Theor Appl Genet (1986) 73:88–93

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Estimates of heritability and correlations of morphometric traits in *Clarkia* (Onagraceae)

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Received April 5, 1986; Accepted May 6, 1986
Communicated by A. R. Hallauer

Summary. Interspecific heritability values were estimated using parent-offspring regression analyses for 11 morphological traits differentiating *Clarkia nitens* and *C. speciosa* subsp. *polyantha*. Estimates ranged from near 0 for anther color and germination percentage, to 0.8 for calyx length and petal tip color. Phenotypic, genetic, and environmental correlation matrices were computed to determine the extent of interspecific correlations of traits. Cluster analyses of the genetic and environmental correlation matrices each resulted in three clusters of correlated traits; however, the clusters derived from the two matrices were different. The clusters produced by analysis of the environmental correlation matrix were similar to the factors obtained from principal component analysis of the phenotypic correlation matrix. Genetic correlations may result from strong linkage due to interspecific chromosomal differences.

Key words: Parent-offspring regression – Multivariate analysis – Cluster analysis – Principal component analysis – *Clarkia*

Introduction

Closely-related species are often differentiated by several morphological traits, which may form suites of characters having functional or developmental significance. Studies of intra- and interspecific variation in plants have demonstrated such interrelationships among morphological characters, using phenotypic correlations and multivariate techniques such as principal component analysis (Clausen and Hiesey 1960; Hiesey et al. 1971; Grant 1979; Grant and Grant 1979; Gilmartin 1980; Holsinger 1985). However, more information could be obtained by partitioning the phenotypic correlations into their genetic and environmental components, as has been accomplished in several studies of character coherence in animals (Leamy 1977; Arnold 1981; Boag 1983). Investigations of genetic and environmental correlations have elucidated the genetic relationships among the traits and have revealed functional assemblages of characters. This study examines the genetic relationships among 11 interspecific morphological differences in two *Clarkia* species by estimating the heritability of each trait and the genetic and environmental correlations among pairs of traits.

Heritability values reflect the proportion of additive genetic variance for a trait and indicate the degree to which the trait is genetically determined. High heritability values indicate a large additive genetic component, relative to dominance, epistatic, and environmental components, in the expression of a trait. Heritability estimates also suggest the degree to which selection acts on a trait. Generally, those traits with lower heritabilities are closely connected with reproductive fitness (Fisher 1958). In contrast, traits with higher heritability values usually are less important as components of fitness. Therefore, heritability estimates may be useful in evaluating the role of selection in populations and species (Arnold 1981; Boag 1983; Stearns 1983).

Correlations of traits have both genetic and environmental sources. The genetic correlation (r_A) is the correlation of breeding values, whereas the environmental correlation (r_E) includes environmental deviations and non-additive genetic factors (Falconer 1981). Correlations of traits are of evolutionary interest for two primary reasons. First, the genetic mechanisms of correlation, such as linkage and pleiotropy, may be elucidated. Although pleiotropy is typically regarded as a more potent cause of genetic correlation (Nagylaki and Crow 1974; Falconer 1981), linkage may also contribute to temporary character associations. Secondly,

correlations of traits may be useful in predicting response to selection. Selection on one trait may cause a correlated response in another. Therefore, understanding the genetic basis of the character association may provide insights as to the direction evolution may take in a population or species.

Taxa

Investigation of a species complex in *Clarkia* (Onagraceae) suggested that examination of the morphological differences between *C. nitens* and *C. speciosa* subsp. *polyantha* might provide insights into the genetics and evolution of interspecific character assemblages. These taxa are closely-related members of Section *Godetia* (Lewis and Lewis 1955), and although superficially very similar morphologically (Lewis and Lewis 1955), *C. nitens* and *C. speciosa* subsp. *polyantha* show significant differences ($P < 0.05$) for 20 of 32 morphological and developmental traits examined (Soltis 1985). Most notable are differences in floral color and floral size. These interspecific differences were evaluated by estimation of heritabilities to determine the degree of genetic control of these traits and by examination and clustering of genetic and environmental correlations to reveal assemblages of traits.

Materials and methods

Plants

Samples from two populations of *Clarkia nitens* and two populations of *C. speciosa* subsp. *polyantha* were grown in a greenhouse located at the University of Kansas, Lawrence, Kansas. All plants were scored for the following 11 traits:

ANTC	Anther color
STYL	Style length
SAD	Stigma-anther distance
FLA	Filament length (large stamens)
CALL	Calyx length
HYPW	Hypanthium width
PETW	Petal width
PETB	Petal base color
GERP	Germination percentage
TIPC	Petal tip color
STIC	Stigma color

These traits showed highly significant differences ($P < 0.001$) between the two taxa (Soltis 1985).

