**Phenotypic Effects of Somatic Mutations Accumulating during Vegetative Growth**

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**Abstract**

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

**Impact Summary**

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

57 **Introduction**

58 Mutation is the source of variation for evolution and adaptation, but organisms differ in whether
59 mutations originating during gamete formation (meiosis) or somatic growth (mitosis) contribute to
60 heritable variation. For the vast the majority of organisms, including viruses, unicellular microbes, and
61 some multicellular eukaryotes, mutations occur only during cell replication, so they primarily exhibit
62 clonal evolution. By contrast, genetic modification occurring during somatic growth of animals is usually
63 limited to the appearance of acquired mutations and the development of cancer (Nowell 1976;
64 Beerenwinkel et al. 2015), neither of which contributes to heritable variation in populations. Plants
65 differ from animals and microbes, because mutations contributing to heritable variation can arise during
66 gamete formation and somatic growth. This is due to the fact that plants lack separate germ lines; the
67 same germ cells in apical meristems are responsible for vegetative growth and sexual reproduction.
68 Consequently, plants have the capacity for clonal evolution, as somatic mutations that occur in plant
69 meristems can accumulate and be passed to the next generation (McKnight et al. 2002; Klekowski 2003;
70 Schultz and Scofield 2009; Bobiwash et al. 2013; Dubrovina and Kiselev 2016; Watson et al. 2016;
71 Schmid-Siegert et al. 2017). This aspect of plant biology is well known (Monro and Poore 2009; Ally et al.
72 2010; Reusch and Bostrom 2011), and somatic mutation accumulation has been important in
73 agriculture, where the origin of many clonally-derived varieties of fruits, including citrus, apples, and
74 wine grapes, have been cultivated by grafting from genetically differentiated bud tips (Aradhya et al.
75 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli et al. 2012; Jarni et al. 2015; Pelsy et al. 2015).
76 However, there is disagreement over the degree and biological importance of somatic mutation
77 accumulation in plants (Schultz and Scofield 2009; Burian et al. 2016; Watson et al. 2016; Schmid-Siegert
78 et al. 2017).

79 Clonal evolution occurs as individual cell lineages accumulate mutations during mitotic cell
80 division (Elena and Lenski 2003; Greaves and Maley 2012; Long et al. 2015). Like mutations occurring
81 during meiosis, somatic mutations are expected to be deleterious more often than beneficial, but the
82 actual distribution of fitness effects of mutations (or their dominance levels) is generally unknown (Orr
83 2010). In diploids, new somatic mutations occur as a single copy within the genome, so their immediate
84 fitness effects will depend on the combination of their selection coefficient (*s*) and their expression in
85 the heterozygous state (dominance; *h*). It is difficult to assess the effects of somatic mutations, because
86 clonal lineages lack recombination, and the phenotypic effects of beneficial mutations are confounded
87 by the co-occurrence of detrimental genetic variants (clonal interference; Long et al. 2015). Similarly,
88 although sexual reproduction does allow mutations to segregate among progeny, it is difficult to detect

89 the phenotypic effects of novel mutations in eukaryotes with separate sexes, because the genomes of
90 two individuals are combined in offspring (Long et al. 2015). In this study, we make crosses within clones
91 of hermaphroditic plants to assess the phenotypic effects of novel mutations accumulating during
92 somatic growth.

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

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

116 When we consider the potential for meiotic and somatic mutations to contribute to the total
117 mutational load of plant populations – particularly for long-lived plants – it becomes evident that not all
118 of the mutations occurring during a plant's lifespan are passed on to the next generation (Fig. 3). First,
119 cell lineage selection will filter mutations displaying some degree of dominance, which will tend to
120 increase the relative frequency of beneficial mutations in the next generation. In addition, some

121 proportion of recessive deleterious mutations will be eliminated during the haploid life stage due to
122 pollen tube attrition and pollen competition (gametophytic selection; Mulcahy 1979; Cruzan 1989;
123 Mable and Otto 1998; Armbruster and Rogers 2004; Arunkumar et al. 2013; Harder et al. 2016). A
124 portion of deleterious mutations also will be homozygous in zygotes, which can lead to higher rates of
125 seed and fruit abortion (Selective Embryo Abortion; Husband and Schemske 1995; Korbecka et al. 2002).
126 Indeed, the effects of deleterious somatic mutations often are apparent as higher rates of embryo
127 abortion after autogamous compared to geitonogamous pollinations, which is referred to as autogamy
128 depression (Schultz and Scofield 2009; Bobiwash et al. 2013). In contrast, mutations that result in faster
129 growth of stem cells will be favored by cell lineage selection and will have an elevated chance of being
130 inherited. The sum effects of this intra-generation selection, which includes cell lineage selection,
131 gametophytic selection, and selective embryo abortion, may result in non-random complements of
132 mutations entering the next generation.
133 In this study, we use autogamous and geitonogamous self-pollinations to estimate the effects
134 of somatic mutations segregating in offspring of *Mimulus guttatus* DC (*Erythranthe guttata* G.L. Nesom;
135 Phrymaceae). Populations of *M. guttatus* display a wide range of life histories and mating systems –
136 from highly selfing annuals to herbaceous perennials that outcross to varying degrees (Wu et al. 2008).
137 In two separate experiments, we characterize the fitness effects of somatic mutations that accumulated
138 during vegetative growth of perenial, self-compatible plants of *M. guttatus* 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, which to our
142 knowledge, has not been done before. We begin by describing our analytical approach in greater detail,
143 followed by providing specifics of the two experiments. We show that somatic mutations accumulate
144 during stem growth, as progeny from autogamous crosses have greater variance in mean fitness
145 compared to progeny from geitonogamous crosses. We further reveal a disproportionate effect of
146 beneficial mutations in some stems, which indicates that intra-generation selection may be filtering
147 mutations to modify the distribution of fitness effects transmitted to progeny. The results from these
148 experiments challenge widely accepted notions concerning mutation accumulation during vegetative
149 growth and the distribution of fitness effects of 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 progeny in the next generation. This is surprising, because somatic mutations affecting
155 levels of embryo abortion also could have effects on the fitness of progeny. We use a comparative
156 approach to evaluate the fitness consequences of somatic mutations that are unique to each stem.
157 For a diploid plant, we can assume that somatic mutations ($a \rightarrow a'$) will be in the heterozygous
158 state when they first arise, so they will segregate in self-fertilized, autogamous progeny as 25%
159 homozygous ($a'a'$), 50% heterozygous ($aa'$), and 25% the original homozygote ($aa$). By contrast, because
160 progeny from geitonogamous crosses contain mutations that arose in separate stems, half of their
161 progeny will be carrying unique mutations in the heterozygous state, and homozygotes for mutations
162 that arose in a single stem will be absent. The difference in fitness of autogamous and geitonogamous
163 progeny will depend on the selection coefficient ($s$) and the dominance ($h$) of somatic mutations unique
164 to each stem and allows us to estimate the fitness effects of somatic mutations. For a single mutation, if
165 we assume that the relative fitness ($w$) of the original homozygote ($aa$) is $1$, then the heterozygote
166 would have fitness $1+hs$, and individuals homozygous for the mutation would have fitness $1+s$ ($s$ ranges
167 between -1 and 1, and $h$ ranges between 0 and 1).
168 If recessive or partially dominant somatic mutations (i.e., $h < 1.0$) accumulate during stem
169 growth, we can use the contrast in fitness between autogamous and geitonogamous progeny to
170 estimate the selection coefficient ($s$) associated with the mutation. Assuming a single mutation, and
171 following the segregation ratios and fitnesses described above, the average fitness of autogamous
172 progeny ($w_A$) due to a single mutation would be the weighted average of the three genotypes ($aa$, $aa'$,
173 and $a'a'$): $w_A = 0.25 + 0.5(1+hs) + 0.25(1+s)$. For geitonogamous progeny, the fitness effects of a single
174 mutation in one stem would be $w_G = 0.5 + 0.5(1+hs)$. However, note that geitonogamy combines
175 mutations that occur in two stems, so the fitness of progeny from geitonogamous crosses would be
176 affected by the combination of mutations that have expression in the heterozygous state from both
177 stems. Regardless, contrasting the fitness of progeny arising from these two cross types provides a close
178 approximation of the fitness effects of somatic mutations that are unique to a stem, which we denote as
179 $s \approx (w_A - w_G)$.
180 While the above calculations are for a single mutation, it is possible that multiple mutations are
181 generated during mitosis in meristem cells during vegetative growth that are subsequently transmitted
182 to progeny. Consequently, the fitness of offspring from autogamous crosses may be affected by the
183 combination of mutations that arose within that stem, which would obscure the effects of individual

