

# Patterns of selection of two North American native and nonnative populations of monkeyflower (Phrymaceae)

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## Summary

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**Key words:** floral evolution, invasive species, local adaptation, mating system, *Mimulus guttatus* (monkeyflower), plant reproduction, selection.

• To better understand invasion dynamics, it is essential to determine the influence of genetics and ecology in species persistence in both native and nonnative habitats. One approach is to assess patterns of selection on floral and growth traits of individuals in both habitats.

• *Mimulus guttatus* (Phrymaceae) has a mixed mating system and grows under variable water conditions across its native and nonnative range in North America. Field investigations of patterns of selection of floral and plant size traits were conducted in two native and two nonnative populations. Field-collected seed was grown and crossed in the glasshouse using a paternal half-sib design. The resulting offspring were grown in saturated and dry-down low-water conditions and the same traits were measured in both environments.

• Patterns of selection varied across years in the native range. Nonnative populations exhibited selection for increased floral size, consistent with the hypothesis that selection favors larger size in nonnative habitats. In the glasshouse, we detected genetic variation for traits across population/treatment combinations. However, size hierarchy in the glasshouse was dependent on water conditions.

• Our results suggest that both variable selection pressures and local adaptation probably influence the persistence of both native and nonnative populations.

## Introduction

The growing literature on invasive species has shown that native and nonnative habitats of the same plant species differ substantially in numerous aspects of the biotic and abiotic environment (for example, Williamson, 1996; Wolfe, 2002; Beckstead & Parker, 2003; Blair & Wolfe, 2004; DeWalt *et al.*, 2004; Bossdorf *et al.*, 2005; Murren *et al.*, 2005; Lavergne & Molofsky, 2007; Maron *et al.*, 2007; Alexander *et al.*, 2009). These ecological differences have been suggested as the underlying drivers of size differences observed between native and nonnative populations (Blossey & Notzold, 1995). In addition to differences in the ecology among populations, nonnative populations represent a different genetic composition from the native range, including impacts of genetic bottlenecks, drift, and genetic mixture caused by multiple introductions (for example, Bossdorf *et al.*, 2005; Lavergne & Molofsky, 2007; Schmid-Hempel *et al.*, 2007). Genetically based phenotypic differences among native and nonnative popu-

lations have frequently been suggested to reflect adaptive evolution (for example, Barrett *et al.*, 2008; Caño *et al.*, 2008). This interaction between environmentally and genetically based differences is critical to understanding the potential adaptive evolution of invasive plant populations.

For species that demonstrate metapopulation dynamics and have a geographic extent outside the native range, the examination of how plant size and floral traits vary among populations is of particular interest in the context of successful population establishment and persistence (Vickery, 1999). From a whole plant perspective, larger size is often cited as a component of invasion success (Bossdorf *et al.*, 2005; Colautti *et al.*, 2009). Larger plants are often dominant in competitive interactions and in nutrient acquisition. Nonnative populations have frequently, but not always, been shown to have larger individuals than the native range counterparts (for example, Blossey & Notzold, 1995; Willis *et al.*, 2000; Bossdorf *et al.*, 2005; Caño *et al.*, 2008; Colautti *et al.*, 2009). Blossey & Notzold (1995) have hypothesized that the size increase is a

result of changes in biotic interactions; escape from enemies results in the evolution of increased competitive ability as resources are used for growth instead of defense. It is unclear whether the increased size hypothesis translates to floral traits, or whether the relationship between increased size and invasion success is present in only vegetative traits. We describe the patterns of plant size and floral size in native and nonnative populations of *Mimulus guttatus*. We do so in a context of patterns of selection on these traits. Thus, we wanted to determine whether the relationship of increased fitness and greater overall plant size translates to increased fitness for plants with larger or smaller individual flowers in the nonnative range. Our study examines the relationship between the size and fitness of populations in native and nonnative environments.

Differential selection among populations may lead to adaptive divergence among populations, whether native or introduced (Endler, 1986; Kingsolver *et al.*, 2001). For floral traits, aspects of the biotic or abiotic environment may lead to evolutionary change in floral size. Floral size may be influenced by environmentally driven resource allocation changes (Alexander *et al.*, 2009). For example, when environmental conditions are such that mates or pollinators are scarce, selection for characters that enhance opportunities for selfing may contribute to population persistence. Mechanisms for such reproductive assurance can vary among populations (for example, Baker, 1955; Dole, 1992; Fenster & Ritland, 1994; Moeller & Geber, 2005; van Kleunen *et al.*, 2007; Lankinen *et al.*, 2007; Fishman & Willis, 2008; Truscott *et al.*, 2008). In addition, under stressful environmental conditions, selfing may minimize energy expenditure, as selfing taxa often have smaller flower size, shorter stigma–anther distance and reduced allocation to male function (for example, Jain, 1976; Murren *et al.*, 2006; Fishman & Willis, 2008).