Twenty individuals from each of the four populations served as parents and were crossed in nearly 400 interspecific combinations to produce F_1 offspring. The F_1 progeny were grown the following spring and summer.

Estimation of heritability

Interspecific heritability values for each trait were estimated by regression analyses of offspring on male parent, female parent, and mid-parent values (Falconer 1981). The slope of the regression of offspring on male or female parent values equals one half the narrow-sense heritability for that trait. The regression coefficient of offspring on mid-parent values directly estimates narrow-sense heritability. The regression of offspring on mid-parent values is most reliable, because it reduces bias in the form of maternal effects (Falconer 1981). However, it can only be used accurately when the parental variances for a trait are equal (Falconer 1981). F_{max} tests for homogeneity of variances indicated that all traits had equal parental variances except anther color, petal base color, and stigma color (Soltis 1985). Standard errors (se) for all heritability (h^2) estimates were calculated using the standard errors of the regression coefficients (Sokal and Rohlf 1981). For estimates based on the regression of offspring on one parent,

$$\text{se}(h^2) = 2\,(\text{se}(b)),$$

where b = the regression coefficient. The standard error for estimates derived from the regression of offspring on mid-parent is

$$\text{se}(h^2) = \text{se}(b).$$

Sixty pairs of parent-offspring data were used to estimate heritability values. Because no segregating generations were involved in the analyses, linkage due to interspecific chromosomal differentiation should have no effect on the results.

Calculation of phenotypic, genetic, and environmental correlations

Phenotypic correlations among pairs of the 11 traits were generated using all individuals in the parental samples. The matrices of genetic and environmental correlations were calculated as outlined by Falconer (1981), using the heritability values estimated by the regression of offspring on mid-parent values. The additive genetic correlations for each pair of traits were calculated by the following formula:

$$r_A = \frac{\text{cov}_{xy}}{\sqrt{\text{cov}_{xx}\text{cov}_{yy}}},$$

where x and y are the two traits under consideration, cov_{xy} is the covariance of the two traits in the parents and offspring, and cov_{xx} and cov_{yy} are the offspring-parent covariances of each trait. Standard errors of the genetic correlations were calculated as follows (Reeve 1955; Robertson 1959; Falconer 1981):

$$\text{se}(r_A) = \frac{1 - r_A^2}{\sqrt{2}} \sqrt{\frac{\text{se}(h_x^2)\,\text{se}(h_y^2)}{h_x^2 h_y^2}},$$

where h_x^2 and h_y^2 equal the heritability estimates of traits x and y, respectively, and $\text{se}(h_x^2)$ and $\text{se}(h_y^2)$ equal the standard errors of these heritability estimates.

Environmental correlations for each pair of characters were calculated from Falconer (1981) by the following:

$$r_P = h_x h_y r_A + e_x e_y r_E,$$

where r_P equals the phenotypic correlation of traits x and y, h_x and h_y are square roots of the heritabilities of traits x and y, and $e = \sqrt{(1 - h^2)}$.

Clustering

A powerful multivariate technique for describing the internal structure of a set of variables is principal component analysis. A PCA was conducted on the phenotypic correlation matrix to

describe the interrelationships of the 11 traits chosen for study. However, this method could not be employed for the genetic and environmental correlation matrices because they generated communalities greater than unity. This was because some values in both matrices exceeded 1, which frequently occurs in studies of genetic and environmental correlations (Hashiguchi and Morishima 1969; Leamy 1977; Arnold 1981). Therefore, a cluster analysis designed to group the 11 variables was performed by BMDP/1M (Dixon 1981) using the genetic and environmental correlation matrices. This method was successfully employed in a similar study of morphological correlations in mice (*Mus domesticus*) by Leamy (1977). Clustering was accomplished using three techniques: single linkage clustering, arithmetic average clustering, and complete linkage clustering.

Results

Heritability estimates

Estimates of heritability of the 11 interspecific differences ranged from 0 to 2 (Table 1). There was considerable variation among the three methods of estimation, particularly for stigma size, petal base color, petal tip color, and stigma color. Because offspring-mid-parent regressions are most reliable when parental variances are equal (Falconer 1981), all subsequent analyses were performed using the offspring-mid-parent estimates.

