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Local adaptation and range boundary formation in response to complex environmental gradients across the geographical range of *Clarkia xantiana* ssp. *xantiana*

Billie Gould[1]*, David A. Moeller[2], Vincent M. Eckhart[3], Peter Tiffin[2], Eric Fabio[4] and Monica A. Geber[1]

[1]*Department of Ecology and Evolutionary Biology, Cornell University, Corson Hall, Tower Rd, Ithaca, NY 14850, USA;* [2]*Department of Plant Biology, University of Minnesota, 250 Biological Sciences, 1445 Gortner Avenue, Saint Paul, MN 55108, USA;* [3]*Department of Biology, Grinnell College, 1116 8th Ave, Grinnell, IA 50112-1690, USA; and* [4]*Department of Horticulture, Cornell University, Tower Rd, Ithaca, NY 14850, USA*

Summary

1. Theory predicts that lack of heritable trait variation and/or maladaptive gene flow can promote the formation of species geographical range boundaries even in the absence of barriers to dispersal. Little is known, however, about the patterns and drivers of differentiation across species' ranges and whether they influence boundary formation in the field.

2. Using field measurements, two common garden studies, and Q_{ST}–F_{ST} analyses, we examined environmental and genetic influences on plant phenotype across the geographical range of *Clarkia xantiana* ssp. *xantiana*. This annual plant endemic to California has an eastern range border that lacks obvious physical barriers to dispersal.

3. We find that across opposing environmental gradients that span the species core range, populations are likely to be locally adapted. Populations are phenotypically differentiated from each other in the field as well as common gardens. Traits are correlated with field environmental variables in both settings, and Q_{ST}–F_{ST} comparisons indicate variation in flowering time, degree of branching, and herkogamy may be adaptive. The strength and direction of environment-trait correlations at the range edge are similar to the range centre, and quantitative genetic variation is not reduced. Genetic correlations between traits are generally weak, also suggesting little constraint on trait evolution at the range edge.

4. *Synthesis.* For traits measured here, it is unlikely that either low heritable variation at the range edge or maladaptive gene flow strongly inhibits eastward range expansion. Our results suggest that across environmentally complex landscapes, patterns of diversity generated by locally adaptive selection do not necessarily contribute broadly to the formation of range boundaries as predicted.

Key-words: *Clarkia*, environmental gradient, gene flow, geographical range boundaries, heritability, landscape, local adaptation, phenotypic variation, plant population and community dynamics, Q_{ST}

Introduction

Understanding the factors that determine species' geographical range limits is a long-standing problem in ecology and evolution (Grinnell 1917; MacArthur 1972; Brown & Lomolino 1998; Gaston 2003; Geber 2011). Why, with ample time for the forces of mutation and selection to produce adaptation, do most native species occupy stable niches at their range boundaries? As Mayr (1963) aptly put it, 'One would expect that a few individuals would survive in a zone immediately outside the species border and form a new local population which becomes gradually better adapted … the species range to grow by a process of annual accretion like the rings of a tree' (p.524). Over time, studies have demonstrated that, from a demographic standpoint, range limits form where death rates

*Correspondence author. E-mail: bag59@cornell.edu

exceed birth rates and where extinction rates exceed colonization rates. From an evolutionary standpoint, populations fail to persist in marginal environments because adaptation fails to occur in response to some (suite of) novel environmental factor(s) (Kirkpatrick & Barton 1997; Bridle & Vines 2007; Geber 2011).

Theoretical work has modelled how demographic and evolutionary factors might cause the formation of stable range limits, but few species have been investigated in enough detail to distinguish among competing ecological and evolutionary mechanisms. On the one hand, peripheral populations may be demographically unstable and maintained largely by immigration. Source-sink or colonization–extinction dynamics from the range centre to periphery may allow edge populations to persist, despite high extinction rates (Pulliam 1988; Hoffmann & Blows 1994; Kirkpatrick & Barton 1997). In this case, peripheral populations may show limited adaptation to range-edge environments and limited adaptive differentiation, from central populations because of continuous introduction of 'centrally adapted' alleles. On the other hand, peripheral populations may be stable without immigration and exhibit adaptive differentiation from central ones. Examining patterns of population differentiation in ecologically important traits in populations from the centre to the periphery of geographical ranges can provide particularly useful insight for differentiating between these possibilities.

For stable peripheral populations, theory suggests that a lack of genetic variation underlying ecologically important traits – due to strong selection, small population size, and/or inbreeding – can hamper adaptive evolution to conditions beyond the range limit. Alternatively, persistent gene flow from central to unstable peripheral populations, while limiting adaptive differentiation, is likely to maintain genetic variation (Hoffmann & Blows 1994). Some studies have investigated population genetic structure and variation between central and marginal populations of plant species (Eckert, Samis & Lougheed 2008; Platt *et al.* 2010; Keller *et al.* 2011; Moeller, Geber & Tiffin 2011), but little is understood about the level of heritable trait variation and its potential correlation with environment. Few studies of plants have examined the relationship between environment, phenotypic differentiation and heritability in combination from the centre to the margin of a species' range.

Here, we use a field study of natural populations combined with population-structured and family-structured common garden studies to examine quantitative trait differentiation and diversity within and between populations, from the centre to the edge of the core geographical range of *Clarkia xantiana* ssp. *xantiana* (Onagraceae). *Clarkia x.* ssp. *xantiana* is an outcrossing annual wildflower that occupies a narrow endemic range in the mountains of inland central and southern California (Lewis & Lewis 1955; Eckhart & Geber 1999). In the Sierra Nevada (the core of its geographical range), it spans a range of elevations where soils, temperature, precipitation and topography vary (Eckhart *et al.* 2010, 2011). The range edge in the eastern part of the mountains has no known physical barrier to dispersal. Demographic work and reciprocal transplant experiments have demonstrated that habitat favourability is low beyond the eastern border (Geber & Eckhart 2005; Eckhart *et al.* 2011). Central populations are more genetically diverse than edge populations, harbouring more private sequence haplotypes and more private microsatellite alleles. One study estimated there is approximately twice as much gene flow (or more) from some central to edge populations than *vice versa* (see Moeller, Geber & Tiffin 2011). Reciprocal transplant experiments of central and range-edge populations have consistently found higher levels of local adaptation at the centre compared with the edge of the range (Geber & Eckhart 2005; Geber *et al.*, in prep).

To address potential limits to adaptation at the eastern range edge, we first quantified population differentiation in ecologically important traits in the field across 30 populations in the species core range in the southern Sierra Nevada, including the eastern range edge. We then tested whether phenotypic differentiation between field populations persisted in the glasshouse, which would indicate differentiation is genetically rather than environmentally based. Secondly, to assess the adaptive significance of trait differentiation, we asked whether trait variation in the field and glasshouse are correlated with key environmental variables measured in the field. If trait–environment relationships are weaker at the range edge versus range centre, this would suggest less adaptive differentiation there. Lastly, we used a subset of six populations distributed from the centre to the range edge in a family-structured common garden to estimate between-family, within-population genetic trait variation. We used the estimates to assess whether range-edge populations have lower levels of trait heritability than central ones and to compare differentiation in phenotypic traits to differentiation at neutral genetic markers (Q-statistics versus F-statistics) as a test of whether differentiation in ecologically important traits is likely due to local adaptation.

