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Original Article

Variation in the effect of repeated intrusions on calling behavior in a territorial toadlet

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Territorial animals must optimize their investment toward exploiting their territory and defending it from competitors. However, the manner in which territorial investment changes in response to repeated interactions with a rival remains unclear. We experimentally exposed nest-defending terrestrial toadlets (*Pseudophryne bibronii*) to playback that simulated an intruder that called and retreated 6 times. We measured the change in a resident's investment toward calls that function to advertise their presence to mates (territory exploitation) or threaten competitors (territory defense) over successive interactions. We found significant independent effects of repeated interactions on each call type, but no trade-off between them. Aggressive call response was initially high, decreasing significantly after the first bout. In contrast, advertisement calls increased linearly over successive bouts. However, the magnitude and direction of changes over time were not consistent among individuals. Instead, individuals appeared to change their calling behavior according to their initial response. Furthermore, there was a decrease in the variability of responses over successive interactions. Together, these results suggest that residents independently optimize their exploitative and defensive behaviors in response to intruders as they gain information over successive interactions. This process will be important to consider when there is variation in the number of times a given intruder will be encountered. *Key words:* anuran calling, familiarity hypothesis, learning, relative threat hypothesis, repeat intruders, territoriality. *[Behav Ecol 23:93–100 (2012)]*

INTRODUCTION

Intruder pressure from nonterritorial individuals (floaters) is arguably one of the most important factors influencing the behavior of a territorial resident (e.g., Brown 1964; Boutin and Schweiger 1988; Chapman and Kramer 1996; Jabłoński 1996; Manteuffel and Eiblmaier 2008). Many models of territoriality assume that intrusion attempts are independent of one another and that residents simply respond to the absolute level of intrusion (for a review and criticism, see Adams 2001). However, residents can face the same intruder on multiple occasions, and it is becoming increasingly apparent that this has important implications for how the resident should respond because both the resident and the intruder have the opportunity to gain information about each other (Sih and Mateo 2001; Morrell and Kokko 2003, 2005). For instance, residents may reduce future intrusion pressure by attacking, and thus "teaching," intruders to avoid their territory (Switzer et al. 2001). Additionally, residents may learn about the likelihood of future intrusions and base decisions regarding space use on this information (Stamps and Krishnan 1999, 2001; Díaz-Uriarte 2001). Floaters, on the other hand, can learn about the likelihood of being met with aggression as well as signal their intentions to settle in an area by repeatedly returning to it in a process analogous to a war of attrition (Stutchbury 1991; Stamps and Krishnan 1999, 2001). Finally, individuals can learn about each other's fighting abilities the more they interact (Enquist et al. 1990; Leiser et al. 2004; Kemp et al. 2006). Information gained during agonistic contests may thus be important for a range of territorial behaviors (e.g., patrolling, advertising, foraging, space use, and aggressive display) beyond deciding when to withdraw from a contest. For example, experiments conducted on birds have demonstrated that simulated intrusions can influence an individual's advertisement of territory ownership hours after the intrusion and aggressive behavior during later intrusions (Amrhein and Erne 2006; Schmidt et al. 2007).

The territorial behaviors that may be affected by repeat intrusions can be broadly classified as being either exploitative (i.e., deriving fitness benefits from territory ownership through mating, predator avoidance, or foraging) or defensive (i.e., minimizing resource loss to intruders). A common assumption is that there is a trade-off between the two (Schoener 1987; Ydenberg and Krebs 1987; Morrell 2004). Theoretically, residents that focus on exploiting their territory may be more vulnerable to having their resources stolen (Díaz-Uriarte 2001; Switzer et al. 2001; Hinsch and Komdeur 2010). On the other hand, residents that invest excessive time and energy in defense may incur the cost of missed opportunities for exploiting their own space (Schoener 1987). Therefore, we expect that a resident's response to repeated intrusions will represent shifts along a continuum between exploitative and defensive behaviors. For example, great tits (*Parus major*) exposed to an intruder reduced their foraging efforts to spend more time being vigilant, whereas those that were not exposed foraged at closer to maximum efficiency (Kacelnik et al. 1981; Ydenberg and Krebs 1987).

A resident's familiarity with an intruder increases with repeated interactions and can be an important determinant of

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its defensive behavior (e.g., Wich and Sterck 2007; Briefer et al. 2008; Rosell et al. 2008; delBarco-Trillo et al. 2009). Individuals that are more familiar with one another are expected to be less aggressive because they have accurate information on the costs and consequences of interaction and are hence less motivated to engage in costly fights (the familiarity hypothesis: Ydenberg et al. 1988; Getty 1989). However, an intruder's familiarity can also indicate the threat it poses to a resident's resources (i.e., likelihood of stealing resources or taking over the entire territory). Consequently, residents can be less, or more, aggressive toward familiar intruders than unfamiliar intruders, depending on which is more threatening (the relative threat hypothesis; Temeles 1994). Overall, residents are expected to reduce their defensive behaviors toward an intruder as it becomes more familiar (the dear enemy phenomenon), except in cases where the intruder is sufficiently threatening, in which case the resident will increase its defensive behaviors (Temeles 1990; Müller and Manser 2007; Schradin et al. 2010). Although the relationship between familiarity and defensive behavior is well studied, our understanding of how exploitative behaviors are affected is limited. This understanding does not generally extend beyond the expectation of a negative correlation between exploitative and defensive behaviors.

