Speciation within the Chorus Frog *Pseudacris triseriata*: Morphometric and Mating Call Analyses of the Boreal and Western Subspecies

**James E. Platz**

Data from mating call and morphological analyses of the boreal and western subspecies of the chorus frog *Pseudacris triseriata* were obtained along a transect from South Dakota to Oklahoma. Two call types are evident. At the northern end of the transect calls are long and low in pulse rate. Both traits had low variability relative to populations further south. The southern end of the transect contained populations with a much shorter call and pulse rates approximately twice those in the north. Identical long and short call types are evident in Colorado as well. Limited data from Oklahoma indicates that the call of *P. triseriata feriarum* is more like that of *P. t. maculata*. Multivariate stepwise discriminant analyses performed on data sets containing only morphological measurements produced two clusters of populations which correspond on geographic grounds to two currently recognized subspecies, *P. t. maculata* and *P. t. triseriata*. When call data are included, the same two clusters are identified suggesting concordance between call type and morphology. The data indicate that the two entities represent separate species and that the zone of overlap is much more extensive than previously depicted.

*Pseudacris triseriata* (Wied) is one of the smallest North American hyliids. According to Tordoff and Pettus (1977), some live in excess of 6 yr. In eastern Colorado, Spencer (1964) found it to be an anuran of limited vagility with few individuals moving more than 750 m from the hatch site. It is also the most widespread species of chorus frog in North America, occurring from the Gulf of Mexico to New York and southern Ontario in the east, and from parts of Texas and Arizona in the southwest, north to Alberta and the Northwest Territory in Canada.

Conant (1975) recognized four subspecies of *P. triseriata*. Three of these have extensive ranges. The Boreal Chorus Frog, *P. triseriata maculata*, occupies the northwestern limits of the range: from northwestern Ontario, east to Winnipeg in Manitoba, and south to New Mexico, and east including parts of Nebraska, South Dakota, Minnesota and Wisconsin. *Pseudacris t. triseriata*, the Western Chorus Frog, replaces *maculata* in the south resulting in extensive sympatry in a broad arc extending through most of Nebraska, eastern South Dakota, central Minnesota and northern Wisconsin and Michigan. Along its southern limit, the western form overlaps with and is then replaced by the Upland Chorus Frog, *Pseudacris triseriata feriarum*: common over most of the Gulf Coast states, extending north along the Atlantic as far as New Jersey. *Pseudacris t. halmi*, the New Jersey Chorus Frog, is restricted in range, occurring in parts of Delaware, Maryland, southern New Jersey, New York and eastern Pennsylvania. According to Conant (1975) it intergrades with *P. t. feriarum* in eastern Pennsylvania and at Staten Island, New York.

The criteria used to distinguish these four subspecies hinge, to a large degree, on variable dorsal skin patterns. The boreal form has shorter legs and often a dorsal pattern of spots in three longitudinal rows distinguishing it from the Western form which has longer legs and three well defined dorsal stripes. The Upland Chorus Frog has thin broken stripes differentiating it from the New Jersey Chorus Frog, which has broad well defined stripes.

Although the present nomenclature has remained stable for the past 25 yr, the literature reveals an earlier history of repeated revision. Since the original description of *Hyla triseriata* (now *P. t. triseriata*) by Wied (1839), four different generic names have been applied, as have numerous specific epithets for this and other subspecies now recognized as *P. triseriata*. For detailed reviews see Smith (1956), Harper (1955), Schwartz (1957) and Cook (1964).

The first serious efforts to understand geographic variation within *P. triseriata* were those
of Smith and Smith (1952) and Smith (1956). At that time boreal, western and upland forms were considered subspecies of *P. nigrita*. Smith (1956) reclassified the Boreal Chorus Frog (*P. nigrita septentrionalis*) as *P. n. maculata*. Based on differences in: 1) breeding habitat; 2) the distinct nature of mating calls; and 3) the lack of intergradation among sympatric populations of *P. n. nigrita* and *P. n. feriarum*, Schwartz (1957) recommended specific status for *P. n. feriarum* as *P. triseriata feriarum*. He further suggested that *P. n. triseriata* and *P. n. maculata* should be treated as subspecies of *P. triseriata* (i.e., *P. t. triseriata* and *P. t. maculata*, respectively). This recommendation is based on evidence interpreted as intergradation between *P. t. feriarum* and *P. t. triseriata* reported by Smith (1956). Conant (1975) followed Schwartz (1957). For convenience each of the four currently recognized subspecies of *P. triseriata* will be referred to in this paper as *maculata*, *triseriata*, *feriarum*, or *kalmi*.

