

# Admixture increases performance of an invasive plant beyond first-generation heterosis

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Handling Editor: Stephen Bonser

## Abstract

1. Through its potential to enhance progeny performance, admixture (between-population crossing) may promote invasiveness of alien plants. The few studies that tested this idea found evidence for heterosis (positive effects of admixture) in the first generation ( $F_1$ ), but have not considered further generations. In this paper, we test whether admixture benefits can be maintained in subsequent generations of an invasive plant.
2. We follow up on a previous study, in which we made crosses between plants of *Mimulus guttatus* from native- (western North America) and invaded-range populations (New Zealand and Scotland), and showed that admixture increases  $F_1$  performance. Here, we performed further crosses to create non-admixed progeny,  $F_1$  progeny resulting from within- and between-range admixture, and subsequent  $F_2$  progeny both through outcrossing and through self-fertilization. As heterosis, out- and inbreeding depression may depend on the environment, we assessed progeny performance under benign (well-watered) and drought-stress conditions in a greenhouse experiment.
3. We found that non-admixed progeny of *M. guttatus* were outperformed by admixed progeny (averaged across  $F_1$  and  $F_2$ ), particularly by progeny from between-range admixture. However, the benefit of admixture was stronger in  $F_1$  than in  $F_2$  progeny, especially when the  $F_2$  was produced by self-fertilization. The benefit of admixture also depended on the range of origin and the test environment.
4. *Synthesis.* Our findings indicate that increased performance of admixed  $F_1$  progeny is partly maintained in the  $F_2$  progeny. Admixture might thus significantly boost performance of an invasive plant across multiple generations.

## KEYWORDS

admixture, alien plant, catapult effect, heterosis, inbreeding depression, intraspecific hybrid, outbreeding depression

## 1 | INTRODUCTION

More than 13,000 vascular plant species have established naturalized populations beyond their native ranges (Pysek et al., 2017; van Kleunen, Dawson, et al., 2015). Some of these naturalized species have become invasive, i.e. they occur at high abundances, and cause ecological and economic damage (Vilà et al., 2011). Historical (e.g.

year of introduction, propagule pressure) and ecological (e.g. disturbance, nutrient enrichment) factors are known to be important drivers of plant invasions (Catford, Jansson, & Nilsson, 2009). However, genetic processes may also play an important role (Dlugosch & Parker, 2008; Perez, Nirchio, Alfonsi, & Munoz, 2006; Rius & Darling, 2014; Schrieber & Lachmuth, 2017; Stebbins, 1985; te Beest et al., 2012). For example, the human-mediated translocation of plant species has

brought together closely related species from different regions, and matings between these species have resulted in novel interspecific hybrids of which some are very vigorous and highly invasive (Ellstrand & Schierenbeck, 2000; Hovick & Whitney, 2014; Schierenbeck & Ellstrand, 2009). Similarly, human actions can result in intraspecific admixture by allowing previously separated populations of the same species to interbreed.

Intraspecific admixture may happen through multiple sequential introductions from different source populations into a single area (Dlugosch & Parker, 2008), or through secondary contact between populations initially introduced to different areas of the invaded range (Facon, Pointier, Jarne, Sarda, & David, 2008; Rosenthal, Ramakrishnan, & Cruzan, 2008). Although intraspecific admixture could slow down or reverse local adaptation (Verhoeven, Macel, Wolfe, & Biere, 2011), it may also facilitate invasion by generating evolutionary novelty, increasing genetic variation, and causing heterosis by alleviating genetic load (Ellstrand & Schierenbeck, 2000). Despite its potential importance for invasiveness, few studies have empirically tested the prediction that admixture can increase performance, and thus invasiveness, of alien species (Rius & Darling, 2014).

The studies that tested whether admixture has beneficial or adverse effects in invasive plants gave mixed results (Keller & Taylor, 2010; Montesinos, Santiago, & Callaway, 2012; Mullarkey, Byers, & Anderson, 2013; van Kleunen, Rockle, & Stift, 2015; Verhoeven et al., 2011; Wolfe, Blair, & Penna, 2007). For example, in *Centaurea solstitialis* and in *Centaurea sulphurea*, reproductive barriers have developed between native and invasive populations, resulting in a lower seed set after between-range outcrossing. On the other hand, invasive populations of *Silene vulgaris* benefited from admixture with native populations (Keller & Taylor, 2010). Similarly, invasive and native populations of *Mimulus guttatus* benefited from admixture (van Kleunen, Dawson, et al., 2015). Thus, admixture is frequently beneficial in invasive species (Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015).

All previous studies on the effects of admixture for invasive species were restricted to the first offspring generation ( $F_1$ ). Benefits of admixture, however, may be transient as a consequence of recombination between the admixed genomes, which only occurs in  $F_2$  and later generations, and could result in the break-up of co-adapted gene complexes (Dlugosch et al., 2015; Lynch, 1991). Theoretically, this could even reduce the fitness of subsequent generations below that of the pre-admixture generation, and thereby reverse initial benefits of admixture and ultimately reduce invasiveness. Furthermore, if a species reproduces predominantly by self-fertilization, heterosis effects in the admixed  $F_1$  may be countered in subsequent inbred generations due to renewed expression of recessive deleterious alleles (i.e. inbreeding depression). Nevertheless, even if heterosis is transient, it can still facilitate establishment of an introduced species by pushing population sizes over or closer to the demographic establishment threshold (Drake, 2006). The strength of this so-called catapult effect (Drake, 2006) will depend on individual performance in later generations. Although some studies made multiple generations of crosses between populations of invasive plant species in their invaded range (Johansen-Morris & Latta, 2006) or native range (Keller, Kollmann, & Edwards,

2000), there have been no such multigeneration studies that assessed the consequences of between-range admixture.

Moreover, the effects of admixture have rarely been assessed in different environments. This is important, because plant performance in general, and inevitably also the relative performance of different individuals, may be environment-specific. For example, the relative performance of self-progeny relative to cross-progeny (inbreeding depression) may be more pronounced under stressful conditions (Dudash, 1990; Fox & Reed, 2011; Yun & Agrawal, 2014). A competitive environment increased fecundity of *Raphanus* hybrids relative to non-admixed plants (Campbell & Snow, 2007) and increased relative fitness of *Helianthus* hybrids (Mercer, Andow, Wyse, & Shaw, 2007). Therefore, it is important to assess the effects of admixture and inbreeding both under benign and stressful conditions.

