

**Natural selection and outbreeding depression suggest
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1 Natural selection and outbreeding depression suggest
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22 rate.

Abstract

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

Background

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

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

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

Materials and Methods

PLANT MATERIAL

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

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

CREATION OF EXPERIMENTAL CROSSES AND F2S

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

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

FIELD EXPERIMENT

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

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

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

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

DEMOGRAPHIC ANALYSIS

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

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

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

COMPARISON OF POPULATION GROWTH RATES

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

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

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

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

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

SELECTION ON FLORAL AND VEGETATIVE TRAITS THROUGH SEXUAL FITNESS

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

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

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

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

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

Results

CHARACTERISATION OF F2 CROSSES

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

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

PERFORMANCE OF F2 CROSSES IN THE FIELD

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

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

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

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

PATTERN OF SELECTION THROUGH SEXUAL FITNESS

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

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

Discussion

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

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

RELATIVE FITNESS OF F2 CROSSES WITH DIFFERENT ADMIXTURE ORIGINS

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

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

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

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

SELECTION IN THE INTRODUCED RANGE

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

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

Conclusion

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

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

Data sharing

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

Competing interests

We have no competing interests.

Authors' contributions

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

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Figure legends

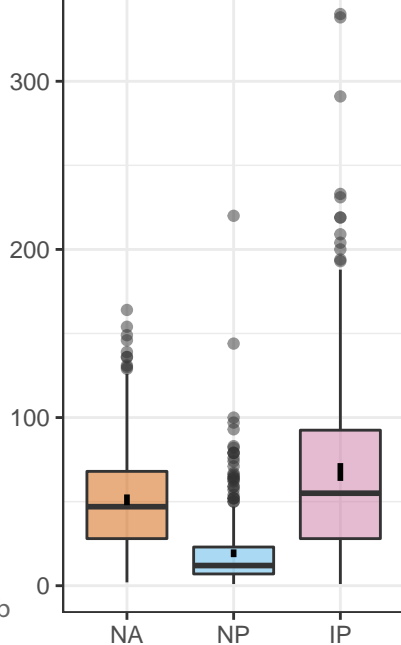
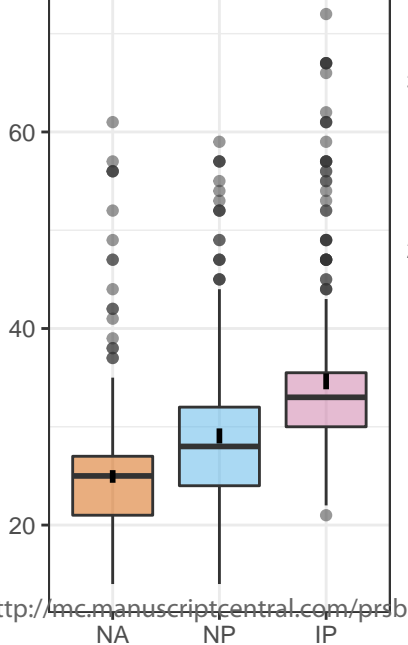
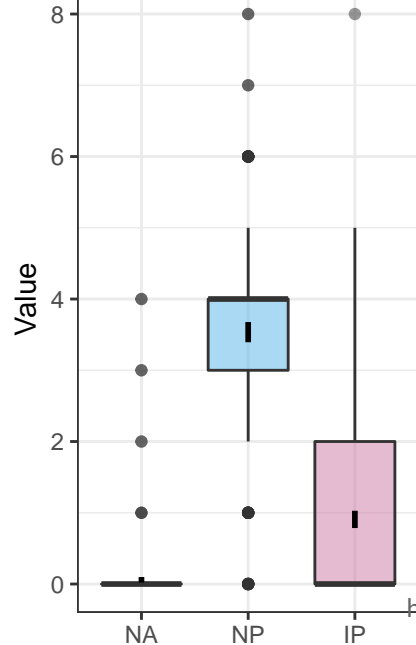
Figure 1. Phenotypic characteristics for some of the traits (stolon number, flowering time, and total number of flowers) measured in the F2 offspring of three crosses between native and introduced populations of *Mimulus guttatus* grown in a field plot in central Scotland. Cross types as follows: NA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