Anuran amphibians (frogs and toads) have provided an excellent model system for exploring changes in exploitative and defensive behaviors in response to intruder pressure (Rose and Brenowitz 1997; Owen and Perrill 1998; Brenowitz and Rose 1999; Bee 2003a; Marshall et al. 2003; Humfeld et al. 2009). This is largely because anuran calling behavior reflects investment toward these competing interests. Typically, males form dense breeding aggregations and defend patches of space required by females for oviposition. They advertise their presence and quality to females using acoustic signals (advertisement calls), which can be considered as investment toward exploiting their territory through breeding. In this regard, residents must maintain a sufficient rate of advertisement calling in order to offset any reproductive interference imposed by nearby callers (Telford 1985; Schwartz and Gerhardt 1989; Dyson and Passmore 1992; Burmeister et al. 1999). Additionally, males benefit from maintaining an exclusive space surrounding their calling site, and males of many species use an aggressive call to deter rivals from settling too close (Wells 1977, 2007; Gerhardt and Huber 2002). Thus, aggressive calling generally reflects investment toward territory defense. These call types are also expected to be involved in a trade-off because females prefer males that give a high rate of advertisement calls, the production of which is impaired by giving aggressive calls (Taigen and Wells 1985; Schwartz et al. 1995; Rose and Brenowitz 1997; Brenowitz and Rose 1999; Marshall et al. 2003). Frogs have also been shown to respond to familiarity and persistent exposure to an unfamiliar intruder. Although by no means the rule (Bee 2003b), frogs in a chorus often exhibit the dear enemy phenomenon, whereby residents respond less aggressively to the calls of a familiar rival than an unfamiliar rival (e.g., Owen and Perrill 1998; Lesbarrères and Lodé 2002; Feng et al. 2009) or become less aggressive toward an unfamiliar intruder with continued exposure (e.g., Rose and Brenowitz 1997; Bee 2003a; Humfeld et al. 2009). This is thought to benefit residents by allowing them to focus their efforts on attracting females rather than on spacing conflicts with males (Brenowitz and Rose 1999).

The aim of this study was to determine how exploitative and defensive behaviors vary in response to repeated intrusions. This was achieved by conducting a playback experiment on the terrestrial brown toadlet, *Pseudophryne bibronii*. We exposed resident males to a speaker that played prerecorded calls presented at intermittent intervals to simulate repeated intrusions by an unfamiliar male. The response of the resident was gauged by the number of calls given in reaction to each simulated intrusion. We expected that aggressive calling would decrease over successive intrusions because the simulated intruder was not a large threat (Temeles 1994). Similarly, we expected that advertisement calling would increase in order for the resident to remain attractive in the face of a new competitor (Burmeister et al. 1999). We also tested whether there was inconsistency among individuals in the direction of change in order to determine if all individuals respond in the same manner. We predicted that any variation in the direction of change for individual responses would be consistent with the relative threat and familiarity hypotheses (Temeles 1994). Finally, we tested the hypothesis that shifts in calling behavior reflect a trade-off between exploitative and defensive behaviors (Schoener 1987; Ydenberg and Krebs 1987; Morrell 2004).

MATERIALS AND METHODS

Study organism and field site

The brown toadlet, *P. bibronii*, is a small (22–36 mm Snout-Vent Length), terrestrial breeding, Myobatrachid frog endemic to temperate regions of southeastern Australia (Tyler and Knight 2009). During the prolonged 4–5 month breeding season, males aggregate along dry creek lines and drainage pans that seasonally inundate. Males construct shallow burrows (nest sites) in moist soil and use advertisement calls to attract females, who deposit their eggs at the call site (Pengilley 1971; Woodruff 1976; Mitchell 2001). Males are territorial and defend the nest site using a distinct aggressive call that is longer and more pulsatile than the advertisement call (Byrne 2008; Figure 1). Most territorial disputes are resolved acoustically, and physical interactions between males are rarely observed (PB, unpublished data). Critically, this means that acoustic investment provides a reliable indicator of exploitation and defense of the nest site. The study was conducted on 2 choruses within a population in remnant *Eucalyptus*, *Banksia*, and *Casurina* woodland in Jervis Bay National Park, on the southeast coast of New South Wales (NSW) in Australia. All work was conducted between 4 May and 20 May 2010.

