

**Natural selection and outbreeding depression suggest
adaptive differentiation in the invasive range of a clonal
plant**

Journal:	<i>Proceedings B</i>
Manuscript ID	RSPB-2018-1091.R1
Article Type:	Research
Date Submitted by the Author:	13-Jun-2018
Complete List of Authors:	Pantoja, Pauline Oliveira; University of Stirling, Biological and Environmental Sciences, Paine, Charles Eliot Timothy; University of Stirling, Biological and Environmental Sciences Vallejo-Marin, Mario ; University of Stirling, Biological and Environmental Sciences
Subject:	Ecology < BIOLOGY, Evolution < BIOLOGY, Plant science < BIOLOGY
Keywords:	Admixture, introduced populations, <i>Mimulus guttatus</i> , natural selection, population growth
Proceedings B category:	Evolution

SCHOLARONE™
Manuscripts

1 Natural selection and outbreeding depression suggest
2 adaptive differentiation in the invasive range of a clonal
3 plant

4 Pauline O. Pantoja^{1*}, C. E. Timothy Paine¹ and Mario Vallejo-Marín¹

5 ¹Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling, FK9
6 4LA, United Kingdom.

7 *p.o.pantoja@stir.ac.uk

8

9

10

11

12

13

14

15

16

17

18

19

20

21 **Keywords:** Admixture, introduced populations, *Mimulus guttatus*, natural selection, population growth
22 rate.

23 **Abstract**

24 Analyses of phenotypic selection and demography in field populations are powerful ways to establishing
25 the potential role of natural selection in shaping evolution during biological invasions. Here we use
26 experimental F2 crosses between native and introduced populations of *Mimulus guttatus* to estimate
27 the pattern of natural selection in part of its introduced range, and to seek evidence of outbreeding
28 depression of colonists. The F2s combined the genome of an introduced population with the genome of
29 either native or introduced populations. We found that the introduced × introduced cross had the
30 fastest population growth rate due to increased winter survival, clonality, and seed production. Our
31 analysis also revealed that selection through sexual fitness favoured large floral displays, large
32 vegetative and flower size, lateral spread, and early flowering. Our results indicate a source-of-origin
33 effect, consistent with outbreeding depression exposed by mating between introduced and native
34 populations. Our findings suggest that well-established non-native populations may pay a high fitness
35 cost during subsequent bouts of admixture with native populations, and reveal that processes such as
36 local adaptation in the invasive range can mediate the fitness consequences of admixture.

37 **Background**

38 The introduction of populations beyond their native range, present individuals with novel environmental
39 challenges. One process that may facilitate biological invasions is admixture between genetically
40 differentiated populations [1, 2]. In the early stages of invasion, admixture can facilitate establishment
41 and spread by allowing introduced populations to benefit from heterosis [1, 3, 4] and from an increase
42 in genetic diversity [2]. However, admixture between genetically differentiated populations can be
43 costly and result in outbreeding depression [5, 6]. The fitness cost of admixture can be environment-
44 independent, for example, when gene flow breaks down co-adapted gene complexes or brings together
45 genetic incompatibilities between previously isolated populations [7]. In addition, admixture can
46 produce phenotypes that are poorly suited to the local ecological conditions [5, 8], resulting in
47 environment-dependent outbreeding depression. As introduced populations become genetically
48 differentiated from native populations due to demographic events, initial admixture, and/or selection
49 [9, 10], the potential cost of subsequent bouts of admixture may increase [11].

50 To date, no studies have attempted to relate natural selection in the introduced range with
51 evidence of outbreeding depression when native and introduced populations come into contact.
52 Previous work has shown that natural selection can be an important force in invasive populations and
53 result in local adaptation to the non-native range [9], which should accentuate environment-dependent
54 outbreeding depression, increasing the cost of admixture [6, 11]. Nevertheless, measuring natural
55 selection and uncovering the fitness costs of admixture can be difficult as it requires studying organisms
56 under field conditions, particularly when outbreeding depression arises due to environment-dependent
57 factors, such as local adaptation. Moreover, the accurate detection of outbreeding depression may
58 depend on incorporating multiple fitness components, especially in perennial organisms combining both
59 sexual and asexual reproduction [12, 13]. A powerful, approach to incorporating multiple fitness
60 components in comparisons among groups of individuals is the use of demographic analyses [13-15].

61 The joint analysis of patterns of selection and comprehensive fitness estimates in the field provide a
62 unique opportunity to assess the extent to which introduced populations may suffer from outbreeding
63 depression when crossed against native populations.

64 Here we estimate patterns of natural selection on introduced populations with different
65 admixed origins. As a study system, we used *Mimulus guttatus* DC. (Phrymaceae), a flowering plant
66 native to western North America that has been introduced to, among other places, eastern North
67 America, Europe, including the UK, and New Zealand [16, 17]. The history of invasion of *M. guttatus* is
68 best documented in the United Kingdom (UK), where it was introduced in 1812, and became naturalised
69 by the 1830s [18]. Although the exact source-of-origin of UK *M. guttatus* is unknown, genetic analyses
70 suggest that it was introduced from the north Pacific, and historical records suggest that it may have
71 originated in Alaska [18, 19]. Admixture between native and introduced populations from the UK and
72 New Zealand of *M. guttatus* has been shown to result in heterosis in the probability of flowering, flower
73 number, clone size and biomass in a previous glasshouse study [3]. Interestingly, the benefits of
74 heterosis in *M. guttatus* are weakened in the F2 generation, as assessed in a similar glasshouse
75 experiment [20]. A separate study of natural selection on a different area of introduction (Eastern North
76 America) showed directional selection for flower size [16], which is consistent with selection playing a
77 role in the phenotypic and genetic makeup of introduced populations [18, 21]. Nevertheless, no studies
78 have yet combined analyses of the pattern of selection in introduced populations with comprehensive
79 fitness comparisons of field-grown individuals with different histories of admixture. Therefore, we still
80 do not know whether selection in the introduced range may be associated with outbreeding depression
81 when measured in field populations.

82 To investigate the effect of admixture on plant fitness under field conditions, and to establish
83 the pattern of selection on individual traits, we generated three arrays of F2 segregant progeny of *M.*
84 *guttatus*, which combined the maternal genome of an introduced individual with the paternal genome

85 of either: (1) a native Alaskan perennial, (2) a native Californian annual, or (3) another introduced British
86 perennial. We used F2 crosses to, first, facilitate the detection of natural selection given the increased
87 phenotypic variation [22], and second, to create admixed individuals and thereby investigate whether
88 population-of-origin shapes the fitness consequences of admixture. The goal of using F2s in this
89 experiment was not to recreate the variation found in natural populations, but to reshuffle the genetic
90 variation of an introduced population among three different backgrounds (two native and another
91 introduced). To this end, the two native populations were chosen to represent contrasting phenotypes
92 that bracket the variation in life history observed in *M. guttatus* (annual vs. perennial), whereas the two
93 introduced populations reflect some of the variation observed in the non-native range. Our study
94 addressed three specific questions: (i) Does source-of-origin affect the fitness of admixed individuals
95 resulting from crosses between native and introduced populations? Given the phenotypic differentiation
96 among the studied populations, we expect that source-of-origin for the admixed individuals has a strong
97 effect on fitness. (ii) Does introduced × introduced admixture result in higher fitness than native ×
98 introduced admixture? Based on previous evidence for adaptive differentiation [21] and of selection at
99 the genomic level in UK *M. guttatus* [18], we predict that introduced × introduced individuals will have
100 higher fitness than native × introduced ones. (iii) What is the pattern of selection acting on floral and
101 vegetative traits of these F2s in the European introduced range? Our overarching goal is to understand
102 the fitness costs of admixture in non-native populations.