Selection patterns on timing and floral size traits relate to pollinator attraction and successful pollen transfer to stigmas on the same or different individuals (for example, Arathi & Kelly, 2004; Fenster *et al.*, 2004). Traditional phenotypic selection studies in the field have provided a valuable snapshot of the patterns of selection within and across environments (for example, Nuñez-Farfan & Schlichting, 2005; Kingsolver & Pfennig, 2007). Recently, Hall & Willis (2006) found differential selection for mean flowering time between a native montane and a coastal population of *M. guttatus* in Oregon. Their results are in line with the long-term differential ecologies at the two sites and provide evidence of local adaptation. In two nonnative naturalized populations of *Arabidopsis thaliana* in Tennessee from contrasting shade habitats, Callahan & Pigliucci (2002) demonstrated directional selection gradients for earlier bolting time, although the date of bolting was earlier in the shade than in the more open field. In the glasshouse, the flowering time was not plastic and there appears to be a complex relationship with the number of rosette leaves at bolting. Together, these examples highlight that contrasting ecological conditions can lead to complex patterns of selection

on traits associated with flowering, whether within or outside the native range of the plant species.

To understand the variable selection pressures in native and nonnative environments, we have taken a combined field and glasshouse approach utilizing *M. guttatus* (see references below). First, in the field, we conducted phenotypic selection analyses on two native populations (previously examined by Fenster & Ritland, 1994) and compared them with two nonnative populations on the east coast of North America. These populations are in a component of the novel range of *Mimulus* that has not yet been examined [see, for example, van Kleunen & Fisher (2008) for a study of Scottish and New Zealand populations, and Truscott *et al.* (2008) for a study in Northern Europe]. We re-located two nonnative *M. guttatus* populations from herbarium records that had been established for more than 10 yr on the east coast of North America. We hypothesize that one of two broad patterns would emerge: (1) patterns of selection in nonnative populations would favor reduced floral traits, following arguments for characters that favor increased selfing and from data presented in Fenster & Ritland (1994), as previously observed in the native range; or (2) following the increased size hypothesis in nonnative populations (for example, Blossey & Notzold, 1995; Caño *et al.*, 2008), selection would favor the increased size of floral traits.

In a companion experiment, we grew plants from our field populations in a common glasshouse environment. We assessed genetic variation for floral and whole plant traits, and examined phenotypic differences between plants grown in two watering regimes that mimic variation in native and nonnative water availability. Common garden studies have been a useful tool in plant ecological genetics since Turesson (Gurevitch *et al.*, 2006), whether in a glasshouse (for example, Blair & Wolfe, 2004), garden plot (Murren *et al.*, 2005) or in the field (for example, Rutter & Rausher, 2004; Hall & Willis, 2006). Although the glasshouse lacks pollinators compared with the field, the aim with our glasshouse experiment was to determine whether genetic variation exists, which would allow selection to influence trait evolution. In addition, we assessed the influence of water on the rank order of whole plant and floral size in these four populations to further assess a potential ecological underpinning for any detected size variation. Unless common garden studies complement field *in situ* studies of native and nonnative populations, the relative influence of ecology and genetics cannot be disentangled. Understanding the influence of ecology and genetics is especially valuable when assessing the increased size hypothesis. Taken together, our studies explicitly combine field studies and glasshouse quantitative genetic studies to examine factors related to population persistence in native and novel habitats.

## Materials and Methods

*Mimulus guttatus* DC (Phrymaceae; Beardsley & Olmstead, 2002), also known as yellow monkeyflower, is a widespread

flowering plant (Vickery, 1974; www.invasivespecies.gov, www.plants.usda.gov) native to western North America and found in moist or seasonally drying open habitats, such as stream edges, seeps and spring ephemeral pools (Fenster & Ritland, 1994; Murren *et al.*, 2006). *Mimulus guttatus* is a hermaphroditic self-compatible species with a mixed mating system (for example, Dole, 1992; Carr & Dudash, 1997; Dudash & Carr, 1998), and selection has been shown previously to operate on mating system characters (for example, Fenster & Ritland, 1994; Willis, 1996). *Mimulus guttatus* is a model organism for mating system and floral evolution (for example, Fenster & Ritland, 1994; Carr & Dudash, 1997; Dudash & Carr, 1998; Ivey *et al.*, 2004; Holeski & Kelly, 2006; van Kleunen, 2007; Fishman & Willis, 2008; Kelly *et al.*, 2008; van Kleunen & Burczyk, 2008; Wu *et al.*, 2008).

For *M. guttatus*, a key abiotic environmental factor, both within and among populations, is water availability, which has been shown to vary among populations in both native and nonnative ranges throughout the season (for example, Hall & Willis, 2006; Murren *et al.*, 2006; van Kleunen & Fischer, 2008; C. J. Murren & M. R. Dudash unpublished; C. A. Wu, Duke University, Durham, NC, USA, pers. comm.). Inland populations of *M. guttatus* are most often annual, whereas, along the coastal areas of western North America, plants are frequently perennial (Fenster & Ritland, 1994; Hall & Willis, 2006). Populations of *M. guttatus* have also established outside the natural range in eastern North America, the UK, northern and central Europe, and New Zealand, and include a worldwide nonnative distribution including eastern North America along creeks or other continuously wet areas (van Kleunen & Fischer, 2008; Truscott *et al.*, 2008; C. C. Chang, C. J. Murren & M. R. Dudash, pers. obs.).

