

Admixture between native and invasive populations may increase invasiveness of *Mimulus guttatus*

Mark van Kleunen, Michael Röckle and Marc Stiff

Ecology, Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany

Self-fertilization and admixture of genotypes from different populations can have major fitness consequences in native species. However, few studies have addressed their potential roles in invasive species. Here, we used plants of *Mimulus guttatus* from seven native North American, three invasive Scottish and four invasive New Zealand populations to address this. We created seeds from self-fertilization, within-population outcrossing, between-population outcrossing within the same range, and outcrossing between the native and invasive ranges. A greenhouse experiment showed that native and invasive plants of *M. guttatus* suffered to similar degrees from inbreeding depression, in terms of asexual reproduction and biomass production. After outcrossing with plants from other populations, *M. guttatus* benefited from heterosis, in terms of asexual and sexual reproduction, and biomass production, particularly when plants from native and invasive populations were crossed. This suggests that, when novel genotypes of *M. guttatus* from the native North American range will be introduced to the invasive ranges, subsequent outcrossing with *M. guttatus* plants that are already there might further boost invasiveness of this species.

Subject Areas:

ecology, evolution, plant science

Keywords:

admixture, heterosis, inbreeding depression, invasive plant, outbreeding depression

Author for correspondence:

Mark van Kleunen

e-mail: mark.vankleunen@uni-konstanz.de

1. Introduction

Many plant species have been introduced from their native range into new regions, where some of them have established naturalized populations and have become invasive. While many plant invasions may be driven by ecological factors such as disturbance (e.g. [1]), or may be caused by pre-adaptations to the new environment [2,3], genetic processes may also play important roles [4–7]. Although many studies have focused on evolutionary change between native and invasive populations (e.g. [5,6,8–10]), relatively few have looked at effects of other genetic processes such as inbreeding depression [11,12] and admixture [12–16].

Self-compatible species are thought to be more likely to become invasive, because they can in principle start a reproductive population from a single individual, and may be less reliant on pollinators [17,18]. Indeed, although there are highly invasive self-incompatible plants, self-compatible and autonomously selfing ones are frequently more widely naturalized and invasive (e.g. [19,20]). A potential negative consequence of self-compatibility that has not received much attention yet in the invasion literature (but see [11,12,16]), and could reduce invasion potential, is inbreeding depression. This arises because selfing increases homozygosity, which may result in the expression of recessive deleterious alleles and a reduced expression of advantageous overdominance effects [21,22]. However, as plants might have higher selfing rates in the invasive than in the native range, as a consequence of stronger mate and pollinator limitation in the invasive range, recessive deleterious alleles may have been purged from the invasive populations [23,24]. Alternatively, these deleterious alleles may have become fixed in the populations by genetic drift [25]. Both processes would reduce the fitness difference between selfed and outcrossed progeny (i.e. the magnitude of inbreeding depression) in invasive populations.

While many non-native species may have undergone strong genetic bottlenecks during naturalization [26,27], some might actually harbour considerable

genetic variation (e.g. [9,28]). The latter is particularly likely when genotypes from previously separated populations come into secondary contact in the non-native range [16,29,30]. Such admixture may occur when genotypes from different native populations are simultaneously introduced into a non-native location, when they are first introduced to different non-native locations and meet after they spread in the landscape, or when genotypes from the native range are introduced into established non-native populations. The increasing evidence that multiple introductions of invasive species are the norm rather than the exception [27,31,32] indicates that at least some of these scenarios of admixture are quite common.

Multiple introductions and subsequent admixture may increase the evolutionary potential of invasive populations, and thereby may have long-term fitness benefits [9]. However, when plants from different source populations interbreed, this can also have immediate fitness consequences – either positive or negative – for the progeny [30]. As mentioned above, populations – particularly the ones that went through recent bottlenecks and are inbred – may suffer from drift load. Outcrossing with a plant from a different population may introduce alleles that are dominant over the recessive deleterious ones that had become fixed, and thus may result in increased progeny fitness. Such heterosis effects are frequently found in studies on genetic rescue of endangered species (e.g. [33,34]), but they might also be important during invasions [30]. Conversely, between-population outcrossing and the resulting genetic recombination can result in reduced fitness of the progeny (i.e. outbreeding depression) when it breaks up adaptation to the local environment and/or adaptive gene complexes [35]. Outcrossing can occur between populations within the native range or within the invasive range. When new propagules from the native range are introduced into previously established invasive populations, or vice versa, outcrossing may also occur between plants from the native and invasive ranges. The heterosis effects of such between-range outcrossing might even be larger than the effects of outcrossing between plants from different populations within a range, particularly when there is limited genetic variation between populations, as one may expect in the invasive range. On the other hand, when native and invasive populations have been separated for a considerable time, genetic incompatibilities may have arisen that could result in strong outbreeding depression [36].

Only a few studies have addressed the fitness consequences of between-population outcrossing in invasive plants [37]. In one of the first of these studies, there was no fitness effect of between-population outcrossing, neither within the native range nor within the invasive range of *Silene latifolia* [14]. In a re-analysis of these data, a heterosis effect was found in the native range when plants from populations that were 600–1000 km apart were crossed, but not when the distances were larger, and also not in the invasive range [16]. Other studies in the invasive ranges of species also found evidence for heterosis after between-population outcrossing [12] or mixed results [13]. The few studies that performed crosses between native and invasive ranges indicate that reproductive barriers might have developed between native and invasive populations [36], and that offspring might be less fit [38]. However, it is hard to draw general conclusions based on these few case studies. Moreover, to the best of our knowledge, no study has yet simultaneously tested the fitness effects of selfing, between-population outcrossing and between-range outcrossing in an invasive plant species.

