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Evolution, Vol. 52, No. 2. (Apr., 1998), pp. 344-354.

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SEED MORPHOMETRICS AND ADAPTIVE GEOGRAPHIC DIFFERENTIATION

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Abstract.—Adaptive geographic differentiation is documented for seed morphology of 36 populations of *Heterosperma pinnatum* Cav. (Asteraceae), a seed heteromorphic annual plant in the central highlands of Mexico. Achenes (single-seeded fruits) vary continuously within heads but are classified by shape and position as central, intermediate, or peripheral morphs and as having adhesive awns or not. Here we quantify shape as a principal component score contrasting log length and width of achenes. Heads and their variation among populations are described in terms of maximum, minimum, and range of shape scores; the number of achenes per head; quantitative indices of the abruptness of shape shift; where in the head the most abrupt change in shape occurs; and what achene shapes have awns. First and second principal components of these descriptors summarize 86% of among-population variation in achene and head morphology and correlate strongly with percent central achenes per head (%C) and percent of achenes with awns (%A), respectively. Awns are associated with greater dispersibility and achene shape is correlated with speed of dormancy loss. We hypothesized that dispersal morphology would be associated with vegetation attributes indicative of population ephemerality and that dormancy morphology would be associated with precipitation patterns during the early germination season. Morphological distance matrices were calculated using Euclidean distances among populations in %A and %C. Geographic distances among populations were calculated, as were genetic distances based on isozyme frequencies from 29 bands of six enzymes. Vegetation was classified as open or closed and early spring (germination season) and summer precipitation means were determined for each site. Closed vegetation was assumed to provide only ephemeral habitats for *H. pinnatum*. Partial matrix correlations between morphology and environment controlled for geographic but not genetic distance among sites, since the latter was not significantly correlated with either morphology or geography. A significant relationship was found between %A and closed vegetation, lower spring, and higher summer precipitation. %C was only correlated with lower spring precipitation. Independence of isozyme and morphological traits is interpreted in terms of selection on the latter but not the former.

Key words.—Adaptation, dispersal, dormancy, ecotypic differentiation, genetic distance, germination, *Heterosperma pinnatum*, morphometrics, seed heteromorphism.

Received March 5, 1997. Accepted December 19, 1997.

Seed heteromorphism, or the production of different seed morphs by individual plants, has been suggested to occur whenever there are advantages for a single plant to pursue multiple reproductive strategies (Venable 1985). Plants that possess seed heteromorphism are ideal study organisms for learning about the evolution of reproductive strategies because seed functions, such as dispersal and germination, often vary with seed morphology (e.g., Sorensen 1978; Tanowitz et al. 1987; Kigel 1992; Telenius 1992; Rocha 1996). Where specific seed morphologies are associated with specific functions, an assessment of reproductive strategies of individuals and populations can be made from seed structure. If variation in the proportions of different seed morphs is heritable, then these proportions may evolve via natural selection in response to local environmental conditions. Adaptive explanations of seed morphology and function can be explored by examining patterns of seed morphology across populations in different environments. Such analyses of intraspecific variation among many populations provide an important complement to functional studies and demographic experiments on selection in individual populations (Venable 1984; Venable et al. 1987, 1995).

Here we describe the patterns of morphometric variation in the achenes (morphologically, single-seeded fruits) of *Heterosperma pinnatum*, a seed heteromorphic composite, across populations in central Mexico. These patterns are related to environmental factors thought to influence the evolution of

dispersal and dormancy strategies. Models of the population dynamics of dispersal suggest that population ephemerality is a dominant selective force favoring dispersal (Venable and Brown 1993). We explore a trait associated with dispersibility (presence of adhesive awns; Venable et al. 1987) in relation to environmental factors indicative of population ephemerality (open vs. closed vegetation). Also, previous demographic experiments on this species suggested that delayed germination within years is favored when early germination is hazardous or when the probability of surviving to reproductive maturity is low (Venable et al. 1995). Achene shape has been shown to influence the within-year timing of dormancy in this species (long, thin achenes lose dormancy faster in the laboratory and germinate earlier in the field than short, wide ones; Venable et al. 1987). We explore a trait associated with within-year timing of germination (achene shape) in relation to environmental factors indicative of the hazards associated with early versus late germination (early germination season precipitation patterns). Genetic studies have shown substantial within- and among-population genetic variation in this species for the proportions of seeds with adhesive awns and with different shapes. This indicates that populations have indeed differentiated and suggests that these traits can respond to natural selection (Venable and Búrquez 1989, 1990).

Care must be exercised in making adaptive interpretations of ecotypic variation in morphology. Nearby populations may

be similar morphologically due to localized gene flow and occur in similar environments due to similarity in factors such as climate or topography. Thus, a correlation between morphology and environment may be due more to the geographic patterning of populations than to adaptive sorting. In this paper we attempted to control for the confounding effects of geographic proximity by using a partial matrix correlation approach (cf. Brown and Thorpe 1991; Castellanos et al. 1994).

A similar problem in the interpretation of correlations across populations will occur if a species is subdivided into a few races or subspecies. Apparently significant adaptive correlations based on many populations may actually represent repeated sampling of only two or three races or subspecies that happen to have different seed morphologies and occur in different environments. In this investigation we carried out an isozyme study to estimate genetic distances between populations. If the structure of genetic relationships among populations is correlated to achene morphology, we can statistically remove genetic distances when comparing morphology and environment. Considering geographic and genetic structuring strengthens any conclusions regarding an adaptive match between morphology and environment.

The relationship between genetic distances based on isozymes, morphological distances, and geographic distances are interesting in their own right. The association between isoenzymes and functional traits is still a matter of debate (Bergmann 1991). Sometimes, relationships between enzyme variation and functional characteristics have been shown (e.g., El-Kassaby 1982; von Wühlisch and Krusche 1991; Beer et al. 1993; Riemenschneider and McMahon 1993), while in others, no significant correlations have been observed (Wolf and Soltis 1992; Borghetti et al. 1993). Also, different geographic structures have been reported for isozymes. For example, in *Avena barbata* there is a strong association between reproductive morphology and genetic markers, and both of these are geographically structured and correlated with environmental variables (de la Vega et al. 1991). Allele frequencies and the color and hairiness of the lemma are correlated regionally with aridity across Californian Mediterranean cool and warm summer zones (Clegg and Allard 1972) and along microgeographic gradients in intermediate zones (Hamrick and Allard 1972). In *Mimulus* there is geographic structure of isozymes that correlates with morphological traits at large geographic scales, that is, the whole continent of America, but no significant structure at smaller geographic scales (Vickery 1990).

