MORPHOLOGICAL VARIATION IN THE
PLETHODON YONAHLOSSEE COMPLEX AND THE
STATUS OF *PLETHODON LONGICRUS*

A Thesis

by

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ABSTRACT

MORPHOLOGICAL VARIATION IN THE
PLETHODON YONAHLOSSEE COMPLEX AND THE
STATUS OF *PLETHODON LONGICRUS*. (December 1983)

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Salamanders of two populations of the *Plethodon yonahlossee* complex were examined for morphological variation to determine if significant differences occur to warrant recognition of *Plethodon longicrus*. Seventeen morphological characters were used in various statistical comparisons between all five samples and between a combination of the four southern samples and the northern sample, respectively. Three taxonomic arrangements were tested; *Plethodon yonahlossee* and *Plethodon longicrus* are conspecific, *Plethodon longicrus* is a subspecies of *Plethodon yonahlossee*, and *Plethodon longicrus* is a separate species.

Analysis of covariance of single characters with SVL as a covariate revealed many more significant differences among populations than would be expected in a homogenous population.

Discriminant analysis tested several significant characteristics simultaneously between groups. Southern populations were found to be much more similar to each other than any of them were to the northern population and a sample of *Plethodon glutinosus*, a related species. Results of the pooled samples showed an 85 percent segregation between northern and southern populations and a virtually total segregation from *Plethodon glutinosus*. These results, along with concordant observation of coloration, indicated *Plethodon longicrus* was a valid taxonomic form.

ACKNOWLEDGEMENTS

In acknowledging any work such as this, it is indeed an egotist who thinks only he deserves credit for the results of such a project. Far too many people play important roles in shaping a life that leads to such an undertaking. It is an impossible task to mention everyone who has provided information, guidance, inspiration, or sacrificed time and energy to help see this project through to completion. The individuals mentioned here have given much more than necessary to what I hope has been an enlightening as well as an academic experience.

The members of my committee, Wayne Van Devender, Richard Henson, and Ed Greene have contributed in more ways than they know. Without their help, this task would have been impossible.

I wish to acknowledge Amy Van Devender for providing me with more than one evening meal and never asking me to wash dishes. Diane Butts deserves mention for similar acts of charity this summer.

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Station during the summer of 1982. Thanks go to Bill Palmer and Alvin Braswell at the North Carolina State Museum of Natural History and Arnold Kluge and Ronald Nussbaum at the University of Michigan for the use of preserved salamanders.

Jon Sole and Barbara Post made a summer's stay in Chimney Rock much more enjoyable as did many of the staff of the Schiele Museum of Natural History. Thanks go to David Cone, Jeff Petty, Walton Conway, Chris Gabriel, Daryl Tate, Chris Matthews, and Steve "Swamp" Morrow for assistance in collection of salamanders. Also, Wayne, thanks for help in this area. The people of Chimney Rock were all friendly and supportive. The residents of Shumont Mountain ignored me while I plundered on their roadside until all hours of the morning. Thanks go to all of you.

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All of the faculty of the Biology Department have made their own contributions. Bill Hubbard, John Bond, Bill Dewel, Jeff Butts, and Frank Randall, in addition to my committee members, have made special impressions. Deanna Bowman was of considerable help in my first attempts at computer assisted statistical analysis. Janice Ashley fulfills the definition of art: beauty and efficiency. She has been a wonderful friend and a great help. David Roye, T. J. Herder, John McIver, Chris Pruneau, Sharon and Joel Winker, Gary Shipley, Wayne Hutchins, Fred Burgin, Grant Banks, Jim Hall, and Wylie Fulton all have my everlasting thanks. Brent and Elois Ann Berlin, John O'Neill, and Gene Beckham have contributed in many ways. Gene did an outstanding job on the illustrations in this text. Carolyn Joines was a great help with Spanish. Thank all of you for your time.

Last, but certainly not least, Charles Fugler gets credit for helping me through undergraduate school. His kindness, warmth, understanding, and guidance will never be forgotten.

DEDICATION

This work is dedicated to Madge F. Justice. She has given me twenty-five years of love, understanding, and been a constant source of inspiration in my life. Everything that is good in me comes from her. She has my undying love and respect and deserves much more than she has ever received.

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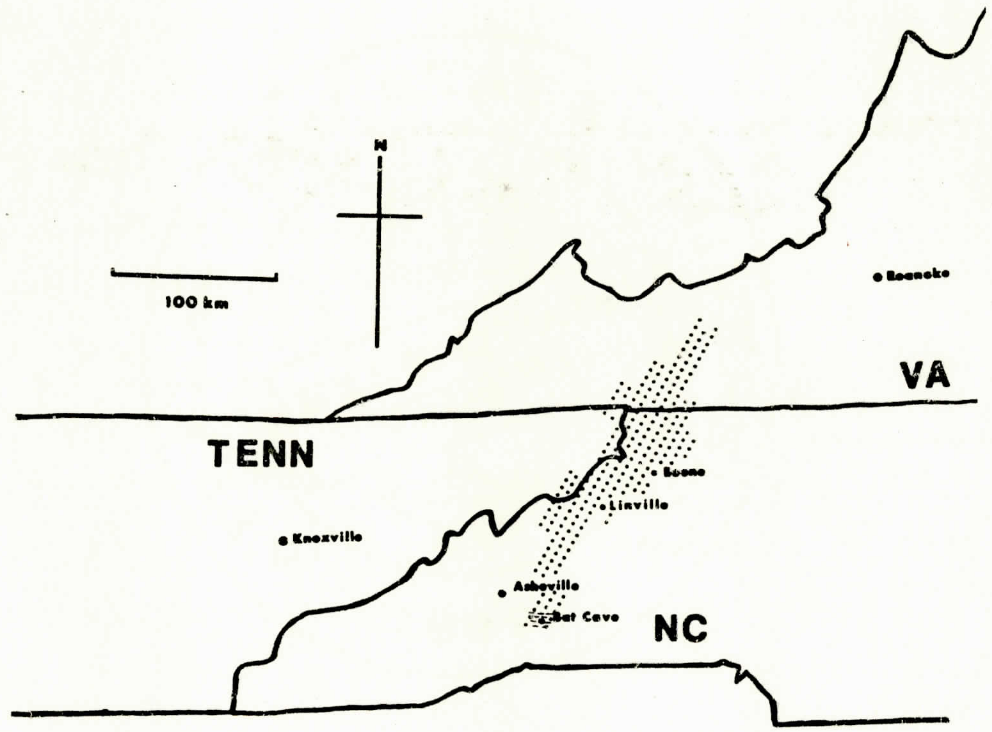
INTRODUCTION

Woodland salamanders of the Family Plethodontidae comprise the largest and most diverse family of urodeles. Of the 25 genera in this family, Plethodon is one of the most successful (Highton, 1962). Many of the 24 species (Behler and King, 1979) are found in the mountains of western North Carolina, making these salamanders the most ubiquitous vertebrates of the forest floor.

Plethodon contains some of the most beautiful salamanders. Plethodon yonahlossee, described by Dunn in 1917, is one of the largest (Highton, 1972) and most primitive salamanders of the genus. The chestnut dorsum and white spotted sides make this a strikingly beautiful salamander. It is found at the higher elevations of forested regions of western North Carolina, northeast Tennessee, and western Virginia (Figure 1).

In 1962, a new species of Plethodon was described from Rutherford County, North Carolina (Adler and Dennis, 1962). This salamander, Plethodon longicrus, was described from a population of salamanders previously considered to be Plethodon yonahlossee or Plethodon glutinosus. The range of Plethodon longicrus is northwestern Rutherford County near Bat Cave, North Carolina extending to Bearwallow Mountain in Henderson and Buncombe counties.

FIGURE 1. Map showing the known range of Plethodon yonahlossee (indicated by dots) and Plethodon longicrus (indicated by dashes).



LITERATURE REVIEW

<u>Plethodon yonahlossee</u> was described in 1917 from Old Yonahlossee Road at Linville, North Carolina (Dunn, 1917). This salamander was described as a large <u>Plethodon</u> with a chestnut dorsum and numerous white lateral spots which form a white "lateral stripe" in adults. It was placed in the <u>Plethodon glutinosus</u> group. <u>Plethodon yonahlossee</u> is considered the most primitive salamander of the Genus <u>Plethodon</u> (Dunn, 1926; Wake, 1966). When described, the range of <u>Plethodon yonahlossee</u> was only known to be western North Carolina. Gray (1939) extended the known range to include northeast Tennessee.