Materials and methods

STUDY SPECIES

Clarkia xantiana ssp. *xantiana* is an outcrossing winter annual that occurs in grassland, pine-oak woodland and chaparral habitats. It occupies a narrow geographical range across approximately 4000 km^2 in the mountains of inland central and southern California (Fig. 1a; Lewis & Lewis 1955; Eckhart & Geber 1999). Populations are mostly discrete, separated by a few hundred metres up to tens of kilometres. Plants are pollinated by both generalist and specialist bees (Moeller 2006; Moeller *et al.* 2012), and seeds are gravity dispersed over short distances.

We studied *C. x. ssp. xantiana* in the core of its geographical range in the southern Sierra Nevada (Fig. 1a). In this area, the southwestern range edge occurs where the environment is heavily altered by human development and habitat changes abruptly. Populations occur along a steep rise into the mountains, on the slopes of the Kern River Canyon above the western Kern Valley, and northward along the North Fork of the Kern River, which parallels the eastern range edge (Eckhart & Geber 1999). There is a gradient of decreasing temperature and spring precipitation from west to southeast across this portion of the range (Eckhart *et al.* 2011). A genealogically distinct self-fertilizing subspe-

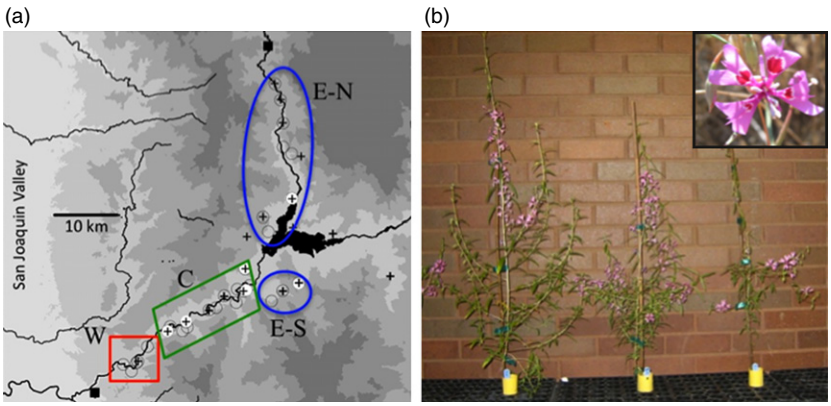

Fig. 1. (a) Location of 30 *C. xantiana* populations (circles) surveyed in the study area near Lake Isabella, CA. Elevation in the region ranges from <500 to >2500 m, indicated by increasing gray shading in 500 m intervals. Filled (white) symbols represent the six populations used in the family-structured common garden study. Crosses show locations of temperature and precipitation sensors and data loggers. Squares show locations of nearby NOAA weather stations. Colored shapes demarcate regions within the range: W, western region, red square; C, central region, green parallelogram; E – N and E – S, northern and southern regions of the eastern range edge, blue ovals. (b) Morphological variation among similar age plants grown in a common garden. Inset: *C. xantiana* flower.

cies, *C. x.* ssp. *parviflora,* is parapatric with *C. x.* ssp. *xantiana* along the latter's eastern range edge (Pettengill & Moeller 2012a). The subspecies are in secondary contact, and hybridization is limited (Pettengill & Moeller 2012b). Hereafter, in the text, the term '*C. xantiana*' will refer to the *xantiana* subspecies only, unless otherwise noted.

ENVIRONMENTAL MEASUREMENTS

In the spring of 2008, we studied 30 populations of *C. xantiana* distributed across its core range in the southern Sierra Nevada (Fig. 1a). The geographical location of each population, in units of easting (longitude) and northing (latitude) (UTM NAD 27, zone 11 north), was recorded with GPS. Populations were divided into four regions: western region near the base of the canyon (five populations), central region from mid-canyon to the upper reaches of the Kern Canyon (14 populations), northeastern and southeastern range-edge regions (eight and three populations, respectively). All eastern edge populations were at, or within 1 km, of *C. xantiana*'s range edge.

Across the area, we estimated three climate variables for the study region for the year 2008: total precipitation and mean daily temperature during the February–June spring growing season and spring equinox solar radiation. To do so, we used a network of data-logging temperature and precipitation sensors (Onset Computer Corporation, Bourne, MA, USA) that we installed on public land and have operated in the study region since 2005, which recorded data at 30 min intervals (Eckhart *et al.* 2011). We supplemented these data with publically available data from two National Oceanic and Atmospheric Administration weather stations just beyond the region's boundaries (Western Regional Climate Center; http://www.wrcc.dri.edu/; Fig. 1). Of 30 study populations, 14 had our sensors operating within them, while others were within 3 km of these or a NOAA station (Fig. 1a). We modelled growing season precipitation at 1 ha resolution (approximately the size of the average natural *C. xantiana* population) by applying inverse distance weighting in ArcGIS 9.3 (ESRI, Redlands, CA) to values from loggers and stations. With this method, estimates at stations and loggers were their actual values, while estimated values between stations were spatially weighted averages of nearby known values. To estimate mean temperature, we used predicted values from an analysis of covariance (using Minitab 16, Minitab Inc., State College, PA) of sensor and station means as a function of year and elevation, applying predictions at 1 ha resolution. Solar radiation (predicted for the spring equinox) was estimated in ArcGIS 9.3 in units of Watt-hours per m^2, under a 12-h day.

For topographic variables, slope was calculated as the average of three measures taken at separate locations within each site. Aspect was measured as the midpoint of compass values from two locations within a site and converted to linear azimuth, a measure of the north–south orientation, with higher values indicating more southern exposure (Warren 2008).

FIELD TRAIT MEASUREMENTS

In each of the 30 *C. xantiana* populations, we measured phenotypes on 61–149 plants (average = 111), along two or three parallel transects spanning each site. Plants were measured every five paces, with more plants sampled from larger populations. We took phenotypic measurements only on plants that lacked heavy damage by herbivores. For each plant, we measured height (cm) and number of primary branches. For plants that had at least one branch, we calculated an index of 'branchiness' as the total number of branches divided by plant height. We counted the total number of flowers on each plant and recorded the maturation stage of each flower. *Clarkia* flowers are protandrous, progressing through five stages, scored as: (1) developing bud, (2) male, when anthers are fully dehiscent, (3) female, when the stigma is receptive, (4) green immature fruit and (5) brown dehiscent fruit. The flowering stage for each plant was calculated as the average maturation score of all flowers on the plant, and then the population average flowering time was calculated as the mean of these values across all plants measured at the site. By this measure, populations with a higher value flowered earlier in the season. Because measuring all 30 populations took 11 days in the field, we adjusted flowering time for differences in sampling date by remeasuring the flowering stage of 13 populations (76–139 plants/population) a second time, 5–14 days following the initial measurement. We calculated the change in average flowering stage per day and used this rate of change to standardize flowering stage to a single date at the middle of the measurement period. For populations that were not remeasured, the average population change (0.104 stages/day, stdev = 0.029) was used for standardization.