In a previous study, we had performed self- and cross-pollinations (within populations, between populations and between ranges) with *M. guttatus* plants from native-range populations (western North America) and invaded-range populations (New Zealand and Scotland). Particularly, the  $F_1$  progeny resulting from between-range crossing produced more biomass, and had more vigorous asexual and sexual reproduction. We therefore concluded that admixture may further boost invasiveness of *M. guttatus* through heterosis (van Kleunen et al., 2015). However, we also identified the need to assess the consequences of admixture in subsequent generations, where outbreeding depression may negate heterotic effects (e.g. Edmands, 2007). Here, to test whether increased performance is maintained in second-generation progeny ( $F_2$ ), we made further crosses with the previously generated  $F_1$  plants to create  $F_2$  plants genetically equivalent to the parental (P) generation, and  $F_1$  and  $F_2$  progeny from within- and between-range crosses (here-after referred to as admixed; Figure 1). The admixed  $F_2$  was created both through outcrossing and through self-fertilization. Then, we assessed the performance of the resulting plants in a greenhouse experiment, in which plants were grown under benign (well-watered) and drought-stress conditions. This setup allowed us to address the following main questions: (1) Does admixture result in a performance benefit, and if so is this stronger for between-range than for within-range admixture? (2) Do the effects of admixture differ between  $F_1$  and  $F_2$ , and does it matter whether the  $F_2$  is formed by outcrossing or self-fertilization? (3) Do the effects of admixture depend on the range of origin and the test environment?

## 2 | MATERIALS AND METHODS

### 2.1 | Study species

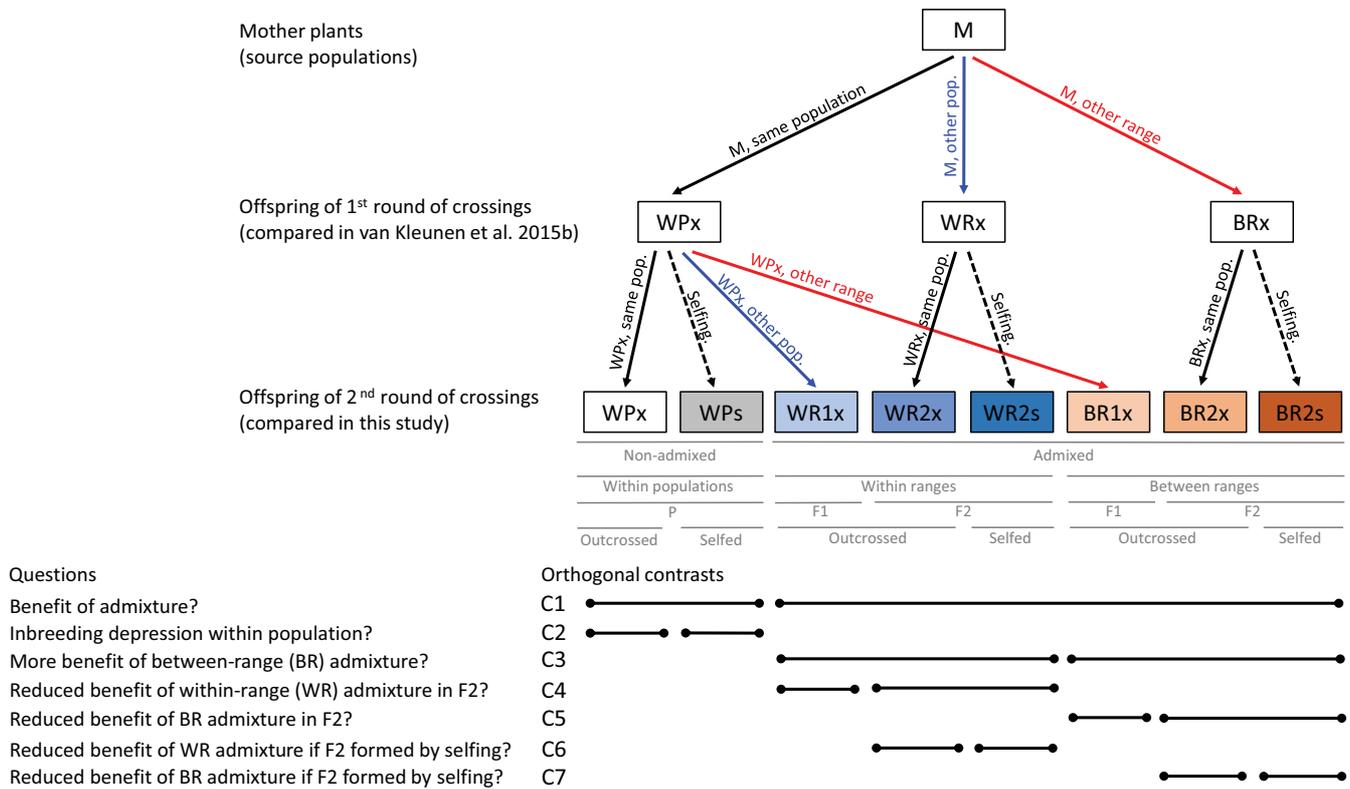
*Mimulus guttatus* DC. (Phrymaceae) is a herbaceous plant native to western North America and invasive in New Zealand and parts of Europe (Truscott, Soulsby, Palmer, Newell, & Hulme, 2006). It can grow up to 1 m high, and can reproduce both clonally (through stolons) and sexually (through seeds). *Mimulus guttatus* is self-compatible, and its population-level outcrossing rates range from about 75% selfing to complete outcrossing (Dudash & Ritland, 1991; Ritland & Ritland, 1989; Willis, 1993). The species grows well in moist to wet soils, and

therefore frequently grows in riparian habitats and ditches, where it tends to have a perennial life cycle. However, in habitats that dry out towards the end of spring, the life cycle can be enforced to be annual (Dole, 1992; van Kleunen, 2007).

