Population Structure of Morphological Traits in *Clarkia dudleyana* I. Comparison of F_{ST} Between Allozymes and Morphological Traits

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ABSTRACT

Studies of genetic variation at allozyme loci, assumed to be selectively neutral, have provided valuable insights into the genetic structure of numerous populations. The degree to which population structure of allozyme variation reflects that of quantitative traits, however, is not well resolved. Here, we compare estimates of population differentiation (F_{ST}) of 11 populations for allozymes with those for nine discrete and nine continuous morphological traits. Overall, the allozymes have the lowest F_{ST} estimates, indicating relatively little population differentiation. Excepting two traits, petal width and long internode length, the continuous morphological traits have estimates similar to those from allozymes. The discrete morphological traits tend to have the highest estimates. On a single trait basis, estimates of F_{ST} for four discrete and two continuous traits are higher than those for allozymes. A more detailed (narrow-sense quantitative) genetic study of two populations suggests that these estimates of F_{ST} may underestimate the true value because of dominance. Clustering analyses show that the pattern of differentiation for the discrete morphological traits strongly reflects the geographical distribution of the populations, whereas the patterns for the continuous traits and allozymes do not. These results suggest that selection has been occurring on the discrete morphological traits, selecting toward a common optimum within each geographic group, and optima differing among geographic groups.

THE spatial distribution of genetic variation within and between populations, or population genetic structure, has long been an active area of research. Knowledge of population genetic structure provides information necessary for understanding the subsequent evolution of populations (WRIGHT 1951) and conserving genetic variability of a species and also reflects recent evolutionary history (*e.g.*, migration). Most empirical studies of population structure have examined the spatial distribution of allelic variation for isozymes (allozymes). HAMRICK and GODT (1990) recently reviewed associations between the population structure of allozymes and organismal attributes such as mating system and life span (*i.e.*, annual *vs.* perennial). Yet, whether these allozyme estimates of population structure are representative of the entire genome remains unresolved. Most allozyme studies assume that allozymes are selectively neutral, but this assumption may not always hold (*e.g.*, CLEGG and ALLARD 1973; CLEGG *et al.* 1978; KOEHN *et al.* 1983; KARL and AVISE 1992). Moreover, to the extent that allozymes are selectively neutral, measures of population structure based on allozymes cannot be expected to reflect the population structure of loci affected by selection. While some evolutionary processes, such as genetic drift and mating system, are expected to influence all loci equally, natural selection can differentially affect particular loci. As LEWONTIN and KRAKAUER (1973) first noted, differences between loci in evolutionary histories, or selective episodes, can be reflected in differences in observed population structure for those loci (LEWONTIN and KRAKAUER 1973; BOWCOCK and CAVALLI-SFORZA 1991; BOWCOCK *et al.* 1991). Of particular interest are the possibilities that selection on individual loci can act either to increase or decrease population differentiation relative to a neutral expectation.

Genetic population structure of quantitative traits summarizes the variation of the potentially many loci contributing to the given trait. The population structure of such polygenic traits is not expected to differ from that of single loci if traits determined by single genes as well as polygenic traits are selectively neutral (ROGERS and HARPENDING 1983; FELSENSTEIN 1986; ROGERS 1986). While the assumption of selective neutrality may likely hold for allozymes, it does not necessarily apply to many quantitative traits of interest (PROUT and BARKER 1993; SPITZE 1993). If selection on quantitative traits differs from the selection on allozymes, then these differences in selection are expected to be evidenced in discordant measures of population structure (SLATKIN 1987; PROUT and BARKER 1993; SPITZE 1993).

In contrast to genetic variation for quantitative traits, allozyme variation is relatively simple to measure. For

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this reason, the ability to predict the genetic structure of quantitative traits from allozyme measures would be valuable (HAMRICK 1983), but evidence of similarity in the population genetic structure of these two kinds of traits is equivocal. Phenotypic comparisons have been made in various organisms including plants (*e.g.*, PRENTICE 1984; PRICE *et al.* 1984), arthropods (*e.g.*, ALLEGRUCCCI *et al.* 1987), fish (*e.g.*, TURNER 1974), birds (*e.g.*, KARL *et al.* 1987), and mammals (*e.g.*, SCHNELL *et al.* 1978), while far fewer genetic comparisons are available (*e.g.*, SCHWAEGERLE *et al.* 1986; COYNE and BEECHAM 1987). Most studies have compared the association between morphological (phenotypic) distance, as estimated by Mahalanobis D^2, and NEI's (1972, 1978) genetic distance based on allozymes. The use of distances in the comparison of population structure is limited, however, for three reasons. First, comparisons based on phenotypic variation may be compromised by the influence of environmental variation (LEWONTIN 1984, 1986). Second, correlation between Mahalanobis D^2 and NEI's genetic distance (1972, 1978) does not indicate that the population structure is the same. Further, the null hypothesis that the morphological and genetic distances are the same has the necessary (but not sufficient) condition that the correlation between these distances is 1, whereas the null hypothesis tested is whether the correlation is 0. Third, these studies do not allow a comparison to determine traits that show unusually high or low levels of differentiation because all the data for either the allozymes or quantitative traits are used to produce the distances between populations.

WRIGHT's F_{ST} (1943, 1951) provides a standardized basis for comparing different types of genetic data in which larger F_{ST} values for some loci or traits indicate that these traits have a higher degree of divergence than those traits with smaller F_{ST} values (ROGERS and HARPENDING 1983; FELSENSTEIN 1986; ROGERS 1986; PROUT and BARKER 1993; SPITZE 1993). Two studies have recently compared F_{ST} between allozymes and quantitative genetic variation (PROUT and BARKER 1993; SPITZE 1993), showing differences between allozymes and quantitative traits in population structure.

In this paper, we estimate F_{ST} using allozymes, and discrete and continuous morphological traits in the California endemic wildflower, *Clarkia dudleyana*. We conducted a broad-sense quantitative genetic study of 11 populations within the Southern California range of *C. dudleyana*. To obtain more accurate estimates of the genetic parameters, we also conducted a narrow-sense quantitative genetic study focussing on two of the 11 populations. A narrow-sense quantitative genetic study is not feasible for studying more than two populations because hundreds of crosses need to be performed per population. We used the results from these studies to compare F_{ST} estimates with those obtained from allozymes. In comparing the estimates of F_{ST}, we ask the following questions. First, are estimates of F_{ST} obtained from allozymes comparable to those obtained from morphological traits? Second, how do broad-sense quantitative genetic estimates compare with narrow-sense estimates? Third, is there evidence for selection affecting any allozyme loci or quantitative traits? Fourth, is there a difference in how selection has affected three different types of genetic variation examined in this study: allozyme, discrete morphological, and continuous morphological?

