EVOLUTION IN THE GRAY TREEFROG COMPLEX: ACOUSTICAL, MORPHOLOGICAL, AND GENETIC VARIATION IN MIDWESTERN POPULATIONS

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"...I hope I get smart soon because I want to lern everything there is in the werld like the collidge boys know. All about art and politiks and god."

"...I see now that the path I chose through the maze makes me what I am. I am not only a thing, but also a way of being --- one of many ways --- and knowing the paths I have followed and the ones left to take will help me understand what I am becoming."

"...intelligence and education that isn't tempered by human affection isn't worth a damn."

Charlie, Flowers for Algernon by Daniel Keyes

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CHAPTER I

INTRODUCTION

This dissertation is composed of two manuscripts written in formats suitable for submission to selected scientific journals. Each manuscript is complete without supporting materials. The arrangement of each manuscript is text, literature cited, tables, and figures. Chapter II, 'Acoustical and morphological variation in the gray treefrog complex', is written in the format for COPEIA. Chapter III, 'Character divergence in Cope's gray treefrog, (Hyla chrysoscelis)', is written in the format for HERPETOLOGICA. The Appendix is a list of the current literature on the gray treefrog complex.

CHAPTER II

ACOUSTICAL AND MORPHOLOGICAL DIVERGENCE IN THE GRAY TREEFROG COMPLEX

George R. Cline

Abstract - Acoustical and morphological divergence were examined in H. chrysoscelis and H. versicolor from the west-central portion of their respective ranges. Univariate call differences were observed between species for 8 of 9 call parameters examined. Multivariate analyses revealed overall call differences between species based on call frequencies (or pitch), pulse repetition rate, and number of pulses. Morphological separation is less extensive. Two of 17 external morphological measurements revealed species differences. MANOVA indicated significant overall morphological differences, but principal components analysis and discriminant functions analysis revealed substantial morphological overlap. Apparent character displacement in call frequencies was observed in H. chrysoscelis, but no morphological changes were observed. Canonical correlation

analysis revealed two major relationships between calls and morphology. As expected, body size and call frequency were similarly related in both species. Three head characters were correlated with pulse repetition rate and number of pulses. Although there is no known functional explanation for this relationship, separation of species along these canonical axes suggests a relationship to the speciation process, or a by-product thereof.

Considerable research has been done on the gray treefrog complex since discovery of the diploid-tetraploid nature of its members. Call variation has played a key role in the study of this complex. Noble and Hassler (1936) noted two call-types, "harsh" and "mellow", in what was then considered Hyla versicolor. Blair (1958) quantified differences in pulse repetition rate between the mellow, slow-trilling form and the harsh, fast-trilling form, and noted distinct geographic distributions of the two call-types across the country. High mortality of between-call-type crosses led Johnson (1959, 1961, 1963) to assign species status to the two forms. The mellow, slow-trilling form was designated H. versicolor (later found to be tetraploid; Wasserman 1970), and the harsh, fast-trilling form was designated H. chrysoscelis (later found to be diploid; Bogart and Wasserman 1972). Ralin

(1977) found significant differences in pulse repetition rate and duration of calls of the two species, and he reported character displacement in pulse repetition rate of sympatric populations of the two taxa. Ralin (1968) observed variation in the dominant, or carrier frequency, used in both species but he offered no statistical analysis.

Morphological analysis of these species is limited, and results from older studies were confounded by taxonomic confusion (Bragg 1947; Flury 1951). Johnson (1961) found no differences in snout-vent length, femur length, tibia length, or relative lengths of femur and tibia between species. Discriminant function analysis of thirteen external morphological characters from thirteen sites across the geographic range of the complex separated eastern and western populations of H. chrysoscelis, but H. versicolor populations were morphologically intermediate (Ralin and Rogers 1979). Morphological character displacement was not observed.

Anuran advertisement calls are important in species recognition (Littlejohn and Michaud 1959; Littlejohn et al. 1960; Littlejohn 1965; Fouquette 1975; Gerhardt 1978a, 1982; and others) and may play a role in sexual selection (Ryan 1980, 1985; Klump and Gerhardt 1987). Females of some species use pulse repetition rate to discriminate against calls of other species (H. versicolor and H. chrysoscelis, Littlejohn et al. 1960; H. ewingi and H. verreauxi, Loftus-Hills and Littlejohn 1971). Pulse repetition rate

increases with increasing temperature (Bellis 1957; Zweifel 1959, 1970; Ralin 1968, 1977; Gayou 1984), but female preference compensates for temperature variation, at least in H. versicolor (Gerhardt 1978b). Rose et al. (1985) demonstrated temperature dependency in midbrain processing of auditory signals in H. chrysoscelis and H. versicolor. Spectral resonant frequency is used as a discriminator in other species (H. gratiosa, H. squirrela, and H. cinerea, Gerhardt 1982; Gastrophryne olivacea and G. carolinensis, Blair 1955a). Frequency is related to body size (Snyder and Jameson 1955; Ramer et al. 1983), but it is unclear whether species recognition is accomplished by size alone, resonant frequency alone, or the two characters in combination (A. P. Blair 1950; W. F. Blair 1955b).

This study examines acoustic and morphological variation of H. chrysoscelis and H. versicolor, and evaluates the influence that morphology has on call characteristics in these species. Theoretical aspects of sound production in anurans is also discussed.

MATERIALS AND METHODS

Data collection - One hundred and fifty-seven treefrogs, (106 H. chrysoscelis and 51 H. versicolor), were collected from twelve sites in Oklahoma, Kansas, Missouri, and Arkansas (Fig. 1). Ten calls were recorded for each individual on a Pearlcorder microcassette tape recorder before capture. Frogs were identified in the field by their calls, and a sub-sample of all specimens collected was

karyotyped by John Wiley of East Carolina University. Field identifications agreed with karyotypes in all instances where comparisons were possible (N = 44). Captured individuals were assigned a code number and frozen at -60° C for later electrophoretic and morphometric analyses. Air temperature at the perch site was measured to the nearest 0.1° C using a Miller & Weber rapid-register thermometer. Call Duration (DUR; measured in seconds), Number of Pulses in the call (NUMP), and Pulse Repetition Rate (PRR; measured in pulses/second) were calculated from sonograms produced by a Kay 6061B Sound Spectrograph (Fig. 2). A Realtime Analyzer was used to determine the first three frequency peaks based on energy levels; these peaks are labeled the Fundamental Frequency (FF; the lowest frequency) and the first two Resonant Frequencies (RF1 & RF2) and measured in Hertz (Hz). Additional calculations included the ratio of the fundamental frequency to each of the resonant frequencies, and the ratio of the resonant frequencies (VR1, VR2, & VR12 respectively; VR12 = RF1/RF2); these ratios determine vowel sounds in humans and are fixed across age and sex (Minifie 1973). As such, these ratios may act as discriminators between species or individuals. All call parameters are described in Table 1.

Dial calipers were used to measure to the nearest 0.01 mm sixteen external characters for each specimen (Table 1): snout-vent length (SVL), snout-urostyle length (SUL), urostyle length (UL), head length (HDL), head width (HDW),

interorbit distance (IOD), internares distance (IND), orbit-naris distance (OND), jaw length (JL), humerus length (HML), radio-ulna length (RUL), thumb length (TBL), 3rd finger length (FL3), femur length (FL), tibiofibula length (TFL), and hind foot length (HFL). Maximum tympanum diameter (TD) was measured using a digitizer linked to a binocular microscope. All bilateral characters were measured only on the right side.

Statistical analyses - All morphological characters and six call characters (DUR, PRR, NUMP, FF, RF1, RF2) were log transformed prior to multivariate analyses to reduce variance due to size and collecting technique. The three frequency ratios (VR1, VR2, VR12) were arcsin transformed prior to analyses. Equality of variances was tested using an F-test; a t-test using pooled variances and adjusted degrees of freedom was used to test for differences between means with significantly different variances; otherwise analysis of variance (ANOVA) was performed. Previous studies indicate that temperature exerts a significant effect on some call parameters including PRR and DUR (Bellis 1959; Ralin 1968, 1976; Zweifel 1970). Call parameters were regressed against temperature and comparisons that produced significant regressions were adjusted to 21° C as per Ralin (1968) prior to analyses. Due to small size of some samples, the species regression rather than that for each population was used to standardize those call parameters affected by temperature. Multivariate analysis of variance

(MANOVA) was used to detect overall differences among groups. Principal Components Analysis (PCA) produces linear combinations of the data in such a manner as to maximize the variance accounted for by each principal component with the restriction that all axes are orthogonal. The correlation matrix was input into the PCA to minimize variance due to differences in relative magnitude among the variables. Additionally I used Canonical Discriminant Analysis (also called Discriminant Functions Analysis, or DFA) to interpret patterns in those data significant by MANOVA. While PCA searches for structure in the data without regard to groups, Canonical Discriminant Analysis uses classification variables, ie. population codes, in an effort to produce linear combinations that best separate groups. Canonical Correlation Analysis was used to evaluate relationships between the call and morphological datasets. This technique creates linear combinations of variables within each dataset in such a way as to maximize the correlation of one linear combination (canonical variate) with a linear combination in the other dataset. Similarly, the second set of canonical variates are linear combinations of the variables in each dataset that are maximally correlated, with the restriction that they are orthogonal to the first set of canonical variates. The sample size of the dataset used for this analysis was reduced to 69 H. chrysoscelis and 32 H. versicolor since both morphological and call data were required from each specimen and some specimens were destroyed prior to morphological measurement.

Due to the large number of statistical tests performed in this study, the significance level was partitioned to "table-wide" or "character-wide" significance of $p < .05$ using the sequential Bonferroni technique (Rice 1989). This technique first requires that the observed significance levels (OSL's) be ranked from smallest to largest. Once a "table-wide" significance level has been chosen, the smallest OSL is compared to p/k, where k = the total number of tests to be run and p = the overall significance level. If the smallest OSL is less than or equal to p/k, then that test is significant at the "table-wide" significance level; if it is greater than p/k, then none of the tests are significant. If the smallest OSL is significant, then the next smallest OSL is compared with $p/(k-1)$. If OSL < $p/(k-1)$, then that test is significant at the table-wide significance level and further comparisons must be made; otherwise this test and all remaining tests are not significant. Each OSL is compared with the inequality, reducing k by one each time the previous test was significant until the inequality is no longer met. Each of the tests is significant at the table-wide significance level.

RESULTS

H. chrysoscelis vs H. versicolor

Calls - As expected, temperature had a significant effect on the calls of both species (Table 2). Pulse repetition rate (PRR) increased with temperature in both species (Fig. 3), but the rate of increase was significantly different for H. chrysoscelis and H. versicolor (using a Multiple Regression model to test for homogeneity of slope), thus precluding Analysis of Covariance. The Johnson-Neyman Technique was used to determine regions of significance between the two regression lines (Huitema 1980). Points on the two regression lines are significantly different down to -12° C; while this extrapolates well beyond the available data, it does indicate that calls are significantly different over the range of temperatures observed in this study. Temperature also significantly affected RF1, and RF2 in H. chrysoscelis, and DUR and NUMP in H. versicolor. Call parameters were standardized to 21° C using the species regressions for those parameters having significant regression coefficients. Parameters not having significant regression coefficients were not adjusted.

Univariate comparisons between species were made using t-tests on temperature-adjusted characters. Significant differences were observed between species for eight of the nine call parameters (DUR, PRR, NUMP, FF, RF1, RF2, VR2, and VR12; $p < 0.05$, sequential Bonferroni; Table 3). MANOVA comparison of calls revealed significant overall differences

(Hotelling-Lawley Trace; $p < 0.0001$) between calls of H. chrysoscelis and H. versicolor, so PCA was employed to determine which characters were the most important sources of variation.

The first five principal components (PC's) explained 99.5% of the observed variance (Table 4). The first PC is weighted by two characters that are size related (FF and RF1), and one size-independent character (PRR). The second PC is a contrast between RF2 and VR2; the RF2 spectral peak is less well defined than the other peaks, and as such it was subject to greater variation. Since VR2 is the ratio of FF/RF2, it is reasonable that a high negative loading of RF2 should cause a high positive loading of VR2. Thus, this PC may represent sample noise that has little or no biological significance. Two ratios (VR1 and VR12) are contrasted on PC3; since RF1 appears in the numerator of one ratio and the denominator of the other ratio this PC most likely represents variation in RF1. RF1 has been called the dominant or carrier frequency of gray treefrog calls (Ralin 1968) and it is the frequency band to which the female's auditory system is tuned (Lombard and Straughan 1974). The fourth PC reflects variation in NUMP. The fifth PC is a contrast between DUR and PRR. The most complete separation of species is obtained by plotting PC scores along PC4 and PC1 (Fig. 4). Principal component 4 reflects variation in NUMP, while PC1 reflects variation in frequency (FF, RF1) and PRR. Since two groups (i.e. species) could be

identified by karyotyping, canonical discriminant analysis was performed on log-transformed, temperature adjusted call data to determine which linear combination of characters provided the best discrimination. The discriminant function provided 100% discrimination between species, largely on the basis of PRR and NUMP (Fig. 5).

Sound waves produced by vibration of the vocal cords are subject to modification by the vocal tract based on size, shape, and resonating qualities of the surrounding tissue. Thus, some frequencies transmitted by the vocal cords may be filtered out or de-emphasized. Those frequencies that are transmitted to the environment are called resonant frequencies (termed vowel formants in human speech). One quantitative measure of complex sound structure is the ratio of the fundamental frequency to the resonant frequencies and the ratio of the resonant frequencies to each other. The ratio of the fundamental frequency and the first two resonant frequencies are unique for different vowel sounds, and the reciprocals of the ratios reveal harmonic structure. In human speech, resonant frequencies of vowel sounds range from the first through the tenth harmonic (Minifie 1973). In this study the reciprocal of the first two frequency ratios approach 2 and 3, respectively, indicating that the resonant frequencies represent nearly pure first and second harmonics of the fundamental frequency, implying that little frequency filtering occurs in this species. Subtle differences in complex structure are still important,

however, as frogs more easily recognize conspecific calls with complex harmonic structure than pure tone calls (lacking harmonics) and inharmonic calls (Simmons 1988).

Morphology - There is little morphological divergence between species (Table 5). There was no difference in length (i.e., SVL) between species even though casual examination suggests that H. versicolor is bigger (Bragg 1947; T. Johnson 1987; pers. observ.). Despite lack of significant differences in HDL or HDW between species, two head characters (IOD, OND) are larger in H. chrysoscelis, suggesting a larger surface area between the eyes and the nares, and thus a larger oral structure.

MANOVA results indicated overall significant morphological differences between species (Hotelling-Lawley Trace; $p < 0.0001$), so PCA was employed to identify important characters. The first four PC's explained 72.6% of the observed variance (Table 6). Nearly equal weighting of all characters on PC1 indicates it is a size vector. Three head characters (IOD, IND, OND) loaded heavily on PC2 and suggest head shape differences between species. A contrast between SUL and UL accounts for most of the variation in PC3; this is probably due to relatively small UL observed in individuals of both species. FL3 loaded heavily on PC4 and may indicate differences in climbing ability that is unrelated to species differences. Plotting PC2 vs. PC1 gave the most complete separation (Fig. 6). Canonical discriminant analysis revealed a significant

Mahalanobis distance between morphological group centroids ($F = 1.986$, $p < .0208$), but there is substantial overlap of canonical scores (Fig. 7). The most important characters in the discriminant function were IOD, RUL, and JL.

Sympatry vs Allopatry

Calls - Small sample sizes of H. versicolor in sympatry with H. chrysoscelis preclude analysis of character displacement in H. versicolor. Sufficient numbers of H. chrysoscelis in allopatry and sympatry with H. versicolor, however, are available to examine character displacement in H. chrysoscelis. Diploid frogs from sympatry produced higher pitched calls (FF, RF1, RF2) than frogs from allopatry (t-test, $P < .05$, table-wide sequential Bonferroni method; Table 7). The direction of change in these characters suggests improved discrimination between these species by reducing acoustical interference (cf. Table 3). Ralin (1977) noted character displacement in PRR in this complex, but he presented no call frequency data. MANOVA revealed significant overall differences between calls of allopatric and sympatric frogs (Hotelling-Lawley Trace; $p < 0.0001$), so PCA was employed to determine the major sources of variance. The first five PC's account for 98.4% of the variance (Table 8). FF, RF1, RF2, VR1, and VR2 load heavily on PC1. DUR and NUMP load heavily on PC2, but these characters are known to be behaviorally influenced by chorus density. VR12, RF2, and VR2 load heavily on PC3, while NUMP and VR1 load heavily on PC4. PRR loads heavily on PC5. The

first PC includes characters that are likely to be influenced by changes in body size and/or shape. NUMP and DUR increase with chorus density in H. versicolor (Wells and Taigen 1986) and they are likely to do the same in H. chrysoscelis. Thus, PC2 suggests differences in chorus densities between groups. Principal component 3 is a contrast between RF2, and two frequency ratios (VR2, VR12). This contrast is expected since, as RF2 increases, both FF/RF2 (VR2) and RF1/RF2 (VR12) decrease; thus PC3 is an RF2 vector. Variation along the third PC axis does not appear to be related to species differences. Principal component 4 contrasts NUMP and VR1. There is no direct relationship between these call variables, and this component reveals no species-related structure. Principal component 5 reflects variation in PRR, which is known to have a genetic basis (Burger 1980). Plotting PC2 vs. PC1 gave the most complete separation (Fig. 8). It is important to note that the best separation comes from using a character that is influenced by chorus density. The Mahalanobis distance between the group centroids was significant (F-test; $p < 0.0001$), but there is considerable overlap of canonical scores (Fig. 9). Discrimination is based mainly on the frequency characters (FF, RF1, RF2) and the first two frequency ratios (VR1, VR2).

Morphology - Despite significant call differences between H. chrysoscelis populations from allopatry and sympatry with H. versicolor, there is no evidence of morphological

character displacement (Table 9). The MANOVA was not significant ($p < .6944$) and the Mahalanobis distance between groups was not significant ($p < .8794$). No structure was revealed by PCA (Fig. 10).

Canonical Correlation Analysis

Canonical correlation analysis was performed on data from frogs with both morphological and call information. The canonical correlation and tests of significance are presented in Table 10. The first two correlations exceed 60% and are statistically significant ($P < .006$). Roy's Greatest Root and Wilk's lambda tested the null hypothesis of no association between datasets; results of these tests were highly significant, therefore, the null hypothesis of no association between calls and morphology was rejected.

The correlations of characters and the canonical axes were used to interpret patterns of covariance between call and morphological variables (Table 11). The correlations reveal interactions between variables and indicate the contribution of each variable to the canonical structure (Miles and Ricklefs 1984). Within-set correlations determine the contribution of each character to the canonical axes, and can be used to "define" the axes. Between-set correlations describe the relationship between each morphological character and each canonical axis of calls, and vice versa. The between-set correlations are the products of the within-set correlations and the canonical correlation; these statistics were generated as part of the

SAS CANCORR procedure (SAS 1982).

The square of each within- and between-set correlation equals the proportion of variance explained by the relationship. Within datasets it is the amount of variance in the original variable that is explained by the canonical variate. Squares of between-set correlations in Table 11 estimate the contribution of each variable in one dataset to each canonical variate of the other dataset. For calls, the first canonical variate explains 91% of the variation in RF1, 47% of the variation in FF, and 37% of the variation in RF2 (squaring the correlation coefficients from Table 11). Thus the first canonical call variate can be considered a frequency axis. The second canonical call variate explains 57% of the variation in PRR, and 28% of the variation in DUR. This is a "mixed" vector and consequently is much harder to interpret, but calls with high pulse repetition rates tended to be shorter in duration. Nine morphological variables had substantial proportions of variation explained by the first canonical variate (RUL 62%; SVL 45%; HDW 41%; SUL 38%; TFL 38%; JL 37%; FL 35%; HFL 32%; HDL 25%); this suggests that the first canonical variate of morphology is a size vector. The second canonical variate of morphology explains 62% of the variation in IOD, 36% of the variation in OND, and 29% of the variation in IND; thus, the second canonical variate defines head shape. The between-set correlations are similar to the within-set correlations. This is largely due to the high correlation between the

first canonical variates. This pattern holds for the second canonical variates as well. Figure 11 is a plot of the first canonical morphological variate versus the first canonical call variate. Individuals of both species have similar, overlapping distributions along these axes. Figure 12 plots the second canonical mophological variate against the second canonical call variate. As expected based on the high canonical correlation, the relationship between calls and morphology is linear. The species, however, have different distributions of scores on both axes.

Canonical redundancy analysis examines the extent to which variation within datasets is related to the canonical variates (Table 12). Inspection of the bottom part of Table 12 shows that the first morphological variate extracts 26% of the morphological variation and 15% of the call variation. All nine morphological variates explain 69% of the morphological variation and 30% of the call variation. The first canonical variate of calls explains 26% of the variation in calls and 15% of the variation in morphology. All nine call variates explain 28% of the variation in morphology. This suggests that morphology is a slightly better predictor of calls than calls are predictors of morphology. Table 12 shows that all the morphological canonical variates combined explain 55% of the variance in RF1, 43% of the variance in PRR, and 41% of the variance in FF. The call variates explain 42% of the variance in RUL.

DISCUSSION

The significant differences between calls of H. chrysoscelis and H. versicolor have been described by other authors and have been observed here. Pulse repetition rates and slopes of pulse repetition rate versus temperature are consistent with previous studies (Ralin 1968, 1977; Gayou 1984; Zweifel 1970). Call duration is a function of numbers of pulses and pulse repetition rate, but numbers of pulses can be influenced by chorus density in H. versicolor (Wells and Taigen 1984). Nevertheless, this study shows that between-species differences in numbers of pulses to be greater than within-species differences due to chorus density. Numbers of pulses in the call may therefore be used as a secondary mechanism for species recognition. Pulse repetition rate and the number of pulses in the call provided excellent separation in both principal component analysis (Fig. 4) and canonical discriminant analysis (Fig. 5).

