
ECOLOGICAL AND REPRODUCTIVE
DIFFERENTIATION IN THE CRYPTIC SPECIES
OF THE *HYLA VERSICOLOR*
COMPLEX (HYLIDAE)¹

DENNIS B. RALIN

Department of Zoology, The University of Texas, Austin

ABSTRACT. The ecological and reproductive behavior of the cryptic species *Hyla chrysoyelis* and *H. versicolor* was examined in two sympatric localities and in one allopatric *H. chrysoyelis* locality. An analysis of the stomach contents of both species at one locality suggests that proportionately more *H. chrysoyelis* are eating arboreal insects, and proportionately more *H. versicolor* are eating terrestrial insects. The two species may be avoiding complete overlap of their niches in this way.

There was a significant statistical, although not absolute, difference between the two species in the positions of the males calling around the breeding ponds; *H. chrysoyelis* tends to call from trees or bushes, while *H. versicolor* tends to call from the ground. A relationship between the ecological and reproductive behavior is discussed in terms of natural selection working in the same direction for both in each species.

Limited relative humidity data, general geographic distribution of the two species, and the ground versus tree differences in food habits and calling position suggest that relative humidity is a key factor involved in these ecological and reproductive differences. It is thought that *H. chrysoyelis* can tolerate or may prefer lower relative humidities than *H. versicolor*.

Two factors that were thought to affect the trill rates of both species were analyzed. High positive correlations between temperature and trill rate were found in regression analyses for both species. No correlation between body size and trill rate was found for either species.

Population differences in the percentage of individuals using either the upper or lower harmonic as the dominant frequency of the mating call may prove to be of significance in the sympatric relations of the two species. Two populations of *H. chrysoyelis* sympatric with *H. versicolor* had mean trill rates 7% and 10% faster than the mean trill rate of an allopatric *H. chrysoyelis* population. It is suggested that reinforcement for differences in trill rate is occurring where the two species are sympatric.

The study presented here deals with the ecological and reproductive interactions of two cryptic species of tree frogs which until recently

¹ Portion of a Master's thesis at The University of Texas at Austin.

were confused under the name *Hyla versicolor*. A detailed account of the nomenclatural and taxonomic history may be found in Johnson (1961, 1966).

The nominal species *Hyla versicolor* has been divided into three subspecies, *H. v. versicolor*, *H. v. chrysozelis*, and *H. v. sandersi*, primarily on the basis of skin texture and color pattern. The validity of the three subspecies is questionable, as individuals attributable to all three subspecies had been collected from the same population (Flury, 1951).

Various authors have noted the occurrence of two mating call types in several parts of the range of the nominal species (Noble and Hassler, 1936; Walker, 1946; Hoffman, 1946; and Mittleman, 1947). The two call types (Fig. 3) were presented objectively by the use of sound spectrograms, and were shown to represent a definite geographic pattern by Blair (1958a). The call types consist of a slow-call type which varies from 17 to 35 trills (notes) per second, and a fast-call type which varies from 34 to 69 trills per second, depending on the temperature (Johnson, 1966). The call types do not overlap in trill rate at any given temperature.

A high degree of incompatibility between the call types was found in hybridization tests using individuals from sympatric populations, as well as in tests using individuals from allopatric populations of the call types (Johnson, 1959). Crosses within the same call type but between geographically widely separated populations resulted in normal viability, whereas crosses involving different call types, whether sympatric or widely separated geographically, resulted in high larval mortality (Johnson, 1963). The F_1 backcrosses demonstrated an even higher degree of incompatibility than the original crosses.

Females taken in amplexus from a pure fast-call population consistently chose the fast-call speaker in preference to the slow-call speaker in a discrimination tank (Littlejohn *et al.*, 1960). There was some evidence that slow-call type females are also able to discriminate in favor of their own call type.

The call types have no correlation with the morphological criteria used to differentiate the subspecies. The call types cannot be differentiated on morphological criteria either, although frogs of the fast-call type are statistically somewhat smaller (Johnson, 1961). Snout-vent measurements of individuals of both species taken at the same locality confirm this (Fig. 1). Only about 3% of the *H. chrysozelis* are not overlapped in snout-vent length by *H. versicolor*, and only about 1% of the *H. versicolor* are not overlapped by *H. chrysozelis*. The

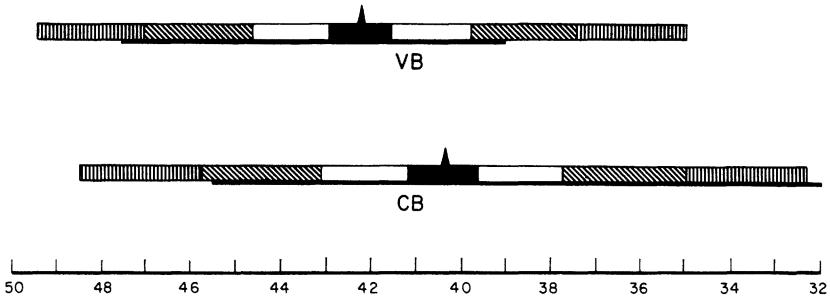


Fig. 1. Comparison of snout-vent lengths of *Hyla chrysocelis* (CB) and *H. versicolor* (VB) males collected at the same locality (Bastrop). Solid triangle is the mean for each species; one-half the solid rectangle is two standard errors of the mean; one half the solid rectangle plus the hollow rectangle is one standard deviation from the mean; the diagonally striped rectangle is two standard deviations; and the vertically striped rectangle is three standard deviations from the mean. Modified from Hubbs & Hubbs (1953). See text for explanation.

difference in mean snout-vent length for the two species, although significant, is useless for species determination.