This is the first plant study to control for the potentially confounding effects of geographic proximity and genetic similarity of populations in tests of adaptive hypotheses using ecotypic variation. Previous studies of quantitative genetic variation (Venable and Búrquez 1989, 1990), function (Venable et al. 1987), and fitness (Venable et al. 1995) for this species strengthens the analyses presented here.

MATERIALS AND METHODS

Species

Heterosperma pinnatum is an herbaceous summer annual of the central highlands of Mexico, with a range extending

from Honduras to the southwestern United States. This heteromorphic composite flowers from August to late October and produces overwintering achenes that typically germinate the following spring. Germination time differs among achene types, usually occurring with rainy weather in April, May, or June, though it has been documented as early as February. *Heterosperma pinnatum* is abundant, both in areas of open vegetation, such as open savannah and desert scrub, and areas of closed vegetation, such as pine-oak forest. In areas of closed vegetation it typically grows in disturbed sites, such as roadsides.

Variation in Achene Morphology

Unlike most heteromorphic composites, achene morphology in *H. pinnatum* is not determined by floret type. Heads typically have from seven to 15 achenes that vary in shape from the center to the periphery of the head. For convenience we have grouped achenes into three categories according to their morphology. Central achenes are five to 15 times as long as wide, possess a beak, and have little or no wing; peripheral achenes are less than twice as long as wide, do not possess a beak, and have broad wings; intermediate achenes represent a range of morphologies that do not fall into either of these two categories (cf. Venable et al. 1987, 1995). Previous experiments have shown that central achenes lose dormancy earlier in the season than peripheral ones and germinate under a broader range of conditions (Venable et al. 1987). Many achenes possess a pair of awns arising from their beak. There is a distinct spatial pattern in the arrangement of awned achenes within a head. Awns begin at some point along the spirals of achenes that converge at the center of the head and are found on all achenes inside that switch point. Previous experiments have shown that the awns aid in adhesive dispersal (Venable et al. 1987).

During the summers of 1984 and 1985, heads were collected from 36 populations in the Eje Volcánico Transversal and Mesa Central region of Mexico (Fig. 1). From each population 40 heads, each from a different individual, were collected at maturity just prior to presentation for dispersal (deflection of the phyllaries). Each head was scored for the number of awned achenes (A) and the number of central achenes (C). These values were expressed as percentages of the total number of achenes per head (%A and %C) and means were calculated for each population. The mean percentages were then logit transformed and the data used to construct two matrices of morphological distance among populations, one for the percentage of central achenes and one for the percentage of awned achenes.

To characterize the morphology of achenes in finer detail, 10 heads were selected from each of 15 populations. Achene length and width were measured using digital calipers and presence of awns was recorded for each achene in these heads. To obtain a univariate measure of achene shape, a principal component analysis was performed on log achene length and width. The first principal component accounts for 80% of the variation in these two variables. While the first principal component in many morphometric analyses has positive loadings on most variables and represents size, the loadings here were positive on log length and negative on log width. Thus, the

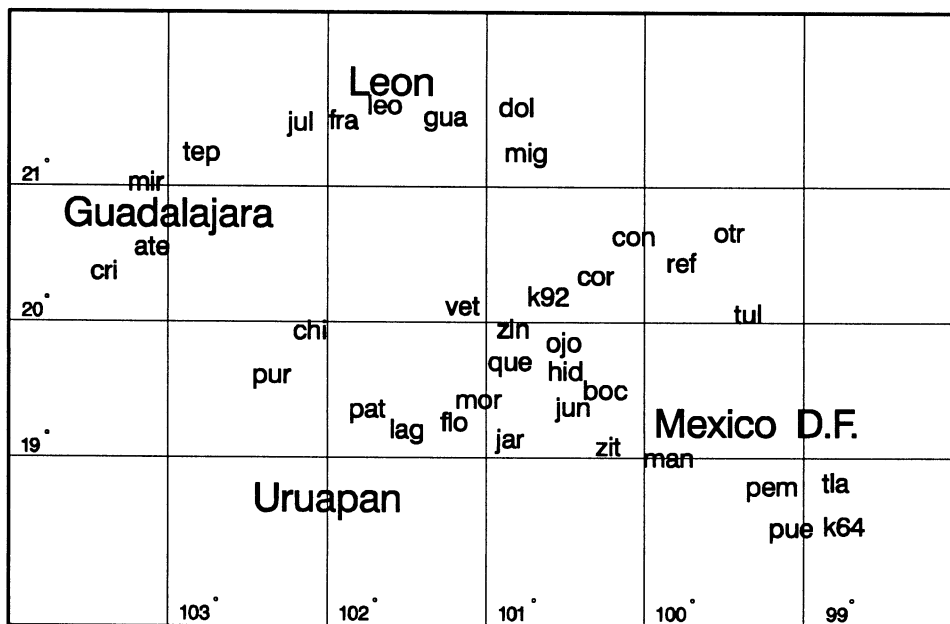


FIG. 1. Map of *Heterosperma pinnatum* population sites used in this study. The region shown is the Mesa Central and Eje Volcánico Transversal of Mexico. Site name codes are: ate, Atequiza; boc, Bocangueo; chi, Chilchota; con, Presa Constitución 1917; cor, Coroneo; cri, San Cristóbal; dol, Dolores Hidalgo, km 30; flo, Nueva Florida; fra, San Francisco del Rincón; gua, Guanajuato; hid, Ciudad Hidalgo; jar, El Jaral; jul, San Julian; jun, Jungapeo; k64, Xochimilco-Oaxtepec, km 64; k92, km 92 ca. Acámbaro; lag, Lagunillas; leo, Leon; man, Mañones; mig, San Miguel de Allende; mir, Mirador; mor, Morelia; ojo, Ojo de Agua; otr, Oromundo; pat, Pátzcuaro; pem, Tepoztlán, Pemex; pue, Tepoztlán, Puente; pur, Purépero; que, Querendaro; ref, El Refugio; tep, Tepatitlán de Morelos; tla, Tlalnepantla; tul, Tula; vet, Escuela Veterinaria; zin, Zinapécuaro; zit, Zitácuaro. The grids represent latitude and longitude.

