1979 JOURNAL OF HERPETOLOGY 13(3):261–269

# A Morphological Analysis of a North American Diploid-Tetraploid Complex of Treefrogs (Amphibia, Anura, Hylidae)

Dennis B. Ralin and James S. Rogers

Department of Biology, Millikin University, Decatur, Illinois 62522 and
Department of Biological Sciences, University of New Orleans, New Orleans, Louisiana 70122

*ABSTRACT*—A multiple discriminant analysis of thirteen external morphological measurements is made on diploid *(Hyla chrysoscelis)* and tetraploid *(H. versicolor)* populations of a cryptic species complex of treefrogs. Eastern diploid populations (South Carolina, Georgia, Ohio, Mississippi) separate from western diploid populations (south-central Texas) when plotted on the first two discriminant function axes. Populations of *H. versicolor* occupy intermediate positions, but are not very distinct from the eastern populations of *H. chrysoscelis*. The same populations are indexed for two qualitative characteristics used to define the three formerly described subspecies in the complex. A bivariate plot of the indices shows a pattern similar to that of the discriminant function plot. The morphological analyses are consistent with non-morphological data in suggesting that there are two groups of populations of *H. chrysoscelis,* and that southern (Texas) populations of the tetraploid, *H. versicolor,* are intermediate. A principal components analysis of genetic (electrophoretic) data is compared with the morphological analyses. The three formerly described subspecies are discussed in relation to current morphological, genetic, and behavioral data that indicate differentiation within both the diploid and tetraploid species.

* * *

## INTRODUCTION

Objective documentation of the existence of two call types (fast-pulsed and slow-pulsed) within the nominal species *Hyla versicolor* was made by Blair (1958). Surprisingly high levels of incompatibility between the call types (Johnson, 1959, 1963; Ralin, 1976a) and female ability to discriminate for call (Littlejohn et al. 1960) confirmed that the call types are distinct species. Johnson (1966) named the slow-call species *H. versicolor,* because the type locality for the former subspecies *H. v. versicolor* (New York City) is well within the range of the slow-call species; and named the fast-call species *H. chrysoscelis* because the type locality of the former subspecies *H. v. chrysoscelis* (Dallas, Texas) is occupied by fast-call populations. The validity of these subspecies and a third subspecies named from south-central Texas, *H. v. sandersi* (type locality Somerset, Atascosa Co., Texas), was questioned because they did not coincide with either call-type (Johnson, 1966). Johnson (1966) also noted that a pair-wise comparison of morphological measurements failed to show any clearcut differences between the call-types.

When corrected for the effects of temperature, the pulse rates of *H. chrysoscelis* and *H. versicolor* do not overlap in samples from either the eastern, western or central portions of the range of the complex (Jaslow and Vogt, 1977; Ralin, 1968, 1977; Zweifel, 1970). Moreover, all specimens identified as *H. chrysoscelis* have proven to be diploid (2N = 24), whereas all specimens identified as *H. versicolor* have proven to be tetraploid (Bogart and Wasserman, 1972). Recently it has been shown that *H. chrysoscelis* populations from south-central Texas and from the eastern United States are behaviorally (Gerhardt, 1974; Ralin 1977) and genetically (Ralin, 1977; Ralin and Selander, 1979) differentiated. Moreover, populations of *H. versicolor* from Texas, which completely divide the range of *H. chrysoscelis* in the southern United States, are intermediate

between the two forms of *H. chrysoscelis* with respect to call duration and distribution of major electrophoretic alleles (Ralin, 1977; Ralin and Selander, 1979).

In light of the above developments, we thought that a multiple discriminant analysis of morphological measurements, using populations rather than species as the basic units, would provide a productive approach to elucidating relationships within this interesting complex. We also re-examine, on a population basis, two of the former subspecific characteristics least subject to alteration in preserved specimens. A numerical and phylogenetic analysis based on allele frequency data from the same populations (Ralin and Selander, 1979) is compared with the morphological analyses.

TABLE 1. Populations of *H. chrysoscelis* (WC and EC) and *H. versicolor* (VE) studied.

| Population Code | Locality | N |
|---|---|---|
| WC01 | Texas: near Fredericksburg, Gillespie Co. | 20 |
| WC03A | Texas: near Palmetto State Park, Gonzales Co. | 20 |
| WC03 | Texas: near Utley, Bastrop Co. | 23 |
| WC04 | Texas: near Elgin, Bastrop Co. | 20 |
| WC05 | Texas: Bastrop State Park, Bastrop Co. | 13 |
| VE05 | Texas: Bastrop State Park, Bastrop Co. | 20 |
| VE08 | Texas: Sam Houston National Forest, Montgomery Co. | 20 |
| VE08A | Texas: near Trawick, Nacogdoches Co. | 5 |
| EC10 | Mississippi: near Starkville, Oktibbeha Co. | 14 |
| EC10A | Ohio: Miami-Whitewater County Park, Hamilton Co. | 15 |
| EC11A | South Carolina: near Tillman, Jasper Co. | 20 |
| EC11B | Georgia: near Savannah, Bryan and Chatham Cos. | 6 |
| VE12 | Northeast: Harriman State Park, N.Y. and Alpine, New Jersey | 3 |

