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Context-dependent concordance between physiological divergence and phenotypic selection in sister taxa with contrasting phenology and mating systems

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Abstract

Premise: The study of phenotypic divergence of, and selection on, functional traits in closely related taxa provides the opportunity to detect the role of natural selection in driving diversification. If the strength or direction of selection in field populations differs between taxa in a pattern that is consistent with the phenotypic difference between them, then natural selection reinforces the divergence. Few studies have sought evidence for such concordance for physiological traits.

Methods: Herbarium specimen records were used to detect phenological differences between sister taxa independent of the effects on flowering time of long-term variation in the climate across collection sites. In the field, physiological divergence in photosynthetic rate, transpiration rate, and instantaneous water-use efficiency were recorded during vegetative growth and flowering in 13 field populations of two taxon pairs of *Clarkia*, each comprising a self-pollinating and a outcrossing taxon.

Results: Historically, each selfing taxon flowered earlier than its outcrossing sister taxon, independent of the effects of local long-term climatic conditions. Sister taxa differed in all focal traits, but the degree and (in one case) the direction of divergence depended on life stage. In general, self-pollinating taxa had higher gas exchange rates, consistent with their earlier maturation. In 6 of 18 comparisons, patterns of selection were concordant with the phenotypic divergence (or lack thereof) between sister taxa.

Conclusions: Patterns of selection on physiological traits measured in heterogeneous conditions do not reliably reflect divergence between sister taxa, underscoring the need for replicated studies of the direction of selection within and among taxa.

KEYWORDS

adaptation, *Clarkia*, climate, divergence, gas exchange, mating system evolution, phenology, phenotypic selection, water-use efficiency

The study of phenotypic divergence between, and phenotypic selection within, closely related taxa provides the opportunity to detect the processes contributing to evolutionary diversification and trait evolution (Hall and Willis, 2006; Anderson and Gezon, 2015; Teixido et al., 2019; Custer et al., 2021). Phenotypic divergence may be generated not only by adaptive evolutionary responses to short-term or sustained differences between populations in the direction of natural selection, but also by plastic responses of populations to temporary or longstanding environmental conditions (which may or may not be adaptive) (Lucek et al., 2014; Frank et al., 2017; Rajkov et al., 2018). In plants, evidence for both mechanisms (adaptive evolution and plasticity) have been detected (Eckhart et al., 2004; Whittall and Hodges, 2007; Mazer et al., 2009, 2010, 2018; Anderson et al., 2012, 2015; Dudley et al., 2012; Briscoe Runquist and Moeller, 2014; Pilote and Donovan, 2016; Brancalion et al., 2018; Ricote et al., 2019).

Here we evaluated the hypothesis that physiological divergence between sister taxa in the wildflower genus *Clarkia* is consistent with taxon-specific phenotypic selection in the field (cf. Hall and Willis, 2006; Dudley et al., 2012; Anderson et al., 2015). For example, when closely related sister taxa differ in a functional trait such as leaf size, evidence that natural selection contributes to this divergence would be either stronger directional selection favoring larger-leaved individuals in populations of the larger-leaved taxon than in the smaller-leaved one, or a qualitative difference between taxa in the sign of the selection coefficient for leaf area, with the larger-leaved taxon experiencing directional selection favoring larger-leaved individuals, and the opposite pattern applying to the smaller-leaved taxon.

Studies examining both divergence between taxa and taxon-specific patterns of selection are rare, particularly for physiological traits (but see Dudley et al., 2012; Anderson et al., 2015), in part because characterizing the strength and direction of selection on any trait in wild species requires observations of both functional traits and lifetime fitness in many individuals (Swenson et al., 2020) and, ideally, in multiple populations. In addition, the phenotype of physiological traits can be both highly dynamic during development and strongly influenced by environmental conditions (Ackerly et al., 2000; Caruso et al., 2020), variation in which is difficult to control in wild populations. Measuring the phenotype of physiological traits should therefore take such dynamism into account by recording the phenotype of individuals multiple times during their life cycle and/or by measuring physiological attributes that reflect an individual's lifetime performance, such as integrated water-use efficiency (WUE). In the current study, to assess whether physiological divergence between sister taxa is both detectable and consistently reinforced (or caused) by phenotypic selection across a heterogeneous landscape, we measured gas exchange traits and estimated reproductive fitness in many individuals in multiple populations of two pairs of sister taxa surveyed during both vegetative growth and flowering.

Even when taxa differ in the mean values of, and the patterns of selection on, fitness-related traits, it can be difficult to identify the factors directly responsible for such differences. Taxa may differ not only in the abiotic conditions and in the composition of co-occurring species (including competitors, pollinators, pathogens, and herbivores) to which their populations are exposed, but also in a suite of ancillary (but unstudied) traits that may influence the pattern of selection on targeted traits. For example, if regionally co-occurring sister taxa differ in flowering phenology and their populations experience intensifying drought as the flowering season progresses, then flowering time may affect the rate of water loss through petals due to seasonal increases in vapor pressure deficit (Teixido et al., 2019). The degree of water loss through flowers, in turn, may influence the strength of phenotypic selection on WUE exhibited by leaves, with higher WUE being favored in populations or taxa that are under the greatest drought stress during flowering. In this case, considering the flowering phenology of the focal taxa may help to identify the mechanism responsible for divergence between them in mean WUE and to understand the effects of WUE or transpiration rates on individual fitness in each taxon. Similarly, sister taxa may differ in mating system, with highly autogamous taxa producing smaller and shorter-lived flowers than their pollinator-dependent sister taxa (Snell and Aarssen, 2005; Dudley et al., 2007). This difference in flower size and longevity may affect the strength and direction of natural selection on gas exchange traits, as higher water loss through the large and longer-lived flowers of outcrossers may drive selection for higher WUE in leaves. In sum, knowledge of differences between taxa with respect to traits other than those being directly examined can inform our understanding of the factors that drive the differences observed in the focal traits.

Here, we use a combination of historical herbarium records and contemporary studies of phenotypic selection and physiological performance to evaluate the ecological factors and evolutionary mechanisms that have led to physiological differences, expressed in wild populations, between congeneric sister taxa that differ in both flowering time and mating system. The genus *Clarkia* is a model system that has been the focus of several decades of research to detect adaptive differentiation between taxa in habitat preferences and in morphological, life history, reproductive, and physiological traits. Two pairs of closely related taxa with contrasting mating systems—the predominantly outcrossing *C. unguiculata* and the highly autogamous *C. exilis*, and the pollinator-dependent *C. xantiana* subsp. *xantiana* and its selfing sister taxon, *C. xantiana* subsp. *parviflora*—have been studied most intensively. In each taxon pair, self-pollinating and outcrossing taxa differ in their geographic distributions and microhabitat preferences (Lewis and Lewis, 1955; Vasek, 1968; Vasek and Sauer, 1971; Eckhart and Geber, 1999). Under experimental conditions, the self-pollinating taxa also flower earlier or produce sequential flowers in more rapid succession than their outcrossing counterparts (Dudley et al., 2007). In addition, each self-pollinating taxon produces smaller flowers with lower pollen to ovule ratios, and smaller leaves and seeds than its outcrossing sister taxon (Runions and Geber, 2000; Delesalle et al., 2008). The selfers have also been found to exhibit higher gas exchange rates during vegetative growth and/or during flowering (Mazer et al., 2010; Dudley et al., 2012). In both taxon pairs, divergence between taxa in vegetative, physiological, or phenological traits is in some cases consistent with the pattern of selection in these traits (Dudley et al., 2012; Anderson et al., 2015). Due to the dynamic nature of plant physiological traits over time and space (Ackerly et al., 2000), a more comprehensive assessment of the degree to which patterns of physiological divergence and selection are concordant is needed.

In the current study, we first used herbarium-derived data to evaluate whether differences in mating system between the focal *Clarkia* sister taxa are associated with intrinsic differences between them in flowering time, independent of the chronic climatic conditions to which populations are exposed. Previous studies of these taxa have reported that the selfing taxa (*C. exilis* and *C. xantiana* subsp. *parviflora*) flower earlier than their outcrossing sister taxa (Lewis and Lewis, 1955; Dudley et al., 2007; Anderson et al., 2015), but this is the first study to control for geographic variation in long-term climatic conditions across each taxon's range when comparing selfing and outcrossing sister taxa with respect to their flowering phenology. For these phenological comparisons, we analyzed hundreds of georeferenced herbarium specimen records of individuals collected across each taxon's geographic range to measure phenological divergence between sister taxa.

We then used physiological observations recorded in spring 2010 for >1800 individuals in 13 field populations to determine whether sister taxa with contrasting mating systems differ consistently throughout the growing season with respect to gas exchange rates and instantaneous WUE (WUE_i). Based on previous observations of the relative flowering times of our focal taxa in the Sierra Nevada foothills of California, along with the seasonal drought that occurs in this region each year as spring progresses, we hypothesized that, during flowering, the earlier-flowering selfing taxa would generally be exposed to lower dehydration risk than their outcrossing sister taxa. This ecological difference between sister taxa should result either in the evolution of higher mean photosynthetic and transpiration rates and lower WUE in populations of the selfing taxa relative to their outcrossing counterparts or in taxon-specific plastic responses that generate the same pattern of divergence. To determine whether patterns of phenotypic selection on physiological traits during vegetative growth and/or flowering are consistent with the divergence between taxa, we then conducted selection gradient analyses to estimate direct and total selection on each focal trait within each life stage and taxon. Finally, the patterns of selection and physiological divergence detected in the current study were compared to those observed in 2008, when a similar study was conducted in the same region (Mazer et al., 2010). The high lability of both divergence and patterns of selection reveals the importance of replicating observations among years, populations, and sampling periods. In addition, examining the combination of phenological and physiological divergence between taxa facilitated the interpretation of the patterns observed.

MATERIALS AND METHODS

Study species

Clarkia unguiculata Lindley and *C. xantiana* subsp. *xantiana* A. Gray are self-compatible winter annuals that flower in late spring, are endemic to California, and occupy oak and pine woodlands, grasslands, and roadside habitats. While *C. xantiana* subsp. *xantiana* is restricted to the southern Sierra Nevada and Transverse ranges to the south, the geographic range of *C. unguiculata* is much more widespread, occupying both the Sierra Nevada and the Coastal and Transverse Ranges (Lewis and Lewis, 1955). In the Lake Isabella region of the southern Sierra Nevada (Kern and Tulare counties, California, USA), both taxa may occur alone or in sympatry with their predominantly selfing sister taxa (*C. exilis* and *C. xantiana* subsp. *parviflora*, respectively; Figure 1). Where sister taxa co-occur, reproductive isolation is reinforced by differences between them in flowering phenology and floral morphology, with selfers flowering earlier and showing lower levels of herkogamy and dichogamy than their bee-pollinated outcrossing counterparts (Eckhart and Geber, 1999; Dudley et al., 2007).

In the southern Sierra Nevada, the sister taxa studied here differ with respect to habitat. *C. exilis* is sometimes sympatric with *C. unguiculata*, but the latter occupies a broader range of habitats, growing in woodlands and encroaching into grasslands. By contrast, *C. exilis* is restricted to low-elevation woodlands and has a narrower, patchier distribution than *C. unguiculata*. Where the species co-occur, *C. exilis* is found near streambeds, boulders, or rocky outcrops that provide some shade and/or that retain more soil moisture (Vasek, 1958). The joint distributions of *C. xantiana* subsp. *xantiana* (hereafter, subsp. *xantiana*) and *C. xantiana* subsp. *parviflora* (hereafter, subsp. *parviflora*) follow a west to east gradient, with subsp. *xantiana* occurring in populations to the west and subsp. *parviflora* occurring toward the east, nearly reaching the Mojave Desert. Although there is a narrow central zone of contact between these subspecies (Eckhart et al., 2004; Geber and Eckhart, 2005), sites occupied solely by subsp. *parviflora* receive less precipitation than those occupied solely by subsp. *xantiana.*

Historical climatic and phenological differences between sister taxa: herbarium-based study

Occurrence data and the GPS coordinates of all specimen records available for each of the focal taxa were downloaded from the Consortium of California Herbarium portal (https://cch2.org) on 31 July 2021 (Figure 2). The date of specimen collection was converted to day-of-year (DOY: 1–365; 366 for leap years). The GPS coordinates of each collection site were then used to extract from the climate database, ClimateNA v.7.01 (Wang et al., 2016), the mean values of 24 annual climatic variables estimated from 1951 to 1980, the 30-year period preceding the point when climate warming is considered to have begun to accelerate (IPCC, 2013). The climate variables estimated are defined in Appendix S1. To estimate climatic variables, ClimateNA uses interpolated values from the PRISM climate database (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu) to generate scale-free estimates of local climatic conditions at a resolution of 4 × 4 km.

