CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

VOCAL REPERTOIRE AND FEMALE CHOICE IN AN ECUADORIAN TREEFROG,

DENDROPSOPHUS CARNIFEX

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science in Biology

By

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ABSTRACT

Vocal Repertoire and Female Choice in an Ecuadorian treefrog, *Dendropsophus carnifex*

By

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Master of Science in Biology

Frogs predominantly communicate acoustically, using advertisement calls to mediate both inter- and intrasexual interactions. In many frogs, these signals comprise a single component; however, in others they are composed of distinct multiple components. Male executioner treefrogs, *Dendropsophus carnifex*, exhibit a multicomponent advertisement call composed of an introductory screech note followed by two or more secondary click notes. However, the vocal repertoire of this species, including spectral and temporal properties, has not been fully characterized. Therefore, the purpose of my study was to identify and characterize the different call types used by male *D. carnifex*, and to determine the effect of the multicomponent advertisement call on both female call recognition and choice. I conducted field recordings of calling males, and carried out three 3-choice female phonotaxis experiments. I tested (1) female phonotaxis to each call component and to a complete call, (2) female choice for differences in the number of click notes, and (3) female choice for differences in call rate. Based on previous literature on multicomponent advertisement calls, I hypothesized that females would recognize the click notes of the advertisement call as the mating signal, and that females would choose calls with more notes and at faster rates. The results demonstrate that both notes,
presented separately or together as a complete call, evoked similar female phonotactic responses, suggesting that either note was sufficient to elicit a mate-recognition response. Additionally, I found that females preferred calls with greater numbers of added click notes and faster call rates. Because females preferred stimuli with greater call output, males calling with more click notes or at faster rates should experience a greater mating advantage. As calling is energetically expensive and possibly indicates male quality, selecting mates based on these call parameters may provide females with indirect benefits, such as good genes. Determining female phonotaxis towards multicomponent calls and call preferences is important in understanding the evolution of signal complexity in light of sexual selection.
CHAPTER 1 – THE VOCAL REPERTOIRE OF AN ECUADORIAN TREEFROG,  

*Dendropsophus carnifex*

INTRODUCTION

Acoustic signals serve as the principal form of communication among most species of anurans. These conspicuous signals are used to mediate social interactions primarily among conspecifics (Gerhardt and Huber, 2002). Males are the more vocal sex in most anurans, primarily calling to attract mates. Additionally, males use calls to assess and maintain the distance between nearby competing males and assist in species recognition (Ryan and Rand, 1993). Other acoustic signals in a male’s vocal repertoire include aggressive and alarm calls; males use aggressive calls to mediate antagonistic encounters with other males, sometimes leading up to combative encounters, whereas alarm calls are used when the signaler is in danger (Andersson, 1994; Gerhardt and Huber, 2002).

In some species, males can incorporate spectral and temporal property changes to their calls, increasing complexity and thus attractiveness to potential mates. These changes can include changes in pulse repetition rate, call rate, or number of notes or frequency modulation (Gerhardt and Huber, 2002; Pallett and Passmore, 1988; Ryan, 1980, 1983; Wagner Jr, 1989; Wells and Schwartz, 2006). In some species, e.g., *Eleutherodactylus coqui* (Narins and Capranica, 1978) and *Geocrinia victoriana* (Littlejohn and Harrison, 1985), advertisement calls are composed of two notes, where the functions of mate attract and competitive assessment are completely separated among them.
The aim of my study was to characterize the vocal repertoire of *Dendropsophus carnifex*, a small treefrog endemic to Ecuador, with populations found along the Pacific slopes of the Andes Mountains (Cisneros-Heredia and Jungfer, 2004) and compare my findings to previous literature. I observed two call types, classified here as Type 1 and Type 2, both containing two acoustically distinct notes. Type 1 consisted of a short introductory “screech” note followed by several brief secondary “click” notes, previously characterized as “wraah-ack-ack” (Duellman and Trueb, 1983); the introductory note of Type 2 is elongated followed by a single secondary note (Figs. 1.1, 1.2). In both call types, the screech note is composed of multiple pulses, and click notes had multiple subnotes varying in amplitude (Figs. 1.1, 1.2).

**METHODS**

I recorded males from Centro de Educación Ambiental, CEA, a private reserve located in Mindo, Ecuador (0.0569ºS, 78.684ºW, elevation 1525 m a.s.l.). Field recordings were made between 1830 h and 2100 h from 22 to 25 June 2011. I recorded males from their original perch site in natural choruses at ponds. An omni-directional microphone (Sennheiser MKE 2-P-C, Sennheiser USA, Old Lyme, Connecticut, USA) connected directly to a laptop (MacBook Pro, Apple Inc., Cupertino, California, USA) with a microphone preamplifier (SP-PASM-2, Sound Professionals, Hainesport, New Jersey, USA) was placed 0.25–0.5 m from the focal male. Animals were allowed several minutes to acclimate to the placement of recording equipment and to resume undisturbed calling.
behavior before recording started. I used CoolEdit 2000 v1.1 (Syntrillium Software, Scottsdale, Arizona, USA) to record calls at 16-bit resolution and at a sample rate of 44.1kHz for at least 5 minutes. Air temperature was noted prior to each recording.

I collected additional call data from a population located in the Maquipucuna Biological Reserve (MBR; 0.0715°N, 78.684°W, elevation 1300 m a.s.l.), approximately 24 km northeast straight-line distance from Mindo. Data were collected during calling activity between 2000 h and 2300 h from 14 to 17 March 2013. I observed calling males in natural choruses at ponds for at least 3 minutes, noting number of calls made and the number of click notes associated with each call. I also took air temperature each night upon arrival to pond sites.