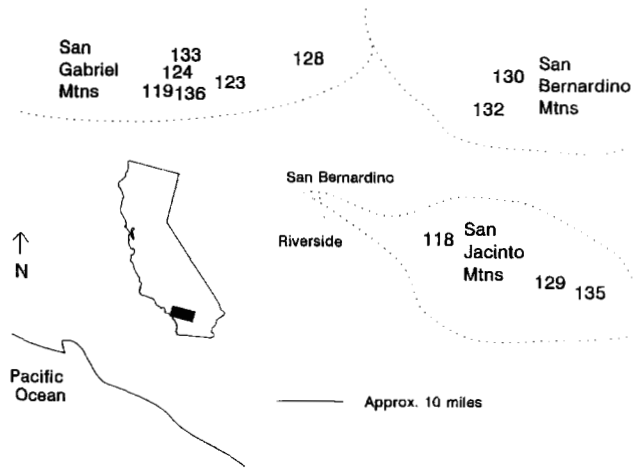

FIGURE 1.—Map of the populations from which seed was collected. The shaded box in the inset map of California shows the approximate location of the larger map. The names of the populations are as follows: 118, Yucaipa; 119, Bell Canyon; 123, Upland; 124, Tanbark Flats; 128, Lytle Creek; 129, Poppet Flats; 130, Running Springs; 132, San Bernardino; 133, Upper San Dimas Canyon; 135, Vista Grande; 136, San Dimas Canyon.

MATERIALS AND METHODS

C. dudleyana (Abrams) Macbr. (Onagraceae) is an annual endemic to California. Southern California populations range in size from a few individuals to thousands of individuals. *C. dudleyana* is an outcrossing species flowering between May and August. The genus Clarkia has been the subject of considerable research including examinations of phylogenetic relationships (*e.g.*, SOLTIS and BLOOM 1986; SYTSMA and SMITH 1990) and examinations of the genetic basis of morphological variation (*e.g.*, GOTTLIEB and FORD 1988; GOTTLIEB 1989).

Seed collections: In the spring and summer of 1991, we collected capsules from plants in each of 11 populations separated by ≥2 km (Figure 1). The size of these populations ranged from ~30 flowering individuals to many more than 1000 individuals flowering. The elevations of the populations ranged from ~600 m to ~1500 m.

At three populations, Tanbark Flats (TF, 124), Running Springs (RS, 130), and Upper San Dimas Experimental Forest (USD, 133), we collected maternal sibgroups of seeds by obtaining at least one capsule (10–120 seeds per capsule) from each of ~350 plants. At the remaining populations, we collected at least one capsule from 25 individuals. All 11 populations were used in both the allozyme and broad-sense experiments. For the narrow-sense experiment, only TF and RS were used.

Germination protocol: For all experiments, seeds were placed on moist, but not soggy, filter paper in 5.5-cm plastic petri dishes. Petri dishes were then placed in a refrigerator

TABLE 1

Electrophoresis systems run and enzymes assayed

Gel buffer system	Current (mA)	Volts (V max)	Enzymes assayed
LiOH-Borate	75	200	PGI, AAT, GDH, ACP
Morpholine citrate, pH 6.0	35	160	SKDH, MDH

and the filter paper allowed to dry. Once dry, the filter paper was again wetted and seeds were checked daily for germination. If the seeds in a petri dish had not germinated after a few days, the dish was removed from the refrigerator and monitored for germination at room temperature. When root radicles had emerged, the seeds were transplanted into pots. Total success rate from germination through establishment in pots was ~90%.

Allozyme experiment: For the populations at which only 25 sibships were collected, we attempted to grow one plant from each sibship. For the other three populations, we attempted to grow one plant from each of 50 sibships. Plants were grown in a greenhouse at the University of Missouri, Columbia.

We followed GOTTLIEB's (1981, 1984) and HOLTSFORD and ELLSTRAND's (1989) procedures for the extraction of plant material and starch gel electrophoresis (Table 1). The following enzyme systems were assayed: glutamate dehydrogenase (GDH, one locus), malate dehydrogenase (MDH, two loci scored, two other putative loci unresolved), phosphogluconate isomerase (PGI, two loci), aspartate/alpha-keto-glutarate transaminase (AAT, one locus), acid phosphatase (ACP, one locus), and shikimate dehydrogenase (SkDH, one locus). Loci were numbered sequentially with the most anodally migrating locus designated as locus 1. Mendelian inheritance of the loci encoding PGI has been documented (GOTTLIEB and WEEDEN 1979; GOTTLIEB 1984). Genetic interpretation of the other loci was straightforward. Allele and genotype frequencies were calculated for each allele and locus. The fit to Hardy-Weinberg expectations was tested over the entire data set as follows. Expected numbers of each genotype were calculated using the population allele frequencies. A chi-square test of goodness of fit was then performed using only those locus/population combinations that were polymorphic. Significant deviation from Hardy-Weinberg expectations ($\chi^2_{906} = 1018.71$; $P = 0.005$) violated an assumption of G_{ST} (NEI 1977; NEI and CHESSER 1983), a commonly used estimator of F_{ST}. We therefore calculated NEI and CHESSER's (1983) F_{ST}, hereafter denoted F_{NC}. COCKERHAM and WEIR (1986, 1993) and NEI (1986) have shown that both F_{NC} and G_{ST} fail to account for the error associated with sampling a subset of populations. Because WEIR and COCKERHAM's (1984) θ estimates an F_{ST} appropriate for the entire series of populations, some of which are not sampled, we also calculated this statistic. F_{NC} was calculated for comparison with other studies. Both F_{NC} and θ were bootstrapped (WEIR 1990) over populations using 5000 replicates to determine 95% confidence intervals.

Broad-sense experiment: We used seed from 20 of the field collected sibships from each population. For the eight populations at which we had collected 25 sibships, we used capsules containing ≥20 seeds. For TF, RS, and USD, we randomly chose 20 sibships.

On 11 March 1992, we began to germinate at least two seeds per sibship per block, and continued at weekly intervals to obtain five blocks. Seeds were transplanted into 5-inch pots filled with U.C. mix No. 3, and grown in a lathhouse at the University of California, Riverside. A week after all the seeds for each block had been transplanted, the pots were randomized. The following week, we thinned the pots to a single plant per pot and recorded pots with no plants. New seed was started for the missing plants and transplanted into a pot at its randomized location. Some deaths occurred due to snail predation and fungal disease and these plants were not replaced. Total survivorship to flowering was 94.4%.

