EVOLUTION OF ARIZONA CHAMAEMYIIDAE (DIPTERA)

by

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I hereby recommend that this dissertation prepared under my direction by Thomas P. Sluss

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be accepted as fulfilling the dissertation requirement for the degree of Doctor of Philosophy

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As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

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SIGNED: Thomas P. Sluss

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ABSTRACT

Species of the acalyptrate Diptera family Chamaemyiidae are investigated for genetic variability using morphometric and electrophoretic techniques. Considerable genetic divergence has occurred within and between species. Geographic speciation is a probable evolutionary mechanism operating upon Arizona chamaemyiids. Discriminant analysis of morphometrical characters in the genus Chamaemyia correctly assigns 97% of the individuals to species. Mahalanobis D^2 estimates of morphological distance grouped C. aridella, C. polystigma, and C. herbarum. C. fumicosta is most widely separated. Species of Pseudodinia are not well discriminated morphologically (78%). Mahalanobis D^2 estimates paired P. nitens with P. sp. 2 and P. sp. 3 with P. sp. 4, placing P. varipes distant from either pair. The genus Leucopis is well discriminated (94%). Estimates of D^2 closely grouped L. ocellaris, L. sp. H, L. pemphigae, and L. bellula, all members of the "ocellaris" species group. Isozyme differences within the "ocellaris" group are greater than their phenotypic differences. The proportion of unshared isozymes between genera shows Leucopis to be the most derived (36% unique), Chamaemyia next (21%), and Pseudodinia least derived (10%). The proportion of shared

isozymes may be a measure of common ancestry. Chamaemyia and Leucopis share only 1%, Pseudodinia and Leucopis share 12%, and Pseudodinia and Chamaemyia share 10%.

Geographic variation in populations of C. herbarum are compared electrophoretically and morphometrically. Populations from isolated mountains in Arizona are compared with continuously distributed populations in the Colorado Rockies. Nei's genetic distance measurement, based upon seven enzyme loci, identifies distinct northern and southern races. A similar separation is seen with the discriminant analysis of morphometric data. Geographic isolation, small population size and inbreeding all tend to randomize isozyme frequencies; however, several patterns of variation were observed. Genetic distance estimates group southern populations according to Dice's biotic provinces. Regional patterns of phosphoglucomutase and adenylate kinase variation, between northern and southern populations, are reproduced microgeographically between meadow populations collected at increasing elevation in a single mountain range. Parallel patterns of isozyme variation are observed between P. varipes and C. herbarum populations. Malic acid dehydrogenase, fumarase, and phosphoglucomutase isozyme frequencies are correlated with elevation in both species. P. varipes populations in southern Arizona were examined to detect seasonal variation. Alpha-glycerophosphate

dehydrogenase isozyme frequencies change significantly between seasonal collections. Significant deviations from Hardy-Weinberg equilibrium suggest nonrandom mating between α-GPDH genotypes. Localized selection for regulatory glycolytic enzyme variation is hypothesized for alpine Diptera.

CHAPTER 1

INTRODUCTION

The acalyptrate Diptera family Chamaemyiidae comprises a relatively small group of flies whose larvae are predators of aphids, mealybugs, and scale insects. The family has received little attention from dipterists, probably because the flies are generally small with few distinguishable characteristics between species. This lack of easily discernible taxonomic characters prompted Aldrich (1914, p. 404) to state that, "specific characters of the genus Leucopis are so obscure and uncertain that one is almost inclined to doubt whether the half dozen species from North America are not really forms of the same one." Yet, Leucopis is the largest genus, with 20 described species and many still undescribed.

Nearctic members of the family are distributed among seven genera and total 38 described species, with many more undescribed. The family is classified into two subfamilies. Cremifaniinae has only one genus, Cremifania, and two described species, one of which was introduced from Europe (McAlpine 1963). The subfamily Chamaemyiinae is divided into two rather divergent evolutionary lines, the tribe Chamaemyiini, with five genera and 16 species; and the tribe

Leucopini, with one genus, Leucopis, which has four subgenera and 20 species. Malloch (1921) wrote the first comprehensive work on North American species and later wrote keys to Pseudoleucopis (1930), Plunomia and Pseudodinia (1940). McAlpine (1960, 1963) has published the most recent and useful keys to the North American subfamilies, tribes, genera, and subgenera. There are no published keys to the North American species of Chamaemyia nor of the largest genus, Leucopis, although 13 Nearctic species of the subgenus Neoleucopis are described by McAlpine (1971).

Morphology has been the basis of taxonomy or species recognition. Traditional methods are not applied easily to the similar appearing species of Chamaemyiidae. Ayala and Powell (1972) have shown that electrophoretic enzyme variants can be used as diagnostic characters between closely related sibling species of Drosophila, species that cannot be distinguished easily by external morphological differences. The electrophoresis of multiple genetic loci has become a useful taxonomic tool as well as an established means of analyzing the genetic variation of populations (for reviews see Wagner and Selander 1974, Avise 1974, Lewontin 1974, and Ayala 1976).

Discriminant analysis of morphological measurements (morphometrics) is useful in the classification of very similar groups (Blackith and Reyment 1971, Sneath and Sokal

1973, and Atchley and Bryant 1975). This technique has, for example, distinguished populations of honeybees (DuPraw 1965) and termites (Sands 1972). Multiple metric measurements can discriminate similar organisms or populations by means of discriminant functions. Discriminant functions are used for purposes of identification and might be thought of as the "modern" Adonsonian taxonomist's equivalent to the "traditional" taxonomist's key. Keys usually weigh certain characters and ignore most correlations between characters. The discriminant function is essentially a weighted average of a set of measurements, the weighting being computed so as to compensate for redundancy of information due to intercorrelations between measurements (Jolicoeur, 1959).

The family Chamaemyiidae is characterized by morphological uniformity. The purpose of this study is to determine whether this morphological conservatism is the result of genetic similarity. Populations and species are analyzed morphometrically on the basis of measurements of external features, and electrophoretically by determination of isozyme variation. Both of these techniques measure genetic variation. The morphological phenotype may be the result of a combination of genes interacting with environmental factors. Isozymes (Markert and Moller 1959) are the products of mutations, usually at single enzyme loci, which differ in net charge while retaining catalytic activity. The

value of these techniques in differentiating populations, discriminating species, and indicating relationships are compared.

Arizona is an excellent site from which to study Chamaemyiidae species relationships and mechanisms of speciation. Ten of the 38 described species of chamaemyiids in America North of Mexico are found in Arizona. In addition, 12 undescribed species have been collected. The state contains the distributional limits of northern, southern, and eastern faunas. The influx of diverse species and genotypes is molded by the region's insular topography, characterized in the southeastern part by desert-isolated mountain ranges. This study attempts to examine the first step toward speciation: the differentiation of populations.

CHAPTER 2

METHODS

All flies were from natural populations. Most were swept from grasses as adults and only two, Leucopis bellula Williston and L. sp. 64, were reared from larvae collected in the field. Specific collection localities are listed with each separate study. Usually, flies measured for the morphological analysis were killed in cyanide vials in the field and pinned within a few hours. Flies to be assayed electrophoretically were placed in an ice cooler and returned to the laboratory where they were sorted and placed in a freezer at -20°C. On longer collecting trips, flies were sorted in the field and immediately placed on dry ice at less than -20°C and kept frozen on dry ice until they could be placed in the laboratory freezer. This use of dry ice was very simple, effective, and prevented the loss of valuable specimens. The only disadvantage was that the specimens frequently lost antennae from vibration in transit and thus could not be used for morphological studies.

Morphometrics

Morphological measurements were taken from pinned specimens with a calibrated Bausch and Lomb Stereo Zoom® dissecting microscope equipped with a foot-pedal operated automatic focusing device (Electra-Focus®, Gomco Corp., Buffalo, N. Y.). Calibration was made at a magnification of 40.5 X using a Leitz stage micrometer and an ocular Whipple disc. Measurements at 40.5 X were made to the nearest 0.01 mm. Initially, 32 randomly chosen structures were measured from each of four species. Data were analyzed and the measurements which gave only "noise" and contributed nothing significant to the discriminant analysis were identified and dropped from further use. For the final morphological analyses, thirteen external morphological characters were used. These characters were as follows: ocellar triangle base, ocellar triangle side, eye length, eye height, cheek width, third antennal segment height, third antennal segment length, arista length, mesonotum length, mesonotum width, wing length, medial-cubital vein length, and radial-medial vein length.

Morphological data were analyzed with the BMD 07M Stepwise Discriminant Analysis computer program (Sampson 1973) run on a CDC 6400 computer. The program performs a multiple group discriminant analysis which provides classification functions that yield the probabilities of each sample

belonging to each preassigned group. Thus, a probability of correct identification is provided based upon morphological measurements. The procedure uses statistics of measured characters in each population as discriminating variables and weights them in order to force each population to be as distinct as possible. Specimens are sorted into groups one step at a time (stepwise) using one additional character at each step until the best possible discrimination is obtained.

Species relationships based upon morphological measurements are given by a distance measurement, Mahalanobis D^2 (Mahalanobis 1936). The D^2 statistic is a sum of squares of differences between corresponding mean values of two sets of weighted measurements. The D^2 statistic has become widely used because it is an actual measure of metric distance between population centroids (Atchley and Bryant 1975). The distance matrix was used to construct a dendrogram with a program called The Dendrograph (McCammon and Wenninger 1970). A listing of this program is given in Appendix A. The output is a two-dimensional diagram depicting the relationships among species by using pairwise distances. Construction of the dendrograph is based upon clustering using the unweighted pair-group method of Sneath and Sokal (1973).

Electrophoresis

Horizontal starch gel electrophoresis after Smithies (1955) was used in combination with histological staining techniques. Two types of hydrolyzed starch were used: 12% w/v Connaught® (Fisher Scientific Co., Chicago, Illinois) and 10% w/v Electrostarch® (Otto Hiller Electrostarch Co., Madison, Wisconsin). Gels were prepared by heating 400 ml. of starch suspension in a vacuum flask. The starch suspension was constantly swirled over a bunsen burner until it became viscous and translucent (near boiling). The flask of heated starch was evacuated to remove air bubbles and poured into a plexiglass mold. After cooling, the gel was wrapped with plastic wrap and stored overnight.

Flies were individually ground in a delrin plastic block with 0.05 ml. of distilled water. Each homogenate was adsorbed by a 4 X 9 mm. piece of Whatman® #1 filter paper. A paper was inserted into a slot made parallel to and 5 cm. from the edge of the gel mold. The gel was attached to the platinum wire electrode trays as shown in Figure 1. Gels were run at 120 volts by a power supply until the front had migrated anodally 12 cm. After the run was completed, the gel was cut horizontally with a fine piano wire into four slices 2 mm. thick. The top slice was discarded and each remaining slice was placed into a plastic box containing 100 ml. of a specific staining solution. The gels in

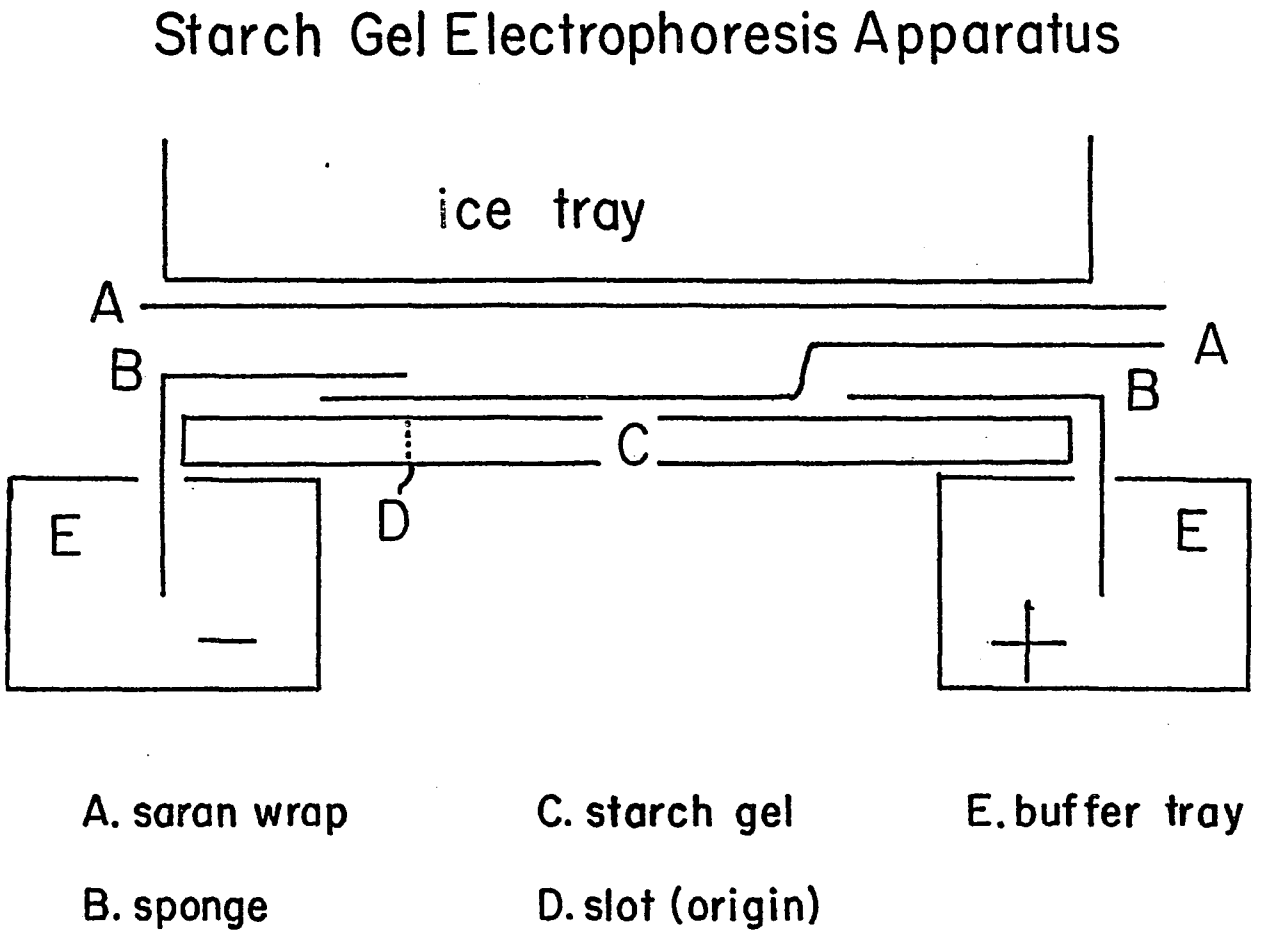

Figure 1. Starch Gel Electrophoresis Apparatus.

staining solutions were placed in an oven at 37°C and allowed to stain overnight. After staining, gels were fixed for five to eight hours in a 1:5:5 mixture of acetic acid, methanol, and water. The gels were wrapped in Saran Wrap® for storage. The composition of buffers and staining solutions are listed below. Additional procedural details can be found in Brewer (1970).

The three buffer systems used are from Ayala et al. (1972) and enzyme assays were modified from Shaw and Prasard (1970) and Brewer (1970).

Buffer system A: gel, 76 mM Tris (Tris(hydroxymethyl) aminomethane) and 5 mM citric acid, pH 8.6; electrode buffer, 300 mM boric acid and 60 mM NaOH (Poulik 1957).

Buffer system B: gel and electrode buffer, 87 mM EDTA (ethylenediamine tetraacetic acid), and 1 mM beta-NAD^+ (nicotinamide adenine dinucleotide), pH 9.0.

Buffer system C: gel buffer, 9 mM Tris, 3 mM citric acid, pH 7.0; electrode buffer, 135 mM Tris and 45 mM citric acid.

Enzyme assays were as follows:

Acid phosphatase (ACPH) (E. C. # 3.1.3.1,2) Electrostarch, buffer B, 6 hrs. at 24 v/cm. Stain: 100 mg. $MnCl_2$, 100 mg. $MgCl_2$, 100 mg. PVP (polyvinyl pyrrolidone), 300 mg. NaCl, 160 mg. Na-alpha-naphthyl acid phosphate, Fast Blue RR

salt; Buffer: 25 ml. 0.2 M tris acid maleate, 13 ml. 0.2 M NaOH, 62 ml. HOH, pH 5.0.

Malic acid dehydrogenase (MDH) (E. C. #1.1.1.37) Electrostarch, buffer B, 6 hrs. at 24 v/cm.; Stain: 20 mg. NBT (nitro blue tetrazolium), 25 mg. beta-NAD^+, 25 mg. malic acid, 1 mg. PMS (phenazine methosulfate); Buffer: 100 ml. 0.1 M tris-HCl, pH 8.4.

Alpha-glycerophosphate dehydrogenase (α-GPDH) (E. C. # 1.1.1.8) Electrostarch, buffer B, 6 hrs. at 24 v/cm.; Stain: 25 mg. beta-NAD^+, 20 mg. NBT, 180 mg. EDTA, 800 mg. alpha-glycerophosphate, 5 mg. PMS; Buffer: 100 ml. 0.1 M tris-HCl, pH 8.4.

Phosphoglucomutase (PGM) (E. C. # 2.7.5.1) Fisher starch, buffer C, 4 hrs. at 15 v/cm.; Stain: 600 mg. $MgCl_2$, 10 mg. $NADP^+$(nicotinamide adenine dinucleotide phosphate), 10 mg. MTT (thiazoyl blue), 80 units glucose-6-phosphate dehydrogenase, 1 mg. PMS (after $1\frac{1}{2}$ hrs. incubation); Buffer: 100 ml. 0.1 M tris-HCl, pH 8.4.

Adenylate kinase (AK) (E. C. # 2.7.4.3) Fisher starch, buffer C, 4 hrs. at 15 v/cm.; Stain: 90 mg. glucose, 20 mg. $MgCl_2$, 25 mg. $NADP^+$, 25 mg. ATP (adenosine-5'-triphosphate), 6 mg. MTT, 80 units glucose-6-phosphate dehydrogenase, 160 units hexokinase, 1 mg. PMS (after $1\frac{1}{2}$ hrs. incubation); Buffer: 100 ml. 0.1 M tris-HCl, pH 8.4.

Hexokinase (HEX)(E.C.#2.7.1.1) Fisher starch,buffer C,4hrs. at 15 v/cm.; Stain: 90 mg. glucose,20 mg. $MgCl_2$,25 mg.ATP,25mg. $NADP^+$,6 mg. MTT, 80 u. glucose-6-PO_4 dehydrogenase, 1 mg.PMS; Buffer: 100 ml. 0.1 M tris-HCl, pH 8.4.

Fumarase (FUM) (E. C. # 4.2.1.2) Fisher starch, buffer A, 4 hrs. at 20 v/cm.; Stain: 50 mg. NAD^+, 20 mg. NBT, 200 mg. fumaric acid, 200 units malic dehydrogenase, 1 mg. PMS (after $1\frac{1}{2}$ hrs. incubation); Buffer: 100 ml. 0.1 M tris-HCL, pH 8.4.

The mechanism of inheritance of most of these enzyme loci is reviewed for Drosophila by Dickinson and Sullivan (1975). In this dissertation, enzyme data are reported as genotypes but the genetic interpretation is inferred. Although the genetic basis of isozyme variation in flies is usually easily demonstrated by mating tests, attempted breeding experiments with parasitoid chamaemyiid species have not been successful. All systems are consistent with patterns of genetic variation such as the presence or absence of hybrid bands as observed in other Diptera.

After staining, isozymes are detected on the starch gel as discrete bands which have migrated a specific distance from the origin. If more than one system of bands (enzyme locus) appears on the gel, the group of bands which migrates farthest is designated A. In each system (locus), the most negatively charged enzyme band (allele) which

migrates farthest is designated 1. The isozymes are numbered in decreasing distance from the origin. In population comparisons, individuals from different populations are placed together on each gel and the mobility position of each isozyme is determined relative to the most common band. For the species comparison, flies of different species were assayed together in the same gel to eliminate differences due to gels, buffers, voltage, or other variables. Whenever slight mobility differences were observed, flies from the two species concerned were assayed in a gel side by side.

Nei's standard genetic distance estimates (Nei and Roychoudhury 1974) were used to compare isozyme frequencies of populations and species within genera. The Edwards and Cavalli-Sforza (1972) E^2 statistic was used to determine genetic distances between genera. A complete listing of the two computer programs is given in Appendix A. Each genetic distance matrix was used to construct a dendrogram by the method of McCammon and Wenninger (1970).

CHAPTER 3

SPECIES COMPARISONS

Both morphological and isozyme differences are used to determine species relationships. Discriminant analysis provides identification and classification analyses and the Mahalanobis D^2 statistic provides a single direct measure of divergence between any two groups. Distance estimates of Nei or Edwards and Cavalli-Sforza provide a measure of differences between any two groups. These distance estimates can be used in phenetic methods of analysis.

For morphometric analysis, 12 specimens were measured for each species except for C. aridella (Fallen) (8 specimens). Equal numbers of females and males were used. The number of individuals assayed electrophoretically varied with each species. Sufficient numbers of live flies were not available to determine species relationships based upon isozyme frequencies within the genera Chamaemyia and Pseudodinia. An electrophoretic analysis of enzyme variability was conducted between species of Leucopis. Genetic distances between genera were determined from both morphological and electrophoretic data.

Chamaemyia

Specimens of Chamaemyia herbarum (Robineau- Desvoidy) and C. fumicosta Malloch were collected in Arizona. C. aridella (Fallen), C. geniculata (Zetterstedt), and C. paludosa Collin were collected in Alaska, and C. polystigma Meigen came from Ohio. The mean and standard deviation for the 13 characters used throughout the study are given in Appendix Table B 1. The morphometric classification of Chamaemyia species is given in Table 1. An excellent 97% discrimination of species was accomplished: only two were misclassified, one C. polystigma as C. geniculata and one C. geniculata as C. aridella. Although 13 characters were analyzed, five characters accounted for 97% of the species discrimination. These characters and their U-statistic, a measure of each character's discriminating power, are as follows: mesonotum length (0.094), cheek width (0.031), third antennal segment height (0.014), eye length (0.008), and ocellar triangle base (0.007).

Mahalanobis D^2 estimates of morphological distance (Table 2) are plotted in the dendrograph shown in Figure 2. The distance between species within a group is given on the horizontal axis. The distance between groups is measured on the vertical axis. C. fumicosta is widely separated from the rest of the species reflecting its larger size (e.g., $\bar{x}$ mesonotum length of C. fumicosta = 1.36 mm. vs. C. herbarum

Table 1. Number of Individuals Classified into *Chamaemyia* Species based upon Morphological Data.

Identifications: ARID,*aridella*; POLY,*polystigma*; HERB,*herbarum*; FUMI,*fumicosta*; GENI,*geniculata*; PALU,*paludosa*.

	ARID	POLY	HERB	FUMI	GENI	PALU
ARID	8					
POLY		11			1	
HERB			12			
FUMI				12		
GENI	1				11	
PALU						12

Table 2. Mahalanobis D^2 Distance between Species of *Chamaemyia*.

Identifications: ARID,*aridella*; POLY,*polystigma*; HERB,*herbarum*; FUMI,*fumicosta*; GENI,*geniculata*; PALU,*paludosa*.

	ARID	POLY	HERB	FUMI	GENI	PALU
ARID	0.000					
POLY	8.847	0.000				
HERB	8.996	11.990	0.000			
FUMI	77.795	63.099	72.753	0.000		
GENI	12.146	17.434	17.648	129.014	0.000	
PALU	15.990	28.523	47.000	102.052	32.377	0.000

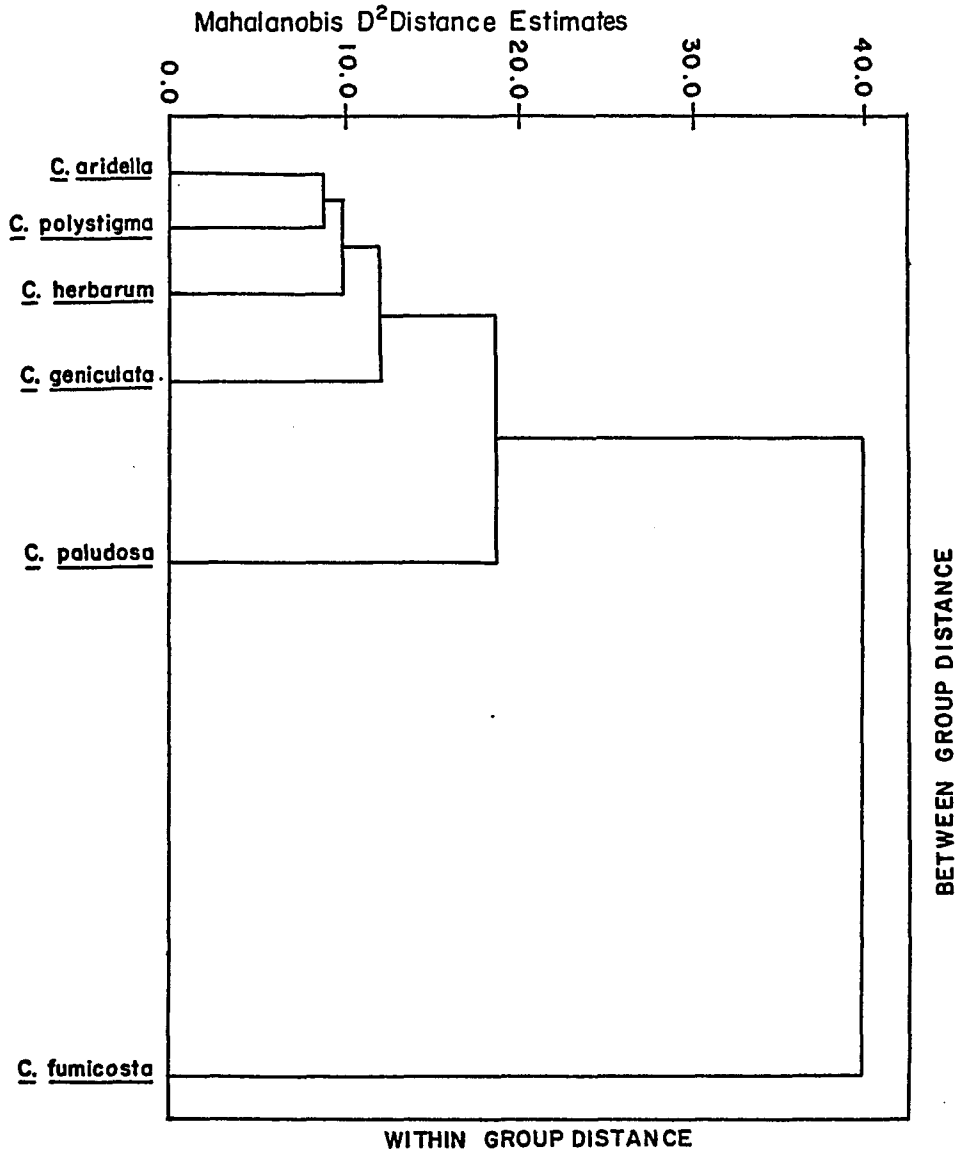

Figure 2. Mahalanobis D^2 Distance Estimates between Species of Chamaemyia.