first principal component represents achene shape. The second component represents achene size and accounts for the remaining 20% of the variation. This analysis confirms a previous conclusion that *H. pinnatum* achenes vary more in shape than size (Venable et al. 1987). Using the PC1 score for each achene to measure shape, the following parameters of achene morphology were calculated for each head: the maximum PC1 score (MAX), the shape of the longest and narrowest achene in a head; the minimum PC1 score (MIN), the shape of the shortest, widest achene in the head; RANGE (= MAX – MIN), the shape difference between the longest, narrowest and shortest, widest achene; the number of achenes in a head (N); the maximum difference (MAXDIFF) between shape scores of adjacent achenes, the largest change in achene shape in the head; the position (PSHIFT) within the head of the maximum difference between scores, which is calculated by counting from the most extreme central achene outward to the achene where maximal shape shift occurs and dividing by the total number of achenes; and the achene shape at the shift between awned and nonawned achenes (SAWN). RANGE tells how much achene morphological divergence there is within a head. MAXDIFF reflects how abrupt the shift in achene shape is. PSHIFT indicates where in the head (how far from the center) the most abrupt change in morphology occurs. SAWN tells whether only long, thin central achenes have awns, or whether awns extend to intermediate and peripheral achenes as well.

To characterize population differentiation in achene and head traits morphologically, a correlation analysis and a principal component analysis were performed on the population means of the traits defined in the previous paragraph. The

means of %A and %C for these 15 populations were also included in these analyses.

Climate and Vegetation

Climate and vegetation data for each site were gathered from maps published by the Mexican government (Anonymous 1981a,b,c,d, 1983a,b). For each site, we found the nearest weather station at an equivalent altitude and obtained the mean precipitation for each month. Precipitation patterns, especially in the spring and early summer germination season, are likely to be important in determining the risk associated with early germination. We also classified each site by vegetation type. Of the 36 sites, eight were classified as subtropical scrub (Matorral Subtropical), three as tropical deciduous forest (Selva Baja Caducifolia), 14 as grassland (Pastizal Natural), eight as pine-oak forest (Bosque de Pino-Encino), and three as cactus scrub (Matorral Crasicaule). Vegetation types were further grouped into “open” (cactus scrub and grassland) and “closed” (tropical deciduous forest, subtropical scrub, and pine-oak forest) based on whether or not a closed canopy of plants much larger than *H. pinnatum* typically exists in the vegetation type. This aspect of vegetation was considered to impinge on the value of dispersal directly because of its impact on habitat permanence for an annual plant. In closed vegetation types, *H. pinnatum* populations only exist in disturbed, open patches that tend to be ephemeral. The open vegetation types have a substantial permanent annual plant component that includes *H. pinnatum* (DLV, pers. obs.). A distance matrix was constructed by assigning a distance of zero between pairs of sites that both had open

or closed vegetation types and a distance of one between sites that differed in this dichotomous variable.

Spatial Correlations

Associations between the distance matrices of morphology and environment were calculated using matrix correlations with a randomization method to determine significance levels (Dietz 1983). Because the association between two matrices can be affected by the association of each with geography, the partial correlation technique for distance matrices developed by Smouse et al. (1986) was used to factor out the effect due to geography. We constructed a matrix of geographic differences based on Euclidean distances between the sites. Then we regressed out the effect due to geography from each matrix of primary interest and used the residuals to calculate a partial matrix correlation coefficient between morphology and the environment. Significance levels were calculated using a Mantel-type randomization procedure with 10,000 randomizations. All matrix correlation statistics were programmed with SAS IML (SAS Institute 1985). We use significance levels to interpret these matrix correlations, since the magnitude and sign of correlations among distance matrices do not have a straightforward correspondence to the magnitude and sign of the correlations of the underlying variables (Dietz 1983).

Genetic Variation among Populations

To determine whether genetic distance between populations confounds correlations between morphology and the environment, an allozyme electrophoretic analysis on specimens from 27 of the populations was performed. Seeds from 10 to 25 plants per population were grown in the greenhouse for six weeks and whole plants, one for each parent, were homogenized in water. The crushed extract was absorbed onto filter paper wicks and inserted on starch gels (10–12 w/v). All enzymes were resolved using two gel/electrode buffer systems, a Histidine system and a Poulik buffer system (Richardson et al. 1986; Piñero and Eguiarte 1988). Gels were run at 4°C for as long as necessary for the colored run marker (red amaranth) to move 8 cm. Six enzymes were identified using standard staining procedures (Soltis et al. 1983). The enzymes phosphoglucose isomerase (Pgi), phosphoglucotomutase (Pgm), ribulose 5'diphosphate carboxylase (Rub) were assayed with the Histidine system and acid phosphatase (AcpH), anodic peroxidase (Apx), and esterase (Est) were resolved with the Poulik system. Because the genetic basis for the allozymes was unknown, the electrophoretic data were analyzed by considering each band to be a separate character having two states (present/absent). There were 29 bands altogether from the six enzymes (AcpH, 6; Apx, 6; Est, 6; Pgi, 4; Pgm, 4; and Rub, 3). The band frequencies within each population were calculated and used to generate a matrix of Roger's distances between populations and a UPGMA dendrogram of populations was produced. The association between the Roger's distances and the morphological distances for %C and %A and between Roger's distances and the geographic distances among populations were calculated using matrix correlations with a randomization test of significance as described in the previous section.

