A Morphometric Comparison of Gray Treefrogs, *Hyla chrysoscelis* and *H. versicolor*, from Ohio[1]

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ABSTRACT. **Thirteen morphometric characters were compared between two cryptic species of gray treefrogs from Ohio. Discriminant function analysis using snout-vent length, shank length, and thigh length correctly classified 76.7% of diploid *Hyla chrysoscelis* and tetraploid *H. versicolor*. Ohio diploids are more similar in size to those in Texas than to those of Michigan and Wisconsin or southeastern states. Tetraploid treefrogs in Ohio are intermediate in size to those of Texas, North Carolina, and Wisconsin. Latitudinal and longitudinal clinal variation in snout-vent length is apparent in both species.**

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INTRODUCTION

Several investigations have addressed morphometric differences between the two currently recognized species within the North American complex of gray treefrogs. Tetraploid *Hyla versicolor* averages slightly larger but is otherwise indistinguishable from its diploid progenitor *H. chrysoscelis*. The two species can be differentiated by measuring nuclear DNA content (Bachman and Bogart 1975), chromosome number (Wasserman 1970), by LDH electrophoresis (Ralin and Selander 1979), and by comparison of the temperature corrected pulse rate of the advertisement call (Zweifel 1970). Johnson (1961) measured three characters on sexually mature individuals and showed that morphometric differences between species exist. Tetraploid male *H. versicolor* were significantly larger on average than diploid male *H. chrysoscelis* based upon snout-vent length (SVL) and ratio of tibia length/femur length. However, overlap in size range between species precluded their differentiation based upon these characters. Ralin (1968) also found overlap between species in SVL, and could not use SVL to separate species in Texas. Jaslow and Vogt (1977) noted a statistically significant difference in SVL between diploids and tetraploids in Wisconsin. However, Little (1983) did not detect differences using discriminant function comparisons of nine "size-free" morphological variables between West Virginia and south-central Ohio populations of *H. chrysoscelis* and *H. versicolor*.

Ralin and Rogers (1979) used discriminant function analysis of 13 morphometric characters on individuals from 12 populations of *H. chrysoscelis* and *H. versicolor* to separate eastern diploid populations (eastern Texas, South Carolina, Georgia, Ohio, and Mississippi) from western diploid populations (central Texas). Southern tetraploid populations (Texas) were intermediate between eastern and western diploid populations but were more similar to eastern diploids. Ralin and Rogers (1979) suggested that populations of *H. chrysoscelis* in the Midwest may be intergrade populations between eastern and western diploids and that Appalachian and northern midwestern populations of *H. versicolor* may resemble tetraploid populations from Texas (southern *H. versicolor*) more than those in the Northeast (New York, New Jersey). The purposes of the present research were to determine the degree of accuracy with which gray treefrogs in Ohio could be classified using morphometric characters, and to compare Ohio material with that available from other portions of the distributional range of the complex.

MATERIALS AND METHODS

Collections of 72 diploid and 100 tetraploid male treefrogs were made between May and July 1985 to 1987 from 13 localities in nine counties of Ohio. Collecting localities approximated a transect extending from Ashtabula County in the northeast to Clermont and Preble Counties in the southwest (Matson 1988). Calling males were identified by sound spectrographic analysis of the temperature corrected pulse rate of the advertisement call. Thirteen external morphological measurements were made on each preserved treefrog, and each measurement was made three times on different days in an attempt to minimize measuring errors. Characters selected are similar to those described by Ralin and Rogers (1979) and were measured on the right side. They are defined as follows: 1) snout-vent length (SVL), distance from tip of snout to posteriodorsal edge of the cloaca; 2) thigh length, distance from lateral edge of vent to knee; 3) shank length, the distance from knee to articulation of tibio-fibula with fibulare; 4) foot length, distance from articulation of tibio-fibula with fibulare to tip of the fourth (longest) toe; 5) upper arm length, distance from articulation of humerus with pectoral girdle to the elbow; 6) forearm length, distance from elbow to articulation of radio-ulna with the carpals; 7) hand length, distance from articulation of radio-ulna with carpals to tip of the third (longest) finger; 8) toepad width, width of toepad on the third finger; 9) head length, distance from tip of snout to posterior edge of rim surrounding the tympanum; 10) eye-to-nostril length, distance from naris to anterior corner of the eye; 11) nostril-to-lip length, vertical distance from nostril to edge of the upper lip; 12) tympanum diameter, horizontal width of tympanum; and 13) head width, widest measurement of the head, usually distance between corners of the mouth. Measurements 8, 11 and 12 were measured to the nearest 0.01 mm with an ocular micrometer; all other

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measurements were made to the nearest 0.1 mm with a dial caliper.

