ORIGINAL ARTICLE

Experimental studies of adaptation in *Clarkia xantiana*. III. Phenotypic selection across a subspecies border

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Sister taxa with distinct phenotypes often occupy contrasting environments in parapatric ranges, yet we generally do not know whether trait divergence reflects spatially varying selection. We conducted a reciprocal transplant experiment to test whether selection favors "native phenotypes" in two subspecies of *Clarkia xantiana* (Onagraceae), an annual plant in California. For four quantitative traits that differ between subspecies, we estimated phenotypic selection in subspecies' exclusive ranges and their contact zone in two consecutive years. We predicted that in the arid, pollinator-scarce eastern region, selection favors phenotypes of the native subspecies *parviflora*: small leaves, slow leaf growth, early flowering, and diminutive flowers. In the wetter, pollinator-rich, western range of subspecies *xantiana*, we expected selection for opposite phenotypes. We investigated pollinator contributions to selection by comparing naturally pollinated and pollen-supplemented individuals. For reproductive traits and for subspecies *xantiana*, selection generally matched expectations. The contact zone sometimes showed distinctive selection, and in ssp. *parviflora* selection sometimes favored nonnative phenotypes. Pollinators influenced selection on flowering time but not on flower size. Little temporal variation in selection occurred, possibly because of plastic trait responses across years. Though there were exceptions and some causes of selection remain obscure, phenotypic differentiation between subspecies appears to reflect spatially variable selection.

KEY WORDS: Contact zone, natural selection, phenotypic selection analysis, range margins, reciprocal transplant, parapatric geographic distributions.

Many sister taxa occupy exclusive geographic ranges to either side of a contact zone, are phenotypically differentiated, and adapted to contrasting environments (Endler 1982; Hewitt 2001). Such patterns suggest that some trait differences between taxa reflect spatially varying selection, yet this hypothesis has rarely been tested (but see Dudley et al. 2012). One powerful approach to determine the adaptive significance of divergent phenotypes is to quantify natural selection on functional traits in reciprocal transplant experiments (Jordan 1991; Nagy 1997). If differentiation between sister taxa occurred in response to divergent selection, native phenotypes should enhance fitness in each taxon's exclusive range but reduce fitness or be selectively neutral in the sister lineage's ex-

clusive range. The use of reciprocal transplants to investigate local adaptation has a long history, including recent studies of sister taxa across range boundaries (Linhart and Grant 1996; Gaston 2003; Hereford 2009; Sexton et al. 2009). However, few studies have combined reciprocal transplants and selection analyses to assess patterns of selection on resident and transplanted phenotypes (Jordan 1991; Bennington and McGraw 1995; Schluter 1995; Nagy 1997; Donohue et al. 2000; Etterson 2004; Hall and Willis 2006).

More generally, though the abiotic and biotic environment clearly shapes adaptive landscapes, the ecological causes of selection are poorly known in most systems (Wade and Kalisz 1990; Kingsolver et al. 2012). Only manipulative experiments

can definitively identify the causal agents responsible for the evolution of complex traits. For example, researchers have used experiments to demonstrate that pollinators and seed predators mediate selection on key floral traits such as flower size, color, and phenology (e.g., Campbell et al. 1994; Parachnowitsch and Caruso 2008; Sandring and Ågren 2009; Sletvold et al., 2010; Hopkins and Rausher 2012).

We conducted a reciprocal transplant experiment of two annual plants that represent sister taxa, to assess whether their divergent phenotypes reflect responses to spatially variable selection. *Clarkia xantiana* ssp. *xantiana* and *C. x*. ssp. *parviflora* have parapatric distributions in the southern Sierra Nevada of California. The combined geographic range of *C. xantiana* spans an environmental gradient of declining precipitation from west to east, with the outcrossing ssp. *xantiana* occupying a wetter, western region, and the self-pollinating spp. *parviflora* a drier, eastern one (Eckhart and Geber 1999). The contact zone is intermediate in climate (Eckhart et al. 2011) and pollinator abundance (Fausto et al. 2001; Moeller 2006; Moeller et al. 2012). In this zone the two taxa frequently grow nearby (Eckhart and Geber 1999); hybridization occurs but is limited by differences in flowering time, with ssp. *parviflora* flowering earlier than ssp. *xantiana* (Eckhart and Geber 1999; Eckhart et al. 2004; Briscoe Runquist et al. 2014). In reciprocal transplant experiments, the two subspecies show strong adaptation to their exclusive ranges but do not differ in lifetime fitness at the contact zone (Geber and Eckhart 2005). The taxa differ in floral form, mating system, and several vegetative, physiological, and developmental life history traits (e.g., flowering time) (Moore and Lewis 1965; Runions and Geber 2000; Eckhart et al. 2004; Mazer et al. 2004; Moeller 2006; Dudley et al. 2012; Moeller et al. 2012; Gould et al. 2014). Trait differences persist in the greenhouse and field transplants, indicating that these differences have a genetic basis (Runions and Geber 2000; Eckhart et al. 2004; Mazer et al. 2004; Gould et al. 2014).

Here, we test whether phenotypic differences reflect adaptation to contrasting environments in the subspecies' exclusive ranges (Mazer et al. 2004; Mazer et al. 2010; Dudley et al. 2012). We take advantage of data on fitness (Geber and Eckhart 2005) and phenotypic variation (Eckhart et al. 2004) in *C. xantiana* in reciprocal transplant experiments conducted over two successive years. Specifically, we ask whether phenotypes characteristic of each subspecies are favored in their respective exclusive ranges, with *xantiana*-like phenotypes favored in the wetter, western region, and *parviflora*-like phenotypes in the drier, eastern region. We also assess patterns of selection on traits in the contact zone to ask whether intermediate phenotypes are favored there. Finally, by adding a supplemental pollination treatment to a phenotypic selection study (e.g., Fishman and Willis 2008; Parachnowitsch and Caruso 2008; Sletvold et al. 2010), we estimate pollen limitation and determine whether reducing it artificially also

relaxes selection for bigger flowers, large petals being the norm in the pollinator-rich exclusive range of ssp. *xantiana*.

Methods
STUDY SPECIES

Clarkia xantiana ssp. *xantiana* and ssp. *parviflora* are winter annual plants with parapatric distributions in the Sierra Nevada of California and adjacent mountain ranges to the south (Eckhart and Geber 1999). In the Sierra Nevada, subspecies *xantiana* occurs from the western foothills eastward up the Kern River Canyon to its shared boundary with ssp. *parviflora* along the North Fork of the Kern River; ssp. *parviflora*'s range extends further east to the Sierra Crest. In the southern Sierra Nevada, the ranges of the two taxa span a west-to-east gradient of declining precipitation that is reflected in declining productivity of herbaceous cover (Eckhart et al. 2004; Eckhart et al. 2011). Higher productivity, especially of annual grasses (many of which are nonnative), suggests stronger light competition in the west. In the east, ground cover is much sparser. Additionally, generalist pollinators decline from west to east (Moeller 2006), and specialist bee pollinators are absent from ssp. *parviflora*'s exclusive range (Fausto et al. 2001, Moeller 2006).

The subspecies of *C. xantiana* differ in reproductive biology. Subspecies *xantiana* has large flowers, pronounced spatial and temporal separation between mature anthers and stigma (herkogamy and protandry, respectively) (Moore and Lewis 1965; Eckhart and Geber 1999; Runions and Geber 2000; Mazer et al. 2004; Moeller 2006), and a predominantly outcrossing mating system (Moeller et al. 2012). This subspecies relies on bee pollinators for reproduction (Moeller 2004; Moeller et al. 2012), including a group of solitary bees that are specialists on the genus *Clarkia* (MacSwain et al. 1973). By contrast, ssp. *parviflora* has small petals and readily self-pollinates because of limited or no herkogamy and protandry (Moore and Lewis 1965; Moeller 2006). Recent work shows that differences in flower form between taxa are more pronounced in the zone of contact than in allopatry (Briscoe Runquist and Moeller 2014).