While sufficient call differences exist for species recognition, this information must be broadcast into a "noisy" environment and must arrive at the receiver, i.e., the female, intact. Partitioning of available broadcast frequencies into species-specific channels is one method of ensuring than call information is accurately broadcast and received (Duellman 1968; Hödl 1977; Drewery and Rand 1983; Duellman and Pyles 1983). The upper limit of the acoustical window available to most anurans is approximately 4000 Hz

(Schiötz 1973; Straughan 1973; Littlejohn 1977). Acoustic partitioning appears to have been partially accomplished by H. chrysoscelis and H. versicolor. All three frequencies measured were significantly different between species (Table 3), but there is overlap in sympatry that could cause acoustic interference.

Analysis of allopatric and sympatric populations of H. chrysoscelis indicate a shift in call frequencies away from the mean frequencies of allopatric populations of H. versicolor; such character displacement would reduce acoustic interference. This frequency shift could be accomplished in two ways. First, an increase in body size would increase the size of the resonating chamber and produce a lower pitched call. Canonical correlation analysis indicates that size and call frequency are related; the first canonical variate of morphology is interpreted as size and the first canonical variate of calls is frequency (Table 11). Plots of these canonical variates against each other suggest that call frequency changes in a similar manner for both species (Fig. 11). Thus, a shift in mean body size of either species when they come into contact could cause a change in call frequency that would decrease call interference. The morphological data for H. chrysoscelis from allopatry and sympatry with H. versicolor does not, however, indicate a significant change in body size in H. chrysoscelis. Further data are required to test if body sizes of the two species are significantly different

in sympatry. Second, frequency changes could also be accomplished by changing the mass of the vocal cords. Martin (1972) demonstrated that frequency changes by members of the genus Bufo were accomplished by adding a fibrous mass of tissue to the middle of the vocal cords, thus changing their fundamental frequency of vibration.

Ratios of resonance frequencies are used by speech scientists to define vowel sounds in human speech. While the absolute frequencies of speech vary across age and sex, the frequency ratios do not (Minifie 1973). Simmons (1988) demonstrated that Hyla cinerea responded to changes in harmonic structure of conspecific calls. Frogs could detect two-tone harmonic calls against a background of white noise more easily than pure tones or two-tone inharmonic calls. Simmons further noted that this sensitivity occurs at the level of the central auditory system. Comparison of frequency ratios in gray treefrogs produces ambiguous results. Two of three frequency ratios are significantly different in univariate tests, but they contribute little to species separation in multivariate space. These ratios also contribute little to canonical structure in comparisons of morphological and call parameters, so their value in understanding anuran vocalizations is unclear.

As might be expected when comparing morphological separation in two cryptic species, discrimination is poor. Apparent qualitative differences in "size" as noted by other authors is not confirmed by data collected in this study.

Perhaps "size" differences are not related to length of these species, but rather to body mass differences. This prospect should be considered in future studies since vocal cord mass (and, possibly total body mass) are related to fundamental frequency. Differences between inter-orbit distance and orbit-naris distance translate into head shape differences with two possible implications. First, head shape differences could reflect diet differences observed by Ralin (1968) in sympatric populations of these species. Changes in head shape will in turn affect the frequency ratios of the calls. These ratios are determined by the size and shape of the resonator(s), in this instance, the vocal sac and oral cavity. Univariate differences were observed in both head morphology (Table 5) and frequency ratios (Table 3), but these differences were not related by canonical correlation analysis (Fig. 12). Thus the frequency ratios are either unaffected by morphological changes or the morphological characters used to test this hypothesis were inappropriate. Care must be taken in interpreting the results of the canonical correlation analysis due to small sample size. Further use of frequency ratios should include an examination of the oral cavity and the vocal sacs.

Results from multivariate analyses of morphological data are inconclusive. Overall morphological differences exist, but species-specific patterns are obscure. Size accounts for most of the variance observed in the dataset, followed

by head shape on the second principal component. The contrast between urostyle length and snout-urostyle length on principal component 3, and length of the third finger on principal component 4 do not relate to species differences, although PC4 does suggest differences in climbing ability. Ralin (1968) noted that H. chrysoscelis from sympatry with H. versicolor called from higher perches and were generally more arboreal. Thus, a more detailed study that incorporates morphology and perch site selection in sympatric populations may be warranted.

Canonical discriminant analysis of morphology revealed significant, but overlapping distributions for the two species (Fig. 7). The discriminant function was based on head shape (inter-orbit distance and jaw length) and length of the forearm (radioulna length). Forearm length approached univariate significance, with H. chrysoscelis having shorter forearms than H. versicolor (Table 5). Forty-two percent of the variation in radioulna length is explained by variation in calls (canonical redundancy analysis). Arm length (radioulna + humerus length) is related to reproductive success in wood frogs, Rana sylvatica (Howard and Kluge 1985). Males of this species fight and attempt to dislodge males already in amplexus, and males with shorter arms are more easily dislodged. Such male-male fighting has not been observed in H. versicolor (Fellers 1979a, 1979b), but males with shorter forearms might be more easily dislodged by unreceptive females. Male

H. chrysoscelis may have mechanical problems remaining clasped with larger (presumably H. versicolor) females, while male H. versicolor may be too large to easily clasp smaller (presumably H. chrysoscelis) females. Thus, forearm length differences may serve partially to isolate the species reproductively.

Morphological results reported herein agree with those of Ralin and Rogers (1979) and Little (1980); both studies report great similarity between species. Ralin and Rogers (1979) were distressed when two eastern populations of H. chrysoscelis were connected in a Prim network, not to other populations of H. chrysoscelis, but to eastern populations of H. versicolor. This result should not be considered problematic considering the populations involved and the nature of this complex. The populations in question were H. versicolor from New Jersey and New York, and two H. chrysoscelis populations from Georgia and South Carolina. All of the populations are from near the coast and likely face similar climatic regimes. Considering that the species have essentially the same genetic background and come from similar environments, it is not unlikely that they face the same selective pressures and have responded in similar fashion.

Ralin (1977) reported character displacement in pulse repetition rate in both members of this complex. Changes in the call frequencies of H. chrysoscelis from allopatry and sympatry with H. versicolor also indicate character

displacement in this study. Since no change in size or head shape was observed, this shift is most likely due to changes in the vocal cords that function to reduce acoustical interference. The direction of change in pulse repetition rate, although not significant, would act to further improve species discrimination. More data on calls and morphology are required before character displacement can be properly assessed in this complex.

Canonical correlation analysis produced results with two diverging interpretations. The first morphological and call variate were highly correlated, and mainly related body size to call frequency. This relationship has been noted by other authors for other species (Snyder and Jameson 1965; Ramer et al. 1983). Plotting individuals along these axes revealed similar call response to morphological change. The second pair of canonical variates relate mainly head shape (interorbit distance, inter-nares distance, and orbit-naris distance) to pulse repetition rate and call duration. Causal functional relationships among these characters are unknown, but plotting individuals along these axes reveals distinct distributions of the species. These variates appear to relate morphological and call differences associated with each species. Hyla chrysoscelis was larger for all head shape measures and produced short calls with high pulse repetition rates. The opposite was true for H. versicolor. The fact that the species separate better along the call axis than along the morphological axis is

consistent with the other multivariate analyses. The low proportion of variance of the call dataset explained by morphology may be a function of the call parameters used in this study. There is no reason to expect relationships between external morphology and pulse repetition rate, number of pulses, and call duration. Characters related to laryngeal morphology might improve the strength of the relationship between morphology and calls.

To summarize, significant differences in call parameters exist between H. chrysoscelis and H. versicolor, but morphological characters exhibit considerable overlap. Calls of H. chrysoscelis had more pulses and higher pulse repetition rates, were shorter in duration, and higher pitched than H. versicolor calls. Morphologically, H. chrysoscelis had longer, broader snouts than H. versicolor. Character displacement in call frequencies is not related to the morphological characters examined and is believed to function to improve acoustic discrimination between these species. Canonical correlation analysis relates body size to call frequency with little interspecific difference. It also relates head shape to call parameters that do show the two species separate from one another.

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TABLE 1. List of call parameters (A) and morphological (B) characters used in this study. All morphological characters were measured in mm; bilateral characters were measured on the right side.

(A) Call Parameters

DUR - length of call calculated from sonogram (s)

PRR - pulse repetition rate (p/s)

NUMP - number of pulses in call (p)

FF - fundamental frequency, lowest frequency peak (Hz)

RF1 - first resonant frequency peak (Hz)

RF2 - second resonant frequency peak (Hz)

VR1 - FF / RF1

VR2 - FF / RF2

VR12 - RF1 / RF2

Table 1 (cont.)

(B) Morphological Characters

SVL - distance from tip of snout to cloaca

SUL - distance from tip of snout to anterior margin of urostyle

UL - length of urostyle

HDL - distance from tip of snout to posterior margin of skull

HDW - head width at center of tympanum

IOD - distance between anterior margins of the eyes

IND - distance between nares

OND - distance between anterior margin of eyes and naris

TD - tympanum diameter

JL - distance from angle of jaw to center of jaw

RUL - distance from elbow to base of thumb (radio-ulna length)

HML - distance from upper arm articulation with scapula and the elbow (humerus length)

TBL - distance from base of thumb to distal margin of last phalanx

FL3 - length of last two phalanxes of largest (3rd) phalange

FL - distance from cloaca to knee (femur length)

TFL - distance from knee to heel (tibiofibula length)

HFL - distance from heel to distal end of last phalanx 4th toe (hindfoot length)

Table 2. Comparison of regression coefficients of individual call parameters against air temperature for H. chrysoscelis and H. versicolor. Regression coefficients and the amount of variance explained by the regression are given. Asterisks indicate significant regression coefficients at $p < .05$ level for species-wide comparisons (sequential Bonferroni method). Sample sizes are as follows: H. chrysoscelis, N = 106, H. versicolor N = 51. Character codes are defined in Table 1.

	H. chrysoscelis		H. versicolor	
Character	Coefficient	R^2	Coefficient	R^2
DUR	-0.006	1.6	-0.048*	51.2
PRR	1.56 *	36.7	0.715*	55.6
NUMP	0.455	3.1	-0.616*	23.8
FF	6.668	4.6	2.694	2.0
RF1	20.131*	15.0	4.389	1.0
RF2	20.838*	6.4	-1.041	0.1
VR1	-0.002	2.7	0.001	0.3
VR2	-0.001	0.1	0.001	2.9
VR12	0.002	1.9	0.002	2.5

Table 3. Comparison of individual temperature-adjusted call parameters of H. chrysoscelis and H. versicolor. Means, standard errors, and t-test results based on table-wide significance level of $p < .05$ (sequential Bonferroni method) are given. Significant comparisons are noted by asterisks. Sample size and character codes are given in Tables 1 & 2.

	H. chrysoscelis		H. versicolor		
Character	Mean	SE	Mean	SE	Results
DUR	0.53	0.01	0.74	0.03	*
PRR	56.7	0.61	25.1	0.4	*
NUMP	30.5	0.8	17.8	0.6	*
FF	1293.87	9.03	1211.07	12.44	*
RF1	2451.00	14.20	2299.82	25.38	*
RF2	3668.24	23.56	3546.69	35.01	*
VR1	0.53	0.01	0.53	0.01	-
VR2	0.35	0.01	0.34	0.01	*
VR12	0.67	0.01	0.65	0.01	*

MANOVA (Hotelling - Lawley Trace, $p < .0001$)

Table 4. Character loadings of the temperature-adjusted call parameters on the first five principal components. Character codes are given in Table 1.

Character	PC1	PC2	PC3	PC4	PC5
DUR	-.2368	.1730	.2518	.4028	.6799
PRR	.4139	-.1021	-.1338	.3598	-.4650
NUMP	.2681	.0318	.0647	.7771	-.0044
FF	.4921	-.0026	.2733	-.1955	.1928
RF1	.4402	-.2551	-.2132	-.1288	.3863
RF2	.2475	-.5861	.2584	-.0927	.1973
VR1	.1602	.3159	.6505	-.1256	-.2006
VR2	.3424	.5557	.0703	-.1431	.0464
VR12	.2460	.3761	-.5478	-.0694	.2319
Eigenvalues	3.300	1.868	1.581	1.229	0.976
% Variance	36.7	20.7	17.6	13.7	10.8
Cummulative % Variance	36.7	57.4	75.0	88.7	99.5

Table 5. Comparison of individual morphological characters of H. chrysoscelis and H. versicolor. Means, standard errors, and t-test results based on a table-wide significance level of $p < .05$ (sequential Bonferroni method) are given. Significant comparisons are noted by asterisks. Sample sizes are as follows: H. chrysoscelis N = 80, H. versicolor N = 42. Character codes are given in Table 1.

	H. chrysoscelis		H. versicolor		
Character	Mean	SE	Mean	SE	Results
SVL	40.23	0.31	40.19	0.05	-
SUL	22.20	0.23	22.48	0.40	-
UL #	15.53	0.22	15.59	0.20	-
HDL#	11.70	0.08	11.70	0.16	-
HDW#	13.90	0.11	14.00	0.21	-
IOD	6.66	0.10	5.94	0.15	*
IND	2.81	0.06	2.56	0.09	-
OND	2.98	0.49	2.72	0.07	*
TD	2.58	0.04	2.60	0.07	-
JL	11.78	0.11	11.75	0.16	-
RUL	8.00	0.07	8.32	0.11	-
HML	11.05	0.15	10.65	0.23	-
TBL	4.85	0.10	5.03	0.12	-
FL3	5.66	0.12	5.94	0.10	-
FL	19.85	0.18	20.28	0.23	-
TFL	18.79	0.16	19.07	0.23	-
TL4	25.65	0.61	26.66	0.30	-

MANOVA (Hotelling - Lawley Trace, $p < .0001$)

Table 6. Character loadings of the morphological data on the first four principal components. Character codes are given in Table 1.

CHARACTER	PC1	PC2	PC3	PC4
SVL	.3036	.0256	-.1662	-.1111
SUL	.2342	-.0728	-.4906	-.1887
UL	.1999	-.1634	.6146	-.0316
HDL	.3009	-.0011	-.0666	-.1445
HDW	.2918	-.0192	-.0944	-.0437
IOD	.2059	.4887	.1773	.0518
IND	.1566	.5848	-.1730	.0853
OND	.1620	.4330	.3240	-.1802
TD	.2004	.0270	-.2906	.0706
JL	.2790	-.1508	.0604	-.2495
RUL	.2683	-.2702	.0878	.1096
HML	.2090	.1682	.0197	-.0820
TBL	.1935	-.0668	.2590	.2720
FL3	.1528	.0143	-.0948	.8462
FL	.2713	-.1565	-.0291	.0865
TFL	.2996	-.1730	.0289	-.0476
HFL	.2968	-.1209	.0186	-.0084
Eigenvalues	9.076	1.382	0.988	0.907
% Variance	53.4	8.1	5.8	5.3
Cummulative % Variance	53.4	61.5	67.3	72.6

Table 7. Comparison of individual temperature-adjusted call parameters of allopatric and sympatric populations of H. chrysoscelis. Means, standard errors, and t-test results based on table-wide significance level of $p < .05$ (sequential Bonferroni method) are given. Significant comparisons are noted by asterisks. Sample sizes are as follows: allopatry N = 50, sympatry N = 56. Character codes are given in Table 1.

	Allopatry		Sympatry		
Character	Mean	SE	Mean	SE	Results
DUR	0.55	0.02	0.51	0.02	-
PRR	55.27	0.90	57.97	0.79	-
NUMP	31.91	1.30	29.20	0.84	-
FF	1257.08	10.41	1326.72	12.91	*
RF1	2400.31	15.91	2496.25	21.49	*
RF2	3575.57	28.02	3750.99	33.42	*
VR1	0.52	0.01	0.53	0.01	-
VR2	0.35	0.01	0.35	0.01	-
VR12	0.67	0.01	0.67	0.01	-

MANOVA (Hotelling - Lawley Trace, $p < .0001$)

Table 8. Character loadings of temperature-adjusted call parameters of H. chrysoscelis from allopatry and sympatry with H. versicolor. Character codes are given in Table 1.

Character	PC1	PC2	PC3	PC4	PC5
DUR	-.0079	.5720	-.0265	.3785	-.2262
PRR	.1643	-.2399	-.0484	.3002	.8515
NUMP	.0526	.5243	-.0611	.5078	.1942
FF	.6074	.0269	-.0577	-.0740	-.0854
RF1	.4318	-.3348	-.0474	.3851	-.3084
RF2	.3193	-.1346	-.5809	.0811	-.1555
VR1	.3794	.3800	-.0241	-.4881	.1987
VR2	.3996	.1569	.5022	-.1578	.0503
VR12	.0958	-.2009	.6303	.2922	-.1322
Eigenvalues	3.300	1.868	1.581	1.229	0.976
% Variance	29.5	24.8	21.6	12.0	10.5
Cummulative % Variance	29.5	54.3	75.9	87.9	98.4

Table 9. Comparison of individual morphological characters of allopatric and sympatric populations of H. chrysoscelis. Means, standard errors, and t-test results based on table-wide significance of $p < .05$ (sequential Bonferroni method) are given. Significant comparisons are noted by asterisks. Sample sizes are as follows: allopatric N = 44, sympatric N = 36. Character codes are given in Table 1.

	Allopatry		Sympatry		
Character	Mean	SE	Mean	SE	Results
SVL	40.40	0.33	40.01	0.55	-
SUL	22.50	0.29	21.85	0.35	-
UL	15.38	0.24	15.71	0.39	-
HDL	11.68	0.09	11.72	0.15	-
HDW	13.97	0.15	13.86	0.18	-
IOD	6.79	0.12	6.50	0.17	-
IND	2.88	0.09	2.71	0.08	-
OND	2.96	0.07	3.00	0.07	-
TD	2.603	0.047	2.540	0.07	-
JL	11.75	0.13	11.83	0.17	-
RUL	8.00	0.08	7.98	0.13	-
HML	11.05	0.18	11.05	0.24	-
TBL	4.87	0.10	4.82	0.18	-
FL3	5.86	0.13	5.41	0.21	-
FL	20.02	0.22	19.64	0.30	-
TFL	18.92	0.18	18.64	0.28	-
HFL	26.70	0.23	24.36	1.30	-

MANOVA (Hotelling - Lawley Trace. $p < .6944$)

Table 10. Results of canonical correlation analysis. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method).

Canonical variates	Canonical Correlation	Canonical R^2	Statistical tests# F	df	P
1	0.77	0.59	1.8	153	.0001*
2	0.67	0.45	1.4	128	.0051*
3	0.59	0.34	1.2	105	.1485
4	0.50	0.25	1.0	84	.5124
5	0.47	0.22	0.9	65	.7394
6	0.43	0.18	0.7	48	.9152
7	0.32	0.10	0.5	33	.9897
8	0.23	0.05	0.4	20	.9939
9	0.18	0.03	0.3	9	.9711

#Tests of the null hypothesis that the correlation in the current row and all that follow are zero.

Table 11. Canonical correlation analysis of morphology and call datasets for H. chrysoscelis and H. versicolor. The correlation of each character with each canonical variate is presented.