$\bar{X}$ = 0.88 mm.). Chamaemyia paludosa is intermediate between C. fumicosta and the remaining four species, which form a tighter group. Chamaemyia aridella and C. polystigma are paired, and C. herbarum and C. geniculata are nearly equally distant from each other and from the former pair.

Pseudodinia

All species of Pseudodinia were collected from Arizona. The results of the discrimination analysis of morphological measurements are shown in Table 3. The mean and standard deviation for the 13 characters are given in Appendix Table B 2. Seven characters were used before 78% of the specimens were discriminated. The U-statistics for the seven characters are as follows: eye length (0.650), mesonotum length (0.403), third antennal segment height (0.274), ocellar triangle side (0.173), medial-cubital vein length (0.124), arista length (0.100), and ocellar triangle base (0.080). Each species had at least one (PS2) or two (varipes Coquillett, PS3, and PS4) specimens misclassified. Pseudodinia nitens (Melander and Spuler), with six specimens misclassified, does not form a morphologically well defined species and accounted for nearly half of those misclassified.

Mahalanobis D^2 estimates (Table 4) were used to form the dendrograph of Pseudodinia spp. (Fig. 3). Surprisingly, although P. nitens and P. varipes are difficult to separate morphologically, P. nitens is shown most distant from

Table 3. Number of Individuals Classified into *Pseudodinia* Species based upon Morphological Data.

Identifications: NITS,*nitens*; VARI,*varipes*; PS 2,*P.* sp.2; PS 3,*P.* sp.3; PS 4,*P.* sp. 4.

	NITS	VARI	PS 2	PS 3	PS 4
NITS	6		3	2	1
VARI	1	10			1
PS 2	1		11		
PS 3	1			10	1
PS 4	2				10

Table 4. Mahalanobis D^2 Distance between Species of *Pseudodinia*.

Identifications: NITS,*nitens*; VARI,*varipes*; PS 2,*P.* sp.2; PS 3,*P.* sp.3; PS 4,*P.* sp.4.

	NITS	VARI	PS 2	PS 3	PS 4
NITS	0.000				
VARI	9.545	0.000			
PS 2	3.798	18.441	0.000		
PS 3	6.849	13.629	17.993	0.000	
PS 4	5.001	13.852	12.281	4.694	0.000

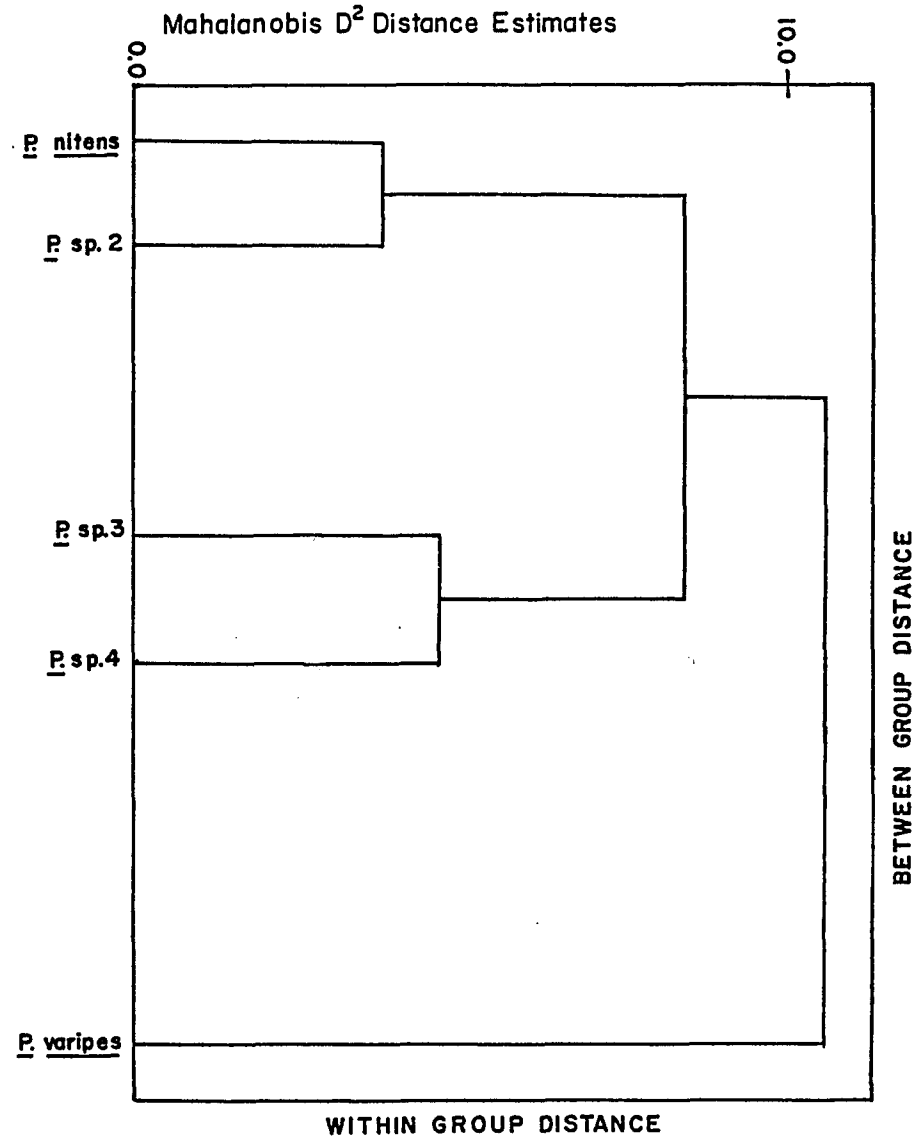

Figure 3. Mahalanobis D^2 Distance Estimates between Species of Pseudodinia.

P. varipes. The characters used in keys, however, involve pruinosity and coloration; none of the metric characters listed above is used. The electrophoretic data (Tables 7 to 12,pp.#25 to 30) also confirmed this separation of P. nitens and P. varipes. Pseudodinia sp. 3 and PS4 are shown together, intermediate between P. nitens, PS2, and P.varipes.

Leucopis

All seven Leucopis species were collected from Arizona except L. pinicola Malloch and L. pemphigae Malloch, both from Ohio. The mean and standard deviations for 13 morphological characters are given in Appendix Table B3. The discrimination of Leucopis spp. was good (Table 5); 94% of the specimens were assigned to the correct species. Two species had specimens misclassified: L. bellula Williston with two as L. ocellaris Malloch, and L. ocellaris with one as L. bellula and two as sp. 64. The four characters providing 94% of the species discrimination and their U-statistics are as follows: ocellar triangle base (0.160), cheek width (0.051), third antennal segment length (0.200), and mesonotum length (0.014). Figure 4 plots the Mahalanobis D^2 morphological distance estimates (Table 6) between species. Little is known taxonomically of relationships within the genus, but the D^2 estimates do reflect some of the known associations. L. ocellaris, L. sp. 64, and L. pemphigae are three members of McAlpine's "ocellaris species group", a

Table 5. Number of Individuals Classified into *Leucopis* Species based upon Morphological Data.

Identifications: BELL, *bellula*; L 82, *L.* sp. 82; BIVT, *bivittata*; OCEL, *ocellaris*; L H, *L.* sp. H; PEMP, *pemphigae*; PINI, *pinicola*.

	BELL	L 82	BIVT	OCEL	L H	PEMP	PINI
BELL	10			2			
L 82		12					
BIVT			12				
OCEL	1			9	2		
L H					12		
PEMP						12	
PINI							12

Table 6. Mahalanobis D^2 Distance between Species of *Leucopis*.

Identifications: BELL, *bellula*; L 82, *L.* sp. 82; BIVT, *bivittata*; OCEL, *ocellaris*; L H, *L.* sp. H; PEMP, *pemphigae*; PINI, *pinicola*.

	BELL	L 82	BIVT	OCEL	L H	PEMP	PINI
BELL	0.000						
L 82	42.524	0.000					
BIVT	18.468	18.835	0.000				
OCEL	5.461	33.945	22.687	0.000			
L H	12.833	47.342	33.048	5.342	0.000		
PEMP	19.751	41.947	39.194	7.855	7.002	0.000	
PINI	28.206	103.530	68.411	25.660	17.551	25.961	0.000

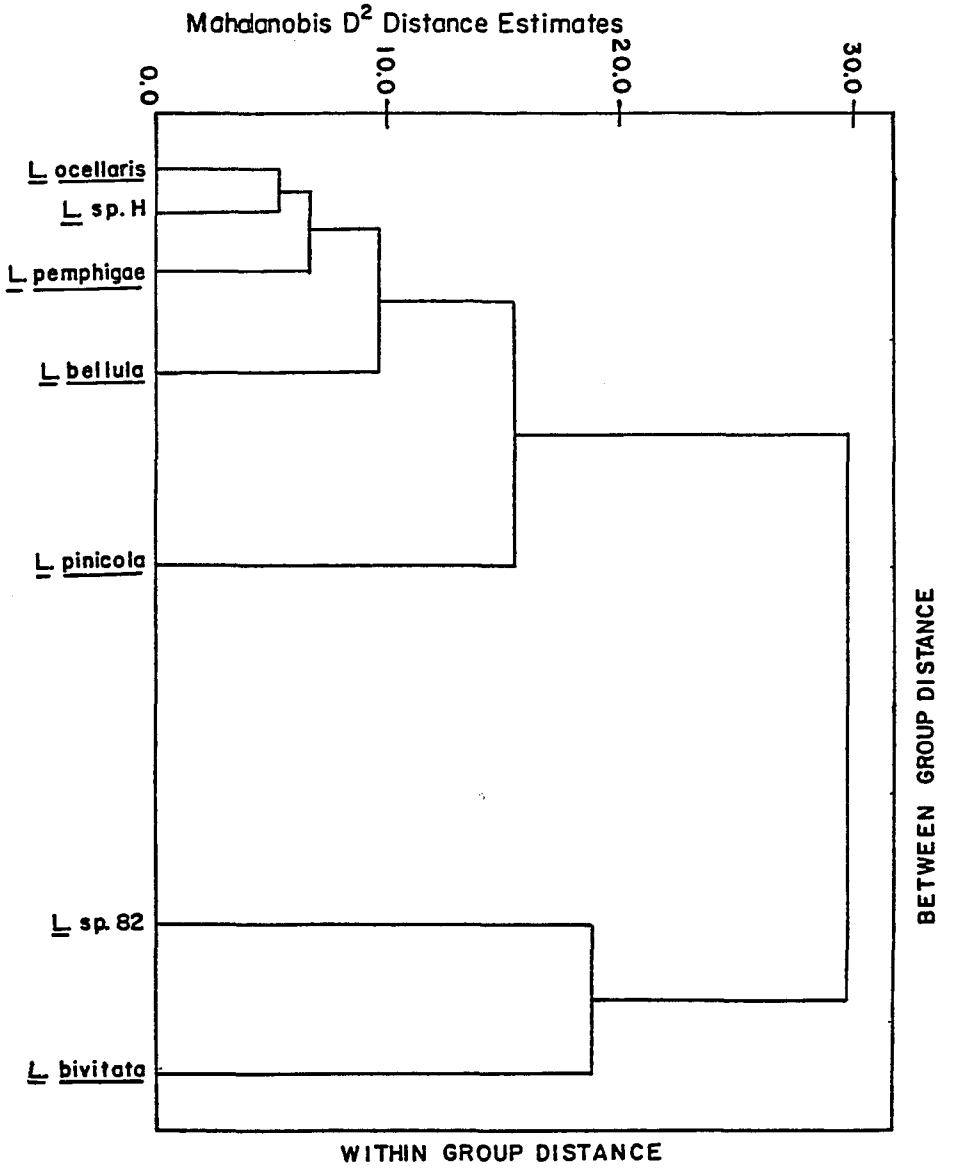

Figure 4. Mahalanobis D^2 Distance Estimates between Species of Leucopis.

relationship that is reflected in their position in the dendrograph. Leucopis pinicola is the only member of the subgenus Neoleucopis studied, and it is not plotted close to any of the other species. L. sp. 82 and L. bivittata Malloch are morphologically the largest species and on this basis are set apart.

Five species of Leucopis were assayed electrophoretically to detect isozyme variation for six enzymes. The same frequencies used for within-genus comparisons were used for between-genera comparisons and are included in Tables 7 to 12. Nei's genetic distances are given in Table 13. The dendrogram based upon Nei's genetic distance estimates is shown in Figure 5. Leucopis sp. E and L. sp. H are closely related, but L. ocellaris, L. sp. 82, and L. bellula are nearly equidistant from each other. The ocellaris group may be morphometrically similar but has diverged at the molecular enzyme level.

Between Genera

The discriminant analysis of species from each of the three genera was conducted to determine if morphometrics can accurately compare different genera. Table 14 shows the results of this analysis. All species were assigned to the correct genus and 82.4% of the specimens were assigned to the correct species. This indicates that discrimination can be obtained between genera. Those misclassified were two

Table 7. Alpha-glycerophosphate Dehydrogenase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Species	Relative Mobility Position of Isozymes 1.2	1.1	1.0	.9	.8	Total Assayed
Chamaemyia						
herbarum	.996	.004				373
fumicosta	1.000					11
juncorum		1.000				9
Pseudodinia						
varipes			.262	.738		227
nitens			.941	.059		59
sp. 3			1.000			10
Leucopis						
ocellaris				1.000		3
bellula					1.000	2
sp. 82			.600	.400		10
sp. E				1.000		8
sp. H				1.000		4

Table 8. Adenylate Kinase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Identifications: HERB, herbarum; FUMI, fumicosta; JUNC, juncorum; VARI, varipes; NITS, nitens; PS 3, P. sp. 3; OCEL, ocellaris; BELL, bellula; L 82, L. sp. 82; L H, L. sp. H; L E, L. sp. E.

	Relative Mobility Positions of Isozymes													To-
Species	3.8	3.7	3.5	3.4	3.3	3.2	3.1	3.0	2.9	2.7	2.5	2.4	1.9	1.5	tal
Chamaemyia															
HERB	.008		.236			.271			.489						375
FUMI			1.000												11
JUNC					1.000										7
Pseudodinia															
VARI		.025	.517	.444		.011		.003							180
NITS		.029	.943	.029											35
PS 3		.055	.944												9
Leucopis															
OCEL						.500				.500					1
BELL												.500	.500		1
L 82							.500	.200				.100	.150	.050	10
L E								.417			.583				6
L H								1.000							4

Table 9. Acid Phosphatase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Identifications: HERB, herbarum; FUMI, fumicosta; JUNC, juncorum; VARI, varipes; NITS, nitens; PS 3, P. sp. 3; OCEL, ocellaris; BELL, bellula; L 82, L. sp. 82; L H, L. sp. H; L E, L. sp. E.

Species	Relative Mobility Positions of Isozymes 1.6	1.5	1.4	1.3	1.2	1.1	1.0	.9	.7	.6	.5	.4	.3	Total
Chamaemyia														
HERB									.028	.297	.484	.124	.067	545
FUMI													1.000	19
JUNC					.300		.600	.100						5
Pseudodinia														
VARI				.375			.625							8
NITS		.083		.083	.833									6
PS 3					.500	.300		.200						5
Leucopis														
OCEL							1.000							1
BELL					1.000									2
L 82	.143		.143			.714								7
L E		.600		.400										5
L H		.500		.500										2

Table 10. Hexokinase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Identifications: HERB, herbarum; FUMI, fumicosta; JUNC, juncorum; VARI, varipes; NITS, nitens; PS 3, P. sp. 3; OCEL, ocellaris; BELL, bellula; L 82, L. sp. 82; L H, L. sp. H; L E, L. sp. E.

Species	Relative Mobility Positions of Isozymes 3.6	3.4	3.2	3.1	3.0	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	1.9	Total
Chamaemyia															
HERB								.074			.548		.374	.004	426
FUMI								.647			.265		.088		17
JUNC									.889		.111				9
Pseudodinia															
VARI					.092				.822	.080		.006			163
NITS					.107				.881	.012					42
PS 3							.111		.778						9
Leucopis															
OCEL		.176		.519	.269	.038									26
BELL						.917		.083							6
L 82	.250	.525	.225												20
L E						.647	.353								17
L H						1.000									

Table 11. Malic Acid Dehydrogenase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Identifications: HERB, herbarum; FUMI, fumicosta; JUNC, juncorum; VARI, varipes; NITS, nitens; PS 3, P. sp. 3; OCEL, ocellaris; BELL, bellula; L 82, L.sp. 82; L H, L. sp. H; L E, L. sp. E.

Species	Relative Mobility Position of Isozymes											Total Assayed
	3.4	3.2	3.1	2.9	2.7	2.6	2.4	2.2	2.1	1.9	1.7	1.5	
Chamaemyia													
HERB		.063		.365		.502		.070					630
FUMI				.932				.068					22
JUNC		.038		.269		.269		.423					13
Pseudodinia													
VARI						.037		.188		.370	.397	.008	189
NITS				.033		.196		.413		.326	.033		46
PS 3								.600		.400			
Leucopis													
OCEL			.227	.523			.205		.045				22
BELL				1.000									2
L 82			.158	.368	.053		.289		.132				19
L E	.227		.682	.091									11
L H	.500		.500										4

Table 12. Phosphoglucomutase Isozyme Frequencies of Chamaemyiidae Used for Species Comparisons.

Identifications: HERB, herbarum; FUMI, fumicosta; JUNC, juncorum; VARI, varipes; NITS, nitens; PS 3, P. sp. 3; OCEL, ocellaris; BELL, bellula; L 82, L. sp. 82; L H, L. sp. H; L E, L. sp. E.

Species	Relative Mobility Positions of Isozymes 2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.0	.9	.8	.7	.6	.5	.3	Total
Chamaemyia																	
HERB				.038		.344		.423		.156		.031		.005			435
FUMI								.071				.286		.500		.143	7
JUNC						.167		.833									6
Pseudodinia																	
VARI				.063		.722		.164		.044		.007					216
NITS				.065		.250		.352		.296		.037					54
PS 3						.045	.364	.500		.091							11
Leucopis																	
OCEL		.077				.115		.673		.135							26
BELL	.083		.792		.083	.042											12
L 82		.262			.310			.071					.333		.024		21
L E		.125					.563			.313							8
L H									.500		.500						5

Table 13. Nei's Genetic Distance Estimates between Species of *Leucopis*.

Identifications: OCEL, *ocellaris*; BELL, *bellula*; L 82, *L.* sp. 82; L E, *L.* sp. E; L H, *L.* sp. H.

	OCEL	BELL	L 82	L E	L H
OCEL	0.000				
BELL	2.038	0.000			
L 82	1.240	1.883	0.000		
L E	1.044	1.814	1.473	0.000	
L H	1.270	1.643	1.562	0.278	0.000

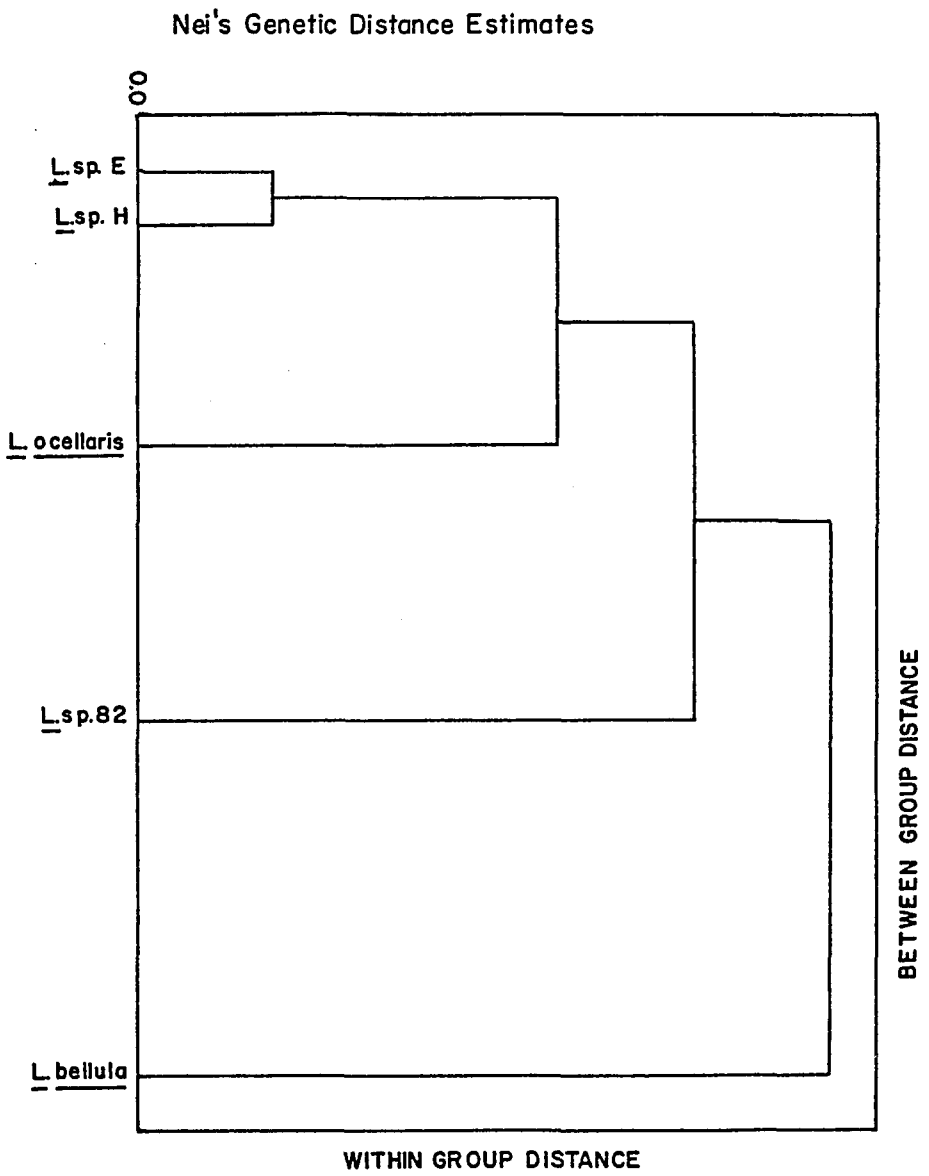

Figure 5. Nei's Genetic Distance Estimates between Species of Leucopis.

Table 14. Number of Individuals Classified into Species of Chamaemyiidae based upon Morphological Data.

Identifications: BELL,*bellula*; OCEL,*ocellaris*; L H,*L.* sp. H; L 82,*L.* sp. 82; VARI,*varipes*; NITS,*nitens*; PS 3,*P.* sp. 3; FUMI, *fumicosta*; HERB,*herbarum*.

	BELL	OCEL	L H	L 82	VARI	NITS	PS 3	FUMI	HERB
BELL	10	1	1						
OCEL	3	6	3						
L H			12						
L 82				12					
VARI					9	3			
NITS					1	7	4		
PS 3						3	9		
FUMI								12	
HERB									12

specimens in the ocellaris group of Leucopis and 11 specimens in the genus Pseudodinia. Nine characters were used to achieve the 82.4% discrimination, those characters and their U-statistics are as follows: mesonotum length (0.141), arista length (0.033), cheek width (0.013), ocellar triangle base (0.009), ocellar triangle side (0.005), third antennal segment height (0.004), eye length (0.003), medial-cubital vein length (0.002), third antennal segment length (0.002).

Figure 6 plots the D^2 estimates (Table 15) for these species. The grouping agrees very well with established genera. P. sp. 3 and P. nitens are very closely grouped with P. varipes near the pair. C. fumicosta, as expected from its enormous size, is morphologically distinct from any of the other species. Excluding this species, the genera Chamaemyia and Pseudodinia are more closely related to each other than they are to Leucopis. This agrees with the tribal concepts of Chamaemyiini (including the former two genera) and Leucopiini (Leucopis). Three species of the "ocellaris" species group, L. sp. H, L. ocellaris, and L. bellula, are closely related. Leucopis sp. 82, a fourth member of the group, is widely separated from the others due to its larger size. Thus, the distance estimates based upon morphological data agree very well with established relationships at the generic level.

