AGGRESSIVE CALLING IN TREEFROGS

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By
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And hereby certify that, in their opinion, it is worthy of acceptance.

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AGGRESSIVE CALLING IN TREEFROGS

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ABSTRACT

Acoustic communication plays a major role in many of the social activities of frogs. A great deal is known about communication in some contexts, such as mate choice. Aggressive communication, however, has received little attention in frogs. Most frog species produce some kind of aggressive vocalization. These aggressive calls are often given in the context of defense of territories or temporary calling spaces. Little is known about how aggressive calls are used to mediate these interactions. In particular, the communicative significance of aggressive calls, in terms of how assessment proceeds via aggressive calling, is unknown. The aim of my dissertation was to document the behavioral significance of aggressive calling behavior in two different treefrog species. In *Dendropsophus ebraccatus*, a Neotropical treefrog, I examined i) the plasticity of the aggressive response; ii) the effects of multiple competitors and a changing social environment on aggressive calling; and iii) the importance of aggressive calls in competitive call timing interactions. In the gray treefrog *Hyla versicolor*, a common North American species, I staged interactions between males in order to examine the determinants of success and the level of escalation in contests. I specifically compared aggressive call characteristics of winners and losers of different types of

aggressive interactions to determine whether or not assessment of aggressive calls may play a role in determining contest outcome.

Chapter 1 is an introduction to the problem of aggressive calling in frogs. I discuss previous studies of aggressive calling and describe why these studies have largely ignored the most significant questions related to aggressive calling behavior. I argue that it is important, nonetheless, to study aggressive calling in frogs because of their general utility as subjects for studies of acoustic communication and the possibility to test assumptions of certain game theory models of aggressive communication.

In chapter 2, I report the results of a study of the plasticity of aggressive calling in *D. ebraccatus*. I measured aggressive thresholds, the minimum signal amplitude required to elicit an aggressive call, in response to advertisement and aggressive calls. Furthermore, I measured the plasticity of aggressive thresholds by examining whether these thresholds change following the presentation of a suprathreshold stimulus. I found that males habituated, or raised their aggressive thresholds, in response to suprathreshold advertisement calls. Males showed the opposite response, which may be an example of sensitization, in response to suprathreshold aggressive calls. These results may point to a proximate mechanism for the extreme amounts of aggressive calling that I observed in choruses of this species.

In chapter 3, I discuss an experiment in which I used playback tests to measure males' aggressive responses to the presence of multiple aggressive competitors in *D. ebraccatus*. Males were sensitive to both the number of simulated aggressive signalers

present and the specific characteristics of their aggressive calls. In particular, males were likely to escalate their own aggressive calling in response to playbacks that did the same, while males de-escalated in response to playbacks in which a more aggressive stimulus was joined by a less aggressive stimulus.

In chapters 4 and 5, I report the results of related experiments on the call timing behavior of *D. ebraccatus*. I used playback experiments with fixed and randomly timed stimuli to determine whether males time their advertisement and aggressive calls nonrandomly. I also presented males with a series of low pulse number stimuli in order to determine if they are capable of synchronizing with extremely brief duration calls, and if they can resolve temporal properties typical of advertisement and aggressive calls in this time period. I recorded the long term interactions of pairs of males in the field in order to quantify natural call timing behavior and the use of advertisement and aggressive calls. Finally, I used a series of phonotaxis tests to test the hypothesis that aggressive calls in certain timing arrangements are attractive to females. I found that males timed their calls nonrandomly and with similar delays whether or not they were responding to advertisement or aggressive calls. Furthermore, since aggressive calls are much longer than advertisement calls, a leading caller is more attractive to females if it gives aggressive calls instead of advertisement calls. I conclude that the primary function of aggressive calls in *D. ebraccatus* is not to mediate aggressive interactions; rather they are strategically used by males engaged in competitive call timing interactions.

In chapter 6, I report the results of a series of staged aggressive interactions in *H. versicolor*. I developed a method to stage aggressive interactions between males in a laboratory setting. I measured body size variables of winners and losers of interactions that either did or did not escalate to aggressive calling or physical fighting. I made comparisons between absolute body sizes of each competitor and the duration and level of escalation of interactions in order to explicitly test predictions of theoretical models of assessment during contests. Larger males had an overall advantage, although this advantage was weak and confined to less escalated interactions. There were no clear relationships between body size and the level of escalation and duration of interactions and I conclude that contest success is likely related to physiological status, a variable that is not directly represented by any measure of body size.

In chapter 7, I continue my discussion of staged interactions in *H. versicolor* by examining the importance of aggressive calling during these interactions. I recorded aggressive calls of males throughout the staged interaction and I compared the aggressive call characteristics of winners and losers to determine if contest success can be predicted by aggressive calling behavior. Indeed, winners of aggressive calling interactions tended to have lower frequency aggressive calls, exhibited a greater decrease in frequency from advertisement to aggressive calls, and had a higher aggressive calling effort. In addition, there was evidence for mutual assessment of aggressive call characteristics. Competitors that engaged in more escalated physical fights tended to be more similar in their aggressive call characteristics than those that engaged in less escalated aggressive calling interactions. I conclude that aggressive calls signal resource holding potential and individuals assess their opponents’ aggressive calls and make the decision to persist or flee based on the magnitude of the relative difference in quality signaled by aggressive calls. Furthermore, the most important characteristics of aggressive calls for determining contest outcome were those that are most likely to be tied to energetic costs. Thus, I conclude that aggressive calls signal energetic state, and energetic state in turn is a major component of an individual’s resource-holding potential.

In chapter 8, I discuss the importance of this dissertation in terms of the broader questions that remain to be studied in the field of anuran aggressive communication. I describe the major topics that should be considered and give recommendations for the methodologies necessary to perform these studies.

CHAPTER 1

General introduction

Acoustic competition in anurans

Both components of sexual selection, intrasexual selection (male–male competition), and intersexual selection (female choice) often involve some form of signaling (Andersson 1994). Signals are used in intrasexual selection to resolve disputes between rival males, maintain territory boundaries, and reinforce dominance hierarchies (e.g., Clutton-Brock and Albon 1979, Kroodsma 1979, Senar 1990, Morris et al. 1995, de Kort et al. 2009). Intersexual selection involves signaling to attract mates and to coordinate reproductive activities (e.g., Crews 1975, Wells 1978a, Borgia 1985, Hill 1991, Márquez and Verrell 1991, Rowland 1994). Many signals may serve both functions (Berglund et al. 1996). Behavioral ecologists have long been interested in how such signals are utilized, and ultimately how signaling translates into variation in reproductive success, and thus, fitness.

For many species of anuran amphibians, the arena in which signal competition takes place is the chorus. Males gather at night and produce conspicuous vocalizations. Some species maintain and defend relatively fixed territories , while in others the situation is more akin to a lek, in which males gather and display from a temporary space and contribute no resources other than genetic material (Wells 1977). Choruses are generally densely packed assemblages of loudly calling males, and in many cases several species may be present (Wells 1977, Duellman and Pyles 1983, Donnelly and

Guyer 1994). Mate choice is primarily mediated through vocalizations in most chorusing anurans (reviewed by Gerhardt and Huber 2002), thus the presence of so many other individuals vocalizing at the same time represents a severe constraint on the ability of any given male to attract a female (Schwartz and Wells 1983a, b, Schwartz and Gerhardt 1989, 1995, Wollerman 1999, Wollerman and Wiley 2002a, b, Marshall et al. 2006, Schwartz and Marshall 2006, Bee and Micheyl 2008, Schwartz and Freeberg 2008, Bee and Schwartz 2009). A major focus of studies of anuran communication is the strategies used by females to select and localize preferred males in the midst of such noise and the strategies used by males to outcompete nearby callers and make their calls more conspicuous to females (reviewed by Gerhardt and Huber 2002).

As with female choice, acoustic competition between males is mediated primarily by vocalizations. In many species, advertisement calls, which are also the primary call type that males give to attract females , serve a function in male–male competition (Wells 1977). Males alter various properties of their advertisement calls including the duration, complexity, effort, rate and frequency in response to changes in the level of acoustic competition (Wells 1988a). Some species also produce aggressive calls, a separate call type that presumably mediates the outcome of competitive interactions between males (Wells 1977, 1988a). Aggressive calls have been described for many species (see Table 8.1, pages 353-356 in Wells 2007), and detailed behavioral investigations of aggressive calling have been carried out for a few species (e.g., Wells and Schwartz 1984b, Wagner 1989a, b, Wells 1989, Wagner 1992). Nonetheless, these studies are few in number compared to the massive amount of studies of female choice of male calls. In addition, few, if any, studies have rigorously confirmed the presumed role of aggressive calls as a means to mediate the outcome of aggressive interactions between males (Wells 2007). This is not a trivial problem, as I describe below. Nonetheless, I argue, and present evidence in the chapters that follow, that aggressive calling in anurans can be studied with the same approaches used to study aggressive signaling in other animals. In addition, as described below, because of their relatively unique signaling behaviors and fighting abilities, anurans can test and challenge the predictions of general models of aggressive signaling in contests in a way that other animal species cannot. Studies of anurans have been key to advancing our knowledge of animal communication in general, and there is no reason not to expect a similar impact of anurans on our knowledge of aggressive behavior.

The problem of aggressive calling

At first glance, it appears relatively straightforward to assign to anuran aggressive calls the role typically ascribed to aggressive signals in most other animals. That is, aggressive calls, because they are observed primarily during close-range male–male competitive interactions, must be signals that communicate information about the signaler’s relative fighting abilities, motivation, or aggressiveness. These general roles have been attributed to aggressive signals in other animals (Maynard Smith and Price 1973, Maynard Smith and Parker 1976, Caryl 1979, Enquist 1985, Hurd and Ydenberg 1996, Enquist et al. 1998, Hurd 2006). It is not necessarily valid to automatically assign these roles to anurans’ aggressive calls, however. Several aspects of anuran communication raise doubts about this presumed use of aggressive calls that must be dispelled in order to speculate about the function of such calling. In fact, I argue that a major question has been ignored in anuran communication research, namely, why do anurans give aggressive calls at all?

Aggressive signals in animals generally are thought to be a less costly means to resolve a dispute than all-out physical fighting (Maynard Smith and Price 1973, Maynard Smith 1974, Parker 1974, Maynard Smith and Parker 1976). When animals resolve disputes through aggressive signaling rather than fighting, both competitors benefit from the avoidance of injuries and other costs. Basic game theory has predicted that the prevalence of fights in a population should be related to the ratio of the value of victory to the cost of an escalated fight (Maynard Smith and Parker 1976). Thus, as the value of victory decreases or the costs of fighting increase, competitors are expected to be more likely to resolve disputes through signals rather than physical aggression. Studies of many anuran species have shown that disputes often are resolved through an exchange of aggressive calls with no physical combat (e.g., Howard 1978, Fellers 1979, Crump 1988, Given 1988, Wagner 1989a, Bastos and Haddad 2002, Wogel et al. 2004, Caldwell et al. 2010). However, anurans in general are characterized by a lack of weaponry capable of inflicting serious injury (Shine 1979) and generally low levels of activity due to a reliance on anaerobic metabolism for most activities other than calling (Bennett and Licht 1973, 1974), although there are some exceptions (e.g., Shine 1979, Channing et al. 1994). Thus the cost of fighting, although this has never been directly measured for any anuran species, is expected to be fairly low. In fact, particularly for smaller anurans, it is unclear if the typical determinants of success in aggressive interactions such as body size would even be expected to give certain competitors an advantage in fights. Given these low costs of fighting and the lack of obvious disparities between successful and unsuccessful competitors, it is unclear why an individual should be expected to back down after an exchange of aggressive calls without at least attempting to defeat its opponent in physical combat. Can individuals that signal that they are superior through aggressive calls actually back up their threat in physical combat? Additional study is necessary to examine costs of fighting and the individual characteristics that influence fighting success (resource-holding potential (RHP) Parker 1974), but for the moment it is unclear what benefits accrue for individuals that assess one another's aggressive calls.

Even assuming that aggressive calls do provide information on a contestant's RHP, several issues remain unresolved. RHP is typically related to some measure of either individual body size or physiological state (Parker 1974, Briffa and Sneddon 2007, Arnott and Elwood 2009). Many studies have shown that these potential measures of RHP are highly correlated with certain characteristics of advertisement calls (e.g., Davies and Halliday 1978, Bucher 1982, Taigen and Wells 1985, Wells and Taigen 1986, Ryan 1988a, Sullivan and Wagner 1988, Wagner 1989c, Wells and Taigen 1989, Penna and Veloso 1990, Wells et al. 1996). Furthermore, males are known to be sensitive to variation in these advertisement call characteristics (e.g., Davies and Halliday 1978, Wells and Schwartz 1984a, Wells and Taigen 1986, Wagner 1989a, Given 1999). Thus, males are fully capable of assessing one another's advertisement calls. If likely determinants of RHP can be assessed by the advertisement calls that males are already giving to attract females, why should an additional call type be used? Presumably, aggressive calls could provide better, or different, information relevant to male RHP and contest resolution than advertisement calls. However, to my knowledge, no study has actually demonstrated that this is the case. Indeed, previous studies of calling assessment in aggressive interactions in frogs have all involved adjustments to properties of the advertisement call, not a switch to aggressive calls (Davies and Halliday 1978, Arak 1983, Wagner 1989a, b, c, 1992, Bee and Perrill 1996, Bee et al. 2000, Bee 2002, Bee and Bowling 2002, Burmeister et al. 2002). Until such a demonstration is made, the presumed function of aggressive calls must be questioned. This is a particularly serious problem given the relationship between aggressive calls and female preferences. Females in many anuran species, including the two discussed in this dissertation, are generally less attracted to aggressive calls than to advertisement calls (Oldham and Gerhardt 1975, Schwartz and Wells 1985, Wells and Bard 1987, Backwell 1988, Grafe 1995, Brenowitz and Rose 1999, Gerhardt et al. 2007, but see Reichert 2011b). Thus, males that engage in aggressive call competition may render themselves less attractive to females than those that simply exchange advertisement calls. Many species partially resolve this problem by concentrating bouts of aggressive calling to the early stages of chorus formation when females are less likely to be present (Wells 1988a). Nonetheless, aggressive calling is by no means restricted to these time periods (e.g., Reichert 2010). Thus, in order for aggressive communication to be evolutionarily stable, the information content of aggressive calling not only must be above and beyond that of advertisement calling but also it must be sufficiently beneficial to overcome the potential costs of reduced ability to attract mates during the aggressive interaction itself.

Study system

Anuran amphibians in general, and Hylid treefrogs in particular, have been major study subjects for a variety of topics in behavioral ecology. Acoustic communication is particularly well described in these species. I studied competitive calling behavior in two species in the family Hylidae: gray treefrogs, *Hyla versicolor*, and pantless (or harlequin) treefrogs, *Dendropsophus ebraccatus*.

Gray treefrogs have long been an important model system for the study of acoustic communication and speciation. A particular point of interest is the repeated speciation events due to polyploidy that have given rise to multiple freely interbreeding clades of *H. versicolor* from other parental species (*H. chrysoscelis* and other presumably extinct lineages; Ptacek et al. 1994, Holloway et al. 2006). The species group is distributed throughout much of eastern North America, with *H. chrysoscelis* occupying a large area of allopatry in the southeastern United States and in the Midwest, while *H. versicolor* occurs in allopatry in much of the northeastern United States and southeastern Canada. In addition, there are large areas of sympatry in the Midwest and possibly other areas in the Mid-Atlantic states (Holloway et al. 2006). Gray treefrogs breed in the early summer months, chorusing and ultimately depositing eggs in fishless woodland ponds and swamps.

Chorusing behavior, acoustic competition for mates and female mate choice have been studied extensively in *H. versicolor* (reviewed by Gerhardt 2001). The male advertisement call consists of a trilled pulse train given with a species specific (and temperature dependent) pulse rate (Gerhardt 1978; see Chapter 7, Figure 1). The pulse rate and frequency properties of advertisement calls are relatively static within males, while other properties such as call duration and call rate are highly variable (Gerhardt 1991, Gerhardt et al. 1996). The latter properties are altered by males with changes in the level of acoustic competition (Wells and Taigen 1986, Schwartz et al. 2002). Advertisement calling is fueled by aerobic metabolism and is energetically expensive; increases in call duration or call rate increase the amount of energy consumed (Taigen and Wells 1985, Wells and Taigen 1986). Nonetheless, males in these studies traded off call rate and call duration such that overall energetic expenditure, in terms of oxygen consumption, did not differ (Wells and Taigen 1986). Energy constraints may limit the amount of time males spend chorusing within a night and the number of nights a male calls during the breeding season; both variables are known to be among the strongest predictors of male mating success in the field (Sullivan and Hinshaw 1992, Runkle et al. 1994, Wells et al. 1995). In laboratory based phonotaxis tests, females are selective for, among other things, species specific pulse rates, long call durations and high call rates (Klump and Gerhardt 1987, Gerhardt 1991, Diekamp and Gerhardt 1995, Gerhardt et al. 1996, Gerhardt et al. 2000, Schwartz et al. 2001, Bush et al. 2002, Gerhardt 2005). These preferences may benefit the female by allowing her to choose a mate with superior genetic quality (Welch et al. 1998), although these benefits may be context dependent

(Welch 2003) and female preferences are expressed far less consistently in the field than in the laboratory setting (Sullivan and Hinshaw 1992, Schwartz et al. 2001). In close-range calling interactions, males sometimes switch to giving aggressive calls (Pierce and Ralin 1972, Fellers 1979). Aggressive calls are acoustically distinct from advertisement calls and are presumably used to mediate disputes over calling spaces. Disputes occasionally escalate to physical fighting (Fellers 1979). Previous studies suggested that residents had an advantage in such agonistic interactions (Fellers 1979, Semsar et al. 1998), although physiological state also appears to play a role (Semsar et al. 1998, Trainor 2003). Females are unresponsive to aggressive calls by themselves (Gerhardt et al. 2007).

D. ebraccatus (formerly *H. ebraccata*; Faivovich et al. 2005) is a small treefrog distributed throughout much of Central America (Duellman 1970). Males call throughout the rainy season in swamps and small ponds in lowland tropical rainforest. These choruses can be especially dense assemblages of both conspecifics and heterospecifics, some with similar call characteristics (Fouquette 1960, Schwartz and Wells 1983a, Schwartz and Wells 1984, 1985, Backwell and Jennions 1993, Donnelly and Guyer 1994, Wollerman and Wiley 2002b). The male advertisement call always contains a pulsed introductory note and often contains one or more click note appendages (Wells and Schwartz 1984a; see Chapter 2, Figure 1). The pulse rate and dominant frequency of advertisement calls are relatively static within males, while the call duration is more variable because males increase the number of click notes with increasing acoustic competition (Wells and Greer 1981, Wells and Schwartz 1984a, Wollerman 1998).

Females prefer calls with more click notes, average pulse rates and lower dominant frequencies (Wells and Schwartz 1984a, Wollerman 1998). Such preferences are expressed less strongly in high levels of background noise (Wollerman 1999, Wollerman and Wiley 2002a), and males may adjust their calling behavior to avoid interference with heterospecific calls (Schwartz and Wells 1983a, b, Schwartz and Wells 1984). An additional element of acoustic competition in this species involves adjustments in call timing. Males time their advertisement calls nonrandomly with respect to those of other nearby males. Specifically, males often time their advertisement calls such that they lag behind, and often overlap, the click notes of a leading caller (Narins 1982, Schwartz and Wells 1984, Wells and Schwartz 1984a, Reichert accepted). Females prefer lagging advertisement calls to leading advertisement calls, likely because a lagging call obscures the click notes of the leading call (Wells and Schwartz 1984a). Click notes appear to be especially important for female attraction, as is the case for acoustic appendages in many other anuran species (Rand and Ryan 1981, Gerhardt et al. 2007).

Although basic questions remain unaddressed, several studies have examined aggressive calling in *D. ebraccatus* (Fouquette 1960, Wells and Greer 1981, Schwartz and Wells 1984, Wells and Schwartz 1984b, Wells and Bard 1987, Wells 1989, Schwartz 1994). Aggressive calls always have higher pulse rates than advertisement calls, but there is overlap between aggressive and advertisement calls for other call characteristics (Wells and Schwartz 1984b). However, aggressive call characteristics tend to be much more variable, and the aggressive calling system has been described as graded because males tend to alter certain characteristics of their aggressive calls with changes in the level of aggressive competition (Wells and Schwartz 1984b, Wells 1989; Reichert, unpublished data). Specifically, as competition escalates, males increase the duration and decrease the pulse rate of the introductory note, and reduce the number of click notes (Wells and Schwartz 1984b, Wells 1989). These responses occur to both general increases in calling activity by nearby conspecific and heterospecific males (Schwartz and Wells 1984, Wells and Schwartz 1984b, Reichert 2011a), and to changes in specific call characteristics of aggressive calls (Wells 1989). Females are generally less attracted to aggressive calls than to advertisement calls, and within aggressive calls they prefer calls with more to those with fewer click notes (Wells and Schwartz 1984b, Wells and Bard 1987). Aggressive calls preferred by females tend to be those given by males in less escalated interactions (i.e., those that involve males that are farther apart), thus the graded aggressive calling system has been interpreted as a means for males to balance the competing demands of mate attraction and calling space defense (Wells and Schwartz 1984b). In this scenario, males reserve the least attractive aggressive calls for only the presumably most escalated aggressive interactions (Wells and Schwartz 1984b, Wells and Bard 1987, Wells 1989). Although this explanation is intuitively appealing, no study has examined the communicative significance to males of aggressive calls (but see Schwartz 1994). For example, it is unknown if the gradation present in aggressive calls provides information on either the likelihood of escalation to actual physical fighting or information on RHP or other male qualities that may be assessed during aggressive interactions. In fact, although aggressive calls are given during physical fights in *D. ebraccatus* (Schwartz and Wells 1984), the function of aggressive calls as an agonistic signal is speculative at best because fights are extremely rare in this species (Schwartz and Wells 1984; Reichert, personal observation).

CHAPTER 2

Aggressive thresholds in *Dendropsophus ebraccatus*: Habituation and sensitization to different call types

ABSTRACT

Males in many chorusing anuran species use aggressive calls during defense of calling spaces from other males. The minimal intensity of another male's vocalizations that elicits an aggressive call response has been termed the aggressive threshold. Previous studies of aggressive thresholds have shown that they are plastic: males habituated (increased their aggressive thresholds) in response to repeated presentation of stimuli above initial threshold levels. Habituation likely contributes to the stable chorus structure of these species, in which aggressive calling is rare compared to advertisement calls. I have observed high levels of aggressive calling in the treefrog *Dendropsophus ebraccatus*, suggesting that males of this species do not habituate. In this study, I investigated the plasticity of aggressive thresholds in *D. ebraccatus.* I measured the aggressive thresholds of males before and after suprathreshold stimulation by both advertisement and aggressive calls. I found that the different call types had different effects: males habituated to advertisement calls but lowered their aggressive thresholds in response to aggressive calls. I consider the latter response to be an example of sensitization, a behavior that has been documented infrequently in

vocalizing anurans. Sensitization is a plausible mechanism responsible for the high levels of aggressive calling observed in this species. Given the high costs of aggressive calling, however, it is unclear why a mechanism that increases aggressive call output would be maintained.

INTRODUCTION

Many of the social interactions of anuran amphibians are mediated by acoustic communication. Anuran acoustic communication often takes place in the context of the chorus, in which males gather, sometimes at great densities, and vocalize to attract females. Studies of calling males have revealed that many properties of their vocalizations are plastic with respect to various aspects of the social environment (Wells 1988a). Males face steep competition from other males when calling to attract females and modify various aspects of their calls including the timing, complexity, rate, duration, frequency, and type of call given in response to such competition (e.g., Rand and Ryan 1981, Wells and Schwartz 1984b, Wells and Taigen 1986, Lopez et al. 1988, Bosch and Marquez 2001, Schwartz et al. 2002). These changes have consequences that affect female choice and, in some cases, may also function to repel rival males (Schwartz 1986, Wagner 1989a, Wells 1989). Thus, understanding how and why males alter their own vocalizations in response to vocal competition is a key to understanding the structure of choruses and male mating success in the complex chorus environment.

One aspect of vocal alteration that has received relatively little attention is the use of different call types by males. In particular, males in many species, in addition to giving advertisement calls that attract females and serve a role in male–male competition, also have a distinct aggressive call (=encounter call, Wells 1977). Aggressive calls are used by males at close proximity to other males and function to mediate male–male interactions. The precise message conveyed by such signals and the mechanisms used by competitors to assess such calls are largely unknown (Wells 2007; but see Wagner 1989b, 1992, Burmeister et al. 1999).