184 mutations. Nevertheless, we can assess the average effects of *n* somatic mutations unique to an
185 individual stem. This is simply the difference in fitness between the autogamous and geitonogamous
186 progeny from a single stem: $\bar{s}_k = \bar{w}_{k(A)} - \bar{w}_{k(G)}$, where $\bar{w}_{k(A)}$ and $\bar{w}_{k(G)}$ are the average relative
187 fitnesses of the autogamous and geitonogamous progeny from stem *k*, respectively. Provided that
188 environmental effects are controlled for with a common garden design, the parameter $\bar{s}_k$ represents the
189 combined effects of one or more mutations that have different magnitudes and/or directions of effects
190 on fitness and serves as an indicator of the average fitness effects of all expressed somatic mutations
191 that accumulated during vegetative growth.

192 In addition, even though autogamy and geitonogamy are both self-pollinations, as long as
193 mutations do not have complete expression in heterozygotes (i.e. $h < 1.0$), then the variance in fitness
194 should be greater for autogamous progeny groups than for geitonogamous progeny groups from the
195 same stem. Therefore, we can generate an independent estimate of *s* based on the variance in the
196 fitness of autogamous offspring (Appendix 1). Somatic mutations that are unique to stems will have
197 different effects on the variance in fitness among autogamous progeny depending on the strength of
198 selection (*s*), their expression in the heterozygous state (*h*), and the number of mutations that
199 accumulated (*n*). To evaluate these effects, we began by considering a single somatic mutation ($n = 1$)
200 occurring in a stem with genotype *aa'* that segregated in offspring in a 1:2:1 ratio. We simulated the
201 fitness of autogamous progeny for different values of *s* and *h*, such that fitness of the *aa*, *aa'*, and *a'a'*
202 genotypes was 1, 1+*hs*, and 1+*s*, respectively (*s* ranges from -1 to 1 and *h* ranges from 0 to 1). The
203 outcome of a multiple regression analysis demonstrated that the large majority of variance in fitness (as
204 measured by the standard deviation, SD) could be explained by the selection coefficient rather than
205 dominance level (Appendix 1; Fig. S1). Moreover, the ratio between estimates of SD and *s* remained
206 constant for different numbers of mutations (*n*) segregating among autogamous progeny (i.e. *s* scales
207 linearly with SD), which allowed us to estimate the selective effects of mutations based on the SD as $s_{SD}$
208 $= c_{n,N}SD$, where $c_{n,N}$ is a constant based on the number of independent mutations of equal effect (n) and
209 the number of progeny sampled from a single autogamous fruit (*N*). For one to four mutations occurring
210 in a stem, values of *c* range from 2.0 to ~5.0, based on sampling between 4 and 256 autogamous
211 progeny (Appendix 1; Fig. S2).

212 From the considerations above, we can make specific predictions about the fitness effects of
213 somatic mutations that occur during stem growth and are passed on to offspring. If individual stems are
214 characterized by unique mutations, autogamous progeny groups should have greater variance in fitness
215 among stems compared to geitonogamous progeny from the same stems, because autogamous progeny

216 will be homozygous for each of these mutations 25% of the time. Consequently, we can make an
217 approximate estimate of the selection coefficient based on the average effect of mutations by
218 comparing the fitness of autogamous and geitonogamous progeny from the same stem. Moreover, if
219 intra-generation selection has the potential to filter mutations, we expect that a non-random subset of
220 the accumulated mutations will be passed on to progeny. For example, cell lineage selection may
221 eliminate deleterious mutations that have effects on cell growth rates, but only if they are expressed in
222 the heterozygous state. In contrast, beneficial mutations would be more likely to be retained if they are
223 expressed in the heterozygous state. While beneficial mutations are generally considered to be rare,
224 they may be detectable in autogamous progeny if mutation rates are high and if cell lineage selection is
225 effective.
226 In addition to having an effect on the mean fitness, the presence of somatic mutations also will
227 increase the variance in fitness of autogamous progeny. Consequently, the second prediction we can
228 make is that the variance in fitness among progeny from single fruits produced by autogamous
229 pollination should be positively associated with the average fitness effects of somatic mutations unique
230 to each stem (Appendix 1). In general, we predict that if somatic mutations with fitness effects are
231 accumulating during vegetative growth, then the variance in fitness of autogamous progeny from
232 different stems would be greater than for geitonogamous progeny. Below, we test these predictions
233 using two different experiments in *Mimulus guttatus*.
234
235 **First Experiment**
236 *Methods* – This experiment originally was designed to test the effects of somatic mutations on
237 seed set and ovule abortion (autogamy depression; Schultz and Scofield 2009; Bobiwash et al. 2013), so
238 we used multiple plants from several populations to obtain a large number of fruits. We grew plants of
239 *M. guttatus* in the Research Greenhouse facility at Portland State University from seed collected in July
240 2013 from three different populations in northern Oregon (Jackson Bottom Wetlands – JB: 45.501794 N,
241 -122.98776 W; and two from Saddle Mountain – SMB: 45.9861 N, -123.6859 W, and SMC: 45.9634 N, -
242 123.6837 W). We assumed that greenhouse conditions were different enough from field environments
243 to provide a novel selection regime for clonal evolution during vegetative growth. In August 2013, seeds
244 were cold stratified on moist paper towels at 2°C for 30 days prior to being sown in soil. Seedlings were
245 transplanted to pots (approximately 10 x 10 x 12 cm) and grown for seven months before the
246 application of pollination treatments. Temperature was maintained between 21–26°C during the day,

247 and 15–21°C at night. Supplemental HID lights ran for 12 hours a day when the seedlings first emerged,
248 and 14 hours a day during adult growth.