TRANSPLANT GARDENS AND CLIMATE GRADIENT

Reciprocal transplants were conducted at three 900 m^2 common gardens distributed from west to east across the combined range (see Fig. 1 in Eckhart et al. 2004). The western transplant site (35°32"N 118°38"W, 750 m elevation) was in ssp. *xantiana*'s exclusive range, the eastern site (35°46"N 118°04"W, 1350 m) in ssp. *parviflora*'s exclusive range, and the contact-zone (edge) site (35°38"N 118°30"W, 800 m) in the zone of overlap. No naturally recruiting *C. xantiana* grew within the fenced gardens, but both subspecies were present within 20–50 m of the gardens
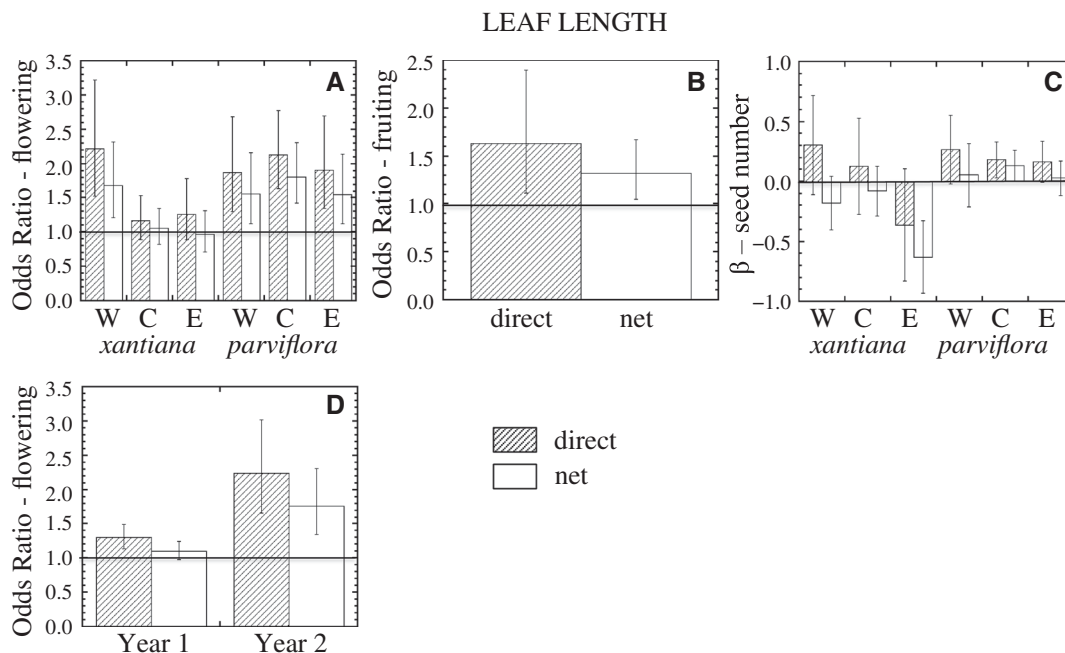
LEAF LENGTH

Figure 1. Patterns of direct (hatched bar) and net (open bar) selection on leaf length via three sequential fitness components, (A) survival to flowering, (B) successful fruiting, and (C) seed number ($\log_e$-transformed) in *Clarkia xantiana*. Patterns illustrate cases where fitness changed significantly with trait value and as a function of site, subspecies or year (see Tables S3–S5). For the first two fitness components, odds ratios (± 95% CI) for fitness change are shown, with ratios greater than 1 (straight line) indicating a positive relationship between fitness and increasing trait values, while ratios less than 1 indicate a negative relationship. For seed number, the directional selection coefficient, β (± 95% CI), is shown. Values greater than 0 (straight line) indicate that fitness increased with trait value.

within their geographic ranges (West and Contact for ssp. *xantiana*; Contact and East for ssp. *parviflora*). On average, 95% of annual precipitation falls between November 1 and June 1 and coincides with *C. xantiana*'s growing season.

EXPERIMENTAL DESIGN

A bulked sample of seed from 50–150 maternal plants was collected from each of six populations of ssp. *xantiana* and 12 populations of ssp. *parviflora* the summer before transplanting to the field. Half of the populations of each taxon came from locations in the zone of contact where the subspecies grow in close proximity (see Fig. 1 in Eckhart et al. 2004). The other populations came from each subspecies' exclusive range (see Eckhart et al. 2004 for additional detail about experimental design and populations).

The experiment began in 1997–1998, a very wet El Niño winter, and replicated in 1998–1999, a much drier La Niña winter. Seeds from each population were sown at the three gardens in a randomized-complete block design in October 1997 and 1998, before cool-season rains began. In each of 10 blocks per year, seeds were planted into 144 square cells (4 cm²) in plastic diffusion screens (i.e., flat lattices with square openings, manufactured for fluorescent light fixtures) affixed to the soil surface. The exact location of blocks differed in the two study years. Natural vegetation was left undisturbed.

Each population was assigned randomly to eight cells per block, with eight seeds per cell (640 seeds per population and 11,520 seeds per transplant site per year). When multiple seeds germinated in a cell, seedling density exceeded natural densities. We thinned seedlings haphazardly to one per cell in March 1998 and 1999, after germination was complete. We assumed that the fitness of the remaining seedling in a cell is a good approximation of any seedling emerging in the cell, when grown at natural densities.

DATA COLLECTION

We collected data on fitness and phenotypes monthly from early December to March, and then continuously from April until June–July of each year. All flowers in five randomly chosen blocks per transplant site were hand pollinated with intrasubspecific pollen from nearby natural populations. This experimental treatment allows us to evaluate the effect of supplemental pollination on reproductive fitness and to estimate pollinator-mediated selection on reproductive traits by comparing selection in hand- versus open-pollinated plants.

Fitness components included: (1) survival from germination to flowering; (2) the probability of fruiting, for the subset of plants that flowered; and (3) the number of seeds produced in the subset of individuals that successfully set fruit. Fruits were collected as they matured and before they dehisced to obtain an accurate

count of total seed production and to prevent nonnative seed from dispersing into sites. The product of the three components is lifetime fitness for seeds that germinated. We could not evaluate selection on individuals that failed to germinate because we lacked phenotypic data.

We chose four traits that (1) differ between the subspecies (Eckhart et al. 2004, Table 1), (2) reflect aspects of vegetative vigor, life history, and floral morphology (mating system), and (3) are not strongly correlated with each other (all correlation coefficients $|r| < 0.53$) (Table S2). We analyzed patterns of selection on the following phenotypic traits measured on all plants left after the March thinning: (1) length of the first true leaf (leaf length); (2) relative expansion rate of the median leaf at the middle node position over a period of 4 days (leaf RGR = ((growth in length)×(initial length)$^{-1}$ ×day^{-1})); (3) the date of first flowering (flowering time = number of days after December 1); and (4) the length of the upper left or right petal on the first or second flower (petal length). In the first year of the experiment, length of the longest leaf, petal length, and the date of first flowering were also measured in 93 natural populations (37 of ssp. *xantiana* and 56 of ssp. *parviflora*) spanning the entire geographic distribution of the species (Eckhart and Geber 1999). As the longest leaf on *C. xantiana* plants occur at higher nodes than the first true leaf, the measure of leaf length in natural populations was, not surprisingly, substantially greater than "leaf length" (of first true leaf) in the experimental gardens. For flowering date and petal length, however, the range of trait values in experimental sites compares well with the range of variation observed among natural populations (Table S1). Thus, experimental plants exhibited a wide and realistic phenotypic range over which to measure selection.

