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Hyla versicolor-chrysoscelis Species Complex of Gray Treefrogs in Arkansas: Histological and Ultrastructural evidence

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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 heterogenous. 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 simmilar 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 was examined. The sample included specimens from 66 sites in 28 counties; the snout-vent 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 Samdri 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 ($\bar{x}$ = 10.9 μm; N = 9); in the presumed *H. chrysoscelis*, nuclear diameters ranged from 7.7 - 8.7 μm ($\bar{x}$ = 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

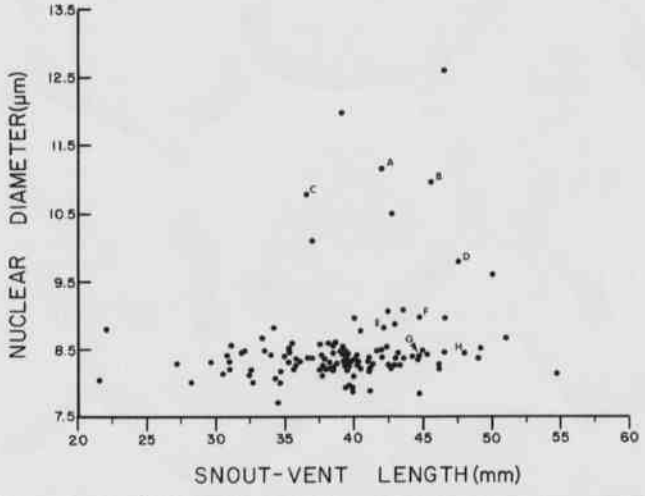

Figure 1. Relationship between average nuclear diameter of eyelid epidermal cells and SVL of gray treefrogs of the *Hyla versicolor-chrysoscelis* species complex from Arkansas. Letters correspond to specimens whose digital toe pad cells are depicted in Fig. 2.

nuclear diameters ranged from 8.7 - 9.1 μm ($\bar{x}$ = 8.9 μm; N = 10). This latter group did not display the large nuclear diameters of presumed *H. versicolor*, yet their diameters were larger than presumed *H. chrysoscelis*. The same existed with respect to their toe pad cells (Fig. 2). With the exception of one animal (from Columbia Co.), all intermediate treefrogs were collected from counties within the Ozark Mountains of northern Arkansas (Fig. 3). Presumed *H. chrysoscelis* specimens were found throughout Arkansas, whereas most of the presumed *H. versicolor* were from the northern regions of the state. In many instances, presumed *H. versicolor* and *H. chrysoscelis* occurred in close proximity to one another. This condition was similar to the situation found in Kansas (Hillis *et al.*, 1987).

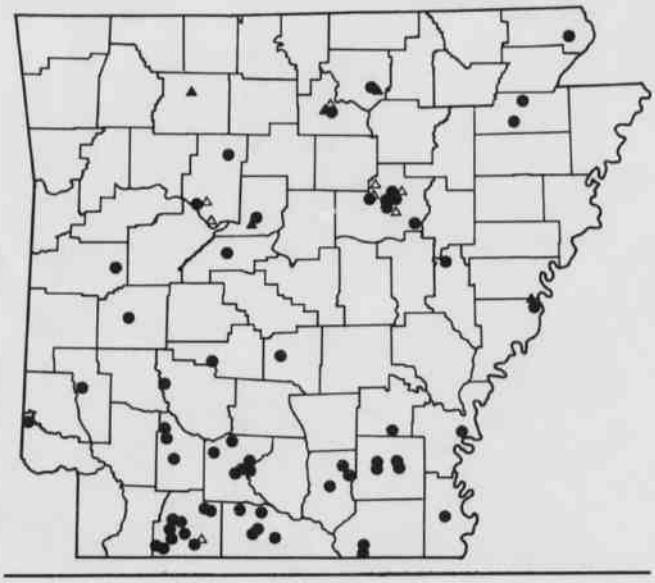

Figure 3. Locations for museum specimens of the *Hyla versicolor-chrysoscelis* species complex of gray treefrogs from Arkansas. Solid circles = presumed *H. chrysoscelis;* solid triangles = presumed *H. versicolor;* open triangles = intermediate males.

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 intermediates (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 decifering 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 integrade 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 Hillis *et al.*, (1987) that additional studies are needed to evaluate the possibility of multiple origins of polyploidy in the gray treefrog complex. These investigations would be especially important in northern Arkansas where we detected intermediate treefrogs.