The DOY of specimen collection does not provide a precise record of the date of first flower or of peak flowering of a specimen because an individual plant collected in flower may have been collected at any time after flowering has begun, and the duration of an individual's flowering period may be several weeks or longer. Nevertheless, the date of specimen collection has been found to be a reliable proxy for flowering time (Davis et al., 2015) and has been used in many studies to detect the factors influencing flowering phenology (Davis et al., 2015; Matthews and Mazer, 2016; Willis et al., 2017; Park and Mazer, 2018, 2019; Ellwood et al., 2019; Love et al., 2019; Park et al., 2019,

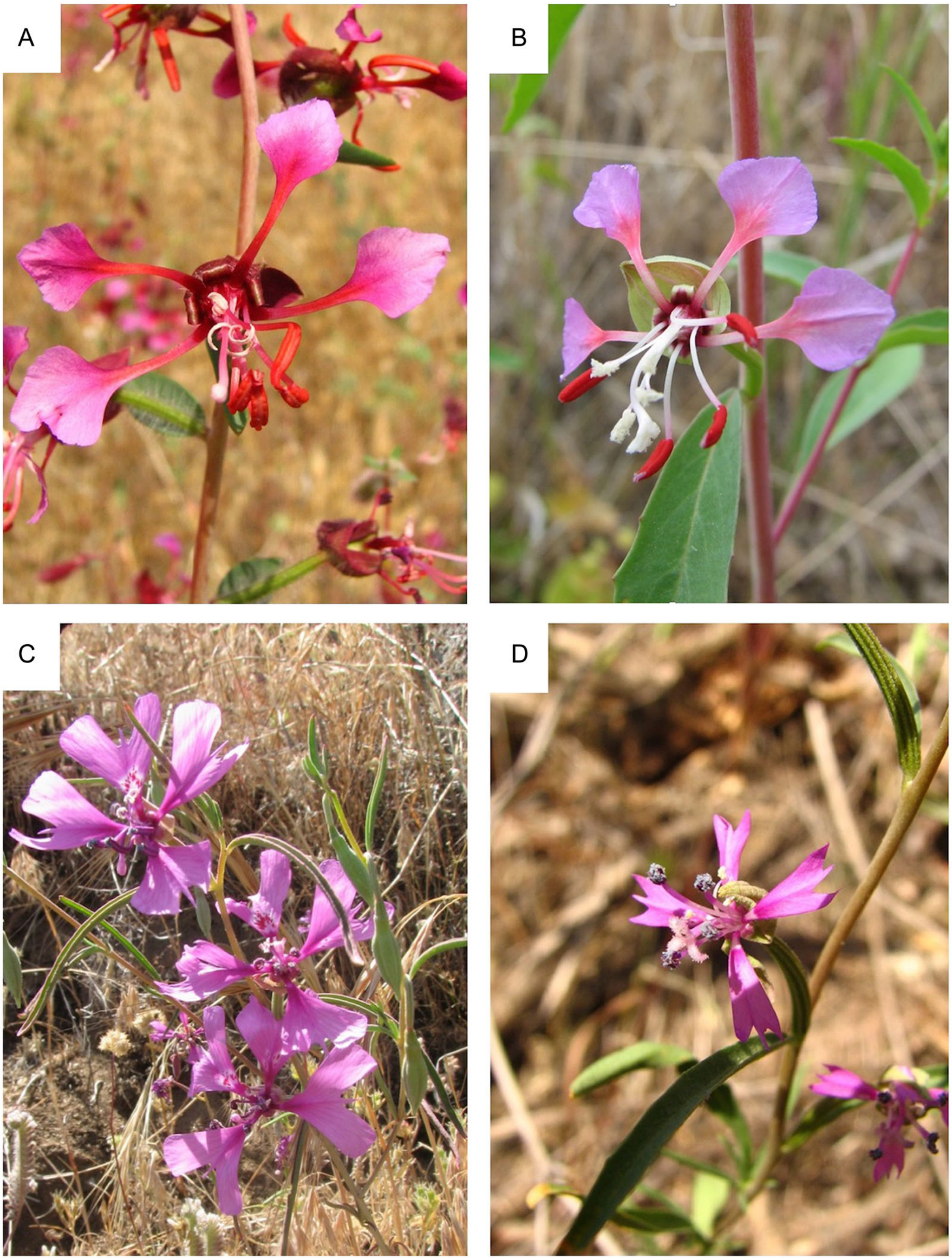

FIGURE 1 Photographs of the study species. (A) *Clarkia unguiculata*. (B) *C. exilis*. (C) *C. xantiana* subsp. *xantiana*. (D) *C. xantiana* subsp. *parviflora*. Within each taxon, mean flower size differs within and among field populations, but autogamous taxa consistently produce smaller petals than their pollinator-dependent sister taxa. The area of individual petals of *C. unguiculata* (mean = 0.52 cm^2, SD = 0.20, range = 0.15–1.14, n = 1302 petals [2/plant] sampled from 12 populations in 2001, unpublished data) is larger than that of *C. exilis* (mean = 0.41 cm^2, SD = 0.09, range = 0.17–0.68, n = 1000 petals [2/plant] sampled from 11 populations in 2001, unpublished data). The area of individual petals produced by *C. xantiana* subsp. *xantiana* (mean = 0.93 cm^2, SD = 0.24, range = 0.47–1.74, n = 366 individuals [1–4 petals/plant] sampled from 7 populations in 2001; unpublished data) is larger than that of *C. xantiana* subsp. *parviflora* (mean = 0.46 cm^2, SD = 0.16, range = 0.15–0.99, n = 180 individuals [1–4 petals/plant] sampled from 4 populations in 2001, unpublished data). Photo credit: Susan J. Mazer.

2021a, 2021b; Mazer et al., 2021; Pearson et al., 2021). Among the focal taxa of this study, herbarium specimens with images that were available for downloading on 25 December 2021 from the Consortium of California Herbaria website (https://www.cch2.org) comprised 26, 679, 10, and 33 specimens of *C. exilis*, *C. unguiculata*, *C. xantiana* subsp. *parviflora*, and *C. xantiana* subsp. *xantiana*, respectively. Each image was examined to determine whether the individual plant(s) on each sheet bore one or more open flowers. Of these, 24 (92%), 659 (97%), 8 (80%),

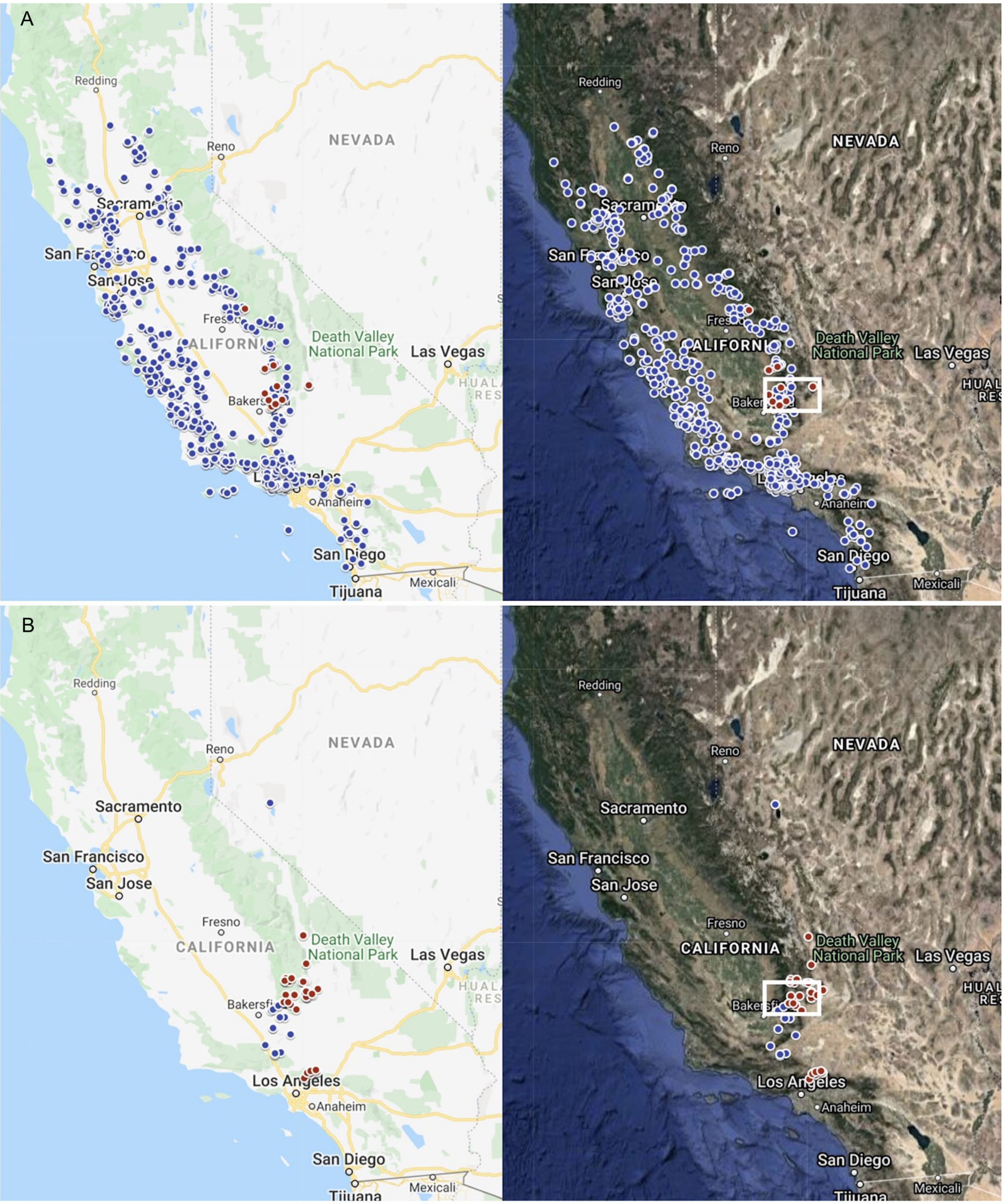

FIGURE 2 Distributions of georeferenced collection sites of herbarium specimens (with and without topographic features) analyzed to detect climatic differences between sister taxa with respect to the mean long-term conditions that they occupy. (A) *Clarkia unguiculata* (blue points) and *C. exilis* (red points); (B) *C. xantiana* subsp. *xantiana* (blue points) and *C. xantiana* subsp. *parviflora* (red points). Boxed areas in maps on right mark the region where field populations were sampled for this study (see Figure 3). Maps created in Google Maps.

and 30 (91%) specimens bore one or more open flowers, indicating that the DOY of specimen collection for these taxa generally reflects the specimen's flowering time at a given site.

Statistical analysis of herbarium-derived data and climate

Closely related taxa might differ with respect to mean flowering time due to evolutionary and/or short-term plastic responses to climate. To test for phenological differences between sister taxa that are independent of variation in local climatic conditions, we controlled statistically for climatic variation among collection sites when testing for differences between taxa in mean flowering time. To characterize each collection site using a multivariate index of its climate, principal components (PCs) for the 24 annual climatic variables were estimated using the pooled data for each pair of sister taxa. In both pairs of taxa, the first two principal components (PC1 and PC2) for the 24 climatic variables together account for >75% of the variance in climate (see Appendices S2 and S3 for contributions and loadings, respectively).

For each data set, the first two PCs were used to determine whether sister taxa differ with respect to their flowering phenology (controlling for site-specific climatic conditions) and in the chronic climatic conditions to which they are exposed. The function prcomp was used to estimate PCs, following centering and scaling of the data to unit variance; the function fviz_pca_var in the package factoextra (v.1.0.7, Kassambara and Mundt, 2020) was used to visualize the contributions of each variable in each PC. These and all subsequent analyses were conducted in RStudio (v. 1.4.1717; RStudio Team, 2020) with R v. 4.1.1 (R Core Team, 2021).

Phenological differences between sister taxa

To determine whether sister taxa differ in their mean flowering date while controlling for the effects of geographic variation in local chronic climatic conditions (which influence DOY; see Results), we conducted four linear regressions on each sister pair (Table 1). In all of these models, the day of year of specimen collection (DOY) was the response variable. For neither taxon pair were either of the two-way interactions (Taxon × PC1 or Taxon × PC2) statistically significant. Below, we present the results of the model with the lowest AIC value. Type III sums of squares were used to detect a significant difference between sister taxa in their mean date of collection, controlling for variation among collection sites with respect to the covariates. The lm function with the default setting for contrasts and the lme4 package (v. 1.1-27.1, Bates et al., 2015) were used.

