Do natural differences in acoustic signals really interfere in conspecific recognition in the pan-Amazonian frog *Allobates femoralis*?

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Summary

The call of the pan-Amazonian frog *Allobates femoralis* shows wide geographical variation, and males show a stereotyped and conspicuous phonotactic response to playback of conspecific calls. We evaluated the capacity of males of *A. femoralis* and a closely related species *A. hodli* to respond aggressively to natural conspecific and heterospecific calls varying in number of notes, by means of field playback experiments performed at two sites in the Brazilian Amazon. The first site, Cachoeira do Jirau (Porto Velho, Rondônia), is a parapatric contact zone between *A. femoralis* that use 4-note calls, and *A. hodli* with 2-note calls, where we performed cross-playbacks in both focal populations. The second site, the Reserva Florestal Adolpho Ducke (Manaus, Amazonas), contained only *A. femoralis* with 4-note calls. There, we broadcast natural stimuli of 2-note *A. hodli*, 3-note and 4-note *A. femoralis,* and 6-note *A. myersi*. We found that the phonotactic behaviour of *A. femoralis* and *A. hodli* males did not differ toward conspecific and heterospecific stimuli, even in parapatry. Our results indicated that the evolutionary rates of call design and call perception are different, because the geographical variation in calls was not accompanied by variation in the males' aggressive behaviour.

Keywords: species recognition, contact zone, playback, Dendrobatoidea, Anura.

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1. Introduction

Advertisement calls are conspicuous acoustic signals emitted by males of most species of anurans. These calls generally have multiple purposes, such as female attraction and territory defence against conspecific males (Duellman & Trueb, 1994), and informing about a male's physical condition, identity, and location (Wells & Schwartz, 2007). Species recognition is especially important for species in which heterospecific mating is very costly (Panhuis et al., 2001), and for territorial males in order to avoid misidentification of competitors, thus saving energy and time (Gerhardt, 1999; Bernal et al., 2009; Lemmon, 2009). The geographical variation in advertisement calls between allopatric populations or sister-species may arise as a result of genetic drift, or from selective pressures exclusive to the geographical range occupied by each population (Littlejohn, 1988; Coyne & Orr, 2004). The occurrence of closely related species in sympatry may facilitate the divergence in signals and signal preferences between and within species (Gerhardt, 1999; Lemmon, 2009), favouring species-recognition traits over mate-quality traits, i.e., by character displacement.

The pan-Amazonian frog *Allobates femoralis* Boulenger (1883) is widely distributed throughout the Amazon non-flooded forests, is diurnal, and males defend a multi-purpose territory (Roithmair, 1992). Previous studies have shown males of *A. femoralis* defending territories for up to 90 days in a Peruvian Amazon population (Roithmair, 1992), and for more than 79 days in a population in French Guyana (Ringler et al., 2009). Geographical variation in number of notes in the advertisement call is known for *A. femoralis* (Hödl et al., 2004; Amézquita et al., 2005, 2006). Calls with 1 note are reported from the Parque Nacional Yasuní in Ecuador (Read, 2000), and along the Rio Juruá in Brazil (Simões & Lima, unpublished data); 3-note calls are known from the Panguana station in Peru (Hödl et al., 2004; Amézquita et al., 2006). Four-note calls are geographically widespread, being described from localities in the central Brazilian Amazon, the Rio Madeira basin, Colombia, and French Guyana (Hödl et al., 2004; Amézquita et al., 2006, 2009; Simões et al., 2008). A related species, *Allobates myersi* (Pyburn, 1981), has its known distribution restricted to the Colombian Amazon (Lötters et al., 2007; Frost, 2010), and was recently found in the municipality of São Gabriel da Cachoeira in the upper Rio Negro, state of Amazonas, Brazil (P. Simões & A. Lima, personal observations). Its advertisement call is very similar to

that of *A. femoralis*, but is composed by a mean number of 6 notes (un-published data) (Figure 1). A 2-note-call population formerly referred to as *A. femoralis*, which recently received species status (*Allobates hodli* Simões, Lima & Farias, 2010), occurs on the left bank of the upper Rio Madeira, and the southeastern part of the state of Acre, Brazil (Simões et al., 2010). On the left bank of the upper course of the Rio Madeira, a 2-note *A. hodli* population encounters a 4-note *A. femoralis* population at a narrow contact zone (Simões et al., 2008, 2010).