In territorial species, aggressive calls serve to identify the territory's owner and defend territory boundaries (Wiewandt 1969). Many species of frogs, however, are non-territorial and instead defend a loosely defined calling space whose location and size can vary within and between nights depending on such factors as the density of calling males in the chorus (Telford 1985, Gerhardt et al. 1989). Aggressive calls appear to be used in the defense of calling spaces to repel intruding males. The ability of a male to maintain a calling space relatively free from interference may improve his mating success because interference from other calling males in a dense chorus can severely reduce the attractiveness and localizability of calls to females (Schwartz 1987, Grafe 1996, Wollerman 1999, Martínez-Rivera and Gerhardt 2008).Thus, the use of aggressive calls in mediating male–male interactions is an important factor that affects male mating success and that must be understood to gain a complete understanding of female mate choice in chorusing frogs.

When males defend a calling space there is presumably a boundary between tolerance and intolerance of other calling males. Males are likely to primarily assess their competitors through acoustic signals, and spacing appears to be mediated by the intensity of neighbors' calls (Brenowitz et al. 1984, Telford 1985, Wilczynski and Brenowitz 1988). Thus, the boundary can be measured in terms of the loudness of calls from neighboring males that a male is willing to tolerate. In the anuran literature, such a boundary has been termed an aggressive threshold (Lopez et al. 1988, Rose and Brenowitz 1991), which is a measure of the minimum amplitude (in decibels, sound pressure level (SPL)) of a neighboring male's call that elicits an aggressive call from the subject male.

Aggressive thresholds have been measured in several species and are well studied in some chorus frogs of the genus *Pseudacris* (Hylidae) (Brzoska 1982, Brzoska et al. 1982, Robertson 1984, Telford 1985, Lopez et al. 1988, Brenowitz 1989, Rose and Brenowitz 1991, Brenowitz and Rose 1994, Rose and Brenowitz 1997, Marshall 2003, Marshall et al. 2003). In addition to simple measurements of thresholds in response to different call types, further observations demonstrated that the thresholds themselves are plastic in response to changes in the local chorus environment. Thresholds in *Pseudacris regilla* and *Pseudacris crucifer* were positively correlated with the density of the chorus (Rose and Brenowitz 1991, Marshall et al. 2003). In addition, these experimenters actively altered male's thresholds by broadcasting stimuli louder than their initial thresholds to them for a period of time. This resulted in a significant increase in aggressive threshold to the presented call type after a relatively brief period of stimulation (Brenowitz and Rose 1994, Rose and Brenowitz 1997, Marshall et al. 2003). Thus, males appear to become more tolerant of neighboring males in denser choruses.

Previous studies of the plasticity of aggressive thresholds have involved measuring threshold change in response to repeated stimulation. It is useful to examine such studies in the context of the dual-process theory of habituation (Bee 2001, Marshall et al. 2003), which proposes a general mechanism for behavioral changes in response to repeated stimulus presentation (Groves and Thompson 1970, Thompson et al. 1973). The observed behavioral response to such stimulation is hypothesized to be a summation of the independent processes of habituation and sensitization. Habituation is characterized by a decreased response to repeated stimulation, while sensitization is characterized by an initially increasing, and later decreasing, response to repeated stimulation (Thompson and Spencer 1966). Thus, previous studies demonstrating an increase in aggressive thresholds following suprathreshold stimulation are demonstrations of short-term response habituation (Brenowitz and Rose 1994, Rose and Brenowitz 1997, Marshall et al. 2003). Response sensitization has been documented infrequently in anurans (Bee 2001) and never in the context of the plasticity of aggressive thresholds.

Aggressive thresholds and their plasticity have been measured in a limited number of anuran species, and it is unclear whether habituation is a universal response to suprathreshold stimulation in chorusing anurans. Aggressive calling is infrequent in the choruses of anurans for which habituation has been demonstrated. In some anuran species, however, aggressive calls are relatively frequent, which suggests that these species do not habituate to the same degree or in the same manner. I have observed very high levels of aggressive calling during field studies of *Dendropsophus ebraccatus* (formerly *Hyla ebraccata*), a Neotropical hylid. In my primary study population in Gamboa, Panama, the density of calling males is extremely high, and males readily and frequently give aggressive calls.

The goal of this study was to examine the plasticity of aggressive thresholds in *D. ebraccatus* and to relate this to the high levels of aggressive calling observed in this species. In order to quantify the overall frequency and temporal distribution of aggressive calls, I analyzed recordings of spontaneous male calling made at different times of night. I also determined whether or not male *D. ebraccatus* habituate to conspecific calls as has been observed in other species. I used playbacks to make measurements of males' initial aggressive thresholds to both advertisement and aggressive calls. I then examined the plasticity of these thresholds by measuring them a second time following the broadcast of suprathreshold stimuli. I predicted that males would not habituate to all stimuli. Males may not only fail to habituate but also in fact may be sensitized by suprathreshold stimulation. The dual-process theory suggests that response sensitization will occur when a stimulus is so strong in eliciting a response that the contribution of the sensitization process outweighs that of the habituation process. Thus, I predicted that if a sensitization response was observed, it would be more likely to occur in response to the presumably stronger aggressive call stimulus.

METHODS

Study site and species

D. ebraccatus is a small treefrog common throughout much of Central and South America. Most calling and breeding occurs in ponds and marshes during the rainy season, which takes place from approximately late May through November at my study sites in Gamboa, Panama. I utilized two sites, a small pond and a flooded field, that were located within 1 km of each other. I did not directly measure male density, but densities can be extremely high at both locations, with males often calling within 10 cm of each other in the most concentrated areas (personal observation). Playback experiments took place nightly at the time of the most intense calling activity (2000–2400 hours) in July and August of 2007 and August of 2008.

Males have distinct advertisement and aggressive calls, which have been described in detail in several studies (Wells and Greer 1981, Schwartz and Wells 1984, Wells and Schwartz 1984a, b, Wells and Bard 1987, Wells 1989, Wollerman 1998). The advertisement call consists of a long introductory note to which shorter click notes may be appended (Figure 1a). The introductory note of the advertisement call is pulsed, and the pulse repetition rate is static and is approximately 95 pulses/second (Wells and Schwartz 1984a, Wollerman 1998). Relatively isolated males often give calls that lack click notes; as the level of acoustic competition increases, males begin giving calls with click notes more frequently (Wells and Schwartz 1984a). The number of click notes per call is almost always between one and four. Females prefer advertisement calls with click notes to calls without clicks (Wells and Schwartz 1984a). The dominant frequency of the call is approximately 3 kHz, and there are no other major harmonics.

The spectral characteristics of aggressive and advertisement calls are similar, but aggressive calls have a higher and more variable pulse rate, ranging between 200 and 500 pulses/second (Figure 1b). There is gradation in many characters of the aggressive call that seems to be associated with the level of escalation (increase in intensity) of the conflict, but this gradation does not encompass the range of the advertisement call. In playback tests, when males are challenged with a louder simulated competitor, they respond by increasing the duration and decreasing the pulse rate and number of click notes of the aggressive call (Wells and Schwartz 1984b, Wells 1989). Although aggressive calls are common, physical fights are rare and appear fairly benign (personal observation). Aggressive calls have been shown to be less attractive to females than advertisement calls in this species (Wells and Bard 1987).

Frequency of aggressive calling

Observations of the *D. ebraccatus* chorus suggested that males give high levels of aggressive calls throughout the nightly calling period. In order to quantify this, I analyzed recordings of spontaneous male calling I had made prior to this experiment. Recordings were made in the field during June and July of 2006 and July of 2007, from the same population described in this study. I used a Marantz PMD-660 digital audio recorder and a Sennheiser ME-67 directional microphone to record approximately ten calls from each male (*N*=104). Density of the chorus was not noted, but the recordings encompass the variation in chorus densities observed nightly and over the breeding season. I analyzed recordings using a computer program (Audacity 1.2.4) and counted the number of advertisement and aggressive calls in order to calculate the proportion of each male’s calls that were aggressive calls. I also noted the time of night at which the recording was made so that I could examine how the level of aggressive calling changes within the nightly calling period.

Playback stimuli

I broadcast synthetic calls representative of different *D. ebraccatus* call types to males in order to measure their aggressive thresholds. Single calls (16-bit digital files with a sampling rate of 20 kHz) were synthesized using a program provided by J. Schwartz. Pulses of both call types were shaped with linear rise and fall times constituting 45% each of pulse duration, and pulse duty cycle (ratio of pulse duration to pulse period) was 50%. Two basic call types were used: a typical advertisement call and an aggressive call with properties typical of a high level of aggression (see above). Parameters of the synthetic stimuli were based on computer analysis of calls from my previous recordings (Audacity 1.2.4) and published work describing the call characters of males from this population (Wells and Schwartz 1984a, b). Temporal and spectral parameters of the synthetic stimuli, along with average values of these properties from recordings made at the study site, are given in Table 1. There is little variation in nightly temperature at the study sites; thus, it was not necessary to correct any of the features of the stimulus calls to account for changes in temperature.

The playback stimuli were created by repeatedly copying and pasting the calls in an audio-editing computer program (Cool Edit Pro 2.0, Syntrillium 2002) to create the appropriate spacing between calls. Both advertisement and aggressive calls were repeated with a call period of 6 s, which approximates the typical *D. ebraccatus* rate of calling. I used the program's amplification function to decrease the SPL of the stimuli in steps of 2 dB. I then arranged these stimuli on a single track so that there was a 2-dB increase in amplitude every 30 s. The tracks used for the playbacks ultimately increased in amplitude by 40 dB. By gradually increasing the intensity over time, I was able to measure the male's aggressive threshold as the intensity at which he first gave an aggressive call. Stimuli were recorded onto a compact disk for playback in the field. I used a portable sound-level meter (Radio Shack 33-2055) to confirm that the stimuli broadcast from the playback system reproduced the range of SPLs and increase in SPL over time generated by the computer program.

Playback procedure

I performed playbacks on calling males in the field. Because male *D. ebraccatus* repeatedly enter into aggressive calling interactions with neighbors, I only performed playbacks on males that were either naturally calling in relative isolation or on males that I transported from denser areas of the chorus to isolated areas. Any male that was moved from a denser chorus was allowed to call for at least 15 min in isolation prior to testing. As males generally enter into aggressive calling interactions much more often than every 15 min (personal observation), I considered this time interval sufficient to allow males' calling behavior to recover from the effects of being in a dense chorus. Performing playbacks with isolated males was necessary to ensure that any aggressive response was to the playback stimulus and not to a neighboring male, but precluded comparisons of aggressive thresholds and chorus density. Although aggressive interactions tend to be less common in low-density areas than in very dense choruses, they are still frequent. In addition, aggressive calling interactions escalate to high levels even in interactions between otherwise isolated males (personal observation). Thus, I consider my choice of an aggressive call stimulus typical of a highly aggressive male to be reasonable.

Playback stimuli were broadcast from a portable compact disk player (Panasonic SLSW940S) through a battery-powered amplified speaker (Saul Mineroff Electronics, SME-AFS) mounted on a tripod. The speaker was adjusted such that the amplitude of the loudest call broadcast from the speaker would have an SPL of 95 dB at 1 m, measured by the portable sound-level meter. I was unable to consistently position the speaker 1 m from the male due to variation in elevation of males at their calling sites and water depth, so that the actual SPLs of the loudest calls that could have been presented to each male varied from 89 to 100 dB SPL at the focal male's position. The initial SPL of playback presentation was 40 dB less than the loudest call that could have been presented. This is presumably near the threshold of hearing in *D. ebraccatus* (Wilczynski et al. 1993), and I considered it unlikely that males' aggressive thresholds would be at a lower SPL than that of the initial playback presentation in most cases.

A series of playbacks was used to measure each male’s aggressive threshold and response to suprathreshold stimulation to the advertisement and aggressive call stimuli. All males in the dataset presented here received both the aggressive call and advertisement call playbacks, presented in random order. It could be argued that males’ responses to the playback of a second call type could be altered by their having already experienced a playback with a different call type. I thus allowed a 15-min timeout period between playbacks of the two different call types. I used statistical tests to ensure that any effects of order on the magnitude of aggressive thresholds or their direction of change following suprathreshold stimulation were accounted for (see below).

The playback methodology followed that of Rose & Brenowitz (1994). Three playback sessions per stimulus were performed with each male. The first measured his initial aggressive threshold to either the advertisement or aggressive call. I broadcast the appropriate call type, which increased in SPL in steps of 2 dB every 30 s. I stopped the playback at the point at which he first gave an aggressive call. The SPL at which this occurred was his “initial aggressive threshold.” Immediately following this playback, I presented the male with a second playback consisting of a suprathreshold stimulus. This allowed me to determine how males’ aggressive thresholds change in response to calls broadcast above their initial threshold. This playback consisted of the same call type being broadcast at an SPL of 4 dB SPL greater than the initial threshold for 5 min, followed immediately by one minute of the same call at 8 dB SPL above the initial threshold. The final playback was a remeasurement of the aggressive threshold using the method described above. I again noted the point at which the male first gave an aggressive call and the SPL at which this occurred was his “final aggressive threshold.” Following a timeout period of 15 min, I repeated these three steps with the other call type. Following playbacks, I measured the SPL of the playback at the position of the frog. I collected males for weighing and measuring and gave each male a unique toeclip to ensure individual identification.

In order to be included in the dataset, males were required to give an aggressive call in response to both attempts to measure his aggressive threshold for at least one call type. Some males (*N*=6) responded to one call type but ceased calling or moved away during the playback of the second call type. These data are included for statistical comparisons within a given call type but are not included for comparisons between the two call types. Occasionally (*N*=3) males instantly gave an aggressive call at the lowest playback level presented. I did not consider this to be his aggressive threshold but instead immediately ceased playback for 5 min and resumed playback at a lower amplitude.

Statistical analysis

Descriptive statistics of sound pressure levels were calculated from the absolute sound pressures in μPa (0 dB SPL = 2×10^{-5} Pa) rather than relative sound pressures in dB SPL because the dB scale is logarithmic. Means and standard errors of aggressive threshold values were calculated from these converted absolute pressure measurements. These values were then reconverted to the dB scale, resulting in standard errors asymmetrical about the mean. Nonparametric inferential statistics, however, were calculated from the unconverted relative dB SPL values.

The primary focus of this study was to determine whether there was a difference in aggressive thresholds measured prior to and following suprathreshold stimulation for the two call types. I used Wilcoxon matched-pairs signed ranks (WSR) tests to test the null hypothesis that there is no difference in the magnitude of aggressive threshold levels before or after suprathreshold stimulation. Sign tests were used to test the null hypothesis that there is no difference in the number of positive and negative deviations when the difference between the final and initial thresholds was calculated. A consistent trend for final aggressive thresholds to be larger than initial aggressive thresholds would constitute evidence for habituation.

I was also interested in testing whether initial aggressive thresholds differed depending on whether the playback stimulus was an advertisement or aggressive call. I used a Wilcoxon matched-pairs signed ranks test to test the null hypothesis that there is no difference in the magnitude of aggressive threshold levels in response to the aggressive and advertisement call stimulus. I only compared initial aggressive thresholds for this test. Finally, I examined the effect of playback order and tested whether males responded differently depending on whether they were first exposed to the aggressive call or the advertisement call playback. I used Wilcoxon–Mann–Whitney (WMW) tests to compare the initial aggressive thresholds and any change in aggressive thresholds to the two different call types between the different playback orders. Statistical tests were performed with SPSS 16.0.1 (SPSS Inc., 2007) software on a PC computer. All statistical tests were carried out at an α=0.05.

RESULTS

Frequency of aggressive calling

Aggressive calls were given throughout the night. Although the modal proportion of aggressive calls in a recording was zero, the majority of recordings contained at least one aggressive call and the median proportion of aggressive calls was 12.1% (Figure 2). In 17.3% of recordings, over 50% of males' calls were aggressive. A linear least-squares regression analysis showed that the proportion of calls which were aggressive declined significantly with time of night (N=104, R^2=0.038, P=0.049, $y = -1.527 \times 10^{-5} x + 1.407$, where x is the time in seconds from 0000 hours), although time of night only explained 3.8% of the variation in levels of aggressive calling (Figure 3). The proportion of calls which were aggressive declined from 20.6% for recordings made between 2000 and 2100 hours to 5.1% for recordings made between 2300 and 2400 hours.

Advertisement call playback

Males showed a habituation response following presentation of suprathreshold advertisement calls (Figure 4). The mean initial aggressive threshold of males to the advertisement calls was 67.2 dB (+2.7, -3.9 dB). Following playback of suprathreshold advertisement calls, the mean final aggressive threshold of males to the advertisement call was 72.7 dB (+2.0, -2.6 dB). This increase in aggressive threshold following suprathreshold stimulation was significant (WSR, *N*=24, *z*=-2.53, *P*=0.012). Sixteen of 24 individuals increased aggressive thresholds in response to suprathreshold stimulation, and five individuals showed no change in threshold. The proportion of individuals habituating to suprathreshold advertisement calls was significant (Sign test, *N*=24, *P*=0.027).

Aggressive call playback

Males did not habituate to suprathreshold stimulation in the form of a highly aggressive call. In fact, males appear to be sensitized by aggressive calls: males significantly decreased their aggressive thresholds following suprathreshold stimulation by this call type (Figure 4). The mean initial aggressive threshold of males to the aggressive call was 70.0 dB (+3.0, -4.6 dB). Following playback of suprathreshold aggressive calls, the mean final aggressive threshold of males to the aggressive call was 61.0 dB (+1.8, -2.2 dB). This decrease in aggressive threshold following suprathreshold stimulation was significant (WSR, *N*=18, *z*=-2.63, *P*<0.01). Fourteen of 18 individuals decreased aggressive thresholds in response to suprathreshold stimulation, and three individuals showed no change in threshold. The proportion of individuals sensitized by suprathreshold aggressive calls was significant (Sign test, *N*=18, *P*<0.001).

Initial thresholds to different call types

There was no evidence that the initial aggressive thresholds to advertisement and aggressive calls differed (WSR, *N*=18, *z*=-0.524, *P*>0.5; Figure 4). Although males responded in quite different ways to suprathreshold stimulation by the two call types, their initial responses were similar.

Effects of playback order

Although direct experimentation is required to rule out effects of playback order (e.g., Brenowitz & Rose 1994), I found no evidence that a male's aggressive threshold to one call type was influenced by previous exposure to the other call type. There was no statistically significant difference in initial aggressive thresholds to either the advertisement (WMW, *N*=16, *n*'=8, *U*=51, *P*=0.425) or aggressive call (WMW, *N*=12, *n*'=7, *U*=33.5, *P*=.471) between the two playback orders. Furthermore, there was no statistically significant difference in the change in aggressive threshold to either the advertisement (WMW, *N*=16, *n*'=8, *U*=54, *P*=0.539) or aggressive call (WMW, *N*=12, *n*'=7, *U*=35, *P*=0.924) between the two playback orders. Thus, the responses of males to the two different playback types appear to be independent of playback order.

DISCUSSION

High levels of aggressive calling were observed in *D. ebraccatus*. Repeated bouts of aggressive calling took place throughout the nightly calling period. With the exception of Stewart and Rand (1992), most descriptions of anuran calling in other species imply much lower levels of aggressive calling than that seen in *D. ebraccatus* (Wells 1988a). Studies of gross temporal patterns of calling within the chorus suggest that for many species, aggressive calling primarily takes place in the initial stages of nightly chorus formation and calling stabilizes to nearly pure advertisement calling thereafter (Wells 1988a). The gross temporal distribution of aggressive calling I observed in *D. ebraccatus* does not match this pattern (but see Wells and Bard 1987). Although levels of aggressive calling did decline as the night progressed, a relatively large proportion of male calls were aggressive throughout the night, even near the end of the nightly calling period.

Behavioral habituation is a decrement in response to repeated stimulation (Thompson and Spencer 1966). Habituation has been invoked as an explanation of the relative lack of aggressive calling following initial chorus formation that has been observed in many species (Marshall et al. 2003). In these species, males give aggressive calls in the early stages of chorus formation because neighbors are calling louder than their initial aggressive thresholds. Later in the evening, however, males habituate to the repeated calls of neighboring individuals, resulting in increased aggressive thresholds and little aggressive calling. This has been demonstrated convincingly in *P. regilla*: males show habituation responses to all call types (Rose and Brenowitz 1997), and choruses are stable, with only rare bouts of aggressive calling beyond the initial stages of chorus formation (Allan 1973). Because male *D. ebraccatus* continually give aggressive calls throughout the night, my primary prediction in this study was that males would not habituate to suprathreshold stimulation.

Indeed, male *D. ebraccatus* did not always habituate to suprathreshold stimulation. As with previous studies of aggressive thresholds, males did habituate to the advertisement call by increasing their aggressive thresholds following suprathreshold stimulation. In response to aggressive calls, however, males actually decreased their aggressive thresholds. I consider this an example of sensitization, an effect that has been documented infrequently in anuran communication (Bee 2001). I had predicted that response sensitization was more likely to be observed in response to the aggressive call stimulus. The aggressive call is a stronger stimulus in terms of eliciting an aggressive calling response: males are more likely to give aggressive calls in response to playbacks of aggressive calls compared to playbacks of advertisement calls (Wells and Greer 1981). Thus, if response to repeated stimulation in this species follows the dual-process theory, the aggressive call, but not the advertisement call, presumably has a stronger effect on the sensitization process than on the habituation process, resulting in the sensitization response of a decreased aggressive threshold. This pattern of response may explain the extreme level of aggressive calling often heard in *D. ebraccatus* choruses, just as the habituation response has been suggested to be responsible for the lack of sustained aggressive calling in choruses of other species (Marshall et al. 2003). Clearly, studies on other species are needed to firmly establish this pattern, but I suggest that for species in which aggressive calling takes place frequently throughout the chorusing period, males are likely to be sensitized by aggressive calls and possibly to advertisement calls as well. However, as discussed below, it is unclear why males would have a mechanism that does not allow them to habituate to all call types.

Habituation in this scenario implies an increased tolerance to the stimulus that initially elicited an aggressive response after repeated, suprathreshold presentation of that stimulus. Although few studies directly measure the thresholds that evoke an aggressive response in frogs, several studies involving repeated stimulus presentation have found that males habituate over time and their aggressive response decreases in response to repeated presentation of a stimulus (Megela and Capranica 1983, Brenowitz and Rose 1994, Rose and Brenowitz 1997, Owen and Perrill 1998, Bee 2001, Marshall et al. 2003). In the most extensively studied species, *P. regilla*, males habituated to both advertisement and aggressive calls, although habituation was slow in response to aggressive calls presented at a very high magnitude (Brenowitz and Rose 1994, Rose and Brenowitz 1997).

Plasticity of aggressive thresholds in the form of habituation has been interpreted as an adaptive response to adjust levels of aggression to varying chorus densities. In particular, if thresholds were fixed, at high densities, males would repeatedly engage in aggressive interactions with nearby neighbors. This in turn could severely reduce a male's ability to attract a mate because females are less attracted or repelled by aggressive calls (Oldham and Gerhardt 1975, Schwartz and Wells 1985, Wells and Bard 1987, Backwell 1988, Grafe 1995, Brenowitz and Rose 1999). Thus, in situations where reductions in spacing are unavoidable, the ability to raise thresholds to neighboring males' calls allows males to balance the ability to defend their calling space while maximizing their time spent giving attractive signals to females.

A similar argument could be made for the response to advertisement calling I observed in male *D. ebraccatus*. In this species, aggressive calls are less effective in attracting females than advertisement calls (Wells and Bard 1987). In two-choice phonotaxis tests, females preferred advertisement calls to aggressive calls and although females showed phonotaxis towards aggressive calls in two-choice tests in which both alternatives were aggressive calls, they responded at a much lower rate to these tests than to tests involving advertisement calls (Wells and Bard 1987). The latter result indicates a lower general motivation to respond to aggressive calls. Therefore, habituation in response to advertisement calls in *D. ebraccatus* would seem a reasonable response to avoid the heavy costs in terms of reduced attractiveness to females of unnecessary extended aggressive calling interactions.

This explanation of habituation as an adaptive response to changes in chorus density does not hold for *D. ebraccatus* once other aspects of its call repertoire are examined. In response to the aggressive call, I observed a decrease in the aggressive threshold following suprathreshold stimulation relative to the initial threshold. Thus, suprathreshold aggressive calls have a sensitizing effect on males: they became more willing to engage in aggressive calling following exposure to loud aggressive calls. Sensitization has not been documented previously in studies of aggressive thresholds in anurans but has been described in the response of *Lithobates catesbianus* to repeated presentations of conspecific advertisement calls at constant intensity (Bee 2001). Similar sensitization responses have been observed in species in other taxa including three-spined sticklebacks, *Gasterosteus aculeatus*, (Peeke 1982) and white-crowned sparrows, *Zonotrichia leucophrys* (Petrinovich and Patterson 1981).

