RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

INVITED PAPER

For the Special Issue: Evolutionary Insights from Studies of Geographic Variation

Geographic variation in climate as a proxy for climate change: Forecasting evolutionary trajectories from species differentiation and genetic correlations[1]

Heather E. Schneider[2] and Susan J. Mazer

PREMISE OF THE STUDY: Climate change models for California predict a warmer, drier future, potentially resulting in shorter growing seasons. If phenotypic differences between closely related species currently distributed across a moisture and temperature gradient represent adaptations to their abiotic environment, then as conditions become warmer and drier, populations presently adapted to cooler and wetter conditions may evolve to become more similar to those adapted to warmer and drier conditions. Two sister species, *Clarkia unguiculata* and *C. exilis*, are distributed across a moisture and temperature gradient in the southern Sierra Nevada, providing an opportunity to predict how this process may occur.

METHODS: In a greenhouse experiment using wild-collected seeds from 11 populations in the southern Sierra Nevada, we examined relationships among elevation, climatic conditions, and population means for each trait, then evaluated bivariate relationships among maternal family means, using raw values and controlling for population and seed mass effects on phenotype.

KEY RESULTS: *Clarkia exilis* occupied warmer, drier conditions, typically at lower elevations, than *C. unguiculata* did and flowered earlier and faster, producing smaller flowers with lower herkogamy. In *C. unguiculata*, petal area, herkogamy, and the rate of flower production were positively correlated with days to first flower.

CONCLUSIONS: If selection favors earlier flowering, smaller petals, or faster flower production in *C. unguiculata*, then the genetic correlations among these traits should reinforce their joint evolution. Moreover, the correlations between these traits and herkogamy may promote the evolution of self-fertilization as an indirect response to selection, a previously unrecognized potential outcome of climate change.

KEY WORDS *Clarkia*; climate change; correlated evolution; geographic variation; life history; Onagraceae; self-fertilization

Climate change is altering natural systems across the globe (Parmesan and Yohe, 2003; Pereira et al., 2010; IPCC, 2014). Understanding the responses of plant populations to climate change is imperative for predicting its long-term effects on wild species. In a changing climate, plant populations may respond in several ways, including migration or dispersal to more suitable habitats; alteration of life history and other traits through phenotypic plasticity; adaptive evolution; and local extirpation or large-scale extinction. Many studies have focused on the effects of climate change on migration and/or dispersal (Huntley, 1991; Davis and Shaw, 2001; Ackerly, 2003; Parmesan and Yohe, 2003; Neilson et al., 2005; Grace et al., 2002; Chen et al., 2011; Zhu et al., 2014), phenology (Parmesan and Yohe, 2003; Parmesan, 2006; Springer and Ward, 2007; Anderson et al., 2012; Mazer et al., 2013; Wolkovich et al., 2013; CaraDonna et al., 2014) and extinction (Midgley et al., 2002; Fordham et al., 2012; Sax et al., 2013). Migration and plastic responses to local changes in climatic conditions may allow many genotypes and populations to persist, but for those that can neither disperse to more hospitable locations nor exhibit plastic responses that allow local persistence, evolutionary adaptation may be required.

The potential for plants to adapt (through natural selection) to climate change has received less attention than dispersal and plasticity (but see Etterson and Shaw, 2001; Davis et al., 2005; Jump and Pañuelas, 2005; Franks and Weis, 2007; Aitken et al., 2008; Franks and Weis, 2008; Salamin et al., 2010; Hoffmann and Sgrò, 2011), but will play a crucial role in determining future community composition, species distributions, and the mean phenotypes of populations for ecologically important traits. While individual traits can evolve by natural selection in

[1] Manuscript received 19 March 2015; revision accepted 6 October 2015.

Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, Santa Barbara, California 93106 USA

[2] Author for correspondence (e-mail: heather.schneider@lifesci.ucsb.edu)

doi:10.3732/ajb.1500108

response to short-term changes in climate (Franks and Weis, 2007, 2008), few studies in plants have addressed how genetic correlations between traits might affect their joint evolution in response to future conditions (Etterson and Shaw, 2001; Etterson, 2004; Savolainen et al., 2004; Burgess et al., 2007; Colautti, et al., 2010; Shaw and Etterson, 2012).

Most models predict that the future climate in California will be warmer and precipitation less predictable. Annual temperatures are expected to increase by 1.5–5.8°C by the end of the 21st century (with more warming in summer than winter), annual precipitation will decrease by 10–20%, and montane snowpack will also decrease (Cayan et al., 2008). These projections mean that California will regularly experience temperatures outside of the current normal range, and annual precipitation levels could become comparable to the lowest third of historical annual totals (Cayan et al., 2008). These changes will increase spring and summer heat and drought stress for local flora and fauna, especially in regions and during seasons that are already warm and relatively dry. Under this increased stress, we expect natural selection to favor traits that promote drought escape or drought avoidance.

To investigate the potential evolutionary consequences of warmer temperatures and earlier onset of late-season drought on late spring-blooming annuals in California, we sampled populations of two sister species in the genus *Clarkia* (Onagraceae): the primarily outcrossing *Clarkia unguiculata* (Lindl.) (hereafter, *unguiculata*) and the facultatively autogamous *C. exilis* (F.H.Lewis & Vasek) (hereafter, *exilis*). *Exilis* typically grows at lower elevations and, in the field, both initiates and completes its flowering before *unguiculata* begins to flower. For both species, their late-season phenology makes them more vulnerable than earlier-flowering species to the end-of-season drought that characterizes many California ecosystems and, potentially, more sensitive to future climate change.

We examined the climatic conditions experienced by 11 populations of these species sampled across an elevation gradient where their distributions overlap in the southern Sierra Nevada of California. In a greenhouse experiment using field-collected seeds from these populations, we confirmed that the phenotypic divergence between these species in several floral and life history traits is genetically based and associated with differences in the climatic conditions they experienced in recent decades. Assuming that the phenotypic differences between these species are the result of adaptive evolution in response to local climatic conditions, then if conditions across their range become warmer and drier, we propose that populations currently occupying relatively cool and mesic conditions will experience direct selection favoring phenotypes that are presently associated with relatively warm and dry conditions. If so, then traits that are genetically correlated with these targeted traits may also evolve as correlated responses to selection.

We also used this greenhouse experiment to address the following questions to evaluate the potential for correlated evolution among life history and floral traits in each of these species: (1) Are the correlations between traits among maternal family means within each species consistent with the genetic divergence between them, suggesting that the correlations may have contributed to their joint evolution? (2) Are the genetic correlations between traits consistent between the two species, or are they evolutionarily labile such that the traits have evolved independently as the species diverged? (3) Within *unguiculata*, are genetic correlations strong enough that selection favoring earlier flowering (which is characteristic of populations in warmer, drier locations) could contribute to the correlated evolution of other traits that affect floral attractiveness, mating system, or the duration of the flowering period?

MATERIALS AND METHODS

Study system—*Clarkia* is a genus of 41 annual, self-compatible species, distributed primarily in the western United States. Self-fertilization has evolved independently at least a dozen times in the genus (Lewis and Lewis, 1955; Vasek, 1958, 1964; Sytsma et al., 1990; Gottlieb and Ford, 1996). In this study, we sampled and cultivated wild-collected seeds from natural populations of a pair of diploid *Clarkia* sister taxa that differ in their reliance on insect pollinators to achieve full fruit set. *Clarkia unguiculata* is a primarily outcrossing species that is endemic to California and occurs in the Coastal Ranges, Transverse Ranges, and the Sierra Nevada foothills in oak woodland and grasslands. We sampled six populations of *unguiculata* along an elevation gradient ranging from 430 to 1139 m a.s.l. in the foothills of the Sierra Nevada (Fig. 1; for additional population information, see Table 1). These sites ranged in latitude from 35.47° to 35.80°N and are subject to late-spring drought (Fig. 2). *Exilis* is a facultatively self-fertilizing species derived from *unguiculata* (Vasek, 1958) and is geographically restricted to a small portion of the range of *unguiculata* in and near the Kern River Valley (in Kern and in Tulare Counties) in the southern Sierra Nevada foothills. We sampled seeds from five populations of *exilis* ranging from 271 to 543 m a.s.l. and from 35.47° to 36.02°N (Table 1). The two taxa co-occur at one location sampled in this study at (443 m).

Previous studies of these species have reported that, relative to *unguiculata*, *exilis* produces sequential flowers more quickly along the primary stem, less pollen but more ovules per flower, and smaller seeds; *exilis* also exhibits faster flower development, shorter floral lifespans, and less protandry (Knies et al., 2004; Delesalle et al., 2007; Dudley et al., 2007). In addition, *exilis* exhibits faster gas exchange rates than *unguiculata* (Mazer et al., 2010). Collectively, these differences suggest that developmental rates in *exilis* at both the whole-plant and individual flower levels are faster than that of *unguiculata*.

Greenhouse methods—We used seeds that were collected in 1997–2010 from wild populations in Kern River Canyon (Kern Co., California, USA) and frozen at −20°C until used for this study. We estimated the mean individual seed mass (to 0.01 mg) of each maternal family by weighing 20–30 full, field-collected seeds per family and dividing by the number of seeds weighed. In the fall of 2013, seeds from each population were placed on agar-filled Petri dishes (15–30 maternal families per population, with seeds from each family germinated in a separate dish). The seeds were vernalized in the dark at 10°C for 14 d. They were then removed from refrigeration and allowed to germinate at room temperature in the laboratory under ambient light conditions.

