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Advertisement Call and Distribution of the Treefrogs *Hyla chrysoscelis* and *Hyla versicolor* in Virginia

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ABSTRACT

The gray treefrog complex consists of two sibling species that are indistinguishable morphologically, the diploid *Hyla chrysoscelis* and the tetraploid *Hyla versicolor*. Identification is possible in the field only by audio recognition of male advertisement call trill rates (pulses/second). During 1979-1983 we evaluated taped calls of these two species taken from 89 populations from throughout Virginia to map their respective ranges and to evaluate differences in call parameters. *Hyla chrysoscelis* occurs in the Coastal Plain, eastern and southern Piedmont, and in southwestern Virginia. *Hyla versicolor* occurs in the Piedmont, Blue Ridge, and Ridge and Valley regions south to Wythe and Tazewell counties. Sympatric sites occur in several locations in the Piedmont and both species are syntopic in several of them. Male trill rates are significantly related to ambient and body temperatures. Rates produced by male *H. chrysoscelis* ($\geq$31/s) are twice as fast as that for *H. versicolor* ($\leq$30/s); they did not overlap in our samples at any temperature. Trill rates and call duration in southwestern Virginia populations of *H. chrysoscelis* differed significantly from those in eastern populations when adjusted for ambient temperature. Adjusted trill rate and duration in *H. chrysoscelis* populations in sympatry with *H. versicolor* were not significantly different from allopatric populations but were for *H. versicolor*.

Keywords: Anura, Cope's Gray Treefrog, ecology, Gray Treefrog, *Hyla chrysoscelis*, *Hyla versicolor*, vocalizations, distribution, Virginia

INTRODUCTION

The diploid *Hyla chrysoscelis* (Cope's Gray Treefrog) and the tetraploid *H. versicolor* (Gray Treefrog) differ in the pulse rate of the trills produced by calling males (Johnson 1959, 1966), their karyotypes (Wasserman 1970, Bogart and Wasserman 1972), and in some cytological parameters, such as size of toe pad cells

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(Green 1980), cell size (Bogart and Wasserman 1972), amount of DNA in the nuclei (Bachmann and Bogart 1975), and size of nuclei and number of nucleoli (Cash and Bogart 1975). Ptacek et al. (1994) recognized five sibling species in the Gray Treefrog complex, two of which were diploid and three tetraploid. Espinoza and Noor (2002) examined evidence for gene flow among *H. versicolor* lineages at various locations using PCR-product cloning techniques. These authors verified distinct mitochondrial lineages in *H. versicolor*, but stated that these lineages hybridize when they exist in sympatry. Halloway et al. (2006) determined that *H. versicolor* originated repeatedly from three diploid ancestors, including *H. chrysoscelis*, and merged through inbreeding to result in a single species. Both of these gray treefrogs occur widely in Virginia.

At present, recognition of male mating trill pulse rates is the only method available to distinguish *H. chrysoscelis* and *H. versicolor* in the field (Elliott et al. 2009). They are indistinguishable morphologically. However, because pulse rates are related to ambient temperature, pulse rate alone may not distinguish a warm *H. versicolor* from a cool *H. chrysoscelis*. Thus, elucidation of their respective distributions has been slow to accumulate. They have been mapped out in several states, including Texas (Johnson 1966), parts of Illinois (Brown and Brown 1972), Wisconsin (Jaslow and Vogt 1977), Michigan (Bogart and Jaslow 1979), and Maryland (Otto et al. 2007). Zweifel (1970) examined the distribution of these species in northeastern Virginia, eastern Maryland, Delmarva and southern New Jersey. Based on these studies and their own work, Ralin (1977) and Gerhardt (1999) extrapolated the range limits of *H. chrysoscelis* and *H. versicolor* for North America and their areas of sympatry. For Virginia, Ralin (1977) hypothesized that *H. chrysoscelis* occurs in the southeastern half of the state and *H. versicolor* in the northwestern half. Gerhardt (1999) illustrated the generally accurate distribution patterns for both species and their known areas of sympatry based, in part, on information supplied by us. Range-wide maps of the two Gray Treefrogs are in Cline (2005a, b) and Halloway et al. (2006).

