AN ABSTRACT OF THE THESIS OF

<u>Joseph J. Beatty</u> for the degree of <u>Doctor of Philosophy</u>

in <u>Zoology</u> presented on <u>11 December 1978</u>

Title: <u>MORPHOLOGICAL VARIATION IN THE CLOUDED SALAMANDER, ANEIDES</u>

<u>FERREUS</u> (COPE) (AMPHIBIA: CAUDATA: PLETHODONTIDAE)

Abstract approved: _____ Signature redacted for privacy. _____

Dr. Robert M. Storm

Multivariate morphometric analyses performed on ten populations of <u>Aneides ferreus</u> indicate that phenotypic expression in this species is exceedingly similar, but that on a fine scale there is considerable significant heterogeneity between most populations as well as between regional groups of populations. In terms of variation between California, Oregon, and Vancouver Island specimens. California is differentiated from Vancouver Island at D^2 of 2.13; Oregon-California $D^2 = 1.35$, and Oregon-Vancouver Island exhibited D^2 of 1.38. These values represent significant F-ratio comparisons at $p \ll .01$.

These slight but significant differences in phenotypic expression
are not unexpected for a species which ranges over such a wide and
varied geographic area, and can be correlated with major topographic
features of the Pacific Northwest. Prior to Pleistocene glaciation,
populations of A. ferreus were probably continuous throughout north-
western California, western Oregon and Washington, as well as Vancouver
Island and British Columbia. As Cordilleran ice advanced and inundated
most of British Columbia, and Vancouver Island, populations became iso-
lated in refugia on Vancouver Island, and were probably extirpated from
British Columbia proper. The slight, but significant levels of hetero-
geneity produced from comparisons of Vancouver Island specimens with
California and Oregon specimens indicate that the observed disjunction
between Vancouver Island populations and those inhabiting coterminous
U.S. is probably a relatively recent occurrence, correlating well with
events produced by the Fraser Glaciation approximately 15,000 yr BP.

Since other plethodontids inhabit Vancouver Island and Washington
it is impossible to utilize Pleistocene glaciation as a direct causal
mechanism which produced the disjunction, accounting for the absence
of A. ferreus in Washington.

On a finer scale, significant phenotypic dissimilarity was found
to exist between a majority of the pairwise comparisons between popu-
lations within each of the three regions. Patterns of heterogeneity
within California populations are consistent with predictions derived

by utilizing a model based upon a gradual latitudinal environmental cline.

No apparent pattern can be seen when pairwise comparisons are made between Oregon populations, although all populations exhibit slight but significant levels of heterogeneity. The effects of Pleistocene climatic events upon California and Oregon populations were probably slight since the maximum southern incursion of Cordilleran ice reached latitudes just south of Olympia, Washington. Montaine glaciers in the Klamath-Siskiyou Mountains provided potential barriers to gene flow between California and Oregon populations until about 10,000 yr BP.

The equable redwood forest ecosystem in northwestern California must be considered to be the ancestral habitat for _Aneides_ in general and _A. ferreus_ in particular. Specimens from this region exhibit high tooth counts, a character that has been considered to be primitive by Wake (1963; 1966). Also, all three western _Aneides_ occur in sympatry in northwestern California redwood associations, and the earliest known fossil _Aneides_ were associated with redwood forests during Eocene times in Montana.

Comparison of morphological relationships with electrophoretic and chromosomal data for each region and population indicates that there is little agreement between morphological and electrophoretic relationships, but that karyotypic variation is present which allows classification of a collection of individuals to one of the three regions with a high degree of certainty.

Morphological Variation in the Clouded Salamander,
Aneides ferreus (Cope)
(Amphibia: Caudata: Plethodontidae)

by

Joseph J. Beatty

A THESIS

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ACKNOWLEDGEMENTS

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MORPHOLOGICAL VARIATION IN THE CLOUDED SALAMANDER, ANEIDES FERREUS (COPE) (AMPHIBIA: CAUDATA: PLETHODONTIDAE

INTRODUCTION

Evolutionary studies of lungless salamanders of the family Pletho-
dontidae have been fruitful in terms of contributions to our under-
standing the process of speciation (Highton, 1977; Highton and Webster,
1976; and Tilley et al., 1978), various aspects of life history theory
(see Houck, 1978, and Tilley, 1978, for reviews), and as general models
to gain insights into the evolutionary process iteself (Highton and
Larsen, ms; Wake and Lynch, 1975).

Plethodontid salamanders are an ancient and diverse group (Lowe,
1950; Wake, 1966 and Wake and Lynch, 1975). Currently, the family is
divided into four tribes and approximately 23 generic groups; there are
over 200 recognized species (Figure 1). The family is thought to have
originated from an ambystomatid-like ancestor in mountain-brook type
habitats of Appalachia where the most primitive members of the family
still reside (Dunn, 1926; Wake, 1966). Early in the history of the
group a major terrestrial adaptive radiation took place culminating in
the Tribe Bolitoglossini. This initial radiation was apparently fol-
lowed by a second terrestrial radiation which produced the Tribe
Plethodontini. Bolitoglossines now survive in North America only as
remnants of what was probably a continuous distribution over the entire
continent. However, this group has successfully invaded the Neotropics

Figure 1.   World distribution of the family Plethodontidae.
            Numbers indicate genera, species at different
            latitudes.   Courtesy of David B. Wake.

1.1

2.2
4.7

5.11
5.9

3.5

4,4
8,12

10, 27
13, 39

Plethodontidae

14,38
6,15

2,12
8, 57

5, 33
4, 38

2,8
1,4

1,2
1,1

3
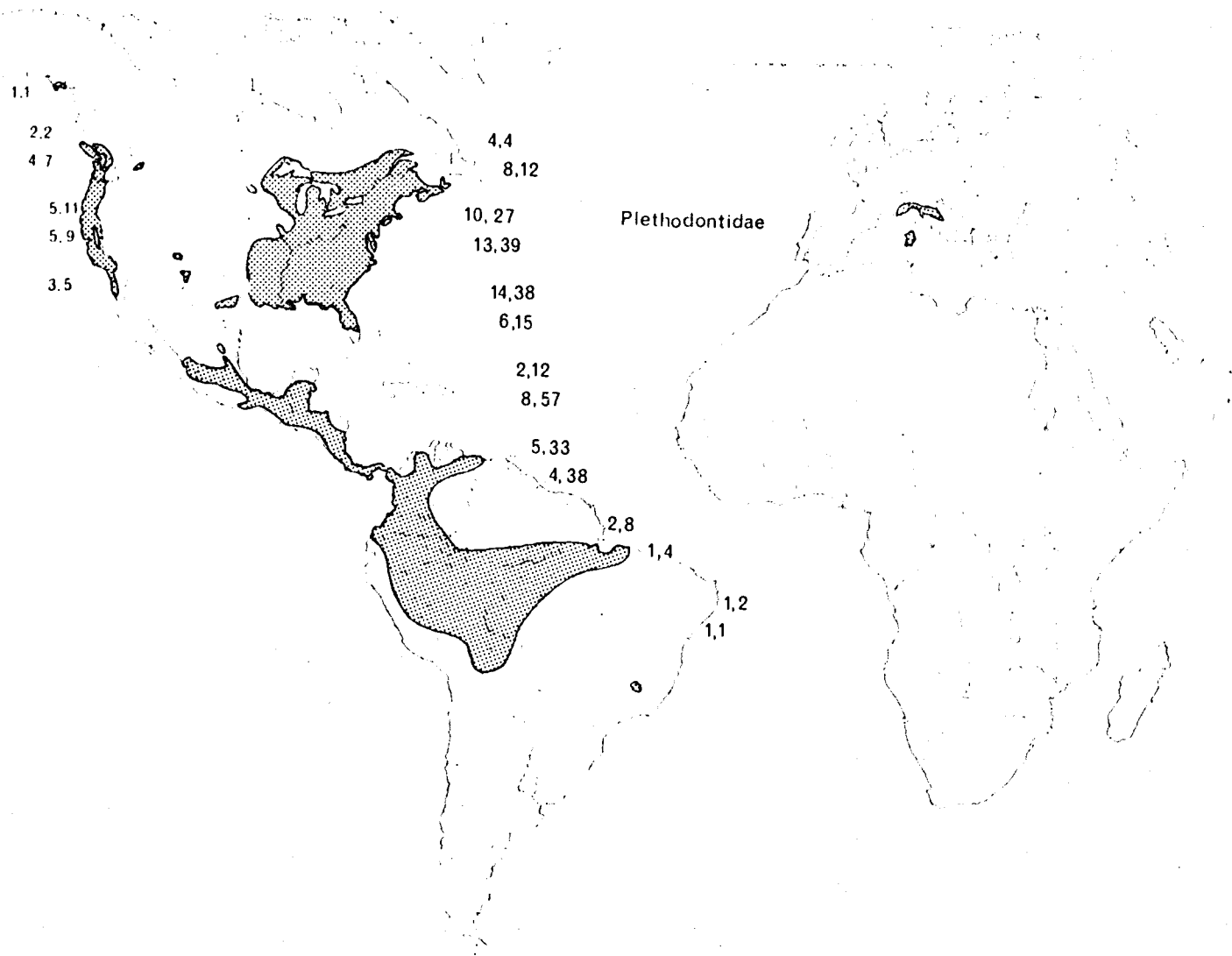
and has undergone tremendous diversification there where nine genera
and over 150 species account for over 50% of the world's caudate amphi-
bians (Wake and Lynch, 1975; and Figure 1).

The Tribe Plethodontini is composed of three closely related
genera: Plethodon, Ensatina, and Aneides. Ensatina is confined to
western North America, but Plethodon and Aneides both have representa-
tives in eastern and western North America, as well as Rocky Mountain
isolates (Highton, 1962; Wake, 1966).

The genus Aneides contains five species and their unique distri-
bution is illustrated in Figure 2. There is an eastern species, aeneus,
which is largely restricted to wet rock-face habitats in Appalachia
(Gordon, 1952; 1967), a Rocky Mountain isolate, hardii, and three
western species: ferreus, flavipunctatus, and lugubris. The evolu-
tionary relationships within the genus have been outlined by Wake
(1963; 1966) who considers A. hardii to be the most primitive member
of the genus. He bases this conclusion on the retention of a suite of
osteological characters which seem to be similar to those of the more
generalized genus, Plethodon.

Evolutionary relationships of the three western species are more
straightforward. Aneides flavipunctatus is the least highly derived
osteologically and ecologically (Wake, 1963; 1966; Lynch, 1974a). In
northwestern coastal California, all three western forms may be collected
syntopically (personal observation). However, for the most part, there

Figure 2. Distribution of the genus _Aneides._

800 km
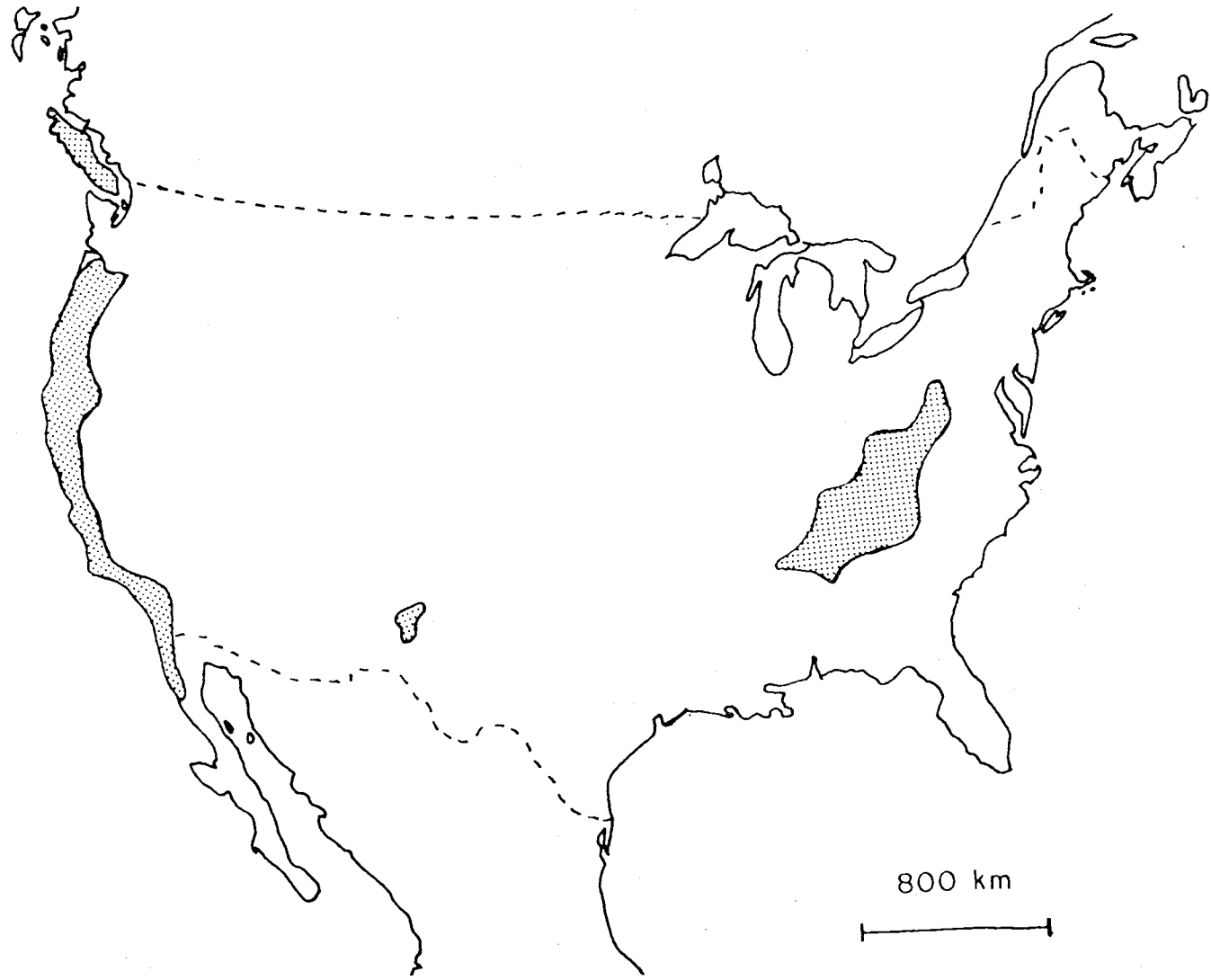
is considerable ecological segregation between the three species.
Aneides flavipunctatus is usually restricted to low (sea level to 1000 m)
mesic habitats in northwestern California and extreme southwestern Oregon
(Lynch, 1974a, 1974b). It is commonly found under bark, logs, and other
surface debris as well as in stabilized talus rock-faces. Aneides lugu-
bris, a highly derived form (Wake, 1963), is largely associated with
more xeric habitats (derivatives of Madro-Tertiary floral units) at
elevations of 600 m or below (Lynch and Wake, 1974). The range of habitat
types in which this species is found is quite great, and can vary from
the extremely mesic redwood (Sequoia sempervirens) forests of northern
California to the xeric oak-chapparal assemblages of northern Baja Cali-
fornia. This salamander tends toward a more arboreal way of life and
possesses anatomical modifications to this end; namely, a prehensile
tail and long digits with terminal expansions (Wake, 1963; 1966; Lynch
and Wake, 1974).

Aneides ferreus is found in coastal northwestern California north
to the Columbia River in Oregon and also on Vancouver Island, British
Columbia (Figure 3). In Oregon it is found at elevations ranging from
sea level to about 1525 m. In all areas of its range, A. ferreus is
associated with two basic habitat types: rock-types and logs. In Oregon
the log-type habitat is virtually always Douglas fir (Pseudotsuga men-
ziesii)in varying degrees of decomposition; in California the species
may also be collected in downed redwood in addition to Douglas fir (per-

Figure 3. Distribution of <u>Aneides</u> <u>ferreus</u> with sampling localities.