Experimental design and procedure

Acoustic playback devices were used to simulate the presence of a novel male that repeatedly calls and retreats from a location within the exclusive space surrounding a focal resident (stimulus callers; Figure 2). Playback experiments were conducted in 2 choruses, less than a kilometer apart. Resident males were located by triangulating on their advertisement calls. A random male was chosen to be a subject from within a subset of residents that had not been within 5 m of a stimulus caller for at least 2 days and that had not been previously used as a subject. A playback trial was conducted by placing an mp3 player (SanDisk Sansa Shaker and attached Cygnett Micro 2 × 2 W speaker), loaded with 1 of 4 stimulus call recordings chosen at random, within 100 cm of a subject in space unoccupied by any current neighbor. We ensured that the stimulus caller used was not based on a recording from any individuals within 5 m of the subject to remove any effect of prior familiarity.

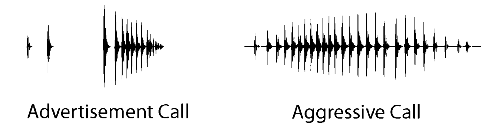

Figure 1
Advertisement and aggressive call types used by *Pseudophryne bibronii* can be distinguished based on their waveform.

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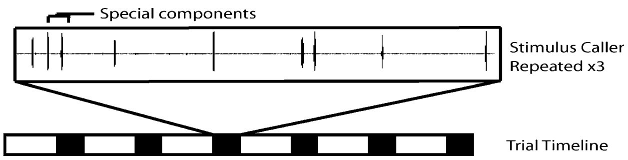

Figure 2
Playback scheme. Each trial lasted for 60 min. White periods represent a 7-min silent period. Black periods represent a 3-min bout period with 1 of 4 stimulus callers (only 1 stimulus caller is presented here as an example). The audio track that comprises a stimulus caller lasts for 1 min but is repeated to produce a 3-min bout. Two special components, comprising either aggressive calls or grouped advertisement calls, are included in the stimulus caller in order to stimulate the resident to respond.

Multiple stimulus callers were used in order to generalize the results across a range of rival identities and calling patterns rather than a specific individual or calling pattern. Playbacks of stimulus calls were conducted at a volume of 60–70 dB sound pressure level (SPL; sound pressure relative to 20 μPa Root Mean Square) at the location of the resident's nest. This value approximates the SPL of a caller located 0.5–2.5 m from the resident (SH, unpublished data), which is consistent with observed distances between natural neighbors and not within the range a resident considers to be a large threat (residents produce a third type of call, which was not recorded, during contests over nest-site ownership). Trials were conducted during the first half of the night (1900–2300 h). We recorded the resident and its interactions with the stimulus caller for a 1-h period using a Maranz PMD660 recorder and Rode NTG-2 directional microphone. Experimenters were not present during trials. Overall, subjects were exposed to six 3-min long exposures to a stimulus caller (a bout period), separated by 7 min of silence (silent period) to simulate the withdrawal of the intruder. Additionally, each trial began with a 7-min silent period before the first bout period to act as a baseline measurement (Figure 2). Seven minutes of silence is a relatively long break from calling in *P. bibronii* compared with the mean call rate of 7.2 ± 4.1 calls/min observed during baseline recordings. A silent period of this duration is thus likely to be representative of the simulated intruder withdrawing from the interaction. Furthermore, a 7-min break may give the resident ample time to readjust its calling behavior to account for the simulated intruder's most recent withdrawal.

Because the calling behavior of anurans is temperature dependent (Oseen and Wassersug 2002; Saenz et al. 2006; Canavera et al. 2008; Navas et al. 2008; Steelman and Dorcas 2010), we measured ambient air temperature at the beginning and end of each trial and calculated the mean. We also noted the number of days since it last rained and the start time for each trial because both the moisture and the time of day are important determinants of anuran calling behavior (Mitchell 2001; Oseen and Wassersug 2002; Saenz et al. 2006; Canavera et al. 2008; Steelman and Dorcas 2010). Following the trial, we measured the volume of 3 of the speaker's calls from directly above the subject's nest, recording the greatest of these values. In 11 cases, we were able to capture the resident male and measure his weight. These values can be used to determine how threatening the speaker was in relation to the resident because speaker volume correlates with the perceived distance of the intruder, and a male's weight correlates with its attractiveness and energy stores. In total, we conducted 21 playback trials, 17 from one chorus and 4 from the other. The experiment was conducted according to the guidelines on animal experimentation produced by the Association for the Study of Animal Behaviour and the Animal Behaviour Society. All of our activities were approved by the Monash University Animal Ethics Committee (permit number BSCI/2007/14) and the NSW National Parks and Wildlife Service (permit number S12552).