103 **Materials and Methods**

104 **PLANT MATERIAL**

105 Introduced populations of *M. guttatus* were collected as seeds from two localities in Scotland: Dunblane
106 (DBL; 56.19° N, 3.96° W), and Coldstream (COL; 55.65° N, 2.24° W). Both populations have a perennial
107 life history, and propagate via clonal reproduction through rooting of lateral stems. To represent an

108 annual life history, we selected a population from Lower Mendocino County, California (LMC; 38.86° N,
109 123.08° W). Individuals from this population come from a seasonally wet habitat that dries over the
110 summer, and do not reproduce clonally. Seeds from LMC were provided by the Willis Lab, Duke
111 University. Previous analysis using genome resequencing show that LMC belongs to a native clade less
112 genetically similar to the UK *M. guttatus* [18]. To represent a perennial life history, we selected a native
113 population collected in the Alaskan peninsula (ALASKA; 59.02° N, 155.85° W). The herbarium specimen
114 shows evidence of clonality. The seeds were sampled from a herbarium specimen collected by the US
115 National Park Service for the University of Alaska Museum Herbarium (ALA; ALAAC accession V142998).
116 Although this specific population has not been previously analysed using genetic markers, our work
117 suggests that Alaskan populations are genetically close to British material [19]. Individuals from this
118 Alaskan population grown in controlled environment facilities produce many long stolons.

119 CREATION OF EXPERIMENTAL CROSSES AND F2S

120 We crossed introduced and native populations to generate three arrays of F2 segregant progeny [23].
121 These arrays combined the maternal genome of an introduced individual (DBL) with the paternal
122 genome of either: (1) a native Alaskan perennial (ALASKA), (2) a native Californian annual (LMC), or (3)
123 an introduced British perennial (COL). A single individual from population DBL formed by two rounds of
124 self-fertilisation and single-seed descent from a wild-collected seed (09-DBL-10-2) was used as the
125 maternal parent to create F1 hybrids. As the paternal parent of the F1 generation we used either an
126 individual grown from a field-collected seed (V142998-5 or 10-COL-24-1, for DBL × ALASKA and DBL ×
127 COL crosses, respectively), or an individual obtained after self-fertilisation of a field collected plant (G-
128 LMC-25; DBL × LMC cross). A single individual from each of these three hybrid lines was self-fertilised to
129 generate three F2 segregant populations. The F2s can be used to increase phenotypic variation in
130 studies of selection in introduced populations, including generating the ancestral variation potentially
131 eliminated by selection [22]. Hereafter, each of these three crosses are referred to using the source-of-

132 origin and life history of their paternal parent: *native-perennial* (DBL × ALASKA; NP), *native-annual* (DBL
133 × LMC; NA), and *introduced-perennial* (DBL × COL; IP).

134 FIELD EXPERIMENT

135 We set up a 15 x 17m field plot at the experimental gardens of the University of Stirling in May 2015.
136 The plot was divided into three spatial blocks each of approximately 15.4 x 4.7m. To mimic more closely
137 the waterlogged environments where *M. guttatus* can be found in the British Isles, we installed a
138 permanent watering system in each block that delivered 1.6 litres per hour at each drip point (spaced
139 every 30cm). Each block was watered in cycles of 48 hours of supplemental water alternated with 24
140 hours of no supplemental water.

141 Seeds from each F2 cross for the field experiment were first germinated in a growth chamber
142 (Snijder, Microclima) in 18h/6h light/dark cycles at 24°C/16°C and 70% relative humidity. Planting
143 *Mimulus* seeds directly in the field is unfeasible due to their very small size, and requirement for surface
144 germination, which make them prone to be washed away by wind or rain and would not allow for the
145 tracking of individual genotypes. Germination rate was assessed for each F2 cross in a separate
146 experiment. After two weeks (two true-leaf stage), seedlings were transplanted into plug trays and
147 placed in a glasshouse with natural light and average day temperature of 18.8°C, for acclimation before
148 transplanting them to the field. On June 17th, we transplanted seedlings with four to six true leaves to
149 their final location in the field plot. Each block consisted of 396 individuals from different crosses
150 planted at random in 11 rows, and separated 0.40 m from each other (1, 188 plants total).

151 We monitored individual plants for survival, growth, and both sexual and clonal reproduction
152 until the end of the growth season (end of September 2015), and measured vegetative and reproductive
153 traits at the onset of flowering for each individual (see *Selection* section). We recorded summer
154 survivorship and total flower and fruit production at the end of the growth season (29 September to 3
155 October 2015), and winter survivorship at the beginning of the following spring (25 March 2016). To

156 estimate seed production, we randomly selected 45 individuals (15 per cross) and collected the seeds of
157 4-21 (average = 11.7) mature, non-dehisced, fruits produced at different times over the summer
158 (August- September) for each individual. We then pooled the seeds from all fruits, weighed them, and
159 divided by the number of fruits used, to obtain an estimate of seed production (in grams) per fruit. To
160 convert this estimate to seed number, we counted and weighted 470-920 seeds per individual (average
161 = 604 seeds), and obtained the number of seeds per gram. The number of seeds per fruit was then
162 calculated as seed production per fruit (g) * number of seeds per gram. The average across the 15
163 individuals from each cross was used as an estimate of seed number per fruit. We estimated
164 germination rates separately by sowing 400 seeds from each F2 cross in 20 9cm-pots, placed in flooded
165 trays in a polythene greenhouse (Tarpaulins, Co. UK) near the experimental plot, and counted the
166 number of seedlings that emerged after eight days. To estimate the average total number of clones
167 produced per individual by the end of the growing season, we germinated and transplanted 24 new
168 plants from each of the F2 crosses. Each individual was placed in a large rectangular container (37 x 24 x
169 6cm) filled with compost and kept in the glasshouse under flooded conditions. Clones were counted
170 weekly for three months, and the average clone production per cross was used in the demographic
171 analysis.

172 DEMOGRAPHIC ANALYSIS

173 To investigate the relative performance of each F2 cross type under field conditions, we built stage-
174 structured matrix population models [14] using the life cycle graph proposed by Peterson et al. [15] to
175 model a perennial, clonal population of *Mimulus guttatus* with an annual time step. This life cycle graph
176 describes a population sampled at the beginning of the growing season, after germination, but before
177 vegetative growth has occurred, which in our experiment occurred in early June. At this point,
178 individuals exist in one of three stages [15]: seeds, seedlings, and rosettes. In our model, seeds that have
179 not germinated by the spring census can remain dormant in the seed bank, and survive to next census

180 with probability D . Seedlings represent individuals that successfully germinated, from seeds produced
181 the previous year or surviving in the seedbank, and established [15]. Rosettes are individuals that persist
182 from the previous year either through survival or through clonal propagation from another rosette.
183 Seedlings and rosettes can contribute to both sexual and clonal reproduction within a growing season.
184 Both sexual (seed) and clonal reproduction (stolons) occur after the census, which in our experiment
185 was approximately from July to September.

186 Transition parameters were estimated from the field and glasshouse experiments. For each F2
187 cross type, we calculated the following vital rates by averaging individual values: germination
188 proportion (G); proportion of individuals that flowered (Gr); survival to the end of the summer (Sn);
189 mean number of clones produced by the end of summer (C); total number of fruits produced (F); mean
190 number of seeds per fruit (Sd); and probability of surviving the winter, estimated as the proportion of
191 individuals alive at the end of summer that were alive the following spring (Sw). Vital rates G , C and Sd
192 were estimated from a subset of the individuals of each cross as described in the previous sections,
193 whereas Gr , Sn , Sw , and F were estimated using all available individuals in the field experiment. Two
194 additional vital rates were obtained from the literature as they were not available for our study: The
195 yearly survivorship of seeds in the seedbank ($D = 0.534$) was obtained from Elderd and Doak [24], who
196 conducted a seed viability experiment of *M. guttatus* using populations in the Sierra Nevada, California.
197 We used the data from Peterson et al. [15, 25] to calculate the relative recruitment success of seeds
198 relative to clonal propagules ($A = 0.00238$), which they calculated in a natural population of clonal,
199 perennial *M. guttatus* in Stanislaus National Forest, California. D and A were treated as constant for all
200 F2 crosses. The projection matrix \mathbf{M} we used is given in the Supplementary Methods.