Standard errors associated with heritability estimates are usually large (Falconer 1981); therefore, heritability values should be interpreted with caution. A *t*-test demonstrated that nine of the 11 traits had heritabilities significantly greater than 0 ($P < 0.05$). These high heritability values indicate a high proportion of additive genetic variance for these traits.

Phenotypic, genetic, and environmental correlations

Phenotypic correlations between pairs of traits ranged from 0.01 to 0.95 and are both positive and negative (Table 2). Particularly high correlations were found between anther color and petal base color, anther color and stigma color, petal base color and stigma color, and filament length and stigma color.

The genetic and environmental correlations are given in Table 3. Standard errors of the genetic correlations are also provided. The genetic correlations generally are positive and relatively large, ranging from −3.25 to 2.65. Several correlations are greater than 1, presumably due to the large standard errors associated with variance and covariance estimates. Environmental correlations are generally negative and are also relatively large, ranging from −4.96 to 5.34. Again, several values exceeded one.

Principal component analysis and clustering

A principal component analysis was performed on the original matrix of phenotypic correlations. It resulted in the extraction of a single major axis (Table 4). Those traits with the highest loadings on Factor 1 are anther color, petal base color, and stigma color, suggesting this is a flower color contrast axis. Factor 2 may be interpreted as a minor axis, with high factor loadings for style length, stigma-anther distance, and hypanthium width, which suggest it is a flower size axis.

Cluster analysis of the genetic and environmental correlation matrices resulted in the dendrograms in Figs. 1 and 2. Clustering produced by arithmetic averaging was chosen because it produced more discrete clusters than did single or complete linkage clustering, although all three methods generated similar results.

Table 1. Heritability (h^2) estimates for 11 traits in *Clarkia*

Trait	No. of pairs	h^2 Estimates		
		Mid-parent	Female	Male
ANTC	63	0.022±0.0	0.022±0.097	0.000±0.0
STYL	60	0.515±0.130***	0.716±0.208***	0.340±0.177*
SAD	58	0.522±0.272*	0.487±0.342	0.627±0.482
FLA	59	0.314±0.039***	0.570±0.378	0.215±0.234
CALL	60	0.802±0.214***	0.666±0.322*	0.682±0.264**
HYPW	59	0.503±0.042***	0.616±0.200**	0.333±0.243
PETW	60	0.650±0.220**	0.566±0.333*	0.884±0.297**
PETB	63	0.602±0.305*	0.602±0.299*	0.000±0.0
GERP	66	0.099±0.163	0.168±0.236	−0.002±0.248
TIPC	60	0.814±0.064***	1.332±0.320***	0.381±0.396
STIC	60	0.698±0.573	0.427±0.690	2.237±1.372

*, ** and *** indicate estimates were significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 2. Phenotypic correlations for the 11 traits measured in interspecific crosses of *Clarkia*

Trait	Trait										
	ANTC	STYL	SAD	FLA	CALL	HYPW	PETW	PETB	GERP	TIPC	STIC
ANTC	1.000										
STYL	−0.388	1.000									
SAD	−0.325	0.704	1.000								
FLA	−0.677	0.556	0.386	1.000							
CALL	−0.466	0.659	0.423	0.690	1.000						
HYPW	−0.473	0.242	−0.007	0.446	0.310	1.000					
PETW	−0.527	0.553	0.416	0.585	0.664	0.421	1.000				
PETB	0.806	−0.429	−0.383	−0.740	−0.560	−0.561	−0.620	1.000			
GERP	−0.406	0.175	0.256	0.356	0.301	0.136	0.330	−0.519	1.000		
TIPC	−0.529	0.456	0.418	0.532	0.517	0.310	0.562	−0.541	0.232	1.000	
STIC	0.868	−0.495	−0.392	−0.791	−0.598	−0.520	−0.632	0.947	−0.514	−0.555	1.000

Table 3. Genetic correlations (above diagonal) and environmental correlations (below diagonal) for the 11 traits studied