Our study sought to determine the distributions of these two species in Virginia and to investigate differences in their vocalizations. The atlas of amphibians and reptiles published by Mitchell and Reay (1999) used the information we present here, in addition to museum specimens and other sources to create maps of both gray treefrogs in Virginia. This paper presents the basis upon which those maps were delineated and presents results of our analyses of call parameters in allopatry and sympatry.

MATERIALS AND METHODS

During March to August 1979-1983, we drove approximately 71,000 km throughout Virginia to locate calling gray treefrogs. We found choruses at 224 localities. Tentative identifications made by ear in the field were later verified and analyzed at 89 of these sites, the results of which are reported here. We recorded vocalizations on portable standard Panasonic® and General Electric® cassette recorders using fresh batteries. When possible, we took cloacal temperatures (to 0.1°C) with Schultheis quick-reading thermometers. Ambient and water temperatures were taken with Schultheis and Webster thermometers placed as close to the calling frog as possible. In the analyses of call parameters, water temperature was used for the ambient measurement instead of air temperature if the frog was sitting in water when calling.

Sonograms of mating calls were produced with a Kay Electronic Sound Spectrograph Model 6061B. From these sonograms we determined trill rates (number of pulses per second) and duration of calls (in seconds) of 326 individual males from the 89 localities. Trill rates were determined directly from the sonograms. Scatterplots of trill rate versus body and ambient temperatures were used to illustrate the relationships between temperature and call parameters. Analysis of covariance, with ambient and body temperatures as covariates, was used to compare differences between species and populations. Statistical tests were performed with SysStat 11® using a Type I error rate of $\alpha = 0.05$. In this paper, means are reported with $\pm$ one sd.

RESULTS

Hyla chrysoscelis occurs in Virginia in two allopatric areas, the Coastal Plain, including the Eastern Shore, and in the Valley and Ridge and Cumberland Plateau regions south of the New River in the southwestern corner of the state (Figure 1). *Hyla versicolor* occurs allopatrically in the western Piedmont, Blue Ridge Mountains, and Valley and Ridge regions south to eastern Wythe County. The two species are sympatric over a wide portion of the eastern and lower Piedmont region and are syntopic in many locations in central and south-central Virginia (Figure 1). We found no evidence that these two species occur in sympatry in southwestern Virginia and could not determine whether the range of *H. versicolor* extends into North Carolina along the Blue Ridge Mountains. Current locality data suggest that eastern and southwestern *H. chrysoscelis* populations are separated by populations of *H. versicolor*. Recent locality records of *H. chrysoscelis* along the Blue Ridge Parkway in Floyd County (Mitchell and Reay, 1999) where they were not known historically (Hoffman, 1996) suggest that this pattern may be changing.

Comparisons of call parameters between species (Table 1) demonstrate that differences in trill rates are readily apparent before adjustment is made for the effects of temperature. Populations of *H. chrysoscelis* from southwestern Virginia have slightly higher average unadjusted trill rates than populations in eastern Virginia and lower average unadjusted call duration. Unadjusted trill rates of *H. chrysoscelis* populations sympatric with *H. versicolor* average higher than that in allopatric populations, but unadjusted call duration is shorter. Sympatric *H. versicolor* populations, however, exhibit similar unadjusted trill rates and shorter unadjusted call durations than allopatric populations (Table 1).

Trill rates of Virginia Gray Treefrogs are significantly related to ambient temperature and body temperature (Figure 2, 3). Note that trill rates for these two species do not overlap at any ambient or body temperature. Between-species comparisons of the ambient temperature range in Figures 2 and 3 suggest that *H. versicolor* is active over a broader range of temperature conditions than *H. chrysoscelis*.

