

EXPERIMENTAL STUDIES OF ADAPTATION IN *CLARKIA XANTIANA*. I. SOURCES OF TRAIT VARIATION ACROSS A SUBSPECIES BORDER

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Abstract.—Both genetic differentiation and phenotypic plasticity might be expected to affect the location of geographic range limits. Co-gradient variation (CoGV), plasticity that is congruent with genetic differentiation, may enhance performance at range margins, whereas its opposite, counter-gradient variation (CnGV) may hinder performance. Here we report findings of reciprocal transplant experiments intended to tease apart the roles of differentiation and plasticity in producing phenotypic variation across a geographic border between two plant subspecies. *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora* are California-endemic annuals that replace each other along a west–east gradient of declining precipitation. We analyzed variation in floral traits, phenological traits, and vegetative morphological and developmental traits by sowing seeds of 18 populations (six of ssp. *xantiana* and 12 of ssp. *parviflora*) at three sites (one in each subspecies' exclusive range and one in the subspecies' contact zone), in two growing seasons (an exceptionally wet El Niño winter and a much drier La Niña winter). Significant genetic differences between subspecies appeared in 11 of 12 traits, and differences were of the same sign as in nature. These findings are consistent with the hypothesis that selection is responsible for subspecies differences. Geographic variation within subspecies over part of the spatial gradient mirrored between-subspecies differences present at a larger scale. All traits showed significant plasticity in response to spatial and temporal environmental variation. Plasticity patterns ranged from spatial and temporal CoGV (e.g., in node of first flower), to spatial CnGV (e.g., in flowering time), to patterns that were neither CoGV nor CnGV (the majority of traits). Instances of CoGV may reflect adaptive plasticity and may serve to increase performance under year-to-year environmental variation and at sites near the subspecies border. However, the presence of spatial CnGV in some critical traits suggests that subspecies ranges may also be constrained by patterns of plasticity.

Key words.—Co-gradient variation, counter-gradient variation, geographic range limits, geographic variation, phenotypic plasticity, reciprocal transplant, species borders.

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In situations in which closely related but phenotypically distinct sets of populations replace each other along environmental gradients (see numerous examples in Endler 1977), natural selection might be expected to influence the location of the border between the sets of populations. Given sufficient genetic variation, selection might be expected to create genetic differentiation in fitness-related traits along the gradient, although selection-mediated range expansion is subject to constraints imposed by gene flow from central to marginal populations and by drift in marginal populations (Endler 1977; Blows and Hoffman 1993; Hoffman and Blows 1994; Kirkpatrick and Barton 1997; Case and Taper 2000; Keitt et al. 2001).

Environmental variation in phenotype, phenotypic plasticity, also might be expected to play a significant role in determining patterns of adaptation and distribution at range limits (Antonovics 1976; Sultan 2000, 2001). Phenotypic plasticity in a given trait with respect to a given environmental variable may take the form of co-gradient variation (CoGV) or counter-gradient variation (CnGV; Levins 1968; Berven and Gill 1983; Conover and Schultz 1995). Co-gradient variation occurs when positive covariance exists between environmental and genetic sources of variation, and it might be expected to evolve as adaptive phenotypic plasticity when populations exist in variable environments (Levin 1968; Sultan and Bazzaz 1993a,b,c; Ackerly et al. 2000).

Cases of CoGV appear frequently in transplant experiments. For example, in Clausen et al.'s (1940) classic transplant experiments across an elevational transect in California, the shoot height of *Achillea* (Asteraceae) individuals decreased with both the elevation of population origin and the elevation of experimental gardens. Transplant experiments also can reveal CnGV, which occurs when genetic and environmental sources of variation correlate negatively (review in Conover and Schultz 1995). For example, Chapin and Chapin (1981) found CnGV in shoot height in the circumpolar sedge *Carex aquatilis* (Cyperaceae); although exceptionally tall in its native site, a hot-spring population of *C. aquatilis* grew shorter in cold soils than did populations native to cold soils. In the above case and some others, CnGV may arise because selection favors rapid potential growth and development in cold or short-season environments that constrain actual growth and development (Conover and Schultz 1995).

When character states in differentiated populations lie near phenotypic optima because of selection, the consequences of CoGV and CnGV on range expansion are expected to contrast sharply. All else equal, CoGV would be expected to facilitate adaptation in environments near or beyond geographic range limits, because it represents phenotypic modification in the direction of character optima (Antonovics 1976). Counter-gradient variation, in contrast, would be expected to restrict adaptation in marginal or novel environments and thus restrict range expansion, because plasticity, in this case, moves phenotypes away from character optima. In this way, CnGV

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may tend to stabilize the geographic boundaries of differentially adapted sets of populations.

Transplant experiments are a powerful way to evaluate the causes of range limits (Connell 1961; Antonovics 1976; Sultan 2001), identify CoGV and CnGV (e.g., Trussell 2000), and to characterize phenotypic variation and adaptation among populations along environmental gradients (e.g., Clausen et al. 1940; Platenkamp 1990; Rice and Mack 1991; Van Tienderen and Van der Toorn 1991; Bennington and McGraw 1995; Nagy and Rice 1997; Donohue et al. 2000; Galloway and Fenster 2000; Donohue et al. 2001). The present study uses reciprocal transplant experiments to tease apart the causes of phenotypic variation and to evaluate the roles of CoGV and CnGV across a border between two plant subspecies, *Clarkia xantiana* ssp. *xantiana* and *C. xantiana* ssp. *parviflora*. These California-endemic winter annuals replace each other along a west–east gradient of declining precipitation (Daly 2000), with ssp. *xantiana* occupying relatively mesic, western areas and both taxa occupying a central contact zone (Eckhart and Geber 1999). Subspecies *parviflora* has several derived features, including self-pollination, early flowering, and small flowers and leaves. Multiple independent evolutionary lineages of annual plants display the same trend, in which populations exhibiting self-pollination, early maturity, and small-stature evolve from outcrossing ancestors that are larger vegetatively and develop more slowly (Moore and Lewis 1965; Guerrant 1989; Eckhart and Geber 1999; Runions and Geber 2000, and references therein). In arid environments, the early onset of drought or other stresses might be expected to favor early maturation at small size in annual plants (Cohen 1971; King and Roughgarden 1982; Ritland and Jain 1984; Hoffman and Parsons 1991; Mitchell Olds 1996; Stanton et al. 2000). The fitness consequences of breeding system, flowering phenology, and vegetative size in *C. xantiana* vary along the precipitation gradient in a way that corresponds closely to trait variation observed in nature along the same gradient (Fausto et al. 2001; M. A. Geber and V. M. Eckhart, unpubl. data).