During the first week of November (2–9 November 2013), seedlings ranging from 4 to 11 d old were transplanted into plastic growing tubes (4 × 20 cm Cone-tainers, Stuewe & Sons, Tangent, Oregon, USA) in a greenhouse. We used a potting mix of 9 parts

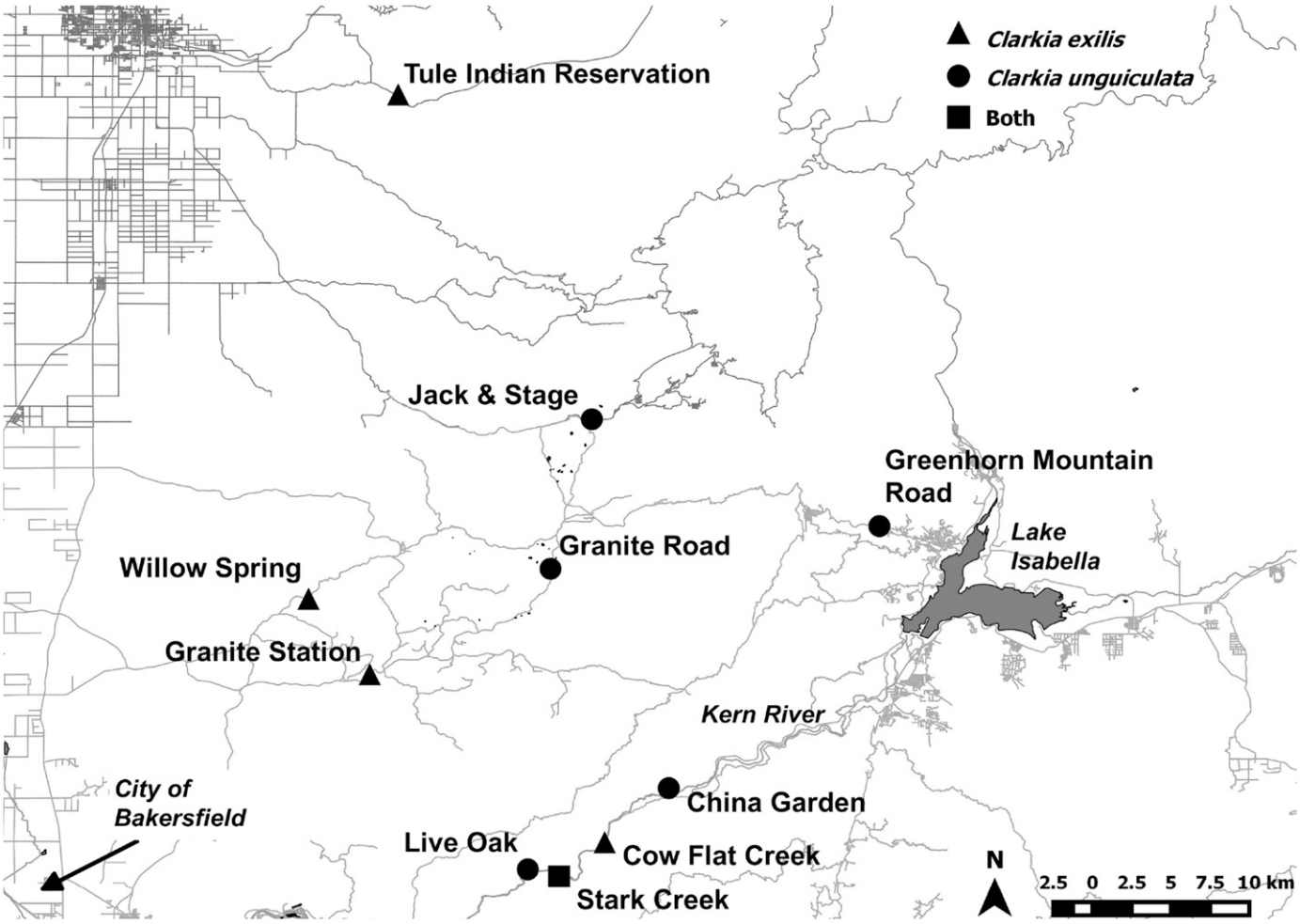

FIGURE 1 Field sites sampled for *Clarkia exilis* and *C. unguiculata* populations in California, United States. Seeds were harvested from these sites by maternal plant and sown in a greenhouse at the University of California, Santa Barbara.

Sunshine #4 potting mix (sphagnum peat moss, perlite, dolomitic limestone and endomycorrhizae; Sun Gro Horticulture, Agawam, Massachusetts, USA) and 1 part worm castings. Four Osmocote slow-release fertilizer pellets (14-14-14 NPK; Osmocote Scotts-Sierra Horticultural Products, Marysville, Ohio, USA) were also added to each growing tube before seedling transplant. Three seedlings per maternal family were sown into each of three tubes. The date of transplant for each pot was recorded. Additional seedlings were transplanted as needed if all of the seedlings in a tube died during the first week following the initial transplant.

TABLE 1. *Clarkia exilis* and *C. unguiculata* population locations and 30-yr climate normals (1981–2010). Sixth-month mean temperatures correspond to the period of germination and growth (November–April) and 3-month means correspond to the general flowering period for *Clarkia* spp. (April–June). Precipitation during the flowering period was negligible and is not shown here. Monthly means were generated by averaging daily values for each parameter across a month.

Population name	Taxon	Elevation (m)	Latitude (DD)	Longitude (DD)	Mean 6-mo precip. (mm)	Mean 6-mo max temp. (°C)	Mean 3-mo max temp. (°C)	Mean 6-mo min temp. (°C)	Mean 3-mo min temp. (°C)
Tule Indian Reservation	*exilis*	271	36.024	−118.839	337.8	18.3	29.0	5.2	11.3
Willow Spring	*exilis*	365	35.670	−118.902	246.1	18.1	28.2	4.8	11.2
Live Oak	*unguiculata*	430	35.480	−118.748	257.6	17.1	26.6	3.1	9.2
Stark Creek	both	443	35.475	−118.726	255.0	18.2	27.7	4.4	10.4
Granite Station	*exilis*	543	35.617	−118.859	257.0	17.2	26.9	3.9	9.5
Cow Flat Creek	*exilis*	518	35.499	−118.694	307.3	17.0	26.3	3.3	9.4
China Gardens	*unguiculata*	641	35.537	−118.649	324.4	17.5	26.7	3.7	10.6
Granite Road	*unguiculata*	869	35.691	−118.732	444.7	15.5	24.2	0.96	6.4
Jack and Stage	*unguiculata*	1006	35.796	−118.703	498.6	14.4	22.7	2.7	7.8
Greenhorn Mountain Road	*unguiculata*	1139	35.721	−118.501	409.4	13.0	22.0	1.7	7.2

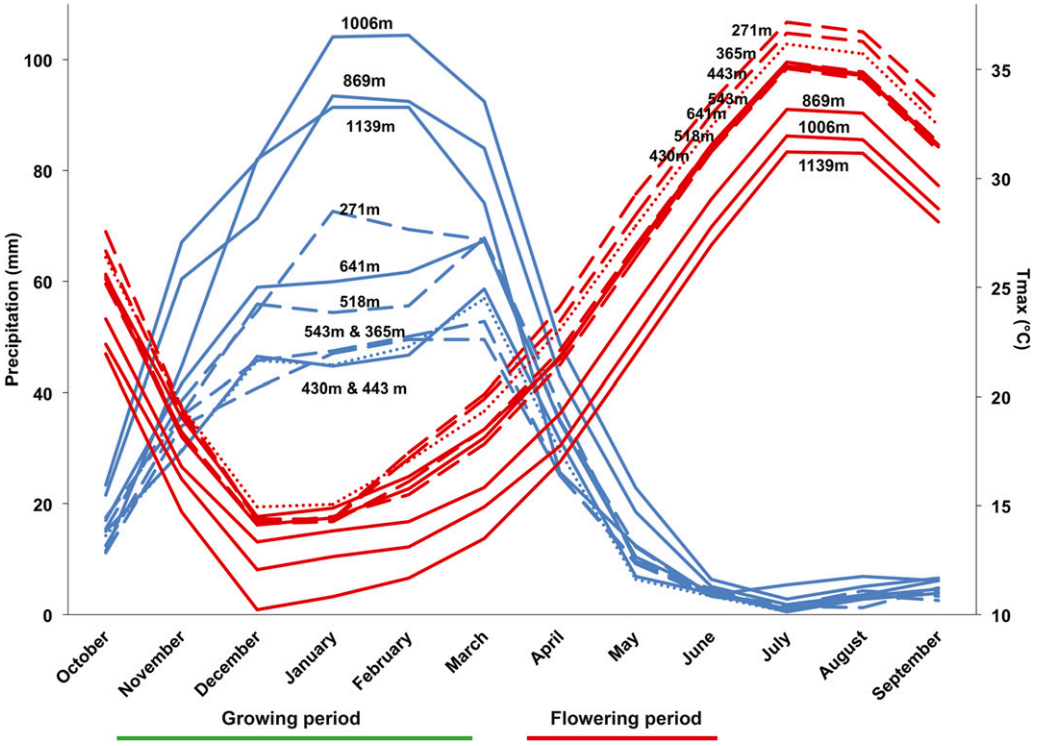

FIGURE 2 Seasonal patterns of monthly precipitation and maximum temperature based on 30-yr normals (1981–2010). Red lines show temperature patterns and blue lines show precipitation patterns. Dashed lines indicate *Clarkia exilis* populations, solid lines indicate *C. unguiculata* populations, and the dotted line indicates the population where the two species are sympatric (Stark Creek).

Growing tubes were arranged in plastic racks, with four maternal families represented in each rack (12 tubes per rack; 4–10 racks per population). The racks were randomly distributed throughout the greenhouse to minimize environmental differences among populations.

On 27 November, each pot was thinned to one healthy seedling. On 13 December 2013, day length was artificially extended to 13-h days using 300 W LED (PAR approximately 1146.8 $\mu mol \cdot s^{-1} \cdot m^{-2}$) grow lights in the greenhouse, and the temperature was maintained between a maximum of 27°C during the day and a minimum of 20°C at night. The plants were watered liberally using deionized water to maintain soil moisture. When plants exceeded 0.5 m in height, each plant was stabilized by attaching its primary stem (using small plastic rings) to a narrow gauge steel wire taped vertically to the outer surface of the growing tube.