201 COMPARISON OF POPULATION GROWTH RATES

202 To compare the performance of populations of different admixed origins, we used the projection matrix
203 for each F2 cross to calculate the population growth rate (λ), which we interpreted as the mean fitness
204 of a population [26]. We obtained a relative measure of fitness for each F2 cross, by dividing each λ by
205 the population growth rate of the *native-perennial* cross. We obtained 95% confidence intervals through
206 a non-parametric bootstrap with 10,000 replicates, resampling with replacement individuals within each
207 cross.

208 To test for differences in absolute λ among F2 crosses, we calculated the pairwise differences
209 between crosses ($\theta^{A-B} = \lambda^A - \lambda^B$) [14]. We then used non-parametric randomisations to assess the
210 statistical significance of each pairwise difference in population growth rates, using 10,000 datasets with
211 cross type randomised across all individuals, but maintaining the original sample size (number of
212 individuals) in each F2 cross. We compared the observed pairwise difference between crosses (θ^{A-B}) with
213 the distribution of differences derived from the randomised datasets and used a two tailed test of the
214 null hypothesis (H_0) that there was no difference between a pair of crosses [14].

215 To further compare F2 crosses and determine the importance of different components of fitness
216 (vital rates) on population growth rate, we took two approaches. First, we carried out a life table
217 response experiment (LTRE), which measured the effect of treatment (F2 crosses) on λ relative to a
218 reference matrix, and quantified how variation in the transition probabilities, P_{ij} , contributed to variation
219 in population growth rates among treatments [13, 14]. As a reference matrix, we used the mean of the
220 three F2 matrices [14]. We obtained 95% bootstrap confidence intervals for the mean values of λ ,
221 elasticities and LTRE contributions using 10,000 bootstrap replicates. Second, we conducted a
222 perturbation analysis that allowed us to establish how small changes in the vital rates influence λ [14].
223 For this, we focus on measures of elasticity of vital rates, as they measure the proportional, rather than
224 absolute, response of λ to changes in individual vital rates, and allow comparisons among vital rates with

225 different scales [14]. All demographic analyses were performed using the package *popbio* [27] in *R* v.
226 3.3.3 [28].

227 Finally, to examine how large variation in the vital rates D and A (obtained from native
228 populations of *M. guttatus*) influenced λ , we conducted a simulation analysis. We generated values of D
229 ranging from -50% to +50% of the estimated value (0.537), obtained a new projection matrix, and
230 calculated λ for each parameter combination. For A , we used values one order of magnitude above or
231 below the observed value (0.00238). These simulations allowed us to establish the potential
232 consequences of under- or overestimating D and A , on population growth rates (λ).

233 SELECTION ON FLORAL AND VEGETATIVE TRAITS THROUGH 234 SEXUAL FITNESS

235 Here, we were interested in estimating the pattern of selection acting on floral traits through
236 individuals' contributions to sexual reproductive fitness to complement the previous demographic
237 approach. A more complete analysis of fitness at the individual level combining sexual and clonal
238 components, was unfortunately not possible as some clonal traits could only be estimated in cohorts of
239 plants. Because our focus was on traits expressed only in individuals that flowered (flower size, flower
240 number, time to flowering), we only included flowering plants in this analysis (94% of experimental
241 plants; 1121/1188). Thus, the analysis of floral selection represents only a temporal snapshot of
242 selection through one of the main components of lifetime fitness.

243 We used Lande and Arnold's [29] approach to estimate phenotypic selection using regression
244 models. As an estimate of sexual fitness, we used total fruit production. For this analysis, we considered
245 the following traits, measured at the onset of flowering: (1) Number of days from date of transplant to
246 the field to the first flower opened; (2) plant height (cm); (3) flowering node, counted from the base of
247 the plant to the first reproductive node; (4) corolla width, (5) corolla height, and (6) corolla tube length

248 (mm) averaged over two flowers; (7) leaf width measured at the midpoint (mm), (8) length of the leaf
249 blade, excluding the petiole, (mm); (9) stem diameter measured above the first node from the ground
250 (mm); and (10) number of stolons. In addition, at the end of the reproductive season (end of
251 September), we measured (11) lateral (clonal) spread, the maximum distance between the two longest
252 horizontal stems (clones; cm); (12) total number of flowers produced; (13) total number of fruits
253 produced. We also estimated (14) average daily floral display (number of flowers open) through weekly
254 surveys from the onset of flowering to the end of the August. Prior to the selection analysis, we carried
255 out a Pearson's correlation analysis to identify strongly correlated variables that could introduce
256 multicollinearity. We identified variables that were strongly correlated ($r \geq 0.70$) and which measured
257 similar traits (e.g., corolla width and corolla height), and only kept one for the remainder of the analysis
258 (see Supplementary Table S4). The variables kept for the selection analyses were: days to flower, corolla
259 width, tube length, daily floral display, number of stolons, plant height, leaf width, and lateral spread.

260 We fitted linear regression models using relative fitness and both linear and quadratic terms
261 [29] using the function *glm* in *R* v. 3.3.3 [R. Development Core 28]. Phenotypic traits were standardised
262 to a mean of zero and standard deviation of one [30]. We fitted separate models for each cross type,
263 which facilitated the interpretation of the selection gradients and took into account the large
264 phenotypic differences observed among the three cross types. Relative fitness was obtained for each
265 cross type separately by log-transforming fruit number ($\log(\text{fruit number}+1)$) to improve the normality of
266 the residuals [cf. 30, 31; but see 32] and then dividing by the mean log-transformed fruit production of
267 the corresponding cross type. We first fitted full models including block as a fixed factor, and all linear
268 and quadratic terms (for stabilising/disruptive selection, only). Then we employed likelihood-ratio tests
269 to eliminate quadratic terms, followed by single-term deletion of non-significant linear terms, following
270 the marginality principle [33]. This approach to model selection produced one regression model for each
271 cross type, keeping only terms that contributed significantly to explain variation in relative fitness.

272 Quadratic selection gradients indicating non-linear, stabilising or disruptive selection were obtained
273 from models including both linear and quadratic terms. The quadratic coefficients were doubled to
274 estimate quadratic selection gradients [34]. Linear selection gradients, indicating directional selection,
275 were obtained from models including only linear terms [30]. Statistical significance of the regression
276 coefficients was assessed using single term deletions and likelihood ratio tests.