Exposure to conspecific calls above 68 dB sound pressure level (re 20 μPa) elicits phonotactic behaviour in *A. femoralis* males, which consist of stopping calling, head and body orientation, and approaching the broadcasting loudspeaker (Narins et al., 2003). The recognition mechanism in *A. femoralis* has been studied in field experiments using synthetic calls directed to males, each time isolating the acoustic trait to be tested. For example, the number of notes and call peak frequency (Amézquita et al., 2005), variations in the frequency modulation of notes (Hödl et al., 2004), and the duration of the silent interval between notes (Göd et al., 2007) have been analyzed previously. The probability of response of *A. femoralis* males matched the main frequency value for a Colombian 4-note-call population, but also matched the range of frequency variation of heterospecific advertisement calls, indicating that the peak frequency alone was not sufficient for males to discriminate between conspecific and heterospecific calls (Amézquita et al., 2005). The changing of the typical ascending-frequency modulation in the advertisement calls of *A. femoralis* to a descending-frequency modulation or to an unmodulated call causes no differences in the response patterns of *A. femoralis* males (Hödl et al., 2004). Males of *A. femoralis* can recognize advertisement calls with a silent interval between notes that varies up to 60% of the population mean value (Göd et al., 2007). Previous studies were not able to determine a particular acoustic trait that elicits accurate species recognition, and demonstrated that not all stereotyped traits are relevant for this purpose. Probably there is no single acoustic trait responsible for species recognition, which is, rather, achieved by the interaction or summing of the distinctive characteristics of multiple traits (Gerhardt & Huber, 2002; Göd et al., 2007).

When considering the variation in the number of notes per call (2–4 notes), differences in the recognition curves toward 2-note calls were found,
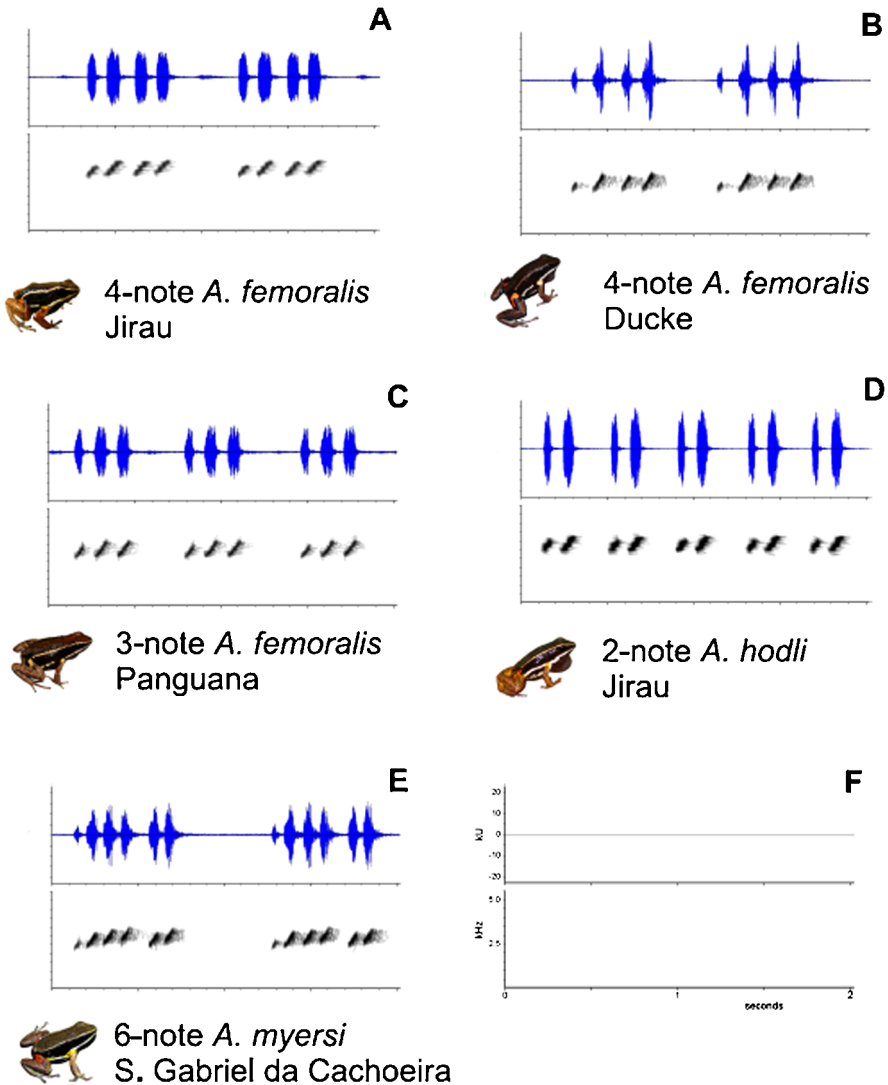
Figure 1. Waveforms and spectrograms of stimuli used in the playback experiments, including the three focal populations. (A) 4-note advertisement calls of *Allobates femoralis* from Jirau, (B) 4-note calls of *A. femoralis* from Ducke, (C) 3-note calls of *A. femoralis* from Panguana, (D) 2-note calls of *A. hodli* from Jirau and (E) 6-note calls of *A. myersi* from São Gabriel da Cachoeira. In (F) the scale of amplitude (kU), frequency (kHz), and time (s) for these acoustic graphs. This figure is published in colour in the online edition of this journal, which can be accessed via http://www.brill.nl/beh