Data collection began on 4 January 2014, when the first flower opened and proceeded until the plants senesced. The date of first flower was recorded for each individual as the date when floral parts (petals, anthers, and styles) were first visible. The number of days to first flower (DFF) was calculated as the number of days from seedling transplant to the date of first flower. The anther–stigma distance (herkogamy) of the first flower was measured as the shortest distance between a newly receptive stigma and the nearest long anther using digital calipers (to the nearest 0.1 mm). Since this measurement was time sensitive, we were unable to record the anther–stigma distance of every individual. An intact lower petal was collected from the third flower whenever possible to measure petal area. In cases where the third flower aborted or wilted before measurement, petals were collected from the fourth or fifth flower. Petals were attached to a data sheet using clear tape, labeled for image analysis, and digitally scanned. Petal area was calculated using the open-source image processing software ImageJ (ImageJ, National Institutes of Health, Bethesda, Maryland, USA). The date that the sixth flower opened on the primary stem was recorded to calculate the number of days between the first and sixth flower opening (Days 1–6). Days 1–6 was used as an estimate of the speed of sequential flower production. After accounting for mortality and missing data, data were recorded from 824 individuals representing 325 maternal families.

Data analysis—All variables were checked for normality; herkogamy was $\log_{10}$-transformed to improve normality. All statistical analyses were performed using JMP Pro (version 11, SAS Institute, Cary, North Carolina, USA).

Climatic variation among sites and between species—Annual and monthly climate data for each seed collection site (at 800 m resolution) were obtained from the PRISM Climate Group (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu). Monthly means are based on modeled daily averages over 30 yr (1981–2010) generated by PRISM. We calculated 6-month (November, December, January, February, March, April) and 3-month means (April, May, June) for mean monthly maximum and minimum temperatures (Tmax and Tmin), and 6-month (November—April) total precipitation for each site. The 6-month means represent the time during which most precipitation occurs in California and winter annual species begin to germinate and grow, while the 3-month means represent the time during which *Clarkia* is flowering.

One-way analysis of variance (ANOVA) was used to detect significant differences among nine of the sampled sites of *exilis* and *unguiculata* with respect to mean 6-month and 3-month precipitation, Tmax, and Tmin. The single site among the sampled populations where *exilis* and *unguiculata* co-occur (Stark Creek) was excluded from this analysis. A Wilcoxon test was used to detect a significant difference in mean elevation between the two taxa because the data were not normally distributed. Bivariate regressions of climatic parameters on elevation were conducted among sites to test whether mean seasonal temperatures declined and total precipitation increased with elevation. Since taxon was not a variable in these regressions, Stark Creek was only included once to avoid duplicating elevation and climate data for the same location. In all analyses of climate and elevation, significance was determined using $\alpha = 0.1$ to compensate for small sample sizes.

Correlations between population mean phenotype and climatic conditions—For each pair of focal traits, we calculated the correlation coefficients between population mean phenotype and historical climatic parameters across sites to evaluate whether recent climatic conditions (over several decades) are associated with floral or life history traits. We tested for the statistical significance of these coefficients using a one-tailed test because in each case we hypothesized that the sign of the correlation would be the same as the sign of the association observed between species (e.g., *exilis* occupies warmer sites and exhibits early flowering, smaller petals, and lower herkogamy than *unguiculata*, so we predicted that, among populations, mean flowering date, petal area, and herkogamy would be negatively correlated with mean monthly Tmax and Tmin).

Phenotypic differences between species—ANOVA tests were used to detect significant differences between species in two ways. First, using the data set that comprised individual observations, we conducted a mixed model ANOVA to detect significant effects on each of our focal traits of taxon (fixed), population nested within taxon (random), and maternal family nested within population (random).

Second, maternal family means for each of the focal traits were calculated from the phenotypes of the 1–3 siblings per family. Using the data set that comprised the maternal family means, we conducted a mixed model ANCOVA to detect significant effects on each trait of taxon (fixed), population nested within taxon (random), and the mean individual seed mass of each maternal family. In this analysis, significant differences detected between taxa were independent of variation among maternal families in the mean individual seed mass of sown seeds. In both analyses, herkogamy was $\log_{10}$-transformed to improve normality and significant differences were determined using $\alpha = 0.05$.

Bivariate relationships among maternal family means—We examined the bivariate relationships among maternal family means in three ways. First, we used the raw phenotypic values for each family; second, we controlled for variation in mean seed mass of sown seeds; third, we controlled for variation both among populations (within each species) and in mean seed mass.

To determine whether initial seed mass influenced the phenotype of traits expressed later in the life cycle, we conducted least square regressions among maternal family means within each species (all populations pooled), using each focal trait as a dependent variable. We then calculated the residuals of maternal family means on mean seed mass for each trait within species. We then used both the raw values and these residuals to examine the bivariate relationships among all genotypes (all populations pooled) between each pair of traits.

To control for variation in mean seed mass and for differences among population means that could influence associations among maternal families, we conducted a fixed model ANCOVA on each focal trait in which population and mean seed mass were included as main effects (the interaction term was always nonsignificant and excluded from the model). The residuals derived from these models were then used to estimate trait values that were independent of variation among populations in the focal trait and of variation in mean seed mass. Within each species, these residuals were used to examine the bivariate relationships between each pair of traits among all maternal families (populations pooled).

For the raw values and for both sets of residuals (i.e., controlling for seed mass only and controlling for population effects and seed mass), we conducted major axis regressions within each species to estimate the genetically based correlation between each pair of traits. For each pair of focal traits, we compared species with respect to the correlation coefficients and the slopes of the regressions to evaluate the lability of these relationships. We used major axis regression because it implies no direction of causality between variables (see Legendre, 2008 for further discussion of its use).

For each pair of traits, the correlation coefficients provided an estimate of the strength of the relation between them. We compared the correlation coefficients derived from the analysis of the raw values to those derived from the analysis of the residuals to evaluate whether the strength of the relationship between any given pair of variables was influenced by variation in initial seed mass (or by differences among population means) and its effects on the phenotypes of subsequently expressed traits. For example, if initial seed mass explained most of the variation in DFF, then the strength of any correlation between DFF and other traits would diminish in analyses that controlled for seed mass. By comparing the slopes of the major axis regressions estimated using the raw values vs. the residuals, one can similarly evaluate whether either the sign or the slope of a given relationship changes when controlling for variation in seed mass or among population means.

RESULTS

Climatic variation among sites and between species—Among the sampled sites, *exilis* populations occurred at lower elevation and at warmer sites than *unguiculata* populations. The mean elevation of the *exilis* populations sampled here was 393 m lower ($P < 0.05$) than that of the sampled populations of *unguiculata*. Mean winter precipitation (1981–2010) at the sampled *exilis* sites was 99.9 mm less than at *unguiculata* sites (Fig. 3A). Tmax and Tmin also differed between each species' sampled sites. Based on 30-yr means, Tmax for November–April at *exilis* sites was >2°C higher than at *unguiculata* sites (Fig. 3B). During the flowering period (April–June), the mean monthly Tmax at *exilis* sites was just over 3°C higher than at *unguiculata* sites (Fig. 3C). Mean monthly Tmin followed a similar pattern, with *exilis* sites experiencing warmer temperatures than *unguiculata* sites during both the winter growing period (November–April) and the flowering period (April–June; Fig. 3B, C). These precipitation and temperature patterns are strongly related to elevation; total seasonal precipitation increases with elevation ($\alpha < 0.10$), while mean monthly Tmax and Tmin decline significantly with increasing elevation (Fig. 4A–E).

Correlations between population mean phenotype and climatic conditions—The correlations among populations between mean phenotype and climatic parameters are consistent with the differences between the species means. Among all sampled populations of both species, climatic conditions are strongly associated with DFF and with herkogamy. Populations at historically warmer sites flower earlier than those at cooler sites; mean DFF is negatively correlated with the mean 3-month Tmax ($r = -0.59$, $P < 0.0258$, $n = 11$). Populations at wetter or cooler sites exhibit floral phenotypes (e.g., more herkogamous) more strongly associated with outcrossing than those at warmer or drier sites; mean herkogamy is positively correlated with mean annual precipitation ($r = 0.53$, $P < 0.0486$, $n = 11$), and negatively correlated with mean 6-month

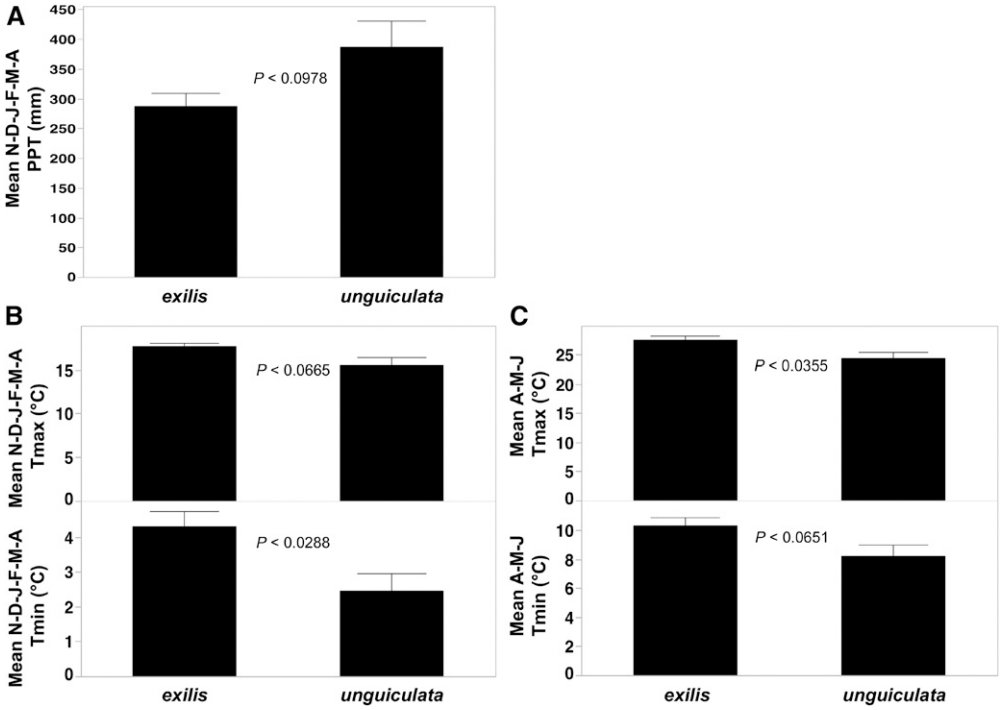

FIGURE 3 Climatic means (±1 SE) among sites from which *Clarkia exilis* and *C. unguiculata* were sampled. Six-month means represent November–April; 3-month means represent April–June. Precipitation during the flowering period was negligible and is not shown. ANOVA was used to determine whether temperature or precipitation regimes differed between mean *C. exilis* and *C. unguiculata* sites. Significant differences were determined using $\alpha = 0.1$. (A) Mean 6-month precipitation. (B) Mean 6-month Tmax and Tmin. (C) Mean 3-month Tmax and Tmin.