A major work by Clifford Pope (1950) dealt with a statistical and ecological study of <u>Plethodon yonahlossee</u>. Pope found, in contrast to previous findings (Dunn, 1926), that <u>Plethodon yonahlossee</u> was quite common. Only at the highest elevations of its range was the salamander rare. The habitat is mostly the forest floor with a significant number found under logs or tree bark. Associations with other species of <u>Plethodon</u> were discussed. Findings of Gordon et al. (1962) comparing microhabitats of some southern species of salamanders were consistent with Pope (1950).

Only two other species have been thought to be closely related to *Plethodon yonahlossee*. One of these forms, *Plethodon ouachitae*, is found exclusively in Arkansas and Oklahoma. *Plethodon ouachitae* resembles *Plethodon yonahlossee* in its general appearance. The chestnut spotted dorsum and lateral white or yellowish spots of *Plethodon ouachitae* are in sharp contrast to its black body. Recent electrophoretic evidence indicates, however, that *Plethodon ouachitae* is more closely related to *Plethodon glutinosus* than to *Plethodon yonahlossee* (Highton and Larson, 1979).

The second salamander related to *Plethodon yonahlossee* is *Plethodon longicrus*. Adler and Dennis (1962) described this new species from the crevices in granite gneiss outcrops in northwest Rutherford County near Bat Cave, North Carolina. This salamander was described from a population previously considered to be *Plethodon yonahlossee* or *Plethodon glutinosus*. They based their description on morphological characteristics and habitat. *Plethodon longicrus* has long legs, large numbers of vomerine teeth, reduced dorsal color, and overlapping adpressed limbs. It lives in crevices of granite rock, hence the common name "crevice salamander" (Conant, 1975). The range of *Plethodon longicrus* is restricted to adjacent portions of the North Carolina counties of Rutherford, Henderson, and Buncombe.

Pope (1965) first suggested that *Plethodon longicrus* should be considered a synonym of *Plethodon yonahlossee*. David Dennis, in a personal communication referred to by Richard Highton (1972), also suggested that *Plethodon longicrus* and *Plethodon yonahlossee* were conspecifics. Dennis based this conclusion on further research on the range of *Plethodon longicrus*. He found that *Plethodon yonahlossee* and *Plethodon longicrus* apparently intergrade in the area between Bat Cave and the Swannanoa Mountains. Biochemical analysis was consistent with a conspecific relationship for *Plethodon longicrus* (Guttman et al., 1978). The latest field guides disagree as to the status of the two forms (Behler and King, 1979, Martoff et al., 1980, and Smith, 1978).

Since relatively little morphological or ecological work has been undertaken on either *Plethodon yonahlossee* or *Plethodon longicrus*, the biological status of the populations remains unclear. By comparing morphological characteristics between populations, I hope to shed new light on the status of these salamanders.

METHODS AND MATERIALS

Introduction

The salamanders Plethodon yonahlossee and Plethodon longicrus have been the subject of relatively few studies and little is known about the morphological and ecological variations in these forms. (See Figure 1 for geographical distribution of the two forms). The objectives of the present study are: 1) to analyze allometric variation in body proportions in Plethodon yonahlossee and Plethodon longicrus; and 2) to determine how these proportions vary with respect to sex, elevation, and habitat. All populations were also compared with a sample of Plethodon glutinosus (Outgroup), a closely related salamander.

Collecting Sites

Collecting sites of typical Plethodon yonahlossee, Plethodon longicrus, and so-called intermediate populations (Guttman et al., 1978) were selected in western North Carolina to allow comparisons of samples from wide ranges of elevation and habitat (Figure 2). With the exception of Boone and the Bat Caves, each site consisted of less than one hectare which was sampled in a non-destructive

manner from May to August 1982. An effort was made to obtain at least 30 individuals from each site (Table 1). These sites included:

BOONE (Watauga County)

The Boone site, average elevation 1600 meters, is located on the north slope of Rich Mountain off of Rainbow Spring Drive, two kilometers north of Boone and 26.4 kilometers northeast of the Plethodon yonahlossee type locality at Linville, Avery County. Salamanders were collected at night along road cuts or in forest litter of a mountainous oak-hickory forest.

THE BAT CAVES (Rutherford County)

The Bat Caves are located in the Hickory Nut Gorge, 1.6 kilometers southeast of the town of Bat Cave, Henderson County, on US 64-74 at an elevation of about 490 meters. This area includes steep slopes on either side of Hickory Nut Gorge. The forest is oak-hickory. All specimens from this location were borrowed from existing museum collections due to the inaccessibility of the Bat Caves site.

FIGURE 2. Distribution of southern collecting sites in southwestern North Carolina.

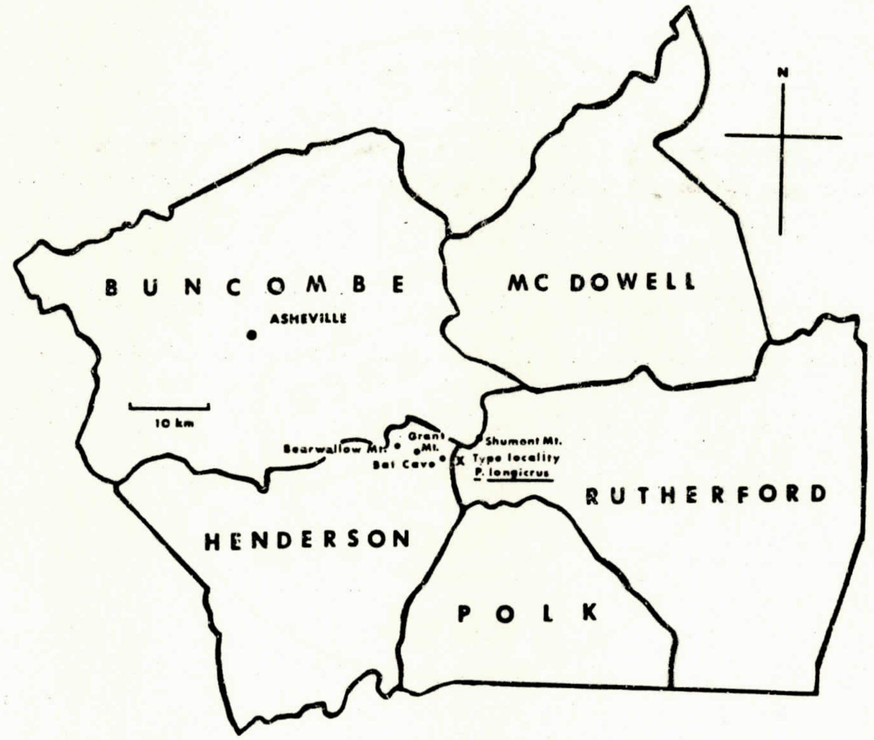


TABLE 1. Collecting sites, elevational ranges, and types of specimens used.

SITE	ELEVATIONAL RANGE (METERS)	CAPTURED SPECIMENS	MUSEUM SPECIMENS	TOTAL
BAT CAVE	436-521	0	66	66
SHUMONT	701-867	96	0	96
BEARWALLOW	1085-1143	52	0	52
GRANT	731-792	30	0	30
BOONE	1590-1610	0	176	176
OUTGROUP	700-1100	30	0	30
TOTALS		208	242	450

GRANT MOUNTAIN ROAD (Henderson County)

With an elevational range of 731-792 meters and located on US 74 3.5 kilometers northeast of the Bat Caves, Grant Mountain Road offered a collecting site with habitat similar to the Plethodon longicrus type locality. Specimens were found at night in the crevices of granite walls and in granite rock formations. Salamanders were also captured on the oak-hickory forest floor in close proximity to burrows.

BEARWALLOW MOUNTAIN (Henderson and Buncombe counties)

The Bearwallow Mountain site is located off of county road 1594, two kilometers south of US 74, 13 kilometers northwest of the Bat Caves. With a maximum elevation of 1290 meters, it is a southern site with elevations similar to Boone. Collection was concentrated, at night, along road cuts leading to the Bearwallow Mountain Fire Tower (1085-1143 meters in elevation). The road cut habitat ranged from rock talus to heavy plant cover.