Smith's (1956) comparison of *maculata* and *triseriata* is critical to our current knowledge of *Pseudacris*. It represents the only published study of morphological variation, based on large numbers of specimens collected over a wide geographic area. His conclusions rely on examination of over 800 individuals, including 403 *maculata* from 112 localities in Canada and the United States, and 343 *triseriata* from 62 localities (Smith and Smith, 1952) as well as 125 presumed intergrades from 42 locations. Based on his examination and measurements of these specimens, he differentiated present day *maculata* from *triseriata* in more specific terms than Conant (1975): *maculata* “differs by the proportionately shorter tibia, femur, and foot; relatively shorter and narrower head; proportionately heavier body; and by the higher frequency of specimens with a spotted or mottled pattern.” Examination of his table I indicates 343 *maculata* and 430 *triseriata* were used to determine tibia-body length ratios. The resulting mean values, given as percent of body length, were 39.3 and 42.6, respectively. Computation of confidence limits for the means (using his published SE of the mean times the appropriate t value) indicates that the means are statistically different (*P* < 0.05). However, in this particular comparison involving over 170 localities (sample sizes were not given), mean values and SE were obtained by pooling all *maculata* vs all *triseriata*. This method resulted in very small SE values but precludes assessment of inter-population variation. To provide such demic information, I have conducted preliminary studies over three breeding seasons. The results indicate that the taxonomic status of *P. triseriata* is not fully resolved.

**Materials and Methods**

**Call analysis.**—In order to assess geographic patterns for the boreal and western subspecies, 221 adult males representing 20 localities were collected. Specific localities are given in Figure 1. At the time of collecting, mating calls were obtained for 259 adults using Sony model TC-150 cassette recorders and microphones. Water temperature was recorded 2 cm posterior to an individual as it called. Temperatures were determined to the nearest 0.1 °C using a Bailey BAT-12 fast reading telemeter thermometer. All recordings were made in night choruses during the breeding season (March–June). All calling individuals at a given locality were within my hearing distance. Each recording generally included a minimum of one additional calling individual, usually calling in a tandem mode. After calls were recorded, each individual was captured and placed in a labeled plastic bag coded against the taped calls and frozen in liquid nitrogen or placed on dry ice for later removal of tissue samples. Specimens were subsequently preserved in formalin and then transferred to 60% alcohol solution.

A Kay Electrometrics Model 6061-B Sonograph was used to generate sonograms. Three calls for each male were analyzed for dominant frequency (DF), pulse number (PN), call duration (CD), and pulse rate (PR). Because CD and PR are temperature dependent, recordings from a test population composed of five adult male *maculata* from an isolated pond (1 km south of Glen Cunningham Lake, Douglas Co., Nebraska) were made over a several week period in order to obtain calls from a known group for a range of temperature values (10.5–17.7 °C). These data (*n* = 39) were then used to determine the relationship between temperature and CD. The least squares regression was employed and the resulting regression equation (*Y* = −0.0974X + 2.277) was used to generate a function, CD1s = CDamb. − (Tamb. − 14) (−0.0974), to permit conversion of duration of all subsequent calls to 14 °C. Here “CD” refers to call duration in seconds and the subscript “amb.” refers to the temperature (T) of a recorded call or the duration (CD) from the son-

Oogram at the recorded temperature. Total PN was counted from each sonogram. DF was measured from the bottom of the base line to the mid-point of the fundamental frequency band in kHz. CD also was measured directly from the sonogram in mm and converted to seconds. PR was obtained by dividing the total number of pulses per call by the projected duration of the call (corrected to 14 C) in seconds.

**Morphometrics.**—Morphometric measurements were made using needle point dial calipers. Each body measurement was made to the nearest 0.05 mm with the exception of body length which was recorded to the nearest 0.1 mm. Measurements were read with the aid of a 3× illuminated magnifying lens. These included: body length (from the tip of the snout to the base of the urostyle), head width (to the posterior margin of the tympanum), lower leg (tibia–fibula) length, head length (from tip of snout to the rear angle of the jaw), snout depth (from external naris to margin of upper lip), tympanum diameter, length of foot, and upper leg (femur) length.