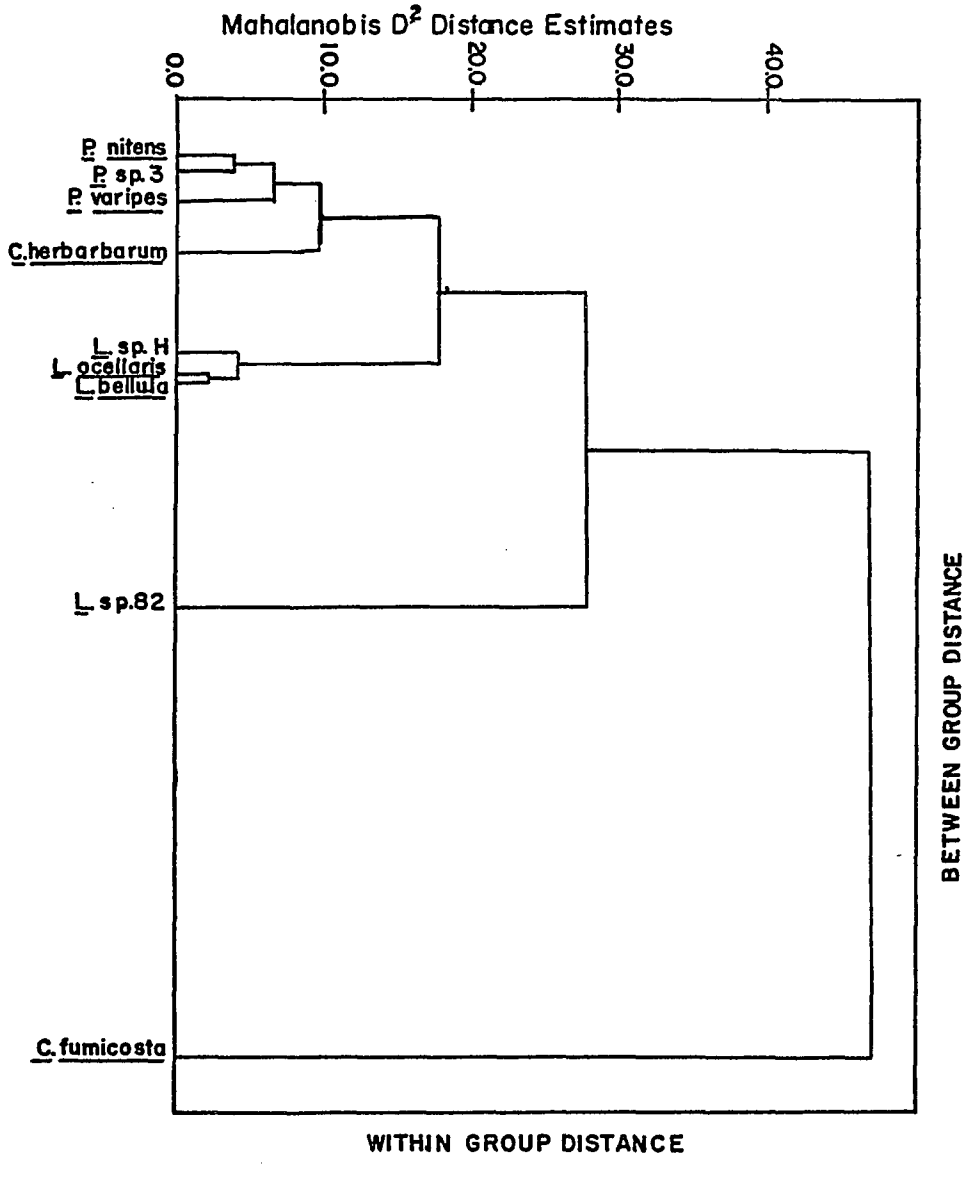

Figure 6. Mahalanobis D^2 Distance Estimates between Species of Chamaemyiidae.

Table 15. Mahalanobis D^2 Distance between Species of Chamaemyiidae.

Identifications: BELL,*bellula*; OCEL,*ocellaris*; L H,*L.* sp. H; L 82,*L.* sp. 82; VARI,*varipes*; NITS,*nitens*; PS 3,*P.* sp. 3; FUMI,*fumicosta*; HERB,*herbarum*.

	BELL	OCEL	L H	L 82	VARI	NITS	PS 3	FUMI	HERB
BELL	0.000								
OCEL	2.285	0.000							
L H	6.256	4.222	0.000						
L 82	36.333	29.337	39.269	0.000					
VARI	31.755	29.004	25.244	88.657	0.000				
NITS	22.432	19.442	14.239	73.817	5.533	0.000			
PS 3	39.343	33.333	28.846	89.865	10.294	3.930	0.000		
FUMI	136.845	123.684	119.596	94.680	139.843	115.860	108.848	0.000	
HERB	25.543	18.196	16.263	46.464	15.910	10.463	12.679	79.849	0.000

After establishing that there are significant morphometric differences between groups, it may be interesting to identify those characters which were most important in separating groups. Table 16 summarizes the characters which were most useful in discriminating populations and species. The importance of characters varies depending upon the genus; but in the three genera compared, the five most important characters always included mesonotum length, ocellar triangle base or side, and third antennal segment length or height.

The same species, with the addition of C. juncorum and L. sp. E, were also compared electrophoretically. Tables 7 to 12 give the isozyme frequencies of ∝-GPDH, ACPH, MDH, AK, HEX, and PGM used in the comparison. Figures 7 to 9 show mobility positions for each genus. The D^2 standard genetic distance estimate of Nei is not suitable for comparing greatly different groups (Nei and Roychoudhury 1974), such as genera of flies. The Edwards and Cavalli-Sforza E^2 statistic may be more appropriate when comparing species that do not share alleles at one or more loci. This statistic was used to compare chamaemyiid isozyme frequencies between genera. Eleven species in three genera were compared for six enzyme loci and the E^2 matrix of genetic distances is given in Table 17. The dendrograph (Figure 10) plots the species according to E^2 estimates. The species

Table 16. A Comparison of the Order of Use of Morphological Characters in Discriminating Chamaemyiid Populations and Species.

	C. herbarum Populations	Chamaemyia	Leucopis	Pseudodinia	Between Genera
1.	eye length	mesonotum length	ocellar triangle base	eye length	mesonotum length
2.	mesonotum length	cheek width	cheek width	mesonotum length	arista length
3.	cheek width	third antennal segment length	third antennal segment length	third antennal segment length	cheek width
4.	medial-cubital vein length	eye length	mesonotum length	ocellar triangle side	third antennal segment length
5.	radial-medial vein length	ocellar triangle base	medial-cubital vein length	medial-cubital vein length	eye length

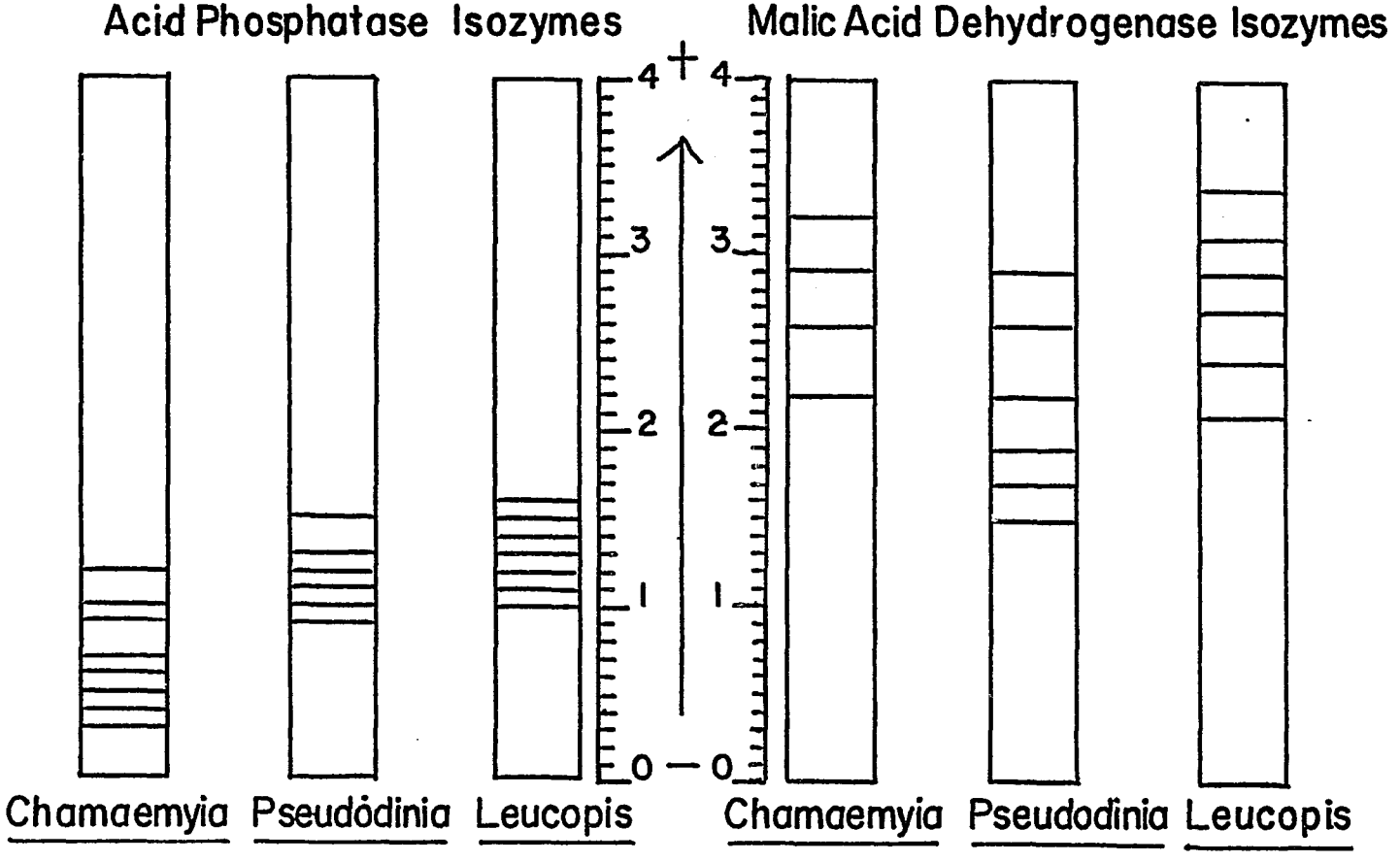

Figure 7. Mobility Positions of Acid Phosphatase and Malic Acid Dehydrogenase Isozymes for Each Genus.

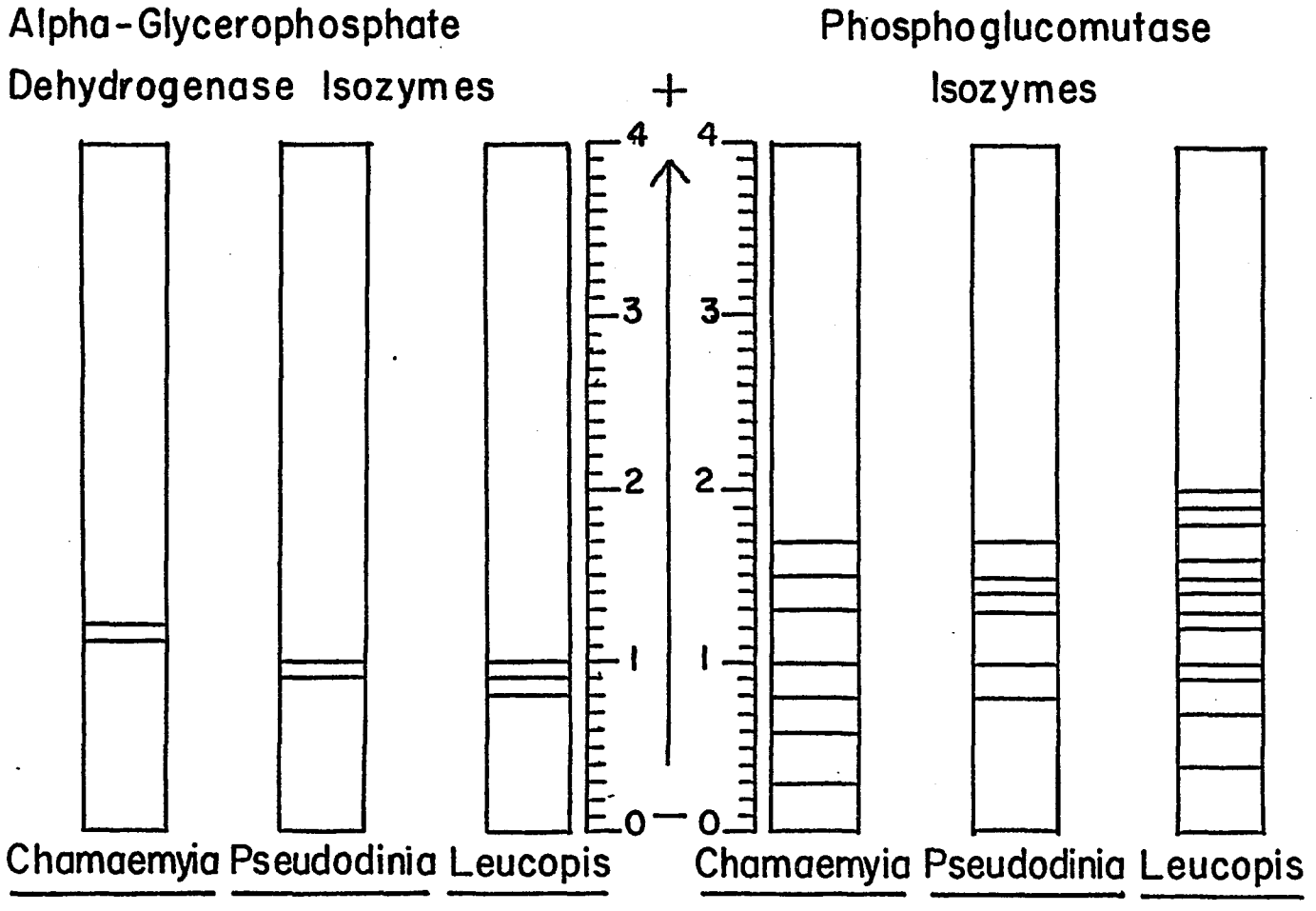

Figure 8. Mobility Positions of Alpha-glycerophosphate Dehydrogenase and Phosphoglucomutase Isozymes for Each Genus.

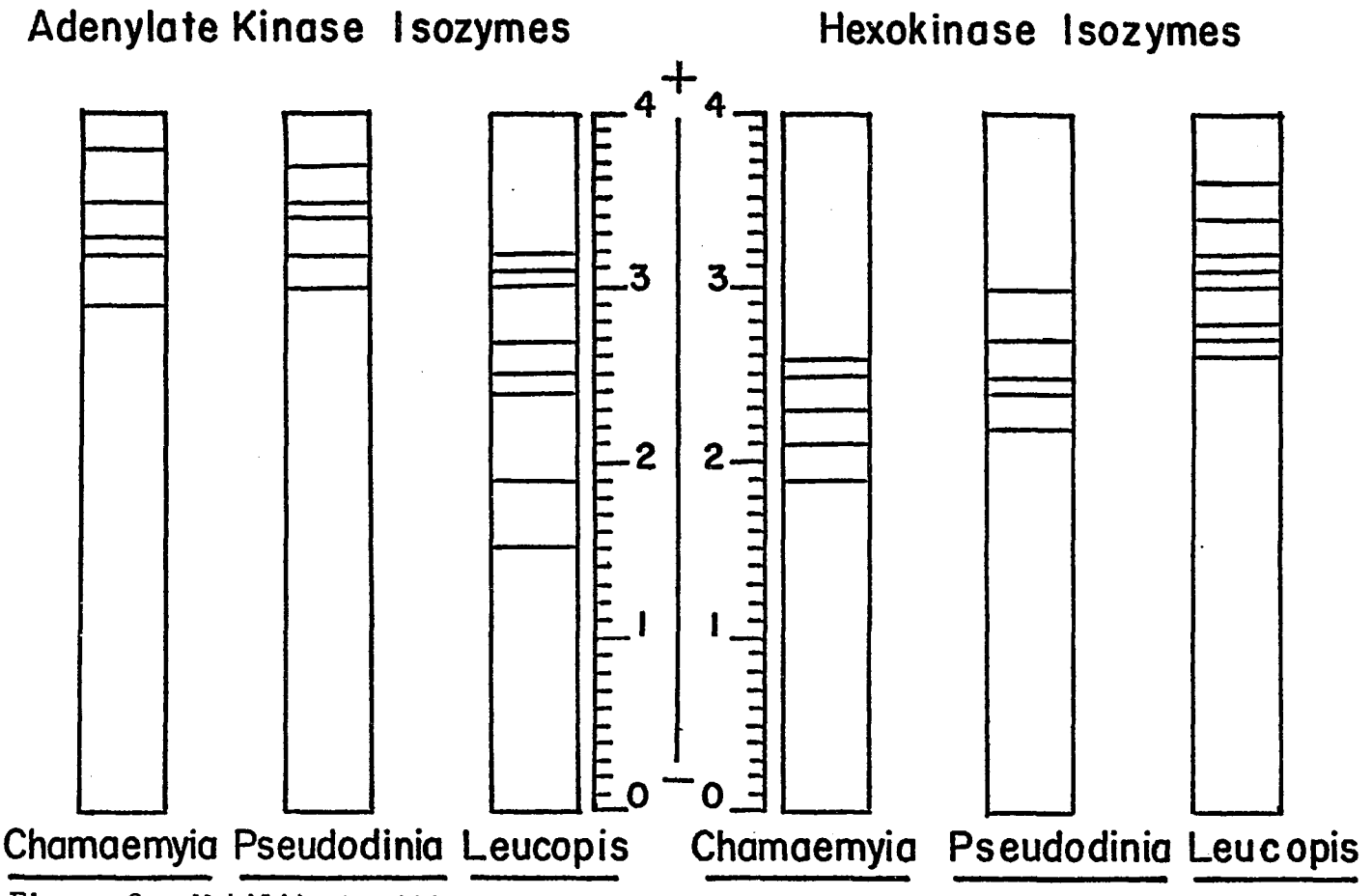

Figure 9. Mobility Positions of Adenylate Kinase and Hexokinase Isozymes for Each Genus.

Table 17. Cavalli-Sforza E^2 Estimate of Genetic Distance between Species of Chamaemyiidae.

Identifications: HERB, C. herbarum; FUMI, C. fumicosta; JUNC, C.juncorum; VARI, P. varipes; NITS, P. nitens; PS 3, P. sp. 3; OCEL, L. ocellaris; BELL, L. bellula; L 82, L. SP. 82; L E, L. sp. E; L H, L. sp. H.

	HERB	FUMI	JUNC	VARI	NITS	PS 3	OCEL	BELL	L 82	L E	L H
HERB	0.000										
FUMI	0.143	0.000									
JUNC	0.223	0.304	0.000								
VARI	0.232	0.285	0.191	0.000							
NITS	0.213	0.252	0.182	0.089	0.000						
PS 3	0.255	0.274	0.204	0.150	0.057	0.000					
OCEL	0.236	0.307	0.231	0.185	0.262	0.306	0.000				
BELL	0.296	0.298	0.300	0.339	0.279	0.321	0.304	0.000			
L 82	0.277	0.298	0.299	0.260	0.258	0.251	0.209	0.261	0.000		
L E	0.308	0.344	0.348	0.261	0.284	0.309	0.234	0.298	0.241	0.000	
L H	0.344	0.377	0.372	0.278	0.318	0.369	0.276	0.315	0.265	0.103	0.000

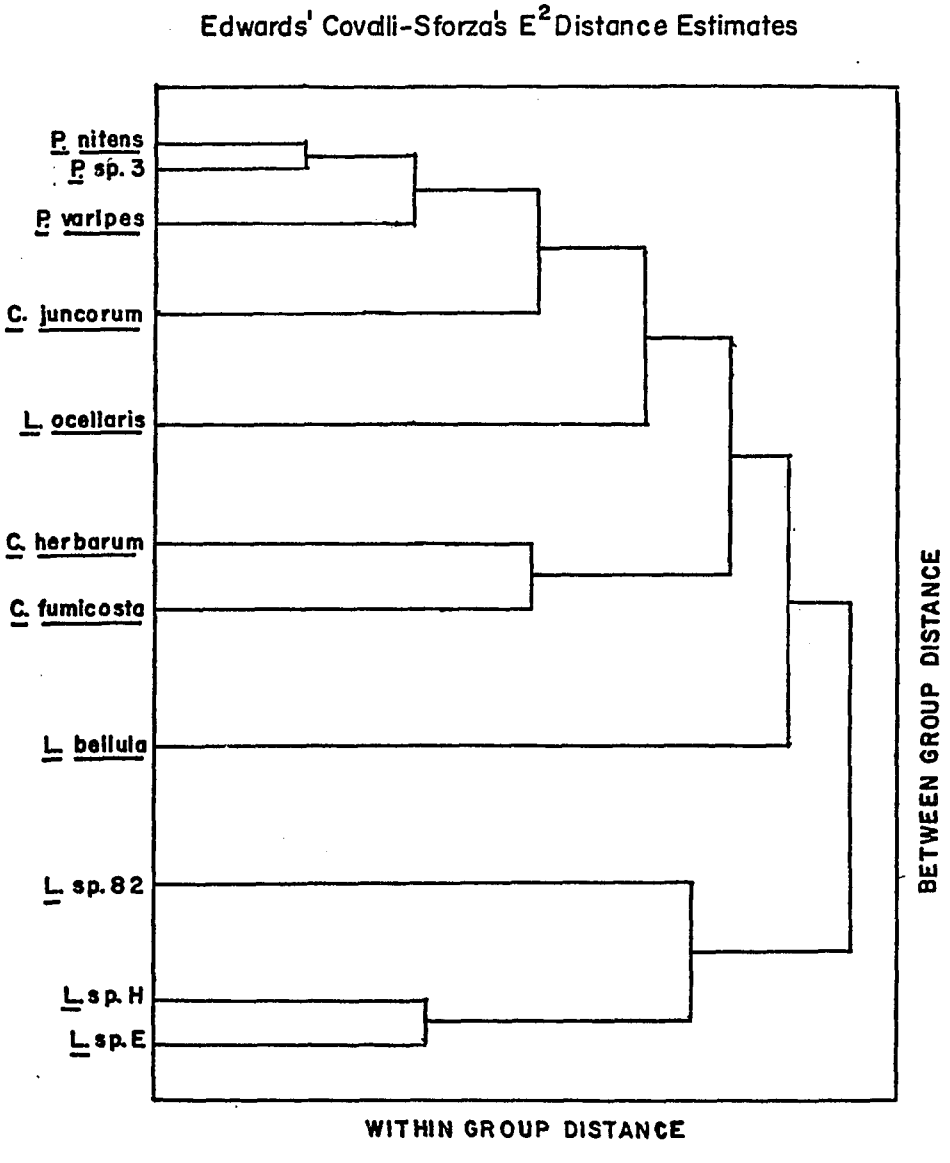

Figure 10. Edwards' and Cavalli-Sforza's E^2 Genetic Distance Estimates Between Species of Chamaemyiidae.

relationships shown agree with the classification of genera. Pseudodinia species are most similar and the genus is grouped with Chamaemyia. Except for L. ocellaris, the genus Leucopis is placed by itself and its species are the most diverse.

The proportion of isozymes unique to a genus is a measure of its divergence. Leucopis has 36% unique isozymes, Chamaemyia has 21%, and Pseudodinia has 10%. The genus Leucopis has seven to ten times more species than any other genus. It is also the most diverse ecologically. Ten percent of the isozyme bands are found in all three genera. The proportion of isozymes shared between genera may be a measure of their common ancestry. Chamaemyia and Pseudodinia share 10%, Pseudodinia and Leucopis share 12%, and Chamaemyia and Leucopis share only 1%.

CHAPTER 4

GEOGRAPHICAL VARIATION OF *C. HERBARUM* POPULATIONS

The center of distribution of *C. herbarum* is in the Rocky Mountains of the western United States and the southernmost extension of its range is in the southeastern mountains of Arizona. Populations in the Colorado Rockies may be classified as semi-isolated; the proximity and continuity of the mountain ranges may allow limited migration between populations or subpopulations. In Arizona and northwestern New Mexico, each population is restricted to a separate mountain range and may be a geographic isolate. The importance of geographic isolation to the differentiation of populations can be determined by analyzing *C. herbarum* populations in Colorado and Arizona.

Figure 11 shows the collection sites of *C. herbarum* used for both morphological and electrophoretic studies. The map also shows Dice's biotic provinces (Dice 1943). The Coloradan Province is represented by Allenspark (Boulder Co., 2653 m.), Bailey (Park Co., 2682 m.), Rampart Range (17.6 km. N. Colorado Springs, El Paso Co., 2743 m.), Redstone (Pitkin Co., 2134 m.), and Gunnison National Monument (Montrose Co., 2438 m.), all in the Colorado Rockies.

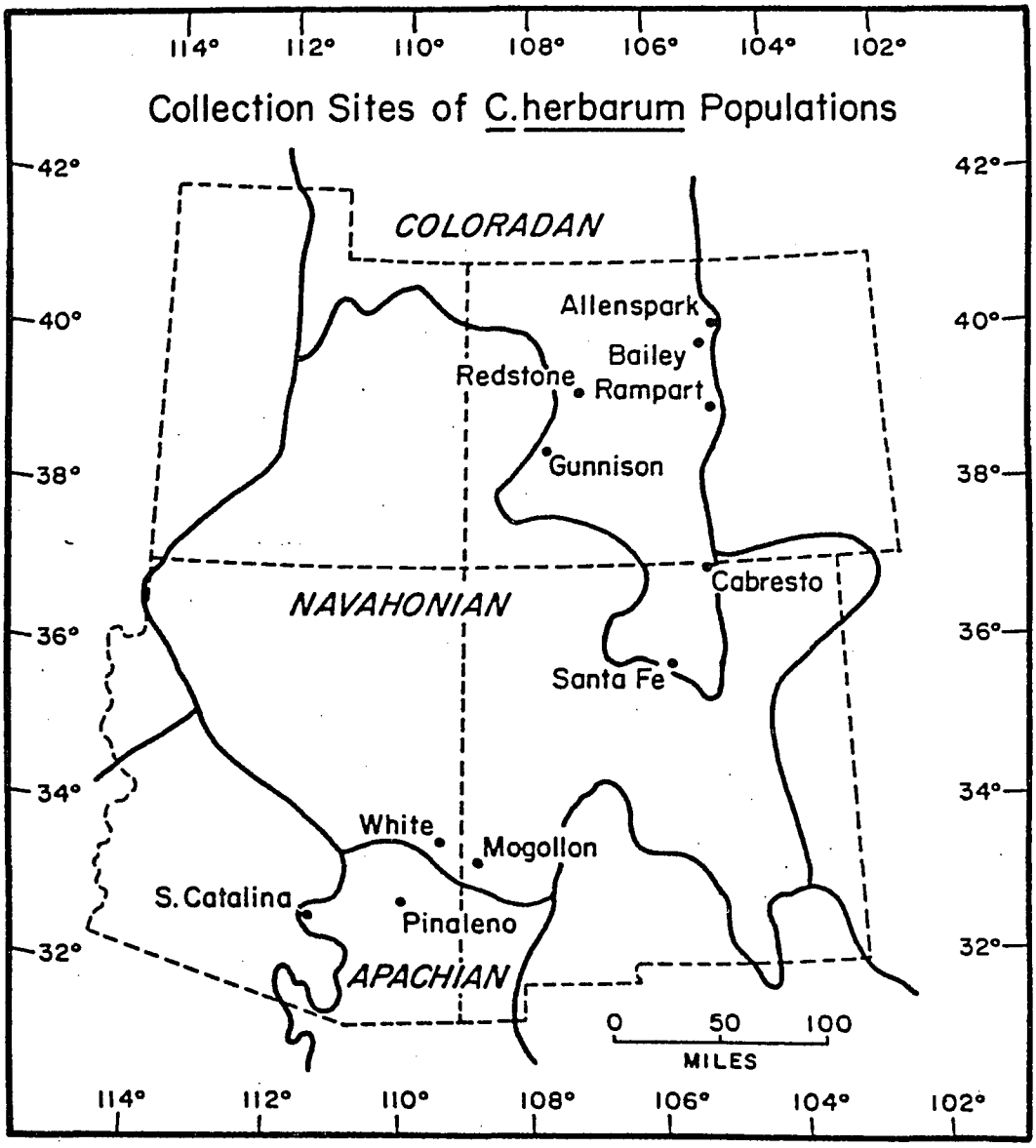

Figure 11. Collection Sites of C. herbarum Populations Overlain with Dice's Biotic Provinces.