We collected one fruit from each of 15 plants per population and weighed four seeds per fruit to calculate an average seed weight. The seeds were then used in a 30 population common garden glasshouse experiment (see below). The census population size of each population was estimated as the product of the average number of fruiting plants across 50–100 haphazardly distributed sampling plots (0.5 m^2) and the site area obtained from GPS coordinates of the circumference of each site.

THIRTY POPULATION COMMON GARDEN

To assess whether phenotypic differences among field populations persist in a common environment, we planted four seeds from each field-collected plant into 16 cm 'cone-tainers' (Stuewe & Sons, Tangent, OR, USA) in MetroMix 360 potting soil (Sun Gro, Bellevue, WA, USA; Fig. 1b). Pots from each plant and source population were randomly assigned to seven blocks in a growth chamber under short 10-h days at cool temperatures (12/10 °C day/night) for 4 weeks to simulate winter conditions preceding germination. Daytime temperature was then increased to 18 °C for an additional 4 weeks to simulate spring warming. Germination rate was fairly even (from 60 to 85% of seeds germinated for 28 of 30 populations), and there was no significant relationship of germination rate with geography (for easting and northing position within the range, significance $P = 0.28$ and 0.37, respectively). We thinned out all but the first emerging seedling from each pot, randomized the pots into six complete spatial blocks in the glasshouse, with two plants per population per block, and grew them at 20/18 °C, 12-h days under supplemental metal halide lighting. For each plant, we measured the date of first flower, and on the same day, we measured the height, the number of primary branches (from which we calculated the index of branchiness) and the number of flowers (including buds over 0.5 cm long). We obtained vegetative measurements on an average of 11.4 plants per population and floral measurements on an average of 11.3 plants per population (total $N = 341$ plants).

FAMILY-STRUCTURED COMMON GARDEN

For 6 of the 30 populations, we generated full-sib families of seeds in the glasshouse. Two populations were used from each of three geographical regions: the range centre, a region intermediate between the centre and the eastern edge, and the eastern edge (Fig. 1a). For each population, a single seed was germinated in a growth chamber (as above) from each of 48 field-collected plants, and seedlings were paired at random (within populations) and crossed reciprocally at flowering across six temporal blocks in the glasshouse to generate 24 full-sib families per population. We measured the average weight of eight seeds per family (four from each reciprocal parent), planted seeds in cone-tainers, and allowed germination in the growth chamber (as above). We transferred the seedlings into each of four spatial blocks in the glasshouse. This was carried out for two separate cohorts of plants (temporal blocks), together totalling 576 plants. For each plant, we measured the date of first flowering, and on the same day, we measured plant height and the number of primary branches from which we calculated branchiness. We also measured two floral characters related to the degree of outcrossing in this subspecies: herkogamy (the distance between anthers and the mature stigma) and the length of the apical petal (a measure of flower size) on the second flower to open on each plant (Moore & Lewis 1965; Runions & Geber 2000; Moeller & Geber 2005).

STATISTICAL ANALYSES

Environment

To detect and characterize differences in local environment experienced by each population across the range, we conducted principal components analysis based on correlations between five environmental variables in JMP Pro 9.0.2 (SAS Institute Inc., Cary, NC; Fig. 2 and Table S1 in the Supporting Information). We also examined differences between regions within the range (western, central, northeastern and southeastern range edge) for each environmental variable separately using one-way analyses of variance with region as the independent variable (Fig. S1). We used Tukey's *post hoc* tests to determine which regions differed from others.

Regional variation in traits and relationships between plant traits and environment

We estimated population trait means using BLUP estimates obtained from general linear models partitioning regional and population sources of phenotypic variation (see next section). We tested for regional differences in population trait means in one-way analyses of variance with region as the independent variable. We used Tukey's *post hoc* tests to determine which regions differed from others (Fig. S2).

One indicator that differentiation between populations is adaptive is a strong relationship between variation in population trait means and environment across the species range, either in the field, the common garden or both. To examine the relationship between the five environmental variables and plant traits, we used stepwise regressions to determine the best predictors of population mean trait values (BLUP estimates) based on the corrected minimum Akaike information criterion (AICc; Hurvich & Tsai 1989; both forward and backward step-wise regression approaches were in agreement, Table S3). Significant predictors for each trait (at $P \leq 0.05$) in the stepwise regressions were retained in the final model. To assess whether eastern range-edge populations show evidence of reduced adaptive differentiation in response to the environment, we performed an analysis of covariance of each trait against the best environmental variables from the stepwise regressions, with geographical region and interactions between geographical region and environmental variables included in the models. For this analysis, we included only two regions: central and eastern range edge (pooling northeastern and southeastern edge populations). There were too few western populations to estimate separate trait–environment relations for this region. A significant region by environment interaction, along with a shallower slope of trait versus environment for eastern edge relative to central populations, would indicate weaker adaptive differentiation at the edge.

Where the best model included only a single significant environmental variable, we plotted population trait means against the environmental predictor, showing the overall regression lines for all 30 populations and separate regression lines for central and edge populations (Figs S3 and S4). Where more than one environmental variable was included in the best model, we used partial linear regression to examine the relationship between the plant trait and each environmental variable separately, holding the other environmental variables constant, again plotting the overall regression lines for all populations and separate lines for central and edge populations (Figs S3 and S4).

Variance components, heritability and trait correlations

For the field study, 30 population common garden study, and the six population family-structured study, we analysed sources of phenotypic

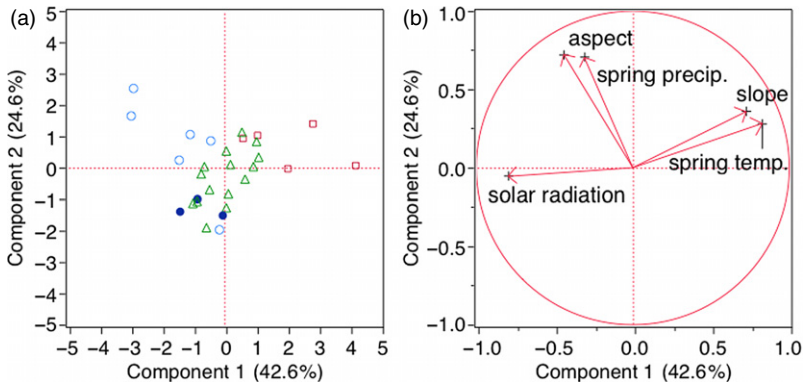

Fig. 2. PCA ordination plots of environmental conditions. Principal component loadings and eigenvectors of environmental data are in Table S1. (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge).

variation for each trait using general linear random effects models using the *nlme* and *lme4* packages in R (R Development Core Team, 2011). Average plant height, branchiness, flower number and seed weight were log-transformed, and normality of residuals was verified.