I analyzed call recordings using CoolEdit 2000 v1.1 (Syntrillium Software, Scottsdale, Arizona, USA) and Audacity v1.13.13-β (Audacity, Boston, Massachusetts, USA). I measured spectral and temporal properties of the screech and click notes that make up both Type 1 and Type 2 calls. In the notes of both call types, individual pulses and subnotes exhibit similar amplitudes, usually characterized by a sharp rise followed by a graded descent. Only subnotes of click notes that were ≥ 50% of maximum amplitude were included in my analyses (Figs. 1.1, 1.2). I measured inter-note time intervals between both the screech and the first click note (INIa) and between click notes (INIb). For Type 1 calls, I only analyzed calls that contained 2–3 clicks. Because the recordings of Type 2 calls only contained a single click note, I only measured screech-click INI. I analyzed 2013 data separately to eliminate the possibility of variation in calling activity recorded at different times of the breeding season.
I report within-individual variation (CV\textsubscript{w}) for call properties that contained individual means (i.e., dominant frequencies of multiple click notes) as well as among-individual variation (CV\textsubscript{a}). These calculations allow us to investigate possible differences in acoustic properties within and among individuals (Bee et al. 2013; Gerhardt and Huber 2002).

I used Kruskal-Wallis tests to determine if acoustic properties differed between notes among calls. All statistics were carried out in Systat v12 (Systat Software Inc., Chicago, Illinois, USA).

RESULTS

The average air temperature during data collection at CEA was 16.34 ± 0.99°C (mean ± SD; range = 15.8–18.1°C) and 19.06 ± 0.22°C (mean ± SD; range = 18.8–19.3°C) at MBR. I analyzed twenty-seven Type 1 calls recorded from five individuals and two Type 2 calls from one individual from my field recordings at CEA. From my MBR data set, I analyzed 531 and 513 calls, surveyed from thirty-two males to obtain average call rates and number of secondary click notes, respectively. Field recordings and observations demonstrated that Type 1 calls were more common than Type 2. Analyses of call recordings showed that dominant frequencies for both notes appeared fairly broadband and were highly stereotyped within and between individuals (Table 1.1). Additionally, data on call rate and number of added secondary click notes differed between collection sites, with higher values for both call properties observed at the MBR (Tables 1.1, 1.2).
Comparative analyses between screech notes and click notes of Type 1 calls showed that although they have similar value ranges in dominant frequencies (screech: 2801.5 ± 114.9 Hz; clicks: 2727.6 ± 114.9 Hz), screech notes had significantly higher dominant frequency ($H(1) = 7.52, P = 0.006$). Additionally, screech notes were significantly longer in duration than click notes ($H(1) = 57.70, P < 0.001$). Type 2 calls showed similar trends, although I lacked the sample size to run analyses.

Type 2 calls comprised a highly pulsed graded introductory screech note, followed by a single secondary click note similar to click notes of Type 1 calls (Table 1.3, Fig. 1.2). However, compared to Type 1 calls, the screech note of Type 2 calls are characterized with similar dominant frequencies ($H(1) = 2.96, P > 0.05$), longer note duration ($H(1) = 5.40, P < 0.05$), and a greater number of pulses per note (pulse number) ($H(1) = 5.40, P < 0.05$).

DISCUSSION

This study identified and characterized two call types within the vocal repertoire of *Dendropsophus carnifex* from two localities, providing data on species-specific spectral and temporal properties including dominant frequency, inter-note intervals, pulse number, pulse duration, pulse rate, subnote number, subnote rate, and call rates, as well as within and among individual variation for these parameters. Although I did not observe other call types, others may exist in these and/or other populations and further comparative studies may illuminate the full diversity of calls in this species. Together
with *D. columbianus*, *D. borgerti*, *D. norandinus*, *D. carnifex* makes up the *D. columbianus* group (Faivovich et al., 2005; Rivera-Correa and Gutiérrez-Cárdenas, 2012). Although Duellman and Trueb (1983) and De la Riva et al. (1997) provide call descriptions for *D. carnifex*, my study is the first to provide a comprehensive characterization of the vocal repertoire of a species within the *D. columbianus* group. Comparatively, I documented an additional call type, and analyzed a greater number of call recordings, which revealed slightly different values for certain call properties, including note duration, dominant frequency, and pulses per note.

In some species, males have multiple calls in their vocal repertoire, using them in different circumstances (Bee et al., 2013). Additionally, few species emit complex calls, containing multiple notes to communicate to both males and females simultaneously (Littlejohn and Harrison, 1985; Narins and Capranica, 1978) or to increase complexity of calls and thus attraction to mates (Ryan, 1983; Wells and Bard, 1987). I observed similar complex calls in the vocal repertoire of *D. carnifex*.

This study shows that while the majority of the call properties analyzed contain a low degree of variability, a finding similar to other studies on anuran call characteristics, dynamic call properties (e.g., the addition of secondary click notes and call rate) were highly variable (Gerhardt and Huber, 2002; Wells and Schwartz, 2006). Males recorded at CEA incorporated fewer secondary click notes to calls and called less than males observed at MBR. Several studies have illustrated that dynamic call properties can be influenced by both abiotic factors (e.g., temperature and rainfall) as well as biotic factors (e.g., calling activity of nearby males) (Gerhardt et al., 1996; Gerhardt and Huber, 2002; Saenz et al., 2006). Although I did not quantify nearest-neighbor distances or chorus size,
I observed higher call output from males that were in denser calling aggregations than males surrounded with fewer calling males (pers. obs.). Thus, differences between my two data sets, may demonstrate that both biotic an abiotic factors may play a role in the variability of these complex calls.