TABLE 1 Predictor variables included in each of four linear models tested using specimen-based data for each taxon pair to detect significant differences between sister taxa in the day of year of specimen collection, controlling for variation in local climatic conditions summarized in the first two principal components. The boldfaced AIC value indicates the model for which the results are presented below (see text). For the analysis of *C. unguiculata* and *C. exilis*, Models 1 and 2 had similar AIC values, but the latter model was selected because neither interaction term was statistically significant at $P < 0.05$.

	Model 1	Model 2	Model 3	Model 4
Taxon	x	x	x	x
PC1	x	x	x	
PC2	x	x		x
Taxon × PC1	x			
Taxon × PC2	x			
AIC (Taxon pair: *unguiculata* and *exilis*)	4692	**4691**	4714	4790
AIC (Taxon pair: *C. xantiana* subsp. *xantiana* and *C. xantiana* subsp. *parviflora*)	320.85	319.23	**317.24**	325.44

Climatic differences between sister taxa

To determine whether sister taxa differ with respect to the chronic climatic conditions at their respective herbarium specimen collection sites, we used linear models to detect statistically significant differences between taxa with respect to the mean values of the first and the second PCs. In these analyses, either PC1 or PC2 was the response variable, and taxon was the predictor variable. The lm function (using the default setting for contrasts: c[contr.treatment, contr.poly]) in the lme4 package (v. 1.1-27.1; Bates et al., 2015) was used.

Contemporary differences between sister taxa: phenotypic divergence in physiological traits

Sampling of extant populations: sample sizes and life stages

Naturally occurring field populations were surveyed in the region of Lake Isabella, California, United States, in the southern Sierra Nevada, where we sampled populations at elevations ranging from 457 to 1628 m. In spring 2010, 11 sites were identified that, at the onset of spring, contained a population of one or more of our focal taxa with a sufficient number of healthy juvenile plants that we judged we would be able to survey at least 90 individuals for their physiological performance during both vegetative and flowering stages (Figure 3; see Appendices S4 and S5 for a summary of each population's GPS coordinates, elevation, sampling dates, and sample sizes). Each population was surveyed once during vegetative growth and once during

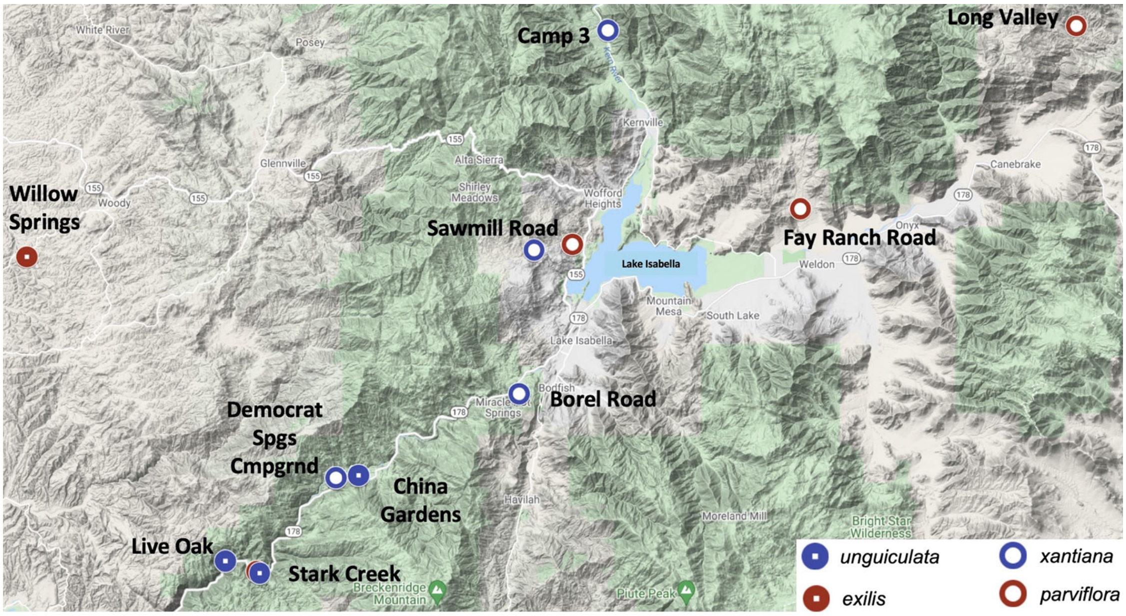

FIGURE 3 Map of populations surveyed for physiological traits and individual fitness in 2010. Map created in Google Maps.

flowering in spring 2010; a different group of individuals was sampled at each life stage. For each survey, 90 individuals were selected at random positions along one or more transects traversing the population, with the restriction that a surveyed plant had to bear at least one leaf long enough to sample with a LI-COR (Lincoln, NE, USA) LI-6400 Portable Photosynthesis System (leaves that were too short did not extend into the leaf chamber). Sampled plants were typically at least 1 m apart. In total, we measured the physiological performance of >1800 individuals. We analyzed and compared these traits while controlling statistically for leaf position on the primary stem and for leaf temperature at the time of measurement, both of which can influence measures of physiological performance (Mazer et al., 2010).

Physiological survey

Gas exchange measurements were recorded on leaves produced by the primary branch on each plant. Measured leaves remained attached to the stem while using a portable infrared gas exchange analyzer (IRGA; LI-COR 6400). We recorded photosynthetic rate (A; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mol H_2O m^{-2} s^{-1}), and the derived variable, A/E, WUE_i μmol_{CO2} $mol_{H2O}{}^{-1}$ × 0.0001). In addition, the position of each measured leaf, recorded as the number of the node that bore it, was recorded; the node just above the cotyledons was referred to as node 1, with subsequently produced nodes counted in sequence. The temperature of the leaf (°C) at the time of measurement was also recorded. Node number and leaf temperature (Tleaf) were included as covariates in our statistical models in order to control for their potential effects on physiological rates, as observed by Mazer et al. (2010). The following LI-COR settings were used: light source, 6400-40 fluorometer; stability, values of CO_2 and H_2O remained stable for at least 15 s with a change in slope of <1; stomatal ratio = 1; flow = 500 µmol s^{-1}; photosynthetically active radiation (PAR_i; PAR in the leaf chamber) = 1500 mol m^{-2} s^{-1}. CO_2 concentration in the IRGA reference chamber was 400 ppm.

For leaves too narrow to fill the entire 2-cm^2 circular area exposed within the leaf chamber of the LI-COR 6400, the actual leaf area exposed was calculated and then the LI-COR 6400 Simulator Software was used to adjust those physiological variables that were influenced by leaf area. To measure the leaf area exposed within the chamber, we used an ink pad to apply black ink to the foam gasket of the chamber; when the chamber was then closed upon the leaf, the gasket stamped ink onto the outer boundary of the leaf's area that was exposed within the chamber. Following gas exchange measurements, each leaf that failed to fill the chamber's circular opening was removed, taped to a piece of paper with transparent tape, and subsequently scanned using a digital scanner. We then used the image analysis program ImageJ (National Institutes of Health, Bethesda, MD, USA; Schneider et al., 2012) to measure the area of each leaf that had been exposed within the LI-COR chamber during gas exchange measurement.

Instantaneous gas exchange measurements and WUE_i provide point estimates of the physiological performance of individual plants and are not equal to the same variables measured over the entire lifespan of individuals (Zelitch, 1982; Serichol-Escobar et al., 2016). In the current study, however, the large sample sizes obtained per taxon, the replication of each taxon within and among 2–4 populations, and the statistical control of variation in other plant attributes that are correlated with instantaneous gas exchange rates (e.g., leaf position on the stem, leaf temperature at the time of measurement, and the life cycle stage at which plants are measured) helped to account for the variance in physiological performance that can obscure differences between taxa. Numerous other recent studies have compared taxa, experimental treatments, or life history stages based on instantaneous gas exchange measurements (Mazer et al., 2010; Franks, 2011; Dudley et al., 2012, 2015; Gorai et al., 2015; Cheng et al., 2017; Gulias et al., 2018; Velikova et al., 2018; Wang et al., 2019; Scalon et al., 2021).

Fitness estimation

In late June to mid-July 2010, all surviving plants were surveyed and, when flower production had ceased, the total number of full-sized or mature fruits borne by each plant was counted to provide an estimate of lifetime reproductive fitness. Many plants, however, experienced high fruit predation by the larvae of *Hyles lineata* (the white-lined sphinx moth: Sphingidae) and, at some locations, by vertebrates, so total fruit production could not be accurately measured on these individuals. Consequently, as plants fully senesced and dried, we collected all aboveground parts (including fruits) from each plant, air-dried each skeleton in a paper bag, and weighed them in the laboratory. All fruits retained by each plant were removed and counted, and the aboveground stem biomass was recorded (in milligrams, without any leaves, flowers, or ovaries). To assess whether aboveground stem biomass is a reasonable proxy for lifetime reproductive success in these *Clarkia* taxa, we measured the relationship between aboveground stem biomass and total fruit production among individuals in each taxon that were sampled during flowering and had no fruit predation. In all taxa, the correlation coefficient between these log-transformed variables was >0.60 (*C. unguiculata*, $r = 0.61$, $n = 151$, $P < 0.0001$; *C. exilis*, $r = 0.\ 90$, $n = 60$, $P < 0.0001$; *C. xantiana* subsp. *xantiana*, $r = 0.76$, $n = 173$, $P < 0.0001$; *C. xantiana* subsp. *parviflora*, $r = 0.84$, $n = 81$, $P < 0.0001$).

In the analyses of phenotypic selection described below, for all individuals, we used aboveground stem biomass as the response variable (i.e., the estimate of individual fitness) (cf. Donovan et al., 2009). The use of final stem biomass as a proxy for fitness resulted in inflated fitness estimates for heavily attacked plants (relative to their actual fitness). However, fruit predation occurred late in an individual's reproductive cycle, when the vast majority of its fruits were developing or fully developed, and new flowers were no longer being produced. Therefore, the final aboveground stem biomass of a harvested individual was not strongly or directly influenced by its predation status, and we assume that the stem biomass of a heavily attacked individual is a good estimate of its potential fitness in the absence of herbivory.

Climatic data

To assess the influence of recent climatic conditions on the physiological rates of populations and taxa, monthly climatic data (cumulative precipitation; mean minimum, maximum and mean temperature; and vapor pressure deficit [VPD]) were extracted from the PRISM climate database (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu) for November 2007–June 2008 (corresponding to the growing season surveyed by Mazer et al., 2010) and for November 2009–June 2010 (the growing season of the current study). These data were examined to determine whether differences between years in the magnitude or direction of physiological divergence between sister taxa was associated with year-specific climate. In addition, these climatic data were used to examine whether physiological differences between sister taxa observed during flowering in the current study were associated with the mean climatic conditions to which their sampled populations were exposed in the month when they were surveyed. When comparing *C. exilis* and *C. unguiculata*, climatic conditions for the months of April and May 2010, respectively, were used; these corresponded most closely to the months when each species were surveyed. When comparing the subspecies of *C. xantiana*, climatic conditions for May 2010 were used for subsp. *xantiana*, except for the Democrat Springs and Saw Mill Road sites, for which conditions in June 2010 were used due to their mid-June survey dates (Appendix S6). For subsp. *parviflora*, climatic conditions for May 2010 were used, except for the Long Valley population, for which June 2010 conditions were used.

Statistical analyses of field data

Physiological divergence between sister taxa

Model structure and parameter estimation

To assess phenotypic divergence, we considered 12 separate linear mixed-effects models, accounting for three gas exchange response variables (photosynthesis, transpiration, and $\mathrm{WUE_i}$) in both vegetative and flowering stages, for each pair of sister taxa (3 traits × 2 stages × 2 taxon pairs). The 12 models are all of the form:

$$\text{GasExchangeTrait} \sim \text{Tleaf} + \text{Node number} + \text{Taxon} + (1|\text{Population})$$

where GasExchangeTrait is either photosynthesis, transpiration, or $\mathrm{WUE_i}$, and population is treated as a random effect nested within taxon. Due to non-normality and heterogeneity in the residuals, we used bootstrap analyses to compute confidence intervals (CI) for the differences between sister taxa in physiological traits, and Bonferroni corrections were applied. Where the 95% CI for the taxon effect does not overlap zero, this is interpreted as a significant difference between taxon means.

Differences between sister taxa in phenotypic selection

Model structure and parameter estimation

We assessed the strength and direction of phenotypic selection on each physiological trait, estimating both total selection (where each physiological trait was used as the sole predictor variable in its own model) and direct selection, in which both photosynthesis and transpiration were included as predictor variables in the same model to detect the independent effects of each of these traits on individual fitness.