Stimulus calls

To create the stimulus calls, 4 independent nest-holding males were recorded for 3 min each. Recordings were made using a Maranz PMD660 portable solid-state recorder and a Rode NTG-2 directional microphone. Recordings were made in mp3 mono format at a sampling rate of 44 kHz and 16 bit (CD quality). Each recording was then edited using the software program Audacity v1.3.5 [cited 2011 July 9]. (http://audacity.sourceforge.net/) to create 4 unique, but similar, stimulus calls. This was done by cutting 9 calls from each recording. Nine advertisement calls were cut from 3 of the recordings, whereas 7 advertisement and 2 aggressive calls were cut from the remaining recording. These calls were then arranged into a 1 min timeline and normalized to 100% amplitude. Calls from different individuals were not mixed together in the same track, so that each track represented a different individual. Each track began with a call being made in the first second and had 2 "stimulating components", as pilot studies indicated that residents often ignored stimuli that did not contain at least one slightly aggressive signal. For one of the stimulus callers, 2 aggressive calls were used as the stimulating component. For the other 3 stimulus callers, 2 advertisement calls given within 1.5 s of each other were used, as grouped calls of this nature appear to stimulate aggression. The remaining 6 calls were distributed randomly throughout the 1 min timeline so as to generalize results across a range of calling patterns and to limit the possibility for residents to be responding to some quirk in calling pattern (for instance, a specific arrangement of calls may act as some kind of signal, which could confound results). The interval between calls for individual residents and stimulus during competitive interactions was not significantly different in terms of mean (residents: 6.30s ± 2.71; stimulus callers: 6.69s ± 0.1; $t_{20.3} = 0.65$, $P = 0.522$) or standard deviation (SD) (residents: 4.68s ± 2.67; stimulus callers: 4.11s ± 0.50; $t_{22.9} = 0.91$, $P = 0.373$). This sequence was made to repeat 3 times, giving a 3-min period of calling (Figure 2). Seven minutes of silence was inserted before the calling period. Each 10-min sequence was loaded onto separate portable mp3 players (SanDisk Sansa Shaker) for use in playback trials. The call rate (9/min), quality (i.e., duration and number of pulses), and type (number of advertisement and aggressive calls) of calls were chosen to reflect slightly below average individuals, based on results reported by Byrne (2008). This was done in order to reduce the threat posed by the intruder to a resident, reducing the risk of the resident abandoning its nest or calling activity.

Statistical analysis

Resident responses

The number of aggressive and advertisement calls given in each 3-min bout period (exposure count) and in each of the 3 min periods immediately preceding a bout (preceding count) were counted from the waveform of the recordings using SoundRuler v0.9.6.0 [cited 2011 July 9]. (http://soundruler.sourceforge.net/main/). Advertisement and aggressive calls were treated separately. We calculated exposure count minus preceding count for each of the 6 bout periods to give values that represented the changes in advertisement and aggressive calling behavior attributable to the stimulus call (these values were termed the call responses). In other words, the call response variables reflect the magnitude (i.e., the difference in call number between the exposure and preceding periods) and direction (i.e., whether this change was positive or

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negative) of the resident's response to the stimulus caller. The call response variables also control for inter- and intraindividual changes in calling behavior (such as those attributable to individual quality, responses to other neighbors and time). These values were divided by the number of minutes in a bout (3) to give the response in units of mean calls/minute.

Do resident calls change over successive interactions?

We used a mixed-model repeated measures analysis of variance (ANOVA) on each call response variable according to the guidelines of Littell et al. (1996) and Logan (2010), using SAS 9.2 (SAS Institute, [cited 2011 July 9]. http://www.sas.com/). For analysis, the stimulus caller, bout period, and their interaction were the fixed factors, with bout period being the repeated measure within each individual toadlet (the blocking factor). Additionally, we included temperature, days without rain, speaker volume, and the starting time of the trial as covariates. We used a restricted maximum likelihood estimation method with Kenward–Roger fixed effects and degrees of freedom approximation. We determined the covariance structure (out of unstructured, correlated symmetry, Toeplitz, and first-order auto correlative) for each model based on the lowest corrected Akaike Information Criterion (AIC). This method resulted in a Toeplitz structure being chosen for the advertisement call response and a first-order autocorrelation structure for the aggressive response. We used planned comparisons to investigate the nature of any differences in the mean call responses at each bout period. For advertisement calls, we used polynomial contrasts (i.e., testing for linear, quadratic, etc., relationships over successive interactions). For aggressive calls, we compared each bout period with the following bout period (i.e., 1 vs. 2, 2 vs. 3 … 5 vs. 6).

Is there individual variation in the direction of change in call response?