	Morphological Variates									Call Variates								
	M1	M2	M3	M4	M5	M6	M7	M8	M9	C1	C2	C3	C4	C5	C6	C7	C8	C9
SVL	.4545	.6120	.6182	.6544	.6657	.6719	.7027	.7049	.7142	.2683	.3393	.3415	.3506	.3531	.3542	.3574	.3575	.3578
UL	.1976	.2502	.3404	.3512	.4982	.5596	.5596	.5800	.6397	.1167	.1404	.1715	.1742	.2066	.2182	.2182	.2192	.2211
SUL	.3785	.4703	.5176	.5403	.5452	.5872	.6100	.6134	.6488	.2236	.2650	.2813	.2870	.2881	.2959	.2982	.2984	.2995
HDL	.2524	.4543	.5119	.5490	.5739	.5756	.6490	.6520	.6523	.1491	.2409	.2599	.2693	.2748	.2751	.2826	.2826	.2826
HDW	.4136	.5920	.6244	.7135	.7548	.7661	.7923	.8051	.8073	.2443	.3248	.3360	.3584	.3676	.3696	.3723	.3730	.3731
IOD	.0878	.7120	.7504	.7561	.7812	.7842	.8121	.8126	.8472	.0519	.3335	.3467	.3481	.3537	.3543	.3571	.3571	.3582
IND	.0128	.3015	.5033	.5182	.5183	.5214	.5727	.5970	.6057	.0076	.1377	.2072	.2110	.2110	.2116	.2168	.2181	.2183
OND	.0004	.3622	.3774	.3778	.3828	.3885	.4182	.4185	.4584	.0002	.1631	.1684	.1685	.1695	.1706	.1736	.1736	.1749
TD	.2110	.3063	.3064	.3474	.3478	.3504	.3761	.3779	.6008	.1247	.1676	.1676	.1780	.1781	.1786	.1812	.1813	.1882
JL	.3736	.5947	.6898	.6951	.7024	.7374	.8227	.8527	.8548	.2207	.3204	.3532	.3545	.3561	.3626	.3713	.3729	.3729
RUL	.6197	.6582	.7060	.7165	.7170	.7766	.7768	.8244	.8443	.3661	.3835	.4000	.4026	.4028	.4138	.4138	.4163	.4170
HML	.0278	.1956	.2350	.2370	.3078	.3135	.3617	.4168	.4179	.0164	.0921	.1057	.1062	.1219	.1229	.1278	.1308	.1308
TBL	.1141	.1493	.2107	.4670	.4919	.5069	.5070	.7430	.7433	.0674	.0832	.1044	.1690	.1690	.1718	.1718	.1844	.1844
FL3	.1568	.1718	.3188	.3486	.5602	.5655	.6473	.7054	.7059	.0926	.0994	.1500	.2079	.2105	.2115	.2198	.2229	.2229
FL	.3472	.4532	.5508	.6525	.6561	.6737	.6812	.6815	.7204	.2051	.2529	.2865	.3121	.3130	.3162	.3170	.3170	.3182
TFL	.3835	.5708	.6798	.7004	.7004	.7110	.7315	.7352	.7388	.2266	.3110	.3486	.3538	.3538	.3557	.3578	.3580	.3581
HFL	.3161	.5659	.6593	.7452	.7577	.7720	.7909	.7975	.8152	.1867	.2994	.3316	.3532	.3560	.3586	.3605	.3609	.3614
DUR	.0442	.1706	.1889	.1889	.2072	.2101	.2168	.2281	.2349	.0748	.3552	.4083	.4096	.4908	.5062	.5723	.7848	1.0000
PRR	.1216	.3803	.3961	.4124	.4220	.4227	.4247	.4257	.4257	.2058	.7792	.8165	.8933	.9364	.9399	.9595	.9792	1.0000
NUMP	.0335	.1217	.1287	.1514	.2186	.2205	.2350	.2395	.2425	.0566	.2522	.2726	.3628	.6658	.6758	.8182	.9036	0.9999
FF	.2786	.3138	.3538	.3876	.3876	.3910	.4042	.4065	.4067	.4716	.5496	.6600	.8000	.8000	.8186	.9478	.9912	1.0000
RF1	.5358	.5368	.5368	.5387	.5416	.5516	.5531	.5531	.5532	.9068	.9090	.9091	.9166	.9297	.9838	.9986	.9986	1.0000
RF2	.2210	.2284	.2326	.2327	.2377	.2626	.2714	.2768	.2847	.3742	.3906	.4028	.4032	.4259	.5602	.6473	.7483	1.0000
VR1	.0690	.1100	.1813	.2123	.2166	.2216	.2556	.2583	.2592	.1167	.2078	.4146	.5377	.5569	.5839	.9188	.9695	1.0000
VR2	.0007	.0089	.0818	.1247	.1266	.1385	.1388	.1539	.1614	.0012	.0195	.2312	.4013	.4102	.4740	.4768	.7617	1.0000
VR12	.0636	.0699	.0882	.0891	.0898	.0981	.1155	.1233	.1376	.1075	.1216	.1747	.1782	.1818	.2262	.3979	.5457	1.0000
	Variance in morphological characters extracted																	
Proportion	.2557	.1808	.0694	.0571	.0230	.0177	.0324	.0296	.0292	.1511	.0815	.0239	.0144	.0051	.0033	.0033	.0016	.0009
Cumulative	.2557	.4365	.5059	.5630	.5859	.6036	.6360	.6656	.6948	.1511	.2325	.2565	.2709	.2760	.2792	.2825	.2841	.2850
	Variance in call characters extracted																	
Proportion	.1520	.0636	.0272	.0170	.0122	.0076	.0109	.0056	.0046	.2572	.1411	.0790	.0674	.0550	.0413	.1076	.1048	.1466
Cumulative	.1520	.2156	.2428	.2598	.2720	.2796	.2906	.2961	.3007	.2572	.3983	.4773	.5447	.5997	.6410	.6410	.7486	1.0000

Table 12. Redundancy analysis of call and morphology data for H. chrysoscelis and H. versicolor.

	Morphological Variates									Call Variates								
	M1	M2	M3	M4	M5	M6	M7	M8	M9	C1	C2	C3	C4	C5	C6	C7	C8	C9
SVL	-.6742	.3968	-.0789	.1903	-.1061	.0786	.1757	-.0470	-.0963	-.5182	.2665	-.0463	.0955	-.0499	.0338	.0560	-.0108	-.0170
UL	-.4445	.2293	-.3005	-.1034	-.3835	.2478	.0007	.1429	.2442	-.3416	.1540	-.1764	-.0519	-.1805	.1066	.0002	.0330	.0432
SUL	-.6152	.3031	-.2174	.1506	-.0702	-.2050	.1510	.0584	-.1879	-.4729	.2035	-.1276	.0756	-.0331	-.0882	.0481	.0135	-.0333
HDL	-.5024	.4493	-.2401	.1927	-.1576	-.0408	.2710	-.0055	-.0159	-.3861	.3017	-.1409	.0967	-.0742	-.0175	.0864	-.0013	-.0028
HDW	-.6431	.4224	-.1799	.2986	.2033	.1062	.1619	-.1129	.0467	-.4943	.2837	-.1056	.1499	.0957	.0457	.0516	-.0261	.0083
IOD	-.2964	.7900	.1960	.0757	-.1585	.0546	-.1670	-.0231	-.1861	-.2279	.5306	.1150	.0380	-.0746	.0235	-.0532	-.0053	-.0329
IND	-.1132	.5373	.4492	.1223	.0062	.0554	-.2266	.1558	.0934	-.0870	.3608	.2636	.0614	.0029	.0238	-.0722	.0359	.0165
OND	.0208	.6015	-.1232	.0207	-.0667	.0790	-.1721	.0185	.1998	.0160	.4039	-.0723	.0104	-.0314	.0341	-.0548	.0043	.0354
TD	-.4594	.3087	.0035	.2025	.0223	.0503	.1603	-.0430	.4721	-.3531	.2073	.0020	.1016	.0105	.0216	.0511	-.0099	.0836
JL	-.6112	.4703	-.3083	.0730	.0855	.1870	.2920	.1732	.0458	-.4698	.3158	-.1809	-.0366	.0402	.0805	.0931	.0400	.0081
RUL	-.7872	.1964	-.2186	.1022	.0233	-.2441	-.0122	.2183	.1410	-.6051	.1319	-.1283	.0513	.0110	-.1050	-.0039	.0504	.0250
HML	-.1667	.4097	-.1983	.0454	.2661	-.0752	.2196	.2347	-.0332	-.1282	.2751	-.1164	.0228	.1253	-.0323	.0700	.0542	-.0059
TBL	-.3378	.1875	-.2478	.5063	-.1578	.1226	-.0097	.4858	.0155	-.2596	.1259	-.1454	.2541	-.0743	.0528	-.0031	.1121	.0027
FL3	-.3960	.1224	.3834	.4794	.1074	-.0730	.2861	.2410	-.0224	-.3044	.0822	.2250	.2406	.0506	-.0314	.0912	.0556	-.0040
FL	-.5892	.3256	-.3125	.3188	.0606	.1324	-.0868	-.0169	-.1972	-.4529	.2186	-.1834	.1600	.0285	.0570	-.0277	-.0039	-.0349
TFL	-.6193	.4327	-.3302	.1437	-.0007	-.1026	.1433	.0612	.0592	-.4760	.2906	-.1938	-.1938	.0721	-.0003	-.0442	.0457	.0105
HFL	-.5622	.4998	-.3056	.2931	-.1120	-.1196	.1375	.0813	.1331	-.4321	.3357	-.1793	.1471	-.0527	-.0515	.0438	.0188	.0236
DUR	-.2102	-.3556	-.1353	.0180	-.1341	-.0535	.0819	-.1064	.0821	-.2735	-.5295	-.2306	.0359	-.2849	-.1243	.2570	-.4610	.4639
PRR	.3487	.5086	.1133	-.1391	-.0978	.0256	.0446	.0324	-.0255	.4536	.7573	.1931	-.2770	-.2077	.0594	.1400	.1404	-.1440
NUMP	.1830	.2970	.0839	-.1507	-.2592	.0430	.1203	-.0674	.0549	.2380	.4422	.1429	-.3003	-.5505	.0999	.3774	-.2922	.3103
FF	.5278	-.1876	.2002	-.1837	.0004	.0587	.1146	.0480	-.0166	.6867	-.2794	.3412	-.3660	.0008	.1364	.3595	.2083	-.0939
RF1	.7320	-.0307	.0074	-.0434	.0540	.1001	-.0388	.0012	.0065	.9523	-.0457	.0125	-.0865	.1146	.2326	-.1216	.0053	.0367
RF2	.4701	-.0860	-.0650	.0094	.0709	.1577	.0941	-.0733	-.0888	.6117	-.1280	-.1108	.0187	.1506	.3665	.2952	-.3178	-.5017
VR1	-.2626	-.2027	.2669	-.1761	-.0654	-.0707	.1844	.0519	-.0310	-.3416	-.3018	.4548	-.3508	-.1388	-.1643	.5787	.2251	-.1749
VR2	.0263	-.0908	.2700	-.2070	-.0444	-.1087	.0167	.1231	.0864	.0343	-.1353	.4601	-.4125	-.0942	-.2526	.0525	.5338	.4883
VR12	.2521	.0797	.1352	-.0296	.0282	-.0907	-.1321	.0881	.1196	.3279	.1187	.2304	-.0589	.0600	-.2109	-.4143	.3819	.6754

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significant ($P < .0001$).

FIG. 6. Plot of PC2 versus PC1 of 19 log transformed morphological characters. Symbols as in FIG. 3

FIG. 7. Histogram of canonical scores generated by discriminant analysis of 19 log transformed morphological characters. The Mahalanobis distance between group centroids was significant ($P < .0208$).

FIG. 8. Plot of PC2 versus PC1 for call parameters of allopatric and sympatric populations of H. chrysoscelis relative to H. versicolor. Solid circles indicate individuals from sympatry, open circles indicate individuals from allopatry.

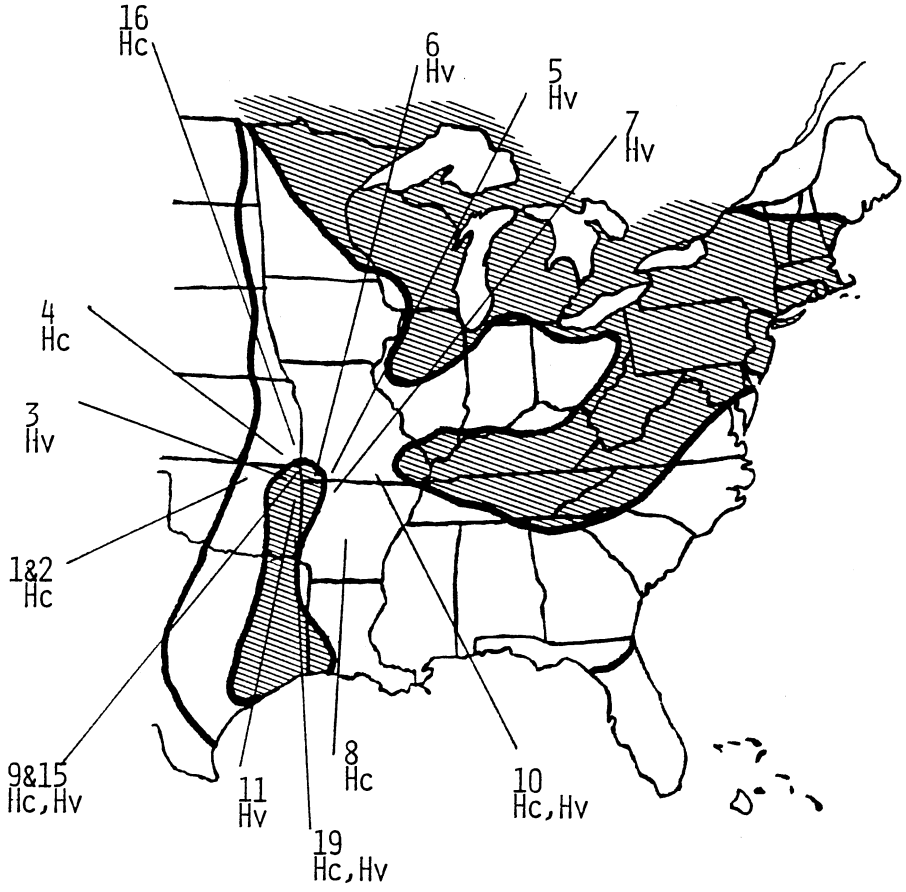
FIG. 9. Histogram of canonical scores generated from discriminant analysis of call parameters of allopatric and sympatric populations of H. chrysoscelis relative to H. versicolor. The Mahalanobis distance between group centroids was significant ($P < .0001$).

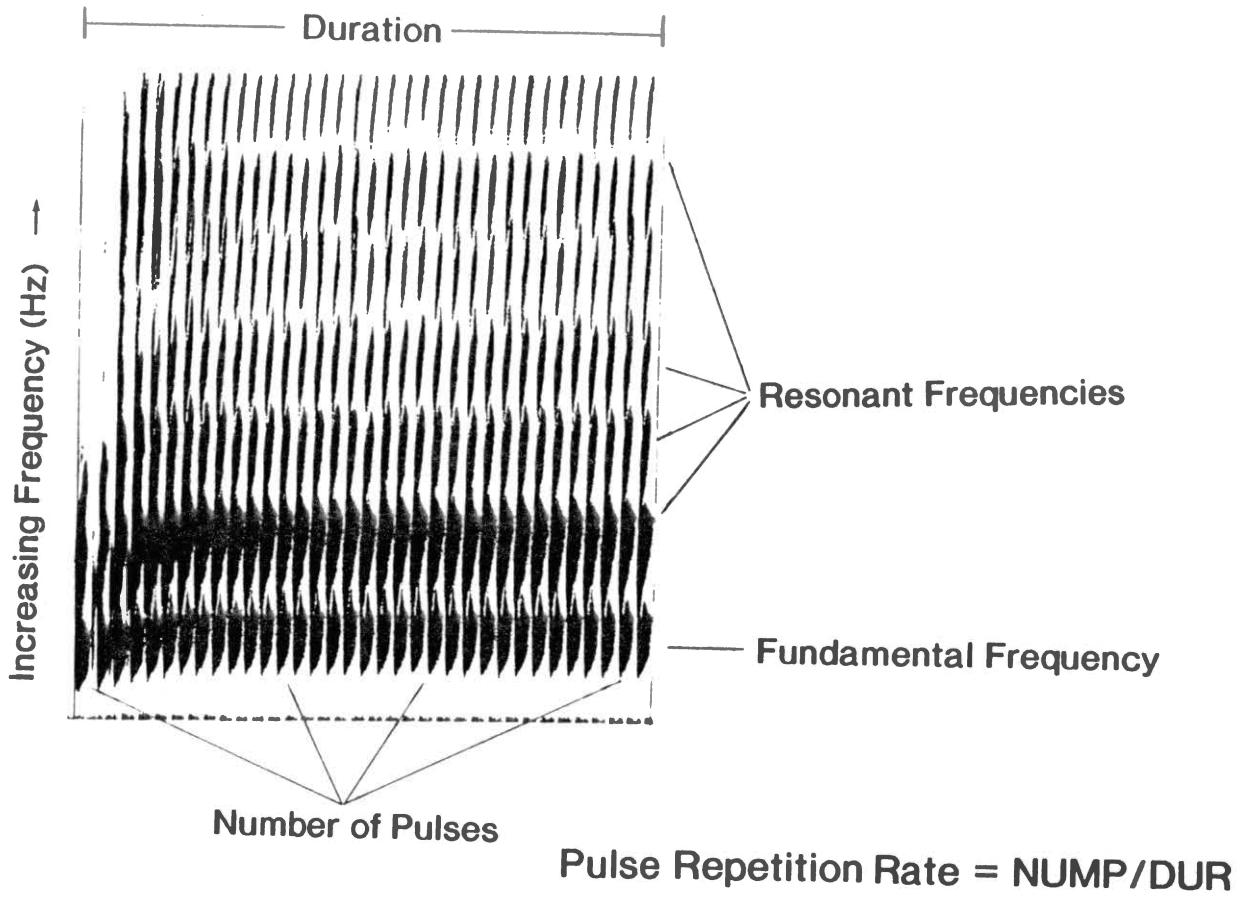
FIG. 10. Plot of PC3 versus PC1 of morphological characters of allopatric and sympatric populations of H. chrysoscelis relative to H. versicolor. The Mahalanobis

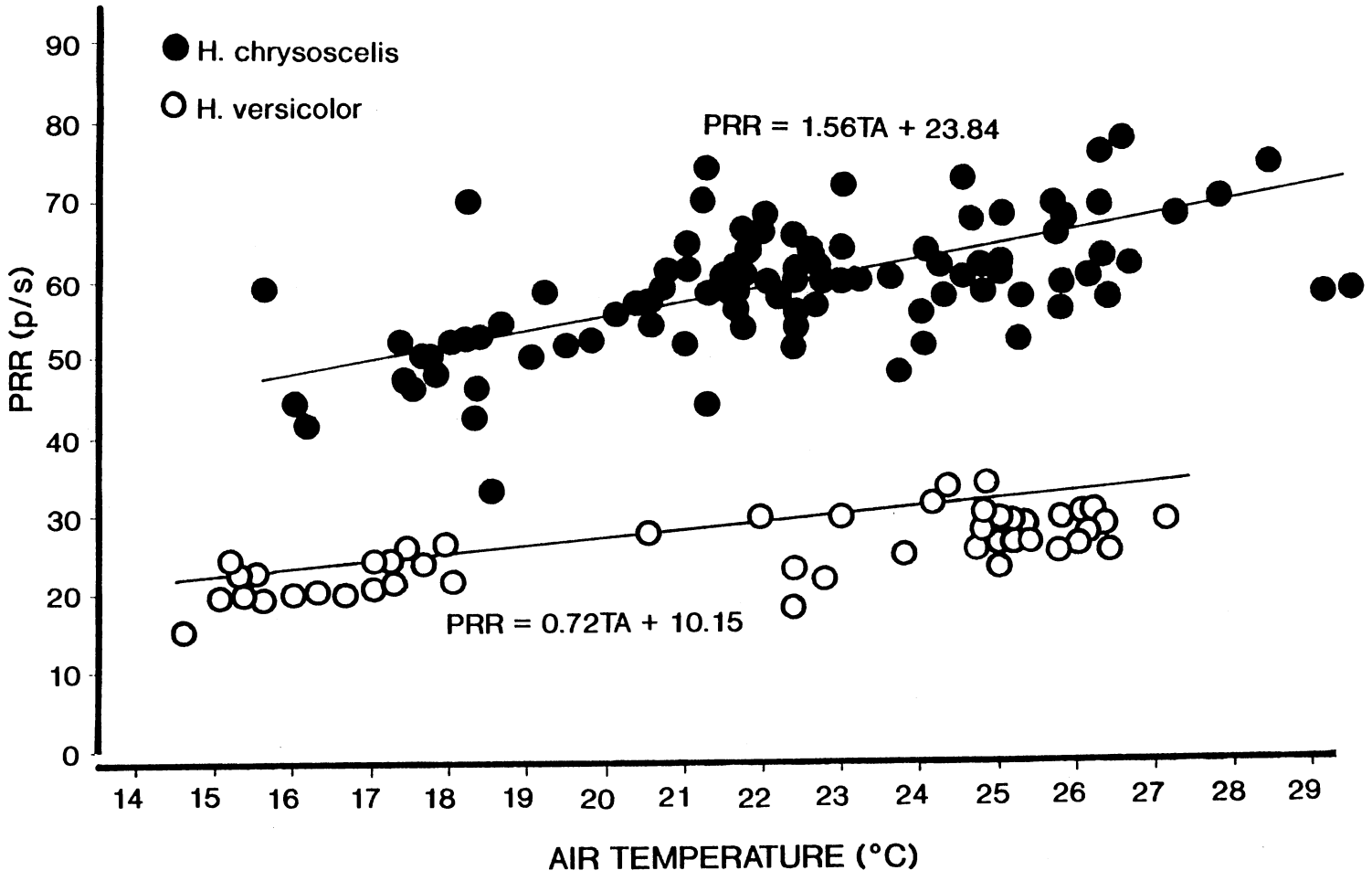
distance between group centroids was not significant ($P < .8794$). Symbols as in FIG. 8.

