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Evidence to Support the Presence of Cope's Gray Treefrog (*Hyla chrysoscelis)* at Green Wing Environmental Laboratory in Northcentral Illinois

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ABSTRACT

We assessed the morphologically indistinguishable gray treefrog complex, *Hyla chrysoscelis* and *Hyla versicolor*, at Green Wing Environmental Laboratory, which is a biological field station in Northcentral Illinois. One source of information used in this assessment was karyotyping since it is known that *H. chrysoscelis* is diploid and *H. versicolor* is tetraploid (n = 12). Our results suggest that only *H. chrysoscelis* was karyotyped since the average number of chromosomes per spread was 23.4 (average range of each spread was 21.3 to 24) and no single spread exceeded 25 chromosomes. We also conducted frog call surveys from 2000-2003 since pulse rate is faster in *H. chrysoscelis* than in *H. versicolor*. Only *H. chrysoscelis* was encountered during standardized acoustic surveys and measured by vocal and temperature recordings in the field. Thus, only Cope's gray treefrog, *H. chrysoscelis*, was found at this site.

INTRODUCTION

Prior to the 1970s, *Hyla versicolor* and *Hyla chrysoscelis* were thought to be the same species (Ralin 1968). However, using karyotyping research, Wasserman (1970) showed that *H. versicolor* is tetraploid and *H. chrysoscelis* is diploid (n = 12). Moreover, acoustic characteristics of male vocalizations are different between these two treefrogs (Littlejohn 2001). At 21˚C, Gerhardt (1982) attained averages of 41 and 17 trill pulses/sec in *H. chrysoscelis* and *H. versicolor*, respectively. This information and mate choice studies (Gerhardt et al. 1994) show that this complex is composed of two different species.

Jaslow and Vogt (1977) used trill rates to determine the distribution of these species in Wisconsin and showed that they are sympatric in the central and southern parts of the state. Oberfoell and Christiansen (2001) studied the distribution of these species throughout Iowa and suggested that these two species are sympatric in central and eastern parts of the state. Hillis et al. (1987) used karyotyping to determine the distribution of these species in Kansas. Their results demonstrated both species coexist in the eastern part of the state. Unfortunately, little published information exists about the geographical ranges of *H. chrysoscelis* and *H. versicolor* in Illinois, although a formal study to address this is currently under way (C. Phillips, pers. comm.).

The goal of this study was to determine which of the species of gray treefrogs occur at Green Wing Environmental Laboratory (GWEL) in Northcentral Illinois. We used karyotyping (a count of chromosomes arrested at metaphase), frog call surveys, and call recordings for species identification.

MATERIALS AND METHODS

Karyotyping

In July and August 2002 twenty individuals (12 metamorphic tadpoles and 8 adults) were captured, 5 from each of 4 ponds, at GWEL. All individuals were taken alive to the laboratory where they were karyotyped. The bodies were fixed in 10% neutral buffered formalin, and each is currently stored in 70% EtOH in the Vertebrate Zoology Collection at Augustana College (voucher numbers: 02-006, Pine Pond, 5 tadpoles; 02-007, Snapper Pond, 5 tadpoles; 02-008, Wet Meadow Complex, 2 tadpoles; 02-012, Wet Meadow Complex, 3 adults; and 02-013, Iris Pond, 5 adults).

Tadpoles were allowed to swim in 0.01% colchicine solution for 12-24 hours (Kligerman and Bloom 1977). Adult frogs were injected with approximately 0.5 cc of colchicine (1 mg/ml deionized water) with a 23 gauge needle (1.9 cm in length). To ensure circulation throughout the adult torso the colchicine solution was injected through the skin of the dorsum and into the abdomen at a depth of ca. 3 mm. Once injected, adult frogs were placed into a container with a moist paper towel at 30˚C for 10-12 hrs (Bogart 1967).

Once the specimens (either tadpole or adult frog) had been subjected to the colchicine solution, they were sacrificed using Orajel® (Altig 1980). Once sacrificed, the specimens were dissected and tissue samples were taken from various locations on the specimen. Leg buds, gills, and intestinal tissue samples were the most useful from tadpoles and intestinal tissue of adult frogs gave the best karyotypes. The tissue sample, which measured ca. 0.5 cm in length, was placed in a small vial containing about 10 times their volume of 0.4% KCl hypotonic solution and allowed to sit for 20-30 minutes (Kligerman and Bloom 1977). The tissue was then transferred into 2-3 changes of fixative (3:1 ethanol acetic:acid solution) for approximately 30 minutes each (Kligerman and Bloom 1977).