200 km
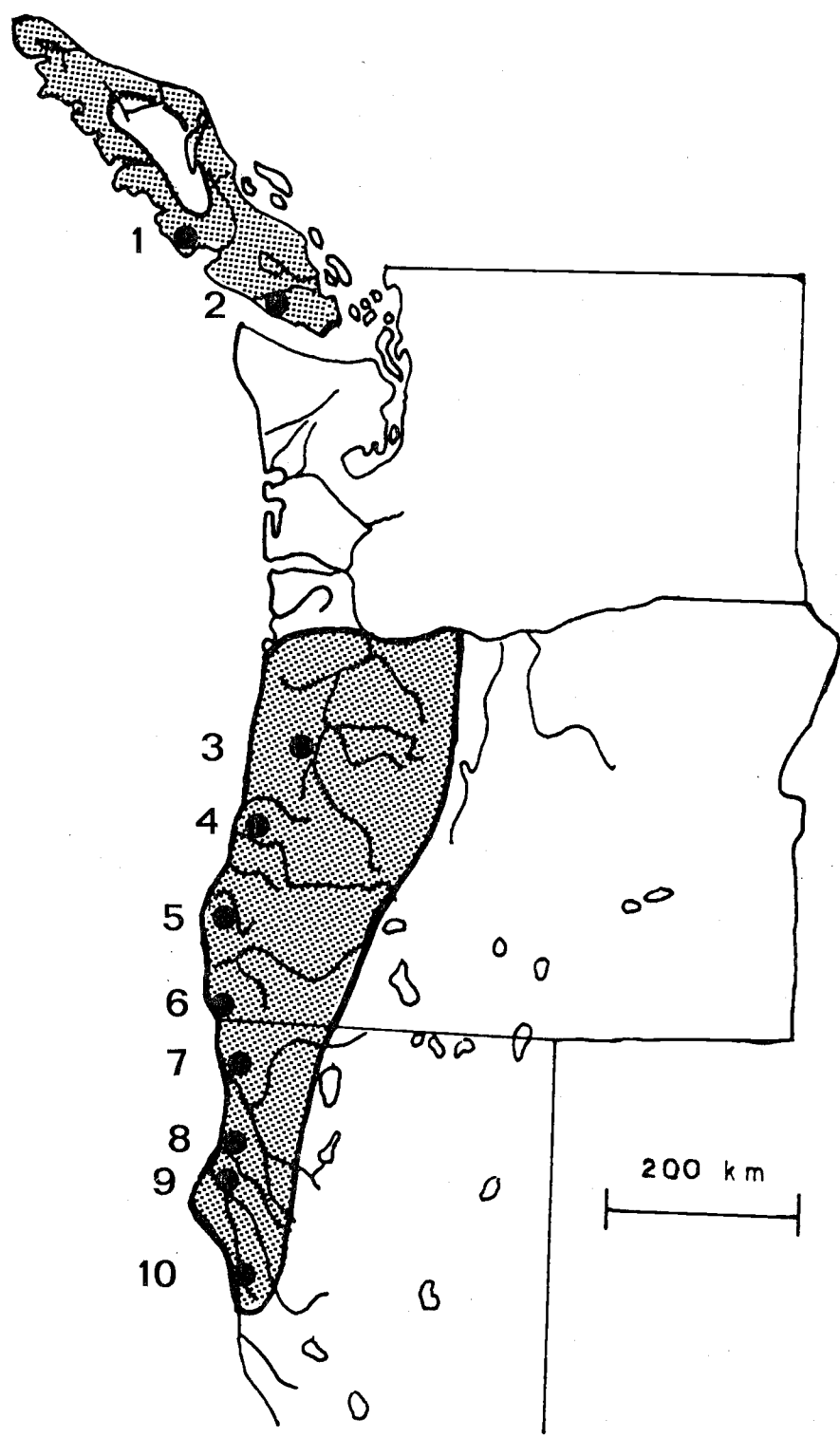
sonal observation). On Vancouver Island, specimens can be taken from Douglas fir as well as western hemlock (Tsuga heterophylla). Storm (1948) has hypothesized that there are two eco-morphs of A. ferreus associated with each of the habitats described above; a dark morph which is associated with the log-type habitat and a lighter colored morph associated with rock-type habitats.

This study was initiated in an attempt to determine levels of morphological differentiation between populations of A. ferreus, with particular emphasis on determining the morphological relationships between the disjunct populations on Vancouver Island and those found in conterminous United States.

ZOOGEOGRAPHICAL SPECULATIONS

The evolutionary history of plethodontid salamanders has been closely associated with elements of temperate Arcto-Tertiary Geofloral units (Lowe, 1950; Wake, 1966; Wake and Lynch, 1975). There is a paucity of fossil plethodontids, but fossil vertebrae from Eocene of Montana (Wake and Lynch, 1975) are assignable to the modern genera Plethodon and Aneides. Paleobotanical studies (Chaney, 1951) indicate that during this time western Montana was covered by a forest which supported dawn redwoods (Metasequoia), indicating that the earliest known populations of Aneides were associated with a redwood-type forest.

Prior to Eocene times, the area which now comprises western Wash-

ington and Oregon was a huge marine embayment (Snavely and Wagner, 1963), but Vancouver Island and California were above water (Jones et al., 1977; MacLeod, et al., 1977; Muller, 1977). Vancouver Island was uplifted during the mid-Mesozoic within 15° latitude of the paleoequator and forms part of an allochthonous terrain that has been named Wrangellia by Jones et al. (1977). Wrangellia is composed of Mesozoic formations in the Wrangell Mountains of Alaska, a band of Triassic aged rocks on western Chichagof and northern Baranof Islands, the Queen Charlotte Islands, Vancouver Island, and possibly portions of Hells Canyon in the Snake River Valley of eastern Oregon, western Idaho, and southeastern Washington. Jones et al. hypothesize that these areas were once much more closely juxtapositioned than at present, and that tectonic events are responsible for their present spatial relationships.

During Tertiary times, ancestral populations of Aneides were confined to latitudes north of their present distribution. Kay and Colbert (1965) suggest that the temperate-tropical ecotone was located at about 52° North latitude during Eocene times, but Eocene fossils assignable to Plethodon and Aneides have been found in Eocene redwood forests of Montana suggesting that 52°N was not an absolute boundary between Arcto-Tertiary and Neotropical-Tertiary floras. Because western Washington and Oregon were part of a marine embayment during Eocene times, and eastern Washington and Oregon supported a Neotropical flora (Snavely and Wagner, 1963), it is probable that Vancouver Island has been popu-

lated by _Aneides_ and other western plethodontids longer than any other region in the Pacific Northwest. Clague (personal communication) has indicated that land bridges between the BC mainland and Vancouver Island existed at one or more times during the Quaternary, but because there are no exposed rocks of Neogene age in the Georgian Depression it is difficult to speculate upon paleogeographical relationships during earlier time periods. However, due to exceedingly small areas of sea water separation between Vancouver Island and mainland BC along the Straits of Georgia, it is quite likely that land bridges existed many times in the past, giving plethodontid salamanders access to Vancouver Island from mainland British Columbia.

A major eugeosyncline developed in Washington and Oregon along the future axis of the Cascade Mountains and volcanism along and west of this axis was responsible for uplifting areas which are now western Oregon and Washington. This process was essentially completed by Miocene times and modern land-water relationships were attained by approximately the Pliocene (Snavely and Wagner, 1963). As climates cooled in the Oligocene due to tectonal rotations of the continents, the temperate-tropic ecotone moved southward, and there is no reason to assume that associated plethodontid faunal elements did not spread southward into western Oregon and Washington along with Arcto-Tertiary floral units. This biogeographic model implies that by Pliocene times when land-water relationships were essentially modern in western Washington

and Oregon, _Aneides_ effected a continuous distribution throughout the Pacific Northwest, British Columbia, and northern California. Electrophoretic and immunological data presented by Highton and Larsen (unpublished manuscript) indicate that western and eastern elements of the Plethodontidae have been genetically distinct units for about 40 my, although avenues of gene flow remained intact until sometime during the Miocene, about 20 my ago (Boucot, personal communication).

Orogenic and tectonic activity increased greatly during the Oligocene, causing the climate of the Pacific Northwest to become progressively cooler and drier. Chaney (1925) has indicated that summer-wet conditions prevailed on exposed land areas from Oligocene time until about the end of the Miocene. This climate supported extensive redwood forests, and little topographic relief was realized until major orogenic activity in the late Miocene initiated the uplifting of the Cascade Mountains.

The newly uplifted Cascade Mountains dramatically altered climatic conditions in the Pacific Northwest. They created a major rainshadow which allowed gradual replacement of redwood-type floral units east of the Cascade axis by more xeric elements of Madro-Tertiary affiliation (Detling, 1968). These drastic environmental changes no doubt restricted ancestral _Aneides_ populations to the humid coastal zones west of the Cascade crests. This readjustment was probably complete by the end of the Pliocene, and _Aneides_ were probably present from British Columbia to California in this zone.

During the Pleistocene, the Pacific Northwest was subjected to a series of five glaciations with intervening interglacial periods (Armstrong et al., 1965; Crandell, 1965). Cordilleran ice, originating in the mountains of British Columbia, spread southward into Washington forming two lobes: an eastern Okanogan Lobe and a western Puget Lobe (Armstrong et al., 1965; Easterbrook, 1978). The Puget Lobe extended south to about the latitude of Olympia, but sent a western lobe across the Straits of Juan de Fuca and completely inundated most of Vancouver Island, except for high mountain peaks in the interior and a small area located midway up the north side of the island (Easterbrook, 1978; Heusser, 1960). Montane glaciers capped the Olympic Mountains, Cascade-Sierra Nevada Ranges, and the Klamath-Siskiyou Mountains; coastal regions in Oregon and California remained unglaciated. Maximum ice cover occurred during the Fraser Glaciation, approximately 15,000 yr BP., and lasted until ±10,000 yr BP (Armstrong et al., 1965; Easterbrook, 1978). During the height of the Fraser Glaciation, temperatures in the Pacific Northwest averaged 6°C below those of today (Heusser, 1972; CLIMAP, 1976) and grasslands, steppes, and deserts greatly increased in area largely at the expense of forests (CLIMAP, 1976; Saltzman and Vernekar, 1975). This drying trend lasted until ±4,000 yr BP and was probably responsible for the extirpation of _Aneides_ and other pletho - dontids from areas east of the Cascade Mountains. Remnant populations of _Plethodon idahoensis_ have survived as isolates in restricted

portions of northern Idaho and northwestern Montana. Amphibians with similar disjunctions such as <u>Dicamptodon ensatus</u> and <u>Ascaphus truei</u> lend strength to this line of reasoning.

. There is little doubt that Pleistocene glacial events have not contributed extensively to the modern distributional pattern exhibited by <u>A</u>. <u>ferreus</u>. Since western Oregon and northwestern California were free of glacial ice there is no problem explaining the persistence through Pleistocene times of <u>A</u>. <u>ferreus</u> in these regions.

Glacial events are still being reconstructed and evaluated for Vancouver Island, but Heusser (1960) indicates the presence of a large unglaciated area on the north side of the Island and Muller (personal communication) implies that while no part of the island was not touched by Pleistocene ice, the ice cover during any one of the five major glaciations was not complete, resulting in habitable areas during each glacial maximum (see also Halstead, 1968). The existence of these potential refugia could account for the persistence of <u>A</u>. <u>ferreus</u> as well as other amphibians on Vancouver Island in spite of Pleistocene glaciation.

An explanation as to why <u>A</u>. <u>ferreus</u> is not found in western Washington is lacking. Every plausible explanation utilizing glacial, geologic, and/or climatic events does not account for the presence of other western plethodontids in western Washington.

MATERIALS AND METHODS

SPECIMENS

The specimens used to form the morphological data base for this study were collected and uniformly preserved by me. They were killed in 0.2% Chlorobutanol, fixed in Alcohol-Formalin-Acetic Acid (AFA) as described by Gallagher and Kozloff (1971), washed in running tap water for 24 hrs., and stored in 63% ethanol. Measurements were made only after specimens had been in alcohol for at least 24 hrs. Figure 3 shows the localities of each of the populations I examined. Populations will be referred to by number and are defined in the Appendix. The primary criterion for choosing these localities was that each proved to be an area where populations were sufficiently large to stand the repeated sampling necessary to generate data for life history comparisons between populations. Most of these data will be presented elsewhere.

CHARACTERS

Populations of A. ferreus show subtle variation in dorsal and ventral coloration; however, I was unable to suitably quantify this character. Further, variation within populations proved to be much greater than variation between populations. This information will therefore be presented only in a very qualitative manner.

The following morphometric and meristic characters were estimated to the nearest 0.1 mm with dial calipers from each post-juvenile

specimen (i.e., specimens $\geq$45.0 mm snout-vent length, the approximate size at which sexual maturity is attained):

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

2. Head width (HW) was measured at the angle of the jaws.

3. Head length (HL) - from the midpoint of the gular fold to the anterior tip of the snout.

4. Body length (BL) was computed by subtracting HL from SVL.

5. Axilla-groin length (AGL) was determined with fore and hind limbs extended perpendicular to the trunk.

6. Forelimb length (FLL) was estimated by measuring from the tip of the longest digit to the axilla when the limb was extended perpendicular to the trunk.

8. The number of coastal folds (CF) between adpressed limbs was estimated to the nearest 0.5 folds for each specimen.

9. Maxillary-premaxillary teeth (MPM) were counted.

10. Vomerime teeth (VT) were counted; counts obtained from the right and left sides were summed to obtain a single value.

The reproductive condition for each animal was assessed by measuring testes and vasa deferentia and by counting and measuring ova and oviducts; these data will be reported elsewhere.

ANALYSIS OF CHARACTERS

Because of the possible problems associated with allometric growth,

differences in population structure due to genetic or environmentally induced effects, and sampling bias, many morphologists and taxonomists resort to the use of ratios in their analyses. This practice has been criticized by many authors (e.g., Christensen, 1954; Marr, 1955; McIntosh, 1955; Simpson, Roe and Lewontin, 1960; Seal, 1965) and the basic objections outlined by Blackith and Reymith (1971) involve the following arguments:

1. Because of allometric growth, even organisms of the same species will vary with respect to a given ratio unless the organisms are the same size.

2. Usually, ratios are contrived using only two characters. The relationship between these characters is often obscure or unknown altogether.

3. By creating a ratio of two characters, there is an implication that only one contrast in form will be studied, and that this contrast can be well represented by the two characters which are both weighted equally.

I elected to use ratios, despite the theoretical difficulties in order to elucidate the differences between the two approaches: the one which utilizes the original 10 variables, and the one utilizing the following ratios:

1. HW/SVL

2. FLL/SVL

9. (FLL + HLL)/BL

10. (FLL + HLL)/SVL

3. HLL/SVL   11. AGL/SVL

4. (FLL + HLL)/AGL   12. HW/BL

5. (HL/BL   13. FLL/HW

6. HW/HL   14. HLL/HW

7. HLL/BL   15. FLL/HL

8. HLL/BL

The method I used to estimate the ratios described above needs
comment. Historically, most morphological studies employ population
means as input variables into the appropriate computer programs in order
to elucidate evolutionary relationships among and between taxa of
various types (e.g. Nussbaum, 1976). This practice might lead to
artificial classifications because by using population means of morph-
ological measurements, much individual variation is sacrificed and
results obtained by this more traditional method may be misleading.
Ratios have been traditionally estimated by the following method:

$$\text{Ratio Estimate} = r = \frac{\sum_{i=i}^{n} y_i}{\sum_{i=i}^{n} x_i} = \frac{\bar{y}}{\bar{x}}$$

I chose to estimate ratios by specifically transforming individual
measurements into respective ratios. An entire population's morpho-
logical measurements, expressed as individual ratios were then input
into various computer programs described below by character matrix.

I also utilized Model I regression analysis to examine a number

of morphological relationships. Assumptions of Model I regression require that the independent variable be measured without error, that the expected value of any variable, Y, for a given value of X can be described by the linear function, $Y = \alpha + \beta x$, that for any value of X, the Y's are independently and normally distributed, and that the variance about the regression line is constant (Sokal and Rolf, 1969).

ESTIMATION OF SIMILARITIES BETWEEN POPULATIONS

Unstandardized means for each of the 10 morphological variables were arranged in a character matrix by population. Populations were then clustered utilizing the single-linkage method of Sneath (1957), employing the Pearson product-moment correlation coefficient (r) as a measure of similarity (see Sneath and Sokal, 1973, for a review of clustering methods).

I also used the OSU version of *CLUSB. This program clusters observations of multivariate data by assigning all observations to the first cluster, or group. A mean vector for the first cluster is then calculated and a second cluster is formed by taking the observation with the largest scaled distance away from that mean and setting it as a second mean; every other observation is examined to see if its scaled distance is less to the new cluster mean or to the old cluster mean. If the distance is less it is moved to the new cluster. Subsequent clusters are formed in the same manner until the desired number of clusters is created. Again, I utilized unstandardized population

means formed from the 10 variable model as input variables.

INTRAPOPULATIONAL VARIATION

In any type of study dealing with geographic variation, it is important not only to determine the level of differentiation between populations or regional groups, but also to attempt to assess the amount of variation which exists within a population or group. The range of phenotypic variation within a population is not a direct indicator of levels of genetic variation, but does indicate the range of possible phenotypes on which natural selection has the opportunity to act.

I used coefficients of variation (CV) and principal components analysis (PCA) to estimate the level of intrapopulational variation in A. ferreus. CV can be used statistically to test hypotheses, but difficulties arise as to the appropriateness and robustness of these tests (Sokal and Rolf, 1969). I employed CV to serve only as qualitative indicators of relative amount of variation present in each population for each of the 10 morphological characters described above and did not attempt any test of significance for between-population or between-group comparisons.

PCA is perhaps one of the most useful methods for determining levels of intra-populational variation. Detailed mathematical formulation of PCA may be obtained from many books dealing with multivariate

statistics (Anderson, 1958; Coohley and Lohnes, 1971; Morrison, 1976; Press, 1972) and there are numerous examples in the literature (see Blackith and Reyment, 1971, for a review).

Blackith and Reyment (1971) note that PCA can be exceedingly useful in elucidating the dependence structure of multivariate data. A smaller number of new variables (latent variables) are derived from linear combinations of the original variables whose directional cosines define orthogonal axes which minimize the covariance components of the dispersion matrix.

The first principal component will account for the largest amount of variation in the data set, and subsequent principal components add more increments of variation until all is accounted. Usually, the variation can be presented by illustrating only the first three or four principal components because they account for most of the variation in the model; including more components adds little information to understanding the dependence structure of the original data set. A general rule (Coohley and Lohnes, 1971) that only eigenvalues equal to or greater than +1 are considered to be significant in terms of their being able to explain the dependence structure of a multivariate data set is often followed.

