The complex calls of *Leptodactylus pustulatus* (Amphibia, Anura, Leptodactylidae)

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Leptodactylus pustulatus is one of the most distinctive species in the genus because of its ventral pattern of large bright red to yellow spots on a dark background. Although the species was described in 1870 and there has been no confusion regarding its taxonomic identity, there has been very little published about the species, particularly with respect to its biology. The lack of biological information for *L. pustulatus* is likely due to the fact that the species occurs in very inaccessible areas (flooded plains) in poorly sampled regions of Brazil at transition zones of Cerrado, Caatinga, and Amazon domains. Herein, we describe the advertisement and response calls of *L. pustulatus*, which turn out to be very different and more complex than for any other species of *Leptodactylus* for which the advertisement calls are known.

We use terms to describe the calls that are meant to be functionally neutral, as we have no evidence for the biological function of the calls. We use the terms advertisement and response calls for the two types of calls we report on. Other authors have ascribed various functions to response calls, terming them as encounter calls or aggressive calls. We also use the term "unit" to describe what we find to be rather discrete portions within the advertisement call. These units are intended only to provide a meaningful way to describe the structure and variation observed in the advertisement call and are specific to this study.

The advertisement and response calls were recorded from Palmas, Tocantins, Brazil, 20 March 2001, relative humidity 96% (±2%), temperature 25.1°C (±1°C), 22:30 h, voucher specimen CHUNB 24009 (Coleção Herpetológica da Universidade de Brasília). The voucher specimen has a snout-vent length of 50.2 mm. Calls were recorded on a Marantz PMD 201 tape recorder using a Sony ECM-Z37C unidirectional microphone. All calls were recorded from a single individual. The response calls were initiated by playing back the recorded advertisement call to the same individual, stopping the playback, then recording the response calls of the same male. Thus, the entire recording consists of a single recording session of advertisement calls (146 s) followed by a single recording session of 45 response calls (60 s). Ten advertisement and 45 response calls were analyzed in detail.

The calls were analyzed using Canary 1.2 software (Charif et al., 1995). The calls were digitized at a sample rate of 22050 Hz, sample size of 16 bits. Call component terminology follows Duellman and Trueb (1986) and Heyer et al. (1990). Call duration was measured from the waveform. Dominant frequencies were determined by two methods. For signal lengths that exceeded the frame length setting, dominant frequencies were determined using spectrum analyses with settings for analysis resolution of filter bandwidth 174.85 Hz, frame length 512 points, grid resolution 256 points, overlap 50%, frequency 43.07 Hz, FFT size 512 points, window function hamming, amplitude logarithmic, clipping level −80 dB. For signal lengths that were shorter than the frame length setting, dominant frequencies were determined from direct measurements of expanded waveforms. Harmonics were determined from wave form structure, spectrum and audiospectrogram displays. Audiospectrogram analyses were made with settings of analysis resolution filter bandwidth 349.70 Hz, frame length 256 points, grid resolution time 128 points (5.8 ms), overlap 50%, frequency 86.13, FFT size 256 points, window function hamming, amplitude logarithmic, clipping level −80 dB, display style smooth. The original signals were bandpass filtered around 400 and 4000 Hz prior to analysis.

Calling is nocturnal. Advertisement calls are given at a rate of 26/min. Each call consists of two notes, together comprising four units (fig. 1). Unit 1 is comprised of the first note and

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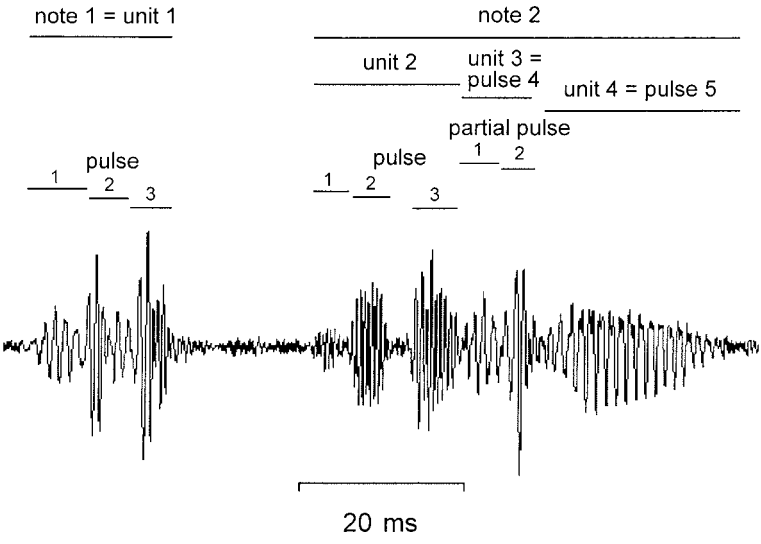

Figure 1. Wave form of advertisement call of *Leptodactylus pustulatus*, showing terminology used in text.

Table 1. Quantitative data for discrete units of the advertisement call of *Leptodactylus pustulatus*. See fig.1 for definition of Units.