Not only does the direction but also the absolute magnitude of the response to suprathreshold stimulation in *D. ebraccatus* differs from that seen in other species. When aggressive thresholds have been measured in other species, aggressive thresholds to aggressive calls were lower than those to advertisement calls (Robertson 1984, Lopez et al. 1988, Rose and Brenowitz 1991). In this study, by contrast, the initial aggressive thresholds to advertisement and aggressive calls in *D. ebraccatus* did not differ. Thus, male *D. ebraccatus* appear to have equal initial tolerances for advertisement and aggressive calls. Repeated suprathreshold stimulation may be necessary for differences in the aggressive response to aggressive calls relative to advertisement calls to emerge, particularly at the somewhat low sound pressure levels used in this study.

Male *D. ebraccatus* clearly are sensitized by exposure to conspecific aggressive calls, and this mechanism may partially explain why they are observed to engage in such high levels of aggressive calling. It is important to keep in mind that the responses described here as habituation and sensitization are short-term measures of the responsiveness to a single presentation of repeated suprathreshold stimulation. The strengths of the habituation and sensitization processes are known to change over time; in particular, the sensitization process generally shows an initial increase but ultimately a decrease in response. The goal of this study was to measure short-term aggressive threshold plasticity, but in order to fully explain gross temporal patterns of the levels of aggressive calling, longer-term stimulus presentations will be necessary.

Nonetheless, my data suggest that complete response habituation does not take place and that sensitization is important in determining the level of aggressive calling in *D. ebraccatus*. This raises some difficult questions. First, if males are sensitized by aggressive calls, it is unclear how advertisement calling resumes once males begin giving aggressive calls. The cyclical nature of *D. ebraccatus* choruses may be a clue. The pattern of chorusing in this species can be roughly described as unison-bout chorusing, in which bouts of calling are separated by bouts of silence (Rosen and Lemon 1974, Whitney and Krebs 1975, Schwartz and Wells 1983a, Schwartz 1991). Unison-bout chorusing has been well described in the closely related *Dendropsophus microcephalus*, although the use of different call types within call bouts was not described for this species (Schwartz and Wells 1983a, Schwartz 1991). Within bouts of calling in *D. ebraccatus*, callers and the chorus as a whole typically progress from initially giving advertisement calls to almost entirely giving aggressive calls to silence (personal observation). The strength of sensitization is known to decay over time with the lack of stimulation (Thompson and Spencer 1966, Thompson et al. 1973). Thus, although it is not clear what cues these transitions, the period of silence should result in reducing the effect of sensitization, allowing males to resume advertisement calling when the chorus restarts.

The more difficult question is why males are sensitized by aggressive calls. Aggressive calling is unquestionably costly. Females are far less responsive to aggressive calls than to advertisement calls, so males would be expected to reduce their levels of unnecessary aggressive calling (Oldham and Gerhardt 1975, Schwartz and Wells 1985, Wells and Bard 1987, Backwell 1988, Grafe 1995, Brenowitz and Rose 1999). Aggressive calling may have additional energetic costs. Energetic costs of calling have been implicated as limiting factors in the ability of males to attract females in several species (Ryan 1988b). Although the mechanism of call production and the energetic costs of calling are not known for *D. ebraccatus*, their aggressive calls have a similar structure and rate of production but are generally of longer duration than advertisement calls. Thus, it is possible to speculate that producing a typical aggressive call involves a greater calling effort than an advertisement call, although this will depend on whether the mechanism of amplitude modulation is shared by both call types. Absolute energy expenditure increases with call effort in a variety of frog species (e.g., Bucher 1982, Taigen and Wells 1985, Wells and Taigen 1986, Wells and Taigen 1989, Grafe 1996); therefore, the aggressive calling of *D. ebraccatus* could conceivably be more energetically expensive than advertisement calling. All speculation aside, even if aggressive calls are less costly than advertisement calls, they undoubtedly have nontrivial energetic costs, and I suggest that such calling is energetically costly because it reduces the energy budget available for advertisement calling that is far more effective at attracting females.

In addition to these costs, it is not clear that there are any strong benefits to aggressive calling in this species. In extensive field observations, I have observed very few adjustments in male spacing following an aggressive calling interaction. Both males generally resume normal advertisement calling after a short amount of time. Thus, aggressive calls appear ineffective in their presumed function of repelling rival males, although more subtle changes in calling behavior following an aggressive interaction have not been examined. Additionally, physical fights are extremely rare, of brief duration, and do not appear to entail a significant risk of injury to either combatant.

We do not understand the communicative significance of the aggressive call or its potential use in the resolution of disputes in this species, but based on the available evidence, it does not seem that males benefit from high levels of aggressive calling. Thus, it is difficult to explain why a sensitizing mechanism, which seems to increase the likelihood of aggressive calling, would be present. It is possible that the sensitization response is simply a consequence of the general stimulus–response system with the aggressive call being particularly effective at producing a strong change in state, as predicted by the dual-process theory (Thompson et al. 1973), and was not selected for its role in adjusting the aggressive thresholds of males to changing social conditions. A sensitization response to aggressive calls combined with high chorus densities could conceivably result in the gross temporal patterns of aggressive call use observed in *D. ebraccatus*. The cyclical nature of chorusing may in part be an adaptation to allow sensitization to decay following the inevitable bouts of aggressive calling. Conceivably, this pattern could maximize, to the extent that is possible, the time spent advertisement calling. These conclusions would be improved by a better general understanding of the function of aggressive calling in this, and other, anuran species.

Table 1. Properties of synthetic stimuli used in playback experiments

	Synthetic stimuli		**Natural calls**		
	Advertisement	**Aggressive**	**Advertisement average**	**Aggressive average**	**Aggressive range**
Total call duration (ms)	301	427	245.2 (50.0)	350.7 (70.6)	138-587
Introductory note duration (ms)	175	300	169.8 (18.4)	143.7 (32.8)	79-399
Pulse number	17	72	16.4 (1.6)	49.5 (17.4)	19-122
Pulse rate (pulses/second)	97.1	240	96.9 (3.3)	340.0 (112.6)	202-565
Dominant frequency (kHz)	3.1	3.1	3.05 (0.13)	3.04 (0.22)	2.54-3.51
Number of click notes	1	1	0.60 (0.35)	1.63 (0.54)	0-4

For comparison, the average values of these call parameters from recordings made in 2007 of naturally calling males are also shown (advertisement call, *N*=67; aggressive call, *N*=38; columns show mean (standard deviation)). Values chosen for the call properties were based on previous recordings and were designed to represent a typical advertisement call and an aggressive call characteristic of a highly escalated interaction. Many of the characters of aggressive calls are graded (see text for details) and mean values do not correspond to those of a highly aggressive call. Thus, I also show the range of values for these call parameters in recordings of natural aggressive calls.

Figure 1. Waveform displays showing change in amplitude over time (in ms) of typical *D. ebraccatus* advertisement and aggressive calls. **a** Advertisement call with long introductory note and a single click note. **b** Aggressive call with single click note typical of a highly escalated interaction. As level of aggression escalates, aggressive calls increase in duration, while decreasing in pulse rate and number of click notes (Wells and Schwartz 1984b).

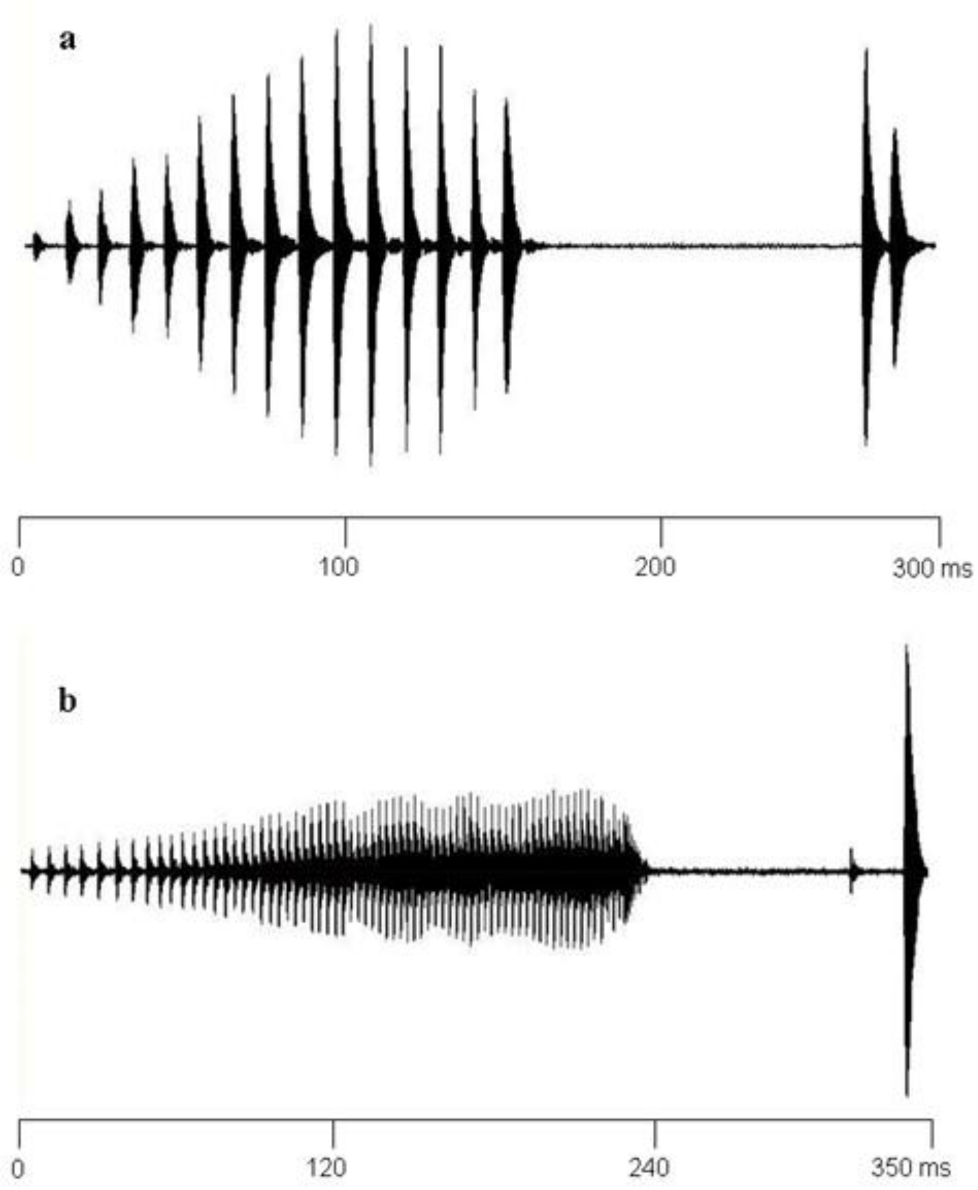


Figure 2. Percentage histogram showing the proportion of calls given by males during baseline recordings that were aggressive calls. Bin size=0.05, *N*=104.

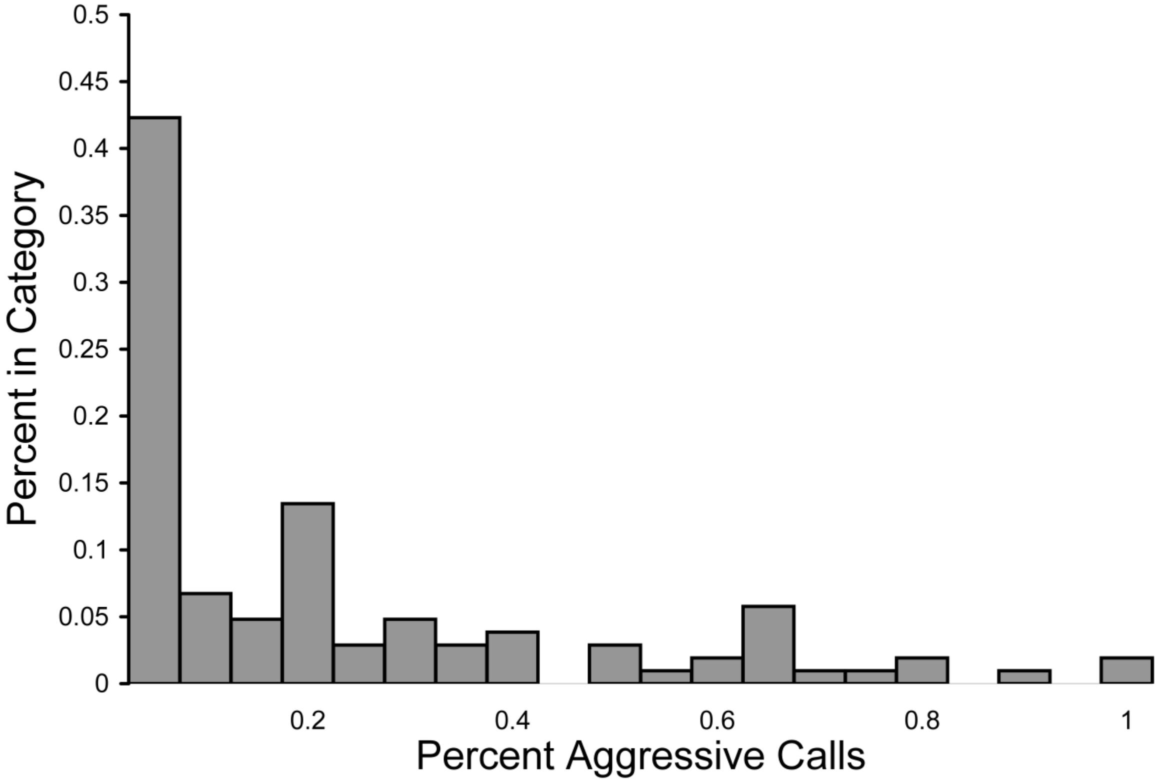


Figure 3. Scatterplot showing the proportion of calls during baseline recordings that were aggressive calls versus time of night in which the recording was made. Trend line was generated by a linear least-squares regression.

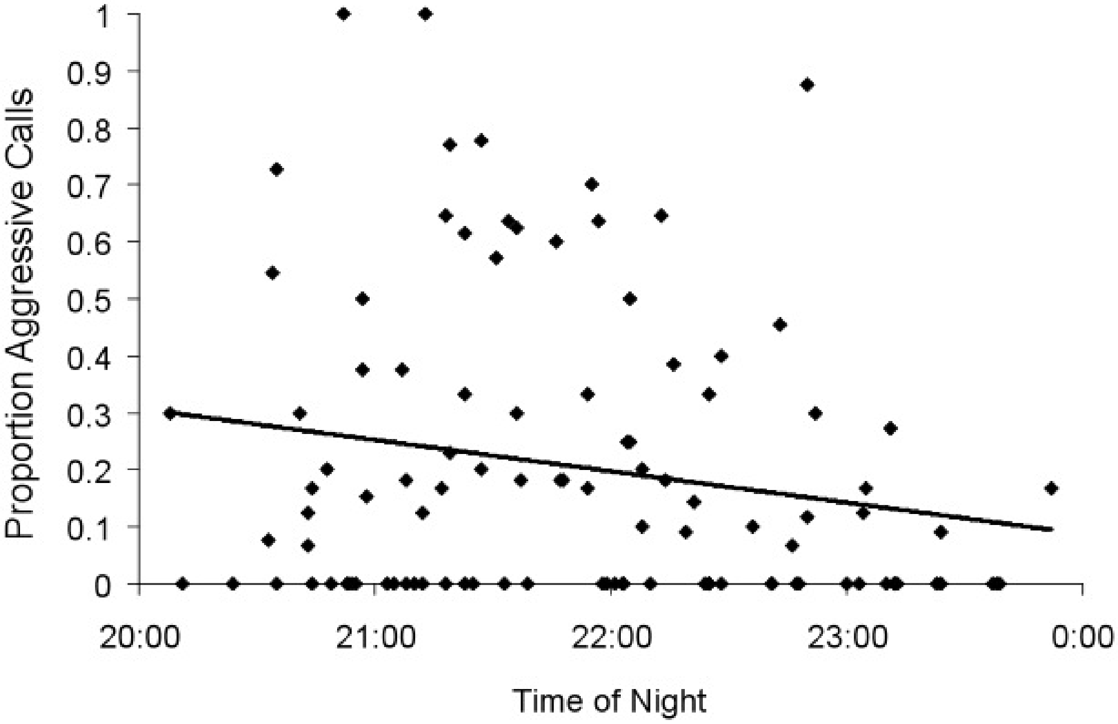
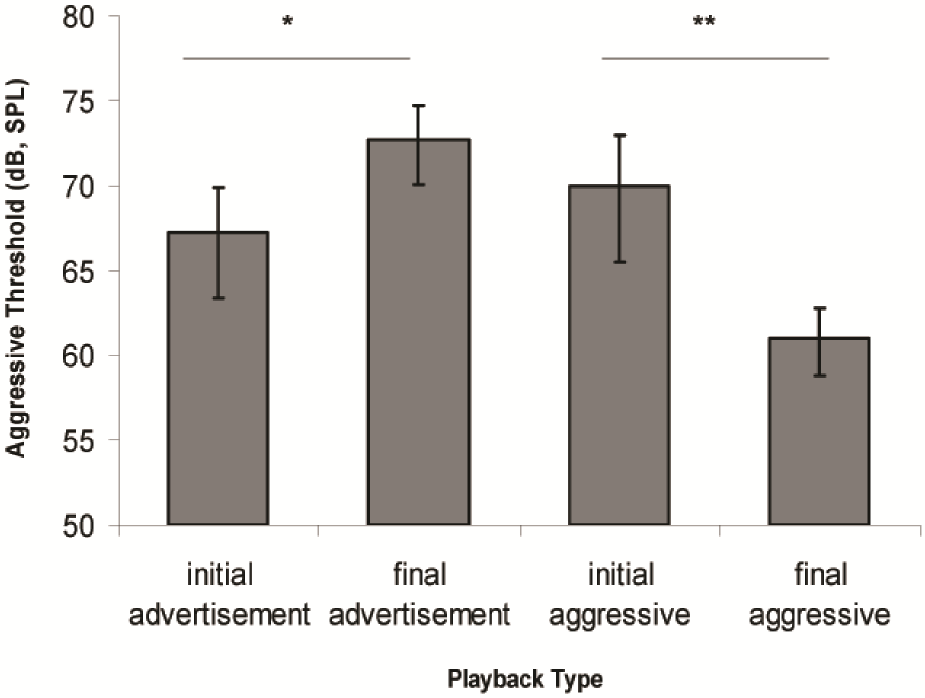


Figure 4. Mean aggressive thresholds for each playback type (advertisement, *N*=24; aggressive, *N*=18). Means and standard errors were converted into dB, SPL after being calculated from absolute pressures in µPa (see text); therefore, standard errors are asymmetric about the mean. *Horizontal bars* show significant differences between initial and final aggressive thresholds for each call type. Wilcoxon signed ranks test, *, *P*<0.05, **, *P*<0.01. There was no significant difference between the initial aggressive thresholds to the advertisement and aggressive call (WSR, *P*>0.5).



CHAPTER 3

Effects of multiple-speaker playbacks on aggressive calling behavior in the treefrog *Dendropsophus ebraccatus*

ABSTRACT

In addition to producing signals, males of chorusing species also act as receivers by adjusting properties of their vocalizations in response to those of other nearby individuals. Although it is likely that males are responsive to more than one other individual, most playback studies investigating male response have involved dyads in which vocal responses are measured to stimuli presented from a single speaker. In this study, I explored changes in both the propensity to give aggressive calls and the temporal properties of those calls in response to the playback of multiple aggressive call stimuli in the treefrog *Dendropsophus ebraccatus*. I found that males were sensitive to both the number of simulated aggressive callers and their specific call characteristics. Males generally gave a highly aggressive response to the first stimulus presented, but their response to the modification of this stimulus by the addition or subtraction of a simulated competitor depended on the degree of aggressiveness of the stimuli. Males tended to decrease their aggressive responses when either a more aggressive call was silenced or a less aggressive call was added and to increase their aggressive responses in the opposite situation. Aggressive calling in this species is clearly affected by complex

changes in the social environment and I suggest that future studies explore these issues in other species to improve the understanding of communication interactions.

INTRODUCTION

The choruses of anurans provide one of the best examples of communication networks (Grafe 2005), in which communication takes place in the presence of multiple signalers and receivers (McGregor and Peake 2000). Within the chorus, males produce conspicuous vocalizations to attract females, but at the same time respond to vocal competition from nearby males. Males function as both signalers and receivers in this context and thus are expected to adjust properties of their vocalizations as the level of acoustic competition changes. Single-speaker playback tests have established that males in many species react to variation in stimulus call properties by altering properties of their own calls (e.g., Wells and Greer 1981, Wells and Taigen 1986, Lopez et al. 1988, Benedix and Narins 1999). Although demonstrating that males are responsive to variation in other males' signals, these studies have often oversimplified the social context in which most communication takes place. In a high density chorus, males are frequently surrounded by many nearby vocalizing males. While studies of female mate choice in anurans have frequently considered the effects of the presence of multiple signalers on response, very few experiments have been conducted in which male response is measured during the presentation of multiple stimuli (Brush and Narins 1989, Schwartz 1993, Greenfield and Rand 2000, Schwartz et al. 2002). A better understanding of male calling behavior in response to vocal competition in more complex situations requires the presentation of multiple stimuli and the simultaneous monitoring of a male's vocal response. In this study, I describe the results of several two-speaker playback tests in which I explored the effects of multiple aggressive stimuli on aggressive calling responses in the neotropical treefrog *Dendropsophus ebraccatus*.

Although recent studies have emphasized the importance of understanding communication networks, even those studies often only considered the effects of a dyad of signalers (but see e.g., Rogers et al. 2004, Mennill 2006, Molles and Waas 2006). For example, social eavesdropping models consider situations in which two individuals signal to each other while the eavesdropper passively observes (Peake 2005). In many communication situations, interactions are not dyadic for either signalers or receivers. In these cases, two-speaker playbacks to a signaling individual represent an initial step in the increase in complexity necessary to examine communication when multiple signalers and receivers are present. These tests will necessarily be more complicated due to the number of stimulus combinations that must be compared with each other. Nonetheless, this playback design will reveal how males respond to multiple competitors and thus give new insights into how males respond to vocal competition. Such studies can also identify the particular features of calls that are important in mediating competition in the complex networks in which communication takes place.

Many anuran species have multiple call types in their repertoires (Wells 1977). The most frequently heard call type is usually the advertisement call, which is the primary call used to attract females. Many species also have a distinct aggressive call that appears to function to mediate male–male aggressive interactions. *D. ebraccatus* is unusual among anurans in producing a large number of aggressive calls (Reichert 2010). In addition, these aggressive calls are by no means limited to occasional close-range dyadic interactions between two males, but instead are given frequently and repeatedly throughout the nightly calling period, and often to no obvious target (personal observation). Sometimes all of the calling males in a chorus produce only aggressive calls for a short period of time. These observations suggested that males in this species are likely to be responsive to the aggressive calls of multiple surrounding males.

Another advantage of this system is that the aggressive call in this species is highly variable, with variation in certain call characters thought to be related to the degree of aggression of the call (Wells and Schwartz 1984b, Wells 1989). While increasing the degree of aggressiveness in the call is presumably more effective at repelling rival males, females are more attracted to low- rather than high-level aggressive calls (Wells and Bard 1987). Thus, males may be forced to balance simultaneous pressures from multiple aggressive callers. On the one hand, males are expected to reduce the aggressiveness of their calls in the presence of a less aggressive caller in order to remain relatively attractive to females. On the other hand, males may be expected to increase the aggressiveness of their calls in order to remain competitive in intrasexual competition in the presence of a highly aggressive caller. In addition, females are more attracted to advertisement calls than aggressive calls (Wells and Bard 1987); thus, in some situations it may be beneficial for males to adjust their overall output of aggressive calls to the level of aggression of nearby neighbors. Therefore, I tested not only whether the presence of multiple simulated aggressive males had an effect on male aggressive vocal response but also how males balanced their aggressive response to specific combinations of the aggressiveness levels of these simulated males. This study was not a systematic attempt to examine aggressive vocal response of *D. ebraccatus* to all possible combinations of relevant stimuli. Rather, I view this study as an initial exploration of how aggressive responses are affected by the simultaneous presentation of multiple playback stimuli and changes in stimulus presentation that mimic relevant events in the social environment in which aggressive calling takes place.

METHODS

Study system

I studied the calling behavior of *D. ebraccatus* at the Smithsonian Tropical Research Institute field station in Gamboa, Panama. Males call nightly from elevated perches on emergent vegetation in and around marshes and ponds. I performed field playback tests with naturally calling males at Bridge Pond, which typically contained a high density of calling males. All playback tests took place between 2000 and 2400 hours, the time of the most intense calling activity. I conducted experiments 1-3 from June to August 2006 and experiment 4 from July to September 2007.