Multivariate stepwise discriminant analyses (Dixon, 1981) were performed on two data sets. The first contained morphometric measure-
ments for 221 adult male specimens. The second set contained morphometric as well as temperature corrected call data for 158 males for which data of both types were available.

**RESULTS**

**Call analysis.**—Sonograms of mating calls (Fig. 2) representing 20 populations were measured to determine the value of each of the parameters (PN, DF, CD, PR). The mean, SD and 95% confidence limits were obtained and plotted for each population in a north–south series. Mean PN varies from 17.4 to 14.3 with no apparent clinial trend \((r = 0.06, F_{1,18} = 0.25, 0.9 > P > 0.5)\), nor are there any apparent geographic patterns correlated with subspecies designation. DF varies clinally \((r = 0.54, F_{1,18} = 7.13; P < 0.05)\) with higher mean frequencies in the northern populations. Means range from 3.58 kHz to 3.28 kHz in South Dakota (pops. 1–2, and 4) to 2.91–3.11 kHz in Kansas at the southern end of the transect (pops. 15–16).

Both the mean CD (Fig. 3) and mean PR (Fig. 4) yielded patterns suggesting two call types. The four most northern sites (pops. 1–4) form a series possessing long calls ranging from 0.91–1.16 sec in length. One hundred km south, at Beemer, Nebraska (pop. 5), the mean call length is 0.59 sec. Populations 6–9 have long calls, population 10 a short call. Population 11 is intermediate between 10 and 12 which again is a long call. The mean values for duration in populations 13–16 are short as are the two lower elevation populations (18 and 19) from Colorado. Population 20 provides striking confirmation in Colorado of the same long and short call types described among populations 1–16.

PR were obtained by dividing the number of pulses per call by the duration in seconds and are therefore temperature dependent and inversely related to duration. In the four most northern populations (1–4) the mean PR range from 16.4 pulses/sec downward to 12.7 pulses/sec at Springfield, South Dakota. The mean of the next locale (pop. 5), Beemer, Nebraska exceeds twice that of population 4. Populations 6–9 have mean values similar to those of the first four. Mean PR in population 10 (23.7 pulses/sec) is again much higher. Populations 11–16 tend to repeat this "low-high" pattern with the exception of population 11 which is intermediate between the two extremes. The three Colorado populations (18–20) again confirm the existence of two call types; a low PR (pop. 20) at Arroyo and a much faster call at Ft. Collins (pop. 18) and Fairplay (pop. 19).

Call data obtained for population 17 at Wagoner, Oklahoma were not typical of the others. Although mean CD is within the range of tris-
Fig. 4. PR (corrected to 14 C) in pulses/sec. Representation of the mean, range, standard deviation, 95% confidence limits, localities and sample sizes are the same as Figure 3.

triseriata populations, PR is very low, as is characteristic of maculata.

Morphometrics.—Stepwise discriminant analysis (BMDP7M; Dixon, 1981) indicated that two axes (K₁ and K₂; Fig. 5) explained 92% of the total dispersion (72% and 20%, respectively). Separation along the horizontal axis (Table 1) is heavily dependent on differences between the two groups in tympanum diameter, head width, and tibia–fivala length. Dispersion along the K₂ axis is largely influenced by head width, tibia–fivala length, and to a lesser extent by tympanum diameter and body length. In the canonical plot of the results (Fig. 5) two clusters of populations within the transect are apparent. The group on the left (pops. 5, 10, 13, 15, and 16) correspond geographically to localities where one would expect to find triseriata (Conant, 1975) and the remaining cluster on the right (pops. 1–4, 7–9, 11–12, and 14) corresponds to maculata. To further clarify this relationship dashed lines on the map in Figure 1 connect populations belonging to the triseriata group and indicate the northern limits (pops. 5 and 10). All remaining populations in Figure 1 (except 17) represent maculata populations. Pseudacris t. triseriata specimens have wider heads, longer legs, and larger tympani than do maculata individuals.

Combined morphometric and acoustic analysis.—Multivariate discriminant analysis was also performed on populations for which both morphological and acoustic data were available and included CD, PN and DF. PR was not included in the analysis reported in Table 1 because it is derived from PN divided by CD and therefore not an independent parameter. When PR was included the canonical plot is much the same as the one in Figure 6, but with somewhat tighter within group clustering.