The southern extremity of the Rockies in New Mexico is represented by Cabresto (Cabresto Lake, Sangre de Cristo Mts., Taos Co., 2743 m.), Mogollon, New Mexico (Mogollon Mts., Catron Co., 2499 m.), and the White Mountains of Arizona (25.6 km. S. Big Lake, Apache Co., 2438 m.) are within the Navahonian Province. The southernmost, smallest and most isolated populations are in the Apachian Province of southeastern Arizona and are from the Pinaleno Mts. (Goudy Creek, Graham Co., 2499 m.) and Santa Catalina Mts. (Mt. Bigelow Lunar Observatory, Pima Co., 2560 m.). All of the collections were made in June and July of 1974 except for the Santa Catalina Mountain sample obtained in July of 1975.

Enzyme Variation

The electrophoretic variation of seven enzymes was determined for the 11 populations of C. herbarum (Figure 11). The isozyme frequencies and total number of individuals assayed are given in Table 18. Appendix Tables C1 to C6 contain the isozyme genotypes observed in each population. Alpha-glycerophosphate was monomorphic in all populations. All of the other loci showed significant differences over all or part of the range. There are two striking examples of single population divergence. ACPH-5, the most frequent ACPH isozyme in the White Mountains (0.756). is absent from six populations and has an average frequency of 0.025 in

Table 18. Isozyme Frequencies of C. herbarum Populations Collected in Arizona, New Mexico, and Colorado.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina.

Enzyme Systems	Populations										
	AP	BA	RP	RS	GN	CB	SF	MG	WH	PL	SC
AK											
Total Isozymes	48	26	36	48	50	42	45	21	38	47	64
1											.008
2						.012		.048			.336
3			.097			.155	.056	.429	.750	.819	.656
4	.021	.077	.875	.063		.274	.589	.476	.224	.160	
5	.979	.923	.028	.938	1.000	.560	.356	.048	.026	.021	
HEX											
Total Isozymes	42	20	47	47	50	44	50	43	47	58	65
1			.011	.085			.310	.081	.043	.034	.008
2	.083		.926	.043	.040	.307	.590	.919	.957	.966	.992
3	.917	1.000	.064	.872	.950	.670	.100				
4					.010	.023					
ACPH											
Total Isozymes	60	21	52	68	93	35	42	62	41	93	61
1	.108	.048	.010	.022	.038	.014		.016		.005	.008
2	.367	.571	.183	.397	.495	.443	.036	.121		.333	.279
3	.508	.357	.644	.574	.452	.500	.548	.387	.073	.602	.418
4	.017	.024	.154	.007	.016	.043	.369	.452	.171	.043	.131
5			.010				.048	.024	.756	.016	

Table 18, Continued

Enzyme Systems	Populations AP	BA	RP	RS	GN	CB	SF	MG	WH	PL	SC
-GPDH											
Total	60	18	48	64	64	33	39	75	34	59	65
Isozymes											
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.985	.992	.985
2									.015	.008	.015
PGM											
Total	43	23	47	49	50	46	54	42	47	58	65
Isozymes											
1			.074			.033	.019	.024	.043	.103	.038
2	.093	.239	.670	.102	.140	.315	.657	.321	.564	.328	.577
3	.628	.370	.213	.745	.630	.337	.287	.369	.340	.405	.362
4	.198	.261	.043	.153	.230	.261	.037	.226	.053	.112	.023
5	.070	.065				.054		.060		.052	
6	.012	.065									
MDH											
Total	52	22	54	83	84	45	55	69	40	81	63
Isozymes											
1	.038	.045		.024	.048	.022		.080	.225	.136	.071
2	.212	.386	.343	.277	.339	.267	.318	.732	.688	.154	.571
3	.692	.500	.639	.669	.577	.689	.527	.145	.088	.667	.349
4	.058	.068	.019	.030	.036	.022	.155	.043		.043	.007
FUM											
Total	63	25	12	36	40	20	2	41	39	47	23
Isozymes											
1	.079	.020		.125					.013		
2	.278	.080	.167	.042	.038	.275		.122	.089	.074	.065
3	.603	.800	.833	.764	.925	.650	1.000	.841	.885	.745	.826
4	.040	.080		.069	.038	.050		.037	.013	.149	.087
5		.020				.025				.032	.022

four others. AK-2, absent from all but two of the populations, has a frequency of 0.336 in the Santa Catalina Mountains. Two of the seven loci showed significant geographic clines. Frequency differences between populations for hexokinase isozymes are shown in Figure 12. HEX-2 is correlated negatively with latitude ($\alpha < .001$) and HEX-3 is correlated positively with latitude ($\alpha = .001$). All correlation tests used a t-test, sign considered. Frequency differences for the adenylate kinase isozymes are shown in Figure 13. AK-3 is correlated negatively with latitude ($\alpha < .001$) and AK-5 is correlated positively with latitude ($\alpha = .001$). MDH-3 and ACPH-1 are also correlated with latitude ($\alpha = .05$), but these enzymes did not show striking geographic clines.

Nei's standard genetic distance measurement was used to determine the genetic divergence between populations. The matrix of genetic distance is given in Table 19, and Figure 14 plots the results of the cluster analysis of the genetic distance data. This plot clearly shows a difference between northern Colorado and southern Arizona-New Mexico populations. Rampart is an exception to this pattern. It is more similar genetically to the southern populations but is geographically northern. It is the only northern population sampled from the Front Range, the most easterly range of the Rockies.

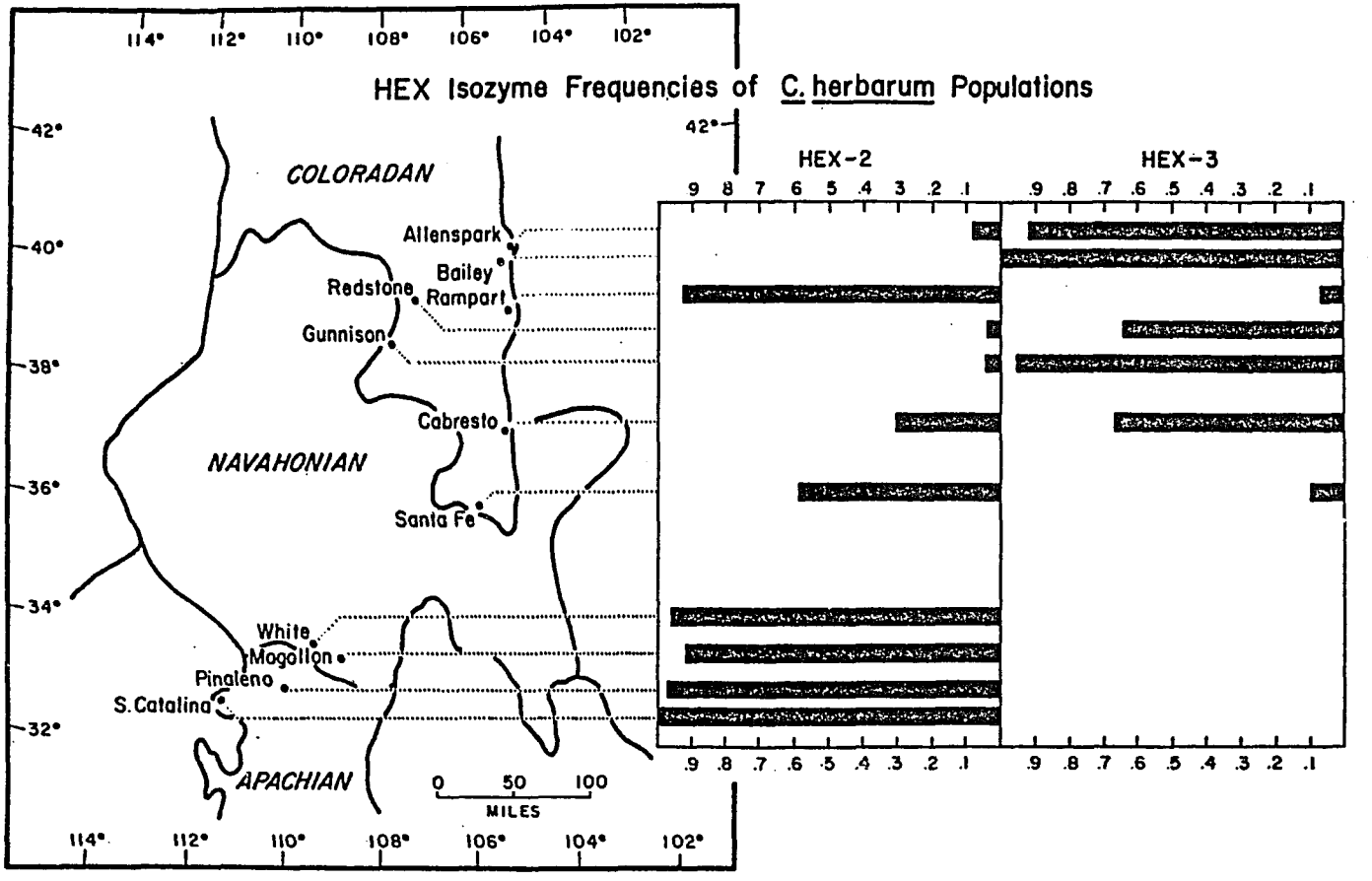

Figure 12. Hexokinase Isozyme Frequencies of C. herbarum Populations.

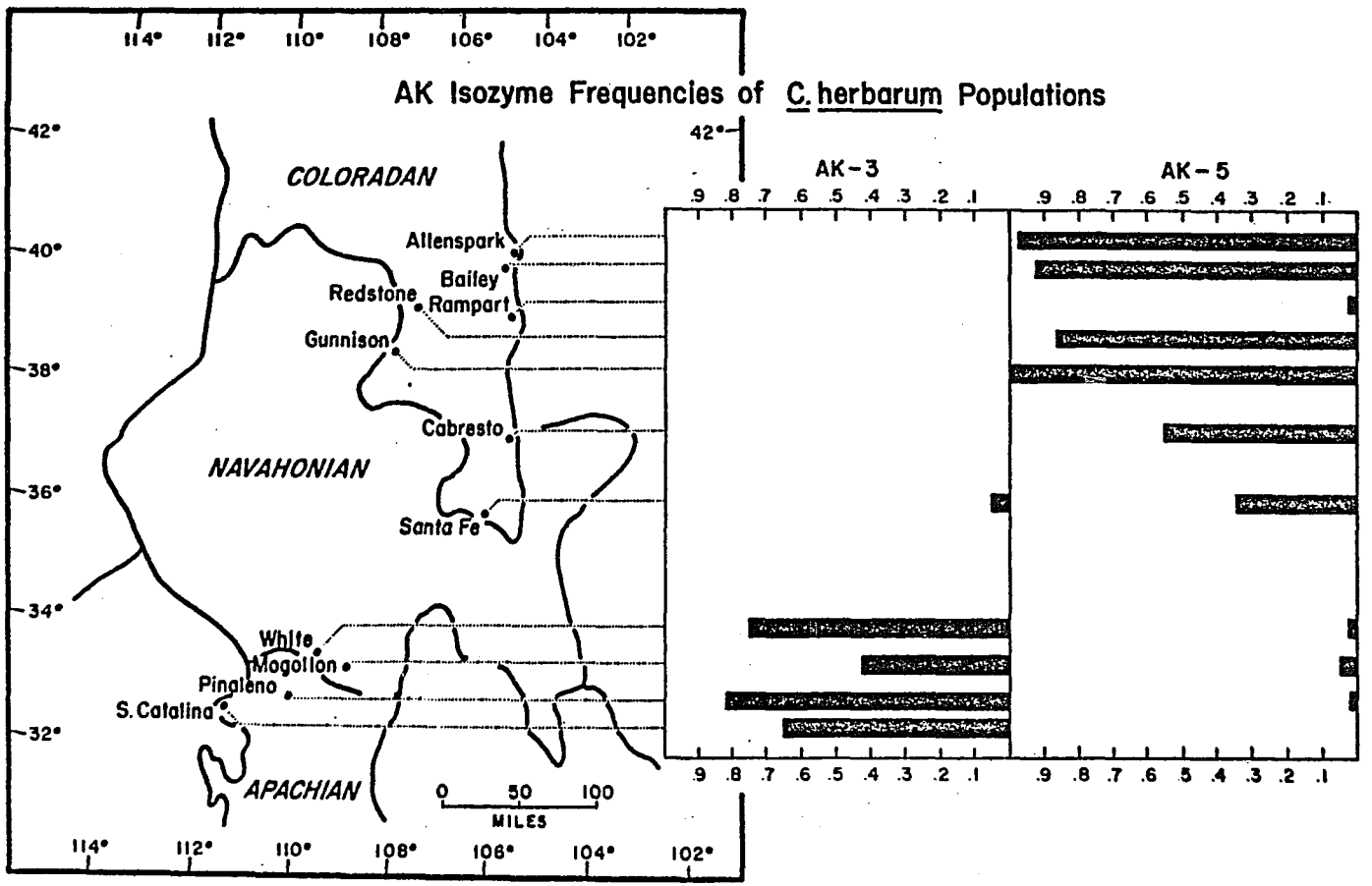

Figure 13. Adenylate Kinase Isozyme Frequencies of C. herbarum Populations.

Table 19. Nei's Standard Genetic Distance between Populations of C. herbarum.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina.

	AP	BA	RP	RS	GN	CB	SF	MG	WH	PL	SC
AP	.000										
BA	.037	.000									
RP	.511	.501	.000								
RS	.015	.036	.474	.000							
GN	.023	.014	.506	.011	.000						
CB	.016	.064	.219	.070	.072	.000					
SF	.366	.343	.062	.312	.331	.182	.000				
MG	.571	.523	.118	.519	.516	.300	.125	.000			
WH	.776	.698	.268	.718	.690	.468	.265	.117	.000		
PL	.480	.519	.153	.447	.481	.239	.204	.145	.201	.000	
SC	.567	.533	.170	.517	.514	.300	.193	.083	.121	.068	.000

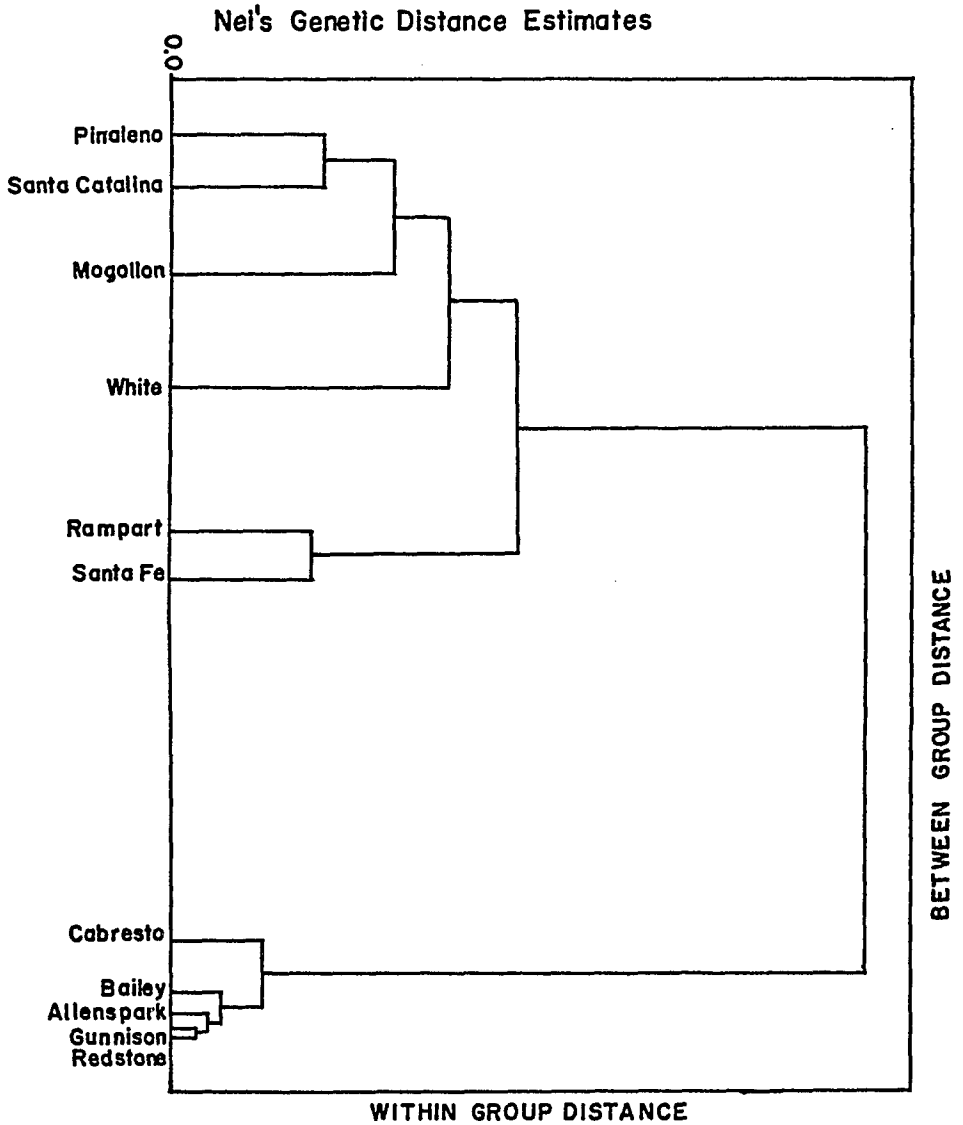

Figure 14. Nei's Genetic Distance Estimates between Populations of C. herbarum.

Rampart and Santa Fe are both marginal populations within the Coloradan Province.

Increased genetic distance between more isolated southern populations is evident in Figure 14. Isolation and reduced population size of southern populations could cause inbreeding which should be detected as deviations from expected Hardy-Weinberg genotypic frequencies. Goodness of fit to the expected Hardy-Weinberg genotypic ratios for each system is indicated in Table 20. The probability levels are included for each population which deviates from Hardy-Weinberg. All deviations are caused by an excess of homozygotes and deficiency of heterozygotes (Appendix Tables C 1 to C 6). In general, the Arizona and New Mexico populations deviate at more loci than do the Colorado populations.

Individuals of C. herbarum were collected June through July, 1976, at three different meadow localities in the Pinaleno Mts., Arizona. These sites were Snow Flat (2591 m.), Hospital Flat (2743 m.), and Chestley Flat (2850 m.). Isozyme frequencies and numbers of individuals assayed are given in Table 21 for each collection site. Cumulative isozyme frequencies are also given in Table 21. With the exception of FUM (Table 22), all of the isozyme genotypes deviated significantly from Hardy-Weinberg in at least one of the meadow populations (MDH, Table 23; AK, Table 24; PGM Table 25; and ACPH, Table 26). All deviations were due to

Table 20. Chi-square Analysis of the Goodness of Fit of the Observed Phenotypic ratios to those Expected from Hardy-Weinberg Equilibrium.

Identification: - , not significant.

	AK	HEX	-GPDH	PGM	MDH	ACPH	FUM
Allenspark	-	-	-	$\alpha < .005$	-	-	$\alpha < .050$
Bailey	-	-	-	-	-	-	-
Rampart	-	-	-	-	-	-	-
Redstone	-	-	-	-	-	-	-
Gunnison	-	-	-	$\alpha = .025$	-	-	-
Cabresto	$\alpha < .005$	$\alpha < .005$	-	-	-	-	-
Santa Fe	$\alpha < .005$	$\alpha < .005$	-	-	-	-	-
Mogollon	-	-	-	-	$\alpha < .005$	-	-
White	$\alpha < .005$	-	-	-	-	-	-
Pinaleno	$\alpha < .005$	-	-	$\alpha = .010$	-	$\alpha < .005$	-
Santa Catalina	-	-	-	$\alpha < .005$	-	$\alpha < .005$	-

Table 21. Isozyme Frequencies of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mountains in 1976.

Enzyme Systems	Snow 2591 m.	Hospital 2743 m.	Chestley 2850 m.	Cumulative
AK A				
Total	37	42	40	119
Isozymes				
2	.068	.024	.063	.050
3	.865	.976	.563	.803
4	.068	.000	.375	.147
PGM				
Total	49	56	60	165
Isozymes				
1	.020	.009	.042	.024
2	.520	.625	.492	.545
3	.398	.313	.283	.327
4	.061	.054	.167	.097
5	.000	.000	.017	.006
MDH				
Total	60	62	61	183
Isozymes				
1	.025	.016	.016	.019
2	.392	.177	.287	.284
3	.533	.484	.426	.481
4	.050	.323	.270	.216
FUM				
Total	68	72	68	208
Isozymes				
2	.037	.028	.037	.034
3	.882	.826	.765	.825
4	.081	.146	.191	.139
5	.000	.000	.007	.002
ACPH				
Total	32	55	62	149
Isozymes				
2	.031	.500	.363	.342
3	.266	.445	.460	.413
4	.391	.055	.089	.141
5	.313	.000	.089	.104

Table 22. Fumarase Isozyme Genotypes of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mts. in 1976.

Collection Site	FUM Genotypes 3-3	3-4	others	Chi Square
Snow	54 (52.9)[a]	7 (9.7)	7 (5.4)	1.248
Hospital	52 (49.1)	11 (17.4)	9 (5.5)	4.7526
Chestley	41 (49.8)	16 (19.9)	11 (8.3)	1.679
Cumulative	147 (141.6)	34 (47.7)	27 (18.7)	7.826[b]

[a]Number of genotypes expected by Hardy-Weinberg formula.
[b]Number significant $\alpha < .025$.

Table 23. Malic Acid Dehydrogenase Isozyme Genotypes of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mts. in 1976.

Collection Site	MDH Genotypes 2-2	2-3	3-3	4-4	others	Chi Square
Snow	15 (9.2)[a]	14 (25.1)	25 (17.0)	3 (.2)	3 (8.5)	13.168[b]
Hospital	6 (1.9)	8 (10.6)	20 (14.5)	14 (6.5)	14 (28.5)	27.602[b]
Chestley	9 (5.0)	9 (15.0)	16 (11.1)	8 (4.0)	19 (25.9)	13.601[b]
Cumulative	30 (14.8)	31 (50.0)	61 (42.3)	25 (8.5)	36 (67.4)	77.756[b]

[a]Number of genotypes expected by Hardy-Weinberg formula.
[b]Number significant $\alpha < .005$.

Table 24. Adenylate Kinase Isozyme Genotypes of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mts. in 1976.

Collection Site	AK Genotypes 1-2	2-2	2-3	3-3	Chi Square
Snow Flat	5 (4.4)[a]	29 (27.7)	1 (4.4)	2 (.2)	.[illegible]0
Hospital Flat	2 (2)	40 (40)			.000
Chestley Flat	5 (2.8)	17 (12.7)	6 (16.9)	12 (5.6)	14.046[b]
Cumulative	12 (9.6)	86 (76.7)	7 (28.1)	14 (2.6)	60.388[b]

[a]Number of genotypes expected by Hardy-Weinberg formula.
[b]Number significant $\alpha < .005$.

Table 25. Phosphoglucomutase Isozyme Genotypes of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mts. in 1976.

Collection Site	PGM Genotypes 2-2	2-3	3-3	others	Chi Square
Snow Flat	20 (13.2)[a]	7 (20.3)	16 (7.8)	6 (7.7)	21.214[b]
Hospital Flat	29 (21.9)	11 (21.9)	11 (5.5)	5 (6.7)	13.658[b]
Chestley Flat	26 (14.5)	6 (16.7)	13 (4.8)	15 (24.0)	24.239[b]
Cumulative	75 (49.0)	24 (58.8)	40 (17.6)	26 (39.6)	67.570[b]

[a]Number of genotypes expected by Hardy-Weinberg formula.
[b]Number significant $\alpha < .005$.

Table 26. Acid Phosphatase Isozyme Genotypes of C. herbarum Populations Collected at Different Elevations in the Pinaleno Mts. in 1976.

Collection Site	ACPH Genotypes 2-2	2-3	3-3	3-4	others	Chi Square
Snow	0 (0)[a]	2 (.5)	5 (2.3)	5 (6.7)	20 (22.5)	8.379[b]
Hospital	25 (13.8)	5 (25.5)	19 (10.9)	6 (2.7)	0 (0)	31.890[c]
Chestley	19 (8.2)	7 (20.7)	24 (13.1)	2 (5.1)	10 (14.9)	35.857[c]
Cumulative	44 (17.2)	14 (42.1)	48 (25.0)	13 (17.4)	30 (47.3)	89.114[c]

[a]Number of genotypes expected by Hardy-Weinberg formula.
[b]Number significant $\alpha < .05$.
[c]Number significant $\alpha < .005$.

excess homozygotes and a deficiency of heterozygotes. Three loci (MDH, AK, and ACPH) showed significant frequency changes ($\alpha < .005$) from 2591 to 2850 meters. HEX and α-GPDH were monomorphic, and PGM and FUM showed no significant differences between sites.

Morphometric Variation

Twenty-four individuals of each population shown in Figure 11 were measured, with the exception of Rampart (11 specimens). The means and standard deviations of the 13 measured characters are given in Appendix Table B 4. The characters and their U-statistic are as follows: eye length (0.658), mesonotum width (0.552), cheek width (0.464), medial-cubital vein length (0.397), radial-medial vein length (0.349), third antennal segment height (0.311), wing length (0.280), arista length (0.255), ocellar triangle base length (0.230), mesonotum length (0.216), eye height (0.204), third antennal segment length (0.193), and ocellar triangle side length (0.183). Each of these characters contributed significantly to the discrimination of populations ($\alpha < .01$).