and it was suggested that this differential response could indicate local adaptation to another species' calls or a reaction to a number of notes that is not typical of the focal population (Amézquita et al., 2005). In this study, we evaluated the male recognition capacity of two different populations of *A. femoralis* and one of *A. hodli*, toward natural conspecific and heterospecific advertisement calls, which have a notable geographical variation in the number of notes, from 2 to 6 notes per call. We expected that the remarkable differences in the advertisement calls would elicit more accurate call recognition, also meaning species or population recognition.

2. Material and methods

To test the effect of natural variation in advertisement calls, here represented as the variation in the number of notes constituting each call (acoustical analysis revealed that the observed variation in call traits between populations and species is related to the number of notes per call, Table 1), on the phonotactic behaviour of *Allobates femoralis* males, we recorded advertisement calls in three distinct populations referred to as *A. femoralis*, in one population of *Allobates hodli* from the southwestern Brazilian Amazon (Simões et al., 2010), and in one population of *Allobates myersi*, a species taxonomically related to *A. femoralis* (Pyburn, 1981; Grant et al., 2006), found in the northwestern Brazilian Amazon (Figure 1). The localities sampled for advertisement calls cover most of the known acoustical variation in number of notes for the species (Amézquita et al., 2006, 2009). Across its distribution, *A. femoralis* shows high levels of genetic differentiation, and it is possible that phenotypic or genetically divergent groups will be assigned species status in the future (Grant et al., 2006; Santos et al., 2009). For purposes of taxonomic consistency, we treat all source and test populations (except the *A. myersi* and *A. hodli* populations) as *A. femoralis.*

Our data set included recordings from 14 individuals of *A. hodli*, which produces a 2-note advertisement call, and 14 individuals of a 4-note advertisement call population found at the extremes of an interpopulation contact zone at Cachoeira do Jirau (Jirau), Rondônia, Brazil (9.3206°S, 64.7225°W). This parapatric contact zone is located on the left bank of the Madeira River and coincides with the boundary between geomorphological units (Simões

Table 1. Acoustic traits of the natural stimuli used in playback experiments at Jirau and Ducke sites.