Tmin ($r = -0.52$, $P < 0.0491$, $n = 11$) and 3-month Tmax ($r = 0.52$, $P > 0.0487$, $n = 11$).

Phenotypic differences between species—*Exilis* and *unguiculata* differed significantly in almost all of the traits observed. In the analysis of individual plant values, *exilis* flowered ~10 d earlier than *unguiculata* (Table 2 and Appendix S1 [see Supplemental Online Data with online version of this article], Fig. 5A: $F_{1, 9.0} = 15.60$, $P < 0.0034$, $n = 819$). *Exilis* flowers had significantly smaller petals (Table 2 and Appendix S1, Fig. 5B: $F_{1, 9.1} = 32.85$, $P < 0.0003$, $n = 801$) and significantly lower herkogamy than *unguiculata* flowers did (Fig. 5C; $F_{1, 8.5} = 148.3$, $P < 0.0001$, $n = 597$). Sequential flower production was faster in *exilis* than in *unguiculata*, but the difference was not statistically significant (Table 2 and Appendix S1, Fig. 5D: $F_{1,8.8} = 1.48$, $P > 0.2552$, $n = 808$). Appendix S1 shows the full ANOVA results.

Differences among maternal families in mean individual seed mass did not influence the detection of differences between species in any of our focal traits. In the ANCOVA using maternal family means and controlling for variation in initial seed mass, *exilis* similarly flowered significantly earlier ($F_{1, 9.1} = 16.46$, $P < 0.0028$, $n = 313$), produced smaller petals ($F_{1, 9.3} = 32.98$, $P < 0.0002$, n = 313), and exhibited lower herkogamy ($F_{1, 9} = 134.13$, $P < 0.0001$, $n = 258$) than *unguiculata* did; the species did not differ significantly with respect to the rate of sequential flower production ($F_{1, 9.1} = 2.12$, $P > 0.1789$, $n = 313$). Appendix S2 (see Online Supplemental Data) shows the full ANCOVA results.

Bivariate relationships among maternal family means—*Relationships among raw maternal family means*—There were significant correlations among maternal family means between several pairs of traits, but the sign of each relationship often differed between taxa (Tables 3A, 4A). In *unguiculata*, mean seed mass was positively correlated with DFF, petal area, herkogamy, and Days 1–6; large-seeded families flowered later, produced larger petals, exhibited higher herkogamy, and produced sequential flowers more slowly than small-seeded families did. These relationships mirror the bivariate association between the means of *unguiculata* and *exilis* (Fig. 5). Within *exilis*, large-seeded maternal families similarly produced larger petals than small-seeded families did; however, large-seeded *exilis* families flowered earlier than small-seeded ones, and seed mass varied independently of both herkogamy and Days 1–6.

In the absence of controlling statistically for variation in mean seed mass, *unguiculata* families that flowered relatively late produced larger petals, exhibited higher herkogamy, and produced sequential flowers more slowly than families that flowered relatively early (Tables 3A, 4A). These associations mirror those observed between the two species' means (Fig. 5). Among maternal families of *exilis*, by contrast, these relationships were statistically significant but opposite in sign (Tables 3A, 4A).

Relationships controlling for initial seed mass—In *unguiculata*, when controlling for variation among maternal families in mean seed mass, the positive correlations among families between DFF and petal area and between DFF and Days 1–6 were maintained but the correlation between DFF and herkogamy was no longer statistically significant (Tables 3A vs. 3B and 4A vs. 4B; Fig. 6A–C). The positive correlation between petal area and Days 1–6 was also maintained (Tables 3B and 4B; Fig. 6D).

In *exilis*, the significant negative relationships between DFF and petal area, and between DFF and herkogamy were similarly maintained (Table 3A vs. 3B; Fig. 6A and B), but the negative relationship between DFF and Days 1–6 was not (Tables 3B and 4B; Fig. 6C). The negative correlations observed in *exilis* contrast qualitatively with the bivariate differences between the species (Fig. 5 vs. 6A and 6A).

Relationships controlling for variation among populations and in seed mass—In *unguiculata*, when controlling for variation among population means and in mean seed mass, the positive correlations between DFF and petal area and between DFF and Days 1–6 remained robust (Fig. 6E and G), but the positive relationship between petal area and Days 1–6 became nonsignificant (Tables 3B vs. 3C and 4B vs. 4C; Fig. 6H).

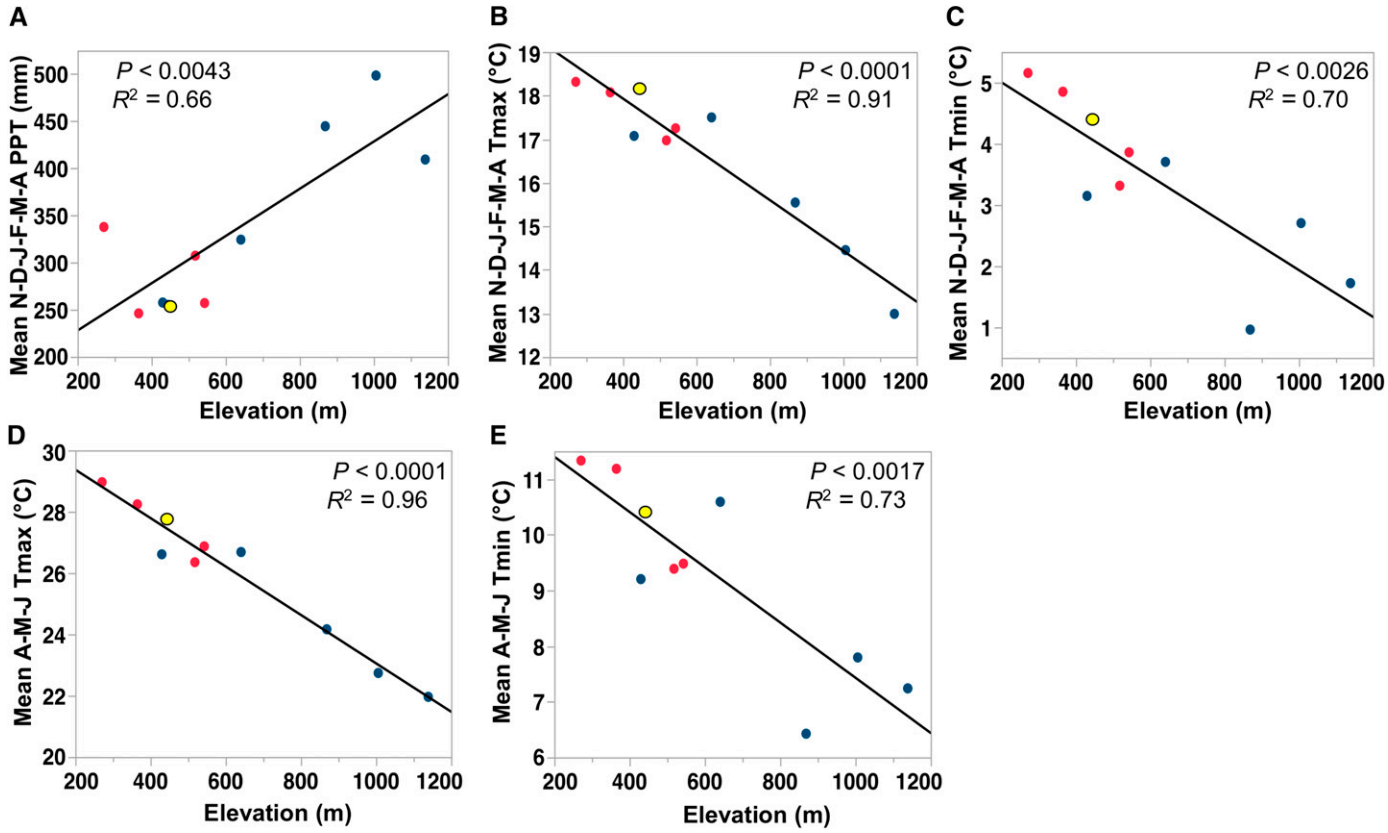

FIGURE 4 Relationships between climatic conditions and elevation across sampled sites. Significant correlations were detected using linear regressions ($\alpha = 0.1$). Red points indicate *Clarkia exilis* sites and blue points indicate *C. unguiculata* sites. Stark Creek, the location where *C. exilis* and *C. unguiculata* are sympatric, was only included in the analysis once and is indicated in yellow. (A) Mean 6-month precipitation vs. elevation. (B) Mean 6-month Tmax vs. elevation. (C) Mean 6-month Tmin vs. elevation. (D) Mean 3-month Tmax vs. elevation. (E) Mean 3-month Tmin vs. elevation.

In *exilis*, the negative relationship between DFF and herkogamy remained strong (Fig. 6F), and a significant negative correlation between petal area and Days 1–6 was expressed (Tables 3C and 4C; Fig. 6G). Within populations of *exilis*, maternal families with large petals produced sequential flowers more rapidly than those with small petals. The sign of this relationship contrasts with the interspecific pattern.