HYPOTHETICAL ADAPTIVE SIGNIFICANCE OF SUBSPECIES DIFFERENCES IN PHENOTYPE

In transplant gardens the subspecies differed in the sizes of leaves and flowers and in whole plant and organ growth rate and phenology (Eckhart et al. 2004). For some annual species, arid environments favor early maturity as a drought escape mechanism (e.g., Donovan et al. 2007; Franks 2011). In our system, the subspecies in the more arid part of the range (*parviflora*) flowers earlier and has shorter flower development time, as might be expected, yet the subspecies in the wetter portion of the range (*xantiana*) tends to exhibit faster rates of leaf growth (Eckhart et al. 2004). We hypothesize that larger, faster growing leaves are favored in the west because higher precipitation there both allows and selects for greater vegetative size. Reduced precipitation in the east increases plant water stress (Eckhart et al. 2010) and should favor smaller plants with smaller leaves (Nicotra et al. 2011).

The evolution of self-pollination in ssp. *parviflora* appears to be favored by reproductive assurance (Moeller and Geber 2005; Pettengill and Moeller 2012a). Pollen limitation in ssp. *xantiana*

increases from west to east (Moeller et al. 2012), and the specialist *Clarkia* bee pollinators are absent and generalist pollinators scarce in the selfing ssp. *parviflora*'s exclusive range (Fausto et al. 2001; Moeller 2006). We expect pollen limitation to reproduction in ssp. *xantiana* to be greater in *parviflora*'s exclusive range than in the west. Hand pollination should have less impact on reproduction in ssp. *parviflora* than *xantiana* because *parviflora* self-pollinates readily. As pollinators are often attracted to larger flowers in this species (Eckhart et al. 2006) and others (reviewed in Krizek and Anderson 2013), we expect selection for larger petals in ssp. *xantiana*. Selection on petal size should be relaxed when pollen is abundant (hand pollinated treatment). Pollinators may also impose selection on flowering phenology (e.g., Sandring and Ågren 2009), which could synchronize pollinator activity with flower availability. While we lack a priori knowledge of pollinator-mediated selection on flowering time in *Clarkia*, differences in selection on this trait between hand- and open-pollinated plants can reveal whether it exists.

ANALYSES

We estimated patterns of selection on traits through each fitness component using SAS (ver. 9.3, SAS Institute, Cary, NC). A combined analysis of selection across the three fitness components using the R package Aster (Geyer et al. 2013) was not possible, because floral traits could not be measured on plants that did not survive to flower and because Aster models of selection on vegetative traits failed to converge. To assess whether selection operated differently between subspecies, we analyzed both subspecies together and modeled interactions between subspecies and traits, testing whether the direction or magnitude of selection differed by subspecies.

Statistical models included the fixed effects of subspecies, site, year, pollination treatment (for fecundity components of fitness), along with 2- and 3-way interactions, to account for their direct influence on fitness. Subspecies, site, and year differences in survival to flowering and in reproductive fitness of open-pollinated plants have been published (Geber and Eckhart 2005). We therefore limit our reporting of results to the influence of pollination treatment on reproductive fitness components. We incorporated random effects for population of origin and block nested within site crossed with year. We included year as a fixed effect because the growing seasons differed distinctly in rainfall and because phenotypic distributions shifted considerably between years (Eckhart et al. 2004).

To assess patterns of selection, the models also included phenotypic trait(s) and interactions between traits and the fixed effects described above. We included vegetative traits and their interactions with subspecies, subspecies × site, and subspecies × year. For reproductive traits, we included the interactions

Table 1. Trait differences between *Clarkia xantiana* subspecies and patterns of trait plasticity across transplant sites and between years from Eckhart et al. (2004).

| Trait | Subspecies differences | Ssp. *xantiana* | | Ssp. *parviflora* | |
| | | Plasticity | | Plasticity | |
		Across sites	Between years	Across sites	Between years
Leaf length	X>P	Contact zone shortest	Cogradient	Contact zone shortest	Cogradient
Leaf RGR	X>P	Contact zone lowest	Cogradient	Contact zone lowest in year 1 Counter-gradient in year 2	Cogradient
Flowering time	X>P	Counter-gradient	Cogradient	Counter-gradient	Cogradient
Petal length	X>P	Contact zone shortest	Cogradient	Counter-Gradient	Cogradient

Cogradient plasticity: Trait phenotypes are more *xantiana*-like at the wetter, western, transplant site in ssp. *xantiana*'s exclusive range or in the first wetter year of transplant experiment and more *parviflora*-like at the drier eastern transplant site in ssp. *parviflora*'s exclusive range or in the second drier year of transplant experiment; Counter-Gradient plasticity: Trait values resemble *xantiana* at the eastern transplant site or in the second year of transplant experiment and *parviflora* at the western transplant site or in the first year of transplant experiment. Contact zone smallest: mean phenotypes are smaller at contact zone site relative to other sites. "Subspecies differences" indicates the relative trait values of *xantiana* (X) versus *parviflora* (P).

between traits and subspecies, subspecies × site, subspecies × year, and subspecies × pollination treatment. In addition, all models included plant size (plant height in April) to control for positive covariance between plant vigor, phenotypic traits, and fitness (Rausher 1992); models without this covariate produced qualitatively similar patterns (results not shown).

For all fitness components, we estimated direct selection from full models including all relevant traits, and net selection (direct + indirect) from reduced models for each trait independently. Phenotypic traits were standardized to a mean of 0 and standard deviation of 1 by subspecies, site, and year to permit comparison of traits measured in different units. We did not evaluate stabilizing selection, correlational selection, or higher order interactions between traits and other main effects. These complex models rarely revealed significant quadratic effects, and substantially decreased our statistical power owing to the large number of model terms.

SURVIVAL TO FLOWERING
Generalized linear-mixed models with a binomial distribution (Proc Glimmix) were used to evaluate selection on vegetative traits (leaf length and leaf RGR) via survivorship. Floral traits could not be measured on plants that did not survive to flowering. We did not include pollination treatment as a predictor, because we scored vegetative traits prior to flowering, and we had no a priori predictions for how pollinators would affect selection on

prereproductive traits. Analysis of this fitness component included data from open- and hand-pollinated individuals.

REPRODUCTIVE FITNESS
The fecundity (seed number) of plants that survived to flowering was highly zero-inflated because many flowering plants failed to set seed. Poisson, zero-inflated Poisson, and zero-inflated negative binomial distributions all showed poor fit to the data. Therefore we divided reproductive fitness into two components: the probability of fruiting in flowering plants, and the number of seeds produced by plants that fruited. The probability of fruiting was analyzed using generalized linear-mixed models, with a binomial distribution (Proc Glimmix). Log transformation of seed number achieved homoscedasticity and normality of residuals, allowing analysis with general linear-mixed models (Proc Mixed). Both vegetative (leaf length, leaf RGR) and reproductive traits (days until flowering, petal length) were included in analyses of reproductive fitness components. Pollination treatment was incorporated as a direct predictor of fitness and in interactions with reproductive traits.

In net selection analyses on leaf length, leaf RGR and flowering time via the probability of fruiting in ssp. *parviflora*, models with the year by site interaction would not converge because of limited data for this subspecies at the western garden in the second year. We excluded the year by site interaction from net selection models, but included it in the full model estimating direct selection.