This study focused on the aggressive call which, although superficially similar in structure to the advertisement call, has a higher pulse rate and (usually) a longer introductory note (Wells and Schwartz 1984b). There is no gradation between the pulse rates of advertisement calls and aggressive calls. The aggressive call consists of a long, pulsed introductory note that is usually followed by one or more click notes (Figure 1). Several characteristics of the aggressive call vary in concert, and this variation appears to be graded with the degree of aggression (Wells and Schwartz 1984b, Wells 1989). Specifically, as aggression increases, males increase the duration of their introductory notes, decrease the number of click notes, and decrease the pulse rate of their aggressive calls (Figure 1; Wells and Schwartz 1984b). In single-speaker playback tests using synthetic aggressive calls, males were shown to be responsive to variation in these characteristics, increasing the level of aggression of their own aggressive calls in response to increases in aggression of stimulus calls (Wells and Schwartz 1984b, Wells 1989). It is not clear how, or even if, variation in aggressive calls is related to the outcome of aggressive interactions in this species. Aggressive calls are given during close-range agonistic interactions and fights, but these are extremely rare (Schwartz and Wells 1984; personal observation). Nonetheless, aggressive calling interactions outside of these contexts are common and likely to be an important component of acoustic competition in this species.

Playback procedure

I generated synthetic *D. ebraccatus* calls using a custom sound synthesis program provided by J. Schwartz (16-bit digital files, 20 kHz sampling rate). Values of call characteristics were chosen to mimic aggressive calls at low, medium, and high levels of aggression, and were based on analyses of previous recordings from this population

(Table 1; Wells and Schwartz 1984b; Reichert, unpublished data). Hereafter, these stimuli will be referred to as "low", "medium", and "high". All stimuli were presented repeatedly with a period of six seconds. When stimuli were presented from two speakers, they were presented antiphonally in order to avoid potential effects of call overlap on male response, and to mimic the natural call timing of aggressive calling interactions in which overlap is rare (Schwartz and Wells 1984). I used an audio-editing computer program (CoolEdit Pro 2.0, Syntrillium 2002) to generate the appropriate call period and spacing between calls on different tracks. The specific stimuli used in each playback depended on the experiment, but all experiments shared the following general structure. Vigorously calling males were chosen as playback subjects, and any other calling individuals within 2 m were removed in order to ensure that the male’s vocal response was to the playback stimuli and not to another frog. I did not begin playbacks for at least 5 min after removing nearby males in order to allow the subject male to resume normal calling, and to allow potential effects of previous experience to dissipate. Playbacks were broadcast from two portable, amplified speakers (Mineroff Electronics, SME-AFS) placed at approximately a 120 degree angle from each other and 1 m from the subject male. Playback stimuli were created as stereo tracks on a compact disk. Stimuli were played back through a compact disk player (Panasonic SLSW940S) via a stereo audio cable so that each track was broadcast through only one speaker. Prior to performing a playback, I used a portable sound-pressure-level meter (Radio Shack 33-2055) to ensure that the SPL outputs of each speaker were identical. Each playback consisted of two halves of 5 min each; the specific stimulus broadcast during each half of the playback depended upon which experiment I was conducting (Table 2). I switched speakers between tests to ensure that male response was not due to any directional bias. During playbacks, I used an audio recorder (Marantz PMD-660) and a directional microphone (Sennheiser ME-67) to record all vocal responses of the male to the playback. Afterwards, I captured the male and gave him a unique toeclip to ensure individual identification.

Experiment 1

The degree of aggressiveness of neighboring males may affect the subsequent aggressive vocal response by a male to an intruder. To test this, I first simulated a distant aggressive calling interaction with different stimuli broadcast from each speaker (both broadcast at 90 dB). I then simulated an "intrusion" onto the focal male's calling space by one of the simulated individuals by increasing the SPL of the playback from that speaker while the other speaker was silenced (Table 2). Here, I am assuming that the increase in SPL represents a simulated male moving towards the focal male rather than simply adjusting its orientation. The large increase in SPL of the "intruding stimulus" makes this a reasonable assumption, although in either case a louder competitor likely represents a greater threat and source of interference than a quieter competitor. The "intruding" stimulus presented in the second half of the playback was the same in all cases (100 dB medium aggressive call), but males differed in whether this stimulus was paired with a more (high aggressive call) or less (low aggressive call) aggressive stimulus in the first half of the playback. The primary comparison of interest was between the 100 dB medium calls that previously had been paired with stimuli with different degrees of aggressiveness. I hypothesized that males would respond differently to the same intruding stimulus if previously they had heard it paired with a less aggressive call than if they had heard it paired with a more aggressive call.

Experiment 2

This experiment was identical to experiment 1 except that during the second half of the playback, the non-intruding speaker was not silenced, but rather continued to broadcast at 90 dB (Table 2). As before, the intruding speaker broadcast the medium aggressive call at 100 dB. This experiment, when combined with the results of experiment 1, allowed me to investigate the importance of increases in SPL, along with changes in the number of aggressively calling males, on aggressive vocal response. This experiment may more realistically simulate natural chorus dynamics. After the two speakers “interacted” with each other during the first half of the playback, the second half simulates a situation in which the medium caller intrudes on the focal male (increases in SPL) while the other caller remains in place (no change in SPL). I predicted that males’ responses in the second half of this experiment would not be affected by the presence of the less intense stimulus and that males’ responses would mirror those seen in experiment 1.

Experiment 3:

In this experiment, I determined the importance of previous stimulation by simulated multiple calling males on the response to a louder, single stimulus (Table 2). I first presented males with a medium call broadcast at 90 dB from a single speaker, or medium calls broadcast in alternation at 90 dB from two speakers. In the second half of these playbacks, all males received the same stimulus: a medium aggressive call broadcast at 100 dB from a single speaker. Thus, males differed in whether the 100 dB medium call was preceded by either one or two speakers broadcasting the 90 dB medium call. I hypothesized that males would respond differently to the identical 100 dB medium call presented during the second half of the playback depending on the number of simulated calling males presented during the first half of the playback.

Experiment 4:

In this experiment, I tested the importance of changes in the number of calling males on a male's aggressive response. Further, I tested the effects of these changes with different combinations of stimulus aggressiveness (Table 2). Single stimuli were used to simulate a single nearby calling male at one of three degrees of aggressiveness: low, medium, and high aggression. Double stimuli simulated two nearby calling males broadcast from different speakers and were made up of all possible combinations of non-identical single stimuli (low-medium, low-high, medium-high). In the *increasing* treatment, the first half of the playback consisted of the broadcast from a single speaker of one of the single stimuli for 5 min. Immediately afterwards, I began playback of one of the double stimuli from two speakers for an additional 5 min. This simulated an increase in the number of nearby calling males. The aggressiveness level of the single stimulus call remained the same during the second half; it was joined by a call of a different aggressiveness level. Thus, there were six total combinations of stimulus presentation for the first and second halves of this playback (Table 2). The *decreasing* treatment used the same sets of stimuli with the same six possible combinations as the increasing treatment, but presented stimuli in reverse: the double stimulus was presented first followed by the single stimulus (Table 2). This simulated a decrease in the number of nearby calling males. All stimuli in both treatments of this experiment were presented at 100 dB SPL.

Call analyses

I measured the following call variables for each call given during the recording of each male’s response to playbacks: call type (aggressive or advertisement), full call duration (duration of the introductory note and any secondary click notes), introductory note duration, number of pulses, number of click notes, and dominant frequency (Figure 1). Call characteristics were measured using sound analysis software (Cool Edit Pro 2.0). From these measurements, I calculated pulse rate (number of pulses per second), call rate (number of calls per second), duty cycle (call rate multiplied by introductory note duration), and the proportion of calls that were aggressive.

Statistical analysis

The general goal of this study was to determine how males' aggressive calls change in response to changes in the number, intensity, and aggressiveness of simulated aggressive neighbors. Previous studies have indicated that multiple call features co-vary with the level of aggression in *D. ebraccatus* (Wells and Schwartz 1984b). Thus, no single call characteristic is an adequate indicator of aggression. I used a principal components analysis to account for the correlations between aggressive call characters and to generate an aggressiveness index that could be used as a single variable representing the level of aggressive response for each male to each playback stimulus. The input variables in the analysis were the following call parameters, averaged over the entire playback for each male used in these experiments: total call duration, introductory note duration, pulse rate, dominant frequency, number of click notes, number of pulses, duty cycle, and call rate. Principal components were extracted from the correlation matrix of these variables (Budaev 2010). I calculated the aggressiveness index for each male's calling by, for each variable, multiplying its corresponding coefficient (coefficient scores given in Table 3; these scores are used to calculate the value of the principal component score for each individual) from the first principal component by the male's standardized mean value of that variable and then summing across variables. This calculation was performed separately for responses to each half of the playback. Because variables were standardized, aggressiveness indices above zero are more aggressive than average while those less than zero are less aggressive than average. As a secondary measure of aggression, I examined the proportion of calls that were aggressive in response to each playback.

Within experiments 1-3, I used repeated measures ANOVAs to compare aggressiveness indices to different playback stimuli as all males were exposed to all stimuli. Post hoc tests were performed to compare levels of aggression between all stimuli presented. In experiment 4, each male was only exposed to one set of playback stimuli within each treatment, although some males were used in both treatments. For this experiment, I first examined the effects of playback stimuli separately for each treatment using the full set of recordings. I used ANOVAs to examine whether there were differences in aggressive response between each of the three single stimuli and each of the three double stimuli. In addition, I compared the difference between the aggressiveness index for each set of stimuli within both the single and double stimulus sets and determined whether there was an overall difference in aggressiveness index between responses to single and double stimuli. In order to determine the effects of treatment itself, I combined the data sets: when males were used in both treatments, I randomly selected whether an individual's response to the increasing or decreasing treatment was included. I used *t* tests to compare the aggressiveness indices to both single and double stimuli. I also used *t* tests to assess the change in aggressiveness index between the single and double stimuli between the two treatments to determine if the order of presentation of single and double stimuli is important.

I performed similar analyses to compare the proportion of aggressive calls that were given to different playback stimuli, but used non-parametric tests because the proportions were not normally distributed, even after transformation. I used an α=0.05 as my criterion for statistical significance and adjusted this value using a sequential Bonferroni correction when multiple comparisons were made. All statistical procedures were performed with the SPSS 16.0.1 (SPSS Inc., 2007) computer program.

RESULTS

Calculation of aggressiveness index

The first principal component explained 36.9% of the variance in the data and loadings were heaviest (component scores farthest from zero; see Table 3) on those call characteristics known to be most related to the aggression level of the aggressive call (Wells and Schwartz 1984b). Remaining components either explained very little variance in the data or had scores that were highest for characteristics that are not known to vary with aggression level such as dominant frequency, a static trait (Wollerman 1998; Reichert, unpublished data).

Experiment 1

Males gave a similar proportion of aggressive calls to the 100 dB medium stimulus regardless of whether that stimulus had been paired previously with the low or high stimulus (Figure 2a; Wilcoxon signed-ranks test, N=22, Z=-0.544, P=0.586). In fact, the proportion of aggressive calls given did not differ between any of the playback stimuli presented (Friedman's test, N=22, χ^2=2.651, df=3, P=0.449), although males tended to give a lower proportion of aggressive calls to the 100 dB medium call than to the preceding 90 dB medium-high playback (WSR, *P*=0.088).

Playback treatment did affect male aggressiveness index (Figure 2d; Greenhouse–Geisser corrected repeated measures ANOVA, $F_{1.92,38.3}$=7.416, *P*=0.002). However, males' aggressiveness indices in response to the identical 100 dB medium aggressive call stimulus by itself did not differ after having heard it paired with either the low or high aggressive call (post hoc, *P*>0.5). Aggressive response to the medium call did not differ from the aggressive response to the low plus medium calls. However, males did respond significantly more aggressively to the high and medium playback than to any of the other three playbacks (Figure 2d; post hoc, all *P* values<0.05).

Experiment 2

Males responded to the different playback stimuli in this experiment by giving different proportions of aggressive calls (Figure 2b; Friedman's test, *N*=17, χ^2=13.3, *P*=0.004). Males gave a significantly higher proportion of aggressive calls in response to either half of the medium-high playback than to the first half of the low-medium playback (WSR, *N*=17, *P*<0.01). The proportion of aggressive calls given to the second half of the medium-high playback was also greater than the proportion given in response to the first half of the medium-high playback (*P*=0.028) and the proportion given to the second half of the low-medium playback (*P*=0.046), although neither of these differences were significant following a Bonferroni correction.

Playback treatment did affect male aggressiveness index in experiment 2 (Figure 2e; Greenhouse–Geisser corrected repeated measures ANOVA, $F_{1.84,29.49}$=6.671, P=0.005). Despite an increase in amplitude of the medium call during the second half of the playback, the aggressiveness index did not differ between the first and second halves of playbacks for either the low-medium or medium-high playbacks (Figure 2e; post hoc, P>0.5). Males' calls were much more aggressive to both halves of the medium-high playback than to either half of the low-medium playback (post hoc, P's<0.05, Figure 2e).

Experiment 3

The proportion of aggressive calls given by males in this experiment was affected by the type of playback stimulus presented (Figure 2c; Friedman's test, N=18, χ^2=13.0, P=0.005). The proportion of aggressive calls given was lower to the single medium call at 90 dB than to any of the other three stimuli (WSR, P<0.05 for all). The other three stimuli did not differ in the proportion of aggressive calls that they elicited.

The aggressiveness indices differed depending on the playback stimulus that was presented in experiment 3, although this difference was not significant following a Greenhouse–Geisser correction (Figure 2f; repeated measures ANOVA, $F_{1.56,\ 21.72}$=3.433, P=0.061). The aggressiveness index to the 100 dB medium call did not differ when this stimulus was preceded by either one or two speakers broadcasting the call at 90 dB (post hoc, P>0.05). Males' calls were less aggressive to the single medium call at 90 dB than to the following 100 dB medium call (post hoc, P=0.009). Differences between aggressiveness indices to the remaining stimuli were not significantly different (post hoc, *P*>0.05).

Experiment 4 increasing treatment

Overall, males gave a significantly higher proportion of aggressive calls in response to the second half of the playback, in which aggressive calls were broadcast from two speakers, than to the first half of the playback, in which aggressive calls were broadcast from only one speaker (Fig 3a; WSR, *N*=44, *z*=-2.663, *P*=0.008). The proportion of aggressive calls given to each of the three single stimuli did not differ (Figure 3b; Kruskal–Wallis, χ^2=1.754, *P*=0.416). In response to the double stimuli, males gave a higher proportion of aggressive calls in response to the low-high than to the low-medium stimuli (Figure 3c; Wilcoxon–Mann–Whitney test, *z*=-2.686, *P*=0.026). Responses to the other double stimuli did not differ.

On average, males did not increase their aggressiveness index from the first half of the playback, in which a single stimulus was played, to the second half of the playback, in which a double stimulus was played (paired *t* test, *df*=43, *t*=-0.237, *P*=0.814). However, when individual sets of stimuli are examined, males did change the aggressiveness of their calls from the first to the second half of the playback; the direction of change depended on whether the initial call was joined by a more or less aggressive call (Figure 3d). Males decreased their aggressive response during the second half of the playback when the initial stimulus was joined by a stimulus that was less aggressive (one-sample *t* test, *df*=19, *t*=-2.622, *P*=0.017). By contrast, males increased their aggressive response during the second half of the playback when the initial stimulus was joined by a stimulus that was more aggressive (one-sample *t* test, *df*=23, *t*=2.591, *P*=0.016). In response to the single stimulus, males' aggressive calls tended to increase in aggressiveness with the aggressiveness of the stimuli (Figure 3e; ANOVA– $F_{2,41}$=3.585, *P*=0.037), although only the difference between the low and high calls was significant (post hoc, *P*=0.034). Males did not respond differently to any of the double stimuli (Figure 3f; ANOVA, $F_{2,41}$=0.713, *P*=0.496).

Experiment 4 decreasing treatment

Overall, males gave a significantly higher proportion of aggressive calls in response to the first half of the playback, in which a double stimulus was played, than to the second half of the playback, in which a single stimulus was played (WSR, *N*=56, *z*=-3.220, *P*=0.001). All six of the combinations of playback stimuli exhibited this decrease during the second half of the playback (Figure 4a). There were no differences in the proportion of aggressive calls given when comparisons were made within single (Figure 4b) and within double stimuli (Figure 4c).

Males' calls had a higher aggressiveness index in response to the first half of the playback, in which a double stimulus was played, than to the second half of the playback, in which a single stimulus was played (paired *t* test, *df*=55, *t*=-9.865, *P*<0.0005). The decrease in aggressiveness index to single stimuli took place in all six stimulus pairs (Figure 4d). Males showed a greater decrease in aggressiveness index to the three combinations of stimuli that involved a decrease in the most aggressive stimulus during the second half of the playback than to the combinations in which the most aggressive stimulus remained the same (*t* test, *df*=54, *P*=0.002). Male responses to the individual single stimuli differed (Figure 4e; ANOVA, $F_{2,53}$=6.33, *P*=0.003), with response being more aggressive to the high than to either the medium or low stimulus (post hoc, *P*=0.012 for both). Aggressiveness to the double stimulus did not differ between the different stimulus pairs (Figure 4f; ANOVA, $F_{2,53}$=0.9, *P*=0.413).

Combined treatments response to single stimuli

There was no difference between experiments in the proportion of aggressive calls that were given to the single stimuli. Males gave high proportions of aggressive calls to a single stimulus whether or not it was the first stimulus they had heard, as in the increasing treatment, or whether it was preceded by a double stimulus, as in the decreasing treatment (Figure 5a; WMW, *N*=52, *z*=-1.418, *P*=0.156). There was, however, a difference between experiments in the aggressiveness index of the aggressive calls that were given. Males' aggressive calls were more aggressive to single stimuli in the increasing treatment than in the decreasing treatment (Figure 5d; *t* test, *df*=50, *t*=3.498, *P*=0.001).

Combined treatments response to double stimuli

There was no difference between experiments in the proportion of aggressive calls that were given to the double stimuli. Males gave high proportions of aggressive calls to the double stimuli in both the increasing and decreasing treatments (Figure 5b;

WMW, *N*=52, *z*=-0.362, *P*=0.717). Likewise, there was no difference between experiments in the aggressiveness index of the aggressive calls that were given to the double stimuli: (Figure 5e; *t* test, *df*=50, *t*=-0.506, *P*=0.615).

Combined treatments difference in response to single and double stimuli

Overall, males gave a higher proportion of aggressive calls in response to the double stimuli than to the single stimuli (Figure 5c; WSR, *N*=52, *z*=-2.847, *P*=0.004), mirroring the response seen when each treatment was analyzed individually. There was no difference between treatments in the change in the proportion of aggressive calls that were given between the single and double stimuli (Figure 5c; WMW, *N*=52, *z*=-1.076, *P*=0.282).

Overall, males' calls were more aggressive in response to the double stimuli than to the single stimuli (paired *t* test, *df*=51, *t*=-3.141, *P*=0.003). The pattern of the change in aggressiveness indices between the double and single stimuli for each of the stimulus combinations was similar for both experiments (Figure 5f), but the difference was larger for the decreasing experiment (*t* test, *df*=50, *t*=-4.143, *P*<0.0005). This difference between experiments arose because males in the decreasing treatment decreased their aggressive response substantially during the second half of the playback (when the number of stimuli decreased from 2 to 1; Figure 4d), while males in the increasing treatment on average maintained a similar aggressive response during the second half of the playback (when the number of stimuli increased from 1 to 2; Figure 3d).

DISCUSSION

These experiments demonstrate that male *D. ebraccatus* are sensitive to both the number of simulated competitors and to the intensity and aggressiveness of their calls. These findings underscore the importance of examining the social network in which signaling takes place and emphasize the perspective of communication network theory that signals often will be received by more than one individual (McGregor and Peake 2000). If some receivers are also competing signalers, then signalers are expected to actively adjust their signaling output based on the level of competition presented by nearby individuals. My findings suggest that males tracked the aggressiveness of simulated nearby males and adjusted their own aggressive calling in a consistent manner. In general, males become more aggressive when multiple, and more aggressive, stimuli were presented and less aggressive when the number of stimuli was reduced or when a new, less aggressive stimulus was introduced. Increasing the number of stimuli presented simultaneously to an individual dramatically increases the number of possible combinations that must be examined before a general understanding of how males respond to multiple stimuli can be obtained. I stress that many additional studies that simulate more realistic signaling environments are needed in this and other chorusing species.

Measures of aggression

In this study, I used two different measures of male aggressiveness: the proportion of its calls that were aggressive calls, and a composite measure of the aggressiveness of specific call characteristics, the aggressiveness index. In some cases, these two measures gave similar results. In the decreasing treatment of experiment 4, for example, males gave a higher proportion of aggressive calls and had higher aggressiveness indices to the double stimuli than to the single stimuli. However, results of comparisons between playback stimuli were not always identical between these two measures of aggressiveness. In general, more differences in response were observed in the aggressiveness index than in the proportion of aggressive calls. These playbacks took place at a relatively high SPL and males mostly responded with uniformly high numbers of aggressive calls regardless of the particular stimuli presented. This observation is consistent with previous studies documenting high levels of aggressive calling and a lack of habituation to aggressive calling in males of this species (Reichert 2010). The aggressiveness index is probably a more sensitive measure of aggression because it captures the gradation observed in aggressive calling. The results presented here extend the findings of previous studies involving single speaker playbacks (Wells and Schwartz 1984b, Wells 1989) by showing that in two-speaker playback tests, males adjust to a fine degree the level of aggression of their aggressive calls based on similar changes in the stimuli presented to them.

Effects of sound pressure level:

Experiments 1-3 involved increases in the SPL of one of the speakers during the second half of the playback test. This design was incorporated to simulate an intrusion upon the focal male's calling space by an aggressive competitor. Overall, SPL was less important than the absolute level of aggression of the playback stimuli. The aggressiveness index was higher for the three stimuli that included the high aggressive call than for any of the other stimuli, which did not differ. In fact, in experiment 2, males showed no change in aggressiveness between the two halves of the medium-high playback in which the high stimulus is broadcast in both halves, while males decreased in aggressiveness during the second half of the medium-high playback of experiment 1 in which the high stimulus was silenced. Thus, it seems that a high level of aggressive response is due to the presence of the high aggressive call, and even a 10 dB increase in SPL of the medium call is insufficient to maintain this aggressive response when the high aggressive call is absent. SPL was not entirely unimportant, however. In experiment 3, males gave a lower proportion of aggressive calls, and these calls were less aggressive, to a medium stimulus played at 90 dB than to the same stimulus played at 100 dB. In addition, the direction of change in the proportion of aggressive calls given to a stimulus seemed to be related to the SPL of calls that preceded that stimulus. For example, males responding to the 100 dB medium call in the second half of playbacks that were preceded by the low-medium stimuli reduced the proportion of aggressive calls compared to the first half of the playback when the low-medium stimuli were broadcast at 100 dB, but increased the proportion of aggressive calls when the low-medium stimuli were broadcast at 90 dB.

Effects of multiple signalers, order of presentation, and aggressiveness of playback stimuli

The synthetic calls used in these experiments aimed to simulate increasing levels of aggression and the aggressive responses obtained from males suggest that males were responsive to this stimulus variation. In response to both single and double stimuli, males generally gave aggressive calls with greater aggressiveness indices to more aggressive than to less aggressive synthetic stimuli (Figures 3 and 4). Further, males tended to respond more aggressively to two-speaker playbacks than to single-speaker playbacks. However, this response depended greatly on the order in which two- and one-speaker stimuli were presented and on the specific levels of aggressiveness involved. The contrasts between response to the increasing and decreasing treatments of experiment 4 are especially illustrative. In the decreasing treatment of experiment 4, males were first presented with a double stimulus that was then followed by a single stimulus. On average, males’ calls had a higher aggressiveness index to the double stimuli than to the single stimuli for all six sets of playback stimuli. In the increasing treatment, however, males were first presented with a single stimulus that was then followed by a double stimulus. In this case, males did not necessarily increase the aggressiveness index of their calls in response to the double stimuli. In fact, the direction of change in the response depended on the level of aggressiveness of the stimulus that was added during the second half of the playback. When the second-half stimulus was less aggressive than the first-half stimulus, males decreased their aggressiveness index; when the second-half stimulus was more aggressive than the first-half stimulus, males increased their aggressiveness index.