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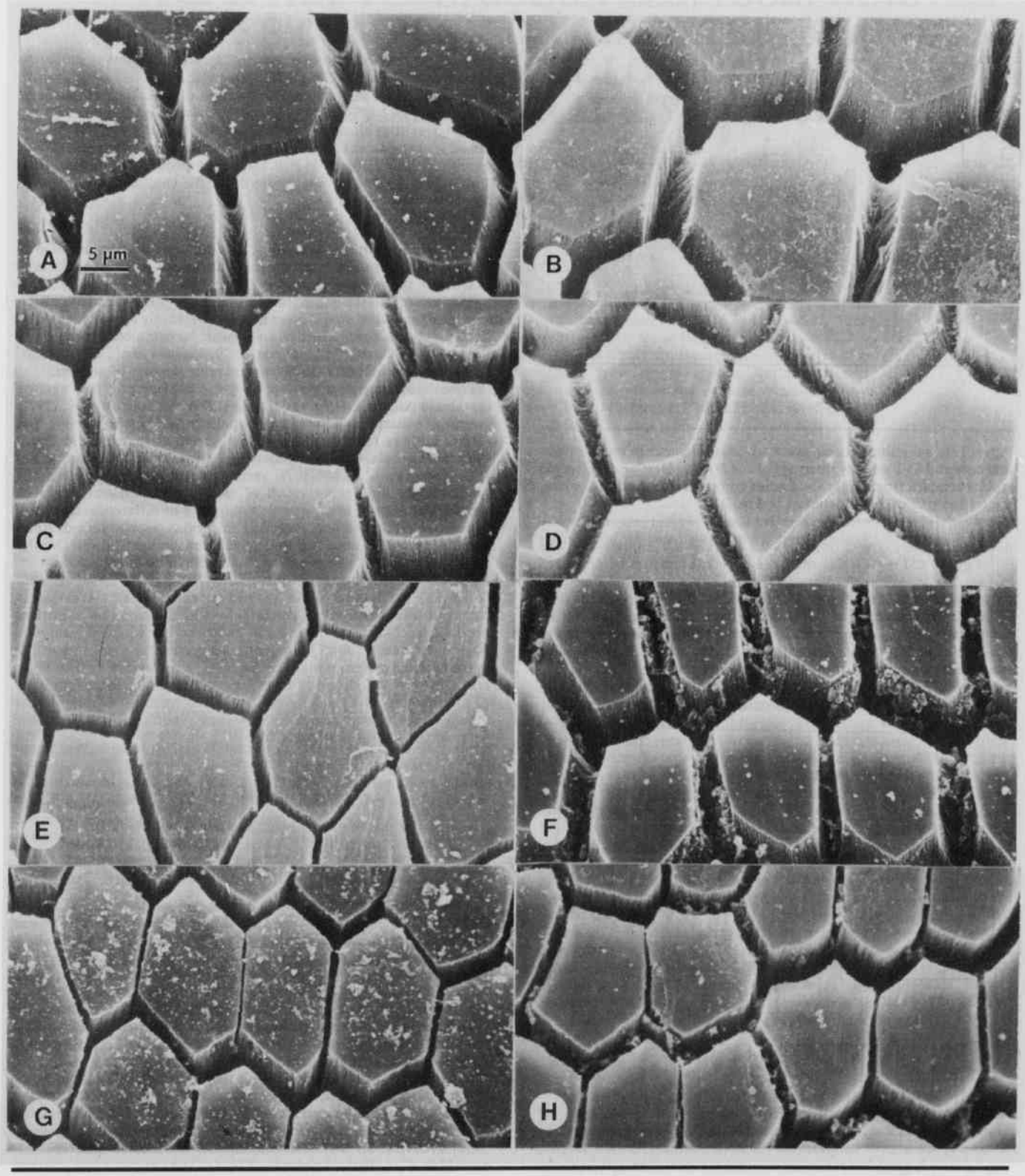

Figure 2. Scanning electron micrographs of toe pad epidermal cells from the hind foot (middle digit) of gray treefrogs of the *Hyla versicolor-chrysoscelis* species complex from Arkansas. X2,000. A - H correspond to A - H of Fig. 1. A-D Polygonal cell surfaces representative of *H. versicolor*. E and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

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