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

266 We assessed the fitness of autogamous and geitonogamous progeny in the same greenhouse
267 environment that was used to grow the parental plants. Seedlings from a subset of ten maternal plants
268 that had fruits from both cross types and at least 20 filled seeds were sown in soil and transplanted to
269 36-cell trays (blocks) in September in a randomized incomplete block design. After three months of
270 growth, the progeny were scored for survival, and above ground biomass was measured after drying at
271 60°C for at least 24 hours. The fitness of progeny was estimated as its final biomass (log transformed to
272 improve normality), weighted by the survival frequency of progeny from the same cross. Biomass is
273 considered to be an appropriate estimate of fitness for perennials (Younginger et al. 2017). These
274 estimates were rescaled relative to the maximum value from all crosses, so that $\overline{w}$ ranged from 0 to 1.
275 Data were analyzed to estimate the mean fitness for autogamous ($\overline{w}_{k(A)}$) and geitonogamous ($\overline{w}_{k(G)}$)
276 progeny from each stem, and the variance in fitness (as measured by the standard deviation; SD) for
277 each group of autogamous and geitonogamous progeny from a single stem.

*Results* – Levels of seed set and germination were similar between cross types, but embryo abortion was greater in autogamous crosses. The mean number of seeds produced after autogamous (56.72 ±2.24, N = 87) and geitonogamous (57.52 ±3.42, N = 52) pollinations was similar ($F_{1,101}$ = 0.22, P = 0.640), and there was little variation among populations ($F_{2,86.5}$ = 0.66, P = 0.517), maternal plants ($F_{18,101}$ = 0.79, P = 0.709), and pollination dates ($F_{11,101}$ = 1.59, P = 0.113). Limited pollinations produced fewer seeds than excess pollinations ($F_{1,101}$ = 4.78, P = 0.031), but the overall model was not significant ($F_{33,101}$ = 1.43, P = 0.089; Table S1). However, the mean number of aborted ovules was greater for autogamous (17.36 ±1.27, N = 87) than for geitonogamous (13.90 ±1.70, N = 52) pollinations ($F_{1,101}$ = 7.52, P = 0.007), and there also were significant differences among populations in the level of ovule abortion ($F_{2,66}$ = 9.76, P < 0.001; Table S2). There were significant differences in ovule abortion among maternal plants ($F_{2,101}$ = 10.60, P < 0.001), but not for pollination treatments ($F_{1,101}$ = 0.01, P = 0.998) or pollination dates ($F_{11,101}$ = 1.77, P = 0.070; model $F_{33,101}$ = 2.76, P < 0.001). Seed germination was similar between autogamous (mean = 13.03 out of 20 planted per fruit) and geitonogamous crosses (mean = 13.28; $F_{1,101}$ = 0.16, P = 0.694). Of the 354 seedlings that germinated, 202 survived, and survival was higher for autogamous (67%) compared to geitonogamous progeny (50%; chi-square = 12.03, P = 0.0005). Nearly all surviving plants flowered by the end of the experiment, and flower production was correlated with above ground biomass ($r$ = 0.58, P < 0.001, N = 282).

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

310 **Second Experiment**

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

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

339 To promote stress recovery, plants were transplanted to soil for six months, which included a
340 two month vernalization period in a growth chamber (4°C and 8 h light; Conviron E8, Controlled
341 Environments Ltd., Winnipeg, Manitoba, Canada). After vernalization, plants were returned to the

342 greenhouse to induce flowering. Autogamous and geitonogamous pollinations were made to pairs of
343 flowers at single nodes or consecutive nodes (seven nodes and 14 pollinations total) on the largest
344 ramets in each of the control and high salt treatments. To account for somatic mutation turnover that
345 may occur due to the effects of cell lineage selection during stem growth, we compared progeny from
346 autogamous and geitonogamous pollinations at pairs of flowers from the same node. Without *a priori*
347 knowledge of the expression of somatic mutations in heterozygotes, it is difficult to determine the best
348 pollen donor for geitonogamous crosses. Consequently, we opted to generate the most diverse
349 geitonogamous progeny possible by pollinating flowers with pollen from a ramet from the other
350 treatment (i.e. salt pollinated with control pollen, and control pollinated with salt pollen). The fruits
351 were collected, and the total number of fertilized, unfertilized, and aborted seeds were counted under a
352 dissecting microscope. Seeds were planted in soil in trays with three seeds per cell. Seeds were cold
353 stratified in moist soil for three weeks before they germinated in the greenhouse.
354 To determine whether autogamous seedlings from ramets exposed to salt stress showed
355 improved performance under the same conditions, all progeny were exposed to high salinity. After
356 germination and establishment in soil, seedlings from autogamous and geitonogamous crosses to
357 control and salt stress ramets were transplanted into pots filled with pea gravel and subjected to high
358 salt in the hydroponics system, as described above. A total of 239 seedlings from 11 fruits (five
359 autogamous and six geitonogamous) were randomly and evenly distributed among 12 hydroponic tubs
360 to ensure equal representation across blocks (tubs). Plant size was measured as the product of the
361 length and width of vegetative spread after two months of growth and was used as a proxy for biomass.
362 Salt concentration increased from 10 mM - 37.5 mM over the course of the experiment to induce
363 mortality (~57% across all progeny groups). Fitness was estimated as plant size (log transformed to
364 improve normality) weighted by the survival frequency for progeny from the same cross.
365 *Results* – Similar to experiment 1, there was greater variation in the fitness of autogamous
366 compared to geitonogamous progeny, as indicted by a significant interaction between cross type and
367 stem/node identity ($F_{4,226}$ = 3.44, P = 0.0095). There was no consistent difference between autogamous
368 and geitonogamous progeny fitness when averaged across stems ($F_{1,226}$ = 0.59, P = 0.444), but there
369 were larger differences among individual stems/nodes ($F_{6,226}$ = 3.44, P = 0.051; Table S4). For
370 autogamous progeny, there was significant variation among stems ($F_{2,89}$ = 3.76, P = 0.028) and nodes
371 (nested within stems; $F_{3,89}$ = 2.59, P = 0.0596), but not for geitonogamous progeny ($F_{1,136}$ = 0.63, P =
372 0.431 and $F_{4,136}$ = 1.19, P = 0.317 for stems and nodes, respectively). There were significant differences in
373 average size among the four tubs ($F_{11,226}$ = 4.51, P < 0.0001), but there was no consistent difference in