Here, we used plants of *Mimulus guttatus* from nine native North American, three invasive Scottish and four invasive New Zealand populations to create seeds from self-fertilization, within-population outcrossing, between-population outcrossing within the same range, and outcrossing between the native and invasive ranges. We grew the first-generation progeny of these crosses in a greenhouse experiment to test for effects of the different cross types on fitness components. We asked the following specific questions: (i) Do plants of *M. guttatus* show inbreeding depression, and if so, is this lower for plants from the invasive ranges? (ii) Do plants of *M. guttatus* show heterosis or outbreeding depression after between-population outcrossing within their ranges, and if so, does the magnitude differ between native and invasive ranges? (iii) Is heterosis or outbreeding depression strongest after intercontinental outcrossing between plants from the native and invasive ranges?

2. Material and methods

(a) Study species

Mimulus guttatus Fischer & DC (Phrymaceae) is native to western North America, and invasive in parts of Europe [39] and New Zealand [40]. The species grows as a perennial herb when grown under permanently wet conditions. However, in habitats that dry out at the end of spring, it has an enforced annual life cycle. *Mimulus guttatus* can vary in height from 0.1 to 1 m, and can spread vegetatively by means of stolons. It produces yellow, funnel shaped flowers, which are visited by hoverflies, bees and bumblebees, and can produce up to 500 small seeds. The species is self compatible and outcrossing rates range from near complete selfing to complete outcrossing [41–44].

(b) Plant material and crossing design

Seeds of *M. guttatus* were collected by the first author and several volunteers in 2002 and 2003 from nine native localities in western North America, three invasive Scottish localities and four invasive New Zealand localities (electronic supplementary material, table S1) [8]. In each population, seeds were collected from at least 19 different plants that were more than 1 m apart. However, as seeds had been used for previous studies [8,43], not all seed families were available for this study. For both the native and invasive ranges, we used seeds from annual and perennial plants (electronic supplementary material, table S1).

To produce progeny with different degrees of inbreeding and outbreeding, we grew plants from the 16 populations in a greenhouse of the Botanical Garden of the University of Konstanz. In November 2011, seeds from at least six maternal plants per population were sown separately in pots filled with commercial seedling soil. At the beginning of December 2011, six or more seedlings from each population were transplanted individually into 2.7 l pots filled with commercial potting soil, and placed in a pollinator free greenhouse compartment. Soon after these plants started flowering (in February 2012), and until our crossing design was completed in April 2012, we performed the following pollinations on each plant: (i) *self pollination*; (ii) *within population outcrossing* (pollination with a randomly chosen other flowering plant from the same population); (iii) *between population outcrossing* (pollination with a randomly chosen flowering plant from one of the other populations from the same range); and (iv) *between range outcrossing* (pollination with a randomly chosen flowering plant from another range). As we were mainly interested in simulating scenarios of ongoing genetic exchange between native and invasive ranges, we only made between range crosses between native (North American)

and invasive plants (Scottish and New Zealand), but not between plants from the two invasive ranges. For the North American range, seven of the nine populations were used as mother plants; plants from the remaining two populations were used as fathers only (electronic supplementary material, table S1). Although we aimed at having six maternal plants per population, some plants died or did not produce seeds for one or more of the four pollination treatments. As a consequence, we had 63 instead of 84 maternal plants with a complete set of crosses (electronic supplementary material, table S2).

In all cross pollinations, to avoid autonomous self pollination of the pollinated flower, we emasculated the recipient flower by removing its anthers before dehiscence. Emasculated control flowers (one on each plant) that were not manually self or cross pollinated did not produce any seeds. All pollinations were made by removing dehisced anthers with clean forceps from a donor plant and rubbing the pollen on the stigma of a flower of the receptor plant. Seeds were harvested when ripe, and stored in paper bags at room temperature until the start of the experiment. As the seeds were produced under the same conditions, and we had all four pollination treatments on each maternal plant, confounding of cross type effects with maternal carry over effects can be excluded.

(c) Experimental set-up

To test for effects of inbreeding and different levels of outbreeding on survival, growth, vegetative reproduction and sexual reproduction, we performed a greenhouse experiment at the Botanical Garden of the University of Konstanz. On 28 November 2012, we sowed for each seed family up to 30 seeds into randomly assigned positions of 54 well quickpot trays (QP 54/5.5R, HerkuPlast Co., Ering am Inn, Germany). Each well was filled with 50 cm³ of commercial seedling soil (Topferde, Einheitserde Co., Sinntal Altengronau, Germany; pH 5.8; 2.0 g l⁻¹ KCl; 340 mg l⁻¹ N; 380 mg l⁻¹ P₂O₆; 420 mg l⁻¹ K₂O; 200 mg l⁻¹ S; 700 mg l⁻¹ Mg). Day length was extended with artificial lighting to 10 h at 21°C, and the night temperature was 18°C. In 242 cases, sufficient seeds (three or more) germinated from a single mother for each of the four cross types (selfing, within population outcrossing, between population outcrossing, between range outcrossing). One week after germination, we transplanted three seedlings of each of these seed families into individual square pots (9 × 9 × 8 cm; Herman Meyer, Westerstede, Germany) filled with a 1 : 1 mixture of sand and vermiculite, and 3.5 g l⁻¹ slow release fertilizer (Osmocote Exact; three to four months; 16% N, 9% P, 12% K, 2% Mg; Scotts International, Geldermalsen, The Netherlands). As 11 seedlings did not survive transplanting, this resulted in a total of 715 instead of 726 plants. Pots were randomly assigned to equidistant positions on a greenhouse table, and were re randomized after one month.