## 2.2 | Plant material and crossing design

As we wanted to compare the parental generation, and  $F_1$  and  $F_2$  progeny without confounding effects of differences in previous environments and age of the seeds, we created seeds resembling these three generations according to the crossing scheme shown in Figure 1. All plants used in the experiment thus had a history of two rounds of crossing in a common greenhouse environment. The starting generation consisted of plants raised from seeds collected in natural populations in 2002 and 2003 (van Kleunen & Fischer, 2008). Both in the native and the invaded ranges, some populations came from permanently wet habitats and some population came from habitats that dry out during part of the year. During spring of 2012, we had performed

a first round of hand pollinations on each plant to generate  $F_1$  cross-progeny with three different degrees of outbreeding (described in detail in van Kleunen et al., 2015). In brief, up to six maternal plants from seven native western North American populations, three invasive Scottish populations and four invasive New-Zealand populations were crossed within each of these populations (WP), between populations within the same range (WR) and between populations from another range (BR; always between a native and an invasive population; Figure 1). Seeds of these crosses were sown in November 2012, and the resulting cross-progeny were grown until flowering. On these plants, we performed self- and cross-pollinations to generate eight different types of progeny per maternal line (Figure 1). With each WP plant, we generated four types of progeny: (1) progeny that resemble outbred plants from the original parental population, by using another WP plant from the same population as pollen donor (WPx); (2) progeny that resemble inbred plants from the original parental population, by manual self-fertilization (WPs); (3) progeny that resemble  $F_1$  within-range admixed plants, using another WP plant from a different



**FIGURE 1** Crossing design employed to generate first and second generation within- and between-range admixed and non-admixed progeny for *Mimulus guttatus* originating from native range and invaded ranges. Each of the seven native-range (North America) populations and the seven invaded range (four from New Zealand and three from Scotland) populations had six individual mother plants. For simplicity, we show one mother plant as an example in the diagram to illustrate the different crossing types that we conducted. In a first round of crossing, each mother plant was used to produce non-admixed plants from within population outcrossing (WPx), and admixed plants from between-population outcrossing, within the same range (WRx) or between ranges (BRx). In a second round of crossing, we used the progeny from the first round to generate the progeny for the present experiment. Non-admixed progeny genetically resembling the parental (P) generation were formed by crossing first round WPx plants from the same population (WPx, white) or by selfing first round WPx plants (WPs, grey). Admixed progeny genetically resembling admixed  $F_1$  were formed by crossing first round WPx plants from different populations within the same range (WR1x, light blue), or between native and invaded range (BR1x, light red). Admixed progeny genetically resembling admixed  $F_2$  were formed by crossing first round WRx plants sharing the same maternal population (WR2x, intermediate blue) or selfing (WR2s, dark blue), or crossing first round BRx plants sharing the same maternal population (BR2x, intermediate red) or selfing (BR2s, dark red) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

population of the same range as pollen donor (WR1x); (4) progeny that resemble  $F_1$  between-range admixed plants, using another WP plant from a different range as pollen donor (BR1x). With each WR plant from the first round of crossings, we generated two more types of progeny: (5) progeny that resemble an outcrossed  $F_2$  after within-range admixture, using another WR plant from the same maternal population as pollen donor (WR2x); (6) progeny that resemble a selfed  $F_2$  after within-range admixture, by manual self-fertilization (WR2s). Similarly, we generated two progeny types for each BR plant from the first round of crossings: (7) progeny that resemble an outcrossed  $F_2$  after between-range admixture, using another BR plant from the same maternal population as a pollen donor (BR2x); (8) progeny that resemble a selfed  $F_2$  after between-range admixture, by manual self-fertilization (BR2s). This crossing scheme (Figure 1) resulted in 401 seed families.

### 2.3 | Experimental design

On 4 February 2014, we sowed 20–30 seeds of each of the 401 seed families into square plastic pots (275 cm<sup>3</sup>, 7 × 7 × 6.5 cm, Pöppelmann, Germany) filled with potting soil (Topferde<sup>®</sup>, Einheitserde Co., Sinntal-Altengronau, Germany; pH 5.8; 2.0 g/L KCl; 340 mg/L N; 380 mg/L P<sub>2</sub>O<sub>5</sub>; 420 mg/L K<sub>2</sub>O; 200 mg/L S; 700 mg/L Mg). Pots were randomly assigned to positions on a bench in a greenhouse in the botanical garden of the University of Konstanz. The temperature ranged between 22°C and 28°C, and supplemental lighting was used to extend the photoperiod to 16 hr. Sixteen days after sowing (20 February 2014), we selected two similarly sized seedlings from each seed family. For three of the 401 seed families, only one seedling was available. We transplanted the seedlings separately into 1 L square pots (12 × 12 × 7 cm, Pöppelmann, Germany), filled with a 1:1 mixture of sand and fine vermiculite with 10 g slow-release fertilizer (Osmocote<sup>®</sup> Exact Standard; 16% N:19% P:12% K + 2% MgO + trace elements, Everris International B.V., Geldermalsen, The Netherlands) mixed into it. The resulting 799 pots were randomly assigned to positions on four greenhouse benches, and watered to saturation.

To test whether performance differences among the eight cross types are exacerbated by drought stress, we applied two watering treatments. One seedling per seed family (401 plants) served as control, receiving sufficient water to keep the substrate moist throughout the entire experiment. The remaining 398 plants were exposed to two periods of drought stress. Whether plants experience drought stress under reduced water supply depends largely on the size of the plants. Therefore, we used plant wilting as an indicator of drought stress. The first period of drought was initiated 18 days after the start of the experiment (i.e. on 10 March 2014), which resulted in the first wilting signs 7 days later. We daily checked all plants in the drought treatment for signs of wilting, and supplied 50 mL of water to each plant that had started to wilt (i.e. had lost leaf turgor) to reduce mortality. Seventeen days after the start of the drought period, when 95% of the plants in this treatment had wilted at least once, we supplied all plants with sufficient water every day, as in the control treatment, to allow the plants to recover (like during a rainy period in nature). A second drought

period was initiated 7 days after the first drought period had ended (i.e. on 4 April 2014), and resulted in the first wilting signs 3 days later. Because plants were now larger than in the first drought period, we supplied 100 instead of 50 ml of water to each plant with wilting signs. Twelve days after the start of the second drought period, when again 95% of the plants had wilted at least once, we supplied all plants with sufficient water every day, as in the control treatment, until the final harvest on 28 April 2014.

### 2.4 | Measurements

To be able to account for differences in initial plant size, we counted the number of leaves, and measured the length and width of the longest leaf 4 days after transplanting. On 28 April 2014, 83 days after the start of the experiment, when at least 30% of the plants had started to flower, we concluded the experiment. We recorded survival (yes/no) and flowering status (yes/no) of all plants. As a measure of vegetative performance, we harvested all above-ground plant parts. All plant material was dried at 70°C for at least 3 days, and then weighed with an accuracy of 0.01 g. In addition, we measured germination success, time to germination, time to flowering, total flower production, ovary size (as a proxy for ovule number) and pollen production, but as these traits are not of main interest, we report them only in the supplements.