For the field data, population was included in the general linear model as the only random factor. For the 30 population glasshouse study, glasshouse block and population were included as random effects. In the family-structured study of six populations, region (centre, intermediate and edge), population, family nested within population and glasshouse block were included as random effects. All analyses were performed using *lmer* in *R*. The significance of the population, family or region, depending on the analysis, was evaluated with a chi-square test (with 1 d.f.) of twice the difference in the log-likelihood of models with versus without the factor of interest, [using *lmer* and restricted maximum likelihood estimation (REML)]. Final estimates of variance components (e.g. between versus within regions, populations or families) were estimated using REML with the *lmer* function. Population mean phenotypes for each trait in each data set were estimated as the random effects coefficients of populations (BLUPs) in the general linear models.

We estimated the upper bound on broad-sense heritability in the family-structured study as twice the intraclass correlation ($H^2 = 2^*(V_{\text{between family}}/(V_{\text{between family}} + V_{\text{within family}}))$) for each trait ($V$ = phenotypic variance; Falconer 1960). Ninety-five percent confidence intervals for heritability were calculated according to Lynch & Walsh (1998). We interpreted overlapping confidence intervals of heritabilities from different populations to signify no difference in levels of genetic variation for a trait. Within- and between-family components of trait variation were obtained from general linear random effects models, with family as a random effect, for each population separately. For each of the six populations, we also estimated pairwise trait genetic correlations as the standard product moment correlation between family means for each trait (Via 1984). Because between-family, within-population trait correlations were based on measurement of 24 families per population (four individuals per family), statistical power is somewhat limited in this analysis.

F- and *Q*-statistics

F_{ST} was calculated from nucleotide variation at eight nuclear loci, each sequenced for 135 individuals (20–23 individuals per population, further details in Moeller, Geber & Tiffin 2011). These loci were chosen on the basis of single copy status in the genome, successful PCR amplification, and not based on level of sequence polymorphism, and there is no evidence these loci are under strong selection. Together, they provide an estimate of background genome-wide differentiation and are hereafter referred to as 'neutral' loci. We examined differentiation between each pair of populations (Hudson, Boos & Kaplan 1992) and hierarchically between geographical regions (F_{CT}; regions: centre, intermediate and edge) and between populations nested within regions (F_{SC}), using AMOVA (Excoffier, Smouse & Quattro 1992).

Q_{ST} is a measure of population differentiation in quantitative genetic traits that is directly comparable with the neutral genetic measure of differentiation, F_{ST} (Spitze 1993; Leinonen & Merilla 2008). For each trait, we calculated an overall (broad sense) Q_{ST}, two hierarchical Q_{ST}'s [between regions (Q_{CT}) and between populations within regions (Q_{SC})], as well as a Q_{ST} for each pair of populations. Variation in complex traits is often mostly additive in diverse organisms (reviewed Hill, Goddard & Visscher 2008) and thus broad-sense Q_{ST} should not greatly overestimate narrow sense Q_{ST} (Pujol *et al.* 2008). With regard to non-additive maternal effects on variance, seeds were generated in the glasshouse from reciprocal crosses, so effects should be much reduced, but are perhaps not entirely eliminated due to differential adaptation of populations to glasshouse conditions. *Q*-statistics were calculated as follows:

$$Q_{ST}(\text{overall, among populations}) : V_{pop}/(V_{pop} + 2V_{family(pop)}),$$

$$Q_{CT}(\text{among regions}) : V_{region}/(V_{region} + V_{pop(region)} + 2V_{family(pop)})$$

$$Q_{SC}(\text{between populations within regions}) : V_{pop(region)}/(V_{pop(region)} + 2V_{family(pop)})$$

$$\text{Pairwise } Q_{ST}, \text{for population pair } i,j : V_{i,j}/(V_{i,j} + 2V_{family(pop\ i,\ j)}),$$

The variances V_{region}, V_{pop}, $V_{pop(region)}$ and $V_{family(pop)}$ were obtained from a nested linear model with three random factors: region (centre, middle, edge), population nested within region and family nested within population. Variances used to calculate Q_{ST} for each population pair (15 combinations) were estimated from a nested linear model with two factors: population and family nested within population.

For neutral quantitative traits that evolve primarily through drift, Q_{ST} is predicted to equal F_{ST} (Lande 1992; Spitze 1993; McKay & Latta 2002; Whitlock 2008). For traits under the influence of diversifying (locally adaptive) selection among populations, Q_{ST} is predicted to be greater than F_{ST}. For traits under stabilizing selection for the same optimum across populations, Q_{ST} will be lower than F_{ST}. In our analyses, we compared each hierarchical Q statistic for each trait, to the average value across loci of each hierarchical F statistic. As a more rigorous test, we also compared the average pairwise Q_{ST} value for each trait to the distribution of all pairwise F_{ST} values for the eight loci. We considered traits with average pairwise Q_{ST} greater than the 95th percentile of the pairwise F_{ST} distribution to be under diversifying selection. We considered average Q_{ST} between the 90th

and 95th percentiles of the F_{ST} distribution as suggestive of diversifying selection (Whitlock 2008).

Results

LANDSCAPE ENVIRONMENTAL VARIATION

The environment experienced by *C. x. xantiana* populations varies in complex ways over the range. There are two major axes of environmental variation in opposing geographical directions (Figs 2 and S1 and Table S1). Temperature and slope decrease in parallel ($r = 0.56$) from southwest to northeast across the study area (Fig. S2). Temperature declines because of increasing elevation. The average slope of study sites declines in part because the Kern Canyon is narrowest and steepest at its base in the southwest (Fig. 1a), but possibly also because the slopes where *C. x. xantiana* populations can persist may vary geographically (Kramer *et al.* 2011). Spring precipitation and linear azimuth are correlated ($r = 0.35$) and vary in an orthogonal direction to the previous gradient, increasing from southeast to northwest. Spring precipitation tends to be higher and occupied sites tend to be more south facing in the northwestern section of the range. A gradient of solar radiation overlies variation in the previous two gradients, increasing strongly from west to east, and south to north, due to the topography of the canyon. Thus, populations experience a wide variety of environmental conditions across the range. At the extremes, some southwestern sites occur deep in the shaded parts of the canyon where precipitation is low, temperatures are high in the spring and slopes are steep and north facing. Other populations occur east of the canyon where it is cooler, slopes are shallower, and there is more sunlight (Figs 2 and S1). There is also a wide variation between population environments within different regions of the range (Fig. S2). Particularly at the range-edge, northern populations experience substantially more spring precipitation than southern populations.

PHENOTYPIC TRAIT DIFFERENTIATION

For 30 populations measured both in the field and glasshouse, almost all traits exhibited significant differentiation between populations for vegetative and reproductive characters (1). The proportion of phenotypic variation attributable to population differences was highest for flowering time (47.4% field and 26.9% common garden) and lowest for branchiness (9.5% field and 0% common garden; Table 1). We found the same result when examining six populations in a family-structured common garden, with larger sample sizes of individuals within populations. Except for seed mass, all traits, including branchiness and two floral characters, exhibited significant differentiation between populations (Table 2). Flowering time, again, was the most differentiated trait. Overall, there were also clear differences between plants growing in the field versus the common garden. First, plants grown in the common garden were much larger, branchier and had more flowers and heavier seeds than field plants (Table S1). Furthermore, population mean phenotypes in the field were not generally predictive of mean phenotypes in the glasshouse, except for flowering time (Fig. 3, $r = 0.67$, $P < 0.0001$). Correlations between height, branchiness and flower number between the field and glasshouse environments were non-significant ($R^2 = 0.01, 0.04, 0.11$, respectively).