RESULTS

Variation in Achene Morphology

The pattern of achene morphologies within a head can be quite variable among populations (Fig. 2). One striking difference among populations is the extent to which achene shape and the presence of awns can be decoupled. In some populations awns only develop on long, thin achenes (e.g., San Miguel, Constitución 1917, Tula). In other populations awns also develop on wider, shorter achenes (e.g., Km 64, Bocanejo, Tlalnepantla).

The mean percentage of central achenes in a population is a good indicator of PSHIFT, the distance of the most abrupt shift in achene shape from the center of the head ($r = 0.95$), and also correlates with shapes of the most extreme achenes (both extremes are longer and thinner in populations with a high percentage of central achenes, $r = 0.74$ with MAX and $r = 0.93$ with MIN). Populations with high %C have fewer achenes per head ($r_{\%C,N} = -0.75$), a greater percentage of awned achenes ($r_{\%C,\%A} = 0.70$), and the shift from awned to nonawned achenes occurring on longer, thinner achenes ($r_{\%C,SAWN} = 0.76$). Populations with a high percentage of awned achenes have longer, thinner extreme peripheral ($r_{\%A,MIN} = 0.76$) but not longer, thinner extreme central ($r_{\%A,MAX} = 0.26$) achenes, some tendency to have fewer achenes per head ($r_{\%A,N} = -0.53$), and a most abrupt shift in morphology far from the center of the head ($r_{\%A,PSHIFT} = 0.60$; $P < 0.05$ for each r greater than 0.50). Populations with very long narrow central achenes have relatively long, narrow, extreme peripheral achenes also ($r_{MAX,MIN} = 0.69$), suggesting a shift in the shape of all achenes toward long and narrow. Such populations also have a broader range of achene shapes (high RANGE, $r_{MAX,RANGE} = 0.66$), fewer achenes per head ($r_{MAX,N} = -0.58$), the most abrupt shift in achene shape farther from the center of the head ($r_{MAX,PSHIFT} = 0.60$), and the shift from awned to nonawned achenes occurring at a longer, thinner shape ($r_{MAX,SAWN} = 0.94$). Populations with the most abrupt shift in achene shape occurring far from the center of the head have longer, thinner achenes ($r_{PSHIFT,MAX} = 0.60$ and $r_{SHIFT,MIN} = 0.83$), fewer achenes per head ($r_{PSHIFT,N} = -0.74$), and a shift from awned to nonawned achenes occurring on long, thin achenes ($r_{PSHIFT,SAWN} = 0.64$). Populations with a more abrupt maximum shift in achene shape have a large range in achene shape ($r_{MAXDIFFERANGE} = 0.69$).

These patterns of correlation in achene morphology are summarized by a principal component analysis (Fig. 2). The first principal component accounts for 58% of the variation in morphology and is strongly positively correlated ($r \geq 0.80$) with the percentage of central achenes, the distance of maximal shape shift from the center of the head, length and narrowness of the most extreme central and peripheral achenes, and the length and narrowness of achenes where the shift from awned to nonawned achenes occurs. This first principal component is also strongly negatively correlated with the number of achenes per head. Thus populations with a high percentage of central achenes have longer and thinner achenes and produce fewer achenes per head. The second principal component accounts for 28% of the variation in achene morphology and is strongly positively correlated to

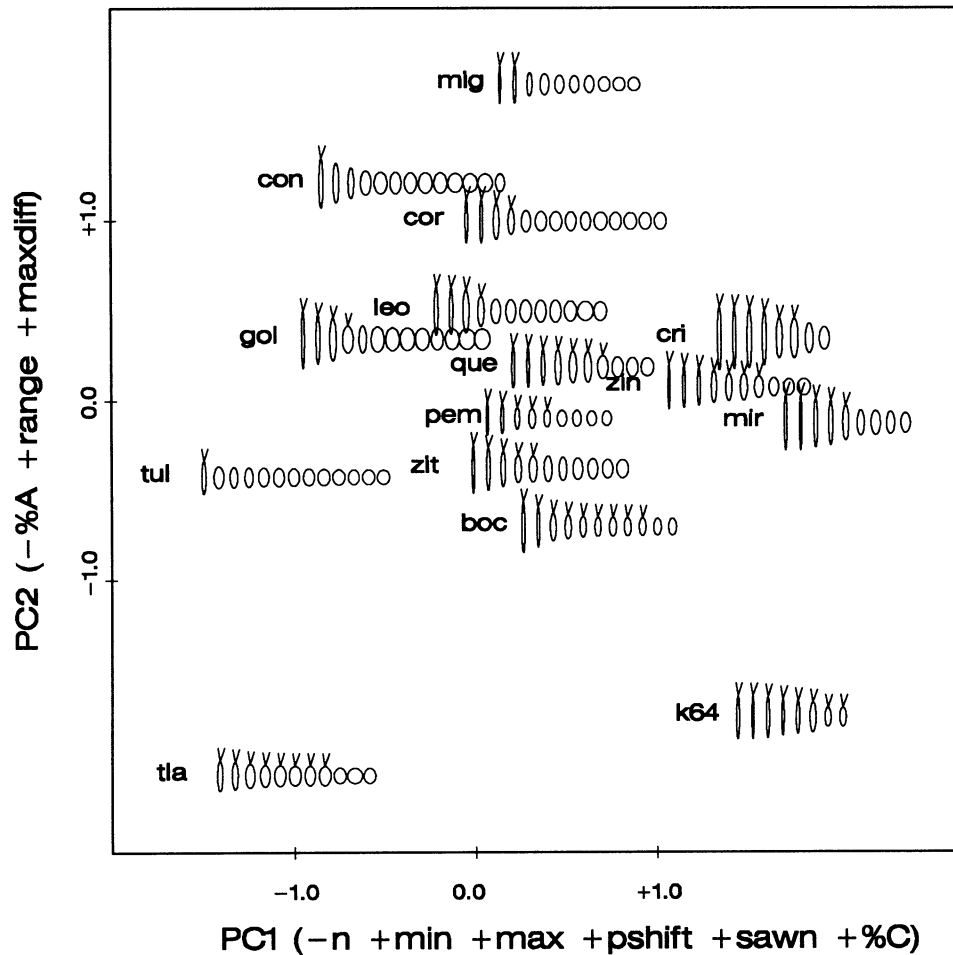


FIG. 2. Achene traits for a typical head are drawn as ellipses with length and width of the achenes and with or without awns for each of the populations used in the detailed morphometric analysis. The three letter site designations referring to the codes used in Figure 1 are centered at the population's position on the principal component space. The principal component analysis was carried out on the following variables, which are defined in the materials and methods: range, min, max, n, pshift, maxdiff, sawn, %C, and %A. The first principal component correlates positively to min, max, pshift, sawn, and %C, and negatively to N. The second principal component correlates positively to range and maxdiff and negatively to %A.