Population	Number of notes	Call duration (s)	Inter-call interval (s)	Peak frequency (Hz)
A. hodli, Jirau	2	0.16 ± 0.011 (0.14–0.18)	0.22 ± 0.032 (0.17–0.28)	3428.6 ± 125.2 (3149.2–3576.3)
A. femoralis, Panguana	3	0.33 ± 0.029 (0.29–0.42)	0.37 ± 0.033 (0.32–0.43)	3238.7 ± 273.9 (2853.1–3707.3)
A. femoralis, Jirau	4	0.49 ± 0.024 (0.46–0.54)	0.44 ± 0.049 (0.36–0.54)	3435.6 ± 162.8 (3075.6–3730.6)
A. femoralis, Ducke	4	0.53 ± 0.03 (0.49–0.57)	0.51 ± 0.08 (0.43–0.65)	2865.4 ± 218.2 (2548.1–3222.8)
A. myersi, São Gabriel da Cachoeira	6	0.61 ± 0.042 (0.53–0.67)	0.79 ± 0.109 (0.64–0.93)	2859.4 ± 138.2 (2662.9–3078.1)

Each trait is represented by the mean ± SD, and the minimum and maximum values. The advertisement calls were analysed in Raven 1.2 using the Blackmann window, 80% overlapping and a fast Fourier transform of frequency resolution of 80 Hz and 2048 points. Note that call duration and inter-call interval, but not peak frequency, increase with the number of notes per call.

et al., 2008). To avoid sampling calls of hybrid individuals, males were recorded at least 1.2 km upstream and downstream from the area where the occurrence of acoustic morphotypes overlaps. Recordings were obtained from November 2004 to January 2005 by P.I. Simões and A.P. Lima.

Ten individuals from another 4-note call population were recorded at the Reserva Florestal Adolpho Ducke (Ducke), in Manaus, Brazil (2.9167°S, 59.9833°W) by A.P. Lima and L.K. Erdtmann in 2002 and 2008. Ten recordings of a 3-note advertisement call population used in this study were obtained by Adolfo Amézquita in 2002, at Panguana Station in Peru (9.6137°S, 74.9355°W). To obtain *A. myersi* calls, ten individuals were recorded at São Gabriel da Cachoeira, Amazonas, Brazil (0.1558°S, 67.0861°W), by A.P. Lima and P.I. Simões in May 2008. Advertisement calls of *A. myersi* used in this study were formed by a first trill of four notes followed by one or more consecutive note pairs, or couplets. The addition of one couplet was the most common call structure, totalling six notes similar to those of *A. femoralis* populations in terms of frequency range and modulation (Table 1). Thus, *A. myersi* recordings were termed a 6-note advertisement call stimulus in the experiments described below.

All recordings were made with a Sony WM D6C (Sony, Tokyo, Japan) cassette tape recorder or a Marantz PMD 660 digital recorder (DM Professional, Wilmington, DE, USA) and AKG D5 (AKG Acoustics, Vienna, Austria) or Sennheiser K6/ME66 (Sennheiser Electronic, Old Lyme, CT, USA) directional microphones. Cassette tape recordings were digitized at 22 kHz using the software Raven 1.2 (Charif et al., 2004). Digital recordings were made at 44 kHz and 16-bit resolution, and analyzed using Raven 1.2 (Charif et al., 2004).

The recordings were used as natural stimuli for field playback experiments performed with three focal populations: the 2-note and 4-note advertisement call populations at Jirau, and the 4-note advertisement call population at Ducke. The populations at Jirau were tested in two short field trips during 11 days in January 2008 and seven days in February 2009. The experiments at Ducke were done weekly from December 2008 to March 2009. The focal populations at Jirau and Ducke are separated by at least 1000 km across the Madeira-Purus interfluve and by the Amazon River, at the southern end of Manaus. Whereas Jirau represents the contact zone between two very distinct populations, the 4-note call population at Ducke is highly allopatric in relation to other *A. femoralis* acoustic phenotypes.

Each stimulus represents a different individual, and all stimuli were edited to control for the number of calls emitted in each calling bout and the duration of silent intervals between calling bouts, using Raven 1.2 (Charif et al., 2004). A calling bout was constituted by 20 calls followed by a 20-s silent interval. In order to construct each stimulus, we used calls from the core of the original calling bout recorded, thus avoiding warm-up and final calls, which generally show wider frequency variation (Gerhardt & Huber, 2002). The number of calls and duration of silent intervals were approximated based on available recordings of complete call bouts and silent intervals for the source populations. Two Ducke recordings that contained excessive background noise were edited further. We used Audacity 1.3.4-beta (Audacity Team, 2008) to filter for frequencies below 300 Hz, and the resulting filtered recordings were used in the playback experiments. We did not control stimuli for any additional acoustic trait, because these represent natural recordings, including the within- and between-individual call variation observed in the source populations.