(d) Measurements

At the end of the experiment (47–52 days after transplanting the seedlings), we first scored the survival of the plants. As a proxy of vegetative reproduction, we measured the cumulative length of all stolons (i.e. creeping branches). As measures of sexual reproduction, we first scored whether plants flowered, and on the ones that did, we counted the combined number of fully opened flowers, flower buds and spent flowers. After these measurements, to measure total biomass, we first harvested the aboveground biomass, including all stolons, and then washed the roots free from the soil. We weighed the above and belowground biomass of each plant after drying it to constant weight at 80°C for at least 3 days. Although the duration of our experiment was relatively short, and might not have given all plants enough time to flower, the length of the growing

season in many natural populations of *M. guttatus* is also less than two months.

(e) Analyses

To test whether cross type significantly affected plant performance (i.e. to test for in- and outbreeding depression, or heterosis), and whether the effect of cross type depended on plant origin (range), we used (generalized) linear mixed models as implemented in the *lmer* function of the *lme4* package (v. 1.1–7 [45]) in R (v. 3.1.0 [46]). As fixed terms, we included mother range (native North America, invasive Scotland, invasive New Zealand), cross type (self, within population, between population within range, between range) and their interaction. As random terms, we included maternal population and mother plant (nested in maternal population). As the fitness of progeny may also depend on the identity of the pollen donor, we also included paternal population and paternal plant (nested in paternal population) in the random model. We used a binomial error distribution for the analyses of survival and flowering. As only a few plants died during the experiment, and we thus had few zeros in the survival data, we used a cloglog link instead of the default logit link in the analysis of survival [47]. To achieve model convergence for the binomial response variables, we used the ‘bobyqa’ optimizer in the *lmer* function. For the number of flowers (including flower buds and spent flowers) on the subset of flowering plants, and for the cumulative length of the stolons and for total biomass of all plants, we used a Gaussian error distribution. To improve normality and homoscedasticity of the residuals, we log_n transformed the number of flowers, and square root transformed cumulative stolon length and total biomass prior to analysis.

We tested the significance of the range × cross type interaction and the main effects mother range and cross type by first removing the interaction, and then the main effects, and performing model comparison using likelihood ratio tests (see notes below table 1). In cases where mother range had a significant effect, we made Tukey contrasts to test which ranges were different from each other using the *glht* function in the *multcomp* package [48]. To test whether there was significant inbreeding depression (self pollinations versus within population crosses), heterosis within range (within population versus between population crosses) and heterosis between range (within population versus between range crosses), we specified a custom contrast matrix and used the *glht* function in the *multcomp* package [48] to perform z tests. To get an indication of the proportion of the variance explained by the fixed and random models, we calculated the marginal R² (variance explained by fixed effects only) and the conditional R² (variance explained by both the fixed and random effects; table 1), following Nakagawa & Schielzeth’s method [49].

3. Results

(a) Survival

The average survival rate was 91.0% and ranged from 72.8% (selfed progeny from Scottish maternal plants) to 97.1% (progeny from between-range crosses with North American maternal plants; figure 1a). Survival, however, did not differ significantly among the mother ranges (table 1). Cross type had a significant effect on survival (table 1). However, although the overall effect of selfing on survival was negative and the overall effects of between-population and between-range outcrossing were positive, these effects were not significant in z-tests (table 1). The effect of cross type did not differ significantly among the three mother ranges (no significant range × cross type interaction; table 1).

Table 1. Summary of (generalized) linear mixed model analysis to test for inbreeding depression and heterosis for performance traits in *Mimulus guttatus*. The full model fixed part included mother range, cross type and their interaction. The random part included maternal population and parent (nested in population), as well as paternal population and parent (nested in population). The marginal R^2 indicates the proportion of the variance explained by the fixed effects, and the conditional R^2 indicates the proportion of the variance explained by both the fixed and random effects. The variances of the random effects are presented in electronic supplementary material, table S3. Specific hypotheses (contrasts) between cross types were evaluated using post-hoc z-tests. Negative estimates indicate that the first cross type in the contrast had a lower value than the second, and positive estimates indicate the opposite. Values in bold are significant at the specified level. Values in italics are marginally significant ($p < 0.1$). S, selfing; WP, within-population outcrossing; BP, between-population outcrossing; BR, between-range outcrossing; MR, mother range; CT, cross type.

trait (error, transformation)	main fixed effects			variance explained		specific hypotheses (contrasts)							
	χ^2 mother range (d.f. = 2) ^a	χ^2 cross-type (d.f. = 3) ^a	χ^2 MR × CT (d.f. = 6) ^b	Marg. R^2	Cond. R^2	S versus WP: inbreeding depression?		WP versus BP: heterosis within range?		WP versus BR: heterosis between range?		BP versus BR: more heterosis between range than within range?	
						estimate	z	estimate	z	estimate	z	estimate	z
survival (binomial with cloglog link, none)	2.51	13.2**	3.79	0.190	0.331	-0.28	-1.66	-0.13	-0.69	-0.25	-1.27	-0.12	-0.60
cumulative stolon length (Gaussian, square root)	2.35	78.6***	7.11	0.158	0.398	-1.90	-4.38***	-0.55	-1.24	-1.55	-2.98*	-1.00	-1.90
total biomass (Gaussian, square root)	1.99	86.1***	7.92	0.172	0.353	-0.29	-3.95***	-0.16	-2.20	-0.31	-3.78***	-0.15	-1.80
flowering rate (binomial with logit link, none)	12.7**	30.7***	<i>10.7</i>	0.208	0.413	-0.21	-0.61	-0.74	-2.27	-1.23	-3.44**	-0.49	-1.35
flower number (Gaussian, log _e)	1.92	13.5**	7.51	0.134	0.488	0.038	0.15	-0.17	-0.80	-0.79	-3.48**	-0.61	-3.03**

^aRemoval of main effects MR and CT compared with random part + MR + CT.