the magnitude of the range in achene shape within heads and the maximum shape shift between adjacent achenes. It is strongly negatively correlated with the percent of awned achenes. Thus populations that have fewer awns are more heteromorphic and this variation is independent of the %C-achene number per head axis. These patterns can be readily visualized when the population mean head morphologies are drawn on the space generated by PC1 and PC2 (Fig. 2).

%C and %A are highly correlated with PC1 and PC2, respectively, thus they provide a good summary of variation in achene morphology. They have clear functional significance in relation to dormancy and dispersal as described above in the introduction and Materials and Methods. They have also been measured on all 40 individuals from the 36 populations and are used in the further analyses.

Genetic Distances

The electrophoretic data was used to determine how *H. pinnatum* populations are structured genetically over the Mexican Plateau. The dendrogram calculated from isozyme

frequencies shows no discrete isozyme races (Fig. 3). On the contrary, there is a continuum of distances with no clear clusters.

The morphological traits, %A and %C, do not show any clear pattern of association with the position of populations in the dendrogram (Fig. 3). This lack of pattern was confirmed statistically by determining that the genetic distance matrix is not significantly correlated to the morphological distance matrices (Mantel's test, $P > 0.25$). Thus isozyme variation is not associated with achene morphological variation in *H. pinnatum*.

Also, populations that are close in the dendrogram are not necessary close on the geographic map of populations given in Figure 1. Mantel's test shows that the geographic distance matrix is not significantly related to the genetic distance matrix ($P > 0.25$).

This lack of significant association of isozyme structure with geography and morphology suggests that it is unnecessary to control for the effects of genetic relatedness of populations when testing for associations of morphology and the environment.

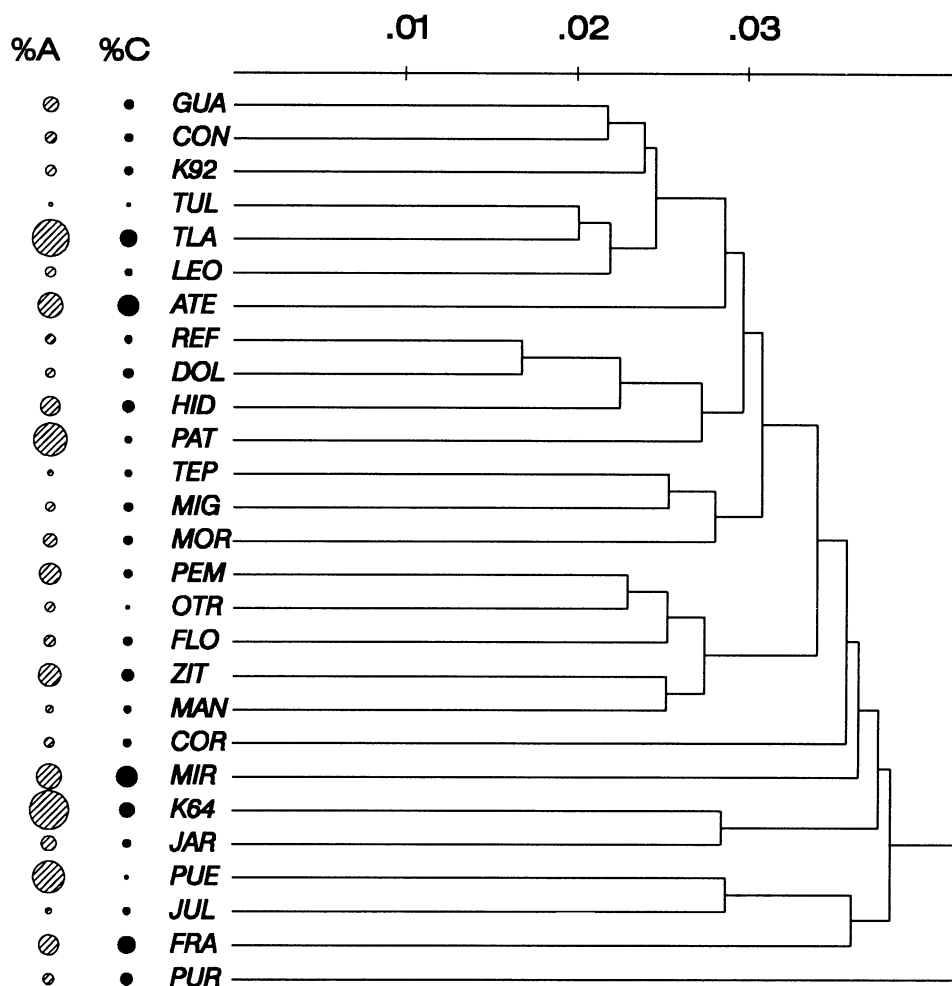


FIG. 3. Dendrogram based on Roger's distances between populations calculated from isozyme band frequencies. Population abbreviations are as in Figure 1. Mean achene morphologies, %A (hashed circles), and %C (dark circles) are plotted for each population, with the diameter of the circle being proportional to the percentage.

Variation in Climate and Vegetation

The spatial pattern of precipitation across the area of study varies as the growing season progresses. Over all the sites, only 3% of the annual precipitation occurs in the early spring months of March and April, while 92% occurs in the summer months of June, July, August, and September (over 20% in each month). During the early spring, precipitation is most abundant in the eastern part of the study area (Fig. 4a), whereas summer precipitation is higher in the southern and western parts of the study area (Fig. 4b). The sites that receive more precipitation in March and April receive a smaller total amount of precipitation over a broader range of months (Figs. 4c vs. 4d).