LEAF RELATIVE GROWTH RATE
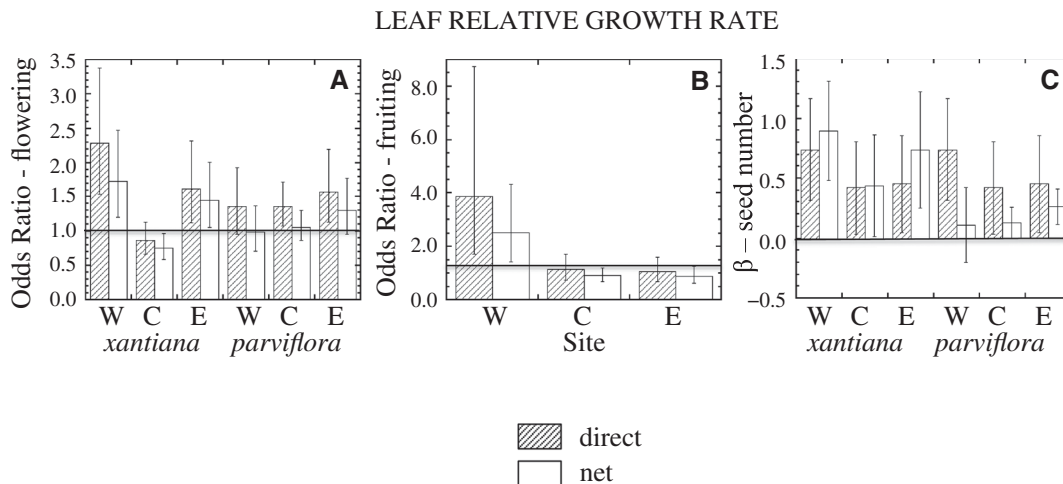
Figure 2. Patterns of direct (hatched bar) and net (open bar) on leaf relative growth rate via three sequential fitness components, (A) survival to flowering, (B) probability of fruiting, and (C) seed number production (log$_e$-transformed) in *Clarkia xantiana*. Patterns illustrate cases where fitness changed significantly with trait value and as a function of site, subspecies or year (see Tables S3–S5). For the first two fitness components, odds ratios (± 95% CI) for fitness change are shown, with ratios greater than 1 (straight line) indicating a positive relationship between fitness and increasing trait values, while ratios less than 1 indicate a negative relationship. For seed number, the directional selection coefficient, β (± 95% CI), is shown. Values greater than 0 (straight line) indicate that fitness increased with trait value. Note that in C) direct selection (β) did not differ between subspecies but varies by site. However, net selection did vary by subspecies and site.

Results

We planted a total of 138,240 seeds. We were able to gather vegetative trait and survivorship data for $N = 1430$ *parviflora* and 948 *xantiana* individuals to examine viability selection via survivorship to flowering, vegetative, and reproductive trait data for $N = 831$ *parviflora* and 427 *xantiana* plants that flowered to estimate selection via the probability of fruiting, and vegetative and reproductive trait data for $N = 646$ *parviflora* and 276 *xantiana* plants that successfully fruited to analyze selection via seed production. Sample sizes declined in the nonexclusive range for each subspecies from survivorship to the probability of fruiting and total seed production (Table 3), which could reduce our power to detect selection. Nevertheless, for most sites and years the range of phenotypes remained broad (Table S1). The proportional representation of each source population remained relatively constant across sites and fitness components (not shown, but data will be available through Dryad).

Selection often differed between subspecies, by geographic region (site), year, and pollinator treatment (Figs. 1–5; Tables S3–S5). Patterns of direct selection (from full models) were generally consistent with patterns of net selection (from reduced models). We found a significantly positive relationship between plant height and fitness for all models, except via the probability of fruiting for direct selection and net selection on leaf RGR and petal length. However, plant height did not appear to influence selection on other traits, as models without height produced

qualitatively similar results. To highlight patterns of selection between subspecies, across space (sites) and time (years), and, for reproductive fitness between pollination treatments, we present results on: (1) site and subspecies differences in selection; (2) temporal differences in selection between years and across the life history; (3) direct effects of hand pollination on fitness; and (4) pollination treatment effects on selection.

SITE AND SUBSPECIES DIFFERENCES IN SELECTION

For some analyses, spatial variation in selection matched expectations. In ssp. *xantiana*, site differences matched the prediction of longer leaves being favored in the west (survival to flowering: Fig. 1A, Table S3; probability of fruiting: Fig. 1B, Table S4; and seed number: Figure 1C, Table S5, Table 2). In full models, the odds of survival in ssp. *xantiana* increased by 122% for every standard deviation (SD) increase in leaf length in the western garden, (odds ratio: 2.22; 95% CI: 1.53, 3.22, Fig. 1A), but leaf length had no effect on survival at other sites. For seed production, direct selection coefficients were nonsignificant at all sites, but net selection favored smaller leaves in the east (Fig. 1C). Direct and net selection on leaf RGR via the probability of fruiting differed among sites, and the pattern met our expectation of selection for faster leaf RGR in the west than at other sites (Fig. 2B, Table S4). Selection via the probability of fruiting favored early flowering in the east in both subspecies (Fig. 3A), while there was no evidence for selection on flowering phenology at the other sites.

Table 2. Summary of patterns of selection across sites and years in two subspecies of *Clarkia xantiana* through three fitness components (P(fruiting): probability of survival from flowering to fruiting, seed no: number of seeds produced in plants setting seed).

		Ssp. *xantiana*		Ssp. *parviflora*	
Trait	Fitness	Across sites	Between years	Across sites	Between years
	Survival	Cogradient (d&n)	Counter-gradient (d&n)	Uniform + (d&n)	Counter-gradient (d&n)
Leaf length	P(fruiting)	Uniform + (d&n)		Uniform + (d&n)	
	Seed no.	Cogradient (n)		Counter-gradient (d)* CZ different (n)*	
	Survival	CZ different (d&n)		Counter-gradient (d)*	
Leaf RGR	P(fruiting)	Cogradient (d&n)		Cogradient (d&n)	
	Seed no.	Cogradient (d)* CZ different (n)*		Cogradient (d)* Counter-gradient (n)*	
Flowering	P(fruiting)	Cogradient (d&n)		Cogradient (d&n)	
Time	Seed no.	Uniform − (n)			
Petal length	P(fruiting)	Cogradient (d&n)		Cogradient (d&n)	
	Seed no.	Uniform + (d) Cogradient (n)		Counter-gradient (d&n)	

In cogradient selection, *xantiana*-like phenotypes are favored in the west in *xantiana*'s exclusive range or in the wet year of the transplant experiment and/or *parviflora*-like phenotypes are favored in the east in *parviflora*'s exclusive range or in the dry year of the experiment. CZ different: Pattern of selection differs at contact zone. Blank cells indicate no significant selection. d: direct selection, n: net selection. *: Confidence intervals of selection coefficients overlap broadly across sites.

Table 3. Sample sizes for each subspecies in the three transplant gardens (West, Central, and East).

|	Subspecies *parviflora*			Subspecies *xantiana*		
	West	Central	East	West	Central	East
Survivorship to flowering	316	720	394	251	462	235
Probability of fruiting	58	471	302	119	195	113
Seed production	33	371	242	98	144	34

These values represent the total numbers of individuals with a complete set of trait and fitness data for each component of fitness.

Both subspecies experienced the strongest selection for longer petals in the exclusive range of ssp. *xantiana*, followed by weaker selection in the contact zone (difference in selection between west and contact zone: $t = 2.54$, $P = 0.011$), and no selection in the exclusive range of ssp. *parviflora,* where pollinators are scarce (Fig. 4A, Table 2). Direct selection favored longer *xantiana* petals in all three locations, while net selection favored longer petals only in the west and contact zone, as expected (Fig. 4B).

In a few analyses, we found no evidence that selection varied across sites. Net selection via seed production universally favored earlier flowering in ssp. *xantiana*, a finding contrary to the expected direction as well as the expected spatial pattern (Fig. 3C, B, Table S5). In ssp. *parviflora*, direct and net selection via survival favored longer leaves everywhere (Fig. 1A, Table S3).