SHUMONT MOUNTAIN (Buncombe, Henderson, and Rutherford counties)

With a summit of 1167 meters, this site provided an ideal location for sampling over a broad elevational range (701-867 meters). Located 3.4 kilometers north of Chimney Rock on state highway 9, the road cut leading to the summit allowed ideal collecting. Road cut habitat ranged from bare ground to dense vegetation of various types. Collecting was restricted to Henderson and Buncombe counties.

Laboratory Procedures

Measurements were made on 450 preserved animals with vernier calipers accurate to 0.1 millimeters. Snout vent length (SVL), tail length, head length, head width, interocular distance, eye length, eye to nose length, head height, interlimb distance, humerus length, ulna length, ulna to phalynx tip length, femur length, fibula length, metatarsal to phalynx tip length, and number of costal grooves between adpressed limbs were recorded for each individual. Measurements of total arm and leg length were obtained by addition of individual arm and leg measurements. In addition to this analytical information, categorical information concerning sex, elevation, and site was noted.

Allometric relationships were used since each measure varied with age and absolute size. These relationships were obtained via regression analysis using SVL as the independent variable and each of the other measures as the dependent variable, respectively (Langley, 1970). Linearity and homoscedasticity of regression were determined through analysis of residuals (Draper and Smith, 1966). Analysis of covariance (ANCOVA) was used to generate

F - and t - tests for significant differences among regression coefficients for subsets of salamander data (Sokal and Rohlf, 1969). Since SVL was the most precise measure and because it is closely related to age, SVL was used as the independent variable and covariate in this study to control for age. Data were partitioned according to sex, site, and habitat to determine if regressions differed among partitioned groups. Stepwise discriminant analysis was used, as a multivariate statistic, to compare combinations of significant variables among partitioned groups (Dixon and Brown, 1979).

The underlying biological assumption to be tested by this procedure is that all the populations belong to the same biological species. Some intrapopulational variation is to be expected, but it should be less common than interspecific variation in the same characters. Conclusions about the relationships among allopatric populations must be somewhat arbitrary but the magnitude and number of differences between partitioned groups will serve as the basis for determining the taxonomic relationships present.

All statistical comparisons were made using the BMDP Biomedical Computer programs, P-Series, 1981 on file at the Appalachian State University (ASU) Computer Center. The primary statistical references used in this study were Dixon and Brown (1979), Draper and Smith (1966), Langley (1970), and Sokal and Rholf

(1969). Specimens collected in this study and a complete data set are on file at the Department of Biology, Appalachian State University.

RESULTS

Introduction

Information from each collecting site was analyzed at the ASU Computer Center using the BMDP Biomedical Computer Programs, P-Series, 1981. Four collecting site names were shortened as follows: The Bat Caves (Bat Cave), Grant Mountain Road (Grant), Bearwallow Mountain (Bearwallow) and Shumont Mountain (Shumont). The Boone site was referred to as Boone and was used as a northern (North) population for comparison with the pooled data for all of the other sites (South). Results from the statistical analyses will be presented in two sections; Single Character Analysis and Multicharacter Analysis.

Single Character Analysis

Model I regressions of the various characters on SVL as the independent variable ranged from highly significant to non-significant. ANCOVA allowed tests for equality of slopes and adjusted group means (AGM) among sites based on these regressions. Interpopulation comparisons were therefore based on a range of different correlations between variables. For the sake of brevity, results will be presented for variables with highest, lowest, and median correlations.

HEAD LENGTH

In both males and females, head length had a significant correlation with SVL (r values ranged from .90 - .99). Slopes of regression lines were less than 1.0 (Figure 3) showing that head length was proportionally smaller as SVL increased. Examination of residuals indicated linear relationships between dependent and independent variables and homoscedastic variance. These data, therefore, were appropriate for use in ANCOVA to compare subsets of data. In females, the AGM in the Bat Cave and Boone sites were found to be significantly larger than all other sites but not different from each other. F - tests indicated no difference in slopes among sites ($F_{4,228}$ = 1.88, P = .11). AGM ranged from 17.5 mm at Shumont to 18.6 mm at Boone and Bat Cave. AGM in males was significantly larger in Boone as compared to all other sites and significantly smaller in Shumont as compared to all other sites. AGM (and related intercepts of regression lines) ranged from 15.9 mm at Shumont to 16.8 mm at Boone. No slope differences among groups was indicated by F - tests ($F_{4,204}$ = 1.36, P = .25).

INTEROCULAR DISTANCE

The correlation of interocular distance and SVL was moderately high. Values ranged from r = .82 to r = .95 among groups. The slopes of regression lines (Figure 4) were less than 1.0 indicating

that interocular distance became proportionally smaller as SVL increased. Examination of residuals allowed an inference of reasonably linear relationships and relatively homoscedastic variance. Among female groups, ANCOVA showed Boone to have a significantly higher AGM than Bat Cave or Shumont. F - tests were consistent with no slope difference among groups ($F_{4,228}$ = .22, P = .93). AGM ranged from 6.4 mm in Shumont to 6.8 mm in Boone. ANCOVA among male groups showed significant AGM differences. Bat Cave was significantly smaller than Grant, Bearwallow, and Boone; Grant and Boone were significantly larger than Shumont. AGM ranged from 5.4 mm at Bat Cave to 6.4 mm at Boone. F - tests indicated no differences in slopes among groups ($F_{4,204}$ = .65, P = .63).

FIBULA LENGTH

The correlation of fibula and SVL was lowest among the measures of length. Correlation coefficients ranged from .73 to .86, respectively. The length of the fibula was proportionally smaller as SVL increased as indicated by the slope of the regression line (1.0, Figure 5). Examination of residuals suggested a slightly curvilinear relationship and small increase in variance with increasing SVL. In females, ANCOVA showed no significant differences in AGM or slopes among tested groups ($F_{4,228}$ = 1.4, P = .23). Ranges of AGM were 4.6 mm in Bearwallow to 4.8 mm in Bat Cave. Among male groups, ANCOVA showed significant AGM differences with Bat Cave larger than Shumont and Boone. AGM ranged from a low of 4.2 mm in Boone and Shumont to a high of 4.4 mm in Bat Cave. No differences in slopes were detected ($F_{4,204}$ = 1.6, P = .18).

TABLE 2. Summary of the number of significant differences for 68 statistical tests using ANCOVA among all populations.

	BAT CAVE	GRANT	BEARWALLOW	SHUMONT	BOONE
BAT CAVE	--				
GRANT	15	--			
BEARWALLOW	11	12	--		
SHUMONT	19	18	7	--	
BOONE	23	20	11	20	--

SUMMARY

Considering males and females separately, two ANCOVA analyses and tests of AGM and slope were made on each of 17 variables for a total of 68 tests for each pair of populations. Significant differences among all combinations of populations were summarized (Table 2). It is noteworthy to remember that each pair of populations should have had about four differences due to chance alone if

areas were part of a single population. Partitioning of data on the basis of altitude showed no significant differences in AGM or slopes.

FIGURE 3. Plot of the relationship between head length and SVL for Shumont females. This plot is representative of all populations and sexes and shows strong correlation between variables.