Thus, the level of aggressiveness exhibited by males appears to be most influenced by changes in the most and least aggressive stimuli presented. Furthermore, when the responses to the decreasing treatment are examined more closely, this conclusion is reinforced. Males decreased their aggressiveness indices in all cases, but the decrease was greater in the second half of the playback when the more aggressive of the two stimuli was silenced than when the less aggressive stimulus was silenced. Experiments 1 and 2 show a similar pattern. There were no differences in aggressiveness index between the two halves of the playbacks in which the most aggressive stimulus remained the same, but in experiment 1, males gave a more aggressive response to the medium-high playback than to the medium playback that followed.

These results suggest that males may give a strong aggressive response to all aggressive stimuli when they are first heard, but over time become sensitive to changes in the aggressiveness levels of nearby individuals. Males in the increasing and decreasing treatments responded with similar aggressiveness indices to the very first stimulus that was presented to them, whether or not it was the single or double stimulus. For males in the decreasing treatment, the second half of the playback may have always represented a de-escalation. Even though the most aggressive simulated competitor remained the same in some cases, there was always a decrease in the number of simulated competitors. By contrast, for males in the increasing treatment, the second half of the playback may not have always represented an escalation. Although there was always an increase in the number of simulated competitors, in some cases the calls of the additional competitor were less aggressive than those of the initial competitor. In these cases, males decreased their aggressive responses, perhaps as an attempt to balance the level of aggressiveness broadcast to these two competitors. This response may enable them to adjust their aggressive calls to most effectively attract females and repel rival males. These results provide evidence that the level of aggressiveness of the simulated aggressive calls itself is a more important determinant of the level of aggressive response exhibited by focal males than the order of presentation or the number of simulated competitors.

Importance of multiple-speaker playbacks

Relatively few studies of male response to multiple signalers have been performed for chorusing species compared to those that have been done on females, almost certainly because of the additional complexity that arises when the study subject is both a signaler and a receiver. The typical behavioral response measured from females exposed to two or more signals is a simple choice response, in which only the passive approach of the female to a stimulus is recorded. Males, however, not only receive signals from multiple sources, but also produce signals of their own. Thus, their response is more interactive, and more complicated, than that of females and it will thus be more difficult to perform controlled experiments that remain relevant to the male's social experience. Some studies examining male response to multiple speakers have avoided these issues by measuring a binary response of male approach to one speaker vs. another (e.g., Naguib and Todt 1997, Leitão and Riebel 2003, Illes et al. 2006, Humfeld 2008). Other studies have eschewed the playback approach altogether by examining natural interactions between multiple signalers in the chorus (e.g., Schwartz et al. 2002, Simmons et al. 2008). The methods used in this study, particularly those of experiment 1, are superficially similar to those of studies of social eavesdropping behavior (e.g., Naguib et al. 2004). I cannot infer whether eavesdropping took place with these experiments, however, because it is unclear whether most aggressive calling interactions have outcomes in this species. Thus, I could not classify my stimuli as simulations of winners or losers of aggressive interactions.

Previous studies in which male signaling response has been measured to a varying number of simulated competitors include studies of duetting and group territorial defense in birds (Hall 2000, Radford 2003, Seddon and Tobias 2003, Fedy and Stutchbury 2005, Mennill and Vehrencamp 2008, Bradley and Mennill 2009). These studies have shown mixed results on the importance of multiple signalers on signal response, but in some cases there is evidence that aggressive response increases with increases in the number of simulated competitors (Hall 2000, Radford 2003, Seddon and Tobias 2003) and to differences in the specific characteristics of the presented signals (sex of individuals; Hall 2000, Seddon and Tobias 2003, Mennill and Vehrencamp 2008). Within chorusing insects and anurans, several researchers have examined questions of call timing in relation to other individuals to determine how many other individuals a male pays attention to when attempting to time his calls to avoid overlapping with his neighbors' calls. These studies have revealed that males pay attention to a small, but not necessarily singular amount of nearby individuals (Brush and Narins 1989, Snedden et al. 1998, Greenfield and Rand 2000, Greenfield and Snedden 2003). My study enhances this past work by showing that males pay attention not only to the presence or absence of signals from multiple individuals but also to specific characteristics of their signals.

The overarching goal of this study was to provide evidence that multiple-speaker playbacks have the potential to generate new insights into animal communication. Indeed, I found that male *D. ebraccatus* responded to multiple simulated aggressive competitors in a complex manner that could not necessarily have been predicted from the results of previous single-speaker playback studies. The situations I simulated in this study are relevant aspects of the male's social environment, as they often are surrounded by multiple other individuals producing aggressive calls. Although most other frog species do not exhibit such behavior with their aggressive calls (but see Wells 1978b, Bee and Perrill 1996), similar questions could be raised with regard to advertisement calling. For example, in species in which females prefer costly long calls, how does the presence of multiple nearby individuals giving calls of different lengths affect the length of a focal male's calls? My experiments show the difficulty involved in multiple-speaker playback studies and indicate that large data sets will be needed to assess the effects of numerous stimulus combinations. I was, however, able to demonstrate the importance of increased playback complexity on male response. I suggest that future studies should continue this line of inquiry to improve our understanding of communication in complex social environments.

Table 1. Characteristics of the synthetic call stimuli used in these experiments

	Introductory note Duration (ms)	Total Call Duration (ms)	Pulse Rate (pulses/s)	Pulse Number	Pulse Duration (ms)	Pulse Duty Cycle	Interpulse Interval	Rise Time (ms)	# Clicks
Low	160	539.7	340	72	2.2	75%	0.7	20	3
Medium	215	468.1	285	61	2.4	67%	1.2	30	2
High	300	426.6	240	55	2.1	50%	2.1	50	1

All calls shared the following characteristics: Dominant frequency=3.1 kHz, click pulse duration=5.311 ms, inter-click interval=100 ms, pulse rise time=0.9 ms, pulse fall time=0.9 ms, call period=6 s, time between calls from opposite speakers=3 s.

Table 2. Stimulus treatments and sample sizes for the playback experiments

Experiment	First half stimulus	Second half stimulus	*N*
1	L (90 dB) + M (90 dB)	M (100 dB)	21
	H (90 dB) + M (90 dB)	M (100 dB)	21
2	L (90 dB) + M (90 dB)	L (90 dB) + M (100 dB)	17
	H (90 dB) + M (90 dB)	H (90 dB) + M (100 dB)	17
3	M (90 dB)	M (100 dB)	15
	M (90 dB) + M (90 dB)	M (100 dB)	15
4: increasing treatment	L	L+M	7
	L	L+H	9
	M	L+M	5
	M	M+H	8
	H	L+H	7
	H	M+H	8
4: decreasing treatment	L+M	L	6
	L+H	L	6
	L+M	M	8
	M+H	M	14
	L+H	H	11
	M+H	H	11

Each playback consisted of two halves in which stimuli were broadcast from one or two speakers. Each row presents an alternative set of stimuli used in each experiment. All stimuli in experiment 4 were presented at an SPL of 100 dB. In experiments 1-3, all frogs were exposed to both sets of playback stimuli. In experiment 4, each frog was only exposed to one set of playback stimuli.

L low aggressive call, *M* medium aggressive call, *H* high aggressive call.

Table 3. Component matrix from the principal components analysis of aggressive call characteristics in *D. ebraccatus*

Call Characteristic	Component score	Coefficient score
Pulse number	0.435453	0.148
Full call duration	0.439734	0.149
Introductory note duration	0.919439	0.312
Duty cycle	0.898648	0.305
Pulse rate	-0.74428	-0.252
Call rate	0.441946	0.15
Dominant frequency	-0.16702	-0.057
Number of click notes	-0.36746	-0.125

The second column shows the component score from the first PC for each call characteristic. The third column shows the coefficients of the first PC for each call type. The latter values were multiplied by the standardized values of each call characteristic to generate the aggressiveness index.

Figure 1. Waveform displays of typical *D. ebraccatus* aggressive calls. Time (in milliseconds) is on the *x*-axis and relative amplitude is on the *y*-axis. **a** A highly aggressive call. **b** A less aggressive call.

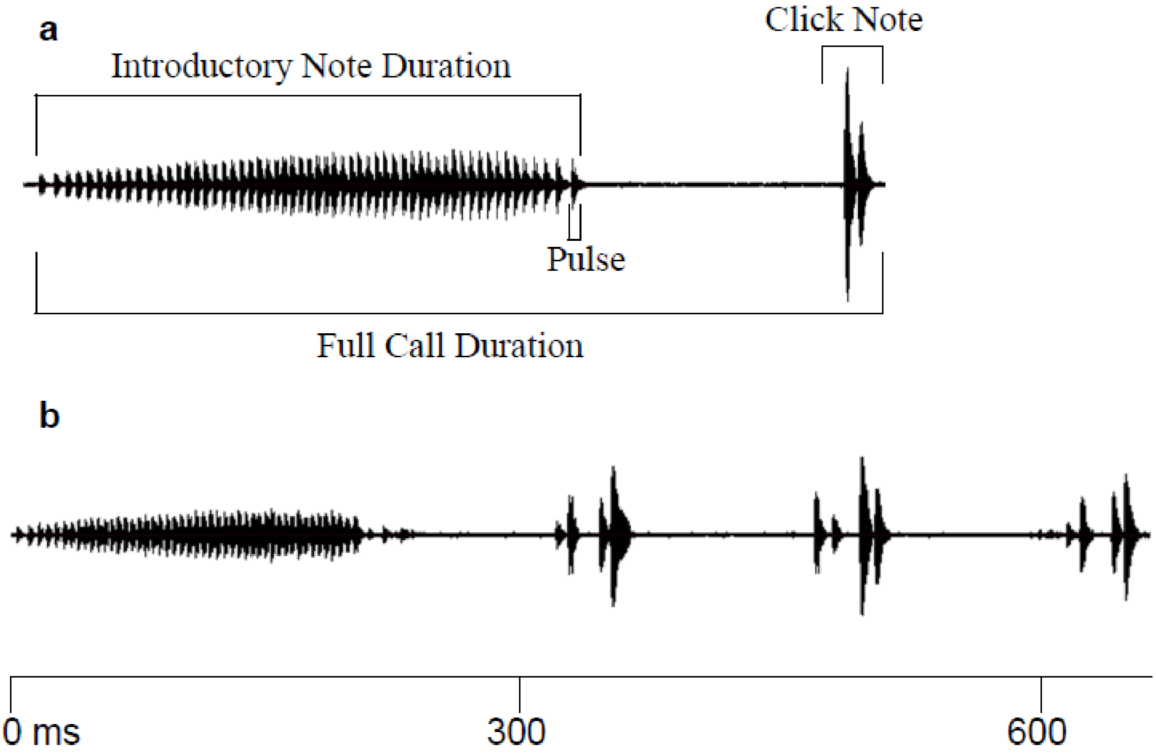


Figure 2. Measures of aggressive calling for experiments 1-3. *White bars*: response to the first half of the playback; *gray bars*: response to the second half of the playback. **a-c** Proportion of calls that were aggressive; **d-f** aggressiveness index. **a,d** Experiment 1; **b,e** experiment 2; **c,f** experiment 3. Specific stimuli are labeled below *x*-axis: *L* low aggressive call, *M* medium aggressive call, *H* high aggressive call. *Numbers* after the stimuli give the SPL of the playback.

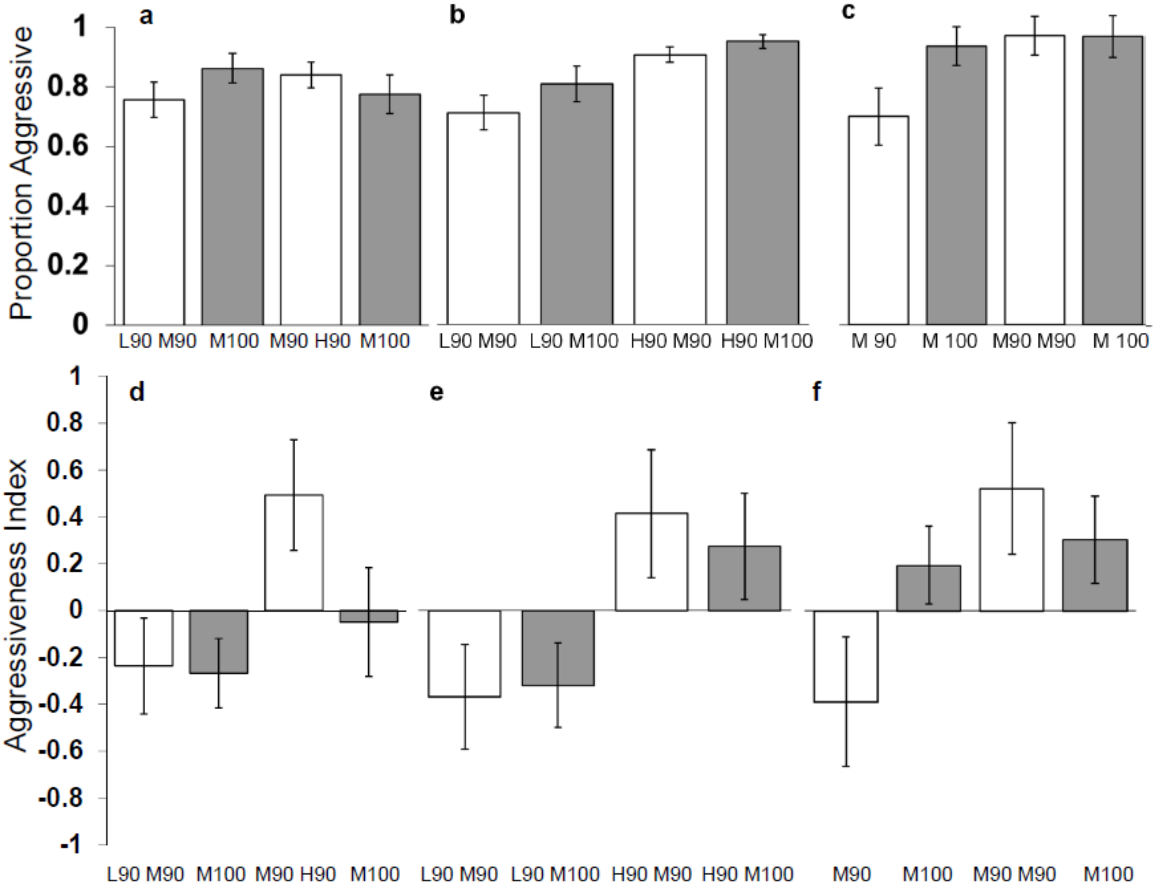


Figure 3. Measures of aggressive calling for experiment 4: increasing treatment. In this treatment, a single stimulus was presented in the first half of the playback and a double stimulus was presented in the second half of the playback. **a-c** Proportion of calls that were aggressive. **d-f** Aggressiveness index. **a,d** Values for each half of each set of playback stimuli; *white bars*: response to the single stimulus; *gray bars*: response to the double stimulus. **b,e** Pooled values for response to the single stimuli. **c,f** Pooled values for response to the double stimuli. *x*-axis as in Figure 2.

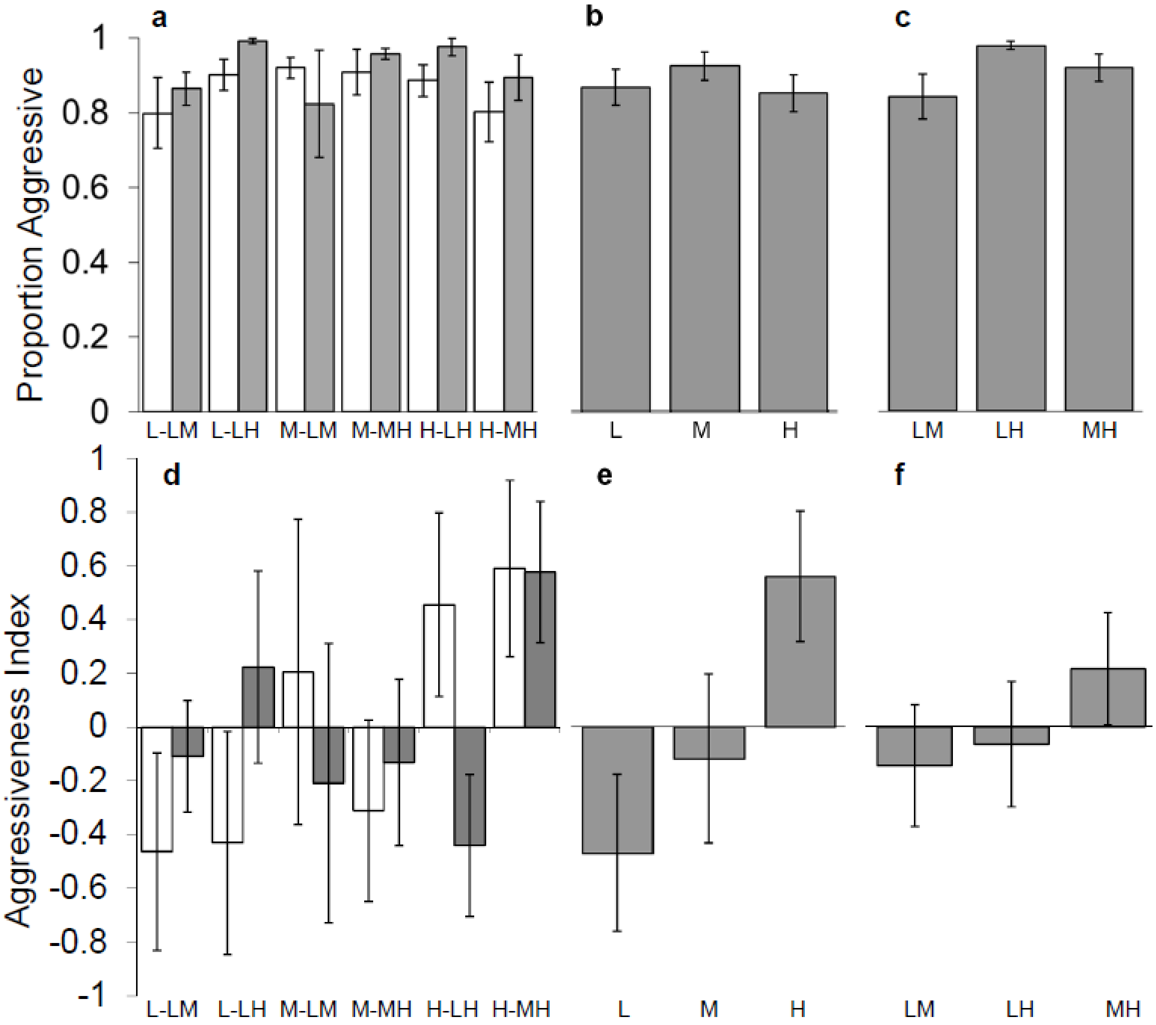


Figure 4. Measures of aggressive calling for experiment 4: decreasing treatment. In this treatment, a double stimulus was presented in the first half of the playback and a single stimulus was presented in the second half of the playback. **a-c** Proportion of calls that were aggressive. **d-f** Aggressiveness index. **a,d** Values for each half of each set of playback stimuli; *white bars*: response to the single stimulus; *gray bars*: response to the double stimulus. **b,e** Pooled values for response to the single stimuli. **c,f** Pooled values for response to the double stimuli. *x*-axis as in Figure 2.

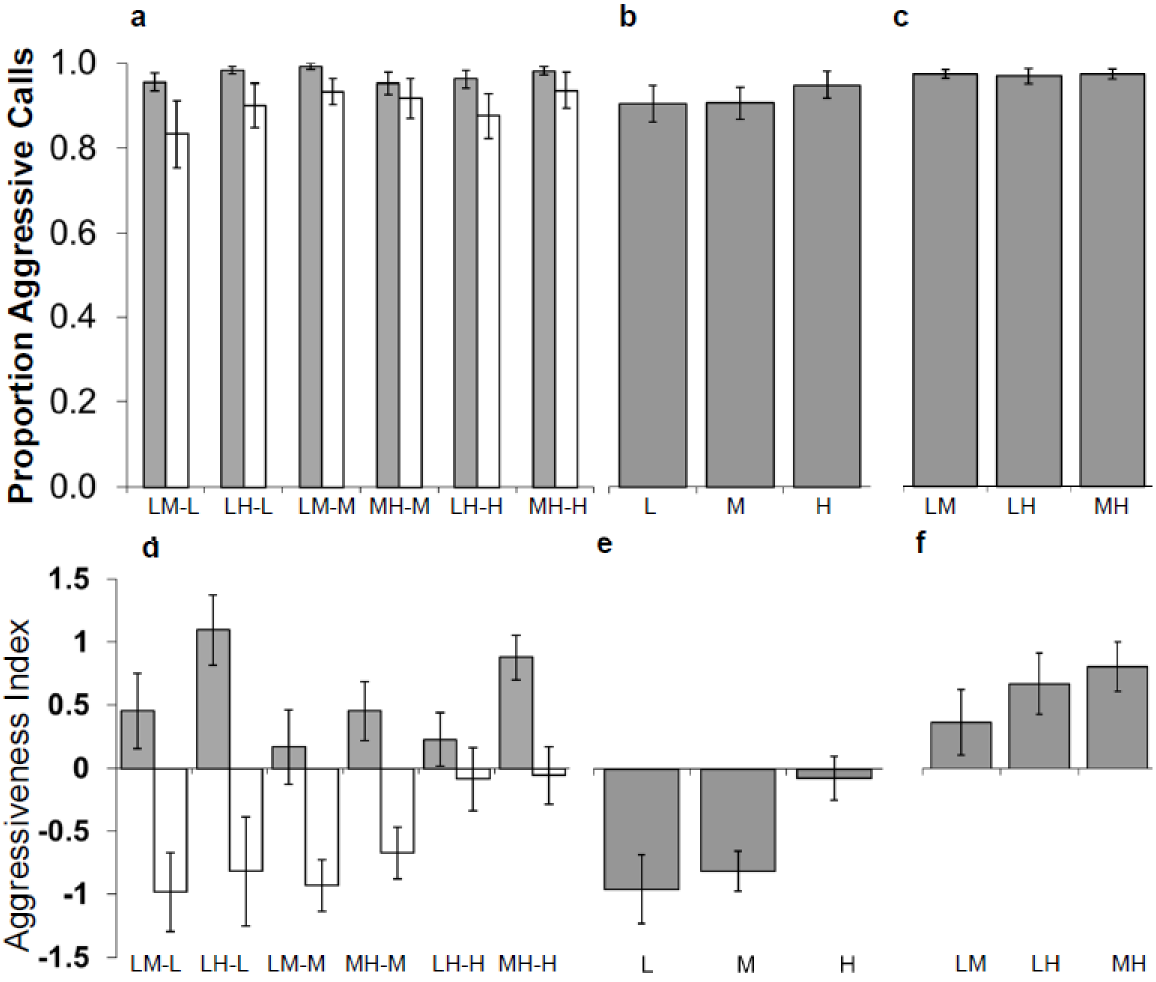
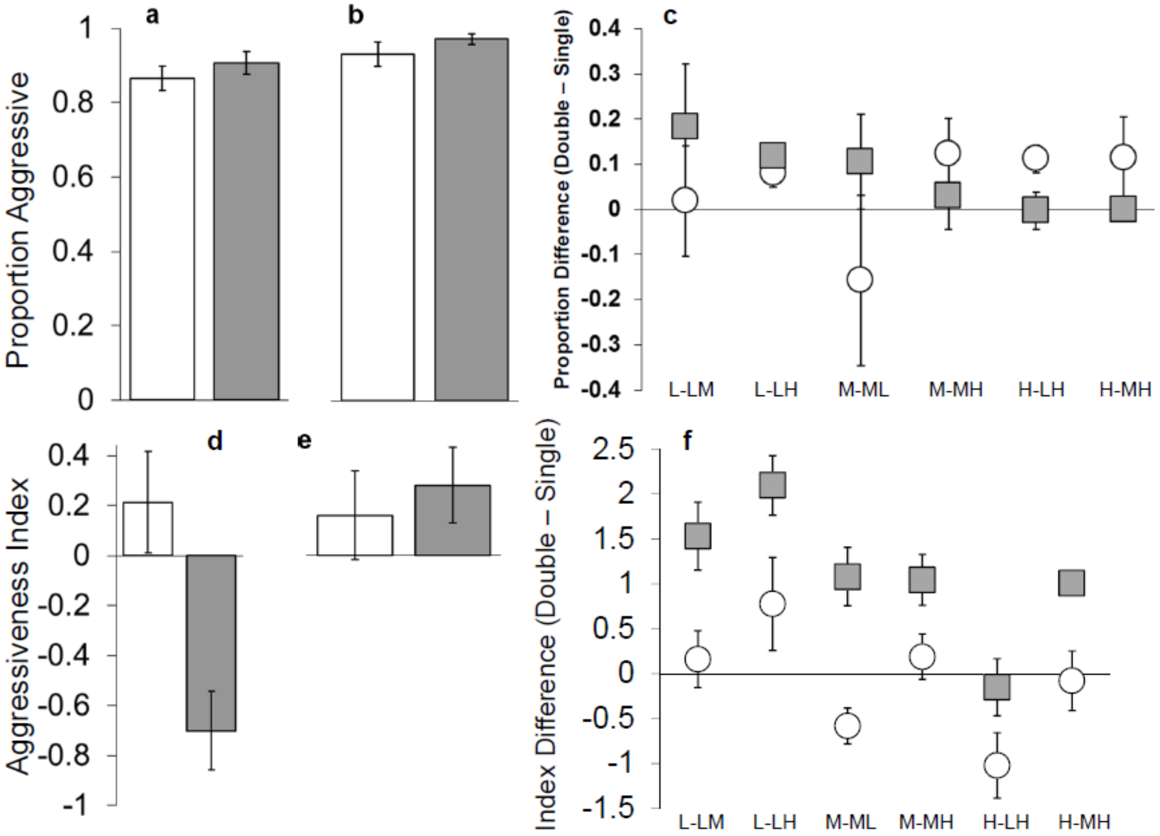


Figure 5. Comparisons of aggressiveness between the increasing and decreasing treatments of experiment 4. **a-c** Proportion of calls that were aggressive. **d-f** Aggressiveness index. *White bars* and *circles*: increasing treatment; *gray bars* and *circles*: decreasing treatment. **a,d** Values for response to all single stimuli combined for each treatment. **b,e** Values for response to all double stimuli combined for each treatment. **c,f** The difference in aggressiveness for each treatment between the double and single stimulus for each set of playback stimuli. *x*-axis for (**c,f**) as in Figure 2.