The phenotypic selection analyses tested five distinct models for each taxon pair and growth stage:

Model A: Total selection on photosynthetic rate (P):
$$\text{Biomass} \sim \text{P} + \text{Taxon} + \text{Node Number} + \text{Tleaf} + \text{Taxon} \times \text{P} + (1|\text{Population});$$

Model B: Total selection on transpiration (T):
$$\text{Biomass} \sim \text{T} + \text{Taxon} + \text{Node Number} + \text{Tleaf} + \text{Taxon} \times \text{T} + (1|\text{Population});$$

Model C: Total selection on WUE:
$$\text{Biomass} \sim \text{WUE} + \text{Taxon} + \text{Node Number} + \text{Tleaf} + \text{Taxon} \times \text{WUE} + (1|\text{Population});$$

Model D: Direct selection on photosynthetic and transpiration rates:
$$\text{Biomass} \sim \text{P} + \text{T} + \text{Taxon} + \text{Node Number} + \text{Tleaf} + \text{Taxon} \times \text{P} + \text{Taxon} \times \text{T} + (1|\text{Population});$$

Model E: Direct selection:
$$\text{Biomass} \sim \text{P} + \text{T} + \text{Taxon} + \text{Node Number} + \text{Tleaf} + \text{Taxon} \times \text{P} + \text{Taxon} \times \text{T} + \text{P} \times \text{T} + (1 \mid \text{Population}).$$

Model E included the P × T interaction to determine whether the effects on individual fitness of photosynthetic or transpiration rates are independent rather than being influenced by the value of the other trait. Quadratic terms were not included in these models because they were always nonsignificant ($\alpha = 0.05$).

In each of the random intercept models analyzed here, we regarded population as a random effect that is nested within taxon, so we adopted a variance structure allowing different residual variances for each taxon. See, for example, Zuur et al. (2009: Chapter 19) and the R code provided in the supplementary material. These models also account for the effects of leaf position and temperature by including them as covariates.

Because the number of populations sampled per taxon was relatively small and the residual patterns for most of these models exhibited non-normality and heterogeneity of variance, the conditions for maximum likelihood or restricted maximum likelihood methods of parameter estimation were violated. In this situation, a more robust estimation procedure is to use nonparametric resampling. Specifically, we used the cases bootstrap method, which resamples the individuals within population clusters (Leeden et al., 2008). Each confidence interval was computed from 20,000 bootstrap samples using the lmeresampler package (Loy et al., 2021).

Concordance vs. discordance between phenotypic divergence and phenotypic selection

We examined patterns of phenotypic divergence between sister taxa and the direction and magnitude of taxon-specific phenotypic selection on each trait during vegetative growth and during flowering to determine whether the direction and statistical significance of phenotypic divergence is consistent with the difference between taxa in the direction or strength of selection. For each trait, we determined whether there is concordance between the taxon-specific patterns of selection vs. phenotypic divergence during vegetative growth; the taxon-specific patterns of selection vs. phenotypic divergence during flowering; and the patterns of selection during vegetative growth vs. phenotypic divergence between sister taxa expressed during flowering. We were interested in the latter comparison because differences between sister taxa in the intensity or direction of selection during vegetative growth could generate phenotypic divergence between them later in the life cycle. For example, if, during vegetative growth, selection favored rapid transpiration rates in *C. exilis* (but was neutral in *C. unguiculata*) resulting in disproportionately high survivorship of *C. exilis* individuals with high transpiration rates, then mean transpiration rates could be higher in *C. exilis* than in *C. unguiculata* during flowering. In this case, taxon-specific patterns of selection early in the life cycle would be interpreted as causing phenotypic divergence between sister taxa later in the life cycle.

Concordance is identified where (1) there is no difference between sister taxa in mean phenotype or in the magnitude of the selection gradients (i.e., there is no Taxon × Trait interaction affecting total biomass) or (2) sister taxa differ in mean phenotype, and the direction of selection on the focal trait differs between sister taxa in a manner that is consistent with the phenotypic divergence between them. Discordance is identified where (1) there is no difference between sister taxa in mean phenotype, but the direction of selection differs qualitatively between them (i.e., there is a significant Taxon × Trait interaction); or (2) sister taxa differ significantly in mean phenotype, but the direction of selection on the focal trait does not differ between them (i.e., there is no Taxon × Trait interaction); or (3) sister taxa differ in mean phenotype, but the direction of selection on the focal trait differs between them in way that is inconsistent with the phenotypic divergence between them (Table 2).

TABLE 2 Types of concordance and discordance between physiological divergence between sister taxa and the direction of phenotypic selection on focal trait

Type of Concordance/ Discordance	Phenotypic divergence between sister taxa?	Difference between sister taxa in selection gradient of the focal trait?
Concordance Type 1	No	No
Concordance Type 2	Yes	Yes; selection gradients **reinforce** phenotypic divergence
Discordance Type 1	Yes	Yes; selection gradients **oppose** phenotypic divergence
Discordance Type 2	Yes	No
Discordance Type 3	No	Yes

RESULTS

Historical phenological and climate differences between sister taxa: herbarium-based study

Phenological divergence between sister taxa

Of the four linear models tested (Table 1) to detect differences between sister taxa in the DOY of specimen collection (controlling for variation in PC1 and PC2), the model with the lowest AIC value was Model 2 for *C. unguiculata* and *C. exilis*, and Model 3 for *C. xantiana* subsp. *xantiana* and subsp. *parviflora* (Table 1). In both pairs of sister taxa, the selfing taxon was collected in flower earlier than the outcrossing taxon (Table 3). *Clarkia exilis* was collected on average 11 days earlier than *C. unguiculata*, and *C. xantiana* subsp. *parviflora* was collected on average 6 days earlier than *C. xantiana* subsp. *xantiana* (Figure 4).

Climatic differences between sister taxa

In the pooled data for *C. unguiculata* and *C. exilis*, PC1 and PC2 account for 46% and 31% of the variance in climate, respectively; in *C. xantiana* subsp. *xantiana* and subsp. *parviflora*, PC1 and PC2 explain 62% and 21% of the variance, respectively (Appendix S2). Across the geographic distributions of both taxon pairs, the loadings of PC1 indicate that it is an axis dominated by temperature, while PC2 is an axis dominated by humidity (Appendix S3).

In each taxon pair, sister taxa differ with respect to long-term (1951–1980) mean climatic conditions at the sites of specimen collection (Table 4, Figure 5). The analysis of the pooled data for *C. unguiculata* and *C. exilis* detected a significant difference between them in the mean value of PC2 (but not PC1), with *C. exilis* exhibiting a lower mean than *C. unguiculata*. On average, *C. exilis* occupies warmer and drier sites than *C. unguiculata*; sites with *C. exilis* are associated with lower relative humidity (RH), a higher mean Hargreaves cumulative moisture deficit (CMD), lower cumulative moisture index (CMI), higher evaporation, and a higher mean annual temperature (MAT) than sites with *C. unguiculata* (Appendix S3). This difference is partly due to *C. unguiculata* having a larger geographic range (Figure 2), particularly its occupation of sites at higher elevations and in more northern and coastal regions.

The analysis of the pooled data for *Clarkia xantiana* subsp. *xantiana* and subsp. *parviflora* detected a significant difference between them with respect to the PC1 (but not PC2), with subsp. *parviflora* exhibiting a higher mean value than subsp. *xantiana* (Table 4). Within its geographic range, the herbarium records suggest that *C. xantiana* subsp. *parviflora* occupies sites that were historically (1951–1980) cooler than those occupied by subsp. *xantiana*. The sites from which subsp. *parviflora* was collected are associated with higher CMI, more degree days <18°C, more degree days <0°C, fewer degree days >5°C, and lower MAT than those from which subsp. *xantiana* was collected (Appendix S3 and Figure 5).

Contemporary differences between sister taxa: phenotypic divergence

In both taxon pairs, the sister taxa differ with respect to all three physiological traits, but the magnitude and the direction of the divergence differed between growth stages (Table 5, Figure 6; see Appendix S7 for application of Bonferroni correction). *Clarkia exilis* and *C. unguiculata* did not differ physiologically during vegetative growth, but during flowering, *C. exilis* exhibited higher photosynthetic and transpiration rates, and lower WUE$_i$, than *C. unguiculata*. *Clarkia xantiana* subsp. *parviflora* differed from subsp. *xantiana* during both growth stages, but the traits and the direction of the difference changed over time. During vegetative growth, subsp. *parviflora* exhibited a higher transpiration rate and lower WUE$_i$ than subsp. *xantiana*, while during flowering subsp. *parviflora* exhibited a lower mean photosynthetic rate and higher WUE$_i$ than subsp. *xantiana*.

Contemporary differences between sister taxa: phenotypic selection

Differences between sister taxa in the strength or direction of phenotypic selection on the three focal traits were common in *C. unguiculata* and *C. exilis*, but undetectable in *C. xantiana* subsp. *xantiana* and subsp. *parviflora*

TABLE 3 Analysis of variance and summary of linear model designed to test for differences between sister taxa in the day of year of specimen collection (DOY), controlling for variation in PC1 and PC2. Parameter estimates and least squares means of the DOY for each taxon are displayed below the analysis of variance table. (A) *Clarkia unguiculata* vs. *C. exilis* (*exilis* is the reference taxon). (B) *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora* (*parviflora* is the reference taxon). Significance levels: ***$P < 0.0001$; **$P < 0.001$; *$P < 0.05$. CL: Confidence limit. Figure 3 illustrates the difference between taxon means.

A. *Clarkia unguiculata* vs. *C. exilis* — Predictor variables: Taxon + PC1 + PC2

Source of variation	df	SS	*F*	*P*
Taxon	1	3367	7.71	0.0056**
PC1	1	46,526	106.47	<2.2e-16***
PC2	1	11,116	25.44	5.71e-07***
Residuals	767	335,175		

Adjusted $R^2 = 0.16$

	Coefficients			
	Estimate	SE	*t*	Pr(>|*t*|)
Intercept	133.82	3.93	34.01	<2e-16***
Taxon (*unguiculata*)	11.15	4.02	2.78	0.0056**
PC1	2.34	0.23	10.32	<2e-16***
PC2	1.45	0.29	5.04	5.71e-07***

	Mean DOY	SE	Lower CL	Upper CL
exilis	134	3.94	126	142
unguiculata	145	0.77	143	146

B. *Clarkia xantiana* subsp. *xantiana* vs. subsp. *parviflora* — Predictor variables: Taxon + PC1

Source of variation	df	SS	*F*	*P*
Taxon	1	630	5.01	0.0289*
PC1	1	1051	8.35	0.0053**
Residuals	62	7807		

Adjusted $R^2 = 0.11$

	Coefficients			
	Estimate	SE	*t*	Pr(>|*t*|)
Intercept	144.63	1.90	76.28	<2e-16***
Taxon(*xantiana*)	6.69	2.99	2.24	0.0289*
PC1	1.12	0.39	2.89	0.0053**

	DOY	SE	Lower CL	Upper CL
parviflora	145	1.9	141	148
xantiana	151	2.2	147	156

(Figure 7). During both growth stages, significant taxon × trait interactions were detected for transpiration and WUE$_{\text{i}}$ in *C. unguiculata* and *C. exilis* (Table 6). During vegetative growth, phenotypic selection favored high transpiration rates and low WUE$_{\text{i}}$ in *C. exilis* but not in *C. unguiculata* (at $P < 0.01$; Figure 7, Table 6). During flowering, selection favored high transpiration rates in *C. unguiculata* and low transpiration rates in *C. exilis*, and selection favored high WUE$_{\text{i}}$ in *C. exilis* but not in *C. unguiculata*. The patterns of direct vs. total selection on photosynthetic and transpiration rates did not differ (Figure 7A vs. 7B).

Concordance between phenotypic divergence and phenotypic selection

All three ecophysiological traits exhibited context-specific concordance between the phenotypic divergence between sister taxa and the direction or strength of selection; the degree and type of concordance depended on taxon pair and developmental stage (Table 7). In both taxon pairs, the patterns of concordance exhibited by direct selection on photosynthesis and transpiration are the same as those exhibited by total selection, so only the latter are described here.