Grassland and Cactus Scrub vegetation dominate in the northern and eastern parts of the study region, pine-oak and tropical deciduous forest are more common in the south and east and subtropical scrub dominates in the west (Fig. 5).

The Association of Morphological and Environmental Variation

The Mantel partial matrix correlations, which remove the effects of geographic proximity, show that the percent of

achenes with awns is more similar for pairs of populations occurring in open vegetation types or closed vegetation types than for pairs of populations that differ in the openness of their vegetation types (Table 1). The percentage of awned achenes is greater in the sites with closed vegetation.

The pattern of variation in the percentage of awned achenes and the percentage of central achenes resembles the pattern of spring and summer precipitation (Fig. 6). When the effects of geographic proximity are held constant, among-site differences in summer precipitation are correlated with among-population differences in the percentage of awned achenes, but not with the percentage of central achenes (Table 1). Populations in sites with more summer precipitation have a greater proportion of achenes with awns.

The association of morphological variation with spring precipitation is somewhat different. Holding geographic distance statistically constant, among-population differences in the percentage of both central and awned achenes are significantly correlated with among-site differences in amount of spring precipitation (Table 1). Both percentages are lower at sites with greater precipitation in March and April. The Bonferroni significance level for the partial correlation of %C

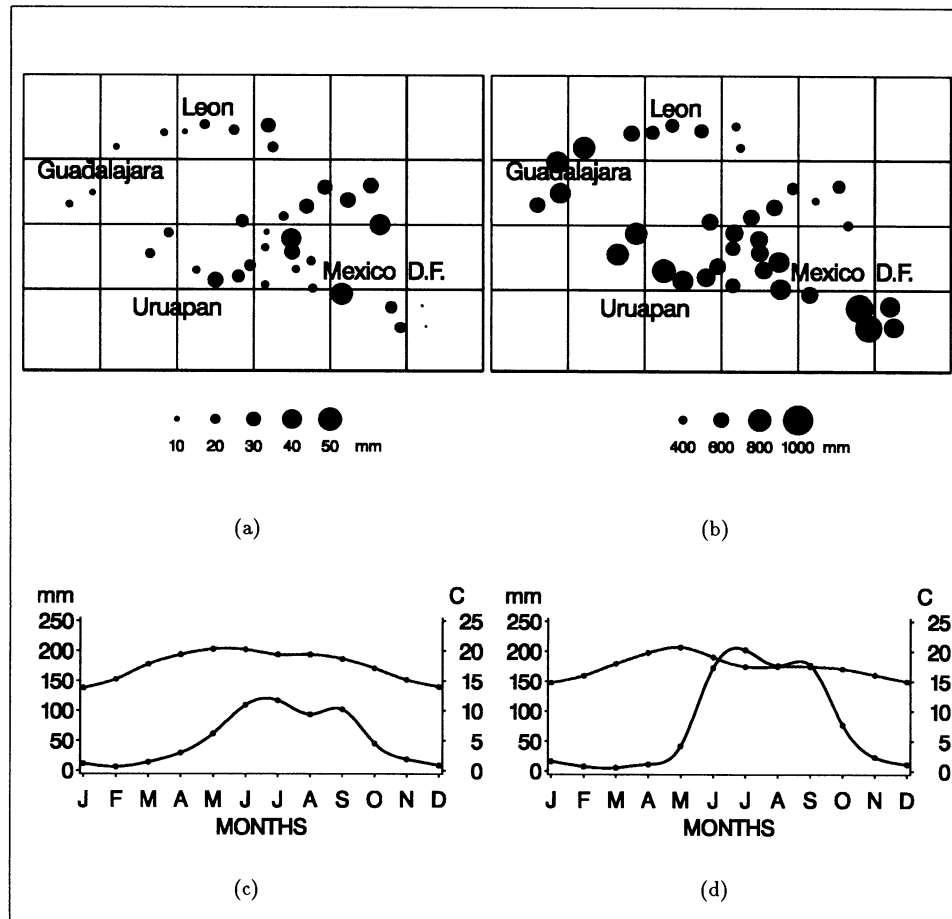


FIG. 4. Geographic pattern of precipitation across the sites: (a) mean sum of March and April precipitation; (b) mean sum of May, June, July, and August precipitation (the diameter of each circle is proportional to precipitation); (c) mean monthly precipitation and temperature at Tula, Hidalgo, a typical site with high spring precipitation; and (d) monthly precipitation and temperature at Zitácuaro, Michoacán, a typical site with high summer precipitation.

with spring precipitation is $P = 0.06$. However, since Bonferroni is an upper bound on the true, but unknown, confidence level, we will consider this a significant association, worthy of interpretation.

DISCUSSION

We hypothesized that natural selection would favor greater dispersibility, and thus higher %A, in more ephemeral populations. Previous studies have shown that *H. pinnatum* achenes with awns can disperse farther via adhesion to animals than those without (Venable et al. 1987) and that the proportion of achenes with awns (%A) has heritable variation, both within and among populations (Venable and Búrquez 1989). Models of dispersal suggest that increasing population ephemerality increases the fitness benefits of dispersal while increasing the risk of nondispersal (Levin et al. 1984; Cohen and Levin 1991; Venable and Brown 1993). *Heterosperma pinnatum* does not grow in the understory of closed vegetation, such as pine-oak forests, subtropical scrub, or tropical deciduous forests. Rather, it is found exclusively in disturbed, open patches in such areas (DLV, pers. obs.). In contrast, grasslands and cactus scrub in central Mexico con-

tain a prominent annual component, including *H. pinnatum*, which is not restricted to highly disturbed patches. The highly significant association of the percentage of awned achenes with open versus closed vegetation supports our prediction of higher %A in more ephemeral populations. We consider this a very robust test of this selective hypothesis, in part, because we have accounted for any spurious effects of geographic proximity (or genetic similarity) of populations. Also, there are prior experimental demonstrations of the dispersal function of awns and genetic variation in the proportion of achenes with awns.