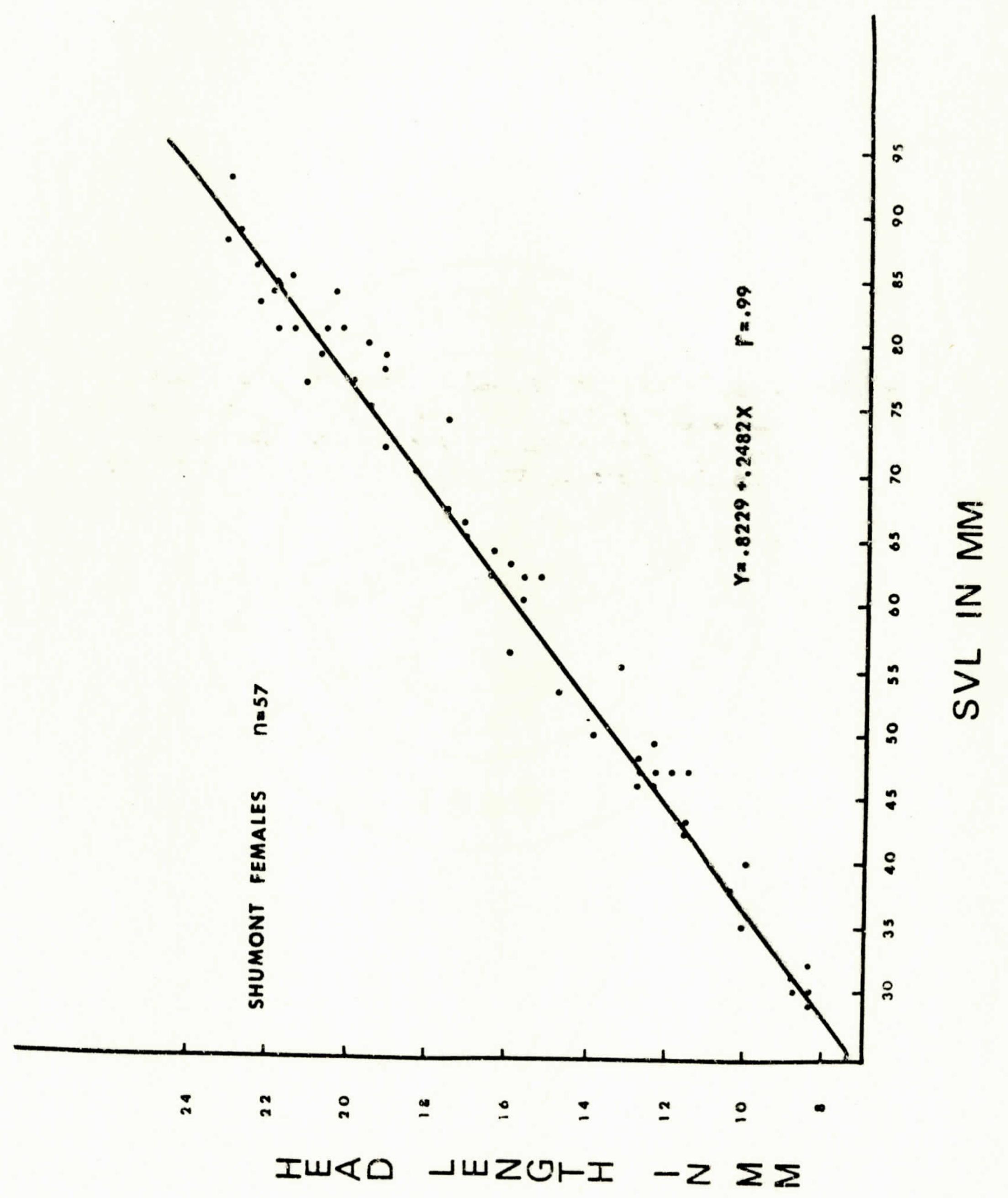


FIGURE 4. Plot of the relationship between interocular distance and SVL for Bat Cave males. This plot is representative of all populations and sexes and shows moderate correlation between variables.

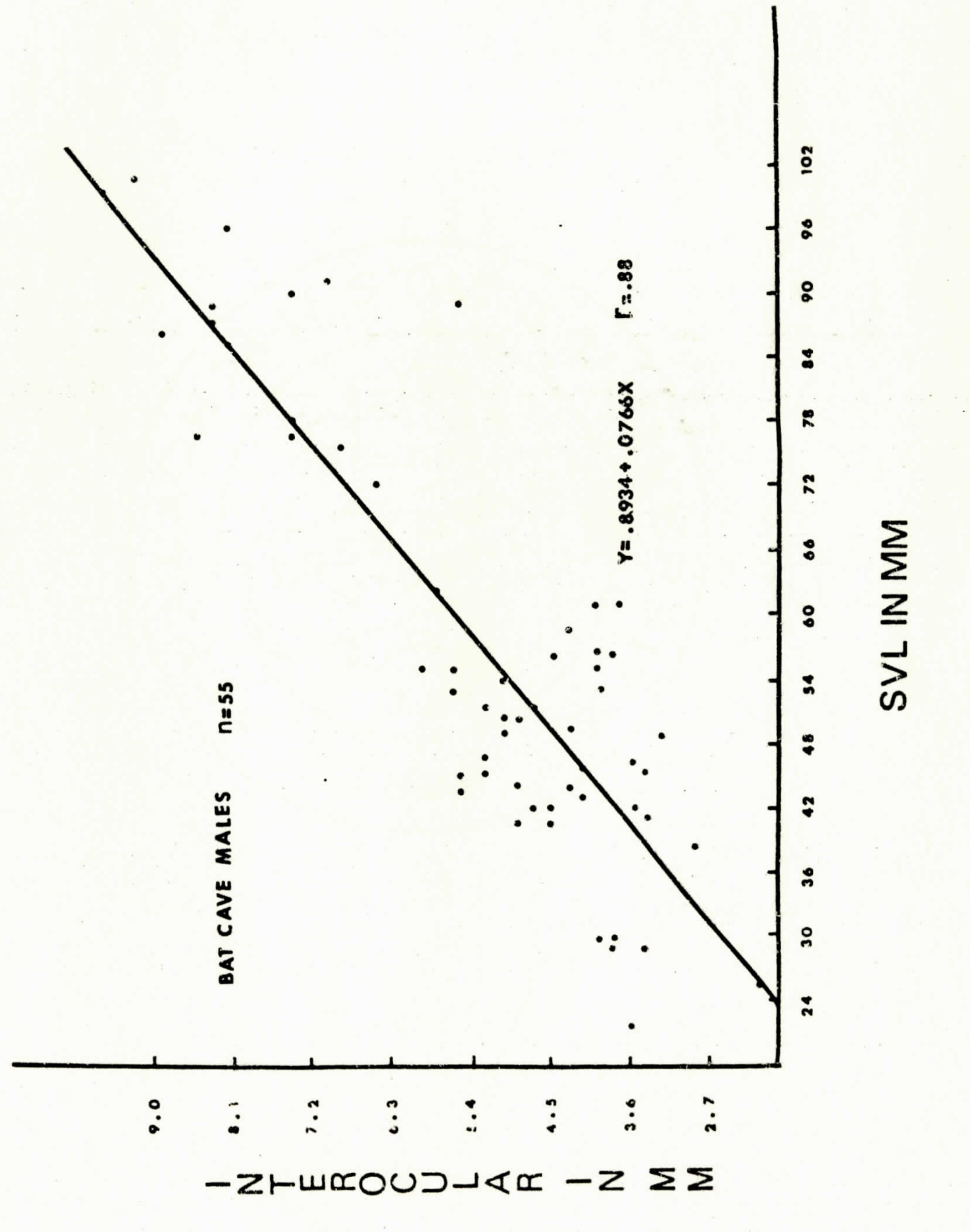
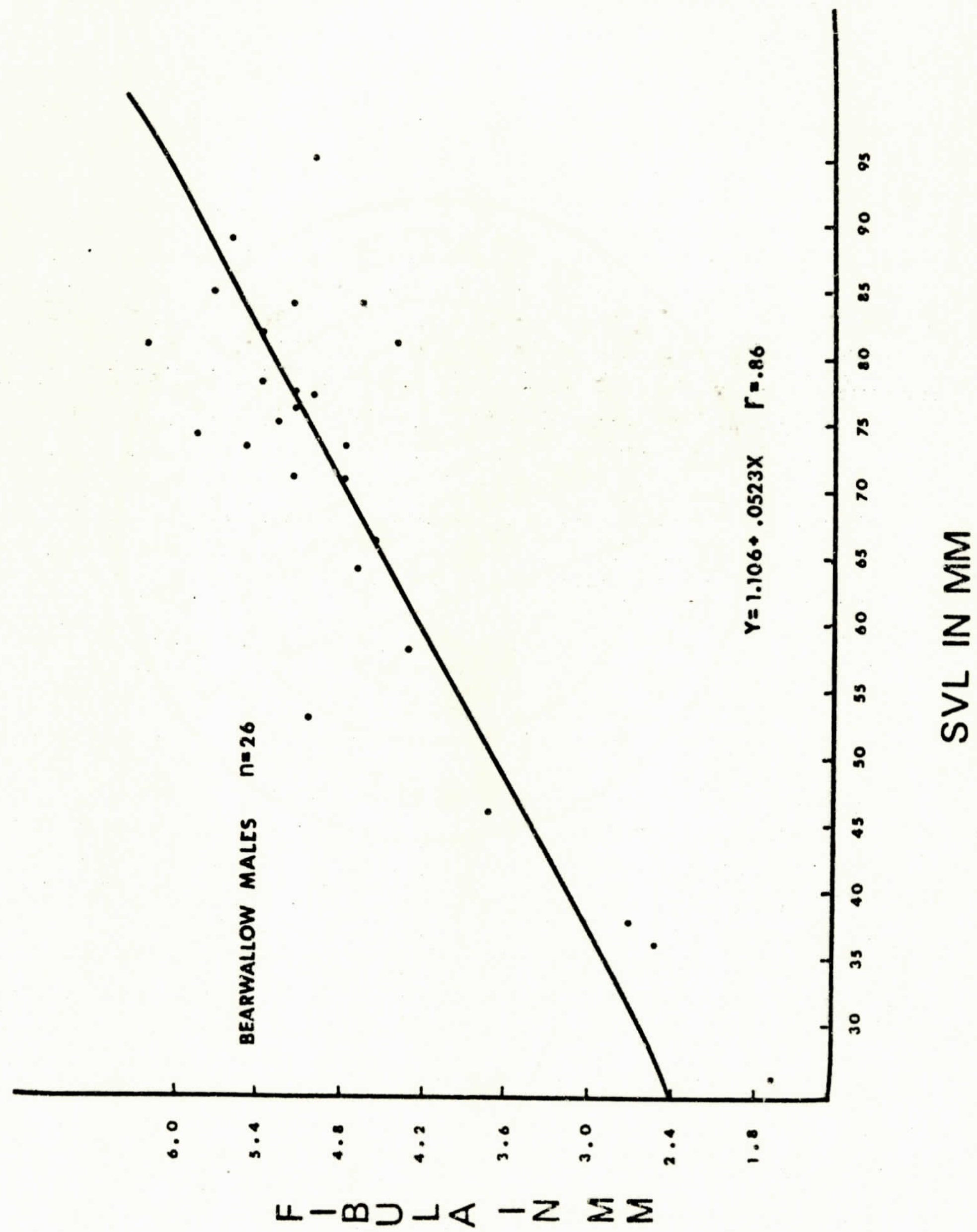