To explore variation in the direction of responses throughout the population, we used a Tukey's test of additivity to determine if there was an interaction between individual toadlets and the effect of bout period (Logan 2010). Additionally, we subtracted each individual's first call response from its last call response (for both advertisement and aggressive calls) as a measure of the direction and magnitude of their ultimate change in behavior (termed delta response indices). To determine if there were any predictors for an individual's change in response or initial reactions to the speaker, we tested for any correlations between delta response indices, call responses in the first bout, temperature, days without rain, resident mass, speaker volume, and trial start time. We also used an ANOVA to test whether the stimulus caller used affected delta response indices or initial call responses. Finally, to determine if responses became more or less similar over successive bouts, we tested for a correlation between the SD of responses and bout period. Note that there is no assumption regarding homogeneity of variance for the mixed model ANOVA, as the covariance structure is able to compensate for a relationship between mean and variance (Littell et al. 2006; Myers et al. 2010). Thus, a correlation between variability and bout period does not invalidate any of the results. All of these tests were conducted using R 2.12.0 (R Project contributors, [cited 2011 July 9]. http://www.r-project.org/).

Is there a trade-off between advertisement and aggressive calls?

To determine whether there was a trade-off between advertisement and aggressive calls, we tested for a negative correlation between advertisement and aggressive call responses at each bout period and for the call responses given by an individual averaged across all 6 bout periods. Additionally, we tested for a correlation between advertisement and aggressive delta response indices. This analysis was conducted using R 2.12.0.

RESULTS

Do resident calls change over successive interactions?

Residents changed their advertisement and aggressive call rates in response to simulated intrusions by significantly different amounts across successive intrusion attempts (Table 1; Figure 3). Planned contrasts indicated that there was a significant linear increase in the mean advertisement call response over successive intrusion attempts ($F_{1,18.8} = 13.96$, $P = 0.001$). In contrast, the mean rate of aggressive calls given in response to an intruder declined after the first bout period ($F_{1,76.7} = 8.96$, $P = 0.004$) and thereafter remained steady (P range = 0.273–0.844). The stimulus call used had no significant effect on the results nor did temperature, speaker volume, time and days since rain (Table 1).

Is there individual variation in the direction of change in call response?

Individuals that substantially increased their call rate when first encountering the intruder gave comparatively lower shifts in call rate during the ultimate bout. Similarly, individuals that reduced their calling effort in response to the first intrusion gave greater call responses in the ultimate bout compared with their initial response. This pattern is described by a significant correlation between initial call responses and delta response indices for both advertisement and aggressive call responses (Table 2; Figure 4). This correlation also shows that the amount an individual changed its calling behavior depended on the magnitude of its initial response, so individuals that showed the most extreme initial responses also showed the most extreme changes. The nature of this variation appears to have led call responses across the population to become more similar over time, as indicated by significant negative decreases in the SDs of advertisement ($F_{1,4} = 12.85$, $P = 0.023$, $r^2 = 0.70$) and aggressive ($F_{1,4} = 18.46$, $P = 0.013$, $r^2 = 0.78$) call responses over successive bout periods (Figure 3). No variables other than initial call responses were found to affect delta response indices, as there were no significant correlations with any covariate (Table 2) and the stimulus call used had no significant effect (advertisement calls: $F_{3,17} = 0.73$, $P = 0.548$; aggressive calls: $F_{3,17} = 0.81$, $P = 0.508$). Despite the influence of initial call response, neither resident mass, speaker volume nor environmental variables (temperature, days since rain, and trial start time) could explain how it varied (Table 3). Furthermore, there were no significant differences in initial call response attributable to the stimulus caller used

Table 1

Mixed-model ANOVA testing for differences in advertisement and aggressive call responses (change in call rate in response to intrusion) over successive intrusions

Call response	Factor	F_{df}	P
Advertisement	Stimulus call	$1.96_{3,13}$	0.170
	Bout period	$4.24_{5,36.6}$	0.004
	Call × Bout period	$1.16_{15,47.9}$	0.333
	Speaker volume	$0.40_{1,13}$	0.537
	Start time	$1.31_{1,13}$	0.273
	Temperature	$1.46_{1,13}$	0.249
	Days without rain	$0.79_{1,13}$	0.390
Aggressive	Stimulus call	$0.90_{3,17.8}$	0.461
	Bout period	$2.34_{5,79}$	0.049
	Call × Bout period	$1.01_{15,80.2}$	0.458
	Speaker volume	$1.13_{1,18.5}$	0.301
	Start time	$0.86_{1,18.5}$	0.367
	Temperature	$3.66_{1,18.5}$	0.071
	Days without rain	$1.35_{1,18.5}$	0.261