### Field data collection

**Native *M. guttatus* field locations** We visited two locations (Tullock and Guenoc, previously examined by Fenster & Ritland, 1994) at the peak of flowering in April 2002 and at fruit maturity in June 2002. These two sites vary in a number of ecological attributes. The Guenoc site is largely open (minimal canopy cover). In 2002, the site was a broad, long, wet seep that ranged from running water and moist soil in April to patchy areas of dry soil in June (unpublished data). The Tullock site has numerous trees in the area, and part of the *M. guttatus* population occurs under a partial tree canopy on a steep slope with a seasonal seep running through it. In April, Tullock had moist soil, yet all soil samples lacked water in June (unpublished data). Thus, the Tullock and Guenoc sites varied in light and water availability during 2002. Native bees were casually observed acting as pollinators at both locations. At each native site, we measured several floral morphological traits: total corolla length (mm), width of the corolla (mm) and stigma–anther separation (mm). We measured 150 individuals at Tullock (hereafter T) and 200 individuals at Guenoc

(hereafter G). Our flower size measurements followed the methods of Fenster & Ritland (1994) (C. B. Fenster, University of Maryland, College Park, MD, USA, pers. comm.). In June, we harvested all marked plants to assess fitness-related traits (total flower and fruit production = total reproductive output, as well as seed biomass in a subsample of plants). We collected seed by maternal plant for use in the glasshouse experiment.

**Nonnative *M. guttatus* field locations** In July 2002, we visited a nonnative *M. guttatus* site in Fly Creek, New York (hereafter NY), identified from herbarium records to have been present since at least 1991. The creek was continuously wet and had a partial canopy of shrubs and ferns. The population numbered in the thousands of plants over an area of several hundreds of meters. We measured floral traits on 200 plants, and obtained a fitness measure: an estimate of total reproductive output (total flower and fruit production) for the season.

We also visited a site just outside St. John, New Brunswick, Canada (hereafter NB), identified to have been present since at least 1967. The population was in a continuously wet creek. The population numbered a few hundred plants, largely in one patch. The only other vegetation at the site was native cattails. In July 2002, we measured 200 plants as described above.

Returning to these nonnative sites in August (NY) and September (NB), the soil was saturated with water in both NY and NB (data not shown). Seeds were collected by maternal plant at each site for use in the glasshouse experiments.

The reproductive output (total flower and fruit production per individual) was used as our measure of fitness because there was a high correlation ( $r = 0.91$ ;  $n = 162$ ) between total seed biomass and reproductive output for a sample of plants from all four study populations in the field. Using the reproductive output allows us to examine reproductive traits in a pollinator-free glasshouse (following, for example, Clay & Levin, 1989).

### Common garden glasshouse experiment

Seeds from a minimum of 40 families from all populations were sown into individual pots for each family in the glasshouse in May 2003 under natural sunlight conditions. Two weeks after sowing, randomly chosen individual seedlings were transplanted singly into separate pots. We employed a paternal half-sib crossing design (Lynch & Walsh, 1998), where one father was randomly crossed with four mothers within the same population using a total of 40 fathers per population. The paternal half-sib design is a powerful model for examining genetic variation, designed to control for maternal and micro-environmental effects (Lynch & Walsh, 1998). The flower and fruit production of the plants from the NB population in the summer conditions of the University of Maryland glasshouse did not meet our minimum number of paternal half-sib families for our design, and thus the NB population was omitted from the glasshouse experiment.

Thirty-six paternal half-sib seed families for T, 34 for G and 30 for NY were sown in the glasshouse in September 2003. Each paternal half-sib family was represented by three dams. Seeds were bulk germinated by paternal half-sib family under natural light for 2 wk. After 2 wk, seedlings were individually transplanted into 6.35 × 6.35 cm pots and supplemental light was provided using sodium vapor lights to extend the day length to 18 h, which has been shown to induce flowering (Carr & Dudash, 1997). Ten replicates of each family were planted per block, using a split-plot design (Sokal & Rohlf, 1995), resulting in an experiment of 3000 plants. All plants were grown in the same glasshouse soil mixture (Metro-Mix 510, Sunagro Horticulture, Bellevue, WA, USA).

After transplanting, we allowed the plants to equilibrate for 1 wk before the onset of watering treatments. The 10 replicates were split into five replicates per saturated (hereafter high) and dry-down (hereafter low) water treatments, across five blocks. For the high-water conditions, each plant received 66 ml of water every 3.5 d, such that plants had access to ample water because the soil was saturated. For the low-water conditions, each pot received 10 ml of water every 3.5 d. At transplant, the soil of plants in both treatments was moist; several weeks after the onset of the treatments, plants in the low-water treatment were dry, such that 10 ml sustained survival. Thus, the low-water treatment simulated dry-down conditions in the field under which, initially, all plants early in the season had access to equal amounts of water, without allowing plants to wilt, as *M. guttatus* does not tolerate wilting (Murren *et al.*, 2006).

