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THE HYLA VERSICOLOR-CHRYSOSCELIS SPECIES COMPLEX OF GRAY TREEFROGS IN ARKANSAS: HISTOLOGICAL AND ULTRASTRUCTURAL EVIDENCE

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ABSTRACT

We investigated the Hyla versicolor-chrysoscelis species complex (tetraploid and diploid species, respectively) of cryptic gray treefrogs from Arkansas using light and scanning electron microscopy. From previous studies of this treefrog complex in other states, H. versicolor has been shown to exhibit larger nuclear diameters and larger toe pad epithelial cells than H. chrysoscelis. Based upon average nuclear diameters of eyelid epithelial cells, we found two or possibly three groups of frogs. The presumed H. versicolor exhibited greatly enlarged toe pad epithelial cells using scanning electron microscopy and were found in four counties, three of which are in the Ozark Mountains. Hyla chrysoscelis occurs throughout the state.

INTRODUCTION

The gray treefrog species complex has long posed as an enigma to researchers. Two cryptic species, Hyla versicolor (Eastern Gray Treefrog) and H. chrysoscelis (Cope's Gray Treefrog), are distinguishable in the field by the male breeding call with H. versicolor exhibiting a relatively slow pulse rate compared to a fast rate in H. chrysoscelis. Wasserman (1970) separated the two species by examination of their karyotypes (H. versicolor, 4n = 48; H. chrysoscelis, 2n = 24).

Recently, a variety of methods have been used to differentiate between the two species. Ralin (1968) examined the stomach contents, calling positions, and effects of temperature and humidity on the trill rates; he concluded that H. chrysoscelis was more arboreal and preferred lower humidity than H. versicolor. Micro-complement fixation was utilized by Maxson et al. (1977) to separate the two species and to estimate the age of origin of tetraploidy in H. versicolor. Noting "east" and "west" populations of H. chrysoscelis, they concluded that one class of Texas H. versicolor was immunologically identical to the eastern H. chrysoscelis, another class was immunologically identical to the western H. chrysoscelis, and a third (and largest) class was immunologically heterogeneous. Ralin and Rogers (1979) performed an analysis of thirteen external morphological characters. They concluded that none of the populations in the study were statistically different from other groups for any one measurement. In addition, they distinguished three groups of populations. One group consisted of five southcentral Texas populations of H. chrysoscelis; the second was three Texas populations of H. versicolor, and the third was from four populations of H. chrysoscelis from different parts of its range.

Green (1979) used scanning electron microscopy of the digital toe pads of a number of frog species (including gray treefrogs). He found that while all Hyla species were very similar in morphology, H. versicolor was distinguished by the greater size of its toe pad cells compared to those of H. chrysoscelis.

Cash and Bogart (1978) theorized that the physical dimensions of the tetraploid nucleus should be greater than those of the diploid. Their study demonstrated that the measurement of nuclear diameters from paraffin histosections was an accurate method for species recognition; the spherical volumes of H. versicolor nuclei were approximately twice the size of H. chrysoscelis.

Although previous studies on the distribution of the H. versicolor-chrysoscelis species complex have been conducted in the border states of Mississippi (Ralin and Rogers, 1979), Missouri (Johnson, 1977), and Texas (Johnson, 1959; 1963) and in Kansas (Hillis et al., 1987), no studies have addressed the distinction of these species in Arkansas. The objectives of the present study were to determine the identity and distribution of gray treefrogs species in Arkansas from museum specimens using histological and ultrastructural techniques.

MATERIALS AND METHODS

A total of 120 gray treefrogs from the Milwaukee Public Museum, University of Arkansas at Monticello, Arkansas Tech University, and Arkansas State University Museum of Zoology were examined. The sample included specimens from 66 sites in 28 counties; the snowvent length (SVL) was recorded for each animal. Eyelids were removed and placed in vials of 70% ethanol. Standard histological techniques were used to prepare tissues for light microscopy (Humason, 1979). Tissues were dehydrated in a graded series of ethanol and toluene, embedded in paraffin, sectioned serially at 10 µm, stained with Harris hematoxylin, and counterstained with eosin. In addition, we oriented eyelids tangentially during sectioning in order to maximize the number of cells in the field of view. Ninety nuclear diameters were measured from a routinely-selected area of each eyelid using an ocular micrometer to the nearest 0.1 µm.