Commonly, however, selection varied in unexpected ways. Spatial patterns of selection on leaf relative growth rate (RGR)

generally did not match the prediction that faster rates would be favored in the west versus the east (Tables S3 and S5). For ssp. *xantiana*, selection via survival favored faster leaf RGR in the west and east to a similar extent ($t = 1.36$, $P = 0.17$; Fig. 2A). There was no direct selection on this trait in the contact zone for ssp. *xantiana*, but net selection favored reduced leaf RGR there (Fig. 2A, Table 2). For ssp. *parviflora*, direct selection via survival tended to be contrary to expectation, with increased leaf RGR favored in the east and the contact site, but no significant selection in the west (Fig. 2A, Table 2); however, confidence intervals on odds ratios overlapped broadly across sites. Direct selection via seed production in both subspecies favored faster leaf RGR at all sites, and although confidence intervals overlapped, selection for faster leaf RGR was stronger in the west relative to the other sites, as expected (Fig. 2C). Contrary to expectations, in ssp. *xantiana*, net selection favored faster leaf RGR at all sites, but

FLOWERING TIME
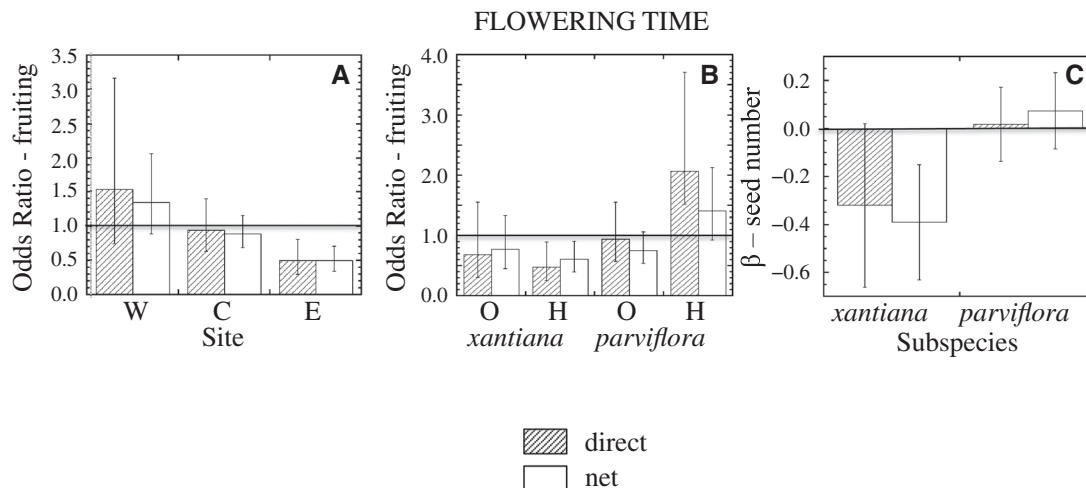
direct
net

Figure 3. Patterns of direct (hatched bar) and net (open bar) selection on flowering time via two sequential fitness components, (A and B) probability of fruiting, and (C) seed number ($\log_e$-transformed) in *Clarkia xantiana*. Patterns illustrate cases where fitness changed significantly (or marginally significantly, direct selection in C) with trait value and as a function of site, subspecies or year (see Tables S3–S5). For the first fitness component, odds ratios (± 95% CI) for fitness change are shown, with ratios greater than 1 (straight line) indicating a positive relationship between fitness and increasing trait values, while ratios less than 1 indicate a negative relationship. For seed number, the directional selection coefficient, β (± 95% CI), is shown. Values greater than 0 (straight line) indicate that fitness increased with trait value.

PETAL LENGTH
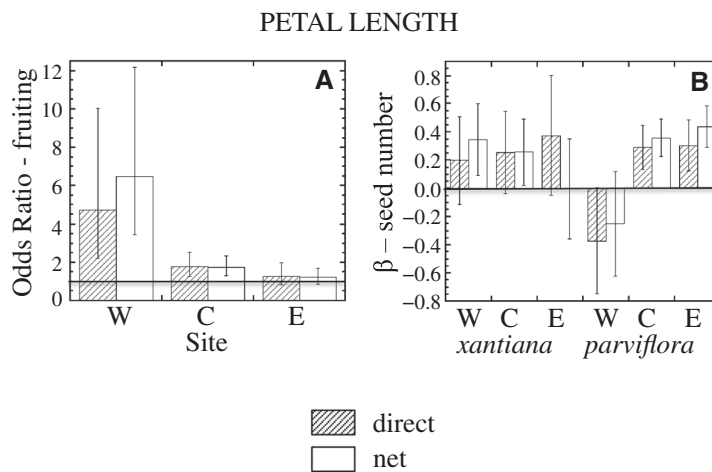
direct
net

Figure 4. Patterns of direct (hatched bar) and net (open bar) selection on petal length via two sequential fitness components, (A) probability of fruiting, and (B) seed number ($\log_e$-transformed) in *Clarkia xantiana*. Patterns illustrate cases where fitness changed significantly with trait value and as a function of site, subspecies or year (see Tables S3–S5). For successful fruiting, odds ratios (± 95% CI) for fitness change are shown, with ratios greater than 1 (straight line) indicating a positive relationship between fitness and increasing trait values, while ratios less than 1 indicate a negative relationship. For seed number, the directional selection coefficient, β (± 95% CI), is shown. Values greater than 0 (straight line) indicate that fitness increased with trait value.

especially in the west and east (Fig. 2C). For ssp. *parviflora*, net selection favored faster leaf RGR in the east and the contact zone, while there was no selection in the west (Fig. 2C). In ssp. *parviflora* spatial variation in selection on petal size was opposite to expectations. Direct and net selection favored longer petals in ssp. *parviflora*'s exclusive range and the contact zone, and there was no selection in the west.

DIRECT EFFECT OF POLLINATION TREATMENT ON REPRODUCTIVE FITNESS

For ssp. *xantiana*, supplemental pollination augmented fitness in the east where pollinators are scarce, but not at other sites (Fig. 5, Table S4). For ssp. *parviflora*, site differences in the effect of supplemental pollination did not conform to expectation of greater pollen limitation in the east. Hand-pollinated plants
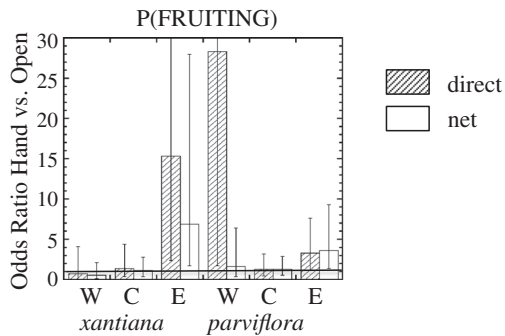
P(FRUITING)

Figure 5. **Direct effect of hand pollination on probability of fruiting in the two subspecies of** *Clarkia xantiana* **at the three transplant sites. Odds ratios of successful fruiting in hand relative to open-pollinated flowers (± 95% CI) are shown from direct selection analysis (hatched bar) of fruiting success as a function of site, subspecies, year, pollination, and all phenotypic traits, and for net selection analysis (open bar) on leaf relative growth rate (see Table 3). Ratios greater than 1 indicate that hand pollination significantly increased fruiting success relative to open pollination.**

were more likely to fruit than open pollinated plants at both the western and eastern sites, but not at the contact zone (Fig. 5). There was no direct effect of hand pollination on seed production in either subspecies (Table S5).

POLLINATOR-MEDIATED SELECTION

Pollination treatment influenced selection on flowering phenology differently for the two subspecies (flowering time by subspecies by pollination interaction; Table S4). Under supplemental pollination selection favored delayed flowering in ssp. *parviflora* and accelerated flowering in ssp. *xantiana* (Fig. 3B). Our analyses did not detect significant selection on flowering phenology in either subspecies in the open-pollination treatment. Contrary to expectation, pollen supplementation did not relax selection for larger petals in either subspecies (Tables S4–S5).

TEMPORAL DIFFERENCES IN SELECTION

Despite contrasting weather between years, selection was rather consistent in time. We found no evidence of temporal variation in selection on leaf RGR, flowering time, or petal size in either subspecies (Tables 2, S3–S5). Leaf length was the exception. Selection via survival for longer leaves was stronger in the dry, second year for both subspecies (leaf length by year interaction; Tables 2 and S3, Fig. 1D). For direct selection, one SD increase in leaf length resulted in a 30.3% increase in the odds of survivorship in year 1 (odds ratio: 1.303; 95%CI: 1.1322, 1.50), and a 124% increase in year 2 (odds ratio: 2.24; 95% CI: 1.66, 3.023). Net selection on leaf length in year 1 was not significant, but strongly favored larger leaves in year 2 (Fig. 1D).