In *C. unguiculata* and *C. exilis*, among the nine comparisons made between physiological divergence and the taxon-specific directions of total selection on each trait, three cases of concordance were detected. Photosynthesis exhibited concordance (Type 1; Table 2) during vegetative growth; the species did not diverge phenotypically nor did their selection gradients differ (Table 7). In this taxon pair, both transpiration and WUE$_{\text{i}}$ exhibited concordance (Type 2) when the phenotypic divergence observed in the flowering stage is compared to the pattern of selection observed during vegetative growth. In *C. xantiana* subsp. *xantiana* and subsp. *parviflora*, among the nine comparisons between divergence and taxon-specific total selection, three cases of concordance were detected. Photosynthesis exhibited concordance (Type 1) during vegetative growth; the sister taxa did not differ phenotypically or in their selection gradients. Transpiration also exhibited concordance (Type 1) when selection and divergence were measured during flowering, and when divergence during flowering was compared to taxon-specific selection during vegetative growth. No cases of concordance were detected for WUE$_{\text{i}}$.

Climatic differences between years and between sister taxa when surveyed during flowering

The qualitative comparison of climatic conditions during the 2007–2008 and 2009–2010 growing seasons for the 11 sites surveyed in this study indicated that the 2009–2010 growing season was wetter and cooler than 2007–2008 (Appendix S8). The mean cumulative precipitation across sites was 399.4 mm from November 2009 to May 2010 vs. 246.5 mm from November 2007 to May 2008. The mean monthly average daily temperature was 10.7°C for 2009–2010 vs. 11.2 for 2007–2008. The wetter and cooler conditions in 2009–2010 resulted in lower mean values

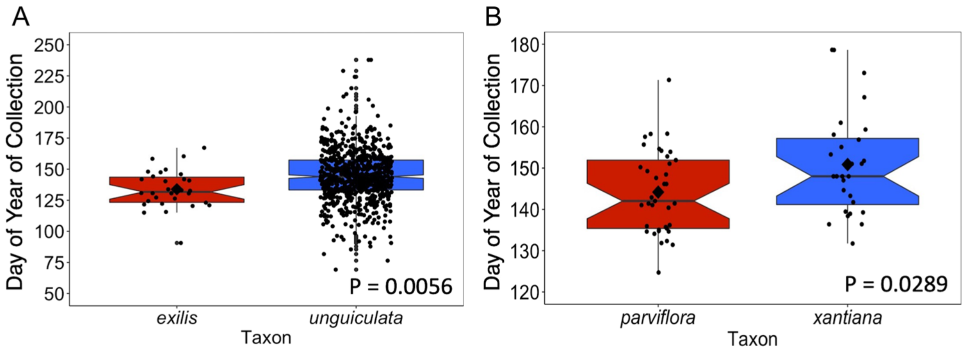

FIGURE 4 Box plots illustrating divergence between sister taxa in the day of year of specimen collection (DOY), independent of variation in long-term climatic conditions among collection sites, based on herbarium data. (A) *Clarkia unguiculata* vs. *C. exilis*. (B) *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora*.

TABLE 4 Summary of linear models designed to test for differences between sister taxa in climatic conditions represented by the mean values of PC1 or PC2. (A) *Clarkia unguiculata* vs. *C. exilis* (*exilis* is the reference taxon). (B) *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora* (*parviflora* is the reference taxon). Significance levels: ****$P < 0.0001$; ***$P < 0.001$; **$P < 0.05$; *$P < 0.10$. CL: confidence limit.

A. *Clarkia unguiculata* vs. *C. exilis* — Response variable: PC2				
Source of variation	**df**	**SS**	***F***	***P***
Taxon	1	329.6	48.66	6.57e-12****
Residuals	769	5209.6		
Adjusted $R^2 = 0.06$				
Coefficients	**Estimate**	**SE**	***t***	***P***
Intercept	−3.25	0.475	−6.84	1.63e-11****
Taxon(*unguiculata*)	3.38	0.485	6.98	6.57e-12****
	Mean value of PC2	**SE**	**Lower CL**	**Upper CL**
exilis	−3.25	0.475	−4.18	−2.32
unguiculata	0.13	0.10	−0.06	0.32
B. *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora* — Response variable: PC1				
Source of variation	**df**	**SS**	***F***	***P***
Taxon	1	103.9	8.33	0.0053**
Residuals	63	785.4		
Adjusted $R^2 = 0.10$				
Coefficients	**Estimate**	**SE**	***t***	***P***
Intercept	1.10	0.581	1.89	0.06
Taxon(*xantiana*)	−2.55	0.884	−2.89	0.0053**
	Mean value of PC1	**SE**	**Lower CL**	**Upper CL**
parviflora	1.10	0.580	−0.06	2.26
xantiana	−1.45	0.667	−2.79	−0.12

across sites for minimum VPD ($\mathrm{VPD}_{\mathrm{min:}}$ 2.5 hPA in 2009–2010 vs. 3.1 hPA for 2007–2008) and maximum VPD ($\mathrm{VPD}_{\mathrm{max}}$: 14.9 hPa in 2009–2010 vs. 17.6 hPa in 2007–2008) in the current study relative to Mazer et al. (2010).

In the current study, sister taxa experienced different conditions during the month closest to the time when physiological traits were measured during flowering (Appendix S6). Across the surveyed populations, *C. exilis* was exposed to 23% lower mean $\mathrm{VPD}_{\mathrm{max}}$ than was *C. unguiculata* (13.3 vs. 17.3 hPa, respectively) and to 55% lower mean $\mathrm{VPD}_{\mathrm{min}}$ (1.11 vs. 2.4 hPa, respectively). The difference in mean VPD between the two subspecies of *C. xantiana* was both smaller and less consistent than that between *C. exilis* and *C. unguiculata*. *C. xantiana* subsp. *parviflora* populations were exposed to a somewhat lower mean $\mathrm{VPD}_{\mathrm{max}}$ than *C. xantiana* subsp. *xantiana* (22.4 vs. 24.2 hPa, or 7% lower) during flowering, but a higher mean $\mathrm{VPD}_{\mathrm{min}}$ (4.9 vs. 4.3 hPa, or 13% higher).

DISCUSSION

In each pair of sister taxa examined here, historical herbarium records revealed that each selfing taxon flowers earlier in the spring than its outcrossing sister taxon, independent of variation among collection sites in local long-term climatic conditions. Due to these intrinsic phenological differences, we hypothesized that because local conditions experienced by these *Clarkia* taxa become increasingly dry as spring progresses, the self-fertilizing taxa experience more mesic conditions and a lower risk of desiccation during flowering than their outcrossing sister taxa do. In turn, we predicted that this ecological difference would promote relatively fast gas exchange rates and low $\mathrm{WUE}_{\mathrm{i}}$ in the self-fertilizing taxa due to adaptive evolution, phenotypic plasticity, or both and that patterns of phenotypic selection would be consistent with this divergence. Sister taxa would not be expected to differ physiologically or with respect to phenotypic selection on physiological traits during vegetative growth (i.e., before late-spring drought), unless there is a strong correlation between physiological rates expressed during vegetative growth and flowering.

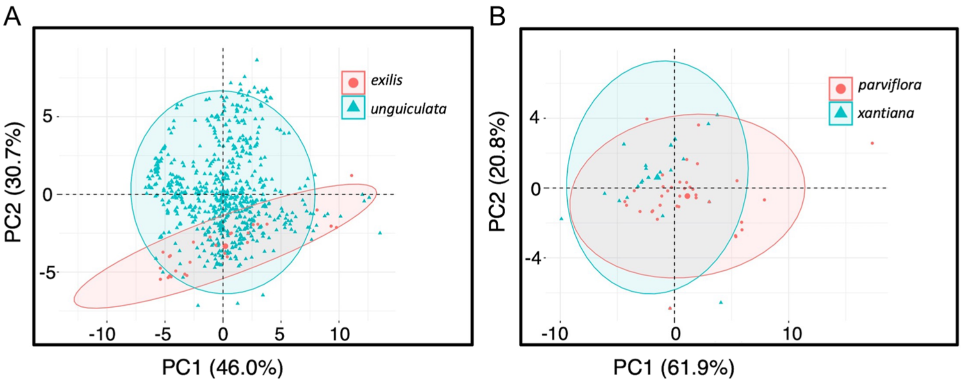

FIGURE 5 Occupation of climate space by sister taxa, as represented by the first two principal components. (A) *Clarkia unguiculata* and *C. exilis*. (B) *Clarkia xantiana* subsp. *xantiana* and *C. xantiana* subsp. *parviflora*. Due to the much broader geographic distribution of *Clarkia unguiculata* relative to *C. exilis* (Figure 2), the former occupies a much wider range of climatic conditions represented by PC2.

TABLE 5 Least squares means (SE, *n*) of photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration (mol H_2O m^{-2} s^{-1}, and WUE (μmol$_{CO2}$ mol$_{H2O}$$^{-1}$ × 0.0001) for each taxon, controlling for node number and Tleaf, and with population treated as a random effect. (A) Trait means during vegetative growth. (B) Trait means during flowering. Boldfaced means with distinct superscripts differ between sister taxa during a given developmental stage.

Response variable	*unguiculata*	*exilis*	*xantiana*	*parviflora*
A. Vegetative growth				
Photo	20.7 (2.3, 306)	21.3 (3.4, 116)	30 (2, 249)	31.2 (2.3, 226)
Trans	0.00965 (0.00073, 306)	0.00902 (0.0011, 116)	**0.0154**^{b} (0.001, 249)	**0.0202**^{a} (0.0012, 226)
WUE$_i$	0.238 (0.02, 306)	0.255 (0.03, 116)	**0.212**^{a} (0.015, 249)	**0.166**^{b} (0.017, 226)
B. Flowering				
Photo	**19.1**^{b} (2.7, 286)	**25.2**^{a} (3.8, 151)	**20.8**^{a} (2.4, 280)	**18.7**^{b} (2.8, 196)
Trans	**0.0102**^{b} (0.0015, 286)	**0.0152**^{a} (0.0022, 151)	0.00865 (0.00098, 280)	0.00801 (0.0012, 196)
WUE$_i$	**0.222**^{a} (0.0051, 286)	**0.165**^{b} (0.0049, 151)	**0.294**^{b} (0.047, 280)	**0.338**^{a} (0.056, 196)

Sister taxa differed in all focal physiological traits, but the degree and (in one case) the direction of divergence depended on life stage (Figure 6). In general, self-pollinating taxa exhibited higher gas exchange rates, corroborating our prediction. In addition, the physiological differences between *C. exilis* and *C. unguiculata* were statistically significant only during flowering, consistent with our prediction. By contrast, in *C. xantiana*, the two subspecies differed physiologically more strongly during vegetative growth than during flowering. In both pairs of sister taxa, the expectation that physiological divergence between sister taxa would be consistent with differences between them in the strength or direction of phenotypic selection was not upheld in most cases. In 6 of 18 comparisons, patterns of total selection on each trait were concordant with the phenotypic divergence (or lack thereof) between sister taxa (Table 7). In sum, patterns of selection on physiological traits did not reliably reflect the observed divergence between sister taxa. This discrepancy may be particularly common in taxa occupying temporally heterogeneous environments, underscoring the need for replicated studies of the direction of selection within and among taxa.

The extent to which seasonal drought drives trait evolution and species divergence in plants has been a topic of longstanding interest. Due to the progressive loss of soil moisture that characterizes late spring in Mediterranean climates, plant species that are adapted to such conditions offer an opportunity to identify the traits that evolve in response to the risk of dehydration. Recent studies of conspecific and interspecific populations exposed to such risk have tested predictions concerning the phenotypes that will evolve when soil moisture is low, revealing the potential for the rapid evolution of traits that promote drought escape (e.g., relative fast germination and growth, earlier flowering, and larger leaf size) (Bazzaz, 1979; Heschel and Riginos, 2005; Franks et al., 2007; Lowry et al., 2008; Wu et al., 2010; Franks, 2011; Kigel et al., 2011; Heschel et al., 2017; Dickman et al., 2019; Metz et al., 2020) or dehydration avoidance (e.g., slower growth, higher water-use efficiency, decreased transpiration, greater succulence, higher trichome density, and smaller leaf size) (Arntz and Delph, 2001; Kooyers et al., 2015; Heschel et al., 2017; Anstett et al., 2021; Burnette and Eckhart, 2021).