^bRemoval of interaction MR × CT compared with random part + MR + CT + MR × CT.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

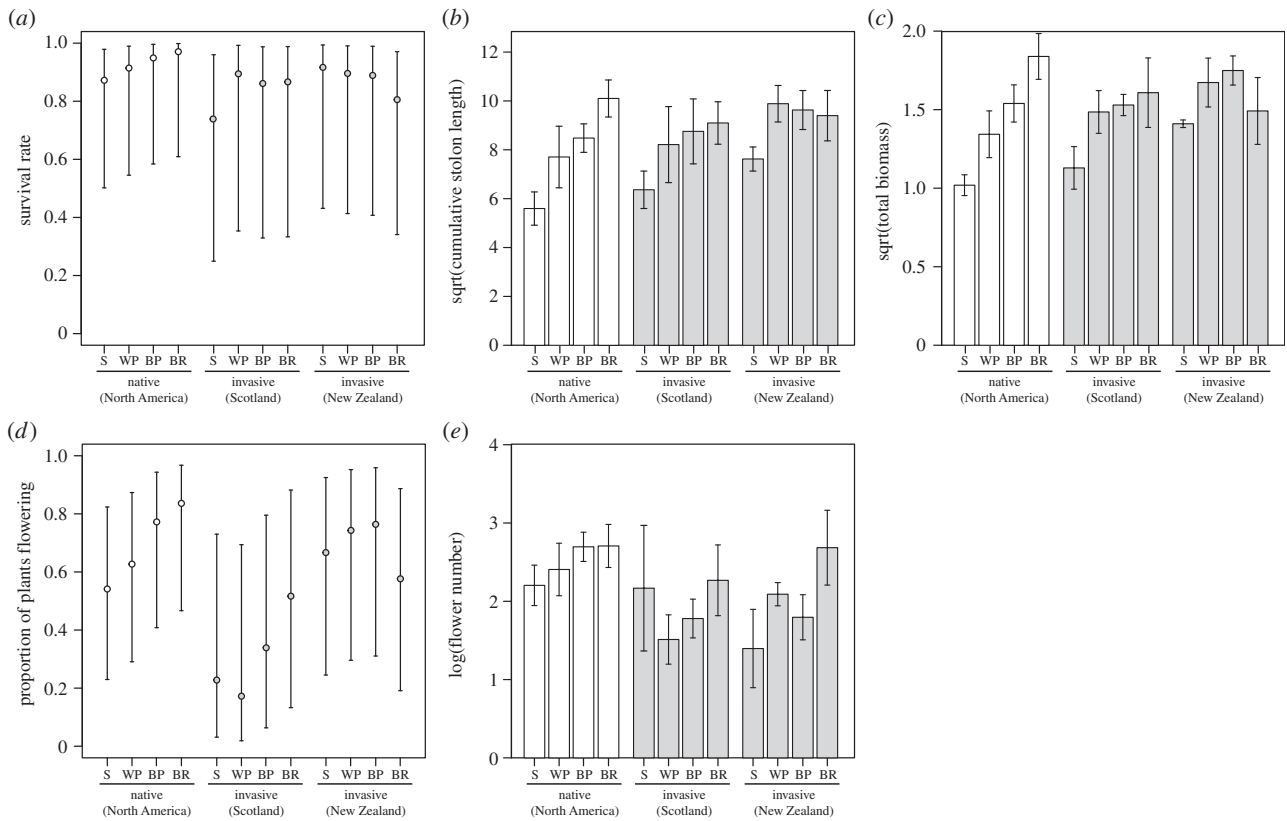


Figure 1. Comparison of life-history traits for *Mimulus guttatus* progeny derived from self-pollination (S) and cross-pollination within populations (WP), between populations (BP) and between ranges (BR). Data are shown for maternal plants from North America (native range), and from Scotland and New Zealand (both invasive ranges). BR crosses were always between native and invasive ranges. Error bars are 95% CIs based on Wilson approximations for the binomial traits seedling survival and flower rate, and standard errors for the approximately Gaussian transformed traits cumulative stolon length and biomass (both square root) and flower number (\log_n). All error bars were calculated with the number of populations on which the range \times cross type means were based as sample size. This means that the error bars are conservative, because they do not reflect that multiple replicate seed families were used for each population, and multiple replicates for each seed family.

(b) Asexual reproduction and biomass production

As the total biomass consists largely of stolon biomass, the results for cumulative stolon length per plant and total biomass were quite similar (table 1 and figure 1). The cumulative stolon length per plant and the total biomass did not differ significantly among the mother ranges (table 1 and figure 1b,c). The effect of cross type on cumulative stolon length and total biomass was significant; selfed progeny produced significantly less stolon length and biomass than progeny from within-population crosses, and progeny from between-range crosses produced significantly more stolon length and total biomass (table 1 and figure 1b,c). Although progeny from between-population crosses also produced slightly more stolon length and total biomass than progeny from within-population crosses (figure 1b,c), this effect was not significant (table 1). Although the effect of cross type on total stolon length and biomass tended to be strongest in the native range (figure 1b,c), it did not differ significantly among the three mother ranges (no significant range \times cross type interaction; table 1).

(c) Sexual reproduction

On average 65.2% of the plants flowered during the experiment, and the flowering rate ranged from 16.7% (selfed progeny of Scottish maternal plants) to 84.8% (progeny from between-range crosses of North American maternal plants; figure 1d). Mother range had a significant effect on

flowering probability (table 1), with Scottish plants significantly less likely to flower than plants from North America (Tukey contrast: $z = 3.74$, $p < 0.001$) and significantly less than plants from New Zealand (Tukey contrast: $z = 3.57$, $p < 0.01$). Cross type also had a highly significant effect on flowering probability. It did not differ significantly between selfed progeny and progeny from within-population crosses (table 1). However, compared with progeny from within-population crosses, the ones from between-population and between-range crosses were more likely to flower, and this difference was significant for the latter (table 1). The effects of cross type on flowering probability were only present in plants from North America and from Scotland, and not in plants from New Zealand, which had a relatively high flowering probability irrespective of cross type (marginally significant range \times cross type interaction; table 1).