The hybridization and discrimination data demonstrate that each call type is behaving as a good biological species. The name *Hyla versicolor* has been retained to represent the slow-call species, which was probably the call type collected at the type locality; the name *chrysocelis* has been elevated to species rank to represent the fast-call species; and the subspecies as formerly described are no longer considered valid (Johnson, 1966).

This situation provides an unusually good opportunity for study in the field of ecological-evolutionary biology. The aims of the present study are: (1) to determine the ecological relationships between the two species, particularly whether or not the competitive exclusion principle is in effect, by examining the stomach contents of both species at a sympatric locality; (2) to determine what sympatric pre-mating isolating mechanisms are operating, and their degree of development; and (3) to determine which environmental stimuli are responsible for any differences that do exist in the ecology and behavior of the two species.

FOOD HABITS AND ECOLOGICAL DIFFERENTIATION. An analysis of the stomach contents was made in the expectation that it might reflect differences in the ecology and microhabitat of the two species in sympatry (Table 1). The frogs were captured at two temporary ponds less than 900 feet apart in Bastrop State Park, Bastrop County, Texas. The digestive tracts of 95 males collected while actually calling were examined. Forty-two of these were *H. chrysocelis*,

TABLE 1

Analysis of stomach contents of H. chrysoscelis and H. versicolor males during the breeding season.

Category of food items	No. and percentage of total food items in each category		No. and percentage of frogs with food items in each category	
	<i>H. chrysoscelis</i>	<i>H. versicolor</i>	<i>H. chrysoscelis</i>	<i>H. versicolor</i>
Insecta*	1 (2.6)	4 (6.3)	1 (5.3)	4(14.3)
Lepidoptera	4(10.5)	4(6.3)	4(21.1)	4(14.3)
Orthoptera	0	1 (1.6)	0	1 (3.6)
Diptera	0	1 (1.6)	4	1 (3.6)
Hymenoptera*	1 (2.6)	1 (1.6)	1 (5.3)	1 (3.6)
Formicidae*	1 (2.6)	1 (1.6)	1 (5.3)	1 (3.6)
<i>Pogonomyrmex</i>	9(23.7)	9(14.3)	3(15.8)	6(21.4)
Coleoptera*	7(18.4)	16(25.4)	6(31.6)	13(46.4)
Cantharidae	1 (2.6)	1(1.6)	1 (5.3)	1 (3.6)
Scarabeidae	0	3(4.8)	0	3(10.7)
Curculionidae	1 (2.6)	0	0	1 (3.6)
Elateridae	11(28.9)	22(34.9)	10(52.6)	12(42.9)
Carabidae	2 (5.3)	0	1 (5.3)	0
	38(99.8)†	63(100)		

* Does not include lower taxa of the particular group which could be identified further; these are listed as separate categories.

† Percentages taken to nearest 0.1%.

of which 19 contained identifiable food matter. Food matter was found in the intestine and cloaca of nearly all those with empty stomachs, or whose stomach contents were considered unidentifiable. Considering only those frogs with identifiable contents, 45.2% of the *H. chrysoscelis* examined had been feeding during the period when they were attempting to breed. Twenty-eight (52.8%) of the 52 *H. versicolor* examined also had food in their stomachs; evidently *H. versicolor* individuals are also feeding during the breeding period.

The diet of *H. chrysoscelis* consists entirely of insects; there was an obvious lack of any other kinds of arthropods, particularly of arachnids. Some mites or ticks were found, but since these measured less than one millimeter in diameter, including the legs, they were discounted as accidentally ingested with other food items. Coleoptera as a whole comprised a large part (58%) of the total food items ingested by *H. chrysoscelis*. Elaterids alone represented approximately one-half of the total Coleoptera, as well as 28.9% of the total food (Table 1). The Elateridae are generally boreal, and are found on the foliage and bark of trees and bushes (Arnett, 1960). The second largest portion of the total diet was composed of *Pogonomyrmex*, the harvester ant genus (Table 1). Examination of some of the litera-

ture (Little, 1963; Creighton, 1950; and Wheeler, 1910) revealed no record of aboreal habits in this genus. Evidently, at least some *H. chrysozelis* individuals are spending time foraging on the ground. This is corroborated by occasional specimens containing gravel, as well as food items, in their stomachs. In terms of the number of *H. chrysozelis* with food items in various categories (Table 1), a larger percentage of frogs contained elaterids as opposed to *Pogonomyrmex*. Three *H. chrysozelis* contained 1, 3, and 5 harvester ants respectively; 9 each had 1 elaterid, and 1 contained 2 elaterids.