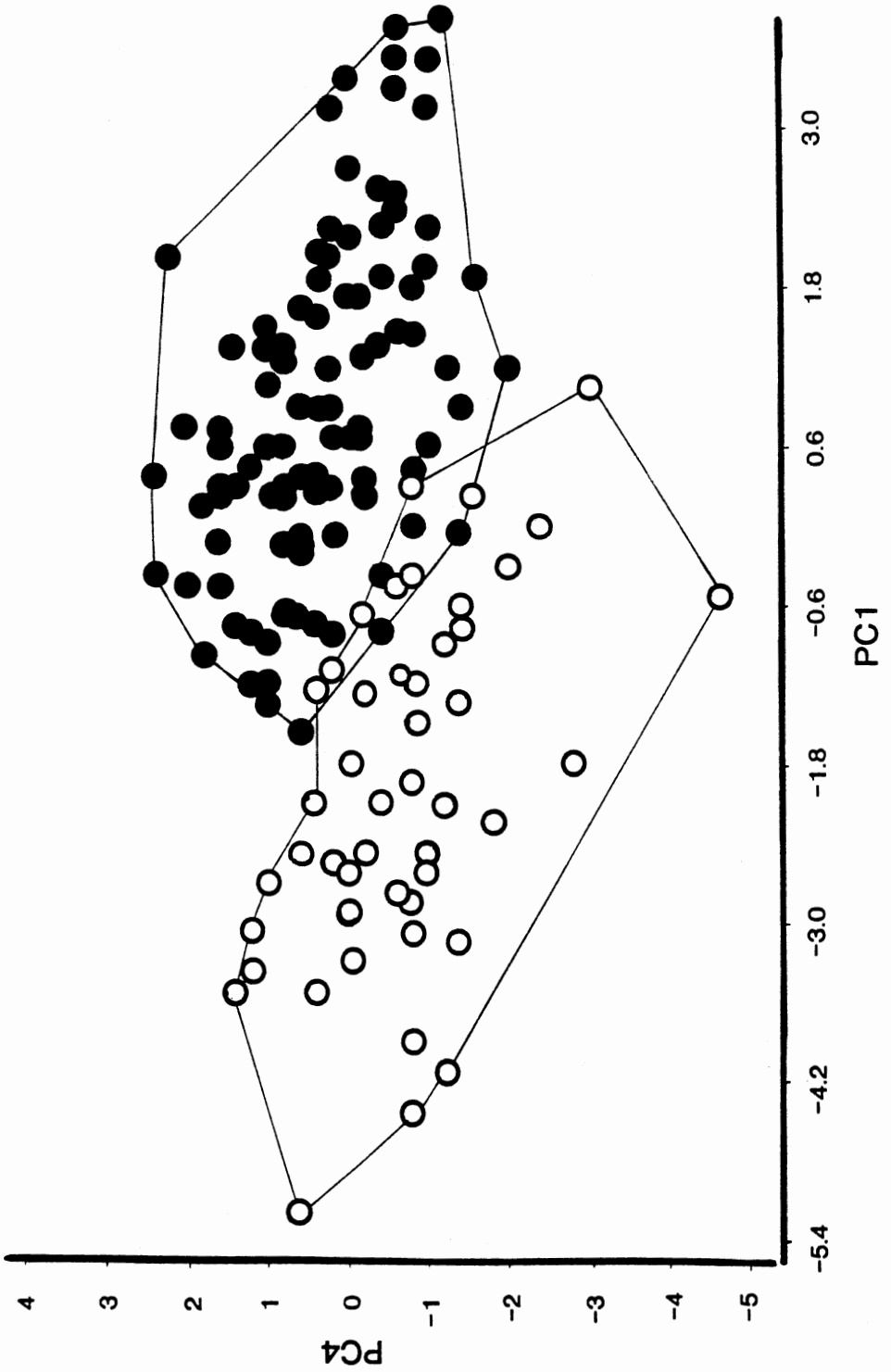
FIG. 11. Plot of morphological and call components of canonical variate 1 of H. chrysoscelis and H. versicolor. Symbols as in FIG. 3.

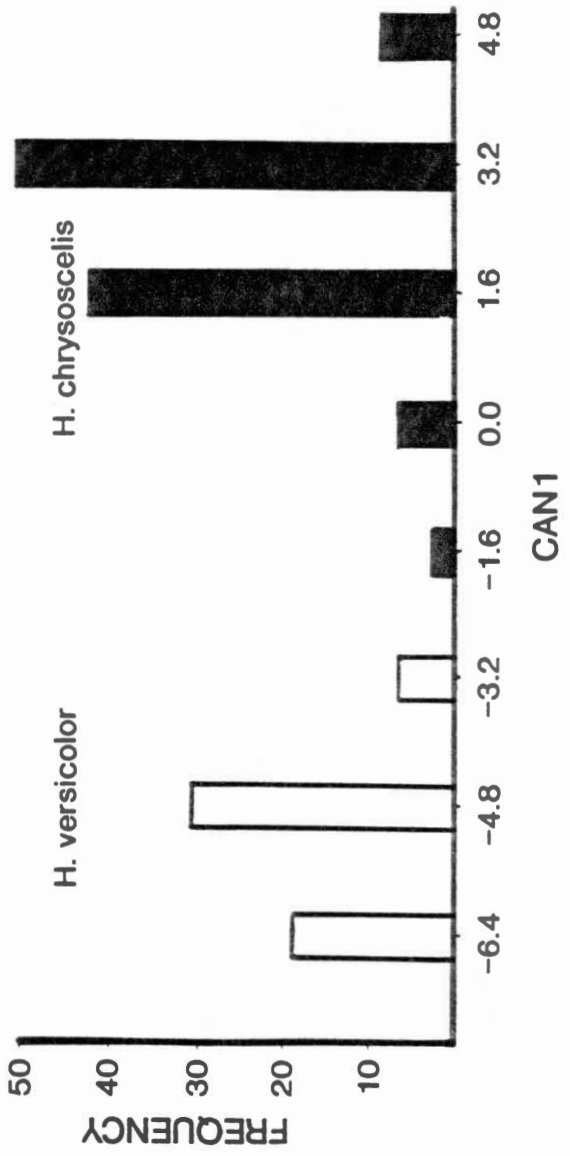
FIG. 12. Plot of morphological and call components of canonical variate 2 of H. chrysoscelis and H. versicolor. Symbols as in FIG. 3.

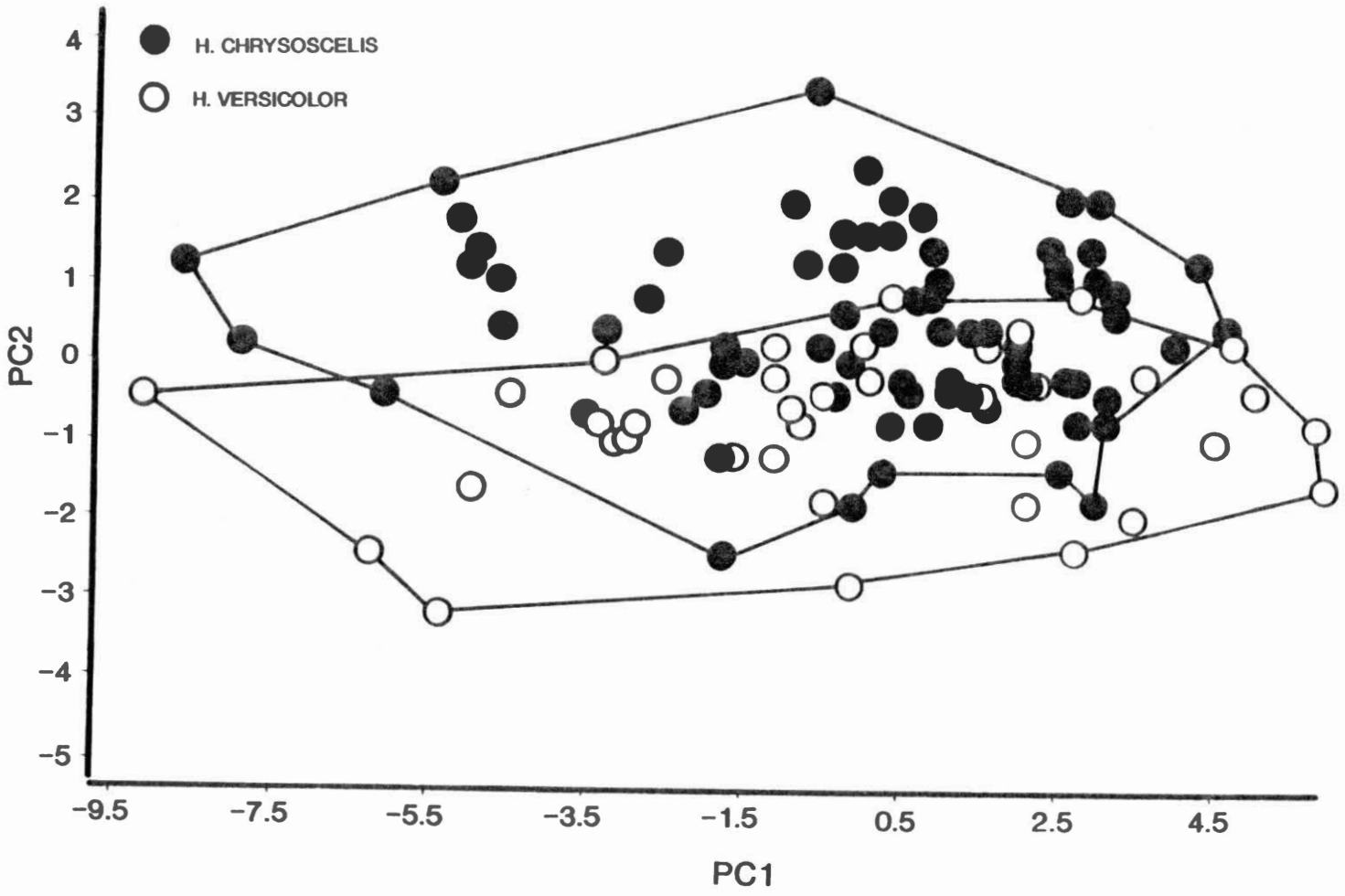

16
Hc
6
Hv
5
Hv
7
Hv
4
Hc
3
Hv
1&2
Hc
9&15
Hc,Hv
11
Hv
19
Hc,Hv
8
Hc
10
Hc,Hv

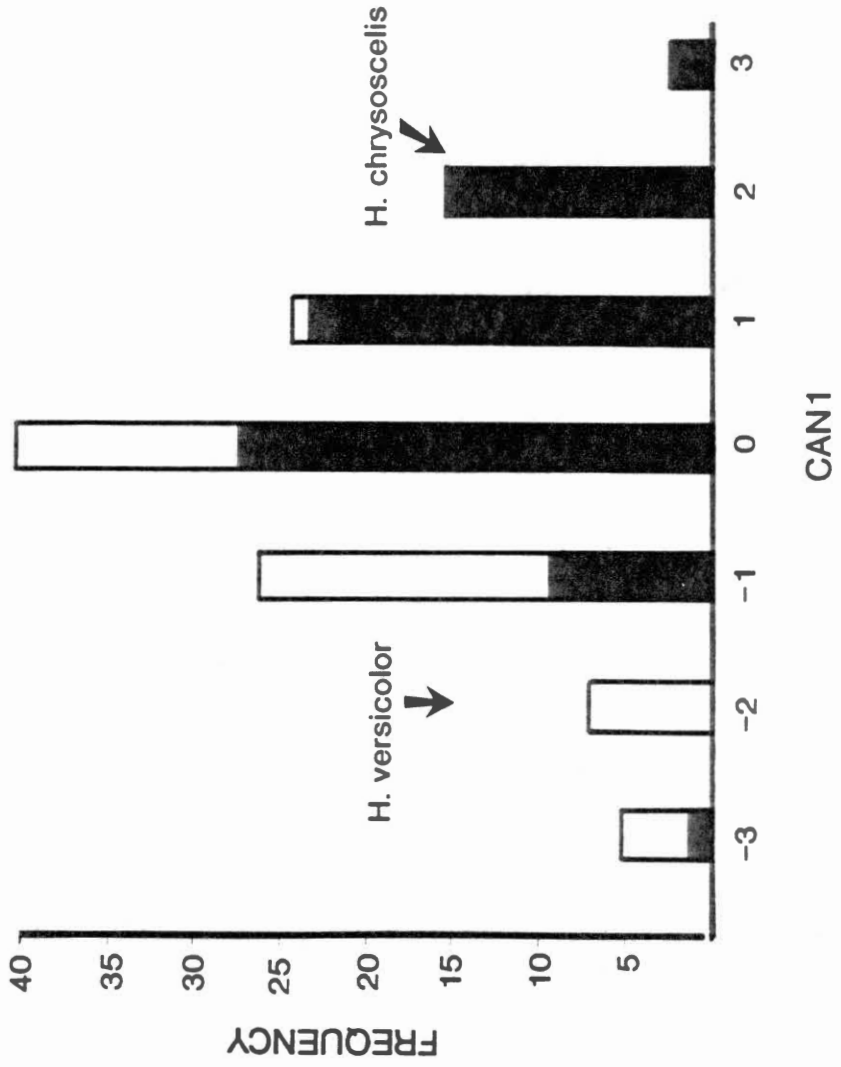

Duration
Increasing Frequency (Hz) →
Resonant Frequencies
Fundamental Frequency
Number of Pulses
Pulse Repetition Rate = NUMP/DUR

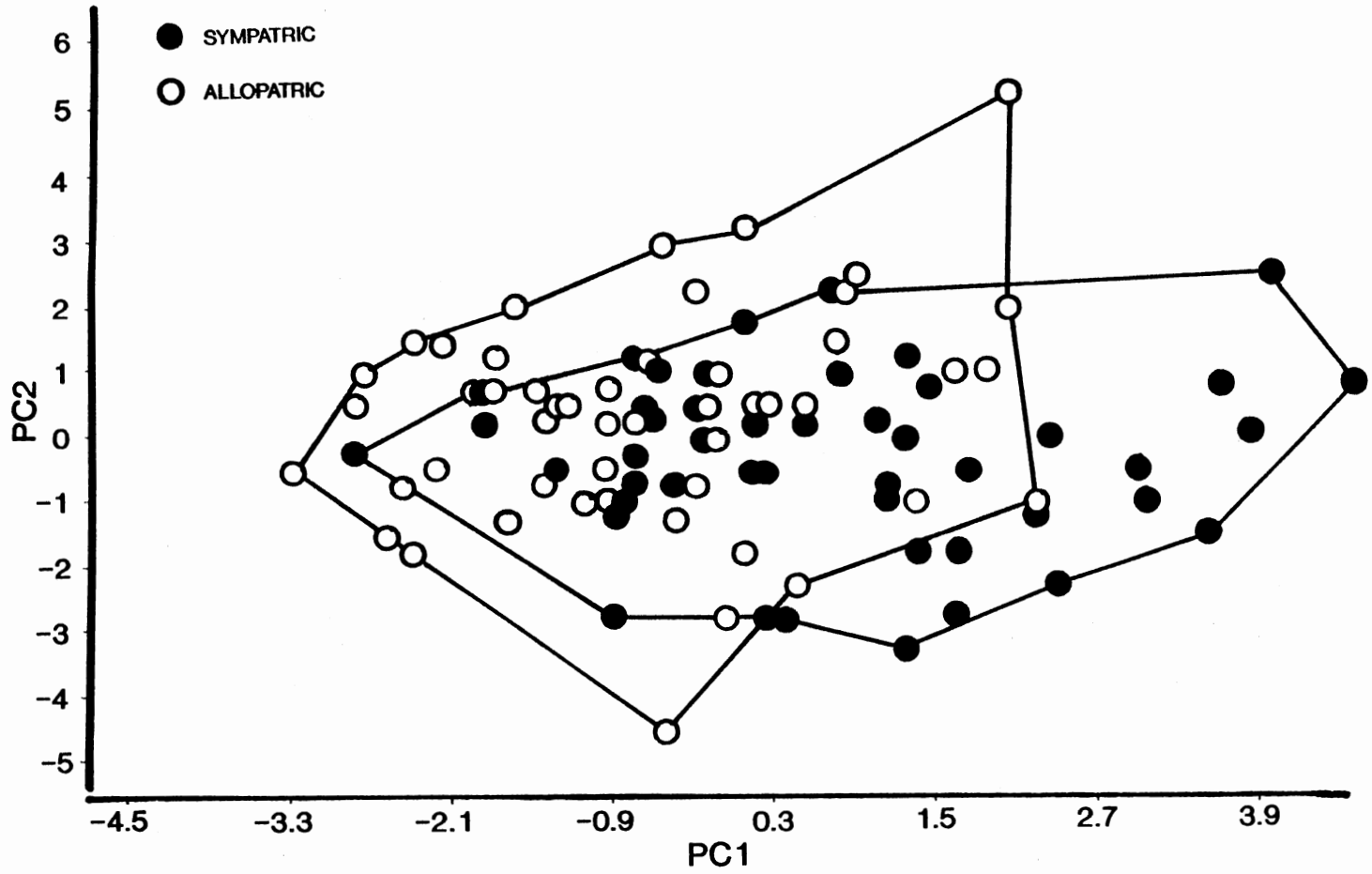

H. chrysoscelis
H. versicolor
PRR = 1.56TA + 23.84
PRR = 0.72TA + 10.15
PRR (p/s)
AIR TEMPERATURE (°C)
0
10
20
30
40
50
60
70
80
90
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29