Mean values of the three data sets were used in all analyses. The Student's *t*-statistic was used to compare means for each character between species. Direct and Mahalanobis methods of discriminant function analysis (DFA) were used to derive an unstandardized canonical discriminant classification function. Normality tests for skewness and kurtosis were significant for three characters in *H. versicolor* and for two different characters in *H. chrysoscelis*; however, skewness was detected only in shank length in *H. chrysoscelis* among characters used in the direct discriminant function presented here. Skewness of the shank measurement was considered to not adversely affect the homogeneity of variance assumption of DFA because the level of significance was only slightly greater than 0.05 ($P > 0.05$). DFA was run on an IBM 3081D mainframe computer using the program Discriminant by SPSS, Inc. (1983). The F to enter and F to remove equalled 1.7, the approximate F-value required for significance with $P = 0.01$. Specimens were deposited in the herpetology collection of The Cleveland Museum of Natural History.

RESULTS

Differences between species were small for the characters measured, and comparison of means between species using the Student's *t*-statistic was significant ($P < 0.05$) for hand length, head width, nostril-to-lip, and SVL only (Table 1). Few females of either species were obtained in the field (n = 2 *H. chrysoscelis*; n = 5 *H. versicolor*); they averaged larger than males in both species but were few in number and were excluded from DFA.

TABLE 1

Mean and standard deviation (mm) for 13 morphometric characters of calling males used in the study of Ohio H. chrysoscelis *(n = 72) and* H. versicolor *(n = 100).*

	H. chrysoscelis		*H. versicolor*	
Character	Mean	S.D.	Mean	S.D.
Thigh	19.56	1.36	19.97	1.57
Shank	19.29	1.28	19.00	1.54
Foot	29.30	1.70	29.72	2.38
Forearm	9.31	0.88	9.22	0.94
Upperarm	11.17	0.91	11.24	1.01
Hand*	12.17	0.82	12.65	0.92
Toepad	2.47	0.32	2.52	0.27
Head length	13.20	0.67	13.41	0.88
Head width*	15.40	0.84	15.80	1.08
Tympanum	2.95	0.30	2.85	0.34
Eye-nostril	3.88	0.32	3.93	0.33
Nostril-lip*	3.08	0.25	3.17	0.31
SVL*	43.61	2.50	44.91	3.05

* Comparison of means between species was significant using the Student's *t*-test for unequal n ($P < 0.05$).

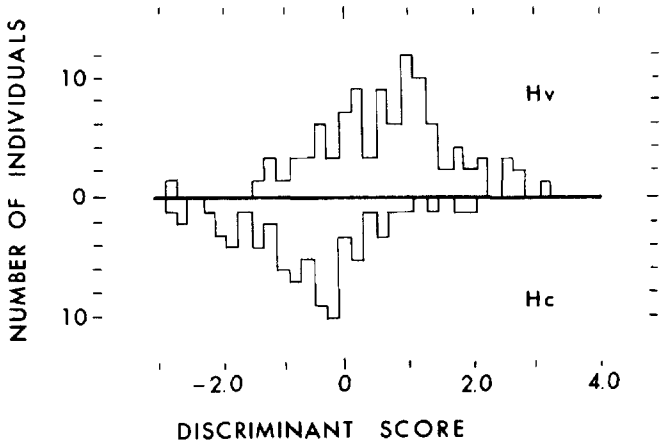

FIGURE 1. Frequency distribution of discriminant scores used in classification of Ohio *Hyla chrysoscelis* and *H. versicolor* based upon thigh length, shank length, and SVL. The midpoint between group centroids (0.01) is the point separating the two species. Nearly 77% were classified correctly using the discriminant function.

