Vocal repertoire and effect of advertisement call intensity on calling behaviour in the West African tree frog, *Leptopelis viridis*

T. Ulmar Grafe, Jan O. Steffen, Carolin Stoll

Department of Animal Ecology and Tropical Biology, Biozentrum, University of Würzburg, 97074 Würzburg, Germany
e-mail: grafe@biozentrum.uni-wuerzburg.de

**Abstract.** The vocal repertoire of the West African tree frog *Leptopelis viridis* was investigated in the Comoé National Park, Ivory Coast. *Leptopelis viridis* had a complex vocal repertoire consisting of short clicks, multinote clicks, trills, and soft calls. Most calls produced were short advertisement clicks. Soft calls were emitted during agonistic interactions between males suggesting that they function as aggressive calls. In contrast, multinote calls and trills were emitted when advertisement call rate was high suggesting that these calls are used by males to increase their attractiveness to females. Even at high densities males were spaced widely with a median nearest neighbour distance of 4.8 m. We also examined the effects of increased sound level of advertisement calls on calling behaviour. Playback experiments using synthetic clicks showed that males increased the proportion of aggressive calls as the stimulus intensity was increased. Males typically responded with aggressive calls when playback levels exceeded 82 dB (re 20 μPa). At high playback levels males either aggressively approached the speaker or silently retreated.

**Introduction**

Intense acoustic competition is a general problem faced by acoustically signalling animals that call in dense choruses. Typically, such choruses consist of males advertising to attract females. In birds, anurans, as well as many grasshoppers and katydids, males show several adaptations that serve to increase their conspicuousness in a chorus. For example, males have been shown to alternate or synchronise their calls with neighbouring males (e.g. Zelick and Narins, 1983; Klump and Gerhardt, 1992; Greenfield and Roizen, 1993; Grafe, 1996; Naguib and Todt, 1997), they may increase their call repetition rate (e.g. Schwartz and Wells, 1985), increase the complexity of their calls (e.g. Wells and Schwartz, 1984), and they typically defend calling sites and acoustic space against other males (reviewed in Ewing, 1989; Gerhardt and Schwartz, 1995). The diversity of call types and their
complexity within a species is a result, at least in part, of the different selective pressures of attracting a mate, repelling rivals and avoiding attracting predators or parasites.

We investigated the call repertoire and spacing mechanisms in the West African tree frog *Leptopelis viridis*. We addressed several questions. First, how extensive is the call repertoire of *L. viridis*? Second, in what contexts are the different calls given. Third, how widely are males spaced and do they defend call sites and acoustic space against other males? Since call amplitude has been identified as being important in mediating spacing between males in numerous anurans (Arak, 1983; Schwartz and Wells, 1985; Wilczynski and Brenowitz, 1988; Brenowitz, 1989; Stewart and Bishop, 1994; Grafe, 1995), we asked, in particular, whether males would tolerate an increase in sound pressure level (SPL) of advertisement calls.

**Materials and methods**

*Study species and study site*

*Leptopelis viridis* (Hyperoliidae) occurs throughout the savannahs of West Africa from Guinea to Cameroon (Schiotz, 1967, 1999; Frost, 1985). In Ivory Coast, *L. viridis* is widespread throughout both the Guinea and Sudan savannah (Rödel, 1996). During the dry season individuals estivate underground where they form a cocoon (Grafe, in press). Males start calling with the very first rains of the wet season that typically start in March or April and can still be heard calling in low numbers and at low rates in September and October. Males call from elevated perch sites 1-4 m above ground mostly on trees and bushes, often at considerable distance from standing water. The large unpigmented eggs are laid in wet mud at the edge of small temporary ponds. We studied *L. viridis* in the Guinea savannah region of the Comoé National Park at 8° 45′ N and 3° 49′ W in March and April 1996 and 1997. This area is characterised by a dry season of 4-6 months. Night-time temperature, measured 1 m above ground, varied between 23-27°C during the study.