CHAPTER 4

Call timing is determined by response call type, but not by stimulus properties, in the treefrog *Dendropsophus ebraccatus*

ABSTRACT

A common form of signal competition in acoustically chorusing animals involves the precise timing of calls relative to those of other nearby individuals. In this study, I present a detailed description of nonrandom timing of both advertisement and aggressive calls in males of the Neotropical treefrog *Dendropsophus ebraccatus*. I used playback tests to measure call delays for both advertisement and aggressive calls given in response to synthetic advertisement and aggressive call stimuli presented with either a fixed or random timing arrangement. Call delays for a given response call type were nonrandomly distributed and did not differ depending on the fixed or random periodicity of stimulus presentation or on the stimulus call type. In general, advertisement call responses overlapped the playback stimuli, while aggressive calls were given with a much longer delay and did not overlap the playback stimuli. A second test involved the presentation of low pulse number advertisement and aggressive call stimuli to determine if males were capable of nonrandom timing to truncated stimuli. These playbacks also assessed whether they responded more aggressively to truncated aggressive call stimuli. Males usually showed synchrony in response to truncated calls of

both types, more commonly in response to truncated advertisement calls. There were no differences in aggressive responses to truncated advertisement or aggressive calls. Call delays appear to be a property solely of the type of call that the male produces, regardless of the kind of stimulus. Thus, there may be a conflict between the ability to discriminate between signals and the ability to rapidly respond to these signals with an appropriate call delay.

INTRODUCTION

Signaling competition can be severe in animals that gather in large aggregations and signal to attract mates. Some of the best examples are the choruses of insects and anurans where males gather in large groups and produce acoustic signals to attract females (reviewed by Gerhardt and Huber 2002). In order to produce a signal that is detectable and also attractive to females, males of many species adjust properties of their calls when in competition with other nearby individuals (e.g., Rand and Ryan 1981, Given 1987, Bee and Bowling 2002, Schwartz et al. 2002, Penna et al. 2005). One of the most striking forms of acoustic competition is the often very precise timing with which males place their calls with respect to those of other males (e.g., Schwartz 1987, Brush and Narins 1989, Klump and Gerhardt 1992, Greenfield 2002). The mechanisms underlying this phenomenon and its consequences for female choice are topics of general interest.

Many acoustically communicating animals show nonrandom patterns of call timing. These patterns can be divided broadly into cases in which males time their calls synchronously, such that there is more overlap between the calls of two individuals than would be expected by chance (e.g., Walker 1969, Greenfield and Roizen 1993, Grafe 2003, Kotiaho et al. 2004, Naguib and Mennill 2010), and in which males alternate their calls such that there is less overlap between calls than would be expected by chance (e.g., Rosen and Lemon 1974, Zelick and Narins 1983, Minckley et al. 1995, Grafe 1996). In a few species, both synchrony and alternation are observed under different conditions (e.g., Jones 1966a, Moore et al. 1989, Sismondo 1990, Hartbauer et al. 2005).

A major topic in studies of call timing is the mechanism by which signalers adjust the timing of their calls in response to those of other signalers, resulting in either synchrony or alternation. These mechanisms are usually inferred indirectly by examining the call timing responses of individual signalers to playback tests (Greenfield 1994a). When responses are proepisodic, an individual's signal timing relative to the concurrent stimulus is determined by adjustments made by that individual in response to a previous stimulus (Walker 1969, Greenfield 1994a). In insects and anurans, various types of phase delay mechanisms have been invoked to describe the proepisodic call timing behavior of a number of rhythmically signaling species (Greenfield 1994a, b). Under a simple phase-delay model, signalers adjust their call periods on a call-by-call basis in response to the relative timing of an external stimulus (Buck 1988). Calling is inhibited when a male receives another signal prior to initiation of its vocalization. The rate of recovery from inhibition determines when the male then resumes calling, and the ratio between the recovery rate and the call period of the external stimulus largely determines whether synchrony or alternation results (Greenfield 1994a, b). The phase delay model has been useful in explaining a wide variety of signaling interactions in insects and anurans, but it may not apply to all species (e.g., Hartbauer et al. 2005), particularly those that do not signal rhythmically. Some non-rhythmic species nonetheless are able to achieve nonrandom call timing (e.g., Tuttle and Ryan 1982). In these cases, a homoepisodic response, in which an individual's signal timing relative to the concurrent stimulus is a direct response to that stimulus, may be responsible (Walker 1969, Greenfield 1994a). The mechanism involved in homoepisodic responses is likely a rapid response to the onset of the concurrent sound (e.g., Ryan 1986b). Few studies have demonstrated that nonrandom timing may be due to a homoepisodic response.

The Neotropical treefrog *Dendropsophus ebraccatus* has an unusual set of call timing behaviors compared to those of other species. In addition to giving advertisement calls to attract mates, males also give relatively high proportions of a second call type, termed the aggressive call (Wells and Schwartz 1984b, Reichert 2010). Aggressive calls differ from advertisement calls in a number of characteristics, including the pulse rate and duration of the introductory note (Wells and Schwartz 1984b). Interacting males show both call synchrony and alternation, with advertisement calls tending to overlap each other and aggressive calls tending to alternate (Schwartz and Wells 1984, Wells and Schwartz 1984a, b). Female preferences are also unusual in that females prefer lagging to leading advertisement calls in the natural call timing arrangement (Wells and Schwartz 1984a). The preference for lagging calls is likely related to the strong female preference for the click notes that are often placed at the end of calls (Wells and Schwartz 1984a). When advertisement calls overlap, the click notes of the leading call tend to be obscured by the lagging call, while the click notes of the lagging call are usually free from interference. Females of relatively few other species of anurans are known to prefer lagging to leading calls (e.g., Grafe 1999).

The research I report here builds upon and extends previous work on call timing in this species (Schwartz and Wells 1984, Wells and Schwartz 1984a, b). I used a series of playback tests to assess whether male call timing is affected by variation in the characteristics of the playback stimuli and to gather evidence on the mechanism responsible for nonrandom call timing in this species. I addressed the following six questions in this study: 1) Is spontaneous calling in *D. ebraccatus* rhythmic? Rhythmic calling is an essential characteristic of the proepisodic phase-delay mechanism of call timing, whereas it is not necessarily required for homoepisodic responses (Greenfield 1994a). 2) Do males entrain (i.e., call with a fixed delay following stimulus onset) to a playback stimulus presented at random intervals, as they do for a stimulus presented at a fixed interval (Wells and Schwartz 1984a)? If males' call delays are proepisodic responses to previous stimulation, then they would not be expected to be able to entrain to a randomly timed stimulus. 3) Do males' call delays differ in response to advertisement and aggressive call stimuli? 4) For the above analyses, did call delays differ when males *responded* with advertisement or aggressive calls? 5) Do call delays vary with the duration of the playback stimulus? If call timing is driven by a homoepisodic response to stimulus onset, then call delays should not vary with the duration of the stimulus. Under certain proepisodic mechanisms, however, males are inhibited for the duration of the stimulus (Greenfield et al. 1997) and call delays would therefore be expected to vary with stimulus duration. 6) Do males give more aggressive calls in response to truncated stimuli with characteristics of typical aggressive calls than they do in response to truncated advertisement calls? Particularly, if call timing is mediated by a rapid homoepisodic response to the onset of sound, in the short term, males may not be able to distinguish between different call types and maintain a rapid timing response at the same time.

METHODS

Study system

I studied call timing behavior in *D. ebraccatus* at the Smithsonian Tropical Research Institute's field station in Gamboa, Panama in June 2007, August 2008, and July to August 2009. Males called on most nights at my study sites: a small pond surrounded by secondary vegetation (Bridge Pond) and a flooded field. I performed field playback tests with naturally calling males at these sites at the time of the most intense calling activity: between 2000 and 2400 hours.

Playback procedure

I generated the synthetic stimuli used for all playbacks with a custom sound synthesis program provided by J. Schwartz (Schwartz 1991; 16-bit digital files, 20-kHz sampling rate). This program created an individual stimulus call, the characteristics of which were based on average values obtained from analyses of previous recordings from this population (Reichert, unpublished data). The specific stimuli used are described below for each experiment. In all cases, I used an audio editing computer program (Cool Edit Pro 2.0, Syntrillium 2002) to copy and paste stimuli with the appropriate spacing between copies to generate entire audio tracks that were used for the playbacks. These audio tracks were placed on an audio compact disk for playback in the field.

I chose males that were calling vigorously as playback subjects and temporarily removed any other calling males within 2 m of the focal male. This may have changed the focal male's context of calling, but was necessary to ensure that males responded primarily to the playback stimuli. I presented the male with one of the six playback stimuli (see experiments, below). Stimuli were played through a portable compact disk player (Panasonic SLSW940S) into a portable amplified speaker (Mineroff Electronics, SME-AFS) mounted on a tripod approximately 1 m from the focal male. For all tests, I used a portable sound pressure level (SPL) meter (Radio Shack 33-2055) to adjust the output of the playback speaker to 95 dB SPL at 1 m (re: 20 µPa; "peak" setting), which is a typical SPL for natural calling at this distance (Wells and Schwartz 1984a). I used portable digital audio recorders (Marantz PMD660 & 661) and a directional microphone (Sennheiser ME-67) to record the male's vocal responses for the entire duration of the playback. On a separate channel, I simultaneously recorded the playback stimulus itself via a cable from the compact disk player to the audio recorder. After being used in a playback test, each male was captured and given a unique toeclip for individual identification.

Call analyses

I used a sound analysis software (Raven Pro 1.3, Cornell Laboratory of Ornithology) to measure call delays in response to the playbacks from waveform displays of the recordings. I calculated call delays to the nearest 1 ms and measured them by subtracting the start time of the playback stimulus on the channel that recorded directly from the cd player from the start time of the male's first call after the playback stimulus on the channel that recorded from the microphone (Figure 1a). I did not adjust the measurements of call delays to account for the time required for the sound to travel from the speaker to the frog and then from the frog to the microphone. Because both the speaker and the microphone were positioned 1 m or less from the frog, any resultant overestimation of call delays would be relatively consistent across recordings and negligible for the purposes of this study. If a male gave more than one call in response to a single stimulus call, I considered only its first response in the analyses. Although this may have biased my results towards lower average call delays, it allowed me to avoid errors associated with measuring responses that were likely not to the playback stimuli. I analyzed baseline recordings by noting the start time of each call and using these times to calculate call periods. I only included advertisement calls in the analyses of baseline recordings. Although the call characteristics of many frog species vary with temperature (e.g., Gayou 1984), I did not correct measurements of any call characteristics I measured in this study for ambient temperature because there was little temperature variation at my study site (range: 23–27°C).

Question 1: Rhythmicity of spontaneous calling

Prior to playback tests, I made baseline recordings of each male's spontaneous calling to assess whether calling is rhythmic in the absence of playback stimulation. I assessed the rhythmicity of baseline calling by calculating the coefficients of variation (CV) associated with the advertisement call periods within individual males. I assumed that if call timing was rhythmic, the deviations in the spontaneous rhythm should be small relative to the mean call period (e.g., Nityananda and Balakrishnan 2007). The use of CVs allowed me to combine these measures from males that may have had different intrinsic call periods.

Question 2: Entrainment to random and fixed stimuli

I measured male call timing in response to synthetic advertisement and aggressive calls with either a fixed call period, or a call period that varied randomly between calls. The fixed stimuli had a constant call period of 6 s. To generate randomly timed call intervals, I calculated random call periods in Microsoft Excel 2007 software by generating pseudorandom numbers from a normal distribution whose mean (7.06 s) and standard deviation (3.71 s; any randomly chosen negative times were thrown out) were based on the call periods of baseline recordings made in July and August of 2006 (Reichert, unpublished data). I created three different stimulus tracks for each call type using three different random drawings of call periods to ensure that the response measured was not due to any specific random arrangement of call periods. Preliminary analyses confirmed that there were no differences in response to the three different exemplar tracks, so I pooled the data for the responses to all three tracks for statistical analyses. A full playback trial consisted of 20 repetitions of the stimulus call at either the fixed or random call period.

Other temporal and spectral properties of the advertisement and aggressive call stimuli were based on average values for the population. Call parameters for these stimuli are given in Table 1. Waveform displays of the individual synthetic stimuli used in this experiment are shown in Figure 1 (advertisement call, lower trace of Figure 1a; aggressive call, lower trace of Figure 1b). Call characteristics of natural aggressive calls vary with the proximity to other calling males and the properties of those males' aggressive calls (Wells and Schwartz 1984b, Wells 1989, Reichert 2011a). The aggressive call stimulus I used in this experiment had call properties typical of the center of this gradation (Table 1).

I used circular statistical methods to describe and analyze these data. I converted each call delay into a phase angle by dividing the delay by the call period of the stimulus and then multiplying by 360°. For the fixed timing stimuli, phase angles were calculated using the constant call period of 6 s. For the randomly timed stimuli, phase angles were calculated using the specific call period of the stimuli between which the male placed his call, as the call periods of these stimuli varied from call to call. This is the standard method to calculate phase angles, but it may not reflect as well the actual degree of overlap in a non-interactive playback test as phase angles that have been calculated under the fixed timing condition. If a male overlaps the playback stimulus with a given call delay, the phase angle for this response depends on the timing of the next stimulus call, which is irrelevant to the male's current response under variable stimulus presentation. Nonetheless, as the call periods of the randomly timed stimuli varied around a normal distribution, there is no reason to expect a bias towards larger or smaller phase angles in my calculations. In fact, I reanalyzed the responses to randomly timed stimuli using a constant value for call period (the average call period of the randomly timed stimuli) and obtained essentially identical results to those presented here (Reichert, unpublished data).

Phase angles near 0° and 360° indicate approximately complete overlap with the stimulus while those near 180° indicate approximately perfect alternation. I used procedures described in Zar (2010) to calculate mean and median phase angles for each male's response to the playback stimulus and to calculate grand means, medians, and the mean vector for all males' responses to the playback stimuli. I calculated these values separately for advertisement and aggressive call responses. The parameter *r* of the mean vector is a measure of concentration; *r*'s close to 0 indicate dispersed data and those close to 1 indicate concentrated data (Zar 2010). Thus, larger *r*'s indicate less variable call delays. For analyses in which the mean or median phase angles of multiple males' calls were combined to calculate grand means and medians, I used second order tests in which each male's contribution to the dataset was adjusted by the magnitude of *r* for that male, following the protocols given by Zar (2010).

Prior to making comparisons between treatment groups, I first confirmed that call timing was nonrandom in each group. To test this, I used nonparametric second order one sample circular statistical tests to test the null hypothesis that the phase angles have a uniform distribution (Zar 2010, page 646). I only proceeded with further testing when the null hypothesis was rejected, that is, when there was a significant directionality in the distribution of phase angles indicating nonrandom call timing. I then used a nonparametric 2 sample circular statistical test to compare the mean phase angles in response to the fixed and randomly timed stimuli (Zar 2010, page 649). When preliminary analyses indicated no difference in response to advertisement and aggressive call stimuli (see results below), I pooled the data across both stimulus call types prior to analysis. However, I performed these tests separately for advertisement and aggressive call responses.

Question 3: Responses to advertisement and aggressive call stimuli

The recordings made in question 2 also allowed me to test whether call timing responses differed between advertisement and aggressive call stimuli. These call types typically differ in a number of parameters such as duration (Table 1; Wells and Schwartz 1984b) that could influence the timing of a call response. Analyses proceeded as in question 2, but in this case, I compared the mean phase angles of responses to the advertisement and aggressive call stimuli. When preliminary analyses indicated no difference in response to fixed and randomly timed stimuli (see results below), I pooled the data across both sets of stimuli prior to analysis. As above, I analyzed advertisement and aggressive call response timing separately.

Question 4: Differences in call timing when producing advertisement and aggressive calls

Previous studies indicated that males' call delays differ depending on whether they gave advertisement or aggressive calls (Schwartz and Wells 1984, Wells and Schwartz 1984a, b). To confirm this, I tested whether the call delays of males' advertisement and aggressive- all responses differed in response to the playbacks described in question 2. When preliminary analyses indicated no difference in response to the stimulus type, or to the fixed or random periodicity of stimulus presentation (see results below), I pooled the data across both sets of stimuli. I compared the average phase angles of males that gave both advertisement and aggressive calls in response to a playback stimulus. Thus, to analyze these data I used a nonparametric paired sample test of angles (Zar 2010, page 654).

Question 5: Differences in call timing with stimulus duration

Previous studies suggested that the synchronous advertisement call responses of males are rapid responses to the onset of sound and the particular call timing is not strongly affected by stimulus duration (Schwartz and Wells 1984, Wells and Schwartz 1984a). If so, then males should not shorten their call delays when responding to stimuli of shorter duration (e.g., Jones 1966b). I tested this hypothesis by presenting males with a series of truncated call playbacks. Stimuli had the same characteristics as the advertisement and aggressive calls used in the previous experiments but lacked click notes and had a reduced number of pulses (PN) in the introductory note (e.g., see lower trace of Figure 1c). Specifically, for advertisement calls, I generated introductory notes with PNs increasing in steps of 1 between 1 and 10 pulses per call (5.8–58.8% of the introductory-note duration of the full advertisement call stimulus). I presented calls at each PN with a fixed call period of 6 s for 2 min, for a total playback duration of 20 min. I presented stimuli in the order of increasing PN. Although this may have confounded duration of the playback with stimulus PN, I used this design to avoid any possible priming effects of exposure to longer PN stimuli on the likelihood of perceiving shorter PN stimuli.

I generated synthetic truncated aggressive call stimuli in a similar manner. Truncated aggressive calls also had no click notes, but in this case the PN increased in steps of 3 between 3 and 33 pulses per call (4.9–54% of the introductory note duration of the full aggressive call stimulus). Because the rate at which pulses are produced in the call is much higher for aggressive calls (Table 1), I used higher PNs for the aggressive call stimulus to keep the durations of the advertisement and aggressive call stimuli approximately the same at each step. These calls were also presented at each PN with a fixed call period of 6 s for 2 min, with PNs arranged in increasing order for a total playback duration of 22 min.

To determine whether males timed their calls nonrandomly to truncated stimuli, I tested the hypothesis of uniform distribution of phase angles in response to each PN of the truncated stimuli using the methods described above for question 2. As above, I calculated separate phase angles for males’ advertisement and aggressive call responses. To test the hypothesis that stimulus duration affected call timing, I compared the mean phase angles of calls given in response to truncated stimuli of various durations. In addition, I compared the mean phase angles given in response to the truncated stimuli and to the full playback stimuli I used in question 2. For the comparisons between stimuli of different durations, I only compared males’ advertisement call delays because few males gave sufficient numbers of aggressive calls in response to the truncated stimuli. In addition, I only performed these comparisons with those truncated stimuli in which males’ call timing was determined to be significantly different from random. Preliminary analyses suggested a high rate of type I errors in 2 sample comparisons of phase angles of responses to full and truncated stimuli because the angular dispersions, used indirectly in these statistical tests, differed significantly between the two groups. Thus, to assess differences in timing between the various truncated and full stimuli, I compared mean phase angles and the associated circular standard errors (Fisher and Lewis 1983) to quantify the overlap between samples.

Question 6: Aggressive call responses to truncated stimuli

An additional goal of the truncated stimuli experiment was to determine if males can discriminate rapidly between the pulse rates typical of advertisement and aggressive calls. Based on previously reported data showing brief median call delays for overlapping advertisement calls (140–200 ms) and entrainment to stimuli lower in duration than typical advertisement calls (Wells and Schwartz 1984a), males appear to rapidly make the decision to respond with an aggressive or advertisement call. In response to calls of normal duration, males tend to respond with advertisement calls to advertisement call stimuli and with aggressive calls to aggressive call stimuli. These call types differ primarily in pulse rate and introductory note duration (Wells and Schwartz 1984b). Because introductory note durations of both are longer than the time that appears to be required to trigger an advertisement call response, I considered it a less likely cue for call type recognition than pulse rate, which can be evaluated from the beginning of the call. Thus, I compared males' aggressive call responses to truncated advertisement and aggressive call stimuli that were similar in duration but differed in pulse rate. To determine whether aggressive responses differed to the truncated aggressive and advertisement calls, I calculated the proportion of aggressive calls given in response to each truncated stimulus as the number of aggressive calls divided by the total number of calls. I used nonparametric tests to compare these proportions between truncated aggressive and advertisement calls and between these calls and the full aggressive and advertisement call stimuli. I predicted that males would give more aggressive calls to the truncated aggressive call stimuli. I presented both the truncated advertisement and aggressive call stimuli to each male and alternated the order of presentation between males. In preliminary analyses, there was no indication that the order of presentation of the truncated stimuli affected the proportion of aggressive calls given in response to each stimulus.

General analysis considerations

For a given playback stimulus and response call type, I only included a male's calls in the analyses if he gave at least five calls of that call type in response to the playback. Not all males produced both advertisement and aggressive calls during all playbacks, and in some cases males gave few calls in response to some stimuli. Thus, sample sizes for each test vary widely. All circular statistical tests were conducted on the mean or median phase angles of the individual males. With the exception of the truncated stimuli playbacks in which each male was presented with two different stimuli and multiple comparisons were made within each stimulus, each male contributed a single data point for inferential statistical testing. All tests were performed at α=0.05. Circular statistics were calculated by hand or with the Circular Statistics Toolbox (Berens 2009) in MATLAB (R2009a, The MathWorks). Other statistical procedures were performed in SPSS 16.0.1 (SPSS Inc., 2007).

RESULTS

Rhythmicity of spontaneous calling

I obtained baseline recordings of 58 males prior to playbacks. Males' spontaneous call periods (i.e., those given before playbacks) were not obviously rhythmic. I found that there was extremely high variability within individual males' call periods (mean CV ± standard deviation, 1.07±0.47, N=58 males). Such high variability suggests that if there is an underlying pacemaker that triggers call production, it is extremely noisy (arrhythmic). There was less variability in call period between males (mean CV = 0.55).

Entrainment to random and fixed stimuli

I obtained responses from 61 males to playbacks of the full call stimuli (fixed timing advertisement N=14, fixed timing aggressive N=9, random timing advertisement N=21, random timing aggressive N=17). Males' advertisement call responses tended to overlap with the click notes of both the fixed and randomly timed stimuli, and there was no difference in phase angles for responses to these two stimuli (Figure 2a; nonparametric two-sample analysis of angles; Zar 2010, page 649; U^2=0.070, N_1=15 males responding to fixed-timing stimuli, N_2=21 males responding to randomly-timed stimuli, P>0.5). The grand median phase angle for advertisement call responses was 21.4° (r=0.69) for the fixed stimuli and 14.2° (r=0.63) for the random stimuli. Males' aggressive call phase angles also did not differ between fixed and randomly timed stimuli (Figure 2b; U^2=0.13, n_1=14 males responding to fixed timing stimuli, n_2=28 males responding to randomly timed stimuli, 0.1<P<0.2). The grand median phase angle for aggressive call responses was 113.1° (r=0.39) for the fixed stimuli and 75.9° (r=0.47) for the random stimuli.