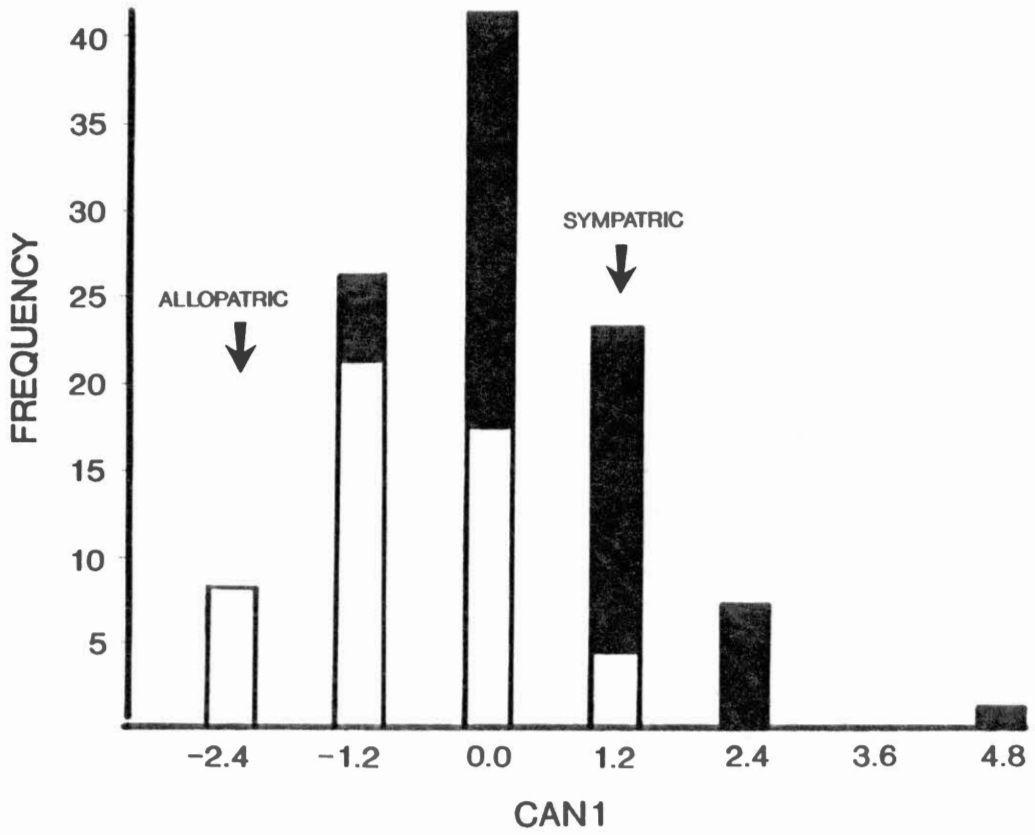

PC4
4
3
2
1
0
-1
-2
-3
-4
-5
PC1
-5.4
-4.2
-3.0
-1.8
-0.6
0.6
1.8
3.0

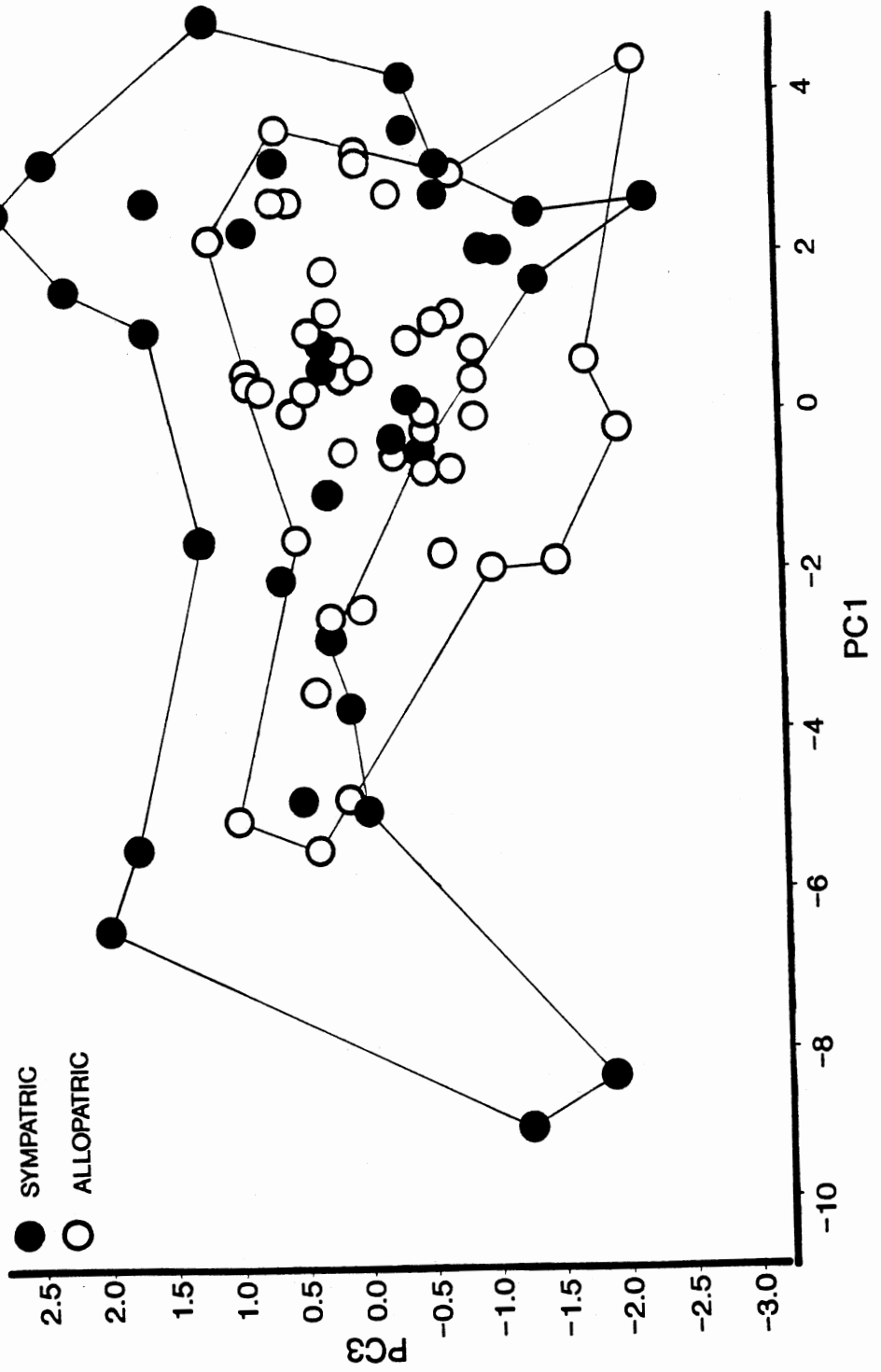

H. versicolor
H. chrysoscelis
FREQUENCY
50
40
30
20
10
-6.4
-4.8
-3.2
-1.6
0.0
1.6
3.2
4.8
CAN1

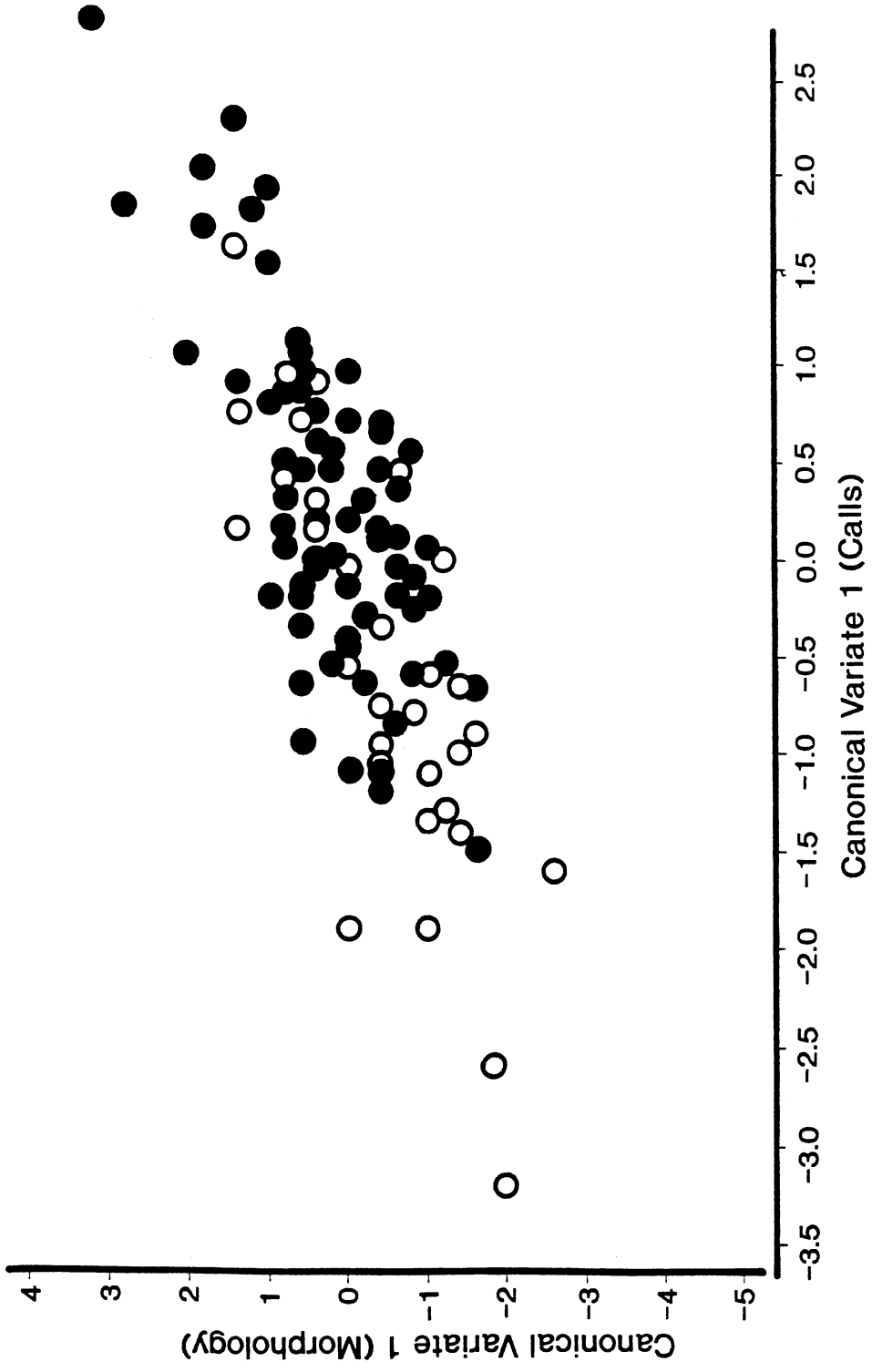

H. CHRYSOSCELIS
H. VERSICOLOR
PC2
PC1
4
3
2
1
0
−1
−2
−3
−4
−5
−9.5
−7.5
−5.5
−3.5
−1.5
0.5
2.5
4.5

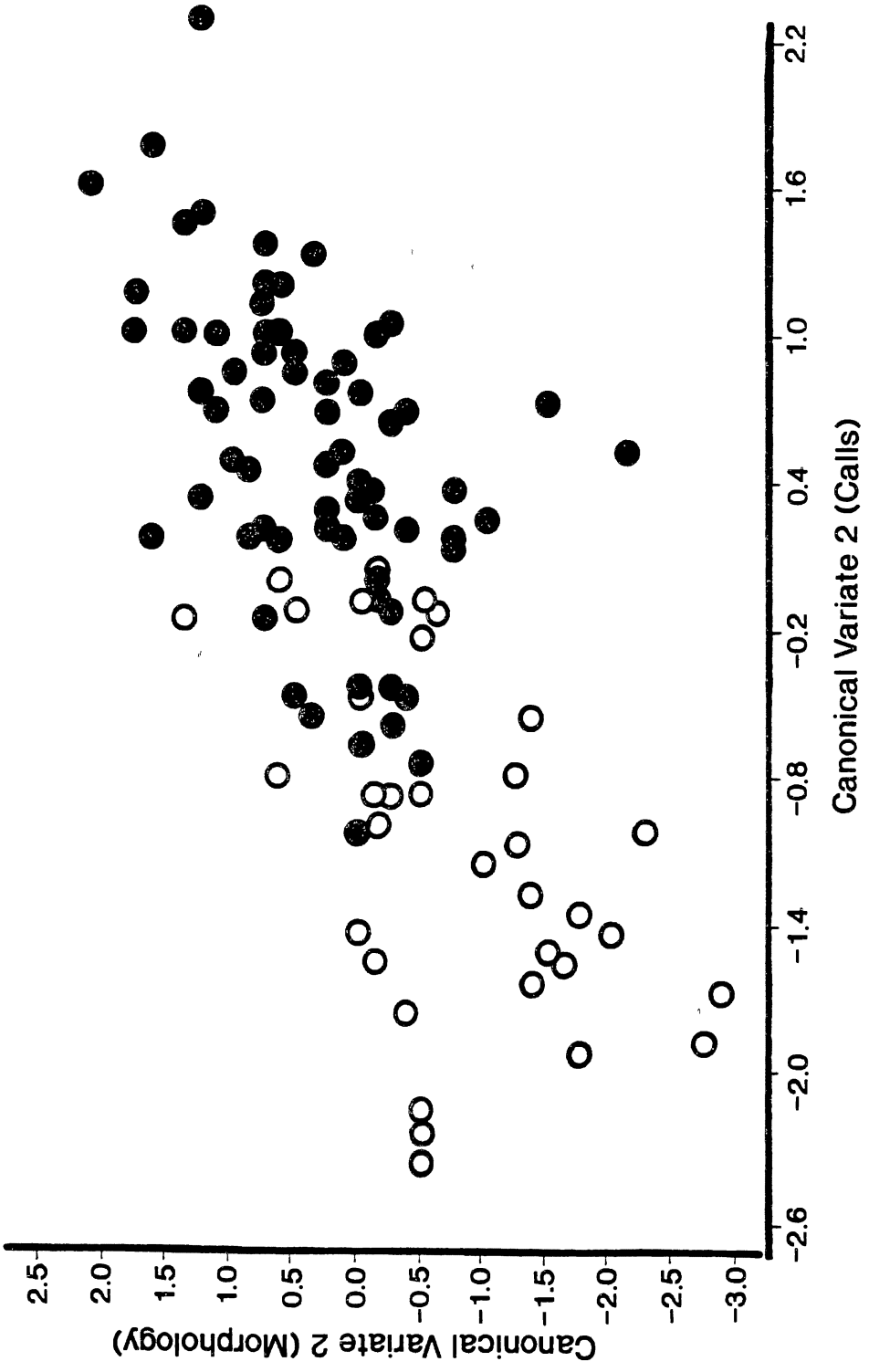

H. chrysoscelis
H. versicolor
FREQUENCY
40
35
30
25
20
15
10
5
−3
−2
−1
0
1
2
3
CAN1


SYMPATRIC
ALLOPATRIC
PC2
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-4.5
-3.3
-2.1
-0.9
0.3
1.5
2.7
3.9
PC1


FREQUENCY
40
35
30
25
20
15
10
5
ALLOPATRIC
SYMPATRIC
-2.4
-1.2
0.0
1.2
2.4
3.6
4.8
CAN1


SYMPATRIC
ALLOPATRIC
PC3
PC1
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-10
-8
-6
-4
-2
0
2
4


Canonical Variate 1 (Morphology)
4
3
2
1
0
-1
-2
-3
-4
-5
Canonical Variate 1 (Calls)
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5


Canonical Variate 2 (Morphology)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Canonical Variate 2 (Calls)
-2.6
-2.0
-1.4
-0.8
-0.2
0.4
1.0
1.6
2.2

CHAPTER III

INTERPOPULATIONAL VARIATION IN CALLS, MORPHOLOGY, AND GENETICS IN COPE'S GRAY TREEFROG (HYLA CHRYSOSCELIS), WITH COMMENTS ON CHARACTER DIVERGENCE

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Abstract: Geographic variation in calls, morphology, and genetics were examined in six populations of the diploid gray treefrog, Hyla chrysoscelis. Significant call differences were observed between sites for 7 of 9 call parameters. Discriminant functions analysis partially separated eastern and western populations. Morphological differences were observed between populations for 4 of 17 external measurements. Again, discriminant functions analysis separated eastern and western populations. Clinal variation in allelic frequencies was observed at 4 of 6 electrophoretic loci. Heterozygotes were rare for 'eastern' and 'western' alleles of LDH-B (sensu Ralin and Selander 1979). Genetic, morphological, and acoustical analyses

indicate the presence of two forms of H. chrysoscelis. Results of tests of five models of gene flow among populations suggest that Hyla versicolor acts as a barrier to direct gene flow between eastern and western gray treefrogs. The taxonomic status of eastern and western forms of H. chrysoscelis is discussed in light of these results.

Geographically structured variation is a common feature among anuran species with wide distributions. A number of these studies have concentrated on call variation (Snyder and Jameson 1965; Capranica et al.1973; Narins and Smith 1987; Sullivan 1989). In some cases, the source of some of the variation has been identified. Ramer et al. (1983) demonstrated an inverse relationship between call frequency and snout - vent length (SVL). Changes in body size (SVL) were accompanied by changes in dominant frequency among Eleuthrodactylus coqui populations along an altitudinal gradient (Narins and Smith 1986). The authors demonstrated that male coquis were less responsive to calls from the opposite ends of the cline than to calls from within their population, and they suggested that calls from the endpoints of the cline are sufficiently different that gene flow between these populations would be restricted. Capranica et al. (1973) demonstrated that geographic changes in dominant

frequency of cricket frog calls (Acris gryllus) are accompanied by concomitant changes in female sensitivity to sound frequencies. Temperature-dependent call variation is well known among anurans (Bellis 1957; Zweifel 1959, 1970; Ralin 1968, 1977; Gayou 1984; Sullivan 1989 and others), and female preference is coupled to temperature change (Gerhardt 1978). Numerous authors (Duellman 1968; Hödl 1977; Duellman and Pyles 1983 and others) have demonstrated channelization of call frequencies in complex anuran communities, while Drewry and Rand (1983) add that specific channels vary depending upon the community composition.

Members of the gray treefrog complex are among the most studied frogs in the world. Ralin (1977) reported call differences among eastern and western populations of the diploid (Hyla chrysoscelis), in addition to call differences between the diploid and the tetraploid (Hyla versicolor). Ralin and Selander (1979) reported clinal variation in electrophoretically detectable allelic frequencies, and fixed differences at the lactate dehydrogenase-B (LDH-B) locus for eastern and western populations of the diploid. Heterozygotes between eastern and western forms appear quite rare. Ralin et al. (1983) reported a single heterozygote from Illinois. Eastern and western diploid populations were fixed for different immunoalleles at nine localities, but two heterozygotes were collected in extreme eastern Texas (Maxson et al. 1977; Ralin 1978). Finally, morphological divergence between eastern and western H. chrysoscelis

populations was demonstrated by Ralin and Rogers (1979). Evidence for divergence of eastern and western diploids has led to considerable controversy over the taxonomic status of H. chrysoscelis (Maxson et al. 1977; Ralin 1978). These questions have remained largely unanswered due to problems of identification in the field and lack of specimens from an area of contact between eastern and western diploids. In this study, call, morphological, and genetic variation are examined in a zone of contact between eastern and western H. chrysoscelis in order to determine if sufficient differences exist to warrant species status for the two forms.

MATERIALS AND METHODS

Data collection - One hundred and fifty-seven treefrogs, (106 H. chrysoscelis and 51 H. versicolor), were collected from twelve sites in Oklahoma, Kansas, Missouri, and Arkansas. Ten calls were recorded for each individual on a Pearlcorder microcassette tape recorder before capture. Frogs were identified in the field by their calls, and a sub-sample of all specimens collected was karyotyped by John E. Wiley of East Carolina University. When the results of both methods were compared, field identifications agreed with karyotypes in all cases (N = 44). Frogs identified as H. chrysoscelis were collected at six sites (Fig. 1). Captured individuals were assigned a code number and frozen at -60° C for electrophoretic and morphometric analyses. Air temperature at the perch site was measured to the nearest 0.1° C using a Miller & Weber rapid register

thermometer. Call Duration (DUR; measured in seconds), Number of Pulses in the call (NUMP), and Pulse Repetition Rate (PRR; measured in pulses/second) were calculated from audiospectrograms produced by a Kay 6061B Sound Spectrograph (Fig. 2). A Realtime Analyzer was used to determine the first three frequency peaks based on energy levels; these peaks are labeled the Fundamental Frequency (FF; the lowest frequency) and the first two Resonant Frequencies (RF1 & RF2) and measured in Hertz (Hz). Additional calculations included ratios of the fundamental frequency to each of the resonant frequencies (FF/RF1 = VR1; FF/RF2 = VR2), and the ratio of the two resonant frequencies (RF1/RF2 = VR12); these ratios determine vowel sounds in humans and are fixed across age and sex (Minifie 1973). As such, these ratios may act as discriminators between species or individuals. All call parameters are described in Table 1A.

Sixteen external characters were measured to the nearest 0.01 mm with dial calipers (Table 1B): snout-vent length (SVL), snout-urostyle length (SUL), urostyle length (UL), head length (HDL), head width (HDW), interorbit distance (IOD), internares distance (IND), orbit-naris distance (OND), jaw length (JL), humerus length (HML), radioulna length (RUL), thumb length (TBL), 3rd finger length (FL3), femur length (FL), tibiofibula length (TFL), and hind foot length (HFL). Maximum tympanum diameter (TD) was measured using a digitizer linked to a binocular microscope. All bilateral characters were measured only on the right side.

Samples of heart tissue were removed from each specimen, ground separately in de-ionized water, and stored at -60° C for later use. Tissues were analyzed using horizontal starch gel electrophoresis using Tris-Citrate pH 5.3 and pH 6.0 buffers. Six loci (lactate dehydrogenase B, malate dehydrogenase A&B, mannose-6-phosphate isomerase, phosphoglucose isomerase, phosphoglucomutase) could be read consistently (Table 2). Loci that exhibited frequency differences between eastern and western populations (Ralin and Selander 1979) were deliberately chosen for study. Alleles at each locus were named alphabetically starting with the most anodal allele.

Statistical analyses - All morphological characters and six call characters (DUR, PRR, NUMP, FF, RF1, RF2) were log transformed prior to multivariate analyses to reduce variance due to size and collecting technique. The three frequency ratios (VR1, VR2, VR12) were arcsin transformed prior to analyses. Equality of variances was tested using an F-test, and a t-test using pooled variances and adjusted degrees of freedom was used to test for differences between means with significantly different variances; otherwise, analysis of variance (ANOVA) was performed. Previous studies indicate that temperature exerts a significant effect on some call parameters including PRR and DUR (Bellis 1959; Ralin 1968, 1976; Zweifel 1970; Cline 1990). Call parameters were regressed against temperature and comparisons that produced significant regressions were

adjusted to 21° C as per Ralin (1968) prior to analyses. Temperature was positively correlated with PRR, RF1, and RF2 (Table 3). Due to small size of some samples, the species regression rather than that for each population was used to standardize those call parameters affected by temperature. Multivariate analysis of variance (MANOVA) was used to detect overall differences among groups. Canonical Discriminant Analysis (Discriminant Functions Analysis) was used to interpret patterns in those data significant by MANOVA. Canonical Discriminant Analysis (DFA) assumes a priori the presence of groups within the data, and it uses these within- and between-group correlations to produce linear combinations that best separate groups. Due to the large number of statistical tests performed in this study, the significance level was partitioned to "table-wide" or "character-wide" significance of $P < .05$ using the sequential Bonferroni method (Rice 1989). Genetic data were analyzed using the Biosys-1 computer package (Swofford and Selander 1981). Results of the genetic analyses were used to partition call and morphological variation between presumptive genetic groups.

RESULTS

Comparisons Among Sites

Calls - Means for temperature adjusted call parameters for each site are given in Table 4. Arranging the sites along north-south or east-west (Table 4) axes reveals no clinal trends for any of the call parameters. Significant

differences between pairs of sites were observed for all call parameters except VR2 and VR12 (Table 5). Each site differed from at least one other site for at least one call parameter. Stillwater had a lower PRR than both Independence and Ottawa. Stillwater had a smaller VR1 than Little Rock, and it had lower values than McCurtain for FF, RF2, and VR1. Independence differed from Willow Springs in FF, and it differed from Little Rock in FF and VR1. Independence differed from McCurtain in FF, RF2 and VR1. Ottawa differed from Little Rock in VR1 and from McCurtain in FF. Willow Springs differed from Little Rock in NUMP and DUR, while it differed from McCurtain in NUMP. Little Rock differed from McCurtain in RF1 and RF2.

Few differences were observed between sites for DUR, NUMP, and PRR. Differences in broadcast frequencies involve high FF for McCurtain and Little Rock versus low FF for Independence. McCurtain also has a relatively high RF2. Differences in VR1 involve relatively high ratios at McCurtain and Little Rock versus relatively low ratios for the three western-most sites (S, I, O). MANOVA revealed significant overall differences among calls for the six sites (Hotelling - Lawley Trace, $p < 0.0001$).

Discriminant functions analysis was performed on the data to determine which characters provided the best separation among populations. Mahalanobis distances among group centroids were calculated and F-approximations were made to test for significant call differences. Calls of each site

were different from every other site in all pairwise comparisons ($p < .05$, sequential Bonferroni; Table 6). The first three discriminant functions were significant (Hotelling - Lawley Trace, $p < .0001$). The first discriminant function is weighted most heavily by FF and RF1 (Table 7). Stillwater frogs score low on this axis and are well separated from all sites except Independence (Fig. 3A). Scores for Independence frogs are evenly distributed across the range of scores. Little Rock and Willow Springs populations have modal scores around 1.2, while Ottawa and McCurtain have modes around 2.4. The second discriminant function is weighted by RF1 and RF2. Willow Springs frogs score low on this axis and are separated from Little Rock frogs (Fig. 3B). The remaining populations have distributions centered around zero. Discriminant function three is weighted by the resonant frequency ratios. There is considerable overlap of scores along this axis, but the distributions appear to be different (Fig. 3C). In general, scores of frogs from Stillwater, Willow Springs, Little Rock, and McCurtain are normally distributed, but each is slightly truncated. Scores from both Stillwater and Willow Springs peak around zero and are truncated on the left. Little Rock and McCurtain populations are truncated on the right and have peaks at 0 and 1, respectively. Scores of Ottawa frogs are skewed to the right with the peak at -1. Scores of Independence frogs have a flat distribution along the negative range of scores. In general, DFA indicates that one or a few populations are discriminated along each

axis.

Sound waves produced by vibration of the vocal cords are subject to modification by the vocal tract based on size, shape, and resonating qualities of the surrounding tissue. Thus, some frequencies transmitted by the vocal cords may be filtered out or de-emphasized. Those frequencies that are transmitted to the environment are called resonant frequencies (termed formants in human speech). One quantitative measure of complex sound structure is the ratio of the fundamental frequency to the resonant frequencies and the ratio of the resonant frequencies to each other. The ratio of the fundamental frequency and the first two resonant frequencies are unique for different vowel sounds, and the reciprocals of the ratios reveal harmonic structure. In human speech, resonant frequencies of vowel sounds range from the first through the tenth harmonic (Minifie 1973). In this study the reciprocal of the first two frequency ratios approach 2 and 3, respectively, indicating that the resonant frequencies represent nearly pure first and second harmonics of the fundamental frequency. Thus, little frequency filtering occurs in this species. Subtle differences in complex structure are still important, however, as frogs more easily recognize conspecific calls with complex harmonic structure than pure tone calls (lacking harmonics) and inharmonic calls (Simmons 1988).