FIGURE 5. Plot of the relationship between fibula length and SVL for Bearwallow males. This plot is representative of all populations and sexes and shows relatively low correlation between variables.



Multicharacter Analysis

Stepwise discriminant analysis was used to compare groups on the basis of several characteristics simultaneously. Sexes were analyzed separately and two taxonomic hypotheses were tested. All sites were included in the preliminary analysis to determine if they were distinguishable from one another. The second analysis pooled the southern populations to determine if northern (North) and southern (South) populations can be distinguished. A sample of Plethodon glutinosus (Outgroup) from the southern sites was included as an outgroup in the second analysis.

FIVE GROUP ANALYSIS

Nine variables were found to contribute significantly to the discriminant function for females (Table 3). This function correctly assigned 66.4 percent of animals to their proper groups (Table 4). Percentage of properly assigned groups ranged from 43.3 in Bearwallow to 78.3 in Boone. All four southern sites had smaller and similar percentages of correct assignment as compared to Boone. If five samples from a single population were compared in this manner only 20.0 percent should be properly assigned. Canonical variables were calculated to show the projection of discriminant functions in two dimensions for each individual (Figure 6). This plot showed considerable overlap in the southern sites.

In males, six variables contributed significantly to the discriminant function (Table 3). Correct group assignment for males was 58.9 percent. Percentage of proper assignment ranged from 45.5 in Bat Cave to 77.8 in Grant (Table 5). The plot of canonical variables showed large areas of group overlap in the southern sites (Figure 7).

NORTH / SOUTH ANALYSIS

Discriminant functions included twelve significant variables for females (Table 5). In total, 84.2 percent of animals were assigned to their correct groups. As expected, Outgroup had the highest percentage of correct assignment (94.7), while North had the lowest percentage (80.7) (Table 7). Canonical variables showed relatively little overlap between groups (Figure 8).

Analysis of males found ten significant variables in the discriminant function (Table 6). A total of 88.0 percent of animals were assigned to the proper groups. Again, Outgroup had the highest percentage of proper assignment (100.0); it was followed by North (88.5) and South (86.2) (Table 8). Canonical variables showed minimal overlap between groups (Figure 9).

SALAMANDER APPEARANCE

While not used in the statistical analysis, observations about color (qualitative and quantitative) and general appearance were recorded (Figure 10). At Boone, the dorsal chestnut stripe was conspicuous and continuous. The color of this stripe was redder than in Bat Cave, Shumont, and Grant individuals. No dorsal white spotting was found on Boone animals.

Bearwallow provided salamanders with continuous dorsal stripes or discontinuous blotches and spots. These salamanders exhibited combinations of continuous reddish stripes with white spots, relatively brownish stripes with white spots, relatively red blotches with white spots, and relatively brown blotches with white spots.

The southernmost sites had salamanders with discontinuous dorsal blotches and white spots. The color of the blotches ranged from somewhat reddish brown to almost totally brown. The incidence of dorsal white spots was much greater than with many of the Bearwallow individuals. The dorsal red/brown blotches were reduced to such an extent on some of these individuals that low power (30X) microscopic observation was necessary to detect the spots. These salamanders were easily confused with Plethodon glutinosus upon casual inspection.

TABLE 3. Summary of the variables contributing significantly to discriminant functions based on Five Group Analysis. Variables are listed in order of importance.

FEMALE VARIABLES	MALE VARIABLES
HEAD LENGTH	HEAD LENGTH
FEMUR LENGTH	EYE TO NOSE LENGTH
GROOVES	INTERLIMB DISTANCE
EYE TO NOSE LENGTH	LEG LENGTH
TAIL WIDTH	METATARSAL LENGTH
METATARSAL LENGTH	INTEROCULAR DISTANCE
EYE LENGTH	
HEAD HEIGHT	
ULNA LENGTH	

TABLE 4. Assignment of animals to groups based on discriminant functions analysis for females.

GROUP	PERCENT CORRECT	NUMBER OF CASES CLASSIFIED INTO GROUP - BAT CAVE	GRANT	BEARWALLOW	SHUMONT	BOONE
BAT CAVE	57.9	11	2	3	0	3
GRANT	50.0	0	6	1	3	2
BEARWALLOW	43.3	4	6	13	4	3
SHUMONT	59.6	2	12	5	34	4
BOONE	78.3	3	10	12	1	94
TOTAL	66.4	20	36	34	42	106

TABLE 5. Assignment of animals to groups based on discriminant functions analysis for males.

GROUP	PERCENT CORRECT	NUMBER OF CASES CLASSIFIED INTO GROUPS - BAT CAVE	GRANT	BEARWALLOW	SHUMONT	BOONE
BAT CAVE	45.5	25	9	7	11	3
GRANT	77.8	0	14	4	0	0
BEARWALLOW	55.6	2	2	15	6	2
SHUMONT	52.6	7	6	11	30	3
BOONE	73.7	1	1	10	3	42
TOTAL	58.9	35	32	47	50	50

TABLE 6. Summary of variables contributing significantly to discriminant functions based on North / South Analysis. Variables are listed in order of importance.

FEMALE VARIABLES	MALE VARIABLES
GROOVES	GROOVES
METATARSAL LENGTH	FEMUR LENGTH
HEAD LENGTH	EYE TO NOSE LENGTH
EYE TO NOSE LENGTH	LEG LENGTH
LEG LENGTH	EYE LENGTH
EYE LENGTH	METATARSAL LENGTH
INTEROCULAR DISTANCE	TAIL WIDTH
INTERLIMB DISTANCE	ULNA LENGTH
HEAD WIDTH	ARM LENGTH
FEMUR LENGTH	
HEAD HEIGHT	