The calls of two other species in the genus *Dendropsophus*, *D. microcephalus* and *D. ebraccatus* have been well characterized and share a complex call structure to that of *D. carnifex*. Compared to the two distinct amplitudinal peaks (biphasic characteristic) that make up the structure of the secondary notes in *D. microcephalus* advertisement call (Schwartz, 1986), the click notes in *D. carnifex*’s Type 1 calls exhibits a “multiphasic” graded structure with many amplitudinal peaks (Fig. 1.1) similar to the click-note structure of *D. ebraccatus* (Ohmer et al., 2009; Wells and Schwartz, 1984a). Although I did not study the functions of Type 1, I infer that Type 1 calls function as advertisement calls based on how commonly they were observed during my study and on the structural similarities to advertisement calls of *D. microcephalus* and *D. ebraccatus*. In comparison, Type 2 calls recorded at CEA share structural similarities with aggressive calls of *D. ebraccatus*: the introductory note is highly elongated with a gradual incline in amplitude, composed of a greater number of pulses compared to the advertisement call (Fig. 1.2; Wells and Schwartz, 1984b). Additionally, based on an event observed at MBR, in which I witnessed two males emitting the elongated screech note of Type 2 calls while grappling each other, I speculate that Type 2 calls may function in close aggressive encounters with conspecific males. However, because data on Type 2 calls was limited to low sampling (*n* = 2 call recordings; *n* = 1 field observation), further research of this call type is needed to fully resolve its function.
Here, I identify and characterize two call types within the vocal repertoire of *D. carnifex*, and compare my findings to previous literature. Comparing previous call descriptions of *D. carnifex* demonstrated slight inconsistencies, suggesting the importance of a more comprehensive analysis of calls. Possible causes for the differences observed between my study and previous call descriptions of *D. carnifex* may include different sampling and analyzing techniques, and call variation among different populations. One major difference between my study and previous characterizations, however, is sample size. In both their call descriptions, Duellman and Trueb (1983) and De la Riva et al. (1997) analyzed calls from 1–2 individuals and from those individuals, analyzed 2–6 notes. Consequently, the values for call parameters obtained in their studies may not properly represent this species.
<table>
<thead>
<tr>
<th>Vocalization</th>
<th>Call property</th>
<th>Mean ± SD</th>
<th>Range (min–max)</th>
<th>CV\textsubscript{a} (%)</th>
<th>Mean CV\textsubscript{w} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Call</td>
<td>Inter-note interval (screech-click; INI\textsubscript{a})</td>
<td>205.5 ± 85.6</td>
<td>110–565</td>
<td>41.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Inter-note interval (click-click; INI\textsubscript{b})</td>
<td>687.9 ± 264.3</td>
<td>330–1507</td>
<td>38.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Call rate (calls per min)</td>
<td>1.8 ± 0.3</td>
<td>1.5–2</td>
<td>13.8</td>
<td>-</td>
</tr>
<tr>
<td>Screech note</td>
<td>Dominant frequency (Hz)</td>
<td>2800.1 ± 117.0</td>
<td>2561.0–2988.6</td>
<td>4.2</td>
<td>3.2</td>
</tr>
<tr>
<td>(N notes = 26)</td>
<td>Note duration (ms)</td>
<td>331.6 ± 67.2</td>
<td>242.0–507.0</td>
<td>20.3</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>Pulse number (pulses per note)</td>
<td>82.7 ± 10.9</td>
<td>61.1–103.5</td>
<td>13.2</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>Pulse duration (ms)</td>
<td>4.0 ± 0.6</td>
<td>2.8–5.3</td>
<td>16.0</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (pulse per sec)</td>
<td>225.4 ± 29.8</td>
<td>166.4–281.9</td>
<td>13.2</td>
<td>11.8</td>
</tr>
<tr>
<td>Click note</td>
<td>Number of click notes per call</td>
<td>2.6 ± 0.5</td>
<td>2–3</td>
<td>19.2</td>
<td>-</td>
</tr>
<tr>
<td>(N notes = 69)</td>
<td>Dominant frequency (Hz)</td>
<td>2727.6 ± 114.9</td>
<td>2517.7–2957.1</td>
<td>4.2</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Note duration (ms)</td>
<td>53.4 ± 11.9</td>
<td>27.0–95.0</td>
<td>22.3</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>Subnote number (subnotes per note)</td>
<td>2.0 ± 0.6</td>
<td>1–3</td>
<td>30.7</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>Subnote duration (ms)</td>
<td>7.2 ± 1.8</td>
<td>4–15</td>
<td>25.4</td>
<td>25.6</td>
</tr>
</tbody>
</table>

Notes: Subnote analyses of click notes are based on subnotes ≥ 50% of maximum amplitude, not on all subnotes comprising a single click note. I report the averages, standard deviations (SD), range, and percentages for both among-individuals variance (CV\textsubscript{a}) and mean within-individual variance (CV\textsubscript{w}).
Table 1.2. Descriptive call properties of call data recorded at MBR. A total of 32 individuals was observed.

<table>
<thead>
<tr>
<th>Call Property</th>
<th>N calls analyzed</th>
<th>Mean ± SD</th>
<th>Range (min–max)</th>
<th>CV_a (%)</th>
<th>Mean CV_w (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Call rate</td>
<td>531</td>
<td>4.9 ± 1.4</td>
<td>2.3–8.5</td>
<td>28.2</td>
<td>-</td>
</tr>
<tr>
<td>(calls per min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of click notes per call</td>
<td>513</td>
<td>5.5 ± 1.8</td>
<td>2–13</td>
<td>33.6</td>
<td>20.0</td>
</tr>
</tbody>
</table>

I report the averages, standard deviations (SD), range, and percentages for both among-individuals variance (CV_a) and mean within-individual variance (CV_w).
Table 1.3. Descriptive call properties of the two notes that make up Type 2 calls. Call data sampled from a single individual.