*Calling behaviour and spacing of males*

Early in the season, in April 1997, we monitored the minute by minute calling behaviour of two males over the course of an evening at high chorus density and calling activity by counting the number and types of calls given. To help identify the function of multitone calls and trills we calculated indices of association between these calls and aggressive calls. We used the following index of association: $N_{AB}/(N_A + N_B + N_{AB})$ where $N_{AB}$ = number of occasions call types A and B are given in the same observation interval; $N_A$ = number of occasions call type A is given without call type B; and $N_B$ = number of occasions call type B is given without call type A, following the procedure by Clutton-Brock et al. (1982). Scores are distributed between 0 (no association) and 1 (complete association). Furthermore, the function of multitone calls and trills was evaluated by examining whether
these calls were given when the rate of advertisement calling was high. We hypothesised that this would indicate that these calls function in mate attraction and less in repelling rival males.

To determine how widely males are spaced in the chorus we measured the linear distance between calling males on one representative night of high calling activity at one aggregation. Eight males were identified between 1900-2000 h and call sites marked with clothespins. Distances between call sites were measured the next day.

Acoustic analyses and playback experiments

The vocal repertoire of *L. viridis* was examined from recordings of males calling undisturbed and in response to playbacks of advertisement calls from 23 March to 19 April 1996 between 1900-2200. We used a Sony WM-DC6 tape recorder and directional microphones (Aiwa CM-Z3 and Sennheiser MZA 14 P48) mounted on a tripod and set near a calling male. Calls were digitised and analysed using Canary (Bioacoustics Research Program, Cornell Laboratory of Ornithology) run on a Macintosh.

Recordings of advertisement calls from ten males were used to generate a synthetic call used for the playback experiments. Ten representative advertisement calls from each individual male were analysed and measurements used to generate synthetic calls on an Apple Macintosh 7100 with the Program SoundEdit 16. Both advertisement and aggressive calls were composed of three or more emphasised frequencies embedded in a broad frequency spectrum. Energy spectrums of advertisement calls showed that the energy of emphasised frequencies were only 1-2 dB above that of surrounding frequencies. Thus, we generated the synthetic advertisement call by bandpass filtering 20 ms of white noise with a lowpass and highpass cut-off frequency of 4 kHz and 1 kHz, respectively, to obtain a broad band call with a dominant frequency of 2.43 kHz. The amplitude envelope of the synthetic call consisted of a 2 ms linear rise time and a 4 ms linear fall time. Preliminary trials indicated that males responded equally to broadcasts of natural and synthetic calls both with respect to call type and frequency of response.

To determine the effect of increases in sound pressure level (SPL) of advertisement calls on calling males we presented males in the field with a broadcast of synthetic calls from an Apple Macintosh PowerBook 5300 amplified by a SONY SRS-67 speaker mounted on a tripod approximately 1 m from the male. Any vegetation between speaker and male was removed to diminish excess attenuation. We also set a directional microphone mounted on a second tripod close to the male to record his response to the playback. Frogs typically stopped calling during instrument set-up but resumed calling within a few minutes. Soon after a frog renewed calling we recorded his calls for two minutes and then presented the frog with single synthetic advertisement calls in an interactive way with a click on the computer track-ball button for each call (advertisement or aggressive) from the male. We presented advertisement calls for periods of 1.5 min at increasing intensities resulting in 3-5 treatments/male depending on his behaviour. The order of presentation was not
randomised because frogs would stop calling if playbacks were started at high intensities. Intensity levels could be varied by 2 dB increments on the amplified speaker. Stimulus intensities at the male call site varied with the distance of the speaker that was placed from 0.63-1.2 m from the male. The peak SPLs (dB re 20 μPa) encountered by males were determined after the experiments for each frog to speaker distance using a precision sound level meter (Brüel & Kjær 2236) set for linear frequency weighting. Variation in distances led to variation in dB levels. We therefore grouped data into three categories: 68-71, 76-80 and 83-88 dB.