The percent of achenes with awns is also positively correlated with summer precipitation and negatively correlated with spring precipitation. These patterns are probably due to the fact that higher summer and lower spring precipitation are found in sites with higher overall precipitation and taller, more closed canopies. Thus these patterns are likely to reflect the same phenomenon documented for vegetation types, that is, greater dispersibility in sites where vegetation is closed and *Heterosperma* populations are more ephemeral.

Kigel (1992) found a similar geographic pattern with seed heteromorphic *Hedypnois rhagadioloides*, which produces a

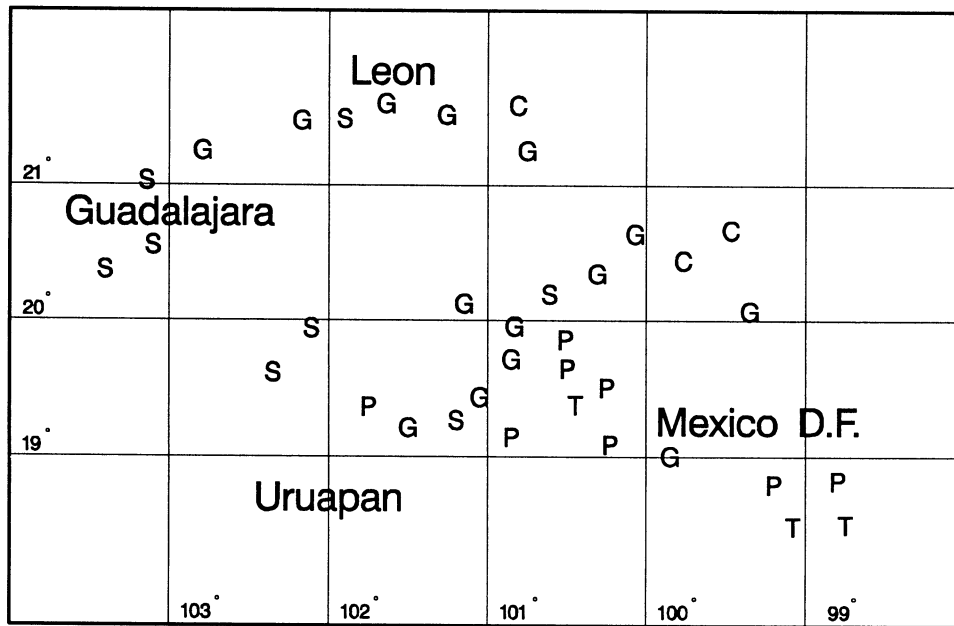


FIG. 5. The vegetation types are plotted for each population given in Figure 1. P, pine-oak forest; G, grassland; C, cactus scrub; S, subtropical scrub; T, tropical deciduous forest.

lower fraction of wind-dispersed, pappose achenes in more open, arid sites than in more closed, Mediterranean sites. These patterns are consistent with Ellner and Shmida's (1981, 1984) arguments about reduced dispersibility in arid habitats. Telenius (1992) found that more winged seeds of *Spergularia media* were produced in closed vegetation and more non-winged seeds were produced in open patches within the same population.

The percentage of central achenes produced in heads of *Heterosperma* is a good indicator of the distribution of achene shapes within heads as was shown by the principal component

TABLE 1. Associations of climate, vegetation, and achene morphology in *Heterosperma pinnatum*. Partial matrix correlations are used to remove geographic associations. Pairwise significance levels (in parentheses) were determined using a Mantel-type randomization procedure with 10,000 iterations. Partial correlations with pairwise $P < 0.0083$ have a Bonferroni global confidence level of at least $P < 0.05$ and are marked with asterisks. One correlation with Bonferroni $P < 0.06$ is marked with an asterisk in parentheses. Matrix correlations with geography are given below.

	Partial matrix correlations		
	Open vs. closed	Spring precipitation	Summer precipitation
%A	0.31* (0.0001)	0.28* (0.0006)	0.19* (0.008)
%C	0.08 (0.023)	0.22(*) (0.010)	-0.05 (0.310)
		Correlations with geography	
		<i>r</i>	<i>P</i>
%A		0.24	0.002
%C		0.37	0.002
Open vs. closed		0.04	0.180
Spring		0.17	0.010
Summer		0.24	0.004

analysis. Achene shape is correlated with the time of dormancy loss during the spring months (Venable et al. 1987). Thus it is interesting to find that %C is significantly associated with precipitation in March and April, but shows no significant relationships to either summer precipitation or open versus closed vegetation types. %C is lower in populations with more early spring precipitation. The fact that precipitation early in the germination season is the only factor correlated with %C suggests that the population differences in %C may have been influenced by selection operating through germination timing. Sites with greater spring precipitation have a more gradual onset of the rainy season and less total rain during the rainy season than sites with lower spring precipitation (cf. Figs. 3c,d). In sites with more early rains, the early rain may often be adequate to trigger germination yet not always sufficient to ensure survival to the reliable onset of the rainy season. Thus, the production of a lower percentage of the more readily germinating, central achenes may be advantageous in such sites. Besides remaining dormant longer and germinating later in the field, peripheral achenes have several other stricter germination requirements. They germinate under a narrower range of temperatures and have a stronger light requirement than central achenes (Venable et al. 1987).