374 the performance of progeny based on the treatment history of their maternal ramets ($F_{1,126}$ = 0.02, P =
375 0.888), and there was no interaction between cross type and historical treatment ($F_{1,136}$ = 0.64, P =
376 0.423). Three nodes were dropped from further analysis, because one of the paired fruits aborted or
377 produced fewer than five seedlings. However, similar to experiment 1, and consistent with the
378 hypothesis that somatic mutations accumulate during stem growth, the variance in fitness among
379 autogamous progeny was greater than for geitonogamous progeny. Also similar to experiment 1, the
380 fitness of autogamous progeny was greater than geitonogamous progeny for half of the stems (Fig. 4).
381 Since results from the two experiments were similar, the data for four nodes from experiment 2 were
382 analyzed together with the first experiment, as described in the next section.
383
384 **Selection Coefficient Estimates**
385 Results from the two experiments described above are consistent with the hypothesis that somatic
386 mutations unique to individual stems can have demonstrable effects on the fitness of progeny when a
387 proportion is made homozygous by autogamous self-pollination. In particular, the variance in fitness
388 among autogamous progeny groups was significantly greater than among geitonogamous progeny from
389 the same set of stems in both experiments. Both experiments also provide us with the unexpected
390 result that the average effects of somatic mutations on fitness are significantly positive for four of the
391 stems (Fig. 4). Since the results from these two experiments are qualitatively similar, we opted to
392 combine them for additional analyses (ten comparisons of autogamy and geitonogamy from Experiment
393 1 and four from Experiment 2, for a total of 14 comparisons). Specifically, we test the prediction that
394 somatic mutations accumulating in stems should lead to a positive relationship between the variance in
395 fitness among progeny from single fruits produced by autogamous pollination ($s_{SD}$) and the selection
396 coefficient estimated from the average fitness effects of autogamous and geitonogamous progeny ($\bar{s}_k$).
397 Estimates of the selective effects of somatic mutations unique to each stem were made based
398 on the fitness of autogamous ($\overline{w}_{k(A)}$) and geitonogamous ($\overline{w}_{k(G)}$) progeny. In both experiments, fitness
399 was estimated as the above ground biomass weighted by the survival of seedlings from the same fruit
400 and scaled to a maximum value of 1.0. The average selective value of mutations ($\bar{s}_k$) for each stem (first
401 experiment) or stem/node combination (second experiment) was calculated as the difference in fitness
402 between autogamous and geitonogamous progeny, as described above ($\bar{s}_k = \overline{w}_{k(A)} - \overline{w}_{k(G)}$). The average
403 fitness of autogamous and geitonogamous progeny from the same stems or nodes was positively
404 correlated (Fig. S3; Table S5). While stems displaying the highest positive selection coefficients tended to
405 have higher fitness for autogamous progeny relative to geitonogamous progeny (Fig. S4), this was not

always the case, as one stem (stem 9) with a value of $\bar{s}_k$ close to zero produced progeny with relatively high fitness after both autogamous and geitonogamous pollination. Overall, comparisons between autogamous and geitonogamous progeny produced a range of estimates of $\bar{s}_k$, eight of which were significantly different from zero (Fig. 4).

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

## Discussion

The observation of higher variation in fitness among stems for progeny from autogamous compared to geitonogamous self-pollinations provides strong evidence for the accumulation of somatic mutations during vegetative growth in *Mimulus guttatus*. Our estimates of the effects of somatic mutations based on differences in the mean fitness between autogamous and geitonogamous progeny ($\bar{s}_k$), and from variation in fitness of autogamous progeny ($s_{SD}$), were consistent between the separate experiments. We found evidence for beneficial mutations being transmitted to progeny, with estimates of $\bar{s}_k$ and $s_{SD}$ exceeding 0.1 in four cases, while estimates for negative selection coefficients were more modest (mostly > -0.15). These results are consistent with the hypothesis that somatic mutation accumulation during vegetative growth is substantial enough to have demonstrable effects on progeny fitness.

438 Furthermore, the observation that autogamous progeny from four of the tested stems had higher
439 fitness than progeny from geitonogamous crosses argues for the accumulation of beneficial mutations
440 and implies that many deleterious mutations are culled by the various types of intra-generation
441 selection prior to seed dispersal. These results suggest that somatic mutations are generated during
442 vegetative growth, but many of them are filtered due to intra-generation selection, which results in a
443 shift in the distribution of fitness effects for the mutations that are passed on to the next generation.
444 Somatic mutations accumulating during vegetative growth had an overall positive effect for four
445 of the stems tested. This surprising result is contrary to widely held views that the accumulation of
446 beneficial somatic mutations should be exceedingly rare (Crow 1993; Charlesworth and Willis 2009).
447 However, a potential explanation for these findings is afforded by the unique biology of plants; somatic
448 mutations in meristem cells could contribute to faster growth and division of cells during stem growth.
449 Thus, cell lineage selection has the potential to retain beneficial somatic mutations, as individual cell
450 lineages outcompete others for limited space and resources. Although there may be few opportunities
451 for beneficial changes to alter basic cellular metabolism, it is becoming apparent from experimental
452 evolution studies with microbes that even basic aspects of cellular metabolism can be sensitive to
453 environmental conditions, which can lead to the appearance of large numbers of beneficial mutations in
454 clonal populations (e.g., Lee and Marx 2013; Maharjan et al. 2015). In this regard, cell lineage selection
455 in a plant meristem is analogous to clonal evolution in microbial populations, and it represents a
456 potentially powerful forum for the filtering of somatic mutations. In addition, gametophytic selection
457 and selective embryo abortion can act as prominent filters, but they are most likely to have effects on
458 culling of deleterious mutations. Regardless, the combined effects of these different forms of intra-
459 generation selection appear to have had a considerable effect on filtering of somatic mutations, such
460 that the distribution of fitness effects among stems has shifted to include more beneficial mutations
461 than expected (Fig. 3).
462 An alternative explanation for the observed phenotypic changes is that exposure to
463 environmental stress has induced heritable epigenetic modifications (Quadrana and Colot 2016).
464 However, epigenetic modifications are generally consistent in direction, are predictable, and are
465 thought to represent an adaptive response to historic exposure to similar stressors (Baulcombe and
466 Dean 2014; Crisp et al. 2016; Itabashi et al. 2018). The results of the experiments described here do not
467 support a role for epigenetics, because phenotypic responses in the next generation were inconsistent
468 in direction and magnitude, and they were not predictable based on environmental exposure of the
469 parent stem. Indeed, among stems and nodes, the mean fitness of autogamous progeny displayed both