The design of playback experiments varied between study sites to explore the simpatry and allopatry condition. At Jirau, 2-note and 4-note stimuli

recorded upstream and downstream from the contact zone were broadcast to 2-note- and 4-note-call focal males in the core area of the parapatric contact zone. At Ducke, 4-note-call focal males were tested with stimuli constructed from recordings of 2-note calls from the Jirau population, 3-note calls from the Panguana population, 6-note calls of *A. myersi*, as well as 4-note calls from males of the same population.

Focal males were located through their advertisement calls. Once spotted by one of the researchers, their initial position was marked with a small wooden stick. The playback experiment started when the focal male resumed calling activity. Stimuli were broadcast with a G-flash wma-mp3 player (Maxfield, Düsseldorf, Germany) connected to Sony SRS-M30 battery-powered loudspeakers (Sony, Bangkok, Thailand), positioned 1.5 m from the focal male. To avoid pseudoreplication, each focal male was tested once. In addition to flagging the initial position of males, differences in lateral lines and patterning of flash marks on the thighs allowed us to discriminate between tested and untested individuals. During the recording of stimuli, the average air temperature at calling sites of recorded males was of $27.3 \pm 1.5°C$ (23.5–29.8°C) at Jirau, and of $26 \pm 1.26°C$ (23–30°C) at Ducke. As air temperatures did not significantly oscillate among recording sessions at each locality or between localities, we did not control for air temperature at the time of playback experiments.

At Jirau, differences in male phonotactic behaviour elicited by playbacks of two classes of stimuli (calls from the same population *versus* calls from the alternate population) were measured as the relative number of males that approached the loudspeakers. We considered that a male approached the loudspeakers when the individual advanced until it reached a 30-cm radius around the loudspeakers. To test for differences between intra- and inter-specific recognition patterns, we performed a Fisher's exact test considering the number of approaches to the loudspeakers in intra-specific and inter-specific categories. Additionally, we measured latency to the first movement (here considered the time elapsed from the start of the experiment to the first orientation, jumping, or antiphonal calling movement), and latency to approach (here considered as the time elapsed between the start of the experiment and the male approaching the loudspeakers). The existence of differences in behavioural responses between different classes of stimuli was tested with the non-parametric Kruskal–Wallis test. The experiment ended when the focal male approached the loudspeakers or, alternatively, 5 min

after the start of the experiment if males did not respond to the playback. Because only a few individuals used antiphonal calls as a response to acoustic stimuli, we did not include the number of antiphonal calls in any of the tests regarding the Jirau and Ducke populations.

At Ducke, the phonotactic response to the different classes of stimuli tested (2, 3, 4 and 6-note calls) was measured as latency to orientation, latency to jump, latency to approach, and number of antiphonal calls produced by focal males during the experiment. The experiments ended when the focal male approached a 30-cm radius around the loudspeakers. In order to control for male motivational state, always when a male did not approach the loudspeakers, we performed a control period, with the broadcast of a natural recording from the Ducke population, subsequent to the stimulus period. After the experiment, the distance between the loudspeakers and the initial focal-male position was confirmed, and the Sound Pressure Level (SPL) at the initial focal-male position was measured in dB with a Voltcraft SL-100 sound-level meter (re 20 μPa, peak intensity, fast time, resolution of 0.1 dB). At Jirau, the distance between the focal male and the loudspeakers (1.5 m) was measured prior to the start of the experiment, and stimuli intensity was controlled by previously fixing the loudspeakers and wma-mp3 player volume controls at around 74 dB. The existence of differences in phonotactic response in relation to stimuli classes at Ducke was tested using the non-parametric Kruskal–Wallis test, considering stimuli intensity measured after the experiment and the distance from the focal male to the loudspeakers as co-variables. Note that the variable 'latency to movement' measured at Jirau and 'latency to orientation' measured at Ducke, can be considered as homologous traits, because at Ducke the first movement in all experiments except one was the orientation. All the statistical analyses were implemented and performed in R 2.9.1 (R Development Core Team, 2009).