DISCUSSION

Geographic patterns in climate and phenotype—We examined geographic variation in climate and in mean phenotype between species and among populations of *unguiculata* and *exilis* to evaluate whether environmental conditions may have influenced the evolution of floral and life history traits. In this study, *unguiculata*, which

TABLE 2. Mean phenotype of floral and life history traits in *Clarkia exilis* and *C. unguiculata* populations based on individual plant data. Least squares means were calculated using a mixed model ANOVA with taxon (fixed), population nested within taxon (random), and maternal family nested within population (random) as independent variables.

		DFF		Petal area (cm^2)		Log_{10} (herkogamy) (mm)		Days 1–6	
Taxon	Population	Mean (SE)	*n*	Mean (SE)	*n*	Mean (SE)	*n*	Mean (SE)	*n*
C. exilis	Tule Indian Reservation	69.1 (0.74)	35	0.46 (0.02)	35	0.23 (0.04)	29	4.0 (0.19)	35
	Willow Spring	77.7 (0.37)	99	0.30 (0.01)	94	0.01 (0.04)	83	1.9 (0.12)	96
	Stark Creek	81.0 (0.47)	62	0.26 (0.02)	56	0.04 (0.04)	54	2.9 (0.15)	59
	Granite Station	75.3 (0.47)	63	0.37 (0.02)	63	−0.1 (0.04)	59	2.5 (0.15)	63
	Cow Flat Creek	80.9 (0.74)	25	0.36 (0.03)	25	0.007 (0.06)	22	2.6 (0.23)	24
C. unguiculata	Live Oak	90.5 (0.39)	90	0.95 (0.01)	89	0.85 (0.02)	84	3.7 (0.12)	89
	Stark Creek	88.5 (0.38)	97	1.0 (0.01)	98	0.81 (0.02)	94	3.4 (0.12)	97
	China Garden	83.0 (0.37)	99	0.68 (0.01)	97	0.72 (0.02)	90	2.9 (0.12)	97
	Granite Road	82.4 (0.36)	108	0.7 (0.01)	108	0.79 (0.02)	105	2.7 (0.11)	108
	Jack and Stage	91.1 (0.45)	70	0.85 (0.02)	66	0.84 (0.02)	68	3.3 (0.14)	70
	Greenhorn Mountain Road	87.5 (0.44)	71	0.64 (0.02)	70	0.77 (0.02)	68	3.5 (0.14)	70

Notes: DFF = Days to first flower; Days 1–6 = number of days between the production of the first and sixth open flower; *n* = number of individuals; least squares mean (SE). Herkogamy data were log_{10}-transformed to improve normality.

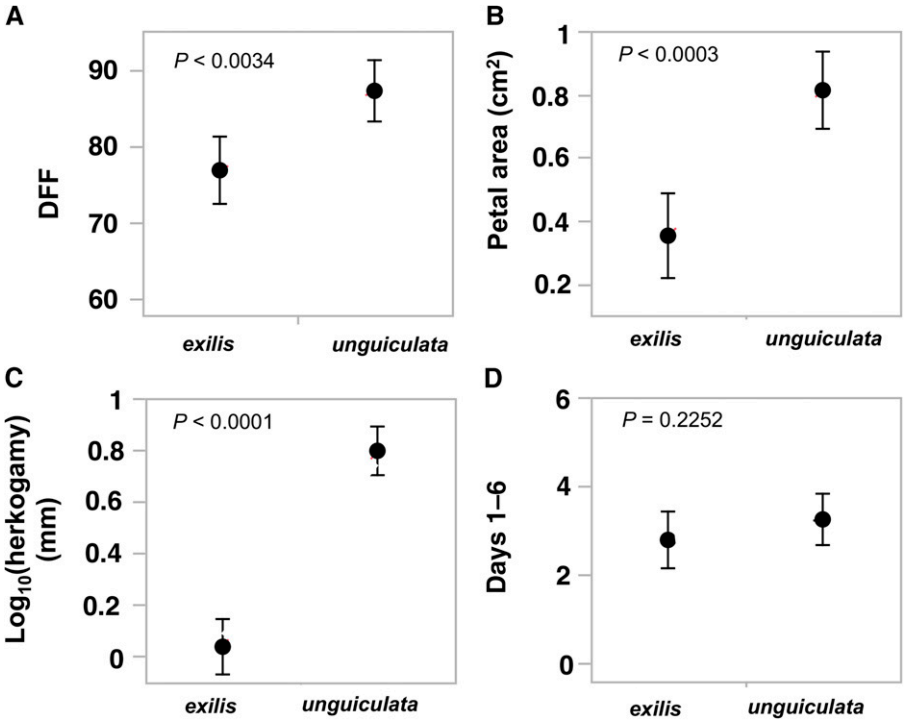

FIGURE 5 Trait means (±1 SE) in greenhouse populations of *Clarkia exilis* and *C. unguiculata*. A mixed model ANOVA using taxon (fixed), population nested within taxon (random) and maternal family (random) as independent variables was used to calculate least squares means and to identify significant differences between the species ($\alpha = 0.05$). DFF = days to first flower; Days 1–6 = number of days between the production of the first and sixth open flower. (A) Mean days to first flower. (B) Mean petal area. (C) Mean herkogamy. (D) Mean number of days between first and sixth flower.

has a much larger geographic range than *exilis*, occupied higher elevations and cooler, wetter sites than *exilis* (Figs. 2 and 3), although there is a region of overlap (Fig. 4).

These climatic differences between the sampled sites occupied by *unguiculata* vs. *exilis* are associated with strong, genetically based differences between them in floral and life history traits (Fig. 5). Moreover, the climatic differences between these species' sampled locations approximate the degree of climate change expected in the near future (Cayan et al., 2008; Loarie et at., 2008). The conditions at the sampled *exilis* sites are a realistic proxy for the climatic conditions that *unguiculata* populations may experience under climate change in the absence of long-distance dispersal. If the observed phenotypic differences between these taxa represent adaptations to the recent environmental conditions they have experienced, then natural selection in *unguiculata* populations in the near future may favor phenotypes more similar to those currently characteristic of *exilis*.

However, it should be noted that the conclusions drawn from the correlations detected between population mean phenotype and climate are somewhat limited by the small sample sizes used in this study (i.e., 5–6 populations per species). Given that the mean phenotypes of conspecific populations are not truly independent, these correlations may be driven by differences between the species with respect to mean phenotype and climate, rather than a strong relationship among populations between mean phenotype and climate. A more robust study with a larger number of populations is needed to verify the correlations between mean phenotype and climate that we detected in this study.

Phenotypic differences between species—*Exilis* flowered earlier, senesced earlier, and had smaller petals and lower herkogamy than *unguiculata*, which is consistent with field observations (Vasek, 1958; Mazer et al., 2010). In contrast to a previous study (Dudley et al., 2007), the rate of sequential flower production did not differ significantly between the two species. The maintenance of these interspecific differences under greenhouse conditions and independent of variation in initial seed mass (Appendix S2) indicates that they are genetically based and not the result of phenotypic plasticity or associated with maternal effects on seed mass.

Evolutionary implications of correlations between traits—Correlations estimated among maternal family means should be interpreted with caution. First, correlations among maternal family means may be unreliable because they may overestimate the magnitude of additive genetic (i.e., highly heritable) correlations (Roff, 1995) and because they may reflect the outcome of selection against alternative combinations of traits rather than underlying pleiotropy or linkage. In this study, the strength and sign of the estimated correlations between traits often differed between species, indicating that the correlations are evolutionarily labile and not strongly governed (if at all) by pleiotropy or linkage.

Second, correlations among maternal family means between life history and/or floral traits can be confounded by correlations among families between initial seed mass and these traits. For instance, environmental heterogeneity in the field populations sampled in this study could have induced the differences in initial seed mass observed among the greenhouse-cultivated maternal families. Alternatively, maternal families may have differed genetically with respect to mean seed mass. Given that the mass of sown seeds was correlated with both DFF and petal area, then DFF and petal area could have appeared to be positively genetically correlated when in fact the correlation was mediated by variation in seed mass. To control for these possibilities, we controlled statistically for seed mass when estimating the correlations among maternal family means between all pairs of traits. Although seed mass influenced the expression of DFF and petal area (Table 3A), several of the correlations observed between traits remained strong whether they were adjusted for seed mass, particularly in *unguiculata*.

It should be noted that controlling for seed mass could potentially obscure important correlations. For example, in *unguiculata*, the correlations among raw maternal family means suggest that early flowering is associated with lower herkogamy (Table 3A). This relationship implies that selection favoring early flowering could promote the evolution of self-fertilization as a correlated response. This correlation disappears when controlling for seed mass

TABLE 3. Correlation coefficients between seed mass, life history, and floral traits among maternal family means (all populations pooled). Correlation coefficients (*r*) for *Clarkia exilis* appear above the diagonal; coefficients for *C. unguiculata* are below the diagonal. DFF = days to first flower; Days 1–6 = number of days between the production of the first and sixth open flower; *n* = number of maternal families. Herkogamy was $\log_{10}$-transformed to improve normality. Statistically significant correlation coefficients are in boldface (α = 0.05).

(A) Correlations among raw values of maternal family means.

	Seed mass	DFF	Petal area	Herkogamy	Days 1-6
Trait	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**
Seed mass		**–0.21 (116)**	**0.38 (116)**	–0.04 (62)	0.08 (116)
DFF	**0.23 (197)**		**–0.56 (122)**	**–0.35 (67)**	**–0.21 (121)**
Petal area	**0.41 (197)**	**0.45 (204)**		0.22 (67)	0.16 (122)
Herkogamy	**0.18 (196)**	**0.15 (203)**	**0.18 (203)**		0.15 (67)
Days 1–6	**0.16 (197)**	**0.36 (204)**	**0.21 (204)**	–0.10 (203)	

(B) Correlations among maternal family means, using the residuals of each trait regressed on mean seed mass to control for variation among families in initial mean seed mass.