Responses to advertisement and aggressive call stimuli

The timing of males' advertisement calls did not differ when those calls were given in response to advertisement or aggressive call stimuli (Figure 3a; U^2=0.06, n_1=6 males responding with advertisement calls to aggressive call stimuli, n_2=30 males responding with advertisement calls to advertisement call stimuli, $P>0.5$). The grand median phase angle for advertisement call responses was 25.2° (r=0.67) for advertisement call stimuli and 25.6° (r=0.84) for aggressive call stimuli. Similarly, when males responded with aggressive calls, the call delays did not differ when responding to advertisement or aggressive call stimuli (Figure 3b; U^2=0.04, n_1=18 males responding with aggressive calls to advertisement call stimuli, n_2=24 males responding with aggressive calls to aggressive call stimuli, $P>0.5$). The grand median phase angle for aggressive call responses was 81.7° (r=0.52) for advertisement call stimuli and 102.8° (r=0.45) for aggressive call stimuli.

Differences in call timing when producing advertisement and aggressive calls

Males' advertisement calls (median values for individual males in response to any of the non-truncated stimuli) were significantly nonrandomly distributed in time with respect to the playback stimuli (nonparametric modification of the Rayleigh test; Zar 2010, page 646; R'=2.96, N=36 males, $P<0.001$). The grand median of advertisement call phase angles was 15.2° with an r=0.66 (Figure 4a). This corresponds to a median call delay of approximately 0.25 s; this delay is shorter than the total call durations of either the advertisement or aggressive call stimuli used in this experiment (Table 1). On average, therefore, males' advertisement call responses overlapped in time with the click notes, but not with the introductory notes, of the playback stimuli (Figure 4a). Thus, I consider advertisement call responses to be roughly synchronous. Aggressive call delays also were significantly nonrandomly distributed (Figure 4b; *R*'=2.72, *N*=42 males, *P*<0.001). The grand median of aggressive call phase angles was 83.6° with an *r*=0.44, corresponding to a median call delay of approximately 1.39 s. Thus, males' aggressive call responses were delayed such that they would not overlap with the playback stimulus but tended to be given in the first half of the stimulus call period (Figure 4b). Although males timed both their advertisement and aggressive calls nonrandomly, the average timing of the two call types differed for males that gave both call types in the same recording (nonparametric paired-sample test of angles; Zar 2010, page 654; *R*'=1.97, *N*=18 males, *P*<0.001).

Differences in call timing with stimulus duration

Nineteen males responded to the truncated stimuli. Males' call delays did not depart from a uniform distribution when giving advertisement calls in response to truncated advertisement call stimuli with between 1 and 3 pulses per call. In response to truncated advertisement calls with 4 or more pulses, however, males did show significant nonrandom timing of their advertisement calls (Table 2). Call delays of advertisement call responses to truncated aggressive call stimuli were more variable. As with responses to truncated advertisement calls, delays did not depart from a uniform distribution for the very shortest aggressive call stimuli. Unlike responses to advertisement calls, however, call delays were non-uniform for only some of the longer-PN aggressive call stimuli. In general, call delays were similar for responses to full stimuli and truncated stimuli (Figure 1; Table 2). Call delays to the truncated advertisement call stimuli with 6 and 9 pulses appeared shorter than those to the other stimuli, which were generally uniform. When males responded with nonrandom call timing to the truncated stimuli, there did not appear to be a difference in call delays between truncated advertisement and aggressive call stimuli of similar duration (Table 2).

Aggressive call responses to truncated stimuli

Males responded with a higher proportion of aggressive calls to the full stimuli than to the truncated stimuli, although this difference was not significant for advertisement call stimuli (pooled across all truncated stimuli for each individual male; Wilcoxon–Mann–Whitney test; advertisement call, Z=-1.865, N(full)=35 males, N(truncated)=17 males, P=0.062; aggressive call, Z=-4.705, N(full)=26 males, N(truncated)=15 males, P<0.0005). Pooling the data was useful for interpretation and did not affect these results; the proportion of aggressive calls given in response to the longest truncated stimulus remained substantially lower than the proportion of aggressive calls given in response to the full stimuli (Table 2). There was no difference in the overall proportion of aggressive calls given in response to the truncated advertisement and aggressive calls (Wilcoxon–signed–ranks test; Z=-0.175, N=13 males, P=0.861). The proportion of aggressive calls given in response to truncated advertisement calls showed a nonsignificant increase with stimulus pulse number (Table 2; Friedman test, χ^2=15.94, df=9, P=0.068). There was no evidence of a change in the proportion of aggressive calls in response to truncated aggressive calls of different pulse numbers (Table 2).

DISCUSSION

Male *D. ebraccatus* showed nonrandom call timing in response to playbacks, but the average call delay, and hence the likelihood that the male's call overlapped with the stimulus, depended on the type of call the male produced. For a given response call type, however, call timing was relatively invariant with regard to the properties of the playback stimulus. The evidence obtained in this study suggests that call timing in this species is governed by a homoepisodic response to the onset of the concurrent stimulus. These general results agree with the findings from previous studies of call timing in this species (Schwartz and Wells 1984, Wells and Schwartz 1984a, b). Below, I discuss the results of these experiments in terms of their contribution towards an understanding of the mechanisms of nonrandom call timing.

Call type and call timing

In comparisons of responses to advertisement and aggressive calls, it is clear that call timing is not a property of the type of call that is responded to, but instead is determined solely by the type of call that is given. In response to either of the full stimuli, advertisement calls tended to overlap while aggressive calls tended to alternate with the stimulus. Although in most anuran species females show preferences for leading males when calls overlap (Grafe 1996, Höbel and Gerhardt 2007, Richardson et al. 2008), in *D. ebraccatus*, females prefer lagging advertisement calls in the typical timing arrangement (Wells and Schwartz 1984a). It is interesting that males do not adjust their call delays when responding with advertisement calls to long duration aggressive calls because they suffer a much higher degree of overlap when responding to an aggressive call than when responding with the same delay to an advertisement call. At these higher overlap levels, females may no longer prefer the lagging call (Reichert 2011b). At the same time, male *D. ebraccatus* are constrained from increasing their call delays. The absolute behavioral refractory period, the time in which a stimulus fails to evoke a vocal response from a male following its own call, roughly corresponds with the typical advertisement call delays observed in this species. For *D. ebraccatus*, the absolute behavioral refractory period was estimated to be 210 ms by Narins (1982). Males calling with delays much greater than this refractory period run the risk of having their own calls overlapped and thus rendered less attractive (Wells and Schwartz 1984a).

When presented with truncated advertisement and aggressive calls, males entrained to many of these stimuli and did not show a difference in the proportion of aggressive calls given in response to these two call types. The failure to detect differences in the propensity to give aggressive calls is likely due in part to shorter duration aggressive calls being less effective at eliciting aggressive calls (Wells 1989). Nonetheless, the rapid entrainment to truncated aggressive calls suggests that evaluations of call duration that may ultimately affect the decision to switch to aggressive calls do not happen on the time scale of response to a single call. Thus, there may be a tradeoff between the necessity to respond rapidly to relevant acoustic signals and the ability to detect variation in signal properties. Adjustments to the latter may only manifest after longer term exposures to a stimulus. The intense and fast paced signal competition within *D. ebraccatus* choruses may result in signal detection errors in which males respond inappropriately to aggressive calls, potentially harming their ability to respond to a threat and reducing the attractiveness of their calls to females (Reichert 2011b). Ryan (1986b) suggested a similar conflict between rapid response and call recognition in another Neotropical hylid, *Smilisca sila*.

Mechanisms of call timing

Several pieces of evidence obtained in this study suggest that the nonrandom call timing I observed in *D. ebraccatus* is based on a rapid response to the onset of the concurrent stimulus. Thus, call timing in *D. ebraccatus* is governed by a homoepisodic mechanism. Males entrained their advertisement call responses as well to the randomly timed stimuli as to the stimuli in which the stimulus call period was fixed. Since the time of stimulus onset was unpredictable for the randomly timed stimuli, males' overlapping responses can only be explained if they timed their calls based on cues from the concurrent stimulus. In addition, I demonstrated in the truncated stimulus experiment that males can entrain to stimuli that are substantially shorter in duration than natural calls. Thus, it appears that the beginning of a simulated conspecific call is sufficient to stimulate an entrained, and relatively synchronous, advertisement call response. This form of synchronization has been described rarely in anurans but appears similar to that observed in *S. sila* (Tuttle and Ryan 1982, Ryan 1986b).

Proepisodic phase-delay mechanisms, although more frequently invoked as responsible for nonrandom call timing arrangements in insects and anurans (Greenfield 1994a, b), do not appear to apply to *D. ebraccatus*. Call timing in isolation was not rhythmic, suggesting that any underlying neural pacemaker that triggers call production does not do so in a rhythmic manner. Despite this lack of rhythmicity, males were able to trigger advertisement call responses rapidly to stimuli with both fixed and random timing. In addition, inhibition does not appear to be responsible for adjustments in call timing. Males were not inhibited by the stimulus because many advertisement call responses were initiated long before the cessation of the playback stimulus. Inhibition would also be expected to produce different call delays for stimuli of different lengths. I found no evidence for this. Although the aggressive call stimulus was over 150 ms longer than the advertisement call stimulus, males' advertisement call delays were nearly identical in response to these two stimuli. Furthermore, males called with similar delays to the full advertisement call stimulus and to truncated advertisement calls that had introductory notes as short as 24% of those of the full advertisement call. The few exceptions to this pattern involved males calling with shorter delays to relatively long PN stimuli (e.g., advertisement call with PN=9; Table 2). Finally, it is unclear how a phase delay mechanism can accommodate the different call timing patterns of the two different call types given by these frogs. There are examples in which phase delay models have successfully explained the occurrence of both synchrony and alternation in the timing of a single call type within the same species (Greenfield 1994a). However, in the absence of extreme differences in the time required to produce these two different call types after they have been triggered by the nervous system, phase delay mechanisms do not seem able to explain rapid switches between these two timing regimes based only on a change in the type of call that is given.

Conclusions

This study highlights the need for further study into the mechanisms responsible for nonrandom call timing in chorusing species. In particular, studies of species with multiple call types in their repertoires such as *D. ebraccatus* have strong potential for new insights. Chorusing behavior involves complex interactions between signal timing, call complexity, call type choice, and other forms of signal competition. The effects of these interactions on the outcomes of male–male competition and female choice remain relatively unexplored.

Table 1. Characteristics of full duration playback stimuli

	Call type	
Call characteristic	Advertisement	Aggressive
Introductory note duration (ms)	175	215
Number of pulses	17	61
Pulse rate (pulses/s)	97	285
Number of click notes	1	2
Total call duration (ms)	301	468
Dominant frequency (Hz)	3100	3100

Both call types consist of a pulsed introductory note followed by click note appendages with 100 ms of silence in between notes. The total call duration is the time from the onset of the introductory note to the offset of the last click note.

Table 2. Advertisement call responses to full duration and truncated stimuli

Stimulus type	PN	Mean angle (±SE)	*R'*	*N*	Proportion aggressive calls (±SE)	*N*
Advertisement	Full	24.31 (4.7)	2.72**	30	0.37 (0.06)	35
	1	229.27	0.41	15	0.06 (0.03)	16
	2	52.02	0.74	16	0.08 (0.03)	17
	3	61.22	0.76	14	0.13 (0.06)	17
	4	32.99 (14.5)	1.59**	16	0.12 (0.05)	16
	5	25.51 (12.3)	1.78**	16	0.09 (0.04)	17
	6	11.46 (10.6)	1.74**	16	0.07 (0.03)	17
	7	25.66 (6.1)	1.88**	14	0.17 (0.03)	15
	8	22.58 (11.0)	1.80**	15	0.11 (0.03)	15
	9	8.58 (5.1)	1.92**	14	0.14 (0.04)	16
	10	26.80 (5.8)	1.91**	14	0.16 (0.04)	15
Aggressive	Full	18.01 (8.6)	1.33*	6	0.85 (0.05)	26
	3	104.17	0.88	15	0.11 (0.03)	15
	6	2.67	0.91	15	0.11 (0.04)	15
	9	34.15 (12.5)	1.59**	15	0.08 (0.04)	15
	12	13.11	0.94	14	0.11 (0.03)	14
	15	56.51	0.34	13	0.11 (0.03)	14
	18	66.94	0.94	15	0.06 (0.03)	15
	21	30.28	0.95	14	0.13 (0.03)	15
	24	48.31	0.92	14	0.06 (0.04)	15
	27	47.90	0.94	14	0.08 (0.03)	14
	30	44.29 (21.8)	1.14*	13	0.18 (0.05)	14
	33	26.15 (8.1)	1.62**	11	0.16 (0.07)	14

Call delays are given as mean phase angles in degrees (± circular standard error) only for advertisement call responses. The responses to the full stimuli presented here are pooled to include both the fixed and randomly timed stimuli. Few males gave advertisement call responses to full aggressive calls, hence the low sample size. Standard errors were only calculated for those mean angles for which there was statistical evidence of a significant mean direction. *R′* is the test statistic for a nonparametric second order test of circular uniformity. Significant *R′* values imply that call delays were nonrandomly distributed. Sample sizes refer to the number of males whose responses were included in the analyses (each male contributed a single data point – its average phase angle). The proportion of aggressive calls is the grand mean of the mean proportion of aggressive calls for each male (± standard error). * $P<0.025$; **$P<0.001$.

Figure 1. Waveform displays of typical call timing delays for advertisement call responses (upper traces) of subject males to the playback stimuli (lower traces). The measurement of call delays is illustrated in **a**. Call delays were calculated by subtracting the start time of the male's call from the start time of the playback stimulus. **a** Advertisement call response overlapping with the full advertisement call stimulus. **b** Advertisement call response overlapping with the full aggressive call stimulus. **c** Advertisement call response to a truncated (PN=4) advertisement call stimulus.

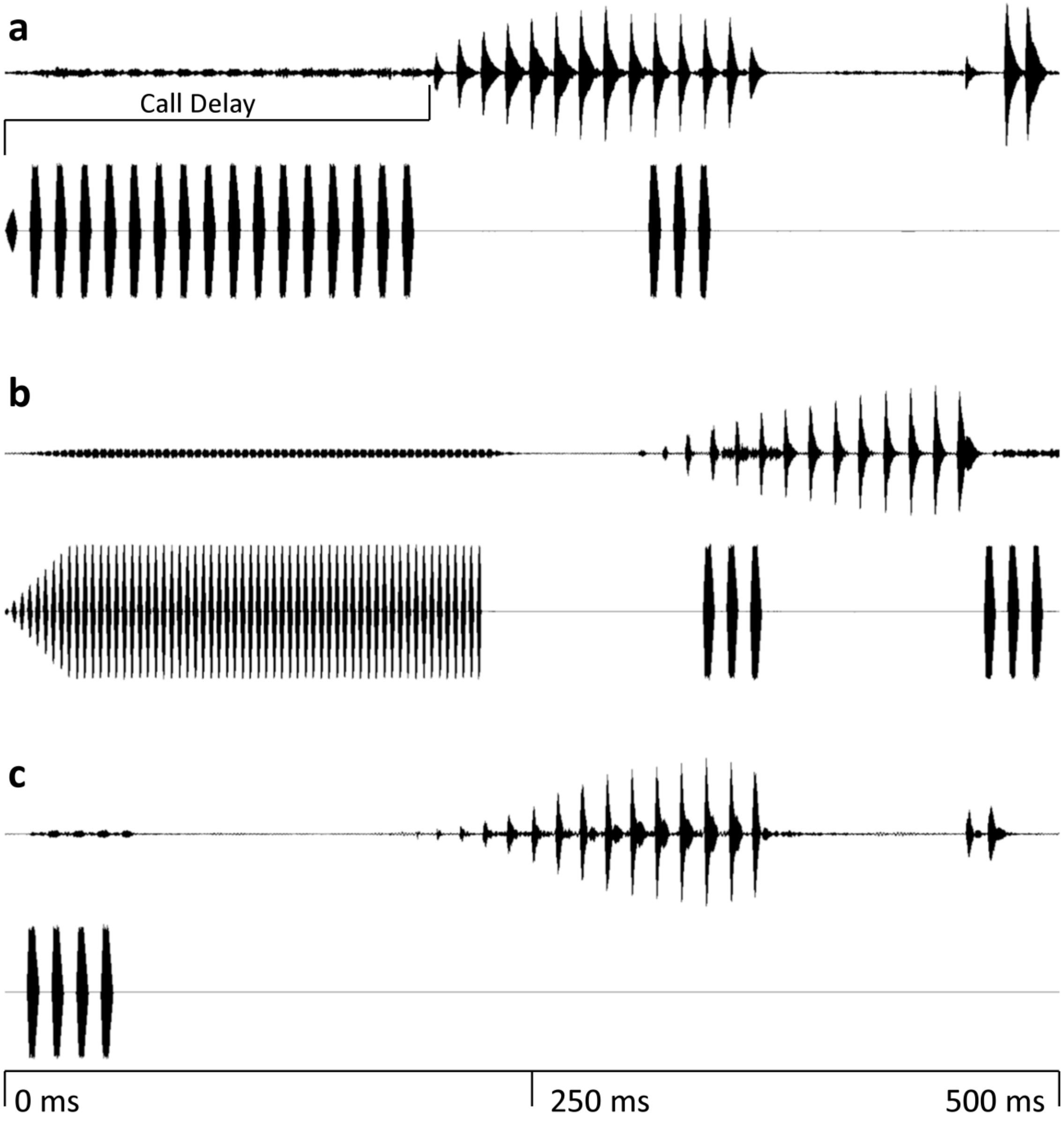


Figure 2. Polar phase plots for **a** advertisement call and **b** aggressive call responses to stimuli with either fixed or randomly timed call periods (combined across stimulus call type). Points represent median phase angles for individual males. Arrow points in the direction of the grand median phase angle. The length of the arrow is *r*, the length of the mean vector.

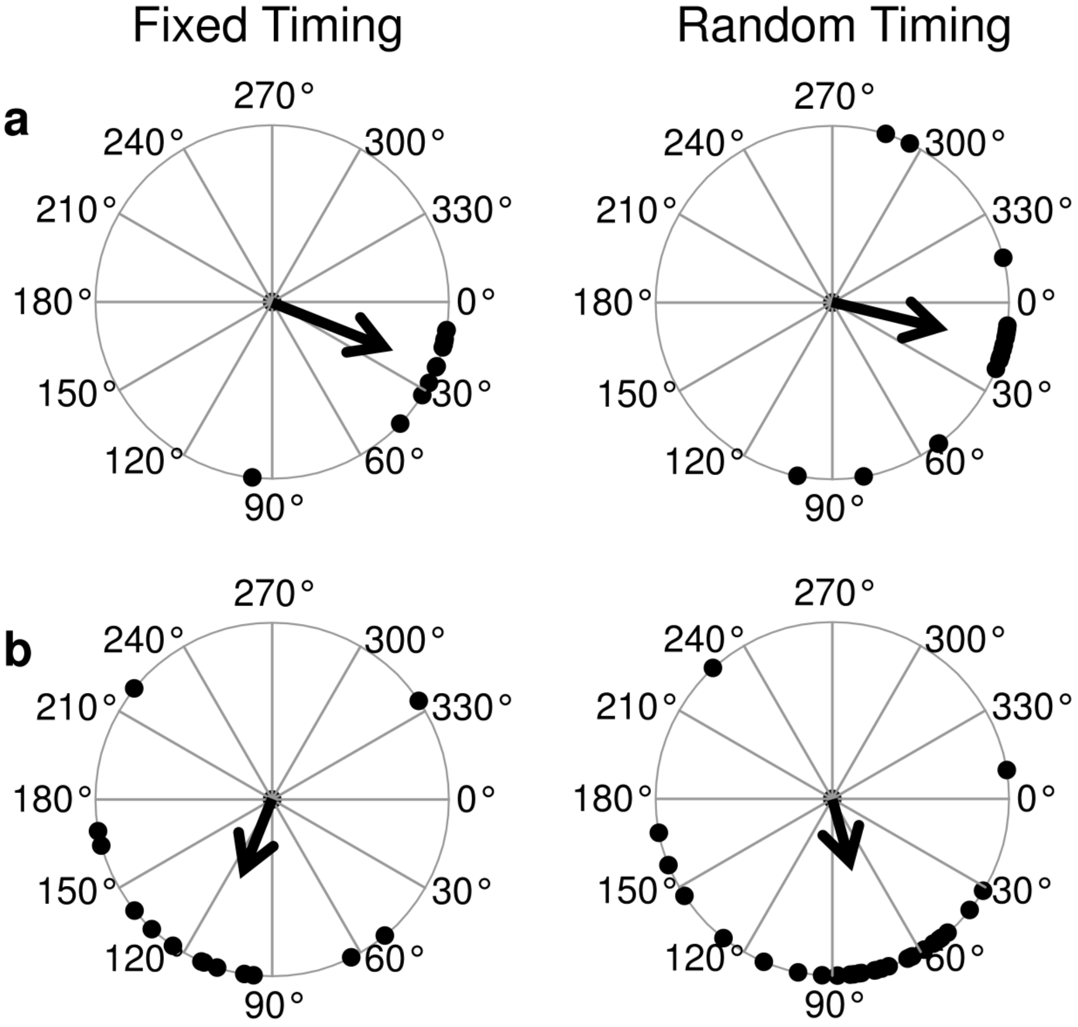


Figure 3. Median phase angles for call responses to either advertisement or aggressive call stimuli (combined across responses to fixed and randomly timed stimuli). **a** Advertisement call responses. **b** Aggressive call responses. Data points and arrows as in Figure 2.

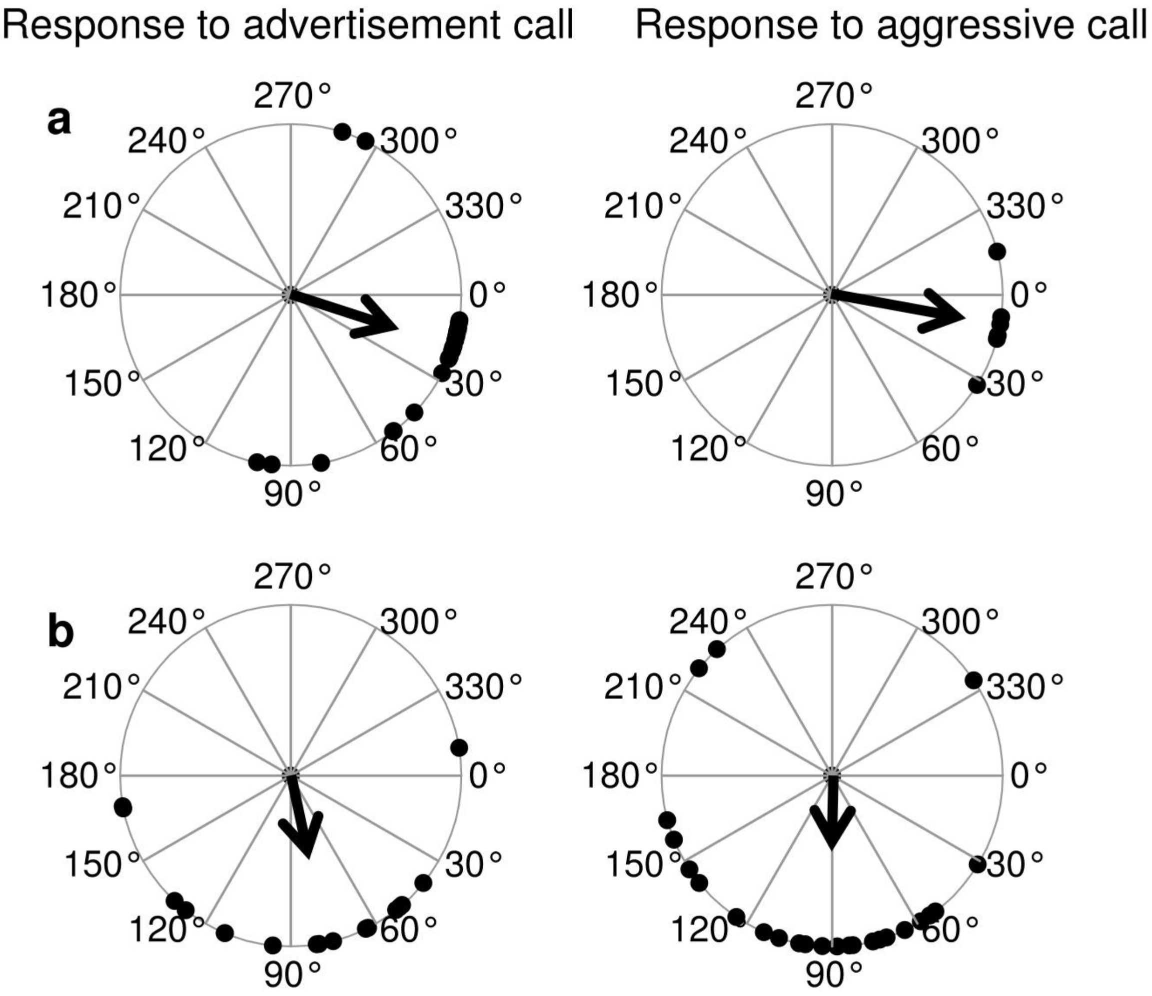
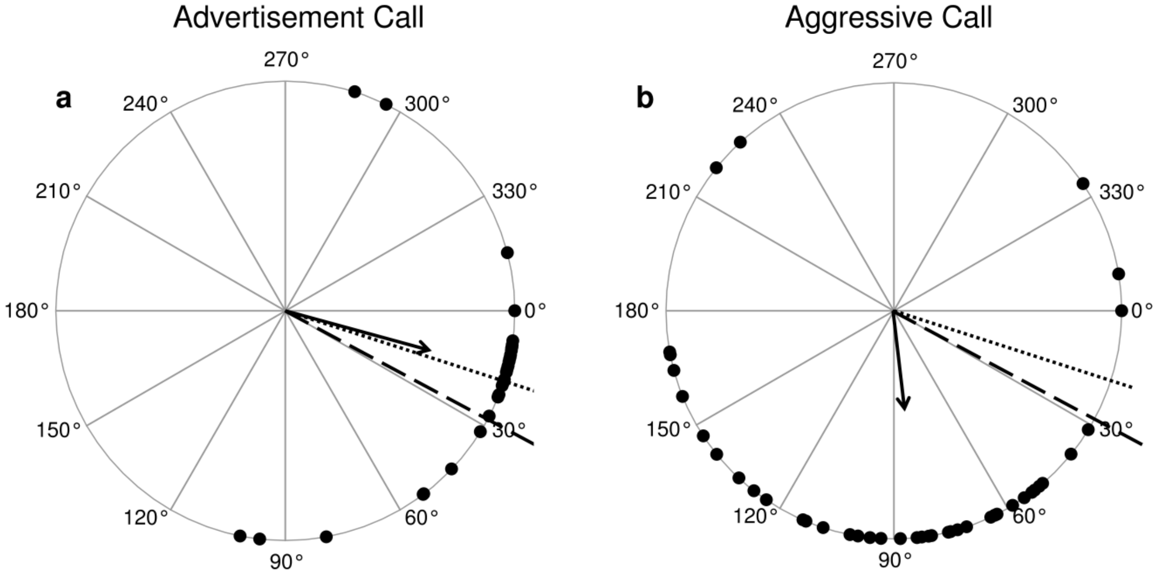


Figure 4. Median phase angles for **a** advertisement call and **b** aggressive call responses to full stimulus playbacks (combined across stimulus call type and call timing regime). Dotted line represents the offset of the advertisement call playback stimulus as a phase angle. Dashed line represents the offset of the aggressive call playback stimulus as a phase angle. The stimulus onset is at 0°; thus, median male responses that actually overlapped the playback stimuli lie between 0° and the line for the respective stimulus. Data points and arrows as in Figure 2.