Chromosomes were mounted on slides using heat fixation. This was accomplished by heating blank slides to 46-48˚C. The tissue sample was then placed into a separate vial containing 100 ul 50% acetic acid (Kligerman and Bloom 1977). The vial was tapped for 60-90 sec to break apart the tissue and form a cell suspension. Using a pipetman, we drew the cell suspension into the tip and expelled it onto the slide, quickly withdrawing the suspension back into the pipet tip, leaving as little liquid on the slide as possible.

The chromosomes were stained with 4% Geimsa in 0.01 M phosphate buffer at pH 7 for 10 minutes. The slides were air-dried and placed in xylene for 10 minutes to remove excess water. Once dried, the slides were completed by using Permount and a cover slip over the fixed sample.

Acoustical data

From 2000-2003, breeding chorus surveys were conducted at GWEL. Mossman and Hine (1984) was consulted for the methods used in these surveys. During this time, a total of six wetland sites was surveyed: Iris Pond, Main Pond, Culvert Swamp, Wet Meadow Complex, Snapper Pond, and Pine Pond. These sites constituted most of the wetland habitat and were scattered throughout GWEL. Species identification was verified by comparing calls to known calls available on a CD-ROM (Cornell Laboratory of Ornithology 1996). Although qualitative in nature, this technique is based on the Wisconsin anuran breeding chorus surveys (Mossman and Hine 1984) which are conducted during optimal weather conditions consisting of warm evenings (water temperatures ≥15˚C), low wind, and high humidity. Despite the fact that call characteristics of both *H. chrysoscelis* and *H. versicolor* vary with environmental and body temperature (Gerhardt 1982), we conducted surveys during weather that would allow one to distinguish each species' call.

In May 2003, one of us (SBH) recorded breeding calls from four frogs at GWEL: two individuals from Wet Meadow Complex and two from Main Pond. These recordings were completed to assess quantitatively and provide a representative oscillogram of the calls. The methods for field recordings of frog calls were borrowed from Heyer (1994). Recordings were completed on a 60 minute, chromium dioxide cassette tape (TDK-PRO, IECII/TYPEII) using a Marantz (PMD222) professional tape recorder (tape speed: 4.75 cm/sec) and a microphone (Shure BETA52), which can record within the frequencies of 20 to 10,000 Hz. Oscillograms (displays of amplitude changes over time) of these calls were generated using Computerized Speech Lab (CSL, Kay Elemetrics Corporation) Model 4300B. During taping, water temperatures at the frog's location in the pond ranged between 15.2 ˚C and 20.2 ˚C, winds were calm, and skies were clear.

RESULTS

Karyotyping

We analyzed between 2 and 8 spreads from each of the 20 individuals (Table 1). The average number of chromosomes per spread was 23.4 (average range of each spread was 21.3 to 25) and no single spread exceeded 25 chromosomes. For each individual, species was determined by comparing chromosome counts to the known chromosome numbers for *H. chrysoscelis* (2n = 24) and *H. versicolor* (4n = 48) (Wasserman 1970). We concluded that that all of the individuals sampled must be *H. chrysoscelis* since none them yielded spreads exceeding 25 chromosomes.

Acoustical data

From 2000-2003, every individual that was heard calling from all wetland sites during breeding chorus surveys matched the recorded call of *H. chrysoscelis*. From our own recordings taken in May 2003, we calculated that the average pulse rate for each individual was 44, 46, 38, and 40 pulses per second (n = 3 calls; SE = 0 for each average). Figure 1 shows a representative oscillogram for one individual's call.

DISCUSSION

We conclude from karyotyping results that all of the individuals sampled from GWEL are *H. chrysoscelis* and not *H. versicolor*, as all specimens examined were diploid rather than tetraploid. Not every spread contained exactly 24 chromosomes; however, a range is expected because it is easy to lose chromosomes during the procedure or to get chromosomes from a nearby cell combined into an adjacent spread. Moreover, if spreads deviated from 24, which happened for 13 of 20 individuals, it appears that chromosomes were lost more frequently than gained from a single spread. Since chromosome numbers of the two species differ by 24, we conclude that average chromosome counts of 21-24 correspond to *H. chrysoscelis*. The breeding chorus surveys and call recordings in the field are consistent with the acoustical characteristics of *H. chrysoscelis*.

There are other methods besides those which we used, chromosome number and call rates, that have the potential to distinguish *H. versicolor* from *H. chrysoscelis*, including toe pad size (Oberfoell and Christiansen 2001), coloration and patterning (Jaslow and Vogt 1977), and size of the individual (Ralin 1968), as well as cytological characteristics such as nucleolar number (Keller 2000). The authors of these studies suggest that chromosome number and call rate offer the most reliable sources of information for species identification relative to the others tested.