Classification of flies within each population using the discriminant analysis of morphological characters is shown in Table 27. Flies from northern populations are classified generally into northern populations and southern flies into southern populations. Regional morphotypes are discriminated. The flies measured from southern populations

Table 27. Number of Individuals Classified into C. herbarum Populations based upon Morphological Data.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; WH, White; PL, Pinaleno; SC, Santa Catalina.

	AP	BA	RP	RS	GN	CB	SF	WH	PL	SC
AP	8	5	0	6	1	3	0	0	1	0
BA	3	5	1	2	0	1	5	3	4	0
RP	0	0	4	0	0	1	3	1	1	1
RS	2	5	1	11	0	4	0	0	1	0
GN	1	4	0	2	14	1	0	2	0	0
CB	2	2	1	2	1	10	0	3	1	2
SF	0	1	0	1	1	2	15	1	3	0
WH	0	1	0	2	1	2	3	11	2	2
PL	1	2	1	1	0	2	2	1	11	3
SC	0	0	0	1	0	0	5	1	0	17

are larger than the flies from northern populations. The only morphometric characters which show significant differences between the northern and southern individuals are eye height and eye length (α = .05). Eye height is correlated with longitude (α = .01) and latitude ($\alpha < .005$). Eye length is correlated with latitude (α = .025).

Santa Catalina is geographically the most isolated southern population and 75% of the individuals in this population are of the southern morphotype. Gunnison is the most discriminated northern population and 87.5% of the measured individuals are of the northern morphotype. Cabresto and Santa Fe populations contain nearly equal proportions of northern and southern morphotypes. Rampart individuals are all southern or Rampart; no northern, non-Rampart morphotypes are observed. Figure 15 plots the results of a cluster analysis of the Mahalanobis D^2 distance estimates (Table 28). The dendrograph groups northern and southern populations with the exception of Bailey Colorado which is paired with Pinaleno Arizona. The Rampart population is set off as morphologically distinct from the others. The regional differences in morphology are not as distinct as the regional isozyme frequency differences.

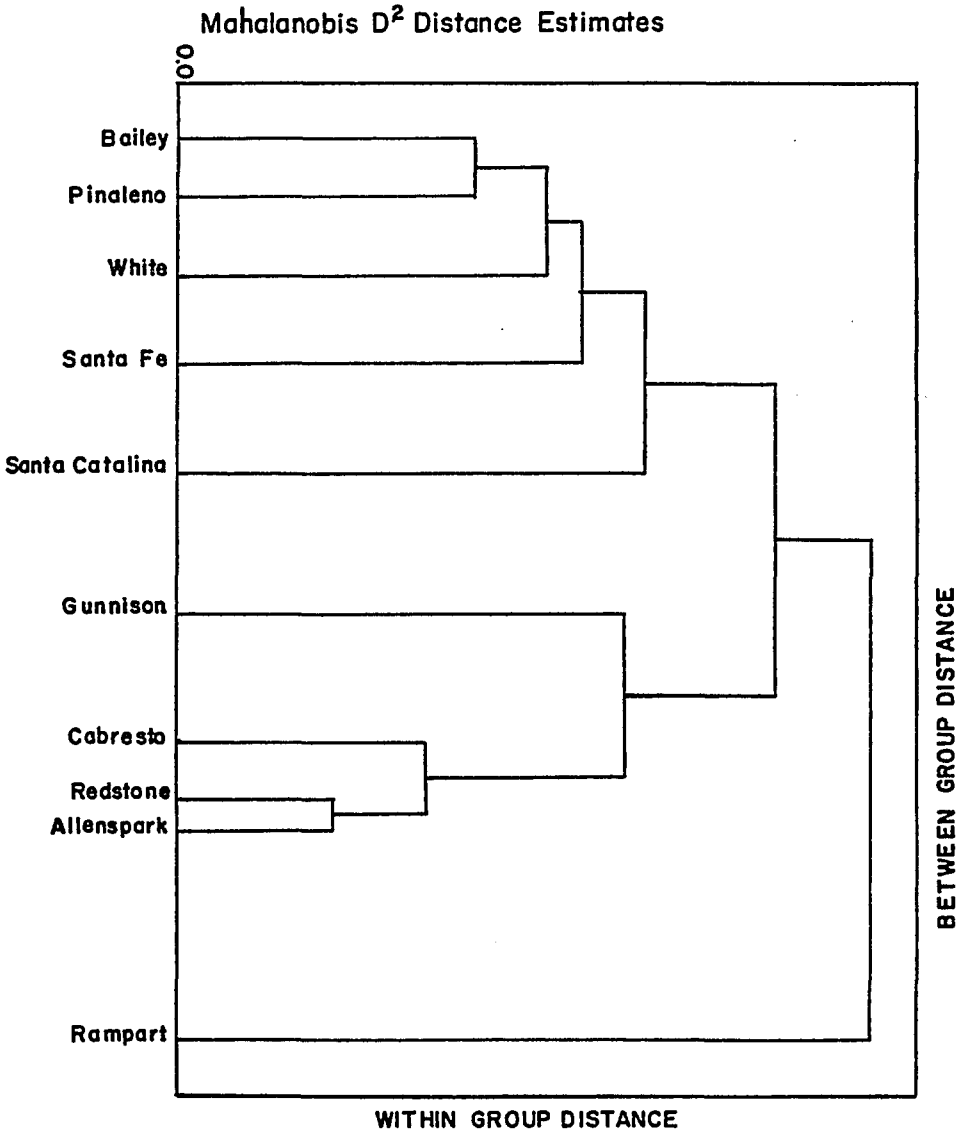

Figure 15. Mahalanobis D^2 Distance Estimates between Populations of C. herbarum.

Table 28. Mahalanobis D^2 Distance between Populations of *C. herbarum*.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; GN, Gunnison; RS, Redstone; CB, Cabresto; SF, Santa Fe; WH, White; SC, Santa Catalina; PL, Pinaleno.

	AP	BA	RP	GN	RS	CB	SF	WH	SC	PL
AP	0.000									
BA	2.431	0.000								
RP	8.356	7.033	0.000							
GN	3.840	4.559	9.969	0.000						
RS	1.033	2.607	8.163	3.680	0.000					
CB	2.467	3.161	6.149	5.333	1.781	0.000				
SF	6.615	2.726	4.548	4.942	5.906	4.596	0.000			
WH	4.474	2.297	6.078	5.539	4.037	1.822	3.281	0.000		
SC	7.037	5.117	5.800	7.619	7.631	5.284	3.468	3.502	0.000	
PL	3.522	1.947	7.074	5.511	3.945	3.477	3.666	2.955	2.732	0.000

CHAPTER 5

ENZYME VARIATION IN *P. VARIPES* AND *P. NITENS* POPULATIONS

Pseudodinia varipes is a common chamaemyiid species in the western United States. It is collected along with *C. herbarum* in the same mountain transition zone on *Muhlenbergia* grasses. Populations of *P. varipes* from four southern Arizona mountain ranges were assayed electrophoretically. Collections were made in the Santa Catalina Mts. (lat. 32° 25', long. 110°43', elev. 2422 m.), Kitt Peak, Quinlan Mts. (lat. 31°58', long. 109°54', elev. 2713 m.), and the Chiricahua Mts. (lat. 31°54', long. 109°17', elev. 2560 m.). The enzyme systems used were HEX, AK-A, AK-B, MDH, FUM, PGM, and ∝-GPDH. Isozyme frequencies are given for each sample of over 30 individuals (Table 29). Appendix Tables D 1 to D 7 give the isozyme genotypes observed in each population. Cumulative frequencies from two or three year collections were used to compare mountain populations. Isozyme frequencies at three of the enzyme loci correlated with elevation: PGM-4 ($\propto < .02$, $r = -.9826$), FUM-3 ($\propto < .05$, $r = -.9757$), and MDH-3 ($\propto < .05$, $r = -.9748$).

Table 29 . Isozyme Frequencies of *P. varipes* Populations in Arizona.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; CUM, cumulative frequency.

Enzyme* Systems	SC 1975	SC CUM	KP 1974	KP CUM	CH 1974	CH 1975	CH CUM	PN CUM
-GPDH								
Total Isozymes	171	224	52	70	72	120	220	15
1	.208	.239	.087	.136	.313	.075	.168	.133
2	.792	.792	.913	.864	.688	.925	.832	.867
FUM								
Total Isozymes	80	105	48	56	77	87	190	10
1	.013	.014	.021	.018	.000	.011	.006	.100
2	.113	.095	.031	.027	.058	.075	.060	.300
3	.813	.820	.875	.875	.779	.741	.785	.550
4	.061	.071	.073	.080	.136	.155	.131	.050
5	.000	.000	.000	.000	.026	.017	.018	.000
MDH								
Total Isozymes	78	102	53	75	70	106	190	15
1	.000	.000	.000	.000	.000	.000	.000	.000
2	.013	.020	.097	.073	.007	.024	.016	.034
3	.147	.147	.292	.380	.121	.160	.142	.234
4	.763	.667	.406	.425	.357	.783	.595	.366
5	.077	.166	.208	.220	.493	.033	.239	.366
6	.000	.000	.000	.000	.021	.000	.008	.000
PGM								
Total Isozymes	112	142	53	75	85	143	254	11
1	.013	.011	.000	.000	.000	.000	.000	.000
2	.036	.042	.038	.033	.082	.018	.045	.045
3	.754	.778	.755	.767	.588	.841	.768	.636
4	.138	.120	.104	.093	.288	.111	.157	.318
5	.058	.049	.104	.087	.035	.031	.028	.000
6	.000	.000	.000	.020	.006	.000	.002	.000

Table 29, Continued

Enzyme Systems*	SC 1975	SC CUM	KP 1974	KP CUM	CH 1974	CH 1975	CH CUM	PN CUM
AK-A								
Total	108	126	87	98	72	148	220	11
Isozymes								
1	.065	.056	.017	.031	.035	.024	.026	.045
2	.907	.921	.080	.158	.882	.916	.905	.409
3	.028	.023	.805	.724	.069	.061	.064	.455
4	.000	.000	.052	.046	.014	.000	.005	.045
5	.000	.000	.046	.041	.000	.000	.000	.045
AK-B								
Total		10		44			64	11
Isozymes								
1		.000		.000			.000	.000
2		.000		.000			.039	.091
3		.000		.045			.148	.045
4		.150		.534			.375	.455
5		.400		.352			.211	.318
6		.450		.068			.227	.091
HEX-A								
Total	109	140	30	52	45	150	239	2
Isozymes								
1	.023	.032	.000	.087	.044	.007	.036	.500
2	.899	.893	.967	.885	.878	.853	.831	.500
3	.078	.075	.033	.028	.067	.140	.130	.000
4	.000	.000	.000	.000	.011	.000	.004	.000

* Isozyme bands are in the same mobility position as in Table 33 .

Nei's genetic distance estimates between mountain populations are given in Table 30 and the dendrograph from these distances is shown in Figure 16. Santa Catalina and Chiricahua populations appear to be more similar genetically. Although Kitt Peak and the Pinaleno populations are paired, the within-group distance (0.127) is almost the same as the distance between groups (0.146). This relationship does not reflect the geographic distance between mountain ranges but may be influenced by population size. Pinaleno and Kitt Peak populations are both small.

Rustler Park, a site in the Chiricahua Mts., was used to investigate seasonal isozyme frequency changes. Collections were made on May 27, July 28, and September 13, 1975, to sample the first, middle, and last generations. Isozyme frequencies for PGM, HEX, AK-A, FUM, and α-GPDH are given in Table 31. Isozyme genotypes are given in Appendix Tables D8 to D12. The only statistically significant seasonal changes are for the α-GPDH isozymes ($\alpha < .005$). Alpha-GPDH-1 had a higher frequency in the spring (0.205) and a lower frequency in late summer (0.031). Parallel seasonal changes are observed in the Santa Catalina Mts. On May 16, 1975, the frequency of α-GPDH-1 in these mountains was 0.295 and the α-GPDH-2 frequency was 0.705 (N = 88). On July 29, 1975, the frequency of α-GPDH-1 was down to 0.114 and the α-GPDH-2 frequency was up to 0.886 (N = 83).

Table 30. Nei's Genetic Distance Estimates between *P. varipes* Populations.

Identifications: SCAT, Santa Catalina Mts.; KITT, Kitt Peak; PINA, Pinaleno Mts.; CHIR, Chiricahua Mts.

	SCAT	KITT	PINA	CHIR
SCAT	0.000			
KITT	0.191	0.000		
PINA	0.190	0.103	0.000	
CHIR	0.044	0.042	0.139	0.000

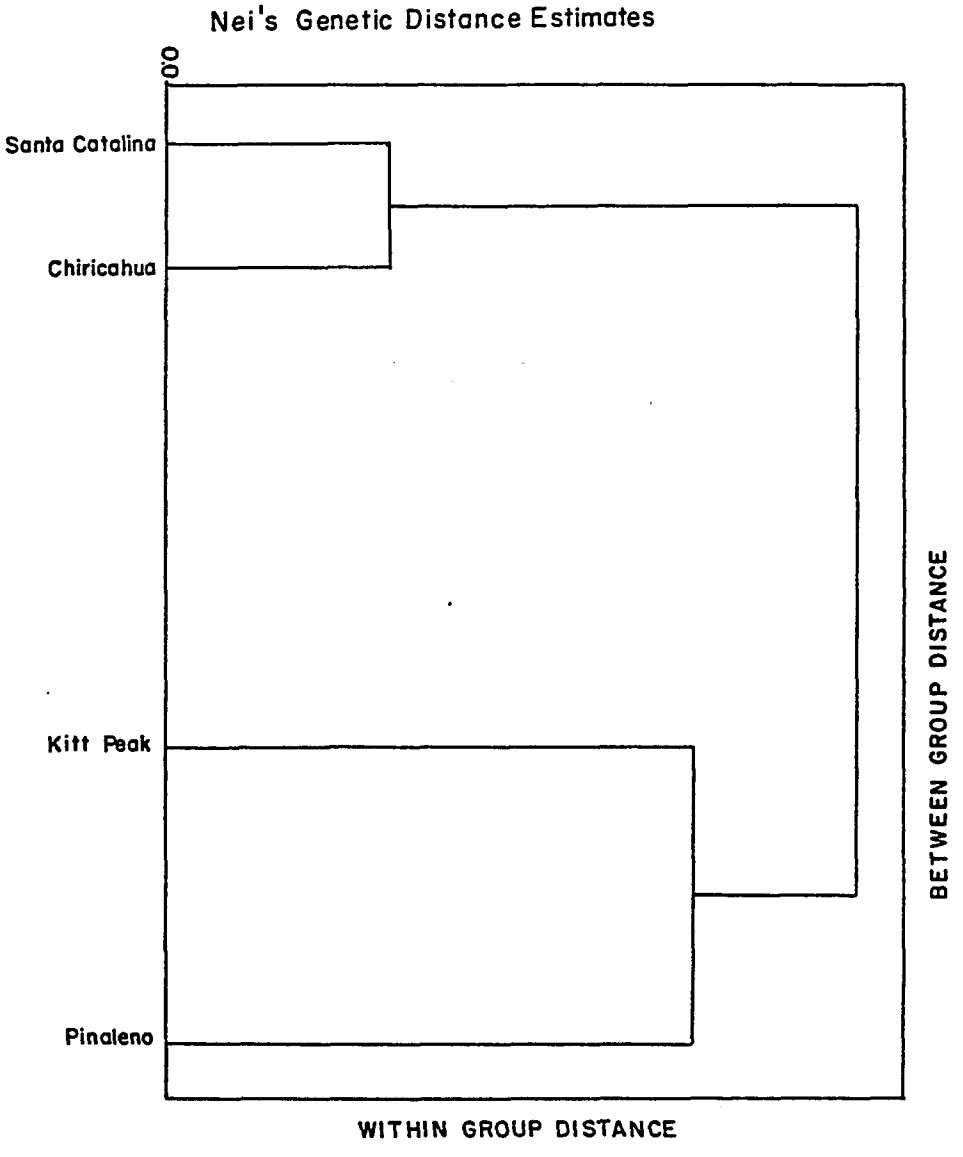

Figure 16. Nei's Genetic Distance Estimates between Populations of P. varipes.

Table 31. *P. varipes* Isozyme Frequencies from Seasonal Collections in the Chiricahua Mts.

Enzyme Systems	May 5/27/75	July 7/28/75	September 9/13/75
PGM			
Total Isozymes	58	69	27
2	.078	.021	.056
3	.776	.876	.815
4	.121	.093	.093
5	.025	.010	.036
HEX			
Total Isozymes	54	72	31
1	.018	.000	.000
2	.917	.792	.919
3	.065	.208	.081
AK A			
Total Isozymes	53	63	32
1	.009	.047	.000
2	.925	.921	.891
3	.066	.032	.109
FUM			
Total Isozymes	27	28	32
1	.000	.018	.000
2	.056	.143	.063
3	.796	.696	.734
4	.130	.125	.188
5	.019	.018	.016
-GPDH			
Total Isozymes	39	47	32
1	.205	.000	.031
2	.795	1.000	.969

Table 32 gives climatic data from the Portal, Arizona, weather station which were compared with the isozyme frequencies observed in seasonal collections at Rustler Park. Both α-GPDH isozymes were correlated significantly with minimum monthly temperature ($\alpha < .05$, $r = +.9994$) and average monthly temperature ($\alpha < .05$, $r = +.9969$). Although none of the other isozymes showed significant seasonal variation, the following correlations were observed: PGM-4 with average monthly temperature, $\alpha < .05$, $r = +.9987$; FUM-2 with total precipitation, $\alpha < .05$, $r = +.9967$.

Significant deviations from the genotypic frequencies predicted by the Hardy-Weinberg equilibrium were observed for α-GPDH isozymes in every sample which was not monomorphic. Every collection showed a deficiency of heterozygotes (Appendix Table D 12). The HEX genotypic frequencies for the July collection at Rustler Park deviated significantly from Hardy-Weinberg equilibrium. A deficiency in HEX 2-3 heterozygotes was observed. The only other deviations from Hardy-Weinberg predictions were AK heterozygote deficiencies in the 1974 Kitt Peak and 1974 Chiricahua samples.

Populations of P. nitens were sampled for enzyme polymorphism. Collections were made in the following localities: Kitt Peak, Quinlan Mts. (lat. 31°58', long. 111°36', elev. 2070 m.), Huachuca Mts. (lat. 31°34', long. 110°20', elev. 1422 m.), Whits Mts. (lat. 33°59', long.

Table 32. Weather Station Data, Portal, Arizona.

Identifications: MAX, maximum temperature; MIN, minimum temperature; AVE, average temperature; PPT, precipitation.

Month	MAX °C	MIN °C	AVE °C	PPT mm.
May	25.1	2.7	13.9	3.05
July	29.1	13.3	21.2	134.90
September	25.4	9.2	17.3	4.72

109°45', elev. 2493 m.), Chiricahua Mts. (lat. 31°54', long. 109°17', elev. 2560 m.) all in Arizona; San Cristobal (lat. 36°11', long. 105°03', elev. 2336 m.) and Mogollon Mts. (lat. 33°24', long. 108°35', elev. 2469 m.) both in New Mexico. The isozyme frequencies for ∝-GPDH, FUM, PGM, AK-A, and HEX-A are given in Table 33. Only two populations, San Cristobal and Kitt Peak, were collected and assayed in sufficient numbers to warrant a comparison between localities. Appendix Tables D 1 to D 7 give the isozyme genotypes observed in San Cristobal and Kitt Peak populations.

The differences between populations are striking. Kitt Peak lost two HEX-A isozymes and tends toward fixation of alternate alleles at ∝-GPDH and AK-A. The Kitt Peak population deviated from all other P. nitens collections at the ∝-GPDH locus. The Kitt Peak population also tended toward fixation for PGM-3. This does not appear to be a regional shift. The Huachuca Mts. are also western and the frequency of PGM-3 and 4 are similar to those of San Cristobal. The observed heterozygosity was 0.134 in the Kitt Peak and 0.356 in the San Cristobal population. Fixation of alternate alleles, loss of alleles, and reduced heterozygosity are typical of random frequency changes caused by founder effects and inbreeding in small populations. Excess PGM homozygotes were observed in the San Cristobal and Huachuca populations, but none of the isozyme genotypic

Table 33. Isozyme Frequencies of P. nitens Populations in Arizona and New Mexico.

Identifications: SA, San Cristobal; KP, Kitt Peak; HU, Huachuca; MG, Mogollon; CH, Chiricahua; WH, White.

Enzyme Systems*	Populations SA	KP	WH	HU	MG	CH
-GPDH						
Total	39	63	7	8	5	3
Isozymes						
1	.949	.024	1.000	.813	1.000	1.000
2	.051	.976	.000	.187	.000	.000
FUM						
Total	35	44	7	3	2	
Isozymes						
1	.000	.000	.071	.000	.000	
2	.114	.068	.214	.000	.000	
3	.800	.909	.715	1.000	.500	
4	.086	.023	.000	.000	.500	
PGM						
Total	35	22	6	54		
Isozymes						
2	.000	.091	.417	.065		
3	.214	.819	.583	.250		
4	.314	.045	.000	.352		
5	.414	.045	.000	.296		
6	.057	.000	.000	.037		
AK A						
Total	35	22				
Isozymes						
1	.029	.000				
2	.943	.023				
3	.029	.818				
4	.000	.159				
HEX A						
Total	35	22	7			
Isozymes						
1	.057	.000	.357			
2	.929	1.000	.643			
3	.014	.000	.000			

*Isozymes are the same as in Table 29.

frequencies deviated from the Hardy-Weinberg equilibrium in the Kitt Peak population. Therefore, the frequency shift may have been a result of founder effects rather than continuous inbreeding.

Nei's genetic distance estimates were calculated between the P. nitens San Cristobal and Kitt Peak populations and the P. varipes populations. The genetic distance matrix is given in Table 34 and the dendrograph is shown in Figure 17. It is interesting that the Kitt Peak populations of P. varipes and P. nitens are most similar. The genetic convergence of both species on Kitt Peak could be accidental or could result from selection in a common environment.

Table 34. Nei's Genetic Distance Estimates between *P. varipes* and *P. nitens* Populations.

Identifications: KVAR, *varipes*-Kitt Peak; PINA, *varipes*-Pinaleno Mts.; SCAT, *varipes*-Santa Catalina; CHIR, *varipes*-Chiricahua Mts.; KNIT, *nitens*-Kitt Peak; SNIT, *nitens*-San Cristobal.

	KVAR	PINA	SCAT	CHIR	KNIT	SNIT
KVAR	0.000					
PINA	0.225	0.000				
SCAT	0.134	0.083	0.000			
CHIR	0.004	0.207	0.115	0.000		
KNIT	0.306	0.015	0.105	0.279	0.000	
SNIT	0.315	0.709	0.621	0.368	1.025	0.000

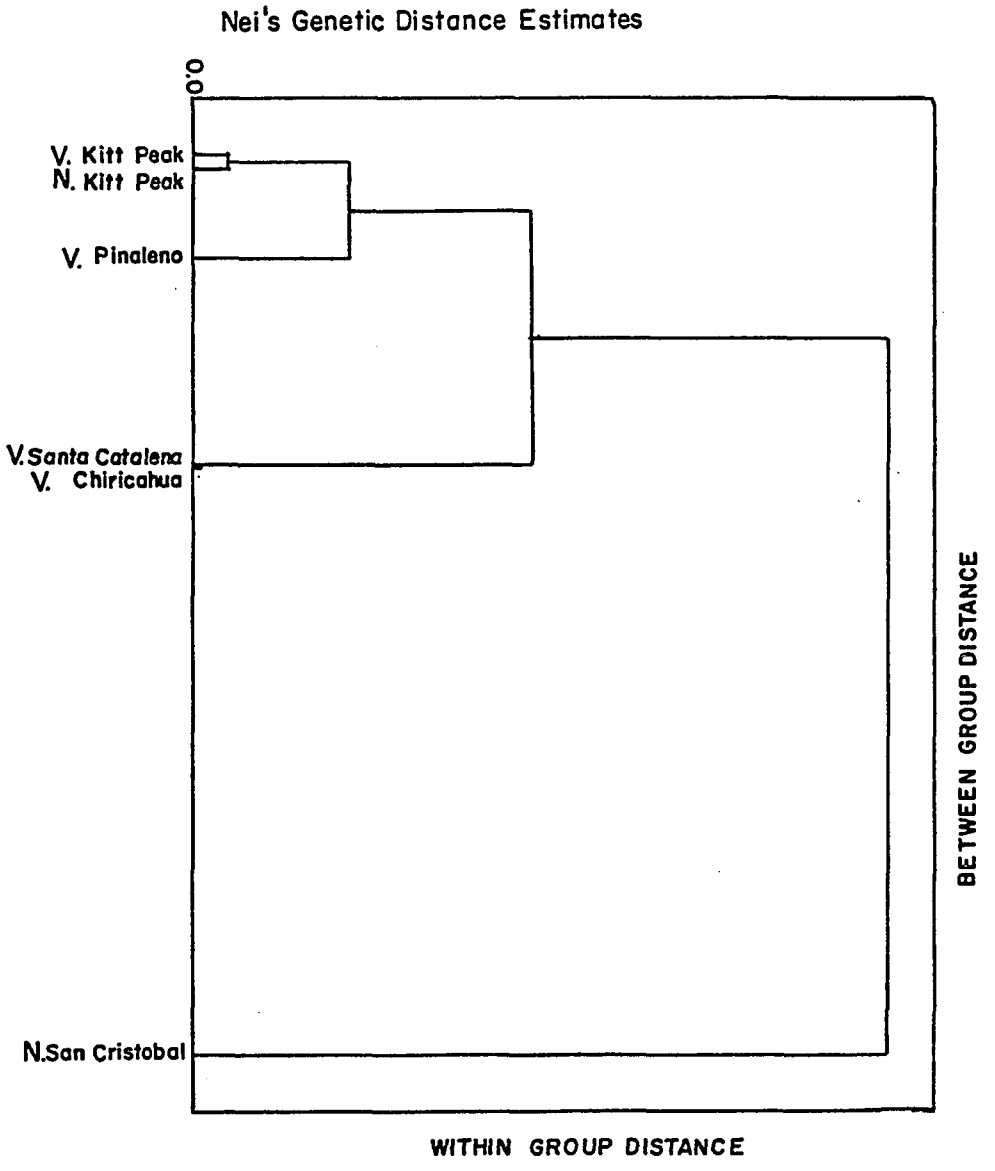

Figure 17. Nei's Genetic Distance Estimates between Populations of P. varipes and P. nitens.