## MATERIALS AND METHODS

Specimens were collected from the localities listed in Table 1 during the breeding season in 1969. A maximum of twenty-three randomly chosen adult males per population were used for the morphological analysis. Specimens had been fixed in 10% formalin and stored in isopropanol. Sample sizes for the electrophoretic analyses are given elsewhere (Ralin and Selander, 1979). Samples of *H. chrysoscelis* from south-central Texas include one population from the western extremity of the range (WCO1), 3 populations within 50 km of sympatry with *H. versicolor* (WCO3, WCO3A, and WCO4), and one sympatric population (WCO5). Samples of *H. versicolor* include one sympatric with *H. chrysoscelis* in central Texas (VEO5), two allopatric populations from eastern Texas (VEO8 and VEO8A), and one small sample from the Northeast (VE12). Samples of *H. chrysoscelis* from the eastern United States consist of specimens from allopatric localities in Mississippi (EC1O), Ohio (EC1OA), and on the Atlantic Coastal Plain (EC11A and EC11B). All specimens have been deposited in the vertebrate collection of the University of Louisville, Louisville, Kentucky.

*Morphological analysis:* Thirteen external morphological measurements were made on each specimen: (1) snout-vent length, distance from tip of snout to dorsal edge of cloaca; (2) femur length, distance from right knee to lateral edge of cloaca; (3) shank length, distance from right knee to articulation of tibiofibula with tarsals; (4) foot length, distance from right tibiofibula-tarsal articulation to tip of second toe; (5) forearm length, distance from right elbow to articulation of radius and ulna with carpals; (6) upper arm length, distance from right elbow to articulation of humerus with pectoral girdle; (7) hand length, distance from articulation of right radius and ulna to tip of third finger; (8) toepad width; (9) head length, distance from tip of snout to posterior edge of tympanum; (10) eye-to-nostril-length, from right naris to anterior edge of eye; (11) nostril-to-lip length, perpendicular distance from right nostril to edge of upper lip; (12) tympanum diameter, horizontal width of right tympanum; and (13) head width, distance between corners of mouth. Measurements (8), (11), and (12) were made to the nearest 0.01 mm with ocular micrometer; all others were made to the nearest 0.1 mm with dial calipers.

The thirteen populations were subjected to multiple discriminant functions analysis on the thirteen morphological measurements using a modified version of Program DISCRM (Cooley and Lohnes, 1971). The eigenvectors were scaled so that the Euclidean distances among the centroids of the populations on the dicriminant axes are proportional to Mahalanobis' generalized distance (Rao, 1952). Generalized distances (D) were calculated for all pairs of populations using only the

eight statistically significant discriminant axes. The total $D^2$ that can be represented by only eight dimensions is thus maximized and variation which may be due simply to chance is eliminated (Gower, 1966; Rao, 1952). The matrix of generalized distances was subjected to cluster analysis by the unweighted pair-group method with arithmetic averages and by the Prim (shortest connected) network method (Sneath and Sokal, 1973). Both analyses were carried out with Program MINT written by F. James Rohlf, Department of Ecology and Evolution, State University of New York at Stony Brook. All data analyses were performed on the DECsystem—10 computer of the Computer Research Center, University of New Orleans.

Roger Barbour of the University of Kentucky sorted specimens, identified by number only, according to thigh pattern and degree of webbing between the fingers. The couplets used were modified from the subspecific descriptions and keys of earlier workers (Brown, 1950; Smith and Brown, 1947; Wright and Wright, 1949). For thigh pattern the alternatives were:

A. Rear of femur with heavy brown-black reticulations usually extending 2/3 or entire length of the femur; yellow-orange areas enclosed by reticulations large (> 1 mm) and irregularly shaped. (Scored as 1.0).

AA. Light black reticulation on rear of femur, if present, confined to anterior 1/3 of femur and enclosing small (< 1 mm) yellow-orange spots; spots, if present, on posterior 2/3 of femur circular or subcircular. (Scored as 0.0).