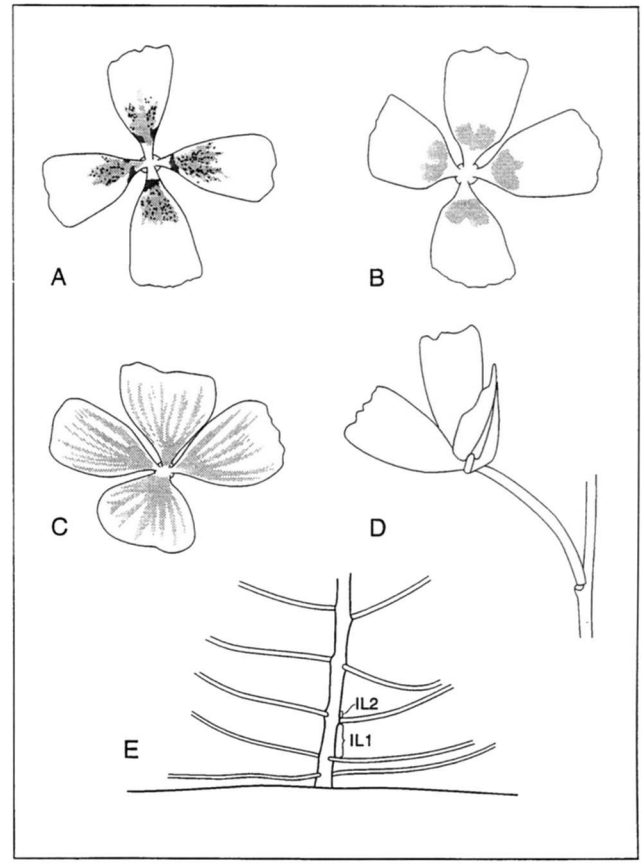

FIGURE 2.—Examples of morphological traits measured. The flower in (A) exhibits the traits PS, WC, DB; (B) shows WS, WC; (C) shows St; (D) exhibits RB; (E) represents the branch arrangement and shows how IL1 and IL2 are measured.

The traits measured and scored are described in Figure 2 and Table 2. We scored leaf spots (LS) three weeks after transplanting. All other traits were scored on the first day of flowering. For each trait, the residuals from an analysis of variance were tested for normality using SAS's Proc Univariate (SAS Institute 1985), and, if possible, transformed to normality as needed. Two of the nine continuous traits, long internode length (IL1) and short internode length (IL2), deviated significantly from normality and two traits, petal width (PW) and claw width (CW), were marginally significant. These traits were transformed to normality as follows: IL1, PW, and CW were square-root transformed; IL2 was transformed using $Y = \mathrm{Ln}[\ln(\mathrm{IL2}) + 1]$. For the discrete traits, residuals of white spot (WS) and stigma color (SC) did not differ significantly from normality. These traits were left untransformed in all analyses. Dark band (DB) had a marginally significant deviation from normality ($P = 0.0406$) and no transformation improved the fit to normality. Therefore, DB was left untransformed in all analyses. The remaining discrete traits all differed significantly from normality and were transformed. LS was transformed using $Y = \sqrt{X} + \sqrt{(X + 1)}$, and the re-

TABLE 2

Morphological traits measured

(a) Continuous morphological traits
- LL: Length of longest leaf
- LW: Width of longest leaf
- IL1: Distance between last pair of opposite branches and first alternate branch (long internode length; see Figure 2)
- IL2: Distance between first two alternate branches (short internode length; see Figure 2)
- PL: Length of petal on first flower
- PW: Petal width of first flower
- CW: Width of the constricted petal base (claw)
- OL: Ovary length
- HL: Hypanthium length

(b) Discrete Morphological Traits (see Figure 2)
- LS: Presence or absence of leaf spots
- PS: Presence or absence of petal specks
- WS: White spot present or absent from cup of petal
- DB: Dark band separating claw and petal present or absent
- WC: Color of the claw is either white or pink
- RB: The ovary is either curved or straight at anthesis
- SC: Color of the nonreceptive stigma is either white, light pink or dark pink
- St: Presence or absence of white streaks on the petals
- Pub: The stem is either pubescent or glabrous

maining traits were transformed using $Y = \sqrt{(X + 1)}$. Throughout, the discrete traits were analyzed as continuous traits.

A nested analysis of variance was conducted for all traits using the model, trait = mean + block + population + family nested within population + residual. For LS and white claw (WC), an interaction term between block and population was also included because including this term eliminated a second mode in the residuals. Variance components were subsequently estimated using the method of moments estimators (Table 3). These observational components of variance were then converted to causal components as follows. While the paternity of the seeds within any capsule is unknown, assuming that all the seeds are full-sibs provides conservative estimates of the genetic variance, barring maternal effects. The genetic variance (V_G) is then equal to twice the observational component of variance for dams within populations. The genetic variance between populations (V_B) was equated to the observational component for populations.

The testing of the variance components assumes that the random effects are normally distributed, which is not the case for most of the discrete morphological traits. Procedures for estimating and making inferences for variance components of discrete data are being developed (McCulloch 1994), but are currently only available for binary data. For the discrete traits, significance tests using method of moments estimators should be regarded with caution.

F_{ST} was calculated following Wright (1951; also Prout and Barker 1989, 1993; Lande 1992; Spitze 1993) as $F_{ST} = V_B/(2V_G + V_B)$. This formulation of F_{ST} for quantitative traits is analogous to Weir and Cockerham's (1984) θ. This calculation assumes that the differences between populations are strictly genetic. When the plants are grown in a common environment, as here, this assumption is plausible. V_B and V_G may, however, be biased by maternal and/or dominance effects, and then F_{ST} will also be biased. The inflated V_G will decrease the estimate of F_{ST}, relative to its true value, while the potentially inflated V_B would tend to increase F_{ST}. The narrow-sense analysis (see below) was conducted to determine the degree to which V_G is inflated because of dominance and maternal effects. Approximate SE of F_{ST} were obtained by the delta method (Appendix; Kendall and Stuart 1976; Rice 1988). Because the distribution of F_{ST} is not known, we used ± 2 SE as an ~95% confidence interval.

Narrow-sense experiment: During Fall 1991, we conducted a standard nested mating design (Comstock and Robinson 1948) to estimate the additive genetic variance (V_A) in TF and RS. Seed was germinated and grown in 5-inch pots filled with U.C. mix No. 3 placed in a greenhouse maintained at 21–26° during the day and 16–21° during the night at the University of California, Riverside. Within each population, plants were randomly designated as either a sire or dam in a ratio of 1:3. There were 495 plants available to serve as dams (300 from TF and 195 from RS) and 165 as sires (100 from TF and 65 from RS). Dams were randomly placed in one portion of the greenhouse, sires were randomly placed in another portion, and extra plants were kept in another portion of the greenhouse. Each sire was mated to a distinct set of three dams chosen at random within its population.

Two to three days before anthesis, two to five buds were emasculated on the dams. Excess flowers were removed from the dams daily. When a stigma became receptive (3–5 days after anthesis), it was brushed with an anther from the designated sire. Forty-four controls in which no pollen was deposited following emasculation yielded a total of five seeds in two fruits. Of these controls, only two contained seeds, with a total of five seeds. In the greenhouse, each capsule from *C. dudleyana* produces a minimum of 60 seeds. On this basis, the contamination rate for crosses was likely to be <0.2%. During the course of the crosses, some plants were killed by a fungus. Whenever possible, these dead plants were replaced with plants grown in the same conditions. However, some sires were mated to only one or two dams. In total, 433 full-sib families (276 from TF and 171 from RS) were produced from this mating design.