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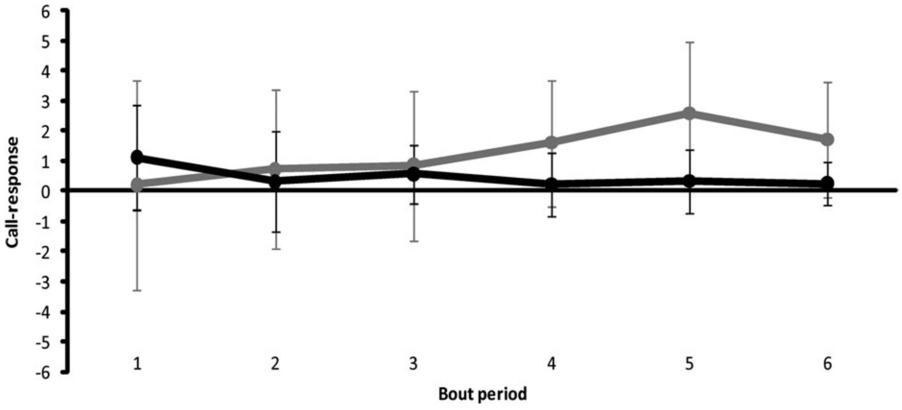

Figure 3
The relationship between unadjusted mean changes in call rate due to intrusion (call response; calls/minute ± SD) and successive intrusion instances (bout period). Advertisement calls are given by gray circles and the gray line; aggressive calls are given by black circles and the black line.

(advertisement calls: $F_{3,17} = 2.36$, $P = 0.108$; aggressive calls: $F_{3,17} = 0.27$, $P = 0.846$). Thus, although residents increased their advertisement call response and decreased their aggressive call response on average (Figure 3), there was significant variation in these directions between individuals for both advertisement (Tukey's test of additivity: $t = 2.87$, $P = 0.004$) and aggressive ($t = 5.21$, $P < 0.001$) call responses.

Is there a trade-off between advertisement and aggressive calls?

Although residents increased their advertisement call response and decreased their aggressive call response on average (Figure 3), there were no significant negative correlations between advertisement and aggressive call responses at any period or averaged across all 6 bout periods (Table 4). Furthermore, the delta response indices for advertisement and aggressive calls were independent of one another ($F_{1,19} = 0.43$, $P = 0.518$, $r^2 < 0.01$). Therefore, investment into either call type was not reduced by investment into the other nor did changes in one call type over time correlate with changes in the other.

DISCUSSION

We aimed to determine whether advertisement and aggressive calling responses increased or decreased over successive interactions with a repeat intruder, whether individuals responded differently, and whether there was a trade-off between the different call types.

In regards to the changes observed in call responses, the mean advertisement call response linearly increased over successive interactions, whereas the mean aggressive call response decreased after the first bout period. These patterns conformed with our expectations, and the results of previous studies of male frogs responding to a persistent caller (Bee 2003a; Marshall et al. 2003; Humfeld et al. 2009). The implication is that individuals benefit from minimizing the costs of aggressive calling after the intruder has signaled its intention to remain in the area. In other words, the intruder has become a neighbor and the resident benefits from having a "dear enemy" relationship with it. Accordingly, the increase in advertisement calling suggests that residents increased their effort toward attracting females in order to compensate for the reproductive interference imposed by their new neighbor. However, contrary to the dear enemy interpretation, there was significant individual variation in the direction of change for both advertisement and aggressive calls, indicating that different individuals showed different patterns of response. Also, unexpected was the correlation between an individual's first response and the direction of its subsequent change. Specifically, individuals that

Table 2
Correlations between advertisement and aggressive delta response indices (difference in change in call rate between last and first interactions) and covariates

Delta response index	Factor	F_{df}	P	r^2
Advertisement	Volume of stimulus call	$0.10_{1,19}$	0.759	<0.01
	Resident mass	$2.00_{1,10}$	0.188	0.08
	Start time	$0.11_{1,19}$	0.747	<0.01
	Temperature	$0.30_{1,19}$	0.591	<0.01
	Days without rain	$1.11_{1,19}$	0.305	<0.01
	First advertisement response	$45.31_{1,19}$	<0.001	0.69
	First aggressive response	$0.77_{1,19}$	0.392	<0.01
Aggressive	Volume of stimulus call	$0.63_{1,19}$	0.436	<0.01
	Resident mass	$<0.01_{1,10}$	0.923	<0.01
	Start time	$<0.01_{1,19}$	0.977	<0.01
	Temperature	$0.87_{1,19}$	0.445	<0.01
	Days without rain	$4.97_{1,19}$	0.708	<0.01
	First advertisement response	$1.18_{1,19}$	0.291	<0.01
	First aggressive response	$94.14_{1,19}$	<0.001	0.82