For finger webbing the alternatives were:

B. Fingers with noticeable webbing. (Scored as 1.0)

BB. Fingers without noticeable webbing. (Scored as 0.0)

Specimens which could not be readily placed in one category or the other for either characteristic received a score of .5 for that characteristic. An index for each characteristic was established by adding up the total score of all specimens examined in a given population, and dividing by the number of specimens. Thus for thigh pattern the possible index ranged from 0.0 (all specimens in the population in category AA) to 1.0 (all specimens in the population in category A). The population index for finger webbing was similarly constructed.

*Analysis of genetic data*—Rogers' (1972) genetic similarity coefficient (S) was calculated for each pair of populations from allele frequency data in Ralin and Selander (1979). Similarities were calculated once using nine polymorphic loci only and a second time with three additional monomorphic loci included. The matrix of similarities was subjected to cluster analysis, by the same method used with the generalized distances, and to principal coordinates analysis (Gower, 1966) with Program MINT.

*Comparison of generalized distance and genetic similarity*— The correlation between the matrices of generalized distance and genetic similarity was calculated. Also, the geographic distance between each pair of sampling sites was estimated from maps. The geographic distances and the genetic similarities were used as independent variables for a multiple regression analysis using the generalized distances as the dependent variable. The analyses were carried out with the multiple regression routine of the STATPACK package written by Richard Houchard of the Western Michigan University Computer Center.

## RESULTS

*Morphological analysis*—Statistical summaries of the measurement data are available on request (JSR). None of the groups of populations (eastern *H. chrysoscelis*, western *H. chrysoscelis*, or *H. versicolor*) were consistently different statistically from the other groups of populations for any one measurement.

The multiple discriminant functions analysis revealed that the first eight statistically significant discriminant functions accounted for 99% of the variance (Table 2). The first disciminant function axis, which accounted for approximately 52% of the variance, had heaviest contributions from head length, toe pad width, and hand length. Heaviest contributions on the second discriminant function

TABLE 2. Standardized weights (X100) and percentages of variance for the eight statistically significant discriminant functions.

| | Standardized Weights | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Measurements | DF 1 | DF 2 | DF 3 | DF 4 | DF 5 | DF 6 | DF 7 | DF 8 |
| Snout-vent | −11 | −22 | 28 | 39 | −10 | 25 | 1 | −62 |
| Femur | 2 | 20 | 30 | −33 | −9 | −58 | −53 | −8 |
| Shank | 0 | −53 | 9 | 27 | −4 | 9 | 13 | 52 |
| Foot | 35 | 34 | 48 | 4 | 24 | −16 | 13 | 10 |
| Forearm | 16 | 27 | −48 | −12 | −27 | −10 | 48 | −23 |
| Upper arm | −6 | 9 | 12 | −9 | 8 | −8 | 35 | 21 |
| Hand | −45 | 56 | −20 | −16 | −32 | 24 | 11 | 37 |
| Toe pad | −50 | 14 | −10 | 43 | 42 | −1 | −10 | −6 |
| Head length | 60 | 24 | −45 | 39 | 27 | 24 | −39 | 21 |
| Eye-to-nostril | −7 | −1 | 13 | −7 | −9 | 46 | 19 | −19 |
| Nostril-to-lip | 10 | 8 | 14 | −33 | −2 | 45 | −8 | 9 |
| Tympanum | −4 | 11 | 21 | 31 | −47 | −16 | −9 | −3 |
| Head width | −5 | −15 | −13 | −26 | 51 | 3 | 32 | −4 |
| % Variance | 52.4 | 17.5 | 11.2 | 5.4 | 4.8 | 3.0 | 2.7 | 2.0 |
| *P* | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.009 | 0.008 | 0.03 |

TABLE 3. Generalized distances among thirteen populations based on the eight statistically significant discriminant functions.

| | WC01 | WC03 | WC03A | WC04 | WC05 | VE05 | VE08 | VE08A | VE12 | EC10 | EC10A | EC11A | EC11B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WC01 | 0.00 | | | | | | | | | | | | |
| WC03 | 0.25 | 0.00 | | | | | | | | | | | |
| WC03A | 0.25 | 0.24 | 0.00 | | | | | | | | | | |
| WC04 | 0.35 | 0.37 | 0.18 | 0.00 | | | | | | | | | |
| WC05 | 0.32 | 0.37 | 0.22 | 0.13 | 0.00 | | | | | | | | |
| VE05 | 0.31 | 0.32 | 0.17 | 0.21 | 0.22 | 0.00 | | | | | | | |
| VE08 | 0.36 | 0.39 | 0.21 | 0.18 | 0.21 | 0.15 | 0.00 | | | | | | |
| VE08A | 0.29 | 0.34 | 0.15 | 0.18 | 0.19 | 0.09 | 0.13 | 0.00 | | | | | |
| VE12 | 0.32 | 0.36 | 0.25 | 0.28 | 0.27 | 0.24 | 0.25 | 0.25 | 0.00 | | | | |
| EC10 | 0.34 | 0.38 | 0.24 | 0.24 | 0.29 | 0.15 | 0.12 | 0.18 | 0.26 | 0.00 | | | |
| EC10A | 0.40 | 0.45 | 0.29 | 0.27 | 0.28 | 0.23 | 0.15 | 0.21 | 0.30 | 0.16 | 0.00 | | |
| EC11A | 0.30 | 0.32 | 0.21 | 0.25 | 0.26 | 0.16 | 0.20 | 0.19 | 0.20 | 0.16 | 0.19 | 0.00 | |
| EC11B | 0.33 | 0.35 | 0.19 | 0.23 | 0.22 | 0.13 | 0.16 | 0.11 | 0.22 | 0.19 | 0.17 | 0.14 | 0.00 |