470 increases and decreases compared to the geitonogamy controls, which is consistent with the hypothesis
471 that individual ramets are accumulating unique complements of somatic mutations. Furthermore, these
472 conclusions are supported by the observation that many low-frequency but few high-frequency unique
473 genetic variants have been found in the transcriptomes of ramets derived from a single genet of *M.*
474 *guttatus* with a history of exposure to salt stress (Schwoch et al. unpublished data). Thus, the current
475 study indicates that somatic mutations accumulating during stem growth can have phenotypic effects.
476 The potential for the acquisition of mutations during vegetative growth is a well-known aspect
477 of plant biology (Klekowski 2003; Schultz and Scofield 2009; Bobiwash et al. 2013), but no previous study
478 has demonstrated the effects of somatic mutations on the fitness of progeny in the next generation.
479 Most studies have focused on the detrimental effects of somatic mutations; chloroplast mutants have
480 been observed in a number of species (Klekowski 2003), declines in pollen fertility were found in older
481 clones of quaking aspen (Ally et al. 2010), and higher rates of seed and fruit abortion after autogamous
482 pollinations were found in several studies (reviewed in Bobiwash et al. 2013). In contrast, agriculturalists
483 have taken advantage of beneficial somatic mutations by conducting clonal selection to improve
484 economically important plants (Aradhya et al. 2003; McKey et al. 2010; Miller and Gross 2011; Vezzulli
485 et al. 2012; Jarni et al. 2015; Pelsy et al. 2015), and a handful of studies report phenotypic responses to
486 selection in asexual lineages. For example, Breese et al. (1965) succeeded in selecting for increased
487 tillering ability (production of new grass stems) within genets of perennial ryegrass (*Lolium perenne*).
488 Similarly, artificial selection on clonal lineages effectively improved branching in the red seaweed,
489 *Asparagopsis armata* (Monro and Poore 2009). The current study on *M. guttatus* contributes to this
490 literature by highlighting the potential for plants to exhibit significant levels of clonal variation within a
491 single generation.
492 Moreover, the unexpected transmission of beneficial mutations in autogamous crosses may
493 explain some heretofore difficult to understand results from mutation accumulation studies. Our results
494 suggest that beneficial somatic mutations are partially dominant, because they would have to be
495 expressed in the heterozygous state to be favored by cell lineage selection. Consequently, we expect
496 autogamy would be more effective for the accumulation of beneficial somatic mutations in populations
497 than geitonogamy or outcrossing. It is striking that high rates of beneficial mutation accumulation have
498 been observed in at least some mutation accumulation studies in the autogamous plant *Arabidopsis*
499 *thaliana* (Shaw et al. 2002; Rutter et al. 2010; Rutter et al. 2012), but not in outcrossing and partially-
500 selfing species of *Amsinckia* (Schoen 2005), or in any animal species (Halligan and Keightley 2009).
501 Similarly, our results suggest that the adaptive potential of autogamous plants may be greater than

502 previously thought, which may help explain the wider geographic ranges of selfing compared to closely-
503 related outcrossing species (Grossenbacher et al. 2015). Although intra-generation selection has the
504 potential to contribute to adaptation in all plants, its effects may be enhanced in autogamous lineages,
505 because beneficial mutations arising during vegetative growth have a greater chance of becoming
506 homozygous in offspring and being retained across generations.

507 As stems elongate, mutations are generated during every mitotic cell division, so the potential
508 for somatic mutation accumulation in plants appears substantial. Thus, understanding how long-lived
509 plants, such as trees, avoid mutational meltdown from the accumulation of deleterious somatic
510 mutations remains a longstanding question. Paradoxically, however, the rate of mutation accumulation
511 observed across generations in plant and animal genomes is similar (Gaut et al. 2011). One explanation
512 for this pattern is that somatic mutations in plants are repressed during vegetative growth, similar to
513 animal germlines, which would protect lineages from the negative effects of mutation accumulation
514 during development of the soma (Burian et al. 2016; Cruzan 2018; page 90). An alternative hypothesis
515 posits that somatic mutations are generated in apical meristems during plant growth, but these
516 mutations are filtered by intra-generation selection occurring prior to the establishment of offspring.
517 Because plants have retained the capacity to undergo clonal evolution from their algal ancestors, the
518 ability to filter mutations during growth and reproduction has existed for some time, and thus intra-
519 generation selection has the potential to skew the distribution of fitness effects of transmitted
520 mutations to include a larger proportion of beneficial mutations than would be expected through
521 random processes. In addition, this provides a reasonable explanation for why longer-lived plants
522 appear to have slower rates of mutation accumulation across generations (Yue et al. 2010; Gaut et al.
523 2011). This is due to the fact that longer generation time leads to more time between recombination
524 events, which can lead to more background selection in non-recombining cell lineages during vegetative
525 growth (Cruzan 2018; pages 94-95). From our results from crosses within plants of *M. guttatus*, it
526 appears that a disproportionate number of beneficial mutations is passed on to offspring in the next
527 generation, likely a consequence of intra-generation selection. Future work that combines information
528 from experiments evaluating the genomic consequences of somatic variation with anatomical estimates
529 of stem cell population dynamics will allow for the development of new models that provide insights
530 into the extent and limitations of somatic evolution in plants.

531 In conclusion, despite the potential for somatic mutation accumulation to generate novel
532 genetic variation in plant populations, its role in their evolution remains almost entirely unexplored.
533 Even though high levels of mutation accumulation are often believed to be detrimental, the basic

biology of plants suggests that the role of somatic mutations in plant evolution should be considered carefully in the future. Moving forward, our results indicate that we must keep in mind that all eukaryotes are not necessarily equivalent, and unique features of the organism's biology may have unexpected consequences for the evolutionary phenomena that we observe. Future lines of investigation will improve our understanding of these fundamental aspects of plant development and evolution that may have contributed to the remarkable diversification of plants, and may help to account for some of the extensive variation in mutation rates detected among lineages.

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Data Archiving

Data will be submitted to DataDryad.

Author Contributions

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

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668 Data Archiving:

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670 Figure Captions

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

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

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

685 Fig. 4. Estimates of $\bar{s}_k$ for fourteen different stems (ramets) from two separate experiments (Experiment
686 1 – blue bars; Experiment 2 – orange bars) based on mean progeny fitness after autogamous $\overline{w}_{k(A)}$ and
687 geitonogamous $\overline{w}_{k(G)}$ self-pollinations ($\bar{s}_k$ = $\overline{w}_{k(A)}$ - $\overline{w}_{k(G)}$). Horizontal lines represent standard errors.
688 Asterisks indicate values of $\bar{s}_k$ that are significantly different from zero based on the *t* value, calculated
689 as $t = \bar{s}_k/\sqrt{SE}$ with *n-1* df, where *n* is the mean of sample sizes for autogamous and geitonogamous
690 progeny. The relationship between $\overline{w}_{k(A)}$ and $\overline{w}_{k(G)}$ across stems is shown in Fig. S3. Means and sample
691 sizes for progeny groups are available in Table S5 and Fig. S4.