CHAPTER 6

THE DIFFERENTIATION OF POPULATIONS

Interbreeding individuals which aggregate in a specific area defined by their common adaptations to a particular set of environmental conditions form a local population. The genetic differentiation of populations is the basis of evolutionary change and may be the first step toward speciation. Genetic divergence is controlled by a combination of factors including the structure of populations (size, migration rate, mating) and environmental variability (selection). The population structure of C. herbarum and P. varipes should be characteristic of mountain dwelling chamaemyiid species. The factors which control gene frequency changes or genetic divergence between populations of these species should be important in speciation.

Migration Rate and Population Size

The desert-isolated mountain ranges of southern Arizona have divided chamaemyiid species into several small isolated populations. But this isolation of C. herbarum and P. varipes populations is not complete. Two mountain populations may have originated from recent wide range

dispersal. On the basis of enzyme variation (Table 18), the Rampart population of C. herbarum is more similar to southern than to northern populations. Rampart is the only Colorado population sampled from the Front Range, the most easterly range of the Rocky Mountains. Wind dispersal from south to north is known to occur in the summer for some insect species (Hodson and Cook 1960). This is the time of year that dispersal would be possible for C. herbarum. It may be pertinent to the fly's dispersal ability that the wing structure of Rampart adults is distinct. The mean wing measurements from northern and southern populations are as follows: radial-medial vein length 0.069 mm. vs. 0.103 mm. for Rampart, medial-cubital vein length 0.232 mm. vs. 0.196 mm. for Rampart, and wing length 2.685 mm. vs. 2.966 mm. for Rampart.

Gene flow into an already established northern population would produce a mixture of north and south morphotypes and intermediate isozyme frequencies. Rampart appears to be the site of a colonization from a southern population. Rampart shows evidence of migration, but not necessarily gene flow into northern populations.

A second example of dispersal is the Kitt Peak population of P. nitens. Kitt Peak was collected in 1972, 1973, and 1974, but no P. nitens were found until 1975. This new population is characterized by a reduction in

heterozygosity and a loss of HEX isozymes. The ∝-GPDH and AK A enzyme loci tend toward fixation of alternate isozymes. The shifts in isozyme frequencies accompanied by a decrease in heterozygosity and loss of alleles would be expected if the population originated from a few founders.

Even infrequent gene flow between differentiated populations can produce clines. These clines can be maintained without selection by an environmental gradient. Intermediate morphometric and electrophoretic variation in New Mexico populations may be evidence of gene flow. The north-south hexokinase (Figure 12) and adenylate kinase (Figure 13) geographic variation could be the result of gene flow between differentiated regions.

Dispersal between southern Arizona mountain ranges, although possible, may not be a yearly occurrence. The importance of reduced migration rates can be demonstrated by comparing genetic distance estimates between continuous Colorado populations of C. herbarum ($\bar{x}$ = 0.040 $\pm$ 0.024) with the discontinuous semi-isolated southern populations ($\bar{x}$ = 0.152 $\pm$ 0.062). The genetic distance estimate between P. varipes populations collected from some of the same southern mountain ranges is similar ($\bar{x}$ = 0.127 $\pm$ 0.063). Increased morphological discrimination of southern C. herbarum populations (Table 27) is additional evidence for the increased differentiation of southern populations.

Small population size also contributes to the differentiation of populations. The increased genetic distance between Kitt Peak and Pinaleno Mountain populations of P. varipes may be due to random genetic changes resulting from small population size. Seasonal or chance reductions in population size may have produced changes observed in the C. herbarum White Mts. (ACPH-5, Table 18) and Santa Catalina Mts. (AK-2, Table 18) populations. Both of these populations have major shifts in isozyme frequencies similar to those observed in the Kitt Peak P. nitens population.

Alternate fixation of alleles is not characteristic of continental populations such as those of Drosophila pseudoobscura (Prakash, Lewontin, and Hubby 1969) or D. willistoni (Ayala et al. 1972) unless gene flow is reduced (Selander and Yang 1969). It is seen in single island Hawaiian Drosophila populations such as D. adiastola (Rockwood et al. 1971) and D. setosimentum (Carson and Johnson 1975). The population size and degree of isolation are similar for both Hawaiian picture wing Drosophila and C. herbarum. Small population size combined with isolation contribute to the differentiation between populations and between regions.

Mating Patterns

The Hardy (1908)-Weinberg (1908) equilibrium (Wallace 1968) predicts the genotypic or zygotic frequencies which will be produced from given gene frequencies. It assumes large populations, equal reproductive success between all genotypes, and random mating. The only consistent deviations from the Hardy-Weinberg equilibrium in P. varipes populations are heterozygote deficiencies at the ∝-GPDH locus. These deviations are consistent and impressive. For example, May 16, 1975 in the Santa Catalina population, four heterozygote individuals were observed and 37 were expected. If these deviations were the result of inbreeding in small populations, more enzyme loci should have deviated. Heterozygote deficiencies are probably caused either by selection against ∝-GPDH heterozygotes or by non-random mating between ∝-GPDH genotypes. It seems unlikely that a polymorphism would persist with strong selection against heterozygotes.

The most likely explanation is non-random mating between ∝-GPDH genotypes. The alpha-glycerophosphate metabolic cycle is essential to insect flight muscle energy production. It oxidizes NADH to NAD^+ which is required for glycolysis (Candy and Kilby 1975). Kinetic differences between ∝-GPDH isozymes in response to temperature have been reported for Drosophila melanogaster (Miller, Pearcy,

and Berger 1975) and Colias butterflies (G. B. Johnson 1976a). The ∝-GPDH-1 individuals of P. varipes may be adapted to lower temperatures. Significant seasonal changes in ∝-GPDH isozyme frequencies are observed in P. varipes populations (Table 29) and the frequency of ∝-GPDH-1 correlates significantly with temperature. As the temperature decreases, the frequency of ∝-GPDH-1 increases.

Temperature selection could cause changes in isozyme frequency and produce non-random mating. If the ∝-GPDH-1 individuals eclose earlier in the spring, they may be reproductively out of phase with the rest of the population. If the ∝-GPDH polymorphism results in temperature-specific flight activity, the mating time of the two genotypes could be different. Either process could lead to sympatric speciation. It is interesting that each of the two sibling pairs of species, C. herbarum + C. juncorum and P. nitens + P. varipes is fixed for alternate ∝-GPDH isozymes (Table 7).

Pinaleno mountain collections of C. herbarum populations were made to identify the microgeographic variation of isozyme frequency. Three of the five polymorphic enzyme loci (AK, MDH, and ACPH) show significant differences between populations in different meadows. Genotype frequencies for four of the five polymorphic loci (AK, PGM, MDH, and ACPH) deviate significantly from

Hardy-Weinberg expectations within single meadows. All deviations are due to an excess of homozygotes and a deficiency of heterozygotes. Selection should cause locus-specific genotypic changes.

Consistent genotypic deviations at all loci could result from sampling. No significant difference between meadow populations is observed for FUM, and FUM isozyme genotypes in each meadow population are in agreement with the Hardy-Weinberg equilibrium. When meadow populations were combined, significant deviations were produced. Deviations will result if several isolated differentiated subpopulations are assumed to represent a freely interbreeding population (Wahlund's principle, in Wallace 1968, pp. 100 - 103). A subdivided population would produce an excess of homozygotes and a deficiency of heterozygotes. Wahlund's principle predicts that homozygotes are increased over expectation by the variance of the gene frequency. When Hardy-Weinberg predictions were corrected for C. herbarum populations, significant deviations were not removed. Wahlund's principle may be producing part but not all of the deviation observed in C. herbarum populations.

Non-random mating could cause parallel genotypic changes at several loci. Inbreeding in small populations would produce excess homozygotes. Significant deviations from Hardy-Weinberg equilibrium in C. herbarum populations

were more frequent in southern Arizona populations. This may relate to the reduction in population size. Although the predatory mobility of C. herbarum adults should mix subpopulations on the same mountain, migration, even between meadows, does not appear to be accompanied by gene flow. Low gene flow is consistent with highly differentiated meadow populations. In summary, C. herbarum populations are characterized by geographic and microgeographic isolation, reduced population size, and non-random mating.

Selection

Isolation, small population size, founder effects, and inbreeding all tend to randomize allele frequencies. Selection favoring specific genotypes produces a pattern of allele frequency variation as populations adapt to local environments.

Genetic distance estimates grouped the southern populations of C. herbarum into a pattern that corresponds to Dice's biotic provinces (Figure 11). These provinces are based upon differences in floral composition; however, they also reflect faunal species boundaries. The clustering which corresponds to biotic province rather than geographic proximity may indicate the importance of environmental interactions in differentiating populations. The specific mealybug associations are not known for C. herbarum; however, southeastern Arizona mealybugs are more closely

related to those of the Mexican fauna than to those of the U. S. If there are, in fact, differences in mealybug prey from north to south, they may contribute to the regional isozyme differences between northern and southern populations. Zouros (1973) has identified two host races of Drosophila mojavensis, one breeding in organ pipe and the other in agria cactus. These races were differentiated at three of five polymorphic enzyme loci.

Parallel patterns of isozyme variation between two species which occupy similar habitats would be strong evidence for selection. Genetic convergence was observed between P. varipes and P. nitens populations collected from Kitt Peak. The Kitt Peak population of P. varipes was differentiated from other P. varipes populations, and the Kitt Peak population of P. nitens was differentiated from other P. nitens populations. The genetic variation of both species was assumed to be due to genetic drift. The genetic similarity could be due in part to selection in a similar environment. Significant correlations between MDH and FUM isozyme frequencies and elevation were observed in both C. herbarum and P. varipes populations.

An excellent example of parallel enzyme variation between two unrelated species has been observed for phosphoglucomutase isozymes. C. herbarum's PGM-3 migrates to the same relative mobility position as P. varipes' PGM-4, 1.3

(Table 12). (The mobility position of P. varipes' PGM-1 was not identified in the species comparison.) These isozymes may be structurally as well as functionally homologous. In both species, this PGM isozyme was significantly correlated with elevation. In the seasonal collections of P. varipes, PGM-1.3 frequency changes were negatively correlated with temperature. The frequency of PGM-1.3 was higher in most Colorado populations of C. herbarum. Although climatic data for specific collection sites of C. herbarum are limited, there are consistent differences (10°C) in the mean low winter temperature between northern and southern collection sites. Significant differences in PGM isozyme frequencies were observed for both P. varipes (1974-1975) and C. herbarum (1974-1976) between year to year collections. Isozyme frequency differences were not in the same direction. Whether these changes are random deviations or adaptations to local temperature differences cannot be determined. There is sufficient evidence that temperature variation is maintaining PGM isozyme polymorphism.

Climatic variables such as temperature reflect latitudinal and elevational gradients. Regional patterns of variation between northern and southern C. herbarum populations are reproduced microgeographically in the Pinaleno Mountains. MDH-3 was correlated with elevation

and latitude. AK-3 was absent from all of the Colorado populations (except Rampart, 0.097). In the Pinaleno Mts., AK-3 was found at a significantly reduced frequency in Chestley meadow compared with populations in lower meadows. Even if the environmental changes between populations are gradual, selection can produce abrupt gene frequency changes (concave fitness set, Levins 1968, Wilson and Bossert 1971).

Although the evidence for selection operating in chamaemyiid populations is circumstantial, it is hard to ignore. North-south patterns of genetic variation of glucose-metabolizing enzymes have been related to temperature differences in other organisms (Prakash 1973, Band 1975). All of the enzymes assayed in this study with the exception of acid phosphatase are glycolytic enzymes. Hexokinase functions in glucose mobilization. Adenylate kinase regulates the ATP/ADP ratio. Phosphoglucomutase functions in gluconeogenesis. All three catalyze non-equilibrium reactions and may be regulatory enzymes (G. B. Johnson 1974). Malic acid dehydrogenase and fumarase function in the tricarboxylic acid cycle and are probably nonregulatory.

The small populations of chamaemyiids would appear to require intense selection pressures to produce the observed patterns of enzyme variation. Patterns of

metabolic regulatory enzyme variation in subpopulations of the alpine butterfly, Colias meadii Edwards, have been observed (G. B. Johnson 1976b). The population size of C. meadii was over 1,000 individuals. Genotypic combinations of alleles were specific and different for five collection sites all located within a distance of 3.2 km. and an elevation difference of 610 m. Johnson hypothesized that specific combinations of alleles were selected at local host plant sites in spite of extensive migration. Organized isozyme variation at several regulatory loci may conbine to produce an integrated metabolic genotype adapted to local environmental variables. Similar selection may be operating in the alpine Diptera.

The distribution of C. herbarum adults in a single meadow is patchy or clumped. Localized selection could produce the observed deviations from Hardy-Weinberg equilibrium. The response to selection, whether in a meadow or in an isolated mountain range, would depend upon the available genetic variation. The founder effect would still be evident between isolated populations. Gene flow prevents the differentiation of populations. It tends to make all populations the same. If gene flow is reduced, selection for localized conditions would be more effective. Dice's biotic provinces which classify local environmental variation correspond to patterns of isozyme variation in C.

herbarum populations. Localized selection pressures would produce parallel patterns of genetic variation between species found in the same environment.

The First Step

Evolutionary divergence by geographical speciation is the progressive differentiation of an ancestral population into local races, geographical races, semispecies, and finally species and species groups. The beginning of the process, local and geographic race formation, was observed in C. herbarum. The desert barricade between southern Arizona mountain ranges has been present from the late Pleistocene, for the last eight to ten thousand years (Martin 1963). Before this time, the mountains were connected during pluvial periods by a mesic environment that would allow the southward invasion of Colorado species. Subsequent isolation favored the genetic differentiation of populations.

This isolation effect upon southeastern Arizona mountain insects is well documented by Ball (1966) for species of a flightless beetle genus. Isolation has been so effective that these beetles have evolved morphological subspecies in each of the isolated mountain ranges. A comparison with C. herbarum suggests a different time scale for speciation events between winged and wingless insect species in southeastern Arizona mountains. Ball (1965)

suggests that eight to ten thousand years are required for the evolution of morphological subspecies from isolated beetle populations. During this same time in this same area, the dipteran C. herbarum populations as a group have diverged morphologically and genetically from the Colorado populations and distinct races have been produced between mountain ranges and possibly between biotic provinces.

Estimates of genetic distance between species in different phyla are extremely variable (King and Wilson 1975). However, genetic distance estimates between northern and southern populations of C. herbarum are two to three times greater than between allopatric subspecies of Drosophila (Ayala et al. 1974). Genetic distance estimates suggest semispecies or sibling species differences between regions, but the hybrid nature of the New Mexico populations and the absence of reproductive isolation tests contraindicate the species level classification. C. herbarum has completed the first steps toward speciation and has reached the level of geographic race formation.

Peripheral isolation of Arizona populations may not be unique to C. herbarum. The process of geographical speciation may be completed for C. fumicosta. This species is known only from Arizona, but recently specimens similar to it but larger have been seen from central Mexico. It is tempting to speculate that an ancestral species was

distributed from Central America to southern Arizona mountains. Present C. fumicosta populations may represent once peripheral populations isolated from the ancestral range.

If the rate of evolution is determined by the amount of genetic variation in populations and the intensity of selection among genotypes, the family Chamaemyiidae should be characterized by rapid evolutionary change.

CHAPTER 7

COMPARISON OF TECHNIQUES

Both electrophoretic and morphometric techniques measure the same gene pool. Morphometric and enzyme variation; however, diverge at different rates as evidenced in the L. ocellaris species group (Figure 4 and 5). Morphometrically the group of species are similar, but they show isozyme divergence. C. juncorum + C. herbarum and P. varipes + P. nitens are pairs of sibling species. Morphological similarity is not the product of genetic similarity.

Neither molecular enzyme evidence nor morphological evidence is more significant for measuring relationships between species. There are advantages and disadvantages in the use of each. Neither technique by itself can be used to establish species relationships unequivocally. The D^2 estimate consistently separated species such as C. fumicosta, L. bivittata, and L. sp. 82 on the basis of size. The genotype-environment interactions evident in population comparisons could result in convergence between species. The reliability of species relationship identification by the electrophoretic analysis of protein variation should increase as the number of loci is increased. In

addition to increasing the number of loci, a random sample of functional enzymes should be used. The discriminant analysis of morphometric characters usually used only selected characters to identify species; therefore, increasing the number of characters may not necessarily increase the reliability of distance estimates between species.

Isozyme data can be and are applied to difficult taxonomic problems and can be very useful at this level. Ayala (1973) has described two new subspecies of Drosophila willistoni on isozymes alone, an interesting exercise, but not particularly useful to other ecologists or taxonomists. Two species of tephritid flies are similar morphologically, but differ in isozyme frequency and karyotype (Bush and Boller 1977). Others are similar both morphologically and karyotypically, but have consistently different isozyme frequencies. These isozyme races differ in host preference and may be incipient species (Bush and Boller 1977). Relating isozyme variation to ecological factors can indicate evolution via ecological speciation. Some of the ecological implications of chamaemyiid isozyme variation have been discussed, such as cold tolerance-PGM, and flight activity or ability - α-GPDH.

Morphometrics may be more readily available to taxonomists because it measures phenotypic variation from

pinned specimens. Living specimens and expensive chemicals or special equipment are not required. Discriminant analysis can be used for identification purposes, giving a probability of correct assignment, as well as indicating the presence or absence of significant differences between groups. It also provides a distance statistic for indicating species relationships.

Morphometric variation could discriminate populations of C. herbarum. Mahalanobis D^2 estimates based on these measurements did differentiate northern and southern populations (Figure 16). The isozyme frequency differences between northern and southern populations (Figure 14) were more consistent. Electrophoretically measured enzyme variation is a more useful tool for studying population differentiation and the effects of isolation, genetic drift, or natural selection. Electrophoresis measures the variation of a specific gene product. Isozyme frequencies can change rapidly in response to selection. Morphometric variation data are obtained from phenotypic characters. Phenotypic variation is due to both genetic and environmental variation. Nongenetic phenotypic variation may buffer the force of selection on the genotype. Therefore, morphometric variation may be less useful for studying the genetic differentiation of populations or the effects of natural selection. The morphometric characters which discriminated

between populations (eye length, mesonotum length, cheek width, medial-cubital vein length and radial-medial vein length) are the characters most often used to discriminate between species and genera (Table 16). Morphometrics may be more useful for measuring relationships between higher taxonomic levels.