At the scale of regions, in the field, differences between western, central and range-edge populations were found in most traits, especially in flowering time and seed weight (Fig. S2). Flowering time was much later in southeastern edge than western populations, and seed weight declined from west to east. In the common gardens, however, none of the regional patterns were statistically significant. In the 30 population common garden (Fig. S2) and the family-structured study (Table 2), region did not contribute significantly to phenotypic variation in traits; rather phenotypic variation was more attributable to variance between populations within regions and between families within populations.

ENVIRONMENT–PHENOTYPE ASSOCIATIONS

All measured traits, except branchiness, co-varied with one or more environmental variables in the field (Table S2). The best environmental predictors of trait variation were frequently different for traits in the field versus in the glasshouse. In the field, plants were taller, had heavier seeds, flowered earlier and had more flowers at warmer, more south facing sites (Fig. S3). By contrast, in the glasshouse, phenotypic variation was more strongly related to site differences in spring precipitation and solar radiation (Table S2 and Fig. S4). Plants from sites with high precipitation were taller, branchier and had more flowers. Plants from sites with more sun flowered earlier were shorter and had fewer flowers (Fig. S4). The only trait with common environmental predictors in the field and common garden was flowering time. In both settings, flowering began earlier in populations from sites on steeper slopes with higher solar radiation. In all cases, the strength and direction of trait–environment relationships did not differ greatly between central and eastern edge populations (Figs S3 and S4; $P > 0.05$ for all region by environment interactions in analyses of covariance).

TRAIT HERITABILITIES AND GENETIC CORRELATIONS BETWEEN TRAITS

In the family-structured common garden experiment, all traits, including two floral characters, exhibited significant heritable variation. Within-population variation exceeded between-population variation for all traits except flowering time (43.5% between populations versus 6.1% between families within populations, Table 2). Broad-sense heritability of traits (H^2) varied widely by population for all traits and was significantly greater than zero in three or more populations (of six) for all traits except flowering time (for which we could detect heritability significantly greater than zero in only two populations, Fig. 4). We did not detect a relationship between

Table 1. Field and common garden variance components of phenotypic traits for 30 populations

	Field 2008			Common garden 2008		
Phenotypic trait	Total	B/w pop (%)	W/in pop	Total	B/w pop (%)	W/in pop
Flowering time*	1.082	**0.512** (47.4)	0.570	44.38	**11.94** (26.9)	32.44
Height (cm) ($\log_e$)	0.258	**0.056** (21.6)	0.202	0.076	**0.016** (20.5)	0.060
Branchiness (no./cm) ($\log_e$)	0.381	**0.036** (9.5)	0.345	0.101	0.000 (0.0)	0.101
Flower number ($\log_e$)	0.861	**0.105** (12.2)	0.755	0.389	**0.056** (14.5)	0.333
Seed weight (mg) ($\log_e$)	0.066	**0.025** (37.4)	0.041	–	–	–

Significant between-population variances are in bold ($P \leq 0.05$) with percentage of total variance shown in parentheses. Total phenotypic variances are the sum of the variance within and between populations. Within-population variance was measured as residual variance which includes within-population environmental variance.
*Measured as flowering stage in the field, as date of first flowering in the common garden.

Table 2. Components of phenotypic variation among six populations in a family-structured common garden

Phenotypic trait	B/w region (%)	B/w pop (%)	B/w families w/in pops (%)	W/in families (%)
Flowering time (days)	1.2 (4.2)	**12.4** (43.5)	**1.7** (6.1)	11.0 (38.7)
Height (cm) ($\log_e$)	0.0 (0.0)	**16.2** (7.6)	**25.1** (11.8)	139.9 (65.6)
Branchiness (no./cm) ($\log_e$)	0.0005 (7.8)	**0.0005** (9.3)	**0.0010** (17.1)	0.0036 (61.5)
Petal length (cm)	0.007 (6.7)	**0.007** (6.7)	**0.017** (16.2)	0.066 (63.6)
Herkogamy (mm)	0.000 (0.0)	**0.009** (17.4)	**0.010** (20.2)	0.032 (61.6)
Seed mass (mg) ($\log_e$)	0.0031 (7.5)	0.0018 (4.3)	**0.0116** (27.9)	0.0250 (60.3)

Phenotypic variances are listed with per cent of total variance in parentheses. Variances are calculated within regions (western, central and range edge), between populations within regions, between families within populations and within families (residual variance). Variance due to block effect in the glasshouse is not shown. Significant values between regions, populations and families are in bold ($P \leq 0.05$).

the amount of trait heritability within populations and their proximity to the range edge (Fig. 4) or their census size.

In the family-structured common garden study, we detected some significant but weak correlations between traits across all populations, but these correlations were not consistent within individual populations (Table S4). Overall, size-related traits were positively correlated across all families measured, and this relationship extended between vegetative and floral characters. However, for no trait, pair was correlation consistently strong or in the same direction in all populations, and no single population had strong correlations for all traits.

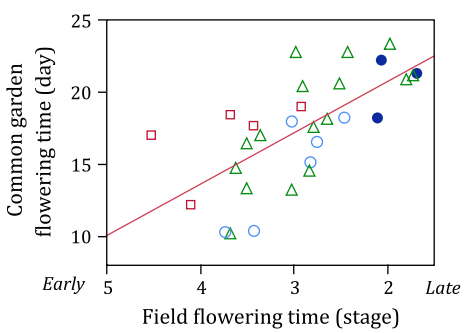

Fig. 3. Population average flowering time in the common garden and the field for 30 *C. xantiana* populations. (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge). (Linear regression line is shown, $P < 0.0001$).

QUANTITATIVE VERSUS NEUTRAL GENETIC DIFFERENTIATION: *F*- AND *Q*-STATISTICS

In general, average Q_{ST}'s (pairwise and overall across populations) were consistently higher than average F_{ST} across neutral loci (Table 3). Average pairwise Q_{ST} (column 2) was significantly greater than average F_{ST} for branchiness, flowering time, herkogamy and seed weight. Differentiation was especially strong for the first three traits, where Q_{ST} exceeded the 90th percentile (herkogamy, branchiness) and 95th percentile (flowering time) of the distribution of average F_{ST} (Fig. 5).

The relative degree of differentiation at different geographical scales was similar when measured using neutral loci and using quantitative traits. Neutral and trait-based differentiation between populations within regions (F_{SC} and Q_{SC}) was higher than differentiation between regions (Q_{CT} and F_{CT}) (Table 3, column 4 vs. 5) for the four traits with the highest average pairwise Q_{ST}'s.