Morphology - Means and standard errors of ten head and body measurements (Table 8) and seven limb measurements

(Table 9) are reported. Patterns of variation along north-south or east-west clines were not observed for any character. Paired t-tests revealed significant differences among groups for three head measurements (IOD, IND, OND) and one body measurement (SUL; Table 10). Little Rock and Willow Springs frogs have shorter, narrower snouts relative to frogs from Independence and McCurtain. Independence frogs had longer SUL than McCurtain frogs, and Little Rock frogs also have shorter snouts than those from Independence. MANOVA indicated significant overall morphological differences among populations (Hotelling - Lawley Trace, $p < .0001$), so DFA was employed to look for separation of populations.

Canonical discriminant analysis identified the characters most useful in differentiating populations. Mahalanobis distances in morphological space were calculated among groups and F-approximations were computed (Table 11). Each site was different from at least three other sites, and two sites (Independence and Ottawa) were different from four other sites. The first two discriminant functions were significant (Hotelling - Lawley Trace, $p < .0001$; Table 12). The first discriminant function separated large frogs (SVL) with long shanks (TFL), short forearms (RUL) and short heads (HDL) from the alternatives. Generally, this separates Stillwater (S), Independence (I), and Ottawa (O) from Little Rock (L) and Willow Springs (W; Fig. 4A.). The second discriminant function separated frogs with long head and

trunk length (SUL), long forearms (RUL), widely spaced eyes (IOD), and short shanks (TFL) from the alternatives. This axis separated Stillwater frogs from the others (Fig. 4B). Morphologically speaking then, two distinct groups (S, I, and O versus L and W) are formed; McCurtain is intermediate, but tends towards the S - I - O group. Additional variation separates Stillwater from the other groups along the second canonical axis.

Genetics - All six loci were polymorphic, but PGM-A and MDH-B were fixed for the same allele in most populations (Table 13). McCurtain County and Little Rock samples shared a unique PGI-A allele, Willow Springs had a unique MDH-B allele, and McCurtain had a unique MDH-A allele.

Two LDH-B alleles were observed. Parallel runs using the Tris-Citrate buffer system and those of Ralin and Selander (1979) confirmed the identity of the LDH-B a-allele as their fast Ldb (= 'eastern') allele and the LDH-B b-allele as their slow Ldb (= 'western') allele. In this study the eastern allele is found in low frequencies in the west (5.5%), and gradually increases to fixation in Little Rock and McCurtain County populations. Four MPI alleles were observed. The a-allele is found in three populations, but generally decreases from west to east. The b- and c-alleles are most common and replace each other along the cline. The b-allele decreases in frequency from west-east, while the c-allele increases from west-east. The d-allele is absent from the three western populations, but it does exhibit an

apparent north-south cline.

Alleles at the MDH-A and PGM-A loci respond inversely along an west-east gradient. The b-allele was the most common form of MDH-A, but it was less frequent in the western populations. A unique a-allele of MDH-A was observed in McCurtain frogs. The b-allele of PGM-A was most common in all populations, but it is found in higher frequencies in the west.

Genotypic analyses are limited by sample size and the method of choosing loci (e.g., average heterozygosity comparisons are meaningless outside this study because polymorphic loci were deliberately chosen). However, examination of the genotypic frequencies of the LDH-B locus proves very interesting (Table 14). Even though eastern and western alleles are found together at four sites, heterozygous individuals were found at only one site (Ottawa). Ralin et al. (1983) reported only a single LDH-B heterozygote in over 250 individuals sampled. Since small sample sizes violate the assumptions of the test statistic for Hardy-Weinberg expectations for each site, the data were combined from the four sites where both eastern and western LDH-B alleles were present (S, I, O, W). Expected frequencies for each genotype were calculated at each site based on the allelic frequencies at that site (Table 15A). Observed and expected genotypic frequencies were tallied separately to produce grand observed and expected frequencies. Chi-square analysis of the combined data

reveal heterozygote deficiency at the LDH-B locus (X^2 = 18.7, df = 1, $p < .01$; Table 15). The data suggest that the alleles are not associating randomly at Stillwater, Independence, and Willow Springs. Mate selection based on calls, call sites, morphology, and/or breeding time, or selection against heterozygotes might explain the non-random association of eastern and western LDH-B alleles.

Nei's (1978) unbiased genetic identity (I) value and Rogers (1972) genetic distance (D) were used to construct a similarity/distance matrix (Table 16). Two groups are evident based on these calculations. A western group consisting of Stillwater, Independence, and Ottawa is linked by $I > 0.980$, while an eastern group of Willow Springs, Little Rock, and McCurtain are linked by $I > 0.970$. The groups are linked by $I = 0.939$ for the Independence - Willow Springs comparison. The same groupings are produced by comparison of D values. Members of the western group are linked by $D < 0.115$, while members of the eastern group are linked by $D < 0.155$. The two groups are joined by $D = .20$ for the Independence-Willow Springs comparison.

The I- and D- values reported here are biased because the loci were chosen to show the greatest differences. We would expect then, that the I-values should be depressed and the D-values exaggerated. Thus, the high within-group levels of genetic similarity suggest that these populations are very similar. The intermediate I- and D-values for between group comparisons are harder to interpret, but they are within the

normal range for within-species comparisons of other amphibians (Avise 1978).

Prim Networks - Prim networks visually demonstrate the relationships among samples by linearly linking nearest neighbors until all groups are included. Comparison of genetic distances among sites reveals two distinct groups (S, I, O versus W, L, M; Fig. 5). Prim networks were also constructed from Mahalanobis distances for call and morphjological data. Calls of Ottawa frogs clustered with calls from Willow Springs, McCurtain, and Independence. Calls from Little Rock and Stillwater are less closely related to this group and are not closely related to each other (Table 6; Fig. 6A). Two pairs of populations are related morphologically. Little Rock and Willow Springs, and Stillwater and Ottawa link together with Mahalanobis distances of 1.598 and 1.602, respectively (Table 11; Fig. 6B). McCurtain frogs are morphologically more similar to frogs from Stillwater than they are to frogs from Little Rock. Finally, Independence frogs are morphologically closer to Stillwater frogs than any other population. Prim networks of genetics and morphology agree most closely.

Gene Flow - Five models were generated to test genetic, morphological, and acoustic relatedness among populations. These models are a subset of all possible models of gene flow among these populations, but they represent three major types of models. The null model (H0) assumes that each population is connected directly to every other population

without any biotic or abiotic barriers (Fig. 7). Three alternative models (H1, H2, H3) assume that a barrier exists in eastern Oklahoma that directs gene flow north through Independence and Ottawa populations. The three models diverge based on gene flow beyond Ottawa. The first of these (H1) assumes that gene flow continues to be directed in a linear fashion through Willow Springs, Little Rock, and finally, to McCurtain. The other two models (H2, H3) assume different patterns of radiation from Ottawa. The final model (H4) assumes that the barrier in eastern Oklahoma is incomplete and allows gene flow directly between Stillwater and Little Rock.

Pairwise measures of genetic distance, genetic similarity, Mahalanobis distance in call space, and Mahalanobis distance in morphological space were regressed against geographic distances between populations as generated by each of the gene flow models. Both genetic measures produced significant coefficients when regressed against geographic distance from each of the gene flow models (Table 17). Regression of call and morphological distances against geographic distance produced no significant regressions for any of the five models. The amount of variance explained by a regression can be calculated by squaring the correlation coefficient (r). Although all five models explained over 45% of the observed genetic variance (rS&'2 = .439 - .782), three models (H1, H2, and H3) explained substantially more of the variance

(rS&'2 = .637 - .782). All three of these models assume the presence of some barrier to gene flow in eastern Oklahoma. Examination of the residuals of genetic distance vs. geographic distance revealed trends common to all models. McCurtain and Willow Springs populations tended to be genetically closer to other populations than geographic distance would predict. Genetic distances between members of the eastern and western groups (described above) tended to be greater than would be predicted by geographic distance alone. This trend may be influenced by relatively large genetic distances between Little Rock and members of the western group.

Eastern vs Western H. chrysoscelis

The genetic analysis indicates two distinct groups of populations. Stillwater, Independence, and Ottawa form a western group, and Little Rock, McCurtain, and Willow Springs an eastern group. Call and morphological differentiation were examined as a function of these genetic groups.

Calls - Eastern and western groups differed significantly in FF, VR1, and VR2 (Table 18). Western frogs had lower-pitched calls and lower ratios of FF/RF1 and FF/RF2 than the eastern group. MANOVA revealed significant overall call differences between groups (Hotelling-Lawley Trace, $p < .0002$), so DFA was employed to identify groups of call characters separating eastern and western populations. The discriminant function was significant (Hotelling - Lawley

Trace, $p < .0002$), and was weighted most heavily by the three frequency characters (FF, RF1, RF2) and two of the frequency ratios (VR2, VR12; Table 19). Discrimination of eastern and western groups is notable, although incomplete (Fig. 8). Eastern H. chrysoscelis are clustered around 0 and 1 on the canonical axis while western H. chrysoscelis are clustered around -1 and 0. Considerable overlap occurs at 0, but the two distributions appear to be complementary; the eastern distribution is truncated on the left and the western distribution is truncated on the right, indicating overall differences between calls of eastern and western populations.

Morphology - Univariate morphological differences between eastern and western populations are limited to a single head shape character (IND; Table 20). MANOVA indicates significant overall differences between groups (Hotelling - Lawley Trace, $p < .0001$). The discriminant function was significant (Hotelling - Lawley Trace; $p < .0001$) and weighted by TFL and RUL (Table 19). Plots of scores along the canonical axis show fairly good separation, suggesting overall morphological differentiation of eastern and western populations (Fig. 8).

DISCUSSION

Every population is faced with a set of biotic and abiotic factors unique to its size, place, and time. To be successful (i.e. survive and grow) members of the population must adapt to the selective pressures of the combined

factors. Soft selection, multiple adaptations to the same set of ecological conditions, and varying selection pressure result in phenotypic variation within a population. Frogs face numerous acoustical problems which must be overcome in order to attract mates and reproduce. Among the factors that affect frog calls are temperature, type and density of vegetation, and community composition. The presence of species-specific call channels in complex anuran communities evinces coevolution within a community to complex acoustical problems (Duellman 1968; Hodl 1977; Drewery and Rand 1983; Duellman and Pyles 1983). In this study, univariate call differences were observed among populations over seven of nine call parameters, but no clinal patterns emerged (Tables 4 & 5). Pairwise comparison of population centroids in call space indicated that each population produced unique calls. Separation of populations is poor along discriminant axes, suggesting that each population responds independently to its own set of acoustical problems. A weak trend toward lower fundamental frequencies in western populations (S, I, O) was observed, however, and is discussed below.

Similarly, univariate morphological differences were observed for three head characters (IOD, IND, OND) and one body length (SUL) character (Table 10), but no clinal patterns were observed (Tables 8 & 9). Significant overall morphological differences are suggested by MANOVA and comparison of Mahalanobis distances among population centroids, but again separation of populations is poor along

discriminant axes. However, the first discriminant function separates Stillwater, Independence, and Ottawa from Little Rock and Willow Springs.

With respect to genetic characters, clinal variation was observed at four electrophoretically detectable loci (LDH-B, MPI, MDH-A, PGM; Table 13). Two groups were identified based on genetic relatedness (Fig. 5; Table 16). Genetic distances among members of the eastern (Little Rock, Willow Springs, McCurtain) and western (Stillwater, Independence, Ottawa) groups were minimal; genetic distance between members of the two groups were greater. Weak support for evidence of these groups comes from divergence of fundamental frequencies of western and eastern calls (Table 4). Stronger support comes from morphological discrimination of Stillwater, Independence, and Ottawa from Little Rock and Willow Springs (Fig. 4).

Given separate eastern and western genetic groups, call and morphological data, when re-analyzed to search for group differences, showed good discrimination between eastern and western H. chrysoscelis. Eastern and western call groups were distinguished by frequency differences (FF, RF1, RF2) and two frequency ratios (VR2, VR12; Table 19A; Fig. 8). Morphological discrimination was based on shank length (TFL) and forearm length (RUL; Table 19; Fig. 8B). Arm length is related to reproductive success in other anurans (Howard and Kluge 1985) and may act as an isolating mechanism in this complex. Western males with short forearms may be less able

to firmly clasp eastern females, thus allowing them to be dislodged by females or rival males. Ralin and Rogers (1979) revealed similar morphological differences between eastern and western H. chrysoscelis. Thus, the call and morphological data reported here, plus the morphological data of Ralin and Rogers (1979), support the presence of eastern and western groups within H. chrysoscelis, but overlap between groups is substantial.

The presence of four heterozygotes for eastern and western LDH-B alleles (sensu Ralin and Selander 1979) is interesting. A single east-west LDH-B heterozygote was collected in Illinois (Ralin et al. 1983) and two heterozygotes for eastern and western immunoalleles were collected in extreme eastern Texas (Maxson et al. 1977). The Ottawa, McCurtain, and Willow Springs populations of H. chrysoscelis examined here are sympatric with H. versicolor. Ralin et al. (1983) did not specifically state that their Illinois heterozygote is from an area of sympatry with H. versicolor, but such is inferred from the description of the study sites and the manner in which the data were treated. Additionally, Romano et al. (1987) report the presence of the western LDH-B allele in low frequency in McCurtain Co., Oklahoma. While not stated by the authors, this low allelic frequency appears to be due to a single heterozygous specimen. The lack of east-west heterozygotes at Independence, Willow Springs, and especially Stillwater, where the eastern allele occurs at low frequency (5.5%), now

becomes problematic because it suggests that eastern and western alleles are not associating randomly at these sites. Positive assortative mating resulting from differences in calls (Gerhardt 1982), choice of call sites (Porter 1964, 1966; Mecham 1965), morphology (A. P. Blair 1950; W. F. Blair 1955), mating behavior, breeding phenology, and/or selection against east-west heterozygotes would explain the heterozygote deficiency. Perhaps eastern and western forms diverged from one another while in isolated refugia during Pleistocene glaciation (Blair 1965). When eastern and western forms contacted each other, genetic differences that arose during isolation or selection against east-west LDH-B heterozygotes caused reduced viability of the "hybrids". These proposed post-mating isolating mechanisms reinforced subtle call and morphological differences between eastern and western forms. Weak pre-mating isolating mechanisms are thought to be responsible for separation of two chromosome morphs of H. chrysoscelis in western North Carolina (J. Wiley, pers. comm.). The two chromosome morphs began to interbreed after completion of a golf course and ponds that are situated in the contact area. Presumably, weak pre-mating barriers broke down when the two morphs contacted each other at these ponds and the morphs began to interbreed. Pre-mating and post-mating isolating mechanisms are well developed between H. chrysoscelis and H. versicolor (Johnson 1961; Gerhardt 1978; Cline 1990), but they appear to be poorly developed among H. chrysoscelis populations. Thus, when eastern and western forms of H. chrysoscelis are

sympatric (and allopatric with H. versicolor) weak pre-mating isolating barriers effectively separate the two forms, but when the two forms are also sympatric with H. versicolor these mechanisms break down and allow the two forms of H. chrysoscelis to interbreed.

Regression of two measures of genetic relatedness (I & D) against geographic distances among H. chrysoscelis populations for five models of gene flow produced significant regressions in all cases. All gene flow models explained over 45% of the genetic variance observed, and four models explained over 50% of the variance. Thus, geographic distance explains most of the genetic differentiation among gray treefrog populations. Lack of significant regressions of call distance and morphological distance against geographic distance suggests that these characters are responding independently to different selective pressures. The three models (H1, H2, H3) that assume the presence of a barrier to gene flow in eastern Oklahoma explained substantially more variance than the other models (Table 17). While physical barriers such as unfavorable habitat are a possibility, the presence of the tetraploid H. versicolor in eastern Oklahoma suggests other interesting possibilities. One theory for the evolution of polyploids calls for the production of unreduced eggs by diploid females and subsequent fertilization by normal haploid sperm to produce a triploid (Bogart and Wasserman 1972). In the next generation, unreduced triploid gametes

are then fertilized by haploid gametes to produce tetraploid offspring. Gene flow in this system is necessarily one way. Should such a system be in place in eastern Oklahoma, the effect would be that of a genetic sink with genes from eastern and western diploids flowing into a polyphyletic H. versicolor population, but not directly to other diploid populations. Romano et al. (1987) rejected this triploid model for the evolution of H. versicolor due to the absence of triploids in any field study to date. An alternative hypothesis is that H. versicolor competitively excludes H. chrysoscelis from large portions of eastern Oklahoma. Ralin (1968) demonstrated that food habits of these species are different in sympatry, suggesting character displacement as a consequence of inter-specific competition.

One unresolved problem is illustrated by examination of the residuals of the regression of genetic distance against geographic distance. If gene flow is in the chain-like fashion illustrated by H1, then McCurtain frogs should be genetically further from Stillwater frogs than all other populations; this is not the case (Table 16). McCurtain and Willow Springs populations are genetically closer to western populations than would be predicted by geographic distance for all of the gene flow models. Heterozygous individuals observed by Maxson et al. (1977) in extreme eastern Texas were dismissed by Ralin (1978) because he assumed that H. versicolor formed a complete barrier to gene flow in eastern Oklahoma and Texas, and gene flow must be directed north

around the tetraploid. Incomplete barriers to gene flow across central and southern Okahoma may be possible, however. Bragg (1950) observed that H. versicolor occurred in upland areas of McCurtain Co., while H. chrysoscelis was restricted to valleys and lowlands. Bragg postulated that H. chrysoscelis invaded Oklahoma from Arkansas along the Little River drainage system. If these observations were correct, then the Arkansas and Red River drainages might act, or have acted in the past, as corridors for dispersal of H. chrysoscelis through an otherwise formidable barrier. One might expect greater gene flow in the downstream direction (Rachuk 1987). Neither numerous dispersers nor numerous dispersal events are required to account for the observed frequency differences. Gene flow between Willow Springs and the western populations is possibly enhanced by relatively homogeneous habitat in northeastern Oklahoma and southern Missouri. Possibly, "hybridization" and subsequent introgression are enhanced when eastern and western forms are sympatric with H. versicolor. An extensive survey of the distribution of members of the gray treefrog complex in eastern Oklahoma is required. Once distributions are better known, models for gene flow can be developed and tested.

The immunological distances between eastern and western H. chrysoscelis led Maxson et al. (1977) to argue for the specific status of the two groups. Ralin (1978) stated that the presence of east - west immunological heterozygotes and the geographic placement of them in eastern Texas argued

against specific status. Discovery of a single electrophoretic heterozygote at a locus that was previously thought to be fixed for eastern and western populations (LDH-B) led Ralin et al. (1983) to re-affirm the conspecific nature of eastern and western diploids. Results of this study indicate partial call, morphological, and genetic divergence of the two diploid groups, but at present these differences would appear to warrant only sub-specific status.

In summary, significant call and morphological differences were observed among six populations of the gray treefrog. Acoustical differences were observed among all sites for seven of nine parameters. Morphological differences in three head characters and one body character were observed among five of six sites. No clinal patterns were observed, but two weakly differentiated east-west groups of populations were resolved. Electrophoretic analysis of four presumptive loci demonstrated east - west clinal variation and confirmed the presence of two groups of diploid treefrogs. Re-analysis of call and morphological data based on these groups produced better resolution. Eastern frogs produced higher pitched calls with higher frequency ratios than western frogs. Morphologically, eastern frogs had narrower snouts than western frogs. While this study does not adequately address the taxonomic questions posed by this complex, it suggests that eastern and western populations of H. chrysoscelis may represent

sub-species, and not full species.

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TABLE 1. List of call parameters (A) and morphological characters (B) used in this study. All morphological characters are measured in mm, and bilateral characters were measured only on the right side.

(A) Call Parameters

DUR - length of call calculated from sonogram (s)

PRR - pulse repetition rate (p/s)

NUMP - number of pulses in call (p)

FF - fundamental frequency, lowest frequency peak (Hz)

RF1 - first resonant frequency peak (Hz)

RF2 - second resonant frequency peak (Hz)

VR1 - FF / RF1

VR2 - FF / RF2

VR12 - RF1 / RF2

Table 1 (cont.)

(B) Morphological Characters

SVL - distance from tip of snout to cloaca

SUL - distance from tip of snout to anterior margin of urostyle

UL - length of urostyle

HDL - distance from tip of snout to posterior margin of skull

HDW - head width at center of tympanum

IOD - distance between anterior margins of the eyes

IND - distance between nares

OND - distance between anterior margin of eyes and naris

TD - tympanum diameter

JL - distance from angle of jaw to center of jaw

RUL - distance from elbow to base of thumb (radio-ulna length)

HML - distance from upper arm articulation with scapula and the elbow (humerus length)

TBL - distance from base of thumb to distal margin of last phalanx

FL3 - length of last two phalynxes of largest (3rd) phalange

FL - distance from cloaca to knee (femur length)

TFL - distance from knee to heel (tibiofibula length)

HFL - distance from heel to distal end of last phalanx 4th toe (hindfoot length)

TABLE 2. Proteins and presumptive loci used in this study. Enzyme nomenclature follows recommendations of the International Union of Biochemistry (1984). Locus designations follow Buth's (1983) recommendations. A Tris - Citrate buffer was adjusted to pH 5.3 or 6.0.

Protein	Locus
Glucose-6-phosphate isomerase (EC 5.3.1.9)	GPI-A
L-Lactate dehydrogenase (EC 1.1.1.27)	LDH-B
Malate dehydrogenase (EC 1.1.1.37)	MDH-A
	MDH-B
Mannose-6-phosphate isomerase (EC 5.3.1.8)	MPI-A
Phosphoglucomutase (EC 5.4.2.2)	PGM-A

Tray buffer: 0.220M Tris, 0.086M Citric Acid

Gel buffer: 0.009M Tris, 0.003M Citric Acid

TABLE 3. Regression of call parameters against air temperature for H. chrysoscelis. Regression coefficients and the amount of variance explained by the regression are given. Asterisks indicate significant regression coefficients at $p < .05$ level for table-wide comparisons (sequential Bonferroni method).