I employed the OSU Computer Center's version of UCLA's BMDO1M program (Dixon, 1974) for PCA using the standardized (mean zero, unit variance) 10 variable data set. The original data set (n=422) was first subdivided by sex and then further divided into three groups, depending

upon whether specimens were collected on Vancouver Island or in Oregon or California. The objectives of the analyses were to determine if the relative magnitude of variation (i.e., the amount of variation accounted for by each principal component was the same or different for each subset. In this way I hope to determine if there were significant effects due to sex, and also to see if patterns and the relative magnitude of variation were the same or different in each of the three regional groups.

DISCRIMINANT ANALYSIS

In order to assess the level of interpopulational differentiation I utilized the OSU version of UCLA's BMD07M program (Dixon, 1974) for stepwise discriminant analysis. This program allows all variables to be considered in the order of their ability to maximize the ratio of between-group to within-group variance matrices. The first variable entered into the model is the one which contributes the most towards discriminating between the groups. Remaining variables are entered at each "step" depending upon their ability to add to the discriminatory power of the model, until all variables have been utilized or there are none left which satisfy a predetermined contribution to intergroup discrimination. After each step, an F-ratio is calculated which indicates a minimum level of significance which must be met in order for the next variable to enter the model; the variable with the highest F-ratio which

exceeds the minimum level will enter before any of the other remaining variables. The probability that a given individual belongs to a particular group is calculated and Confusion matrices (Massey, 1965) are constructed which summarize the number of correct and incorrect classifications made by the discrimination procedure. Canonical coefficients are calculated and plots of the first two canonical variables are given so that the position of all specimens in two-dimensional space may be evaluated.

Pairwise comparisons of centroid (=group) means may be evaluated statistically utilizing an F-ratio with m and $(t_j + t_k + t_L -2)$ degrees of freedom, calculated as follows (Sneath and Sokal, 1973):

$$F = \frac{D^2_{jk}(t_j t_k)\ (t_j + t_k - m - 1)}{(t_j + t_k)\ (t_j + t_k - 2)m}$$

where m= the number of variables in the model; t= group sample sizes for the jth, kth, and Lth groups, and D^2= Mahalanobis' generalized distance measure (Mahalanobis, 1936) between the jth and kth groups.

I manipulated the data in several different ways in these analyses: In the first set of analyses, sample size was 422, and I used the 10 standardized morphological characters outlined above. I wanted to test

to determine if there were any general trends in morphological diver-
gence which could be detected on a regional basis, so the data were
divided into three groups: Vancouver Island, Oregon, and California,
depending upon where specimens were collected. A second analysis
was made with the data grouped into the discrete populations that I
samples (n=10; see Appendix). This same scheme was applied to the
15 ratios outlined above (i.e., a three group analysis and a 10 group
analysis). Lastly, I made a three group analysis using population
means for the 10 original variables (standardized to mean zero and
unit variance); where sample sizes allowed, I analyzed sexes separately.

RESULTS

INTRAPOPULATIONAL VARIATION

The coefficients of variation (CV) for each of the 10 morphological characters utilized in this study were modestly large in all populations I examined. CV for SVL averaged 0.08 (range: 0.06-0.14) and the other six metric characters exhibited CV of similar magnitude. The three meristic characters (CF, VT, and MPM) exhibited high CV. This is to be expected for CF counts as considerable error might be associated with straightening limbs and trunks; also gravid females always exhibit higher CF counts than non-gravid individuals and males. These obvious problems indicate that great care must be taken when interpreting these data. On the other hand, I feel my tooth counts are accurate and their CV (VT=0.21, range: 0.14-0.34; MPM=0.22, range: 0.14-0.33) indicate a substantial amount of intrapopulational variation.

Table 1 gives means, standard errors, and within group correlation coefficients for MPM and VT for the 10 populations of A. ferreus I examined. Since the significance of these correlations was sporadic, and tooth counts seemed to show an increase in number with decreasing latitude (Table 1), I used least-squares regression in an attempt to demonstrate a significant linear relationship between tooth counts and latitude. Latitude was designated as the independent variable and tooth counts the dependent variable. The northern most population (1) was arbitrarily assigned as zero and distances in km to the other populations

Table 1. Mean number of maxillary-premaxillary and vomerine teeth, standard errors, and within-group correlation coefficients between MPM and SVL and HW, and VT and SVL and HW; * indicates p < .05; ** indicates p<.01.

Population	$\bar{X}$	SE	MPM r_{SVL}	r_{HW}	$\bar{X}$	SE	VT r_{SVL}	r_{HW}
1	13.41	0.42	0.31*	0.51**	9.78	0.24	0.05	0.04
2	11.88	0.39	0.23	0.33*	7.76	0.26	-0.14	-0.02
3	13.64	0.34	0.08	0.24	10.32	0.29	0.07	0.10
4	12.98	0.35	0.35*	0.46**	9.22	0.29	0.18	0.27*
5	14.72	0.42	0.21	0.40**	8.98	0.26	-0.02	0.13
6	14.38	0.37	0.14	0.36**	9.54	0.31	-0.04	-0.14
7	15.21	0.76	-0.34	-0.37	11.26	0.44	-0.07	-0.17
8	16.00	0.42	0.41**	0.47**	10.98	0.27	0.48**	0.47**
9	15.53	0.53	0.01	0.27	10.74	0.34	0.05	-0.05
10	14.37	0.59	0.54**	0.49**	10.83	0.31	0.37*	0.31*

Figure 4. Variation in VT and MPM tooth counts. Means indicated by horizontal lines; solid bars represent ± 2 standard errors. Populations are numbered according to Figure 3. Lines fitted by least-squares regression on individuals' tooth counts and are significant at $p < .01$.

MPM = 12.95 + .002 (LAT)

R^2 = .048

VT = 9.33 + .0007 (LAT)

R^2 = .021

LATITUDE
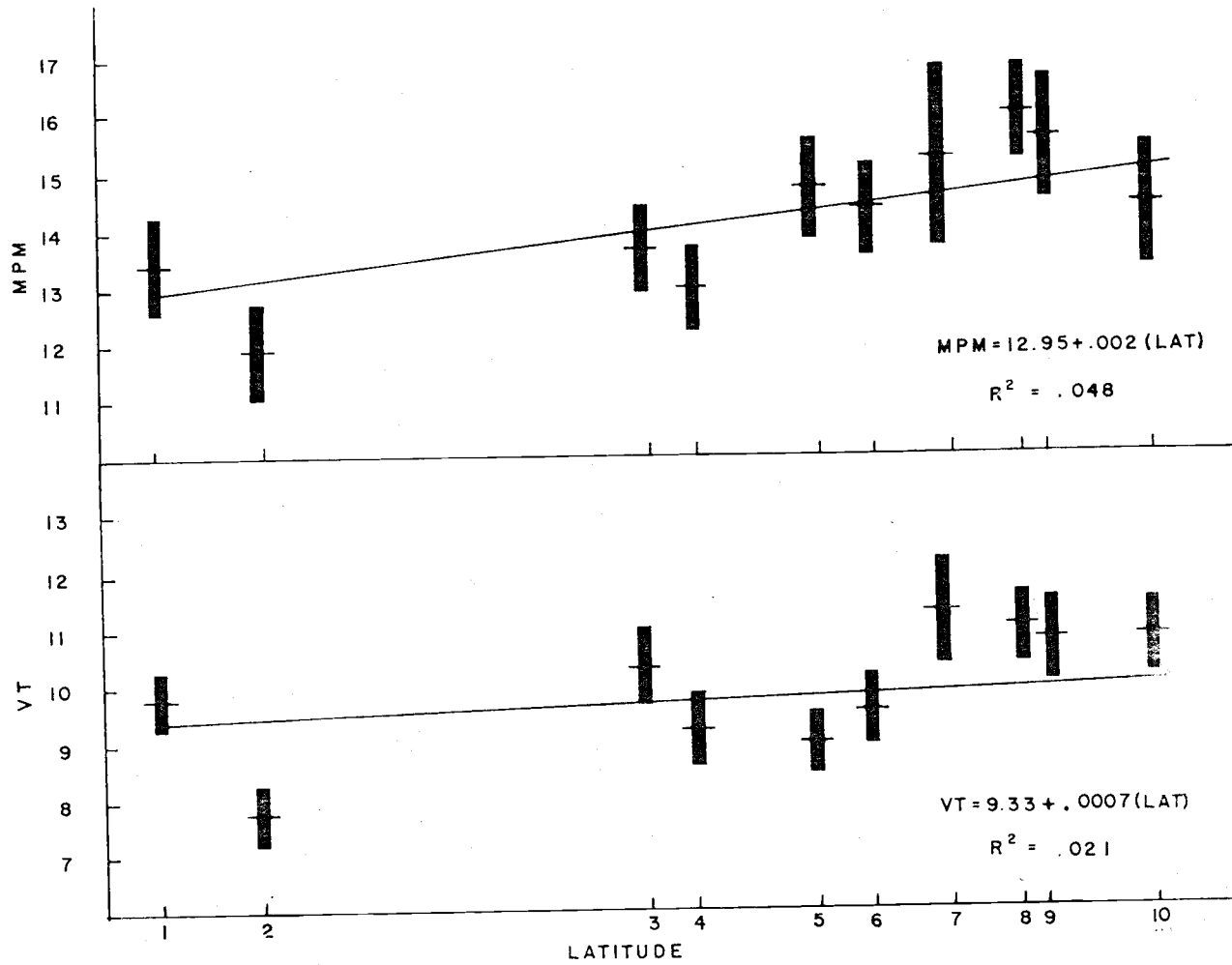
Table 2. Costal folds between adpressed limbs for 10 populations of A. ferreus; (-) denotes lack of limb overlap, (+) indicates limbs overlap.

Population	Number of Costal Folds												$\bar{X}$
	-4.0	-3.5	-3.0	-2.5	-2.0	-1.5	-1.0	-0.5	0.0	+1.0	+1.5	+2.0	
1			3	5	10	9	16	4	2				-1.49
2		1	4	5	11	11	14	1	3				-1.62
3	1	6	9	7	9	9	3	5	1				-2.14
4			1	6	12	8	11	9	2				-1.29
5				4	8	8	10	5	23	9	2		-0.50
6			2	1	10	4	15	8	11				-1.05
7					1	2	2	1	5	6	2		-0.11
8					3	2	14	7	16	3	1		-0.53
9				1	2	2	5	2	5	2			-0.74
10						2	2	11	6	11	3		-0.06

Table 3. Eigenvalues and the cummulative proportion of variation each explains in the 10 variable data set.

	Vancouver Island		Oregon		California	
	Males	Females	Males	Females	Males	Females
PC1	6.3097(0.63)	6.3334(0.63)	5.9470(0.59)	5.0637(0.51)	5.8228(0.58)	5.7641(0.58)
PC2	1.2146(0.75)	1.4010(0.77)	1.3672(0.73)	1.7112(0.68)	1.3754(0.72)	1.8986(0.77)
PC3	1.0158(0.85)	0.8702(0.86)	0.9416(0.83)	1.3558(0.81)	1.0987(0.83)	0.8807(0.85)
PC4	0.8578(0.94)	0.7377(0.93)	0.5541(0.88)	0.6365(0.88)	0.7246(0.90)	0.6091(0.92)
PC5	0.1841(0.96)	0.2184(0.96)	0.4528(0.99)	0.5445(0.93)	0.3392(0.94)	0.2984(0.95)
PC6	0.1476(0.97)	0.1880(0.97)	0.2897(0.96)	0.3876(0.97)	0.2424(0.96)	0.2363(0.97)
PC7	0.1435(0.99)	0.1027(0.99)	0.2010(0.98)	0.1602(0.99)	0.1796(0.98)	0.1703(0.99)
PC8	0.0910(1.00)	0.0792(0.99)	0.1570(0.99)	0.0899(0.99)	0.1440(0.99)	0.0962(1.00)
PC9	0.0351(1.00)	0.0689(1.00)	0.0886(1.00)	0.0492(1.00)	0.0715(1.00)	0.0378(1.00)
PC10	0.0007(1.00)	0.0004(1.00)	0.0008(1.00)	0.0011(1.00)	0.0017(1.00)	0.0069(1.00)

were then estimated using map and ruler. Figure 4 illustrates the results of these analyses. In both regression models, there is a highly significant (p <0.1) linear relationship between the number of VT and MPM with decreasing latitude. The R^2 values (the coefficient of multiple determination) were exceedingly small, suggesting that other factors besides latitude are also important influences on tooth counts.

Variation in CF is depicted in Table 2. Despite the previously mentioned shortcomings, it appears that the longest legged forms of A. ferreus have been drawn from populations in California, while Oregon and Vancouver Island populations generally tend to have shorter legs and/or longer bodies. Examination of the ratio, FLL+HLL/ SVL, the reciprocal of which has been termed the coupling value (Peabody, 1959; Brame and Murray, 1968) revealed no significant pattern that could be construed to be related to any aspect of latitude or physiography with respect to the relationship between relative limb and body length.

The results of PCA indicate that on a regional and sex specific basis, populations of A. ferreus vary in body proportions in the same manner and magnitude (Table 3). In each of the six analyses the first principal component accounted for an average of 59% of the total variability (range: 58-63%). The second principal component accounted for about 15% of the observed variability (range: 12-19%) and the third about 10% (range: 8-13%). It is important to note that, in each

analysis, the relative magnitude of the amount of variation explained by each of the principal components is nearly equal, even when sexes are separate.

DISCRIMINANT ANALYSIS

Figures 5 and 6 and Table 4 summarize the results of stepwise discriminant analysis obtained from the 10 variable model utilizing standardized morphological data. These data were divided into three groups depending upon where specimens were collected (i.e., Vancouver Island, Oregon or California). Figure 5 illustrates the positions of centroid means constructed from plots of the first two canonical variates in two dimensional space. The three centroid means differ significantly from one another (average F ratio ($\bar{F}$) = 9.93; df=10, 410; p<<.01), indicating a high degree of heterogeneity between the three regional groups. Mahalanobis' generalized distance measure (D^2) shows that Vancouver Island-California populations exhibited the greatest amount of morphological differentiation (D^2=2.13), while Vancouver Island-Oregon comparisons were intermediate between California-Oregon comparisons (D^2=1.38 and 1.35 respectively).

Figure 6 illustrates the plots of canonical scores for each specimen on the first two canonical axes. Due to the dense scatter of the data points, I calculated the percent composition, by geographic region, of each of the four quadrats. These data are illustrated by

Figure 5. Group means plotted on first two canonical axes; 3-group, 10 variable model. C = California, O = Oregon; V = Vancouver Island.

SECOND CANONICAL AXIS

V

C

O

FIRST
CANONICAL
AXIS

Figure 6. Individual canonical scores plotted on first two
           canonical axes; 3-group, 10 variable model.
           C = California specimens, O = Oregon specimens,
           V = Vancouver Island specimens. Histograms indicate
           percent of individuals from each of the three regions
           in each of the four quadrats.
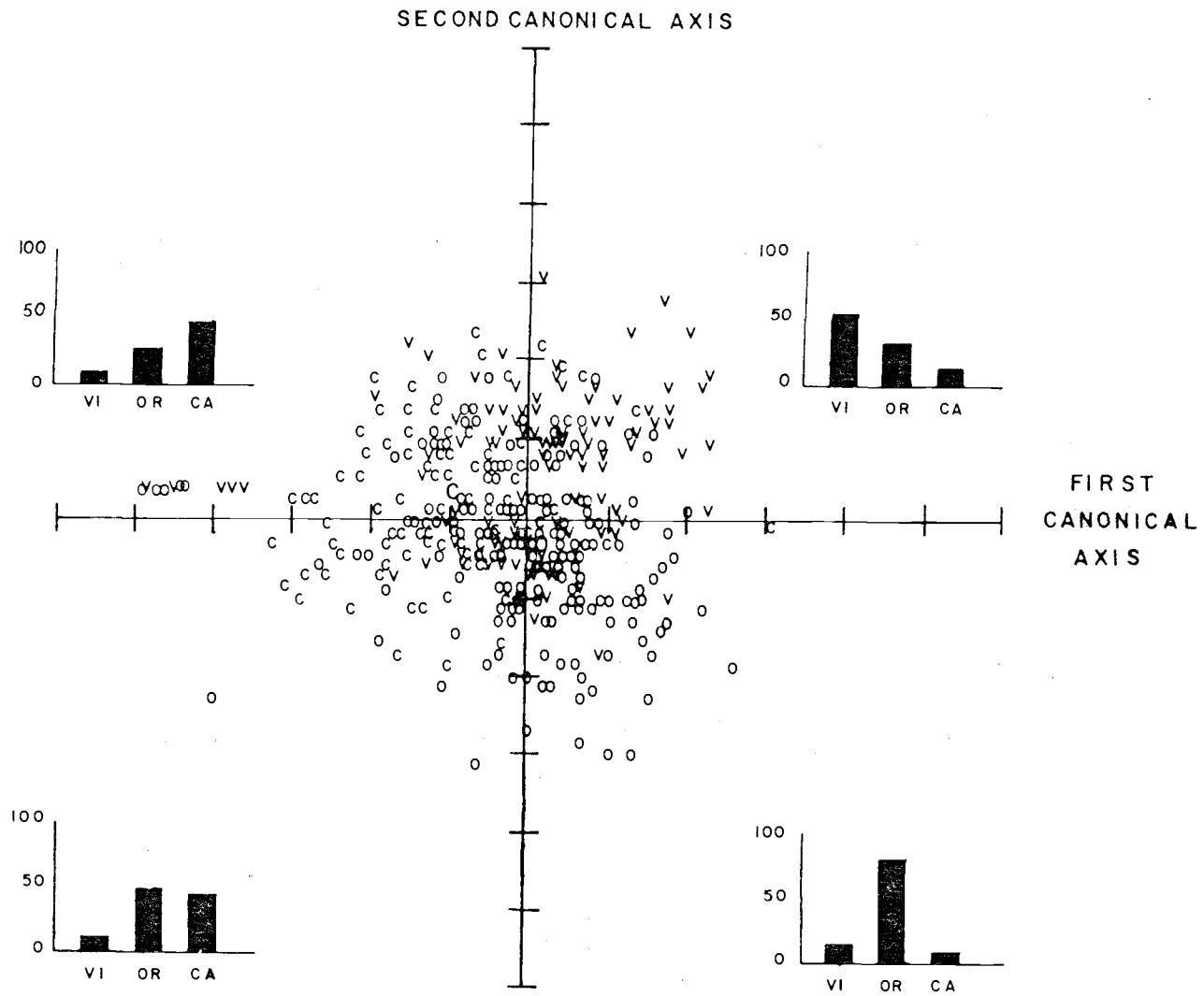
Table 4. Confusion matrix generated by 3-group, 10 variable stepwise
discriminant model. Diagonal elements represent correct
classifications; off-diagonal elements incorrect
classifications.