<table>
<thead>
<tr>
<th>Vocalization (N = 2)</th>
<th>Call property</th>
<th>Mean ± SD</th>
<th>Range (min–max)</th>
<th>CVw (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screech note</td>
<td>Dominant frequency (Hz)</td>
<td>2970.0 ± 140.0</td>
<td>2871–3069</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Note duration (ms)</td>
<td>1954.0 ± 277.2</td>
<td>1758–2150</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>Pulse number (pulses per note)</td>
<td>254.9 ± 10.3</td>
<td>247.6–262.2</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Pulse duration (ms)</td>
<td>7.7 ± 0.8</td>
<td>7.1–8.2</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (pulse per sec)</td>
<td>131.4 ± 13.4</td>
<td>122.0–140.8</td>
<td>10.2</td>
</tr>
<tr>
<td>Click note</td>
<td>Number of notes per call</td>
<td>1 ± 0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(N = 2)</td>
<td>Dominant frequency (Hz)</td>
<td>2864.5 ± 91.3</td>
<td>2737.8–2866.9</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Note duration (ms)</td>
<td>62.75 ± 0</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Subnotes number (subnotes per note)</td>
<td>1.5 ± 0.7</td>
<td>1–2</td>
<td>47.1</td>
</tr>
<tr>
<td></td>
<td>Subnote duration (ms)</td>
<td>2.75 ± 1.2</td>
<td>3–5</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Notes: Click note analysis based on one click note for each Type 2 call recorded. Since only one individual used a Type 2 call, only CVw was calculated.
Fig. 1.1. Oscillogram (A) and spectrogram (B) of a single natural representative Type 1 call of male *Dendropsophus carnifex*, consisting of the introductory screech note followed by three secondary click notes. An oscillogram of the call to show the pulses comprising the screech note (C) and the subnotes comprising the click note (D). Call was recorded on 22 June 2011 with an air temperature of 15.9°C.
Fig. 1.2. Oscillogram (A) and spectrogram (B) of a single natural representative Type 2 call of male *Dendropsophus carnifex*, consisting of the introductory screech note followed by three secondary click notes. An oscillogram of the call to show the pulses comprising the screech note (C) and the subnotes comprising the click note (D). Call was recorded on 22 June 2011 with an air temperature of 15.9°C.
Numerous studies have documented female mate choice based on the assessment of sexual signals in a variety of taxa (Andersson, 1994). These signals, often conspicuous and energetically expensive, may indicate some fitness-related quality of the signaler from which females may benefit (Andersson and Iwasa, 1996; Andersson, 1994). Additionally, certain characteristics of these signals can vary among individuals, with females often showing a strong preference for extreme signals (Andersson, 1982; Kokko et al., 2003). For instance, females choose mates that exhibit brighter breeding coloration (Houde and Torio, 1992; Waitt et al., 2003), highly exaggerated ornamentation (Karino et al., 2011; Möller, 1988), or more elaborate courtship displays (Barske et al., 2011; Gibson and Uetz, 2008). Females may also choose a mate based on signals that serve as honest indicators of genetic quality, or “good genes,” that can increase the fitness of her offspring, in survival and/or reproductive success (Good Gene Hypothesis; Andersson, 1994; Jennions and Petrie, 2000; Johnstone, 1995). It is often the case that only males in good condition, and/or free of parasites, for example, can produce the most exaggerated signals (Andersson, 1982). For example, Milinski and Bakker (1990) showed that in sticklebacks (*Gasterosteus aculeatus*), male breeding coloration serves as an indication of parasite infection; males that are infected display less intense red coloration and are less likely to be chosen by females. Similar signaling of mate quality, and “good genes,” has
also been documented in anurans (Forsman and Hagman, 2006; Madelaire et al., 2013; Welch et al., 1998).

Acoustically communicating animals have been the subject of many studies of sexual selection (Gerhardt and Huber, 2002). Acoustic signals can serve not only for species recognition (Ryan and Rand, 1993), but can also indicate a signaler’s location, individual identity, as well as other biologically relevant information (Gerhardt and Huber, 2002). For example, acoustic signals may indicate size (Gray, 1997; Pfefferle and Fischer, 2006) and competitive ability (Wells, 1977). Wyman et al. (2012) demonstrated that female American bison (Bison bison) assess a male’s size through his vocalizations and show a strong preference for larger males. Such preferences in mate choice have been well documented in anurans (Castellano and Giacoma, 1998; Gingras et al., 2013; Ryan, 1980).

Most early work on signal variation focused on single components of signals, however, female mate assessment may be affected by sexual signals comprising multiple components and/or multiple modalities (Candolin, 2003; Johnstone, 1996; Møller and Pomiankowski, 1993). Multicomponent signals can either (i) provide different information about the signaler (multiple message hypothesis), (ii) repeat information among different components to lessen transmission errors (redundant ‘backup’ signal hypothesis), or (iii) contain some signals that do not inform of current fitness of the signaler, thus the need to use multiple cues for overall fitness (unreliable signal hypothesis) (Møller and Pomiankowski, 1993). For example, male wolf spiders use both visual and seismic (vibratory) cues to increase mate detection, although depending on the species, the use of both signals may provide redundant information or different
information altogether (Uetz et al., 2009). Grether et al. (2004) demonstrated that in mating systems where visual cues serve an important role in acquiring mates, individual color patches or contrasting color ornamentation can reveal different cues of male fitness, serving as unimodal multicomponent sexual signals.

In many anurans, acoustic signals serve as the dominant form of communication. Among them, many anurans can produce different acoustic signals depending on the social context (Gerhardt and Huber, 2002; Wells 1977), with the conspicuous advertisement call being the most understood. Generally, the advertisement call serves two functions: to attract potential mates and as an indicator of competitive ability to nearby conspecific males (Wells, 1977). In many anurans, these functions are integrated into a single component advertisement call, transmitting information to both males and females simultaneously. However, in other anuran species, advertisement calls are composed of two distinct notes and are therefore described as “diphasic” (Littlejohn and Harrison, 1985). Among these species, a few are known to exhibit functional partitioning of call notes, whereby the female-directed and male-directed components are temporally separated into the different notes of the call (Littlejohn and Harrison, 1985). For example, the advertisement call of male *Eleutherodactylus coqui* has two notes (i.e., “co-qui”), which differ in spectral properties. The introductory “co” note functions in male-male interactions and the secondary “qui” note functions in mate attraction (Narins and Capranica, 1978). Similarly, *Geocrinia victoriana* exhibits a functional partitioned diphasic call, with the male’s elongated introductory note directed towards other males, and the secondary short repeated notes for mate attraction (Littlejohn and Harrison, 1985). Both of these components exhibit similar spectral properties, but differ in their
duration and pulse repetition rate. In *Physalaemus pustulosus*, however, males call with a “whine” note and can add “chuck” notes (up to seven) to increase attractiveness to potential mates, albeit at the cost of increased predation (Bernal et al., 2009; Ryan, 1983). Ryan (1980) demonstrated that the spectral properties of the chuck note reveal a signaler’s size, and that females distinguish males by this property, showing a strong preference for larger males.