The number of playbacks was defined based on previous studies which found significant results with similar number of experiments (e.g., 9–15 experiments in Narins et al., 2003; 14 positive and/or negative results experiments in Hödl et al., 2004; 10 males tested in Luna et al., 2010). In this way, the total number of experiments executed in this study was 10 tested males in 2-note population at Jirau, five of them were presented with 4-note stimuli and five with 2-note stimuli, and 18 males were tested in the 4-note population, nine of them presented with 4-note stimuli and nine with 2-note stimuli. At Ducke, we tested 37 males, 10 males per class of stimulus (2-note, 3-note and 6-note), and 7 males to the control (4-note calls from Ducke).

3. Results

We found no difference between the phonotactic behaviour of males from the two tested *A. femoralis* populations and one *A. hodli* population and the natural calls from different populations of *A. femoralis* and *A. myersi*. At Cachoeira do Jirau, 2-note and 4-note males showed similar phonotactic responses to calls from their own population and calls from the parapatric population (Fisher's exact test $p = 0.45$, $N = 14$), considering either the latency to movement ($\chi^2 = 1.11$, $p = 0.77$, $N = 28$, 10 males tested in 2-note population and 18 males tested in 4-note population) or the latency to approach ($\chi^2 = 2.66$, $p = 0.45$, $N = 15$, 10 males tested in 2-note population and 18 males tested in 4-note population) (Figure 2).

At Ducke, the male response patterns did not differ between the 2-note, 3-note, and 6-note stimuli and the control (4-note calls from Ducke) for latency to orientation ($\chi^2 = 19.25$, $p = 0.74$, $N = 37$), latency to jump ($\chi^2 = 17.73$, $p = 0.82$, $N = 37$) and latency to approach ($\chi^2 = 25.01$, $p = 0.40$, $N = 33$), these analyses included 10 males per stimulus class and seven for the control (Figure 3).

At Jirau, 28 experiments were performed. In all of them, the males presented any behavioural change, orientation or jump, from the initial be-
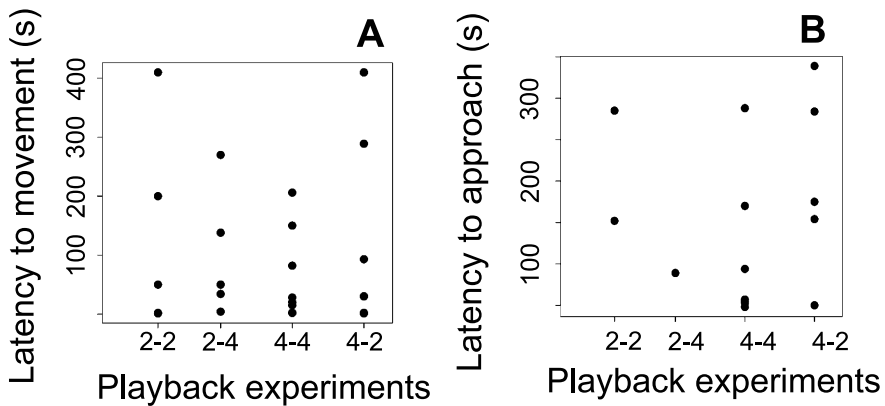