277 **Results**

278 CHARACTERISATION OF F2 CROSSES

279 As expected, the F2 crosses differed in several phenotypic and life history traits when grown under
280 identical field conditions (Tables S1, S2; Figure 1, Figure S2). The *native-annual* cross flowered most
281 quickly (24.92 ± 0.34 days after being transplanted into the field; mean \pm SE), and produced many (5116
282 ± 1.63), but relatively small, flowers (Table S1). Vegetatively, the *native-annual* cross had smaller leaves,
283 thinner stems, and much less lateral spread compared to the other F2 crosses. The *native-perennial*
284 cross took relatively long to flower (29.07 ± 0.41 days), and produced fewer flowers (19.21 ± 1.17) of
285 intermediate size (Table S1). The *native-perennial* plants produced leaves of similar size compared to
286 *native-annual* plants, but it was strongly clonal, and had the largest lateral spread of all crosses ($67.87 \pm$
287 0.98 cm). Finally, the *introduced-perennial* cross took the longest to flower (34.62 ± 0.41 days), but
288 produced the most flowers (67.45 ± 2.74). Vegetatively, the *introduced-perennial* cross had the largest
289 leaves and thickest stems, and had large lateral spread (43.76 ± 0.82 cm). Most vital rates were different
290 among F2 crosses, except the probability of surviving to produce at least one flower, which was very
291 high across all crosses (92-99%; Table S2). In general, the *native annual* cross was characterised by
292 investment into early sexual reproduction (early and abundant flowering and fruiting), low year-to-year
293 survivorship, and sparse clonality, while the *native-perennial* invested less in sexual reproduction (few
294 fruits), had high summer and winter survivorship, and produce many, and large clones (Table S2). The

295 *introduced-perennial* cross, delayed investing in reproduction but achieved high fruit number, high
296 clonality, and high year-to-year survivorship. Thus, the three F2 crosses encompassed a range of life
297 strategies from highly sexual to highly clonal.

298 PERFORMANCE OF F2 CROSSES IN THE FIELD

299 The demographic analysis compared the performance of the three F2 crosses in the field, and identified
300 transitions associated with population growth rates (Supplementary Figure S3). Population growth rates
301 (λ) varied significantly among F2 crosses. The lowest population growth rate was for the *native-annual*
302 cross ($\lambda = 19.16$; 95% confidence interval = 14.08 – 23.99; $\lambda_{\text{relative}} = 0.58$), while the *native-perennial* had
303 an intermediate value ($\lambda = 32.91$; 95% CI = 28.39 – 36.86; $\lambda_{\text{relative}} = 1.00$), and the *introduced-perennial*
304 cross had the highest ($\lambda = 99.22$; 95% CI = 83.56 – 116.50; $\lambda_{\text{relative}} = 3.01$). The analysis of pairwise
305 differences in population growth rates confirmed that the introduced \times introduced cross performed
306 better in the field than the other two cross types (Supplementary Figure S4). The stable stage structure
307 also varied among F2 crosses (Supplementary Figure S5). At equilibrium, most individuals from the
308 *native-perennial* cross would occur as seedlings and adult rosettes at the census stage (67% and 28%,
309 respectively), whereas for the *introduced-perennial* the population would consist mostly of newly
310 emerged seedlings (85%), and a small fraction of the population would persist as rosettes (9%) and
311 seeds in the seed bank (6%). For the *native-annual* cross, a larger fraction (31%) would occur as seeds in
312 the seedbank, and the majority (62%) as seedlings.

313 Elasticity analysis showed that the effect of small changes in individual transition rates (P_{ij}) to
314 population growth varied among F2 crosses (Table S3). Elasticities (e_{ij}) associated with transitions
315 involving the contribution of sexual reproduction to new seedlings are higher for both *native-annual* and
316 *introduced-perennial* ($e_{22} + e_{23} = 0.899$ and 0.905, respectively) than for *native perennial* ($e_{22} + e_{23} =$
317 0.706). In contrast, in the *native-perennial* cross, the elasticities of transition rates involving survival and

318 clonal reproduction were larger ($e_{32} + e_{33} = 0.291$) than in both *native-annual* and *introduced-perennial*
319 ($e_{32} + e_{33} = 0.083$ and 0.093 , respectively). In all F2 crosses, the elasticities of transition rates from (e_{11}
320 and e_{21}) and to the seed bank (e_{12} , and e_{13}) were negligibly small (Table S3). The analysis of the elasticity
321 of lower-level vital rates shows that in all the three crosses, sexually-related components had the
322 highest elasticities, although summer and winter survivorship (S_n and S_w) and clonality (C) were higher
323 in the *native perennial* cross as compared to the other two crosses (Figure S1).

324 The life-table response experiment (LTRE) showed that the *introduced-perennial* cross
325 outperforms the two other F2 cross types (Figure 2). The decomposition of LTRE into individual
326 transition rates (P_{ij}) indicates that the greater contribution for variation in λ among the cross types, can
327 be attributed to seedling to seedling transitions (P_{22}); in other words, to the contribution of newly
328 merged seedlings via sexual reproduction (Supplementary Figure S6). The *introduced-perennial* had
329 positive contributions for both P_{22} and for transitions from established adults to seedling production
330 (P_{23}). These two transition rates are a function of vital rates related with sexual reproduction and
331 germination. In addition, the variation in population growth rates among crosses was also explained by
332 differences in the transition from seedling to rosette (P_{32}) and from rosette to rosette (P_{33}). In both
333 cases, *introduced-perennial* had positive contributions of both transition rates to λ , while *native-annual*
334 had negative contributions. The *native-perennial* had the highest contribution of P_{33} to variation in λ ,
335 which probably reflects its higher investment in clonal growth (Tables S1, S2). Calculation of λ using a
336 range of values for seed bank survival ($D \pm 50\%$) and recruitment rate (one order of magnitude on either
337 side of A) did not alter the rank order of λ among crosses (results not shown).

338 PATTERN OF SELECTION THROUGH SEXUAL FITNESS

339 We found positive selection on daily floral display and plant height across all cross types (Table 1; full
340 model results in Table S6). The significant quadratic selection gradients on floral display indicate that

341 selection for larger floral displays decelerates as floral display increases. Selection on plant height also
342 had a quadratic component for two of the three crosses (*introduced-perennial* and *native-annual*),
343 indicating decelerating gains in fitness with increased height. In the *native-perennial* cross, we found
344 positive directional selection on corolla width and leaf width, and negative directional selection for
345 flowering time. In this cross type, we also found selection gradients favouring individuals that start
346 producing flowers at intermediate nodes (Table 1). In the *introduced-perennial*, selection through fruit
347 production favoured earlier flowering, and increased lateral spread. In the *native-annual* cross, selection
348 also favoured increased investment in lateral spread, although in both cross types selection on lateral
349 spread was decelerating. In the *native-annual*, selection on flower size favoured larger corollas (Table 1).
350 Overall, our results suggest that natural selection favours individuals with large vegetative and
351 reproductive size, and early flowering, but that the pattern of selection on individual traits varies with
352 the phenotypic architecture characterising each cross type.

353 **Discussion**

354 Our study shows that the source-of-origin of admixed populations of *M. guttatus* strongly influences
355 their fitness under field conditions in the non-native range. We found that admixture within the
356 introduced range resulted in the highest fitness compared to admixture between native and introduced
357 populations estimated using demographic models, which integrated multiple components of fitness,
358 including survivorship, and clonal and sexual reproduction. In comparison, admixture between native
359 and introduced populations conferred lower fitness, particularly when admixture occurred between
360 introduced perennial and native annual ecotypes. Further work is needed to determine the degree to
361 which outbreeding depression is caused by intrinsic genetic interactions (i.e., is environment
362 independent), or by environment-dependent factors mediated by local adaptation. Analysis of natural
363 selection revealed that phenotypic traits, including flowering time, flowering node, daily floral display,

364 plant size, and lateral spread are under selection in the introduced range. Natural selection in field
365 populations of *M. guttatus*, combined with previous evidence of adaptive differentiation [21], and
366 selective sweeps in introduced populations [18], indicate a role of adaptive evolution in shaping
367 populations of *M. guttatus* in UK. We expect that as introduced populations become locally adapted,
368 environment-dependent outbreeding depression should accumulate. Our findings suggest that
369 admixture in introduced species is not necessarily beneficial, particularly when introduced populations
370 have evolved to adapt to the new environment and when admixture occurs between potentially
371 maladapted populations.