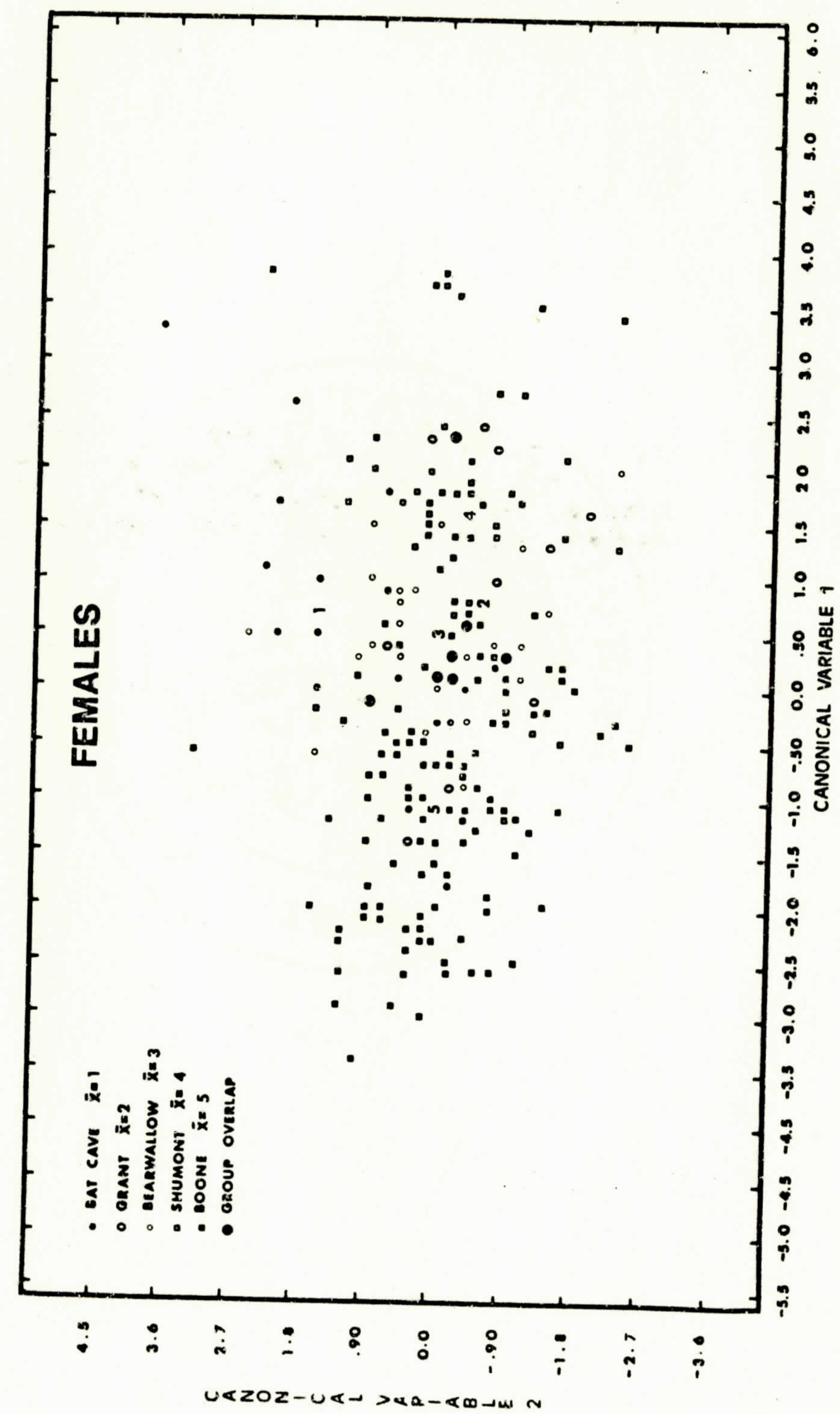
TABLE 7. Assignment of animals to groups based on discriminant functions analysis for females.

GROUP	PERCENT CORRECT	NUMBER OF CASES CLASSIFIED INTO GROUP - NORTH	SOUTH	OUTGROUP
NORTH	80.7	46	8	3
SOUTH	84.2	18	133	7
OUTGROUP	94.7	0	1	18
TOTAL	84.2	64	142	28

TABLE 8. Assignment of animals to groups based on discriminant function analysis for males.

GROUP	PERCENT CORRECT	NUMBER OF CASES CLASSIFIED INTO GROUP - NORTH	SOUTH	OUTGROUP
NORTH	88.3	106	14	0
SOUTH	86.2	15	100	1
OUTGROUP	100.0	0	0	13
TOTAL	88.0	121	114	14

FIGURE 6. Plot of canonical variables for five populations of females. Each point represents one or more animals. Large solid circles are areas of group overlap. Numbers represent mean population values.



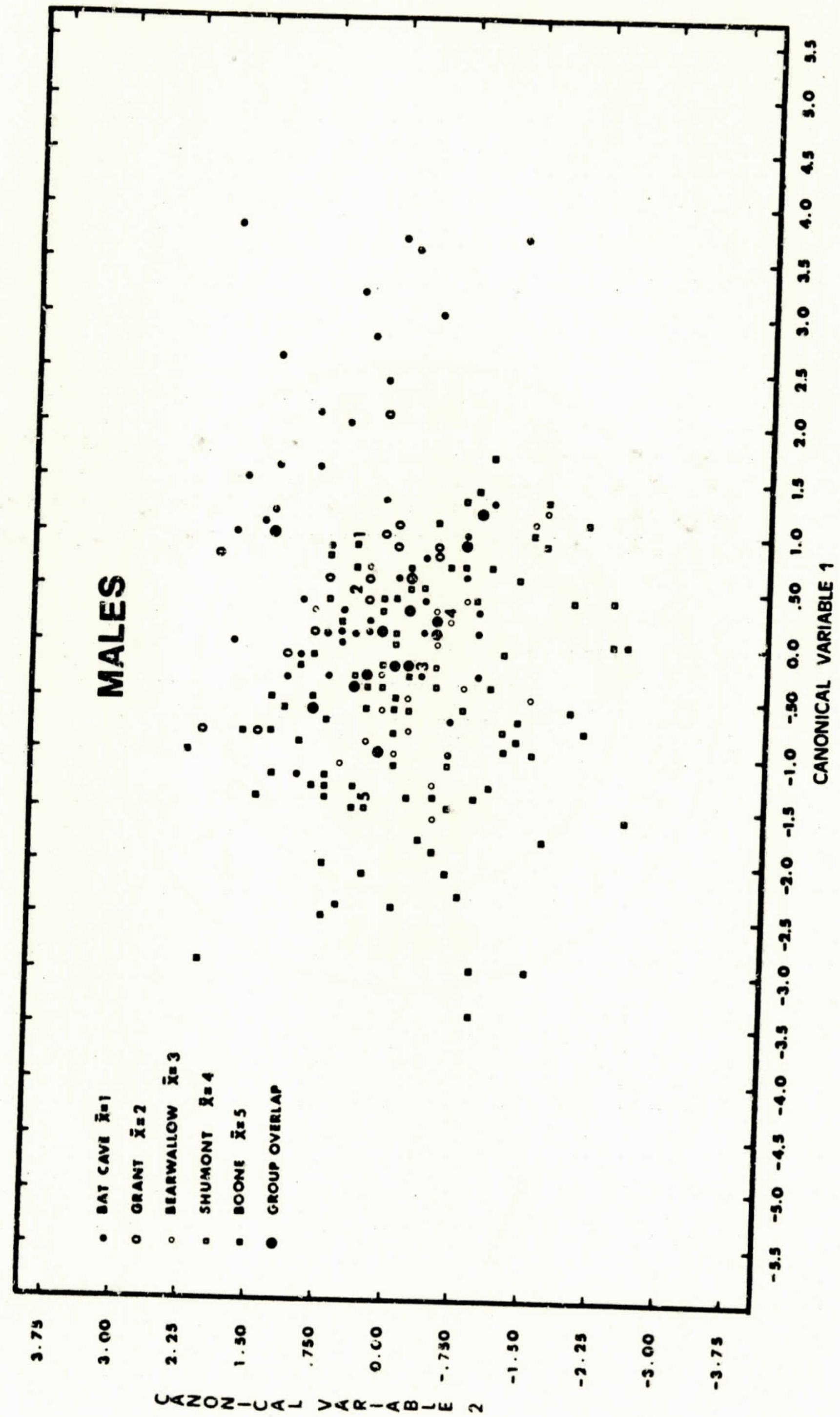


FIGURE 7. Plot of canonical variables for five populations of males. Each point represents one or more animals. Large solid circles are areas of group overlap. Numbers represent mean population values.

FIGURE 8. Plot of canonical variables for North and South populations of females. Each point represents one or more animals. Large solid circles are areas of group overlap. Numbers represent mean population values.