We observed males throughout the playback trial with a dim red light to determine whether they moved toward or away from the speaker, or remained stationary on their calling perch. Temperature and relative humidity were recorded before and after each trial. At the end of each trial we captured the test frog and measured his snout-vent length, tibiafibula length and mass. Males were then toe-clipped for individual recognition to prevent re-testing the same frog on subsequent nights.

Statistical analyses

The Friedman ANOVA for nonparametric dependent data was used to determine if playback intensity had an effect on male calling behaviour. We used the Wilcoxon-Wilcoxon test as a post hoc test to examine differences between groups. Mean values ± s were used as descriptive statistics for parametric data unless stated otherwise.

Results

Vocal repertoire

We recorded three distinct call types. First, the most common call was the advertisement call (fig. 1a; see also Schiøtz, 1967, 1999; Rödel, 1996 p. 162). This call is a short click with an average duration of 22.6± 4.5 ms (range 15-31 ms, n = 18) and a broad frequency band with a mean dominant frequency of 2.1 ± 0.31 kHz (range 1.5-2.6 kHz). The highest call repetition rates on wet nights early in the evening averaged 10.4± 5.0 calls/min (range 4-19 calls/min, n = 14). On dry nights call rates dropped substantially with calls being emitted only sporadically. Occasionally, 2-3 clicks were produced in short succession (interclick interval 212.8± 14.4 ms; n = 8). Second, a trill was given only in conjunction with one or more advertisement clicks (fig. 1b). The number of trills within a call varied from 2-5 with an average intertrill interval of 42.3 ms (± 5.0 ms; n = 8). Trills were produced during peak calling activity when several males were chorusing. Third, a soft call was produced much like the advertisement call but significantly longer in duration (40.8± 9.0 ms, range 24.9-59.2 ms, n = 13; Mann-Whitney U-test, Z = -2.13, N₁ = 13, N₂ = 18, P < 0.05) and somewhat lower in frequency (1.4 ± 0.14 kHz, range 1.28-
Figure 1. Sonogram and waveform of a typical advertisement call (a) followed by a typical trill composed of 4 pulses (b) of *Leptopelis viridis* from Comoé National Park, Ivory Coast. Analysing filter bandwidth was 171 Hz. Temperature during recording was 25°C.

Figure 2. Sonogram and waveform of a typical aggressive call of *Leptopelis viridis* from Comoé National Park, Ivory Coast. Analysing filter bandwidth was 171 Hz. Temperature during recording was 25°C.
Figure 3. Number and types of calls given within the course of a night for two male *Leptopelis viridis*: male 1 (a) and male 2 (b). Bin width is ten minutes. Sunset was at 1823 for both days.

1.79 kHz; Mann-Whitney $U$-test, $Z = -2.25, N_1 = 13, N_2 = 18, P < 0.05$; fig. 2). Two soft calls were also occasionally emitted in short succession.

From initial observations in which males gave soft calls in response to our vocal imitations of advertisement calls we hypothesised that soft calls are aggressive calls used by males during agonistic encounters with other males. This was confirmed by our playback experiment (see below) and the observation of a fight between two males that lasted for approximately 1.5 hours during which soft calls were given nearly exclusively. During this encounter males engaged in intense wrestling and pushing bouts in which a male was kicked or shoved until he fell from his perch only to return immediately to continue the fight. Interestingly, this was the only observation of males fighting that we observed.

Figure 3 shows the complete record of the vocal behaviour of two male tree frogs over the course of one night. Most of the calls given within the 5-7 hour calling period were advertisement calls. Trills, multitone calls and aggressive calls were produced only sporadically. Association indices between these calls were low (table 1). In the one minute intervals sampled, trills and multitone calls were never emitted alone without advertisement calls whereas this was not the case for aggressive calls. For both males,
Table 1. Association indices between call types. Top line: male 1, bottom line: male 2.