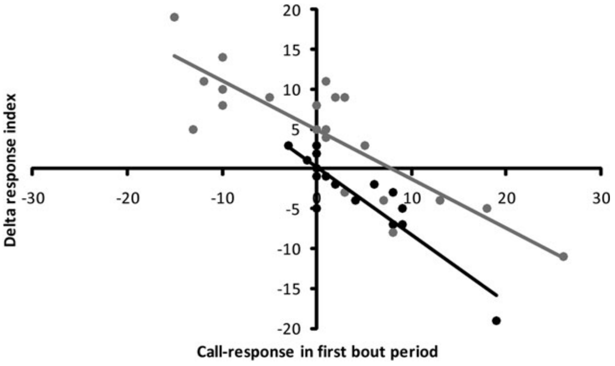

Figure 4
The relationship between an individual's change in call rate on initial contact with a simulated intruder (call response in the first bout; calls/minute) and its change in response over multiple interactions (delta response index; calls/minute) for advertisement (gray) and aggressive calls (black). Positive values for call response in the first bout represent individuals that increased their call rate during the first playback, whereas negative values represent individuals that reduced their call rate. Positive values for delta response index represent individuals that showed a greater shift in call rate during the final bout compared with the first, whereas negative values represent individuals that showed a greater shift in the first bout period compared with the last.

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Table 3

The correlations between advertisement and aggressive call responses (change in call rate in response to intrusion) in the first bout period and covariates

First call response	Factor	F_{df}	P	r^2
Advertisement	Volume of stimulus call	$0.06_{1,19}$	0.807	<0.01
	Resident mass	$1.54_{1,10}$	0.243	<0.01
	Start time	$0.19_{1,19}$	0.664	<0.01
	Temperature	$0.42_{1,19}$	0.523	<0.01
	Days without rain	$0.34_{1,19}$	0.570	<0.01
Aggressive	Volume of stimulus call	$1.19_{1,19}$	0.290	<0.01
	Resident mass	$<0.01_{1,10}$	0.974	<0.01
	Start time	$0.13_{1,19}$	0.721	<0.01
	Temperature	$0.74_{1,19}$	0.401	<0.01
	Days without rain	$0.08_{1,19}$	0.779	<0.01

responded strongly in the first interaction reduced the magnitude of their call response by the ultimate interaction, whereas those that responded weakly increased their efforts. This resulted in a convergence of call responses toward a similar level by the ultimate bout. The establishment of a dear enemy relationship is not sufficient for explaining these 3 important findings (individual variation in the direction of shifts in calling behavior, a correlation between these shifts and an individual's first call response, and convergence of calls over time).

A large body of work suggests that territorial residents alter their aggressive behavior in response to an intrusion based on the threat posed by the intruder and that the perceived threat of the intruder varies with repeated interactions (the relative threat hypothesis; e.g., Temeles 1994; Wich and Sterck 2007; Briefer et al. 2008; Akçay et al. 2009; Booksmythe et al. 2010). This would suggest that although most individuals were not threatened by the intruder (and hence decreased their aggressive signaling), some were threatened enough to attempt expelling the intruder by increasing aggressive call rate. However, we did not detect any effect of resident mass (although the sample size was only 11) or speaker volume, which should be related to the threat of the stimulus in relation to the resident, on the changes in call responses. Additionally, the relative threat hypothesis cannot explain the correlations between initial call responses and their subsequent change nor the convergence of call responses to a similar level. The familiarity hypothesis (which is often associated with relative threat) is also insufficient for explaining the results, as it only predicts aggression to decrease with increasing familiarity (Ydenberg et al. 1988; Getty 1989).

One explanation for the variation in response direction is that residents were initially uncertain about the intruder's quality and intentions but were able to more accurately assess the intruder with each interaction and thus gradually adjust their calling behavior to an optimal level. That is, a resident may need to give a certain number of advertisement and aggressive calls in response to an intruder in order to remain attractive and maintain adequate spacing while minimizing calling costs (Burmeister et al. 1999; Marshall et al. 2003; Wells 2007; Byrne 2008; Humfeld et al. 2009). For instance, chorusing frogs have been observed to gradually match the number of calls produced by rivals in order to optimize their calling behavior (Ayre et al. 1984; Gerhardt et al. 2000). However, residents may initially be uncertain about the optimal response (this would explain the large degree of variation in, and lack of predictor for, call responses in the first bout period). Residents may thus require information gathered over repeated interactions in order to determine how best to alter their behavior. The ability to gather information and use it as the basis for behavioral changes is suggested by the familiarity hypothesis (Ydenberg et al. 1988; Getty 1989) and some models of contest behavior (Parker and Rubenstein 1981; Hammerstein and Parker 1982; Enquist and Leimar 1983). Furthermore, resident frogs are expected to continually assess their opponents and alter their calling appropriately in order to remain closer to optimal levels. For instance, spring peepers (*Pseudacris crucifer*) have been observed to reduce their aggression over time toward speakers that give purely advertisement calls but immediately respond with aggressive calls once the playback changes to a mixture of advertisement and aggressive calling (Humfeld et al. 2009). Overall, the call adjustment hypothesis can explain why the initial call response predicted the ultimate change in response because individuals that gave too many calls to begin with benefited by giving fewer, whereas those that responded weakly benefited by giving more. Furthermore, initial reactions with the greatest error required the greatest adjustment. It would also explain why resident calls tended to converge because residents may have ultimately made similar assessments of the quality and threat posed by the simulated intruders. Therefore, variation in the direction of change in call responses may not be due to an increase or decrease in the perceived threat of the intruder over successive interactions, but differences in how calling had to be adjusted from initial uncertainty in order to give an optimal response.