Southwestern Virginia populations of *H. chrysoscelis* differed significantly from eastern populations in trill rate ($F= 10.24$, $P = 0.002$) and call duration ($F = 4.86$, $P = 0.029$) when adjusted for ambient temperature. Adjusted trill rate and duration in *H. chrysoscelis* populations sympatric with *H. versicolor* were not significantly different from allopatric populations ($F = 3.38$, $P = 0.068$, $F = 1.084$, $P = 0.300$, respectively). Sympatric *H. versicolor* had a significantly lower adjusted trill rate than allopatric populations ($F = 4.93$, $P = 0.029$). There was no significant difference in adjusted call duration between these two groups ($F = 1.25$, $P = 0.269$).

Ambient temperature does not affect dominant frequency (KHz) in Virginia gray treefrogs ($F = 0.177$, $P = 0.675$). Mean dominant frequency of *H. chrysoscelis* and *H.*

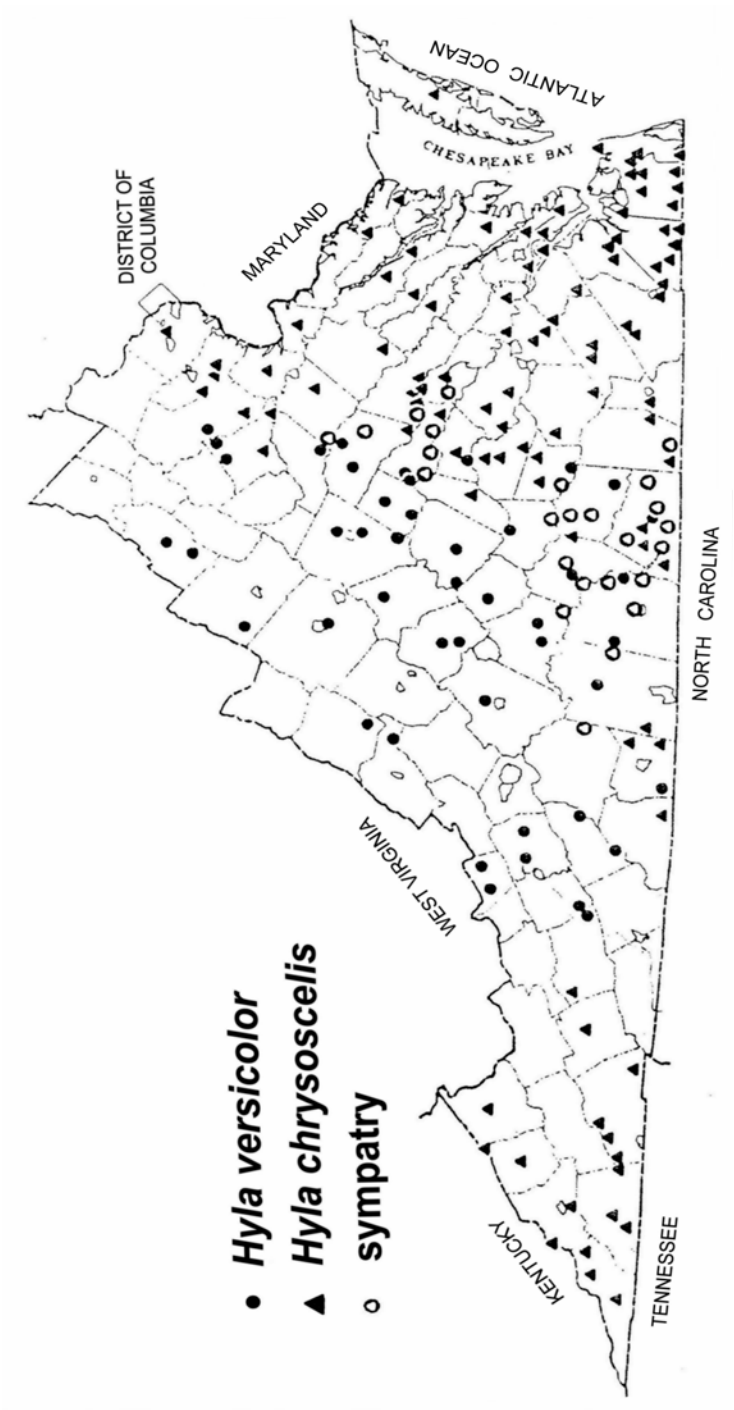

FIGURE 1. Distribution patterns for *Hyla chrysoscelis* and *H. versicolor* in Virginia based on analysis of trill rates in breeding choruses.