	DFF	Petal area	Herkogamy	Days 1–6
Trait	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**
DFF		**–0.49 (116)**	**–0.34 (62)**	–0.13 (116)
Petal area	**0.40 (197)**		0.13 (62)	0.08 (116)
Herkogamy	0.12 (196)	0.11 (196)		–0.001 (67)
Days 1–6	**0.33 (197)**	**0.16 (197)**	–0.13 (203)	

(C) Correlations among maternal family means, using the residuals of each floral and life history trait on population means and on mean seed mass to control for variation in these parameters.

	DFF	Petal area	Herkogamy	Days 1–6
Trait	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**	***r* (*n*)**
DFF		–0.02 (116)	**–0.40 (62)**	0.07 (116)
Petal area	**0.18 (196)**		0.12 (62)	**–0.28 (116)**
Herkogamy	–0.06 (195)	0.02 (195)		–0.22 (62)
Days 1–6	**0.20 (196)**	0.05 (196)	–0.03 (195)	

(Tables 3B and 3C). Natural selection, however, may act on flowering time independent of seed mass, generating the correlated evolution of DFF and herkogamy.

Here, the most consistent correlations observed in *unguiculata* suggest that prospective future selection on traits that may promote the avoidance of drought stress—early flowering, small petal size, and rapid flower production—will result in correlated responses of other traits, reinforcing their joint evolution. For example, maternal families that flowered relatively early had smaller petals and less-herkogamous flowers, and they produced successive flowers more rapidly than late-flowering genotypes (Tables 3A and 4A; Fig. 6). These relationships support the prediction that, if selection favors earlier flowering, then smaller petals and more rapid sequential flower production will evolve as correlated responses to selection. Indeed, given that smaller petals and rapid flower production may also be directly favored under xeric conditions, the simultaneous evolution of all three traits might be highly probable. If the positive correlation between DFF and seed mass observed in Table 3A is strongly genetically based, then smaller seeds would also be expected to evolve in response to selection favoring early flowering (interestingly, *exilis* both flowers earlier and produces significantly smaller seeds than *unguiculata*). When controlling for mean seed mass, the strongest correlations among maternal families expressed in *unguiculata* were maintained (Tables 3A vs. 3B and 4A vs. 4B).

Several bivariate correlations differed markedly between species. For example, when controlling for variation among populations and in initial seed mass, petal area and DFF varied independently in *exilis*, but were positively correlated in *unguiculata*; herkogamy and DFF were negatively correlated in *exilis*, but varied independently in *unguiculata*; petal area and Days 1–6 were negatively correlated in *exilis*, but varied independently in *unguiculata;* and DFF and Days 1–6 varied independently in *exilis* but were positively correlated in *unguiculata* (Table 3C). The differences observed between these taxa in the direction and strength of these correlations indicate that they are not governed by strong linkage or pleiotropy and are likely to be disrupted easily by natural selection (or genetic drift). In cases where the sign of the regression slopes differs significantly between taxa (e.g., Table 4), selection may have consistently favored different combinations of traits in each taxon such that the genotypes retained in each species now occupy distinct regions of bivariate phenotypic space.

Predictions for correlated trait evolution in a warmer, drier future—Several studies have investigated the joint evolution of mating system and floral or life history traits (Moore and Lewis, 1965; Ornduff, 1969; Sato and Yahara, 1999; Mazer et al., 2004; Dudley et al., 2007; Goodwillie et al., 2010; Ivey and Carr, 2012). Selfing is often accompanied by a suite of morphological and physiological traits relative to closely related outcrossers (Darwin, 1876; Ornduff, 1969; Richards, 1986), including early flowering (Mazer et al., 2004; Martin and Willis, 2007), a decrease in flower size and herkogamy (Ornduff, 1969; Wyatt, 1988), and more rapid floral development (Armbruster et al., 2002; Mazer et al., 2004; Dudley et al., 2007). In some cases, self-fertilization is also associated with shifts toward rapid maturation, high gas exchange rates and low water-use efficiency (Mazer et al., 2010; Wu et al., 2010). Few studies, however, have considered how climate change might affect this kind of joint evolution.

TABLE 4. Slopes estimated from major axis regressions (see Fig. 6) between floral or life history traits among all maternal family means. Regressions that were not significant for either species are not shown. CL = confidence limit; DFF = days to first flower; Days 1–6 = number of days between the production of the first and sixth open flower; *n* = the number of maternal families. Boldface slopes are significantly different from zero (α = 0.05).

(A) Raw values of maternal family means used in regressions.

	Clarkia exilis				*Clarkia unguiculata*			
Bivariate relationship (*y* vs. *x*)	***n***	**Slope**	**Lower CL**	**Upper CL**	***n***	**Slope**	**Lower CL**	**Upper CL**
Petal area vs. DFF	116	**−0.010**	−0.012	−0.007	197	**0.018**	0.013	0.022
Herkogamy vs. DFF	62	**−0.022**	−0.036	−0.007	195	**0.004**	0.0005	0.009
Days 1–6 vs. DFF	116	**−0.048**	−0.089	−0.008	196	**0.071**	0.0452	0.097
Petal area vs. herkogamy	116	0.061	−0.005	0.128	197	**3.43**	1.869	13.251
Petal area vs. Days 1–6	62	0.013	−0.001	0.028	195	**0.044**	0.015	0.072

(B) Residuals of maternal family means on mean seed mass used in regressions.

	Clarkia exilis				*Clarkia unguiculata*			
Bivariate relationship (*y* vs. *x*)	***n***	**Slope**	**Lower CL**	**Upper CL**	***n***	**Slope**	**Lower CL**	**Upper CL**
Petal area vs. DFF	116	**−0.009**	−0.011	−0.006	197	**0.015**	0.010	0.020
Herkogamy vs. DFF	62	**−0.025**	−0.042	−0.008	195	0.003	−0.001	0.008
Days 1–6 vs. DFF	116	−0.031	−0.075	0.013	196	**0.068**	0.041	0.096
Petal area vs. Days 1-6	116	0.006	−0.008	0.020	197	**0.030**	0.004	0.057

(C) Residuals of maternal family means on population mean and mean seed mass used in regressions.

	Clarkia exilis				*Clarkia unguiculata*			
Bivariate relationship (*y* vs. *x*)	***n***	**Slope**	**Lower CL**	**Upper CL**	***n***	**Slope**	**Lower CL**	**Upper CL**
Petal area vs. DFF	116	−0.0003	−0.004	0.003	197	**0.006**	0.001	0.011
Herkogamy vs. DFF	62	**-0.046**	−0.073	−0.018	195	−0.002	−0.007	0.003
Days 1–6 vs. DFF	116	0.021	−0.034	0.077	196	**0.05**	0.015	0.085
Petal area vs. Days 1–6	116	**-0.019**	−0.031	−0.007	197	0.008	−0.013	0.029

Many traits commonly associated with selfing may facilitate drought escape (e.g., by enabling individuals to shorten their life cycle or to reduce evaporative water loss through petals), which is likely to be an important ecological or evolutionary response to future climatic conditions (Cayan et al., 2008). The projected combination of hotter and drier conditions in California will likely increase drought stress in late-flowering species such as *unguiculata*, leading to selection favoring traits associated with drought escape. Assuming that *exilis* is adapted to warmer and drier conditions than *unguiculata*, if climate change intensifies such conditions at the sampled *unguiculata* sites, and if *unguiculata* evolves more *exilis*-like traits, then the correlated evolution of floral and life history traits in *unguiculata* could lead to increased selfing rates under climate change, even if self-fertilization is not directly beneficial.

The two clearest examples of genetically based correlations observed in *unguiculata* in this study were the positive correlations between (1) petal area and DFF and (2) Days 1–6 and DFF (Tables 3A–C). Flowering earlier and producing smaller flowers can each result in reduced exposure to water stress under seasonal drought conditions (Geber and Dawson, 1990; Galen, 1999; Arntz and Delph, 2001; McKay et al., 2003). Similarly, rapid sequential flower production may reduce exposure to drought and water loss through the petals if it shortens the flowering period. Given the lack of rainfall after March among the *unguiculata* sites sampled here (Fig. 2), we predict that if spring conditions become warmer and drier, then earlier flowering, a reduction in flower size, and a shorter flowering period may evolve in *unguiculata*.

Previous studies have evaluated adaptive responses of life history and/or floral traits under water-limited conditions and have detected cases where such traits may evolve independently. In two greenhouse populations each of *Mimulus guttatus* and *M. nasutus* grown in wet and dry soil treatments, natural selection favoring early flowering was stronger than selection for floral traits that influence selfing rates (e.g., flower size and herkogamy), especially in dry soil (Ivey and Carr, 2012). Galen (1999) reported that small flowers have slower evapotranspiration rates than large flowers due to the water demand required to maintain fresh petals, providing a mechanism for the evolution of self-fertilization independent of flowering date.

Potential for the evolution of selfing in a warmer, drier future—If natural selection favors early flowering in *unguiculata*, then the genetically based combination of early flowering, rapid flower production, and small petals could lead to the evolution of increased selfing rates by affecting pollinator visitation. For example, if selection for early flowering leads to smaller petals (due to a genetic correlation of the kind observed in *unguiculata* in Fig. 6), then flowers may become less attractive to pollinators and receive fewer visits than other coflowering species (Conner and Rush, 1996; Button et al., 2012). Likewise, if natural selection favoring early flowering leads to more rapid sequential flower production, then the amount of time available for pollinator visitation could be reduced. A sufficiently large reduction in insect-mediated pollen deposition due to less attractive flowers, a shorter flowering period, or both could result in a direct advantage to selfing among early-flowering genotypes.