### 2.5 | Statistical analyses

To analyse the effects of cross type and its interactions with mother range and watering treatment on plant performance traits, we used linear and generalized linear mixed effects models implemented in the *lme* function of the “nlme” package (Pinheiro, Bates, DebRoy, & Sarkar, 2013) and the *glmer* function of the “lme4” package (Bates, Machler, Bolker, & Walker, 2015) in R 3.0.2 (R Core Team, 2013). The model fixed part always included mother range (western North America, Scotland, New Zealand), cross type (WPx, WPs, WR1x, WR2x, WR2s, BR1x, BR2x, BR2s; see Figure 1) and their interactions with each other (see below for coding). As it was not always possible to select two similar-sized seedlings, for traits that were assessed after transplanting (all except germination), the models accounted for initial variation in plant size by including a covariate in the fixed part that represented initial plant size at transplanting (leaf number × length of longest leaf × width of the longest leaf). For those traits that were assessed after the drought treatment (above-ground biomass and flowering status), the fixed part further included watering treatment (control, drought) and its interaction with mother range and cross type. The model random part always included maternal population of origin and maternal line (i.e. the mother plant [M] in Figure 1, nested in maternal population). We did not include paternal line in the random part of the model, because some paternal plants were used in multiple crossings and others only in one, these data are too unbalanced to include in the models. For those traits that were assessed both in the control and drought treatment (above-ground biomass and flowering success), and for which we thus had two individuals per seed family, the random

part also included seed family (nested in mother plant) to account for non-independence of the two individuals. Survival and flowering success were analysed with a binomial error term. For above-ground biomass, we used a Gaussian error distribution. To improve normality and homogeneity of the residuals, we square-root transformed above-ground biomass prior to analysis.

Because we had one native range (western North America) and two invaded ranges (Scotland and New Zealand), we coded the three mother ranges as two dummy variables (MR1 and MR2, Table S1; see Schielzeth, 2010 for an explanation of dummy variables). A model including both dummies MR1 and MR2 is equivalent to a model with one-three-level fixed effect (North America, Scotland, New Zealand), but allows making specific contrasts. The dummy variable MR1 explains differences between the native range (North America) and the average of the two invaded ranges. The dummy variable MR2 explains differences between the two invaded ranges: Scotland vs. New Zealand. Thus, removing MR2 from a model including MR1 and MR2 tests whether there is a difference between Scotland and New Zealand, while accounting for any differences between the native and invaded range. Removing MR1 from a model including MR1 (and not including MR2) tests whether there is a difference between the native and invaded range.

Similarly, we coded the eight cross types as seven dummy variables (Table S2) to allow addressing a specific set of seven questions (Figure 1) through orthogonal contrasts (following Schielzeth, 2010). We tested the significance of each dummy term and its interaction terms by removing the term of interest from a specific reference model (Table S3). For example, to test whether there was a reduced benefit of within-range admixture that depended on whether the mother range was invasive or native ( $C4 \times MR1$  interaction), the reference model included the main effects mother range (MR1) and watering treatment (WT), the dummy terms C1, C3 and C4, and the two-way interactions  $MR1 \times WT$ ,  $C1 \times MR1$ ,  $C1 \times WT$ ,  $C3 \times MR1$ ,  $C3 \times WT$ ,  $C4 \times MR1$  and  $C4 \times WT$ . The reference model was then compared to a model from which the term of interest  $C4 \times MR1$  was removed, using a likelihood-ratio test.

Only six out of 401 plants died before the final harvest in the control treatment, but 166 out of 398 plants died in the drought treatment. Therefore, for survival, we only tested whether there were significant effects of mother range and cross type (dummy variables; Tables S1 and S2) in the drought treatment. None of the WPx and WPs progeny with the maternal origin in Scotland flowered before our harvest. Regardless of maternal origin, very few plants (46 out of 234 surviving plants) flowered in the drought treatment. Therefore, the analyses of the floral traits (except flowering status) only included plants with a maternal origin in western North America (native) and New Zealand (invasive), and only those grown in the control treatment.

## 3 | RESULTS

### 3.1 | Drought survival

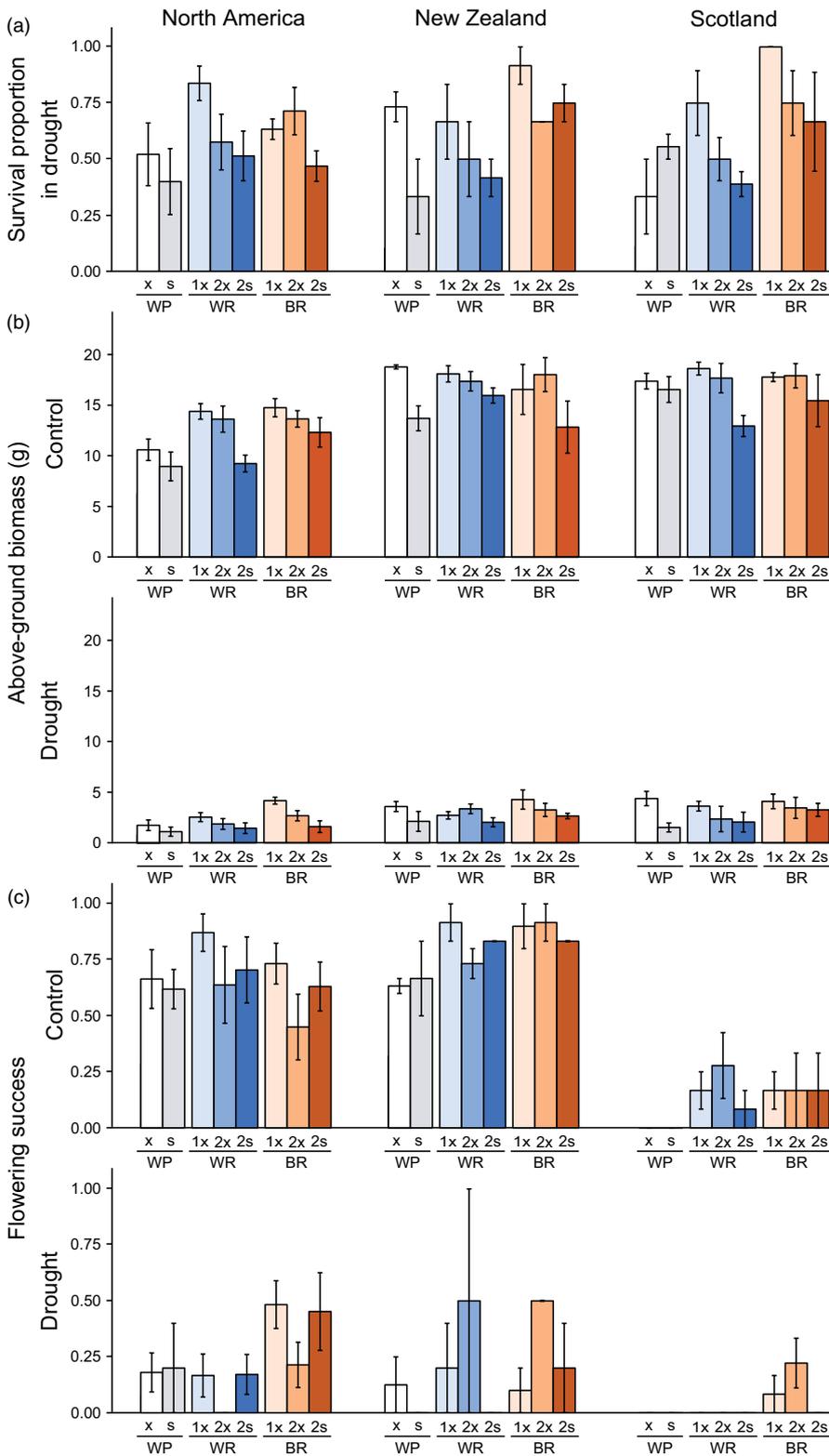
Because almost all plants (98.5%) survived in the well-watered control treatment, we restricted the survival analysis to the plants in the