TABLE 1. Unadjusted call character and temperature statistics for *Hyla chrysoscelis* and *H. versicolor* samples in allopatric and sympatric regions in Virginia. Eastern Virginia samples of *H. chrysoscelis* include allopatric and sympatric data from sites east of the Blue Ridge Mountains. Sympatric samples for both species are from the eastern Piedmont. The first number in each column is the sample size followed by the mean ± one SD.

	Body Temp °C)	Ambient Temp (°C)	Trill Rate	Duration (s) (pulse/s)	Dominant Frequency
H. chrysoscelis					
E. VA	40 22.65±1.99	194 21.91±2.06	197 45.63±4.75	119 0.841±0.272	46 1882.39±625.42
SW VA	– –	14 21.55±2.06	14 48.29±6.17	9 0.679±0.159	7 1441.43±484.85
Allopatric	14 22.79±1.39	100 21.6±1.96	101 44.95±4.64	65 0.855±0.306	28 1913.93±636.62
Sympatric	24 22.46±2.25	103 22.11±2.18	104 46.62±4.99	59 0.789±0.199	24 1748.33±604.51
All samples	38 22.65±1.99	217 21.88±2.06	209 45.81±4.89	133 0.830±0.268	53 1824.15±633.16
H. versicolor					
Allopatric	11 24.51±1.80	34 21.96±2.48	34 24.85±3.29	21 0.809±0.379	10 1793.40±438.59
Sympatric	9 22.21±3.28	71 22.67±3.15	73 24.12±3.51	40 0.722±0.319	21 1914.67±500.25
All samples	20 23.71±2.53	105 22.5±2.90	107 24.54±3.42	61 0.729±0.327	31 1785.55±477.35

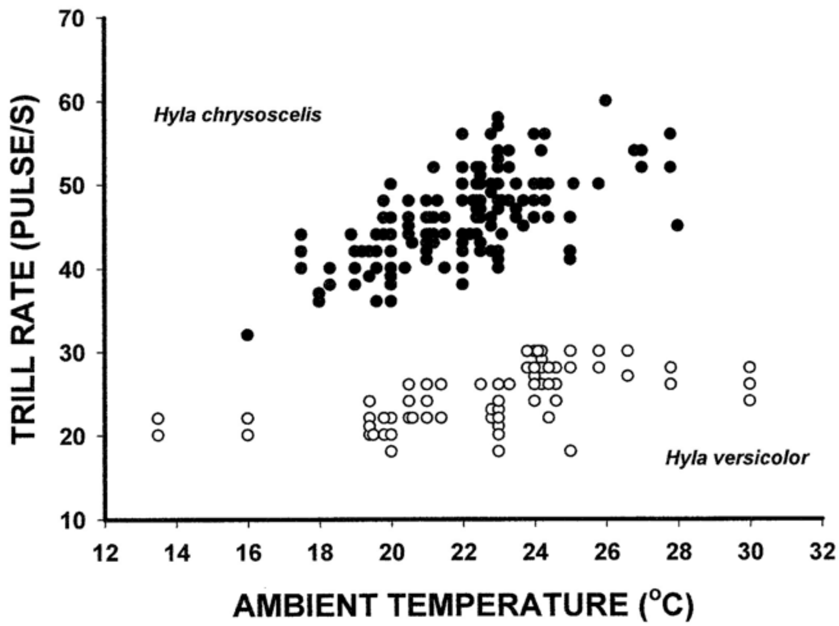

FIGURE 2. Relationship of trill rate to ambient temperature for *Hyla chrysoscelis* and *H. versicolor* in Virginia.

versicolor calls (Table 1) do not differ significantly (t = 0.424, P = 0.673). The dominant frequency for *H. chrysoscelis* calls in southwestern Virginia populations are not significantly different from those in eastern populations (t = 1.780, P = 0.081).