Regional Groups	Vancouver Island	Oregon	California
Vancouver Island	67	14	18
Oregon	44	114	46
California	23	19	77

frequency histograms in each quadrat. The bulk of the specimens located in the first quadrat are from populations on Vancouver Island. Oregon specimens tend to be found in greatest frequency in the third and fourth quadrats, while California specimens occur predominantly in quadrats two and three.

While statistically significant differences exist between centroid means, there is extensive overlap between groups as evidenced by the Confusion matrix (table 4). Correct classifications ranged between 56 and 68% ($\bar{x}$ = 63%). Extensive overlap is also shown by the frequency histograms in each quadrat (Figure 6).

The results obtained by employing the 15 standardized ratios and standardized CF, VT, and MPM counts in the stepwise discriminant model did not differ significantly from those obtained utilizing the 10 standardized variables (Figures 7 and 8). Average correct classifications (Table 5) ranged from 56-68% ($\bar{x}$ = 63%) and it is readily apparent from Figure 8 that there is again considerable overlap between groups as evidenced by plots of the first two canonical variates, even though centroid group means differ significantly ($F=7.30$; df 10, 410; $p \ll .01$). The pattern and magnitude of the distribution of individuals in each of the four quadrats, as depicted by the histograms, is exceedingly similar to those obtained from the 10 variable data set. I used these results to justify the contention that the more complicated data set constructed from ratios derived from the original 10 variables offers little in the

Figure 7. Group means plotted on first two canonical axes;
3-group, 17 variable model (ratios). C = California,
O = Oregon, V = Vancouver Island.
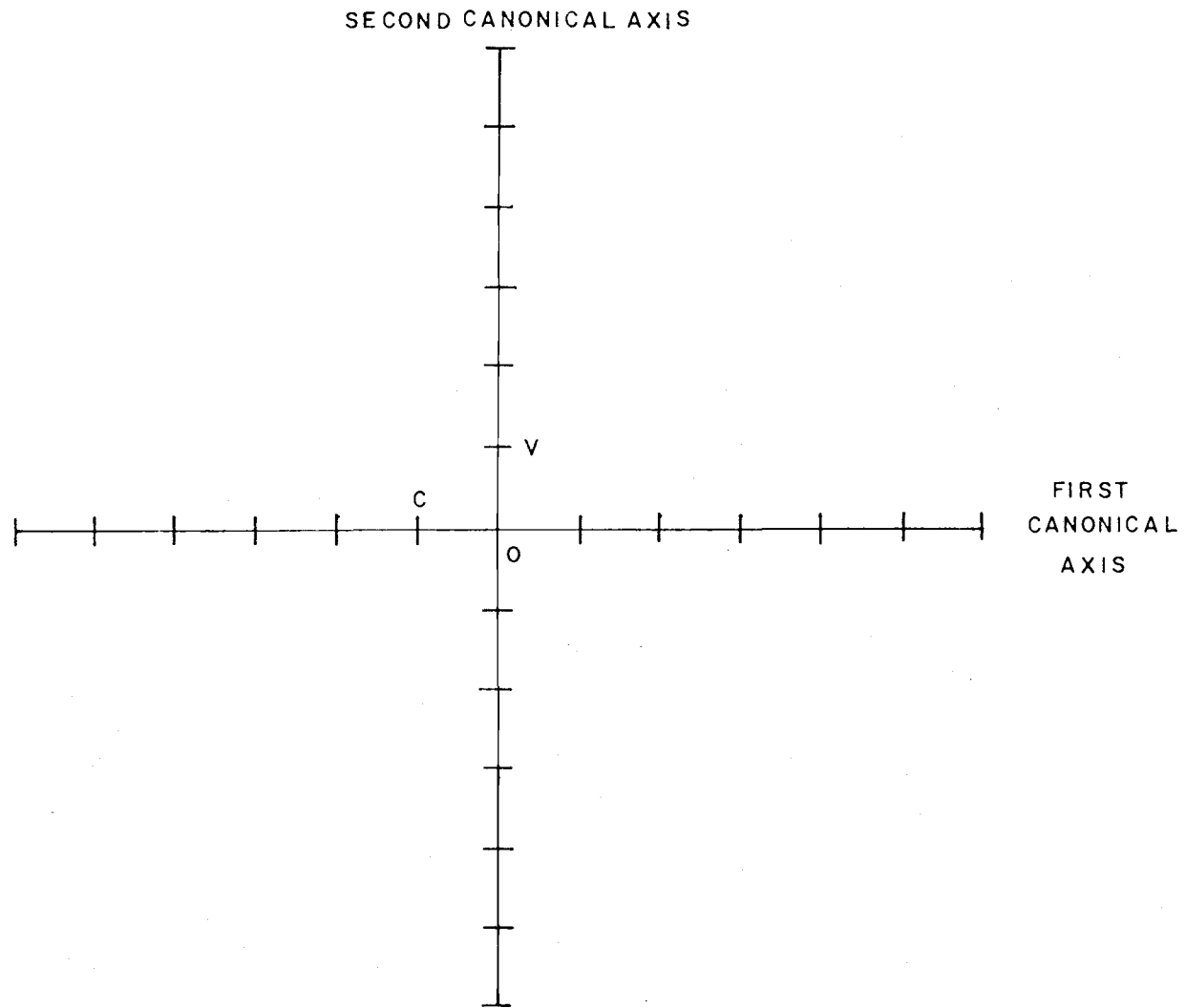
SECOND CANONICAL AXIS

C   V

O

FIRST
CANONICAL
AXIS
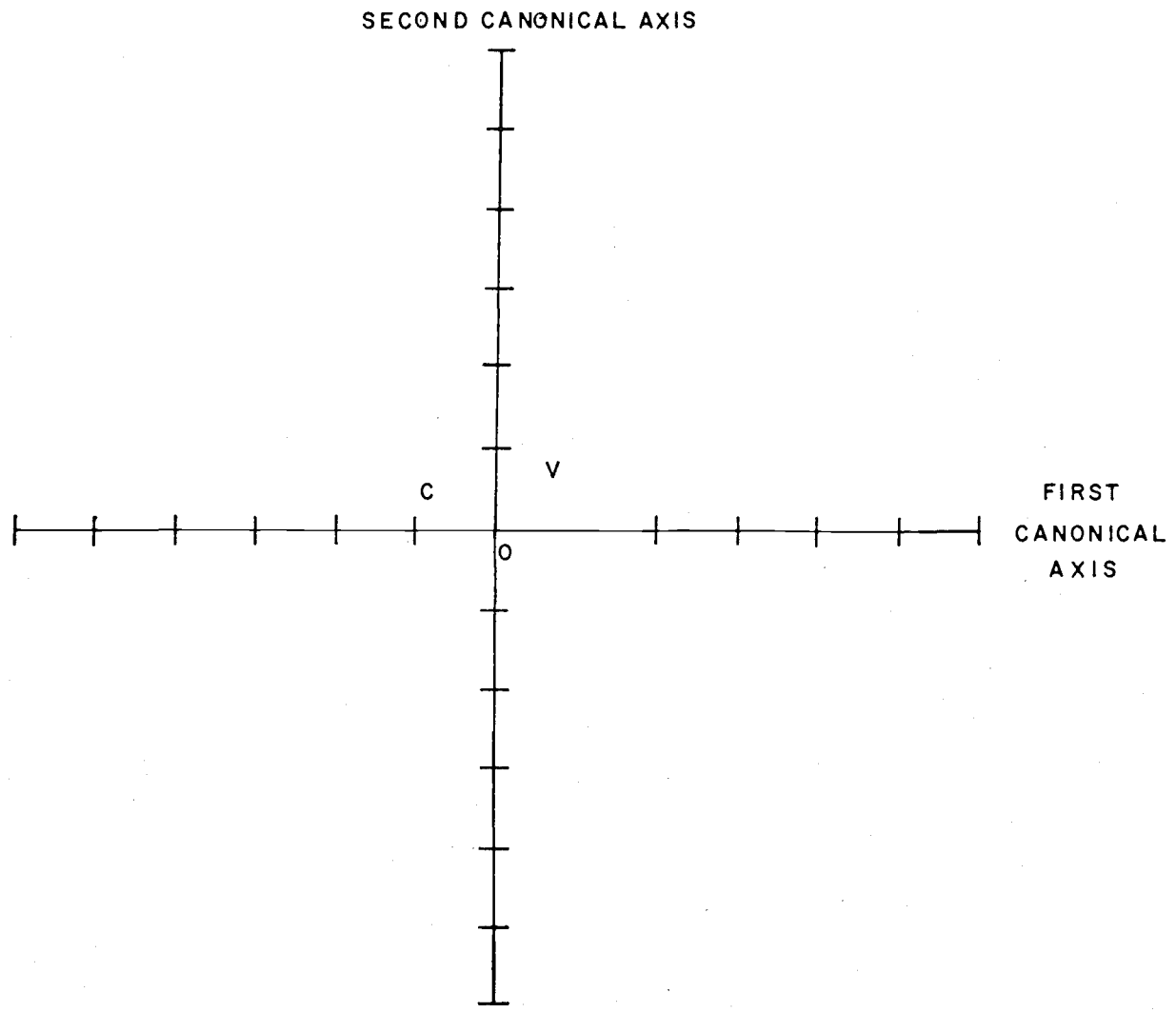
Figure 8. Individual canonical scores plotted on first two
canonical axes; 17 variable (ratio) model. C =
California specimens, O = Oregon specimens,
V = Vancouver Island specimens. Histograms
indicate percent of individuals from each of the
three regions in each of the four quadrats.

SECOND CANONICAL AXIS

FIRST CANONICAL AXIS
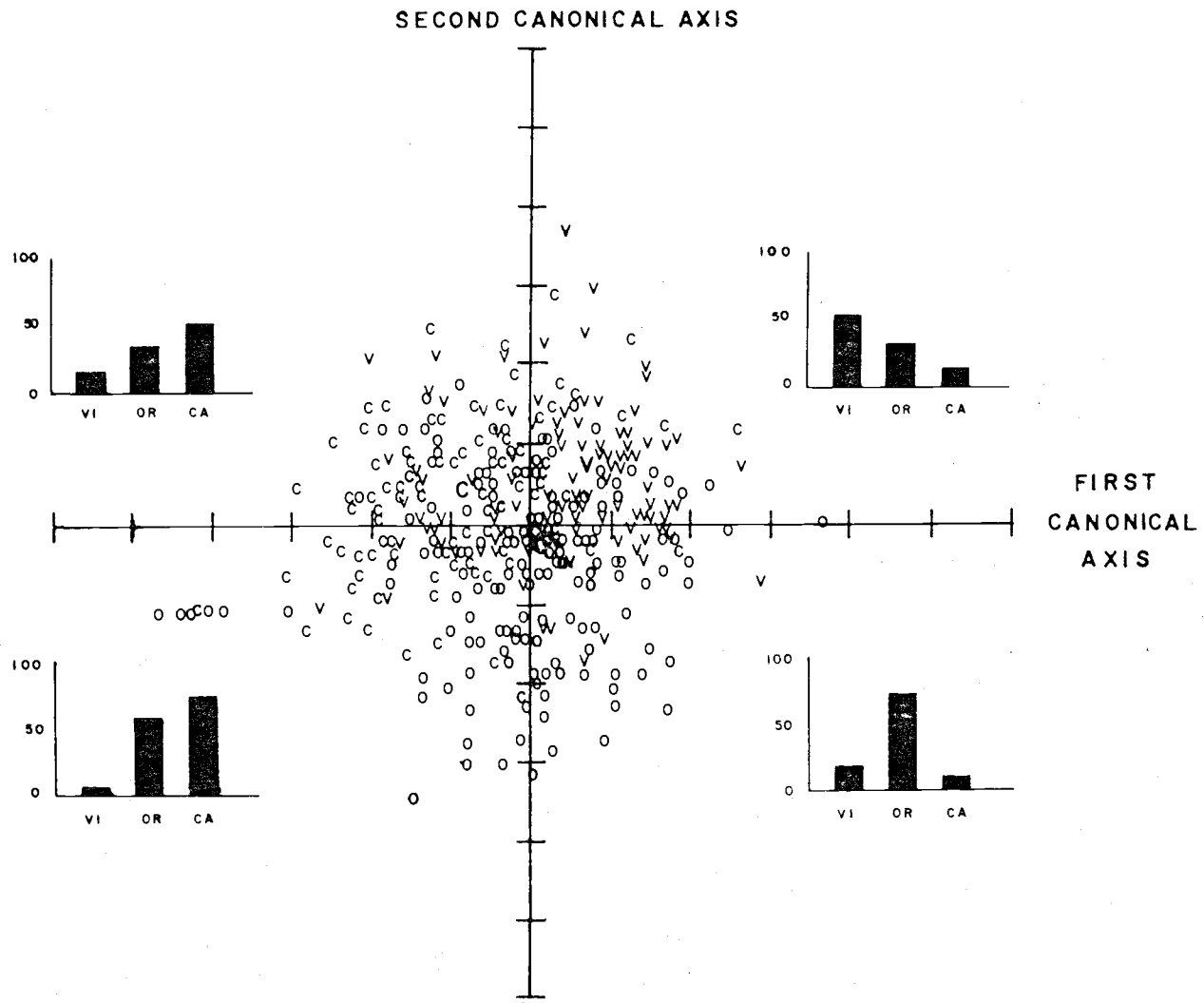
Table 5. Confusion matrix generated by 3-group, 17 variable (ratios) stepwise discriminant model. Diagonal elements represent correct classifications; off-diagonal elements incorrect classifications.

Regional Groups	Vancouver Island	Oregon	California
Vancouver Ialand	67	19	13
Oregon	43	114	47
California	19	23	77

Figure 9. Group means (higher case) and individual canonical scores (lower case) plotted on first two canonical axes; 3-group, 10 variable model utilizing only males. C = California, O = Oregon, V = Vancouver Island.

SECOND CANONICAL AXIS

FIRST
CANONICAL
AXIS
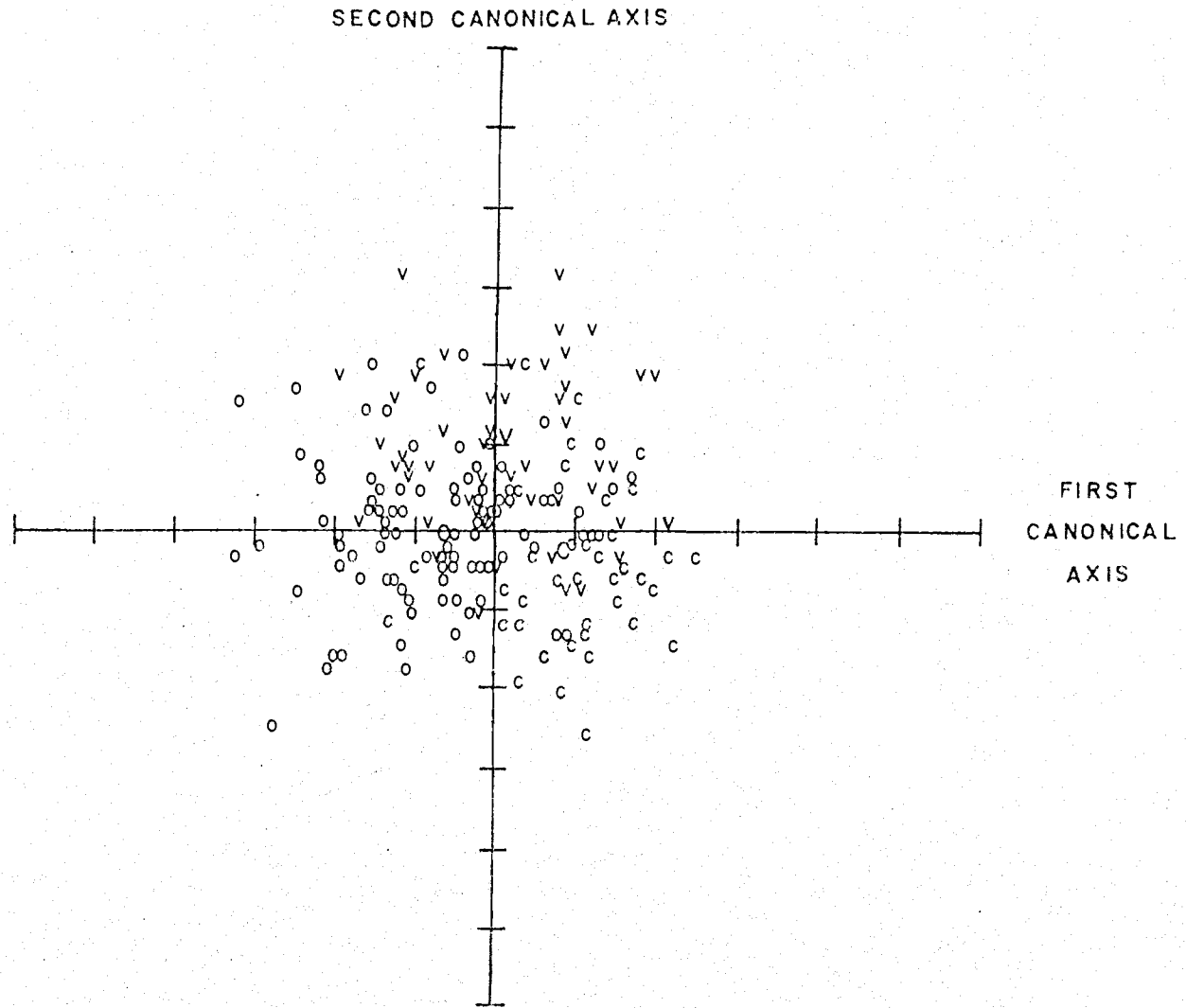
Table 6. Confusion matrix generated by 3-group, 10 variable stepwise
discriminant model utilizing only males. Diagonal elements
represent correct classifications; off-diagonal elements
incorrect classifications.