Discussion

The goals of this study were to assess potential limits to eastward range expansion in the southern Sierra Nevada for *Clarkia xantiana* ssp. *xantiana*, a California endemic plant, because of limited adaptive differentiation or lack of genetic diversity in edge populations. Theory suggests that adaptation to conditions beyond the range edge may be caused by limited genetic variation in, or strong and unfavourable genetic corre-

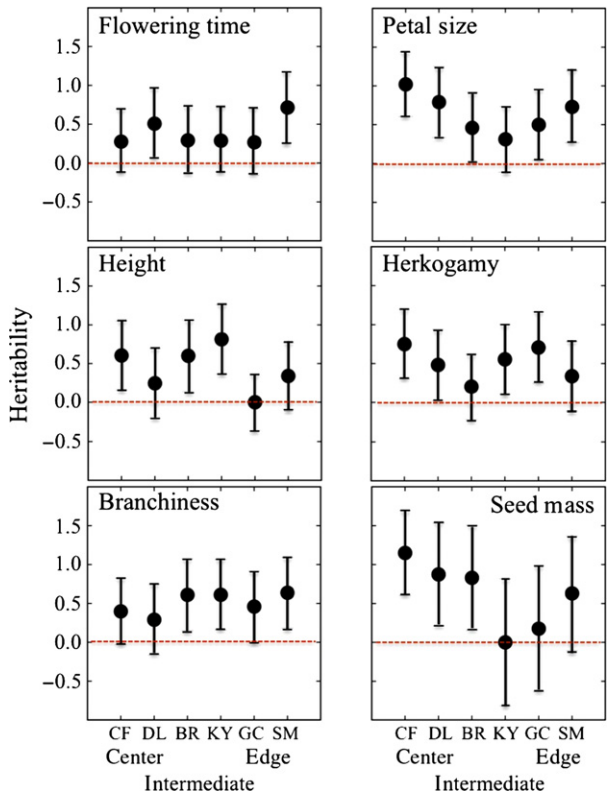

Fig. 4. Broad-sense heritability (H^2) estimates for six plant traits for six *C. xantiana* populations. Data are shown for two central (CF, DL), two intermediate (BR, KY) and two eastern range edge populations (GC, SM). Error bars represent 95% confidence intervals (CI). Estimates whose CI are above the dotted line are significantly different from zero. For all traits, confidence intervals of heritability estimates from the three regions overlap. (Linear regression line is shown, P < 0.0001).

Table 3. Pairwise and hierarchical *Q*- and *F*-statistics among six glasshouse populations

Trait	Average pairwise Q_{ST}* and F_{ST}	Between populations Q_{ST} and F_{ST}	Between populations within regions Q_{SC} and F_{SC}	Between regions Q_{CT} and F_{CT}
Flowering time	**0.532**	0.697	0.69	0.084
Height ($\log_e$)	0.187	0.180	0.182	0.000
Branchiness ($\log_e$)	*0.246*	0.312	0.301	0.000
Petal length	0.192	0.229	0.078	0.192
Herkogamy	*0.256*	0.297	0.291	0.000
Seed weight ($\log_e$)	0.171	0.159	0.058	0.130
F_{ST}	0.109	0.082	0.049	0.034

*Average pairwise Q_{ST} values above the 95th percentile of all pairwise F_{ST} values are in bold; those above the 90th percentile are in italic.

lations between, ecologically important traits. Low or primarily maladaptive genetic variation in edge populations may also be caused by biased gene flow from central to marginal populations if central populations are locally adapted to conditions at the range centre. Two previous reciprocal transplant studies of central and eastern edge populations of *C. x. xantiana*, spanning three transplant years (1997–1998 and 1998–1999 [Geber & Eckhart 2005;]; 2009–2010 [Geber *et al.*, in prep]) consistently found evidence of greater levels of local adaptation in central populations than edge populations. In two of the three transplant years (1997–1998 and 2009–2010), central populations had highest fitness and outperformed edge populations at the centre site, whereas edge populations often had higher fitness transplanted away from the edge and did not outperform central populations at the edge. Only in one relatively dry year, 1998–1999, did edge populations exhibit a slight, although non-significant, fitness advantage over centre populations when transplanted to the edge. Population genetic studies further indicate asymmetric gene flow from central to eastern edge populations in this species (Moeller, Geber & Tiffin 2011).

Using trait measurements from 30 populations in the field, the same 30 populations grown in a glasshouse common garden, and from a subset of six family-structured populations from across the range, we tested whether vegetative and reproductive traits show evidence of local adaptation, and whether patterns of quantitative genetic trait diversity are consistent with the above hypothesized drivers of range boundary formation. We tested (i) whether plant traits show evidence of correlation with environment, indicative of locally adaptive selection; (ii) whether local adaptation (trait–environment correlations) is weaker at the range edge than centre; and (iii) whether range-edge adaptation could be limited by low quantitative genetic diversity or strong genetic trait correlations within populations.

POPULATION DIFFERENTIATION AND ADAPTATION

In *C. xantiana*, we found that quantitative genetic trait differentiation between populations is ubiquitous. Among a sample of 30 populations spanning the core of the species range, there was strong evidence for phenotypic and genetic differentiation in vegetative, phenological and reproductive characters among populations. The pattern was replicated in a second common garden study of a subset of populations where flowering time, height, branchiness and two floral characters were also differentiated between populations (Table 2). Results of the family-structured study also indicate that differentiation is much greater both between and within populations rather than between regions (centre, intermediate and edge regions, Fig. S2, Tables 2 and 3). Although we might have expected low regional differentiation due to gene flow (Moeller, Geber & Tiffin 2011), it is noteworthy that gene flow between populations within regions does not similarly homogenize differentiation in ecologically important quantitative traits.

Population differentiation has likely been driven by local adaptation to climate variables given the strong trait–environment relationships for most traits both in the field and glasshouse (Figs S3 and S4), and the fact that the

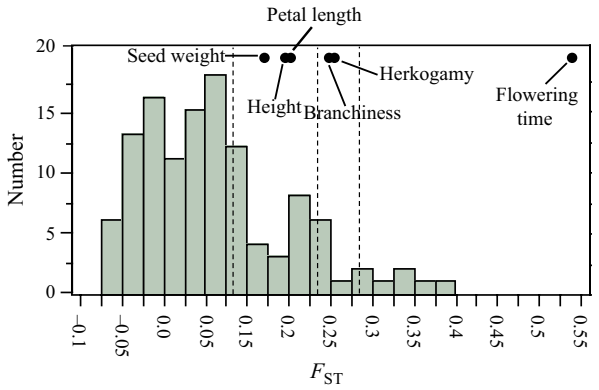

Fig. 5. Frequency distribution of pairwise F_{ST} values for eight molecular markers in six populations of *C. xantiana* used in the family-structured common garden study (N = 121 values, see Methods). The dotted lines mark the mean, the 90th, and the 95th percentiles of the distribution (0.109, 0.235 and 0.285, respectively). Dots represent the average pairwise Q_{ST} values for each trait.

environmental factors measured in this study are known to affect plant growth, survival and reproduction (Eckhart *et al.* 2010, 2011; Kramer *et al.* 2011). We did not find weaker trait–environment relationships among edge populations suggestive of lower levels of adaptive differentiation compared with central populations, even though this was the expectation given the findings from reciprocal transplant experiments summarized above (Geber & Eckhart 2005; Geber *et al.*, in prep). We had low power to detect small differences in the strength of trait–environment relationships between regions; however, large differences would have been evident. Also the direction of correlations in this study did not differ between the centre of the range and the range edge (Figs S3 and S4). This suggests that populations in both regions have diverged to similar extents in response to diversifying environmental selection.