DISCUSSION

Twenty years before it was determined that gray treefrogs were, in fact, two separate species, Hoffman (1946), following differences in calls defined by Noble and Hassler (1936) and Walker (1946), delineated their ranges in Virginia with considerable accuracy. The patterns he found, based only on the relative locations of gray treefrogs with "harsh" and "mellow" voices, are essentially the same as those we report here. He noted a population in the central Virginia Piedmont (an area of sympatry) that apparently had calls somewhat intermediate between the two voice types and with longer durations than those he had noted in southeastern Virginia (*H. chrysoscelis* only). We suspect that this was a function of temperature because most of his southeastern records were obtained in summer (based on museum records); his Piedmont record was taken in late September. Hoffman's paper has apparently been overlooked in all the previous literature on the calls and biogeography of these species except Zweifel (1970).

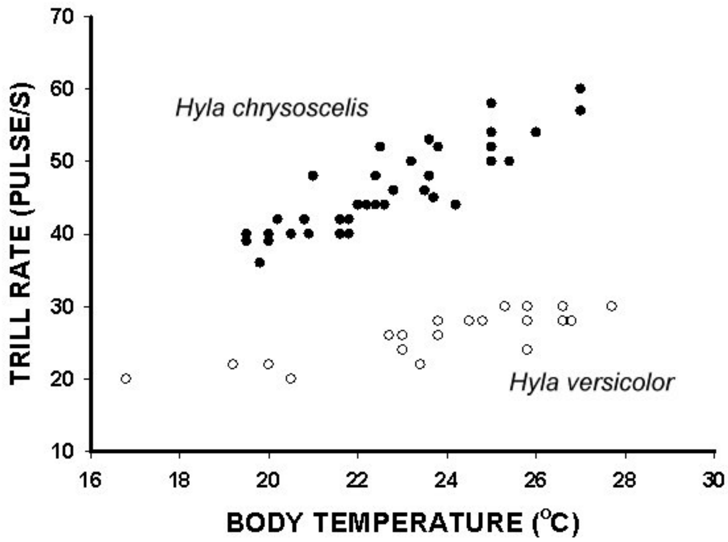

FIGURE 3. Relationship of trill rate to body temperature for *Hyla chrysoscelis* and *H. versicolor* in Virginia.

The distribution pattern hypothesized by Ralin and Sealander (1979) for *Hyla chrysoscelis* and *H. versicolor* in Virginia was essentially correct for these two species east of the Blue Ridge Mountains, although the broad zone of overlap was not predicted. The primary modification of their pattern occurs in southwestern Virginia where only *H. chrysoscelis* is found; they had predicted only *H. versicolor* in that area. Hoffman and Kleinpeter (1948) described what appeared to be a sympatric population of the two species near Burkes Garden, Tazewell County, although R.L. Hoffman and J.A. Fowler heard only *H. versicolor* there three years later (R.L. Hoffman, pers. comm.). This unvouchered record, along with the one for eastern Wythe County, may delineate the range boundary of this species in southwestern Virginia. *Hyla chrysoscelis*, which occurs in southwestern Virginia, also ranges northward into West Virginia (Green and Pauley 1987, M. Little, pers. comm.) and Kentucky (J. MacGregor, pers. comm.) and southwestward throughout Tennessee (Redmond and Scott 1996, Niemiller and Reynolds 2011). The eastern form of *H. chrysoscelis* occurs throughout North Carolina (A. Braswell, pers. comm.) and extends northward into eastern Maryland and New Jersey in the Coastal Plain (Zweifel 1970). In Maryland, *Hyla chrysoscelis* occurs throughout most of the eastern half of the state and upper Delmarva, whereas *H. versicolor* occurs primarily in central and western Maryland; sympatry occurs in several locations (Otto et al. 2007, D. Forester and R. Miller, pers. comm.).