Discussion

As rainfall (Eckhart et al. 2004; Eckhart et al. 2011) and pollinator abundance and diversity (Moeller 2006) decline from west to east across *C. xantiana*'s range, we predicted cogradient selection across the landscape, with selection favoring phenotypes of native subspecies where they occur alone, and intermediate patterns of selection in the contact zone. Knowing that pollen limits *C. xantiana* reproduction (Moeller et al. 2012) and that pollinators prefer large flowers in *C. xantiana* (Eckhart et al. 2006), we also hypothesized that pollen supplementation would relax selection for longer petals. Findings matched some of these expectations. Selection on reproductive traits in both subspecies, plus selection on leaf traits in *xantiana*, was often cogradient with respect to one or more fitness components (Table 2). One lesson of the study is therefore that subspecies differences in reproductive morphology and phenology, and leaf traits of *xantiana*, may represent environment-specific adaptations. Selection also showed other patterns for some combinations of traits and fitness components (Table 2), including: (1) uniform selection across sites and/or subspecies, favoring *xantiana*-like phenotypes; (2) counter-gradient selection, favoring *parviflora*-like phenotypes in *xantiana*'s exclusive range (or vice versa); and (3) similar selection in each subspecies' exclusive range but distinctive selection in the contact zone. Selection generally favored longer petals, but supplemental pollination did not alter selection on petal length. Instead it altered selection on flowering phenology. Below we highlight lessons of the expected and unexpected findings.

SPATIAL PATTERNS OF SELECTION IN RECIPROCAL TRANSPLANTS

Variation in the magnitude and direction of selection across natural populations is common (Siepielski et al. 2013), partly as a function of environmental gradients (e.g., Caruso et al. 2003). Some studies of reciprocal transplants have detected selection favoring native phenotypes in the respective habitats (Jordan 1991; Bennington and McGraw 1995; Schluter 1995; Dudley 1996; Nagy 1997; Donohue et al. 2000; Etterson 2004; Hall and Willis 2006). Others, however, have found similar selection across environments, favoring trait values of one lineage at all sites (Nagy 1997; Ludwig et al. 2004; Gardner and Latta 2006; Wright and Stanton 2007; Latta 2009). The contrast in results between studies is not readily explained by degrees of divergence among taxa, populations, or environments; evidence for and against selection favoring native phenotypes can be found both among populations and subspecies, and across habitat or geographic range boundaries.

The present study found a mixture of the above patterns as well as distinctively different selection in a spatially central location. Selection on leaf length illustrates a complex outcome.

Subspecies *parviflora* has shorter leaves in nature and in common environments (Eckhart et al. 2004). Leaf length showed cogradient selection in *xantiana* with respect to survival and seed number, and uniformly upward selection with respect to the probability of fruiting. In *parviflora*, directional selection favored longer leaves in all sites, in contrast with predictions of selection for shorter leaves in the eastern exclusive range. In both subspecies, selection on leaf length was more strongly upward in the drier second year.

Except for leaf length, we saw little temporal variation in selection, consistent with a recent metaanalysis that found it to be rare (Morrissey and Hadfield 2012), but not consistent with our predictions for this system. Interannual variation in conditions would be expected to alter patterns of selection experienced by sequential generations of annual species. Few studies can attribute temporal changes in selection to the underlying causal mechanisms (Siepielski et al. 2009). In that respect, our work provides a useful opportunity, as we conducted this study over two years that differed dramatically in precipitation (a wet El Niño winter followed by a dry La Niña winter). Across the range, it would be reasonable to expect selection for *xantiana*-like traits in the wet first year and *parviflora*-like traits in the dry second one.

One possibility is that similar selection between years occurred because traits in both subspecies exhibited strong interannual cogradient plasticity, being more *xantiana*-like in the wet year and more *parviflora*-like in the dry year (Eckhart et al. 2004, Table 1). If phenotypic shifts to drought were adaptive, selection might not differ between years. This is in contrast to spatial patterns of plasticity, which were never cogradient (Table 1), but where selection patterns more often did conform to expectations, at least in *xantiana* (Table 2). The ability to detect expected patterns of varying selection on traits across space or time may depend on whether plasticity in those traits is co- or countergradient. Unexpectedly stronger directional selection for longer leaves in the drier year may have occurred because (nonadaptive) plasticity shifted phenotypic distributions below the optimum.

In *parviflora*, consistent selection for longer leaves suggests that the trait might be below its optimum even in its native environment. At least two mechanisms could explain the maintenance of apparently maladaptive trait variation in ssp. *parviflora*. First, there might be introgression from subspecies *xantiana*. However, barriers to gene flow between the subspecies of *Clarkia xantiana* have evolved in sympatry (Briscoe Runquist et al. 2014), and the little introgression that does exist seems to be biased in the direction of subspecies *parviflora* to *xantiana* (Pettengill and Moeller 2012b). Furthermore, introgression from subspecies *xantiana* would cause *parviflora* leaves to be longer, and would not explain why *parviflora* has short leaves despite selection for long ones. A second possibility is that the evolution of leaf length is constrained by genetic correlations with other traits that confer

a fitness advantage. Genetic correlations do not appear to constrain adaptive evolution in subspecies *parviflora* (Dudley et al. 2007), but we cannot entirely dismiss this hypothesis. It may be most likely that upward selection on leaf length was partly spurious, arising from environmental covariance between leaf length and fitness components. We attempted to control for this environmental covariance by including plant height as a covariate in the statistical models. However, low-performing individuals may exhibit low fitness and short leaves, particularly during stressful conditions. The fact that stronger directional selection for longer leaves occurred in both subspecies in the dry year represents circumstantial evidence in favor of this hypothesis.

Distinctive selection at the contact zone site might be explained partly by locally stressful conditions. Environmental conditions such as precipitation and pollinator availability are intermediate between the western and eastern sites, but the substrate consists of more compacted soil, on average, throughout the contact zone, which reduces *C. xantiana* performance (Eckhart et al. 2010). Perhaps as a result of stressful edaphic conditions, the average phenotypes of vegetative and other size-related traits (e.g., petal size) were reduced at the contact zone relative to other sites (Table 5 of Eckhart et al. 2004). Other reciprocal transplant studies have typically included only parental environments; therefore, it is unclear how often contact zones are not intermediate in terms of selection. In our system, distinctive attributes of the contact zone might expose subspecies to different patterns of selection than present in the exclusive ranges, not only because of the physical environment but also because of the presence of the other subspecies (Briscoe Runquist and Moeller 2014).

VARIATION IN SELECTION WITH RESPECT TO DIFFERENT FITNESS COMPONENTS,

Individuals often express different phenotypes across their life history because of seasonal change in the environment and size-dependent physiological requirements (Farnsworth 2004; Donohue et al. 2010). Thus the magnitude, direction, and the mode (directional, disruptive, stabilizing) of selection on traits can vary through ontogeny (Farnsworth 2004).

We found that patterns of phenotypic selection sometimes differed across fitness components. For example, in *xantiana*, accelerated flowering increased seed set of plants that successfully fruited (Fig. 3C), but there was no selection on flowering phenology for open-pollinated *xantiana* via the probability of producing seeds. In some cases, patterns of spatially varying selection matched predictions only when we evaluated multiple fitness components. For example, our prediction of spatial variation in selection on leaf length is supported by the combination of viability selection for long leaves in the west (Fig. 1A) and fecundity selection for short leaves in the east (Fig. 1C). In other cases, combining results of multiple fitness components hindered

generalizations. For example, we expected stronger selection for larger flowers in the west, as found in selection via the probability of fruiting (Fig. 4A), but selection via seed production favored longer *parviflora* petals in the east, but not the west (Fig. 4B). Selection that occurs at an early life history stage could deplete phenotypic variation from the population, potentially diminishing the strength of selection on a trait via subsequent fitness components.

