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Plethodon dunnii Bishop (Amphibia: Caudata: Plethodontidae)

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Life history attributes and apparent distributional patterns suggest that Plethodon dunnii exists as small, semi-isolated populations throughout its range. Isozyme and morphological variation in this species was examined to determine any possible geographic trends.

Starch gel electrophoresis was utilized to examine genetic variation at 24 isozyme loci in 287 individuals from 12 populations to determine the genetic structure of populations of this species. Low total genetic variability was observed. However, this variability is partitioned into relatively low genetic heterozygosity within populations and proportionally high diversity between populations. It is concluded that this geographically restricted species is characterized by small local populations with little gene flow. Comparisons with previous reports of Plethodon species indicate that the high genetic divergence between local populations is apparently general for species in this genus.

There is marked reduction in heterozygosity with peripheral populations of P. dunni, which may result from the reduction in genetic exchange from surrounding populations in peripheral as compared to central populations. No indication of additional structural genes associated with the increased DNA in P. dunni was found.

Morphological variation in 10 populations was examined via stepwise discriminant analyses. Considerable overlap of individuals' canonical scores and only moderately correct classifications into groups indicate that phenotypic expression in this species is exceedingly similar. However, on a fine scale there is significant heterogeneity between all populations. Generalized distance values (D^2) between the P. dunni populations are high, particularly when compared to those of Aneides ferreus, a wider-ranging, more ubiquitous sympatric species. Clinal variation in morphological divergence was observed and was highly correlated with a similar cline in allele frequencies of EST-2. It is concluded that the phenotypic structuring of populations of P. dunni is consistent with the structuring observed via electrophoresis and that this species is characterized by small, localized populations throughout its range.

Geographic Variation in Dunn's Salamander
Plethodon dunnii Bishop
(Amphibia: Caudata: Plethodontidae)

by

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GEOGRAPHIC VARIATION IN DUNN'S SALAMANDER, PLETHODON
DUNNI BISHOP (AMPHIBIA: CAUDATA: PLETHODONTIDAE)

I. Introduction

The following thesis consists of two distinct parts. This structure is primarily due to the preparation of each part as a separate manuscript. The parts are related, however, in that different tools are utilized to test my hypotheses concerning the distribution and structure of populations of Plethodon dunni.

In the first part the genetic structure of populations is examined in collaboration with Drs. Fred Allendorf and Charles Daugherty, Department of Zoology, University of Montana, Missoula, and Dr. Joseph Beatty of this department. We found, among other things, that the variability in this species, although low, is as much due to variation between populations as within.

In the second part I determine the levels of morphological differentiation between populations of P. dunni utilizing stepwise discriminant analyses. The results are consistent with the notion that significant heterogeneity exists in this species.

II. Genetic Structure of Populations of Plethodon dunnii Bishop

The amount and distribution of genetic variation within a species are fundamental evolutionary information. Gel electrophoresis provides the opportunity to assess this genetic variation. In the last decade electrophoretic studies have proliferated (see Lewontin, 1974; Ayala, 1976). However, only rarely has it been possible to examine in detail the genetic population structure of a species over its entire range.

Several authors have electrophoretically examined salamanders of the genus Plethodon (Highton and Webster, 1976; Highton, 1977; Feder et al., 1978). However, their goals were primarily systematic. We undertook this study in an attempt to examine the relative contributions of gene flow, effective population size, genetic drift, and natural selection to the genetic structure of populations of P. dunnii. This species is particularly suitable because populations tend to be extremely localized, exhibit limited dispersal, and are exceedingly sedentary, as is the case with many other plethodontid salamanders (Gordon, 1952; Huheey and Brandon, 1973; Highton, 1956; Fraser, 1976). These characteristics have important evolutionary implications. The restricted total range and the patchy distribution of populations should serve to alter the genetic

structure of P. dunni, i.e., to reduce the genetic diversity of the species as a whole and to increase inter-population divergence via the effects of genetic drift and natural selection. While it is difficult to distinguish the effects of these crucial evolutionary forces, we intend to examine variation within the species to test the above predictions (i.e., low total variability and high inter-population divergence).

Plethodon dunni inhabits rocky outcrops and stabilized talus slopes from sea level to approximately 1000 m (Dumas, 1956; Storm and Brodie, 1970). Although these favorable habitats are quite abundant throughout much of the Pacific Northwest, P. dunni occurs over only a limited area, primarily in the Coast and Cascade Ranges of western Oregon (Fig. 1). Storm and Brodie (1970) suggest that its absence from the Willamette Valley in Oregon is due to the paucity of suitable rocky substrates. However, it is absent from apparently suitable habitats and is less widely distributed than some other western plethodontids (see Stebbins, 1966). This distribution is probably the result of Pleistocene glacial events (see Armstrong et al., 1965) and reflects a contraction of what was probably once a much wider range that extended further into Washington and California.

The present restricted distribution of P. dunni provides the opportunity to sample a sufficiently large number

of populations to obtain a relatively complete description of the genetic variation across the entire range of a species. In this study we examine protein variation in 12 populations. We compare our results with relevant previous findings, particularly those of Highton and Webster (1976).

Two corollary considerations are also addressed in the present paper. First, the taxonomic status of Plethodon gordonii is reviewed and new evidence assessed. Second, the possible nature and function of the relatively large amounts of DNA in western Plethodon species are discussed.

MATERIALS AND METHODS

Samples

A sample of approximately 20 individuals was collected from each of 12 sites throughout the range of P. dunni between 19 November 1977 and 7 January 1978 (Fig. 1). At each site the animals were collected from the smallest possible area, often one rock face, to ensure that they could reasonably be considered to have come from only one randomly breeding population or deme. In the few instances where enough animals could not be obtained within a small area, samples from within a radius of a few km were combined after statistical examination for genetic homogeneity. Animals from the following sites (with abbreviations) were

used in this study; Abiqua Cr., SE of Silverton, Marion Co., OR (AB); Crabtree Cr., E. of Lacombe, Linn Co., OR (CR); Elk River Rd., 19.7 mi. E. of Hwy 101 Curry Co., OR (ER); Horsetail Falls, Multnomah Co., OR (HF); Humbug Mtn., Curry Co., OR (HM); Marys Peak, Benton Co., OR (MP); Nehalem River Rd., near Clatsop-Tillamook Co. line, OR (NR); 1.4 mi. SE of South Bend, Pacific Co., WA (SB); Siuslaw River Rd., 5.3 mi. S. of Junction of Wolf Cr., Douglas Co., OR (SI); confluence of Wolf Cr. and Siuslaw R., Lane Co., OR (WC); Smith River Road, E. of Vincent Cr. Guard Station, Douglas Co., OR (SM); West Fork Rd., 7.6 mi. from junction with Smith River Road, Douglas Co., OR (WF).

Electrophoresis

Whole animals were stored at -40°C or lower until use. Samples of liver and tail muscle from each individual were subjected to horizontal starch gel electrophoresis using the techniques of Utter et al. (1974) and Allendorf et al. (1977). Thirteen enzymes were examined for electrophoretically detectable variation. A locus was identified and chosen for use on the basis of consistency and clarity of resolution.

All results were obtained using one of two buffer systems described by Allendorf et al. (1977): Buffer A-- Gel: 0.03 M Tris, 0.0005 M citric acid, pH 8.5; Electrode: 0.06 M lithium hydroxide, 0.3 M boric acid, pH 8.1. Gels

were made using 99% gel buffer and 1% electrode buffer. Buffer B--Gel: 0.002 M citric acid, pH 6.0; Electrode: 0.04 M citric acid, pH 6.1. Both buffers were pH adjusted with n-(3-Aminopropyl)-morpholine.

Nomenclature

The nomenclature is that of Allendorf et al. (1977). A presumed genetic locus is designated in italics by the abbreviation of the enzyme, plus a hyphenated numeral for those enzymes coding for multiple loci (in order of increasing mobility). The most common allele at a locus is designated 100, representing its migration distance. Other alleles are identified by a numerical value representing their mobility relative to the common allele, i.e., MDH-2 (120) identified a variant allele at the second MDH locus which has a mobility 20% greater than the most common allele.

RESULTS

Genetic Variation

We examined genetic variation at 24 presumed loci (Table 1). Electrophoretic phenotypes occurred in accordance with the known enzymatic sub-unit structure and pattern of inheritance observed in other vertebrates (Utter et al., 1974): PGM and PMI showed the two-banded

heterozygous pattern expected of a monomer, while AGP, IDH, and MDH showed the three-banded heterozygous pattern expected of a dimer. EST-2 showed a two-banded homozygous pattern and a three-banded heterozygous pattern.

Genetic variation was detected at 7 of 24 loci examined: AGP, EST-2, IDH-1, MDH-1, MDH-2, PGM-1, PMI. At all loci except EST-2, the amount of variation was limited; all populations possessed a common allele at a frequency of 0.84 or greater (see Table 2). At EST-2 three alleles were found with a large amount of variation in frequency over the entire range (see Table 3 and Fig. 1).

Deviations from Hardy-Weinberg (H-W) proportions can be represented by the generalized inbreeding coefficient, F (Crow and Kimura, 1970, p.65)

$$F = \frac{H_e - H_o}{H_e} \quad (1)$$

where H_e is the expected H-W proportion of heterozygotes, and H_o is the observed proportion of heterozygotes (see Tables 2 and 3). This value of F is related to the χ^2 value used in testing for deviation from H-W proportions by the relationship

$$\chi^2 = F^2 N (k-1) \quad (2)$$

(Li and Horvitz, 1953), where k is the number of alleles and N is the number of individuals. Significant deviation from H-W proportions was discovered only at PGM-1 in

population SM ($\chi^2 = 4.24$, $p > 0.05$).

The total amount of variation detected in P. dunni is low in comparison to other vertebrates (Nevo, 1978). The average heterozygosity per locus (H) was calculated by

$$H = \frac{r}{\sum_{l=1}^r} (1 - \sum p_i^2) / r, \quad (3)$$

where l refers to the l th locus, r is the number of loci studied, and p_i is the frequency of the i th allele at a particular locus. The average heterozygosity per locus and the proportion of loci polymorphic are presented in Table 4.