APPENDIX A

COMPUTER PROGRAM

LISTINGS

```
C THE DENDROGRAPH

      PROGRAM DKGRAF(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT,TAPE16)
      REAL MATR,NAME
      INTEGER SEARCH
      COMMON MATR(820),NGR(40),NN(40),NOM(40),WIGR(40),
     1BEGR(40),NP(40),IORD(40),NAME(2,40),ALIN(40),A(8),FMT(8),
     2T1(8),T2(8)
C     1BEGR(200),NP(200),IORD(200),NAME(4,200),ALIN(200),A(18),FMT(18)
C 999 READ(5,10)          M,KLAM,(A(I),I=1,18)
      CALL INITIAL(8H23001561,10,0.5,0,0)
  999 READ(5,10)          M,KLAM,(A(I),I=1,8)
      IF(EOF(5)) 100,2
C     MM=M
    2 MM=M
      READ(5,20) T1,T2
      MT=M
      M=IABS(M)
      READ(5,40) AENG,AHI,FNCR
C     READ(5,30)((NAME(I,J),I=1,4),J=1,M)
      READ(5,30)((NAME(I,J),I=1,2),J=1,M)
C     READ(5,20)(FMT(J),J=1,18)
      READ(5,20)(FMT(J),J=1,8)
C     WRITE(6,11)(A(I),I=1, 8),M
      WRITE(6,11) A,T1,T2,M
      DO 15 J=1,M
      JJ=(J*(J-1)) /2
      WIGR(J)=0.0
      BEGR(J)=0.0
      NN(J)=J
      NP(J)=J
      NOM(J)=1
      NGR(J)=J
      IORD(J)=0
C
C      LOWER HALF OF MATRIX IS READ ROW WISE FROM LEFT TO RIGHT
C
   15 READ(5,FMT)(MATR(JJ+K),K=1,J)
      WRITE(6,91)
  102 DO 25 I=1,M
      JJ=(I*(I-1))/2
   25 WRITE(6,21) I,(MATR(JJ+J),J=1,I)
      IF(MM.GT.0) GO TO 107
      MM=(M*(M+1))/2
      DO 105 I=1,MM
      IF(MATR(I).GT.1.) MATR(I)=1.
  105 MATR(I)=ACOS(MATR(I))
      WRITE(6,101)
      GO TO 102
  107 MH=M+1
      ML=M-1
C
C      MAIN LOOP FOR CALCULATING CLUSTERS
C
      DO 95 MAIN=1,ML
      LCT=MH-MAIN
      LCM=LCT-1
      AMIN=100000.
```

```
C
C      MATRIX IS SEARCHED FOR SMALLEST WITHIN GROUP MEASURE
C
      DO 35 I=1,LCM
      K=I+1
      IMA1=NN(I)+(NN(I)*(NN(I)-1))/2
      DO 35 J=K,LCT
      IT=(NN(J)*(NN(J)-1))/2
      IMA2=NN(I)+IT
      IMA3=NN(J)+IT
      LA=NOM(I)+NOM(J)
      ANOM=(LA*(LA-1))/2
      TEMP=(MATR(IMA1)+MATR(IMA2)+MATR(IMA3))/ANOM
      IF(TEMP.GT.AMIN) GO TO 35
      ITI=I
      ITJ=J
      AMIN=TEMP
   35 CONTINUE
      LA=NOM(ITI)+NOM(ITJ)
C
C      BEGINNING AND ENDS OF GROUPS TO BE JOINED ARE FOUND BY SEARCH
C
      JKL=NN(ITI)
      IBEG1=SEARCH(NGR,JKL)
      JKL=NN(ITJ)
      IBEG2=SEARCH(NGR,JKL)
      IEND1=IBEG1+NOM(ITI)-1
      IEND2=IBEG2+NOM(ITJ)-1
      IT=(NN(ITJ)*(NN(ITJ)-1))/2
      IMA1=NN(ITI)+(NN(ITI)*(NN(ITI)-1))/2
      IMA2=NN(ITI)+IT
      IMA3=NN(ITJ)+IT
      JKL=NOM(ITI)*NOM(ITJ)
      BEGR(IBEG2)=MATR(IMA2)/FLOAT(JKL)
      WIGR(IBEG2)=AMIN
      IORD(IBEG2)=MAIN
      TEMP=MATR(IMA1)+MATR(IMA2)+MATR(IMA3)
C
C      OUTPUT ARRAYS ARE SHIFTED INTO THE NEW ORDER
C     THE RIGHTMOST GROUP IS SHIFTED LEFT
C
      LDIF=IBEG2-IEND1-1
      KLIM=NOM(ITJ)
      IF(LDIF) 77,17,18
   18 DO 45 I=1,KLIM
      DO 45 J=1,LDIF
      KS=IBEG2+I-J
      MST=NGR(KS)
      NGR(KS)=NGR(KS-1)
      NGR(KS-1)=MST
      MST=NP(KS)
      NP(KS)=NP(KS-1)
      NP(KS-1)=MST
      MST=IORD(KS)
      IORD(KS)=IORD(KS-1)
      IORD(KS-1)=MST
      GW=WIGR(KS)
      WIGR(KS)=WIGR(KS-1)
      WIGR(KS-1)=GW
      GW=BEGR(KS)
```

```
      BEGR(KS)=BEGR(KS-1)
   45 BEGR(KS-1)=GW
   17 IF(NOM(ITI)-NOM(ITJ)) 131,141,141
  141 KNO2=NOM(ITJ)
      KS=IEND1+1
      GO TO 78
  131 KNO1=NOM(ITI)
      KNO2=NOM(ITJ)
      DO 125 I=1,KNO1
      DO 125 J=1,KNO2
      KS=IEND1 + J - I
      MST=NP(KS+1)
      NP(KS+1)=NP(KS)
      NP(KS)=MST
      MST=IORD(KS+1)
      IORD(KS+1)=IORD (KS)
      IORD(KS)=MST
      GW=WIGR(KS+1)
      WIGR(KS+1)=WIGR(KS)
      WIGR(KS)=GW
      GW=BEGR(KS+1)
      BEGR(KS+1)=BEGR(KS)
  125 BEGR(KS)=GW
      KS=IBEG1+KNO2
      IORD(KS)=IORD(IBEG1)
      WIGR(KS)=WIGR(IBEG1)
      BEGR(KS)=BEGR(IBEG1)
      IORD(IBEG1)=0
      WIGR(IBEG1)=0.0
      BEGR(IBEG1)=0.0
      KNO2=NOM(ITI)
C
C       IF THE NEW RIGHT GROUP HAS MORE THAN TWO ELEMENTS
C          ITS ORDER IS REVERSED
C
   78 IF(KNO2.LE.2) GO TO 111
      KNO1=KNO2/2
      KS=KS-1
      IEND2=IBEG1+NOM(ITI)+NOM(ITJ)
      DO 135 I=1,KNO1
      MST=NP(IEND2-I)
      NP(IEND2-I)=NP(KS+I)
      NP(KS+I)=MST
      MST=IORD(IEND2-I)
      IORD(IEND2-I)=IORD(KS+I+1)
      IORD(KS+I+1)=MST
      GW=BEGR(IEND2-I)
      BEGR(IEND2-I)=BEGR(KS+I+1)
      BEGR(KS+I+1)=GW
      GW=WIGR(IEND2-I)
      WIGR(IEND2-I)=WIGR(KS+I+1)
      WIGR(KS+I+1)=GW
  135 CONTINUE
C
C        LATEST GROUP IS PARTIALLY RELABELED IN *NGR*
C
  111 DO 55 I=1,KLIM
   55 NGR(IEND1+I)=NN(ITI)
C
C          ROW AND COLUMN NN(ITJ) ARE ADDED ON TO ROW AND COLUMN NN(ITI)
```

```
C
      DO 75 I=1,M
      KLA=IPOS(NN(ITI),I)
      KLE=IPOS(NN(ITJ),I)
   75 MATR(KLA)=MATR(KLA)+MATR(KLE)
      MATR(IMA1)=TEMP
      LDIF=LCT-ITJ
      IF(LDIF) 77,27,28
   28 DO 85 I=1,LDIF
      NN(ITJ+I-1)=NN(ITJ+I)
   85 NOM(ITJ+I-1)=NOM(ITJ+I)
   27 NOM(ITI)=LA
      IF(KLAM.EQ.0) GO TO 95
      KS=MAIN-(MAIN/KLAM)*KLAM
      IF(KS.NE.0) GO TO 95
      WRITE(6,31) MAIN
      WRITE(6,61) NP(1),NGR(1)
      DO 65 J=2,M
      WRITE(6,71) IORD(J),WIGR(J),BEGR(J)
   65 WRITE(6,61) NP(J),NGR(J)
      WRITE(6,41)(NN(I),I=1,LCM)
      WRITE(6,41)(NOM(J),J=1,LCM)
   95 CONTINUE
C
C      ********      END OF MAIN LOOP      ********
C
      WRITE(6,121)
      KS=NP(1)
C     WRITE(6,81)(NAME(L,KS),L=1,4),KS
      WRITE(6,81)(NAME(L,KS),L=1,2),KS
      DO 205 I=2,M
      WRITE(6,181) IORD(I),WIGR(I),BEGR(I)
      KS=NP(I)
C 205 WRITE(6,81)(NAME(L,KS),L=1,4),KS
  205 WRITE(6,81)(NAME(L,KS),L=1,2),KS
      WRITE(6,1)
C     CALL DENDRO(WIGR,BEGR,NP,IORD,NAME,ALIN,M,FNCR,AENG,AHI,MT,A)
      CALL DENDRO(WIGR,BEGR,NP,IORD,NAME,ALIN,M,FNCR,AENG,AHI,MT,A,
     $T1,T2)
      CALL PLOT(AENG+5.0,0.0,-3)
      GO TO 999
   77 WRITE(6,51) LDIF
      GO TO 999
C 100 STOP
  100 CALL PLOT(0.,0.,999)
      STOP
    1 FORMAT(*1*)
C   10 FORMAT(2I4,18A4)
   10 FORMAT(2I4,7A10,A2)
C   11 FORMAT(*1*,30X,18A4,//* NUMBER OF GROUPS IS *,I3)
C   11 FORMAT(*1*30X,7A10,A2,//* NUMBER OF GROUPS IS *I3)
   11 FORMAT(*1*30X,7A10,A2,/,2(30X,8A10,/),* NUMBER OF GROUPS IS *I3)
C   20 FORMAT(18A4)
   20 FORMAT(8A10)
   21 FORMAT(*0*,I3,(1X,16F8.4))
C   30 FORMAT(20A4)
C   30 FORMAT( 5(A10,A6))
C   30 FORMAT(A10,A6,64X)
   30 FORMAT(2A10,60X)
   31 FORMAT(*1 CLUSTERING STAGE NO. *I3,//* NP*5X,*NGR ORD*,4X,*WGR
```

```
     1    BGR*)
    40 FORMAT(2F10.3,F5.2)
    41 FORMAT(*0*,33I4)
    51 FORMAT(*0 LDIF = *I4)
    61 FORMAT(* *,I3,5X,I3)
    71 FORMAT(* *,12X,I3,2F8.4)
C   81 FORMAT(* *,4A4,I4)
    81 FORMAT(* *A10,A6,I4)
    91 FORMAT(*0*,20X,*ORIGINAL INPUT MATRIX  (LOWER HALF BY ROWS)*)
   101 FORMAT(*1 TRANSFORMED INPUT MATRIX*)
   121 FORMAT(*1*,30X,*FINAL RESULTS*//* NAME*,14X,*NP*,5X,*ORDER*,5X,
     1*WGR      BGR*)
   181 FORMAT(* *,27X,I3,2X,2F8.4)
       END
       INTEGER FUNCTION IPOS(IA,IB)
       IF(IA.GT.IB) GO TO 1
       IPOS=IA+(IB*(IB-1))/2
       GO TO 2
     1 IPOS=IB+(IA*(IA-1))/2
     2 RETURN
       END
       INTEGER FUNCTION SEARCH(NAR,NINT)
       DIMENSION NAR (1)
       DO 5 J=1,200
       IF(NINT.EQ.NAR(J)) GO TO 10
     5 CONTINUE
       J=-1
    10 SEARCH=J
       RETURN
       END
       SUBROUTINE DENDRO(WIGR,BEGR,NP,IORD,NAME,ALIN,M,FNCR,AENG,AHI,MT,
      $A,T1,T2)
C      1A)
       REAL NAME
       INTEGER SEARCH
       DIMENSION WIGR(1),BEGR(1),NP(1),IORD(1),NAME(2,1),ALIN(1),A(1),
      $T1(1),T2(1)
       MM=M-1
       IORD(1)=M+1
       IORD(M+1)=M+1
       AENG=AENG-1.
C
C        SCALE X-AXIS IN INCHES
C
       DO 15 J=3,M
    15 BEGR(J)=BEGR(J)+BEGR(J-1)
       SCAX=AENG/BEGR(M)
       UPI=BEGR(M)/AENG
       DO 25 J=1,M
    25 BEGR(J)=BEGR(J)+UPI/2.0
       TOTX=BEGR(M)+UPI/2.0
       DO 35 J=1,M
    35 BEGR(J)=BEGR(J)*SCAX
C
C         SCALE Y-AXIS IN INCHES
C
       IF(MT.LT.0) TOTY=1.-COS(WIGR(IH))
       SCAY=AHI/TOTY
       TOTY=WIGR(IH)
       IH=SEARCH(IORD,MM)
```

```
      DO 45 I=1,M
      IF(MT.LT.0) GO TO 44
      WIGR(I)=WIGR(I)*SCAY
      GO TO 45
   44 WIGR(I)=(1.-COS(WIGR(I)))*SCAY
   45 CONTINUE
C
C     SET X-AXIS LIMIT
C
      XLEN=48.
C     CALL LIMIT(XLEN)
C
C     SET ORIGIN
C
      XORG=0.
      YORG=2.
C
C     PRINT TITLE
C
C     X=XORG-2.
      X=XORG-1.5
C     Y=.5
      Y=0.1
C     CALL SYMBOL(X,Y,.125,A(1),90.,72)
      CALL SYMBOL(X,Y,.200,A(1),90.,72)
C    DRAW THE SUBTITLES
      X=XORG-1.1
      CALL SYMBOL(X,Y,0.125,T1(1),90.,80)
      X=XORG-0.85
      CALL SYMBOL(X,Y,0.125,T2(1),90.,80)
C
C     DRAW IN BOUNDARY LINES
C
      CALL PLOT(XORG,YORG,3)
      X=XORG+AENG+1.
      CALL PLOT(X,YORG,2)
      Y=YORG+AHI+.4
      CALL PLOT(X,Y,2)
      CALL PLOT(XORG,Y,2)
      CALL PLOT(XORG,YORG,2)
C
C     PEN NOW LOCATED AT ORIGIN
C
C
C     PLACE TIC MARKS ALONG X-AXIS
C
      IAX=TOTX
      AX=XORG
      DO 145 I=1,IAX
      AX=AX+SCAX
      Y1=YORG+.06
      Y2=YORG-.07
      CALL PLOT (AX,Y1,3)
  145 CALL PLOT(AX,Y2,2)
C
C     LABEL AND PLACE TIC MARKS ALONG Y-AXIS
C
      IF(MT.LT.0) GO TO 36
      IF(FNCR.EQ.0.) FNCR=1.
      NTOT=TOTY/FNCR
```

```
      FNMB=0.
      FDIS=FNCR*SCAY
      GO TO 37
   36 IF(FNCR.EQ.0.) FNCR=.1
      NTOT=TOTY/FNCR
      FDIS=FNCR*SCAY
      FNMB=1.
      FNCR=-FNCR
   37 X=XORG-.425
      Y=YORG-.125
      CALL NUMBER(X,Y,.125,FNMB,0.,1)
      AI=FNMB
      AY=YORG
      IF(NTOT.EQ.0) GO TO 27
      DO 85 I=1,NTOT
      AI=AI+FNCR
      AY=AY+FDIS
      X1=XORG+.125
      X2=XORG-.125
      CALL PLOT(X1,AY,3)
      CALL PLOT (X2,AY,2)
      AYY=AY-.125
      X=XORG-.425
   85 CALL NUMBER(X,AYY,.125,AI,0.,1)
C
C     PRINT OBJECT NAMES
C
   27 DO 55 I=1,M
      AX=XORG+BEGR(I)
      IL=NP(I)
C     Y=YORG-2.54
C  55 CALL SYMBOL(AX,Y,.125,NAME(1,IL),90.,16)
C  55 CALL SYMBOL(AX,0.0,0.125,NAME(1,IL),90.,16)
   55 CALL SYMBOL(AX,0.1,0.090,NAME(1,IL),90.,20)
      DO 65 I=1,M
   65 ALIN(I)=0.0
C
C     GENERATE DENDOGRAPH
C
      DO 75 I=1,MM
      IH=SEARCH(IORD,I)
C   THIS IS ADDED TO BRING THE YORG DOWN 0.54 INCH
C     YORG=YORG-0.54
      BAR=YORG+ALIN(IH)
      BAL=YORG+ALIN(IH-1)
      TOP=YORG+WIGR(IH)
      SR=XORG+BEGR(IH)
      SL=XORG+BEGR(IH-1)
      CALL PLOT (SR,BAR,3)
      CALL PLOT(SR,TOP,2)
      CALL PLOT(SL,TOP,1)
      CALL PLOT(SL,BAL,1)
      K=IORD(IH)
      J=IH
   67 J=J+1
      IF(K-IORD(J)) 68,67,67
   68 IEND=J-1
      J=IH
   77 J=J-1
      IF(K-IORD(J)) 78,77,77
```

```
      78 IBEG=J
         ALIN(IBEG)=WIGR(IH)
         ALIN(IEND)=WIGR(IH)
         CENT=(BEGR(IH)+BEGR(IH-1))/2.0
         BEGR(IBEG)=CENT
      75 BEGR(IEND)=CENT
C        CALL ENDPLT
         RETURN
         END
7/8/9
  11   OE SQUARE ESTIMATE OF GENETIC DISTANCE
BETWEEN 11 SPECIES OF CHAMAEMYIIDAE
BASED UPON SIX ENZYME LOCI
       9.0       6.0       0.0
C HERBARUM
C FUMICOSTA
C JUNCORUM
PVARIPES
P NITENS
P SP3
L OCELLARIS
L BELLULA
L SP82
L SPE
L SPH
(12F7.4)
00.0000
  .1429  .0000
  .2233  .3039  .0000
  .2319  .2852  .1906  .0000
  .2133  .2519  .1822  .0887  .0000
  .2552  .2735  .2039  .1503  .0573  .0000
  .2362  .3070  .2310  .1854  .2620  .3060  .0000
  .2962  .2984  .3002  .3391  .2792  .3214  .3044  .0000
  .2773  .2977  .2987  .2604  .2582  .2514  .2089  .2671  .0000
  .3081  .3442  .3480  .2605  .2840  .3048  .2340  .2980  .2414  .0000
  .3439  .3766  .3718  .2776  .3182  .3686  .2764  .3152  .2653  .1028  .000
7/8/9
6/7/8/9
```

```
C  PROGRAM FOR AVERAGE HETEROZYGOSITY AND GENETIC DISTANCE
C  NO. OF SUBPOPULATIONS  = M
C  NO OF POLYMORPHIC LOCI IN EACH POPULATION = N
C  NO OF MONOMORPHIC LOCI IN EACH POPULATION =RNM
C  TOTAL NO. LOCI STUDIED = RN
C  NO OF ALLELES IN ITH LOCUS = NN(I)
C  X(I,J,K) = GENE FREQUENCY OF KTH ALLELE OF ITH LOCUS IN JTH SUBPOPULATIO
C  CN(I,J) = NO. OF ALLELES OF ITH LOCUS IN JTH SUBPOPULATION
C
      PROGRAM NEI GS(INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION NN(20),X(20,15,15),CN(20,15),SX(20,15,15),DX(20,15,15),
     1 TX(15,15),TXX(15,15),TXXY(15,15),TXYY(15,15),TXXYY(15,15),
     2TM(15,15),DM(15,15),VHX(15),SHX(15),D(20,15,15),DMI(15,15),
     3AVH(15),TDM(15,15),VDM(15,15),SDM(15,15),SST(15,15),CST(15,15),
     4VST(15,15),VHXY(15,15),CHXXY(15,15),CHXYY(15,15),CHXY(15,15)
      DIMENSION NAME(20),NAME1(20)
C
      INTEGER ZFMT2(6),NUM(15)
      DATA ZFMT2/4H(2X,,4H     ,10H(4HPOP= I2,10H,2X))     /
      DATA NUM/4H   1,4H   2,4H   3,4H   4,4H   5,4H   6,4H   7,4H   8,
     14H   9,4H  10,4H  11,4H  12,4H  13,4H  14,4H  15/
C
  700 READ (5,790) NAME, NAME1
  790 FORMAT (20A4)
      IF  (EOF(5)) 800,998
  998 READ (5,1) M, N, RNM, RN, (NN(I), I=1,N)
    1 FORMAT(2I2,2F2.0/40I2)
      WRITE (6,100) NAME,NAME1,M, N, RNM, RN
  100 FORMAT (5X,*POPULATION NAME IS*,1X,20A4,//,5X,*SYSTEM STUDIED IS*,
     11X,20A4,//5X,*NO.OF SUBPOPULATION M = *,I3,/,5X,*NO.OF POLYMORPH*,
     2 *IC LOCI(N)=*,I3,/,5X,*NO. OF MONOMORPHIC LOCI(RNM)=*,F3.0,/,5X,
     3*TOTAL NO.OF LOCI(RN)=*,F3.0)
C
      DO 40 I=1,N
      M1=NN(I)
      DO 20 J=1,M
      READ(5,2)  (X(I,J,K),K=1,M1),CN(I,J)
    2 FORMAT (8F10.0)
   20 CONTINUE
   40 CONTINUE
C
      ZNN = RN*(RN-1.)
C
      DO 45 J =1,M
      DO 45 J1 = 1,M
      TDM(J,J1) = 0.
      TX(J,J1) = 0.
      TXX(J,J1) = 0.
      TXXY(J,J1) = 0.
      TXYY(J,J1) = 0.
      TXXYY(J,J1) = 0.
   45 CONTINUE
C
      DO 50 I=1,N
      M1 = NN(I)
      DO 50 J=1,M
      DO 50 J1=1,M
      SX(I,J,J1) = 0.0
      DO 48 K = 1,M1
```

```
   48 SX(I,J,J1) = SX(I,J,J1) + X(I,J,K)*X(I,J1,K)
      DX(I,J,J1) = 1. - SX(I,J,J1)
   50 CONTINUE
C
      DO 70 I=1,N
      DO 70 J=1,M
      J2=J
      DO 60 J1=1,J2
      TX(J,J1) = TX(J,J1) + SX(I,J,J1)
      TXX(J,J1) = TXX(J,J1) + SX(I,J,J1)*SX(I,J,J1)
      TXXY(J,J1) = TXXY(J,J1) + SX(I,J,J1)*SX(I,J,J)
      TXYY(J,J1) = TXYY(J,J1) + SX(I,J,J1)*SX(I,J1,J1)
      TXXYY(J,J1) = TXXYY(J,J1) + SX (I,J,J)*SX(I,J1,J1)
      TM(J,J1) = (TX(J,J1) + RNM)/RN
      DM(J,J1) = 1. - TM(J,J1)
   60 CONTINUE
C
C  AVERAGE HETEROZYGOSITY PER LOCUS OF JTH SUBPOPULATION
      AVH(J) = DM(J,J)
C  VARIANCE OF AVERAGE HETEROZYGOSITY
      VHX(J) = ((TXX(J,J) + RNM) - RN*TM(J,J)*TM(J,J))/ZNN
      SHX(J) = 0.
      IF(VHX(J) .GT. 0.) SHX(J) = SQRT(VHX(J))
   70 CONTINUE
      DO 80 I = 1,N
      DO 80 J = 2,M
      J2 = J-1
      DO 80 J1 = 1,J2
C MINIMUM GENETIC DISTANCE BET J AND J1 POPULATIONS
      D(I,J,J1) = DX(I,J,J1) - (DX(I,J,J) + DX(I,J1,J1))/2.
      DMI(J,J1) = DM(J,J1)-(DM(J,J)+DM(J1,J1))/2.
C  STANDARD GENTIC DISTANCE BET J AND J1 POPULATIONS
      DXX = -ALOG(TM(J,J))
      DYY = -ALOG(TM(J1,J1))
      DXY = -ALOG(TM(J,J1))
      SST(J,J1) = DXY - (DXX+DYY)/2.
C
C  VARIANCE OF MINIMUM GENETIC DISTANCE BET J AND J1 POPULATIONS
      TDM(J,J1) = TDM(J,J1) +D(I,J,J1)*D(I,J,J1)
      VDM(J,J1) = (TDM(J,J1) - RN*DMI(J,J1)*DMI(J,J1))/ZNN
      SDM(J,J1) = 0.
      IF (VDM(J,J1) .GT. 0.) SDM(J,J1)= SQRT(VDM(J,J1))
C
C  VARIANCE OF STANDARD GENETIC DISTANCE BET J AND J1 POPULATIONS
      VMXY(J,J1) = ((TXX(J,J1)+RNM) - RN*TM(J,J1)*TM(J,J1))/ZNN
      CMXXY(J,J1)=((TXXY(J,J1)+RNM) -RN*TM(J,J1)*TM(J,J))/ZNN
      CMXYY(J,J1)=((TXYY(J,J1)+RNM) -RN*TM(J,J1)*TM(J1,J1))/ZNN
      CMXY(J,J1)=((TXXYY(J,J1)+RNM)-RN*TM(J,J)*TM(J1,J1))/ZNN
      VST(J,J1) = VHX(J)/(4.*TM(J,J)**2)+VHX(J1)/(4.*TM(J1,J1)**2.)+
     1  VMXY(J,J1)/(TM(J,J1)**2.)+CMXY(J,J1)/(2.*TM(J,J)*TM(J1,J1))-
     2  CMXXY(J,J1)/(TM(J,J1)*TM(J,J))-CMXYY(J,J1)/(TM(J,J1)*TM(J1,J1))
      CST(J,J1) = 0.0
      IF(VST(J,J1) .GT. 0.) CST(J,J1) = SQRT(VST(J,J1))
   80 CONTINUE
C
      WRITE (6,105)
  105 FORMAT (1H1)
      WRITE (6,110)
  110 FORMAT (50X,*AVERAGE HETEROZYGOSITY PER LOCUS*,//)
      ZFMT2(2)=NUM(M)
```

```
      WRITE(6,ZFMT2) (I, I=1,M)
      WRITE(6,120)  (AVH(J), J=1,M)
  120 FORMAT (1HO,3X,14F9.4)
      WRITE(6,130)
  130 FORMAT(//,40X,*S.E.S. OF AVERAGE HETEROZYGOSITIES*,//)
      WRITE(6,120)  (SHX(J), J=1,M)
C
      WRITE(6,105)
      WRITE(6,140)
  140 FORMAT(2X,*MINIMUM GENETIC DISTANCE BET TWO POPULATIONS*,//)
      DO 150 J = 2,M
      J2 = J-1
  150 WRITE(6,160)  (DMI(J,J1),J1=1,J2)
  160 FORMAT(1H ,1X,14F9.5)
C
      WRITE(6,105)
      WRITE(6,170)
  170 FORMAT(2X,*S.E. OF MINIMUM GENETIC DISTANCE BET TWO POPULATIONS*,
     1 //)
      DO 180 J= 2,M
      J2 = J-1
  180 WRITE(6,160)  (SDM(J,J1), J1=1,J2)
C
      WRITE(6,105)
      WRITE(6,190)
  190 FORMAT(2X,*STANDARD GENETIC DISTANCE BET TWO POPULATIONS*,//)
      DO 200 J=2,M
      J2 = J-1
  200 WRITE(6,160)  (SST(J,J1),J1=1,J2)
C
      WRITE(6,105)
      WRITE(6,210)
  210 FORMAT(2X,*S.E. OF STANDARD GENETIC DISTANCE BET TWO POPULATION*
     1,//)
      DO 220 J = 2,M
      J2 = J-1
  220 WRITE(6,160)  (CST(J,J1), J1=1,J2)
      IF (M .GT. 16)  GO TO 999
C
      WRITE(6,105)
      WRITE(6,330)
  330 FORMAT(1X,*EACH BLOCK REPRESENTS GENE FREQUENCIES OF ONE LOCUS*,
     1 * OF EACH  POPULATION*,//)
      ZFMT2(2)=NUM(M)
      WRITE(6,ZFMT2) (I, I=1,M)
      DO 90 I = 1,N
      WRITE(6,305) I
  305 FORMAT (40X, *LOCUS=*,I3)
      M1 = NN(I)
      DO 89 K =1,M1
   89 WRITE(6,9)  (X(I,J,K), J=1,M)
    9 FORMAT(1X, 16(1X,F6.4,1X))
      WRITE(6,11)  (CN(I,J), J =1,M)
   11 FORMAT (1HO, 1X, 16(F7.0,1X))
   90 WRITE (6,102)
  102 FORMAT(//)
C
  999 WRITE(6,105)
      GO TO 700
  800 CONTINUE
```

```
          STOP
          END
7/8/9
     NEI NEW D VARIPES POP 1SANTA CATALINA 2KITT 3PINALENO 4CHIRI 5NITKIT 6NITSC
     AKA ALPHAGPDH PGM PUM
     06040004
     05020605
     .056      .921      .023      .000      .000      252.
     .031      .158      .724      .046      .041      196.
     .045      .409      .455      .045      .045      22.
     .026      .905      .064      .005      .000      440.
     .000      .023      .818      .159      .000      44.
     .029      .943      .029      .000      .000      70.
     .239      .761      448.
     .186      .814      140.
     .133      .867      30.
     .168      .832      440.
     .024      .976      126.
     .949      .051      78.
     .042      .778      .120      .049      .000      .011      284.
     .033      .767      .093      .087      .020      .000      150.
     .045      .636      .318      .000      .000      .000      22.
     .045      .768      .157      .028      .002      .000      508.
     .091      .819      .045      .045      .000      .000      44.
     .000      .214      .314      .414      .057      .000      70.
     .014      .095      .820      .071      .000      210.
     .018      .027      .875      .080      .000      112.
     .100      .300      .550      .050      .000      20.
     .006      .060      .785      .131      .018      380.
     .000      .068      .909      .023      .000      88.
     .000      .114      .800      .086      .000      70.
7/8/9
6/7/8/9
```

```
      PROGRAM ESQUARE(INPUT,OUTPUT,TAPE1=INPUT,PUNCH)
      COMMON FREQ(50,75),ALL(50),NAME(50),DIST(50,50)
      INTEGER Q,FMT(8),TOTALL,ALL
      REAL NUM

      KOUNT=61
    1 READ(1,10) NG,NL,TOTALL
      IF(EOF,1)99,5
    5 READ(1,10) (ALL(I),I=1,NL)
      READ(1,11) (NAME(I),I=1,NG)
   11 FORMAT(A10,70X)
      READ(1,12) FMT
   12 FORMAT(8A10)
   10 FORMAT(40I2,/,10I2)
      READ(1,FMT) ((FREQ(I,J),J=1,TOTALL),I=1,NG)
      PRINT 30,NG,NL,TOTALL,(ALL(I),I=1,NL)
   30 FORMAT(*1  E-SQUARE ALGORITHM OF EDWARD,S AND CAVALLI-SFORZA,S*,
     $//,*0 NUMBER OF GROUPS =*,I4,/,
     $*0 NUMBER OF LOCI =*I4,/,
     $*0 NUMBER OF ALLELES=*I4,/,
     $*0 NUMBER OF ALLELES IN EACH LOCUS=*50I2)
      PRINT 32,FMT
   32 FORMAT(*0 FORMAT USED TO READ FREQUENCIES =*,8A10)
      PRINT 31
   31 FORMAT(*  GROUP         ALLELE FREQUENCIES*)
      DO 34 I=1,NG
      PRINT133,NAME(I),(FREQ(I,J),J=1,TOTALL)
  133 FORMAT(1X,A10,15F8.4,(/,11X,15F8.4))
   34 CONTINUE
      IX=NG-1
      DO 1000 I=1,IX
      J2=I+1
      DO 900 J1=J2,NG
      ISTOP=SUM=SUMK=0.
      DO 800 J=1,NL
      SUMTOP=SUMBOT1=SUMBOT2=0.0
      ISTOP=ISTOP + ALL(J)
      Q=ISTOP-ALL(J)+1
      DO 700 M=Q,ISTOP
C REMOVE THIS CARD SET WHEN RUNNING
      SUMTOP=SUMTOP+SQRT(FREQ(I,M)*FREQ(J1,M))
      SUMBOT1=SUMBOT1+SQRT(FREQ(I,M)/FLOAT(ALL(J)))
      SUMBOT2=SUMBOT2+SQRT(FREQ(J1,M)/FLOAT(ALL(J)))
  700 CONTINUE
      NUM=8*(1-SUMTOP)
      BOT1=1+SUMBOT1
      BOT2=1+SUMBOT2
      DENOM=BOT1*BOT2
      ESQ=NUM/DENOM
      SUM=SUM + ESQ
      SUMK=SUMK + FLOAT(ALL(J)-1)
      IF(KOUNT .GT. 60)2,6
    2 PRINT 3
    3 FORMAT(*1 GROUP VS GROUP AT LOCUS"INCREMENT TO E-2" E-SQUARE*,
     $/,*  -----    -----    -----  --------------  --------*/)
      KOUNT = 0
    6 PRINT 4,I,J1,J,ESQ,SUM
    4 FORMAT(1X,I4,5X,I4,6X,I3,8X,F8.4,5X,F8.4)
      KOUNT = KOUNT + 1
```

```
  800 CONTINUE
      DIST(I,J1)=SUM/SUMK

  900 CONTINUE
 1000 CONTINUE
      DO 2000 M=1,NG
      DO 2500 N=1,NG
      DIST(N,M)=DIST(M,N)
 2500 CONTINUE
 2000 CONTINUE
      PRINT 20
   20 FORMAT(*1 DISTANCE MATRIX, LOWER TRIANGLE + MAIN DIAGONAL*,///)
      DO 600 I=1,NG
      PRINT 15, NAME(I),(DIST(I,J),J=1,I)
   15 FORMAT(1X,A10,15F8.5,(/,11X,15F8.5))
      PUNCH 16, NAME(I),(DIST(I,J),J=1,I)
   16 FORMAT(A10/,7F10.8,(/,8F10.8))
  600 CONTINUE
      GO TO 1
   99 STOP
      END
7/8/9
6/7/8/9
```

APPENDIX B

MEANS AND STANDARD DEVIATIONS OF MORPHOMETRIC CHARACTERS

Table B1. Means and Standard Deviations of 13 Morphological Characters in Species of Chamaemyia.