	Entire call	Unit 1 (= Note 1)	Unit 1, Pulse 1	Unit 1, Pulse 2	Unit 1, Pulse 3	Note 2	Unit 2	Unit 2, Pulse 1	Unit 2, Pulse 2	Unit 2, Pulse 3	Unit 3	Unit 4
Duration (ms)	81-88	16-20	5-8	4-7	4-6	49-54	18-21	3-5	4-8	5-7	7-12	17-24
Pulse rate/s		149-183				92-101	145-174					
Dominant frequency (Hz)	775-861	812-1120	724-796	996-1146	996-1146	775-861	1895-2022	1948-2128	1760-2030	1348-1759	776-903	776-865
Second harmonic frequency (Hz)												1551-1717
Third harmonic frequency (Hz)												2289-2416

consists of three pulses. Unit 2 consists of the first three pulses of note 2. Units 3 and 4 consist of the third and fourth pulses of the second note.

Quantitative characteristics of the advertisement call are presented in table 1. The third pulse of Unit 1 is partially pulsed in 9 of the 10 calls analyzed. Unit 3 is pulsatile in 3 of the calls analyzed. Unit 3 varies from having distinct harmonic structure to not showing clear harmonic structure. Unit 4 consistently has pronounced harmonic structure (figs. 2c, 3). There is slight frequency modulation from the beginning to the end of Unit 4, ranging from 29-138 Hz. There is no pattern as to whether the beginning, middle, or end of Unit 4 has the highest or lowest frequencies. The pulsed nature of the advertisement call is reflected by regularly spaced loudness peaks in some spectrum analyses (fig. 2a) and by sidebands in the audiospectrograms (fig. 3).

The rate of the 45 response calls analyzed is extremely variable, from a minimum of 0.09 s between calls to a maximum of 4.80 s. Just under half (47%) of the calls had inter-call values between 0.09 and 0.50 s. Call duration ranges from 0.005 to 0.205 s. Forty percent of the calls consist of 1 note, 48% of 2 notes, 6% of 3 notes, and 6% of 4 notes. Note duration ranges from 0.004-0.086 s. The number of pulses per note ranges from 1-11. Forty percent of the notes have a single pulse; the next highest occurrences are for 2 pulses/note (20%) and

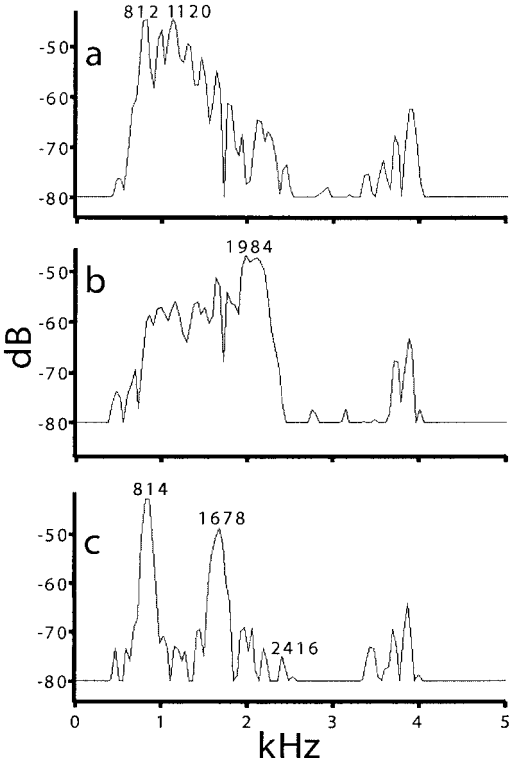

Figure 2. Spectrum analyses of advertisement call of *Leptodactylus pustulatus*. 2a – spectrum of Unit 1; 2b – spectrum of Unit 2; 2c – spectrum of Unit 4 showing harmonic structure.

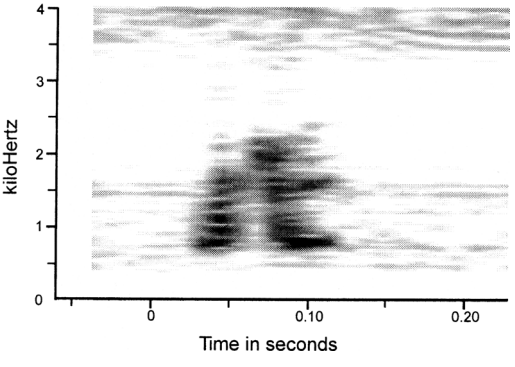

Figure 3. Audiospectrogram of advertisement call showing pulse and harmonic structures.

5 pulses/note (14%). Pulse duration ranges from 0.002-0.052 s. The dominant frequencies of individual call elements (individual pulses) range from 433-2200 Hz.