The relatively low amount of genetic variation is associated with high genetic identity (Nei, 1975) between populations (Table 5). The lowest similarity between populations is 0.958, and the majority of pairwise relationships shows a similarity of 0.990 or greater. Nonetheless, there is evidence of substantial genetic divergence among populations, as indicated by the number of loci showing significant differences in allele frequencies (2x2 contingency χ^2 tests) between populations (above the horizontal Table 5). Altogether, 49 of 66 pairwise comparisons of populations show significant divergence at one or more loci. Furthermore, the four populations with the highest H are found within 21.1 km of each other (SI, WC, WF, SM), yet five of their six pairwise combinations exhibit significant divergence at a minimum of one locus.

Taxonomic Status of *Plethodon gordonii*

Our results provide information concerning the taxonomic status of *P. gordonii*. Stebbins (1951) considered *P. gordonii* to be a melanistic color morph of *P. dunni*, but Brodie (1970), on the basis of color and other morphological characters, elevated *P. gordonii* to specific status, with closest evolutionary affinities to *P. dunni*. Using electrophoretic evidence, however, Feder et al. (1978) found no genetic differentiation between these species and concluded that *P. gordonii* is probably a color morph of *P. dunni*.

Our collections from Marys Peak contain both *P. dunni* and *P. gordonii* found syntopically, including both forms from Dinner Cr., the type locality of *P. gordonii*. Electrophoretic results fail to show these two groups to be genetically differentiable (Table 6). Other *Plethodon* species examined via electrophoresis have shown clear and substantial genetic divergence (Highton and Webster, 1976).

Starch gel electrophoresis is a powerful tool for direct identification of reproductive relationships of sympatric, reproductively isolated populations (Makela and Richardson, 1977). Because we find negligible isozyme differences, and because color is the only morphological character showing non-overlapping distribution between

P. dunni and P. gordonii, we conclude that P. gordonii represents only a variant color morph of P. dunni and, in accordance with Stebbins (1953) and Feder et al. (1978), does not deserve specific status.

DISCUSSION

The restricted distribution of P. dunni provides an unusual opportunity to investigate the amount of genetic variation present in a species across its entire range without the need of examining an extremely large number of population samples. We intend to compare the genetic variation within and between populations of P. dunni with that in other amphibian species. Such comparisons will initially depend on the available literature. However, we are presently extending our study to a variety of amphibians in the Pacific Northwest with the goal of understanding the evolutionary forces acting on these species.

The average proportion of heterozygotes per locus in the 12 populations included in this study is 0.012. This value is low in comparison to heterozygosity estimates in other amphibian species (Nevo, 1978) and may reflect the restricted range and the relatively small number of individuals in this species. A more detailed analysis of genetic variation within this species is necessary to begin to understand the evolutionary forces of interest.

Nei (1975, 1977) presents a method to partition

genetic diversity among different hierarchical levels of population structure. This method is a modification of Wright's F statistic analysis (Wright, 1965) extended to multiple alleles. We used this method to estimate the total genetic variability in P. dunni resulting from genetic diversity within, as opposed to between, populations.

The mean gene diversity within each population (H_S) is equal to the average proportion of heterozygotes per locus found in the individual populations: $H_S = 0.0123$. The total gene diversity (H_T) is estimated by the expected H-W proportion of heterozygotes, using the mean allele frequencies at each locus for the twelve populations averaged over 11 loci: $H_T = 0.0245$. This low total gene diversity can be partitioned into the gene diversities within and between populations:

$$H_T = H_S + D_{ST}, \quad (4)$$

where D_{ST} is the average gene diversity between populations ($D_{ST} = 0.0122$). The relative magnitude of gene differences among populations can then be estimated by

$$G_{ST} = D_{ST}/H_T = 0.498. \quad (5)$$

Thus, approximately 50% of the estimated gene diversity detected in P. dunni is attributable to genetic differences between populations.

The value of G_{ST} is high relative to estimates available in other species (Nei, 1975). For comparison,

Nei and Roychoudhury (1974) estimate that only 7% of the gene diversity in the three major human races is attributable to gene differences between the races. The pattern of genetic diversity in P. dumni is one of relatively low overall genetic variability; this variability can be separated into low genetic variability within populations and proportionally high genetic divergence between populations.

What genetic population structure of this species is suggested by these findings? The amount of genetic diversity within and between populations is a function of the local effective population sizes, the migration rates (i.e., gene flow) and natural selection (Kimura and Maruyama, 1971). The three major conclusions which follow from this analysis are (1) the low total genetic diversity in P. dumni is compatible with its limited range and relatively small total number of individuals; (2) the relatively low genetic variability within local populations supports our hypothesis that local effective population sizes are small; and (3) the relatively high genetic diversity between populations supports our hypothesis that gene flow is restricted between local populations. These conclusions are based primarily on the theoretically predictable effects of population size and gene flow and do not directly incorporate the effects of natural selection.

The validity of these conclusions can be strengthened by comparing these results to those reported for other

Plethodon. Highton and Webster (1976) have described the genetic variation in two eastern representatives of this genus: P. cinereus and P. serratus. We have re-analyzed their published data and present the results in Table 7.

There are substantial differences in the absolute amount of genetic variation in the three species. The widespread species P. cinereus has the greatest amount of genetic variation both within (H_S) and between (D_{ST}) local populations. A comparison of the relative amount of total diversity within each species attributable to divergence between populations (G_{ST}) reveals similar values for all three species. However, the value D_{ST} which is used to compute G_{ST} includes the comparisons of populations with themselves (Nei, 1973). To compare these species we should use a measure which is independent of the different H_S values found for each species. We can estimate interpopulational gene diversity independently of H_S by

$$\bar{D}_m = \frac{1}{s} D_{ST} (s-1), \quad (6)$$

where s is the number of populations. The interpopulational gene diversity relative to the intrapopulational gene diversity can then be computed by

$$R_{ST} = \bar{D}_m / H_S. \quad (7)$$

The comparison of R_{ST} for all three species reveals striking similarity. A relatively high proportion of the total genetic variability results from genetic differences between

populations in all three species.

Highton and Webster suggest this large interpopulational genetic divergence in P. cinereus could be caused by the existence of undetected regional types (i.e., semi-species). Our analysis of P. dunni is not in agreement with this explanation. The presence of continuous variations within a small area is not compatible with the concept that regional types exist which account for the high estimates of interpopulational diversity. Rather, we suggest that the consistent high divergence among populations in these species stems from similarities in life history parameters affecting local population size and gene flow. Thus we predict that a more detailed analysis of the continuously distributed P. cinereus will not yield distinct regional types.

Geographical Pattern of Variation

Although the concept of a circular cline is old (see Mayr, 1963), actual examples are rarely documented. EST-2 shows marked clinal variation with the exception of the MP population (see Fig. 1). EST-2(90) is fixed at the northern limit of distribution (SB) and shows a regular decrease in frequency to the south through the Coast Range. EST-2(100) shows a corresponding increase in frequency, reaching fixation at the southern limit (HM). The cline also extends into the Cascades, showing a second arm of

decreasing EST-2(90) frequency. This arm extends from SM around the southern end of the Willamette Valley, north through CR, reaching fixation for EST-2(100) at HF in the Columbia River Gorge. We see no obvious explanation for the failure of the MP population to fit the observed continuous pattern. It is worth noting that Marys Peak is the highest point in the Oregon Coast Range and is the area in which the melanistic "gordoni" morph occurs. This area might possess some unique environmental features or history affecting selection at EST-2 or local patterns of gene flow.

This bifurcating cline does not have a simple latitudinal basis; in fact, it is unusual in that it runs in both directions with respect to any major selective forces which might be associated with latitude. Distinguishing the effects of gene flow and selection in analyzing clinal distributions is difficult (Clarke, 1975). We have already hypothesized that there is reduced genetic exchange between populations of P. dunni as well as between populations of other western plethodontids. Since allele distribution appears to be independent of latitudinal selective gradients, we believe that geographical patterns of reduced gene flow and genetic drift are sufficient to explain the EST-2 cline (see Kimura and Maruyama, 1971). However, Endler (1973) presents evidence suggesting that the effect

of reduced gene flow on the development of geographic differentiation may be small. Since environmental differences between collecting sites in the Cascade and Coast Ranges exist and we cannot predict which factors may serve as selective forces influencing the frequency of EST-2, we cannot discount the possibility that selection may contribute to the observed cline.

A second noteworthy feature of the geographical pattern of genetic variation in P. dunni is the higher amount of genetic variation in central (i.e., SI and SM) as compared to peripheral (i.e., SB, HF, and HM) populations. This difference is reflected both in the average heterozygosity per locus and the proportion of loci polymorphic (see Table 4). Although these peripheral populations are marginal in a geographical sense, they may not be marginal in an ecological sense (Lewontin, 1974, p. 148; Soulé, 1973). The low heterozygosity in the peripheral populations may be the result of a reduction in genetic exchange from surrounding populations. Karyological information obtained by Kezer and Sessions (1979) for Aneides ferreus, another western plethodontid with similar life history, tends to strengthen our conclusions regarding the relative importance of gene flow in contributing to the reduced genetic variation in peripheral versus central populations of plethodontid salamanders. They found "central" populations of A. ferreus were polymorphic for

chromosome 13($n=14$) and that populations inhabiting the periphery of the species' range tended to be monomorphic for the 13th chromosome. They conclude that one possible mechanism which might be responsible for this phenomenon is reduced gene flow between peripherally located populations.

Karyotypic Evolution in Plethodon Species

Western North American Plethodon have been found to possess much greater amounts of DNA than those in the East (Macgregor et al., 1973; Mizuno and Macgregor, 1974). This difference results from changes in the amount of moderately repetitive DNA. This increase in DNA is associated with an increase in the number of chromomeres in the lampbrush chromosomes, i.e., it is due to the duplication of pre-existing chromomeres. These observations led to the conclusion that there "cannot be a relationship between chromomeres and unique sequences," i.e., structural genes (Vlad and Macgregor, 1975). White (1978) has offered an alternative suggestion: only some of the structural genes in western Plethodon species have undergone extended duplication and are "represented 4, 8, 16....times," and therefore contribute to the increase in the moderately repetitive DNA.