The stomach contents of *H. versicolor* are quite similar to those of *H. chrysozelis*. Again, the diet consists exclusively of insects. In terms of the total diet (Table 1) 13.6% of the food items were *Pogonomyrmex* and 34.9% were elaterids. However, when analyzed in terms of number of frogs containing food items in these categories (Table 1), *H. versicolor* had a higher percentage of frogs containing *Pogonomyrmex* and a lower percentage of frogs containing Elateridae than did *H. chrysozelis*. Five *H. versicolor* had 1 harvester ant each, and a sixth contained 4 harvester ants. Five individuals contained 1 elaterid each, 6 contained 2 elaterids each, and 1 individual contained 5 elaterids.

Elaterids were the only definitely arboreal food item found in relatively good numbers, and *Pogonomyrmex* the only definitely terrestrial item found in comparable numbers. It was assumed that one occurrence of a particular food item in a frog indicated tree or ground foraging as well as the occurrence of two or four of the same item. Due to the small sample size, a statistical analysis was not attempted.

Although both species favor aboreal foraging, it appears that proportionately more *H. chrysozelis* forage above the ground and proportionately more *H. versicolor* forage on the ground (Table 1). In accordance with the competitive exclusion principle, complete overlap of niches would not be occurring. The hypothesis that *H. chrysozelis* forages and lives at generally higher levels in the forest is also supported by some observational and statistical data to be presented later. There are two alternative hypotheses: (1) either the competitive exclusion principle is simply not valid in this particular case or at this particular time, or (2) adult food is not a limited resource for which the two species are competing.

DIFFERENTIAL UTILIZATION OF THE AVAILABLE REPRODUCTIVE ENVIRONMENT: RELATION TO FOOD HABITS. In the course of this field work, Johnson (1966) formed the impression that *Hyla versicolor* males were calling from higher positions than *H. chrysozelis* males in areas of overlap, but lacked objective data to

TABLE 2

Totals of actively calling males of H. chrysoxcelis and H. versicolor collected on the ground and their distances from the edge of the pond.

Species	Distance in feet from edge of pond					Total no. of individuals on ground
	0-1	1.1-3	3.1-5	5.1-10	>10	
<i>H. chrysoxcelis</i>	6	0	0	2	0	8
<i>H. versicolor</i>	4	1	3	4	6	17

support this. During the present study the calling positions of 58 males of both species were noted while the calls were being recorded. The data combine observations at three ponds where both species were found calling together. Two of these ponds were the ponds in Bastrop State Park, and the third was a shallow farm pond four miles northwest of Caldwell, Burleson County, Texas. The data were collected throughout the breeding season in 1966, from late March to early June.

The numbers of ground-calling and tree-calling individuals of both species are given in Tables 2 and 3, respectively. The significance of the data was tested by a 2 X 2 contingency table (Bailey, 1959), and the differences were significant ($p < .005$ at one degree of freedom) for *H. chrysoxcelis* calling from above-ground positions and *H. versicolor* calling from terrestrial positions. The relationship of these differences to the inconclusive food habit data will be discussed later in this section.

The data were further analyzed in terms of measurements in feet of the distances of ground-calling individuals from the pond margins and the heights of tree-calling individuals. The comparatively few *H. chrysoxcelis* that do call on the ground appear to call at very short distances from the edge of the pond (Table 2). In contrast, *H. versicolor* appears to be more spread out in terms of distances from the pond edge, and distances greater than 5 feet seem to be favored (Table 2). Larger numbers might prove this to be another significant difference in areas of overlap.

There also appears to be some difference in the preferred heights above ground level of both species (Table 3). *H. chrysoxcelis* exhibits a marked preference for the 1.1-3.0 foot range, while the small number of *H. versicolor* calling above the ground seem to demonstrate a slight preference for the 1.1-5.0 foot range.

The differences in both ground versus above-ground calling positions, and preferences for particular heights above the ground and/or distances from the pond margin, would obviously be of importance

TABLE 3

Totals of actively calling males of *H. chrysofelis* and *H. versicolor* collected above ground level and their heights above the ground.

Species	Height in feet above ground level				Total no. of individuals above ground level
	0-1	1.1-3	3.1-5	>5	
<i>H. chrysofelis</i>	1	15	7	1	24
<i>H. versicolor</i>	0	4	3	2	9

with respect to reproduction in areas of overlap. The concentration of calling males of one species in one or two portions of the available reproductive environment would increase the chances for mating with a conspecific female. This would be particularly applicable to the genus *Hyla*, as the females appear to select a specific male to approach and contact (Blair, 1958c; Littlejohn, 1959a). Accidental contacts with other males, on the way to the selected male, are more likely to be conspecific if the males of the right species are concentrated in a particular part of the environment.