Our reciprocal transplant studies address two central questions. (1) In common environments, do populations of *C. xantiana* from different positions along the precipitation gradient express mean trait differences that correspond to changes in the traits' fitness optima along the gradient? (2) Does phenotypic plasticity in *C. xantiana* generally follow co-gradient patterns, which would be expected to facilitate range expansion, or is there also CnGV, which could constrain adaptation and limit range expansion? A history of geographic variation in natural selection might be expected to generate corresponding genetic variation in fitness-related traits among *C. xantiana* populations along the precipitation gradient. Meanwhile, interannual variation in precipitation and spatial variation in water availability might be expected to favor reaction norms that increase performance in wet and dry years and wet and dry sites, for those traits that confer adaptation in wet or dry environments. The latter idea is a specific version of the hypothesis that organisms are expected to evolve adaptive phenotypic plasticity, given: (1) reliable and detectable external signals of environmental quality; (2) the absence of absolute developmental and genetic constraints; and (3) benefits of plasticity that exceed its costs

(Schmalhausen 1949; Bradshaw 1965; Sultan 1987, 2000; Moran 1992; Getty 1996; Donohue et al. 2000). We make the following specific predictions regarding this study's transplant experiments. (1) Significant main effects of subspecies will be found in all traits, with the direction of subspecies differences corresponding to observed differences in nature. (2) Genetic differentiation will also be found within subspecies between wetter and drier regions across the precipitation gradient, with differentiation occurring in the same direction as the larger scale differences between subspecies. (3) Phenotypic plasticity exhibited among sites and between years will tend to represent CoGV.

MATERIALS AND METHODS

Study Organisms

The recognized subspecies of *C. xantiana* are phenotypically distinct and related as sister clades. Flowers of ssp. *xantiana* possess traits associated with outcrossing (Ornduff 1969; Wyatt 1984, 1986; Holtsford and Ellstrand 1992), including relatively large petals, pronounced protandry, and stigmas that are exerted several millimeters beyond the anthers (Moore and Lewis 1965; Eckhart and Geber 1999; Runions and Geber 2000). Flowers of *C. xantiana* ssp. *parviflora* (Eastw.) Harlan Lewis possess small petals, little or no protandry, and little or no or anther-stigma separation (Moore and Lewis 1965; Lewis and Raven 1992; Eckhart and Geber 1999; Runions and Geber 2000), and ssp. *parviflora* self-pollinates by autonomous autogamy (sensu Lloyd 1992), selfing automatically without external agents (Moore and Lewis 1965; V. A. Eckhart and M. A. Geber, pers. obs.). Leaves of ssp. *parviflora* also tend to be smaller than in ssp. *xantiana* (Eckhart and Geber 1999), and ssp. *parviflora* flowers earlier and at a lower node (leaf position) than ssp. *xantiana* (Moore and Lewis 1965; Eckhart and Geber 1999; Runions and Geber 2000). Chromosome organization (Moore and Lewis 1965), allozyme frequencies (Gottlieb 1984), and variation in nuclear (M. A. Geber and L. D. Gottlieb, unpubl. data) and chloroplast (M. A. Geber, unpubl. data) DNA sequences indicate the sister status of ssp. *parviflora* and ssp. *xantiana*.

Companion studies to the present analysis show that *C. xantiana* character variation in nature corresponds to contrasting patterns of selection and adaptation along a west–east precipitation gradient. In each subspecies' exclusive native range, the lifetime seed production of the native *C. xantiana* subspecies averages an order of magnitude higher than the non-native subspecies (M. A. Geber and V. M. Eckhart, unpubl. data). Geographic variation in pollinator abundance and plant density (Fausto et al. 2001) and supplemental hand pollination experiments (M. A. Geber and V. M. Eckhart, unpubl. data) indicate a benefit to automatic self-pollination (and presumably to the traits that enable it: small anther-stigma separation and short protandry) in arid, eastern parts of the range. Finally, phenotypic selection favors large flower organs, high nodes of first flower, long duration of flower development, and late flowering in the exclusive (western) range of ssp. *xantiana*, whereas contrasting traits are favored in the exclusive (eastern) range of ssp. *parviflora* (M. A. Geber and V. M. Eckhart, unpubl. data).

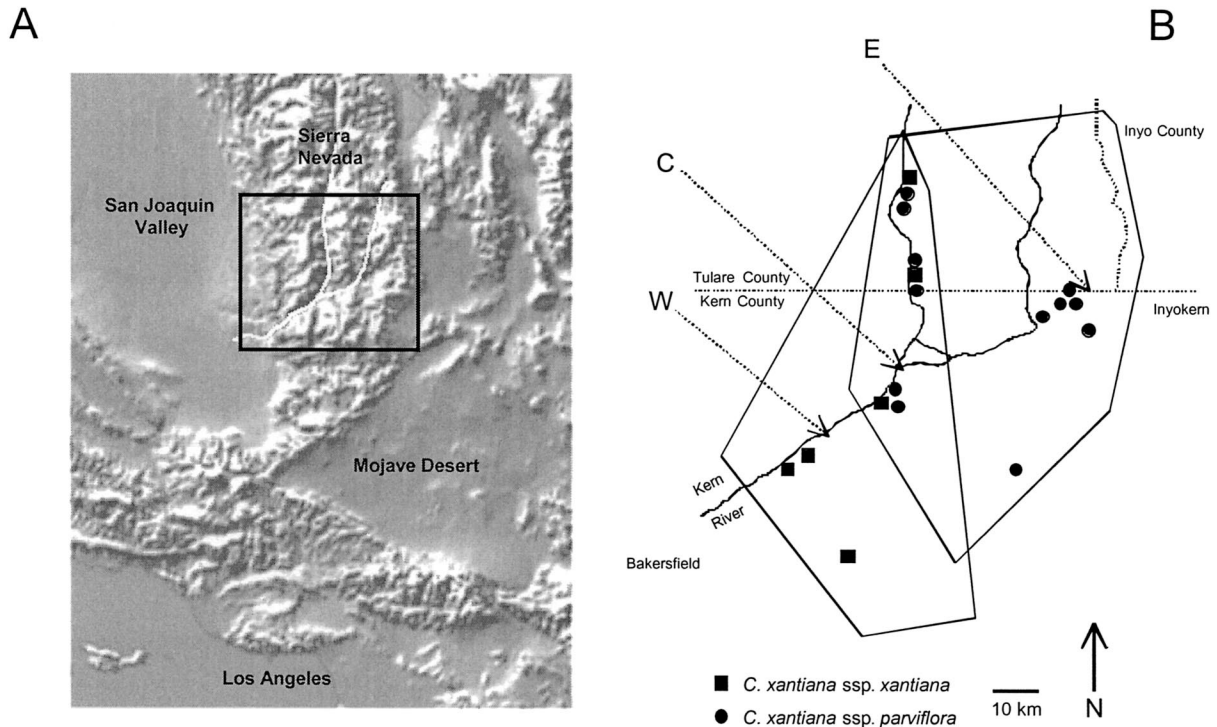


FIG. 1. Geography of the reciprocal transplant experiment. (A) Section of the Kern River drainage in central California. (B) Map of boxed area in (A), giving locations of west (W), central (C), and east (E) transplant sites. Symbols represent the native locations of the populations used in the experiment; these are populations 5X, 6X–96X, 9X, 17X, 51X, 52X, 5p, 5w, 6p–31p, 6w–96w, 7p–9p, 9w, 20w, 23w, 24p, 24w–29w, and 48p from Eckhart and Geber (1999).