372 RELATIVE FITNESS OF F2 CROSSES WITH DIFFERENT ADMIXTURE 373 ORIGINS

374 Our results show that in F2 individuals under field conditions, admixture between native and introduced
375 populations results in lower fitness than admixture within the introduced range. The reduced fitness of
376 native x introduced F2 crosses is consistent with outbreeding depression expressed in the introduced
377 range of *M. guttatus*. Our results contrast with previous work showing heterosis on F2 recombinants of
378 *M. guttatus*. Vickery [35] found that several F2 recombinants of a cross between annual and perennial
379 native populations of *M. guttatus* retain high vigour and performed well under a broader range of
380 environments than their parents. Similarly, Li et al. [20] found that heterosis still occurs in some F2s
381 product of crossing native and introduced populations of *M. guttatus*. However, both studies were
382 conducted under relatively benign glasshouse conditions, where the fitness costs of admixture can be
383 masked [6]. The mechanistic causes of outbreeding depression observed in native x introduced crosses
384 remains unknown, and both environment-dependent, and environment-independent factors could be at
385 play [1, 5, 8]. Increased genetic and phenotypic distance between the maternal parent (DBL) and the
386 native populations could explain the observed outbreeding depression [2, 36]. Both ALASKA and LMC

387 populations are more genetically and phenotypically different from introduced population DBL than the
388 other introduced population COL [18, 19; R. Cumming and M. Vallejo-Marin, unpublished]. Increased
389 evolutionary distance (genetic and phenotypic differentiation) can be magnified by adaptation to
390 different environments and increase outbreeding depression [37]. Indirect evidence in introduced
391 populations of *M. guttatus* is consistent with some role of adaptive evolution and selection in mediating
392 the observed outbreeding depression. For example, common garden experiments in introduced *M.*
393 *guttatus* from New Zealand and the UK suggest that phenotypic differentiation in floral production and
394 clone size is structured along latitudinal clines, consistent with adaptive, rather than non-adaptive
395 differentiation [21]. At the genomic level, there is also evidence that selection has acted in introduced
396 UK populations. Using genome resequencing of 10 native and 10 introduced populations, Puzey and
397 Vallejo-Marin [18] detected selective sweeps in 5 of the 14 chromosomes of *M. guttatus*. These selective
398 sweeps were absent in the native populations studied, lending support to the hypothesis that selection
399 occurred after the introduction of *M. guttatus* into Europe. Future work comparing the consequences of
400 admixture over a larger range of genetic and phenotypic distances [36], will help disentangling the
401 contribution of environment-independent and environment-dependent factors (including local
402 adaptation) to outbreeding depression in invasive species.

403 Although our experimental design can confidently distinguish the fitness differences of the three
404 F2 crosses analysed here (Supplementary Figure S3), further studies are needed to make generalisations
405 about why particular native populations produce, on average, fitter admixed individuals than others.
406 Here we observed that the *native-perennial* cross had higher fitness than the *native-annual* cross. It is
407 tempting to speculate that the lower fitness of the *native-annual* cross reflects maladaptation of the
408 annual LMC parental phenotype when grown in the ecological environment found in the British Isles.
409 Annual populations of *M. guttatus* are typically found in seasonally dry inland areas of the native range
410 [38]. Drought during the summer favours short life spans and investment in sexual reproduction instead

411 of clonality [38, 39]. In contrast, the wet cool summers and mild winters of the British Isles may favour
412 perennial life cycles and investment in clonal growth [21, 38]. Indeed, our analysis of selection showed
413 positive, but decelerating selection, on lateral spread in two of the three crosses studied, including the
414 *native-annual* cross. Moreover, the LTRE analysis indicates that transition rates that involve clonality and
415 survival contribute positively to the difference in fitness between cross types (P_{32} and P_{33} ; Supplemental
416 Figure S6). The *native-perennial* cross had higher fitness than the annual cross, which in part is explained
417 by the higher reproductive contribution of surviving adult rosettes through both sexual (P_{23}) and clonal
418 reproduction (P_{33}) compared to the annual cross (Supplemental Figure S6). In a study of native *Mimulus*,
419 Peterson et al. [15] also found that vital rates for rosette reproduction (including both sexual and clonal
420 components) contributed to local adaptation of perennial vs. annual forms. Although tentative, our
421 results may help explaining why perennials, but not annuals, have become established in the UK. These
422 results also raise the possibility that niche matching between native sources and the introduced habitats
423 may make some lineages more likely than others to become established and spread following
424 introduction [40]. Species with variation in life history and broad ecotypic differentiation in the native
425 range, such as *M. guttatus* [41], could be a fruitful system to test hypotheses about the role of pre-
426 adaptation and maladaptation during biological invasions.

427 SELECTION IN THE INTRODUCED RANGE

428 Our analysis of selection provides the first attempt in quantifying and characterising natural selection in
429 introduced populations of *M. guttatus* outside of North America. Consistent with the general
430 observation of the ubiquity of natural selection [42], we find that that several floral and vegetative traits
431 in *M. guttatus* are under selection in the invasive range. In particular, selection in the introduced range
432 favours larger plants that reproduce early, with larger and more numerous flowers, and increased
433 investment in clonal reproduction (Table 1). Importantly, the pattern of selection on some traits (i.e.,
434 flowering node, daily floral display, plant height, and lateral spread), is non-linear, indicating diminishing

435 fitness returns with higher trait values. Our analysis of selection supports the hypothesis that selection
436 should favour larger size in the introduced range [43]. Consistent with our findings, Murren et al. [16]
437 detected positive selection on flower size and plant height in non-native populations of *M. guttatus* in
438 eastern North America. Studies of native populations of *M. guttatus* have often found positive or
439 stabilising selection on flower size [44-46] and flowering time [47], indicating that flower size and time
440 of flowering continue to be under selection after the dispersal of *M. guttatus* beyond its native habitats
441 to both Eastern North America [16] and the British Isles. Native populations of *M. guttatus* harbour
442 considerable levels of genetic variation [48], and both vegetative and reproductive traits often display
443 significant heritabilities [e.g., 16, 49]. Genomic analysis of introduced populations suggest that, although
444 diversity is reduced, there is still considerable variation within the introduced range [18]. Therefore,
445 introduced populations may be capable of rapid adaptive evolution [10]. Our results suggest that
446 adaptive evolution caused by natural selection in the introduced range in a genetically variable taxon,
447 such as *M. guttatus*, may be a key mechanism in facilitating the naturalisation and spread of non-native
448 species when faced with novel ecological challenges.

449 **Conclusion**

450 Biological invasions are often characterised by multiple introduction across both space and time, often
451 resulting in admixed populations in the non-native range [2, 5]. Understanding the fitness consequences
452 of admixture is therefore key in predicting the dynamics of invasive populations. Our results indicate
453 that, well-established non-native populations may pay a high fitness cost during subsequent bouts of
454 admixture with native populations. This is a key result because it reveals that processes such as local
455 adaptation in the invasive range can mediate the fitness consequences of admixture [8]. To the extent
456 that evolution in non-native populations yields local adaptation [10], we expect that admixture between
457 introduced and native populations should result in relatively lower fitness than outcrossing within the

458 introduced range. Importantly, the fitness costs of admixture may only be exposed when measured
459 under field conditions and when incorporating multiple fitness components (e.g., seed set, survival,
460 clonal reproduction). Because local adaptation in non-native populations is more likely to accumulate
461 with time (number of generations) since introduction, we predict that the negative effects of admixture
462 on the fitness of non-native populations should be higher in older invasions. As populations introduced
463 around the world in the last 500 years come of age, the role of natural selection in shaping non-native
464 populations should be on the rise.