Beginning 8 September 1992, we began to germinate at least two seeds per full-sib family per block. We continued every other week to obtain three blocks. Germinated seeds

TABLE 3

Method of moments estimators for variance components

Source	Expected mean squares[a]	Observational components
Population	$\sigma^2_R + b\sigma^2_{Dam} + c\sigma^2_{Pop}$	$\hat{\sigma}^2_{Pop} = [MS_{POP} - MSR - b/a(MS_{Dam} - MSR)]/c$
Dam (population)	$\sigma^2_R + a\sigma^2_{Dam}$	$\hat{\sigma}^2_{Dam} = (MS_{Dam} - MSR)/a$
Residual	σ^2_R	$\hat{\sigma}^2_R = MSR$

[a] a, b, and c are coefficients dependent on structure of data. With balanced data these coefficients are as follows: a = b = number of replicates; c = number of replicates * number of dams within a population. $\hat{\sigma}^2_{Pop} = V_B$, $\hat{\sigma}^2_{Dam} = 1/2V_G$; $\hat{\sigma}^2_R = 1/2V_G + V_E$.

TABLE 4

Estimates of F_{ST} obtained from allozymes

Locus	F_{NC}	95% confidence interval	θ	95% confidence interval
ACP	0.016	−0.033, 0.030	0.004	−0.026, 0.040
GDH	0.051	−0.039, 0.089	0.023	−0.032, 0.101
MDH4	0.089	−0.001, 0.156	0.097	0.016, 0.171
MDH1	0.041	−0.016, 0.088	0.037	−0.011, 0.086
PGI2	0.185	0.059, 0.278	0.239	0.078, 0.353
PGI3	0.139	0.069, 0.174	0.181	0.078, 0.201
SKDH	0.072	0.015, 0.110	0.098	0.012, 0.137
AAT	−0.068	−0.641, −0.025	−0.079	−0.222, 0.012

were transplanted to 5-inch pots filled with U.C. mix No. 3 and grown in a lathhouse at the University of California, Riverside. A week after all the seeds had been transplanted for each block, the pots were randomized. The following week, we thinned the pots to a single plant per pot and started new seed for the missing plants. Initial mortality due to fungus was high and 4 weeks after planting each block, new seed was started for the missing plants. Any subsequent deaths were not replaced. Total survivorship over the three blocks was ~50%. To compensate for this excessive mortality, a fourth block was grown in the greenhouse beginning 2 September 1993.

The morphological traits were measured on the first day of flowering, except LS, which was not scored. A nested analysis of variance was conducted on all traits and residuals examined for normality. Five of the continuous traits did not differ significantly from normality: LL, PL, PW, OL, and HL. Following transformation, the other continuous traits did not differ significantly from normality and were transformed as follows: LW, $Y = \sqrt{LW}$; IL1, $Y = \ln [\ln (IL1 + 1)]$; IL2 and CW, $Y = \ln (X)$. The residuals for none of the discrete morphological traits were normally distributed. Regardless, the discrete traits were transformed as $Y = \sqrt{X} + \sqrt{(X + 1)}$, which is expected to stabilize variances (FREEMAN and TUKEY 1950). The realized experimental design necessitated an assumption that genotype-environment interaction between the greenhouse and lathhouse was negligible.

Variance components were estimated for populations, sires within populations, dams within sires, and residual using Proc Mixed in SAS (SAS Institute 1992) for the continuous traits and Proc Varcomp (SAS Institute 1985) for the discrete traits. These observational components of variance were then related to causal components as follows: $\hat{\sigma}^2_{Pop} = V_B$; $\hat{\sigma}^2_{Sire} = 1/4V_A$; $\hat{\sigma}^2_{Dam} = 1/4V_A + 1/4V_D$. Where the dam components were less than the sire components, we estimated V_A as $2(\hat{\sigma}^2_{Dam} + \hat{\sigma}^2_{Sire})$. F_{ST} was calculated as in the broad-sense experiment with $V_G = V_A$, and SE determined using the delta method (KENDALL and STUART 1976; RICE 1988).

UPGMA clustering: UPGMA clustering analyses were conducted on the discrete and continuous morphological traits (using all 11 populations) and the allozymes to examine the relationship of morphological and genetic distance with geographical distance. Mahalanobis D^2 between populations was calculated using either the continuous or discrete morphological traits using Proc Candisc in SAS (SAS Institute 1985). REYNOLDS *et al.* (1983) suggest using F_{ST} as a measure of genetic distance. For the allozymes, we calculated F_{ST} pairwise between all populations, using the program GENDIST from the PHYLIP package (FELSENSTEIN 1989). F_{ST} was then used as a measure of genetic distance. These calculated distances were used to cluster the populations using a UPGMA procedure from FELSENSTEIN's PHYLIP package (FELSENSTEIN 1989). Morphological and genetic distance matrices were then tested for correlation with a geographic distance matrix using a Mantel test from the program NTSYS (ROHLF 1993).

RESULTS

Allozyme experiment: Estimates of F_{ST} obtained from the allozymes using either F_{NC} or θ were not large, indicating little differentiation between populations for the allozymes. F_{NC} ranged from −0.068 to 0.185 and θ ranged from −0.059 to 0.239 (Table 4). F_{NC} and θ yielded similar results overall. Four loci, ACP, GDH, MDH-1, AAT, had F_{ST} estimates which did not significantly differ from 0 when θ and F_{NC} were used, and one additional locus, MDH-4, had F_{NC} not significantly different from 0. Estimates of θ for two of the loci, PGI-2 and PGI-3, had bootstrap confidence intervals that did not overlap those of some of the other allozyme loci, suggesting that these loci exhibit increased differentiation between populations relative to the other allozymes. When F_{NC} was used, all confidence intervals overlapped. F_{NC} for AAT was significantly negative suggesting that assumptions underlying the calculation of F_{NC} as an estimate of F_{ST} may have been violated.

