SHORT COMMUNICATION

The influence of geographic heterogeneity in predation pressure on sexual signal divergence in an Amazonian frog species complex

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Abstract

Sexual selection plays an important role in mating signal divergence, but geographic variation in ecological factors can also contribute to divergent signal evolution. We tested the hypothesis that geographic heterogeneity in predation causes divergent selection on advertisement call complexity within the *Engystomops petersi* (previously *Physalaemus petersi*) frog species complex. We conducted predator phonotaxis experiments at two sites where female choice is consistent with call trait divergence. *Engystomops* at one site produces complex calls, whereas the closely related species at the other site produces simple calls. Bats approached complex calls more than simple calls at both sites, suggesting selection against complex calls. Moreover, bat predation pressure was greater at the site with simple calls, suggesting stronger selection against complex calls and potentially precluding evolution of complex calls at this site. Our results show that geographic variation in predation may play an important role in the evolution and maintenance of mating signal divergence.

Introduction

Signals used to attract mates are some of the most remarkably diverse traits in nature. Closely related species often diverge more in their mating signals than in other morphological, behavioural or genetic traits (Irwin, 2000; Boughman, 2002). Although the theoretical causes of mating signal divergence have been widely discussed (West-Eberhard, 1983; Price, 1999; Schluter, 2001), there is still a need for empirical studies that relate mating signal variation in wild populations to the processes involved in generating and maintaining this variation. Understanding these processes can illuminate how signal diversity is shaped and provide insights into the forces that contribute to reproductive isolation and ultimately speciation.

Mating signal evolution is shaped by interactions between sexual and ecological selective forces (West-

Eberhard, 1983; Boughman, 2002; Cocroft *et al.*, 2010). Several studies show that signal divergence can result from ecological selection on traits that are not directly involved in communication but which influence signal production or reception (Podos, 2001; Nosil *et al.*, 2007). Selection on traits associated with feeding ecology, such as beak morphology, has been shown to indirectly affect signal divergence by modifying morphological features used during sound production (Podos, 2001; Ballentine, 2006). Ecological factors can also directly affect traits involved in communication. Habitat-mediated differences in signal transmission, for instance, generate signal divergence by influencing the detection and optimum tuning of mating signals in local populations (Endler, 1992; Marchetti, 1993; McNett & Cocroft, 2008). Likewise, interspecific interactions, such as predation, parasitism, pollination, mimicry, and competition for signal space, can lead to signal divergence (Hoskin & Higgie, 2010).

Predators and parasites exert selection on mating signals when they exploit the visual, auditory or chemical displays of prey (Zuk & Kolluru, 1998; Haynes &

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Yeargan, 1999). They achieve this by eavesdropping on prey signals (Ryan *et al.*, 1981; Tuttle & Ryan, 1981; Zuk *et al.*, 2006) or by mimicking the signals themselves (Lloyd, 1975; Saul-Gershenz & Millar, 2006). In such cases, female influence on signal evolution is often directly at odds with that of predators because both females and predators generally respond preferentially to signals that are conspicuous in their shared environment (Ryan *et al.*, 1982; Stoddard, 1999; Bernal *et al.*, 2006; Akre *et al.*, 2011). The locally adapted signal will then be the result of trade-offs between selection imposed by female preferences and that imposed by predation pressure. This balance may shift in areas where signallers suffer differing amounts of predation. Given that predator spatial distributions are not uniform and the strength of predation can change over a small geographic range, such interspecies interactions may play an important role in the generation, maintenance or exaggeration of mating signal divergence among populations and species.

Although the idea of geographic variation in predation pressure as an important factor in the divergence of mating signals has persisted in the literature for several decades (Haskins *et al.*, 1961; Endler, 1980), it has rarely been tested empirically (Verrel, 1991). While many studies show a trade-off between conspicuous sexually selected signals and predation pressure (Zuk & Kolluru, 1998; Hedrick, 2000; Rosenthal *et al.*, 2001; Fowler-Finn & Hebets, 2011), few studies outside of poeciliid fish test whether variation in predation pressure is, in fact, associated with variation in these signals (Endler, 1983; Basolo & Wagner, 2004; Zuk *et al.*, 2006). In this study, we examine variation in mating call complexity in a cryptic species complex of Amazonian *Engystomops* toadlets and investigate the role of predation in this call variation.