465 **Data sharing**

466 Phenotypic data is deposited in Dryad doi:10.5061/dryad.m1r0356.

467 **Competing interests**

468 We have no competing interests.

469 **Authors' contributions**

470 MVM and POP developed the project idea and experimental design. POP collected the data, and POP
471 and MVM analysed the data with input from CETP. POP and MVM drafted the manuscript with
472 comments by CETP. All authors gave final approval for publication.

473 **Acknowledgements**

474 We thank D. Lowry and K. Wright for collecting the LMC population, to John Willis and his lab for access
475 to their seed collection, and to S. Ickert-Bond for herbarium material. We thank J. Weir and the Gardens
476 and Grounds staff for providing assistance, and J. Betts, K. Zapata, M. Wuerges, N. Craw, P. Gonzales, S.
477 Meeus, S. Richie and Z. Pattinson for fieldwork help. We thank three anonymous reviewers and the
478 Associate Editor for valuable and constructive suggestions on a previous version of the manuscript.

479 Funding

480 POP was supported by a PhD studentship from CAPES, Brazil.

481 References

- 482 1. Hahn M.A., Rieseberg L.H. 2017 Genetic admixture and heterosis may enhance the invasiveness
483 of common ragweed. *Evolutionary Applications* **10**(3), 241-250. (doi:10.1111/eva.12445).
- 484 2. Dlugosch K.M., Anderson S.R., Braasch J., Cang F.A., Gillette H.D. 2015 The devil is in the details:
485 genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology* **24**(9),
486 2095-2111. (doi:10.1111/mec.13183).
- 487 3. van Kleunen M., Rockle M., Stift M. 2015 Admixture between native and invasive populations
488 may increase invasiveness of *Mimulus guttatus*. *Proceedings of the Royal Society B-Biological Sciences*
489 **282**(1815). (doi:ARTN 20151487
490 10.1098/rspb.2015.1487).
- 491 4. Keller S.R., Taylor D.R. 2010 Genomic admixture increases fitness during a biological invasion.
492 *Journal of Evolutionary Biology* **23**(8), 1720-1731. (doi:10.1111/j.1420-9101.2010.02037.x).
- 493 5. Rius M., Darling J.A. 2014 How important is intraspecific genetic admixture to the success of
494 colonising populations? *Trends in Ecology & Evolution* **29**(4), 233-242. (doi:10.1016/j.tree.2014.02.003).
- 495 6. Waser N.M., Price M.V. 1994 Crossing-distance effects in *Delphinium nelsonii*: outbreeding and
496 inbreeding depression in progeny fitness. *Evolution* **48**(3), 842-852.
- 497 7. Etterson J.R., Keller S.R., Galloway L.F. 2007 Epistatic and cytonuclear interactions govern
498 outbreeding depression in the autotetraploid *Campanulastrum americanum*. *Evolution* **61**(11), 2671-
499 2683. (doi:10.1111/j.1558-5646.2007.00234.x).
- 500 8. Verhoeven K.J.F., Macel M., Wolfe L.M., Biere A. 2011 Population admixture, biological invasions
501 and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society*
502 *B-Biological Sciences* **278**(1702), 2-8. (doi:10.1098/rspb.2010.1272).
- 503 9. Colautti R.I., Lau J.A. 2015 Contemporary evolution during invasion: evidence for differentiation,
504 natural selection, and local adaptation. *Molecular Ecology* **24**(9), 1999-2017.
- 505 10. Colautti R.I., Barrett S.C. 2013 Rapid adaptation to climate facilitates range expansion of an
506 invasive plant. *Science (New York, NY)* **342**(6156), 364-366. (doi:10.1126/science.1242121 [doi]).

- 507 11. Verhoeven K.J., Macel M., Wolfe L.M., Biere A. 2011 Population admixture, biological invasions
508 and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society*
509 *of London B: Biological Sciences* **278**(1702), 2-8.
- 510 12. Shaw R.G., Geyer C.J., Wagenius S., Hangelbroek H.H., Etterson J.R. 2008 Unifying life-history
511 analyses for inference of fitness and population growth. *The American Naturalist* **172**(1), E35-E47.
- 512 13. Angert A.L. 2006 Demography of central and marginal populations of monkeyflowers (*Mimulus*
513 *cardinalis* and *M. lewisii*). *Ecology* **87**(8), 2014-2025.
- 514 14. Caswell H. 2001 *Matrix Population Models*. Sunderland, Massachusetts, Sinauer; 722 p.
- 515 15. Peterson M.L., Kay K.M., Angert A.L. 2016 The scale of local adaptation in *Mimulus guttatus*:
516 Comparing life history races, ecotypes, and populations. *New Phytol* **211**(1), 345-356.
517 (doi:10.1111/nph.13971).
- 518 16. Murren C.J., Chang C.C., Dudash M.R. 2009 Patterns of selection of two North American native
519 and nonnative populations of monkeyflower (Phrymaceae). *New Phytologist* **183**(3), 691-701.
- 520 17. Vallejo-Marin M., Lye G.C. 2013 Hybridisation and genetic diversity in introduced *Mimulus*
521 (Phrymaceae). *Heredity* **110**, 111-122.
- 522 18. Puzey J., Vallejo-Marin M. 2014 Genomics of invasion: diversity and selection in introduced
523 populations of monkeyflowers (*Mimulus guttatus*). *Molecular ecology* **23**(18), 4472-4485.
524 (doi:10.1111/mec.12875).
- 525 19. Pantoja P.O., Simon-Porcar V.I., Puzey J.R., Vallejo-Marin M. 2017 Genetic variation and clonal
526 diversity in introduced populations of *Mimulus guttatus* assessed by genotyping at 62 single nucleotide
527 polymorphism loci. *Plant Ecology & Diversity* **10**(1), 5-15. (doi:10.1080/17550874.2017.1287785).
- 528 20. Li Y., Stift M., van Kleunen M., Bonser S. 2018 Admixture increases performance of an invasive
529 plant beyond first-generation heterosis. *Journal of Ecology*. (doi:10.1111/1365-2745.12926).
- 530 21. van Kleunen M., Fischer M. 2008 Adaptive rather than non-adaptive evolution of *Mimulus*
531 *guttatus* in its invasive range. *Basic and Applied Ecology* **9**(3), 213-223.
- 532 22. Conner J.K. 2003 Artificial Selection : A Powerful Tool for Ecologists. *Ecology* **84**, 1650-1660.
- 533 23. Lynch M., Walsh B. 1998 *Genetics and Analysis of Quantitative Traits*. Massachusetts, USA,
534 Sinauer; 980 p.
- 535 24. Elderd B.D., Doak D.F. 2006 Comparing the direct and community-mediated effects of
536 disturbance on plant population dynamics: flooding, herbivory and *Mimulus guttatus*. *Journal of Ecology*
537 **94**(3), 656-669. (doi:10.1111/j.1365-2745.2006.01115.x).
- 538 25. Peterson M.L. 2017 Corrigendum. *New Phytologist* **216**(3), 956-957. (doi:10.1111/nph.14768).