Broad-sense experiment: All the continuous traits had significant variation among sibships and between populations except IL2 (Table 5a). The F_{ST} estimates for the continuous morphological traits ranged from 0.190 to 0.611 (Table 6a). Only IL2 had an estimate of F_{ST} that did not significantly differ from 0 (Table 6a; Figure 3). The environmental component (V_E) for this trait was the predominant component, which resulted in the large SE of F_{ST} for this trait. While all of the continuous morphological traits had two SE ranges of F_{ST} estimates overlapping, IL1 and PW had much larger estimates, and the two SE ranges for these two traits did not include most of the other point estimates for the continuous traits (Figure 3). These two continuous traits had confidence intervals which did not overlap seven of the eight allozyme confidence intervals, using θ. When F_{NC} is used as an estimate of F_{ST}, none of the confidence intervals overlap when comparing either IL1 or PW with the allozymes. These results indicate that F_{ST} is significantly different between the allozymes

TABLE 5

Broad-sense variance component estimates for morphological traits

Trait	V_B	V_G	V_E
	(a) Continuous traits		
LL	23.38***	32.62***	64.37
LW	1.83***	1.64***	4.56
IL1	0.33***	0.10***	0.60
IL2	2.38[a]	3.86[a]	0.15
PL	0.98***	1.09***	3.18
PW	0.033***	0.015***	0.033
CW	4.26[b]***	5.95[b]***	35.83[b]
OL	1.38***	2.94***	6.35
HL	0.037***	0.049***	0.175
	(b) Discrete traits		
PS	0.013***	0.035***	0.037
WS	0.009***	0.090***	0.293
DB	0.168***	0.134***	0.317
RB	4.55[a]***	3.13[a]*	0.034
SC	0.193***	0.112***	0.328
St	3.90[a]***	6.02[a]***	0.020
Pub	0.015***	4.83[a]***	0.019
LS[c]	0.104***	0.053**	0.255
WC[a]	5.75[a]***	5.34[a]*	0.032

* $0.01 < P < 0.05$; ** $0.005 < P < 0.01$; *** $P < 0.005$.
[a] $\times 10^3$.
[b] $\times 10^4$.
[c] Due to inclusion of population * block interaction term and unbalanced design, the test for the population effect is not feasible.

TABLE 6

Broad-sense F_{ST} estimates for the morphological traits

Trait	F_{ST}	SD	>0[a]
	(a) Continuous traits		
LL	0.264	0.095	Yes
LW	0.358	0.114	Yes
IL1	0.611	0.135	Yes
IL2	0.235	0.384	No
PL	0.310	0.108	Yes
PW	0.518	0.118	Yes
CW	0.264	0.119	Yes
OL	0.190	0.078	Yes
HL	0.273	0.105	Yes
	(b) Discrete traits		
PS	0.158	0.065	Yes
WS	0.500	0.125	Yes
DB	0.385	0.114	Yes
RB	0.421	0.188	Yes
SC	0.462	0.122	Yes
St	0.244	0.097	Yes
Pub	0.606	0.122	Yes
LS	0.204	0.084	Yes
WC	0.350	0.137	Yes

[a] This column indicates whether the 2 SD range excludes 0.

and both IL1 and PW, with populations showing more differentiation for these two continuous traits. The F_{ST} estimate for LW differed significantly from five of the allozyme F_{NC} estimates and three of the θ estimates (Tables 5a and 6a).

All the discrete morphological traits had significant variation among sibships and between populations (Table 5b). While the SE of F_{ST} are large, all estimates are significantly >0 (Table 6b; Figure 3). The estimate of F_{ST} for Pub was significantly larger than the allozyme estimates (Figure 3). Estimates of F_{ST} for WS and SC were significantly larger than F_{ST} for seven allozymes, estimated either by F_{NC} or by θ, and F_{ST} for DB was significantly larger than F_{ST} (θ) for five allozymes.

Narrow-sense experiment: None of the morphological traits, either continuous or discrete, had significant variation between the two populations examined (Table 7). Four continuous (LL, CW, OL, HL) and four discrete traits (PS, DB, St, Pub) had dam components smaller than sire components. For these traits, we assumed that the dominance and maternal effect variances are zero, and the larger dam component was obtained due to sampling error. Of these traits, PS and St had significant variation due to both sires and dams. Although the sire component of variation for these traits was larger than the dam, it was not markedly so (PS, $\hat{\sigma}^2_{Sire} = 0.068$; $\hat{\sigma}^2_{Dam} = 0.065$; St, $\hat{\sigma}^2_{Sire} = 0.052$; $\hat{\sigma}^2_{Dam} = 0.042$), suggesting that dominance and maternal variance may in fact be 0 and the differences between these two components was due to sampling error. The results for these traits imply that V_A was significantly different from 0. The remaining traits with larger sire than dam components, except LL, had sire components that differed significantly from 0. The dam component of variance was significantly different from 0 for five traits when the sire component was not significantly different from 0: LW, IL1, PL, PW, and WS (Table 7). These results imply that the dominance variance (V_D), and/or maternal effects variance, is significantly greater than 0 for these traits. For SC, both the sire and dam components were significantly different from 0, indicating that V_A is significantly greater than 0. The estimates of V_D and V_A for this trait were similar (Table 7). The estimates of V_A for two discrete traits, WC and RB, were 0, but estimates of V_D were greater than 0 (Table 7).

Although no traits had significant variation between the two populations used for this experiment, some estimates of F_{ST} were significantly >0 (Table 8). Traits with estimates of $V_A = 0$ (IL2, WC, RB) had estimates of $F_{ST} = 1$ due to the lack of genetic variation within populations (Table 8). Estimates of F_{ST} for two discrete morphological traits, WS and Pub, differed significantly from 0 (Table 8) with genetic variation also being found within populations. The remaining traits had estimates of F_{ST} that did not differ significantly from 0 (Table 8).

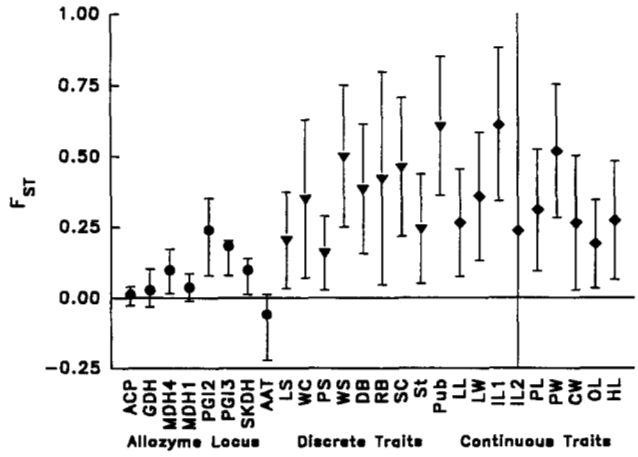

FIGURE 3.—Comparison of F_{ST} between all traits/loci measured. θ is estimated (●) from the allozyme loci; discrete morphological traits (▲) and continuous morphological traits (◆) are shown.

The narrow-sense estimates of F_{ST} for both the continuous and discrete traits did not appear to match the broad-sense estimates closely. However, broad-sense estimates of F_{ST} that included only RS and TF showed results that were similar to the narrow-sense results. The difference in the narrow-sense and broad-sense results was due to the fact that means of the morphological traits for the populations used in the narrow-sense experiment did not differ substantially. Based on the UPGMA clustering results below, which were obtained after the start of the narrow-sense experiment, this result was to be expected for the continuous traits since these two populations clustered closely together (Figure 4).