Regional Groups	Vancouver Island	Oregon	California
Vancouver Island	30	13	7
Oregon	17	69	16
California	6	4	36

Figure 10. Group means for the 10 populations of A. ferreus plotted on first two canonical axes. Numbers correspond to sampling localities of Figure 3.

SECOND CANONICAL AXIS

FIRST CANONICAL AXIS
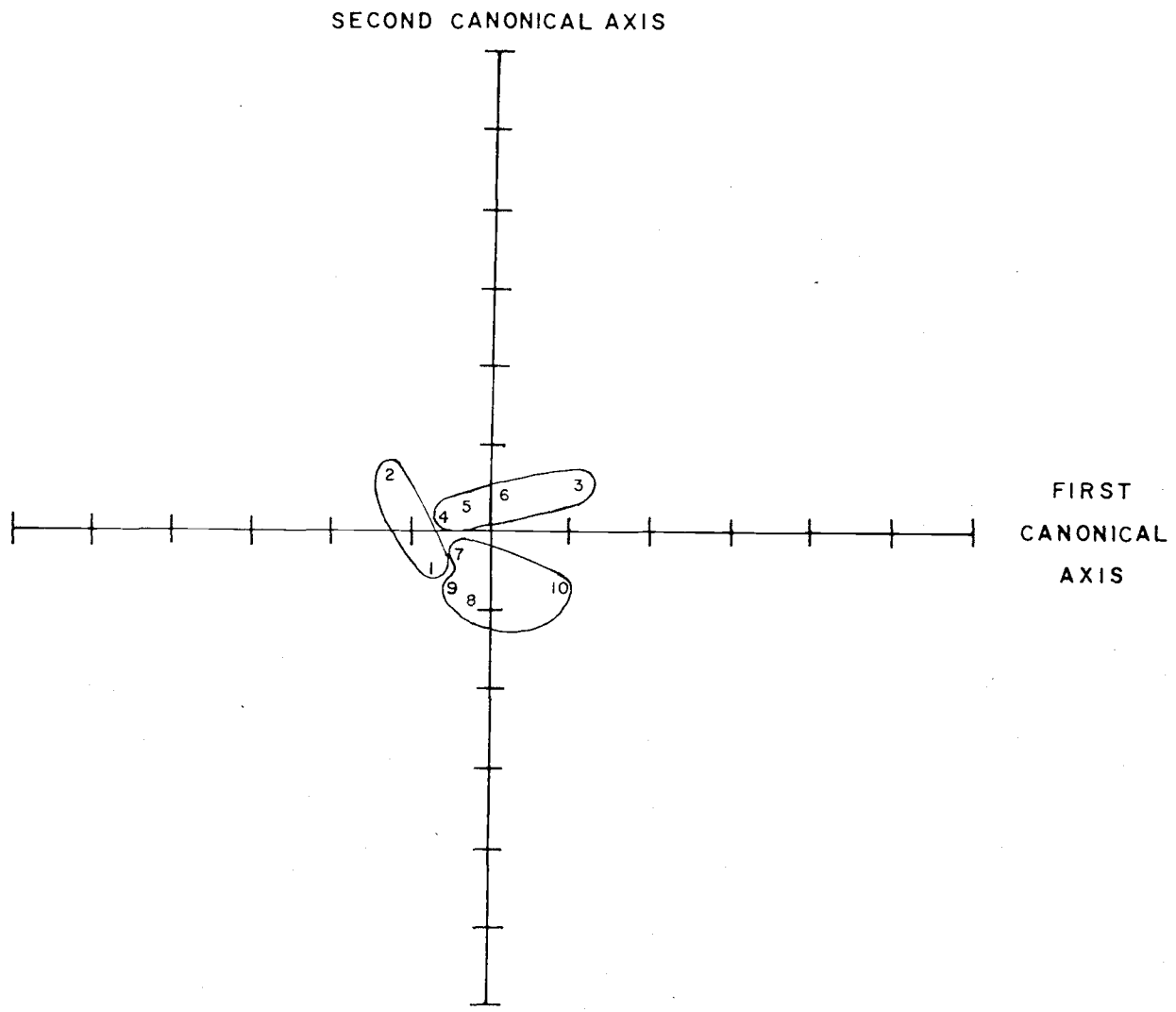
Figure 11. Individual canonical scores plotted on first two can-
            onical axes. Numbers correspond to sampling localities
            of Figure 3.

SECOND CANONICAL AXIS

FIRST
CANONICAL
AXIS
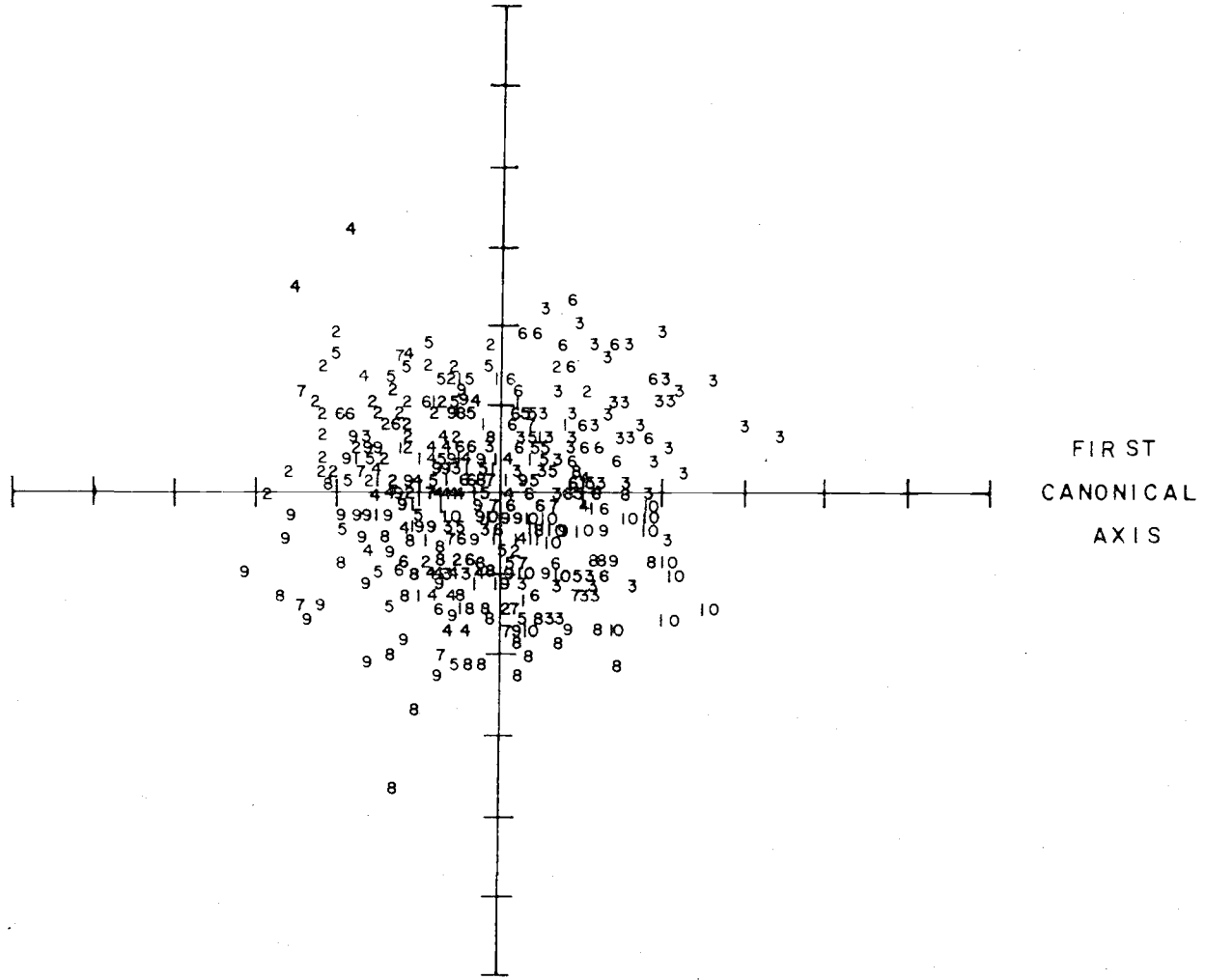
Table 7. Confusion matrix generated by 10-group, 10 variable stepwise discriminant model. Diagonal elements represent correct classifications; off-diagonal elements incorrect classifications.

Population	1	2	3	4	5	6	7	8	9	10
1	13	10	0	2	6	3	4	3	5	3
2	4	35	1	2	2	3	1	1	0	1
3	3	1	34	1	1	4	4	1	0	1
4	3	8	1	13	10	3	6	2	2	5
5	2	7	4	4	12	5	5	3	5	3
6	1	3	9	3	2	19	5	2	3	4
7	2	2	2	0	1	1	5	4	0	2
8	4	1	1	2	1	6	3	17	4	7
9	1	2	1	0	0	0	3	4	5	3
10	1	0	6	1	1	3	3	0	4	16

Table 8. Generalized distance measures (Mahalanobis' D^2) (above diagonal) and F-ratios (below diagonal) for pairwise comparisons between populations of $\underline{A}$. ferreus. * represents non-significant values.

Population	1	2	3	4	5	6	7	8	9	10
1	-	2.08	6.20	1.26	1.47	3.24	1.49*	1.35	0.57*	5.17
2	4.67	-	7.83	1.70	2.39	3.40	4.02	5.20	3.93	9.21
3	13.92	17.78	-	5.45	4.30	2.78	5.59	5.92	6.19	4.37
4	2.83	3.99	12.76	-	1.03	2.05	2.27	2.82	1.89	4.33
5	3.30	5.43	9.77	2.42	-	1.42	1.50	3.07	1.98	4.13
6	7.34	7.80	6.37	4.85	3.26	-	2.72	7.91	3.09	3.60
7	1.77*	4.80	6.66	2.77	2.40	3.26	-	2.21	0.86*	5.71
8	2.89	11.27	12.83	6.30	6.65	7.15	2.54	-	0.72*	3.43
9	0.67*	4.69	7.38	2.60	2.36	3.71	0.62*	0.68*	-	4.76
10	9.39	16.91	8.01	8.17	7.57	6.68	5.54	6.04	4.85	-

way of resolution of the objectives of this study, so I concentrated remaining stepwise discriminant analyses on the simpler 10 variable data set as its adoption should be more parsimonious.

Since the analyses I have just outlined incorporate postjuveniles of both sexes, I attempted to determine if better fit to the three group model could be obtained if sexes were analyzed separately. Figure 9 and Table 6 present the results of stepwise discriminant analysis when only postjuvenile males were utilized.

Centroid means differed significantly from one another ($\bar{F} = 6.71$; df = 10, 186; p <<.01), but $\bar{F}$ and F values are not as great as those observed in the preceeding two analyses and only slightly better classifications were obtained (average correct classification = 69%; range: 60-78%).

I also employed the discriminant model in an attempt to assess the level of phenotypic differentiation between populations of A. ferreus. These results are summarized in Figures 10 and 11 and Table 7. In this analysis, I designated as groups the 10 populations I sampled. The first two canonical variates accounted for 71% of the variation in the model. The average correct classification was 39% (range: 26-70%). Pairwise D^2 comparisons between the 10 populations of A. ferreus are presented above the diagonal in Table 8; pairwise F values below. Non significant F values are asterisked as well as their corresponding D^2 values. The only non-significant pairwise

comparisons between populations are seen when a Vancouver Island population (1) was compared to two California populations (7 and 9) and when three California populations were compared to each other (7 and 9; 8 and 9). Average D^2 value ($\bar{D}^2$) between Vancouver Island and California was 3.86, and $\bar{D}^2$ between Vancouver Island and California was 3.86, and $\bar{D}^2$ comparisons between California and Oregon populations was 3.84. These values indicate that there is roughly the same degree of differentiation between California and Oregon populations as there is between Vancouver Island and California populations. Between-population F values (Table 8) also indicate that a significant amount of heterogeneity exists between populations in the three regional groups.

I also substituted the standardized means of the original 10 morphological variables for individual measurements in the regional three group stepwise discriminant analysis. In this analysis I separated sexes, where sample sizes allowed and the results are presented in Table 9 and Figure 12. It is readily apparent that excellent discrimination between groups and populations was attained by substituting population means for individual measurements. Table 10 gives posterior probabilities (i.e., the liklihood that each population "fits" in its preassigned group); it is clear from these data that by utilizing population means to characterize morphological variation between populations of A. ferreus a much neater "fit" to the discriminant classification model has been attained. The only misclassifica-

Table 9. Confusion matrix generated by 3-group, 10 variable stepwise discriminant model utilizing population mean values for each of the variables. Diagonal elements represent correct classifications; off-diagonal elements incorrect classifications.

Regional Groups	Vancouver Island	Oregon	California
Vancouver Island	4	0	0
Oregon	0	7	1
California	0	0	5

Figure 12. Results of discriminant analysis when population means
were used instead of individual measurements utilizing
the 10 variable model. Letters denote centroid means.
Sexes separate except for populations 13, 16, and 17
which correspond to populations 7, 9, and 10 of Figure
3; 1-4 correspond to populations 1 and 2; 5 and 6 to
3; 7 and 8 to 4; 9 and 10 to 5; 11 and 12 to 6; 14
and 15 to 8.

SECOND CANONICAL AXIS

FIRST
CANONICAL
AXIS

O

5 11

10
6 9 12
7

17

3
4

13

V.I.

8

C
16

15

2
1

14

58
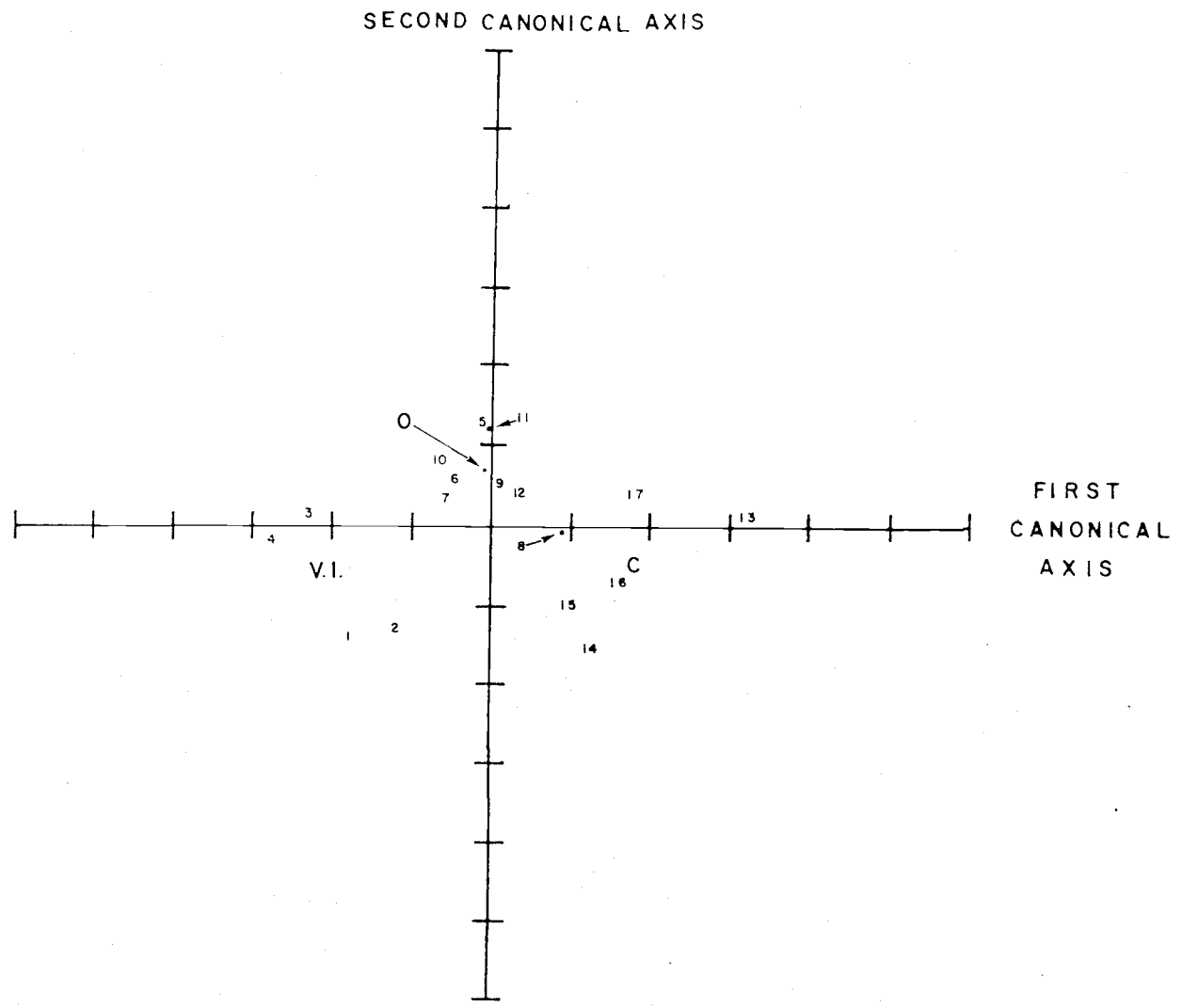
Table 10. Posterior probabilities generated by discriminant model when population means for 10 morphological characters were used as input variables. (M) or (F) designate males or females.