Experimental Design

Source material for the transplant experiments came from a subset of the natural populations analyzed by Eckhart and Geber (1999; Fig. 1). Each population's seeds consisted of bulked samples of 50–150 maternal families. We stored seeds dry at 4°C until sowing.

We constructed transplant gardens on public lands at three sites along a west–east transect across the subspecies' contact zone (Fig. 1). The west (35°32'N; 118°38'W; 750 m elevation) and central sites (35°38'N, 118°30'W; 800 m) were in Sequoia National Forest, and the east site (35°46'N, 118°04'W; 1350 m) was in the Caliente Resource District (U. S. Bureau of Land Management). Each garden was approximately 900 m² in area. We surrounded the west and east sites with barbed-wire fences to exclude grazing cattle. Each garden was positioned in what we determined to be suitable *C. xantiana* habitat (i.e., steep slopes of loose soil, with abundant bare ground), at least 50 m from the nearest sizable natural populations. Except for removing a small number of *C. xantiana* individuals that appeared at the sites in the season prior to the first planting, we left native vegetation intact.

We set up each transplant garden in a randomized complete block design, with 10 blocks per garden. Each block was subdivided into four plots made of white plastic diffusion screens (i.e., flat lattices with square openings, manufactured for fluorescent light fixtures) set down approximately 1 cm into the soil. Each plot consisted of a 7 × 7 grid of cells, each cell being 2 cm long × 2 cm wide, and having walls

approximately 1 cm high. Seeds were sown only in the inner 36 cells, to reduce possible edge effects.

We randomly assigned cells to populations, with the restriction that each plot had to contain two cells for each population. Thus, each block contained eight cells per population, and each of the three gardens contained 80 cells per population. We sowed eight seeds in each cell in October of each year, before cool-season rains began. In the experiment's second year we changed block locations.

Clarkia xantiana germinates with the onset of the wet season (November–February) and completes its life cycle during the following (usually rainless) late spring and summer. Precipitation during the two growing seasons of this experiment contrasted sharply, with 1997–1998 being an exceptionally wet rainy season (associated with an El Niño event) and 1998–1999 being much drier. Weather stations at Bakersfield, CA (to the west of the west site) and Inyokern, CA (to the east of the east site) recorded more than twice as much November–June precipitation in 1997–1998 (373 mm and 114 mm, respectively) than in 1998–1999 (163 mm and 46 mm, respectively; National Climatic Data Center, <http://www.ncdc.noaa.gov>). Plant fitness, measured as lifetime seed production, averaged tenfold higher in the first, wetter year compared to the second, drier year (M. A. Geber and V. M. Eckhart, unpubl. data). Thus, the two-season experiment fortuitously captured strikingly different levels of resource availability.

Data collection took place at regular intervals as soon as

the winter rains began. We visited transplant gardens monthly from December through March to score germination (out of eight seeds sown per cell), survival, cotyledon length, and leaf (node) number in each cell. All linear measurements we made to the nearest 0.5 mm. At the March census we thinned any squares containing multiple individuals to a single focal individual to be tracked from then on. We returned to the focal individuals in April to measure the length of the first true leaf and leaf growth rate, the latter by measuring the length of the median leaf (i.e., the leaf at the middle node position) on the central stem twice over a four-day interval. We transformed this rate into relative leaf growth rate (leaf RGR) by dividing by initial leaf length. We also estimated total leaf length at this time by multiplying the fully expanded median leaf length times total leaf number. From March until the last individuals senesced (June–July), we made monthly estimates of leaf (node) number on the central stem and of total leaf number. Using these variables we estimated total plant size as the sum of the lengths of all leaves (total leaf length) on the periodic census dates, and estimated whole-plant relative growth rate (size RGR) as the rate of increase in total leaf length between the April and March census dates, divided by the March value. We estimated plastochron interval (the reciprocal of the rate of vegetative node production) on the central stem for each individual as the slope of a regression of time on node number on consecutive monthly censuses. Beginning in early April of each year, we visited each site at two-day intervals, recording: (1) the node position of the first flower bud; (2) the anthesis (opening) date (flowering time) and date of stigma receptivity of the first flower, measured in days since the first germinants appeared at that site (which turned out to be the same in the two years); and (3) the ovary length, petal length, petal width, style length (on the first day of receptivity), and anther–stigma distance (on the first day of receptivity) of the first flower. Flower development time we estimated as the period between the first visible appearance of the lowest (first) bud and the anthesis date of the flower that developed from that bud. Protandry we estimated as the difference between the date a flower's stigma became receptive and that flower's anthesis date. We chose to record morphological and phenological data on each plant's first flower to standardize measurements across plants; scoring additional flowers would have been impossible for many plants, because mean flower production was low (overall means were 2.4 in 1997–1998 and 1.3 in 1998–1999).

Statistical Analysis

We analyzed variation in three sets of traits. The first set comprised flower dimensions: petal length and width, style length, ovary length, and anther–stigma distance. The second included three phenological traits: flowering time, protandry, and flower development time. The final set comprised traits describing vegetative morphology and development: node of first flower, plastochron interval, leaf length, leaf RGR, and size RGR. The correlation coefficient between petal length and petal width was very high (0.94, $N = 1275$). Therefore, we report results for petal length but not petal width, out of concern for these traits' nonindependence. Because all of the

remaining 77 (of 78 possible) pairwise trait correlations among the original 13 traits had absolute values below 0.85, and our sample sizes were very large, we did not eliminate any other traits from the analysis. We did not transform variables, as raw values and residuals fit or closely approached the normality and variance assumptions of ANOVA, and because transformations did not substantially improve the fit.

Survivorship differences between the two growing seasons led us to divide the statistical analysis into two parts. In 1997–1998, at least one seed germinated in 81% of 4320 cells, and an individual survived to flower in 30% of 4320 cells, compared to 44% germination and 4% survival the following year. The first part of the analysis (the space-time analysis) used both growing seasons' data but did not evaluate variation among populations within subspecies. We designed the second part of the analysis (the regional analysis, described below) specifically to evaluate differentiation at that level.

In the space-time analysis, values for individuals were analyzed by an ANOVA model that included subspecies, site, block (nested within site), year, and all interactions between fixed factors (i.e., all except block, which was considered random). This model is, in a sense, an analysis of subspecies norms of reaction. We tested hypotheses about specific mean differences with single degree of freedom independent contrasts, constructed with the SAS MIXED procedure (SAS Institute 2000).