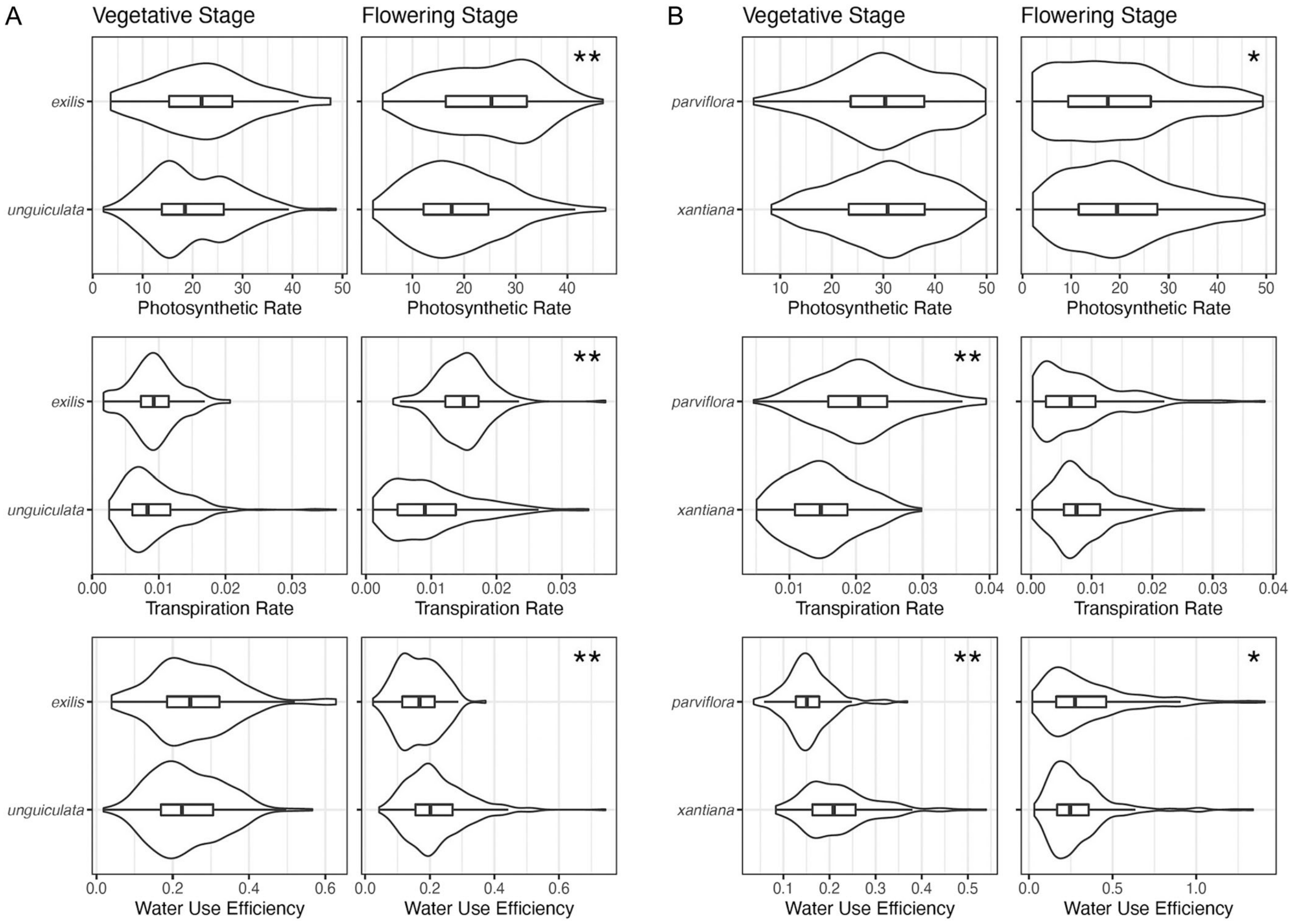

FIGURE 6 Differences in physiological traits between sister taxa; each pair of taxa was analyzed separately. (A) *Clarkia unguiculata* vs. *C. exilis*. (B) *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora*. The significance of the differences between taxa was assessed using 90% and 95% bootstrap CIs for the taxon effects. Single asterisks (*) indicate cases where the 90% CI for the taxon effect does not contain zero, while the double asterisks (**) indicate cases where the 95% CI for the taxon effect does not contain zero. All tests for which the difference between the means of the two sister taxa was significant at $\alpha = 0.05$ withstood Bonferroni correction (Appendix S7). The small vertical bars in each violin plot indicate the medians. Units for physiological traits are as follows: photosynthesis, μmol CO_2 m^{-2} s^{-1}; transpiration, mol H_2O m^{-2} s^{-1}; instantaneous water-use efficiency (WUE_i, μmol CO_2/mol H_2O × 0.0001).

Studies of the genus *Mimulus* have provided particularly clear cases in which differences between populations or higher taxa in their exposure to drought are associated with phenotypic divergence in flowering time, with early flowering associated with more arid conditions (Lowry et al., 2008, 2009; Kigel et al., 2011; Ivey and Carr, 2012; Wolfe and Tonsor, 2014; Anderson et al., 2015; Kooyers et al., 2015; Metz et al., 2020). For example, in a greenhouse study of 14 pairs of coastal and inland populations of *Mimulus guttatus*, Lowry et al. (2008) found that inland populations, which experience greater summer drought than the coastal populations do, flowered earlier and produced smaller leaves and corollas than their coastal counterparts. Evidence that this geographic pattern is the result of adaptive evolution is provided by a complementary reciprocal transplant experiment, in which Lowry et al. (2008) found that early flowering contributes to population persistence where soil moisture is limiting. Consistent with these observations, in a study of selection in two populations of *M. guttatus* native to a montane habitat with dry midsummer soils vs. a temperate coastal site, Hall and Willis (2006) found that the difference between sites in the pattern of selection on flowering time reinforced the phenotypic divergence between the parent populations.

The patterns above are also consistent with subsequent studies. Ivey and Carr (2012) examined phenotypic selection in greenhouse populations of *M. guttatus* and its self-pollinating, earlier-flowering, shorter-lived, and summer drought-adapted congener, *M. nasutus*. Two populations per species were raised in wet and in dry conditions, and phenotypic selection was measured; early flowering contributed most positively to individual fitness where the risk of dehydration was elevated, particularly in *M. nasutus*. Similarly, in a study of two sympatric *Mimulus* species that differ in their microhabitat preferences, Ferris and Willis (2018) measured selection on flowering time, flower size,

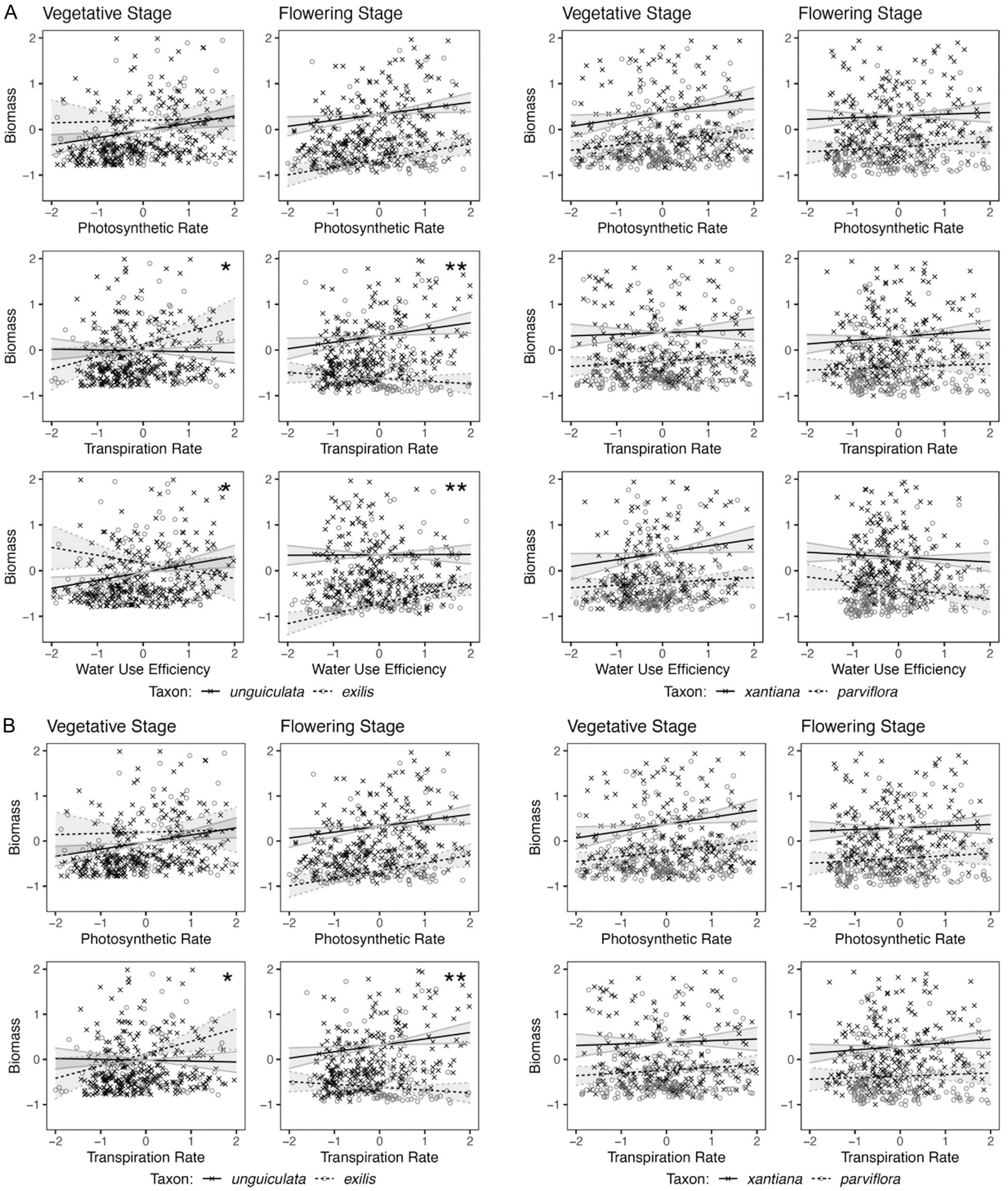

FIGURE 7 Taxon-specific selection gradients during vegetative growth and flowering for photosynthesis, transpiration, and WUE_i. (A) Total selection gradients for each trait (Models A, B, C). (B) Direct selection gradients for photosynthesis and transpiration (Model D). The significance of the differences between taxa in the slope of the relationship between the physiological trait and biomass (i.e., the taxon × trait interaction) was assessed using 90% (*) and 95% (**) bootstrap CIs for the taxon effects. The shaded regions indicate the range of slopes captured by the 95% bootstrap CI for the gradient. Units for physiological traits are as follows: photosynthesis, μmol CO_2 m^{-2} s^{-1}; transpiration, mol H_2O m^{-2} s^{-1}; instantaneous water-use efficiency (WUE_i, μmol CO_2/mol H_2O × 0.0001).

plant height, and leaf shape in a hybrid population grown in the dry soils of the granite outcrops occupied by the early-flowering *M. laciniatus* and in the relatively mesic meadows occupied by *M. guttatus*. They found that the direction of habitat-specific selection was consistent with the differences between species in flowering time and plant size.

Similar patterns have been found at broader geographic scales. In a study of intraspecific variation in *Arabidopsis thaliana*, 48 lineages were collected in Spain across an elevation gradient. When raised under controlled conditions, low-elevation genotypes, which were historically exposed to relatively low precipitation, exhibited earlier bolting, lower net photosynthesis, slower transpiration rates, and higher WUE than those sourced from high elevations (Wolfe and Tonsor, 2014). A link between flowering time and adaptation to drought was also observed among populations of 10 species of annuals distributed across a Mediterranean-desert rainfall gradient (Kigel et al., 2011); when grown in a common environment, each species exhibited a positive relationship among populations between flowering date and rainfall.

In *Clarkia*, two studies have measured both divergence between sister taxa in fitness-related traits and taxon-specific selection on these traits. The first is a comprehensive study of phenotypic selection on flowering time and leaf and floral traits in *C. xantiana* subsp. *xantiana* and *C. xantiana* subsp. *parviflora* (Anderson et al., 2015). In this study, 6–12 populations of each subspecies were raised at three field sites: two sites at which only one of the two subspecies naturally occurs, and one site located in the contact zone between them. For some traits, variation among sites in the direction of selection was concordant with predictions based on the phenotypic divergence between, and the geographic range of, the subspecies. For example, selection favored individuals with relatively long leaves, higher leaf relative growth rate, and longer petals in the home site of *C. xantiana* subsp. *xantiana*, which also exhibits higher mean values of these traits than subsp. *parviflora*. In addition, selection favored earlier flowering only in the home site of *C. xantiana* subsp. *parviflora*, which flowers earlier on average than subsp. *xantiana*. In many cases, the strength and significance of the selection coefficients depended on the fitness component (survival, probability of fruiting, or seed production) examined and, as in the current study, they were sometimes context or taxon-specific. Another study examined selection and physiological divergence in nine field populations of *C. unguiculata* and *C. exilis* measured in situ during vegetative growth and at the onset of flowering (Dudley et al., 2012). While patterns of selection on photosynthetic rates were generally concordant with interspecific divergence in this trait (when populations were pooled), populations and sampling periods differed in the strength and direction of selection on photosynthetic rate, and there was low concordance between divergence and selection for either transpiration rate or WUE$_i$. The context-specific relationships observed in these two studies underscore the importance of replicating estimates of selection gradients and differentials in multiple populations, developmental stages, and years.