Trait	Trait										
	ANTC	STYL	SAD	FLA	CALL	HYPW	PETW	PETB	GERP	TIPC	STIC
ANTC		0.122	0.079	−0.347	0.373	0.051	−0.014	−0.204	0.427	−0.049	0.235
STYL	−0.554		0.420*	−0.253	0.978***	1.863**	0.385*	0.764***	0.806***	−0.463**	1.692***
SAD	−0.483	0.960		0.176	0.294	0.808***	−0.542**	1.232***	0.133	0.613	−0.182
FLA	−0.858	0.962	0.370		0.199	1.698**	0.294	2.647	0.480	−0.218	−0.038
CALL	−1.314	−0.076	0.649	1.517		0.641***	0.555***	0.052	1.642*	−0.322*	−0.755***
HYPW	−0.640	−1.684	−1.062	−0.418	−0.469		−3.252	2.489*	1.076***	0.130	−1.162***
PETW	−0.813	0.518	1.560	0.733	0.989	5.340		0.601***	−0.057	0.556***	0.413*
PETB	1.438	−1.692	−2.231	−3.540	−2.032	−4.309	−2.515		0.128	0.083	0.499**
GERP	−0.548	−0.100	0.259	0.294	−0.451	−0.112	0.594	−0.947		0.594*	0.712**
TIPC	−1.097	2.071	−0.316	1.566	4.014	0.417	0.069	−1.953	0.089		0.872***
STIC	1.661	−3.787	−0.556	−1.654	−0.106	0.420	−2.678	1.794	−1.360	−4.961	

*, ** and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively, for the genetic correlations

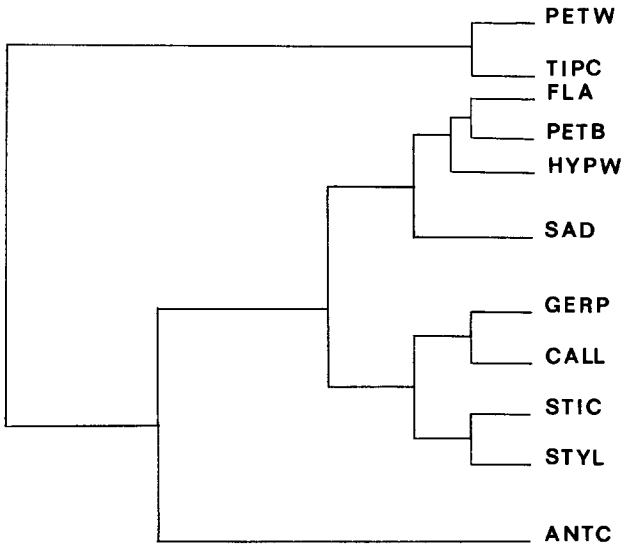

Fig. 1. Dendrogram derived from complete linkage clustering of the genetic correlation matrix

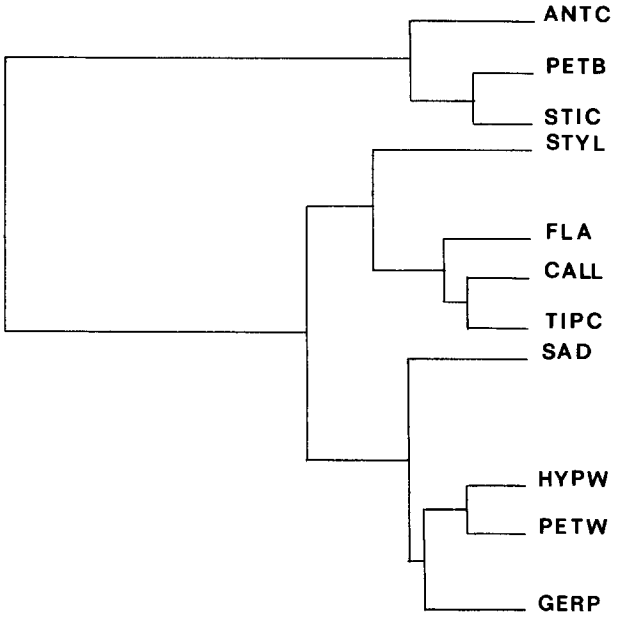

Fig. 2. Dendrogram derived from complete linkage clustering of the environmental correlation matrix

Table 4. Phenotypic structure of interspecific morphological differences, as revealed by principal component analysis. Two factors were extracted from the phenotypic correlation matrix

Trait	Factor 1	Factor 2
ANTC	−0.817	0.301
STYL	0.690	0.569
SAD	0.562	0.654
HYPW	0.544	−0.490
PETB	−0.893	0.292
STIC	−0.921	0.249
Variance explained	0.55	0.12

Three general groups of traits are evident in the dendrogram derived from the cluster analysis of the genetic correlation matrix (Fig. 1). The first cluster contains petal width and petal tip color. The second is composed of filament length, petal base color, hypanthium width, and stigma-anther distance, while the third contains germination percentage, calyx length, stigma color, and style length. No obvious functional groups emerge from this analysis.