drought treatment (58.3% survival). Admixture had a positive effect overall on drought survival (64.2% of admixed plants vs. 46.9% of non-admixed plants survived; significant effect of C1 in Table 1; Figure 2a). Between-range admixed plants had 25.6% higher survival than within-range admixed plants from the invaded ranges, but not so (-3.9%) in the native range (significant  $MR1 \times C3$  interaction in Table 1; Figure 2a). The benefit of within-range admixture was smaller in the  $F_2$  than in the  $F_1$  (50.6% in  $F_2$  vs. 78.8% in  $F_1$ ; significant effect of C4 in Table 1; Figure 2a). The benefit of between-range admixture was also reduced in the  $F_2$  of the invaded-range populations but not in the native range (significant  $MR1 \times C5$  interaction in Table 1; Figure 2a). It made, however, no difference whether the second generation was formed by outcrossing or selfing (no significant effects of C6 and C7 in Table 1).

### 3.2 | Above-ground biomass

Plants from the invaded range produced significantly more above-ground biomass, mainly consisting of stolons used for vegetative spread, than those from the native range (10.5 vs. 7.5 g; significant effect of MR1 in Table 1; Figure 2b). Drought resulted in a reduction in biomass relative to the control (14.3 vs. 2.6 g; significant effect of WT in Table 1; Figure 2b), and this effect was stronger for plants from the invaded (17.0 vs. 3.1 g) than from the native range (12.4 vs. 2.2 g; significant  $MR1 \times WT$  interaction in Table 1; Figure 2b).

Overall, admixture had a positive effect on biomass (8.9 g in admixed vs. 8.0 g in non-admixed progeny; significant effect of C1 in Table 1; Figure 2b). However, this effect depended on mother range as, relative to non-admixed plants, admixed plants from the native range produced 1.7 g (27.4%) more biomass, whereas admixed plants from the invaded range produced 0.2 g (1.9%) less biomass (significant  $MR1 \times C1$  interaction in Table 1; Figure 2b). There was significant inbreeding depression for the non-admixed progeny of the parental populations ( $\delta = 0.189$ , significant effect of C2 in Table 1; Figure 2b). Progeny of between-range admixture produced more biomass than progeny of within-range admixture in the drought treatment (3.2 g vs. 2.5 g), but this effect was weaker in the well-watered control treatment (15.2 g vs. 14.8 g; significant  $WT \times C3$  interaction in Table 1; Figure 2b). For native-range plants, between-range admixture was more beneficial than within-range admixture (1.0 g [13.4%] more above-ground biomass), whereas this difference was smaller for invaded-range plants (0.4 g [4.0%] more above-ground biomass; marginally significant  $MR1 \times C3$  interaction in Table 1; Figure 2b). The benefit of within- and between-range admixture was smaller in the  $F_2$  than in the  $F_1$  (7.9 g in  $F_2$  vs. 9.6 g in  $F_1$  for within-range admixture, 8.8 g in  $F_2$  vs. 10.2 g in  $F_1$  for between-range admixture; significant effects of C4 and C5 in Table 1; Figure 2b). This reduction could be partly attributed to inbreeding depression, because the  $F_2$  progeny produced by selfing had a significantly lower above-ground biomass than those produced by outcrossing (significant effects of C6 and C7 in Table 1; Figure 2b).



**FIGURE 2** The effect of admixture on drought survival, above-ground biomass production and flowering success of  $F_1$  and  $F_2$  *Mimulus guttatus* plants. Non-admixed plants resulted from within population (WP) outcrossing (X, white) or selfing (S, grey). Admixed  $F_1$  plants (1X, lightest tones) resulted from crosses between previously non-admixed plants from different populations either within the same range (WR, blue) or between the native and invaded range (BR, red). Admixed  $F_2$  plants resulted from crosses between previously admixed plants (2X, intermediate tones), or from selfing of previously admixed plants (2S, darkest tones). See Figure 1 for further details of the crossing design. Results are grouped by mother range (North America [native range], New Zealand and Scotland [invaded ranges]). Error bars are standard errors, which were calculated from the mean values of each population in a range [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### 3.3 | Flowering success

Flowering probability did on average not differ between plants from the native range and plants from the invaded ranges (no significant effect of MR1 in Table 1). However, plants from the invaded range Scotland were significantly less likely to flower than those from the

invaded range New Zealand (8.5% vs. 50.3%; significant effect of MR2 in Table S4; Figure 2c). Drought significantly reduced flowering probability from 63.5% to 19.7% (Table 1; Figure 2c), and this effect was stronger for the plants from the invaded range New Zealand (80.4% vs. 20.3%) than for those from the invaded range Scotland (12.8% vs. 4.0%; significant MR2  $\times$  WT interaction in Table S4; Figure 2c).