Toadlets in the *Engystomops petersi* (Jiménez de la Espada 1827, previously known as *Physalaemus petersi*) species complex are nocturnal, pond breeding and found in rainforest throughout the Amazon Basin (Cannatella & Duellman, 1984; Funk *et al.*, 2007, 2008). A recent systematics study found that the two currently recognized species in this complex (*E. petersi* and *E. freibergi*) are actually 5–7 cryptic species (Funk *et al.*, 2012). There is extraordinary divergence in mating calls across the *E. petersi* complex. In two species (C and D), males produce a simple call, which consists of a 'prefix' and a frequency-modulated 'whine'; in one species (A), males produce facultative complex calls, in which the whine can be followed by a harmonic burst, or 'squawk'; and in another species (*E. freibergi*), males from some populations can produce complex calls, whereas males from other populations only produce simple calls (Boul & Ryan, 2004; Boul *et al.*, 2007). Ancestral trait reconstructions suggest that complex calls originated twice in this species complex, once in species A and once in

E. freibergi (Ron, 2008). The spectral properties of whines also vary across the species complex (Guerra & Ron, 2008). In a previous study, Boul *et al.* (2007) showed that the pattern of divergence found in male call types across species was due to selection, rather than drift.

Variation in the strength of sexual selection appears to play a role in call divergence within the *E. petersi* species complex. Females from a species with complex calls (species A) significantly prefer complex over simple calls in two-choice preference experiments, whereas in a species with simple calls (species D), females do not significantly prefer complex calls, although preferences for complex calls were not significantly different between the two species (Boul *et al.*, 2007). Sexual selection, however, is likely not the only factor affecting call divergence in these species. Geographic variation in predator communities may also be important for the pattern of call divergence in the *E. petersi* species complex. In the related túngara frog, *E. pustulosus*, males produce similar complex calls and females prefer complex to simple calls (Ryan, 1980; Gridi-Papp *et al.*, 2006). However, several predators and parasites of *E. pustulosus* 'eavesdrop' on calls to locate their prey (Ryan *et al.*, 1981; Tuttle & Ryan, 1981; Tuttle *et al.*, 1981; Bernal, 2006; Bernal *et al.*, 2006), and among these, bats and blood-sucking flies are also more strongly attracted to complex calls than to simple calls (Ryan *et al.*, 1982; Bernal *et al.*, 2006). In a previous study, bats showed improved localization performance when responding to complex calls in some, though not all, levels of localization task complexity (Page & Ryan, 2008). Variation among *Engystomops* breeding sites in the presence or abundance of predators that are more strongly attracted to complex calls could result in divergence in ecological selection against complex calls and influence the pattern of mating call divergence.

Here, we investigate the role of heterogeneity in predation pressure in the evolution and maintenance of mating signal variation in the *Engystomops petersi* species complex. We used predator phonotaxis experiments to test whether variation in predation pressure is consistent with mating call variation between the two *Engystomops petersi* complex species, A and D, for which the role of sexual selection has already been studied. We used predator phonotaxis as a proxy for predation pressure to examine: (i) whether there was ecological selection against complex calls in these species due to predation and (ii) whether ecological selection against complex calls was greater at the site with the species that produces only simple calls than at the site with the species that produces complex calls. We discuss our results in light of previous studies of sexual selection in these species and explore the interactions between ecological and sexual selection on mating signals at these sites.