Table 4

The correlation between advertisement and aggressive call responses (change in call rate in response to intrusion) given by an individual at each bout period and for all bout periods pooled together

Period	$F_{1,19}$	P	r^2
1	1.41	0.249	0.02
2	0.24	0.632	<0.01
3	0.32	0.580	<0.01
4	0.40	0.535	<0.01
5	0.01	0.915	<0.01
6	0.01	0.933	<0.01
Pooled	1.55	0.228	0.03

Fatigue is an alternative to optimization for explaining changes in behavior related to repeated intrusions (Peeke 1984). Specifically, the observed decreases in call response may have reflected prohibitively high metabolic costs of sustaining call production. By contrast, individuals that increased call response with each interaction may have been able to do so because they were initially conservative. However, even though calling is energetically expensive for frogs (Taigen and Wells 1985), reduced call rate is not necessarily indicative of depleted metabolic resources (Schwartz et al. 1995). Instead, frogs are expected to manage energy allocation to enable them to maintain calling throughout the night (Schwartz et al. 1995) and to give intense displays when the situation demands it (Byrne 2008). Furthermore, other playback experiments conducted on frogs have ruled out fatigue as a contributing factor by demonstrating that individuals are able to respond at full intensity if the stimulus is changed (Owen and Perrill 1998; Bee 2003a; Humfeld et al. 2009). If fatigue was important, we would expect a relationship between call rate and environmental factors such as days since rain, temperature, or the starting time of the trial as these are all expected to influence fatigue thresholds. However, we found no effect of these factors on either call response. Taken together, this evidence suggests that fatigue is unlikely to explain the observed changes in calling behavior by *P. bibronii.*

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Finally, our expectation that shifts in behavior would reflect a trade-off between exploitative and defensive interests was not supported. This trade-off is expected to occur in frogs because an individual that gives an aggressive call does so at the cost of not producing an advertisement call and vice versa (Brenowitz and Rose 1999; Marshall et al. 2003). There are at least 3 alternatives to the lack of a trade-off to explain why we did not detect any correlation. First, advertisement and aggressive calls may have some functional overlap (i.e., advertisement calls may weakly repulse males and aggressive calls may weakly attract females). For example, Marshall et al. (2003) found evidence that suggests preference for advertisement over aggressive calls in female spring peepers (*P. crucifer*) depends on the frequency and volume at which the calls are given in relation to one another. However, in species that produce these 2 types of call, each call type is superior to the alternative at performing the hypothesized function, so some degree of trade-off is still expected. The second potential explanation is that residents were not calling at their maximum output, allowing them to vary each call type independently. Finally, the trade-off may be reflected in the quality of calls (e.g., call duration, call volume, and energetic requirements) rather than in quantity. In any case, changes in calling behavior in this species and at the observed competitive intensity do not necessarily reflect a shift along a continuum between exploiting and defending a territory.

In conclusion, our results suggest that changes in a resident's response to a repeated intruder over successive interactions may reflect corrections made to an initially uncertain response. Frogs benefit by responding to changes in their social environment by minimizing the costs of calling and reproductive interference imposed by others, such as the simulated intruder in this study. Our results imply that a learning period is necessary for residents to assess the relative costs and benefits of interacting with an intruder and to arrive at this optimal level of calling. This is important to consider in cases where there is variation in the number of times a given intruder will be encountered. Additionally, unlike the predictions of the familiarity hypothesis, the results of this study imply that the optimal shift in an aggressive response to increasing familiarity is not always negative. Instead, individuals may tune their aggressive response to an appropriate level, which could be greater or less than their initial investment. Therefore, we may need to reconsider the prediction that increasing familiarity leads to a decrease in aggression when the threat of the intruder is low. Instead, we may consider that increasing familiarity leads to decreased uncertainty regarding the threat of the intruder. Finally, models of contest behavior have long incorporated increasing accuracy of opponent assessment over time as contestants gain more information about their relative abilities (Parker and Rubenstein 1981; Hammerstein and Parker 1982; Enquist and Leimar 1983; Enquist et al. 1990). Our study highlights that a similar process is likely to influence the structure of long-term competitive interactions. Furthermore, behaviors related to territory exploitation, in addition to aggressive behaviors, can be influenced by the gradual acquisition of information through interaction.

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