Population	Vancouver Island	Oregon	California
1(M)	1.000	0.000	0.000
1(F)	0.991	0.009	0.000
2(M)	0.997	0.003	0.000
2(F)	1.000	0.000	0.000
3(M)	0.000	0.999	0.001
3(F)	0.015	0.984	0.001
4(M)	0.024	0.975	0.001
4(F)	0.000	0.431	0.569
5(M)	0.001	0.993	0.006
5(F)	0.012	0.998	0.000
6(M)	0.000	0.999	0.001
6(F)	0.000	0.959	0.040
7	0.000	0.000	1.000
8(M)	0.000	0.001	0.999
8(F)	0.000	0.024	0.975
9	0.000	0.005	0.995
10	0.000	0.034	0.996

tion occurred with Douglas County females (Population 4), but the probability that this population belongs with other Oregon populations is quite high (0.43 versus 0.57 for California). Pairwise comparisons of populations utilizing the F ratio show that centroids do not differ significantly from one another (F = 1.74; df = 10,5; p>>.05).

Table 11 presents a summary of the differences between four of the analyses I utilized in terms of the order each morphological variable enters the stepwise model. Variables enter the model in the order of their ability to maximize the ratio of between group variance matrix to within group variance matrix. Critical F ratios to enter the model appear in parentheses next to each variable. There is considerable variation as to the order that each variable enters a particular model; when individual's measurements are used VT and BL always enter first and second, respectively. These two variables are not strongly correlated ($r_{VT \cdot BL}$ averaged 0.11 and ranged from -0.12 - +0.27). Other variables enter the model in the order of their ability to discriminate between groups and no discernible relationships exist between the step at which variables enter the model and the manner in which groups were delineated.

CLUSTER ANALYSIS

Both methods of cluster analysis provided no meaningful insights concerning potential phenetic relationships between populations; both *CLUSB analysis and Single Linkage clustering of product-moment corre-

Table 11. Summary of stepwise discriminant analyses on 10 populations of A. ferreus. Order in which variables enter each of the analyses are given; F-scores to enter the model are given in parentheses.

3-Group Population Means	3-Group Individual	3-Group Individual Males	10-Group Individual
VT(8.74)	VT(30.50)	VT(15.14)	VT(12.02)
CG(5.67)	BL(13.86)	BL(13.57)	BL(7.69)
HL(0.53)	AGL(13.43)	AGL(6.21)	HL(8.11)
BL(2.58)	HL(6.84)	CG(3.83)	HW(14.67)
HW(1.42)	HW(14.38)	MPM(3.11)	MPM(4.00)
FLL(0.17)	MPM(7.30)	HW(3.97)	FLL(3.69)
HLL(0.35)	SVL(2.68)	HL(10.44)	HLL(5.37)
MPM(0.42)	FLL(1.69)	HLL(1.54)	AGL(2.61)
AGL(0.59)	CG(1.40)	FLL(2.99)	SVL(1.86)
SVL(0.12)	HLL(0.92)	SVL(1.68)	CG(0.85)

lation coefficients showed no significant morphological relationship between populations associated with any aspect of latitude or physiography. The Single Linkage method showed average pairwise comparisons between populations to be 0.9937 (range: 0.9551-0.9998) indicating that all populations I examined covaried together in the same manner and relative magnitude. These results point to a high degree of phenetic resemblance between populations.

DISCUSSION

GEOGRAPHIC VARIATION

Interpretation of the results of morphometric analyses performed on 10 populations of _Aneides ferreus_ presents some difficulties. On one hand there is considerable variation within populations as evidenced by relatively high CV for morphological characters and by considerable overlap of individuals' canonical scores (Figures 6, 8 and 9). Principle components analysis was employed to elucidate the dependence structure of the 10 morphological variables and the results indicate that morphological variation within populations is similar in magnitude for each of the variables I considered; these analyses also indicate that variation between sexes is exceedingly similar in magnitude and direction. These data, along with those obtained from the similarity matrix of correlation coefficients generated during cluster analysis, indicate that there is a high degree of phenotypic resemblance between populations of this species. These results are consistent with current taxonomic conclusions which indicate that all populations are conspecific.

Classically, morphologists and taxonomists employ means of variables at the population level in order to compare populations and estimate evolutionary divergence. Many studies may be cited as examples of this practice: Clover, 1974; Crump, 1978; Ellis et al., 1978; Hafner, 1978; Hoffmiester and Diersing, 1978; Nussbaum, 1976 are but a few. My own work provides a contrast with this more traditional

method of analysis, in that each individual's suite of morphological characters is considered. Figures 11 and 12, and Tables 7 and 8 clearly indicate that by utilizing population means in the stepwise discriminant model, a much more effective separation in two dimensional space is achieved as well as a much better fit to the Confusion matrix. However, when population means are utilized, centroids do not differ significantly from one another and it is clear that while populations are delineated more clearly, they are still positioned quite close to each other in two dimensional space. The stepwise discriminant model allows large sample sizes with many variables to be considered simultaneously. When each specimen's morphological characters are used instead of population means it presents a more realistic, but certainly more complicated interpretation of the morphological divergence between populations of A. ferreus.

The results of stepwise discriminant analysis indicate there is considerable morphological heterogeneity between regional groups as well as populations of A. ferreus in spite of the fact that there is considerable overlap between groups and populations as indicated by Confusion matrices and graphical representation of individual canonical scores on the first two canonical axes. Despite poor fit to the classification models generated by the program and considerable overlap on graphs, centroids were found to differ signifcantly from one another in most of the pairwise comparisons (Table 9). Since these data may

or may not conform with a multivariate normal distribution, all standard statistical tests are, strictly speaking, invalid; however, the results do indicate that most populations and all regions exhibit morphological heterogeneity.

Patterns of morphological differentiation between populations inhabiting Vancouver Island, Oregon, and California are difficult to interpret. Examination of tooth counts with respect to latitude (Figure 4) reveals that as one proceeds south from Vancouver Island there is a general tendency for VT and MPM counts to increase within populations. On a broad scale, morphological variation appears to be correlated with latitude indicating that environmental selective regimes may be selecting for phenotypes which are adapted to localized as well as regional environments. A more simplistic viewpoint would argue that obvious differences along a latitudinal environmental gradient only modify homeostatic mechansims so that in the absence of environmental differences phenotypic expression would be more similar.

I constructed climographs (Ricklefs, 1974) for eight of the 10 populations which were examined (Figures 13, 14 and 15). These data were taken from U.S. and Canadian Weather Bureau data and represent average values taken over at least a 20 yr period. These figures illustrate that as one proceeds south from Vancouver Island, variation in rainfall decreases markedly. Mean monthly rainfall for the two collecting stations on Vancouver Island never falls below 6 cm; during

Figure 13. Climograph for populations 1 (◯) and 2 (△) on Vancouver Island. Numbers indicate months of the year; cm on abcissa; °C on ordinate.
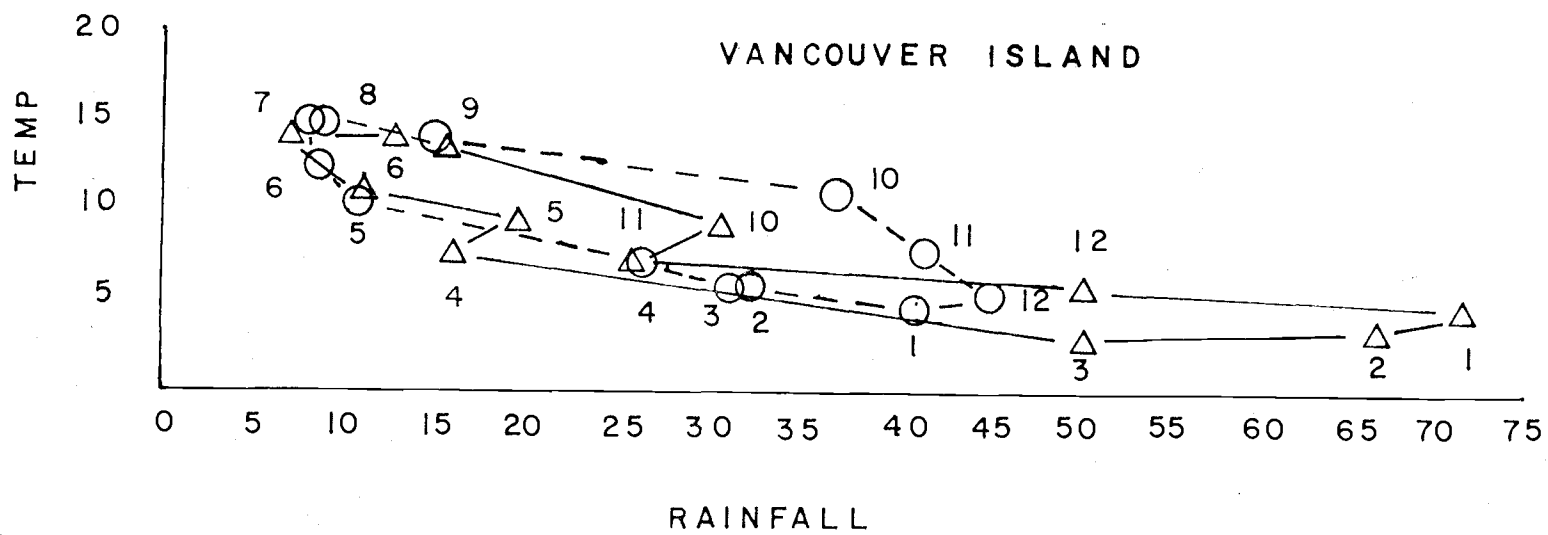
VANCOUVER ISLAND

Figure 14. Climograph for populations 3 (◯), 5 (△), and
6 (⬡). Numbers indicate months of the year; cm on
abcissa; °C on ordinate.

OREGON

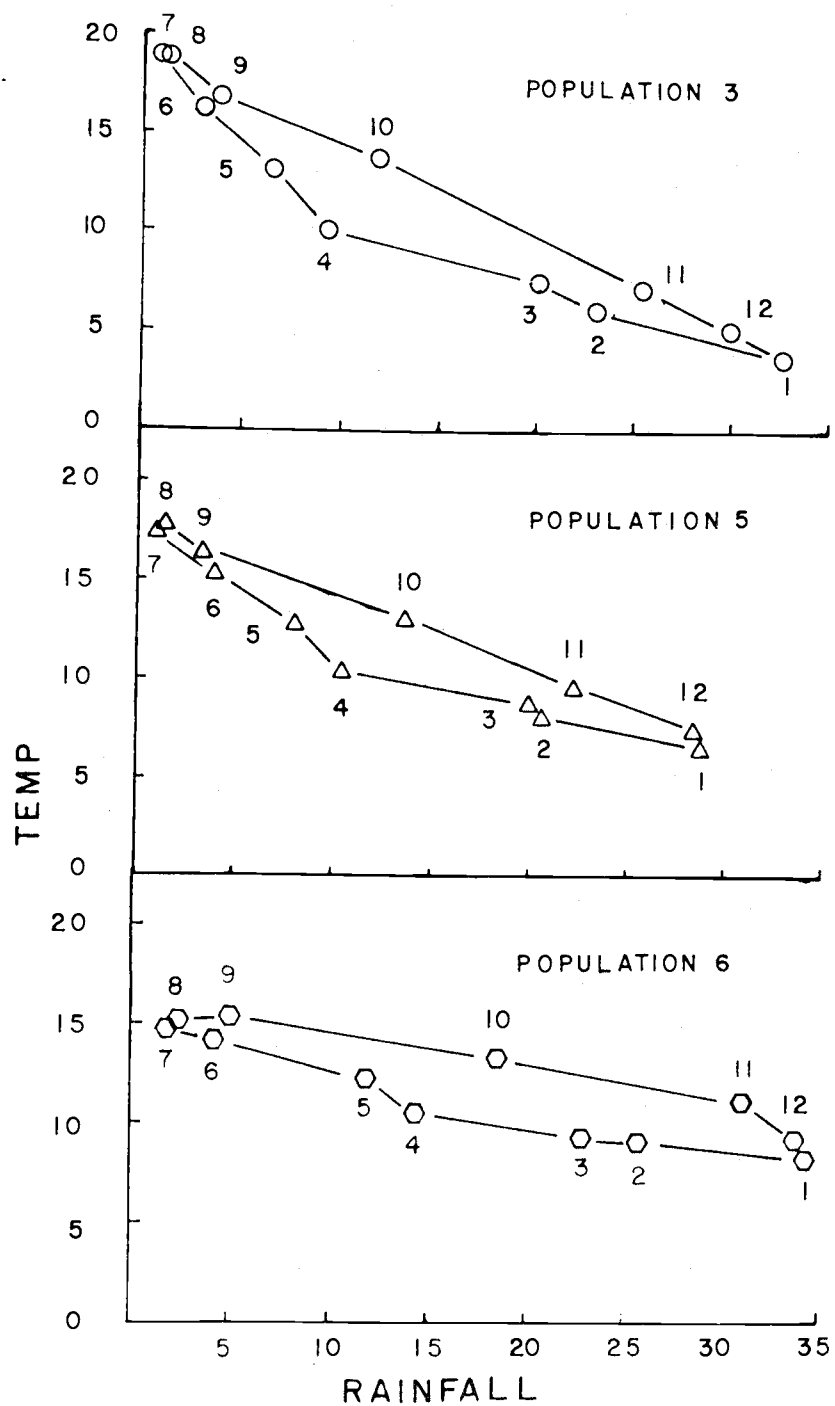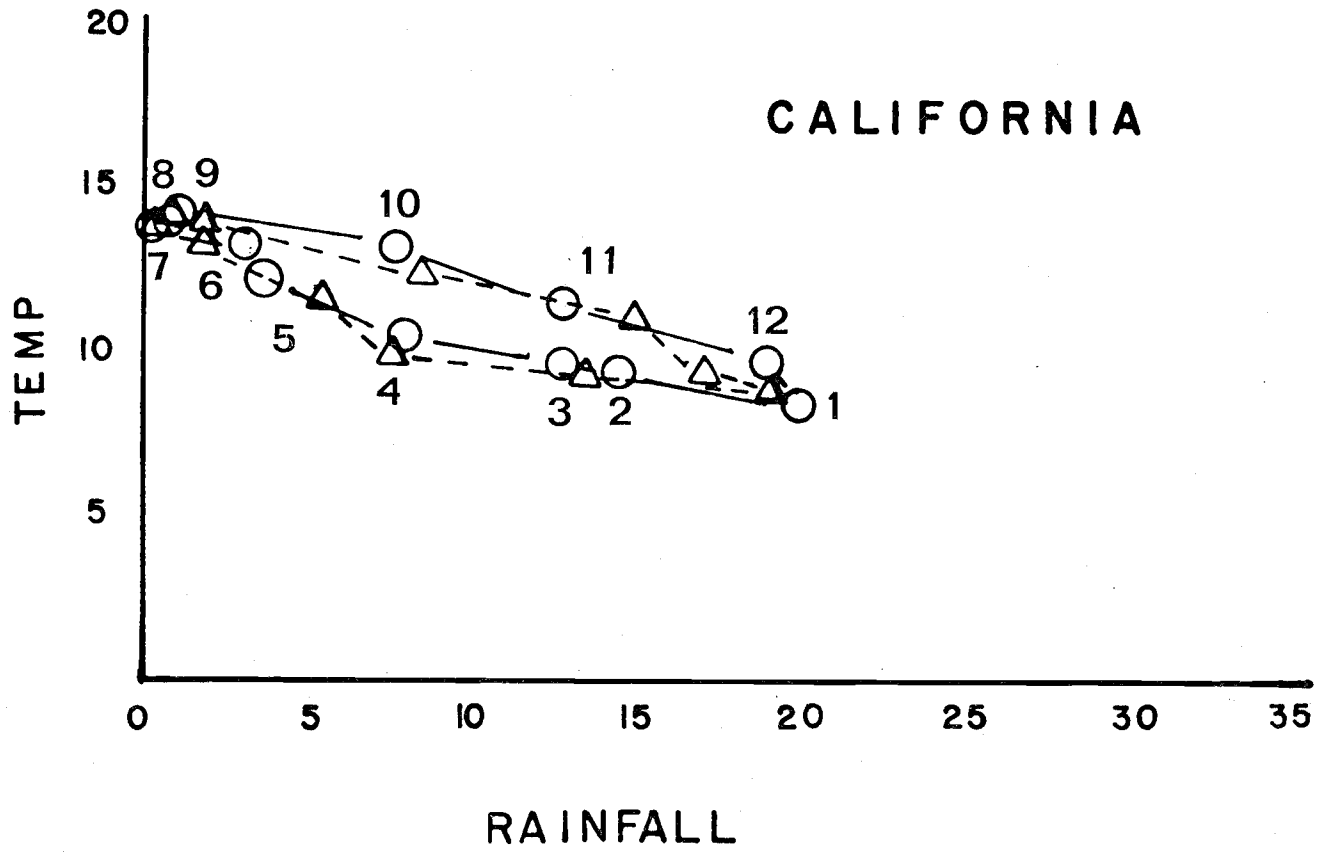Firgure 15. Climograph for populations 8 ($\triangle$) and 10 ($\bigcirc$).
            Numbers indicate months of the year; cm on abcissa;
            ^{o}C on ordinate.