## Materials and methods

Study sites and species

We conducted predator phonotaxis experiments in two neighbouring locations of the Ecuadorian Amazon where female choice for complex versus simple calls had already been assessed: the Yasuní Scientific Station in Yasuní National Park (cryptic species A, males produce complex calls, females prefer complex calls) and La Selva Lodge (cryptic species D, males produce simple calls, females show no preference), an ecotourism lodge 21 km north of Yasuní, across the Napo River (Boul *et al.*, 2007). Species A and D are sister species (Funk *et al.*, 2012), and ancestral trait reconstruction suggests that species A gained complex calls since the divergence of these two species (Ron, 2008).

Field methods

Between February 2009 and December 2010, we conducted predator phonotaxis experiments to determine which predators were attracted to mating calls, the frequency of visits to speakers and whether predators were more strongly attracted to complex over simple calls. Predators such as bats (Tuttle & Ryan, 1981), caimans (Bernal, 2006), opossums (Tuttle *et al.*, 1981) and *Corethrella* flies (Bernal *et al.*, 2006) approach speakers playing frog calls, and this behaviour can be exploited to quantify predator attraction to different calls.

For each location, we randomly selected one of six different positions around the perimeters of ponds with known breeding choruses and played complex and simple calls from speakers on non-rainy nights. Each night, we set up two Sony Portable Speakers (SRS-M30; frequency response = 250–20 000 Hz; Sony, New York, NY, USA) facing each other, 3 m apart. Each speaker was mounted to a 25 × 20 cm black wooden platform with an *Engystomops* model 5 cm in front of the speaker (Fig. 1). We played complex and simple calls alternately from either speaker at an amplitude of 82 dB SPL (re. 20 μP, measured at 1 m) and a rate of one call every 2 s. Call rate and amplitude were similar to those in nature (Boul *et al.*, 2007). Each night, we switched the locations of the speakers playing complex and simple calls.

We used 40 simple and 40 complex call stimuli for each site. Simple calls were natural calls recorded from the local species, and complex calls were generated by appending one of 10 squawks from Yasuní to the end of simple calls (Boul *et al.*, 2007). All complex calls, including those for Yasuní (site with species with complex calls), were generated using this method to standardize the complex call stimulus. Each series of 40 calls was repeated for a total of 2 h. Two infrared digital video cameras were positioned 0.5 and 1 m away from each speaker and focused on the platform to film incoming
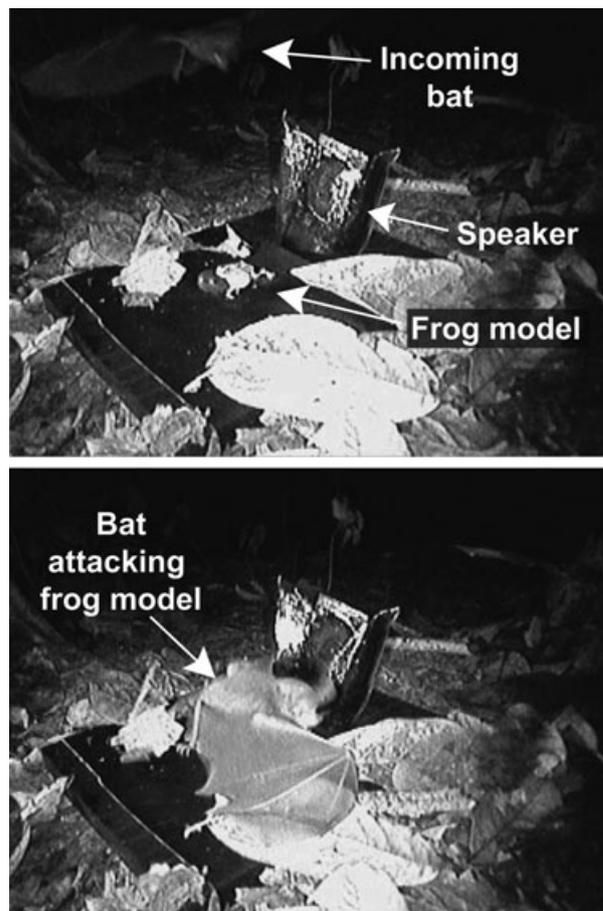
Fig. 1 Fringe-lipped bat (*Trachops cirrhosus*) approaching the speaker and frog model (upper panel) and attacking the model 0.2 s later (lower) in predator phonotaxis experiment.