Among the plants that flowered, the number of flowers produced per plant ranged from 1 to 232, with a mean of 21. The number of flowers did not differ significantly among the three mother ranges (figure 1e and table 1). Cross type had a significant effect on flower number; progeny from between-range crosses produced significantly more flowers than progeny from within- and between-population crosses (figure 1e and table 1). Although the effect of cross type on flower number tended to be strongest in New Zealand (figure 1e), it did not differ significantly among the mother ranges (no significant range \times cross type interaction; table 1).

4. Discussion

We found evidence that native and invasive plants of *M. guttatus* suffered to similar degrees from inbreeding depression, in terms of asexual reproduction and biomass production. Moreover, we showed that after outcrossing with plants from other populations, *M. guttatus* benefited from heterosis, in terms of asexual and sexual reproduction, and growth, particularly when plants from native and invasive populations were crossed. The latter suggests that if novel genotypes of *M. guttatus* from the native North American range were to be introduced to the invasive ranges, subsequent outcrossing with *M. guttatus* plants that are already there might boost invasiveness of this species.

(a) Inbreeding depression in native and invasive populations

The inbred offspring of *M. guttatus* in our study suffered from significant reductions in clonal reproduction and biomass production (most biomass was stolon biomass) compared with outbred offspring (figure 1). As inbreeding depression requires genetic variation [22], the presence of inbreeding depression in the invasive populations indicates that there is considerable genetic variation in the invasive ranges. Indeed, a previous study on the same populations showed that most allozyme alleles from the native range were also present in the invasive ranges, and that there was little allozyme differentiation between the invasive and native ranges [8]. Although we did not find statistically significant inbreeding depression in terms of sexual reproduction, previous studies on inbreeding depression in native populations of *M. guttatus* found significant reductions in flower production of inbred offspring [50–52]. However, as asexual reproduction increases the number of flower-bearing shoots, the negative effect of inbreeding on stolon production might ultimately also decrease reproductive fitness. Our seven-week experiment may not have been long enough to detect this effect.

Inbreeding depression in *M. guttatus* is mainly caused by recessive deleterious alleles, at least in two native populations of another study [51], and thus could be purged. Indeed, genetic load was purged after five generations of selfing in some maternal lines of *M. guttatus* in a previous study [50]. The absence of significant differences in inbreeding depression between native and invasive populations in our study suggests that if there has been purging of genetic load, it has not been stronger in the invasive range than in the native range. This would imply that selfing rates in the invasive populations are not higher than in the native populations. While *M. guttatus* might initially have been mate limited in its invasive ranges, the species is unlikely to be pollinator limited as the flowers readily attract generalized pollinators, such as hoverflies, honeybees and bumblebees, at least in its invasive European range [53] (M. van Kleunen 2004, personal observation). Therefore, the most likely explanation for the lack of a difference in inbreeding depression, and thus purging, between the native and invasive populations of *M. guttatus* is that the invasive populations do not rely more on selfing than the native ones do. Furthermore, because overall the invasive populations did not have lower performance than the native ones, with the exception of the lower likelihood of flowering in the Scottish populations, the absence of a difference in inbreeding

depression also suggests that the invasive populations have not accumulated more drift load than the native ones.

(b) Admixture between plants from different populations and ranges

On average, offspring from crosses between parents from different populations produced more stolons, biomass and flowers than offspring from within-population crosses (figure 1). While these effects were statistically not (or only marginally) significant for crosses between populations within the same range (WP versus BP in table 1), they were significant for crosses between native and invasive populations (WP versus BR in table 1). The fact that the increases in performance traits after between-population outcrossing within the same range were not statistically significant suggests that there is already considerable genetic variation both within native and within invasive populations of *M. guttatus*. Hence, gene flow among populations within the invasive ranges, as well as within the native range, will not (or will only marginally) increase performance traits beyond that of progeny from within-population outcrosses. Indeed, although a previous study on the same populations found significant allozyme differentiation among populations in the native range, population differentiation in the invasive ranges was not significant [8]. Nevertheless, the fact that performance traits increased considerably after between-range outcrossing suggests that both native and invasive ranges suffer to some extent from drift load, which can be reduced by admixture through heterosis [4].

Although none of the range \times cross type interactions was statistically significant (table 1), the magnitude of the heterosis effects appeared to differ among the ranges (figure 1). The effect of heterosis on the proportion of flowering plants was apparent in the native range and in Scotland, but not in New Zealand (this interaction was marginally significant). Similarly, the heterosis effect on stolon and biomass production appeared to be strongest in the native range, weak in Scotland and absent in the New Zealand range. However, the reverse pattern, particularly with regard to between-range outcrossing, appeared to hold for flower production. The lack of significance of the range \times cross type interactions could reflect low statistical power for detecting such interactions. Nevertheless, even if the apparent (but non-significant) differences are real, the fact that for some performance traits the heterosis effects are stronger in the native range and for others in the invasive range still suggests that both native and invasive populations will benefit from between-range outcrossing.