CALIFORNIA

RAINFALL

the summer months in Oregon and California, mean monthly rainfall approaches zero. Values for maximum monthly rainfall are over 70 cm for Population 2 on Vancouver Island and decrease to about 35 cm in Oregon and less than 20 cm for populations inhabiting southern portions of the species' range in northern California. Mean monthly temperatures fluctuate only 7°C for California populations, but this fluctuation increases to 14°C for Oregon localities and 12°C for populations inhabiting Vancouver Island. In a general way these data are consistent with the idea that gradual latitudinal environmental gradients could lead to the observed levels of slight but significant morphological heterogeneity between California, Oregon, and Vancouver Island populations, as well as the differentiation observed between populations inhabiting each of the three regions.

Macrogeographic characterization of climatic variation may be an oversimplification since populations are consistently encountered in exceedingly similar habitats (i.e., rotten logs or rock faces). If animals are able to select habitats within a region which are identical in terms of their microclimates, then the argument I have outlined above becomes much weaker. Ontogenetic changes in habitat preferences are discussed by McKenzie and Storm (1970), but data which characterize microclimatic variation between habitats within California, Oregon and Vancouver Island are lacking. A potential problem which eventually must be reconciled is a consideration of the selective differences and/or similarities between the two distinct habitat types in which this species is found (i.e., rock faces and logs). Clearly, quantitative

data are needed so that each of these two microenvironments may be characterized in terms of their selective influences upon this species.

On a fine scale, significant patterns of heterogeneity between most of the populations I examined were demonstrated by the stepwise discriminant model. Within each of the three geographical regions, levels of morphological differentiation between populations have been detected which are consistent with predictions derived from general considerations of the life history of the species coupled with regional topographic features of the Pacific Northwest.

Life history patterns characteristic of A. ferreus which are pertinent to this discussion may be summarized as follows:

1. Low dispersal rates. Gordon (1952) has shown for A. aeneus that adults within specific populations are very sedentary. This has also been shown for some Plethodon (Fraser, 1976a, 1976b), and for Desmognathus ochrophaeus (Huheey and Brandon, 1973; Tilley, 1974).

2. Relatively low rates of reproductive output. Female A. ferreus oviposit bienially (McKenzie, 1970; Beatty, unpublished). Low metabolic rates (Feder, 1976; 1977), coupled with harsh seasonality characteristic of north-temperate environments probably makes it impossible for females to channel sufficient amounts of energy into annual production of their Type

III eggs (Salthe, 1969). Vial (1968) and Houck (1978) have

shown that Neotropical bollitoglossines oviposit annually, and

that the testes of males are in meiosis at all times during the

year. Highton's (1962) work with Plethodon glutinosus supports

this notion as females from southern latitudes effected annual

oviposition; those from northern latitudes, biennial.

These life history attributes lead to the prediction that mechanisms

such as random genetic drift, lack of gene flow between populations,

and natural selection play important roles in structuring differential

phenotypic responses to varying environmental selective regimes in this

species.

Topographic barriers to dispersal which could potentially play

important roles in reducing gene flow between populations of A. ferreus

are abundant in each of the three regions I have delineated.

California. Topography and drainage patterns in northwestern

California are diverse and complex. A major topographic feature which

serves to delineate the southern boundary of the species' range has

been termed the North Coast Divide by Nussbaum (1976). This uplifted

terrain appears near Guala, southern Mendocino County, and runs east-

ward just south of the Garcia River, turning north at the headwaters

of the Navarro River, then turns southwest, separating the Russian and

Eel River drainages. It then turns north to join the Coast Range crest.

The area to the north and west of the North Coast Divide is drained by a series of rivers: the Garcia, Navarro, Big, Ten Mile, Mattole, Eel, Van Duzen, Mad, Redwood Creek, Trinity, Klamath, and Smith Rivers. In general, these all flow in a northwesterly direction and define a deeply dissected topography which might serve to semi-isolate small local populations which have low dispersal rates. This might account for slight but significant morphological heterogeneity between populations brought about by phenomena such as the relative lack of gene flow between populations acting in concert with differential selective regimes caused by subtle variation in microclimate.

The Klamath-Siskiyou Mountains form potentially a major east-west topographic discontinuity between California and Oregon populations of A. $ferreus$. This geologically old and ecologically complex area contains many relictual plant forms and ancient floral assemblages (Franklin and Dyrness, 1969; Stebbins and Major, 1965; Waring, 1969; Whitaker, 1961); Remington (1968) has discussed the area's impact upon faunal elements of southwestern Oregon and northwestern California.

Populations of A. $ferreus$ inhabiting the region between the North Coast Divide and the Klamath-Siskiyou Mountains show varying degrees of morphological differentiation; D^2 comparisons between populations range between 0.86 and 5.71; only two comparisons correspond to non-significant F-ratios (Populations 7 and 9; 8 and 9). Clearly, within California populations, the most morphologically distinct speci-

mens have been drawn from Population 10 from near Rockport, Mendocino County. In terms of color, these specimens are also quite distinct as melanophores overwhelm all other pigment cells in adults leaving live specimens uniformly dark in appearance. White irridiophores along the lateral sides of the body are also conspicuous and do not appear in these concentrations in other populations I examined.

The other three California populations are located between the peneplain of the Klamath River and the Van Duzen River; pairwise comparisons between these three populations are again consistent with the idea that morphological heterogeneity is a function of latitude or distance, or that climatic differences between latitudes may be exerting slightly different selective regimes on individuals within populations thereby eliciting a differential phenotypic response.

Oregon. Oregon populations are geographically delimited to the north by the Columbia River, although populations from extreme north-western Oregon are not known; this does not appear to be a collecting artifact and therefore leads to the prediction that the Columbia River, per se, does not limit the distribution of this species. Topography and drainage patters in Oregon Oregon north of the Klamath-Siskiyou Mountains are characterized by rivers which, as in northern California, have cut complex and deeply dissected paths through the Coast Range Mountains. Major drainage systems from south to north are: Chetco, Rogue, Coquille, Coos, Umpqua, Smith, Siuslaw, and Alsea Rivers.

Pairwise comparisons of D^2 values between populations of A. ferreus within Oregon all exhibited significant levels of heterogeneity, but no clean latitudinal morphoclines are apparent. Mary's Peak, Benton County, is the highest point in the Coast Range of Oregon and supports populations which appear to be the most morphologically distinct of any populations I examined. The Mary's Peak area supports extensive populations of western plethodontids including completely melanistic populations of Plethodon dunni. Additionally, electrophoretic work (Hanlin, et al., ms) shows that P. dunni from Mary's Peak are quite distinctive in terms of their patterns of allozyme variation. Whether or not the morphological distinctness exhibited by Mary's Peak A. ferreus is related to any peculiar selective regime associated with this area is a question which is unanswerable at this time.

In terms of coloration, Oregon populations resemble specimens from northwestern California. Storm (1947) and others (e.g., Wake, 1965) hypothesize that two ecomorphs exist in this species: one (dark) associated with log-type habitats, and a lighter morph which is found exclusively in rock-face and talus-type habitats. Qualitative impressions derived from my own field work lead to the conclusion that background coloration is exceedingly variable within populations, regardless of the habitat in which they were collected, and that the general appearance of a specimen is largely temperature dependent as McKenzie (1970) has intimated.

Vancouver Island. Vancouver Island is 451 km in length and spans 126 km at its widest point. It is separated from the U.S. by the Straits of Juan de Fuca, a distance of about 70 km; its separation from the British Columbia (BC) mainland is considerably less. Proceeding northward from the southeastern tip of the island, the Haro Straits are only 15 km from the San Juan Islands. The Georgia Straits separate Vancouver Island from the BC mainland by about 44 km at their widest point and by only 3 km at Discovery Passage. Further northwestward, the Johnstone Straits provide a sea water separation of about 5 km, and the island is isolated at its extreme northwest reaches by the Queen Charlotte Straits which range from 2.4 km at Goletus Channel to about 5 km at their widest point. The island encompasses approximately 3650 km^2.

The Vancouver Island Mountains form the major topographic relief on the island. They are centrally located, running along the northwest-southeast axis of the island and their highest peaks range from 1700 to 2460 m. This dramatic rise from sea level has left much of the margin of the island deeply dissected by the sea, forming major fjord systems. These mountains have had a strong influence upon the climate of the island, creating a major rainshadow along the northeastern margin.

The two populations which I sampled on Vancouver Island exhibited a significant level of morphological heterogeneity; however, since there were only two samples it is not possible to determine if patterns of heterogeneity resemble those determined for Oregon A. ferreus (i.e.,

essentially random) or those exhibited by California populations (a gradual morphocline). Pairwise comparisons between the two Vancouver Island populations and populations in Oregon and California revealed that non-significant levels of differentiation were seen when Population 1 (near Tofino) was tested against Population 7 (Klamath River, California) and Population 9 (Fortuna, California).

Specimens comprising the collection represented by Population 2, from River Jordan, appear to be quite distinct morphologically. It is worth noting here, that in each of the three regions I sampled, one population stood out in terms of its morphological uniqueness. This uniqueness may be attributed to stochastic processes such as drift, or it may be explained by taking a viewpoint which would argue that environmental selective regimes at these three localities may be responsible for the distinct phenotypic differentiation exhibited by these populations. River Jordan, Vancouver Island, is located in an area which receives exceedingly high amounts of precipitation (ca 380 cm/yr), but it is ludicrous to suggest that rainfall alone is responsible for this differentiation; I only use it as an example to illustrate the existence of possible environmental heterogeneity which could account for variation in phenotypic expression. An hypothesis which asserts that this differentiation could be due to random events cannot be rejected.

In terms of coloration, populations of A. ferreus from Vancouver Island appear to have a more reticulated pattern of dorsal guanophores than specimens collected from Oregon and California, but I am left with the impression that variation in color within populations is present in great magnitude and precludes comparisons until some method is devised to quantify color variation.

It is interesting to compare the results of this work with those obtained by utilizing starch-gel electrophoresis and karyology. Recent electrophoretic analyses (Cadle and Wake, personal communication) have shown that Vancouver Island Aneides ferreus collected from Populations 1 and 2 are more closely related to California populations (average Nei distance ($\bar{D}$) = 0.006) than to Oregon populations ($\bar{D}$) = 0.513). The two groups (Vancouver Island - California, and Oregon can be distinguished by fixed differences at three loci and by frequency differences at other loci as well. Electrophoretic differentiation of this order usually indicates that two species are involved (e.g., Tilley et al., 1978). Morphological comparisons reveal no such discrepancies and in no way support electrophoretic results.

Kezer and Sessions (Chromosoma, in press) have found karyological differences which also suggest that Vancouver Island populations are more similar to populations in California than those in Oregon. Figure 16 illustrates an idiogram constructed by Kezer and Sessions for A. ferreus. An unusual chromosomal polymorphism is evident for the 13th

Figure 16. Idiogram for <u>Aneides</u> <u>ferreus</u> illustrating polymorphism at 13th chromosome.
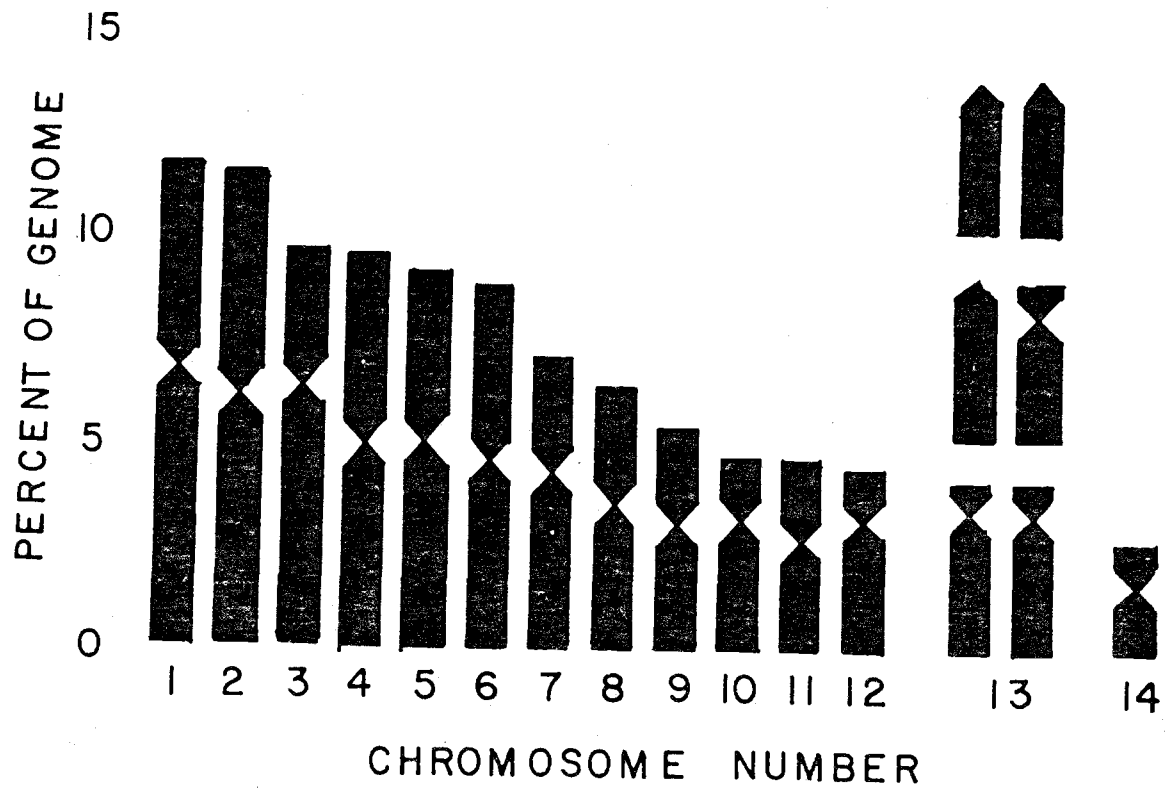
chromosome (n=14). Vancouver Island females always exhibit a hetero-morphic pair at the 13th chromosome (one telocentric(t); one subtelo-centric (st)), while males are always t/t. Oregon populations show considerable variation on this theme, both within and between popula-tions (see Figure 17). Populations located near the center of the species' range in Oregon exhibit all possible combinations of chromo-some types for the 13th chromosomes. Furthermore, there is no effect attributable to sex, as on Vancouver Island where females are always st/t. Males drawn from California populations are characterized by t/t arrangements and females are usually st/t, but t/t females appear at low frequency (Figure 17).

As a result of this work, it is often possible to assign a collec-tion of individuals to a specific region (i.e., Vancouver Island, Oregon, or California) if the frequencies of (t) and (st) within each sex are known. These data are consistent with my morphological work, at least where Oregon populations are concerned. Karyotypic variation within Oregon populations is great, if enough individuals from enough populations are examined it is possible that populations may be unique in terms of their frequencies of (t) and (st) 13th chromosomes. This parallels the results of the stepwise discriminant analysis which showed that all pairwise comparisons between Oregon populations re-vealed significant levels of morphological heterogeneity.