The canonical plot (Fig. 6) shows tighter grouping within and greater separation between the two clusters than the morphological data treated alone (Fig. 5). The K₁ and K₂ axes (Table 1) account for 82% (K₁ = 68%; K₂ = 14%) of the dispersion. Snout–urostyle distance has only minor importance and tibia length is absent. Although head width and tympanum diameter continue to contribute substantially in separation along the K₁ axis, CD becomes the major influence. Separation along the K₂ axis is still dependent upon head width and tympanum diameter and to a lesser extent snout–urostyle distance. However, CD continues to be the dominant influence along the K₂ axis. Membership within the two clusters is the same as
Fig. 6. Canonical plot from multivariate stepwise discriminant analysis (BMDP7M, Dixon, 1981) based on the eight body measurements and three call parameters (PN, CD and DF) for each of 158 adult males. Populations are identified by number and correspond to localities listed in Figure 1. The $K_1$ and $K_2$ axes are the same as Figure 5. Note that the two clusters of population means depicted here remain the same as Figure 5 in terms of membership with the exception of the separation of population 14 which is intermediate between the two clusters.

that of Figure 5 with the exception of population 14 which shows equally low affinity to either of the two groups.

**Discussion**

Call analyses (CD and PR) alone demonstrate the existence of two call types, one replacing the other across an extensive zone of north to south overlap. The differences are sometimes abrupt between populations (e.g., populations 4–5 and 5–6). This pattern of marked differences (pops. 18–19 vs 20) is seen in Colorado as well.

Multivariate morphometric analysis of mensural characters produced two clusters of populations, independent of the call data indicating the existence of two morphotypes. When mating call data are incorporated in the analysis, the same two clusters occur hence call type and morphotype are concordant.

In several recent studies involving similar zones of overlap among North American leopard frogs, marked changes in morphology over short geographic distances have proved to represent sympatric species pairs (Littlejohn and Oldham, 1968; Brown and Brown, 1972; Platz and Platz, 1973; Platz, 1976; Frost, 1977). Doc-

umenting numerous sympatric populations of chorus frogs possessing the characteristics of one or the other of two forms is consistent with the conclusion that each represents a separate species. The data thus far support recognizing the boreal subspecies, $P. t. maculata$, as $P. maculata$, and the western subspecies, $P. t. triseriata$ as $P. triseriata$. Preliminary data of Platz and Forester (1988), based on mating CD and PR of feriarum (see this paper’s discussion of population 17) and kalmi suggest that the same may be true for each of these two subspecies as well. This conclusion is further supported by recent electrophoretic findings involving three of the four subspecies (excluding maculata). Hedges (1986) employed 33 loci to assess the genetic affinities of North American hylids. A modification of the Cavalli-Sforza distance method resulted in distance values between triseriata and feriarum of 0.33 and between triseriata and kalmi of 0.32. These are twice the magnitude of the difference between feriarum and $P. brachyphona$ (0.14) and of the same order as that between $P. brachyphona$ and kalmi (0.14). Specific status for feriarum was suggested much earlier by Ralin (1970) on the basis of laboratory crosses.

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<th>Variable</th>
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<td>DF</td>
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**Table 1. BMDP7M—Coefficients for Canonical Variables.** The upper set of coefficients contain values for the morphometric data set (n = 221) individuals alone. The lower set (n = 158) are for those individuals for which call data and morphometric data were both available. The abbreviations for each variable are as follows: SVL = body length; TFL = tibia-fibula length; HW = head width; TYP = tympanum diameter; CD = duration of call; PN = number of pulses in a call; DF = dominant frequency of a call. Each of the morphometric features is further defined under Methods and Materials. $K_1$ and $K_2$ represent the first and second canonical axes respectively.
In light of my findings and those of Hedges (1986), the call data presented in Figures 3 and 4 representing the South Dakota–Oklahoma transect warrant further discussion and interpretation. If a conservative approach to current range maps is adopted which presumes population 1 to represent allopatric *maculata* and population 19 to be allopatric *triseriata*, and further, that the two are behaving as species, then the pattern of call variation, both in terms of means and population variability, is of special interest.