Studies of mate choice in anurans have demonstrated that females prefer species-specific call characteristics when selecting a potential mate. As demonstrated in other acoustically communicating animals, females in many anuran species use signals that indicate signaler size, i.e., fundamental or dominant frequency, showing preference for larger males (Castellano et al., 1999). In other anuran species, females demonstrate a preference for temporal call characteristics including variations in pulse repetition rate or call duration, adding additional call notes, or increasing call rates (Gerhardt and Huber, 2002). Because vocalizations in anurans are energetically expensive to produce, variation in temporal call characteristics may indicate the quality of the signaler (Wells and Taigen, 1989).

*Dendropsophus carnifex* is a chorusing treefrog endemic to Ecuador, with populations found along the Pacific slopes of the Andes Mountains (L. A. Coloma, pers. comm.). It inhabits lower montane and cloud forest habitats, as well as in disturbed areas, like plantations. Breeding occurs in temporary and permanent ponds (Cisneros-Heredia and Jungfer, 2004), with males aggregating on surrounding vegetation, calling at the start of the evening. Females arrive later in the evening and exhibit mate choice. Male *D. carnifex* exhibit a diphasic-type multicomponent advertisement call. The call comprises
two distinct note-components: an introductory “screech” note followed by two or more secondary “click” notes (Fig. 1.1; Duellman, 1969; pers. obs.). In chapter 1, I showed that the two notes differ significantly in note duration, and while they exhibit similar ranges in dominant frequencies, they are significantly different (see Table 1.1). Moreover, the number of clicks added to the advertisement call is highly variable, both within and among individuals, and may be a function of male-male interaction as well as mate attraction, as observed in the congeners *D. microcephalus* (Schwartz and Wells, 1985) and *D. ebraccatus* (Wells and Schwartz, 1984b).

The purpose of this study was to determine the effect of the male *D. carnifex* multicomponent advertisement call on female call recognition and choice. Experiment 1 was an investigation of call recognition. I investigated whether males exhibit functional partitioning of their call by testing which call component(s) are necessary and/or sufficient for female phonotaxis. Based on previous research done in other anuran taxa, I hypothesized that only the secondary click note-component would be both necessary and sufficient to elicit phonotaxis. Experiments 2 and 3 then tested female choice based on (i) variation in numbers of secondary click notes and (ii) call rate. Because previous studies in many taxa have demonstrated that females often prefer high energy calls (Gerhardt and Huber, 2002) I predicted that females would demonstrate preferences towards calls with more secondary click notes and higher call rates.
METHODS

Study site:
I conducted this study at the Thomas H. Davis Research Station (TDRS), located within the Maquipucuna Biological Reserve in Pichincha, Ecuador (0.0715°N, 78.684°W, elevation 1300 m a.s.l.). Data collection occurred from March to April, during the wet season when *D. carnifex* exhibits increased breeding activity, compared to relatively drier parts of the year (Wells, 2010; pers. obs.). Experiment 1 on call recognition was conducted in 2012; experiments 2 and 3 on call preference were conducted in 2013.

Collecting and handling:
I collected unamplexed females by hand from a natural chorus area in the evenings and conducted phonotaxis experiments with them between 2300 h and 0200 h on the same night they were collected. Captured females were placed in 50 ml conical tubes for transport from the natural chorus back to TDRS, where I subsequently transferred them into half-pint plastic containers with a small amount of pond water (ca. 20 mL). I then housed them within a Styrofoam cooler, which was placed in an enclosed room, away from the experimental area to prevent acoustic and visual exposure to experimental stimuli. In experiment 1, females were returned to the Styrofoam box in their individual plastic containers after testing, but this led to two females releasing their egg clutches. To reduce the number of oviposition events, I modified animal storage procedures for experiments 2 and 3 by placing females in a domestic refrigerator (6.5°C) immediately after testing (Gerhardt, 1995). Females that released their eggs prior to phonotaxis were
not tested. All females were released within 24 hours and females were tested only once. To prevent re-use of females, individuals from all experiments were measured (mass and snout-vent length) and marked immediately prior to their release at the site of capture. I marked females by excising a single toe pad on either forelimb (toe-tipping, a modified version of toe-clipping; Lüddecke and Amézquita, 1999).

Phonotaxis arena design:
I used two different arenas to conduct three three-choice phonotaxis experiments. In experiment 1, I tested females in a hexagonal arena 0.4 m × 2 m (height × diameter) made of wooden dowels and a black acoustically porous canvas cloth (Fig. 2.1A). This arena was placed in an open area under a roof structure in the vicinity of TDRS. Females were thus exposed to natural ambient noise throughout experimental trials, as a previous study showed that the absence of noise may indicate imminent danger by predation (Dapper et al., 2011). In experiments 2 and 3, I replaced the wooden dowels with PVC pipes to increase stability; the same canvas cloth from experiment 1 was used, resulting in a circular arena of the same height and diameter as the hexagonal arena (Fig. 2.1B). This arena was placed in an enclosed space (testing room) within the TDRS building; a recording of natural chorus noise was provided (see below). To facilitate observations, I covered the floor of both arenas with light-colored towels to contrast the dark-colored bodies of females. Three Eco Extreme Aux Speakers (Grace Digital, San Diego, CA, USA) were positioned on the outside of the arenas facing the center, equidistant to each other and 1 m from the center of the arena (Fig. 2.1).
Experimental stimuli preparation:

To create the experimental stimuli, I used recordings of calling males from a natural chorus \((n = 4)\) obtained in 2011 from a population located in Mindo, Pichincha, Ecuador \((0.0569^\circ S, 78.684^\circ W, \text{elevation 1525 m a.s.l.})\), approximately 24 km straight-line distance from the TDRS study site. Based on the relatively continuous suitable habitat between the two locations, their close proximity and lack of obvious differences in their advertisement calls, I assumed there was no difference in acoustic properties of the calls of frogs from the two locations.