- 539 26. Lande R. 1982 A quantitative genetic theory of life history evolution. *Evolution* **37**, 607-615.
- 540 27. Stubben C., Milligan B. 2007 Estimating and analyzing demographic models using the popbio
541 package in R. *Journal of Statistical Software* **22**(11), 1-23.
- 542 28. Team R.D.C. 2017 R. A language and environment for statistical computing. R Foundation for
543 Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>.
- 544 29. Lande R., Arnold S.J. 1983 The measurement of selection on correlated characters. *Evolution* **37**,
545 1210-1226.
- 546 30. Stinchcombe J.R., Rausher M.D. 2001 Diffuse selection on resistance to deer herbivory in the
547 ivyleaf morning glory, *Ipomoea hederacea*. *American Naturalist* **158**(4), 376-388.
- 548 31. Vallejo-Marin M., Rausher M.D. 2007 Selection through female fitness helps to explain the
549 maintenance of male flowers. *American Naturalist* **169**(5), 563-568.
- 550 32. Stanton M.L., Thiede D.A. 2005 Statistical convenience vs biological insight: consequences of
551 data transformation for the analysis of fitness variation in heterogeneous environments. *New*
552 *Phytologist* **166**(1), 319-337.
- 553 33. Fox J. 2016 *Applied regression analysis and generalized linear models*. 3. ed. Los Angeles, SAGE;
554 xxiv, 791 s. p.
- 555 34. Stinchcombe J.R., Agrawal A.F., Hohenlohe P.A., Arnold S.J., Blows M.W. 2008 Estimating
556 nonlinear selection gradients using quadratic regression coefficients: Double or nothing ? *Evolution*
557 **62**(9), 2435-2440. (doi:10.1111/j.1558-5646.2008.00449.x).
- 558 35. Vickery R.K. 1974 Growth in artificial climates. Indication of *Mimulus* ability to invade new
559 habitats. *Ecology* **55**(4), 796-807.
- 560 36. Edmands S. 1999 Heterosis and outbreeding depression in interpopulation crosses spanning a
561 wide range of divergence. *Evolution* **53**(6), 1757-1768.
- 562 37. Frankham R., Ballou J.D., Eldridge M.D.B., Lacy R.C., Ralls K., Dudash M.R., Fenster C.B. 2011
563 Predicting the probability of outbreeding depression. *Conservation Biology* **25**(3), 465-475.
564 (doi:10.1111/j.1523-1739.2011.01662.x).
- 565 38. Lowry D.B., Rockwood R.C., Willis J.H. 2008 Ecological reproductive isolation of coast and inland
566 races of *Mimulus guttatus*. *Evolution* **62**(9), 2196-2214. (doi:10.1111/j.1558-5646.2008.00457.x).
- 567 39. Kooyers N.J., Greenlee A.B., Colicchio J.M., Oh M., Blackman B.K. 2015 Replicate altitudinal
568 clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus*
569 *guttatus*. *New Phytologist* **206**(1), 152-165.

- 570 40. Holt R.D., Barfield M., Gomulkiewicz R. 2005 Theories of niche conservatism and evolution:
571 could exotic species be potential tests? In *Species Invasions: Insights into Ecology, Evolution, and*
572 *Biogeography* (pp. 259-290. Sunderland, Sinauer Associates.
- 573 41. Grossenbacher D.L., Veloz S.D., Sexton J.P. 2014 Niche and range size patterns suggest that
574 speciation begins in small, ecologically diverged populations in North American monkeyflowers
575 (*Mimulus* spp.). *Evolution* **68**(5), 1270-1280. (doi:10.1111/evo.12355).
- 576 42. Kingsolver J.G., Diamond S.E., Siepielski A.M., Carlson S.M. 2012 Synthetic analyses of
577 phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary*
578 *Ecology* **26**(5), 1101-1118.
- 579 43. Blossey B., Notzold R. 1995 Evolution of increased competitive ability in invasive nonindigenous
580 plants: A hypothesis. *Journal of Ecology* **83**(5), 887-889. (doi:10.2307/2261425).
- 581 44. Hall M.C., Willis J.H. 2006 Divergent selection on flowering time contributes to local adaptation
582 in *Mimulus guttatus* populations. *Evolution* **60**(12), 2466-2477.
- 583 45. Fishman L., Willis J.H. 2008 Pollen limitation and natural selection on floral characters in the
584 yellow monkeyflower, *Mimulus guttatus*. *New Phytologist* **177**(3), 802-810.
- 585 46. Fenster C.B., Ritland K. 1994 Evidence for natural selection on mating system in *Mimulus*
586 (*Scrophulariaceae*). *International journal of plant sciences* **155**(5), 588-596.
- 587 47. Ivey C.T., Carr D.E. 2012 Tests for the joint evolution of mating system and drought escape in
588 *Mimulus*. *Annals of Botany* **109**(3), 583-598. (doi:10.1093/aob/mcr160).
- 589 48. Puzey J.R., Willis J.H., Kelly J.K. 2017 Population structure and local selection yield high genomic
590 variation in *Mimulus guttatus*. *Molecular Ecology* **26**(2), 519-535. (doi:10.1111/mec.13922).
- 591 49. Fenster C.B., Ritland K. 1994 Quantitative genetics of mating system divergence in the yellow
592 monkeyflower species complex. *Heredity* **73**, 422-435.

593

594

595 **Figure legends**

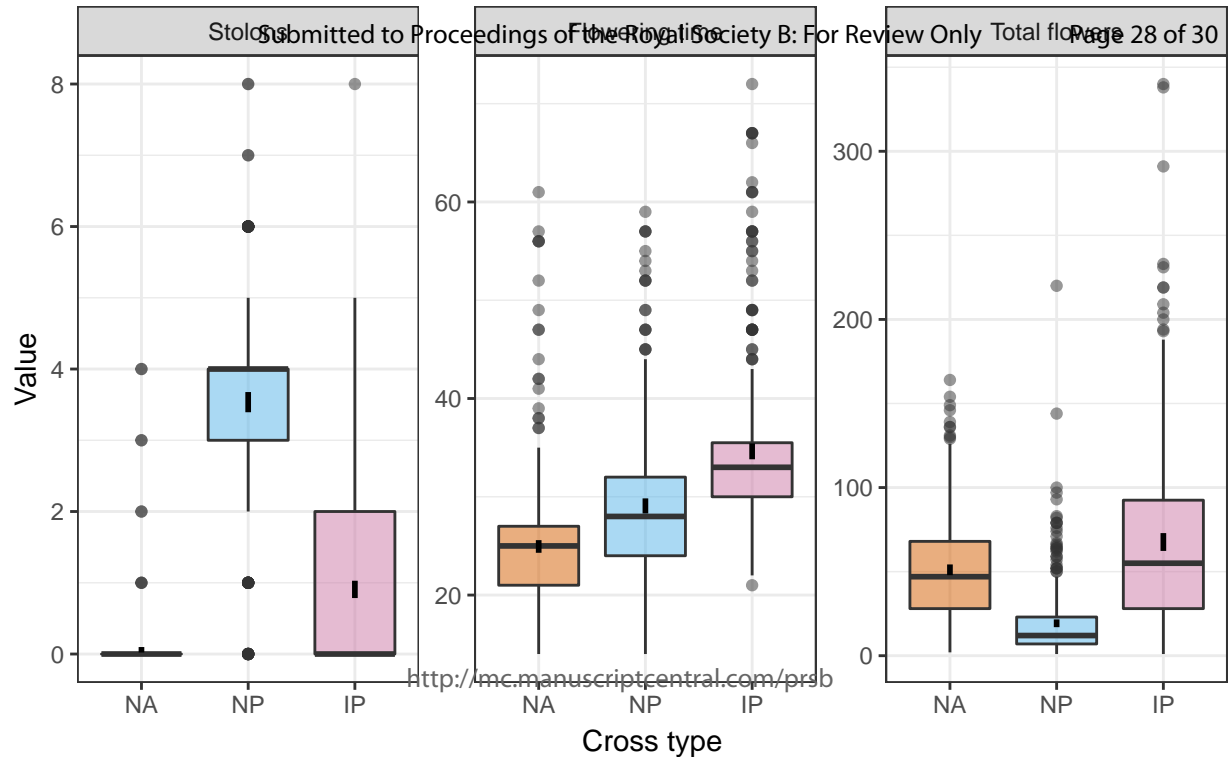
596 **Figure 1.** Phenotypic characteristics for some of the traits (stolon number, flowering time, and
597 total number of flowers) measured in the F2 offspring of three crosses between native and
598 introduced populations of *Mimulus guttatus* grown in a field plot in central Scotland. Cross types
599 as follows: NA = *native-annual* (DBL × LMC); NP = *native-perennial* (DBL × ALASKA); IP =
600 *introduced-perennial*, (DBL × COL). Units for each trait are provided in Table S1. Vertical lines:
601 bootstrap-estimated 95% confidence intervals. Plots for all measured traits are given in Figure
602 S2.

