

CALL ANALYSIS OF TRIPLOID HYBRIDS RESULTING FROM DIPLOID-TETRAPLOID SPECIES CROSSES OF HYLID TREE FROGS

BARBARA K. MABLE AND JAMES P. BOGART

Department of Zoology, College of Biological Sciences, University of Guelph, Guelph, Ont.,
Canada N1G 2W1

ABSTRACT

Calls produced by hybrids resulting from laboratory crosses of tetraploid *Hyla versicolor* females and either diploid *Hyla chrysoscelis* (type I) or *Hyla arborea* (type II) males were induced through manipulation of environmental conditions. Type I hybrids produced trilled calls similar in note repetition to *H. versicolor*, but more similar in dominant frequency to *H. chrysoscelis*. Mean duration was shorter than in both parent calls. Type II hybrids produced calls which were longer in duration and lower in note repetition rate than *H. versicolor*, but shorter in duration and higher in note repetition rate than *H. arborea*. Dominant frequency of type II hybrids was lower than in *H. arborea* but not significantly different than in *H. versicolor*. Hybrid calls were not strictly intermediate, and may provide information regarding parental relationships.

INTRODUCTION

Polyploidy is a phenomenon usually associated with plants. Vertebrate polyploids are rare and most are unisexual (Dawley & Bogart 1989). Several species of frogs, however, exist as polyploids with normal bisexual reproduction acting to maintain this condition (Bogart & Wasserman 1972, Bogart 1980). Most of the known polyploid frog species are associated with a diploid species from which they are thought to be derived (Bogart & Wasserman 1972, Bogart 1980).

The grey tree frog, *Hyla versicolor*, identified as a tetraploid through chromosome analysis (Wasserman 1970), has an extensive range throughout North America much of which overlaps that of its diploid counterpart, *Hyla chrysoscelis* (Bogart & Jaslow 1979, Bogart 1980, Hillis et al. 1987). The most reliable basis for comparison of frog species is through analyses of the specific advertising calls they produce (Blair 1958a). *Hyla versicolor* and *H. chrysoscelis* were primarily distinguished as distinct species by acoustic differentiation (Johnson 1966), *H. versicolor* having a much reduced pulse rate compared with *H. chrysoscelis*. Zweifel

(1970) reported the call from a single individual which appeared to be intermediate to the slow-trilled call of *H. versicolor* and the fast-trilled call of *H. chrysoscelis*. He suggested that this might represent a hybrid between the two. Such an individual would have a triploid chromosome complement. Ploidy was not confirmed by Zweifel and no further studies have confirmed the existence of intermediate calls or hybrids in any natural populations.

Laboratory crosses using *H. versicolor* females and diploid males often result in very viable triploid offspring, even if the parents are distantly related (Mable 1989). Examination of the calls produced by male hybrids provides a means to determine if a triploid call could be distinguished from the diploid and tetraploid call and to investigate the genetic basis for acoustic dissimilarities between the parental species. Identification of hybrids by acoustic means in the field would help to assess the potential for hybridization to occur in natural, sympatric populations. The objectives of this study were to obtain and examine calls produced by triploid hybrids from crosses involving *H. versicolor* and its closest diploid relative, *H. chrysoscelis*, as well as a distantly related European species, *H. arborea*. *Hyla arborea* has a distinctly different call, so the genetic components of the call could be more easily assessed.

METHODS AND MATERIALS

Male triploids (3n) resulting from crosses between a tetraploid (4n) female *H. versicolor* from Aberfoyle, Ontario, and either a diploid (2n) male *H. chrysoscelis* from Cadiz Springs, Wisconsin, or a diploid male *H. arborea* from France, were selected from a population of two-year old males and females maintained at 24°C under artificial light set on a 12L:12D photoperiodic schedule. Prior to recording, the hybrids were placed in an incubator at 10°C under continuous darkness for two weeks, after which they were placed in aquaria at 22°C on an increasing photoperiod schedule (2 hours per week). The animals were fed crickets, *ad libitum*. Although control crosses were performed using *H. versicolor* males and females at the time of the hybrid crosses, none survived to sexual maturity for call analysis.

Calls were recorded using a Uher 4000S recorder and a Uher M517 microphone attached to an Akustomat, which switched the recorder on only when sound was produced near the microphone. Calls were analyzed using a Kay Elemetrics Sound Spectrograph (7030 A) using effective filter band widths of 45 Hz and 300 Hz. Note repetition rate, mean duration and dominant frequency were determined from the sonographs of five calls from each of the crosses and from representative calls of the parent types using a SAC (Science Accessories Corporation) GP-6 sonic digitizer interfaced with an IBM PC. For comparison with previously established

relationships between parental calls, the method of analysis follows that of Ralin (1969). Since *H. versicolor* and *H. chrysoscelis* both produce calls containing two main harmonics with only slight differences in energy (Blair, 1958b), the term dominant frequency was used to distinguish these harmonics from those of lesser intensity. The dominant frequency was determined by sectioning the energy spectrum using the sectioning function of the Kay Sonagraph as well as the digital signal which was displayed on the monitor of a Uniscan II (Multigon Corp.) spectral analyzer. Both methods provided identical frequencies for the most intense (or dominant) frequency. Averages and standard errors were determined, and comparisons between call types made using Student's *t*-tests (Steele and Torrie 1980).