The difference in calling positions may reflect some basic differences in the non-breeding ecology of these two species. There may be a basic ecological difference which has been and is being utilized and perhaps enhanced in a reproductive context, as well as in an ecological context. Specifically, *H. chrysofelis* calls from above-ground positions in part because it is somewhat more boreal than *H. versicolor* when not breeding, and *H. versicolor* in sympatry calls from ground positions because in part it is somewhat more terrestrial than *H. chrysofelis* when not breeding. The converse of this would be more difficult to formulate in terms of natural selection. For example, on one hand natural selection in a reproductive context would be working in favor of *H. chrysofelis* calling from above-ground levels, and on the other hand natural selection in an ecological context would be working in favor of *H. chrysofelis* foraging on the ground. An equally good case can be made for any of four likely hypotheses at the present time: (1) an ecological difference, either already present or developed as a result of reinforcement, was preadaptive and/or reinforced in a reproductive context; (2) a reproductive difference, either already present or developed as a result of reinforcement, was preadaptive and/or reinforced in an ecological context; (3) both ecological and reproductive differences are "innate" and were developed before the species became sympatric; or (4) both types of differences were developed concurrently, due to reinforcement when the species became sympatric. The absence of definitive ecological differences (probably due to small

sample size) and the presence of significant reproductive differences do not indicate which hypothesis would be most likely. Whether the differences are inherent or the result of reinforcement can only be determined by studies of allopatric populations of both species.

The conclusions reached here regarding calling position in areas of overlap do not agree with previous impressions (Johnson, 1966). Two aspects of the field work suggest that workers would tend to concentrate on those frogs calling from vegetation, conceivably leading to the impression that *H. versicolor* generally calls from higher positions than *H. chrysosecelis* (Table 3). Firstly, in areas where dense vegetation capable of supporting tree frogs comes to the edge of the pond, most recording and collecting work would necessarily be concentrated primarily on those frogs calling above the ground. Secondly, frogs of either species appeared more wary when calling on the ground, and would often discontinue calling when approached. However, when calling from vegetation, individuals of both species appeared less concerned and often would even continue calling after the vegetation had been disturbed.

The data presented here raise important questions as to (1) the physiological differences that exist between the two species, and (2) the environmental stimuli that are being translated through these physiological differences to cause the ecological and reproductive behavioral differences that exist. Additional data bearing on these problems will be presented in the next section.

RELATIVE HUMIDITY AS A KEY FACTOR IN SYMPATRY.

Enough observations have been accumulated to justify a discussion of questions regarding the environmental stimuli important to the two species, particularly in terms of their microdistributional differences in sympatry.

The general distributions of both species (Blair, 1958a; Chantrell, 1966; Conant, 1958; Hoffman, 1946; Johnson, 1961, 1966; Mittleman, 1947; Noble and Hassler, 1936; and Walker, 1946) in the United States (Fig. 2) suggests a difference in moisture requirements between the two species. *H. chrysosecelis* is generally more western in distribution, and is presumably exposed to drier climates than *H. versicolor*. *H. versicolor* is not present on the Atlantic Coastal Plain of the eastern United States or in the prairie peninsula region of Illinois, Indiana, and Ohio. Both are present in the deep South, where moisture levels are high. The general distributions suggest that *H. chrysosecelis* may be able to tolerate lower moisture levels than *H. versicolor*.

Some relative humidity data, although incomplete, are also indica-

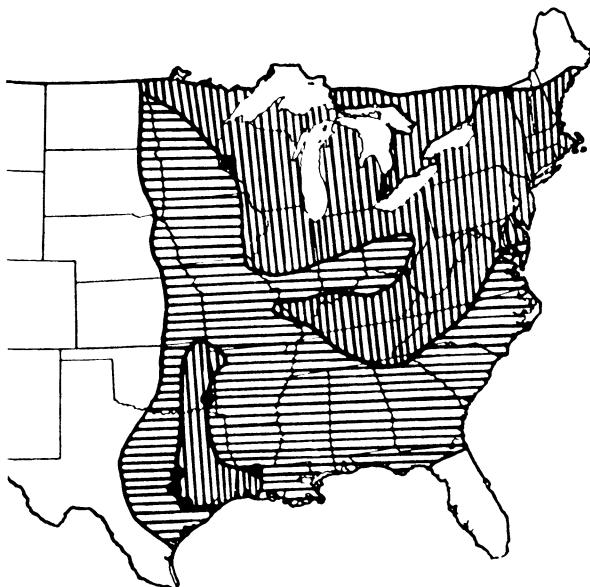


Fig. 2. Tentative distribution map for *Hyla chrysoscelis* and *H. versicolor* in the United States. Modified from Blair (1958a) and Johnson (1961, 1966). Horizontal hatching represents the distribution of *H. chrysoscelis*, vertical hatching the distribution of *H. versicolor*. Black dots are known sympatric localities.

tive. The relative humidity was recorded for several nights in June, 1966, and the numbers of calling individuals of each species were noted at the same time. On two nights with relative humidities of 75% and 82% there were 7 *H. chrysoscelis* and 1 *H. versicolor* calling; on two nights of 95% relative humidity there were 8 *H. chrysoscelis* and 11 *H. versicolor* calling. There is a trend towards greater numbers of *H. chrysoscelis* calling at lower humidities and greater numbers of *H. versicolor* at higher humidities. More observations of this type are needed before any definite conclusions may be drawn.