From each call recording, I randomly chose an advertisement call containing two secondary click notes. To avoid pseudoreplication (Kroodsma et al., 2001), I used Adobe Audition v. 2.0 (Adobe Systems, Inc., San Jose, CA, USA) to pitch shift each advertisement call \(\pm 1, 2, 3, \text{and } 4\) semitones using the Pitchshifter plugin, generating a total of 36 unique sound file exemplars. Based on call properties observed in a previous study (see Table 1.1; Chapter 1), I created the test stimuli from the exemplars using Audacity 1.3.13-beta (Audacity, Boston, MA, USA) and CoolEdit 2000 v1.1 (Syntrillium Software, Scottsdale, AZ, USA). These test stimuli were subsequently used to create three tracks played in each trial. Therefore, within each trial, all stimuli a female was exposed to were made from a single exemplar. Additionally, in each trial, stimuli were arranged across tracks to broadcast with little to no overlap. The same exemplars were used in all three experiments, however no two females received the same exemplar within an experiment.
Playback and experimental procedure:

I broadcast the test stimuli using three iPods (Apple Inc., Cupertino, CA, USA) through speakers that were calibrated to 75 dB SPL at 1 m. I calibrated speakers using a sound level meter (RadioShack 33-055, Fort Worth, Texas) to similar natural sound levels of calling *D. carnifex* males at 1 m distance (pers. obs.). For each experiment, I randomized the playback of stimuli among the speakers in each trial to avoid speaker bias. I also randomized the order of acoustic stimuli for each trial to control for any order effect.

At the start of each trial, I played the stimuli antiphonally and placed a female in an acoustically transparent release container positioned in the center of the arena. Females were given 2 min to acclimate and orient themselves within the container while the experimental stimuli were being broadcast. At the end of the acclimation period, I removed the lid of the container using a rope and pulley system (Bee and Swanson, 2007) and monitored their behavioral response for 10 min. In experiment 1, I made direct observations under dim red light from a headlamp (Black Diamond Equipment, Salt Lake City, UT, USA). In experiments 2 and 3, I observed females from outside the testing room using a webcam (HD Webcam C615, Logitech Int’l S.A., Romanel-sur-Morges, Switzerland) connected to a laptop (MacBook Pro, Apple Inc., Cupertino, CA, USA) with a red light source (25 watt bulb). The webcam and the red light were placed on one side of the arena, 2.5 m and 2 m above the ground, respectively, with the webcam positioned at an angle to facilitate visualization.

A choice was scored if, within the 10-min observation period, a female moved to the wall within 10 cm of a speaker. I recorded speaker choice and latency to choice (time recorded once the container lid was removed to speaker). If a female did not leave the
release container within the allotted observation period, the trial was considered a failed trial and was excluded from analyses and the female was not retested. If the female left the release cage but did not make a choice within 10 min., the trial was scored as a no choice trial and analyzed. The experimental stimuli used in either failed or no choice trials were reused to test new females on subsequent nights.

Female choice tests:

*Experiment 1: Which call component(s) are necessary and sufficient to elicit female phonotaxis?*

The three stimuli presented to females in each trial were: (a) a single screech note, (b) two click notes, and (c) a complete call (i.e., a screech followed by two click notes). Each track was 45 s in duration and was looped for the entirety of each trial (Fig. 2.2).

*Experiment 2: Do females prefer more click notes?*

Females were presented with three advertisement calls containing 2, 5, and 8 secondary click notes. I produced the 5-click and 8-click calls by duplicating the natural click notes from the 2-click calls using the observed inter-note intervals of calls analyzed (see Chapter 1). Each track contained the same number of calls and was 2 min in duration. Tracks were broadcast as a loop for the entire trial period (Fig. 2.3).

*Experiment 3: Do females prefer faster call rates?*

I presented three otherwise identical stimuli with call rates of 2, 5, or 8 calls/min. The advertisement calls in each track contained five secondary click notes, the average
number of click notes observed in the 2012 field season (see Table 1.1). The 5-click stimuli produced in experiment 2 were reused to create the stimulus tracks for this experiment. Each track was 2 min in duration and looped for the entire trial period (Fig. 2.4).

For experiments 2 and 3, I broadcast natural chorus sounds recorded from the Mindo population with a portable recorder (Olympus Linear PCM Recorder LS-10, Olympus Co., Tokyo, Japan). Chorus noise was adjusted -10 dB relative to the test stimuli using the aforementioned audio software.

**Body condition on latency to choice:**
Female condition has been shown to affect their behavior in choosing a mate (Baugh and Ryan, 2009). Accordingly, to determine if female condition plays a role in phonotaxis behavior in *D. carnifex*, I analyzed latency to stimulus choice on female body condition. I calculated body condition by obtaining the residual values from a linear regression of the cubed root body mass on snout-vent length (SVL) and the divided those values by SVL, following the method used by Baugh and Ryan (2009) and Leary et al. (2008).

**RESULTS**

*Experiment 1: Which call component(s) are necessary and sufficient to elicit female phonotaxis?*
I caught 29 females and used 19 in analyses (n = 2 released eggs prior to trials; n = 8 failed to leave the release container within the allotted observation time). Females were responsive to the playbacks, as only 1 of 19 did not make a choice (p < 0.001, exact binomial test). There was no difference between numbers of females attracted to the screech alone (n = 5), secondary clicks alone (n = 7), or screech with clicks (n = 6) (G = 0.33, df = 2, p = 0.85, n = 18; Fig. 2.5). In some trials, females were observed to make brief pauses between movements towards speaker choice. Consequently, latency to choice was independent of female choice (one-way ANOVA: F2,15 = 1.40, p = 0.28, n = 18; Fig. 2.5). Body condition did not influence latency to choice (r²=0.21, df = 16, p = 0.055; Fig. 2.8).

**Experiment 2: Do females prefer more click notes?**

I tested 37 females and excluded three females because they failed to leave the release container within the allotted time. Females demonstrated responsiveness to playbacks, as only 5 of 34 did not make a choice (p < 0.0001, exact binomial test). Females that made a choice showed a preference for 8-click calls (n = 15) over both 5-click calls (n = 10) and 2-click calls (n = 4) (G = 6.80, df = 2, p = 0.03, n = 29; Fig. 2.6). Some females exhibited momentary pauses during phonotaxis, and thus stimulus choice did not affect time to choice (one-way ANOVA: F2,26 = 0.77, p = 0.47, n = 29; Fig. 2.6). Additionally, body condition did not affect latency to choice (r²=0.02, df = 27, p = 0.519; Fig. 2.8).
Experiment 3: Do females prefer faster call rates?