**TABLE 1** Linear mixed model analysis to test effects of admixture (and selfing) on drought survival, above-ground biomass production and flowering success of  $F_1$  and  $F_2$  progeny of *Mimulus guttatus*. For drought survival, the fixed part of the model included initial plant size as a covariate, mother range coded as dummy variables MR1 and MR2 (dummy variables were coded to allow testing difference between native and invaded range listed in Table S1), cross type coded as dummy variables (C1, C2, C3, C4, C5, C6, C7; dummy variables were coded to allow testing of the specific orthogonal contrasts listed in Figure 1 and all listed interactions). As the contrast between the native and invasive ranges (MR1) was of more interest than the contrast between the two invaded ranges (MR2), the results for the latter are only shown in Table S4. The random part always included maternal population of origin and maternal line. For the random terms, the table shows the estimated SD. For above-ground biomass and flowering success, the model was the same, but the fixed part additionally included watering treatment (WT) and all its interactions with other fixed effects, and the random part also included seed family. We tested the significance of the fixed effects (including the dummy variables) and all the listed interactions by removing the term of interest from a specific reference model (Table S3), and performing model comparison using likelihood-ratio tests. Significant effects ( $p < .05$ ) are in bold

Source	df	Drought survival (binomial)		Above-ground biomass (Gaussian, square root)		Flowering success (binomial)	
		$\chi^2$	P	$\chi^2$	p	$\chi^2$	p
Initial plant size	1	<b>31.12</b>	<b>&lt;.001</b>	0.61	.436	1.96	.161
Mother range (MR1)	1	1.00	.318	<b>7.92</b>	<b>.005</b>	3.00	.083
Watering treatment (WT)	1	-	-	<b>962.12</b>	<b>&lt;.001</b>	<b>139.83</b>	<b>&lt;.001</b>
C1 (WP vs. WR + BR)	1	<b>0.01</b>	<b>.012</b>	<b>20.99</b>	<b>&lt;.001</b>	<b>5.17</b>	<b>.023</b>
C2 (WPx vs. WPs)	1	1.98	.159	<b>9.35</b>	<b>.002</b>	0.53	.466
C3 (WR vs. BR)	1	0.10	.747	3.36	.067	1.43	.232
C4 (WR1 vs. WR2)	1	<b>7.98</b>	<b>.005</b>	<b>11.22</b>	<b>&lt;.001</b>	<b>4.52</b>	<b>.033</b>
C5 (BR1 vs. BR2)	1	3.54	.060	<b>8.95</b>	<b>.003</b>	0.72	.396
C6 (WR2x vs. WR2s)	1	1.15	.285	<b>11.90</b>	<b>&lt;.001</b>	0.41	.521
C7 (BR2x vs. BR2s)	1	<0.0001	.984	<b>7.34</b>	<b>.007</b>	0.25	.616
MR1 × WT	1	-	-	<b>12.06</b>	<b>&lt;.001</b>	1.28	.258
MR1 × C1	1	0.20	.655	<b>7.65</b>	<b>.006</b>	<b>4.97</b>	<b>.026</b>
MR1 × C2	1	0.42	.519	0.03	.854	0.14	.706
MR1 × C3	1	<b>8.51</b>	<b>.004</b>	3.74	.053	0.39	.530
MR1 × C4	1	0.26	.609	0.58	.448	0.61	.434
MR1 × C5	1	<b>4.17</b>	<b>.041</b>	3.12	.077	2.02	.155
MR1 × C6	1	0.10	.750	0.05	.818	1.85	.174
MR1 × C7	1	1.19	.275	0.18	.670	<b>5.13</b>	<b>.024</b>
WT × C1	1	-	-	0.03	.854	0.17	.679
WT × C2	1	-	-	0.05	.828	0.13	.724
WT × C3	1	-	-	<b>3.86</b>	<b>.049</b>	<b>12.20</b>	<b>&lt;.001</b>
WT × C4	1	-	-	2.88	.090	1.47	.225
WT × C5	1	-	-	2.84	.092	0.72	.398
WT × C6	1	-	-	1.76	.185	0.20	.656
WT × C7	1	-	-	1.24	.266	0.01	.941
MR1 × WT × C1	1	-	-	2.90	.088	0.04	.844
MR1 × WT × C2	1	-	-	0.06	.813	0.93	.335
MR1 × WT × C3	1	-	-	1.62	.203	2.68	.109
MR1 × WT × C4	1	-	-	0.35	.554	0.39	.531
MR1 × WT × C5	1	-	-	0.80	.371	0.83	.362
MR1 × WT × C6	1	-	-	0.60	.441	<b>6.08</b>	<b>.014</b>
MR1 × WT × C7	1	-	-	3.84	.050	2.03	.154
Random effects		SD		SD		SD	
Maternal population		0.3043		<0.0001		0.6718	
Maternal line		<0.0001		<0.0001		0.5663	
Seed family		-		0.1640		1.1245	
Residual		-		0.5050		-	

Admixture had a positive effect overall on flowering success (38.8% of admixed plants vs. 31.6% of non-admixed plants; significant effect of C1 in Table 1; Figure 2c), and this effect was stronger for plants from the invaded than for plants from the native range (28.8% vs. 15.0% for plants from the invaded range; 44.2% vs. 46.1% for plants from the native range; significant MR1  $\times$  C1 interaction in Table 1; Figure 2c). The benefit of within- and between-range admixture on flowering success was similar overall (no significant effect of C3, Table 1). However, it depended on the watering treatment as in the control treatment within-range admixture was slightly more beneficial than between-range admixture (61.2% vs. 53.6%), whereas in the drought treatment the reverse was true (10.8% vs. 28.8%; significant WT  $\times$  C3 interaction in Table 1; Figure 2c). The benefit of within-range admixture was significantly smaller in the F<sub>2</sub> than in the F<sub>1</sub> (33.7% in F<sub>2</sub> vs. 41.7% in F<sub>1</sub>; significant effect of C4 in Table 1; Figure 2c). Overall, it did not make a difference whether the second generation was produced through outcrossing or selfing (no significant effects of C6 and C7 in Table 1), but it depended on mother range and watering treatment (significant MR1  $\times$  WT  $\times$  C6 interaction in Table 1). In the drought treatment, within-range admixed selfed F<sub>2</sub> had a lower flowering success than within-range admixed outcrossed F<sub>2</sub> from the invaded range (0.0% vs. 20.0%; Figure 2c), whereas the reverse was true from the native range (17.1% vs. 0.0%; Figure 2c). In the control treatment, the pattern was the same but of smaller magnitude (38.3% vs. 46.0% for selfed and outcrossed F<sub>2</sub> progeny from the invaded range, and 70.4% vs. 63.8% from the native range; Figure 2c). Between-range admixed selfed F<sub>2</sub> progeny had a lower flowering success than outcrossed F<sub>2</sub> progeny when they came from the invaded range (25.7% vs. 40.0%), whereas the opposite was true when they came from the native range (54.1% vs. 33.2%; significant MR1  $\times$  C7 interaction in Table 1; Figure 2c).