In the glasshouse, we measured the corolla length, corolla width and stigma–anther separation, as in the field, to assess differences in trait means under common environmental treatments. We also evaluated the genetic variation of these traits within and among populations. Throughout the course of the experiment, we noted the date of the first flower, the node at which flowering was initiated and measured the first flower of each plant. Plant height (cm) and reproductive output (total flower and fruit production) were measured after harvest.

### Field data analysis

We compared phenotypes between coasts and among populations for the traits described above using PROC MIXED to assess a restricted maximum-likelihood analysis of variance (SAS version 9.13; SAS Institute Inc., 2003; Little *et al.*, 1996). The model response was phenotypic traits (described above) and the main effects were coast and population nested within coast. Both coast and population factors were considered to be fixed, given that we chose specific Californian populations that had been studied previously by Fenster & Ritland (1994).

Phenotypic selection analyses (following Lande & Arnold, 1983) for stigma–anther separation, corolla length and corolla width were completed separately for each population

using the General Linear Model (GLM) procedure (SAS version 9.13; SAS Institute Inc., 2003), with traits standardized to a mean of zero and a standard deviation of unity and relative fitness. We calculated relative fitness by dividing the reproductive output by the site mean, separately for population. Selection differentials ( $S$ ), or the strength of total directional selection, including both direct and indirect selection through correlations with other traits, were determined using a univariate regression model to estimate the covariance between a single standardized trait and relative fitness. We used multiple regression to obtain the partial regression coefficients for each of the measured and standardized traits on relative fitness (Lynch & Walsh, 1998). The partial regression coefficients, interpreted as selection gradients ( $\beta$ ), are estimates of direct selection controlling for all correlated selection acting through other measured traits. A negative  $\beta$  value is interpreted as a negative directional selection, whereas a positive  $\beta$  value is interpreted as a positive directional selection; in both cases, associations with all measured traits are considered in the model. We restricted our analyses to directional selection to maximize statistical power to assess multiple traits. Analysis of covariance was used to assess differences between populations in patterns of directional selection (for example, as in Donohue *et al.*, 2000).

### Glasshouse experiment analysis: analysis of genetic variation and phenotypic differences in different watering treatments

To test for differences in trait means among populations and between watering treatments, we utilized a mixed model in SAS with Tukey *post hoc* tests. To test for genetic variation, we used the MIXED procedure of the SAS statistical software package (version 9.13; SAS Institute Inc., 2003) to perform a mixed-model restricted maximum-likelihood analysis individually for each population, trait and treatment combination. All traits for the G population were logarithmically transformed, whereas the NY and T populations required no transformations to meet model assumptions. Block, pollen parent (sire) and seed parent (dam nested within sire) were considered as random effects. The significance level of each of the random effects was determined using the likelihood-ratio statistic, and evaluated via a chi-squared test. A significant sire effect for a trait indicates the presence of genetic variation for that trait, and maternal line nested within sire effects are suggestive of genetic variation (following Rutter & Rausher, 2004).

## Results

### Field results

In comparisons between coasts, significant differences in trait means were found for corolla length ( $F_{1,743} = 1914.92$ ;  $P < 0.0001$ ), corolla width ( $F_{1,741} = 191.6$ ;  $P < 0.0001$ ),

**Table 1** Field trait means in native and nonnative populations

Trait	Native populations		Nonnative populations	
	G <i>n</i> = 200	T <i>n</i> = 150	NY <i>n</i> = 200	NB <i>n</i> = 200
Corolla length (mm)	13.1 <sup>a</sup> (0.2)	15.8 <sup>b</sup> (0.2)	19.6 <sup>b</sup> (0.2)	25.3 <sup>c</sup> (0.1)
Corolla width (mm)	15.9 <sup>a</sup> (0.3)	22.4 <sup>b</sup> (0.4)	22.5 <sup>b</sup> (0.2)	23.0 <sup>c</sup> (0.1)
Stigma–anther separation (mm)	1.5 <sup>a</sup> (0.07)	1.3 <sup>a</sup> (0.06)	1.5 <sup>a</sup> (0.05)	1.9 <sup>b</sup> (0.04)
Plant height (cm)	13.52 <sup>a</sup> (0.7)	19.6 <sup>b</sup> (0.7)	n/a	34.4 <sup>c</sup> (0.34)
Total number of reproductive units	8.9 <sup>a</sup> (1.1)	4.32 <sup>b</sup> (0.2)	22.2 <sup>c</sup> (1.3)	14.9 <sup>d</sup> (0.5)

Trait means (1 standard error) at two native and two nonnative geographic locations in the field in 2002. Significant differences among the populations in trait means in 2002, based on a Tukey *post hoc* test, are indicated by different lowercase superscript letters.