In all four *Clarkia* taxa examined in the current study, the focal physiological traits had been recorded previously during vegetative growth and flowering in spring 2008 (Mazer et al., 2010), when conditions were both drier and warmer than those of the current study (Appendix S8). In both 2008 and 2010, the magnitude or direction of physiological divergence between sister taxa changed between vegetative growth and flowering, revealing the lability of these physiological traits (Table 8). The detection of year-, taxon- and developmental stage-specific patterns of divergence and selection draws attention to the importance of repeating such studies when aiming to generate general conclusions concerning their concordance.

Patterns of physiological divergence between sister taxa and their relation to mean flowering time

In both pairs of *Clarkia* sister taxa examined here, the herbarium specimen-based analyses confirmed that when the effects on flowering time of the long-term climatic conditions at the sites of specimen collection were controlled statistically, the self-pollinating taxa have historically flowered earlier than their outcrossing sister taxa (Figure 4). Moreover, the specimen-based analyses corroborate patterns based on phenological observations in the field of the two subspecies of *C. xantiana* (Moore and Lewis, 1965; Eckhart and Geber, 1999; Eckhart et al., 2004; Guerrant, 2019), and field and greenhouse observations of *C. exilis* and *C. unguiculata* (Vasek, 1958; Vasek and Sauer, 1971; Schneider and Mazer, 2016).

The particularly early mean collection date for herbarium specimens of *C. exilis* (day 134 [14 May) relative to *C. unguiculata* (24 May), *C. xantiana* subsp. *parviflora* (24 May) and *C. xantiana* subsp. *xantiana* (31 May; Table 3) and the low values for vapor pressure deficit (VPD) observed when the two field populations of *C. exilis* in the current study began to flower (Appendix S6) support the inference that *C. exilis* may be the taxon most likely to escape the desiccating effects of intensifying late-spring drought. If *C. exilis* reliably escapes drought, this could explain its high photosynthetic and transpiration rates (and low WUE$_i$) relative to *C. unguiculata* (Figure 6). However, the previous study of physiological divergence in these taxa (Mazer et al., 2010), reported that *C. exilis* exhibited a faster photosynthetic rate than *C. unguiculata* only during vegetative growth and did not detectably differ from *C. unguiculata* in transpiration rate during either growth stage (Table 8). This kind of intertaxon inconsistency was also observed for *C. xantiana*. In spring 2008, *C. xantiana* subsp. *parviflora* had higher mean photosynthetic and transpiration rates than subsp. *xantiana* during both vegetative growth and flowering, but in spring 2010, subsp. *parviflora* had a higher transpiration rate than subsp. *xantiana* during

TABLE 6 Selection coefficients and 95% CIs for predictor variables in each of five models per taxon pair and growth stage (vegetative vs. flowering). For each model, filled cells in its column indicate the predictor variables included in the model. (A) Vegetative growth, *Clarkia unguiculata* vs. *C. exilis*. (B) Vegetative growth, *Clarkia xantiana* subsp. *xantiana* vs. subsp. *parviflora*. (C) Flowering, *Clarkia unguiculata* vs. *C. exilis*. (D) Flowering, *Clarkia xantiana* subsp. *xantiana* vs. subsp. *parviflora*. Units for physiological traits are as follows: Photosynthesis, μmol CO_2 m^{-2} s^{-1}; Transpiration, mol H_2O m^{-2} s^{-1}; instantaneous water use efficiency (WUE_i, μmol CO_2/mol H_2O × 0.0001). Significance: **P* < 0.10. ***P* < 0.05. Models A, B, and C estimate total phenotypic selection on each physiological trait; Models D and E measure direct selection on photosynthetic and transpiration rates, with the effect of each variable estimated independent of the effect of the other.

Predictor	Model A	Model B	Model C	Model D	Model E
A. *Clarkia unguiculata* vs. *C. exilis* (*unguiculata* is the baseline), Vegetative growth stage					
Photo	0.158** (0.045, 0.265)			0.201** (0.088, 0.324)	0.220** (0.095, 0.346)
Trans		−0.020 (−0.139, 0.057)		−0.106** (−0.219, −0.032)	−0.102** (−0.220, −0.020)
WUE_i			0.175** (0.065, 0.298)		
Taxon *exilis*	0.220** (0.032, 0.524)	0.146* (−0.007, 0.421)	0.205** (0.039, 0.500)	0.284** (0.090, 0.647)	0.287** (0.082, 0.648)
Taxon × Photo	−0.131 (−0.381, 0.147)			−0.347 (−0.779, 0.131)	−0.342 (−0.765, 0.105)
Taxon × Trans		0.293* (−0.037, 0.645)		0.453* (−0.043, 0.951)	0.418* (−0.030, 0.901)
Taxon × WUE_i			−0.344* (−0.663, 0.008)		
Photo × Trans					−0.075 (−0.156, 0.060)
Node number	0.081** (0.054, 0.113)	0.090** (0.068, 0.127)	0.086** (0.062, 0.116)	0.077** (0.053, 0.109)	0.076** (0.052, 0.108)
Tleaf	0.031** (0.007, 0.093)	0.032** (0.008, 0.096)	0.039** (0.025, 0.101)	0.035** (0.020, 0.109)	0.036** (0.017, 0.103)
B. *Clarkia xantiana* subsp. *xantiana* vs. subsp. *parviflora* (*xantiana* is the baseline), Vegetative growth stage					
Photo	0.153** (0.027, 0.279)			0.201** (0.044, 0.359)	0.207** (0.046, 0.366)
Trans		0.036 (−0.072, 0.140)		−0.089 (−0.221, 0.041)	−0.096 (−0.228, 0.035)
WUE_i			0.150** (0.029, 0.271)		
Taxon *parviflora*	−0.600** (−0.890, −0.324)	−0.619** (−0.913, −0.340)	−0.652** (−0.956, −0.358)	−0.615** (−0.907, −0.337)	−0.617** (−0.907, −0.338)
Taxon × Photo	−0.038 (−0.200, 0.125)			−0.072 (−0.261, 0.118)	−0.074 (0.261, 0.117)
Taxon × Trans		0.025 (−0.117, 0.171)		0.068 (−0.094, 0.229)	0.074 (−0.091, 0.238)
Taxon × WUE_i			−0.095 (−0.234, 0.044)		
Photo × Trans					0.023 (−0.043, 0.089)
Node number	0.137** (0.094, 0.183)	0.142** (0.098, 0.189)	0.137** (0.092, 0.187)	0.134** (0.090, 0.182)	0.133** (0.090, 0.180)
Tleaf	0.004 (−0.022, 0.033)	−0.003 (−0.029, 0.025)	0.013 (−0.016, 0.043)	0.009 (−0.019, 0.039)	0.010 (−0.018, 0.040)
C. *Clarkia unguiculata* vs. *C. exilis* (*unguiculata* is the baseline), Flowering					
Photo	0.131** (0.036, 0.228)			0.089 (−0.027, 0.207)	0.089 (−0.027, 0.204)
Trans		0.142** (0.012, 0.282)		0.083 (−0.078, 0.260)	0.078 (−0.070, 0.249)
WUE_i			0.005 (−0.078, 0.083)		
Taxon *exilis*	−0.985** (−1.483, −0.474)	−0.927** (−1.370, −0.434)	−1.068** (−1.588, −0.547)	−0.873** (−1.281, −0.333)	−0.885** (−1.311, −0.350)
Taxon × Photo	0.038 (−0.112, 0.183)			0.153 (−0.034, 0.339)	0.153 (−0.038, 0.343)
Taxon × Trans		−0.204** (−0.384, −0.041)		−0.238** (−0.462, −0.033)	−0.228** (−0.462, −0.026)
Taxon × WUE_i			0.212** (0.025, 0.403)		
Photo × Trans					0.019 (−0.090, 0.093)
Node number	0.077** (0.057, 0.100)	0.082** (0.062, 0.104)	0.080** (0.059, 0.103)	0.078** (0.058, 0.100)	0.077** (0.058, 0.100)
Tleaf	0.019 (−0.034, 0.069)	0.008 (−0.040, 0.054)	0.018 (−0.033, 0.067)	0.031 (−0.013, 0.082)	0.028 (−0.017, 0.082)

(Continues)

TABLE 6 (Continued)

Predictor	Model A	Model B	Model C	Model D	Model E
D. *Clarkia xantiana* subsp. *xantiana* vs. subsp. *parviflora* (*xantiana* is the baseline), Flowering					
Photo	0.037 (−0.067, 0.141)			−0.009 (−0.120, 0.105)	−0.008 (−0.121, 0.106)
Trans		0.079 (−0.039, 0.194)		0.083 (−0.047, 0.212)	0.082 (−0.039, 0.203)
WUE_i			−0.053 (−0.172, 0.073)		
Taxon *parviflora*	−0.675** (−0.841, −0.496)	−0.659** (−0.815, −0.493)	−0.677** (−0.838, −0.502)	−0.657** (−0.823, −0.483)	−0.655** (−0.826, −0.474)
Taxon × Photo	0.017 (−0.171, 0.207)			0.063 (−0.179, 0.305)	0.063 (−0.179, 0.307)
Taxon × Trans		−0.044 (−0.182, 0.090)		−0.083 (−0.262, 0.091)	−0.085 (−0.283, 0.103)
Taxon × WUE_i			−0.069 (−0.286, 0.153)		
Photo × Trans					0.005 (−0.085, 0.097)
Node number	0.077** (0.055, 0.099)	0.078** (0.056, 0.100)	0.080** (0.058, 0.103)	0.078** (0.055, 0.100)	0.078** (0.054, 0.100)
Tleaf	−0.001 (−0.024, 0.020)	−0.002 (−0.025, 0.018)	−0.006 (−0.028, 0.015)	−0.002 (−0.026, 0.021)	−0.002 (−0.026, 0.020)

vegetative growth but a lower photosynthetic rate during flowering (Table 8). Collectively, these patterns indicate that the historical mean flowering dates of these taxa do not reliably predict that the relatively early-flowering taxa will consistently achieve higher gas exchange rates than their later-flowering sister taxa. These inconsistencies would not have been detected in the absence of repeated measures of the physiological performance of these taxa.

When pronounced, the patterns of physiological divergence between *C. exilis* and *C. unguiculata* support the prediction that, where drought intensifies as the flowering season progresses, taxa that flower early (when soil moisture is relatively high and VPD is low) will have faster gas exchange rates and lower WUE_i than those that flower late. The patterns of divergence between the two subspecies of *C. xantiana*, however, are not wholly consistent with this prediction. Trait divergence recorded in spring 2008 supports the prediction; the earlier-flowering taxon, *C. xantiana* subsp. *parviflora*, had higher photosynthetic and transpiration rates than subsp. *xantiana* during flowering (Table 8). However, the patterns of divergence recorded during flowering in spring 2010 do not; *C. xantiana* subsp. *parviflora* had a lower mean photosynthetic rate than in subsp. *xantiana*. The weak support for the "drought escape" hypothesis in *C. xantiana* may have been due to a lower degree of ecological segregation between the two subspecies than between *C. unguiculata* and *C. exilis*. For example, among the populations in the current study, the two subspecies had much more similar values for mean VPD_{min} and VPD_{max} in the months of their flowering than *C. exilis* and *C. unguiculata* (Appendix S6).

Context-specific physiological divergence between sister taxa: variation between developmental stages and years

In both taxon pairs, the direction or degree of divergence in the focal physiological traits differed between 2008 and 2010 and/or between developmental stages. In spring 2008, during vegetative growth, *C. exilis* (self-pollinating) had a significantly higher photosynthetic rate and higher WUE_i than *C. unguiculata* (outcrossing), but these differences disappeared during flowering. In spring 2010, these sister species did not differ in any of the focal traits during vegetative growth, but during flowering *C. exilis* had higher photosynthetic and transpiration rates and lower WUE_i than *C. unguiculata* (Table 8). Despite the early flowering of *C. exilis*, its WUE_i relative to that of *C. unguiculata* changed qualitatively between years; in 2008, *C. exilis* had higher WUE_i than did *C. unguiculata* during vegetative growth, but in 2010, their relative WUE_i reversed during flowering.