Calls from *H. versicolor* and *H. chrysoscelis* were obtained from tapes of previous field recordings from the parent populations during breeding site choruses. Calls from *H. arborea* were induced by injecting an adult male with 0.5 ml (500 IU) of Human chorionic gonadotropin (HCG) diluted with Hank's balanced salt solution.

Ploidy of the hybrids was verified at the conclusion of the experiment by counting chromosomes obtained from corneal epithelial squashes of animals injected with colchicine, using the methods outlined by Bogart (1981).

RESULTS

Type I hybrids produced trilled calls with a note repetition rate which was not significantly different from *H. versicolor* but significantly lower than *H. chrysoscelis* ($p < 0.001$). Typical calls of both parental species and the hybrid call are provided in Figure 1. As in the parents, the hybrid calls contained two major frequency bands. The upper band was significantly higher (in frequency) than that of *H. versicolor* ($p < 0.001$) but not significantly different from that of *H. chrysoscelis*, while the lower band was significantly higher than in both parent types ($p < 0.001$) and fell between the upper and lower frequency bands of the parents. Mean call duration was shorter in the hybrids than in both parent types (Table 1).

Type II hybrids produced calls with a note repetition rate which was lower than *H. versicolor* ($p < 0.001$) but higher than *H. arborea* ($p < 0.001$) (Fig. 2). The upper frequency band in the hybrid calls was lower than that of *H. arborea* ($p < 0.001$) but not significantly different from that of *H. versicolor*, while the lower frequency band was once again higher than in both parent types and intermediate between their upper and lower bands. Mean duration was longer than in *H. versicolor* ($p < 0.001$) and shorter than in *H. arborea* (Table 2).

All hybrids from both crosses had triploid ($3n=36$) chromosome complements (Figure 3).

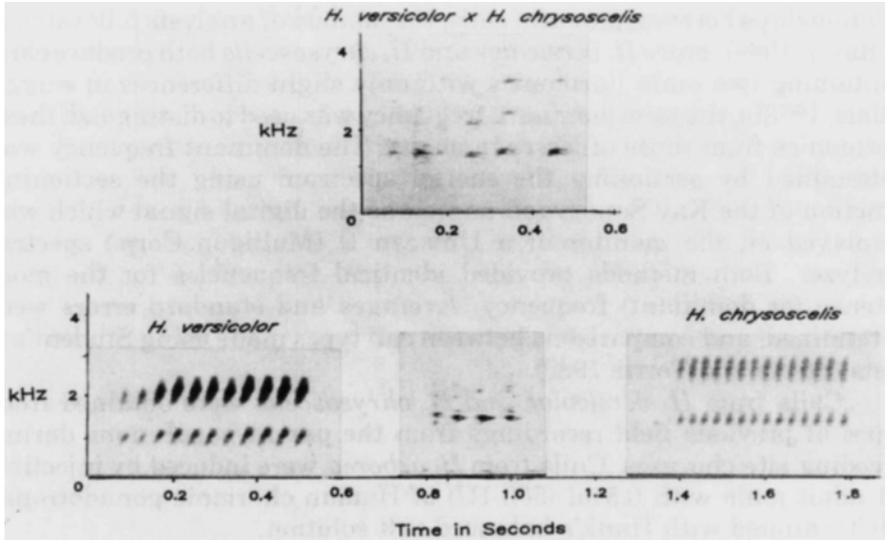


Figure 1. Sonograms of calls from *Hyla versicolor*, *H. versicolor* x *H. chrysoceles*, and *Hyla chrysoceles* at 22°C. Note that the hybrids produced two call types—a short trill (lower figure) and a longer, more varied call (upper figure).

TABLE 1

Comparisons between call parameters of type I hybrids and their parent species at 22°C. Values are expressed as means \pm SEM. Comparisons between calls made using Student's t-tests.