To interpret patterns of plasticity as representing CoGV or CnGV required us to evaluate whether plasticity along the precipitation gradient was congruent with genetic differentiation and adaptation. In nature, ssp. *xantiana* has significantly greater values of flower dimensions (except for ovary length, which is greater in ssp. *parviflora*), protandry, flower development time, flowering time, node of first flower, and leaf length (Moore and Lewis 1965; Eckhart and Geber 1999; Runions and Geber 2000). This experiment confirmed that subspecies differences in these traits were maintained in the transplant gardens (see below; leaf RGR also was greater in ssp. *xantiana*), and companion studies showed that selection favors these patterns of differentiation (Fausto et al. 2001; M. A. Geber and V. M. Eckhart, unpubl. data). Thus evidence of CoGV in the above traits, plus leaf RGR, was present when changes in phenotypic expression of different populations among sites followed the pattern “west > central \geq east” or the pattern “west \geq central > east” (e.g., Fig. 2). Plasticity in ovary length needed to follow the opposite ranking (i.e., “east \geq central > west” or “east > central \geq west”) to be interpreted as CoGV. Plastochron interval did not exhibit genetic differentiation, so we could not evaluate CnGV or CnGV. Size RGR correlated closely with lifetime fitness, with the fitness of each subspecies being greater than the other in its exclusive range (M. A. Geber and V. M. Eckhart, unpubl. data). Thus we interpreted size RGR's plasticity as CoGV if it declined from west to east for ssp. *xantiana* and/or declined from east to west in ssp. *parviflora*. We interpreted as CnGV all cases in which changes in phenotypic expression of different populations among sites were opposite to the pattern of genetic differentiation among geographic regions. Temporal variation in traits we categorized as being co- or counter-gradient in a fashion analogous to the analysis of space, with wet year versus dry year effects treated in the

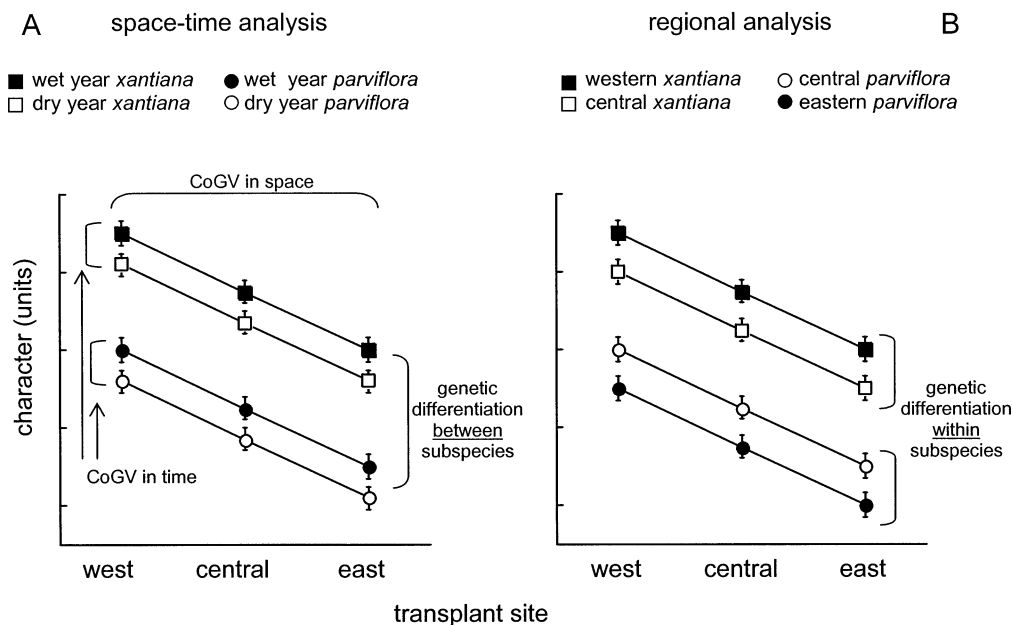


FIG. 2. Findings expected under idealized patterns of genetic differentiation and CoGV. (A) Figure depicting analysis of both 1997–1998 and 1998–1999 data showing genetic differentiation between subspecies, CoGV in space, and CoGV in time for the same character. (B) Figure depicting analysis of 1997–1998 data, showing spatial genetic differentiation within subspecies (also subspecies differences and CoGV in space) for a trait that is larger in *ssp. xantiana* than in *ssp. parviflora*. Symbols and error bars represent (hypothetical) means \pm one standard error.

same way as wet site versus dry site differences. For example, for the majority of traits, in which the subspecies from more mesic environments (*ssp. xantiana*) had higher mean values overall, we interpreted higher means in the wetter year as CoGV in time (see Fig. 2A). Any significant interaction terms involving subspecies indicated genotype-by-environment interaction at the level of subspecies.

In the second part of the statistical analysis (the regional analysis), we first calculated trait means for each population at each site in the 1997–1998 season, the year in which survivorship was high. Sample sizes of mature individuals for each population-site-trait combination in that season were generally $\gg 20$, dispersed among most or all of the blocks. Thus, these means estimated, with a high degree of confidence, each population's trait values in each of the three sites in 1997–1998. We then analyzed population mean values ($N = 18$ populations per site) with an ANOVA model that included subspecies, region (contact zone or native zone) nested within subspecies, site, and interactions between site and subspecies and between site and region within subspecies. We used the model to evaluate specifically the nested effect of region within subspecies, which when significant revealed significant genetic differentiation within subspecies (Fig. 2B), and the interaction effect of site by region within subspecies. Because the space-time analysis (described above) shared all other main effects and interactions of the regional analysis, we do not present hypothesis tests of those shared effects (e.g., main effects of site and subspecies) for the regional analysis. To do so would have been to test the same hypotheses twice, with (mostly) the same dataset. We used SAS procedure GLM to carry out the regional analysis.

RESULTS

Flower Dimensions

Patterns of variation in flower dimensions were strikingly similar among the four traits. For all four, main effects of subspecies, site, and year were large and significant, whereas interaction effects tended to be small (Table 1; Fig. 3). Subspecies *xantiana* had higher mean values in all traits except ovary length, in which *ssp. parviflora* had larger values (Fig. 3). Thus subspecies differences were concordant in direction with subspecies differences in nature. Site effects in these traits were not consistent with CoGV or CnGV. Instead, the central site tended to have smaller trait values than either the west or the east sites, whereas west and east sites did not differ significantly (Table 1). In contrast, year effects revealed CoGV (i.e., smaller trait values in the dry year) for three of four traits: petal length, style length, and anther-stigma distance. Plasticity patterns in ovary length were distinctive, as they exhibited CnGV in time (longer ovaries in the wet year, Fig. 3E). All traits except petal length exhibited genetic differentiation at the regional level (Table 2; Fig. 3 right column). Subspecies *xantiana* native to the central region showed a shift in trait values in the direction of *ssp. parviflora*. Unlike traits in other categories (see below), flower dimensions did not exhibit significant three-way interactions.

Phenology

Patterns of variation among the three phenological traits were more complex and diverse than those in flower dimensions. Flowering time was later in *ssp. xantiana* than *ssp.*

TABLE 1. Analysis of variance summary of flower dimensions in the space-time analysis. *F*-tests constructed by the SAS MIXED procedure; denominator df determined by the "satterth" option. Abbreviations: W, west site; C, central site; E, east site; wet y, 1997–1998. Site rankings assessed by independent contrasts.