The differences in calling positions and stomach contents constitute a third line of evidence. There are indications that *H. chrysoscelis* is more aboreal than *H. versicolor* in terms of these differences. Humidity studies done in pine woods and fir plantations in Europe (Geiger, 1965) have shown that the daily average of relative humidity decreases with height above ground level. This would also fit the hypothesis that *H. chrysoscelis* tolerates and may prefer lower relative humidity levels than *H. versicolor*.

Confirming this hypothesis would do much to clarify the sympatric relations and explain the distributions of the two species. In addition

to being an intrinsic difference in humidity sensitivity, this may also be acting as a partial isolating mechanism.

FACTORS AFFECTING THE TRILL RATE OF THE MATING CALL. A total of 694 mating calls was analyzed, representing 125 individuals from three localities. The trill rate (number of notes per second in the call) and dominant frequency (the frequency with the most energy) for each individual were obtained by averaging these parameters of the 2–8 calls available per individual. Air temperatures, in degrees centigrade, were taken to the nearest half degree at the time of recording. The calls were recorded with a Stancil-Hoffman Co. Minitape recorder, and analyzed by means of a Kay Electric Co. Sona-Graph.

The effects of temperature and body size on the trill rates were determined by least-squares regression analysis. In these analyses only the frogs from the two ponds in Bastrop State Park were utilized. It was thought that including populations from other localities would obscure the analysis by introducing any existing populational differences as a source of error for the regression coefficient values.

In the temperature regression analysis, the effects of body size were minimized as much as possible for *H. chrysoscelis* by using only those individuals of snout-vent length 41.0 ± 1.0 mm. This happened to be the range with the largest number of individuals (17). The regression coefficient was $+2.24$ notes/second/ $^{\circ}\text{C}$, with a correlation coefficient (Moroney, 1951:186) of 0.97 between temperature and trill rate. Previous call work done with this species (Johnson, 1961) indicates that this is a reasonable figure. The maximum range in trill rate for all the *H. chrysoscelis* recorded was 35 notes/second, with a maximum temperature variation of approximately 13°C . Dividing the maximum change in trill rate by the maximum range in temperature yields a temperature coefficient of approximately 2.5 notes/second/ $^{\circ}\text{C}$. Considering the rough nature of this approximation, the value of 2.24 notes /second/ $^{\circ}\text{C}$ found by finer calculation here is thought to be valid.

Twenty-two individuals with a snout-vent length of 41.0–42.0 mm inclusive were used for the *H. versicolor* regression analysis. The temperature regression coefficient was $+.89$ notes/second/ $^{\circ}\text{C}$ ($r = 0.74$). This value does not agree with the temperature coefficient of $+.44$ notes/second/ $^{\circ}\text{C}$ previously found for this species (Blair, 1958a). However, another rough calculation from Johnson (1961) may help to clarify the matter. Through about the same temperature range, the maximum range in trill rate for *H. chrysoscelis* was approximately 35 notes/second, while the maximum range for *H. versicolor* was

about 18 notes/second. This indicates that the temperature coefficient should be about half that of *H. chrysoxcelis*, or about +1.3 notes/second/°C. On the basis of the *H. chrysoxcelis* coefficient found in this study (+ 2.24 notes/second/°C), that of *H. versicolor* should be about +1.1 notes/second/°C. It is assumed that the *H. versicolor* temperature coefficient of .89 notes/second/°C found in this study is close to being the correct one.

The *H. versicolor* temperature coefficient of .89 notes/second/°C found here is about .20 below that expected on the basis of the *H. chrysoxcelis* coefficient. The probable source of this error is the fact that many of the *H. versicolor* were recorded on the ground, while the temperatures used were often those of the air. This means that many of the frogs recorded on the ground may have actually been calling at temperatures higher than that of the air, thus yielding trill rates higher than they should be for the recorded temperature. This would result in the smaller than expected slope found here.

Since most of the *H. chrysoxcelis* recorded were above the ground, the air temperatures should be quite close to those of the frogs. The lower *r* value for the *H. versicolor* regression (0.74), compared to *r* = 0.97 for *H. chrysoxcelis*, also indicates more variability than would be expected. On the basis of rough calculations from Johnson's call data and the *H. chrysoxcelis* temperature coefficient presented here, it is thought that the temperature coefficient of *H. versicolor* should be 1.13 notes/second/°C.

The second type of regression analysis possible was one involving the effect of body size on trill rate. In this case, the effect of temperature was minimized by using individuals recorded at the same or very similar temperatures (19.0°–20.0°C for both species). The regression coefficient was + .68 (*r* = 0.14) for *H. chrysoxcelis*, and + .14 (*r* = 0.16) for *H. versicolor*. Body size and trill rate are not correlated, and the coefficients are meaningless.

COMPARISON OF TWO PARAMETERS OF THE MATING CALL IN SYMPATRY AND ALLOPATRY. One of the primary premating isolating mechanisms in anurans is the mating call of the male (Blair, 1958a, Mecham, 1961). Female response to the call of the male in hylids has been reported in field observations (Blair, 1958c; Littlejohn, 1959a) as well as in discrimination tests (Littlejohn, 1960, 1961; Littlejohn and Michaud, 1959; Littlejohn *et al.*, 1960; Michaud, 1962; and others). There is also some evidence demonstrating or suggesting reinforcement of call differences between similar species of anurans in areas of sympatry (Blair, 1955; Littlejohn, 1959b, 1965).