**Table 2** Field patterns of selection

Trait	Field populations							
	Native field sites				Nonnative field sites			
	G		T		NY		NB	
	S	β	S	β	S	β	S	β
Corolla length	<b>0.37</b> (0.13) <i>P</i> = 0.004	−0.19 (0.11) <i>P</i> = 0.08	<b>0.23</b> (0.05) <i>P</i> < 0.0001	0.05 (0.07) NS	0.005 (0.06) NS	−0.08 (0.06) NS	<b>0.18</b> (0.03) <i>P</i> < 0.0001	−0.0006 (0.04) NS
Corolla width	<b>0.51</b> (0.12) <i>P</i> < 0.0001	0.08 (0.11) NS	<b>0.28</b> (0.05) <i>P</i> < 0.0001	0.08 (0.07) NS	<b>0.18</b> (0.06) <i>P</i> = 0.002	<b>0.19</b> (0.07) <i>P</i> = 0.004	<b>0.23</b> (0.03) <i>P</i> < 0.0001	<b>0.12</b> (0.04) <i>P</i> = 0.002
Stigma–anther separation	<b>0.47</b> (0.12) <i>P</i> = 0.0002	−0.10 (0.10) NS	<b>0.15</b> (0.06) <i>P</i> = 0.008	<b>0.10</b> (0.05) <i>P</i> = 0.05	0.11 (0.06) <i>P</i> = 0.07	0.04 (0.06) NS	0.02 (0.03) NS	0.02 (0.02) NS
Plant height	<b>1.34</b> (0.09) <i>P</i> < 0.0001	<b>1.42</b> (0.10) <i>P</i> < 0.0001	<b>0.40</b> (0.05) <i>P</i> < 0.0001	<b>0.34</b> (0.05) <i>P</i> < 0.0001	n/a	n/a	<b>0.12</b> (0.03) <i>P</i> < 0.0001	<b>0.27</b> (0.03) <i>P</i> < 0.0001

Standardized selection differentials (*S*), estimating total direct selection, and standardized selection gradients (β), estimating strength of direct selection where correlated traits are accounted for, are reported separately for each of the field populations. Standard errors of the parameter estimates are given in parentheses. Parameter estimates where *P* < 0.05 are given in bold. NS is indicated for *P* > 0.09.

stigma–anther separation ( $F_{1,743} = 23.49$ ;  $P < 0.0001$ ), plant height ( $F_{1,436} = 75.15$ ;  $P < 0.0001$ ) and total reproductive output ( $F_{1,747} = 476.26$ ;  $P < 0.0001$ ; Table 1). Plants were generally larger for all traits measured for the nonnative NY and NB populations than for the native G and T populations (Table 1). Comparison among populations nested within coasts revealed significant differences for height ( $F_{1,436} = 9.17$ ;  $P < 0.0001$ ), stigma–anther separation ( $F_{2,743} = 14.55$ ;  $P < 0.0001$ ), corolla width ( $F_{2,741} = 150.3$ ;  $P < 0.0001$ ), corolla length ( $F_{2,743} = 313.01$ ;  $P < 0.0001$ ; Table 1) and total reproductive output ( $F_{2,747} = 17.01$ ;  $P < 0.0001$ ; Table 1).

### Field phenotypic selection results

Selection differentials reflecting total directional selection were positive and significant for all traits for the G and T populations in the native habitat. We also detected positive and significant selection differentials for all traits, except stigma–anther separation, for NB. However, only corolla width was positive and significant for NY (Table 2). Selection gradients measuring direct selection, which account for correlations among measured traits, were positive and significant for corolla width in the nonnative sites (NB and NY), but no

**Table 3** Glasshouse genetic variation analyses

Trait	Population	Water treatment	Block	Sire	Dam (sire)
Corolla length	G	High	<b>0.002</b>	<b>0.03</b>	< <b>0.0001</b>
		Low	0.50	<i>0.08</i>	<b>0.015</b>
	T	High	0.13	<b>0.004</b>	<b>0.001</b>
		Low	~	~	~
	NY	High	0.19	0.22	0.29
		Low	<b>0.002</b>	<b>0.057</b>	0.24
Corolla width	G	High	0.16	0.24	< <b>0.0001</b>
		Low	0.50	<b>0.05</b>	<b>0.03</b>
	T	High	0.50	<b>0.02</b>	<b>0.01</b>
		Low	<b>0.003</b>	~	<b>0.002</b>
	NY	High	0.50	0.16	<i>0.10</i>
		Low	0.13	0.29	<b>0.04</b>
Stigma–anther separation	G	High	0.19	<b>0.0007</b>	< <b>0.0001</b>
		Low	0.50	0.50	< <b>0.0001</b>
	T	High	0.50	0.29	<b>0.0002</b>
		Low	0.12	0.26	<b>0.0002</b>
	NY	High	< <b>0.0001</b>	0.14	0.38
		Low	0.38	0.5	< <b>0.0001</b>
Days to first flower	G	High	< <b>0.0001</b>	<b>0.01</b>	< <b>0.0001</b>
		Low	< <b>0.0001</b>	0.20	< <b>0.0001</b>
	T	High	< <b>0.0001</b>	<b>0.01</b>	<b>0.005</b>
		Low	< <b>0.0001</b>	0.38	< <b>0.0001</b>
	NY	High	< <b>0.0001</b>	0.38	<b>0.06</b>
		Low	< <b>0.0001</b>	0.22	<b>0.0002</b>
Reproductive output	G	High	~	~	~
		Low	0.38	<b>0.02</b>	< <b>0.0001</b>
	T	High	< <b>0.0001</b>	<b>0.021</b>	<b>0.0005</b>
		Low	0.5	<b>0.05</b>	<b>0.02</b>
	NY	High	< <b>0.0001</b>	<b>0.01</b>	0.2
		Low	0.11	0.5	< <b>0.0001</b>