The pattern of trait differentiation across *C. xantiana*'s range is complex, and only a few traits (flowering time in field and glasshouse, and seed weight in the field) exhibit clinal variation from west to east. We attribute this to the ability of populations to adapt to environmental variation at subregional scales. (Most environmental factors vary widely between populations within regions and only temperature appears to vary more between regions themselves, Fig. S1). Despite linear changes in temperature from west to east, there are countervailing gradients in growing season precipitation and southerly aspect (Figs 2 and S1 and Table S1; Eckhart *et al.* 2011). Temperature and precipitation as well as aspect and slope largely vary in orthogonal directions to each other rather than in parallel across the range. The eastern range edge itself is complex. Temperatures are generally cooler at the range edge than at the range centre, but precipitation varies widely from north to south (Fig. S2). Range-edge populations are even differentiated north to south for flowering time (Fig. S3). As a result of all these factors, trait differentiation between populations within regions was as great as, or greater than, differentiation between regions of the geographical range.

Because the environmental variable that best predicts phenotype is different for traits measured in the field versus traits measured in a common garden, it is difficult to say whether trait plasticity observed in the field or genetically based phenotypic differences observed in the common gardens (or both) reflect adaptation. In earlier reciprocal transplant studies of central and peripheral *C. xantiana* populations, plasticity (large transplant site and year effects on phenotype) was found in some of the same phenotypic traits measured here (Eckhart, Geber & McGuire 2004), as was evidence of local adaptation (Geber & Eckhart 2005). Measurements of selection on traits in reciprocally transplanted populations may help discern the relative importance of plasticity versus intrinsic genotypic differences to adaptation (Anderson *et al.*, in prep).

Additional evidence that trait divergence between populations is adaptive comes from comparisons of the level of differentiation at quantitative traits (Q_{ST}) versus neutral loci (F_{ST}) in a sample of six central to peripheral populations. Q_{ST} was significantly higher than average F_{ST} for four of six traits, and for flowering time, branchiness and herkogamy, range-wide Q_{ST} exceeded the 90th percentile of the distribution of pairwise F_{ST}'s. The fact that locally adaptive selection commonly promotes interpopulation trait differentiation, particularly in wild plants, has been demonstrated across studies (Linhart & Grant 1996; McKay & Latta 2002; Leinonen & Merilla 2008), and here, this conclusion is supported even across an exceptionally narrow geographical range.

The best evidence for strong genetic determination and common environmental drivers of adaptive divergence in *C. xantiana* is for flowering time. It is the trait that: (i) is most strongly differentiated between populations both in the field and glasshouse, (ii) shows a strong correlation between the two growth environments and (iii) is correlated with common environmental predictors – spring solar radiation and slope – in the two growth environments. In contrast to other traits, flowering time is differentiated between populations more so than between families within populations. In annuals, early flowering is thought to be adaptive as a means of completing the life cycle before the onset of severe environmental stress, such as heat or drought (Levitt 1980; Rice & Mack 1991; Aronson *et al.* 1992; Stanton, Roy & Thiede 2000). Indeed, in the genus *Clarkia,* populations and species from hot and/or dry environments consistently flower earlier, when grown in a common environment, than those from cooler and/or wetter locations (Moore & Lewis 1965; Vasek 1971; Vasek & Sauer 1971; Jonas & Geber 1999; Eckhart, Geber & McGuire 2004; Dudley, Mazer & Galusky 2007). Our data support this hypothesis: we find earlier glasshouse flowering in populations from sunnier, steeper slopes, environments which are likely to be warmer and experience more variable water availability (experience faster drainage) throughout the range.

Q_{ST}/F_{ST} comparisons also suggest, to a lesser degree, that population differences in herkogamy, a trait related to mating system, reflect adaptive divergence. Previous work has shown that herkogamy is reduced in eastern peripheral populations relative to central populations (Moeller 2006), although petal (flower) size does not differ (Eckhart, Geber & McGuire

2004). At a smaller sample size, in this study, we did not detect significant regional differences in herkogamy; however, we did find that range-wide Q_{ST}/F_{ST} is high for herkogamy but does not differ significantly from one for petal size. This suggests variation in herkogamy in *C. xantiana* populations is locally adaptive. Small flowers and limited herkogamy are both generally associated with evolution of the selfing habit in plants (reviewed, Wyatt 1988) and are characteristic of selfing taxa in the genus *Clarkia* (Lewis & Lewis 1955) including the subspecies *C. x.* ssp. *parviflora* (Fausto, Eckhart & Geber 2001; Eckhart, Geber & McGuire 2004). Here, we find that, range-wide, plants with greater herkogamy are taller and branchier (another character that has relatively high Q_{ST}). This is consistent with the idea that selection on herkogamy is indirect due to correlated selection on plant size and growth rate, rather than due to selection for an accelerated life cycle in drought-prone habitats. However, conclusions from physiological studies of *C. xantiana* flower development have been mixed (Runions & Geber 2000; Mazer, Paz & Bell 2004), and the nature of selection on herkogamy remains ambiguous.

Q_{ST} analysis also suggests local selection on plant architecture (branchiness). Branching is a complex trait that can be influenced by selection for light and nutrient-use efficiency or selection on root to shoot allocation ratio, among others (Bonser & Aarssen 1996; Baker, Hileman & Diggle 2012). In our common garden study, branchiness was greater in populations from sites with higher precipitation. In years when water and other resources are abundant in the field (a condition simulated by the glasshouse environment), plants that produce more branches, and hence, more flowers may have higher fitness than less branched plants.

COULD LACK OF GENETIC VARIATION OR PRESENCE OF STRONG GENETIC CORRELATIONS LIMIT RANGE EXPANSION?

A potential evolutionary limit on range expansion is a low level of genetic variation in ecologically important traits at the range edge. If peripheral populations are demographically stable, they may diverge adaptively from central populations, but adaptation to environments beyond the range edge may be limited because a history of strong selection, small population size and/or inbreeding has reduced levels of genetic variation. In *C. xantiana*, more than one measure of overall genetic diversity (haplotype richness at nine nuclear loci and allelic richness at four microsatellite loci) are lowest in peripheral populations (and subsequently increase towards the range centre; Moeller, Geber & Tiffin 2011). However, in this study, we found no evidence that the amount of genetic variation (heritability) for traits or the number of traits with heritable variation was lower in peripheral compared with central populations. Levels of genetic variation were also not correlated with census population size. We found that almost all heritability values and their confidence intervals are broadly overlapping between regions. Our statistical power to compare heritability between the range centre and edge was limited. However, large differences in heritability between central and edge populations would have been evident here.