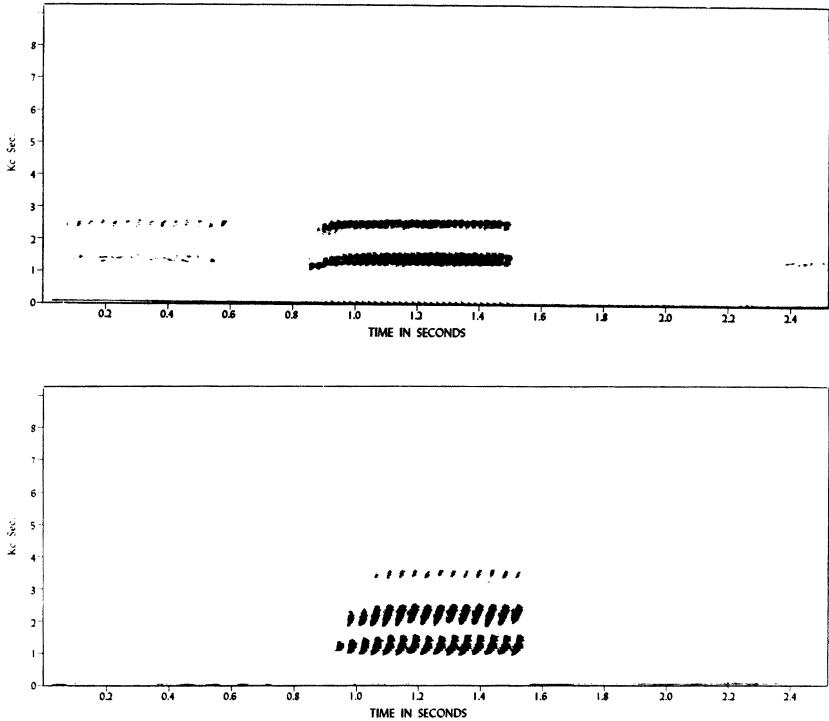


Fig. 3. Narrow band sound spectrograms of the mating calls of *H. chrysoscelis* (upper) and *H. versicolor* (lower) recorded at approximately the same temperature.

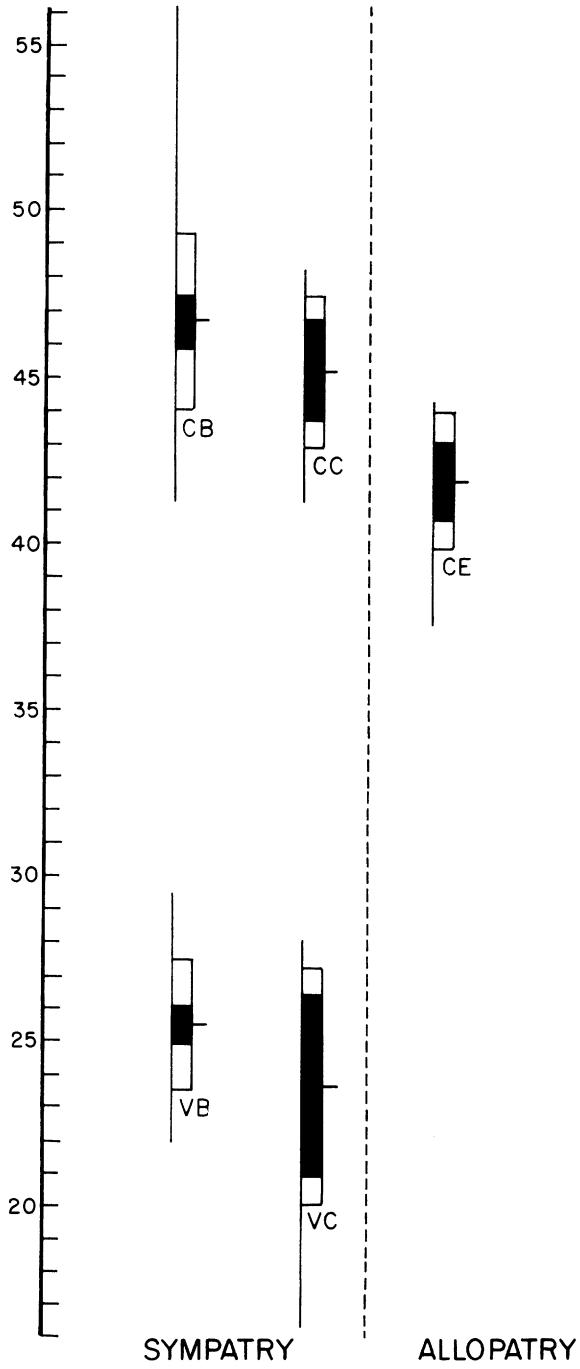
From inspection of typical sound spectrograms of calls of the two species (Fig. 3), it is apparent that the calls are similar in structure. The structure of the mating calls has already been described (Blair, 1958b; Johnson, 1961). There are two main harmonics: an upper that varies between 1,900 and 3,000 cycles/second, and which usually contains the most energy (the dominant, or carrier frequency); and a lower, with slightly less energy, between 1,000 and 1,600 cycles/second. Since the dominant frequencies have the same degree of variability in both species in and out of sympatry, Johnson (1961) suggested that trill rate was the primary premating isolating mechanism in sympatry.