<table>
<thead>
<tr>
<th>Call type:</th>
<th>Trills</th>
<th>Aggressive calls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multinote calls</td>
<td>0.21</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Trills</td>
<td>0.02</td>
<td>0.04</td>
</tr>
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intervals that contained multinote calls had significantly higher advertisement call rates than intervals without such calls (Mann-Whitney U-test: $Z = -5.27, N_1 = 28, N_2 = 359, P < 0.0001$ and $Z = -2.75, N_1 = 35, N_2 = 259, P < 0.01$; male 1 and male 2, respectively). Likewise, for both males, intervals that contained trills had significantly higher advertisement call rates than intervals without such calls (Mann-Whitney U-test: $Z = -6.73, N_1 = 29, N_2 = 358, P < 0.0001$ and $Z = -5.01, N_1 = 16, N_2 = 278, P < 0.0001$; male 1 and male 2, respectively). In contrast, for both males, intervals that contained aggressive calls did not have significantly higher (or lower) advertisement call rates than intervals without such calls (Mann-Whitney U-test: $Z = -0.81, N_1 = 18, N_2 = 370, \text{n.s. and } Z = -0.41, N_1 = 6, N_2 = 288, \text{n.s.; male 1 and male 2, respectively}$). Results did not differ qualitatively when the same analyses were performed on five minute sampling intervals. These results suggest that multinote calls and trills are used by males to increase their attractiveness to females and are not used in association with aggressive calls to repel intruders.

Distance between calling males

All calling sites were elevated between 1-4 m above ground. The median nearest-neighbour distance (NND) in an aggregation of eight males was 4.8 m (range 3.5-6 m).

Playback experiments

The vocal response of 12 males to increasing playback levels of advertisement calls was evaluated for three categories of calls: advertisement calls, soft aggressive calls and compound calls, the latter a category in which multinote calls and trills were pooled. Advertisement calls were emitted throughout the experiment. In contrast, no aggressive calls were produced during the non-stimulus period and at low playback intensities (68-73 dB). One male responded with aggressive calls at a playback intensity of 76 dB, two males at an intensity of 80 dB. At playback levels above 83 dB, all males but one responded by producing predominantly aggressive calls. Four males gave only aggressive calls at the highest playback intensity (83-88 dB). The median aggressive threshold, defined as the lowest amplitude of playback that evoked aggressive calls, was 84 dB. Compound calls were produced routinely during playback of advertisement calls, whereas only one male gave such calls during the non-stimulus period. Two males never produced compound calls.
during the experiment. At the final playback level, six males approached the speaker giving aggressive calls, five males silently retreated from the speaker whereas one male remained at his call site but fell silent. Only nine males were tested in the non-stimulus control and at three different playback levels and thus only those nine individuals are included in further analyses.

When presented with synthetic advertisement calls of increasing intensity, males significantly increased the number of calls emitted (Friedman ANOVA: $\chi^2 = 9.9$, df = 3, $P < 0.05$). The proportion of advertisement calls decreased whereas the proportion of aggressive calls increased significantly with increasing playback intensity (Friedman ANOVA: $\chi^2 = 12.0$, df = 3, $P < 0.01$ and $\chi^2 = 9.9$, df = 3, $P < 0.05$, respectively; fig. 4). The proportion of advertisement calls decreased significantly between the second (76-81 dB) and the third playback level (83-88 dB; Wilcoxon-Wilcoxon, $P < 0.05$). Concomitantly, a significant increase in the proportion of soft aggressive calls occurred between the second (76-80 dB) and third (83-88 dB) playback level (Wilcoxon-Wilcoxon, $P < 0.05$). In contrast, there was no significant change in the proportion of compound calls with increasing playback levels (Friedman ANOVA: $\chi^2 = 3.9$, df = 3, $P > 0.05$; fig. 4).