Trait	<i>F</i> for fixed sources of variation (denominator df)							Site mean rankings
	Subspecies	Site	Year	Subspecies × site	Subspecies × year	Year × site	Three-way interaction	
Numerator df	1	2	1	2	1	2	1	
Petal length	1107*** (1321)	17.87*** (98.7)	112.5*** (123)	6.67** (1330)	4.06* (1344)	1.06 (106)	1.2 (1344)	W ≈ E > C (wet y)
Style length	215.0*** (1148)	20.66*** (129)	39.94*** (364)	0.96 (1192)	6.83** (1148)	2.66 (129)	0.05 (1192)	W ≈ E > C
Ovary length	5.87* (1102)	23.00*** (96.1)	10.88** (225)	3.86* (1214)	0.02 (1102)	2.03 (96.1)	0.76 (1214)	W ≈ E > C
Anther–stigma distance	242.0*** (1126)	4.10* (152)	5.21* (568)	5.37* (1238)	5.65* (1226)	1.67 (152)	2.28 (1238)	W ≈ E > C

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

parviflora at all sites in both years (Table 3; Fig. 4A), and *ssp. parviflora* varied geographically in flowering time, with central populations flowering later than eastern populations (Table 2; Fig. 4B). This trait exhibited CnGV in space, with later flowering at the east site than at the others, but it displayed CoGV in time, with earlier flowering in the dry year (Table 3; Fig. 4A). A significant site-by-subspecies interaction for flowering time (Table 3) arose from the fact that *ssp. parviflora* varied more across sites than did *ssp. xantiana* (Fig. 4A).

Protandry differed consistently between the subspecies, with *ssp. xantiana* having longer protandry, as expected, than *ssp. parviflora* (Table 3; Fig. 4C). Environmental effects on protandry were complicated. There was no main effect of site or year. There was, however, a significant interaction between site and year and a significant subspecies-by-site-by-year interaction. Overall, protandry was shortest at the central site and similarly higher at the west and east sites, but in the wet year, *ssp. xantiana* showed greatest protandry in the east (Table 3; Fig. 4C). There was no significant geographic variation within subspecies for this trait (Table 2; Fig. 4D).

Flower development time exhibited the most complicated patterns of variation of any trait. Subspecies *xantiana* had longer flower development times than *ssp. parviflora* (Table 3; Fig. 4E). Flower development time exhibited CnGV overall, being longest at the east site (Table 3; Fig. 4E), but in the wet year, *ssp. xantiana* exhibited CoGV in this trait (Table 3; Fig. 4E). Main factor effects were not independent, as there were significant interactions of almost every possible form (subspecies by site, subspecies by year, and subspecies by site by year; Table 3). Though it is difficult to summarize succinctly the tangle of interactions, it is noteworthy that flower development in *ssp. xantiana* took longer in the dry year than the wet year, whereas flower development in *ssp. parviflora* proceeded faster in the dry year than the wet year (Fig. 4E). There was no evidence of genetic differentiation within subspecies in flower development time (Table 2; Fig. 4F).

Vegetative Morphology and Development

Like phenological traits, vegetative morphological and developmental traits exhibited complicated patterns of variation. Evidence of genetic differentiation in these traits was

common but not ubiquitous. Main effects of subspecies with greater values in *ssp. xantiana* were detected in node of first flower, leaf length, and leaf relative growth rate (Table 4; Figs. 5A, 6A, 6C). Node of first flower and leaf length also varied geographically within subspecies (Table 2). Relatively western populations began to flower at higher nodes than relatively eastern populations within both subspecies (Fig. 5B), and leaf length varied in this way in *ssp. xantiana* (Fig. 6B). Plastochron interval did not exhibit significant main effects or interactions involving subspecies or region (Tables 2, 4; Fig. 5C,D). In size RGR, genetic differentiation appeared only as interactions (see below).

Patterns of plasticity were highly variable among vegetative morphological and developmental traits. Overall, node of first flower was lowest at the central site (Table 4; Fig. 5A), but this trait exhibited spatial CoGV in the dry year, when node of first flower was significantly higher at the west site than at the others (Table 4; Fig. 5A). Plastochron interval was highly environmentally sensitive, being longest at the central site, especially in the dry year (Fig. 5C). All traits except plastochron interval displayed CoGV in time, having lower values in the dry year (Table 4; Figs. 5, 6). Size RGR was greater in the subspecies that performed better in a particular year: *ssp. xantiana* in the wet year and *ssp. parviflora* in the dry year (Table 4; Fig. 6E). Within each subspecies, size RGR exhibited CoGV in space (Table 4; Fig. 6E).

DISCUSSION

A summary of this study's findings (Table 5) indicates substantial genetic differentiation between *C. xantiana* subspecies (see also Moore and Lewis 1965; Runions and Geber 2000) and geographic variation within *C. xantiana* subspecies, along with substantial and diverse environmental responses to environmental variation. We evaluate below these findings' implications with regard to the questions and predictions presented in the introduction, as well as to more general issues of the role of phenotypic plasticity at (sub)species borders, plasticity in flowering time in arid-land annual plants, and the causes of genotype-by-environment interactions.

Question 1. In common environments, do populations of C. xantiana from different positions along the precipitation gradient express mean trait differences that correspond to chang-

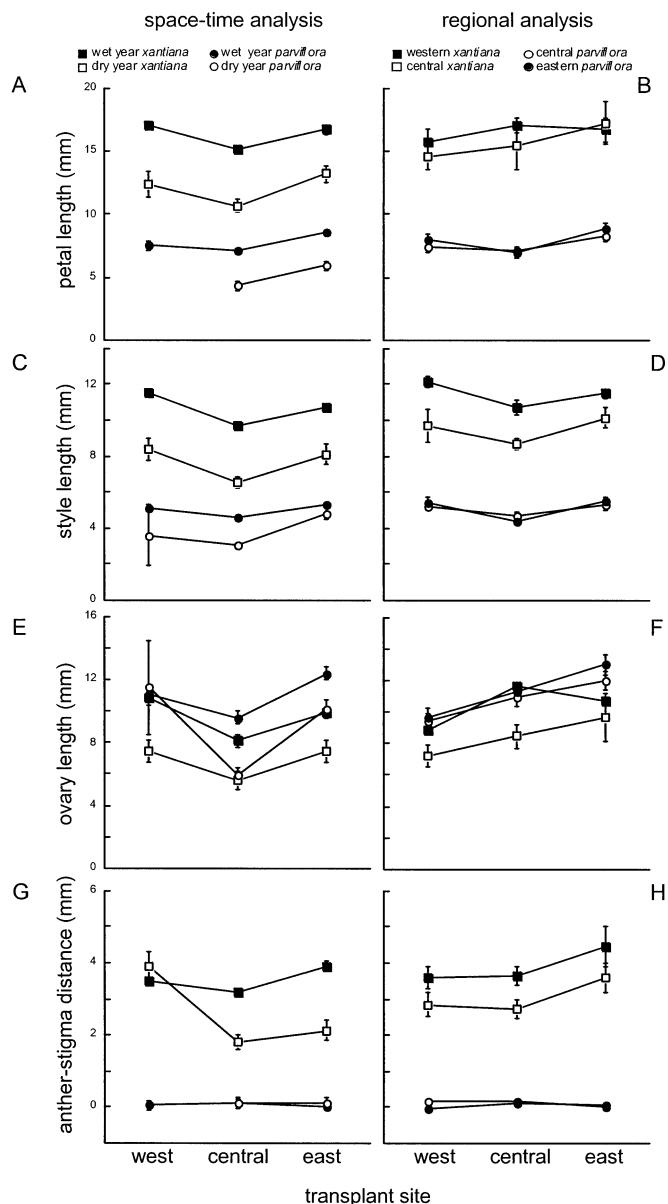


FIG. 3. Flower dimensions in the reciprocal transplant experiment. Left column (parts A, C, E, and G) depicts analyses of both 1997–1998 and 1998–1999 data (space-time analysis). Right column (parts B, D, F, and H) depicts analyses of 1997–1998 data (regional analysis). Symbols as in Fig. 2.