However, analysis of the calls recorded in this study indicate that which harmonic is dominant varies from population to population. In sympatry with *H. versicolor* (Bastrop and Caldwell localities), 28 *H. chrysoscelis* had the upper harmonic dominant, 24 had the lower dominant, and 2 individuals were indistinguishable as to which was dominant. *H. versicolor* from the same two localities had 34 individuals

using the upper as dominant, 16 using the lower, and 9 indistinguishable. In contrast, an allopatric population of *H. chrysosecelis* located about four miles south of Elgin, Texas, had 11 individuals using the upper harmonic as the dominant, and 1 with the lower harmonic dominant. Any inferences regarding these data must be drawn with caution, as (1) the number of populations is very small, and (2) it has not been demonstrated that the frogs are capable of perceiving the difference of a few decibels involved. If the number of populations was increased, and (2) above shown, as an additional pre-mating difference this would have at least a slight positive value in terms of natural selection. From the standpoint of conservation of energy, switching to the lower harmonic would require little modification of existing structures if carrier frequency production in these animals is similar to that of *Bufo* (Martin, 1967). A slight modification such as thickening a membrane in the larynx could accomplish the change quite readily.

It was stated previously that trill rate was thought to be the primary pre-mating isolating mechanism in sympatry. Therefore, it seemed likely that if reinforcement of call differences was occurring, the mean trill rate of *H. chrysosecelis* populations sympatric with *H. versicolor* should be faster than the mean trill rate of allopatric populations of *H. chrysosecelis*; and the mean trill rate of *H. versicolor* populations sympatric with *H. chrysosecelis* should be slower than the mean trill rate of allopatric *H. versicolor* populations.

For purposes of comparison it was necessary to correct the trill rate of all individuals to one arbitrary temperature (21.0°C). This was done by subtracting the actual temperature at which an individual was recorded from 21.0°C, multiplying this difference by + 2.24 for *H. chrysosecelis* or + 1.13 for *H. versicolor*, and then adding or subtracting the figure thus obtained from the raw trill rate of the given individual. The mean corrected trill rate of each population was obtained by totaling the corrected trill rates of all the individuals in the population and then dividing by the number of individuals in that population. A graphical comparison (Hubbs and Hubbs, 1953) of the data for two *H. chrysosecelis* populations sympatric with *H. versicolor*, one allopatric *H. chrysosecelis* population, and two *H. versicolor*, one allopatric *H. chrysosecelis* population, and two *H. versicolor* populations sympatric with *H. chrysosecelis*, corrected to 21.0°C, is presented in Figure 4. Since it was difficult to determine by inspection (Fig. 4) whether the difference in mean trill rate between Bastrop and Caldwell *H. chrysosecelis* was significant, a Student's *t* test was run on these two populations. The difference in means was not significant. This was expected in a comparison of populations which are both sympatric



with *H. versicolor*. In contrast, it is obvious by inspection that the difference in means between Bastrop and Elgin *H. chrysoseleis* is significant, with the allopatric population (Elgin) being considerably slower in trill rate. The difference in means between Caldwell and Elgin, respectively sympatric and allopatric localities, is also significant. The Elgin population is considerably slower than the Caldwell *H. chrysoseleis* population, as well as the Bastrop population. Unfortunately, I was unable to record any allopatric populations of *H. versicolor* to compare with the Bastrop and Caldwell *H. versicolor*. In addition, the Caldwell *H. versicolor* population is based on a small sample size which must be increased before any comparisons may be made. Although the number of populations so far sampled is exceedingly small, it is suggested that reinforcement for a higher trill rate is occurring in *H. chrysoseleis* populations which are sympatric with *H. versicolor*.

There are several ways in which this selection for higher (or lower) trill rates may be operating. It has already been shown that body size does not affect the trill rate in either species, eliminating the possibility of changing the trill rate in sympatry by selection for a larger or smaller body size. A second possibility is that *H. chrysoseleis* populations sympatric with *H. versicolor* have a larger temperature regression coefficient than allopatric *H. chrysoseleis* populations, resulting in a higher average trill rate when they are all corrected to the same temperature. The third and most attractive possibility is simply that in sympatry the *H. chrysoseleis* individuals with slower trill rates are less likely to breed successfully, and over a period of time the average populational trill rate would increase due to the operation of selection upon individual variation in the trill rate originally present.

I wish to thank Dr. W. F. Blair for reading the manuscript, as well as for his advice and encouragement throughout the course of this study. This research was supported in part by a Grant-in-Aid of Research from the Society of the Sigma Xi.

LITERATURE CITED

ARNETT, R. H. JR. 1960. The beetles of the United States. Catholic Univ. of America Press, Washington, D.C. 1112 p.

