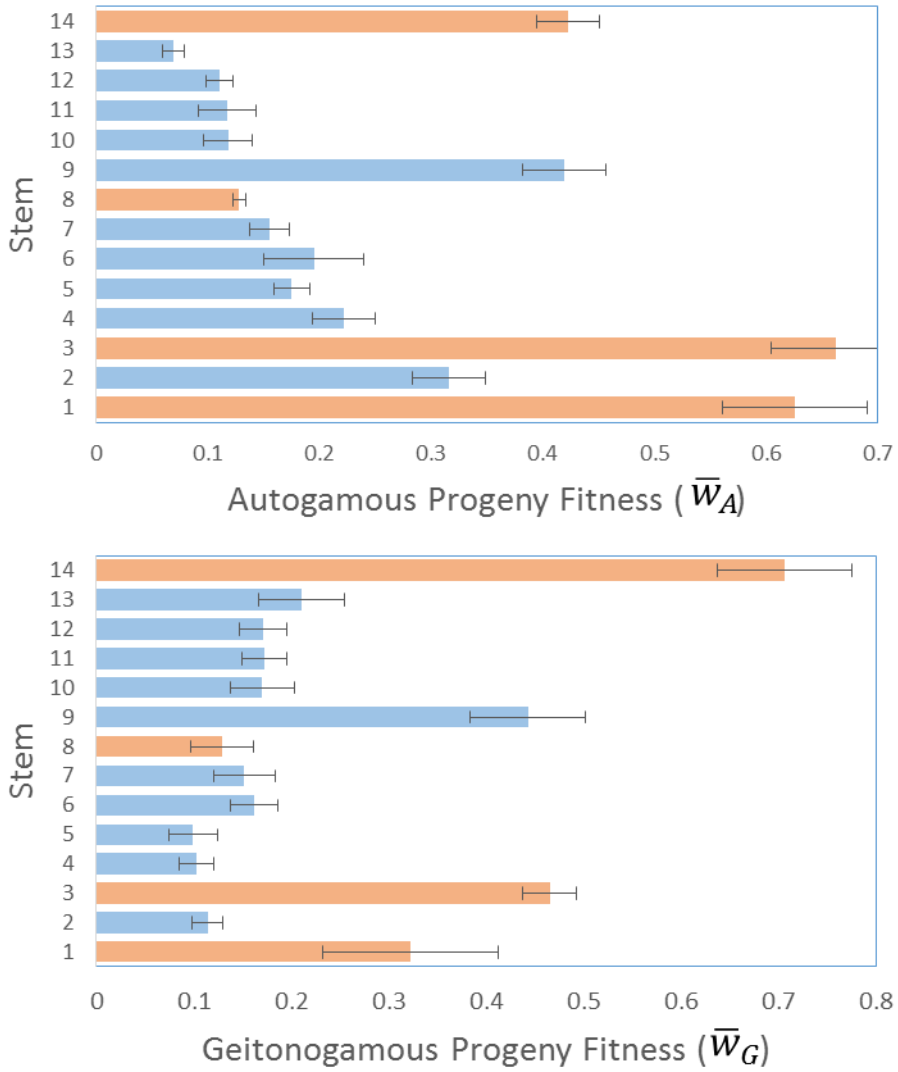


Fig S3. Means and standard errors (lines) for fitness estimates of autogamous (upper panel) and geitonogamous (lower panel) progeny groups from experiment 1 (blue bars) and 2 (orange bars) listed in Table S6. Progeny groups are ordered as in Fig. 4.