axis were from hand length and shank length. On the third discriminant function axis major and approximately equal contributions were made by foot length, forearm length, and head length.

A matrix of generalized morphological distances is given in Table 3. Morphological relationships in the complex are close, ranging from 0.09 to a maximum distance of only 0.45. Largest distances are generally between eastern and western populations of *H. chrysoscelis*. Distances between eastern populations of *H. chrysoscelis* are small relative to the large geographic distances involved. Distances between Texas populations of *H. versicolor* are also small. Western populations of *H. chrysoscelis* are quite variable with respect to generalized distances.

Figure 1 plots all of the populations on the first two discriminant function axes. Except for some of the smaller samples, eastern populations of *H. chrysoscelis*, populations of *H. versicolor,* and western populations of *H. chrysoscelis* separate out on these two axes. Clearest separation is between the eastern and western populations of *H. chrysoscelis*. Populations of *H. versicolor* occupy intermediate positions, but are not very distinct from the eastern populations of *H. chrysoscelis*. Within the western population group of *H. chrysoscelis*, samples WCO1 and WCO3 are quite distinct from the other samples. The Prim (shortest connecting) network shows that the shortest connecting link for VE12, the sample of *H. versicolor* from the Northeast, is via the Coastal

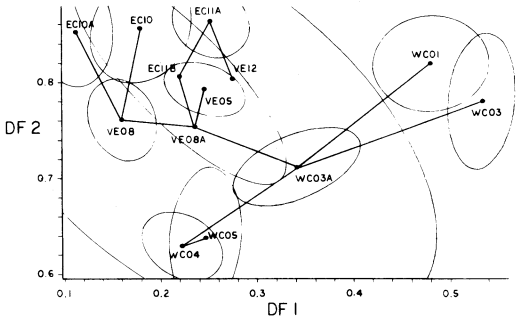


Figure 1. Ordination of thirteen *Hyla* populations on the first two discriminant axes. Solid dots are the centroids of the populations. Lines connecting the dots are a Prim network derived from the generalized distances of Table 3. The ellipses are the 95% confidences ellipses for the centroids (not for individual variation). The ellipse for population VE12 is not shown since it is beyond the boundaries of the graph.

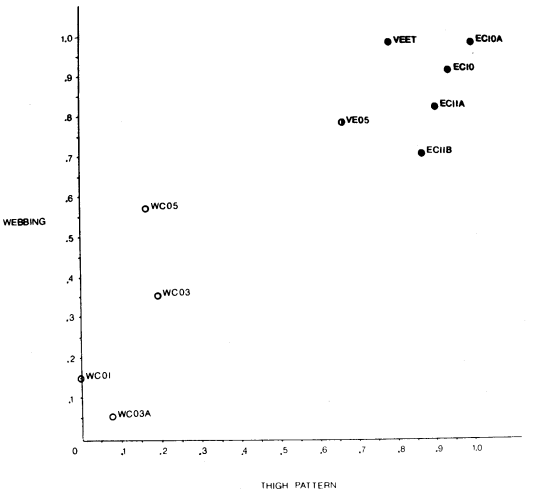


FIGURE 2. Ordination of populations of *H. chrysoscelis* and *H. versicolor* based on indices for thigh pattern and finger webbing. (VEET = VE08 + VE08A)

Plain populations of *H. chrysoscelis* (EC11A and EC11B) rather than through the other populations of *H. versicolor*.

The distributions of thigh pattern and finger webbing in populations of *H. chrysoscelis* and *H. versicolor* are given in Table 4. The distributions were used to construct indices for these two characteristics as described earlier. A bivariate plot of the indices for each population is given in Figure 2. The populations of *H. versicolor* plot between the eastern and western populations of *H. chrysoscelis*, but are closer to the eastern populations.