Hyla versicolor and *H. chrysoscelis* may have different ecological requirements, such as humidity levels and habitat type, which may explain why only *H. chrysoscelis* was found at GWEL. Ralin (1968) suggested that *H. chrysoscelis* is a hardier species, tolerating and maybe even preferring more arid conditions, whereas *H. versicolor* prefers more humid surroundings. Oberfoell and Christensen (2001) showed this in their study of distribution patterns of the species in Iowa, where *H. versicolor* was found in the eastern portion of the state, nearer the Mississippi River, and *H. chrysoscelis* was found in the drier, more western loess hill regions. This pattern may also be gleaned from Hillis et al. (1987), who suggested that *H. chrysoscelis* is more aridly adapted and lives in the more western regions of Kansas. There have been no published reports describing these particular species in conjunction with relative humidity levels within the northcentral Illinois area; however, it is possible that that region containing GWEL is not humid enough for *H. versicolor*.

Ideal living and breeding habitats for both species includes forest edges and areas with accessible water sources (Northern Prairie Wildlife Research Center 2002). As a result, the distribution of the gray treefrog complex covers the entire eastern portion of the US with the exception of the tip of Florida and all of Maine, to the West down the middle of Texas, Oklahoma, Kansas, and North and South Dakota. However, differences in habitat preference exist as well, where *H. chrysoscelis* prefers more open habitats such as prairies, open grasslands, and old field habitat, and *H. versicolor* prefers coniferous and deciduous woodlands and is rarely found in prairies and savannahs (University of Wisconsin Sea Grant 2001; Cofrin Center for Biodiversity at the University of Wisconsin – Green Bay 2004). Upland and lowland oak and pine forests, sedge meadows, and old-field habitat, as well as five temporary ponds characterize GWEL. Based on this evidence, it is unclear as to why only *H. chrysoscelis* would be found there, as the area contains the apparent habitat of both species. It is likely that other ecological requirements

and historical biogeographical constraints may play a greater role in the distribution of these species than simply habitat type.

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Figure 1. Oscillogram of Frog #1 recorded at Wet Meadow Complex. A total of 44 pulses per second was counted.

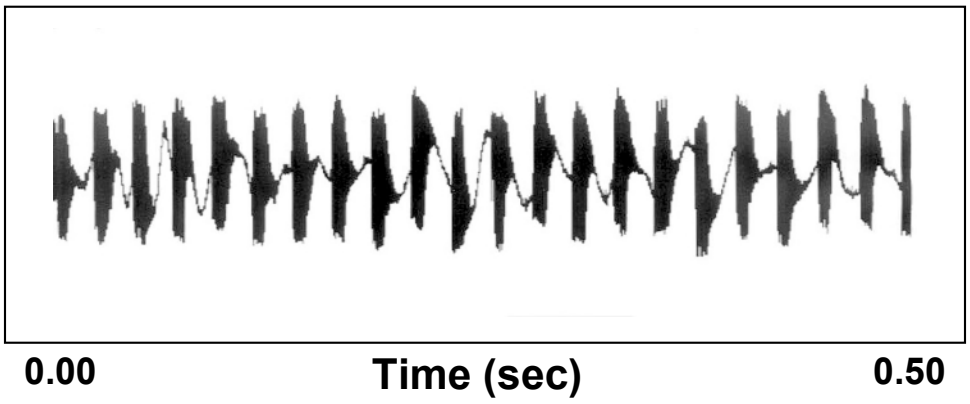

Table 1. Sites sampled, chromosome spread values, and mean spread for each individual sampled at Green Wing Environmental Laboratory.

Wetland site	Individual	Spread Values	Mean Spread
Pine Pond	1	23, 24, 24, 24	23.8
	2	19, 24, 24, 24, 24, 24	23.2
	3	24, 24, 24	24.0
	4	19, 19, 24, 24, 25	22.2
	5	23, 24, 24, 24, 24, 25	24.0
Snapper Pond	1	24, 24, 24	24.0
	2	22, 24, 24	23.3
	3	23, 24, 24, 24, 24, 24	23.8
	4	24, 24, 24, 24	24.0
	5	14, 18, 24, 24, 24, 24	21.3
Wet Meadow Complex	1	24, 24, 24, 24	24.0
	2	23, 23, 24	23.3
	3	23, 23, 24, 24	23.5
	4	24, 24, 24	24.0
	5	24, 24	24.0
Iris Pond	1	19, 21, 24, 24, 24, 24, 24, 24	23.0
	2	23, 24, 24, 24	23.8
	3	24, 24, 24	24.0
	4	23, 24	23.5
	5	22, 24, 24	23.3
		Total Mean	23.4