Figure 2.   Latency of response of *A. femoralis* and *A. hodli* to natural conspecific and heterospecific stimuli in field cross-playback experiments at Jirau. (A) Latency to movement and (B) latency to approach the loudspeakers, where 2-2 represents the response of *A. hodli* males when presented with its own calls, 2-4 the response of *A. hodli* males when presented with the parapatric *A. femoralis* calls, 4-4 that of *A. femoralis* males presented with *A. femoralis* calls, and 4-2 that of *A. femoralis* males presented with *A. hodli* calls.
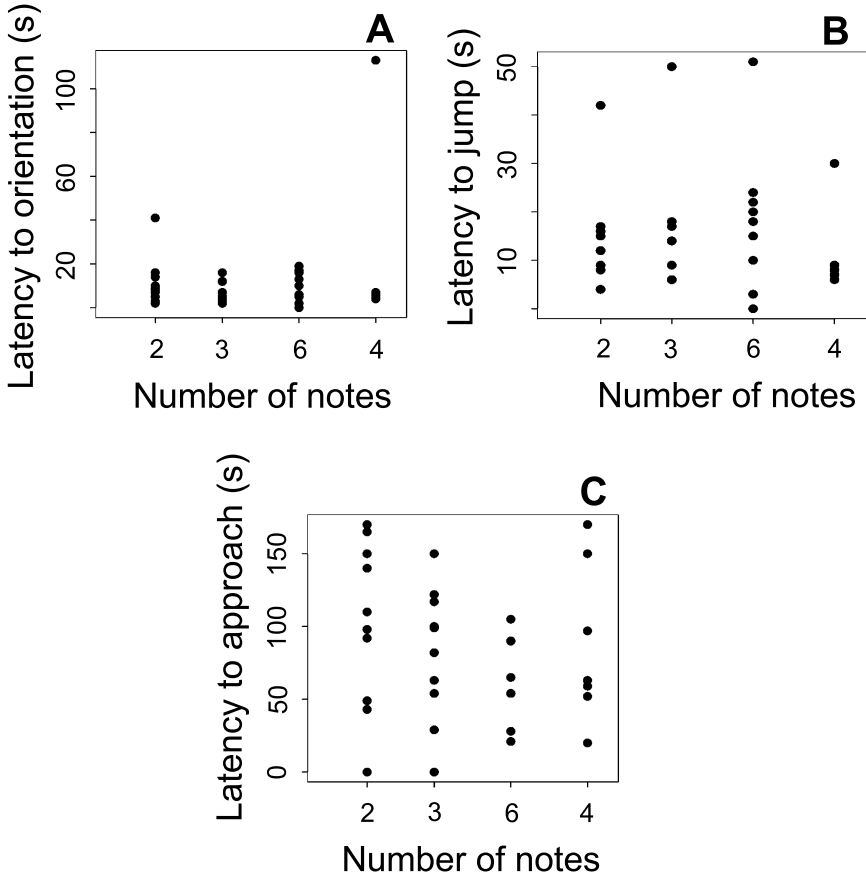
Figure 3. Latency of reaction of *A. femoralis* males to natural conspecific and heterospecific stimuli in field playback experiments at the Ducke field station. (A) Latency to orientation, (B) latency to jump and (C) latency to approach the loudspeakers, toward advertisement calls of 2-note *A. hodli*, 3-note *A. femoralis*, 6-note *A. myersi,* and the control call, 4-note Ducke's *A. femoralis.*

haviour, just calling. 13 resulted in males that did not approach the loud-speakers, and of these, five showed antiphonal behaviour (emission of adver-tisement calls from its own population) in response to the broadcast stimulus, four of them belonged to a 2-note population, and called in response to 2-note ($N = 2$) and 4-note stimuli ($N = 2$). The number of antiphonal calls varied from 35 to 100 during the playback experiments. At Ducke, we per-formed 37 playback experiments, 10 for each type of stimulus and 7 for the control. Four males did not approach the loudspeakers during the 5 min of

stimulus playback (two for 6-note, one for 2-note, and one for 3-note call stimulus), however all these males approached the loudspeakers during the subsequent control period. Only one male was recorded as having antiphonal behaviour; it did not approach the loudspeakers but emitted 56 calls during the 6-note stimulus playback. Another calling male was observed, but it approached the loudspeakers and emitted only five calls during the stimulus (3-note advertisement call) period.

4. Discussion

We found that males of the Amazonian frog *A. femoralis* showed similar phonotactic behaviour toward natural stimuli from conspecific and heterospecifc populations, varying mainly in the number of notes emitted per call. This result suggests that evolutionary rates are different for signal production and male signal perception, because the geographical variation in the advertisement call was not related to a concomitant variation in signal recognition.