Mahalanobis stepwise variable selection incorporated six of 13 characters entered into the analysis and correctly classified 77.9% of sample treefrogs. However, three characters were not normal, and assumptions of the homogeneity of variances of DFA were not met. The next highest percent correct classifications (76.7%) was achieved by direct analysis using thigh length, shank length, and SVL. Similar analysis substituting hand length for thigh length classified 75.6% of the treefrogs correctly. All other combinations produced greater classification error. The unstandardized canonical discriminant function $Z = 0.64T - 1.41S + 0.45SVL - 5.53$ where T = thigh length, S = shank length, and SVL when compared with 0.10, the midpoint between group centroids, correctly classified 80.6% of *H. chrysoscelis* and 74.0% of *H. versicolor* examined (Fig. 1). Frogs with discriminant scores lower than 0.10 would be classified as *H. chrysoscelis*, whereas those with higher scores would be identified as H. versicolor.

DISCUSSION

Because of the morphometric similarity between species, no single morphometric character or array of morphometric characters has been detected that accurately separate geographically variable *H. chrysoscelis* and *H. versicolor*. Jaslow and Vogt (1977) were able to correctly classify 74% of gray treefrogs from Wisconsin by measuring SVL alone. In Ohio, only 54.1% of the treefrogs measured were correctly identified using SVL. The numbers of individuals in samples obtained by Bogart and Jaslow (1979) were inadequate to draw conclusions about the size of *H. chrysoscelis* based on SVL. However, they stated that Michigan *H. chrysoscelis* appeared more similar in size to those of Wisconsin (Jaslow and Vogt 1977) than to those of Texas (Ralin 1977). *Hyla versicolor* in Wisconsin and Michigan are similar in size to those reported in Texas by Ralin (1968) and do not appear to exhibit the decrease in size from southern to northern latitudes apparent in *H. chrysoscelis* (Bogart and Jaslow 1979).

Hyla chrysoscelis males in Ohio are morphometrically intermediate to those measured in Texas (Johnson 1961, Ralin 1968) and eastern states (Johnson 1961) and to those

TABLE 2

Comparison of mean snout-vent length (SVL) in Hyla chrysoscelis *and* H. versicolor *throughout portions of their respective distributional ranges. M = male; F = female. Sample number in parentheses.*

	SVL (mm)		
Locale (Sex)	*H. chrysoscelis*	*H. versicolor*	Reference
Texas (M)	42.2 (187)	49.8 (104)	Johnson 1961*
Texas (F)	47.6 (45)	53.4 (26)	Johnson 1961*
Texas (M)	40.5	42.5	Bogart & Jaslow 1979 extrapolated from Ralin 1968
Oklahoma (M)	40.1 (17)	52.4 (57)	Johnson 1961*
Oklahoma (F)	50.4 (4)	54.9 (10)	Johnson 1961*
Missouri (M)	46.0 (5)		Johnson 1961*
Arkansas (M)	45.9 (4)		Johnson 1961*
North Carolina (M)	47.3 (47)	52.9 (54)	Johnson 1961*
North Carolina (F)	51.2 (4)	54.5 (8)	Johnson 1961*
Wisconsin (M)	35.2 (25)	42.6 (26)	Jaslow & Vogt 1977
Ohio (M)	43.6 (72)	44.9 (100)	Present study
Ohio (F)	52.2 (2)	50.4 (5)	Present study

*Average of means from the sites described by Johnson 1961.

measured in Wisconsin (Jaslow and Vogt 1977, Table 2). Ralin (1976, 1977) referred to *H. chrysoscelis* of central Texas as "western" *H. chrysoscelis* and to those from eastern Texas to the Atlantic coast as "eastern" *H. chrysoscelis*, based upon advertisement call analysis and LDH electrophoresis. Hamilton County, OH treefrogs were included in the latter grouping (Ralin 1977, Ralin and Rogers 1979). Sound spectrographic analysis of advertisement calls of Michigan and Wisconsin *H. chrysoscelis* suggest that the diploid is the western form (Bogart and Jaslow 1979, Jaslow and Vogt 1977). In a later publication, Ralin et al. (1983) suggested that the geographic location of the origin of the tetraploid may have been from an intermediate population of *H. chrysoscelis* west of Ohio, north of Mississippi, and east of central Texas. Based upon electrophoretic evidence, populations of *H. chrysoscelis* occurring in the Midwest states may be intergrades between eastern and western forms (Ralin et al. 1983). Accumulated morphometric data, based primarily upon SVL of males, indicate that mean SVL of the diploid in Ohio is intermediate in size between the western form which averages smaller and the far eastern frogs which average larger (Table 2).