The toe pads of 20 specimens were examined using scanning electron microscopy (SEM). Toes from the left hind foot were excised, dehydrated in a graded series of ethanol and amyl acetate, dried with Sadmi critical point dryer, coated with gold/palladium with a Hummer IV sputter coater, and viewed with a JEOL 100 CXII TEM-SCAN electron microscope at an accelerating voltage of 40 kV.

RESULTS

Two distinct groups of frogs were observed in our sample (Fig. 1). Those which exhibited higher average nuclear diameters were presumed to be H. versicolor, whereas those with lower average nuclear diameters were presumed to be H. chrysoscelis. Within the presumed group of H. versicolor, nuclear diameters ranged from 9.6 - 12.6 µm (x = 10.9 µm; N = 9); in the presumed H. chrysoscelis, nuclear diameters ranged from 7.7 - 8.7 µm (x = 8.4 µm; N = 101). We found no significant correlation between the average nuclear diameter and SVL (P > 0.05). In addition, we noted a third (intermediate) group whose average

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red in close proximity to one another. This condition was similar to the situation found in Kansas (Hillis et al., 1987).

Variation in the size of digital toe pad cells of the complex using SEM is shown in Fig. 2. Cells of presumed H. versicolor (Fig. 2 A-D) are much larger than those of intermediate (Fig. 2 E and F) or of presumed H. chrysoscelis (Fig. 2 G and H). A pairing of toe cell size with average nuclear diameter in Fig. 1 yielded a proper species identification in 88% (excluding intermediates) of the specimens.

**DISCUSSION**

In our study of the H. versicolor-chrysoscelis species complex of gray treefrogs, we only used data on average nuclear diameter of eyelid cells to separate the two species mainly because Green (1979), using SEM, was able to match toe pad cell size with the proper species 79% of the time. Earlier studies used specimens from previously identified populations and then sought to further distinguish the two species (Brown and Brown, 1972; Gayou, 1984; Green, 1979; Maxson et al., 1977; Ralin, 1968; Ralin and Rogers, 1979) with varying degrees of success.

Previous investigations were not helpful in deciphering the gray treefrog complex in Arkansas. Ralin and Rogers (1979) and Maxson et al., (1977) mention “eastern” and “western” populations of H. chrysoscelis. The former authors refer to the “eastern” group as occurring in South Carolina, Georgia, Ohio, and Mississippi, whereas the “western” group were from Texas. Although H. versicolor and H. chrysoscelis occur sympatrically throughout much of their ranges, current usage of geographic terms to imply genetic similarities or differences only tend to obfuscate the complex, especially in regions poorly studied. Ralin and Rogers (1979) suggested that populations in the Midwest, border states, and western portions of the South might be intermediate or integrate populations of H. chrysoscelis. While Ralin’s (1968) distributional map for the two species showed the entire state of Arkansas as having almost exclusively H. chrysoscelis (with the exception of extreme northwestern Arkansas), our study indicates that what we assume to be H. versicolor is found throughout northcentral Arkansas as well as in the extreme eastern portion of the state. We also concur with Hills et al., (1987) that additional studies are needed to evaluate the possibility of multiple origins of polyplolyd in the gray treefrog complex. These investigations would be especially important in northern Arkansas where we detected intermediate treefrogs.

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**LITERATURE CITED**


Figure 2. Scanning electron micrographs of toe pad epidermal cells from the hind foot (middle digit) of gray treefrogs of the *Hyla versicolor-chrysoscelis* species complex from Arkansas. X2,000. A - H correspond to A - H of Fig. 1. A-D Polygonal cell surfaces representative of *H. versicolor*. E and F. Cells from intermediate males. G and H. Cells from *H. chrysoscelis*. 