predators at distances up to ~150 cm from separate angles. Throughout the night, we removed frogs attracted to the speakers. Videos were subsequently analysed blind to call treatment to record the number of bat visits to each call type. Bats entering the field of view of each speaker's cameras were recorded as visiting that call. Because it was not possible to identify individual bats, a single bat may have been attracted to the speaker multiple times. Nonetheless, as we were testing whether there was greater predation pressure against complex calls than simple calls, we focused on the nightly difference between visits to each call type, and this relative difference should be maintained even if multiple visitations increase the absolute number of attacks recorded.

Statistical analyses

To determine whether there was selection against complex calls due to bat predation, we first examined relative predation rates on complex versus simple calls at Yasuní and La Selva separately. For each site, we tested

whether the number of bat visits to complex calls minus the number of visits to simple calls per night (Δ_{c-s}) was significantly greater than zero using a one-sample sign test. For this analysis, we only included nights when at least one bat visited a speaker because our objective was to determine whether bats were attracted to complex more than simple calls; thus, nights without bats were not relevant for this question.

Next, to examine whether there was greater overall predation pressure at La Selva than at Yasuní, we calculated the total number of nights in which at least one bat visited a speaker and the number of nights when no bats came. We then tested for differences between La Selva and Yasuní in the proportion of nights that bats visited speakers using a Fisher's exact test.

Finally, to examine whether overall selection against complex calls caused by bat predation was greater at La Selva than at Yasuní, we tested whether Δ_{c-s} values across all nights were greater at La Selva than at Yasuní using a Mann–Whitney U-test. For this analysis, we included all nights, whether or not bats visited speakers (where $\Delta_{c-s} = 0$ for nights without bats), because our goal was to test for differences between the sites in overall selection against complex vs. simple calls across the entire study period. Given the specific directionality of our hypotheses (increased predator attraction to complex over simple calls and higher predation pressure against complex calls in simple calling species), we report one-tailed probabilities for all tests.

Results

Predation phonotaxis experiments were highly successful at attracting predators to the speakers. The most common predators were bats. These were likely to be *Trachops cirrhosus* (Spix, 1823), identified in some videos by their large ears and wing pattern (Fig. 1). Bats approached the speakers first, but if they landed on the platform, they landed close to or on the model, as opposed to the speaker, suggesting that bats recognized the model as prey. At La Selva (species D with simple calls), bats approached the speakers on 20 of 34 nights (59%), and at Yasuní (species A with complex calls), bats approached the speaker on 21 of 75 nights (28%). Other less common visitors to the speakers were snakes (three nights in La Selva and three nights in Yasuni) and spiders (two nights in La Selva and one night in Yasuni). We focus on bat predation, as bats were the dominant predator approaching speakers at both sites. We found no evidence for habituation to our playbacks across nights at either location.