I tested 37 females and excluded four females because they failed to leave the release container within the allotted time. The majority of females were responsive to playbacks, with 3 of 33 not making a choice (p < 0.0001, exact binomial test). Females that chose stimuli showed a strong preference for faster call rates, with most females choosing a stimulus containing 8 calls/min \( (n = 18) \) over stimulus of either 5 calls/min \( (n = 10) \) or 2 calls/min \( (n = 2) \)(G = 14.72, df = 2, p < 0.001, n = 30; Fig. 2.7). Similarly to the other experiments, some females were slow in their movements towards a speaker choice. Thus, choice did not affect latency to choice (one-way ANOVA: F\(_{2,27} = 0.25, p = 0.78, n = 30\); Fig. 2.7). Also, body condition did not affect latency to choice \( (r^2 = 0.02, df = 28, p = 0.501; \) Fig. 2.8).

DISCUSSION

My study demonstrated that the note components of a multicomponent sexual signal, although distinct in both spectral and temporal acoustic properties, elicit a behavioral response from females. In fact, all call components either separately or in combination were equally effective at eliciting phonotaxis. Additionally, I demonstrated that females preferred greater call output, with a strong preference for greater number of added click notes and faster call rates. The results of my first experiment did not support my hypothesis that only the secondary click notes would be necessary and sufficient for female phonotaxis, thus eliminating the possibility that male D. carnifex exhibit a fully
functionally partitioned advertisement call. Rather, my results demonstrated that each
note is sufficient to elicit female phonotaxis, at least over short distances. Additionally,
the results of my second and third experiments demonstrated that, as predicted, females
show a strong preference for more secondary notes in a call, as well as faster call rates.
Here, I discuss these findings with respect to redundancy signal hypothesis and female
choice.

Redundant signal hypothesis

The equal phonotaxis response of females to both distinct notes observed in my
study may indicate that each note of *D. carnifex* advertisement call communicates the
same fitness-related quality of the signaler, a pattern consistent with the redundant signal
hypothesis (Møller and Pomiankowski, 1993). Additionally, data suggest that the
response I observed does not encode new information, nor does it enhance behavioral
response, but rather demonstrates an equivalent behavioral response (Partan and Marler,
1999). The redundant, equivalent signals of the multicomponent call of *D. carnifex* may
function to increase detection of a signal and/or decrease signal transmission error in
complex environments, such as an anuran chorus. (Candolin, 2003; Johnstone, 1996).
Thus, the production of redundant signals may facilitate localizing potential mates for
females entering a densely populated chorus, especially those containing other vocalizing
species.
Female preference for temporal call properties

When presented simultaneously with three different stimuli varying in either the number of click notes appended to the advertisement call or call rate, the majority of females displayed positive phonotaxis towards speakers that broadcast stimulus of calls containing the most click notes or the fastest call rate. Consequently, males that call with more clicks or produce more calls over time should experience a greater mating advantage over those that produce calls with fewer clicks or call less frequently. These findings are consistent with previous literature, demonstrating that females prefer greater call output in the form of calls with more appended notes or faster call rates (Forsman and Hagman, 2006; Lopez and Narins, 1991; Taylor et al., 2007; Ward et al., 2013).

Anuran vocalizations are the most energetically expensive activity in which males participate (Gerhardt and Huber, 2002; Prestwich, 1994). Although energetic cost of calling has not been studied in *D. carnifex*, Wells and Taigen (1989) found that the congener *D. microcephalus* exhibits energetic costs of about 19 times higher when calling than during rest. Additionally, the cost of calling was positively correlated with the number of notes added to calls, a result that can probably be generalized to *D. carnifex*, since both exhibit a similar call structure. As such, increased call output in the form of adding more notes to a call or faster call rates is likely to increase total energetic costs and thus may serve as an indicator of current male quality (Prestwich, 1994; Stoddard and Salazar, 2011; Sullivan, 1983). For example, female *Dendrobates leucomelas* benefit from choosing high-quality males based on high-cost vocalizations, i.e., increased call rates and chirp duration, resulting in greater offspring quality (Forsman and Hagman, 2006). Therefore, the increased call output by male *D. carnifex* may
similarly demonstrate higher mate quality, and females that choose them may similarly
gain some form of benefit (direct and/or indirect).

On the other hand, female preference for increased call output may be explained
by some form of sensory bias. In their review of call properties that affect female choice,
Ryan and Keddy-Hector (1992) suggested that females prefer sexual signals of greater
intensity because it elicits greater reproductive stimulation. Thus, adding more attractive
notes to a call or increasing call rate, as investigated in my study, may similarly have
caused greater stimulation in females, causing them to show preferences for stimuli of
greater call output. Lastly, increasing call output may enhance detection and thus
localizability of a potential mate in noisy environments, as is the case of many chorusing
anurans (Wells and Schwartz, 2006). Price (2013) suggested that similar calling patterns
occur in birds and is consistent with signal detection theory, in which repetitive bird
songs allow for increased detection. Preference for increased detection in noisy
environments may be beneficial for females in finding a mate in noisy environments
considering the energetic cost they would experience by traveling and/or predation risk.

Latency to choice

Interestingly, regardless of the stimulus chosen, latency to choice did not differ in
all three experiments. Given the strong call preference observed in both temporal call
characteristic experiments (experiments 2 and 3), these results were unexpected. It is
worth mentioning that in several females, the experimental stimulus ultimately chosen
was not the stimulus females initially moved towards. Furthermore, some females paused
between movement towards stimuli, a similar pattern observed with P. pustulosus (Baugh
and Ryan, 2010). In their study, they demonstrated female phonotaxis behavior is more variable and flexible than previously postulated, and influenced by continual updating of signals in a complex acoustic environment throughout the travel period. Therefore, the pauses observed in some females during phonotaxis may similarly demonstrate continual updating of sexual signals and consequently affected latency analyses. Additionally, they showed that commitment to stimuli may vary based on female size, with larger females less likely to change their commitment to a stimulus once it has been made, decreasing travel time and thus decreasing overall latency (Baugh and Ryan, 2009). This explanation is unlikely, however, as female body condition, which is calculated from size (SVL) and mass, were not significantly correlated with latency periods for all three experiments.