Our genetic results relate to this question. We observed no indication of additional gene copies in the

enzymes we examined in P. dunni as compared to other amphibian species, including the eastern P. cinereus and P. serratus examined by Highton and Webster (1976). Although we have observed only a very small sample of structural genes, our results agree with the contention that there is no increase in the number of structural genes associated with the increased DNA in western Plethodon species. Any duplications as suggested by White might represent only a small proportion of the structural genes.

The detection of gene duplication is a potential problem. Duplicate loci possessing common alleles with identical electrophoretic mobility can only be detected through the examination of genetic variants (Allendorf et al., 1975). We have conservatively identified only 7 loci in P. dunni which have not undergone detectable duplication. A more comprehensive analysis is needed, including larger sample sizes and additional species, to provide a more substantial search for the duplication of structural genes in western Plethodon.

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Table 1. Genetic loci studied.

Enzyme	Locus designation	Buffer*	Alleles identified
α -glycerophosphate dehydrogenase	<u>AGP</u>	B	<u>AGP</u> (100,30)
Esterase	<u>EST-2</u>	A	<u>EST-2</u> (100,110,90)
	<u>EST-3</u>	A	<u>EST-3</u> (100)
Glyceraldehyde-3-dehydrogenase	<u>GAPDH</u>	B	<u>GAPDH</u> (100)
Glucose-6-phosphate dehydrogenase	<u>G6PDH-1</u>	A	<u>G6PDH-1</u> (100)
	<u>G6PDH-2</u>	A	<u>G6PDH-2</u> (100)
Isocitrate dehydrogenase	<u>IDH-1</u>	B	<u>IDH-1</u> (100,50)
	<u>IDH-2</u>	B	<u>IDH-2</u> (100)
Lactate dehydrogenase	<u>LDH-1</u>	A	<u>LDH-1</u> (100)
	<u>LDH-2</u>	B	<u>LDH-2</u> (100)
Malate dehydrogenase	<u>MDH-1</u>	B	<u>MDH-1</u> (100,200)
	<u>MDH-2</u>	B	<u>MDH-2</u> (100,125)
Malic enzyme	<u>ME-1</u>	A	<u>ME-1</u> (100)
	<u>ME-2</u>	A	<u>ME-2</u> (100)
6-phosphogluconate dehydrogenase	<u>6PGDH</u>	A	<u>6PGDH</u> (100)
Phospho glucose isomerase	<u>PGI-1</u>	B	<u>PGI-1</u> (100)
	<u>PGI-2</u>	B	<u>PGI-2</u> (100)
Phosphoglucomutase	<u>PGM-1</u>	A	<u>PGM-1</u> (100,200)
	<u>PGM-2</u>	A	<u>PGM-2</u> (100)
Phosphomannose isomerase	<u>PMI</u>	A	<u>PMI</u> (100,120,140)
General protein	<u>GP-1</u>	A	<u>GP-1</u> (100)
	<u>GP-2</u>	A	<u>GP-2</u> (100)
	<u>GP-3</u>	A	<u>GP-3</u> (100)
Sorbitol dehydrogenase	<u>SDH</u>	A	<u>SDH</u> (100)

* Buffer formulas given in Materials and Methods.

Table 2. Gene frequencies at polymorphic loci with the exception of Est-2.

Locus	Population	2N*	Allele			f†
			A1	A2	A3	
<u>AGP</u>	NR	44	.977	.023	--	.000
	SI	44	.932	.068	--	-.049
	SM	58	.914	.086	--	-.075
	WF	20	.850	.150	--	-.120
	All others	402	1.000	0		.000
<u>IDH-1</u>	CR	56	.964	.036	--	-.019
	MP	92	.989	.011	--	.000
	SI	44	.841	.159	--	-.162
	All others	382	1.000	0	--	.000
<u>MDH-1</u>	SI	44	.977	.024	--	.000
	All others	530	1.000	0	--	.000
<u>MDH-2</u>	MP	92	.967	.033	--	-.022
	SM	64	.984	.016	--	.000
	WC	22	.909	.091	--	-.050
	All others	396	1.000	0		.000
<u>PGM-1</u>	SI	44	.955	.045	--	1.000
	SM	64	.859	.141	--	.364
	All others	466	1.000	.000		.000
<u>PMI</u>	HM	52	.981	.019	.000	.000
	SI	44	.976	.000	.024	.000
	All others	386	1.000	.000	.000	.000

*2N is the number of genes examined; †F is the generalized inbreeding coefficient (see text).

Table 3. Gene frequencies at Est-2.

Population	2N	<u>EST-2(100)</u>	<u>EST-2(110)</u>	<u>EST-2(90)</u>	F [†]
AB	42	.952	0	.048	1.000
CR	56	.875	0	.125	-.071
ER	46	.913	.043	.043	-.071
HF	50	1.000	0	0	0
HM	10	1.000	0	0	0
MP	92	1.000	0	0	0
NR	44	.045	0	.955	-.024
SB	42	0	0	1.000	0
SI	44	.452	0	.548	-.032
SM	62	.710	0	.290	-.078
WC	22	.136	0	.864	-.060
WF	20	.900	0	.100	-.056

*2N is the number of genes examined; †F is the generalized inbreeding coefficient (see text).

Table 4. Average heterozygosity and proportion of polymorphic loci in P. dunni.

Population	N	H \pm s.e	Proportion of loci polymorphic ¹	Proportion of loci polymorphic ²
AB	21	0.0038 \pm 0.0038	0	0.042
CR	28	0.0120 \pm 0.0094	0.042	0.083
ER	23	0.0068 \pm 0.0068	0.042	0.042
HF	25	0	0	0
HM	26	0.0016 \pm 0.0016	0	0.042
MP	46	0.0036 \pm 0.0028	0	0.083
NR	22	0.0055 \pm 0.0040	0	0.083
SB	21	0	0	0
SI	22	0.0446 \pm 0.0232	0.125	0.167
SM	32	0.0351 \pm 0.0202	0.125	0.167
WC	11	0.0167 \pm 0.0117	0.083	0.083
WF	10	0.0181 \pm 0.0127	0.083	0.083

1 - common allele having frequency of 0.95 or less;

2 - common allele having frequency of 0.99 or less.

Table 5. Genetic similarities (Nei's I) (below diagonal) and number of loci showing significant allele frequency differences (above diagonal).

	AB	CR	ER	HF	HM	MP	NR	SB	SI	SM	WC	WF
AB	-	0	0	0	0	1	1	0	2	2	2	1
CR	1.000	-	0	1	1	1	1	0	3	3	2	1
ER	1.000	1.000	-	1	1	1	1	0	2	3	2	1
HF	1.000	.999	1.000	-	0	0	1	0	2	3	2	2
HM	1.000	.999	1.000	1.000	-	0	1	0	2	3	2	2
MP	1.000	.999	1.000	1.000	1.000	-	0	0	4	3	1	2
NR	.966	.971	.967	.962	.962	.962	-	0	2	2	0	1
SB	.962	.968	.963	.958	.958	.958	1.000	-	1	1	1	1
SI	.988	.991	.989	.986	.986	.986	.992	.990	-	2	3	1
SM	.996	.998	.997	.995	.995	.995	.980	.978	.996	-	1	1
WC	.972	.977	.973	.968	.968	.968	.999	.999	.994	.985	-	1
WF	.999	.999	.999	.999	.999	.999	.969	.965	.990	.974	.997	-

Table 6. Gene frequencies in P. dumni and P. gordonii for loci showing genetic variation.

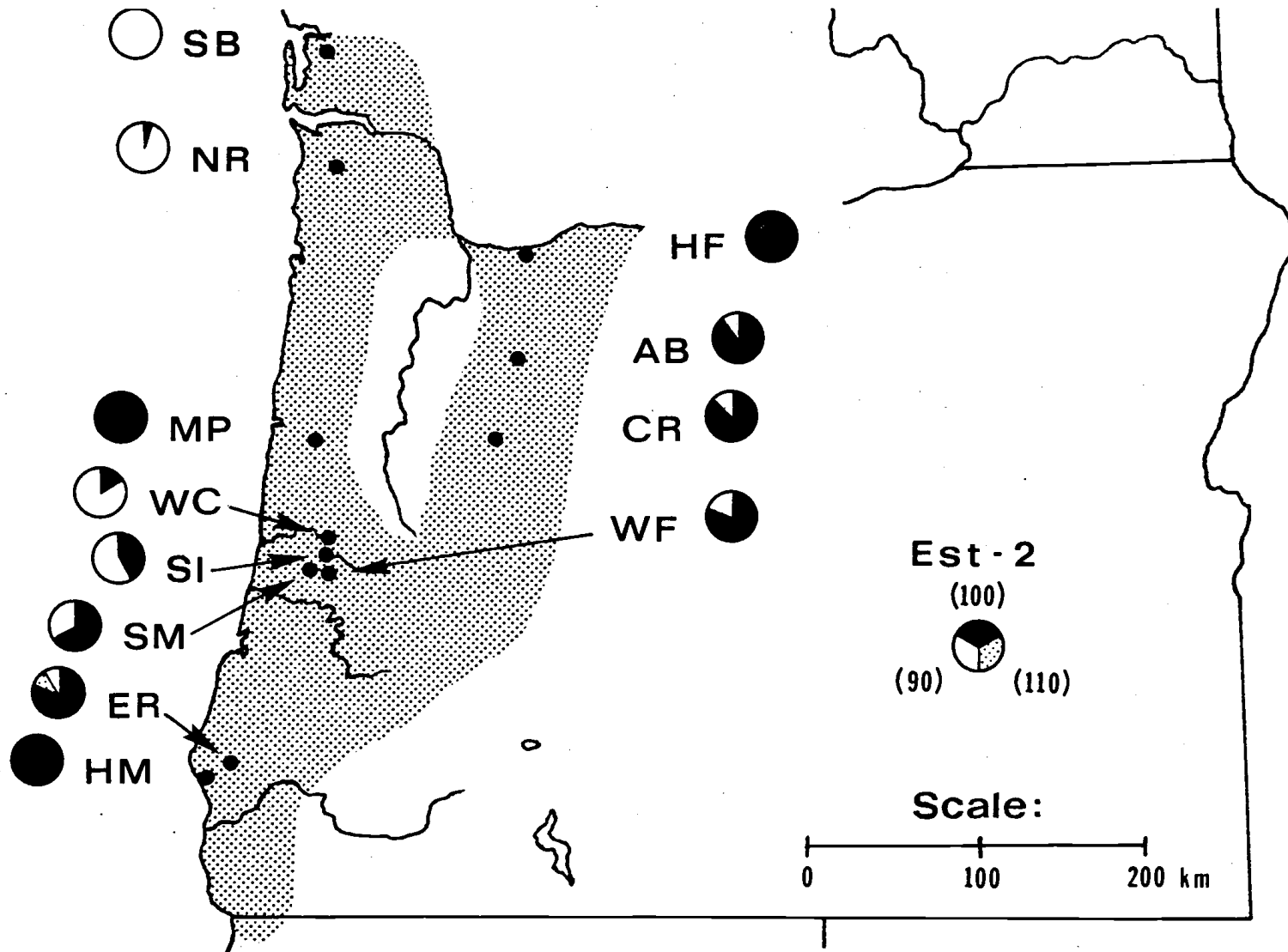
Locus (allele)	<u>P. dumni</u> (2N=70)	<u>P. gordonii</u> (2N=24)
<u>IDH-1</u> (100) (50)	1.000 0.000	0.960 0.040
<u>MDH-2</u> (100)	0.971 0.029	0.960 0.040