*Genetic analysis*—Allele frequency data for twelve enzyme loci (Ralin and Selander, 1979) were used to construct the matrix of genetic similarities (Table 5). Similarities are quite high, ranging between 0.66 and 0.98. In an attempt to accentuate differences among the populations, we re-ran the coefficients of genetic similarity using only the nine polymorphic loci (Table 5). Although this tended to lower the S values somewhat, they still remained fairly high (range 0.63–0.97).

In Figure 3 the populations are plotted on the two largest principal coordinate axes resulting from a principal coordinates analysis of the genetic data. The populations separate very distinctly into three groups, with the exception of VE12. One group consists of the five southcentral Texas

TABLE 4. Distribution of thigh pattern and finger webbing in populations of *H. chrysoscelis* (EC and WC) and *H. versicolor* (VE).

| | Thigh pattern | | | Webbing | |
|---|---|---|---|---|---|
| Population code | Reticulated | Not reticulated | Intermediate | Noticeable | Not noticeable |
| WC01 | 0 | 20 | 0 | 3 | 17 |
| WC03A | 0 | 17 | 3 | 1 | 19 |
| WC03 | 3 | 17 | 3 | 8 | 15 |
| WC05 | 2 | 10 | 0 | 7 | 5 |
| VE05 | 12 | 6 | 2 | 16 | 4 |
| VE08 + VE08A | 15 | 4 | 1 | 20 | 0 |
| EC10 | 13 | 1 | 0 | 13 | 1 |
| EC10A | 15 | 0 | 0 | 15 | 0 |
| EC11A | 17 | 2 | 0 | 16 | 3 |
| EC11B | 6 | 1 | 0 | 5 | 2 |

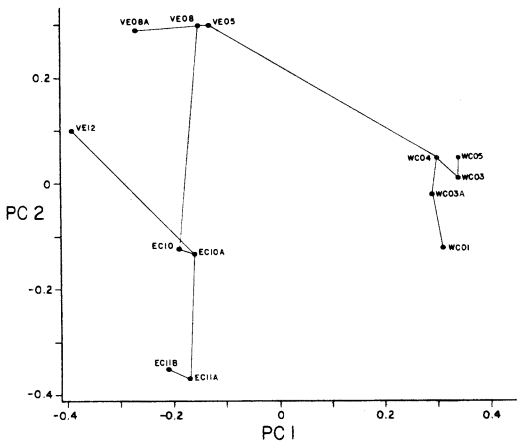


FIGURE 3. Ordination of 13 *Hyla* populations on the first two principal coordinates (Gower, 1966) derived from the matrix of genetic similarities calculated for nine polymorphic loci. Solid lines are a Prim network derived from the matrix of genetic similarities.

populations of *H. chrysoscelis*, with the Fredericksburg population (WCO1) from the western edge of the range most distinct; a second group consists of the three populations of *H. versicolor* from Texas; and the third group consists of the four populations of *H. chrysoscelis* from localities east of the Mississippi River. Within the latter group the Mississippi (EC10) and Ohio (EC10A) populations are particularly close to each other, as are the South Carolina (EC11A) and Georgia (EC11B) populations. On the Prim network the Texas populations of *H. versicolor* occupy intermediate positions. The sample of *H. versicolor* from the Northeast (VE12) is closer to the Ohio population of *H. chrysoscelis* than it is to the other populations of *H. versicolor.* However, on the basis of polymorphic loci, its average similarity to the other populations of *H. versicolor* is only slightly lower than its similarity to EC10A; and its average similarity to other populations of *H. versicolor* exceeds its average to all of the eastern populations of *H. chrysoscelis* (Table 5).

*Comparison of generalized distance and genetic similarity.*—A comparison of phenograms (Figs. 4 and 5) based respectively on cluster analysis of generalized distances ($D_m$) and genetic similarity (S) reveals few differences from the respective Prim networks of Figure 1 and 3. On the genetic similarity phenogram (Fig. 5) VE12 branches from the line leading to the other populations of *H. versicolor*, rather than branching from the line leading to eastern populations of *H. chrysoscelis*.

The multiple regression of D on S and geographic distance was not significant. Although the signs of the correlation coefficients were as expected (*D* vs. *S* negative; *D* vs. geographic distance positive), both correlations were small and non-significant.