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

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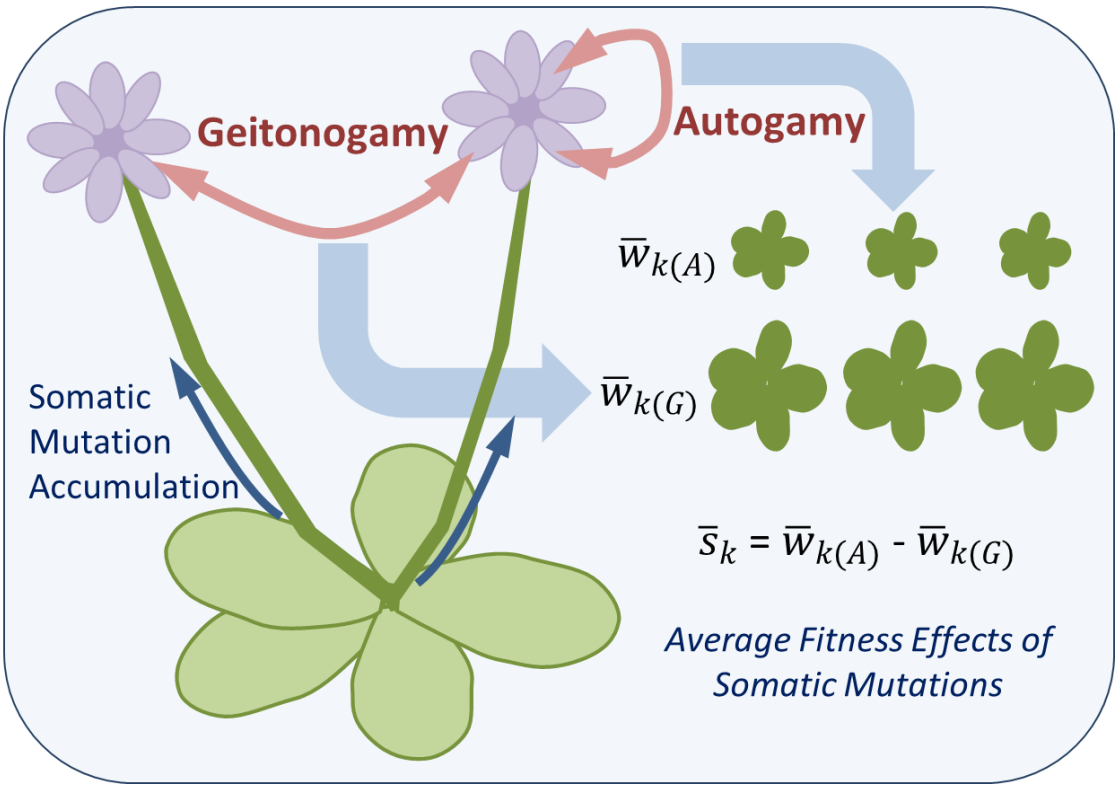
Fig. 1


Geitonogamy
Autogamy
$\overline{w}_{k(A)}$
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Somatic
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Average Fitness Effects of
Somatic Mutations