POLLINATOR-MEDIATED SELECTION ON REPRODUCTIVE TRAITS

Pollinator behavior can impose selection on flower morphology, nectar rewards, and phenology (e.g., Galen and Cuba 2001; Fenster et al. 2004; Fenster et al. 2006; Sandring and Ågren 2009; Parachnowitsch and Kessler 2010; Dudash et al. 2011). In response to selection exerted by pollinators, outcrossing species generally maintain larger floral displays than autonomously self-pollinating species (Goodwillie et al. 2010). In *C. xantiana*, the outcrossing *xantiana* has larger flowers and pronounced herkogamy and protandry compared with the autonomously self-pollinating *parviflora* (Runions and Geber 2000; Moeller 2006). Bee pollinators are more abundant in *xantiana*'s exclusive western range, whereas in the eastern exclusive range of *parviflora*, specialist pollinators are absent and generalists are rare (Fausto et al. 2001; Moeller 2006). The floral morphology of *parviflora* confers reproductive assurance by facilitating self-fertilization (Moeller and Geber 2005; Moeller 2006).

Pollinator-mediated selection occurred in this system, but not always as expected. Selection on petal length via the probability of fruiting fit the idea that selection on petal length would be strongest in the west (Figure 4a), but selection via seed set favored increased petal length at all sites, except in the west for *parviflora*, where we found counter-gradient selection (i.e., for shorter petals). Natural populations of *parviflora* in the contact zone have shorter petals than populations in *parviflora*'s exclusive range, possibly as a result of selection to avoid the low-fertility consequences of interbreeding with *xantiana* where they cooccur (i.e., reinforcement) (Briscoe Runquist and Moeller 2014). Downward selection on *parviflora* petal length at the western experimental site might have mimicked such selection that favors increased divergence in phenotypic characters between the subspecies where they cooccur.

Because *xantiana* depends on pollinators for reproduction more strongly than *parviflora*, we predicted supplemental pollination would increase reproductive fitness (which did happen) and alter selection on reproductive traits more strongly in *xantiana* than *parviflora* (which did not happen). In *xantiana* hand pollination increased the probability of fruiting in the eastern transplant garden where pollinators are scarce. In *parviflora* hand pollination improved the probability of fruiting both in the west and

east (though more strongly so in the west), but not in the contact zone. These results are consistent with evidence for pollinator limitation in natural *parviflora* populations, despite *parviflora*'s capacity for autonomous self-pollination (Briscoe Runquist and Moeller, 2014).

Finally, pollinators altered selection on flowering phenology in surprising ways. In *parviflora*, selection via the probability of fruiting favored delayed flowering under supplemental pollination, but there was no selection on flowering phenology under open pollination. We found the opposite pattern in *xantiana*. Supplemental pollination favored accelerated flowering via the probability of fruiting, but, as in *parviflora*, there was no evidence for selection via this fitness component in the open pollination treatment. The mechanisms that account for these findings remain obscure, but it is clear that pollinators can impose selection on flowering phenology in both subspecies.

Conclusions

Divergent phenotypes in sister taxa occupying parapatric geographic distributions that span environmental gradients are generally thought to reflect adaptive divergence resulting from spatially varying selection. Reciprocal transplant experiments of the divergent lineages can examine whether selection favors native phenotypes. Our test of this idea in two parapatric subspecies of *C. xantiana* supports the hypothesis that some phenotypic differences are adaptive (particularly in reproductive characters), while also revealing unexpected spatial patterns of selection that may have arisen from phenotypic plasticity and from a distinctly stressful environment at the central transplant site. As predicted, pollen-limitation of reproductive success was greater in the exclusive range of the outcrossing *xantiana* than the selfing *parviflora*, and selection for larger flowers was stronger in *xantiana*'s exclusive range. However, we also found evidence of pollen limitation in the selfing subspecies transplanted in the outcrosser's range and evidence that pollinators influence selection on flowering phenology. Subspecies *parviflora* and *xantiana* originally diverged in allopatry (Pettengill and Moeller 2012b) and now are strongly locally adapted (Geber and Eckhart 2005). By exposing recently diverged sister taxa to their native and nonnative ranges, we were able to document that natural selection sometimes favors native phenotypes and can contribute to taxon divergence in allopatry and possibly to current reproductive isolation in sympatry.

LITERATURE CITED

Arendt, J. D., and D. Sloan Wilson. 1999. Countergradient selection for rapid growth in pumpkinseed sunfish: disentangling ecological and evolutionary effects. Ecology 80:2793–2798.

Bennington, C., and J. McGraw. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. Ecol. Monogr. 65:303–324.

Briscoe Runquist, R. D., and D. A. Moeller. 2014. Floral and mating system divergence in secondary sympatry: testing an alternative hypothesis to reinforcement in *Clarkia*. Annal. Bot. 113:223–235.

Briscoe Runquist, R. D., E. Chu, J. L. Iverson, J. C. Kopp, and D. A. Moeller. 2014. Rapid evolution of reproductive isolation between incipient outcrossing and selfing *Clarkia* species. Evolution 68:2885–2900.

Byars, S., W. Papst, and A. Hoffmann. 2007. Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. Evolution 61:2925–2941.

Campbell, D. R., N. M. Waser, and M. V. Price. 1994. Indirect selection of stigma position in *Ipomopsis aggregata* via a genetically correlated trait. Evolution 48:55–68.

Caruso, C. M., S. B. Peterson, and C. E. Ridley. 2003. Natural selection on floral traits of *Lobelia* (Lobeliaceae): spatial and temporal variation. Am. J. Bot. 90:1333–1340.

Conover D. O., T. A. Duffy, and L. A. Hice. 2009. The covariance between genetic and environmental influences across ecological gradients: reassessing the evolutionary significance of countergradient and cogradient variation. Annal. NY Acad. Sci. 1168:100–129

Donohue, K., D. Messiqua, E. H. Pyle, M. S. Heschel, and J. Schmitt. 2000. Evidence of adaptive divergence in plasticity: density- and site-dependent selection on shade-avoidance responses in *Impatiens capensis*. Evolution 54:1956–1968.

Donohue, K., R. Rubio de Casas, L. T. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, postgermination adaptation, and species ecological ranges. Ann. Rev. Ecol. Syst. 41:293–319.

Donovan, L., S. A. Dudley, D. M. Rosenthal, and F. Ludwig, 2007. Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers. Oecologia 152:13–25.

Dudash, M. R., C. Hassler, P. M. Stevens, and C. B. Fenster. 2011. Experimental floral and inflorescence trait manipulations affect pollinator preference and function in a hummingbird-pollinated plant. Am. J. Bot. 98:275–282.

Dudley, S. A. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. Evolution 50:92–102.

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. J. Evol. Biol. 20:2200–2218.

Dudley, L. S., A. A. Hove, and S. J. Mazer. 2012. Physiological performance and mating system in *Clarkia* (Onagraceae): does phenotypic selection predict divergence between sister species. Am. J. Bot. 99:488–507.

Eckhart, V. M., and M. A. Geber. 1999. Character variation and geographic distribution of *Clarkia xantiana* (Onagraceae): flowers and phenology distinguish two subspecies. Madroño 46:117–125.

Eckhart, V. M., M. A. Geber, and C. M. McGuire. 2004. Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. Evolution 58:59–70.

Eckhart, V. M., M. A. Geber, W. F. Morris, E. S. Fabio, P. Tiffin, and D. A. Moeller. 2011. The geography of demography: long-term demographic studies and species distribution models reveal a species border limited by adaptation. Am. Nat. 178:S36–S53.