Table 7. Gene diversity in Flethodon species.

Species	No. pop.	No. loci	H_T^*	H_S	D_{ST}	G_{ST}	R_{ST}	\bar{D}_m
<u>P. dunni</u>	12	24	.024	.012	.012	.498	1.083	.013
<u>P. serratus</u>	9	24	.099	.033	.066	.667	1.121	.074
<u>P. cinereus</u>	15	24	.134	.045	.089	.664	1.067	.095

*See text for explanation of H_T , H_S , D_{ST} , G_{ST} , R_{ST} , \bar{D}_m .

Figure 1. Range of P. dumni and variation at Est-2.



III. Morphological Variation in Dunn's Salamander,
Plethodon dunnii Bishop

In a recent study (Hanlin et al., ms) it was shown that Dunn's salamander, Plethodon dunnii, exhibits low isozyme variability. However, there was about as much variation between populations as within. It was suggested that this species occurs as semi-isolated, localized populations throughout its range. Highton and Larson (ms) found Plethodon species to be very distinct electrophoretically but noted low morphological divergence between the species. In the present study I used stepwise discriminant analysis to examine an array of morphological characters to determine the extent of morphological variation in P. dunnii. In doing so, I tested the prediction that the restricted total range and the patchy distribution of populations should serve to alter the phenotypic structure of this species, i.e., to reduce the morphological diversity of the species as a whole and to increase inter-population divergence.

Stepwise discriminant analysis is a powerful statistical tool for examining morphological variation. Recent works in which it was employed include those of Montanucci (1978), Lynch and Wake (1978), and Iverson (1979). Beatty (1978) utilized the stepwise analysis to determine the level of phenotypic divergence between regional groups and populations of the western plethodontid salamander,

Aneides ferreus. Although Brodie (1970) used univariate statistics to examine morphological relationships in all the western Plethodon, the morphological differentiation of those species has not been examined via multivariate techniques.

It is generally agreed (Highton, 1962; Wake, 1966; Brodie, 1970; Highton and Larson, ms) that P. dumni has closest affinities with P. vehiculum, a sympatric western species. It is probable that its life history patterns resemble those of P. vehiculum (see Peacock and Nussbaum, 1973) and other plethodontids (see Houck, 1978, and Tilley, 1978, for reviews) in that it exhibits limited dispersal and relatively low fecundity (Hanlin, unpublished data)!

P. dumni inhabits localized rocky outcrops and stabilized talus slopes primarily in western Oregon. Its distribution is largely dependent upon adequate moisture (Dumas, 1956) and suitable rocky substrates (Storm and Brodie, 1970). These favorable habitats are distributed as quite abundant but very localized patches throughout its range. This type of habitat distribution coupled with the above-mentioned life history attributes leads to the prediction that populations should diverge morphologically in response to such evolutionary forces as random genetic drift, reduced rates of genetic exchange between populations, and natural selection.

A corollary consideration is also addressed in this

study. Brodie (1970) assigned specific status (P. gordonii) to a melanistic form sympatric with P. dunni. However, Feder et al. (1978) and Hanlin et al. (ms) found negligible isozyme differentiation between P. dunni and P. gordonii and suggested that the latter did not deserve specific status. In this study the morphological relationships of P. gordonii and P. dunni are reassessed utilizing discriminant analysis, and the taxonomic status of P. gordonii, based on those findings, is reviewed.

METHODS AND MATERIALS

Samples

Specimens of Plethodon dunni were collected and uniformly preserved by me. They were killed in 0.2% chloro-butanol, fixed in AFA as described by Gallagher and Kozloff (1971), washed in running tap water for 24 h, and stored in 65% ethanol. Some specimens of P. gordonii were collected by me and prepared as described above for P. dunni. Others were ^{lent} ~~loaned~~ to me by the University of Michigan Museum of Zoology (UMMZ). Although the fixatives used for these specimens may have varied, all were stored in 65% ethanol. Measurements on all animals were made only after they had been in alcohol for at least 24 h.

Animals were sampled from 10 sites across the range of P. dunni. At each site the animals were collected from the smallest possible area, often one rock face. In the

few instances where enough animals could not be obtained within a small area, samples from within a radius of a few kilometers were combined. The sites (with abbreviations and sample sizes) follow: 1.4 mi. SE of South Bend, Pacific Co., WA (SB, N=27); Nehalem River Rd., near Clatsop-Tillamook Co. line, OR (NR, N=41); Marys Peak, Benton Co., OR (MP, N=48); Siuslaw River Rd., near Douglas-Lane Co. line, OR (SI, N=25); West Fork Rd. between Smith River Rd. and Lane Co. line, Douglas Co., OR (WF, N=52); Smith River Falls, Douglas Co., OR (SM, N=50); along Elk River Rd., Curry Co., OR (ER, N=50); Horsetail Falls, Multnomah Co., OR (HF, N=55); Crabtree Cr., E. of Lacombe, Linn Co., OR (SR, N=46). Figure 1 shows the location of these sites.

Specimens of P. gordonii were collected sympatrically with MP P. dunnii and will be referred to as population G (N=46). Some of the paratypes of P. gordonii ^{ent} loaned by UMMZ were among the animals included in this study.

Characters

A variety of morphometric characters were estimated to the nearest 0.1 mm with dial calipers from post-juvenile specimens (i.e., specimens ≥ 50 mm snout-vent length). They include:

- (1) Snout-vent length (SVL) - measured from the tip of the snout to the anterior edge of the vent

- (2) Head length (HL) - measured from the midpoint of the gular fold to the anterior tip of the snout
- (3) Axilla-groin length (AGL) - measured from axilla to groin with fore- and hind-limbs extended perpendicular to the trunk
- (4) Forelimb length (FLL) - measured from the tip of the longest toe to the axilla when the limb was extended perpendicular to the trunk
- (5) Hindlimb length (HLL) - measured from the tip of the longest toe to the groin when the limb was extended perpendicular to the trunk
- (6) Head width (HW) - measured at the angle of the jaw
- (7) Neck width (NW) - measured at the gular fold.

Two morphometric characters were estimated to the nearest 0.1 mm by using a standard dissecting microscope with an ocular micrometer. They were

- (1) Interorbital distance (IOD) - measured at the point of minimum distance between the eyes
- (2) Internasal distance (IND) - measured at the point of minimum distance between the nostrils

Four meristic characters included:

- (1) Costal grooves (CG) - the total number of costal grooves counted between the axilla and groin

- (2) Costal folds (CF) between adpressed limbs - estimated to the nearest 0.5 fold
- (3) Vomerine teeth (VT) - combined count of vomerine teeth on left and right sides
- (4) Maxillary - premaxillary teeth (MPT) - combined count of maxillaries and premaxillaries.

Populations of P. dunni show some variation in the width of the dorsal stripe and in the degree of melanophore pigmentation within that stripe. In an attempt to assess the importance of that variation, two other characters were estimated and included as input variables for an analysis of the nine P. dunni populations:

- (1) Stripe width (SW) was measured to the nearest 0.1 mm with dial calipers. Maximum stripe width was taken at mid-dorsum.
- (2) The degree of melanophore pigmentation (P) was estimated on a one-to-five scale similar to that proposed by Brodie (1970). The "standard" animals for each numerical category are shown in Figure 2.

Analysis of Characters

For various reasons morphologists have often resorted to the use of character ratios as input variables in statistical analyses. However, Beatty (1978) found that the results from using character ratios did not differ significantly from those obtained utilizing the original variables

in a stepwise discriminant analysis of morphological variation in Aneides ferreus. Additionally, Atchley et al. (1975, 1976) have criticized the use of ratios on theoretical grounds. I chose to use a standardized (mean zero, unit variance) 13 variable, 10 group (=population) model. I manipulated the data in two ways: (1) sexes were combined to determine the overall morphological divergence between populations and to try to detect any general trends of differentiation across the range of the species, and (2) sexes were analyzed separately to determine if variables differ between sexes in their relative discriminatory power.

The level of interpopulation differentiation was analyzed by the OSU version of UCLA's BMD07M stepwise discriminant analysis program (Dixon, 1974). In this program canonical variates are created from linear combinations of the original variables whose directional cosines define orthogonal axes which maximize the ratio of between-group variance to within-group variance matrices. The original variable for which the mean values of all populations are most different (i.e. the best discriminator) is identified using a one-way analysis of variance and is the first variable to enter the model. After each "step," an F-value is calculated which sets a minimum level of significance which must be met for the next variable to enter the model. Each remaining variable is entered in the order which reflects its ability to add to the discriminatory power of the model

based on its F-value. The program also computes the probability that a given individual belongs to a particular group (i.e., its posterior probability) and constructs a confusion matrix (Massey, 1965) which enumerates the correct and incorrect classifications. Canonical coefficients are computed, and plots of the first two canonical variables are given to show the position of each specimen in two-dimensional space and, thereby, a visual representation of the separation of the groups.