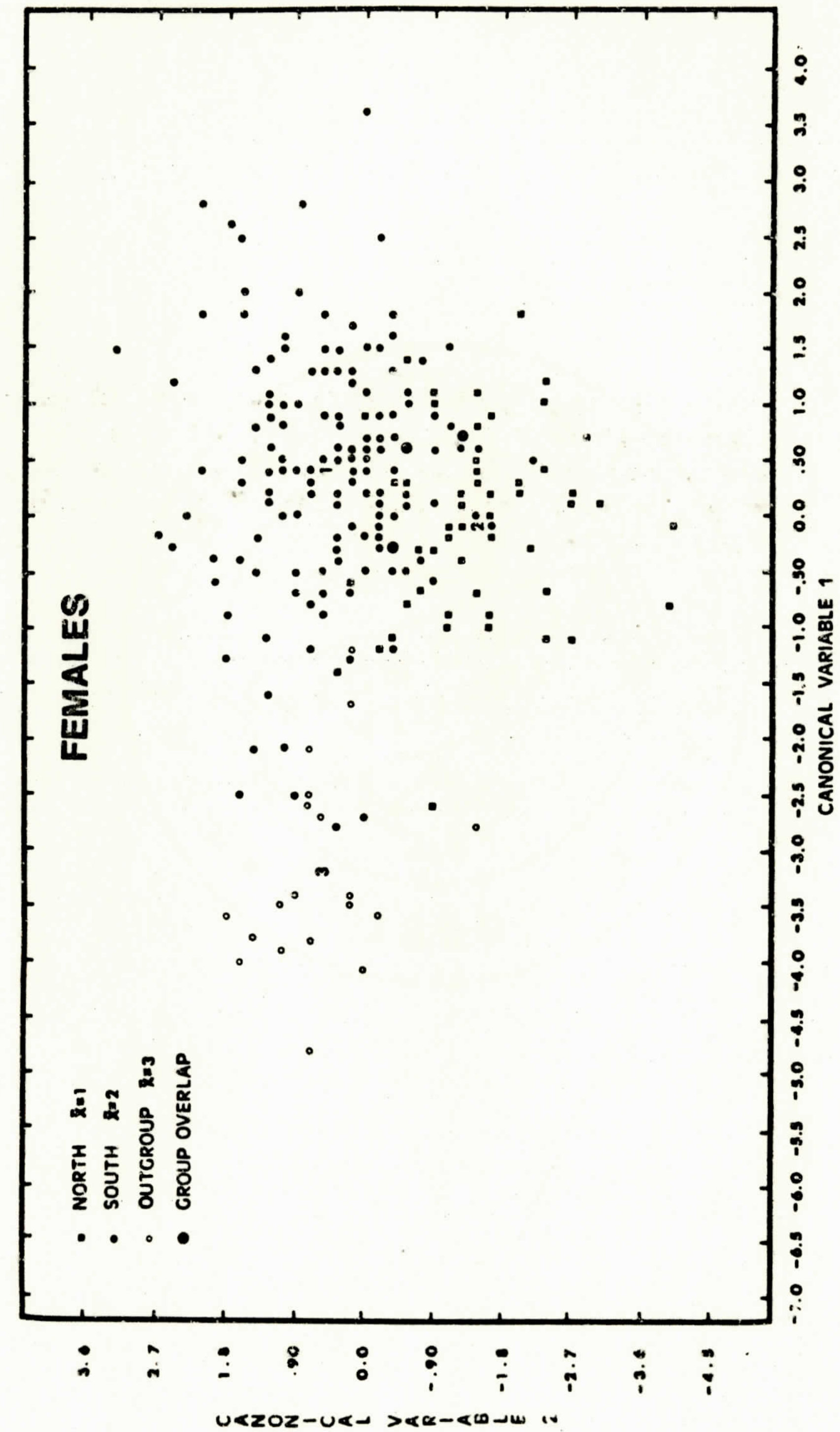
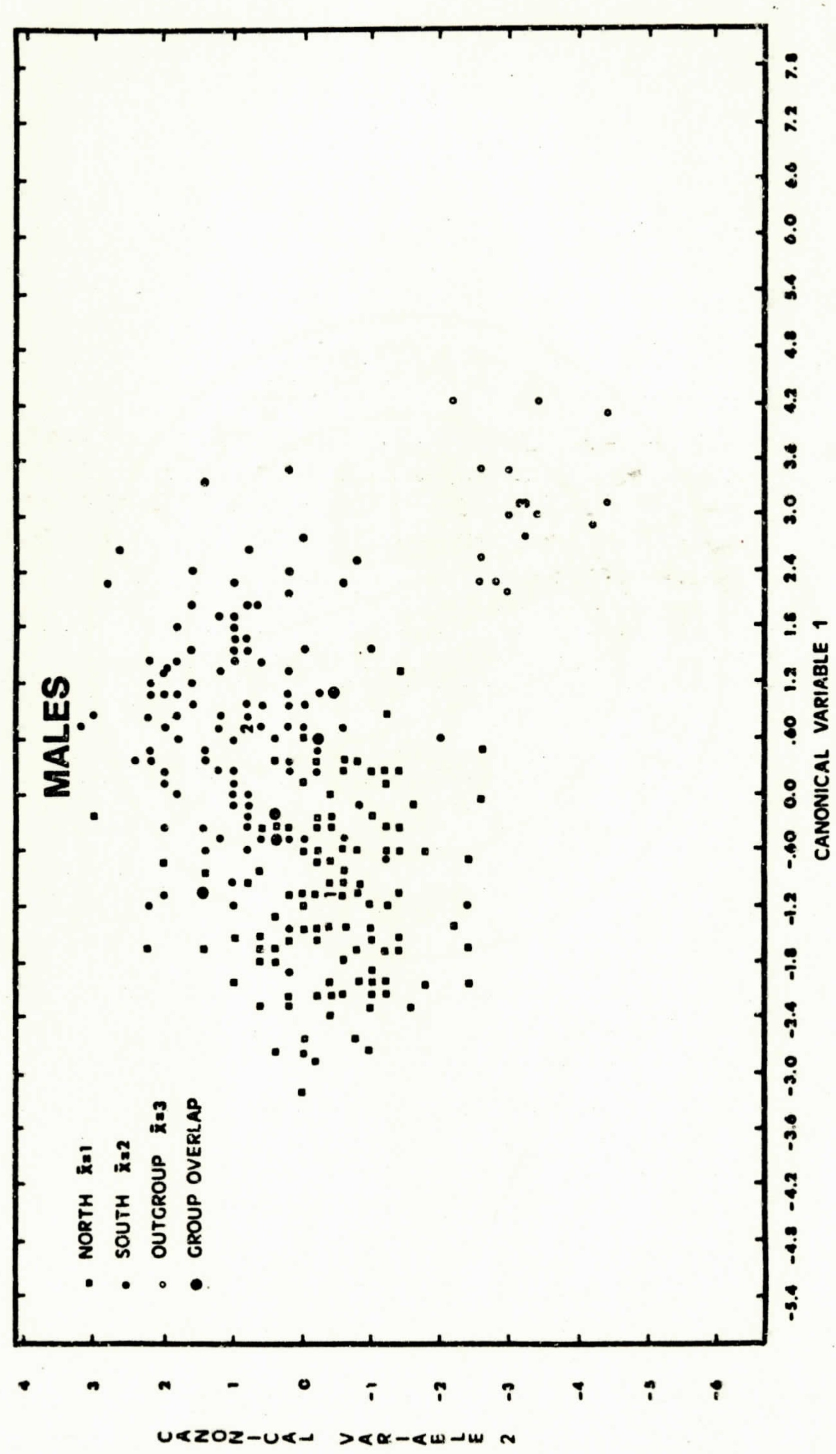


FIGURE 9. Plot of canonical variables for North and South populations of males. Each point represents one or more animals. Large solid circles are areas of group overlap. Numbers represent mean population values.