es in the traits' fitness optima along the gradient?—The short answer to this question is “Yes.” Phenotypic differences between subspecies were expressed consistently within transplant sites, and differences observed at transplant sites were of the same sign as in nature. Of 12 traits, only plastochron interval did not exhibit any evidence of subspecies differentiation. This study's findings agree with those of previous common-environment studies of these subspecies of *C. xantiana*, which have found that the directions of population trait differences in nature are generally maintained in cultivation (Moore and Lewis 1965; M. A. Geber and V. M. Eckhart, unpubl. data). Subspecies distinctions in nature clearly rep-

TABLE 2. Summary of the regional analysis (tests of spatial genetic differentiation within subspecies in the 1997–1998 reciprocal transplant experiment): F -values and associated exact probabilities (or ns, if $P > 0.05$) from the regional analysis ANOVA.

Trait	Source of variation			
	Region within subspecies		Site \times region within subspecies	
	$F_{(2,4)}$	P	$F_{(4,42)}$	P
Petal length	1.37	ns	0.38	ns
Style length	23.50	0.0062	0.56	ns
Ovary length	8.36	0.0373	0.87	ns
Anther–stigma distance	66.50	0.0009	0.16	ns
Flowering time	32.91	0.0033	0.29	ns
Protandry	2.32	ns	0.43	ns
Flower development time	1.50	ns	0.88	ns
Node of first flower	19.55	0.0086	0.45	ns
Plastochron	0.37	ns	0.42	ns
Leaf length	7.51	0.0442	0.79	ns
Leaf RGR	1.43	ns	2.16	ns
Size RGR	5.60	ns	0.22	ns

resent substantial genetic differentiation, not phenotypic plasticity alone.

Six of 12 traits also exhibited significant geographic variation within subspecies (Table 5). In most cases; patterns of variation coincided in direction with the larger scale subspecific differences along the geographic gradient. For example, central populations of ssp. *xantiana* had smaller leaves than western populations, and they began to flower at a lower node. Central populations of ssp. *parviflora* began to flower later and at a higher node than eastern populations. Our findings substantially supported our specific predictions about genetic differentiation.

Correspondence between geographic variation within and between subspecies could have two nonexclusive causes: (1) parallel responses to selection within subspecies to that observed between subspecies; and (2) gene flow between subspecies in the contact zone. The fact that phenotypic selection at the central site is intermediate in direction and magnitude compared to the extreme sites (M. A. Geber and V. M. Eckhart, unpubl. data) is consistent with the notion of parallel responses to selection. Allozyme data suggest that there is little or no gene flow between subspecies where they co-exist (Gottlieb 1984), presumably because ssp. *parviflora*'s early flowering and self-pollination would be expected to act as premating isolation barriers. Postmating barriers are not strong, however (M. A. Geber, unpubl. data), and we have occasionally observed what appear to be subspecies-hybrid individuals in the contact zone. Thus, the potential for gene flow between subspecies exists and should be evaluated more thoroughly.

Question 2. Does phenotypic plasticity in C. xantiana generally follow co-gradient patterns, which would be expected to facilitate range expansion, or is there also CnGV, which could constrain adaptation and limit range expansion?—This question lacks a short answer, and our findings were not congruent with the simple prediction that CoGV would be common. Phenotypic plasticity was ubiquitous in the traits we examined. Evidence of CoGV, which might be interpreted as adaptive plasticity, was not ubiquitous. There was evi-

TABLE 3. Summary of ANOVA of phenological traits in the space-time analysis. CoGV is bold, CnGV is underlined.

Trait	F for fixed sources of variation (denominator df)							Site mean rankings
	Subspecies	Site	Year	Subspecies × site	Subspecies × year	Year × site	Three-way interaction	
Numerator df	1	2	1	2	1	2	1	
Flowering time	420.2*** (1298)	106.4*** (123)	265.4*** (427)	4.09* (1359)	0.17 (1298)	1.87 (123)	1.90 (1354)	<u>E > C ≈ W</u>
Protandry	185.0*** (1246)	0.41 (239)	1.61 (758)	0.05 (1252)	<0.01 (1246)	4.46* (239)	3.92* (1252)	W ≈ E > C (wet y)
Flower development time	54.67*** (910)	17.23*** (70.6)	0.03 (136)	3.32* (1032)	42.98*** (910)	2.13 (70.6)	12.58*** (1036)	<u>E > C ≈ W</u> W > C ≈ E (wet y) (ssp. <i>xantiana</i>)

* $P < 0.05$, *** $P < 0.001$.

dence of CnGV in several traits, and the most frequent pattern was plasticity that did not fit the expectations of either CoGV or CnGV (Table 5).

When CoGV was detected, it was more likely to occur in time rather than in space. Spatial CoGV occurred in three traits: node of first flower, flower development time, and size RGR. Co-gradient variation in time occurred in six traits (Table 5). This contrast represents a minor paradox. The rar-

ity of CoGV in space might suggest there is little potential for phenotypic plasticity to facilitate adaptation at the subspecies border or expansion of geographic range, but the relative commonness of CoGV in time suggests that plasticity can enhance performance. We warn that not all temporal CoGV should be interpreted as adaptive. For example, reductions in growth rates and organ sizes in the dry growing season may represent inevitable constraints imposed by resource limitation (cf. Bradshaw 1965). Nevertheless, these findings beg the question of why CoGV was so rare in general in the present study, and why it was more often detected in time than in space.

A partial resolution to the above question may lie in the experimental design, which compelled us to apply a conservative definition of spatial CoGV. Site availability, safety, and other logistical issues limited our experiments to single sites within each zone and to administrative sites on National Forest lands (west and central sites). For several traits, phenotypic values were smallest at the central site, but for plastochron interval the central site had the largest values, which means that the slowest rates of leaf production occurred at the central site, especially in the dry year. Any case in which the central site exhibited extreme values for trait means, relative to the other sites, ruled out a simple interpretation of CoGV or CnGV. The central site appears to have been a relatively poor growth environment that had extreme effects on phenotypic variation in this set of traits. We are reluctant to remove the central site from the analysis, as we prefer to test our predictions conservatively. It is instructive, however, that an exploratory analysis (not shown) in which the central site data were omitted revealed spatial CoGV in four traits that the original analysis interpreted as not showing spatial CoGV: petal length, ovary length, flowering time, and leaf length. Thus spatial CoGV, which presumably represents adaptive phenotypic plasticity, may be more common in this system than our conservative analysis indicates.