Character	Coefficient	R^2
DUR	-0.006	1.6
PRR	1.56 *	36.7
NUMP	0.455	3.1
FF	6.668	4.6
RF1	20.131*	15.0
RF2	20.838*	6.4
VR1	-0.002	2.7
VR2	-0.001	0.1
VR12	0.002	1.9

TABLE 4. Temperature-adjusted means and standard errors in the calls of six treefrog populations arranged along an east-west cline. Site codes are given in Fig. 1, character codes are given in Table 1, sample sizes are as follows: S - 19; I - 14, O - 19, W - 18, L -17, M -19.

Call	Sites					
Parameter	S	I	O	M	L	W
DUR	0.516	0.487	0.550	0.532	0.633	0.441
	(0.029)	(0.034)	(0.027)	(0.021)	(0.046)	(0.028)
PRR	51.38	59.54	59.36	57.04	56.10	57.47
	(1.57)	(1.67)	(1.47)	(0.95)	(0.61)	(1.64)
NUMP	28.22	29.95	31.40	31.53	37.64	24.42
	(1.19)	(1.91)	(1.21)	(0.87)	(2.79)	(1.65)
FF	1244	1220	1288	1375	1303	1317
	(17.3)	(14.0)	(19.8)	(20.6)	(15.9)	(22.8)
RF1	2433	2393	2467	2545	2369	2476
	(27.9)	(35.1)	(36.1)	(36.7)	(18.2)	(36.5)
RF2	3609	3564	3687	3854	3547	3710
	(29.0)	(40.2)	(55.5)	(47.0)	(69.2)	(65.7)
VR1	0.511	0.510	0.522	0.541	0.550	0.532
	(0.005)	(0.007)	(0.004)	(0.006)	(0.006)	(0.006)
VR2	0.345	0.343	0.357	0.349	0.370	0.355
	(0.004)	(0.005)	(0.003)	(0.004)	(0.010)	(0.004)
VR12	0.674	0.673	0.669	0.660	0.673	0.668
	(0.007)	(0.016)	(0.003)	(0.006)	(0.017)	(0.007)

TABLE 5. Pairwise comparisons of H. chrysoscelis calls among sites by t-tests. Asterisks indicate character-wide significance at the $p < .05$ level (sequential Bonferroni method). Site codes are given in Fig. 1, character codes are given in Table 1, sample sizes are given in Table 4.

Paired	Call Parameters								
Comparison	DUR	PRR	NUMP	FF	RF1	RF2	VR1	VR2	VR12
S - I	NS	*	NS	NS	NS	NS	NS	NS	NS
S - O	NS	*	NS	NS	NS	NS	NS	NS	NS
S - W	NS	NS	NS	NS	NS	NS	NS	NS	NS
S - L	NS	NS	NS	NS	NS	NS	*	NS	NS
S - M	NS	NS	NS	*	NS	*	*	NS	NS
I - O	NS	NS	NS	NS	NS	NS	NS	NS	NS
I - W	NS	NS	NS	*	NS	NS	NS	NS	NS
I - L	NS	NS	NS	*	NS	NS	*	NS	NS
I - M	NS	NS	NS	*	NS	*	*	NS	NS
O - W	NS	NS	NS	NS	NS	NS	NS	NS	NS
O - L	NS	NS	NS	NS	NS	NS	*	NS	NS
O - M	NS	NS	NS	*	NS	NS	NS	NS	NS
W - L	*	NS	*	NS	NS	NS	NS	NS	NS
W - M	NS	NS	*	NS	NS	NS	NS	NS	NS
L - M	NS	NS	NS	NS	*	*	NS	NS	NS

MANOVA (Hotelling - Lawley Trace, $p < .0001$)

TABLE 6. Mahalanobis distances among treefrog populations based on discriminant functions analysis of calls. Mahalanobis distances are given above the diagonal and results of significance tests for differences between group centroids are given below the diagonal. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method).

	1	2	3	4	5	6
1 Stillwater	-	2.139	2.633	2.595	2.642	2.362
2 Independence	*	-	1.619	2.096	2.042	2.266
3 Ottawa	*	*	-	1.547	2.004	1.594
4 Willow Springs	*	*	*	-	2.540	1.831
5 Little Rock	*	*	*	*	-	1.812
6 McCurtain	*	*	*	*	*	-

TABLE 7. Standardized canonical coefficients from discriminant functions analysis of call parameters. Character codes are given in Table 1.

Character	CAN1	CAN2	CAN3
DUR	2.5322	-0.9328	-0.7416
PRR	1.7574	-0.4107	-0.8780
NUMP	-2.1694	1.7443	0.5541
FF	9.1841	-0.5582	0.8149
RF1	-6.0415	-3.0108	-1.1138
RF2	-1.5257	3.7356	1.6106
VR1	-2.9212	-0.7498	-2.9957
VR2	-4.1575	1.8231	3.8010
VR12	2.5032	1.8566	-2.2851

TABLE 8. Means and standard errors of ten external head and body measurements of six treefrog populations arranged along an east-west cline. Site codes are given in Fig. 1, character codes are given in Table 1, sample sizes are as follows: S - 18, I - 11, O - 13, W - 11, L - 15, M - 9.

	Sites					
Character	S	I	O	W	L	M
SVL	40.96	41.07	40.83	38.91	39.22	40.86
	(0.593)	(0.475)	(0.998)	(0.932)	(0.453)	(0.998)
SUL	22.04	23.73	22.21	22.26	22.14	20.84
	(0.539)	(0.454)	(0.559)	(0.842)	(0.380)	(0.413)
UL	15.58	15.06	16.14	14.36	15.36	17.16
	(0.348)	(0.617)	(0.527)	(0.601)	(0.353)	(0.832)
HDL	11.63	11.67	11.95	11.48	11.75	11.94
	(0.131)	(0.201)	(0.258)	(0.288)	(0.148)	(0.220)
HDW	14.15	13.78	14.00	13.57	13.89	14.21
	(0.270)	(0.280)	(0.291)	(0.327)	(0.210)	(0.392)
IOD	6.83	7.54	6.63	5.89	6.20	7.31
	(0.116)	(0.232)	(0.232)	(0.250)	(0.138)	(0.322)
IND	2.99	3.25	2.81	2.51	2.46	3.01
	(0.144)	(0.174)	(0.088)	(0.170)	(0.114)	(0.164)
OND	2.92	3.20	2.91	2.81	2.84	3.32
	(0.128)	(0.100)	(0.104)	(0.140)	(0.081)	(0.113)
TD	2.69	2.52	2.46	2.60	2.56	2.56
	(0.077)	(0.087)	(0.115)	(0.101)	(0.076)	(0.143)
JL	11.78	11.29	12.10	11.52	12.04	12.12
	(0.220)	(0.284)	(0.267)	(0.322)	(0.183)	(0.329)

TABLE 9. Means and standard errors of seven external limb measurements from six treefrog populations arranged along an east-west cline. Character codes are given in Fig. 1, site codes are given in Table 1, sample sizes are given in Table 8.

Character	S	I	O	W	L	M
RUL	7.87	8.05	7.86	8.06	8.13	8.21
	(0.125)	(0.152)	(0.219)	(0.257)	(0.127)	(0.271)
HML	11.21	11.54	11.08	10.50	10.51	11.89
	(0.248)	(0.473)	(0.408)	(0.339)	(0.224)	(0.486)
TBL	4.87	5.15	4.60	5.00	4.67	5.34
	(0.146)	(0.138)	(0.220)	(0.158)	(0.184)	(0.215)
FL3	5.57	6.04	5.25	5.69	6.07	5.99
	(0.207)	(0.305)	(0.244)	(0.236)	(0.200)	(0.256)
FL	20.36	20.09	20.41	19.10	19.58	19.63
	(0.294)	(0.605)	(0.499)	(0.580)	(0.292)	(0.541)
TFL	19.24	19.05	19.21	18.05	18.44	19.03
	(0.338)	(0.330)	(0.417)	(0.511)	(0.237)	(0.520)
HFL	26.72	27.39	26.89	25.81	26.17	27.04
	(0.321)	(0.509)	(0.623)	(0.803)	(0.368)	(0.825)

TABLE 10. Pairwise comparisons of H. chrysoscelis morphology among sites by t-tests. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method). Site codes are given in Fig. 1, character codes are given in Table 1, and sample sizes are are given in Table 8.

Paired	Morphological Characters			
Comparison	SUL	IOD	IND	OND
S - I	NS	NS	NS	NS
S - O	NS	NS	NS	NS
S - W	NS	NS	NS	NS
S - L	NS	*	NS	NS
S - M	NS	NS	NS	NS
I - O	NS	NS	NS	NS
I - W	NS	*	NS	NS
I - L	NS	*	*	NS
I - M	*	NS	NS	NS
O - W	NS	NS	NS	NS
O - L	NS	NS	NS	NS
O - M	NS	NS	NS	NS
W - L	NS	NS	NS	NS
W - M	NS	*	NS	NS
L - M	NS	*	NS	*

MANOVA (Hotelling - Lawley Trace, $p < .0001$)

TABLE 11. Mahalanobis distances among treefrog populations based on discriminant function analysis of morphology. Mahalanobis distances are given above the diagonal and results of significance tests for differences between group centroids are given below the diagonal. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method).

	1	2	3	4	5	6
1 Stillwater	-	3.328	1.602	3.421	3.449	2.715
2 Independence	*	-	3.882	4.101	4.347	3.412
3 Ottawa	NS	*	-	3.601	3.303	2.928
4 Willow Springs	NS	*	*	-	1.598	3.488
5 Little Rock	*	*	*	NS	-	3.254
6 McCurtain	*	NS	*	*	NS	-

TABLE 12. Standardized canonical coefficients from discriminant functions analysis of morphological characters. Character codes are given in Table 1.

Character	CAN1	CAN2
SVL	0.9739	-0.6394
SUL	-0.3011	0.9676
UL	0.1152	-0.3131
HDL	-0.7871	-0.2538
HDW	-0.3476	-0.2915
IOD	0.5861	0.8471
IND	0.5327	0.0740
OND	0.1049	0.3014
TD	-0.3399	-0.1761
JL	-0.4627	-0.4836
RUL	-1.0487	0.8845
HML	-0.0687	-0.0377
TBL	0.0358	0.3971
FL3	-0.5237	0.4654
FL	0.0745	-0.5967
TFL	1.2578	-0.7485
HFL	0.4821	0.6184

TABLE 13. Allelic frequencies at six loci in six treefrog populations. Enzyme codes are given in Table 2, site codes are given in Fig. 1. Sample sizes are as follows: S -18, I - 11, O - 12, W - 14, L - 13, M - 10.

	Site					
Locus	S	I	O	W	L	M
LDH-B						
a	5.5	27.3	33.3	64.3	100.0	100.0
b	94.5	72.7	66.7	35.7	-	-
MPI						
a	19.4	-	12.5	7.1	-	-
b	80.6	72.7	70.8	32.1	23.1	35.0
c	-	27.3	16.7	46.4	65.4	60.0
d	-	-	-	14.3	11.5	5.0
PGM-A						
a	11.2	15.0	-	30.8	34.6	30.0
b	88.8	85.0	100.0	69.2	65.4	70.0
MDH-A						
a	-	-	-	-	-	15.0
b	63.8	50.0	54.2	82.1	92.3	85.0
c	36.2	50.0	45.8	17.9	7.7	-

Table 13 (cont.)

Locus	S	I	O	W	L	M
MDH-B						
a	100.0	100.0	100.0	82.1	100.0	100.0
b	-	-	-	17.9	-	-
PGI						
a	-	-	-	-	13.0	5.0
b	100.0	100.0	100.0	100.0	87.0	95.0

TABLE 14. Genotypic frequencies at six loci in six treefrog populations. Site codes are given in Fig. 1, enzyme codes are given in Table 2, sample sizes are given in Table 13.

	Site					
Locus	S	I	O	W	L	M
LDH-B						
aa	5.5	27.3	16.7	64.3	100.0	100.0
ab	-	-	33.3	-	-	-
bb	94.5	72.7	50.0	-	-	-
MPI						
aa	-	-	8.3	7.1	-	-
ab	38.9	-	8.3	-	-	-
ac	-	-	-	-	-	-
ad	-	-	-	-	-	-
bb	61.1	54.5	58.3	14.2	-	20.0
bc	-	36.4	16.6	35.7	46.2	30.0
bd	-	-	-	-	-	-
cc	-	9.1	8.3	21.4	38.5	40.0
cd	-	-	-	14.2	7.7	10.0
dd	-	-	-	7.1	7.1	-
PGM-A						
aa	-	10.0	-	7.7	7.7	20.0
ab	29.4	10.0	-	46.2	53.8	20.0
bb	70.6	80.0	100.0	46.2	38.5	60.0

TABLE 14 (cont.)

Locus	S	I	O	W	L	M
MDH-A						
aa	-	-	-	-	-	10.0
ab	-	-	-	-	-	10.0
ac	-	-	-	-	-	-
bb	33.3	9.1	16.7	64.3	84.6	80.0
bc	61.1	90.9	75.0	28.6	15.4	-
cc	5.6	-	8.3	7.1	-	-
MDH-B						
aa	100.0	100.0	100.0	85.8	100.0	100.0
ab	-	-	-	7.1	-	-
bb	-	-	-	7.1	-	-
GPI						
aa	-	-	-	-	-	-
ab	-	-	-	-	23.1	10.0
bb	100.0	100.0	100.0	100.0	76.9	90.0

TABLE 15. Chi-square analysis of genotypic frequencies for LDH-B. Observed (O) and expected (E) frequencies were determined by site (A) and combined prior to analysis (B).

(A)

	Genotype						
	bb		ab		aa		
Site	O	E	O	E	O	E	
S	17	(16.1)	0	(1.9)	1	(0.1)	
I	8	(5.8)	0	(4.4)	3	(0.8)	
O	6	(5.3)	4	(5.3)	2	(1.3)	
W	5	(1.8)	0	(6.4)	9	(5.8)	
Totals	36	(29)	4	(18)	15	(8)	= 55

(B)

Genotype	O	E	$(O-E)^2$	$(O-E)^2/E$
bb	36	29	49	1.69
ab	4	18	196	10.89
aa	15	8	49	6.12
Totals	55	55	X^2 = 18.70	

Table 16. Genetic relatedness among H. chrysoscelis populations. Nei's (1978) unbiased genetic identity (I) is above the diagonal and Rogers (1972) genetic distance (D) is below the diagonal.

	1	2	3	4	5	6
1 Stillwater	-	0.988	0.983	0.870	0.707	0.732
2 Independence	0.093	-	1.000	0.939	0.809	0.830
3 Ottawa	0.111	0.055	-	0.928	0.801	0.825
4 Willow Springs	0.249	0.200	0.220	-	0.973	0.977
5 Little Rock	0.363	0.311	0.334	0.151	-	1.000
6 McCurtain	0.335	0.281	0.305	0.134	0.057	-

TABLE 17. Results of regression of measures of genetic, call, and morphological distances against geographic distance from five gene flow models discussed in the text. The measures include Nei's unbiased genetic similarity (I), Rogers genetic distance (D), and the Mahalanobis distances for calls and morphology. Asterisks indicate character-wide significance at the $p < .05$ level (sequential Bonferroni method).

	Distance Measure			
Model	I	D	Call	Morphology
H0	.458*	.439*	.120	.071
H1	.787*	.782*	.067	.004
H2	.672*	.672*	.250	.012
H3	.643*	.637*	.097	.003
H4	.544*	.526*	.061	.002

TABLE 18. Means and standard errors of temperature-adjusted call means of eastern and western H. chrysoscelis. Results of t-tests are reported at the right. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method). Sample sizes are as follows: western N = 54, eastern N = 52. Character codes are given in Table 1.

	western		eastern		
Character	Mean	SE	Mean	SE	Significance
DUR	0.521	0.017	0.533	0.021	NS
PRR	56.50	1.04	56.89	0.66	NS
NUMP	29.85	0.81	31.08	1.29	NS
FF	1253.53	10.86	1332.91	12.21	*
RF1	2434.78	19.23	2466.61	20.80	NS
RF2	3625.62	25.81	3709.28	38.42	NS
VR1	0.515	0.003	0.541	0.004	*
VR2	0.346	0.002	0.360	0.004	*
VR12	0.672	0.004	0.667	0.006	NS

MANOVA (Hotelling - Lawley Trace, $p < .0002$)

TABLE 19. Standardized canonical coefficients from discriminant analysis of eastern and western H. chrysoscelis calls (A) and morphology (B). Character codes are given in Table 1.

(A) Calls		(B) Morphology	
Character	Coefficient	Character	Coefficient
DUR	0.4891	SVL	-0.6890
PRR	0.2749	SUL	-0.0518
NUMP	-0.5892	UL	0.0124
FF	0.8063	HDL	0.6304
RF1	-1.8834	HDW	0.2640
RF2	1.8769	IOD	-0.3169
VR1	-0.0840	IND	-0.4508
VR2	0.7667	OND	0.1508
VR12	0.9648	TD	0.1958
		JL	0.5375
		RUL	1.0287
		HML	0.2266
		TBL	0.1353
		FL3	0.6637
		FL	-0.5437
		TFL	-1.3794
		HFL	-0.2724

TABLE 20. Means and standard errors of morphological characters of eastern and western H. chrysoscelis. Results of t-tests are given at the right. Asterisks indicate table-wide significance at the $p < .05$ level (sequential Bonferroni method). Sample sizes are as follows: western N = 42, eastern N = 35. Character codes are given in Table 1.

	western		eastern		
Character	Mean	SE	Mean	SE	Significance
SVL	40.95	0.41	39.54	0.44	NS
SUL	22.54	0.32	21.84	0.33	NS
UL	15.62	0.27	15.51	0.36	NS
HDL	11.74	0.11	11.71	0.12	NS
HDW	14.00	0.16	13.87	0.17	NS
IOD	6.95	0.12	6.39	0.16	NS
IND	3.00	0.08	2.62	0.09	*
OND	2.99	0.07	2.96	0.07	NS
TD	2.57	0.05	2.57	0.06	NS
JL	11.75	0.15	11.90	0.15	NS
RUL	7.91	0.09	8.13	0.12	NS
HML	11.26	0.20	10.86	0.21	NS
TBL	4.86	0.10	4.95	0.12	NS
FL3	5.59	0.14	5.93	0.13	NS
FL	20.30	0.25	19.44	0.26	NS
TFL	19.18	0.21	18.47	0.23	NS
HFL	26.95	0.27	26.28	0.36	NS

MANOVA (Hotelling - Lawley Trace, $p < .0002$)

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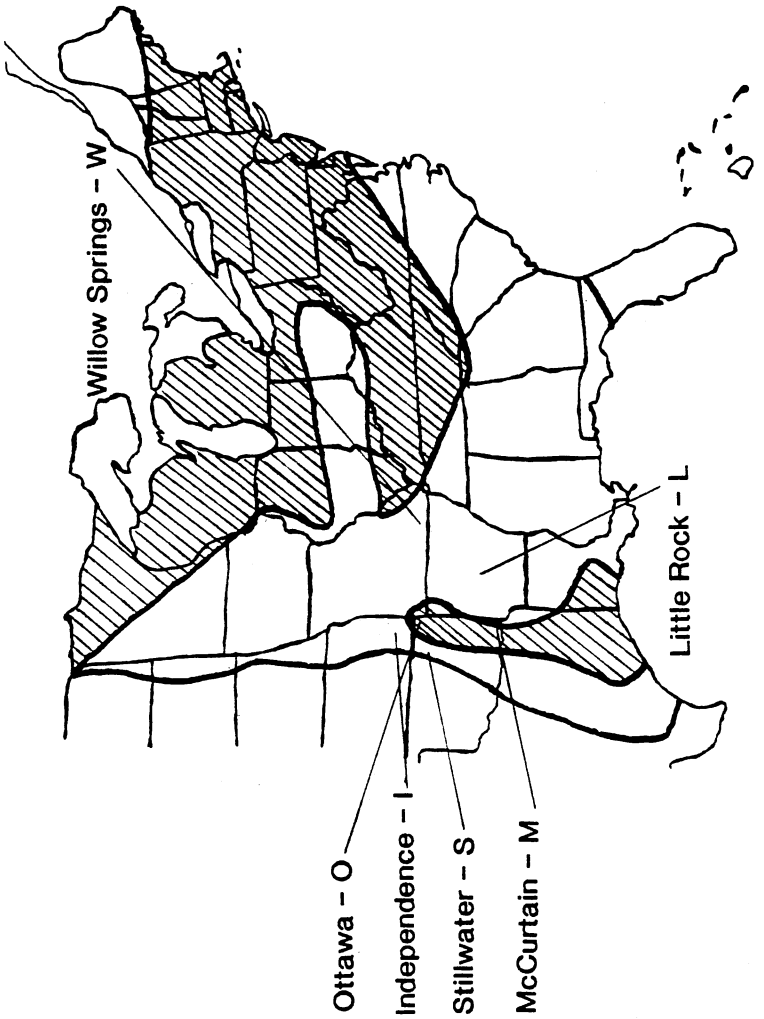
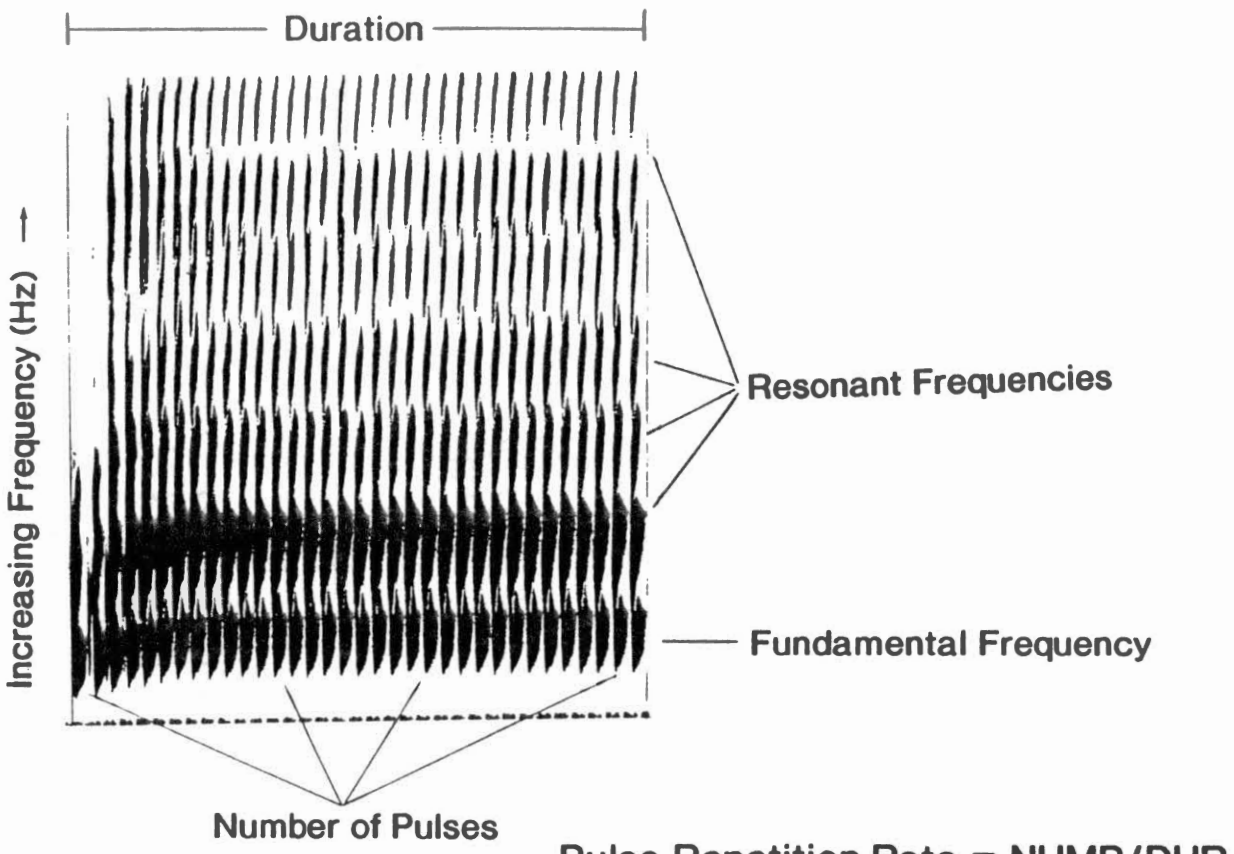
and morphology (B) among H. chrysoscelis populations.