**Discussion**

The vocal repertoire of *L. viridis* is more complex than reported by Schiøtz (1967, 1999) and Rödel (1996). In addition to clicks that serve as advertisement calls, males in our...
study area produced soft aggressive calls, mutinote calls and trills. We never heard any buzzing sounds reported by Schiøtz (1967, 1999) or Passmore and Carruthers (1979) for other *Leptopelis* species. The context in which clicks and soft calls were produced suggest that they function as advertisement and aggressive calls, respectively. The fact that single notes of mutinote calls resemble advertisement calls and since one component of the trill is an advertisement call it appears that these calls are used by males to increase their attractiveness to females. This assessment is underscored by the observation that mutinote calls and trills were given when advertisement call rate and overall chorus activity was high and show only very low association with aggressive calls. Overall, this suggests that compound calls are not used to repel rival males. It seems likely that compound calls are energetically more expensive to produce and are thus emitted only in competitive social environments to maintain one’s attractiveness to females. Interestingly, there was also a very low association in timing between mutinote calls and trills suggesting that each call type serves a different function in mate attraction or can complement the other in different situations. A full understanding of the function of compound calls can be gained only by testing females for their preference for each call type and call variant.

In our study area, *L. viridis* males call throughout the wet season for several months. In a 27 day pilot study, individually marked males were present and active in the chorus up to 23 consecutive nights even when other syntopically occurring species fell silent during dry nights. By laying their eggs in mud *L. viridis* is less dependent on rains and is able to expand its reproductive activity window to times and localities unsuitable to other species, thus reducing interspecific acoustic competition with possible effects on its vocal activity and vocal repertoire.

We have experimentally demonstrated that an increase in sound pressure level of advertisement calls stimulates male *L. viridis* to increase the proportion of aggressive calls. In addition, at high advertisement playback levels males changed their behaviour from stationary advertisement calling to aggressive approach, silent retreat, or in one case, call suppression. This behavioural response suggests that, despite calling within loose and expanded aggregations (median NND of 4.8 m), *L. viridis* males probably use advertisement call intensity to mediate spacing between individuals. This parallels findings for other widely spaced acoustically signalling anurans, for example the Neotropical frog *Eleutherodactylus coqui* (mean NND of 3.7 m and 14.7 m, Stewart and Bishop, 1994), the Sri Lankan treefrog *Philautus leucorhinus* (mean NND of 5.5 m, Arak, 1983) in which males responded to increased sound levels of advertisement calls by increased levels of aggressive calling or in the bush-cricket *Mygalopsis mariki* (mean NND’s in two distinct habitats were 6.2 and 11.5 m; Römer and Bailey, 1986) and *E. diastema* (mean NND of 14.7 m, Wilczynski and Brenowitz, 1988) in which the sound pressure level has been shown to mediate spacing.

There was considerable variation between individuals in the threshold at which aggressive calls were given (range 76-88 dB) and the proportion of aggressive calls produced. This probably reflects the different social situations experienced by males, in particular the
varying numbers of simultaneously chorusing males and their distance to the focal male. A shift in the threshold of aggressive response is a function of the call amplitude of the loudest neighbour in *Hyla regilla* (Rose and Brenowitz, 1991) and shows high plasticity (Brenowitz and Rose, 1994). The plasticity in aggressive call threshold most likely allows males to balance the benefits and costs of aggressive calling. While aggressive calls serve to repel rivals, male *L. viridis* probably incur costs in reduced attractiveness towards females while producing aggressive calls. Such a cost has been shown to occur in other hyperoliid frogs in which aggressive calls are less attractive to females than advertisement calls (Backwell, 1988; Grafe, 1995).

There was additional variability in the response of males to high playback levels: one male stopped calling, five retreated, while six approached the speaker. In our sample, there was no correlation between either body size or other morphometric measures (tibiafibula, mass) with this behavioural response. It remains to be seen if males convey information about their resource holding potential, particularly their fighting ability, and which, if any, acoustic parameters might serve that purpose.

**Acknowledgements.** We thank Eduard Linsenmair for providing the opportunity and the logistical support to carry out this study. Annette Schmidt and Katja Ueberschaer assisted in collecting data. Rafael Marquez and Peter Narins made helpful comments on the manuscript. The Ministre des Eaux et Forêts and the Ministre de la Recherche Scientifique, Republique de Côte d’Ivoire granted the research permit for field work in the Parc National de la Comoé.

**References**


Calls of *Leptopelis viridis*


*Received: January 27, 1999. Accepted: May 31, 1999.*