A variety of other species have been shown to have ecotypic variation in germination response. Meyer et al. (1995a) demonstrated ecotypic variation in germination rate of the grass *Leymus cinereus* under simulated snow pack that correlated with the timing of snow melt at the sites of origin. *Penstemon* species and populations vary in germination rate, the production of seed banks, and the presence of dormancy in ways that appear to correlate adaptively with the environment in which they grow. For example, low- and midelevation species and populations appear to produce between-year seed

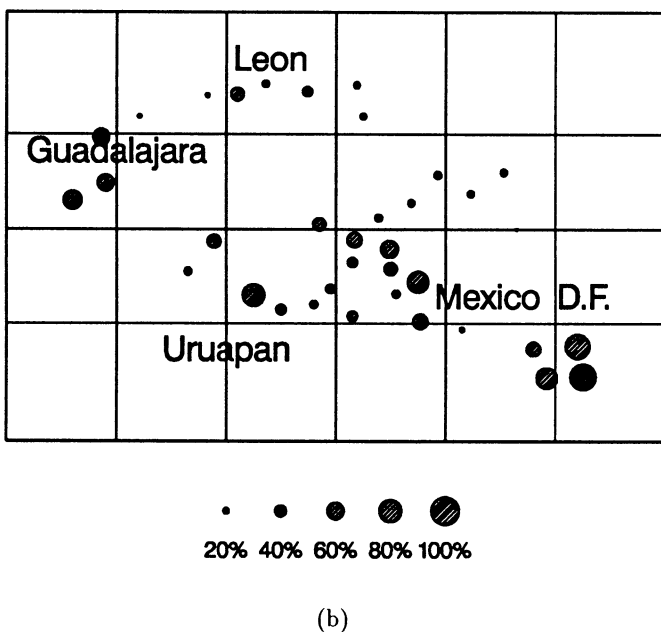
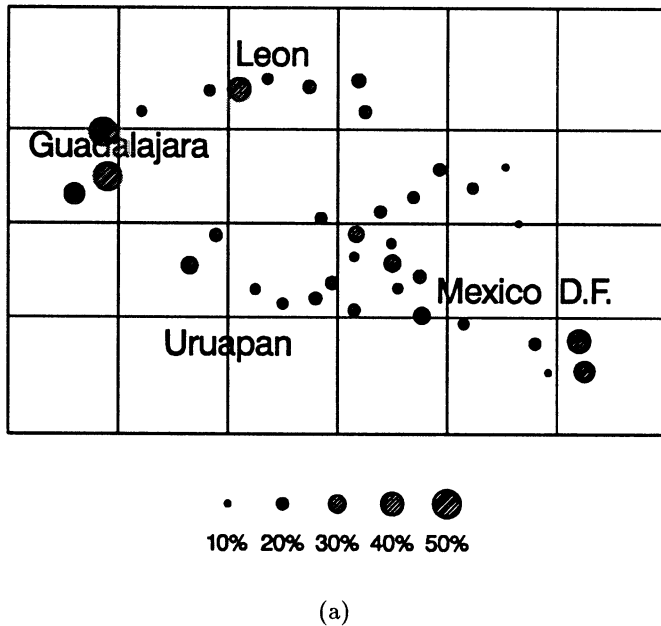


FIG. 6. The percentage of central (a) and awned (b) achenes is plotted for each population in the study area. Diameters of the circles are proportional to the relevant percentage.

banks and have variable growing conditions, while more predictable, high-elevation environments have some populations that apparently do not produce seed banks (Meyer 1992; Meyer and Kitchen 1994a; Meyer et al. 1995b). In *Linum perenne* high-elevation populations are spring germinating, whereas low-elevation populations germinate in spring and fall and have between-year carry over. These ecotypic differences were maintained in common garden experiments that also revealed among-sibship variation in germination, suggesting

within-population genetic variation (Meyer and Kitchen 1994b). A variety of other studies have also documented apparently adaptive ecotypic variation in germination or dormancy (Barclay et al. 1984; Meyer et al. 1990; Philippi 1993), yet none have controlled for the potentially confounding effects of geographic proximity or genetic relatedness of populations.

We wanted to consider the possible role of historical constraints on adaptive ecological patterns among populations. In comparative studies among species or higher taxa, methodologies exist for taking phylogeny into account (Harvey and Pagel 1991). We deemed these methods inappropriate for intraspecific comparisons in *H. pinnatum*, due to likely networks of colonization and gene flow characteristic of a cohesive species. Thus we chose to analyze the structure of genetic distances among populations and, if necessary, control for it via partial matrix correlations of morphology and the environment. The isozyme data indicate a lack of strong spatial structure of genetic distances between populations and a lack of correlation of achene morphology with genetic distances. This suggests that convergent achene and head morphologies are present in a mixture of genetically distinct populations. Thus, it is unlikely that the ecological associations documented here are due to confounding of historical constraints with adaptive patterns.

Previous studies have documented a strong genetic basis for among-population variation in %C and %A, indicating that we are indeed dealing with ecotypic variation in achene structure and function. The among-population distribution of isozyme genetic markers is not related to the distribution of morphological variation, probably due to recurrent migration combined with selection on morphology, but not isozymes. Such decoupling of isozymes and morphological traits has been found in some studies (e.g., Wolf and Soltis 1992; Borghetti et al. 1993). Others, such as that of seed morphology of *Avena barbata* or *Mimulus* have found correlations of morphology and isozymes (Vickery 1990; de la Vega et al. 1991).

Molecular markers and particularly isozymes have been shown to have a range of contrasting geographic structures in different species. Sometimes linear clines have been found, whereas in other cases geographic structure is related to multiple environmental factors (e.g., Beer et al. 1993). An additional group of studies shows no significant relationship to geography (e.g., Arevalo et al. 1994). We found no evidence for population subdivision into geographic races in *H. pinnatum*. This pattern is expected for neutral markers with recurrent migration. Achenes with awns are well adapted for dispersal (Venable et al. 1987), but little is known about the extent of dispersal among sites. Pollen dispersal among sites is perhaps less likely given that *H. pinnatum* readily sets selfed seeds without the aid of pollinators and has small, nonshowy flowers visited irregularly by small dipterans and hymenopterans not known for tremendous feats of migration (DLV, pers. obs.). The data presented here support the contention that one should not expect to find a significant correlation between neutral genetic markers and adaptive morphological traits, because while selection may be operating on the latter, it cannot determine the geographic patterns of the former.

In conclusion, this study of morphological, functional, environmental, genetic, and geographic variation in a seed heteromorphic species suggests some of the adaptive functions of seed traits and the role of natural selection in population differentiation.

ACKNOWLEDGMENTS

The authors thank N. Perez and E. Morales for help on this project. The research was partially funded by National Science Foundation grants BSR-8516971, DEB-9419905, and INT-9505941 and was written by DLV during his Fulbright Fellowship in Mexico.

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