The BMD07M package also calculates an F-matrix which presents the F-values for pairwise comparisons of centroid (= group) means. Mahalanobis' generalized distance measure (D^2 , Mahalanobis, 1936) between the groups can be obtained from the following equation (Sneath and Sokal, 1973):

$$F = \frac{D_{JK}^2 (t_J t_K) (t_J + t_K - m - 1)}{(t_J + t_K) (t_J + t_K - 2)m}$$

where m = the number of variables in the model, t = group sample sizes for the Jth and Kth groups, and D^2 = the generalized distance between the Jth and Kth groups.

RESULTS

Figures 3-5 and Tables 1 and 2 show the results of stepwise discriminant analysis obtained from the 13 variable model utilizing standardized morphological data from males and females combined. Figure 3 shows the plots of

canonical scores for each specimen on the first two canonical axes. Because the scatter of the data points is dense, I located the most dispersed canonical variates for each centroid and outlined them in Figure 4. This figure clearly illustrates that there is extensive overlap between centroids as is also evidenced by the confusion matrix (Table 1). Correct classifications ranged from 48 - 88% ($\bar{x} = 60\%$).

Figure 5 depicts the positions of the centroid means on the first two canonical axes. Although extensive overlap of centroids exists, the centroid means for all 10 groups differ significantly from one another ($\bar{F}=13.30$; $df=13, 418$; $p<.01$; Table 2) indicating a high degree of heterogeneity between the groups. Mahalanobis' generalized distance measure (D^2) shows that the greatest amount of morphological differentiation exists between SB and ER ($D^2=21.69$), HF ($D^2=18.11$), and CR ($D^2=25.28$).

Since the plots of the first two canonical axes depict only 59% of the total dispersion, I plotted the second and third canonical variates (Figure 6). This plot revealed similar results; there was extensive overlap of centroids, although 74% of the total dispersion was accounted for by the first three canonical variates.

In the above described analyses I assessed the level of phenotypic differentiation between populations by incorporating post-juveniles of both sexes. To determine if a

better fit to the model could be obtained, I also analyzed the sexes separately. The results of the stepwise discriminant analysis for males are given in tables 3 and 4. With the exception of SI x WF, all centroid means differed significantly from one another ($\bar{F}=6.80$; $df=13$, $p < .01$), but \bar{F} and individual F-values were not as great as when sexes are combined, and only slightly better classifications were obtained (range of correct classifications: 56 - 92%, $\bar{x}=68\%$).

The results of the analysis of females are given in tables 5 and 6. With the exception of SI x WF and SI x SM, all pairwise comparisons of centroid means were significantly different ($\bar{F}=8.02$; $df=13, 200$; $p < .01$). F-values were generally larger than those of the comparisons of males but smaller than when sexes were combined. Correct classifications (range 50 - 96%; $\bar{x}=66\%$) were generally lower than classifications with males but slightly higher than when sexes were combined.

In table 7 the three treatments are summarized in terms of the order in which each morphological variable enters the stepwise model. In each analysis, the variable VT (vomerine teeth) most effectively maximizes the ratio of between-group variance to within-group variance matrices. CG (costal grooves) and CF (costal folds between adpressed limbs) are relatively poor discriminators for males and for

the combined sexes. However, those variables appear to be quite important in discriminating between females of different populations. Of the analyses of separate sexes, the stepwise analysis of the males more closely reflects the analysis of both sexes combined than does the analysis of females.

To determine if stripe characteristics provided better discrimination between the P. dumni populations, the 15 variable, 9 group model was employed. With the exception of WF x SI, all pairwise comparisons of centroid means were significantly different ($\bar{F}=12.72$; $df=15, 369$; $p<.01$). \bar{F} and individual F-values were generally lower than the 13 variable model, but slightly better classifications were obtained (range of correct classifications: 56 - 94%; $\bar{x}=70\%$). VT (vomerine teeth) was, again, the first variable to enter the model, but P (stripe pigmentation) entered second. SW (stripe width) was a relatively poor discriminator (10th variable to enter model). In spite of the importance of P (stripe pigmentation) as a discriminator, it appears that no better separation was obtained with this model than with the 13 variable, 10 group model.

Status of Plethodon gordonii

In his description of the species, Brodie (1970) stated that "P. gordonii is more similar to some allopatric

populations of dunni than it is to sympatric populations of dunni," (i.e., MP) and suggested that "character displacement may be taking place in the zone of sympatry." The results of my stepwise discriminant analyses do not support this contention. In all analyses, the population with closest affinities to G was MP, the sympatric P. dunni population (see Tables 1-6). However, to reexamine their relationship I utilized a 13 variable, 3 group model using G, MP, and SM as a representative "central" population of P. dunni. The results are presented in Tables 8 and 9. G, quite clearly, is more similar morphologically to MP ($D^2=4.68$) than to SM ($D^2=8.12$).

In this analysis VT (vomerine teeth) was replaced by HL (head length) as the most important discriminator. This is interesting in that Brodie (1970) found only one significant difference ($p<.01$) between P. gordonii and sympatric P. dunni; the slope of the regression line for HW vs. HL was significantly lower for P. gordonii, indicating a longer, narrower head. In this analysis, however, with HL most effectively maximizing the ratio of between-group variance to within-group variance matrices, G showed closer affinities to MP than to SM.

Since stepwise discriminant analysis is a very powerful tool, it is not unreasonable to suggest that possible differences in preservation techniques may have been, at least in part, responsible for the significant F-values for

the G x MP pairwise comparisons. In spite of the risk of reducing sample size, G was divided into those collected by me and those provided by UMMZ, and a 13 variable, 3 group stepwise analysis was made with MP. The results are presented in Table 10. The only significant ($p < .01$) F-value was that of the comparison of the MP population and P. gordonii provided by UMMZ. The closest affinity was between MP and P. gordonii collected by me. Since UMMZ specimens accounted for 74% of G in the previous analyses, I contend that the morphological relationships between Marys Peak P. dunni and P. gordonii are even closer than those analyses suggest. Because of these findings, and because of the isozyme similarities presented by Feder et al. (1978) and Hanlin et al. (ms), I conclude that P. gordonii is a melanistic morph of P. dunni and, in accordance with Stebbins (1951), does not deserve specific status.

DISCUSSION

Western Oregon was largely an inland sea when the Tribe Plethodontini was undergoing its explosive radiation in the Tertiary (Wake, 1966; Snavely and Wagner, 1963). Since then important orogenic events such as the uplifting of the Cascade and Coast ranges and numerous strike-slips have created a deeply dissected terrain exposing many areas of rocky outcrops that are presently extensively inhabited by plethodontid salamanders. However, the complex drainage

patterns and dissected topography may also serve to semi-isolate small local populations of plethodontids which have low dispersal rates and relatively narrow habitat requirements. I suggest that such is the case for P. dumni.

The results of the three stepwise discriminant analyses in the present study support this hypothesis. In spite of considerable overlap of centroids, pairwise comparisons of centroid means consistently show significant differences (see Tables 2, 4, and 6). This indicates that there is considerable morphological heterogeneity between populations of P. dumni. This phenotypic structuring is very consistent with the apparent genotypic structuring described by Hanlin, et al. (ms). In that study it was shown that almost 50% of the isozyme diversity detected in P. dumni was attributable to divergence between populations.

The validity of the above interpretation of the morphological variation can be strengthened by comparing these results to those reported for a more ubiquitous western plethodontid, Aneides ferreus. Beatty (1978) utilized the BMD07M program to compare populations across the range of that species (average straight-line distance between populations sampled \approx 125 km). The average morphological differentiation between 10 populations was quite low ($D^2 = 3.48$; range = 0.57-9.21) relative to the differentiation of P. dumni populations ($D^2 = 9.68$; range = 2.05-25.28). The high D^2 values for P. dumni are even more striking when one

considers that the range of this species is much more restricted than A. ferreus (average straight-line distance between populations sampled ≈ 85 km).

A consideration of life history patterns is important in elucidating the causal factors of the phenotypic and genotypic structure of populations of a given species. Both A. ferreus and P. dunni are thought to have low dispersal rates, as do many plethodontids, and both exhibit relatively low fecundity (McKenzie, 1970; Hahlin, unpublished data). However, it has been shown (Storm, 1948; McKenzie, 1970; McKenzie and Storm, 1970) that A. ferreus may be found in a variety of habitats: exposed rock faces, talus slopes, fallen logs, and under surface debris. P. dunni, on the other hand, is restricted to rocky substrates (Brodie, 1970; Storm and Brodie, 1970) and has more limited moisture requirements (see Dumas, 1956, and McKenzie, 1970). This suggests that, in spite of its more restricted range, P. dunni may indeed exist as small, localized populations within suitable habitat patches. If so, this might explain the existence of significant morphological and isozyme heterogeneity between populations due to phenomena such as reduced gene flow and genetic drift acting in concert with subtly different selective regimes associated with varying microclimates.

Geographical Pattern of Variation

Overlapping centroids (see Figures 4 and 6) and only moderately correct classifications (see Tables 1, 3, and 5) indicate that, in spite of significant F-values for pairwise comparisons of centroid means, considerable similarities between populations exist (i.e., there is a marked amount of variation within populations). This does not contradict results of our isozyme studies (Hanlin et al., ms). We found that within-population variation was low relative to that found in other species but it was about equal to that of between-population variation. Similarities between populations of a species with a relatively restricted range and narrow habitat requirements are not unexpected. All populations of P. dumni are exposed to the winter-wet, summer-dry climate characteristic of western Oregon and are, therefore, exposed to similar broad selective regimes. The low isozyme variability and morphological similarities in P. dumni are consistent with current taxonomic conclusions that all populations are conspecific. The question of including the melanistic "gordoni" morph in this taxon has already been addressed.