In three traits we detected significant CnGV. Ovary length exhibited CnGV in time, and flower development time and flowering time exhibited CnGV in space. (The exploratory analysis that removed the central site data also detected spatial CnGV in anther-stigma distance.) Do the above cases represent maladaptive phenotypic plasticity? Perhaps not in the case of ovary length, in which the counter-gradient temporal shift may actually have increased performance. Selec-

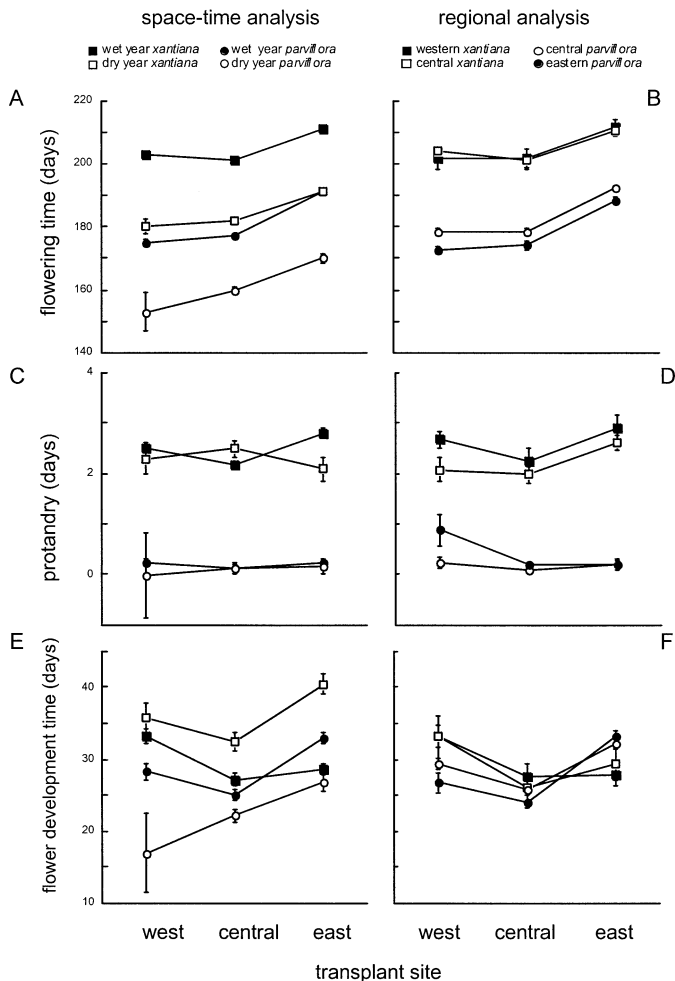


FIG. 4. Phenological traits in the reciprocal transplant experiment. Organization and symbols as in Figure 3.

TABLE 4. Summary of ANOVA of vegetative traits in the space-time analysis. CoGV is bold.

Trait	F for fixed sources of variation (denominator df)							Three-way interaction	Site mean rankings
	Subspecies	Site	Year	Subspecies × site	Subspecies × year	Year × site	Year × site × year		
Numerator df	1	2	1	2	1	2	1		
Node of first flower	190.2*** (2009)	65.46*** (61.8)	71.60*** (68.9)	3.30* (2000)	18.84*** (2009)	1.80 (61.8)	4.78** (2000)	W > E > C (wet y) W > C ≈ E (dry y) C > W ≈ E	
Plastochron	2.64 (3662)	24.1*** (129)	0.83 (146)	0.57 (3659)	<0.01 (3662)	12.46*** (129)	0.08 (3659)	W ≈ E > C W ≈ E > C	
Leaf length	186.7*** (2491)	10.88*** (64)	13.94*** (67.4)	1.03 (2487)	0.38 (2491)	1.21 (64)	1.31 (2487)	W ≈ E > C	
Leaf RGR	29.37*** (2385)	5.65*** (70.6)	52.20*** (73.7)	10.76*** (2381)	4.85 (2385)	5.27*** (70.6)	0.78 (2381)	W ≈ E > C	
Size RGR	0.09 (4228)	13.14*** (73.2)	397.3*** (75.4)	0.79 (4228)	5.60*** (4228)	5.26*** (73.2)	5.82*** (4228)	W > C ≈ E (spp. <i>xantiana</i>) E > C ≈ W (ssp. <i>parviflora</i>)	

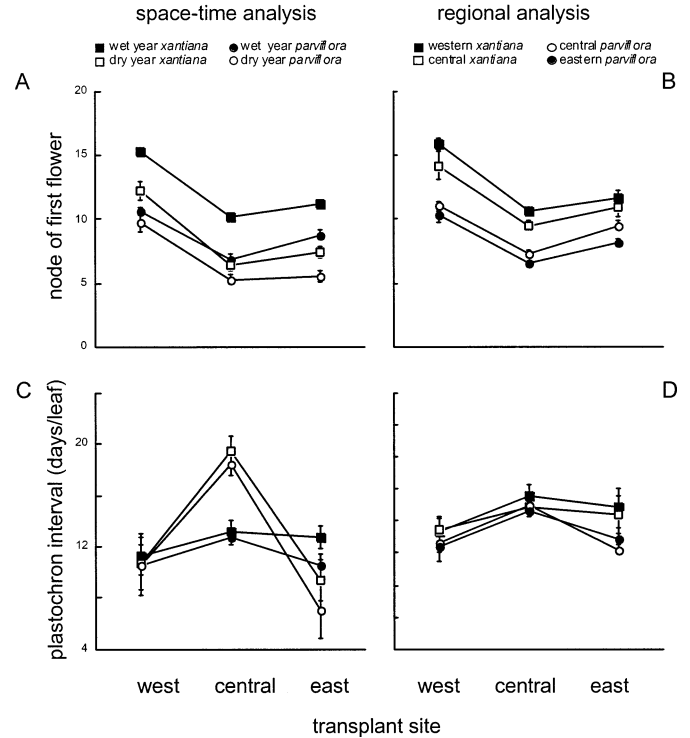
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

FIG. 5. Node of first flower and plastochron interval in the reciprocal transplant experiment. Organization and symbols as in Figure 3.

tion favored long ovaries at the relatively mesic west site (M. A. Geber and V. M. Eckhart, unpubl. data).

The observed CnGV in space of flowering time, however, may represent a significant limit to adaptation and geographic range expansion. Yet there also is evidence of a compensatory response in another character, a response that reduced the magnitude of CnGV in flowering time. All else being equal, flowering time in semelparous plants would be expected to correlate positively with node of first flower and with flower development time; flowering time would be expected to correlate negatively with plastochron interval, as long plastochron intervals would delay the production of the node of first flower (see Guerrant 1989; Geber 1990; Diggle 1992). In this study, flowering time was latest at the east site. This pattern probably did not arise from variation in plastochron interval, which was longest at the central site, but it may have arisen from variation in flower development time, which, like flowering time, displayed CnGV in space. Note, however, that node of first flower exhibited CoGV in space. Thus flowering time at the east site would have been even later if node of first flower had not declined in concert. This exemplifies the idea that if one trait's unavoidable response to the environment is indeed maladaptive, harmful effects may be ameliorated by compensatory responses in other traits (Levins 1968; Berven and Gill 1983; Conover and Schultz 1995; Parsons 1997; Preston 1999).