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Fig. 2

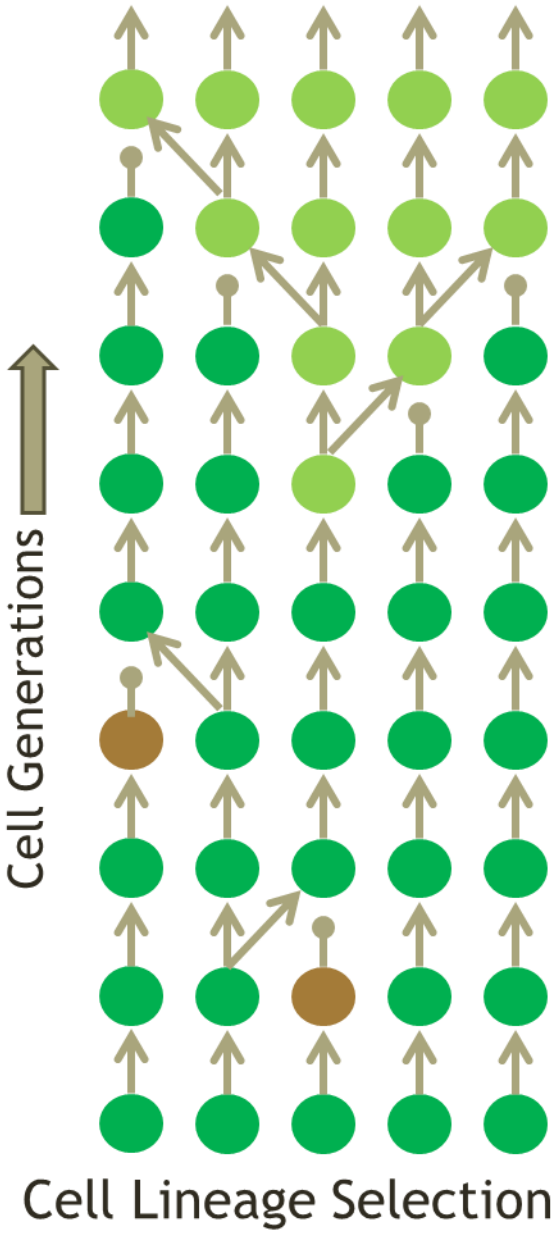


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Fig. 3

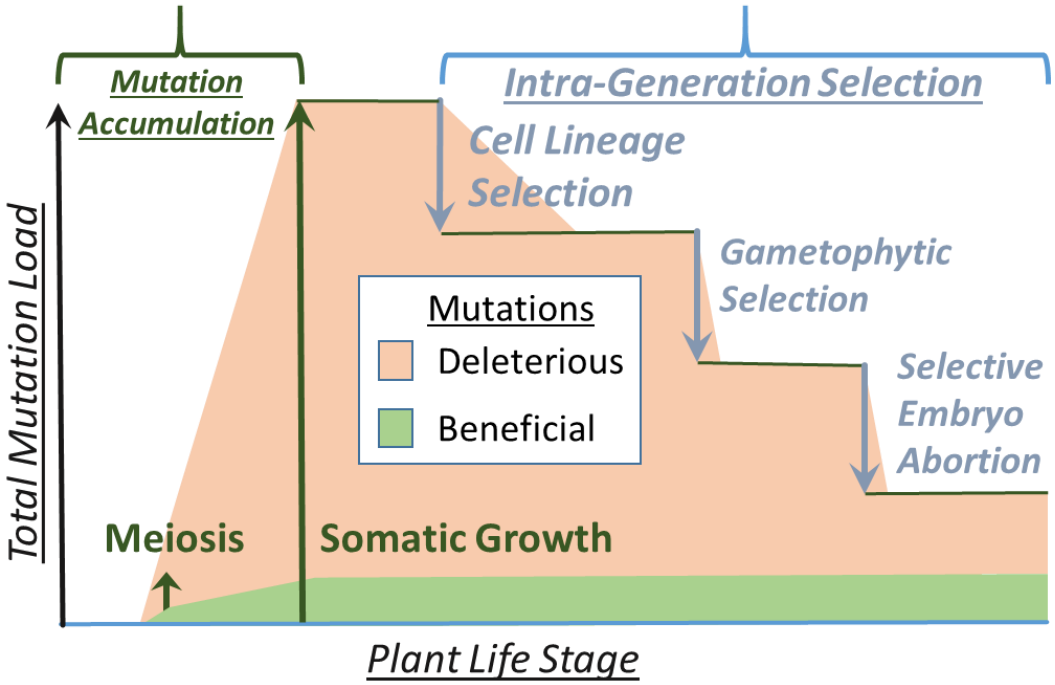


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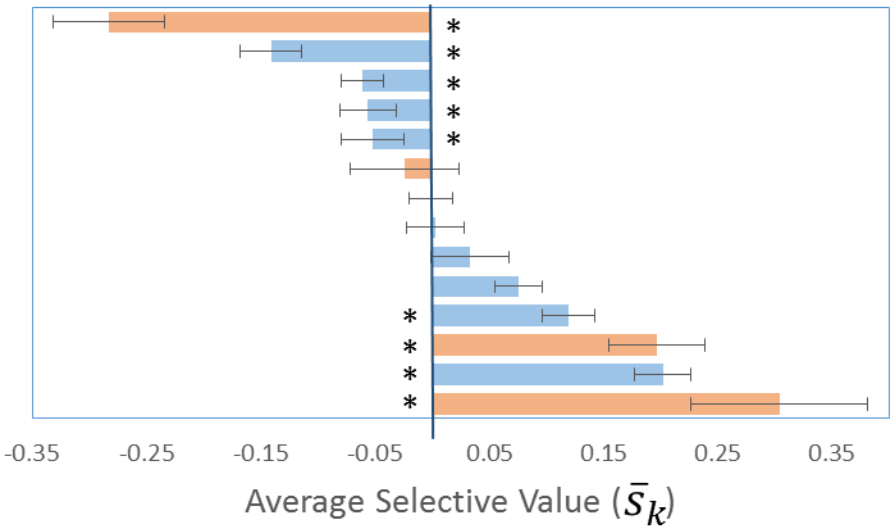
Fig. 4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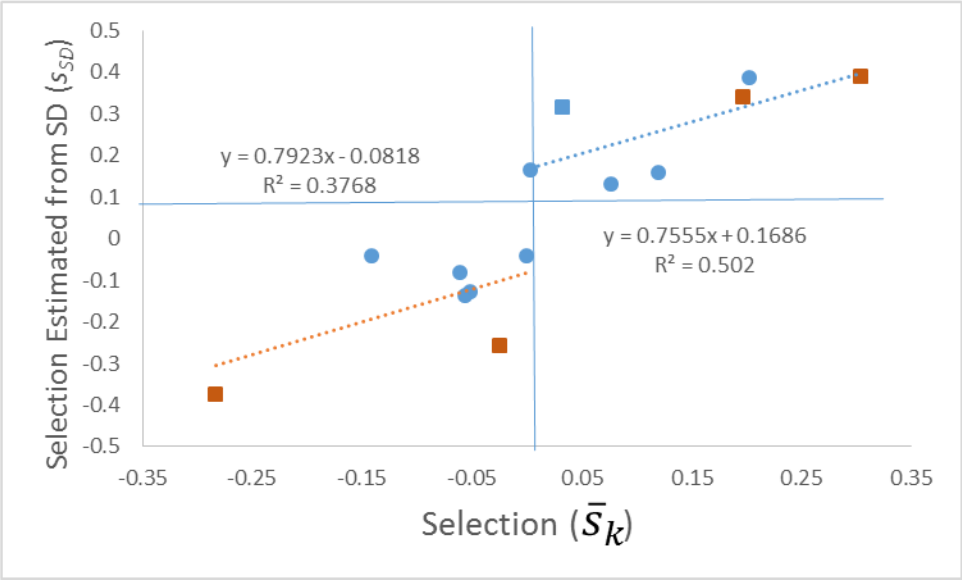
*
-0.35
-0.25
-0.15
-0.05
0.05
0.15
0.25
0.35
Average Selective Value ($\bar{S}_k$)

Fig. 5


Selection Estimated from SD ($s_{SD}$)
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
y = 0.7923x - 0.0818
R² = 0.3768
y = 0.7555x + 0.1686
R² = 0.502
-0.35
-0.25
-0.15
-0.05
0.05
0.15
0.25
0.35
Selection ($\bar{s}_k$)

**Appendix 1. Estimating the selection coefficient due to somatic mutations from the variance in fitness among progeny.**

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

| *Regression Statistics* | | | | | | |
|---|---|---|---|---|---|---|
| Multiple R | 0.9945883 | | | | | |
| R Square | 0.9892059 | | | | | |
| Adjusted R Square | 0.9889634 | | | | | |
| Standard Error | 0.0045317 | | | | | |
| Observations | 92 | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| | *df* | *SS* | *MS* | *F* | *Significance F* | |
| Regression | 2 | 0.1674981 | 0.083749 | 4078.1284 | 2.998E-88 | |
| Residual | 89 | 0.0018277 | 2.054E-05 | | | |
| Total | 91 | 0.1693258 | | | | |
| | | | | | | |
| | *Coefficients* | *tandard Erro* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* |
| Intercept | -0.0020422 | 0.0011426 | -1.7872509 | 0.0773012 | -0.0043125 | 0.0002282 |
| s | 0.439797 | 0.0048812 | 90.099736 | 3.127E-89 | 0.4300981 | 0.4494959 |
| h | 0.0040666 | 0.0016018 | 2.5387309 | 0.0128633 | 0.0008838 | 0.0072494 |

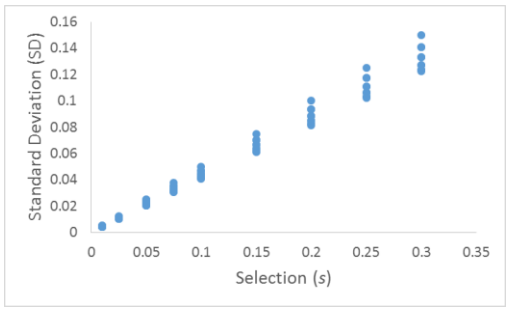



**Fig. S1.** The effects of selection (*s*) and dominance (*h*) on the standard deviation (SD) among progeny. The spread of points at each level of *s* represents the effects of dominance with the lowest point corresponding to *h = 0.5* and the highest point corresponding to *h = 0.0* and *h = 1.0* for each level of selection.

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

Genotype (*n=1*)

| aa | aa' | aa' | a'a' | SD |
|---|---|---|---|---|
| 1.0000 | 1.3000 | 1.3000 | 1.3000 | 0.1500 |

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

Genotype (*n=2*)

| aa bb | aa bb' | aa bb' | aa b'b' | aa' Bb | aa' bb' | aa' bb' | aa' b'b' | aa' bb | aa' bb' | aa' bb' | aa' b'b' | a'a' bb | a'a' bb' | a'a' bb' | a'a' b'b' | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.00 | 1.00 | 1.00 | 1.00 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | |
| 1.00 | 1.30 | 1.30 | 1.30 | 1.00 | 1.30 | 1.30 | 1.30 | 1.00 | 1.30 | 1.30 | 1.30 | 1.00 | 1.30 | 1.30 | 1.30 | |
| 1.00 | 1.15 | 1.15 | 1.15 | 1.15 | 1.30 | 1.30 | 1.30 | 1.15 | 1.30 | 1.30 | 1.30 | 1.15 | 1.30 | 1.30 | 1.30 | 0.0949 |

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

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

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

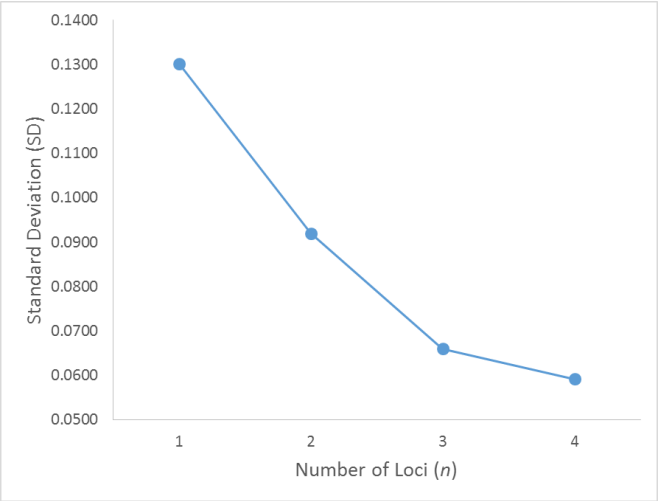



Fig. S2. The standard deviation (SD) in fitness among autogamous progeny from a single self-pollination declines rapidly with the number of independently segregating loci.