Although considerable overlap of centroids is shown in Figure 4, canonical variates of animals from HF, CR, SB, and ER are more widely dispersed in two dimensional space. In fact, SB and ER (the northernmost and southernmost

populations) show no overlap of centroids at all. The morphological distinctness of these peripheral populations is further demonstrated by their number of correct classifications (see Tables 1, 3, and 5). There are two possible explanations for their distinctness: (1) being spaced widely enough geographically, selective forces are sufficiently different to induce measurable differences in their morphology or (2) the separation of habitat patches is sufficiently great in peripheral areas to create isolation and allow differentiation by reduced gene flow and drift. I suggest that a plausible explanation is a combination of the two: the peripheral populations are, indeed, more isolated spatially than are central populations, and selective forces, possibly in concert with genetic drift, influence their morphological divergence. Regardless of causal factors, it is noteworthy that the same peripheral populations also showed marked reduction in isozyme variation relative to central populations (Hanlin, et al., ms).

A more striking trend in the level of morphological divergence is depicted in Figure 1. D^2 values for all populations relative to SB tend to increase markedly to the south through the Coast Range, indicating clinal variation in phenotypic expression. A corresponding southward increase in D^2 occurs in the two Cascade populations, although the D^2 values of those populations are much larger than the values of Coast Range populations at comparable latitudes.

One's initial impression is that the variation is based on an environmental gradient associated primarily with latitude. However, this clinal morphological differentiation should be considered in light of previous electrophoretic results (Hanlin et al., ms). One type of esterase isozyme, EST-2, was found to show variation in allele frequencies across the range of P. dunni in the form of a bifurcating cline. EST-2(100) increased in frequency southward from SB reaching fixation at the southernmost Coast Range population at Humbug Mountain State Park. A second arm of increasing frequency extended from SM around the southern end of the Willamette Valley and northward through the Cascades reaching fixation at HF. When allele frequencies of EST-2(100) are compared to the generalized distance values (D^2) for morphological characters, the two clines are found to be highly correlated (Spearman's rank correlation coefficient, $r_s = .76$, $n=9$; $p < .01$; see Sokal and Rohlf, 1969).

Based primarily on (1) the hypothesis that there is reduced gene flow between populations of P. dunni and (2) the fact that the variation appeared to be independent of latitudinal gradient, the variation in EST-2 was thought to be sufficiently explained by geographical patterns of reduced gene flow and genetic drift. The high correlation of the two clines suggests that similar factors may influence the morphological cline as well. The fact that D^2 values

for the Cascade population are comparable to that of ER even though they are geographically closer to SB implies that the morphocline may also extend around the southern end of the Willamette Valley. However, of the two Cascade populations, HF shows closer affinities to SB. This indicates that selective forces, either associated with the Columbia River or with latitude, may contribute to the morphological cline. To assess more accurately the Cascade arm of the morphocline would require analyses of samples from additional populations.

SUMMARY

Life history and distributional evidence indicate that P. dunni is characterized by low dispersal and fecundity and that populations exist in relatively small, semi-isolated, localized patches throughout its range. I have predicted that these characteristics should serve to reduce the diversity of this species as a whole and to increase inter-population divergence via the evolutionary forces of reduced gene flow, drift, and natural selection.

Morphological differentiation was examined by stepwise discriminant analyses, and the results were compared to those of a previous study (Hanlin, et al., ms) where isozyme variation was examined. Apparent genotypic and phenotypic differentiation in P. dunni supports the above-mentioned evolutionary model. The striking similarities

of phenotypic and genotypic structuring in this species follow.

(1) Extensive overlap of centroids (i.e., scatter of canonical variates for each population in two dimensional space) indicates considerable morphological similarity between populations. This is consistent with the electrophoretic findings that only 7 of 24 presumed loci showed variation and, of those, 6 possessed a common allele at a frequency of 0.84 or greater. The results of both studies indicate low overall diversity in this species.

(2) Although I found extensive overlap of centroids, the centroid means for all populations were significantly different. This indicates subtle but significant phenotypic heterogeneity between groups. Similarly, in spite of low overall isozyme diversity in P. dunnii, it was estimated that almost 50% of the detected isozyme variation was attributable to differences between populations.

(3) The canonical variates of animals from peripheral populations are more widely dispersed in two-dimensional space, indicating that these peripheral populations are more distinct morphologically. Enzymatically the peripheral populations showed the lowest average heterozygosity and the lowest proportion of polymorphic loci. This reduced variation in peripheral populations is thought to reflect the more isolated nature of those populations.

(4) Mahalanobis' generalized distance measure (D^2)

for all populations relative to the northernmost (SB) increased markedly to the south and into the Cascades. This reveals clinal variation in phenotypic expression. The morphocline is highly correlated with a cline in allele frequencies at one esterase locus. Although it would be highly speculative to suggest any specific factors which might influence both morphology and the allele frequencies for the isozyme, the similarity of these two clines supports my model of geographical patterns of reduced gene flow and drift, with selective forces also possibly contributing to the clines.

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Table 1. Confusion matrix generated by the stepwise discriminant model for 10 populations of P. dunni (sexes combined). Diagonal elements represent correct classifications, off-diagonal elements incorrect classifications.

NUMBER OF CASES CLASSIFIED INTO GROUP

GROUP	SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB	22	2	0	1	1	1	0	0	0	0
NR	8	20	0	0	3	2	2	0	6	0
MP	2	4	22	5	2	3	3	4	2	1
G	0	1	9	26	1	0	3	1	5	0
SI	0	4	3	1	13	2	1	0	0	1
WF	0	2	5	1	7	31	5	0	1	0
SM	0	3	7	6	5	4	24	0	1	0
ER	0	0	2	1	2	0	0	44	1	0
HF	0	5	0	3	3	1	1	2	33	7
CR	0	5	0	3	0	0	0	2	7	29

Table 2. Generalized distance measures (Mahalanobis' D^2) (above diagonal) and F-values (below diagonal) for pairwise comparisons between populations of P. dunni when sexes are combined. All F-values are significant, $p < .01$.

	SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB	--	8.23	12.59	15.05	13.23	13.44	12.80	21.69	18.11	25.28
NR	8.44	--	7.06	9.43	5.49	7.77	5.62	13.56	6.64	11.48
MP	13.98	10.35	--	3.15	4.16	4.24	2.33	8.59	7.20	11.05
G	16.37	13.50	4.94	--	7.37	8.34	4.76	12.69	5.42	7.87
SI	10.04	5.33	4.37	7.58	--	2.05	3.01	11.22	7.36	9.28
WF	15.51	11.89	7.14	13.70	2.23	--	3.78	13.11	8.75	13.31
SM	14.50	8.76	3.83	7.65	3.22	6.53	--	12.39	9.34	9.64
ER	24.57	20.33	14.16	20.40	12.02	22.62	20.91	--	16.86	17.31
HF	21.45	10.47	12.51	9.17	8.23	15.93	11.69	30.00	--	3.51
CR	27.49	16.43	17.36	12.06	9.55	21.87	15.49	27.83	5.94	--

Table 3. Confusion matrix generated by the stepwise discriminant model for males of 10 populations of P. dumni. Diagonal elements represent correct classifications, off-diagonal elements incorrect classifications.

NUMBER OF CASES CLASSIFIED INTO GROUP

GROUP	SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB	5	0	0	0	1	1	0	0	0	0
NR	2	14	1	0	2	2	0	0	1	0
MP	0	3	15	1	0	1	1	2	0	0
G	0	2	2	17	0	0	0	2	3	0
SI	0	2	1	1	8	1	0	0	0	0
WF	0	0	1	0	3	17	3	0	0	1
SM	1	0	3	1	0	1	18	0	1	0
ER	0	0	0	0	0	0	1	23	1	0
HF	0	3	1	0	2	1	0	1	15	4
CR	0	1	1	1	0	0	1	1	2	18

Table 4. Generalized distance measures (Mahalanobis' D^2) (above diagonal) and F-values (below diagonal) for pairwise comparisons between males of 10 populations of P. dunni. *represents non-significant values. All other values are significant, $p < .01$.

	SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB	--	16.08	23.40	19.23	31.11	21.00	19.45	29.64	28.55	42.15
NR	3.65	--	8.93	9.22	6.20	9.76	11.15	16.15	6.74	12.44
MP	5.52	5.57	--	5.61	6.58	5.81	4.24	11.54	7.12	13.60
G	5.00	6.25	4.08	--	9.95	12.57	10.63	15.83	6.40	13.56
SI	3.63	2.48	2.72	4.48	--	2.76*	7.62	13.70	7.41	11.61
WF	5.30	6.44	3.96	9.31	1.21*	--	6.64	14.85	10.12	16.26
SM	4.91	7.36	2.89	7.87	3.34	4.79	--	14.76	11.06	15.61
ER	7.48	10.66	7.86	11.72	6.01	10.68	10.62	--	16.79	22.02
HF	7.63	4.68	5.10	4.99	3.42	7.68	8.38	12.72	--	5.02
CR	10.64	8.21	9.26	10.04	5.09	11.70	11.23	15.84	3.80	--

Table 5. Confusion matrix generated by the stepwise discriminant model for females of 10 populations of P. dunni. Diagonal elements represent correct classifications, off-diagonal elements incorrect classifications.

		NUMBER OF CASES CLASSIFIED INTO GROUP									
GROUP		SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB		18	1	0	0	0	0	0	1	0	0
NR		4	11	0	0	1	0	1	0	2	0
MP		2	0	13	3	1	2	2	1	0	1
G		0	0	4	14	0	0	0	0	2	0
SI		0	0	1	0	6	2	2	1	0	0
WF		0	1	2	1	4	18	1	0	0	0
SM		0	1	3	1	2	1	13	0	1	3
ER		0	0	1	0	0	0	0	24	0	0
HF		0	2	0	4	1	0	2	0	17	2
CR		0	3	0	1	0	0	0	0	3	14

Table 6. Generalized distance measures (Mahalanobis' D^2) (above diagonal) and F-values (below diagonal) for pairwise comparisons between females of 10 populations of P. dunni. * represents non-significant values. All other values are significant, $p < .01$.