	Note repetition rate (notes/s)	Mean duration (s)	Dominant frequency (kHz)
<i>H. versicolor</i>	22.02 \pm 0.39	0.87 \pm 0.15	1.018 \pm 0.034 2.002 \pm 0.956
<i>H. chrysoceles</i>	37.77 \pm 0.38*	0.76 \pm 0.50	1.473 \pm 0.047* 2.796 \pm 0.042*
<i>H. versicolor</i> x <i>H. chrysoceles</i>	23.73 \pm 2.91*	0.31 \pm 0.11*	1.773 \pm 0.123** 2.468 \pm 0.319*

*p < 0.001 compared with *H. versicolor*

** p < 0.001 compared with *H. chrysoceles*

DISCUSSION

Previous studies (Zweifel 1968, Gerhardt 1974, 1982, Ralin 1977) quantified the trills produced by diploid and tetraploid treefrogs as a "pulse rate" parameter. The calls of *H. arborea* and the type II hybrid consist of longer notes with an internal pulsed structure (Figure 2)

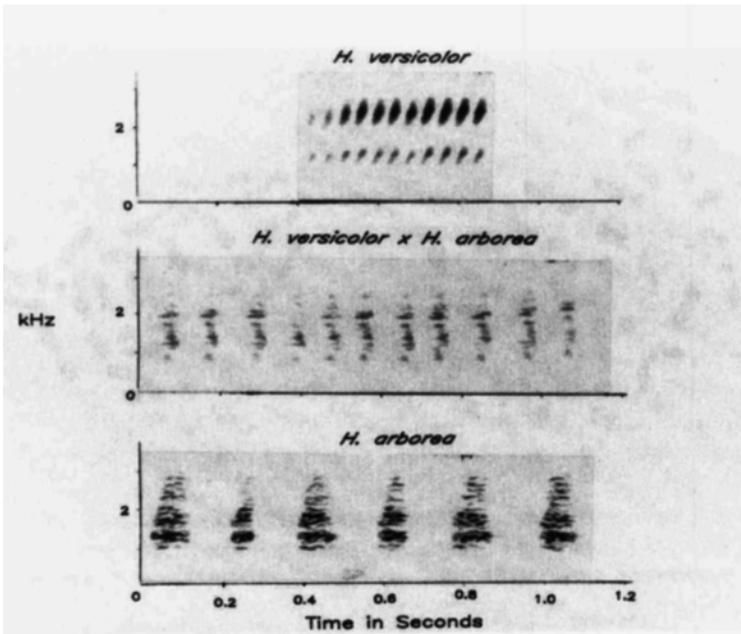


Figure 2. Sonograms of calls from *Hyla versicolor*, *H. versicolor* x *H. arborea*, and *Hyla arborea* at 22°C. For the *H. arborea* and hybrids, dominant frequency was determined from sections of the calls.

TABLE 2

Comparisons between call parameters of type II hybrids and parent types at 22°C. Values are expressed as means \pm SEM. Comparisons between values made using Student's t-tests.

	Note repetition rate (notes/s)	Mean duration (s)	Dominant frequency (kHz)
<i>H. versicolor</i>	22.02 \pm 0.39	0.87 \pm 0.15	1.018 \pm 0.034 2.002 \pm 0.096
<i>H. arborea</i>	5.30 \pm 1.01*	4.57 \pm 0.40*	1.373 \pm 0.142* 2.274 \pm 0.100*
<i>H. versicolor</i> x <i>H. arborea</i>	9.77 \pm 0.56**	2.67 \pm 0.41**	1.738 \pm 0.047** 2.022 \pm 0.218*

*p < 0.001 compared with *H. versicolor*

**p < 0.001 compared with *H. arborea*

produced in a repeated sequence rather than the rapid trills characteristic of *H. versicolor*. But it is evident, from the structure of the calls in *H. versicolor* and the transition provided by the hybrid call, that the "pulse rate" of these previous authors actually measures the number of notes per second. Therefore, note repetition rate would be a more accurate terminology to quantify these calls than would 'pulse rate' or 'trill rate'.

Type I hybrids produced trilled calls similar to *H. versicolor* in note repetition rate, but more similar to *H. chrysoscelis* in dominant frequency