## DISCUSSION

The analysis of the eight mensural characters (Fig. 1), the two qualitative characteristics (Fig. 2), and the genetic data (Fig. 3) are in substantial agreement. They indicate that three groups of

TABLE 5. Rogers' (1972) coefficient of genetic similarity (S) among the thirteen populations, based on nine polymorphic loci (below diagonal) and nine polymorphic and three monomorphic loci (above diagonal).

| | WC01 | WC03 | WC03A | WC04 | WC05 | VE05 | VE08 | VE08A | VE12 | EC10 | EC10A | EC11A | EC11B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WC01 | | 0.94 | 0.94 | 0.91 | 0.91 | 0.79 | 0.78 | 0.74 | 0.66 | 0.81 | 0.87 | 0.84 | 0.85 |
| WC03 | 0.93 | | 0.97 | 0.97 | 0.98 | 0.84 | 0.81 | 0.77 | 0.68 | 0.82 | 0.87 | 0.82 | 0.83 |
| WC03A | 0.94 | 0.96 | | 0.97 | 0.95 | 0.84 | 0.83 | 0.78 | 0.71 | 0.84 | 0.89 | 0.84 | 0.85 |
| WC04 | 0.90 | 0.96 | 0.96 | | 0.96 | 0.85 | 0.84 | 0.79 | 0.70 | 0.83 | 0.89 | 0.82 | 0.83 |
| WC05 | 0.90 | 0.97 | 0.95 | 0.96 | | 0.84 | 0.81 | 0.76 | 0.69 | 0.81 | 0.87 | 0.81 | 0.82 |
| VE05 | 0.77 | 0.82 | 0.82 | 0.84 | 0.82 | | 0.95 | 0.89 | 0.81 | 0.85 | 0.88 | 0.80 | 0.82 |
| VE08 | 0.75 | 0.79 | 0.81 | 0.81 | 0.79 | 0.94 | | 0.92 | 0.82 | 0.86 | 0.89 | 0.81 | 0.83 |
| VE08A | 0.70 | 0.74 | 0.75 | 0.76 | 0.73 | 0.88 | 0.91 | | 0.80 | 0.85 | 0.88 | 0.79 | 0.82 |
| VE12 | 0.63 | 0.65 | 0.68 | 0.68 | 0.66 | 0.80 | 0.81 | 0.78 | | 0.82 | 0.87 | 0.82 | 0.84 |
| EC10 | 0.79 | 0.80 | 0.83 | 0.81 | 0.79 | 0.83 | 0.84 | 0.83 | 0.81 | | 0.97 | 0.90 | 0.92 |
| EC10A | 0.80 | 0.81 | 0.84 | 0.83 | 0.80 | 0.82 | 0.83 | 0.82 | 0.81 | 0.96 | | 0.90 | 0.92 |
| EC11A | 0.79 | 0.76 | 0.78 | 0.76 | 0.74 | 0.73 | 0.74 | 0.72 | 0.74 | 0.88 | 0.88 | | 0.94 |
| EC11B | 0.77 | 0.75 | 0.77 | 0.74 | 0.72 | 0.73 | 0.75 | 0.73 | 0.77 | 0.88 | 0.87 | 0.94 | |

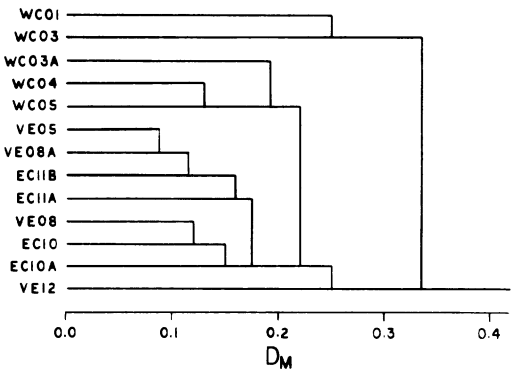


FIGURE 4. Phenogram generated by the unweighted pair-group method with arithmetic averages derived from the matrix of generalized distances ($D_M$) in Table 3.

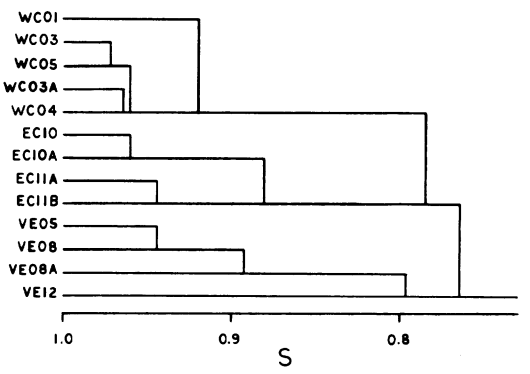


FIGURE 5. Phenogram generated by the unweighted pair-group method with arithmetic averages derived from the matrix of genetic similarities above the diagonal in Table 5.

populations can be discerned in the complex: one group consisting of the five southcentral Texas populations of *H. chrysoscelis*; a second group consisting of the three Texas populations of *H. versicolor*; and a third group consisting of the four eastern populations of *H. chrysoscelis*.

However, the details vary. Both the qualitative characteristics and the morphometric analysis plot the Texas populations of *H. versicolor* closer to the eastern populations of *H. chrysoscelis*. The overall genetic analysis plots the Texas populations of *H. versicolor* more nearly equidistant between the western populations of *H. chrysoscelis* and the two westermost populations of eastern *H. chrysoscelis*. Call durations shows a similar pattern of three groups of populations (Ralin, 1977), but Texas populations of *H. versicolor* are somewhat closer to the western populations of *H. chrysoscelis*.