Analysis of the phonotaxis videos indicated ecological selection via bat predation against complex calls at La Selva, where bats visited complex calls significantly more than simple calls (one-sample sign test, $N = 20$ nights with bats, $P = 0.007$; Fig. 2). Bats at Yasuní also
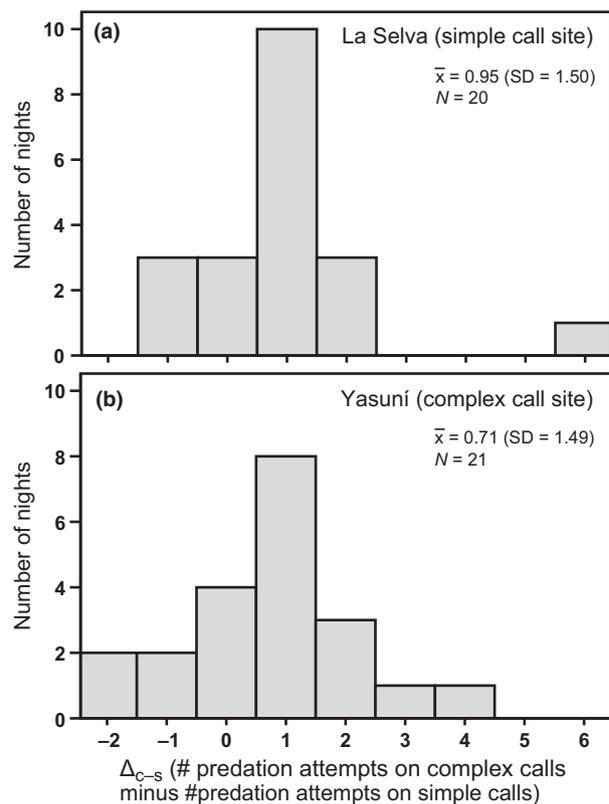

Fig. 2 Frequency histograms of the number of predation attempts on complex calls minus the number of attempts on simple calls (Δ_{c-s}). Larger values of Δ_{c-s} indicate greater predation pressure on complex vs. simple calls.

visited complex calls significantly more than simple calls ($N = 21$, $P = 0.025$; Fig. 2).

In addition, bat predation against complex calls was greater at La Selva (species D, with simple calls) than at Yasuní (species A, with complex calls). There were significantly more nights with bat visits at La Selva than at Yasuní (Fisher's exact test, $P = 0.002$; Fig. 3). The Δ_{c-s} values were also greater at La Selva than at Yasuní (Mann–Whitney U-test, $U = 1031$, $Z = -1.95$, $P = 0.025$), indicating that overall ecological selection against complex calls due to bat predation was greater at La Selva than at Yasuní across the entire study period.

Discussion

Our phonotaxis experiments show significantly higher predator visitation rates to complex calls than to simple calls in La Selva and Yasuní. This is consistent with field measures of bat visitation in the túngara frog (Ryan *et al.*, 1982). We also found that both the proportion of nights when bats visited speakers and the overall predation pressure against complex calls were greater at La Selva than at Yasuní. Together, these results indicate that: (i) there is ecological selection, via
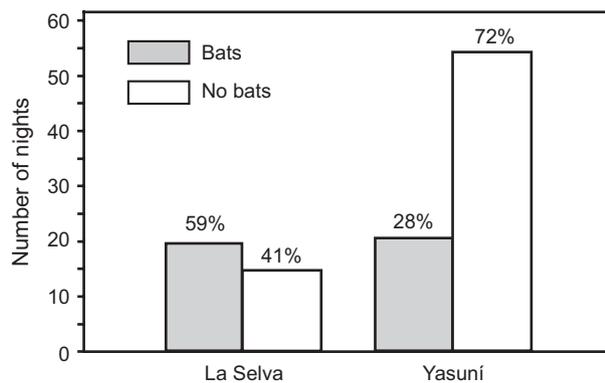
Fig. 3 Number of nights that bats visited or did not visit speakers at La Selva (site with simple calls, $N = 34$) and Yasuní (site with complex calls, $N = 75$).

bat predation, against complex calls in both species and (ii) the strength of ecological selection against complex calls differs between the two sites as predicted: stronger selection against complex calls occurs at the site where males of *E. petersi* species D only produce simple calls.

These results are consistent with heterogeneity in predation playing a role in signal divergence. For example, high predation pressure at La Selva may prevent the evolution of complex calls in species D, whereas the relatively low predation pressure at Yasuní has allowed the evolution of complex calls in species A. Alternatively, signals may have diverged between these two species elsewhere, but the high risk of predation at La Selva has prevented species with complex calls from colonizing this location. Regardless of the mechanism for the initial gain of the squawk at Yasuní, complex calls have evolved multiple times in *Engystomops* (Ron, 2008), and heterogeneity in predation pressure has the capacity to enhance and maintain variation in signal complexity.