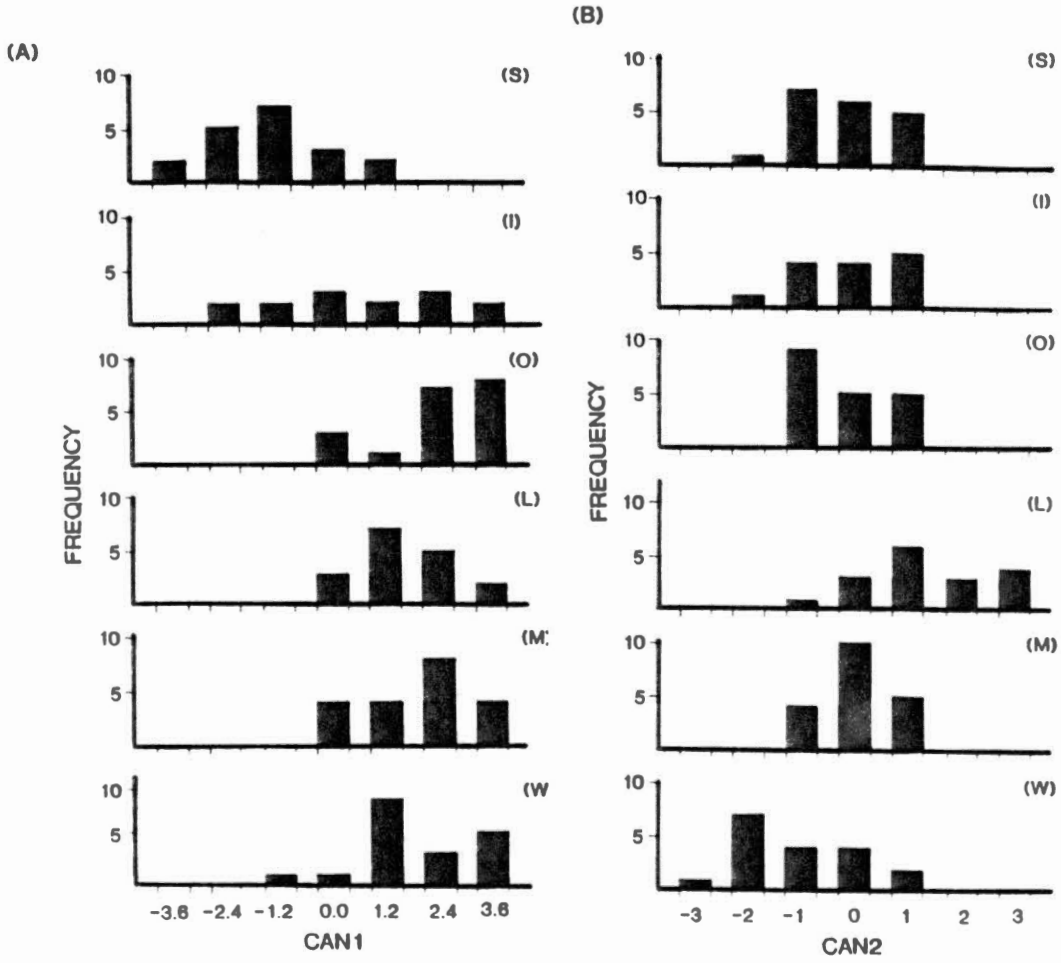
FIG. 7. Five models of gene flow among gray treefrog populations. Assumptions of each model are discussed in the text.

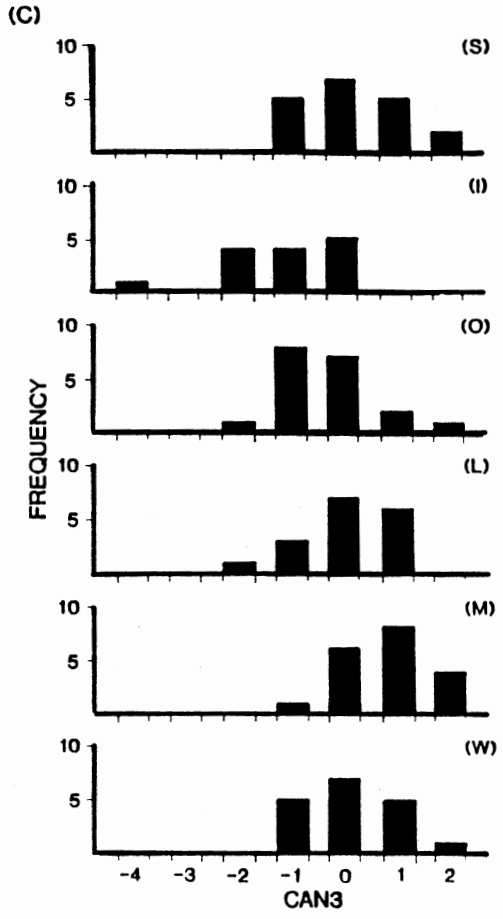
FIG. 8. Frequency histograms of canonical discriminant scores for calls (A) and morphology (B) comparing eastern and western treefrog groups.

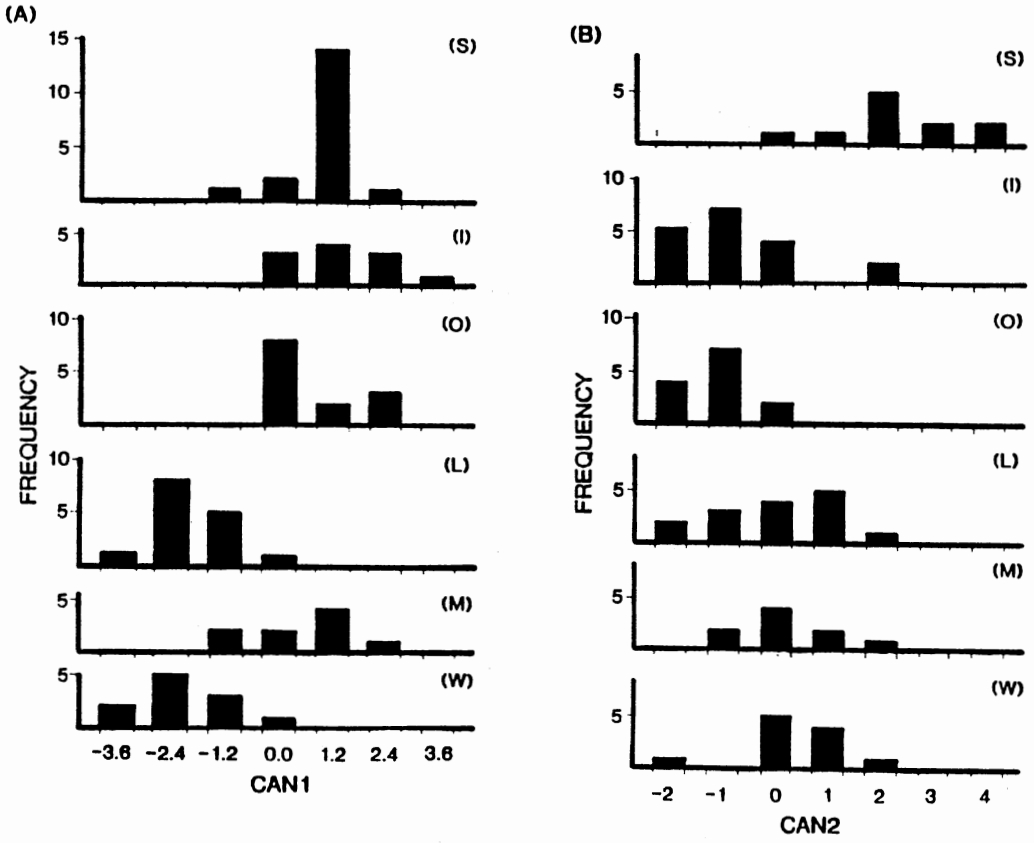

Willow Springs - W
Little Rock - L
Ottawa - O
Independence - I
Stillwater - S
McCurtain - M

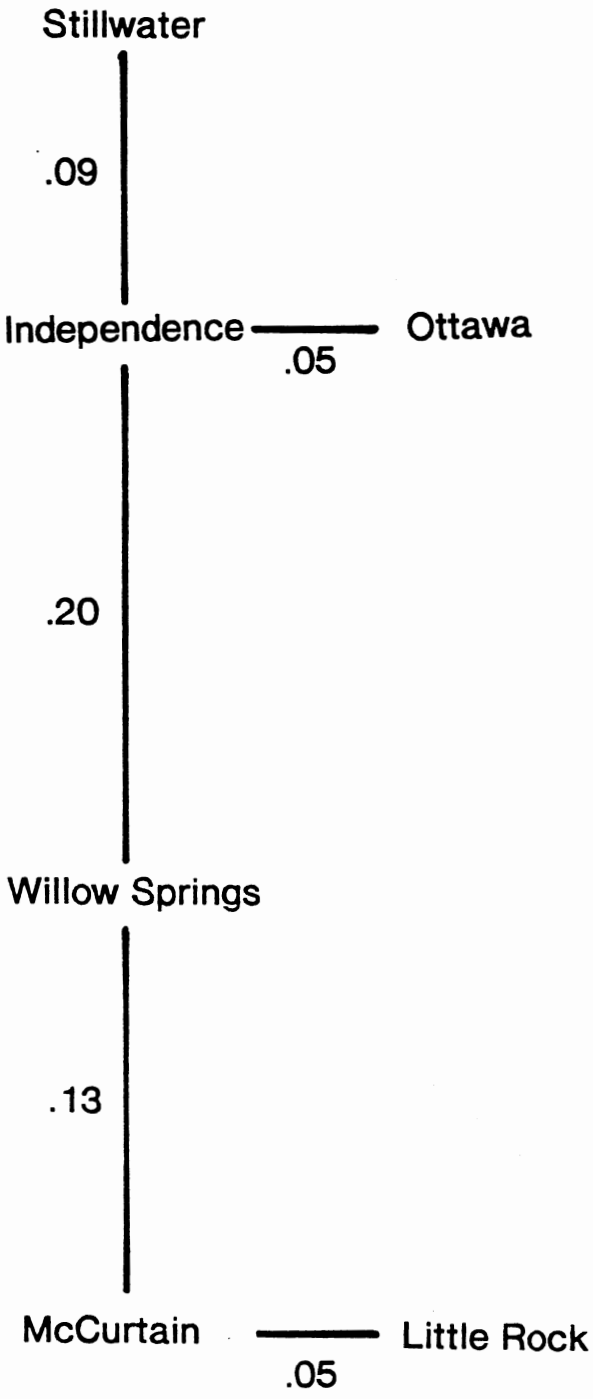


Pulse Repetition Rate = NUMP/DUR

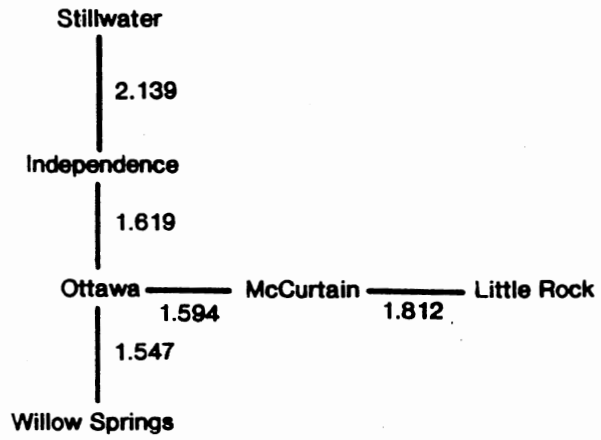

(A)
(B)
(S)
(I)
(O)
(L)
(M)
(W)
10
5
FREQUENCY
-3.6 -2.4 -1.2 0.0 1.2 2.4 3.6
CAN1
-3 -2 -1 0 1 2 3
CAN2


(C)
(S)
(I)
(O)
(L)
(M)
(W)
10
5
FREQUENCY
-4
-3
-2
-1
0
1
2
CAN3


(A)
(S)
(I)
(O)
(L)
(M)
(W)
FREQUENCY
-3.6 -2.4 -1.2 0.0 1.2 2.4 3.6
CAN1
(B)
(S)
(I)
(O)
(L)
(M)
(W)
FREQUENCY
-2 -1 0 1 2 3 4
CAN2


Stillwater
.09
Independence
Ottawa
.05
.20
Willow Springs
.13
McCurtain
Little Rock
.05

(A)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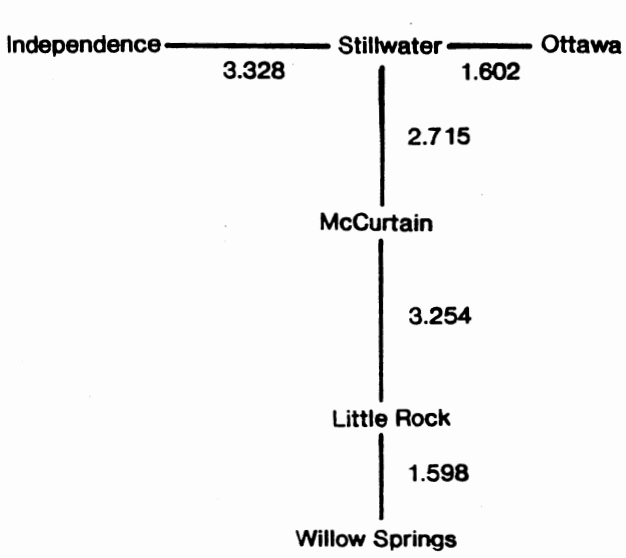
2.139
Independence
1.619
Ottawa
1.594
McCurtain
1.812
Little Rock
1.547
Willow Springs

(B)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Stillwater
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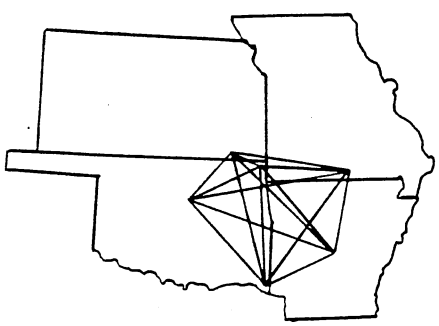
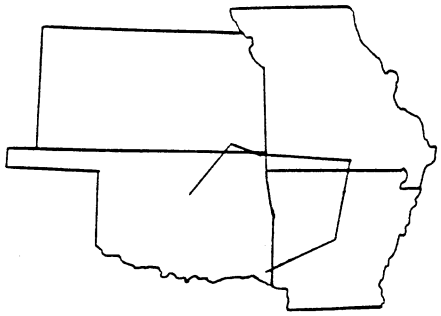
Ottawa
2.715
McCurtain
3.254
Little Rock
1.598
Willow Springs



H_0



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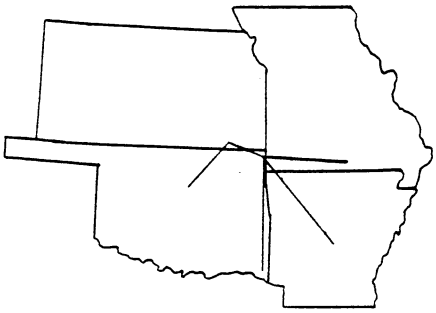
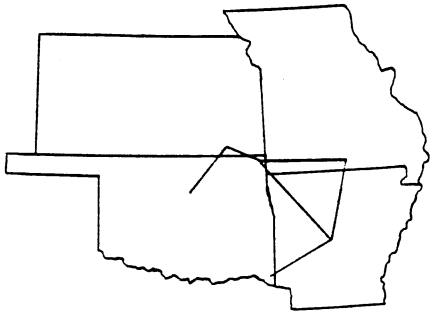
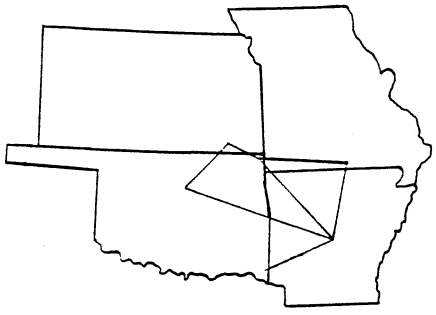


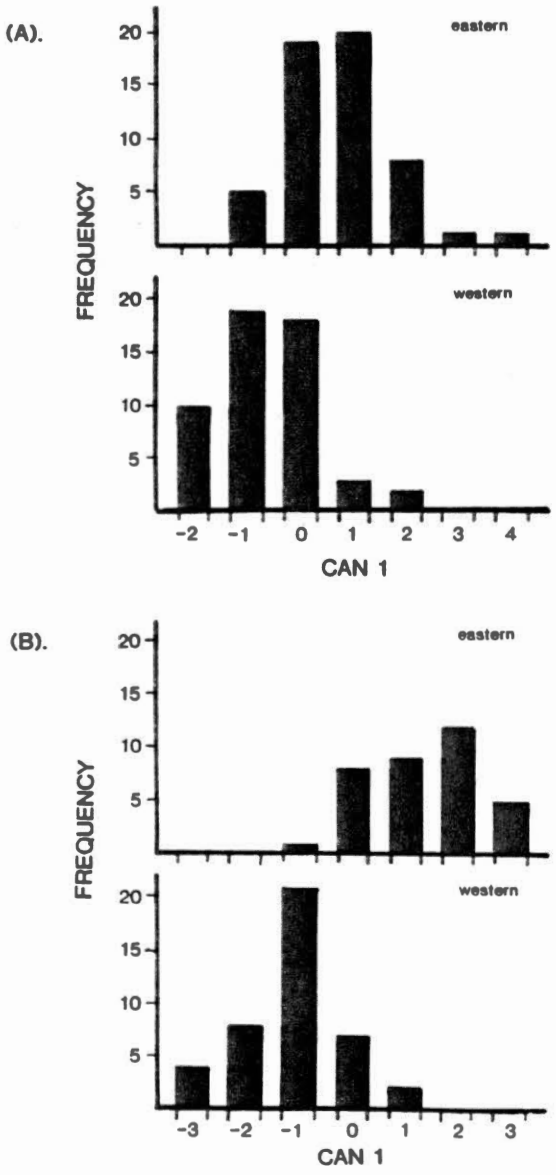
H_2



H_3



H_4


(A).
eastern
western
FREQUENCY
20
15
10
5
-2
-1
0
1
2
3
4
CAN 1
(B).
eastern
western
FREQUENCY
20
15
10
5
-3
-2
-1
0
1
2
3
CAN 1

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APPENDIXES

APPENDIX.
BIBLIOGRAPHY OF THE GRAY TREEFROG COMPLEX

The following is an annotated bibliography of the gray treefrog complex. Articles are listed alphabetically by author and year. Articles are listed under subject headings by author and year.

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Subject Listing

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Paleontology

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Vocalization and Audition

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Distribution

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Behavior

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Morphology

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Evolution

Blair 1965; Bogart and Wasserman 1972; Hedges 1986; Maxson and Wilson 1975; Maxson et al. 1977; Mecham 1961; Ralin 1977, 1978; Ralin and Rogers 1979; Ralin and Selander 1979; Ralin et al. 1983; Romano and Vaughn 1986; Romano et al. 1987; Toivonen et al. 1983; Wiley 1982, 1983; Wiley et al. 1989.

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