Fig. 4. Comparisons of trill rate in *H. chrysoseleis* and *H. versicolor* populations. Solid vertical line is range for each population; one-half the solid rectangle is two standard errors of the mean; and one-half the solid rectangle plus the hollow rectangle at its end is one standard deviation from the mean (Hubbs & Hubbs, 1953). CB, CC, and CE are *H. chrysoseleis* at Bastrop (259 calls, 45 individuals), Caldwell (48 calls, 9 individuals), and Elgin (63 calls, 12 individuals), respectively. VB and VC are *H. versicolor* at Bastrop (285 calls, 52 individuals) and Caldwell (39 calls, 7 individuals). All populations are corrected to 21.0°C.

- BAILEY, N. T. J. 1959. Statistical methods in biology. English Univ. Press Ltd., London, 200 p.
- BLAIR, W. F. 1955. Mating call and stage of speciation in the *Microhyla olivacea*—*M. carolinensis* complex. *Evolution* 9: 469–480.
- 1958a. Mating call in the speciation of anuran amphibians. *Amer. Nat.* 42: 27–51.
- 1958b. Call structure and species groups in U.S. tree frogs (*Hyla*). *Southwest. Nat.* 3: 77–89.
- 1958c. Response of a green tree frog (*Hyla cinerea*) to the call of the male. *Copeia* 1958: 333–334.
- CHANTRELL, C. J. 1966. Late Cenozoic hylids from the Great Plains. *Herpetologica* 22: 259–264.
- CONANT, R. C. 1958. A field guide to reptiles and amphibians of eastern North America. Riverside Press, Cambridge, Mass. 366 p.
- CREIGHTON, W. S. 1950. The ants of North America. *Bull. Mus. Comp. Zool. Harvard* 104: 569 p.
- FLURY, A. G. 1951. Variations in some local populations of *Hyla versicolor* in Central Texas. M. A. thesis, Univ. of Texas, Austin, Texas.
- GEIGER, R. 1965. The climate near the ground. Translated by Scripta Technica, Inc. from the 4th German Ed. Harvard Univ. Press, Cambridge, Mass. 611 p.
- HOFFMAN, R. L. 1946. The voice of *Hyla versicolor* in Virginia. *Herpetologica* 3: 141–142.
- HUBBS, C. L., and C. HUBBS. 1953. An improved graphical analysis and comparison of series samples. *Syst. Zool.* 2: 49–57.
- JOHNSON, C. 1959. Genetic incompatibility in the call races of *Hyla versicolor* Le Conte in Texas. *Copeia* 1959: 327–335.
- 1961. Cryptic speciation in the *Hyla versicolor* complex. Ph.D. dissertation, Univ. of Texas, Austin, Texas. 121 p.
- 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.
- LITTLE, V. A. 1963. General and applied entomology. Harper & Row. New York. 543 p.
- LITTLEJOHN, M. J. 1959a. Mating behavior in the treefrog, *Hyla versicolor*. *Copeia* 1959: 222–223.
- 1959b. Call differentiation in a complex of seven species of *Crinia* (Anura, Leptodactylidae). *Evolution* 13: 452–468.
- 1960. Call discrimination and potential reproductive isolation in *P. triseriata* females from Oklahoma. *Copeia* 1960: 370–371.
- 1961. Mating call discrimination by females of the spotted chorus frog (*Pseudacris clarki*). *Texas J. Sci.* 13: 49–50.
- 1965. Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution* 19: 234–243.

- , and T. C. MICHAUD. 1959. Mating call discrimination by females of Strecker's chorus frog (*Pseudacris streckeri*). Texas J. Sci. 11: 86–92.
- , M. J. FOUQUETTE, and C. JOHNSON. 1960. Call discrimination by female frogs of the *Hyla versicolor* complex. Copeia 1960: 47–49.
- MARTIN, W. F. 1967. The mechanism and evolution of sound production in the toad genus *Bufo*. M. A. thesis, Univ. of Texas, Austin, Texas. 131 p.
- MECHAM, J. S. 1961. Isolating mechanisms in anuran amphibians. p. 24–61, *In* W. F. Blair, Ed. Vertebrate Speciation. Univ. Texas Press, Austin.
- MICHAUD, T. C. 1962. Call discrimination by females of the chorus frogs *Pseudacris clarki* and *Pseudacris nigrita*. Copeia 1962: 213–215.
- MITTLEMAN, M. B. 1947. Miscellaneous notes on Indiana amphibians and reptiles. Amer. Midl. Nat. 38: 466–484.
- MORONEY, M. J. 1951. Facts from figures. Penguin Books, Baltimore, 472 p.
- NOBLE, G. K., and C. HASSLER. 1936. Three Salientia of geographic interest from southern Maryland. Copeia 1936: 63–64.
- WALKER, C. F. 1946. The amphibians of Ohio. Part 1. The frogs and toads (order Salientia). Ohio State Mus. Bull. 1: 1–109.
- WHEELER, W. M. 1910. Ants: their structure, development, and behavior. Columbia Univ. Press, New York. 663 p.