Comparisons of adjusted trill rate and duration for *H. chrysoscelis* populations revealed significant differences between eastern populations and those in southwestern Virginia but not in populations in sympatry with *H. versicolor*. The regional differences may be due to a combination of the large difference in sample sizes for these two areas and the narrow range of ambient temperatures recorded in southwestern counties (20-24ºC) compared to the much wider range in eastern samples (17.5-28ºC). Additional sampling over a broader range of temperatures in southwestern counties may provide a different result. Lack of a difference in adjusted trill rate and duration for *H. chrysoscelis* and *H. versicolor* in sympatry suggests that there is no species effect. The significant difference in adjusted trill rate between allopatric and sympatric populations in *H. versicolor* may have been influenced by several factors. Differences in trill rate between sympatric populations of these two species in Missouri are greatest at high temperatures (Gerhardt 1982) suggesting that higher temperatures in the Piedmont compared to those in higher elevations contributed to this result.

Despite the fact that trill rates are temperature-dependent, complete lack of overlap at most body and ambient temperatures in Virginia populations suggests that this parameter may be used as an identifying field character once the differences become recognized through experience and training. Trill rates $\geq$31 pulses per second are *H. chrysoscelis* and rates $\leq$ 30 pulses per second are *H. versicolor*. Johnson (1966) used trill rate differences to construct a key to these species, although there was a 2-pulse overlap in the minimum and maximum values in his study. Other studies (e.g., Zweifel 1970, Ralin 1977, Gerhardt 1982) found a small range overlap in trill rates between cool *H. chrysoscelis* and warm *H. versicolor*. Because individuals have been found to exhibit trill rates intermediate between the two species (Zweifel 1970, Gerhardt 1982), we caution the use of this parameter rate for final species identification without correction for temperature affects. Trill rates are non-overlapping at 20ºC (diploid species mean = 35.7+1.4ºC, tetraploid species mean = 22.9+1.5ºC) (Gerhardt 2005). Mean breeding body temperatures in Virginia (22.7ºC for *H. chrysoscelis*, 23.7ºC for *H. versicolor*) are higher than the 20ºC. Holloway et al. (2006) concur that this is the middle of the range of breeding temperatures for these species range-wide.

Comparisons of the call parameters for Virginia populations of *H. chrysoscelis* with values of these parameters in Ralin (1977) indicate that the populations in eastern and southwestern Virginia correspond with his eastern form. Virginia *H. versicolor* trill rates correspond more closely with Ralin's northern *H. versicolor*, but call duration is considerably longer than those noted for either northern or southern *H. versicolor*. Jaslow and Bogart (1979) found a similar result for gray treefrogs in Michigan. Using our data, it is not possible to determine whether the eastern Virginia populations of *H. chrysoscelis* differ genetically from the southwestern populations. Electrophoretic (e.g., Ralin and Sealander 1979), immunological (e.g., Maxson, et al. 1977), and genetic analyses (Espinoza and Noor 2002, Holloway et al. 2006) have elucidated the historical biogeographical patterns and origins of this diploid-tetraploid species pair in North America. Distribution patterns illustrated in Figure 1a in Halloway et al. (2006) for the two species show sympatry in a continuous range from southwestern Virginia (Wythe County to about Giles County) to about Mecklenburg County in the southern Piedmont and along the western edge of the Piedmont. Our results do not show sympatry west of the Blue Ridge Escarpment and places the overlap zone of these two species in the eastern Piedmont. Their distribution of *H. versicolor* is well to the west of the range in

Virginia that we elucidated. Additional records of these two species in southwestern Virginia and along the eastern side of the Blue Ridge Mountains are needed to clarify this discrepancy.

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