Even in the absence of genetically based correlations between flowering date and floral traits, changes in flowering time alone could lead to pollinator mismatches (Kiers et al., 2010; Rafferty and Ives, 2011), decreasing insect-mediated outcrossing in *unguiculata* and resulting in selection favoring autogamous selfing among early-flowering genotypes. The effects of such mismatches could be exacerbated

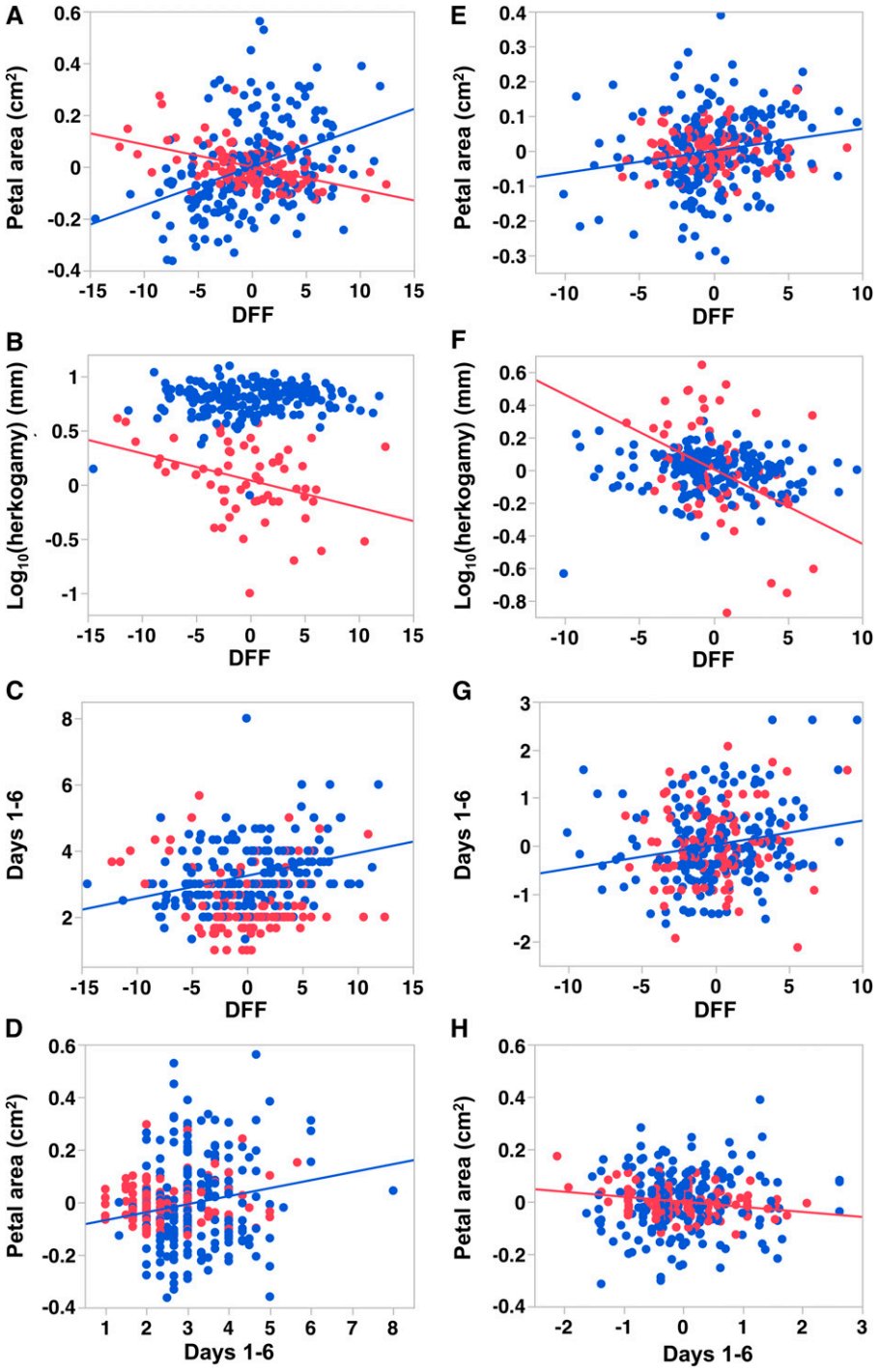

FIGURE 6 Major axis regressions between floral and life history traits. *Clarkia exilis* points are shown in red and *C. unguiculata* points are shown in blue; regression lines indicate slopes that are significantly different from zero ($\alpha = 0.05$). (A–D) Major axis regressions using pooled maternal family means, controlling for seed mass. DFF = Days to first flower; Days 1–6 = number of days between the production of the first and sixth open flower. (A) Petal area vs. DFF. (B) Herkogamy vs. DFF. (C) Days 1–6 vs. DFF. (D) Petal area vs. Days 1–6. (E–H) Major axis regressions among maternal family means using the residuals of each floral and life history trait calculated from ANCOVAS including population (nested within species) and mean seed mass as independent variables. (E) Petal area vs. DFF. (F) Herkogamy vs. DFF. (G) Days 1–6 vs. DFF. (H) Petal area vs. Days 1–6.

if earlier flowering is genetically correlated with shorter flowering periods or smaller, less attractive flowers. In either case, delayed selfing is one mechanism that could compensate for a lack of pollen deposition; similarly, increased autogamous selfing rates could be achieved through the evolution of lower protandry or herkogamy.

The most common explanation for the evolution of selfing is reproductive assurance—the ability to produce seed when pollinators are absent or unreliable or where the availability of mates is low. However, reproductive assurance may not be the only mechanism by which selfing evolves. In environments with short growing seasons that are terminated by drought (as in the American Southwest and other arid habitats throughout the world), natural selection might favor individuals with early flowering, small flowers, and short flowering periods simply because these traits help plants to avoid exposure to drought (Andersson, 1997; Eckhart and Geber, 2000; Mazer et al., 2004). The evolution of selfing in response to drought could be the result of direct or indirect selection under these conditions. For example, natural selection could directly favor selfing genotypes if the ability to escape drought via rapid floral development increases fitness (e.g., by reducing water loss), but lowers attractiveness to pollinators. In this case, selfing genotypes may be directly favored. Alternatively, if natural selection under hot and dry conditions favors early-flowering genotypes, and if early flowering is genetically correlated with traits that increase the probability of selfing (e.g., small flowers, lower herkogamy, and rapid sequential flower production), then selfing rates could increase as a correlated response whether selfing is advantageous. Other studies have shown that increases in floral development rates can lead to reductions in herkogamy (anther–stigma distance) or dichogamy (temporal separation between male and female receptivity) (Fenster et al., 1995; Armbruster et al., 2002; Mazer et al., 2004; Dudley et al., 2007), which are likely to increase selfing rates.

Although reproductive assurance via selfing is advantageous in the short-term, the long-term evolutionary potential of selfing lineages may be limited (Wright et al., 2013). Increased selfing rates are often accompanied by a loss of genetic variation (Charlesworth and Charlesworth, 1995; Hamrick and Godt, 1996; Ashman and Majetic, 2006) and inbreeding depression (Charlesworth and Wright, 2001), which could reduce the ability of

populations to adapt to future environmental change. Extinction rates have also been found to be higher for selfing than outcrossing taxa (Goldberg and Igic, 2012), and climate change is expected to increase the risk of extinction at both the local and global scale (Malcolm et al., 2006; Bellard et al., 2012). Thus, the prospective benefits of the evolution of selfing in *unguiculata* and similarly late-spring blooming annuals under climate change also come with stark genetic risks. The evolution of selfing, especially in scenarios where it may not be directly adaptive, represents a potential and unforeseen consequence of climate change and could threaten the genetic diversity and adaptive potential of wild plant populations over time.

However, the detection of genetic correlations in this study is not sufficient to predict with confidence how the sampled populations and traits will evolve under climate change or to predict how other species will respond. Further research is needed to determine the expression, under field conditions, of the genetic associations we examined in this study and to understand the consequences of the rapid evolution of selfing in *unguiculata* and in other species that are vulnerable to the effects of swift environmental change.

ACKNOWLEDGEMENTS

The authors thank D. Taber for technical support in the greenhouses at the University of California, Santa Barbara. The Mazer Laboratory undergraduates in 2014, especially L. Barley, C. Hannah-Bick, R. Lambert, E. Maul, and K. Rodriguez for assistance with data collection and data entry. The manuscript was greatly improved by comments from anonymous reviewers.

LITERATURE CITED

Ackerly, D. D. 2003. Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences* 164: S165–S184.

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* 1: 95–111.

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society, B, Biological Sciences* 279: 3843–3852.

Andersson, S. 1997. Genetic constraints on phenotypic evolution in *Nigella* (Ranunculaceae). *Biological Journal of the Linnean Society* 62: 519–532.

Armbruster, W. S., C. P. H. Mulder, B. G. Baldwin, S. Kalisz, B. Wessa, and H. Nute. 2002. Comparative analysis of late floral development and mating system evolution in tribe Collinsieae (Scrophulariaceae). *American Journal of Botany* 89: 37–49.

Arntz, A. M., and L. F. Delph. 2001. Pattern and process: Evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127: 455–467.

Ashman, T.-L., and C. J. Majetic. 2006. Genetic constraints on floral evolution: A review and evaluation of patterns. *Heredity* 96: 343–352.

Bellard, C., C. Bertelsmeier, P. Keadly, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity. *Ecology Letters* 15: 365–377.

Burgess, K. S., J. R. Etterson, and L. F. Galloway. 2007. Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity* 99: 641–648.

Button, L., A. L. Villalobos, S. R. Dart, and C. G. Eckert. 2012. Reduced petal size and color associated with transitions from outcrossing to selfing in *Camissoniopsis cheiranthifolia* (Onagraceae). *International Journal of Plant Sciences* 173: 251–260.

CaraDonna, P. J., A. M. Iler, and D. W. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences, USA* 111: 4916–4921.

Cayan, D. R., E. P. Maurer, M. D. Dettinger, M. Tyree and K. Hayhoe. 2008. Climate change scenarios for the California region. *Climatic Change* 87(Supplement 1): 21–42.

Charlesworth, D., and B. Charlesworth. 1995. Quantitative genetics in plants: Effect of the breeding system on genetic variability. *Evolution* 49: 911–920.

Charlesworth, D., and S. I. Wright. 2001. Breeding systems and genome evolution. *Current Opinion in Genetics & Development* 11: 685–690.

Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333: 1024–1026.

Colautti, R. I., C. G. Eckert, and S. C. H. Barrett. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proceedings of the Royal Society of London, B, Biological Sciences* 277: 1799–1806.

Conner, J. K., and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105: 509–516.

Darwin, C. 1876. The effects of cross and self fertilization in the vegetable kingdom. John Murray, London, UK.

Davis, M. G., and R. G. Shaw. 2001. Range shifts and adaptive responses to quaternary climate change. *Science* 292: 673–679.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to climate change. *Ecology* 86: 1704–1714.

Delesalle, V. A., S. J. Mazer, and H. Paz. 2007. Temporal variation in the pollen:ovule ratios of *Clarkia* (Onagraceae) taxa with contrasting mating systems: Field populations. *Journal of Evolutionary Biology* 21: 310–323.

Dudley, L. S., S. J. Mazer, and P. Galusky. 2007. The joint evolution of mating system, floral traits and life history in Clarkia (Onagraceae): Genetic constraints vs. independent evolution. *Journal of Evolutionary Biology* 20: 2200–2218.

Eckhart, V. M., and M. A. Geber. 2000. Character variation and geographic range in *Clarkia xantiana* (Onagraceae): Breeding system and phenology distinguish two common subspecies. *Madroño* 46: 117–125.

Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate chane. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the Great Plains. *Evolution* 58: 1459–1471.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294: 151–154.

Fenster, C. B., P. K. Diggle, S. C. H. Barrett, and K. Ritland. 1995. The genetics of floral development differentiating two species of *Mimulus*. *Heredity* 74: 258–266.

Fordham, D. A., H. Resit Akçakaya, M. B. Araújo, J. Elith, D. A. Keith, R. Pearson, T. D. Auld, et al. 2012. Plant extinction risk under climate change: Are forecast range shifts alone a good indicator of species vulnerability to warming? *Global Change Biology* 18: 1357–1371.

Franks, S. J., and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences, USA* 104: 1278–1282.

Franks, S. J., and A. E. Weis. 2008. A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *Journal of Evolutionary Biology* 21: 1321–1334.

Galen, C. 1999. Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* 118: 461–470.

Geber, M. A., and T. E. Dawson. 1990. Genetic variation and covariation between leaf gas exchange, morphology and development in *Polygonum arenastrum*, an annual plant. *Oecologia* 85: 153–158.

Goldberg, E. E., and B. Igic. 2012. Tempo and mode in plant breeding system evolution. *Evolution* 66: 3701–3709.

Goodwillie, C., R. D. Sargent, C. G. Eckert, E. Elle, M. A. Geber, M. O. Johnston, S. Kalisz, et al. 2010. Correlated evolution of mating system and floral display traits in flowering plants and its implications for the distribution of mating system variation. *New Phytologist* 185: 311–321.

Gottlieb, L. D., and V. S. Ford. 1996. Phylogenetic relationships among sections of *Clarkia* (Onagraceae) inferred from the nucleotide sequences of *PgiC*. *Systematic Botany* 21: 45–62.

Grace, J., F. Berninger, and L. Nagy. 2002. Impacts of climate change on the tree line. *Annals of Botany* 90: 537–544.

Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plants. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 351: 1291–1298.

Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470: 479–485.

Huntley, B. 1991. How plants respond to climate change: Migration rates, individualism and the consequences for plant communities. *Annals of Botany* 67: 15–22.

IPCC. 2014. Climate change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, et al. [eds.], 1–1131. Cambridge University Press, New York, New York, USA.

Ivey, C. T., and D. E. Carr. 2012. Tests for the joint evolution of mating system and drought escape in *Mimulus*. *Annals of Botany* 109: 583–598.

Jump, A., and J. Pañuelas. 2005. Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters* 8: 1010–1020.

Kiers, E. T., T. M. Palmer, A. R. Ives, J. F. Bruno, and J. L. Bronstein. 2010. Mutualisms in a changing world: An evolutionary perspective. *Ecology Letters* 13: 1459–1474.

Knies, J. L., V. A. Delesalle, and A. R. Cavaliere. 2004. Seed mass and morphology in outcrossing and selfing species of *Clarkia* (Onagraceae): An SEM study. *International Journal of Plant Sciences* 165: 85–96.

Legendre, P. 2008. lmodel2: Model II regression. R package version 1.6-3. Website http://CRAN.R-project.org/package=lmodel2.

Lewis, H., and M. E. Lewis. 1955. The genus *Clarkia*. University of California Press, Berkeley, California, USA.

Loarie, S. R., B. E. Carter, K. Hayhoe, S. McMahon, R. Moe, C. A. Knight, and D. D. Ackerly. 2008. Climate change and the future of California's endemic flora. *Plos ONE* 3: e2502.

Malcolm, J. R., C. Liu, R. P. Neilson, L. Hansen, and L. Hannah. 2006. Global warming and extinctions of endemic species from biodiversity hotspots. *Conservation Biology* 20: 538–548.

Martin, N. H., and J. H. Willis. 2007. Ecological divergence associated with mating system causes nearly complete reproductive isolation between sympatric *Mimulus* species. *Evolution* 61: 68–82.

Mazer, S. J., L. S. Dudley, A. A. Hove, S. K. Emms, and A. S. Verhoeven. 2010. Physiological performance in *Clarkia* sister taxa with contrasting mating systems: Do early-flowering autogamous taxa avoid water stress relative to their pollinator-dependent counterparts? *International Journal of Plant Sciences* 171: 1029–1047.

Mazer, S. J., H. Paz, and M. D. Bell. 2004. Life history, floral development and mating system in *Clarkia xantiana* (Onagraceae): Do floral and whole-plant rates of development evolve independently? *American Journal of Botany* 91: 2041–2050.

Mazer, S. J., S. E. Travers, B. I. Cook, T. J. Davies, K. Bolmgren, N. J. B. Kraft, N. Salamin, and D. W. Inouye. 2013. Flowering date of taxonomic families predicts phenological sensitivities to temperature: Implications for forecasting the effects of climate change on unstudied taxa. *American Journal of Botany* 100: 1381–1397.

McKay, J. K., J. H. Richards, and T. Mitchell-Olds. 2003. Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. *Molecular Biology* 12: 1137–1151.

Midgley, G. F., L. Hannah, D. Millar, M. C. Rutherford, and L. W. Powrie. 2002. Assessing the vulnerability of species richness to anthropogenic climate change in a biodiversity hotspot. *Global Ecology and Biogeography* 11: 445–451.

Moore, D. M., and H. Lewis. 1965. The evolution of self-pollination in *Clarkia xantiana*. *Evolution* 19: 104–114.

Neilson, R. P., L. F. Pitelka, A. M. Solomon, R. Nathan, G. F. Midgley, et al. 2005. Forecasting regional to global plant migration in response to climate change. *Bioscience* 55: 749–759.

Ornduff, R. 1969. Reproductive biology in relation to systematics. *Taxon* 18: 121–133.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* 37: 637–669.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.

Pereira, H. M., P. W. Leadly, V. Proenca, R. Alkemade, J. P. W. Scharlemann, J. F. Fernandez-Manjarres, M. B. Araújo, et al. 2010. Scenarios for global biodiversity in the 21st century. *Science* 330: 1496–1501.

Rafferty, N. E., and A. R. Ives. 2011. Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters* 14: 69–74.

Richards, A. J. 1986. Plant breeding systems. George Allen & Unwin, London, UK.

Roff, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: A test of Cheverud's conjecture. *Heredity* 74: 481–490.

Salamin, N., R. O. Wüest, S. Lavergne, W. Thuiller, and P. B. Perman. 2010. Assessing rapid evolution in a changing environment. *Trends in Ecology & Evolution* 25: 692–698.

Sato, H., and T. Yahara. 1999. Trade-offs between flower number and investment to a flower in selfing and outcrossing varieties of *Impatiens hypophylla* (Balsaminaceae). *American Journal of Botany* 86: 1699–1707.

Savolainen, O., F. Bokma, R. García-Gil, P. Komulainen, and T. Repo. 2004. Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management* 197: 79–89.

Sax, D. F., R. Early, and J. Bellemare. 2013. Niche syndromes, species extinction risks, and management under climate change. *Trends in Ecology & Evolution* 28: 517–523.

Shaw, R., and J. R. Etterson. 2012. Rapid climate change and the rate of adaptation: Insight from experimental quantitative genetics. *New Phytologist* 195: 752–765.

Springer, C., and J. Ward. 2007. Flowering time and elevated atmospheric CO_2. *New Phytologist* 176: 243–255.

Sytsma, K. J., J. F. Smith, and L. D. Gottlieb. 1990. Phylogenetics in *Clarkia* (Onagraceae): Restriction site mapping of chloroplast DNA. *Systematic Botany* 15: 280–295.

Vasek, F. C. 1958. The relationship of *Clarkia exilis* to *Clarkia unguiculata*. *American Journal of Botany* 45: 150–162.

Vasek, F. C. 1964. The evolution of *Clarkia unguiculata* derivatives adapted to relatively xeric climates. *Evolution* 18: 26–42.

Wolkovich, E. M., T. J. Davies, H. Schaefer, E. E. Cleland, B. I. Cook, S. E. Travers, C. G. Willis, and C. C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407–1421.

Wright, S. I., S. Kalisz, and T. Slotte. 2013. Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society, B, Biological Sciences* 280: 20130133.

Wu, C. A., D. B. Lowry, L. I. Nutter, and J. H. Willis. 2010. Natural variation for drought-response traits in the *Mimulus guttatus* species complex. *Oecologia* 162: 23–33.

Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. *In* L. D. Gottlieb and S. K. Jain [eds.], Plant evolutionary biology, 109–131. Chapman and Hall, New York, New York, USA.

Zhu, K., C. W. Woodall, S. Ghosh, A. E. Gelfand, and J. S. Clark. 2014. Dual impacts of climate change: Forest migration and turnover through life history. *Global Change Biology* 20: 251–264.