603 **Figure 2.** Life table response experiment (LTRE), showing the effect of cross identity on
604 variation in population growth rate (λ , fitness) in three crosses between native and introduced
605 *Mimulus guttatus*. Values on the y-axis indicate the sum of the contribution of all transition rates
606 to population growth rate, relative to the average of the matrices of the three cross types.
607 Whiskers show the 95% CI estimated using 10,000 bootstrap replicates. Cross types as in
608 Figure 1.

Table

Table 1. Standardised linear (β) and quadratic (γ) selection gradients estimated in the F2 generation of three crosses of *Mimulus guttatus* in a field population in central Scotland (Stirling). The subscript indicated the paternal parent of each cross: ALASKA = Alaskan Peninsula, U.S.A. (*native-perennial*); COL = Coldstream, Scotland (*introduced-perennial*); LMC = California, U.S.A. (*native-annual*) All crosses had the same maternal parent (DBL, Dunblane, Scotland). For each cross type, traits that were not statistically significant (assessed with a likelihood ratio test of nested models) were dropped from the model, except if the quadratic coefficient was significant. Statistical significance of individual coefficients was assessed via single-term deletions and likelihood ratio tests. Linear selection gradients were calculated in a model with linear terms only [30]. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Trait	β_{ALASKA}	γ_{ALASKA}	β_{COL}	γ_{COL}	β_{LMC}	γ_{LMC}
Flowering time	-0.046***	–	-0.037***	–	–	–
Flowering node	-0.056***	-0.026**	–	–	–	–
Daily floral display	0.307***	-0.226***	0.118***	-0.058***	0.138***	-0.064***
Corolla width	0.034**	–	–	–	0.031**	–
Leaf width	0.041**	–	–	–	–	–
Plant height	0.079***	–	0.059***	-0.030***	0.005	-0.032**
Lateral spread	–	–	0.053***	-0.108***	0.055*	-0.196**



<http://mc.manuscriptcentral.com/prsb>

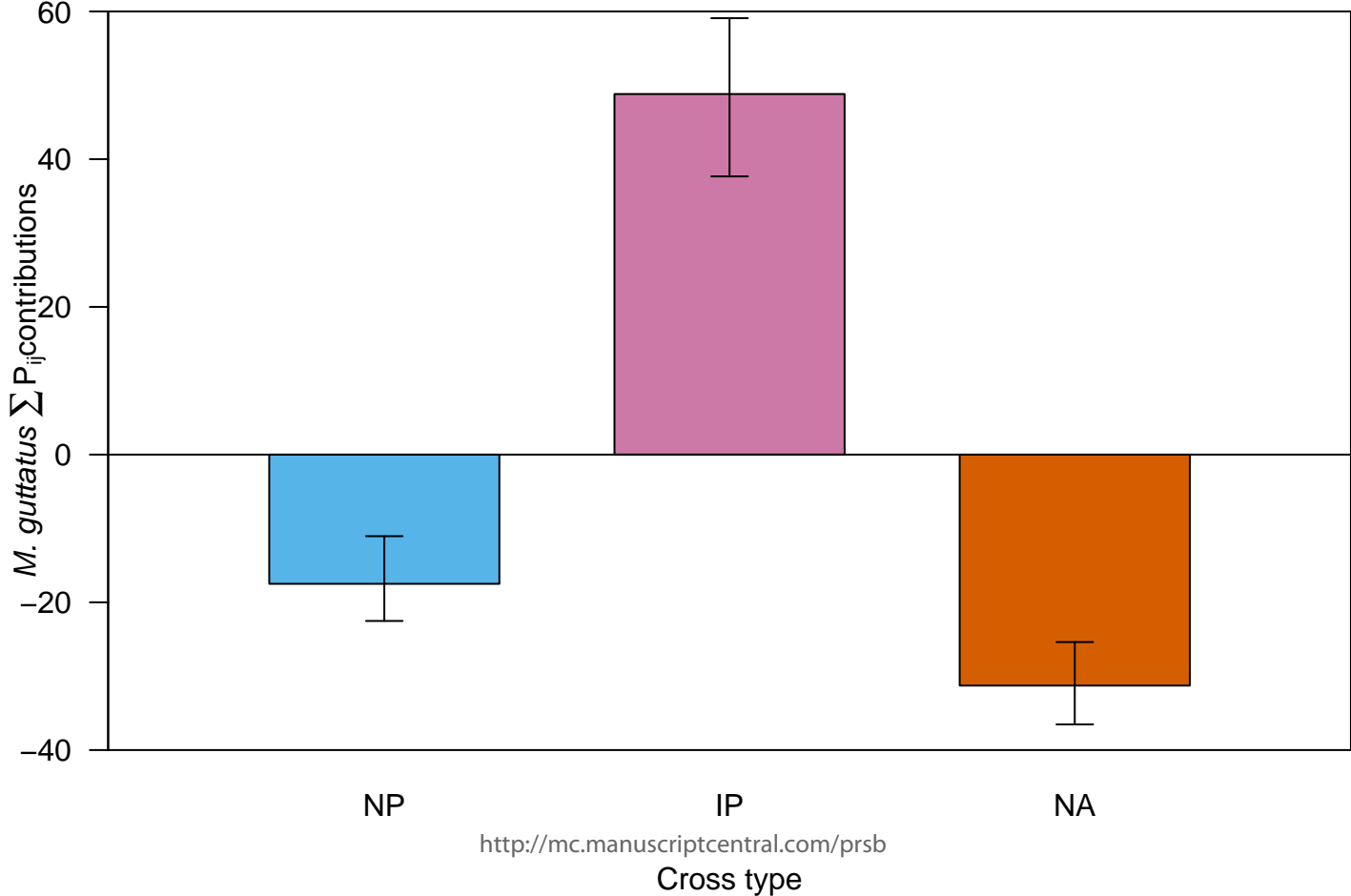


Table 1. Standardised linear (β) and quadratic (γ) selection gradients estimated in the F2 generation of three crosses of *Mimulus guttatus* in a field population in central Scotland (Stirling). The subscript indicated the paternal parent of each cross: ALASKA = Alaskan Peninsula, U.S.A. (*native-perennial*); COL = Coldstream, Scotland (*introduced-perennial*); LMC = California, U.S.A. (*native-annual*) All crosses had the same maternal parent (DBL, Dunblane, Scotland). For each cross type, traits that were not statistically significant (assessed with a likelihood ratio test of nested models) were dropped from the model, except if the quadratic coefficient was significant. Statistical significance of individual coefficients was assessed via single-term deletions and likelihood ratio tests. Linear selection gradients were calculated in a model with linear terms only [30]. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Trait	β_{ALASKA}	γ_{ALASKA}	β_{COL}	γ_{COL}	β_{LMC}	γ_{LMC}
Flowering time	-0.046***	–	-0.037***	–	–	–
Flowering node	-0.056***	-0.026**	–	–	–	–
Daily floral display	0.307***	-0.226***	0.118***	-0.058***	0.138***	-0.064***
Corolla width	0.034**	–	–	–	0.031**	–
Leaf width	0.041**	–	–	–	–	–
Plant height	0.079***	–	0.059***	-0.030***	0.005	-0.032**
Clonal spread	–	–	0.053***	-0.108***	0.055*	-0.196**