UPGMA clustering: The UPGMA clustering tree of the 11 populations based on the allozymes superficially looked very different from the clustering trees based on the discrete and continuous morphological traits (Figure 4). This difference resulted from the difference in Mahalanobis D^2 and F_{ST}. The F_{ST} distances for the allozymes were very small ranging from 0.07 to 0.43. Mahalanobis D^2 ranged from 1.24 to 12.16 for the continuous traits and from 0.42 to 14.30 for the discrete morphological traits. A common feature of the clustering trees for the discrete and continuous morphological traits was that the populations occurring in the San Jacinto mountains clustered together (Figure 4). Considering the remaining populations, clustering based on the discrete morphological traits most closely reflected the geographic distribution of the populations (Figure 4), with populations within each mountain range clustering together without exception. Distances based on the continuous and discrete morphological traits were positively correlated with geographic distance, with the discrete traits showing the highest correlation ($r = 0.256$, $P = 0.046$ for continuous traits; $r = 0.485$, $P = 0.003$ for discrete traits). The F_{ST} distance matrix based on the allozymes showed no correlation with a geographic distance matrix ($r = 0.085$, $P = 0.722$).

DISCUSSION

The distribution of genetic variation within and among populations differed for the three types of traits. While the estimates of F_{ST} obtained using allozymes did not differ from those for many quantitative traits, three morphological traits had estimates of F_{ST} that significantly exceeded those of the allozymes. The most common estimate of F_{ST}, over all traits and loci, was approximately 0.1 and this estimate can be assumed to represent the approximate value for a trait or locus diverging among these populations in the absence of selection (SLATKIN 1987). At least two continuous and four discrete morphological traits had much higher F_{ST} estimates than this value. These results suggest that natural selection has increased the observed differentiation for these six traits. The relative rankings of the three types of traits suggest that, overall, the discrete traits have been most affected by differentiating selection, and the continuous traits and allozymes only slightly affected if at all.

Two recent studies conducted similar comparisons. PROUT and BARKER (1993) found that F_{ST} for body size exceeded that for allozymes in *Drosophila buzzatii* sampled from 19 cactus cladodes. These authors concluded that differentiating selection had a larger effect on body size. SPITZE (1993) showed that fitness had a significantly lower, and body size a significantly larger F_{ST} than allozymes in *Daphnia obtusa* sampled from eight populations. Estimates of F_{ST} for growth, clutch size, and reproductive age did not differ significantly from those obtained for allozymes. SPITZE (1993) concluded that fitness was affected by selection unifying populations, body size by differentiating selection, and the other three traits were selectively neutral. In addition, information to calculate F_{ST} is available for several traits in two coniferous tree species. Estimates of F_{ST} for *Abies concolor* cover the entire range of this measure (HAMRICK 1976). Data from J. H. HAMRICK, W. J. T. PLATT, and M. HESSING (personal communication), using *Pinus palustris* show small quantitative trait estimates of F_{ST} that are just slightly larger than those observed for allozymes (DUBA 1985). These studies demonstrate that estimates of F_{ST} based on allozymes do not necessarily coincide with quantitative genetic estimates, although a number of quantitative traits exhibit population structure similar to that of allozymes. Thus, allozymes do not reliably reflect the population structure for any given quantitative trait.

Several assumptions underlie any inferences of selection based on F_{ST}. First, a neutral F_{ST} must be assumed. BOWCOCK *et al.* (1991) examined the neutral distribution of F_{ST} in human populations of known evolutionary history. Using simulation, these authors were able to

TABLE 7

Narrow-sense variance component estimates for the morphological traits

Trait	V_B	V_A	V_D	V_E
	(a) Continuous traits			
LL	5.60	22.65[a]	0.00	122.72
LW	0.504	2.636	1.870*	7.885
IL1	9.971[b]	2.585[c]	3.802[c]**	6.972[c]
IL2	1.704[c]	0.000	0.000	0.288
PL	1.550	0.545	2.213**	2.547
PW	0.340	0.685	1.358***	1.880
CW	0.294[c]	2.768[a,c]	0.000	11.994[c]
OL	2.197	2.869[a]	0.000	5.671
HL	0.127	0.081[a]	0.000	0.128
	(b) Discrete traits			
PS	0.027	0.267[a]***	0.000	0.171
WS	0.815	0.056	0.197**	0.206
DB	0.091	0.084[a]	0.000	0.435
WC	0.324[c]	0.000	0.034	0.159
RB	0.194	0.000	0.020	0.380
SC	1.005[c]	0.098*	0.081	0.175
St	0.076	0.189[a]**	0.000	0.249
Pub	0.816	0.027[a]	0.000	0.058

[a] Dam and sire components combined for estimate of V_A.
[b] $\times 10^5$.
[c] $\times 10^3$.
* $0.05 > P > 0.01$; **$0.01 > P > 0.005$; ***$P < 0.005$.

TABLE 8

Narrow-sense F_{ST} estimates for the morphological traits

Trait	F_{ST}	SD	$>0^a$
	(a) Continuous traits		
LL	0.110	0.160	No
LW	0.087	0.131	No
IL1	0.019	0.039	No
IL2	1.000	0.000	Yes
PL	0.587	0.436	No
PW	0.199	0.256	No
CW	0.051	0.083	No
OL	0.277	0.293	No
HL	0.442	0.384	No
	(b) Discrete traits		
PS	0.049	0.071	No
WS	0.879	0.181	Yes
DB	0.349	0.336	No
WC	1.000	0.000	Yes
RB	1.000	0.000	Yes
SC	0.005	0.017	No
St	0.168	0.205	No
Pub	0.939	0.084	Yes

show that, among their empirical estimates of F_{ST}, there were more small and more large estimates of F_{ST} for DNA polymorphisms than would be expected by chance under neutrality. Thus, they concluded that selection unifying populations influenced the loci with small F_{ST} estimates, and selection differentiating populations influenced the loci with large estimates. However, because long-term effective population sizes and the degree of isolation of populations are generally not known, it is not possible to determine the amount of differentiation that is expected due to genetic drift alone. PROUT and BARKER (1993) indicated that their allozyme estimate does not represent a strictly neutral F_{ST} based on evidence that selection has acted on those allozymes in *D. buzzatii*. SPITZE (1993) found no association between any fitness component and allozyme genotype and therefore assumed the allozymes were neutral. In the present study, we assumed the most common F_{ST} estimate represented a close to neutral value. Selection is not likely to affect all loci the same way, indicating that if many loci and traits have similar F_{ST} estimates then it is reasonable to assume that these traits and/or loci are nearly neutral (SLATKIN 1987).