Latency to choice, which may imply mate choosiness, can also be affected by possible costs incurred during mate sampling. Indeed, females may experience certain trade-offs, e.g., risk of predation or time costs, either in the form of mate access and availability, in an attempt to optimize mate choice among the choices she has (Jennions and Petrie, 1997). For example, when faced with increased predation risk, female fiddler crabs (Booksmythe et al., 2008) and green swordtails (Johnson and Basolo, 2003) demonstrated a decrease in choosiness, opting to travel shorter distances in search of a mate, thus spending less sampling time. On the other hand, females may increase vigilance, decreasing both travel speed and conspicuous movements that would increase predator detection (Bonachea and Ryan, 2011; Rand et al., 1997), thus increasing overall latency to choose a mate. Other possible factors that can influence female mate sampling time and choosiness include mate availability and competition. Lindström and Lehtonen (2013) demonstrated that in female sand gobies, Pomatoschistus minutus, mate sampling
time was greatly influenced by the costs of delayed mating opportunities as well as competition for mate access by nearby receptive females, as females decreased their time to mate in both cases. The costs of predation risk, mate availability, and mate competition are unlikely to play a role in the latency to choice observed in the females of this study. Although predation risk has not been investigated in *D. carnifex*, it is unlikely as (i) females were tested away from their natural breeding sites where possible predation risk is high and (ii) females did not differ in latency times among the three experiments, regardless of choice. However, the effect of predation risk on choosiness cannot be entirely ruled out. In regard to mate availability and female competition, in most chorusing treefrogs, the operational sex ratio is male-biased with females migrating to choruses long after males have begun calling (Wogel et al., 2006). Thus, delayed mating opportunities and competition by females in acquiring a mate would be unlikely and therefore less likely to affect mate sampling time.

The reproductive state of females may also influence phonotaxis behavior. Lynch et al. (2005) showed that latency to choice toward mate signals was strongly affected by time to oviposition. In their study, female *P. pustulosus* that were closer to depositing their eggs exhibited decreased latency times in choosing a mate (speaker) once they were released from their container. Although I did not monitor oviposition events, the reproductive state of the females tested could have affected their phonotaxis behavior in my study, resulting in the variation in latency times I recorded. The lack of difference in latency to choice observed in this study may suggest that these costs may not be as strongly correlated with mate choosiness in *D. carnifex*. However, because my
experiments were conducted in a controlled space, it is possible that these factors may play a more significant role in mate choosiness in natural conditions.

Conclusions

Here, I demonstrate that both notes of the multicomponent advertisement call are sufficient and necessary for mate recognition, with females demonstrating a strong preference for calls of greater acoustic output. Additionally, latency to choice, regardless of the strength of preference, appears to be unaffected by costs of choosiness, e.g., predation (Booksmythe et al., 2008; Johnson and Basolo, 2003) and mate availability (Johnstone, 1996). Because females fail to show increased phonotaxis behavior towards complete calls over each note, a pattern contrary to previous studies in which increased complexity increases attraction (Bernal et al., 2009), yet prefer greater number of call notes and faster call rates, a comprehensive understanding of the functionality and significance of maintaining a complex advertisement call is needed. In particular, conducting experiments on male responses to these advertisement calls may provide insight into why a complex advertisement call exists. Determining female phonotaxis towards multicomponent calls and the direction of call preferences is important in understanding the evolution of signal complexity in light of sexual selection.
Fig. 2.1. Schematic diagrams represent the phonotaxis arenas used in female choice experiments. The center circles of each arena represent the release cage females started in at the beginning of each trial. Diameter for both arenas is 2 m. Hexagonal arena (A) was used for experiment 1—mate signal recognition. Circular arena (B) was used for experiments 2 and 3—preference for temporal acoustic characteristics: number of added click notes and call rate, respectively.
Fig. 2.2. Stimulus tracks of a single representative experimental trial for experiment 1. Females received all three tracks simultaneously and tracks were looped for 10 min. Shown (A–C) is a 45 s track of the full call, screech components, and click component.
Fig. 2.3. Stimulus tracks of a single representative experimental trial for experiment 2. Females received all three tracks simultaneously and tracks were looped for 10 min. Shown (A–C) is a 2 min track of eight calls containing 5 click notes, 2 click notes, and 8 click notes.
Fig. 2.4. Stimulus tracks of a single representative experimental trial for experiment 3. Females received all three tracks simultaneously and tracks were looped for 10 min. Shown (A–C) is a 1 min track of advertisement calls containing 5 click notes at 2, 5, and 8 calls/min.
Fig. 2.5. Number of females (white bars) choosing each stimulus of complete call (screech note + click notes), screech note, or click notes. Mean latency (gray bars) to chosen stimulus. Error bars are SD. Females did not show strong preference for any of the three stimuli. Latency to choice was not affected by stimulus choice.
Fig. 2.6. Number of females (white bars) choosing each stimulus of advertisement calls containing 2 clicks, 5 clicks, or 8 clicks. Mean latency (gray bars) to chosen stimulus. Error bars are SD. Females showed strong preference for calls containing more click notes. Latency to choice was not affected by stimulus choice.
Fig. 2.7. Number of females (white bars) choosing each stimulus of 2, 5, or 8 calls/min. Mean latency (gray bars) to chosen stimulus. Error bars are SD. Females showed strong preference for faster call rates. Latency to choice was not affected by stimulus choice.
Fig. 2.8. Relationship between body condition index and latency to choice for each experiment: call component (○), click notes (□), and call rates (▲). Body condition of females did not affect choice of stimulus in all three experiments.
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