The variation in the advertisement calls of *A. femoralis* has been attributed, at least in part, to stochastic processes, although not excluding the hypothesis that selective effects could act on some call traits, generating the remaining call variation (Amézquita et al., 2009). Regarding the evolutionary mechanism underlying call selection in *A. femoralis*, Grether et al. (2009) proposed that the best model is the divergent Agonistic Character Displacement (ACD), based on the sympatry with *Amereega trivittata* (see Amézquita et al., 2006). However, our results for species that are more closely related to *A. femoralis* do not support the ACD hypothesis. The recognition of heterospecific and conspecific calls suggests that the advertisement calls of *A. femoralis* and *A. hodli* have little effect on the male species recognition mechanism. A similar pattern is observed among males of the field cricket *Gryllus texensis*, which courted equally conspecific and heterospecific females in sympatry and allopatry (Gray, 2004). Although females of *G. texensis* and males and females of *G. rubens* preferred conspecifics, again there were no differences between the response pattern in sympatry and allopatry (Gray, 2004). It has been postulated that heterospecific competition in *A. femoralis* is regulated by the competition for acoustic space and oviposition sites (Göd et al, 2007), once the reproductive success in this species is related to the size and ownership of one territory (Roithmair, 1992). At Jirau,

the simpatric species, *A. hodli*, presents a similar ecology and use of space of *A. femoralis*. However, the only contact zone known for this species-group is located at Jirau. At Reserva Ducke, *A. femoralis* males are not sympatric to any closely-related species with similar ecology.

Signal detection and perception can vary according to the receiving gender, because the associated cost of an error in recognition can differ between the sexes (Searcy & Brenowitz, 1988). According to this theory, females would show a more accurate response to signals than would males (Searcy & Brenowitz, 1988; Bernal et al., 2007; but see Espinedo et al., 2010). Our experiments were performed only with males of *A. femoralis*, because this species has a very complex courtship behaviour, where the female can remain in a male's territory for two, and up to four days before mating (Roithmair, 1994). After that, the male guides the female through his territory for at least 1 day before oviposition (Montanarin et al., 2011). In contrast to males, *A. femoralis* females do not exhibit a stereotyped phonotaxis behaviour, restricting the utility of simple playback experiments in tests of sex differences in call recognition. Field observations provide support to the development of experimental designs that are more appropriate for tests of female *A. femoralis* recognition, as well as possible selection between conspecific and heterospecific signals. These questions remain unclear, and deserve more attention in order to establish the relative role of sexual selection in speciation within this species group.

The acoustic environment to which an individual is exposed includes much more than its own species' calls, and the individual must be able to identify conspecific calls, predators, heterospecifics and any potential danger (Dall et al., 2005). The ability to detect and discriminate between distinct environmental sounds can be evolutionarily advantageous (Hödl et al., 2004). A good example is the European smooth newt *Lissotriton vulgaris*, which may access habitat-quality information during migration by the heterospecific calling of the common toad *Bufo bufo* (Pupin et al., 2007). In this sense, the recognition space will be as wide as the amount and variety of sounds found in the local environment, constrained by the species' physiology and neurology.

Although often proposed as a key trait promoting or reinforcing species isolation and diversification in contact zones between related taxa (e.g., Höbel & Gerhardt, 2003; Hoskin et al., 2005; Lemmon, 2009), the role of acoustic signals in impairing the recognition of interspecific individuals as conspecifics may be overestimated. Our results suggest that advertisement call

variation can contribute to the behavioural reproductive isolation between closely related species, without necessarily being reflected in the tuning of aggressive response toward more similar acoustic signals, even when the defense of food and reproductive resources within a male's territory is at stake. Although the stochastic process may be related to call evolution, the pattern of recognition of sound cues by *A. femoralis* and *A. hodli* males did not concord with the call evolution observed within the *A. femoralis* species group. Identifying the precise role of each acoustic trait in the long-range and short-range communication between *A. femoralis* males and, importantly, between males and females, is a key step that will help us to disentangle patterns of advertisement-call evolution from the evolution of call perception, providing valuable insights into the evolution of this communication.

Acknowledgements

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