797 **Appendix 2. Supplemental Tables and Figures**

798

799 Table S1. Analysis of variance results for seed set after autogamous and geitonogamous pollinations
800 (Cross) with limited and excess pollen (Pollen). Population (Pop), maternal plant nested within
801 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 | | |

802

803 Table S2. Analysis of variance results for aborted ovules after autogamous and geitonogamous
804 pollinations (Cross) with limited and excess pollen (Pollen). Population (Pop), maternal plant nested
805 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 | | |

806

807 Table S3. Analysis of variance for progeny fitness (biomass weighted by survival of seedlings) after
808 autogamous and geitonogamous pollinations (Cross) for ten maternal plants (Mat) and their interaction
809 (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 | | |

810

811

812 Table S4. Analysis of variance for progeny fitness (biomass weighted by survival of seedlings) after
813 autogamous and geitonogamous pollinations (Cross) for seven paired pollinations at individual nodes
814 (Stem/Node) and their interaction (Stem/Node*Cross). Hydroponic planting block (Tub) was declared as
815 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 |
| **Tub** | 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 | | |

816

817

818 Table S5. Means and standard deviations (SD) for progeny groups from autogamous and geitonogamous
819 pollinations in experiments 1 and 2. Experiment 1 included ten different genets. Experiment 2 was
820 conducted with two ramets (C1 and D1) from the same genet.

821

| 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 | | |

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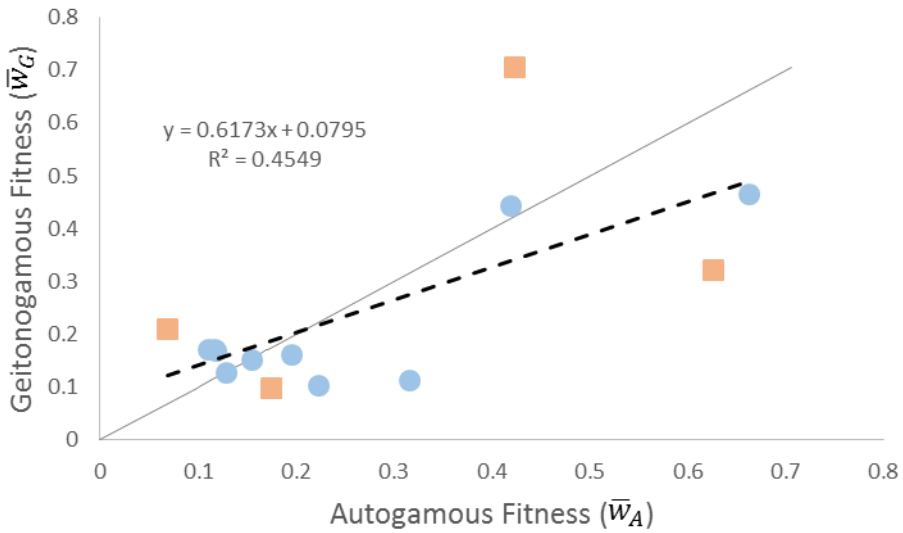


Fig. S3. The relationship between the autogamous and geitonogamous progeny from the 14 comparisons from experiments 1 (blue circles) and 2 (orange squares) as listed in Table S5. The solid line represents a 1:1 relationship and the dashed line represents the best-fit to the data.

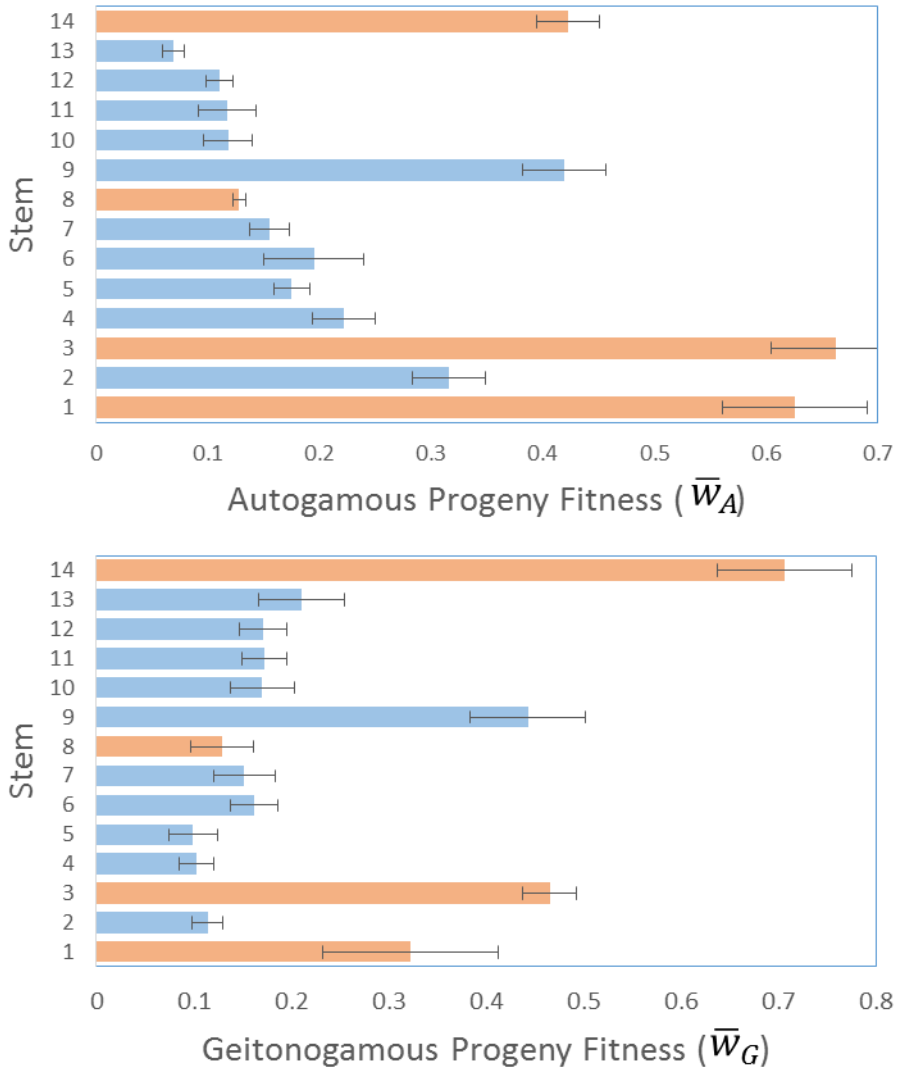


Fig S4. Means and standard errors (lines) for fitness estimates of autogamous (upper panel) and geitonogamous (lower panel) progeny groups from experiment 1 (blue bars) and 2 (orange bars) listed in Table S5. Progeny groups are ordered as in Fig. 4.