Similarly, adaptation may be limited by a lack of diversity in the way that traits are correlated with each other, that is, if there are strong maladaptive genetic correlations between traits within a population. We tested whether size and flowering-related traits are strongly correlated within populations, an indicator that lack of flexibility in trait covariation could constrain adaptive evolution, particularly in range-edge populations. Genetic correlations between traits, both vegetative and floral, showed range-wide positive correlations: bigger plants are bigger overall. However, most genetic trait correlations between families within populations were not strong, and the pattern of trait correlation was not consistent within populations (Table S4). (For 7 of 15 trait correlations, the highest and lowest population values were different from each other at $P \leq 0.10$, comparisons of Fisher's z-transformed correlation coefficients, data not shown). Trait correlations also did not appear to be consistently stronger in range-edge populations, suggesting that the genetic architecture of traits measured here is free to evolve and is not likely to limit adaptation.

It is of note that genetic constraints of other types may still contribute to lack of range expansion in *C. xantiana*. In particular, if many traits must be selected simultaneously to allow range expansion, lack of variation in some traits (particularly those not measured here) may still be low enough to prohibit colonization beyond the range edge despite high levels of variation in other required traits. Whether genetic constraints in general play a role in restricting adaptation varies widely by species (Etterson & Shaw 2001; Futuyma & Agrawal 2009; Futuyma 2010), and a role for genetic constraints cannot be entirely ruled out for *C. xantiana*.

COULD MALADAPTIVE GENE FLOW LIMIT RANGE EXPANSION?

Source-sink or colonization/extinction dynamics also have the potential to limit adaptation if populations at the range edge persist solely through immigration from central ones. Edge populations may be perpetually maladapted and genetically similar to more central populations because of repeated or swamping gene flow from the range centre. For *C. xantiana,* the results are not definitive, but certainly do not strongly support this hypothesis. Despite gene flow between populations (Moeller, Geber & Tiffin 2011), edge populations are well-differentiated from central populations. Trait–environment relationships are as strong among edge populations as central populations. Indeed, it is clear that gene flow is not sufficient to restrict local adaptation even between populations within regions. It is possible the selective forces restricting trait homogenization at this scale may be the same ones that prohibit adaptation of populations to conditions beyond the range edge. In this study, we can say generally that lack of population structure across the range (low F_{ST}) implicates very strong diversifying selection in maintaining interpopulation trait differences rather than genetic drift, but more analyses are forthcoming (Andersen *et al.* in prep). The fact remains that

we cannot explicitly rule out that gene flow has any role at all in inhibiting range-edge adaptation using current theoretical models. The most well-known theoretical models address the dynamics of adaptation across linear environmental gradients (Lande 1992; Dias 1996; Kirkpatrick & Barton 1997) but have not, to our knowledge, addressed adaptation across more complex landscapes or selection scenarios. For example, an assumption of the Kirkpatrick & Barton (1997) model is that trait optima across populations follow a linear gradient. In the present study, we find that the most differentiated trait, flowering time, varies significantly along two or more environmental axes in both the glasshouse and the field, and these axes are themselves not parallel but sometimes orthogonal. Range-edge populations themselves vary widely in average flowering time between the north and south.

It is true that across simple linear environmental gradients, a low amount of gene flow from range centre to edge may allow population differentiation but still prevent trait variation in range-edge populations from attaining the right magnitude to allow adaptation to conditions beyond the edge (Dias 1996; Kirkpatrick & Barton 1997). The same might be predicted for plants distributed across landscapes encompassing more than one significant environmental gradient, as in this study. We might reasonably expect the genetic trait variation and covariation required for adaptation in such cases is much greater than that required for adaptation at the extreme of a single gradient. Few studies have directly tested whether the magnitude or rate of change of phenotypic variation from centre to edge populations excludes the possibility of adaptation at the range edge (but see Angert & Schemske 2008). However, because for *C. xantiana* the amount of heritable trait variation in edge populations is within the same range of values and varies in some cases more than in central populations, we find it unlikely that adaptation would be limited because of maladaptive gene flow in *C. xantiana*.

Conclusion

Overall, our results are inconsistent with the hypotheses that peripheral *C. xantiana* populations are depauperate in genetic variation due to locally strong selection, or that high rates of gene flow swamp out the ability of peripheral populations to become locally adapted to range-edge environments. Local adaptation across the range was verified in that we find strong differentiation in phenotypic traits in *C. xantiana*, in the field and glasshouse, and that in both environments, most population trait means are correlated with important environmental variables. Indeed, it is clear that gene flow is not sufficient to restrict local adaptation even between populations within regions. Lack of population structure across the range (low F_{ST}) implicates very strong diversifying selection in maintaining interpopulation differences. Q_{ST}–F_{ST} comparisons of a subset of populations indicate that differentiation in traits, particularly flowering time and to a lesser extent branchiness and herkogamy, is the result of diversifying selection. We found no evidence that trait–environment relationships are weaker at the range edge compared with the centre, in spite of evidence from prior work indicating higher levels of local adaptation in central compared with edge populations. Furthermore, edge populations do not have lower levels of heritable variation or stronger genetic correlations between traits than central populations, which suggests they do not have more limited adaptive potential than central populations.

Previous work has shown that population growth rates decline towards the range edge, and conditions beyond the edge are clearly unfavourable (Geber & Eckhart 2005; Eckhart *et al.* 2011): the range edge is almost certainly restricted due to lack of adaptation. But we have demonstrated here that lack of adaptation is not likely to be caused by low diversity at the edge or by maladaptive gene flow, at least with regard to the traits measured in this study and their close correlates. The possibility remains that the diminished local adaptation of range-edge populations observed in previous transplant studies is driven by variation in traits that are not strongly correlated with the plant size, phenology and reproductive characters we measured here. The overall weakness of trait correlations we observed within populations supports the hypothesis that such important but as yet unmeasured traits could exist. These may provide potential targets for future investigation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Principal components and eigenvectors of environmental data in Fig. 1.

Table S2. Ranges of population average trait values.

Table S3. Environmental variables of significant effect on plant traits in stepwise regression models.

Table S4. Genetic correlations between pairs of traits within and across six populations. Values in bold are significant at $P < 0.05$, values in italic are significant at $P < 0.10$. *residual value accounting for greenhouse block effect.

Figure S1. Variation in environmental variables across the range of *C. xantiana*. (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge). Different lettering (a–d) indicates differences between regional means are statistically significant (Tukey's HSD test).

Figure S2. Population mean traits across the range of *C. xantiana* measured in the field and greenhouse. (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge). Different lettering (a–c) indicates differences between regional means are statistically significant (Tukey's HSD test). Larger flowering stage in the field indicates *earlier* flowering.

Figure S3. Field regression and partial regression plots of average population plant trait values versus field environmental variables. Partial regressions were used where more than one environmental variable was a significant predictor of plant traits in a step-wise regression. The black line represents the regression including all 30 populations (*P*-value shown). The lighter green and blue lines represent regressions for central and eastern edge populations respectively (*P*-values for a difference in slope between regions non-significant). *Larger residual flowering stage indicates later flowering. (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge).

Figure S4. Common garden regression and partial regression plots of average population plant trait values versus field environmental variables. Partial regressions were used where more than one environmental variable was a significant predictor of plant traits in a step-wise regression. The black line represents the regression including all 30 populations. The lighter green and blue lines represent regressions for central and eastern edge populations respectively (*P*-values for a difference in slope between regions are non-significant). (Populations: squares, western; triangles, central; open circles, northeastern edge; closed circles, southeastern edge).