### 3.4 | Additional traits

In the supplements, we report on the results for time to flowering and total flower production (Figure S1; Table S5), germination success and time to germination (Figure S2; Table S6), and ovary size and pollen production (Figure S3; Table S7).

## 4 | DISCUSSION

Our greenhouse study showed that admixture between plant populations of *M. guttatus* increased the probability of surviving drought, biomass production and flowering. Overall, the positive effects were stronger after admixture between the native and invasive range than after within-range admixture. Although we found evidence that the benefits of admixture decreased in the F<sub>2</sub>, this was mainly found for within-range admixture, and sometimes for F<sub>2</sub> produced by selfing. Moreover, it depended largely on the maternal range of the plants and the test environment. Our results suggest that admixture can boost invasions by increasing performance, and that this effect can, under certain conditions, be maintained in the F<sub>2</sub> generation.

### 4.1 | Admixture increases performance

Admixed progeny had higher drought-survival rates, produced more above-ground biomass and were more likely to flower than non-admixed progeny. While admixture increased drought survival both for the native and invasive populations, it increased biomass mainly for the native populations and flowering probability mainly for the invasive populations. This may reflect that the non-admixed invaded-range populations tended to produce more biomass, and the non-admixed native populations tended to have a higher flowering likelihood. Therefore, introgression of invaded-range genes into the native range increased biomass production, and introgression of native-range genes into the invaded range increased flowering likelihood. Although a previous study revealed a trade-off between sexual and clonal reproduction (van Kleunen, 2007), we here did not find that an increase in biomass production (mainly due to clonal growth) or flowering coincided with a decrease of the other trait. In other words, overall performance of *M. guttatus* increased after admixture.

Benefits of admixture are likely to increase with genetic dissimilarity between the populations until the genetic dissimilarity is so high that incompatibilities arise and outbreeding depression occurs (Lynch, 1991; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008). Many studies have revealed genetic differences between invasive and native populations (Bossdorf et al., 2005). However, an allozyme study that partly included the same populations as our study indicated that there is little genetic differentiation among the native- and invaded-range populations of *M. guttatus* (van Kleunen & Fischer, 2008). Nevertheless, in a previous study on the effects of admixture in *M. guttatus*, we found that the performance benefits of one generation of admixture were stronger after between-range outcrossing than after within-range outcrossing (van Kleunen et al., 2015). The current study shows that the increased benefit of between-range admixture also persists in the second generation.

Benefits of between-range admixture have not been found for several other invasive plant species. For example, within *C. solstitialis* and within *C. sulphurea*, between-range outcrossing resulted in a lower seed set than within-range outcrossing, which suggests that reproductive barriers may have developed between native and invasive populations. Such within-species reproductive barriers have also been reported in other studies (Hughes & Hollingsworth, 2008; Scopece, Lexer, Widmer, & Cozzolino, 2010; Twyford, Kidner, & Ennos, 2014). However, as we could easily produce seeds of the different experimental crosses in our study, we have no indications that reproductive barriers between the native and invaded ranges have developed yet in *M. guttatus*.

### 4.2 | Admixture benefits persist but were reduced in the second generation

Although the overall effects of admixture over two generations were positive, some of the benefits were smaller in the F<sub>2</sub> than in the F<sub>1</sub>, particularly after within-range admixture (compare WR1 vs. WR2 and BR1 vs. BR2 in Table 1). After within-range admixture, F<sub>2</sub> progeny had

lower drought survival and biomass production, were less likely to flower, and the ones that flowered produced fewer flowers (Table S5; Figure S1). After between-range admixture, similar patterns were found, but these depended partly on the watering treatment and the maternal range of the plants (interactions with WT and MR1 in Table 1). The reduced benefits of admixture in  $F_2$  progeny are likely to reflect outbreeding depression caused by the break-up of co-adapted gene complexes (Fox & Reed, 2011; Lynch, 1991). However, because part of the  $F_2$  progeny, in contrast to the  $F_1$  progeny, were created by self-fertilization, reduced  $F_2$  performance could also reflect inbreeding depression. For drought survival, both the outcrossed and selfed  $F_2$  progeny had reduced values compared to the  $F_1$  progeny (Figure 2a). This suggests that at least part of the reduced performance in  $F_2$  reflects outbreeding depression.

On the other hand, for biomass production, the outcrossed  $F_2$  progeny performed quite similar to the  $F_1$  progeny, whereas the selfed  $F_2$  progeny had reduced biomass (Figure 2b). This suggests that the benefit of admixture could be maintained in the  $F_2$  generation when introduced populations reproduce predominantly by outcrossing, but not when introduced populations reproduce predominantly by selfing. However, selfing may nevertheless be advantageous when introduced populations experience mate or pollinator limitation (Baker, 1955; Razanajatovo et al., 2016). When introduced species, on the other hand, have large founding populations or have undergone multiple introduction events, and attract pollinators, they might maintain high outcrossing rates (Shirk & Hamrick, 2014; Verhoeven et al., 2011). This could be the case for *M. guttatus*, as there was most likely no or only a minor role for founder effects in *M. guttatus* invasion (van Kleunen & Fischer, 2008), and *M. guttatus* has most likely undergone multiple introductions from closely related native populations (Puzey & Vallejo-Marín, 2014).