The response calls are much more variable than the advertisement calls (fig. 4). Seventy

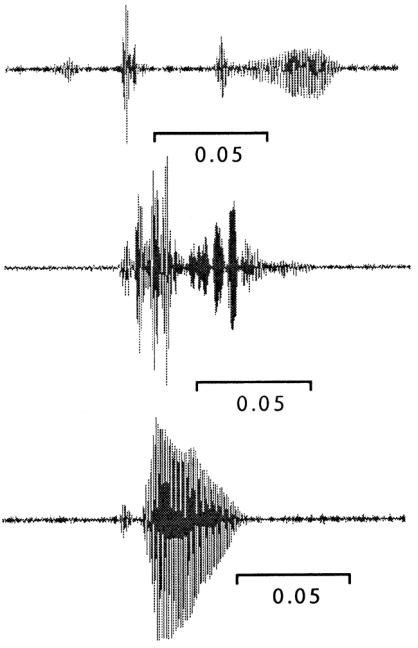

Figure 4. Wave forms of response calls of *Leptodactylus pustulatus*. Vertical scales unmodified in analysis and reflect comparable amplitudes.

eight of 218 (36%) pulse elements of the response calls exactly match the pulse elements of the advertisement call. All pulse elements of the advertisement calls appear somewhere in the response calls analyzed. Whereas the advertisement call is highly structured and composed of elements that are consistently packaged in all calls, no two response calls are identical to each other and most are very different from each other. It appears that not only are individual advertisement call pulses separated and mixed in all possible combinations, but most response calls differ from the advertisement call pulses both in terms of dominant frequencies and durations, with all extreme parameter measurements occurring in the response calls. Response calls are typically more variable than advertisement calls in frogs (Wells, 1988). The variability of *L. pustulatus* response calls is much greater than observed in most frogs we are aware of, however.

The calling habitat of *L. pustulatus* is of variable complexity. Typically, males call from

floating plants over the deepest portions of large ponds (>1 ha in area), with about 80% of the body exposed to the air. The large ponds are almost completely covered by several kinds of aquatic or semi-aquatic plants (Cyperaceae, Musaceae, Nymphaceae, Poaceae, Pontederidaceae), along with algae, but there are open areas of the ponds as well. The plant cover is less than 50 cm to more than 2 m in height. When close to forests, these large ponds may have tall trees, dead trunks and logs, and buriti palms (*Mauritia flexuosa*). This same calling habitat is shared with *Lysapsus caraya* and *Pseudis tocantins*. Rarely, males call from small roadside ponds, which are shallower and have fewer aquatic plants than the more typical calling habitats of large ponds.

Calling males of *L. pustulatus* are never abundant and are widely spaced. In large ponds, calling males are never less than 2 m from each other and often more than 5 m apart. Females are rarely seen and are larger than the males. Males readily give response calls to adjacent calling males, even in the presence of potential predators. At the individual pond level within the cerrados, almost 20 species of frogs can occur together at a single time, including up to 5-6 species of *Leptodactylus.* For example, at the large pond at Palmas, *L. pustulatus* occurs syntopically with *L. fuscus*, *L. labyrinthicus*, *L. mystacinus*, *L. ocellatus*, and *L. podicipinus*.

The advertisement and response calls described herein for *Leptodactylus pustulatus* are much more complex than for any other species of *Leptodactylus* for which calls are known. All but one other species of *Leptodactylus* have advertisement calls solely comprised of single notes (Straughan and Heyer, 1976; Heyer, 1978, 1979, 1994, 1998, in press; Lescure, 1979; Heyer and Morales, 1995; Marquez et al., 1995; Heyer et al., 1996; Heyer and Carvalho, 2000; Lescure and Marty, 2000; Heyer and Juncá, 2003). There is variation within single notes of the advertisement call in terms of whether the call is pulsed or not and whether the call is frequency modulated. Frequency modulation is usually a rising frequency throughout the call, but may also include both rising and falling frequencies within a given single-noted call. The exception is the advertisement call of *L. riveroi* (Heyer and Pyburn, 1983). *Leptodactylus riveroi* is the only other species of *Leptodactylus* that has multiple notes per call. The advertisement call consists of 9-28 notes per call. Each note of the *L. riveroi* call has a similar structure that consists of an initial falling frequency followed by a rising frequency. The advertisement call of *L. pustulatus* is more complex than the call of *L. riveroi* in that the two notes of the *L. pustulatus* call each contain very different temporal and frequency information.

We only have recordings from a single individual of *L. pustulatus* available to analyze. The first author saw and heard several *L. pustulatus* males calling from Palmas, Britânia, and Ananás (State of Tocantins) in July 2003 but was unable to record the calls. All of the advertisement and response calls heard in July 2003 sounded very similar to those analyzed herein. Further recordings are needed in order to determine the range in inter-individual variation of both advertisement and response calls. Based on what is known about frog calls in general and *Leptodactylus* calls in particular, we anticipate that additional recordings will support the findings reported herein for stereotyped advertisement calls consisting of the four units characterized herein and the same magnitude in response calls observed in the calls we analyzed.

Determining why the calls of *Leptodactylus pustulatus* are as complex as they are requires additional data. We suggest the following may be involved: (1) species coding, (2) territorial defense, (3) sexual selection, and/or (4) habitat, specifically the potential differential roles of air-transmitted versus water-transmitted call components.

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