These studies are limited to making inferences concerning the effect of selection on population differentiation. Selection occurring within populations could result in an estimate of F_{ST} that is similar to a neutral value. For instance, balancing selection occurring in all populations for a number of traits could result in similar estimates of F_{ST} for those traits. One interpretation of our results is that the allozymes and morphological traits with similar estimates of F_{ST} are actually the targets of such balancing selection. We agree with SLATKIN (1987), however, that this scenario is unlikely due to the fact that all traits would have to be subject to the same type and strength of selection as well as similar mutation. COHAN (1984) also suggests that uniform selection, such as balancing selection occurring in all populations, may in fact lead to increased divergence of finite populations. Only selection which has increased or decreased population differentiation can be detected by comparing estimates of F_{ST}.

The second assumption made by these comparative studies is that V_B is purely due to genetic causes. For quantitative traits, differences between populations need not be purely genetic and could partially be due to maternally inherited environmental effects. Although the progeny from different populations were ultimately randomized within a common environment, they may have been differentially affected by their source environments during their initial development. Such an effect would bias the quantitative genetic estimates of F_{ST} upward. This form of potential bias is common to all the studies cited above. The magnitude of such effects needs to be investigated.

A limitation of SPITZE's (1993) and of our study involving 11 populations is that broad-sense genetic variances are used. These variances include such components of variation as dominance and maternal effects. The relevant genetic variance for these comparisons is

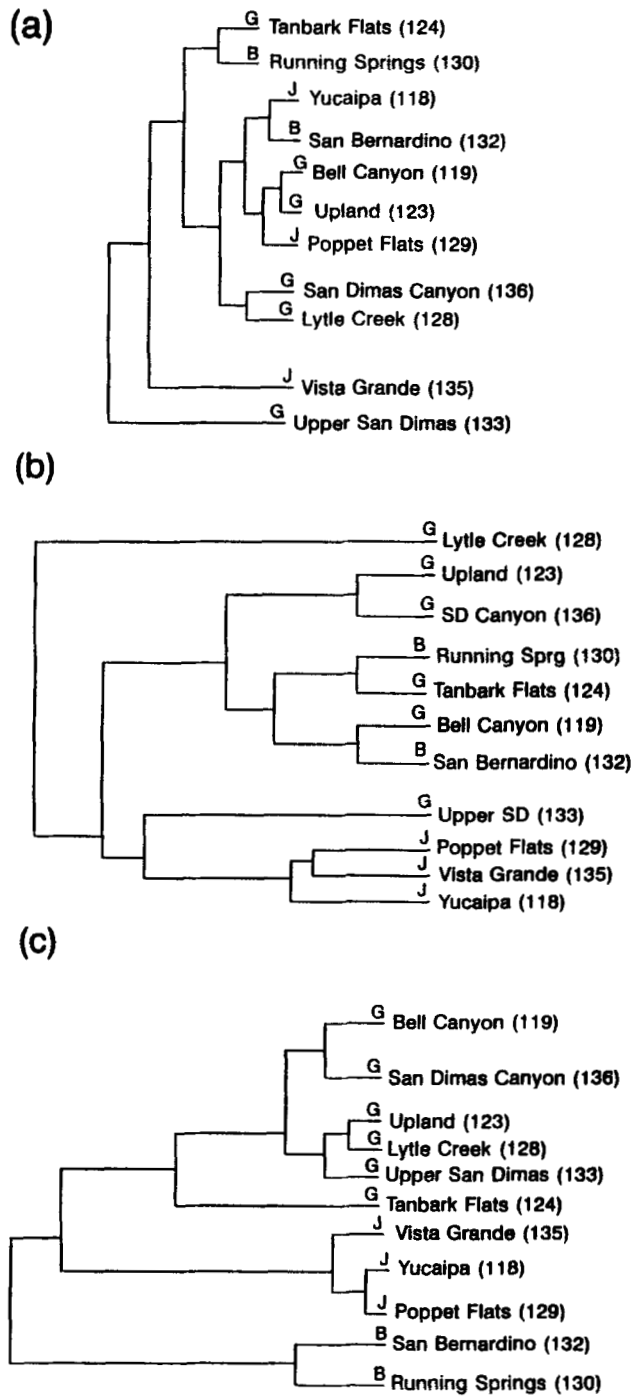

FIGURE 4.—UPGMA clustering trees for the three types of traits measured. The allozyme UPGMA tree is shown in (a), the continuous morphological tree in (b), and the discrete morphological tree in (c). Letters on the branches represent the mountain ranges to which the population, or groups of populations on a branch, belong. B, San Bernardino; G, San Gabriel; J, San Jacinto.

V_A, which is confounded with these other effects in V_G. Inflated estimates of V_G will bias the quantitative genetic estimates of F_{ST} downward. Our narrow-sense study provides evidence that dominance and/or maternal effects have affected many traits. All continuous traits that had a smaller estimate of the sire component than the dam component had $V_D > 0$. Likewise, the discrete traits had estimates of V_D that were similar to estimates of V_A. Thus, our broad-sense estimates of F_{ST} have probably been biased downward because of dominance. A comparison of the broad- and narrow-sense estimates of F_{ST} for the continuous traits does not reflect the influence of V_D because the two populations examined in the narrow sense were morphologically similar for these traits. The two discrete traits, WS and Pub, which did have narrow-sense estimates of F_{ST} significantly different from 0 were larger than the broad-sense estimates, although not significantly so.

A related limitation of this study is that V_G, in the broad-sense study, was estimated as twice the variance component among dams. This estimate is based on the assumption that all the seeds from a single capsule were full-sibs. The seeds from a single capsule were probably not only full-sibs but represented a mixture ranging from full- to half-sibs. We do not currently have enough data to determine the relationship among progeny from a single capsule and therefore prefer the conservative estimates of V_G. If the progeny obtained from a single capsule were in fact all half-sibs, V_G should be estimated as four times the variance among dams. This increase in V_G would effect a decrease in our F_{ST} estimates. Estimates of F_{ST} based on a half-sib assumption, however, do not change the qualitative results.

The third assumption made by all studies is that V_G (V_A) as measured in the laboratory is similar to that measured in the field. It is well known (FALCONER 1989) that inferences concerning quantitative genetic parameters are strictly applicable only to the environment in which they were estimated. In another experiment, we are examining field-based V_A estimates, which will provide insight into how comparable these estimates are.

Our analyses of the discrete morphological traits need to be viewed with caution. We have preliminary data suggesting that variation for our discrete traits is attributable to variation at single nuclear genes. Based on this information, we calculated allele frequencies for four of the discrete traits and used these frequencies to estimate F_{ST} using the method outlined in WEIR (1990). For all four discrete morphological traits examined in this fashion, θ was somewhat larger than the estimate of F_{ST} based on variance components in which the discrete traits are treated as being continuous. Further, the conclusions would change slightly for only one of these four discrete morphological traits, SC. This trait had a quantitative genetic estimate of F_{ST} that was only marginally significantly larger than that of the allozymes, but the θ estimate of F_{ST} was significantly larger than all allozyme estimates. These results suggest that an approach that treats the discrete traits as continuously distributed provides conservative estimates of F_{ST}.