	SB	NR	MP	G	SI	WF	SM	ER	HF	CR
SB	--	11.14	13.32	23.43	18.32	14.97	12.93	24.05	21.90	30.28
NR	5.64	--	10.98	15.88	10.88	9.99	8.58	20.32	11.23	23.47
MP	8.21	6.51	--	4.98	6.61	5.22	4.12	10.14	11.56	15.18
G	12.33	8.04	3.07	--	12.97	9.57	7.51	19.22	8.34	14.37
SI	6.34	3.61	2.71	4.49	--	3.06*	4.05*	8.71	13.62	17.92
WF	9.70	6.23	3.96	6.20	1.32*	--	5.39	16.91	11.97	19.39
SM	7.97	5.09	2.97	4.63	1.66*	4.09	--	15.72	8.41	11.43
ER	14.82	12.05	7.31	11.84	6.85	12.83	11.34	--	24.25	20.69
HF	14.53	7.17	8.98	5.53	6.02	9.79	6.53	18.94	--	7.64
CR	16.52	12.32	9.69	7.84	6.45	13.02	7.30	13.21	5.25	--

Table 7. Summary of stepwise discriminant analyses of 10 populations of P. dunni. The order in which variables enter are given; F-values to enter the model are given in parentheses. * represents non-significant values. All other values are significant, $p < .01$.

<u>Step</u>	<u>MALES ONLY</u>	<u>FEMALES ONLY</u>	<u>SEXES COMBINED</u>
1	VT (11.79)	VT (13.27)	VT (23.34)
2	IOD (7.72)	CG (9.49)	IOD (16.15)
3	HW (7.73)	CF (8.36)	HW (20.94)
4	FLL (11.34)	IOD (8.26)	FLL (13.61)
5	AGL (8.96)	HW (14.02)	NW (13.32)
6	HL (8.26)	NW (8.65)	HL (11.83)
7	NW (4.94)	HL (6.31)	CG (11.30)
8	MPT (4.80)	SVL (6.69)	SVL (10.77)
9	CG (4.66)	MPT (4.43)	MPT (12.41)
10	SVL (3.43)	AGL (3.61)	AGL (6.55)
11	HLL (3.57)	HLL (3.61)	HLL (5.26)
12	IND (3.19)	IND (3.01)	CF (4.11)
13	CF (1.27*)	FLL (1.93*)	IND (2.28*)

Table 8. Confusion matrix generated by the stepwise discriminant model for Marys Peak P. dumni, P. gordonii, and a representative "central" population of P. dumni from Smith River Falls. Diagonal elements represent correct classifications, off-diagonal elements incorrect classifications.

NUMBER OF CASES CLASSIFIED INTO GROUP

GROUP	G	MP	SM
G	28	12	6
MP	9	34	5
SM	5	8	37

Table 9. Generalized distance values (Mahalanobis' D^2) (above diagonal) and F-values (below diagonal) for pairwise comparisons between Marys Peak P. dunni, P. gordonii, and a representative "central" population of P. dunni from Smith River Falls. All F-values are significant, $p < .01$.

	G	MP	SM
G	--	2.90	5.05
MP	4.68	--	3.47
SM	8.12	5.58	--

Table 10. Generalized distance measures (Mahalanobis' D^2) (above diagonal) and F-values (below diagonal) for pairwise comparisons between samples of Marys Peak P. dumni, sympatric P. gordonii collected and preserved by the author, and sympatric P. gordonii provided by the University of Michigan Museum of Zoology. All F-values are significant (* $p < .05$, ** $p < .01$).

	<u>P. gordonii</u> (author's)	<u>P. gordonii</u> (UMMZ)	MP <u>P. dumni</u>
<u>P. gordonii</u> (author's)	-	13.41	12.77
<u>P. gordonii</u> (UMMZ)	2.81*	-	18.41
MP <u>P. dumni</u>	2.68*	3.86**	-

Figure 1. Range map of P. dumni indicating the location of populations sampled and their generalized distance measure (D^2) from SB. (See text for an explanation of D^2 .)

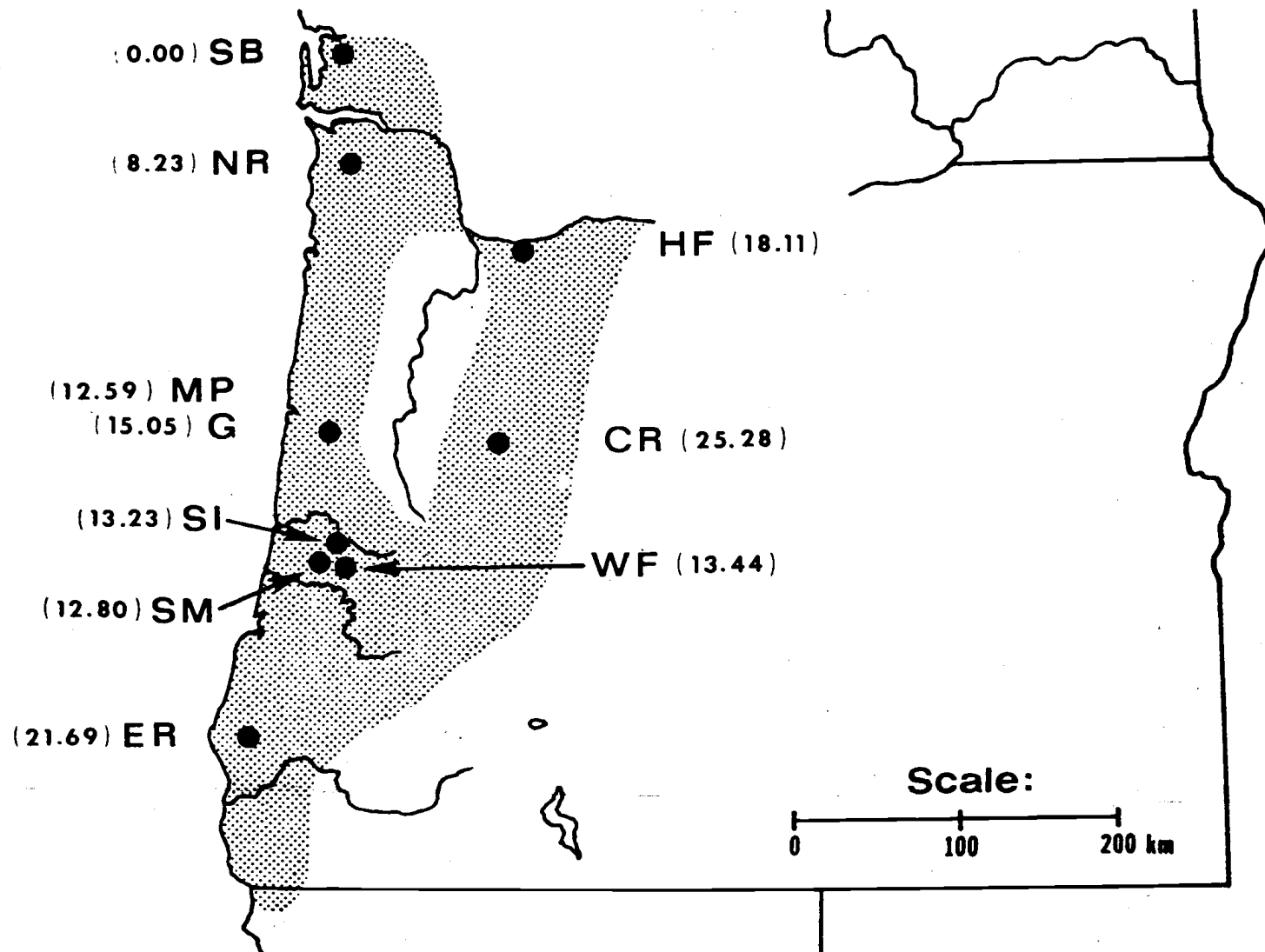


Figure 2. The "standard" specimens of P. dumni used to estimate the degree of melanophore pigmentation (P) within the dorsal stripe. From left to right the animals represent the numerical categories 1-5. The sixth animal is a specimen of P. gordonii.



Figure 3. Canonical scores for each specimen plotted on the first (C_1) and second (C_2) canonical axes. Canonical scores for animals from each population are indicated as follows: SB=B, NR=N, MP=M, G=G, SI=I, WF=W, SR=S, ER=E, CR=C, HF=H.