In natural populations, admixture does not always lead to heterosis, but can also have negative fitness consequences when it results in a reduction of local adaptation [38]. This outbreeding depression, however, is more likely to appear in the second and later offspring generations, after recombination has broken up co-adapted gene complexes [21]. Moreover, it has been suggested that during the invasion process, when populations have not yet become locally adapted to their new environments, outbreeding depression is less likely to offset the benefits of heterosis [16]. Therefore, the heterosis effects found in our greenhouse experiment indicate that introduction of novel genotypes from the native range into invasive ranges might boost fitness. This effect is known from endangered native species, where artificial introduction of novel genotypes into genetically impoverished

populations reduces inbreeding depression and drift load [33,54]. For some native species, it has been shown that heterosis effects in the first offspring generation were swamped by negative effects of outbreeding depression in later generations [55,56]. Therefore, it is important that future studies on the effects of admixture in invasive plants also assess the fitness of plants in subsequent generations (e.g. [13]). Nevertheless, even when the fitness benefit of admixture is ephemeral, the temporary boost in performance might have a catapult effect that could increase invasiveness [57]. Furthermore, introducing genotypes from the invasive ranges back to the native range would also boost performance there. Although we are not aware of any examples, such an introduction of invasive alien genotypes back into the native range could result in invasive behaviour there.

So far, only a few other studies have assessed the importance of admixture in invasive plant species [12–16]. Nuclear and chloroplast markers provided evidence for a considerable degree of admixture in invasive North American and in native European populations of *Silene vulgaris* [15]. An accompanying greenhouse experiment showed that invasive populations benefitted more from admixture in terms of fruit production than native populations, which could be due to increased evolutionary potential of admixed populations and/or heterosis [15]. Based on offspring from self-fertilization and from outcrossing within populations, between populations and between regions in its invasive North American range, there was evidence for strong inbreeding depression in *S. vulgaris*, but the effect of admixture depended on the regions; some regions showed heterosis while others showed outbreeding depression [13]. On the other hand, in an invasive North American population of *Alliaria petiolata* offspring fitness increased after between-population outcrossing, but there was no evidence for inbreeding depression [12]. Results on the effects of admixture between populations within the non-native ranges of invasive species are thus variable, and more studies are required to see whether there are any general patterns.

Few studies have crossed plants from the native and invasive ranges to assess the fitness consequences. For a native Swiss provenance of *Silene alba*, heterosis was observed for progeny from crosses with three other European provenances, but outbreeding depression was observed for progeny from a cross with a non-native North American provenance [38]. In two *Centaurea* species, there was even evidence that reproductive barriers may have arisen between invasive and native populations, as between-continent crosses resulted in fewer seeds per inflorescence than within-continent crosses [36]. In our study, we did not systematically score seed set of the crosses that we made, but as most of the crosses resulted in sufficient numbers of seeds and the offspring had increased performance, we have no reason to believe that reproductive barriers have arisen yet between native and invasive *M. guttatus* plants, nor that between-continent outcrossing will result in outbreeding depression.

5. Conclusion

While selfing might provide reproductive assurance when plants are mate and/or pollinator limited after introduction

to non-native ranges [17], our findings indicate that the contribution of selfing to invasiveness may be hampered by significant inbreeding depression. As a consequence, factors that increase outcrossing in non-native populations can potentially boost individual fitness, and consequently population growth rates and invasiveness. Therefore, species that manage to attract sufficient pollinators after introduction to a non-native range might have an advantage over those species that can only reproduce by selfing, unless the latter have effectively purged genetic load. So, although selfing might increase the likelihood of becoming invasive by providing reproductive assurance, it will still be beneficial for plants in the invasive range to maintain some outcrossing.

In the invasive range, a further benefit of outcrossing could be that it allows for admixture when novel genotypes are introduced. Although admixture between populations within the native range or within the invasive ranges had no significant (or only marginal) benefits, we showed that heterosis can be considerable after crosses between the native and invasive ranges. While some countries have strict import regulations for novel alien species, this legislation frequently does not prohibit the import of new genetic material of species that had already established before the legislation became active (e.g. the Biosecurity Act 1993 [58] and the Hazardous Substances and New Organisms Act 1996 of New Zealand [59]). As a consequence, the current legislation is not sufficient to prevent an increase in invasiveness through admixture in alien species that have already established.

Studies that found increased fitness of non-native over native populations of invasive species have frequently implied adaptive evolutionary processes [10]. However, our results suggest that such fitness differences could also arise if heterosis effects persist for multiple generations, and if admixture is more common in non-native than in native populations of invasive species. The latter could be the case when invasive populations occur mainly in well-connected anthropogenic environments, while native populations occur in more isolated natural habitats. Most importantly, even if the effect of heterosis turns out to be ephemeral, the temporary increase in performance might be sufficient to overcome stochastic effects, and when this translates into increased population growth rates [60], it will boost invasiveness.

Data accessibility. The datasets have been uploaded as part of the electronic supplementary material.

Authors' contributions. M.v.K. conceived of the study and contributed natural resources (seeds). M.v.K. and M.S. designed the study and drafted the manuscript; M.R. carried out the experiment and participated in data analysis. M.S. carried out the statistical analyses. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. This study was supported by the Deutsche Forschungsgemeinschaft (project KL1866/3 1 to M.v.K.).

Acknowledgements. We thank Allison Butlen, Annie Truscot, Elizabeth Parnis, Jessica Ruvinsky, Linda Jennings, Marilyn Barker, Brian Rance, Chris Woolmore, Lawrence Janeway, Lowel Ahart, Nick Page, Nishanta Rajakaruna, Tom Belton and Tony Labanca for collection of seeds, Anna Kesberg for help with the crossings, Wayne Dawson for statistical advice, Carolin Bogs, Carolina Wackerhagen, Janosch Sedlacek, Gregor Müller, Timo Scheu, Katya Mamonova, Verena Geyer and Otmar Ficht for help with the experiment, and three anonymous reviewers and the editors for constructive advice.