The four northernmost populations of *maculata* each have higher mean values for CD (Fig. 3) which might be indicative of clinal variation, sampling error, or character displacement. If clinal variation were operating one might then expect to see even higher means further south, but this is not the case. Alternatively, it is possible by chance to obtain four samples whose means are each larger than the one to the north ($P = \frac{1}{2^4} = 0.0625$). However, this explanation seems even less likely when the rest of the transect is considered, including the fact that variability (as measured by the larger SD) is much higher in sympathy. Increased variability itself may be the result of hybridization or mixed species sampling at single locales because the nature of the zone of overlap (highly variable dorsal pigment patterns) precluded assigning individual specimens to subspecies. It is also possible that this pattern (mean values) among northern populations is the result of natural selection within *maculata* favoring longer calls, suggesting that character displacement has reduced the likelihood of mating with *triseriata*. This last possibility merits consideration since it is within the zone of sympathy that the extremes in high (*maculata*) and low (*triseriata*) individual values are encountered. These observations are more easily explained in this manner than by assuming that matings between two species would produce hybrids with higher or lower values than either parent species, rather than the more likely case of producing intermediate ones.

PR (Fig. 4) reveals a similar overall pattern with the lowest means and individual limits for *maculata* occurring in sympathy (pops. 4, 9, and 12) and in general even greater variability than the data for CD.

The arguments advanced to explain the CD patterns seen in populations 1–16 also apply to PR. PR is an important parameter permitting females to choose between call types of conspecifics and non-conspecifics. According to Littlejohn (1960, 1965), and Loftus-Hills and Littlejohn (1971), to permit effective discrimination, at least one parameter must differ by a factor approaching a two-fold difference, but in some cases discrimination may still be efficient with smaller differences (Gerhardt, 1978). Assuming this is true for *maculata* and *triseriata*, then the displacement of mean values for *maculata* PR downward in sympathy and the presence of *triseriata* individuals in the zone of sympathy with extremely high PR (above 30 pulses/sec in pops. 5, 10 and 16) suggests character displacement.

The concept of character displacement in the original (ecological) sense of Brown and Wilson (1956) has also been applied to those reproductive qualities which would retard gene exchange between diverging sympatric populations or what Blair (1955) referred to as “reinforcement.” Evidence for character displacement in this second sense has been sought among anurans for 30 yr during which a limited number of cases have been recorded.

Fouquett(1975) provided an additional report involving *fieriaram* and *nigrita* in Alabama and Georgia. His results differ from those of this study in that differences between the two taxa in mean CD, PN, and PR are more marked. In sympathy, PR show no overlap in range values. PR means meet the minimum two-fold difference in value in all cases and some exceed a three-fold difference. Each of the two species can be readily identified visually in the field and no evidence of hybridization was found. Fouquet’s (1975) study involved two taxonomically well known congeners and a substantial number of sympatric populations thus eliminating two criticisms leveled at several earlier reports of presumed cases of reproductive character displacement. Clinal variation is an alternative explanation for the marked upward shift in PR for the more southern populations of *fieriaram* along the Alabama-Georgia border. However, this seems less parsimonious on two grounds: 1) there is no evidence of clinal variation among allopatric populations ranging from central Kentucky south as far as central Alabama or approximately two-thirds of the latitude encompassed in his study; and 2) the shift toward higher PR is abrupt and coincident with sympathy between *nigrita* and *fieriaram*. Therefore, his results currently represent the strongest evidence for reproductive character dis-

The evidence presented here clearly suggests secondary contact between maculata and triseriata, each having diverged to a measurable degree and therefore best explained by postulating reduced gene exchange between the two entities. Limited data involving mating calls (see pop. 17, Figs. 3–4) is consistent with Fouquette’s (1975) characterization of allopatric feriarum. Both findings are an indication that the call of feriarum is also distinct from that of triseriata.

Although it is likely that some hybridization is occurring in the contact zone between maculata and triseriata, my findings indicate that two call types are present and that the highest and lowest values for PR and CD occur within what I interpret to be a large area of overlap between the two forms. When hybrid and parental types can be individually identified from these populations, differences in the means and range limits for call parameters are likely to be more marked than they appear at present. The results from my call data are consistent with the inferences drawn from the morphometric analysis in that each approach suggests the same two assemblages of populations. Taken together they identify a broader area of overlap than currently recognized in the literature, extending as far west as Fort Collins, Colorado, and as far south as Lawrence, Kansas.

Known zones of overlap involving large numbers of populations of each of the four subspecies pairs invite comparative studies involving biochemical, behavioral, morphological and acoustical approaches. Studies now underway are expected to yield results pertinent to the issues discussed here.

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