Additionally, results stemming from electrophoretic and karyological

Figure 17. Map to show frequency of telocentric and subtelocentric 13th chromosomes in populations sampled for morphological analyses.
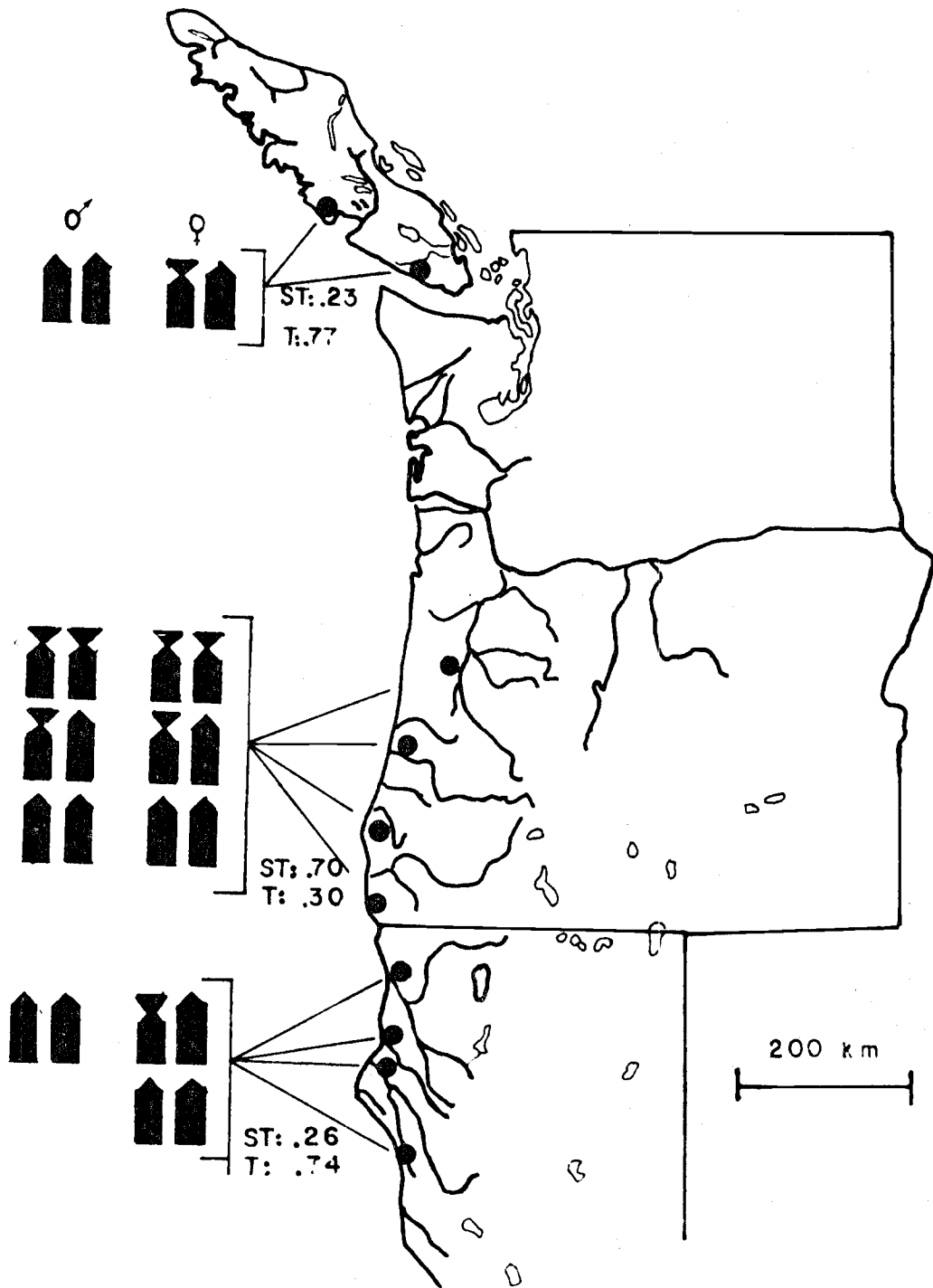
studies are not totally consistent with morphological analyses, but are consistent with the notion that natural selection, lack of gene flow between populations, and random genetic drift are important evolutionary forces acting on populations of A. ferreus. An important challenge remains which is to determine the relative importance of each in the structuring of genotypes and phenotypes in natural populations.

CONCLUSIONS

Results of morphological analyses indicate that there is a high degree of phenotypic resemblance between populations of A. ferreus, but, on a fine scale, significant heterogeneity exists between populations in different goegraphic regions. These data can be explained in two ways:

1. Since the general trend of morphological differentiation is somewhat dependent upon latitude it may be the result of slightly different environmental conditions associated with latitude. Heterogenous environments may be modifying homeostatic mechanisms resulting in slightly different phenotypic expressions which characterize and differentiate populations and regional groups. This hypothesis could be tested by raising specimens from each of the three regions under identical environmental conditions and then subjecting them to morphological analyses to determine the level of heterogeneity realized under a constant environment.

2. The other explanation invokes natural selection acting differentially upon populations producing slightly disparate phenotypic expressions which are genetically based. This explanation is testable only if the differences in phenotypic expression are actual representations of heritable genetic variation and can be demonstrated as such. This would require breeding experiments, which have never been done with plethodontid salamanders.

BIBLIOGRAPHY

Anderson, T. W. 1958. An introduction to Multivariate Statistical Analysis. John Wiley and Sons, Inc., New York, 374 pp.

Armstrong, J. E., D. R. Crandell, D. J. Easterbrook, and J. B. Noble. 1965. Late Pleistocene stratigraphy and chronology in southwestern British Columbia and northwestern Washington. Geol. Soc. Amer. Bull. 76:321-330.

Blackith, R. E., and R. A. Reyment. 1971. Multivariate Morphometrics. Academic Press, London and New York. 412 pp.

Brame, A. H., Jr., and K. F. Murray. 1968. Three new salamanders (Batrachoseps) with a discussion of relationships and speciation within the genus. Bull. Los Ang. Co. Mus. Nat. Hist. Sci. 4:1-35.

Bretz, J. H. 1920. The Juan de Fuca lobe of the Cordilleran ice sheet. J. Geol. 28:333-339.

Chaney, R. W. 1925. A comparative study of the Bridge Creek flora and the modern redwood forest. Carnegie Inst. Wash. Publ. 349:1-27.

Chaney, R. W. 1951. A revision of fossil Sequoia and Taxodium in western North America based on the recent discovery of Metasequoia. Trans. Am. Phil. Soc. 49(3):171-263.

Christensen, K. 1954. Ratios as a means of specific differentiation in Collembola. Ent. News 65:176-177.

CLIMAP. 1976. The surface of the Ice-Age earth. Science 191(4232): 1131-1137.

Crandell, D. R. 1965. The glacial history of western Washington and Oregon. In: The Quaternary of the United States, eds., H. E. Wright and D. G. Frey, Princeton, New Jersey, Princeton University Press, pp. 341-353.

Clover, R. C. 1974. Morphological variations in populations of Lacerta from islands in the Adriatic Sea. Ph.D. Thesis, Oregon State University, Corvallis. 141 pp.

Cooley, W. W., and P. R. Lohnes. 1971. Multivariate Data Analysis. John Wiley and Sons, Inc., New York. 364 pp.

Crump, M. L. 1977. Intrapopulation and interspecific variation of "standard" morphological characters of four closely related South American salamanders (Bolitoglossa), with description of habitat preferences. Herpetologica 33(4):415-426.

Dietling, L. E. 1968. Historical background of the flora of the Pacific Northwest. Bull. Mus. Nat. Hist. Univ. Oregon 13:1-57.

Dixon, W. J. 1976. BMD Biomedical Computer Programs. Univ. Calif. Press, Berkeley. 773 pp.

Dunn, E. R. 1926. The salamanders of the family Plethodontidae. Smith College, Northampton, Mass. 441 pp.

Easterbrook, D. J. 1977. Quaternary geology of the Pacific Northwest. In: Quaternary Stratigraphy of North America, ed., W. C. Mahaney, Halstead Press, pp. 441-462.

Ellis, L. S., V. E. Diersing, and D. F. Hoffmeister. 1978. Taxonomic status of short-tailed shrews (Blarina) in Illinois. J. Mamm. 59(2):305-311.

Feder, M. E. 1976. Oxygen consumption and body temperature in neotropical and temperate zone lungless salamanders (Amphibia: Plethodontidae). J. Comp. Physiol. 110:197-208.

_____. 1977. Oxygen consumption and activity in salamanders: Effect of body size and lunglessness. J. Exp. Zool. 202(3): 403-414.

Franklin, J. F., and C. T. Dyrness. 1969. Vegetation of Oregon and Washington. U.S. Dept. Ag. For. Serv., Res. Pap. PNW-80, 216 pp.

Fraser, D. F. 1976a. Coexistence of salamanders in the genus Plethodon: A variation of the Santa Rosalia theme. Ecology 57(2):238-251.

_____. 1976b. Empirical evaluation of food competition in salamanders of the genus Plethodon. Ecology 57(3):459-471.

Gallagher, A. E., and E. N. Kozloff. 1971. Essentials of Practical Microtechnique. 2 ed., Lea and Febiger, Philadelphia. 523 pp.

Gordon, R. E. 1952. A contribution to the life history and ecology of Aneides aeneus (Cope and Packard). Amer. Midl. Nat. 47(3):666-701.

_____. 1967. Aneides aeneus. Catalogue of American Amphibians and Reptiles. p. 30.

Hafner, J. C. 1978. Evolutionary relationships of Kangaroo mice, genus Microdipodops. J. Mamm. 59(2):354-365.

Halstead, E. C. 1968. The Cowichan ice tongue, Vancouver Island. Can. J. Earth Sci. 5:1409-1415.

Hanlin, H. G., F. W. Allendor, C. H. Daugherty, and J. J. Beatty. 1978. Genetic structure of populations of Plethodon dunni Bishop. ms.

Heusser, C. J. 1960. Late-Pleistocene environments of North Pacific North America. Am. Geogr. Soc. Spec. Publ. 35, 308 pp.

_____. 1972. Palynology and phytogeographical significance of a late Pleistocene refugium near Kalaloch, Washington. Quat. Res. 2:189-201.

Highton, R. 1962a. Geographic variation in the life history of the slimy salamander. Copeia 1962:597-613.

_____. 1962b. Revision of North American salamanders of the genus Plethodon. Bull. Florida State Mus., Biol. Sci. 6(3):235-367.

_____. 1977. Comparison of microgeographic variation in morphological and electrophoretic traits. Evol. Bio. 10: 397-436.

_____, and A. Larsen. 1978. The genetic relationships of salamanders of the genus Plethodon. ms.

_____, and T. P. Webster. 1976. Geographic protein variation and divergence in populations of the salamander Plethodon cinereus. Evol. 30:33-45.

Hoffmeister, D. F., and V. E. Diersing. 1978. Review of the tassel-eared squirrels of the subgenus Otosciurus. J. Mamm. 59(2):402-413.

Houck, L. D. 1977. Life history patterns and reproductive biology of neotropical salamanders. In: The Reproductive Biology of Amphibians, eds., D. H. Taylor and S. H. Guttman, New York, Plenum Press, pp. 43-72.

Huheey, J. E., and R. A. Brandon. 1973. Rock-face populations of the mountain salamander, Desmognathus ochrophaeus, in North Carolina. Ecol. Monogr. 43(1):59-77.

Jones, D. L., N. J. Silberling, and J. Hillhouse. 1977. Wrangellia - A displaced terrane in northwestern North America. Can. J. Earth Sci. 14:2565-2577.

Kay, M., and E. H. Colbert. 1965. Stratigraphy and Life History. John Wiley and Sons, Inc., New York. 736 pp.

Kezer, J., and S. K. Sessions. 1978. Chromosome variation in the plethodontid salamander, Aneides ferreus. Chromosoma, in press.

Lowe, C. H., Jr. 1950. The systematic status of the salamander Plethodon hardii, with a discussion of biogeographical problems in Aneides. Copeia 1950(2):92-99.

Lynch, J. L. 1974a. Ontogenetic and geographic variation in the morphology and ecology of the Black Salamander (Aneides flavipunctatus).

_____. 1974b. Aneides flavipunctatus. Catalogue of American Amphibians and Reptiles. pp. 158.1-158.2.

_____, and D. B. Wake. 1974. Aneides lugubris. Catalogue of American Amphibians and Reptiles. pp. 159.1-159.2.

MacLeod, N. S., D. L. Tiffin, P. D. Snavely, Jr., and R. G. Currie. 1977. Geologic interpretation of magnetic and gravity anomalies in the Strait of Juan de Fuca, U.S.-Canada. Can. J. Earth Sci. 14:223-228.

Mahalanobis, P. C. 1936. On the generalized distance in statistics. Proc. Nat. Inst. Sci. India 2:49-55.

Marr, J. C. 1955. The use of morphometric data in systematic, racial, and relative growth studies. Copeia 1955(1):23-31.

Massey, W. F. 1965. On methods: Discriminant analysis of audience characteristics. J. Advert. Res. 5:39-48.

McIntosh, W. B. 1955. The applicability of covariance analysis for comparison of body and skeletal measurements between two races of the deer mouse, Peromyscus maniculatus. Contrib. Lab. Vert. Biol., Univ. Mich. 72:1-55.

McKenzie, D. S. 1970. Aspects of the autecology of the plethodontid salamander, Aneides ferreus (Cope). Ph.D. Thesis, Oregon State University, Corvallis. 98 pp.

_____, and R. M. Storm. 1970. Patterns of habitat selection in the clouded salamander, Aneides ferreus (Cope). Herpetologica 26(4):450-454.

Morrison, D. F. 1976. Multivariate Statistical Methods. McGraw-Hill Book Co., San Francisco. 415 pp.

Muller, J. E. 1977. Evolution of the Pacific margin, Vancouver Island, and adjacent regions. Can. J. Earth Sci. 14:2062-2085.

Nussbaum, R. A. 1976. Geographic variation and systematics of salamanders of the genus Dicamptodon Strauch (Ambystomatidae). Misc. Pub. Mus. Zool., Univ. Mich. 149. pp.

Peabody, F. E. 1959. Trackways of living and fossil salamanders. Univ. Calif. Publ. Zool. 63(1):1-72.

Press, S. J. 1972. Applied Multivariate Analysis. Holt, Rinehart, and Winston, Inc. 521 pp.

Remington, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. In: Evolutionary Biology Vol. 2, eds., Th. Dobzhansky, M. K. Hecht, and W. C. Steere, New York, Merideth Corp., pp. 321-428.

Ricklefs, R. E. 1973. Ecology. Chiron Press, Inc., Newton, Mass., 861 pp.

Salthe, S. W. 1969. Reproductive modes and the number and sizes of ova in the urodeles. Am. Midl. Nat. 81:467-490.

Saltzman, B., and A. D. Vernekar. 1975. A solution for the northern hemisphere climatic zonation during a glacial maximum. Quat. Res. 5:307-320.

Seal, H. 1966. Multivariate Statistical Analysis for Biologists. Methuen and Co., Ltd., London. 209 pp.

Simpson, G. G., A. Roe, and R. C. Lewontin. 1960. Quantitative Zoology. Harcourt, Brace, and World., New York. 440 pp.

Snavely, P. D., Jr., and H. C. Wagner. 1963. Tertiary geologic history of western Oregon and Washington. Wash. St. Div. of Mines Geol. Rep. Investig. 22:1-25.

Sneath, P. H. A. 1957. The application of computers to taxonomy. J. Gen. Microbiol. 17:201-226.

_____, and R. R. Sokal. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W. H. Freeman and Co., San Francisco. 573 pp.

Sokal, R. R., and F. J. Rolf. 1969. Biometry, the Principles and Practice of Statistics in Biological Research. W. H. Freeman and Co., San Francisco. 776 pp.

Stebbins, G. L., Jr., and J. Major. 1965. Endemism and speciation in the California flora. Ecol. Monogr. 35:1-35.

Storm, R. M. 1948. The herpetology of Benton County, Oregon. Ph.D. Thesis, Oregon State University, Corvallis. 280 pp.

Tilley, S. G. 1974. Structures and dynamics of populations of the salamander Desmognathus ochrophaeus in different habitats. Ecology 55(4):808-817.

_____. 1978. Studies of life histories and reproduction in North American plethodontid salamanders. In: The Reproductive Biology of Amphibians, eds., D. H. Taylor and S. H. Guttman, New York, Plenum Press, pp. 1-41.

_____, R. B. Merrit, B. Wu, and R. Highton. 1978. Genetic differentiation in salamanders of the Desmognathus ochrophaeus complex. Evol. 32:93-115.

Vial, J. L. 1968. The ecology of the tropical salamander, Bolitoglossa subpalmata in Costa Rica. Rev. Biol. Trop. 15:13-115.

Wake, D. B. 1963. Comparative osteology of the plethodontid salamander genus Aneides. J. Morph. 113(1):77-118.

_____. 1965. Aneides ferreus. In: Catalogue of American Amphibians and Reptiles. pp. 16.1-16.2.

_____. 1966. Comparative osteology and evolution of lungless salamanders, family Plethodontidae. Mem. So. Calif. Acad. Sci. 4:1-111.

_____, and J. L. Lynch. 1975. The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. Nat. Hist. Mus. Los Angeles Co., Sci. Bull. 25:1-65.

Waring, R. H. 1969. Forest plants of the eastern Siskiyous: Their environmental and vegetational distribution. North. Sci. 43(1):1-17.

Wittaker, R. H. 1961. Vegetation history of the Pacific Coast states and the "central" significance of the Klamath Region. Madrono 16(1):5-23.

APPENDIX

APPENDIX

Population Localities

1. Long Beach Penninsula, Vancouver Island, British Columbia; 9.7 and 17.2 km southeast of Tofino; western hemlock, Sitka spruce, western red cedar; n = 49.

2. River Jordan, Vancouver Island, British Columbia; Douglas fir, western hemlock, western red cedar, Sitka spruce; n = 50.

3. Mary's Peak, Corvallis Watershed, Benton Co., Oregon; Douglas fir, n = 50.

4. Douglas Co., Oregon; vicinity of Smith River Falls; Douglas fir; n = 53.

5. Coos Co., Oregon; Daphne Grove Campground; Douglas fir; n = 50.

6. Curry Co., Oregon; 9.5 km south of Pistol River; Douglas fir; n = 51.

7. Humboldt Co., California; Klamath River; redwood; n = 19.

8. Humboldt Co., California, east of Arcata; redwood, n = 46.

9. Humboldt Co., California; 13.5 km west of Grizzly Creek Campground; n = 19.

10. Mendocino Co., California; 3 km north of Rockport; redwood; n = 35.