It could be argued that more weight should be given to the biochemical data, since they are closer to the gene level than are any of the other data. In all four data sets we are dealing with rather small differences, and the mathematical treatments are such that the differences in detail are not statistically significant. For example, the biochemical data consist of the series of *S* values calculated from allele frequency distributions at twelve loci, and the patterns of variation differ from locus to locus (Ralin and Selander, 1979). At four loci all three groups of populations share one allele in major frequency, but additional alleles at polymorphic frequencies are found in only one of the diploid groups and in Texas populations of *H. versicolor (Sod-1, Pgi-2, Got-2,* and *Lda)*. Two loci are virtually clinal across the three groups with respect to the major allele and minor alleles *(Mdh-1* and *Pgm-2);* and at two other loci different minor alleles appear only in the easternmost and westernmost populations of *H. chrysoscelis (Idh-1* and *Idh-2)*. In most cases the confidence limits for the allele frequency estimates (see sample sizes in Ralin and Selander, 1979) for any one population are large enough to include most of the other diploid and tetraploid populations in the complex. It is only at the *Ldb* locus that Texas populations of *H. versicolor* appear to be truly intermediate to all populations of eastern and western *H. chrysoscelis*, because alternative alleles apparently fixed in eastern and western diploid populations are found at intermediate frequencies in the Texas tetraploid populations (Ralin and Selander, 1979).

Maxson et al. (1977) applied the immunological technique of micro-complement fixation to the serum albumins of 3-5 individuals of *H. chrysoscelis* from the same populations that were studied here and by Ralin and Selander (1979). In a striking parallel to the *Ldb* locus, they found that the albumins of eastern and western *H. chrysoscelis* were different, that some *H. versicolor* from Texas had albumins indistinguishable from the western type, and the majority were apparent heterozygotes. However, see Ralin (1978) and Ralin and Selander (1979) for critiques of the interpretations and conclusions drawn from these data, particularly the claims that *H. versicolor* is an allopolypoid and that eastern and western *H. chrysoscelis* merit specific status. The latter inference contradicted the data presented (Maxson et al., 1977), since two of two individuals of *H. chrysoscelis* from a

population in extreme eastern Texas, nearly sympatric with *H. versicolor*, were both albumin heterozygotes.

LeConte (1825) named *H. versicolor* from the New York City vicinity, well within the range of the tetraploid species presently called *H. versicolor*. The smooth-skinned subspecies *H. femoralis chrysoscelis* was described by Cope (1880) from a specimen collected in north-central Texas near Dallas. Strecker (1910) pointed out that this specimen was a smoother-skinned form of *H. versicolor*, and that the reticulation pattern on the rear of the femur that Cope (1880) described was quite typical of western examples of *H. versicolor*. He agreed that *H. versicolor chrysoscelis* was worthy of subspecific status. The third subspecies, *H. v. sandersi,* was applied to south-central Texas populations (Smith and Brown, 1947). Brown (1950) stated that *chrysoscelis-sandersi* intergrades could be found along the Balcones fault line in south-central Texas, and that many specimens in extreme eastern Texas appeared to be *H. v. chrysoscelis—H. v. versicolor* intergrades. These areas are now known to be areas of parapatry or sympatry respectively between the western diploid and Texas tetraploid and eastern diploid and Texas tetraploid. (Ralin, 1968, 1977; Ralin and Selander, 1979).

The application of the modern techniques of electrophoresis, sound spectrographic analysis, micro-complement fixation, chromosome analysis and multivariate statistics has begun to clarify the status of this interesting complex, although much remains to be done. The earlier workers who used a combination of intuition and subjective treatment of qualitative characteristics appear to have been pointed in the right direction. It seems probable that the diploid populations in north-central and extreme eastern Texas are intergrades between the eastern and western diploid forms ("eastern" and "western" *H. chrysoscelis*, Ralin, 1976b). Since Texas populations of the tetraploid ("southern" *H. versicolor*, Ralin 1976b) appear to have been derived from a genetically intermediate population of *H. chrysoscelis* (Ralin and Selander, 1979), the inclusion of diploid intergrades and Texas tetraploids under the name of *H. v. chrysoscelis* on the basis of qualitative morphological characteristics is now understandable. Since a small northeastern sample of the tetraploid ("northern" *H. versicolor*, Ralin, 1976b) exhibits biochemical (Maxson et al., 1977; Ralin and Selander, 1979), behavioral (Ralin, 1977) and morphological (this study) parallelism or convergence with eastern *H. chrysoscelis*, the inclusion of these two forms under the name *H. v. versicolor* is also understandable. At this point it would be premature to advocate subspecific rank for either the two forms of *H. versicolor* or the two forms of *H. chrysoscelis*. Most populations of *H. chrysoscelis* in the Midwest, border states, and western portions of the South (western Mississippi, Louisiana, extreme eastern Texas) may prove to be intermediate or intergrade populations. The populations of *H. versicolor* in the northern Midwest and Appalachian region may also resemble Texas populations of *H. versicolor* more than they resemble northeastern populations.