Identifications: ( ), standard deviation; 1, ocellar triangle base; 2, ocellar triangle side; 3, eye length; 4, eye height; 5, cheek width; 6, mesonotum length; 7, mesonotum width; 8, wing length; 9, radial-medial vein length; 10, medial-cubital vein length; 11, third antennal segment length, 12, third antennal segment height; 13, arista length.

	aridella	polystigma	herbarum	fumicosta	geniculata	paludosa
1.	0.140 (.011)	0.158 (.014)	0.130 (.015)	0.204 (.033)	0.107 (.016)	0.150 (.021)
2.	.107 (.014)	.110 (.015)	.100 (.015)	.142 (.029	.093 (.019)	.115 (.016)
3.	.459 (.025)	.551 (.052)	.465 (.027)	.746 (.066)	.428 (.041)	.491 (.035)
4.	.439 (.038)	.460 (.033)	.465 (.033)	.721 (.034)	.392 (.051)	.355 (.115)
5.	.146 (.017)	.128 (.019)	.118 (.009)	.163 (.022)	.135 (.019)	.194 (.026)
6.	.869 (.053)	.878 (.077)	.878 (.039)	1.358 (.082)	.671 (.101)	.878 (.058)
7.	.688 (.052)	.690 (.050	.750 (.046	1.246 (.564)	.536 (.080)	.533 (.188)
8.	3.000 (.310)	2.500 (.300)	2.798 (.792)	4.338 (.187)	2.451 (.282)	2.090 (.938)
9.	.079 (.004)	.080 (.003)	.065 (.012)	.125 (.026)	.080 (.040)	.025 (.087)
10.	.239 (.089)	.258 (.036)	.253 (.020)	.421 (.045)	.191 (.038)	.234 (.046)
11.	.190 (.031)	.222 (.029)	.180 (.018)	.252 (.053)	.188 (.026)	.204 (.024)
12.	.138 (.010)	.147 (.008)	.119 (.003)	.145 (.012)	.122 (.017)	.161 (.026)
13.	.479 (.045)	.473 (.052)	.435 (.050)	.713 (.091)	.403 (.073)	.482 (.042)

Table B2. Means and Standard Deviations of 13 Morphological Characters in Species of Pseudodinia.

Identifications: ( ), standard deviation; 1, ocellar triangle base; 2, ocellar triangle side; 3, eye length; 4, eye height; 5, cheek width; 6, mesonotum length; 7, mesonotum width; 8, wing length; 9, radial-medial vein length; 10, medial-cubital vein length; 11, third antennal segment length; 12, third antennal segment height; 13, arista length.

	nitens	varipes	P. sp.2	P. sp. 3	P. sp. 4
1.	0.138 (.019)	0.149 (.080)	0.128 (.009)	0.135 (.009)	0.118 (.015)
2.	.108 (.027)	.148 (.082)	.099 (.003)	.098 (.006)	.088 (.010)
3.	.480 (.039)	.487 (.052)	.443 (.027)	.521 (.050)	.517 (.023)
4.	.471 (.065)	.476 (.072)	.458 (.033)	.508 (.059)	.513 (.030)
5.	.097 (.008)	.101 (.020)	.101 (.003)	.088 (.010)	.098 (.006)
6.	.774 (.093)	.690 (.049)	.790 (.042)	.773 (.091)	.760 (.041)
7.	.621 (.058)	.634 (.064)	.590 (.029)	.600 (.067)	.591 (.036)
8.	2.538 (.197)	2.729 (.228)	2.436 (.138)	2.518 (.303)	2.044 (.108)
9.	.061 (.013)	.063 (.015)	.078 (.015)	.077 (.021)	.055 (.012)
10.	.196 (.022)	.186 (.022)	.198 (.014)	.181 (.026)	.192 (.010)
11.	.137 (.019)	.129 (.029)	.129 (.003)	.138 (.021)	.129 (.011)
12.	.131 (.012)	.138 (.023)	.131 (.003)	.126 (.019)	.115 (.016)
13.	.418 (.057)	.435 (.065)	.382 (.024)	.508 (.097)	.428 (.031)

Table B3. Means and Standard Deviations of 13 Morphological Characters in Species of Leucopis.

Identifications: ( ), standard deviation; 1, ocellar triangle base; 2, ocellar triangle side; 3, eye length; 4, eye height; 5, cheek width; 6, mesonotum length; 7, mesonotum width; 8, wing length; 9, radial-medial vein length; 10, medial-cubital vein length; 11, third antennal segment length; 12, third antennal segment height; 13, arista length.

	bellula	L.sp.82	bivittata	ocellaris	L.sp. H	pemphigae	pinicola
1.	0.188 (.015)	0.247 (.049)	0.259 (.027)	0.167 (.018)	0.133 (.018)	0.147 (.008)	0.105 (.012)
2.	.103 (.009)	.113 (.015)	.095 (.009)	.085 (.009)	.085 (.019)	.151 (.189)	.072 (.017)
3.	.388 (.029)	.530 (.109)	.494 (.057)	.440 (.054)	.405 (.052)	.443 (.028)	.367 (.023)
4.	.585 (.030)	.578 (.086)	.565 (.043)	.533 (.049)	.533 (.025)	.541 (.031)	.378 (.033)
5.	.116 (.021)	.201 (.020)	.151 (.011)	.128 (.023)	.133 (.014)	.140 (.010)	.093 (.010)
6.	.853 (.090)	1.059 (.103)	1.006 (.088)	.869 (.069)	.878 (.049)	.909 (.052)	.639 (.052)
7.	.694 (.086)	.829 (.062)	.725 (.084)	.654 (.054)	.646 (.033)	.690 (.029)	.483 (.081)
8.	2.163 (.190)	2.939 (.158)	2.511 (.129)	2.313 (.122)	2.418 (.140)	2.305 (.150)	1.773 (.144)
9.	.048 (.006)	.123 (.134)	.053 (.009)	.053 (.009)	.055 (.012)	.033 (.009)	.060 (.008)
10.	.218 (.031)	.282 (.067)	.240 (.033)	.218 (.021)	.225 (.020)	.215 (.021)	.142 (.019)
11.	.150 (.025)	.150 (.017)	.151 (.011)	.148 (.006)	.151 (.011)	.128 (.009)	.118 (.018)
12.	.168 (.023)	.178 (.019)	.151 (.011)	.174 (.023)	.153 (.009)	.160 (.015)	.125 (.012)
13.	.304 (.040)	.373 (.033)	.337 (.031)	.336 (.035)	.315 (.023)	.325 (.024)	.267 (.026)

Table B4. Means and Standard Deviations of 13 Morphological Characters in C. herbarum Populations.

Identifications: ( ), standard deviation; 1, ocellar triangle base; 2, ocellar triangle side; 3, eye length; 4, eye height; 5, cheek width; 6, mesonotum length; 7, mesonotum width; 8, wing length; 9, radial-medial vein length; 10, medial-cubital vein length; 11, third antennal segment length; 12, third antennal segment height; 13, arista length; AL, Allenspark; BA, Bailey; RA, Rampart; GU, Gunnison; RE, Redstone; CA, Cabresto; SA, Sante Fe; WH, White; SC, Santa Catalina; PI, Pinaleno.

	AL	BA	RA	GU	RE	CA	SA	WH	SC	PI
1.	0.121 (.016)	0.127 (.022)	0.140 (.028)	0.128 (.013)	0.124 (.017)	0.134 (.014)	0.142 (.013)	0.135 (.016)	0.140 (.013)	0.124 (.020)
2.	.088 (.015)	.090 (.013)	.094 (.016)	.092 (.014)	.089 (.010)	.088 (.010)	.090 (.010)	.093 (.013)	.096 (.014)	.090 (.013)
3.	.430 (.036)	.467 (.046)	.494 (.048)	.462 (.033)	.434 (.040)	.454 (.035)	.505 (.043)	.495 (.048)	.517 (.027)	.468 (.043)
4.	.411 (.040)	.436 (.037)	.434 (.043)	.447 (.043)	.417 (.037)	.426 (.035)	.452 (.039)	.458 (.035)	.472 (.034)	.444 (.044)
5.	.120 (.020)	.115 (.017)	.133 (.022)	.119 (.015)	.118 (.019)	.133 (.018)	.118 (.018)	.137 (.017)	.133 (.018)	.123 (.023)
6.	.730 (.057)	.761 (.073)	.804 (.080)	.830 (.073)	.738 (.059)	.747 (.067)	.829 (.083)	.794 (.088)	.823 (.067)	.779 (.072)
7.	.566 (.071)	.596 (.060)	.623 (.059)	.675 (.095)	.570 (.058)	.584 (.066)	.650 (.096)	.618 (.054)	.643 (.053)	.603 (.058)
8.	2.368 (.689)	2.693 (.245)	2.966 (.244)	2.773 (.562)	2.593 (.174)	2.671 (.208)	2.945 (.305)	2.887 (.309)	2.615 (.477)	2.623 (.161)
9.	.076 (.017)	.079 (.013)	.103 (.061)	.073 (.014)	.070 (.016)	.070 (.017)	.078 (.015)	.078 (.010)	.074 (.012)	.068 (.015)
10.	.221 (.025)	.248 (.049)	.196 (.077)	.244 (.031)	.216 (.030)	.222 (.030)	.243 (.033)	.249 (.030)	.219 (.023)	.227 (.062)
11.	.154 (.020)	.155 (.017)	.158 (.030)	.167 (.021)	.147 (.013)	.159 (.018)	.161 (.021)	.166 (.027)	.165 (.020)	.155 (.019)
12.	.120 (.017)	.131 (.015)	.136 (.016)	.121 (.010)	.119 (.017)	.129 (.017)	.136 (.015)	.134 (.021)	.138 (.015)	.138 (.013)
13.	.402 (.041)	.424 (.038)	.457 (.053)	.425 (.041)	.393 (.032)	.413 (.049)	.456 (.039)	.439 (.062)	.475 (.036)	.443 (.039)

APPENDIX C

ISOZYME GENOTYPES OF *C. HERBARUM* POPULATIONS

Table C1. Adenylate Kinase Isozyme Genotypes of *C. herbarum* Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	AK Genotypes								
Site	1-2	2-2	2-3	3-3	3-4	4-4	4-5	5-5	Total
AP						1		47 (46)	48
BA						2		24 (22)	26
RP				2	3	30 (28)		1	36
RS						2	2	44 (42)	48
GN								50 (50)	50
CB			1	4	4	8 (3)	3 (13)	22 (13)	42
SF				2	1	25 (16)	2 (19)	15 (6)	45
MG		1		7 (4)	4	8 (5)		1	21
WH				21 (21)	1	8		1	38
PL				37 (32)	3 (12)	6		1	47
SC	1	10 (7)	22 (28)	31 (28)					64

Table C2. Hexokinase Isozyme Genotypes of C. herbarum Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	HEX Genotypes							
Site	1-1	1-2	2-2	2-3	2-4	3-3	3-4	Total
AP			1	5		36 (35)		42
BA						20 (20)		20
RP		1	42 (40)	2		2		47
RS	4		1	2		40 (36)		47
GN				4		45 (45)	1	50
CB			11 (4)	3 (18)	2	28 (20)		44
SF	13 (5)	5 (18)	26 (17)	2		4		50
MG	3	1	39 (36)					43
WH	1	2	44 (43)					47
PL	1	2	55 (54)					58
SC		1	64 (64)					65

Table C3. Malic Acid Dehydrogenase Isozyme Genotypes of *C. herbarum* Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	MDH Genotypes									
Site	1-1	1-2	1-3	2-2	2-3	2-4	3-3	3-4	4-4	Total
AP		3	1	3	13 (15)		26 (25)	6 (4)		52
BA		2		3	9 (12)		5 (6)	3		22
RP				9 (6)	19 (23)		24 (22)	2		54
RS		3	1	5 (6)	33 (31)		36 (37)	5 (3)		83
GN	1	4	2	10 (10)	32 (33)	1	29 (28)	5 (4)		84
CB		2		4	14 (17)		24 (21)		1	45
SF				8 (6)	18 (18)	1	18 (15)	4	6	55
MG	2	7		44 (37)	6 (15)		6	2	2	69
WH	3	12 (12)		19 (19)	5 (5)		1			40
PL	4	6 (4)	8 (15)	3	12 (16)	1	41 (36)	6 (5)		81
SC	2	5 (5)		23 (21)	21 (25)		11 (8)	1		63

Table C4 . Fumarase Isozyme Genotypes of C. herbarum Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	FUM Genotypes											
Site	1-1	1-2	1-3	2-2	2-3	2-4	3-3	3-4	3-5	4-4	4-5	Total
AP	4	2		8 (5)	16 (21)	1	28 (23)	4				63
BA			1		4		16 (16)	2	1	1		25
RP					4		8 (6)					12
RS	4	1			2		29 (25)	4 (5)	1	1		42
GN					3		34 (34)	3				40
CB				1	9 (7)		7 (9)	2	1			20
SF							2					2
MG				1	8 (8)		29 (29)	3				41
WH		1		1	4		32 (31)	1				39
PL				2	3		30 (26)	6 (10)	1	3	2	47
SC					3		16 (16)	3				23

Table C5. Acid Phosphatase Isozyme Genotypes of *C. herbarum* Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampark; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	ACPH Genotypes											
Site	1-1	1-2	1-3	2-2	2-3	2-4	3-3	3-4	3-5	4-4	4-5	5-5	Total
AP	3	1	6 (7)	9 (8)	25 (22)		15 (16)			1			60
BA		1	1	7 (7)	9 (9)		2	1					21
RP		1		2	13 (12)	1	23 (22)	8 (10)		3	1		68
GN		4	3	26 (23)	36 (42)		21 (19)	3					93
CB		1		7 (7)	16 (16)		8 (11)	3					35
SF					3		12 (13)	18 (17)	1	6 (6)	1	1	42
MG		2		2	8 (6)	1	13 (9)	14 (22)		20 (13)	1	1	62
WH							2		2	2	10 (11)	25 (23)	41
PL		1		19 (10)	21 (37)	2	44 (34)	3		1	1	1	93
SC		1		8 (5)	16 (14)	1	23 (14)	9 (7)		3			61

Table C6. Phosphoglucomutase Isozyme Genotypes of C. herbarum Populations.

Identifications: AP, Allenspark; BA, Bailey; RP, Rampart; RS, Redstone; GN, Gunnison; CB, Cabresto; SF, Santa Fe; MG, Mogollon; WH, White; PL, Pinaleno; SC, Santa Catalina; (number of genotypes expected by Hardy-Weinberg equilibrium).

	PGM Genotypes															
Site	1-1	1-2	1-3	1-4	2-2	2-3	2-4	2-5	3-3	3-4	3-5	4-4	4-5	5-5	5-6	6-6	Total
AP					1	6 (5)			22 (17)	4 (10)		6 (2)	1	2	1		43
BA					1	8 (4)	1		4 (3)	1		5 (2)		1	1	1	23
RP		5 (5)	2		22 (21)	14 (13)			2 (2)			2					47
RS					1	8 (7)			30 (27)	5 (11)		5					49
GN					2	9 (9)	1		23 (20)	8 (14)		7 (3)					50
CB	1	1			9 (5)	6 (10)	4		8 (6)	9 (8)		5 (3)	1	2			46
SF	1				27 (23)	17 (20)			6 (4)	2		1					54
MG		2			9 (4)	6 (10)	1		9 (6)	6 (7)	1	6 (2)		2			42
WH	1	2			18 (15)	15 (18)			8 (6)	1		2					47
PL	3	5 (4)		1	12 (6)	8 (15)		1	16 (10)	6 (8)	1	3		2			58
SC	2	1			34 (22)	5 (27)	1		21 (9)			1					65

APPENDIX D

ISOZYME GENOTYPES OF *P. VARIPES* AND *P. NITENS* POPULATIONS

Table D1. Alpha-glycerophosphate Dehydrogenase Isozyme Genotypes of *P. varipes* and *P. nitens* Populations.

Identifications: SC, Santa Catalina; KP Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; WH, White; HU, Huachuca; MG, Mogollon; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

Site	∝-GPDH Genotypes 1-1	1-2	2-2	Total
P. varipes				
SC				
1975	33 (7)	5 (56)	133 (107)	171
CUM	51	5	168	224
KP				
1974	3	3 (8)	46 (43)	52
CUM	8	3	57	70
CH				
1974	22 (7)	1 (31)	49 (34)	72
1975	9 (1)		111 (103)	120
CUM	36	2	182	220
PN				
CUM	2		13	15
P. nitens				
SA	37 (35)		2	39
KP		2	61 (60)	63
WH	7			7
HU	6	1	1	8
MG	5			5
CH	3			3

Table D2. Hexokinase Isozyme Genotypes of *P. varipes* and *P. nitens* Populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; WH, White; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	HEX Genotypes							
Site	1-1	1-2	1-3	2-2	2-3	2-4	3-3	Total
P. varipes								
SC								
1975		5		90	11		3	109
		(5)		(88)	(15)			
CUM		9		113	15		3	140
KP								
1974				28	2			30
				(28)	(2)			
CUM	2	5		42	3			52
CH								
1974	1	2		35	6	1		45
		(4)		(35)	(5)			
1975		2		114	26		8	150
				(109)	(36)		(3)	
CUM	4	8	1	174	39	2	11	239
PN								
CUM	1			1				2
P. nitens								
SA		4		30	1			35
		(4)		(30)				
KP				22				22
				(22)				
WH	1	3		3				7

Table D3. Fumarase Isozyme Genotypes of *P. varipes* and *P. nitens* Populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; WH, White; HU, Huachuca; MG, Mogollon; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	FUM Genotypes									
Site	1-2	1-3	2-2	2-3	3-3	3-4	4-4	4-5	5-5	Total
P. varipes										
SC										
1975	1	1	1	15 (15)	53 (53)	8 (8)	1			80
CUM	1	2	1	17	71	11	2			105
KP										
1975		2 (2)		3 (3)	37 (37)	5 (6)	1			48
CUM		2		3	43	7	1			56
CH										
1974				9 (7)	50 (47)	11 (16)	4	2	1	77
1975	1	1		12 (10)	49 (48)	18 (20)	3	3		87
CUM	1	1		22	123	29	8	5	1	190
PN										
CUM	1			4	3	1				10
P. nitens										
SA				8 (6)	21 (22)	6 (5)				35
KP			2	2 (5)	38 (36)	2 (2)				44
WH	1			2	4					7
HU					3					3
MG					1		1			2

Table D4. Adenylate Kinase A Isozyme Genotypes of *P. varipes* and *P. nitens* populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	AK-A Genotypes							
Site	1-2	2-2	2-3	3-3	3-4	4-4	4-5	Total
P. varipes								
SC								
1975	14 (13)	89 (89)	4	1				108
CUM	14	107	4	1				126
KP								
1974	3	4	3 (11)	68 (56)	1		8 (1)	87
CUM	6	11	3	69	1		8	98
CH								
1974	5 (4)	61 (56)		5		1		72
1975	7 (7)	127 (124)	10 (17)	4				148
CUM	12	188	10	9		1		220
PN								
CUM	1	3	2	4			1	11
P. nitens								
SA	2 (2)	32 (31)		1				35
KP			1	15 (15)	5 (6)	1		22

Table D5. Adenylate Kinase B Isozyme Genotypes of P. varipes and P. nitens Populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	AK-B Genotypes													
Site	1-2	2-2	2-4	3-3	3-4	3-5	3-6	4-4	4-5	4-6	5-5	5-6	6-6	Total
P. varipes														
SC														
CUM									3		2	1	4	10
KP														
CUM				1	2			16	13		7	4	1	44
CH														
CUM		2	1	3	12		1	14	6	1	8	5	11	64
PN														
CUM		1			1			1	7				1	11
P. nitens														
SA	1			1	1			14	2		3	2		24

Table D6. Malic Acid Dehydrogenase Isozyme Genotypes of P. varipes and P. nitens Populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; WH, White; HU, Huachuca; CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	MDH Genotypes												
Site	1-2	1-3	2-2	2-3	2-4	3-3	3-4	3-5	4-4	4-5	4-6	5-5	5-6	Total
P. varipes														
SC														
1975				2		3	15 (18)		46 (46)	12 (9)				78
CUM				4		9	[illegible]	2	49	20		6		102
KP														
1974			1	6 (3)	2	[illegible]	[illegible]	2	7 (9)	12 (9)		4		53
CUM			1	7	2	[illegible]	23	4	12	15		7		75
CH														
1974				1			11 (6)	5 (8)	5 (9)	28 (25)	1	17 (17)	2	70
1975			1	3		6	18 (27)	1	71 (65)	6 (6)				106
CUM			1	4		6	31	7	77	40	1	21	2	190
PN														
CUM				1		1	1	3	2	6		1		15
P. nitens														
SA	2	1	1	8 (7)	5 (5)	4 (7)	17 (11)	1	2			1		42
WH				1			9		1					11
HU								3	2	3		1		9

Table D7. Phosphoglucomutase Isozyme Genotypes of P. varipes and P. nitens Populations.

Identifications: SC, Santa Catalina; KP, Kitt Peak; CH, Chiricahua; PN, Pinaleno; SA, San Cristobal; WH, White; HU, Huachuca;CUM, cumulative; (number of genotypes expected by Hardy-Weinberg equilibrium).

	PGM Genotypes																	
Site	1-2	1-3	1-4	2-2	2-3	2-4	2-5	3-3	3-4	3-5	3-6	4-4	4-5	4-6	5-5	5-6	6-6	Total
P. varipes																		
SC																		
1975	1	1	1	1	4	1		70 (64)	20 (23)	4 (3)		4 (2)	1		4			112
CUM	1	1	1	3	4	1		95	21	5		5	1		4			142
KP																		
1974				1	1		1	35 (31)	8 (8)	1 (8)		1	1		4			53
CUM				1	1		2	51	11	1		1	1		4	1	1	75
CH																		
1974				3	4 (8)	4 (4)		31 (29)	29 (29)	4 (4)	1	8 (7)			1			85
1975				1	2 (4)	1		109 (101)	21 (35)	3 (8)		3 (4)	2		1			143
CUM				6	6	5		162	51	8	1	11	2		2			254
P. nitens																		
SA								3	1	7 (6)	1	8 (4)	5 (9)		8 (6)	1	1	35
KP				1	2 (3)			15 (15)	2 (2)	2 (2)								22
WH				2	1			3										6
HU				3	1			7 (3)	3	8 (8)	1	15 (7)	5 (11)		9 (5)	1	1	54

Table D8. Hexokinase Isozyme Genotypes from Seasonal Collections of P. varipes in the Chiricahua Mountains.

	HEX Genotypes				
Collection	1-2	2-2	2-3	3-3	Total
May 5/27/75	2 (2)[a]	45 (45)	7 (6)		54
July 7/28/75		50 (45)	14 (24)	8 (3)	72
September 9/13/75		26 (26)	5 (5)		31

[a]Number of genotypes expected by Hardy-Weinberg equilibrium.

Table D9. Adenylate Kinase A Isozyme Genotypes from Seasonal Collections of P. varipes in the Chiricahua Mountains.

	AK A Genotypes				
Collection	1-2	2-2	2-3	3-3	Total
May 5/27/75	1 (1)[a]	47 (45)	3 (7)	2	53
July 7/28/75	6 (6)	53 (53)	4 (4)		63
September 9/13/75		27 (25)	3 (6)	2	32

[a]Number of genotypes expected by Hardy-Weinberg equilibrium.

Table D10. Phosphoglucomutase Isozyme Genotypes from Seasonal Collections of P. varipes in the Chiricahua Mountains.

Collection	PGM Genotypes 2-2	2-3	2-4	3-3	3-4	3-5	4-4	4-5	5-5	Total
May 5/27/75	4		1	39 (35)[a]	10 (11)	2 (2)	1	1		58
July 7/28/75	1	1		56 (53)	6 (11)	1	3	1		69
September 9/13/75	1	1		19 (18)	5 (4)				1	27

[a] Number of genotypes expected by the Hardy-Weinberg equilibrium.

Table D11. Fumarase Isozyme Genotypes from Seasonal Collections of P. varipes in the Chiricahua Mountains.

Collection	FUM Genotypes 1-2	2-2	2-3	3-3	3-4	4-4	4-5	Total
May 5/27/75			3 (2)[a]	18 (17)	4 (6)	1	1	27
July 7/28/75	1	1	5 (6)	14 (14)	6 (5)		1	28
September 9/13/75			4 (3)	18 (17)	7 (9)	2	1	32

[a] Number of genotypes expected by the Hardy-Weinberg equilibrium.

Table D12. Alpha-glycerophosphate Dehydrogenase Isozyme Genotypes from Seasonal Collections of P. varipes.

Collection	∝-GPDH Genotypes 1-1	1-2	2-2	Total
Chiricahua				
May 7/28/75	8 (2)[a]	(13)	31 (25)	39
July 7/28/75			47 (47)	47
September 9/13/75	1		31 (30)	32
Santa Catalina				
May 5/16/75	24 (8)	4 (37)	60 (44)	88
July 7/29/75	9 (1)	1 (17)	73 (65)	83

[a] Number of genotypes expected by the Hardy-Weinberg equilibrium.

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