In spring 2008, during vegetative growth, *C. xantiana* subsp. *parviflora* had higher photosynthetic and transpiration rates, but lower WUE_i, than in subsp. *xantiana*; the differences in gas exchange rates persisted during flowering, but the difference in WUE_i disappeared. In spring 2010, during vegetative growth, there was no difference between these subspecies in photosynthetic rate, but subsp. *parviflora* had a higher mean transpiration rate and lower WUE_i than subsp. *xantiana*. By contrast, during flowering, subsp. *parviflora* had a lower photosynthetic rate and a higher WUE_i than subsp. *xantiana*. Of particular note is that, in spring 2010, the two subspecies of *C. xantiana* differed in WUE_i during both developmental stages, but the direction of the divergence changed between vegetative growth and flowering (Figure 6, Tables 7 and 8).

The two taxon pairs differed in the direction of within-season temporal change in WUE_i during spring 2010. *Clarkia unguiculata* and *C. exilis* declined in mean WUE_i between vegetative growth and flowering, associated with an increase in their mean transpiration rates. By contrast, both *C. xantiana* subsp. *xantiana* and subsp. *parviflora* increased their WUE_i between vegetative growth and flowering due to reductions in their mean transpiration rates.

TABLE 7 Occurrences and types of concordance and discordance between the direction of phenotypic divergence between sister taxa in gas exchange rates and WUE$_i$, and the taxon-specific selection gradients; see Table 2 for the definitions of each type of concordance and discordance. (A) *Clarkia unguiculata* (*Cu*) vs. *C. exilis* (*Ce*). (B) *Clarkia xantiana* subsp. *xantiana* (*Cxx*) vs. subsp. *parviflora* (*Cxp*). Cells indicate whether sister taxa differ in mean phenotype (> and < indicate the taxon with the higher phenotypic value; see Figure 4) and the type of concordance or discordance observed between the directions of phenotypic divergence and selection in each taxon pair.

	Vegetative stage: divergence vs. selection	**Flowering stage: divergence vs. selection**	**Flowering stage divergence vs. Vegetative stage selection**
A. *Clarkia unguiculata* vs. *C. exilis*			
Total selection			
Photosynthesis	**Concordance Type 1** *Ce* = *Cu*	Discordance Type 2 *Ce* > *Cu*	Discordance Type 2 *Ce* > *Cu*
Transpiration	Discordance Type 3 *Ce* = *Cu*	Discordance Type 1 *Ce* > *Cu*	**Concordance Type 2** *Ce* > *Cu*
WUE$_i$	Discordance Type 3 *Ce* = *Cu*	Discordance Type 1 *Ce* < *Ce*	**Concordance Type 2** *Ce* < *Cu*
Direct selection			
Photosynthesis	**Concordance Type 1** *Ce* = *Cu*	Discordance Type 2 *Ce* > *Cu*	Discordance Type 2 *Ce* > *Cu*
Transpiration	Discordance Type 3 *Ce* = *Cu*	Discordance Type 1 *Ce* > *Cu*	**Concordance Type 2** *Ce* > *Cu*
B. *Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora*			
Total selection			
Photosynthesis	**Concordance Type 1** *Cxp* = *Cxx*	Discordance Type 2 *Cxp* < *Cxx*	Discordance Type 2 *Cxp* < *Cxx*
Transpiration	Discordance Type 2 *Cxp* > *Cxx*	**Concordance Type 1** *Cxp* = *Cxx*	**Concordance Type 1** *Cxp* = *Cxx*
WUE$_i$	Discordance Type 2 *Cxp* < *Cxx*	Discordance Type 2 *Cxp* > *Cxx*	Discordance Type 2 *Cxp* > *Cxx*
Direct selection			
Photosynthesis	**Concordance Type 1** *Cxp* = *Cxx*	Discordance Type 2 *Cxp* < *Cxx*	Discordance Type 2 *Cxp* < *Cxx*
Transpiration	Discordance Type 2 *Cxp* > *Cxx*	**Concordance Type 1** *Cxp* = *Cxx*	**Concordance Type 1** *Cxp* = *Cxx*

Context-specific concordance between physiological divergence and phenotypic selection in each taxon pair

In this study, selection sometimes reinforced the physiological differences between sister taxa, with the self-pollinating taxa experiencing phenotypic selection favoring individuals with a high transpiration rate and low WUE$_i$ (Concordance Type 2; Table 7). Concordance between sister taxa was also observed in several cases in which neither physiological divergence nor directional selection were detectable (Concordance Type 1). In a few cases, the direction of selection differed between sister taxa in a pattern that opposed the phenotypic difference between them (Discordance Type 1; Table 7). In no case did the type of concordance or discordance and the direction of statistically significant divergence between sister taxa remain constant between vegetative growth and flowering. One limitation of the current study is that our fitness estimates were based entirely on stem biomass, a correlate of reproductive success. We could not examine the effects of the focal physiological traits on small seedlings or juveniles, so we cannot assert that these traits did not influence early survivorship and affect individual fitness. This is a common constraint in studies of phenotypic selection on physiological traits or on traits (e.g., flowering time, flower size, pollen production) that cannot be measured on juveniles (Lowry et al., 2008; Dudley et al., 2012; Quezada et al., 2017).

As a consequence of these inconsistencies and uncertainties, the mechanism(s) contributing to the observed divergences between sister taxa remains obscure. Phenotypic plasticity of physiological traits in response to local conditions, responses to direct or total selection on each physiological trait, and/or indirect responses to selection on unstudied, correlated traits may all have played a role. Nevertheless, the many cases in which a self-pollinating taxon exhibited higher photosynthetic or transpiration rates than its outcrossing sister taxon in 2008 or 2010 (Table 8) support the hypothesis that rapid gas exchange is required to achieve early maturation, which may be independently favored by selection for any number of reasons, including the escape from drought-stress.

TABLE 8 Divergence in gas exchange rates and WUE$_i$ between sister taxa recorded in the current study and in Mazer et al. (2010). Within each cell, the operator (>, <) indicates the direction of significant differences reported between sister taxon means. Values shaded in light orange illustrate the case in which the direction of divergence between sister taxa differs qualitatively between studies (2008 vs. 2010). Values shaded in yellow illustrate the case in which the direction of divergence between sister taxa differs qualitatively between life stages. Values shaded in blue illustrate the case in which the direction of divergence between sister taxa differs qualitatively between life stages and studies. In all other cases, divergence between sister taxa either does not change between life stages or studies, or changes from statistically significant to nonsignificant ($\alpha = 0.05$).

	Clarkia unguiculata* vs. *C. exilis			
	Mazer et al. (2010) **Field season: 2007–2008** **Life stage**		**Current study** **Field season: 2009–2010** **Life stage**	
Physiological trait	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**
Photosynthesis	**Ce > Cu**	Ce = Cu	Ce = Cu	**Ce > Cu**
Transpiration	Ce = Cu	Ce = Cu	Ce = Cu	**Ce > Cu**
WUE$_i$	**Ce > Cu**	Ce = Cu	Ce = Cu	**Ce < Cu**
	Clarkia xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora			
	Mazer et al. (2010) **Field season: 2007–2008** **Life stage**		**Current study** **Field season: 2009–2010** **Life stage**	
	Vegetative	**Flowering**	**Vegetative**	**Flowering**
Photosynthesis	**Cxp > Cxx**	**Cxp > Cxx**	Cxp = Cxx	**Cxp < Cxx**
Transpiration	**Cxp > Cxx**	**Cxp > Cxx**	**Cxp > Cxx**	Cxp = Cxx
WUE$_i$	**Cxp < Cxx**	Cxp = Cxx	**Cxp < Cxx**	**Cxp > Cxx**

If so, then the divergence in physiological traits observed in the current and in the previous study (Mazer et al., 2010) may represent the outcome of adaptive evolution.

Phenotypic plasticity in response to conditions during flowering may also have contributed to the high gas exchange rates and low WUE$_i$ exhibited by *C. exilis* relative to *C. unguiculata*. In the current study, the conditions at each field site (Appendix S6) indicate that the populations of *C. exilis* surveyed in flower on 25–26 April were exposed (in April) to a 23% lower mean VPD$_{max}$ and a 55% lower mean VPD$_{min}$ than the populations of *C. unguiculata* experienced in May (they were surveyed in flower on 13, 15, 18, and 21 May). The more mesic conditions during which *C. exilis* was surveyed may have induced its more rapid gas exchange rates. The lower photosynthetic rate exhibited by *C. xantiana* subsp. *parviflora* relative to subsp. *xantiana* cannot be explained as easily by phenotypic plasticity. During flowering in spring 2010, populations of subsp. *parviflora* experienced a 13% higher mean VPD$_{min}$, but a 7% lower mean VPD$_{max}$ than subsp. *xantiana*. This small environmental difference between them may account for the lack of divergence in transpiration rate (Table 8), but not the significantly lower photosynthetic rate and higher WUE$_i$ of subsp. *parviflora*.

CONCLUSIONS

Snapshots of selection measured in heterogeneous conditions do not reliably reflect physiological divergence between taxa, highlighting the need for replicated studies of the factors influencing both divergence and the direction of selection within and among taxa. Regardless of the mechanism (selection, drift, or plasticity) generating the observed phenotypic divergence between sister taxa, the current study holds several implications for the persistence and evolution of these *Clarkia* taxa in response to projected climate warming and increasingly unpredictable drought (IPCC, 2013). First, earlier flowering of the self-pollinating taxa may enable them to reproduce more reliably than their outcrossing sister taxa if climate warming results in higher VPD toward the end of spring. Second, within outcrossing taxa, selection may progressively favor earlier-flowering genotypes (along with the physiological traits associated with earlier maturation), which may escape the negative effects of seasonally increasing VPD. If selection simultaneously operates in sympatric species to increase the frequency of early-flowering genotypes, then, at the community level, the overlap in flowering time among taxa may increase, intensifying intra- and interspecific competition for pollinators. This could provide self-fertilizing taxa with a short-term advantage, but where selfing populations and lineages fail to produce sufficiently genetically variable offspring due to the absence of recombinants, their adaptive capacity will be compromised, limiting their ability to adapt to a rapidly changing environment.

AUTHOR CONTRIBUTIONS

S.J.M. and L.S.D. were awarded funding from the National Science Foundation to conduct the work described here; S.J.M., L.S.D., and A.A.H. designed and conducted the fieldwork; all authors contributed to planning the statistical analyses; D.J.H. and S.J.M. conducted the analyses; all authors discussed and reached consensus on the interpretation of the results; S.J.M. wrote the manuscript, with editorial contributions from all co-authors.

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DATA AVAILABILITY STATEMENT

The data sets and the R code used to generate the analyses, figures, and tables included in the main text and in supplementary material have been deposited in the Dryad Digital Repository: https://doi.org/10.25349/D91318 (Mazer et al., 2022a); the software is published at Zenodo: https://doi.org/10.5281/zenodo.5866848 (Mazer et al., 2022b).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Annual climatic variables (1951–1980) extracted from ClimateNA for use in principal component analyses.

Appendix S2. Climatic variables contributing to the first two principal components for 24 long-term (1951–1980) mean climatic variables at the collection sites of the herbarium specimens of each pair of sister taxa.

Appendix S3. Sorted loadings for principal components 1 and 2 for each pair of sister taxa. (A) *Clarkia unguiculata* and *C. exilis*. (B) *C. xantiana* subsp. *xantiana* and &QJ0;*C. xantiana* subsp. *parviflora*.

Appendix S4. Names and GPS coordinates of populations surveyed for physiological traits and total aboveground stem biomass in 2010.

Appendix S5. Dates and sample sizes (n) for physiological data recorded for each taxon, life history stage, and field population in 2010.

Appendix S6. Interpolated monthly climatic variables extracted from the PRISM database for each site where populations were surveyed during the 2009–10 growing season.

Appendix S7. Application of Bonferroni correction to the tests designed to detect significant differences between sister taxa (*Clarkia unguiculata* vs. *C. exilis* and *C. xantiana* subsp. *xantiana* vs. *C. xantiana* subsp. *parviflora*) with respect to physiological traits measured during vegetative growth and during flowering. All of the 12 tests (3 physiological traits × 2 life history stages × 2 pairs of sister taxa) that were statistically significant at $\alpha = 0.05$ remained statistically significant at $\alpha = 0.05/12 \approx 0.004$. See Figure 6 for comparison of means between sister taxa.

Appendix S8. Cumulative precipitation and means of monthly climatic parameters from November 2007–June 2008 and November 2009–June 2010 at each of the 11 field sites where populations were surveyed in the current study.

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