- Davis MA, Grime JP, Thompson K. 2000 Fluctuating resources in plant communities: a general theory of invasibility. *J. Ecol.* **88**, 528–534. (doi:10.1046/j.1365-2745.2000.00473.x)
- Schlaepfer DR, Gattli M, Fischer M, van Kleunen M. 2010 A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *New Phytol.* **185**, 1087–1099. (doi:10.1111/j.1469-8137.2009.03114.x)
- Petitpierre B, Kueffer C, Broennimann O, Randin C, Daehler C, Guisan A. 2012 Climatic niche shifts are rare among terrestrial plant invaders. *Science* **335**, 1344–1348. (doi:10.1126/science.1215933)
- Lee CE. 2002 Evolutionary genetics of invasive species. *Trends Ecol. Evol.* **17**, 386–391. (doi:10.1016/S0169-5347(02)02554-5)
- Cox GW. 2004 *Alien species and evolution: the evolutionary ecology of exotic plants, animals, microbes, and interacting native species*. Washington, DC: Island Press.
- Muller-Scharer H, Schaffner U, Steinger T. 2004 Evolution in invasive plants: implications for biological control. *Trends Ecol. Evol.* **19**, 417–422. (doi:10.1016/j.tree.2004.05.010)
- Barrett SC, Colautti RI, Eckert CG. 2008 Plant reproductive systems and evolution during biological invasion. *Mol. Ecol.* **17**, 373–383. (doi:10.1111/j.1365-294X.2007.03503.x)
- van Kleunen M, Fischer M. 2008 Adaptive rather than non-adaptive evolution of *Mimulus guttatus* in its invasive range. *Basic Appl. Ecol.* **9**, 213–223. (doi:10.1016/j.baae.2007.03.006)
- Lavergne S, Molofsky J. 2007 Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc. Natl Acad. Sci. USA* **104**, 3883–3888. (doi:10.1073/pnas.0607324104)
- Blossey B, Notzold R. 1995 Evolution of increased competitive ability in invasive nonindigenous plants—a hypothesis. *J. Ecol.* **83**, 887–889. (doi:10.2307/2261425)
- Daehler CC. 1999 Inbreeding depression in smooth cordgrass (*Spartina alterniflora*, Poaceae) invading San Francisco Bay. *Am. J. Bot.* **86**, 131–139. (doi:10.2307/2656962)
- Mullarkey AA, Byers DL, Anderson RC. 2013 Inbreeding depression and partitioning of genetic load in the invasive biennial *Alliaria petiolata* (Brassicaceae). *Am. J. Bot.* **100**, 509–518. (doi:10.3732/ajb.1200403)
- Bailey MF, McCauley DE. 2006 The effects of inbreeding, outbreeding and long-distance gene flow on survivorship in North American populations of *Silene vulgaris*. *J. Ecol.* **94**, 98–109. (doi:10.1111/j.1365-2745.2005.01090.x)
- Wolfe LM, Blair AC, Penna BM. 2007 Does intraspecific hybridization contribute to the evolution of invasiveness? An experimental test. *Biol. Invasions* **9**, 515–521. (doi:10.1007/s10530-006-9046-0)
- Keller SR, Taylor DR. 2010 Genomic admixture increases fitness during a biological invasion. *J. Evol. Biol.* **23**, 1720–1731. (doi:10.1111/j.1420-9101.2010.02037.x)
- Verhoeven KJF, Macel M, Wolfe LM, Biere A. 2011 Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proc. R. Soc. B* **278**, 2–8. (doi:10.1098/rspb.2010.1272)
- Baker HG. 1955 Self compatibility and establishment after long distance dispersal. *Evolution* **9**, 347–349. (doi:10.2307/2405656)
- Baker HG. 1965 Characteristics and modes of origin of weeds. In *The genetics of colonizing species* (eds HG Baker, GL Stebbins), pp. 147–172. New York, NY: Academic Press.
- van Kleunen M, Johnson SD. 2007 Effects of self-compatibility on the distribution range of invasive European plants in North America. *Conserv. Biol.* **21**, 1537–1544. (doi:10.1111/j.1523-1739.2007.00765.x)
- Hao JH, Qiang S, Chrobock T, van Kleunen M, Liu QQ. 2011 A test of baker's law: breeding systems of invasive species of Asteraceae in China. *Biol. Invasions* **13**, 571–580. (doi:10.1007/s10530-010-9850-4)
- Lynch M. 1991 The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**, 622–629. (doi:10.2307/2409915)
- Charlesworth D, Willis JH. 2009 The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**, 783–796. (doi:10.1038/nrg2664)
- Lande R, Schemske DW. 1985 The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. *Evolution* **39**, 24–40. (doi:10.2307/2408514)
- Crnokrak P, Barrett SC. 2002 Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**, 2347–2358. (doi:10.1111/j.0014-3820.2002.tb00160.x)
- Lynch M, Conery J, Burger R. 1995 Mutation accumulation and the extinction of small populations. *Am. Nat.* **146**, 489–518. (doi:10.1086/285812)
- Brown AHD, Marshall DR. 1981 Evolutionary changes accompanying colonization in plants. In *Evolution today* (eds GE Scudder, JS Reveal), pp. 351–363. Pittsburgh, PA: Hunt Institute for Botanical Documentation.
- Dlugosch KM, Parker IM. 2008 Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* **17**, 431–449. (doi:10.1111/j.1365-294X.2007.03538.x)
- Kolbe JJ, Glor RE, Rodriguez Schettino L, Lara AC, Larson A, Losos JB. 2004 Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **431**, 177–181. (doi:10.1038/nature02807)
- Ellstrand NC, Schierenbeck KA. 2000 Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl Acad. Sci. USA* **97**, 7043–7050. (doi:10.1073/pnas.97.13.7043)
- Rius M, Darling JA. 2014 How important is intraspecific genetic admixture to the success of colonising populations? *Trends Ecol. Evol.* **29**, 233–242. (doi:10.1016/j.tree.2014.02.003)
- Facon B, Pointier JP, Jarne P, Sarda V, David P. 2008 High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Curr. Biol.* **18**, 363–367. (doi:10.1016/j.cub.2008.01.063)
- Uller T, Leimu R. 2011 Founder events predict changes in genetic diversity during human-mediated range expansions. *Glob. Change Biol.* **17**, 3478–3485. (doi:10.1111/j.1365-2486.2011.02509.x)
- Madsen T, Shine R, Olsson M, Wittzell H. 1999 Conservation biology—restoration of an inbred adder population. *Nature* **402**, 34–35. (doi:10.1038/46941)
- Willi Y, Van Kleunen M, Dietrich S, Fischer M. 2007 Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. *Proc. R. Soc. B* **274**, 2357–2364. (doi:10.1098/rspb.2007.0768)
- Templeton AR. 1986 Coadaptation and outbreeding depression. In *Conservation biology: the science of scarcity and diversity* (ed. ME Soule), pp. 105–116. Sunderland, MA: Sinauer Associates.
- Montesinos D, Santiago G, Callaway RM. 2012 Neo-allopatry and rapid reproductive isolation. *Am. Nat.* **180**, 529–533. (doi:10.1086/667585)
- Dlugosch KM, Anderson SR, Braasch J, Cang FA, Gillette HD. 2015 The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Mol. Ecol.* **24**, 2095–2111. (doi:10.1111/mec.13183)
- Keller M, Kollmann J, Edwards PJ. 2000 Genetic introgression from distant provenances reduces fitness in local weed populations. *J. Appl. Ecol.* **37**, 647–659. (doi:10.1046/j.1365-2664.2000.00517.x)
- Truscott AM, Soulsby C, Palmer SCF, Newell L, Hulme PE. 2006 The dispersal characteristics of the invasive plant *Mimulus guttatus* and the ecological significance of increased occurrence of high-flow events. *J. Ecol.* **94**, 1080–1091. (doi:10.1111/j.1365-2745.2006.01171.x)
- Howell CJ, Sawyer JWD. 2006 *New Zealand naturalised vascular plant checklist*, pp. 1–60. Wellington, New Zealand: New Zealand Plant Conservation Network.
- Fenster CB, Ritland K. 1994 Quantitative genetics of mating system divergence in the Yellow Monkeyflower species complex. *Heredity* **73**, 422–435. (doi:10.1038/Hdy.1994.190)
- van Kleunen M, Ritland K. 2004 Predicting evolution of floral traits associated with mating system in a natural plant population. *J. Evol. Biol.* **17**, 1389–1399. (doi:10.1111/j.1420.9101.2004.00787.x)