Restricted maximum-likelihood analyses examining genetic variation and micro-environmental variation of measured traits for each population and water treatment. Values presented are *P* values: bold type indicates  $P < 0.05$ ; italic type indicates  $P < 0.10$ ; ~ indicates that no variation was detected.

selection was detected for this trait in the native populations (G and T). We found significant positive selection gradients for plant height in both native and nonnative habitats. We found a marginally significant negative selection gradient for corolla length in the G population. In the native T population, we found a significant positive selection gradient for the stigma–anther separation (Table 2).

Comparing patterns of selection using analysis of covariance (ANCOVA) between native and nonnative populations, we found differences between G and NB for stigma–anther separation ( $F = 2.07$ ;  $P < 0.0005$ ), corolla width ( $F = 5.15$ ;  $P = 0.02$ ) and height ( $F = 133$ ;  $P < 0.0001$ ). We found differences in patterns of selection between G and NY for stigma–anther separation ( $F = 7.01$ ;  $P = 0.0084$ ), corolla length ( $F = 6.93$ ;  $P = 0.0088$ ) and corolla width ( $F = 5.98$ ;  $P = 0.01$ ). Finally, differences in selection were detected between T and NY for corolla length ( $F = 7.51$ ;  $P = 0.0065$ ), and between T and NB for stigma–anther separation ( $F = 4.46$ ;  $P = 0.0355$ ).

We also detected some differences in patterns of selection between pairs of populations within each coast. Between native

populations G and T, we found differences in the patterns of selection for stigma–anther separation ( $F = 4.47$ ;  $P < 0.035$ ) and height ( $F = 75.38$ ;  $P < 0.0001$ ).

### Glasshouse results

**Genetic variation and between-treatment comparisons** We detected significant genetic variation (differences among sire means and among means of dams nested within sires) for most trait and treatment combinations (Table 3). Significant block effects were detected for some treatment and population combinations (Table 3).

We found significant differences among population and watering treatments for plant size (Table 4). Treatments and populations differed significantly for all traits measured (corolla length: treatment  $F = 87.1$ ,  $P = 0.0007$ , population  $F = 518.34$ ,  $P < 0.0001$ ; corolla width: treatment  $F = 177.1$ ,  $P < 0.0002$ , population  $F = 518.34$ ,  $P < 0.0007$ ; stigma–anther separation: treatment  $F = 45.64$ ,  $P = 0.002$ , population  $F = 3.23$ ,  $P = 0.04$ ; height: treatment  $F = 86.07$ ,  $P = 0.0008$ , population  $F = 402.3$ ,

**Table 4** Glasshouse trait means

Trait	Native populations				Nonnative population	
	G		T		NY	
	Treatment		Treatment		Treatment	
	Low	High	Low	High	Low	High
Corolla length (mm)	17.7 <sup>a</sup> (1.9)	17.5 <sup>a</sup> (2.2)	20.2 <sup>b</sup> (2.5)	22.7 <sup>c</sup> (3.1)	18.5 <sup>d</sup> (2.5)	19.6 <sup>e</sup> (2.3)
Corolla width (mm)	16.9 <sup>a</sup> (2.6)	16.9 <sup>a</sup> (2.8)	19.9 <sup>b</sup> (3.5)	23.8 <sup>c</sup> (4.5)	18.9 <sup>d</sup> (3.5)	20.4 <sup>b</sup> (3.4)
Stigma–anther separation (mm)	2.3 <sup>a</sup> (0.8)	2.6 <sup>b</sup> (0.9)	2.1 <sup>a</sup> (0.8)	2.5 <sup>b</sup> (0.9)	2.5 <sup>b</sup> (0.8)	2.2 <sup>a</sup> (0.8)
Time to first flower (d)	32.7 <sup>a</sup> (5.1)	37.4 <sup>b</sup> (8.2)	42.0 <sup>c</sup> (10.2)	45.9 <sup>d</sup> (10.0)	45.0 <sup>d</sup> (7.8)	49.9 <sup>e</sup> (8.7)
Plant height (cm)	16.3 <sup>c</sup> (5.1)	29.7 <sup>d</sup> (9.4)	10.5 <sup>b</sup> (6.2)	25.2 <sup>e</sup> (12.6)	6.9 <sup>a</sup> (4.0)	11.4 <sup>b</sup> (7.7)
Total number of reproductive units	10.1 <sup>c</sup> (4.4)	21.0 <sup>d</sup> (12.8)	5.3 <sup>b</sup> (3.5)	11.3 <sup>c</sup> (8.9)	3.9 <sup>a</sup> (2.2)	5.0 <sup>a,b</sup> (3.8)

Trait means (1 standard error) in the glasshouse common garden, in both the high and low treatments, of two native and two nonnative populations are presented for each trait. Significant differences among the populations in trait means, based on a mixed model followed by Tukey *post hoc* test, are indicated by different superscript letters.