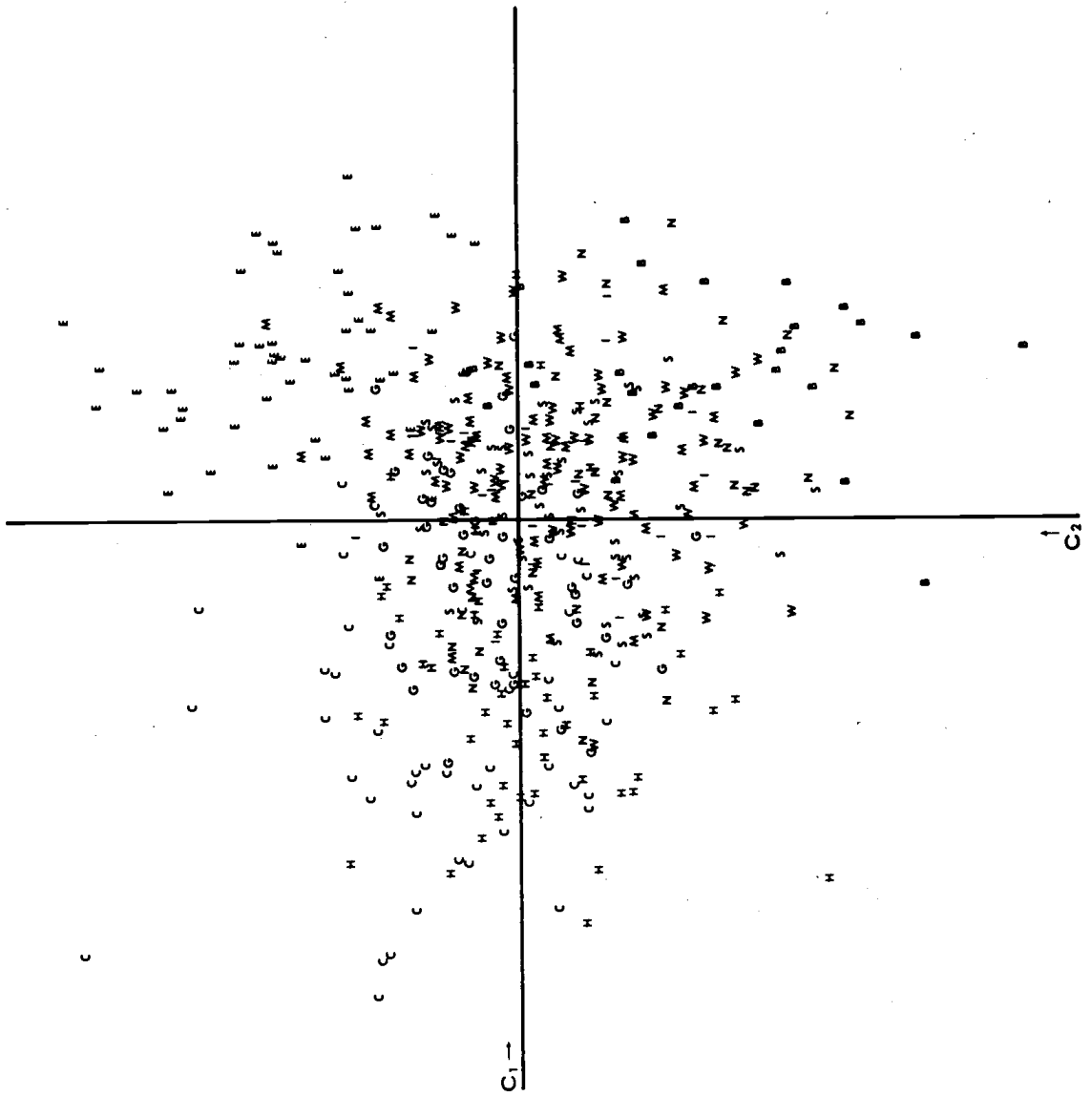


Figure 4. Dispersion plots for each sample on the first (C_1) and second (C_2) canonical axes.

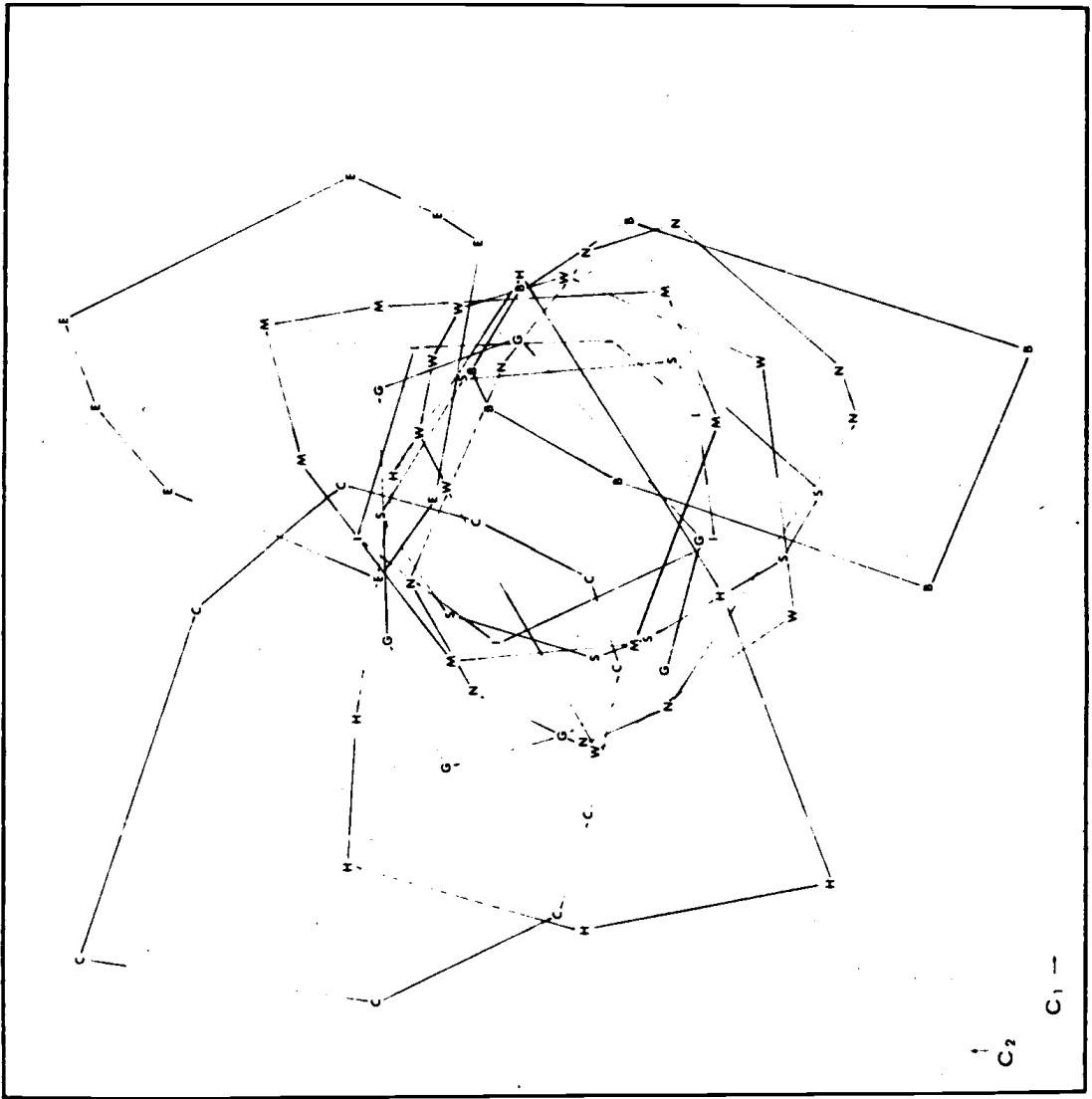


Figure 5. Centroid means plotted on the first (C_1) and second (C_2) canonical axes.

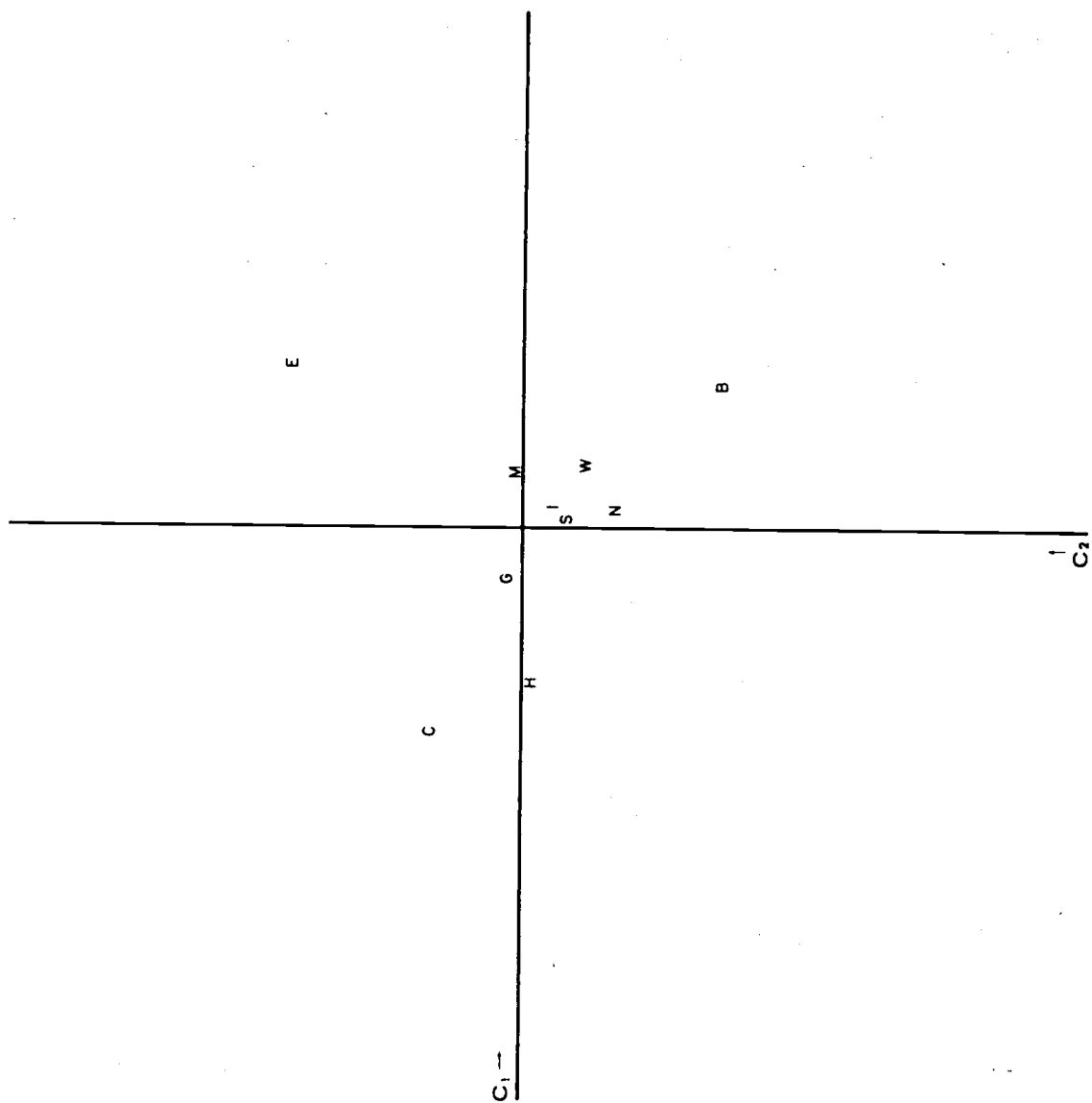


Figure 6. Dispersion plots for each sample on the second (C_2) and third (C_3) canonical axes.