In addition to sexual reproduction, *M. guttatus* can also have vigorous clonal reproduction, particularly in habitats that remain permanently wet (van Kleunen, 2007). By analysing 62 SNP markers in 14 populations of *M. guttatus*, Pantoja, Simón-Porcar, Puzey, and Vallejo-Marín (2017) found that reproductive strategies of *M. guttatus* in its invaded range in the United Kingdom vary from mostly clonal to mostly sexual. Clonal reproduction might allow *M. guttatus* to rapidly colonize a new habitat, after long-distance dispersal of its small seeds (Truscott et al., 2006). In addition, clonal reproduction might allow the species to maintain favourable gene combinations. In other words, even if heterosis disappears due to outbreeding depression or inbreeding depression in subsequent sexual generations, it may be maintained in predominantly clonally reproducing populations. Therefore, admixture could contribute to invasiveness of *M. guttatus* for an extended period of time.

### 4.3 | Effects of the maternal range of origin and the test environment

A previous allozyme study revealed no evidence that the invaded-range populations used in this study had undergone a major bottleneck (van Kleunen & Fischer, 2008). This might partly reflect the

low resolution provided by allozymes. Indeed, a recent genome-sequencing study showed that genetic diversity in populations of *M. guttatus* across the United Kingdom was reduced by about 50% compared to native-range populations (Puzey & Vallejo-Marín, 2014). This indicates a mild bottleneck during introduction that in turn may have resulted in an increased genetic load. Furthermore, during subsequent expansion after introduction, low genetic variation in populations at the invasion front might have resulted in the rapid fixation and spread of mutations (i.e. gene surfing; Klopstein, Currat, & Excoffier, 2006). Since deleterious mutations are more frequent than beneficial ones, plants at the invasion front might carry a so-called “expansion load” (Peischl, Dupanloup, Kirkpatrick, & Excoffier, 2013; Peischl & Excoffier, 2015). The increase in genetic load (including expansion load) comes with a prediction of increased inbreeding depression, unless load has become fixed within populations or has been purged. The finding that inbreeding depression of the non-admixed plants (i.e. the fitness difference between WPx and WPs) did not differ between native-range and invaded-range populations, suggests that this is not the case for *M. guttatus*. If genetic load has become fixed within invaded-range populations, one would expect that one generation of admixture between populations within the same range followed by a second generation of selfing (WR2x vs. WR2s) should result in particularly severe inbreeding depression of the  $F_2$  progeny of invaded-range populations. With regard to flowering likelihood in the drought treatment, we found that this might indeed be the case. However, as there was no evidence for such an effect in any of the other performance traits, we conclude that the native- and invaded-range populations used in our study had overall a comparable genetic load.

While the positive admixture effect on biomass production was most pronounced in the native populations, the positive effect on flowering was most pronounced in the invasive populations. In addition, while admixture (regardless of whether within- or between-range) in native populations resulted in faster germination and faster flowering, this was not the case for invasive populations (Tables S5 and S6; Figures S1 and S2). One possible explanation could be that admixture between populations originating from different parts of the native range might already have occurred in the introduced range of *M. guttatus* (Puzey & Vallejo-Marín, 2014), so that further admixture had smaller effects there. Furthermore, as invasive populations overall produced more biomass and germinated earlier, introgression of alleles conferring increased biomass and earlier germination from the invasive populations may explain the benefits of admixture for the native populations, which were most pronounced for between-range admixture. This suggests that if plants from the invaded range make it back to the native range, introgression of their genes into the native gene pool could potentially increase weediness of the species in its native range. The extent to which introgression from the invasive back to the native range has happened in nature in *M. guttatus* or other invasive species requires further research.

While most *M. guttatus* plants in the well-watered control treatment survived, drought caused significant mortality. Drought also had strong negative effects on above-ground biomass production

and flowering success of the surviving plants. As it has been suggested that environmental stress might enhance inbreeding depression (Dudash, 1990; Fox & Reed, 2011; Yun & Agrawal, 2014), we also tested whether drought stress affects the benefits of admixture. While this was not the case for above-ground biomass, drought revealed a benefit of admixture in terms of survival, as almost all well-watered plants (394 out of 399) survived regardless of admixture status, whereas under drought stress admixed plants survived better than non-admixed plants. Additionally, between-range admixed plants were more likely to flower under drought than within-range admixed plants, but not so under control conditions. We therefore conclude that drought stress revealed some additional benefits of admixture, but only for survival and flowering probability.

## 5 | CONCLUSIONS

Even when the immediate fitness benefits of admixture are ephemeral, a temporary boost in individual plant performance in admixed progeny might have a catapult effect on population dynamics that could increase invasiveness (Drake, 2006). Admixture might also increase genetic variation, which in the long term might provide the population with a higher potential for adaptive evolution. However, the lack of a clear difference in the size of the error bars between admixed and non-admixed plants (Figure 2) suggests that an increase in variation due to admixture might have been limited. The frequent occurrence of local adaptation in invasive plants suggests that genetic variation is frequently not limiting evolution (Oduor, Leimu, & Kleunen, 2016). It would nevertheless be interesting to test in future studies how heritabilities of important traits of invasive plants change after admixture.

Our results show that overall performance of *M. guttatus* increased after admixture, particularly after between-range admixture. These benefits of admixture partly persisted in the second generation, but decreased when  $F_2$  progeny were produced by selfing. Overall, our results thus indicate that the increase in individual performance of admixed  $F_1$  progeny, as found previously (van Kleunen et al., 2015), may be maintained by further outcrossing, with only limited evidence for hybrid breakdown in later generations. Our results thus provide the first multigenerational empirical support for the catapult effect (Drake, 2006). The implications of our findings for the management of plant invasions are that gene flow between populations in the invaded range should be limited, and that introduction of new genotypes from other ranges should be prevented. As admixture between ranges has a higher risk of boosting invasion than admixture within ranges, legislation should not only prevent the introduction of new genotypes from the native to the invaded range but also introduction of invasive genotypes back to the native range.

## 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 and Carolin Bogs for help with the crossings, and Ayub Oduor, Stefanie Lemmermeyer, Yanhao Feng, Paula Rotter, Samuel Fernandes, Noëlie Maurel, Mialy Razanajatovo, Martin Mavrogenis, Timo Scheu, Katya Mamonova, Otmar Ficht, Claudia Martin and Yanjie Liu for help with the experiment. Y.L. was funded by a scholarship from the China Scholarship Council.

## AUTHORS' CONTRIBUTIONS

M.S. and M.v.K. designed the study; Y.L. did the experiment and collected data; Y.L. led the statistical analyses and writing; M.S. and M.v.K. advised on the statistical analyses and contributed to the writing.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.v88q2> (Li, Stift, & van Kleunen, 2017).

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## SUPPORTING INFORMATION

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