A common feature of this study and those of PROUT and BARKER (1993) and SPITZE (1993) is that the allozyme estimates of F_{ST} are not large. The mean allozyme

F_{ST} for the three organisms examined are as follows: *D. buzzatii*, 0.032; *Daphnia obtusa*, 0.276; and *C. dudleyana*, 0.087. *C. dudleyana* and *D. buzzatii* have similar spatial distributions of genetic variation based on these F_{ST} estimates, and *D. obtusa* has more genetic variation distributed among populations than either of the other two. In the current study, the allozyme estimates of F_{ST} were smaller than the means for any of the applicable categories considered by HAMRICK and GODT (1990). For example, the mean G_{ST} for plants that outcross and are animal pollinated was 0.197 and for annuals was 0.357 (HAMRICK and GODT 1990). However, our F_{ST} values are similar to others measured for Clarkia. SOLTIS and BLOOM (1986) found that G_{ST} ranged from 0.003 to 0.127 in *C. speciosa* subsp. *polyantha*. HAMRICK (1983) reported mean G_{ST} values for *C. biloba* (G_{ST} = 0.121), *C. lingulata* (G_{ST} = 0.136), and *C. rubicunda* (G_{ST} = 0.060). This survey shows that the comparisons of F_{ST} between quantitative traits and allozymes have used organisms that have little substructuring, although this structure is not atypical. A comparison between quantitative traits and allozymes for an organism that has high allozyme estimates of F_{ST}, such as a self-pollinating (selfing) plant (HAMRICK and GODT 1990), remains to be conducted.

SCHEMSKE (1984) examined population structure of quantitative traits in a selfing plant, *Impatiens pallida*, and showed significant genetic differentiation occurred on a small spatial scale within this species, as is expected for a selfer. SCHEMSKE also provided data from a reciprocal transplant experiment that indicated that selection could have affected the observed population structure. Similarly, ARGYRES and SCHMITT (1991) examined the small scale population structure of morphological and life-history traits in the largely inbreeding *I. capensis*. Their study found genetic differentiation on a small scale, but as with SCHEMSKE (1984) the population structure of the traits examined cannot be compared with allozyme estimates of population structure.

On a larger scale, the geographical correlates of the spatial distribution of genetic variation represented by the UPGMA trees provide another comparison between the allozymes and the morphological traits. The UPGMA results show the clustering depends on the types of traits examined. The trees from all three types of traits show some pattern of geographic associations of variation. Only the discrete morphological traits produce clusters of the three main geographic groups, mountain ranges, sampled.

The F_{ST} results suggested that the allozymes were selectively neutral and the discrete morphological traits, overall, were subject to selection increasing population differentiation. Assuming that the allozyme tree represents a neutral clustering, the UPGMA results together with the F_{ST} results suggest that selection has affected the discrete traits in such a way that unifying selection has acted within the geographic groups but this unifying selection has differed between the geographic groups. This suggestion provides a basis for addressing the question of whether current selection is acting in the manner described above.

Based on field observations, we have noted that the potential pollinators differ between the mountain ranges. At Tanbark Flats, a variety of insects have been recorded visiting the flowers. No insect visitation to Clarkia has been noted at Running Springs. Only honey bees, *Apis mellifera*, have been seen visiting Clarkia at Vista Grande. These three populations occur in the three separate mountain ranges. These observations suggest that pollinator mediated selection may account for our morphological results. Forthcoming results from an experiment examining current selection within two populations will begin to address such possibilities.

Regardless of whether the proposed selection scenario actually holds, the fact that the continuous morphological and discrete morphological traits show different patterns of differentiation and different levels of substructuring is interesting. The discrete traits are mainly floral polymorphisms and are similar to other single gene polymorphisms found in Clarkia by GOTTLIEB (1989; GOTTLIEB and FORD 1988; *e.g.*, WS is the same phenotype as White Cup in *C. gracilis*). These sorts of floral polymorphisms have been found to be subject to some form of selection (*e.g.*, CLEGG and EPPERSON 1988; RAUSHER and FRY 1993) further suggesting selection may have a larger impact on such discrete traits. The question remains, however, whether the larger divergence observed here is due to larger selection intensities on discrete traits or rather is due to the response of traits determined by a few genes as opposed to a large number of genes. Such questions are just beginning to receive critical empirical attention.

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APPENDIX

To obtain the variance for the quantitative genetic estimates of F_{ST}, we used the delta method (RICE 1988; KENDALL and STUART 1976). Using this method, the variance of any function of one or more variables,

$$Y = f(X_i)$$

is approximately

$$\text{Var}\ (Y) = \sum \left(\frac{\partial Y}{\partial X_i}\right)^2 \text{Var}\ (X_i) + 2 \sum\sum \frac{\partial Y}{\partial X_i}\frac{\partial Y}{\partial X_j} \text{Cov}\ (X_iX_j)$$

The (co)variances of the variance components, V_B and V_G, are then needed to find the variance of F_{ST}. These (co)variances can be obtained using the mean squares from the analysis of variance because the method of moments estimators are used and the variance components are simple linear functions of the mean squares. The variance of any given mean squares is equal to

$$\text{Var}\ (MS) = \frac{2MS^2}{d.f. + 2}$$

and all mean squares are independent (SEARLE *et al.* 1992). Therefore, the (co)variances of the variance components are as follows:

$$\text{Var}\ (V_G) = \frac{8}{a^2}\left[\frac{MS^2_{\text{Dam}}}{d.f._{\text{Dam}} + 2} + \frac{MSE^2}{d.f._E + 2}\right]$$

$$\text{Var}\ (V_B) = \frac{1}{c^2}\left[2\frac{MS^2_{\text{Pop}}}{d.f._{\text{Pop}} + 2} + 2\frac{MSE^2}{d.f._E + 2} + \frac{b^2}{4}\text{Var}\ (V_G)\right]$$

$$\text{Cov}\ (V_GV_B) = \frac{4}{ac}\left[\left(1 - \frac{b}{a}\right)\frac{MSE^2}{d.f._E + 2} - \frac{b}{a}\frac{MS^2_{\text{Dam}}}{d.f._{\text{Dam}} + 2}\right]$$

where a, b, and c are coefficients obtained from the expected mean squares from an analysis of variance (Table 3). Using these (co)variances of the variance components, the approximate variance of F_{ST} is

$$\text{Var}\ (F_{ST}) = \frac{4}{(2V_G + V_B)} \times [V_B^2\,\text{Var}\ (V_G) + V_G^2\,\text{Var}\ (V_B) - 2V_GV_B\,\text{Cov}\ (V_GV_B)]$$