By using the mean extrapolated from Ralin (1968), Bogart and Jaslow (1979) showed that male *H. versicolor* from Wisconsin are nearly equal in SVL to those in Texas. However, the mean SVL from Ralin (1968) is much smaller than that determined from data of Johnson (1961). *H. versicolor* exhibits a latitudinal cline in size from Texas through Ohio to Wisconsin as does *H. chrysoscelis* (Table 2). Tetraploid populations in North Carolina average larger than those in Ohio. Ohio tetraploids, then, are intermediate in size to those in Texas, North Carolina, and Wisconsin.

Morphometric differences between *H. chrysoscelis* and *H. versicolor* in Ohio are among the smallest reported, and classification based solely upon this method of identification must be regarded as predictive and not confirmative. The classification discriminant function derived here has not yet been tested on many frogs from other than the original data sets and, because of apparent geographic variation in size in both species, is expected to have limited, regional applicability. Also, the discriminant function was derived from advertising males and should not be expected to differentiate between individuals that have not attained sexual maturation or between females. The method probably has greatest utility applied to preserved material where nucleoli count and size (Cash and Bogart 1978, Little 1983) and toepad cell size (Green 1980) are the predictive taxonomic methods available.

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LITERATURE CITED

Bachman, K. and J. P. Bogart 1975 Comparative cytochemical measurements in the diploid-tetraploid species pair of hylid frogs *Hyla chrysoscelis* and *H. versicolor*. Cytogenet. Cell Genet. 15: 186–194.

Bogart, J. P. and A. P. Jaslow 1979 Distribution and call parameters of *Hyla chrysoscelis* and *Hyla versicolor* in Michigan. Life Sci. Contri. Royal Ontario Mus. 117: 1–13.

Cash, M. N. and J. P. Bogart 1978 Cytological differentiation of the diploid-tetraploid species pair of North American treefrogs (Amphibia, Anura, Hylidae). J. Herpetol. 12: 555–558.

Green, D. M. 1980 Size differences in adhesive toe-pad cells of treefrogs of the diploid-polyploid *Hyla versicolor* complex. J. Herpetol. 14: 15–19.

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

Johnson, F. C. 1961 Cryptic speciation in the *Hyla versicolor* complex. Ph.D. Dissertation, Univ. of Texas, Austin, TX. 121 p.

Little, M. L. 1983 The zoogeography of the *Hyla versicolor* complex in the central Appalachians, including physiological and morphological analyses. Ph.D. Dissertation, University of Louisville, Louisville, KY. 94 p.

Matson, T. O. 1988 The *Hyla chrysoscelis* - *H. versicolor* complex in Ohio. Ph.D. Dissertation, Kent State University, Kent, OH. 154 p.

Ralin, D. B. 1968 Ecological and reproductive differentiation in the cryptic species of the *Hyla versicolor* complex (Hylidae). Southwest. Nat. 13: 283–299.

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

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

———— and J. S. Rogers 1979 A morphological analysis of a North American diploid-tetraploid complex of treefrogs (Amphibia, Anura, Hylidae). J. Herpetol. 13: 261–269.

———— and R. K. Selander 1979 Evolutionary genetics of diploid-tetraploid species of treefrogs of the genus *Hyla*. Evolution 33: 595–608.

————, M. H. Romano, and C. W. Kilpatrick 1983 The tetraploid treefrog *Hyla versicolor*: Evidence for a single origin from the diploid *H. chrysoscelis*. Herpetologica 39: 212–225.

SPSS, Inc. 1983 SPSSX user's guide. McGraw-Hill Book Co., New York, NY. 806 p.

Wasserman, A. O. 1970 Polyploidy in the common tree toad *Hyla versicolor* LeConte. Science 167: 385.

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