The dendrogram resulting from cluster analysis of the environmental correlation matrix (Fig. 2) is notably different from that derived from the genetic correlation matrix. Again, three clusters emerge, but they are composed of different groups of variables. The first cluster contains germination percentage, petal width, hypanthium width, and stigma-anther distance. The second is composed of petal tip color, calyx length, filament length, and style length, while the third is made up of stigma color, petal base color, and anther color. This third cluster contains three of the four flower color traits and appears to represent a functionally related suite of traits. The second cluster contains three floral size characters, and may also represent a functionally related group of traits.

Discussion

Heritability of interspecific morphological differences

Clarkia nitens and *C. speciosa* subsp. *polyantha* differ by several morphological features, most notably those traits examined in this study. Nine of the 11 traits exhibited heritability estimates significantly greater than 0, suggesting a relatively large additive genetic component to the phenotypic differences between *C. nitens* and *C. speciosa* subsp. *polyantha* for these nine traits. The genetic control of these interspecific morphological differences is reported by Soltis (1985).

Because there is generally a high inverse correlation between the magnitude of intrapopulational heritability estimates and the effect of a trait on fitness (Fisher 1958; Falconer 1981), the adaptive value of individual traits may be inferred. This assumes a model of selection such that a single genotype has an optimum selective value in a population and that selection eliminates additional variation. However, more complicated selection models indicate that variation may be maintained by selection, suggesting that inferences of a trait's selective value based on heritability estimates may not accurately reflect the trait's contribution to the fitness of the organism. In this study, such speculation may not be appropriate due to the interspecific nature of the comparisons.

Interpretation of cluster analyses

The cluster analyses of the genetic and environmental correlation matrices were performed to elucidate genetic or functional relationships among traits. No obvious functional groups were revealed by clustering of the genetic correlations among traits, as have been demonstrated in similar studies of *Mus domesticus* (Leamy 1977), *Thamnophis elegans* (Arnold 1981), and *Geospiza scandens* and *G. fortis* (Boag 1983). This could be due to the less integrated developmental systems of plants, as compared with animals. Pleiotropic gene action may be responsible for the character associations produced, but because the clusters contain anatomically and developmentally distinct characters, linkage may be a more likely explanation.

The clusters of traits derived from analysis of the environmental correlation matrix differed from those produced from clustering of the genetic correlations. This suggests that at least two separate gene complexes may affect each trait, possibly through different physiological mechanisms (Falconer 1981). The observation that the genetic and environmental correlations tend to differ in sign is consistent with this hypothesis. The single suite of traits which exhibit obvious functional relationships resulted from clustering of the environmental correlations. This cluster of flower color traits, which corresponds to the traits of Factor 1, suggests a biochemical relationship among these traits.

The clusters produced by analysis of the genetic correlation matrix bear little resemblance to the principal components extracted from the matrix of phenotypic correlations. This is in contrast to previous studies of animal morphology (Leamy 1977; Arnold 1981), which reported a high correspondence between the character groupings derived from analyses of the genetic and environmental correlation matrices. However, Factor 1 is strongly influenced by flower color traits (anther color, petal base color, and stigma color), which comprise one of the three character assemblages produced by clustering of the environmental correlation matrix. In addition, the second principal component contains the traits of floral size, similar to those of cluster 2 derived from analysis of the environmental correlations among traits. It is, therefore, apparent that the associations of traits produced by principal component analysis of the phenotypic correlation matrix more accurately reflect the environmental component of correlation than the genetic sources.

Several factors may account for the closer correspondence of phenotypic and environmental correlation matrices observed in this study than in previous studies (Leamy 1977; Arnold 1981). First, the traits investigated by Leamy (1977) and Arnold (1981) are probably under polygenic control, whereas the traits examined here are under relatively simple genetic

control (Soltis 1985). Secondly, the heritability estimates provided here are interspecific heritabilities, whereas Leamy (1977) and Arnold (1981) each dealt with a single species. The effects of these differences on the structure of the correlation matrices are unknown; therefore, comparisons between this study and other investigations should be interpreted with caution.

This investigation demonstrated that the morphological differences between two closely-related species are genetically related, forming suites of traits that presumably evolve as units. Whether these character associations result from pleiotropic gene action or linkage remains to be determined, although linkage could play a major role.

Acknowledgements. I thank Doug Soltis, Bill Bloom, and Jim Hamrick for helpful comments on the manuscript. The financial support of the AAUW Educational Foundation is appreciated.

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