43. van Kleunen M. 2007 Adaptive genetic differentiation in life-history traits between populations of *Mimulus guttatus* with annual and perennial life-cycles. *Evol. Ecol.* **21**, 185–199. (doi:10.1007/s10682-006-0019-7)
44. Fishman L, Willis JH. 2008 Pollen limitation and natural selection on floral characters in the yellow monkeyflower, *Mimulus guttatus*. *New Phytol.* **177**, 802–810. (doi:10.1111/j.1469-8137.2007.02265.x)
45. Bates D, Maechler M, Bolker B. 2014 *Linear mixed-effects models using Eigen and S4*. See <http://CRAN.R-project.org/package=lme4>.
46. R Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
47. Egli P, Schmid B. 2001 The analysis of complex leaf survival data. *Basic Appl. Ecol.* **2**, 223–231. (doi:10.1078/1439-1791-00048)
48. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous inference in general parametric models. *Biometrical J.* **50**, 346–363. (doi:10.1002/bimj.200810425)
49. Nakagawa S, Schielzeth H. 2013 A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133–142. (doi:10.1111/j.2041-210x.2012.00261.x)
50. Dudash MR, Carr DE, Fenster CB. 1997 Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. *Evolution* **51**, 54–65. (doi:10.2307/2410960)
51. Dudash MR, Carr DE. 1998 Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* **393**, 682–684. (doi:10.1038/31468)
52. Murren CJ, Dudash MR. 2012 Variation in inbreeding depression and plasticity across native and non-native field environments. *Ann. Bot.* **109**, 621–632. (doi:10.1093/Aob/Mcr325)
53. Robertson AW, Mountjoy C, Faulkner BE, Roberts MV, Macnair MR. 1999 Bumble bee selection of *Mimulus guttatus* flowers: the effects of pollen quality and reward depletion. *Ecology* **80**, 2594–2606. (doi:10.1890/0012-9658)
54. Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN. 1998 Tracking the long-term decline and recovery of an isolated population. *Science* **282**, 1695–1698. (doi:10.1126/science.282.5394.1695)
55. Edmands S. 1999 Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**, 1757–1768. (doi:10.2307/2640438)
56. Fenster CB, Galloway LF. 2000 Population differentiation in an annual legume: genetic architecture. *Evolution* **54**, 1157–1172. (doi:10.1111/j.0014-3820.2000.tb00551.x)
57. Drake JM. 2006 Heterosis, the catapult effect and establishment success of a colonizing bird. *Biol. Lett.* **2**, 304–307. (doi:10.1098/rsbl.2006.0459)
58. New Zealand Government. 2015 *Biosecurity Act 1993 No 95 (Reprint as at 28 May 2015)*. Wellington, New Zealand: Ministry for Primary Industries. See <http://www.legislation.govt.nz/act/public/1993/0095/latest/DLM314623.html>.
59. New Zealand Government. 2014 *Hazardous Substances and New Organisms Act 1996 No 30 (Reprint as at 24 June 2015)*. Wellington, New Zealand: Ministry for Primary Industries. See <http://www.legislation.govt.nz/act/public/1996/0030/latest/DLM381222.html>.
60. Hovick SM, Whitney KD. 2014 Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. *Ecol. Lett.* **17**, 1464–1477. (doi:10.1111/Ele.12355)