## ACKNOWLEDGMENTS

We thank Sylvia S. Colbert for providing valuable technical assistance. Roger Barbour (University of Kentucky) sorted the specimens on the basis of thigh pattern and finger webbing, a task for which we are deeply grateful. Computer time was provided by the Computer Research Center, University of New Orleans.

## LITERATURE CITED

Blair, W. F. 1958. Mating call in the speciation of anuran amphibians Amer. Nat. 42:27–51.

Bogart, J. P. and A. O. Wasserman. 1972. Diploid-polyploid species pairs: a possible clue to evolution by polyploidization in anuran amphibians. Cytogenetics 11:7–24.

Brown, B. C. 1950. An annotated checklist of the reptiles and amphibians of Texas. Baylor University Press, Waco, Texas. 257 p.

Cooley, W. W. and R. R. Lohnes. 1971. Multivariate data analysis. John Wiley and Sons, New York. 364 p.

Cope, E. D. 1880. On the zoological position of Texas. Bull. U.S. Nat. Mus. No. 17:1–51.

Gerhardt, H. C. 1974. Mating call differences between eastern and western populations of the treefrog *Hyla chrysoscelis.* Copeia 1974:534–536.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325-338.

Jaslow, A. P. and R. C. Vogt. 1977. Identification and distribution of *Hyla versicolor* and *Hyla chrysoscelis* in Wisconsin. Herpetologica 33:201–205.

Johnson, C. 1959. Genetic incompatibility in the call races of *Hyla versicolor* LeConte in Texas. Copeia 1963:327–335.

————. 1963. Additional evidence of sterility between the call types in the *Hyla versicolor* complex. Copeia 1963:139–143.

————. 1966. Species recognition in the *Hyla versicolor* complex. Texas J. Sci. 18:361–364.

LeConte, J. 1825. Remarks on the American species of the genera *Hyla* and *Rana*. Annals Lyc. Nat. N.Y. 1:278–282.

Littlejohn, M. J., M. J. Fouquette Jr. and C. Johnson. 1960. Call discrimination by female frogs of the *Hyla versicolor* complex. Copeia 1960:47–49.

Maxson, L. R., E. Pepper and R. D. Maxson. 1977. Immunological resolution of a diploid-tetraploid species complex of treefrogs. Science 197:1012–1013.

Ralin, D. B. 1968. Ecological and reproductive differentiation in the cryptic species of the *Hyla versicolor* complex. Southwestern Natur. 13:283–299.

————. 1976a. Comparative hybridization of a diploid-tetraploid cryptic species pair of treefrogs. Copeia 1976:191–196.

————. 1976b. Behavioral and genetic differentiation in a diploid-tetraploid species complex of treefrogs. Herp. Rev. 7:97–98.

————. 1977. Evolutionary aspects of mating call variation in a diploid-tetraploid species complex of treefrogs (Anura). Evolution 31:721–736.

————. 1978. "Resolution" of diploid-tetraploid treefrogs. Science 202:335–336.

Ralin, D. B. and R. K. Selander. 1979. Evolutionary genetics of diploid-tetraploid sibling species of treefrogs of the genus *Hyla.* Evolution, in press.

Rao, C. R. 1952. Advanced statistical methods in biometric research. John Wiley and Sons, N.Y. 390 p.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Stud. Genet. VII. Univ. Texas Publ. 7213:145–153.

Smith, H. M. and B. C. Brown. 1947. The Texas subspecies of the treefrog, *Hyla versicolor.* Proc. Biol. Soc. Wash. 60:47–50.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco. 573 p.

Strecker, J. K. 1910. Description of a new solitary spadefoot *(Scaphiopus hurterii)* from Texas, with other herpetological notes. Proc. Biol. Soc. Wash. 23:115–122.

Wright, A. H. and A. A. Wright. 1949. Handbook of frogs and toads of the United States and Canada. Comstock Publ. Co., Ithaca, N.Y. 640 p.

Zweifel, R. G. 1970. Distribution and mating call of the treefrog, *Hyla chrysoscelis,* at the northeastern edge of its range. Chesapeake Sci. 11:94–97.

Accepted 5 Apr 1979