Many females throughout the *Engystomops* genus (both within the *E. petersi* complex and more broadly) prefer complex calls (Ryan, 1980, 1985; Ryan & Rand, 2003; Ron, 2008). Our results provide a plausible explanation as to why some species have simple calls despite common sexual selection for complex calls. High and sustained levels of bat predation may select against complex calls in certain locations. Thus, as predation intensity varies, we expect the balance between ecological and sexual selection to shift, such that at locations with lower predation, sexual selection predominates, favouring conspicuous, complex signals, and in locations with higher predation, ecological selection predominates favouring less conspicuous signals (Endler, 1983; Basolo & Wagner, 2004).

Moreover, in locations with high predation, we might expect a positive feedback between ecological and sexual selection, because female preference for complex calls may decrease or may not evolve if females incur

an increased predation risk by approaching complex calling males or there is an increased risk of predation to male offspring that produce complex calls (Stoner & Breden, 1988; van Dorn *et al.*, 2009). In guppies, differences in female preferences between sites can be influenced by predation risk. Studies show that, although females from high predation localities can still choose conspicuous males in binary choice experiments (Evans *et al.*, 2004), the strength of female preference for conspicuous coloration is reduced (Stoner & Breden, 1988; Houde & Endler, 1990; Endler & Houde, 1995). We find a similar relationship in the *Engystomops* species complex, where there is weaker predation and a trend for stronger female preference for complex calls at Yasuní and stronger predation and a trend for weaker female preference for complex calls at La Selva (Boul *et al.*, 2007)

The geographic variation in predation pressure found in this study is likely to be common across many species or species groups that vary in mating signals. Given that abundance of many taxa can change over small spatial scales (Tuomisto *et al.*, 1995; DeVries & Walla, 2001; Doan & Arizábal, 2002), it is not unexpected for predator abundance to differ between neighbouring sites. Many studies have focused on the effects of sexual selection on mating signal divergence, but less is known about the importance of other selective processes, such as predation, in the evolution and maintenance of mating trait divergence (Verrel, 1991; Hoskin & Higgie, 2010). Aside from the well-known examples of predation on guppies (Endler, 1980, 1983, 1995), there are few studies documenting the influence of geographic variation in predation on mating signal divergence. Nevertheless, several examples suggest that this process may be widespread. In a *Xenotoca* fish species, males from populations where predatory snakes are abundant show less conspicuous coloration than those of populations with low predation (Moyaho *et al.*, 2004). It has also been suggested that electric fish with cryptic signals are more likely to occur in locations with high predation (Stoddard, 1999). In fireflies, Lewis & Cratsley (2008) propose that Old World species have evolved in the absence of specialist predators, allowing for more conspicuous flashing signals than those of New World species.

Understanding the processes that generate mating signal divergence lies at the core of speciation research. Geographic variation in predation can generate direct selection on mating traits, which could drive rapid speciation (Verrel, 1991). This direct selection and rapid speciation could generate cryptic species that differ little apart from mating traits, as is the case of the *E. petersi* complex (Funk *et al.*, 2012). Our results suggest that environmental heterogeneity in predation may be an important mechanism shifting the balance between the forces of ecological and sexual selection, thus contributing to the origin and maintenance of signal divergence. Moreover, our study gives us insight into effects of predation pressure on variation in signal elaboration and

signal complexity. Future studies should focus on the quantification of variation in predation pressure across populations or closely related species that differ in mating signals in a greater range of taxa. If predation pressure is indeed contributing to the evolution of mating signal elaboration, then geographic variation in predation pressure should be generally associated with variation in signal complexity.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Video S1 Infrared video of a bat visiting a playback speaker in the field, shown from two camera angles. The bat attacks the frog model sitting in front of the speaker, rather than attacking the speaker itself. Video is shown at one half natural speed.

Data deposited at Dryad: doi:10.5061/dryad.5cv40