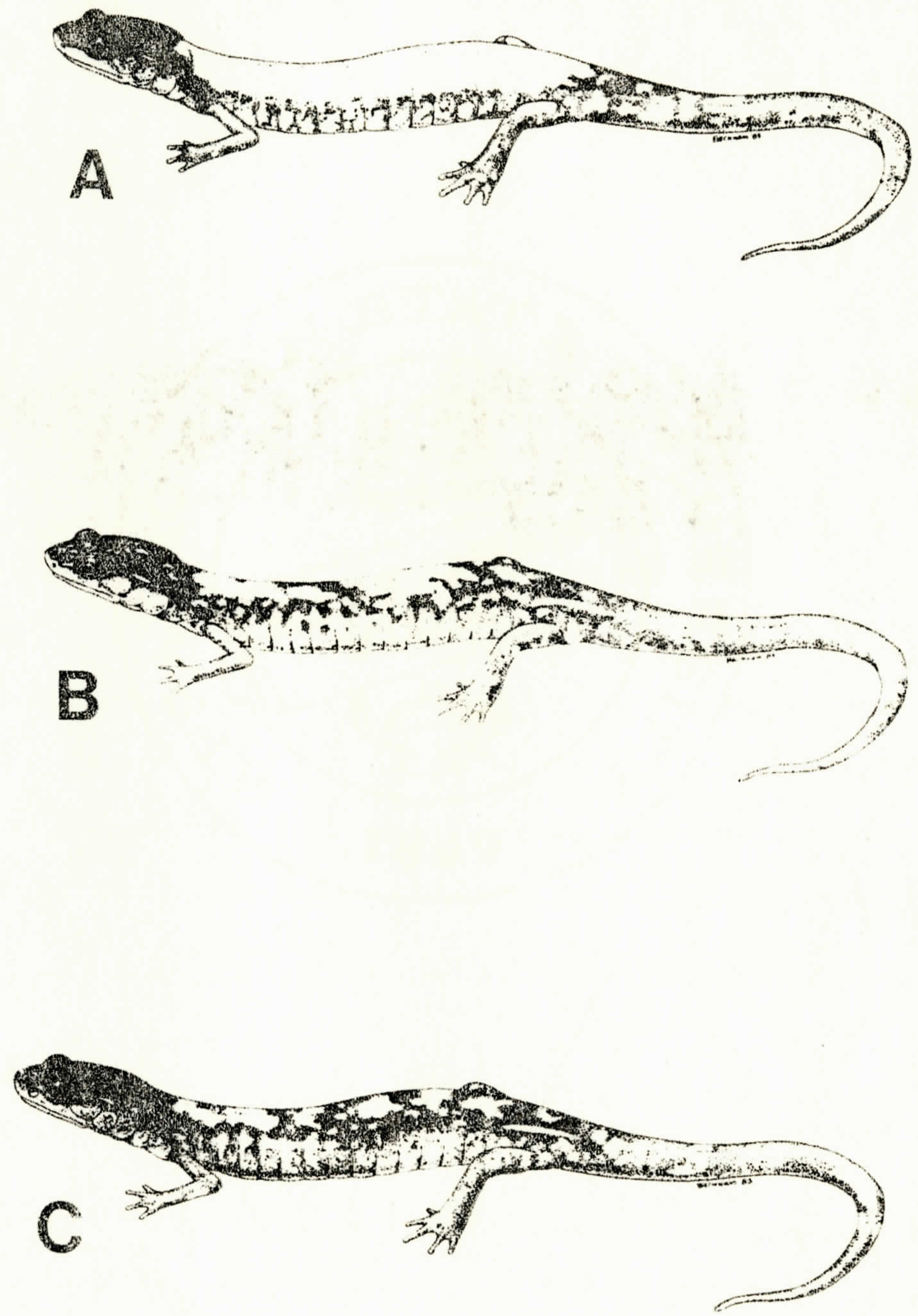


FIGURE 10. Representative members of the <u>Plethodon yonahlossee</u> complex. A - <u>Plethodon yonahlossee</u> type from Boone. B - <u>Plethodon longicrus</u> type from Shumont. C - <u>Plethodon longicrus</u> type from Grant.

DISCUSSION

Currently, the relationships of the salamanders *Plethodon longicrus* and *Plethodon yonahlossee* are not clear. Described by Dunn (1917), *Plethodon yonahlossee* was found to have a large SVL, a chestnut colored dorsal stripe, and large numbers of vomerine teeth. These characteristics distinguished it from the other salamanders in the region. Adler and Dennis (1962) described *Plethodon longicrus* on the basis of reduced dorsal coloration, larger numbers of vomerine teeth, overlapping adpressed limbs, and specialization for crevice environments. The most recent study, an electrophoretic comparison of these two forms, concluded that they were conspecific (Guttman et al., 1978); yet there has not been total acceptace of this conclusion by the scientific community (Behler and King, 1979). There are several possible relationships between *Plethodon yonahlossee* and *Plethodon longicrus*. Each of these models places emphasis on different characteristics and produces different predictions about patterns of variation.

The two forms may be conspecific. Guttman et al. (1978) suggested that *Plethodon longicrus* was a poorly distinguished low elevation form of *Plethodon yonahlossee*. In doing so, they placed

little value on color or morphological characteristics used by Adler and Dennis (1962). If this hypothesis is correct, there should be (A) little consistent regional variation, ie. there should be a relatively uniform population, (B) possible variation with altitude, and (C) no zone(s) of intergradation.

A second possible relationship would be subspecific. If two forms are subspecies, there should be distinct populations at the extremes of the range with gradual transition between these extremes. Emphasis has been placed on Bearwallow Mountain as such a transitional area since its salamanders exhibit seemingly intermediate characteristics (Guttman et al., 1978).

The third possible relationship is that *Plethodon yonahlossee* and *Plethodon longicrus* are separate species. Different species should show greater interpopulational morphological variation than intrapopulational variation. These differences could also be behavioral or biochemical in nature. For sympatric species, gene flow would be restricted due to these differences, ie. isolating mechanisms (Mayr, 1965). In areas where these isolating mechanisms are incomplete, interbreeding of populations may occur and areas of hybridization may result. These areas exhibit varied forms of hybrids, including animals with extreme forms of variation and characteristics uncommon to either interbreeding population.

Hybrid zones are commonly narrow as gene flow between populations is restricted to areas of sympatry. If Bearwallow Mountain is an area of hybridization, the offspring of these crosses should show morphological extremes.

Analysis of single variables revealed more variation than would be expected due to sampling error from a homogeneous population (Table 2). Also, no definite pattern of variation could be found upon scrutiny of the data. This would imply a great deal of morphological variation among subpopulations or more than one different population contributing to the variation. In other words, hypothesis one from above does not seem to be borne out.

Multivariate analysis for all sites revealed somewhat different patterns for males and females. Considerable overlap was seen among the four southern sites. The Boone site, conversely, had a relatively low overlap with the southern populations as a whole. Overlap with Boone females was largely restricted to the Bearwallow and Grant sites and minimal with the Shumont site that was geographically closest to Boone (Table 4, Figure 6). In males, there was considerable overlap between Boone and Bearwallow but very little overlap between Boone and any other site (Table 5, Figure 7). It is curious that the Boone females showed similar overlap with both Bearwallow (12) and Grant (10) while Boone males

show little overlap with Grant (1). This could be due to the smaller sample of females (12) at Grant as compared to males (18, a 33.0 percent larger sample).

Data from North / South analysis showed less overlap between populations than did the previous analyses. The female Outgroup overlapped with the South site at only one point (Table 7, Figure 8). Overlap between North and South females was greatly restricted (12.6 percent). Males showed a similar overlap (Table 8, Figure 9) between North / South groups (12.3 percent). Outgroup males totally segregated from both North and South animals.

Overlap with the Boone site was highest at Bearwallow. This overlap is consistent with observations made at Bearwallow (Guttman et al., 1978) where <u>Plethodon yonahlossee</u> and <u>Plethodon longicrus</u> types are found. The Boone type urodele has the characteristic dorsal stripe seen further north, but also exhibits the dorsal iridophores almost universally found on southern salamanders. The dorsal blotches found on some Bearwallow <u>Plethodon longicrus</u> types are somewhat redder than Shumont or Bat Cave.

An understanding of the status of the Bearwallow site is of paramount importance to determine if there is a zone of hybridization or intergradation present and to elucidate the actual interaction of the northern and southern forms. There seems to be no broad range of intergradation between Bearwallow and any other site. Morphologically, Bearwallow does not seem to be transitional. Morphological characteristics of the salamanders do not seem to show concordant variation. The presence of both color types is restricted to this small area. The Shumont site is actually closer to the Yonahlossee type locality than is the Bearwallow site but relatively little overlap occurs between Shumont and Boone. Because there is no evidence indicating a broad intergrade zone and since the morphological types found at Bearwallow represent extreme examples of morphology as well as intermediates, Bearwallow should be considered a hybrid zone.

Based on the data presented, Plethodon longicrus should be considered a valid taxonomic form for the following reasons: 1) the small overlap of discriminant functions between the North and South populations, 2) the probable hybrid zone at Bearwallow and 3) the coordination of the statistical information with observations of color and habitat.

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VITA

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