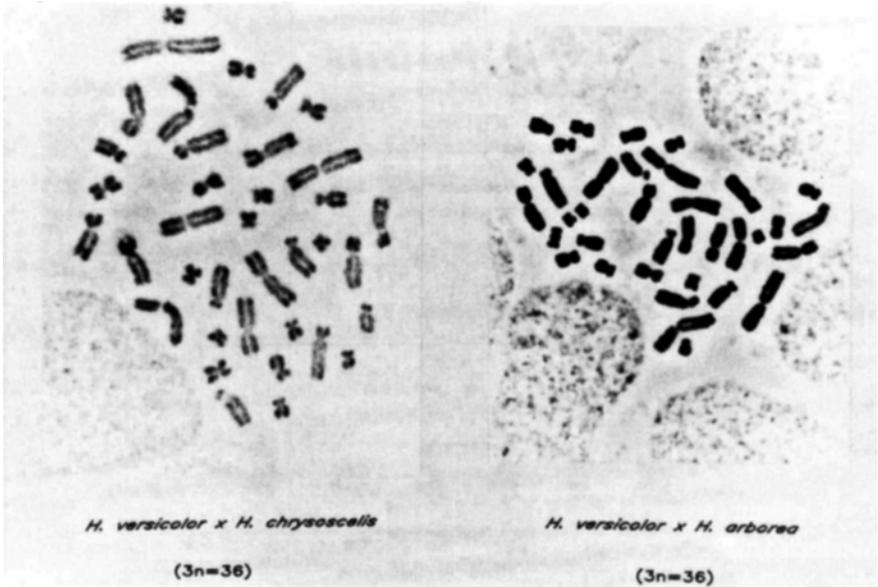


Figure 3. Chromosome spreads from each of the two cross types, *H. versicolor* x *H. chrysoscelis* (Type I) and *H. versicolor* x *H. arborea* (Type II), verifying triploid status. Both spreads are at the same magnification.

(Table 2). Discrimination experiments have shown that females are able to distinguish between the calls of *H. versicolor* and *H. chrysoscelis* on the basis of differences in note repetition rate (Gerhardt 1974) and that they show selective phonotaxis towards their own type (Gerhardt 1981). Since dominant frequency varies with body size (Licht 1976, Zweifel 1968), note repetition rate may provide a more consistent basis for comparison. If call characteristics are genetically determined, a greater similarity of the hybrid calls to those of *H. versicolor* in this feature might be predicted, since the triploids have a diploid *H. versicolor* and a haploid *H. chrysoscelis* chromosome complement.

However, if note repetition rate is the main call feature to which females respond in mate selection, the similarity of the hybrid call to that of *H. versicolor* would create a potential for backcross matings to occur. Such pairings would not be expected to produce viable offspring because hybrids between the two species of grey treefrogs have been shown consistently to be sterile (Bogart & Wasserman 1972, Johnson 1959, Johnson 1963). It would be expected that hybridization would be selected against through a postmating isolation mechanism. Since this results in wasted reproductive effort if mismatings do occur, it would be more energetically efficient to prevent pairing errors through some premating isolation mechanism. This could be achieved if other features of the hybrid call are used in discrimination by females.

The hybrid calls were shorter than those of both parent types, but duration is highly variable and probably does not provide a reliable measure, although Gerhardt (1974) suggested that duration may be important in distinguishing between eastern and western populations of *H. chrysoscelis*. Dominant frequency, however, showed an interesting pattern in both the type I and type II hybrids which may be distinctive. The upper frequency band was similar to that of one of the parents, while the lower band was intermediate between the upper and lower bands of both the parent types (Tables 1 and 2). In green treefrogs (*H. cinerea*), Gerhardt (1981, 1986) found both the upper and lower bands to be important for female attraction, and therefore it is possible that the change in the lower band noted in the hybrids results in a "deficient" call which would not be recognized by females. Such a system of both premating and postmating isolation of hybrids from parent populations would be consistent with Blair's (1964) finding that hybrids between *Bufo woodhousei* and *Bufo valliceps* produced deficient mating calls and were sterile. Verification of this hypothesis would require discrimination experiments involving hybrid males and both parental and hybrid females.

Since the calls induced in the hybrids were not intermediate in note repetition rate to the parental types, as suggested by Zweifel (1970), it is possible that such hybrids have been acoustically "hidden" in sympatric populations. This non-intermediacy of hybrid calls may be related to physiological constraints rather than character displacement or selection. The ratio of DNA content in *H. versicolor* to that in *H. chrysoscelis* is not 2:1 as might be expected on the basis of doubling ploidy, but is regulated at 1.7:1 (Bachman & Bogart 1975). This corresponds well with the ratio of note repetition rates between the calls of the two species, suggesting that note repetition rate is determined by simple cell composition (Ralin 1977). Therefore, it is possible that further DNA regulation in the triploids is responsible for the call produced, and that call characteristics are simply a by-product of polyploidy.

Hedges (1986) used *H. arborea* as an outgroup for phylogenetic comparison of holarctic hylids, indicating that it should be separated from the North American grey treefrogs to an extent that should allow for few shared characters. Calls of the type II hybrids in this experiment support this relationship, as they were intermediate to the parent types in all variables examined. Since a triploid hybrid between the diploid *H. arborea* and the tetraploid *H. versicolor* should have an asymmetrical distribution of parental genes in favour of the female, it is surprising that more dosage effects were not apparent in the call parameters, as was noted in the crosses with *H. chrysoscelis*. Backcrosses between each of the hybrid types and female *H. versicolor* show that the type II hybrids are more reproductively successful as well: Type I crosses resulted in 100% mortality; while type II crosses produced a number of viable offspring

(Mable 1989). Analysis of calls produced by these backcross individuals should help to clarify genetic relationships and further exemplify the effects of polyploidy on phenotypic characters such as mating call production.

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