$P < 0.0001$ ; total reproductive output: treatment  $F = 32.5$ ,  $P = 0.004$ , population  $F = 568.5$ ,  $P < 0.0001$ ). Within populations, plants grown in the high-water treatment were generally larger in corolla dimensions, had a greater height and a greater number of reproductive units than in the low-water treatment (Table 4). However, for the NY population, the stigma–anther separation was larger in the low- than high-water treatment. In the G population, corolla length and width were not found to be different between water treatments. Flowers in the native G population were the smallest in flower size overall of the three populations. In addition, the native T population had a larger corolla length and corolla width than the NY population when comparing within low- and high-water treatments. The NY population had a greater stigma–anther separation than the T population in the low-water treatment. Yet, in the high-water treatment in the T population, the stigma–anther separation was greater than in the NY population (Table 4). For corolla length, stigma–anther separation and reproductive output, T plants in low-water conditions were generally similar in size to NY plants in high-water conditions. Although one native population (G) was the smallest for floral morphological traits, whether a particular trait of the T or NY population was smaller was dependent on which water treatments were compared.

## Discussion

Our broad aims in this set of field and glasshouse studies were to examine the patterns of selection on floral traits in native

and nonnative field populations, and to investigate the genetic variation and differences in performance of these populations in the glasshouse under manipulated water environments that mimic conditions in the field. Patterns of selection in the field for this pair of nonnative east coast *Mimulus* populations favored increased floral size, and differed in the size and patterns of selection from the native populations studied. Our findings both in the field and in the glasshouse are consistent with the observation that, in nonnative habitats, plants are often larger than in their native populations (Blossey & Notzold, 1995), and our findings are in line with many of the studies reviewed by Bosdorf *et al.* (2005) and Colautti *et al.* (2009). Here, we expand the scope of this previous work to floral traits, and demonstrate that the increased size hypothesis extends to floral traits and may be an important component of success in nonnative populations. Although these broad patterns of performance exist, the local abiotic environment influences overall floral size, as revealed in the glasshouse, and affects the size rank order of populations. We found little evidence for selection favoring traits often considered to be associated with selfing (as in Fenster & Ritland, 1994) in either the native or nonnative range. In the glasshouse, genetic variation was detected for all traits, including the floral traits observed in our populations. We demonstrate that, in nonnative as well as native populations, evolutionary change via selection is possible given the detectable quantitative genetic variation in these populations. These findings demonstrate important ecological genetic parameters for population persistence, particularly in novel environments.

## Patterns of selection in two populations from native California and in two east coast North American nonnative environments

In this study, we were interested in investigating selection as a possible mechanism behind the morphological differences observed in the field in the native and nonnative populations of *M. guttatus* and other species. In the field, we observed that selection consistently favored a larger size of floral and vegetative traits in both native and nonnative populations. Only a few recent studies have examined patterns of selection of combinations of native and nonnative accessions in common gardens exclusively in the nonnative range (for example, Callahan & Pigliucci, 2002; Maron *et al.*, 2007; Wolfe *et al.*, 2007; Caño *et al.*, 2008; Franks *et al.*, 2008). These studies provide growing evidence that selection contributes to phenotypic evolution in invasive and nonnative taxa. In our field investigations in natural or naturalized populations, we detected selection on floral traits, yet the particular traits of the flower on which selection is operating are distinct among our four populations, suggesting divergent evolutionary trajectories among populations in both native and nonnative habitats. These divergent patterns could be a result of multivariate changes between native and nonnative environments that include pollinators, herbivores, water availability and soil quality (for example, Williamson, 1996; DeWalt *et al.*, 2004; Bossdorf *et al.*, 2005; Murren *et al.*, 2005; Maron *et al.*, 2007; Mitchell & Ashman, 2008; Alexander *et al.*, 2009).

### Comparison of trait means and patterns of selection across time

The patterns of selection observed in the field were not consistent with the patterns observed previously in the same two native populations (Fenster & Ritland, 1994), where negative directional selection gradients were detected in the Guenoc, CA population for corolla length and in the Tullock, CA population for stigma–anther separation. These historic patterns were consistent with the hypothesis of selection under low pollinator availability, favoring selfing. By contrast, we found a marginally significant negative selection gradient for corolla length in the G population, and selection for positive stigma–anther separation in the T population (Table 2). The patterns of selection found in these two Californian populations in 2002 are similar to the patterns of selection on floral size that have been reported from the field in Oregon and British Columbia (van Kleunen & Ritland, 2004; Hall & Willis, 2006; Fishman & Willis, 2008). However, in the observations of the trait means in 2002, we found trait means in the field that would be consistent with a response to selection patterns described in 1988 (Fenster & Ritland, 1994).