The importance of CnGV in constraining geographic ranges in general is hard to estimate at present. Patterns of plasticity in plant flowering time, a trait which frequently exhibits genetic differentiation in response to selection in populations of contrasting environments, vary from little plasticity at all

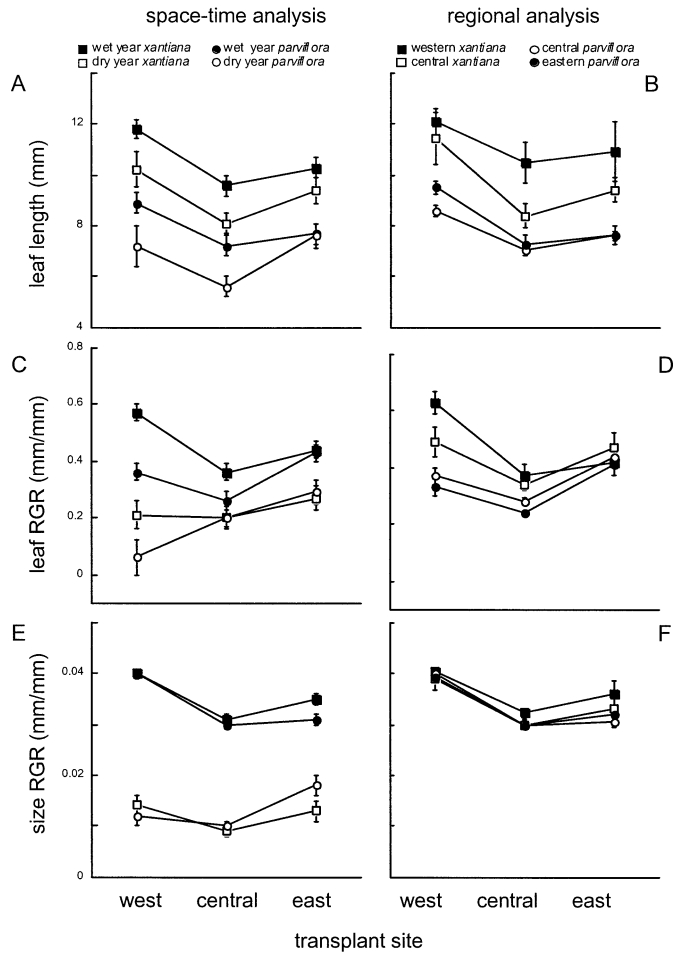


FIG. 6. Leaf length, leaf relative growth rate, and size relative growth rate in the reciprocal transplant experiment. Organization and symbols as in Figure 3.

(e.g., *Plantago major* [Lotz 1990] and *Impatiens pallida* [Bennington and McGraw 1995]) to apparent co-gradient variation (e.g., *Achillea millefolium* [Clausen et al. 1940]), to CnGV (this study). The commonness of CnGV in fitness-related traits appears to be poorly known in general, though it has

been found, especially in animal systems, that CnGV in growth may commonly accompany (and underlie) invariance or CoGV in mature size and form (Conover and Schultz 1995; Parsons 1997; Trussell 2000).

Plasticity of flowering time in arid environments.—Environmental responses of flowering time in annual plants from arid and otherwise stressful environments have been the subject of considerable research. The present findings suggest a reevaluation of this issue is needed. Fox (1990) reviewed evidence concerning the idea that arid-land annuals flower early in response to drought stress, a view of adaptive plasticity in flowering time that Fox ascribed to Went (1948). Early flowering in response to drought and other stresses is often favored by selection (e.g., Fox 1989, 1990; Bennington and McGraw 1995; Stanton et al. 2000 and references therein, M. A. Geber and V. M. Eckhart, unpubl. data). Thus one might expect the evolution of norms of reaction in which drought accelerates flowering. However, Fox’s (1990) review suggested that although there are cases of modest acceleration of flowering under drought, annual plants’ plastic response of flowering time to drought stress is more commonly a delay or no change. The finding of spatial CnGV of flowering time in this study fits the pattern Fox (1990) describes, but the plastic acceleration of flowering found in the dry year does not. We can offer two hypotheses to account for these findings. First, delayed flowering at the east site may have arisen partly from a factor other than precipitation. Lower growing season temperatures at the relatively high-elevation east site may have constrained flowering time to be somewhat later at that site within years. Consistent with this idea is the fact that flowering onset increases with elevation in natural populations of *C. xantiana* (Eckhart and Geber 1999). Alternatively, however, accelerated flowering in dry growing seasons may actually be relatively common (see Aronson et al. 1992; Bennington and McGraw 1995; Stanton et al. 2000).

Genotype-by-environment interaction.—At the level of subspecies or sets of populations within subspecies, genotype-by-environment interaction occurred in 11 of 12 traits (Table 5). This finding indicates evolutionary differentiation in phenotypic plasticity, presumably arising from similar interactions that occur among genotypes within populations

TABLE 5. Summary of genetic differentiation and phenotypic plasticity in all traits in both analyses. Columns 2–7 indicate: (2) main effects or interactions involving subspecies; (3) region within subspecies effects; (4) co-gradient variation; (5) counter-gradient variation; (6) plasticity that does not fit co- or counter-gradient variation; and (7) genotype-by-environment interactions of subspecies with time or space.

Trait	(2) Differentiation between subspecies	(3) Differentiation within subspecies	(4) CoGV	(5) CnGV	(6) Plasticity ≠ CoGV or CnGV	(7) G × E
Petal length	X		time		X	X
Style length	X	X	time		X	X
Ovary length	X	X		time	X	X
Anther–stigma distance	X	X	time		X	X
Flowering time	X	X	time	space		X
Protandry	X				X	X
Flower development time	X		space	space	X	X
Node first flower	X	X	space, time		X	X
Plastochron					X	
Leaf length	X	X	time		X	X
Leaf RGR	X				X	X
Size RGR	X		space			X

(Schmalhausen 1949; Bradshaw 1965). Population differentiation in plasticity has been shown to affect the magnitude of CoGV (and hence performance in foreign environments) in *Impatiens capensis* (Donohue et al. 2001).

A careful look reveals an interesting possible source for the genotype-by-environment interaction found in this study, a source that also might apply in other situations. In the present study, phenological traits consistently exhibited subspecies-by-environment interaction. For example, flower development time in ssp. *xantiana* increased from the wet year to the dry year, whereas the same trait in ssp. *parviflora* decreased between years. We hypothesize that these contrasting responses to the same environmental change arose from the subtle fact that the subspecies' flowering time difference caused them to experience contrasting environments within the same locations. In the dry year, ssp. *parviflora* individuals accelerated flower development, which was an adaptive response, because early flowering was at a premium in that year (M. A. Geber and V. M. Eckhart, unpubl. data). However, by the time ssp. *xantiana* individuals' flowers began to develop a few weeks later, the increasingly dry, warm conditions may have constrained flower development to occur unusually slowly. This kind of situation—in which genetic differentiation in life history alters the environmental conditions experienced while other traits develop—may represent a serious constraint on adaptation to novel environments.

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