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



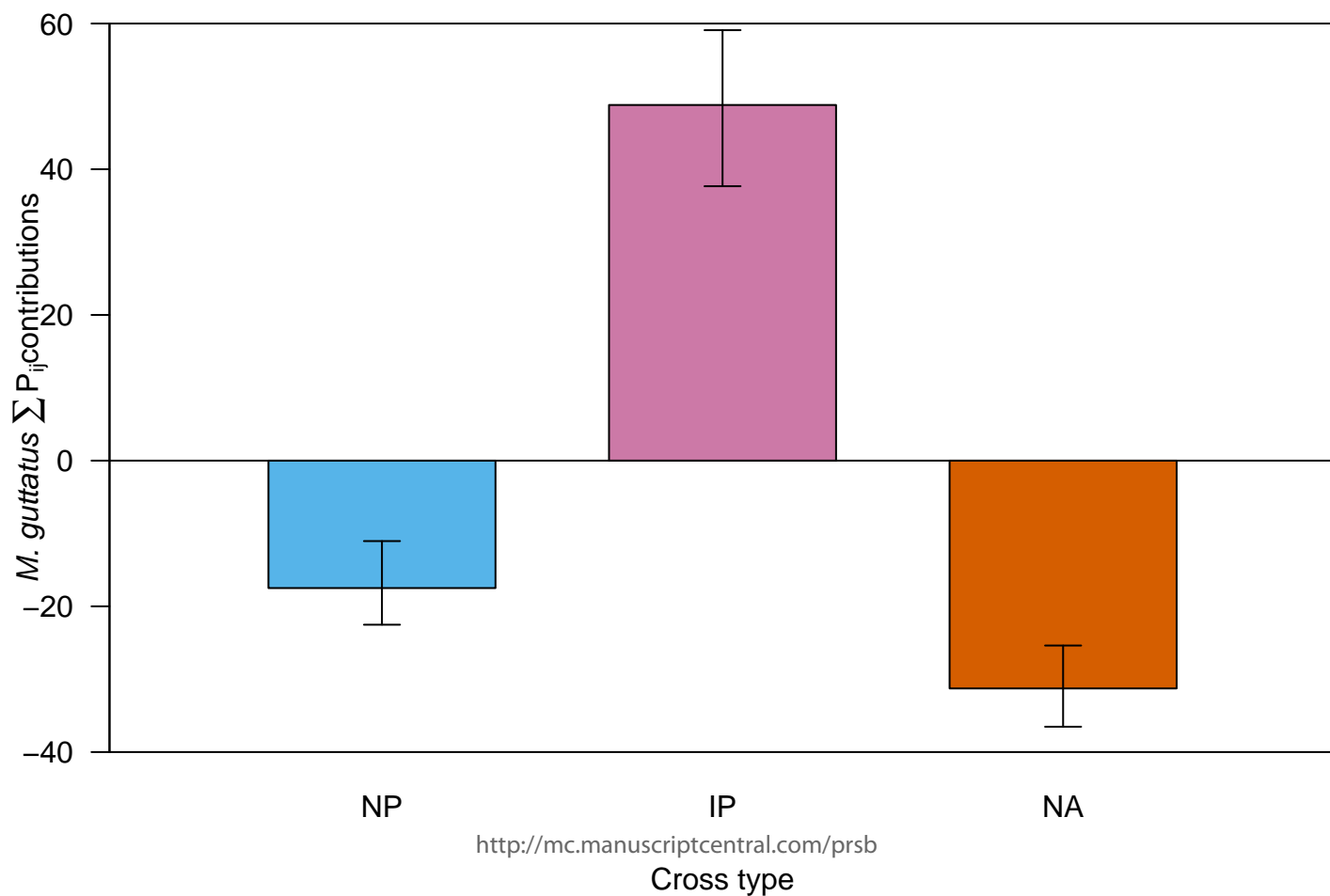


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Leaf width	0.041**	—	—	—	—	—
Plant height	0.079***	—	0.059***	-0.030***	0.005	-0.032**
Clonal spread	—	—	0.053***	-0.108***	0.055*	-0.196**