Context-dependent selection has long been recognized as important (for example, Stanton *et al.*, 2000; Moeller & Geber, 2005). Both temporal and spatial (both within and

among sites) variation in selection on timing of germination has been described previously for the annual *Collinsia verna* (Kalisz, 1986). Therefore, taken together, these studies and our demonstration of variable spatial and temporal patterns of selection on floral form in two native populations provide further evidence of the complexities of selection in natural environments. These observations also have implications for populations in nonnative environments. We consider that, as the invasion process transitions between the lag and logarithmic phases, patterns of selection may vary. Patterns of selection in the lag phase may favor resource allocation to seed production, and thus traits associated with selfing. Yet, once populations are established, selection may favor larger floral displays that attract particular pollinators in the nonnative range. Offspring derived from outcrossing events may lead to greater success during the logarithmic phase of invasion dynamics. Our results are consistent with this lag/logarithmic pattern of selection, suggesting that we may have measured selection during an expansion phase of native metapopulation dynamics. Thus, temporal patterns of selection are an important consideration for further study and may likewise inform the application of management regimes.

Our field studies, as well as those of Fenster & Ritland (1994), are phenotypic selection analyses (*sensu* Lande & Arnold, 1983). Thus, there could always be some countervailing selection on unmeasured correlated traits if differences in expression were environmental (see Rausher, 1992; Stinchcombe *et al.*, 2002), as suggested by differences in patterns between selection differentials and selection gradients. If selection differentials are highly significant, but selection gradients are not detected, this pattern may suggest environmentally influenced covariance between the trait and fitness. Unmeasured traits might be correlated with the traits for which significant selection differentials are found. These unmeasured correlated traits may also be environmentally sensitive. In addition, measures of fitness may differ in their environmental sensitivity (van Kleunen & Burczyk, 2008). To begin to elucidate the component of trait variation that may be caused by environmental variation, we assessed performance in a glasshouse setting (see also Fuller *et al.*, 2005).

### Genetic variation in native and nonnative populations

For the patterns of selection observed to result in an evolutionary response, there must be underlying genetic variation. In a glasshouse common garden, we detected a significant amount of quantitative genetic variation for all floral and vegetative traits measured, populations and water treatment combinations. Quantitative genetic variation for floral size in *Mimulus* has also been reported elsewhere (for example, Hall & Willis, 2006; van Kleunen, 2007; Kelly *et al.*, 2008). Notably, we detected genetic variation present in the east coast nonnative populations, allowing the potential for selection on floral size in a long-established nonnative population. During the

bottleneck associated with the establishment of new populations, quantitative and neutral genetic variation may be very low (for example, Briggs & Goldman, 2004; Dudash *et al.*, 2005) and, if selfing occurs, loss of lines may reduce the initial genetic variation further. With repeated introductions or local population establishment, hybridization and metapopulation dynamics (gene flow from seed and pollen), new genetic variation can be introduced into populations, whether in the native or nonnative range (for example, Durka *et al.*, 2005). Time since invasion may be an important attribute to consider in both selection and genetic variation. Our nonnative NY population may have already utilized reproductive assurance and experienced subsequent inbreeding depression (for example, Dudash & Carr, 1998; Dudash & Murren, 2008), such that we have captured its floral biology and mating system trajectory at a new optimum.

### Variation in trait expression between water environments

In *Mimulus*, we hypothesized that variation among populations in water availability may be an important component in the patterns of phenotypic variation observed and responsible for the performance rank differences between native and nonnative environments (see also Galloway, 1995; Caruso, 2006; Murren *et al.*, 2006). We observed that trait responses to water availability varied within and among populations (Murren *et al.*, 2006; C. J. Murren & M. R. Dudash, unpublished), which was also noted by others (for example, C. A. Wu, pers. comm.). In the field, we found that, for nearly all floral and vegetative traits measured, the nonnative plants were larger than the plants from the native Californian populations. In the glasshouse, the broad pattern was upheld with two caveats: (1) overall plant size and floral traits were generally larger in the glasshouse than in the field; and (2) the water treatments influenced the rank order among populations. G (a native population) remained consistently smaller in flower size, yet T (native) and NY (nonnative) populations varied in terms of whether there were floral trait differences based primarily on water availability. Our conclusions indicate that the similar phenotypes observed in the field appear to be dependent on different underlying conditions that include water availability.

Understanding how selection operates on a wide range of phenotypes through time warrants further attention, as we grapple with the effects of global warming, increased species introduction and human alteration of the natural landscape. Such data will ultimately help us to outline effective management strategies in native and invaded habitats to preserve biodiversity and functional ecosystems.

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