Eckhart, V. M., N. C. Rushing, G. M Hart, and J. D. Hansen. 2006. Frequency dependent pollinator foraging in polymorphic *Clarkia xantiana* ssp. *xantiana* populations: implications for flower colour evolution and pollinator interactions. Oikos 112:412–421.

Eckhart, V. M., I. Singh, A. M. Louthan, A. J. Keledjian, A. Chu, D. A. Moeller, and M. A. Geber. 2010. Plant-soil water relations and species border of *Clarkia xantiana ssp. xantiana* (Onagraceae). Int. J. Plant Sci. 171:749–760.

Endler, J. A. 1982. Problems in distinguishing historical from ecological factors in biogeography. Am. Zool. 22:441–452.

Etterson, J. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. Evolution 58:1446–1458.

Farnsworth, E. J. 2004. Hormones and shifting ecology throughout plant development. Ecology 85:5–15.

Fausto, J. A., V. Eckhart, and M. Geber. 2001. Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). Am. J. Bot. 88:1794–1800.

Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. Ann. Rev. Ecol. Syst. 35:375–403.

Fenster, C. B., G. Cheeley, M. R. Dudash, and R. J. Reynolds. 2006. Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). Am. J. Bot. 93:1800–1807.

Fishman, L., and J. Willis. 2008. Pollen limitation and natural selection on floral characters in the yellow monkeyflower *Mimulus guttatus*. N. Phytol. 177:802–810.

Franks, S. 2011. Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. N. Phytol. 190:249–257.

Galen, C., and J. Cuba. 2001. Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. Evolution 55:1963–1971.

Gardner, K. M., and R. G. Latta. 2006. Identifying loci under selection across contrasting environments in *Avena barbata* using quantitative trait locus mapping. Mol. Ecol. 15:1321–1333.

Gaston, K. J. 2003. The structure and dynamics of geographic ranges. Oxford Univ. Press, Oxford, UK.

Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in *Clarkia xantiana*. II Fitness variation across a subspecies border. Evolution 59:521–531.

Geyer, C. J., C. E. Ridley, R. G. Latta, J. R. Etterson, and R. G. Shaw. 2013. Local adaptation and genetic effects on fitness: calculations for exponential family models with random effects. Annal. Appl. Stat. 7:1778–1795.

Goodwillie, C., R. Sargent, C. G. Eckert, E. Elle, M. A. Geber, M. O. Johnston, S. Kalisz, D. A. Moeller, R. H. Ree, M. Vallejo-Marin 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. N. Phytol. 185:311–321.

Gould, B., D. A. Moeller, V. M. Eckhart, P. Tiffin, E. S. Fabio, and M. Geber. 2014. Local adaptation and range boundary formation in response to complex environmental gradients across the geographical range of *Clarkia xantiana* ssp. *xantiana*. J. Ecol. 102:95–107.

Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. Evolution 60:2466–2477.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness tradeoffs. Am. Nat. 173:579–588.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. Mol. Ecol. 10:537–549.

Hopkins, R., and M. D. Rausher. 2012. Pollinator-mediated selecton on flower color allele drives reinforcement. Science 335:1090–1092.

Jordan, N. 1991. Multivariate analysis of selection in experimental populations derived from hybridization of two ecotypes of the annual plant *Diodia teres* W. (Rubiaceae). Evolution 45:1760–1772.

Kingsolver, J., S. E. Diamond, A. M. Siepielski, and S. M. Carlson. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. Evol. Ecol. 26:1101–1118.

Krizek, B. A., and J.T. Anderson. 2013. Control of flower size. J. Exp. Bot. 64:1427–1437.

Lambrecht, S. C. 2013. Floral water costs and size variation in the highly selfing *Leptosiphon bicolor* (Polemoniaceae). Int. J. Plant Sci. 174:74–84.

Latta, R. G. 2009. Testing for local adaptation in *Avena barbata*: a classic example of ecotypic divergence. Mol. Ecol. 18:3781–3791.

Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. Ann. Rev. Ecol. Syst. 27:237–277.

Ludwig, F., D. M. Rosenthal, J. A. Johnston, N. Kane, B. L. Gross, S. A. Dudley, L. H. Rieseberg, and L. A. Donovan. 2004. Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early-generation hybrids. Evolution 58:2682–2692.

MacSwain, J. W., P. Raven, and R. W. Thorp. 1973. Comparative behaviour of bees and Onagraceae. IV. Clarkia bees of the western United States. Univ. Calif. Publ. Entomol. 70:1–80.

Mazer, S., 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? Int. J. Plant Sci. 171:1029–1047.

Mazer, S., 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? Am. J. Bot. 91:2041–2050.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology 85:3289–3301.

——. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. Ecology 87:1510–1522.

Moeller, D. A., and M. Geber. 2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. Evolution 59:786–799.

Moeller, D. A., M. A. Geber, V. M. Eckhart, and P. Tiffin. 2012. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. Ecology 93:1036-1048.

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

Morrissey, M. B., and J. D. Hadfield. 2012. Directional selection in temporally replicated studies is remarkably consistent. Evolution 66:435–442.

Nagy, E. S. 1997. Selection for native characters in hybrids between two locally adapted plant subspecies. Evolution 51:1469–1480.

Nicotra, A. B., A. Leigh, C. K. Boyce, C. S. Jones, K. J. Niklas, D. L. Royer, and H. Tsukaya. 2011. The evolution and functional significance of leaf shape in the angiosperms. Funct. Plant Biol. 38:535–552.

Parachnowitsch, A. L., and C. M. Caruso. 2008. Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. Ecology 89:1802–1810.

Parachnowitsch, A. L., and A. Kessler. 2010. Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. N. Phytol. 188:393–402.

Pettengill, J. B., and D. A. Moeller. 2012a. Phylogeography of speciation: allopatric divergence and secondary contact between outcrossing and selfing *Clarkia*. Mol. Ecol. 21:4578–4592.

Pettengill, J. B., and D. A. Moeller. 2012b. Tempo and mode of mating system evolution between incipient *Clarkia* species. Evolution 66:1210–1224.

Rausher, M. D. 1992. The measurement of selection on quantitative traits—biases due to environmental covariances between traits and fitness. Evolution 46:616–626.

Runions, C. J., and M. A. Geber. 2000. Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. Size and development of floral organs. Am. J. Bot. 87:1439–1451.

Sandring, S., and J. Ågren. 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. Evolution 63:1292–1300.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–90.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. The evolution and ecology of geographic range limits. Ann. Rev. Ecol. Evol. Syst. 40:415–436.

Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol. Lett. 12:1261–1276.

Siepielski, A. M., K. M. Gotanda, M. B. Morrissey, S. E. Diamond, J. D. DiBattista, and S. Carlson. 2013. The spatial patterns of directional phenotypic selection. Ecol. Lett. 16:1382–1392.

Sletvold, N., J. M. Grindeland, and J. Ågren. 2010. Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. N. Phytol. 188:385–392.

Wade, M. J., and S. Kalisz. 1990. The causes of natural selection. Evolution 44:1947–1955.

Wright, J., and M. L. Stanton. 2007. *Collinsia sparsiflora* on serpentine and non-serpentine soils: using F2 hybrids to detect the potential role of selection on ecotypic differentiation. N. Phytol. 173:354–366.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1: Mean (± stdev) and range of phenotypic traits across natural population in 1998 (37 populations of ssp. *xantiana*, 56 populations of ssp. *parviflora*), and for experimental plants at the three transplant gardens in both transplant years (1998 and 1999).

Table S2: Pearson Correlation Coefficients (r) for phenotypic traits included in the analyses.

Table S3: Direct (full model) and net (reduced models) selection on vegetative traits via survivorship to flowering.

Table S4: Direct (full model) and net (reduced models) selection on vegetative and floral characteristics via the probability of fruiting.

Table S5: Direct (full model) and net (reduced models) selection on all traits via seed production (log transformed).