CHAPTER 5

Aggressive calls improve leading callers' attractiveness in the treefrog *Dendropsophus ebraccatus*

ABSTRACT

In complex acoustic choruses, competitive call timing interactions are often important in female mate choice. In the treefrog *Dendropsophus ebraccatus*, neighboring males' advertisement calls tend to overlap and females prefer lagging calls. Males that produce leading advertisement calls are thus at a disadvantage relative to lagging males. In this study, I propose a novel strategy by which leading males may overcome this problem: the production of aggressive calls. Aggressive calls are longer than advertisement calls. Therefore, if a lagging male responds with an advertisement call to a leading aggressive call, the leading aggressive call may end after the lagging call. If females prefer calls that end last, then leading aggressive calls may be more attractive. I compared female preferences for advertisement and aggressive calls when they either overlapped, with the aggressive call leading, or alternated. Females preferred the advertisement call in alternation, but this preference was abolished when it overlapped with the aggressive call. I recorded interactions between pairs of males to determine whether leading males utilized aggressive calls as predicted. Among leading calls, aggressive calls were more likely to end after lagging calls than were

advertisement calls. When switches to aggressive calling occurred after a bout of overlapping advertisement calls, it was more likely that the male that switched to aggressive calling had been in the leading position previously. These experiments suggest a strategy for leading males to reduce their disadvantage in call timing interactions and provide an explanation for this species' high levels of aggressive calling.

INTRODUCTION

Competitive signaling interactions between males are a hallmark of the chorusing behavior of many species of acoustically signaling animals. Such interactions, in addition to mediating competition for calling space or territories among males (Wells 1977), are also important in influencing female choice of mates. Within chorusing anurans, female choice is often based solely on characteristics of the acoustic signals of males (Gerhardt 1994), including the very characteristics that are most likely to be adjusted by males in the course of signal competition (Gerhardt 1991). Thus, understanding how vocal competition works between naturally interacting individuals is essential to understand how the acoustic structure of the chorus, in which female choice takes place, is formed.

Nearly every measurable call characteristic has been implicated as being important to female choice in at least some species of anurans. Most of these characteristics (e.g., call duration, frequency, rate, etc.) are properties of individual males' calls, although females may assess some of these characteristics relative to those of other males (Ryan et al. 1992, Gerhardt et al. 1996, Wollerman 1998, Murphy and Gerhardt 2000, Schwartz et al. 2001, Bush et al. 2002, reviewed by Gerhardt and Huber 2002). One characteristic is of particular interest for studies of chorusing individuals because it can arise only through interactions with others: the often precise timing of calls by a male with respect to those of other nearby individuals (Greenfield 1994a, b). Such nonrandom call timing can result in choruses in which signalers largely synchronize or alternate calls with one another (e.g., Walker 1969, Zelick and Narins 1983, Greenfield and Roizen 1993, Minckley et al. 1995, Grafe 2003). For the most part, synchrony and alternation appear to arise either directly or indirectly through acoustic competition related to female preferences for calls in certain timing arrangements (Greenfield 1994a). Neither synchrony nor alternation is exact, however, and males compete for position as either the leading or the lagging individual (Greenfield 1994b, Grafe 1999, 2003). Under these circumstances, females of most species that have been studied have shown preferences for leading calls (e.g., Greenfield 1994a, Grafe 1996, 1999, Höbel and Gerhardt 2007, Richardson et al. 2008).

Another form of acoustic competition observed in many anuran species is the production of aggressive calls. These calls are distinct acoustically from advertisement calls (the primary female attracting call) and presumably are used to mediate the outcomes of agonistic male–male interactions as this is the context in which they tend to be given in most species (Wells 1977). Despite extensive research, relatively little is known about how aggressive calls are actually used to resolve disputes between male anurans, much less which call characters may be involved (Wells 2007). Although aggressive calls may be useful in repelling rival males, production of aggressive calls comes at a cost: Aggressive calls are usually less attractive to females than advertisement calls (Oldham and Gerhardt 1975, Schwartz and Wells 1985, Wells and Bard 1987, Backwell 1988, Grafe 1995, Brenowitz and Rose 1999). Thus, in many species, production of aggressive calls is limited to early in the nightly chorusing period and males quickly habituate to their neighbors, giving few aggressive calls if the chorus remains stable (Wells 1988a, Brenowitz and Rose 1994, Grafe 1995).

The Neotropical treefrog *Dendropsophus ebraccatus* is unusual among anuran species whose aggressive calling and call timing behavior have been analyzed. Males give relatively high levels of aggressive calls throughout the night when calling spontaneously (Reichert 2010). The frequency (of occurrence) of aggressive calls given in response to playbacks is even higher, and males are actually sensitized over the short term to aggressive calls so that thresholds for the aggressive response become lower (Reichert 2010). Although aggressive calls are given during close-range agonistic interactions (Schwartz and Wells 1984, Wells and Schwartz 1984b), these types of interactions are rare and do not appear to be the context in which most aggressive calls are given. Even though males (and sometimes entire choruses) give bouts of aggressive calls frequently and repeatedly throughout the nightly chorusing period, such high levels of aggressive calling do not appear to be related to more direct consequences of aggression such as adjustments of the size of calling spaces, chasing and retreat behavior, or physical fighting (personal observation). The high levels of aggressive calling are especially unusual because previous studies have shown that females of this species prefer advertisement calls to aggressive calls (Wells and Bard 1987).

The advertisement and aggressive calls of *D. ebraccatus* consist of pulsed introductory notes that are usually, although not always, followed by one or more acoustic suffixes termed click notes (Wells and Schwartz 1984a, b). Aggressive calls differ from advertisement calls in several temporal characteristics: They tend to be longer in duration and always have higher pulse rates in comparison with advertisement calls (Wells and Schwartz 1984a, b). Both advertisement calls and aggressive calls are variable with respect to changes in the social environment. Males tend to increase the number of click notes in their advertisement calls in response to acoustic competition (Wells and Schwartz 1984a). Aggressive calls vary in a graded fashion with the proximity and call characteristics of nearby males. Relatively isolated males tend to give aggressive calls with short introductory notes, high pulse rates and many click notes (Wells and Schwartz 1984b). When males are in denser areas of the chorus or are responding to relatively long aggressive calls, their aggressive call responses have longer introductory notes, lower pulse rates and few, if any, click notes (Wells and Schwartz 1984b, Wells 1989).

Competitive call timing is clearly an important factor in male–male interactions and female choice in *D. ebraccatus* (Schwartz and Wells 1984, Wells and Schwartz 1984a, b; Chapter 4). The diversity of call timing interactions in this species is particularly noteworthy: Males synchronize their advertisement calls, whereas aggressive calls are given in alternation (Schwartz and Wells 1984, Wells and Schwartz

1984a; Chapter 4). These call timing arrangements were not dependent on the kind of playback stimulus: Advertisement calls were produced with the same degree of synchrony and aggressive calls with the same degree of alternation, in response to both aggressive and advertisement calls. Responses to low pulse number calls were also similar, despite the large differences in duration and other call characters among these different stimuli (Chapter 4). Unlike most other species that prefer leading calls, female *D. ebraccatus* have been shown to prefer lagging advertisement calls when there is overlap (Wells and Schwartz 1984a). Lagging advertisement calls tend to obscure the click notes of leading calls; the click notes of lagging calls remain unobscured because the refractory period is too long for the leading male to reciprocate (Narins 1982). Thus, leading males are at a disadvantage in competitive call timing interactions.

This study addresses these 2 major questions: 1) Why do males give so many aggressive calls? and 2) How can males overcome the disadvantage of calling in the leading position? I hypothesize that these 2 questions are in fact related and propose a novel explanation for the function of aggressive calls in *D. ebraccatus*. Namely, aggressive calling allows leading males to retake the advantage in competitive call timing interactions. In the case of advertisement calling, lagging calls both start later and end later than leading calls. Either of these characteristics could underlie female preferences for lagging over leading advertisement calls, but the more likely criterion is a preference for the call that ends last because of the attractiveness of the terminal click notes (Wells and Schwartz 1984a). Aggressive calls are typically longer (in terms of full call duration, see below) than advertisement calls (Wells and Schwartz 1984b, this study) and males synchronize with similar delays when responding to both advertisement calls and aggressive calls (Chapter 4). Thus, it is likely that leading aggressive calls will often end after lagging advertisement calls. If female preferences favor the call that ends last, then they may prefer a leading aggressive call to a lagging advertisement call. Thus, males in the leading position could equalize or possibly regain the advantage in competitive call timing interactions through the production of aggressive calls. As bouts of synchrony are quite common in this species, this hypothesis would also account for the relatively high levels of aggressive calling that have been observed. In this study, I describe an analysis of a series of recordings of natural interactions between pairs of males to further characterize the nature of call timing competition in *D. ebraccatus*. I used these recordings, along with a series of female choice tests, to test the hypothesis that leading males may use aggressive calls to reduce their disadvantage in call timing interactions.

METHODS

Natural interactions

I recorded the natural calling interactions of 16 pairs of males in August 2009 at the Smithsonian Tropical Research Institute’s field station in Gamboa, Panama. Recordings were made in the sites described by Reichert (2010) as well as the Experimental Pond, an artificial pond located in the Santa Cruz neighborhood. I identified pairs of males that were close to each other and relatively isolated from other individuals (i.e., no other individuals within twice the distance between the focal males). For each pair, I recorded 30 min of their natural calling interactions. I recorded each male’s calling on a separate channel of a digital audio recorder (Marantz PMD-661; 16-bit pcm files, 44.1 KHz sampling rate) using directional microphones (Sennheiser ME-66 and ME-67), creating a stereo recording that contained each male’s entire calling output for the recording period. After recording, I used a tape measure to measure the distance between the males’ calling sites. Some males moved during the recording (N=5); thus the distances I present correspond to the males’ positions at the end of the recording. I was unable to measure initial between-male distances because such measurements would have disturbed the males and caused them to retreat from their calling sites. When males moved, I noted whether the net movement was toward or away from each other. Three recordings had a duration of less than 30 min because one of the males either mated or retreated very far from its original calling site. After the recordings, I captured each male, measured its snout–vent length and mass and gave it a unique toeclip for individual identification.

The major aim of this study was to test the hypothesis that leading males use the aggressive call as a means of equalizing the advantage in competitive call timing interactions in which females prefer males whose calls end last. I was able to test 3 major predictions that follow from this hypothesis with these data. First, aggressive calls should be longer in duration on average than advertisement calls. Leading aggressive calls should also be more likely to end after a synchronized lagging call than leading advertisement calls. By duration, I am referring to the full duration of the call from the beginning of the introductory note to the end of the last click note. Call duration could also be quantified as the duration of the introductory note or as the total number of notes, but neither of these measures are as suitable as the full call duration for the quantification of call overlap, which usually involves the overlap of the click notes of the leading male by the introductory note of the lagging male (Wells and Schwartz 1984a). Second, leading males should give a higher proportion of aggressive calls than lagging males. There should be a negative correlation between the proportion of calls given in the lagging position and the proportion of aggressive calls given. Third, males should be more likely to transition to aggressive calling after being overlapped than after overlapping when giving advertisement calls. Preliminary analyses indicated that the responses of interest in these 3 predictions were independent between individuals in a pair. Thus, when appropriate (predictions 1 and 3), I considered each male's response as a separate data point to improve the clarity and power of statistical analyses.

Call analyses

I analyzed temporal and spectral parameters of every call given by each male using sound analysis software (Raven Pro 1.3; Cornell Laboratory of Ornithology). I noted whether each call was an advertisement or aggressive call and measured the following: start time of call, full call duration, duration of the introductory note, number of pulses in the introductory note, number of click notes and the dominant frequency. From these measures, I calculated call period as the time between a male's consecutive calls, pulse rate as the number of pulses divided by the duration of the introductory note, duty cycle as the ratio of introductory note duration to call period, and the proportion of each male's calls that were aggressive. Detailed descriptions of these call parameters are given by Wells and Schwartz (1984a, b).

I used the starting and ending times of each male's calls to assess the timing of its calls relative to its neighbor. I calculated call delays each time there was a transition in which male was calling (i.e., I only calculated call delays for a male's first response to the calls of its neighbor. Any consecutive uninterrupted responses to that call were excluded). I generated additional categorical measures of call timing as follows: I considered calls to be in synchrony if the onset of the lagging call was within 0.6 s of the offset of the leading call. Otherwise, calls were considered to have alternated. For each synchronous call, I noted whether the call was in the leading or lagging position (i.e., whether it occurred first or second in time). I also noted the number of calls that actually overlapped in time.

Data analyses

I generated a "lagging index" (LI) as the proportion of the male's synchronized advertisement calls that were in the lagging position. I used the LI values to determine, for each interaction, the male that more consistently called in the leading (henceforth, the leading male) and lagging (henceforth, the lagging male) position. I used separate correlation analyses for leading and lagging males to determine if there were correlations between LI, any of the call characters, the distance between males, and the size variables. I also ran a correlation analysis of the difference between leading and lagging males for these variables. Finally, I used paired *t* tests to determine if leading and lagging males differed in size or call characters.

Female choice tests

I used a standard 2-speaker phonotaxis design to determine female preferences for aggressive calls in the leading position. I obtained responses from 12 females in the Gamboa population in August and September 2009. I tested an additional 10 females in August and September 2010 from a population at La Selva Biological Research Station in Costa Rica. These latter females were captured at Cantarrana Swamp, the Experimental Pond and a flooded field located just outside of the station's entrance.

I captured gravid females in amplexus and returned them to the phonotaxis arena for testing. All tests took place between 2200 and 0300 h. The layout and dimensions of the phonotaxis arena were the same in both years, but the La Selva arena was located within a fully screened shadehouse, whereas the Gamboa arena was under a roofed hut that was open on the sides. Neither arena was soundproof, but both were located far from any audible *D. ebraccatus* chorus noise. I placed 2 portable amplified speakers (Mineroff SME-AFS) 1.5 m from the female release point such that they formed an angle of approximately 90° with respect to this point. The sound pressure levels (SPLs) of the stimuli broadcast from the speakers were equalized to 87 dB SPL ("fast" peak setting) at the female release point using a Radio Shack 33-2055 portable SPL meter. Stimuli were broadcast from a compact disk player (Panasonic SLSW940S) through a stereo cable so that each stimulus could be played through a separate speaker. For each test, I began playback of stimuli for 30 s while the female was restrained in an acoustically transparent cage. I then raised the cage remotely to release the female and allow her to move freely about the arena. I observed female movements using the night vision feature of a handheld video camera (Sony DCR-SR85). I defined a choice as a deliberate movement by the female to within 10 cm of one of the speakers. Females that failed to reach a speaker within 10 min were tested again later in the night. I required females to respond to both test stimuli as well as to a control stimulus: An advertisement call played from a single speaker to ensure that her movements were in response to sound rather than simply attempts to escape.

The 2 test stimuli presented to females both involved presentation of an advertisement call from one speaker and an aggressive call from the other but differed in the relative timing of these calls. The individual call stimuli were generated using a sound synthesis computer program provided by J. Schwartz. The advertisement call stimulus had properties like those of the average call in the population and consisted of a pulsed introductory note 175 ms in duration with 17 pulses given at a pulse rate of 97 pulses/s. This introductory note was followed, after a delay of 100 ms, by a single click note that gave the advertisement call a full duration of 301 ms. The aggressive call stimulus used in this experiment approximated the mid–point of the gradation typical of *D. ebraccatus* aggressive calls (Wells and Schwartz 1984b, Wells 1989). The introductory note of the aggressive call was 215 ms in duration with 61 pulses given at a pulse rate of 285 pulses/s. This introductory note was followed by 2 click notes, each separated by 100 ms. The full duration of the aggressive call stimulus was 468 ms. The dominant frequency of both call types was 3100 Hz. I used a sound editing computer program (Cool Edit Pro 2.0; Syntrillium 2002) to create stereo files with the appropriate spacing and repetition rate between calls on each channel. The alternating stimulus consisted of the advertisement and aggressive calls arranged such that there was no overlap between them; they were in perfect alternation with one another (Figure 1a). The overlapping stimulus consisted of the same calls, but in this case there was overlap between the 2: The advertisement call began 150 ms after the aggressive call. In this scenario, although the aggressive call began first, it was long enough so that it ended after the advertisement call (Figure 1b). Stimuli were repeated with a period of 6 s. I compared the proportion of females responding to each stimulus with the null expectation of no preference using binomial tests. All statistical analyses were carried out with SPSS 16.0.1 software (SPSS Inc., 2007) using an alpha value of 0.05.

RESULTS

Description of natural interactions

In the 30-min recording period, males spent a substantial proportion of time giving aggressive calls (Figure 2; mean percentage of calls that were aggressive ± SD: 10.6±8.1%, *N*=32 males). The percentage of a male's advertisement calls that were in physical overlap (either leading or lagging) with its neighbor's calls ranged from 7% to 82% (Figure 2; mean ± SD: 41.6±21.9%, *N*=32 males). The degree to which one male tended to lag the other in its advertisement calls (LI) varied from near equality to highly consistent overlap of one male's calls by the other (Figure 2; LI of lagging males; mean±SD: 69.9±11%, *N*=16 pairs).

Males were relatively stationary during the recordings. In 11 trials, neither male moved. In the remaining trials, 3 involved movements of males toward each other and 2 involved movements of males away from each other. Although the sample size of interactions that involved movement was small, there was no evidence for a difference in the amount of aggressive calling given during these interactions than during those in which no movement was observed (*t*-test: *t*=0.832, *df*=14, *P*=0.419). The average distance (±SD) between males was 318 (±25) cm. This distance is larger than the typical spacing in the densest areas of the study choruses and is reflective of the generally low abundance of individuals of this species during the study period in relation to those observed in previous years (personal observation). Despite this low density, the patterns of call timing and aggressive calling clearly demonstrate that individuals interacted acoustically with their neighbors during the recordings.

Distance between males had an effect on measures of acoustic competition. There was no relationship between distance and advertisement call total duration (*r*=-0.185, *N*=15 pairs, *P*=0.509), but males that were closer together tended to have shorter call periods (averaged over both males in a pair; correlation analysis: *r*=0.581, *N*=15 pairs, *P*=0.023) and higher duty cycles (*r*=-0.565, *N*=15 pairs, *P*=0.028). Thus, there was a higher overall level of calling effort when males were closer together. At these low densities, there were no relationships between distance and any aggressive call character or between distance and the proportion of calls given during the interaction that were aggressive. There were indications that distance had an effect on the nature of call timing competition. Distance was negatively correlated with the proportion of advertisement calls that overlapped (Figure 3a; *r*=-0.643, *N*=15 pairs, *P*=0.01). There was a negative, although nonsignificant, relationship between the distance between males and the difference between their LI's (Figure 3b; *r*=-0.45, *N*=15 pairs, *P*=0.09). Thus, success in call timing competition in terms of placing advertisement calls in the lagging position was more equal between males that were spaced farther apart.

Descriptives of some temporal call characteristics are given in Table 1. Temporal characters of the advertisement calls of the 2 males in a pair tended to be highly correlated with each other (Table 2). As a consequence, there were few differences in calling characteristics between leading and lagging males. Leading males had longer total duration aggressive calls (paired *t* test, *t*=3.96, *df*=12, *P*=0.002) and aggressive calls with more clicks (*t*=3.87, *df*=12, *P*=0.002) than lagging males. The difference between lagging and leading males in a pair in LI was positively correlated with the difference in the call periods of their advertisement calls (*r*=0.531, *N*=16 pairs, *P*=0.034). Thus, the greater the degree to which one male consistently lagged another's calls when overlap occurred, the longer its average call period was in relation to that of the leading male. Nonetheless, there was no direct relationship between LI difference and the difference in the proportion of singleton calls (i.e., calls given directly after their own call with no prior response from the other male; *r*=-0.4, *N*=16 pairs, *P*=0.13), nor was there a difference in the proportion of singleton calls between leading and lagging males (*t*=0.211, *df*=15 pairs, *P*=0.836). This suggests that leading males did not necessarily recoup the advantage in the call timing interaction by producing more calls entirely unobscured from overlap.

The total duration of advertisement calls that overlapped was greater than that for calls that did not overlap (Figure 4; $t=4.641$, $df=31$, $P<0.0005$). There was no difference in the total duration of overlapping advertisement calls between calls that were in the leading and lagging positions (Figure 4; $t=1.311$, $df=31$, $P=0.2$).

Use of aggressive call in call timing competition

The first prediction stemming from my hypothesis that leading males use aggressive calls in call timing competition is that the full duration of aggressive calls should be longer than that of advertisement calls. Indeed, aggressive calls were on average 178 ms longer than advertisement calls (Table 1; paired *t* test, $t=14.0$, $df=28$, $P<0.0005$). In fact, the full duration of aggressive calls was significantly longer than that of advertisement calls for each of the 29 males that gave both call types during the recordings. Importantly, leading aggressive calls were much more likely to finish after a lagging advertisement call than were leading advertisement calls (Figure 5a; paired *t* test, $t=5.174$, $df=29$, $P<0.0005$). Thus, aggressive calls are much better at allowing a leading male's calls to finish last when there is overlap.

The second prediction was that leading males should give a higher proportion of aggressive calls than lagging males. There was no evidence that this was the case (Figure 5b; paired *t* test, $t=1.192$, $df=15$, $P=0.252$). Interactions that were characterized by a higher degree of overlap (*r*=-0.019, *N*=16 pairs, *P*=0.94) or by a greater disparity in LI (*r*=-0.108, *N*=16 pairs, *P*=0.69) did not involve a greater amount of aggressive calling.

The third prediction was that transitions to aggressive calling from advertisement calling should be more likely after overlap when the male is in the leading rather than lagging position. This was, in fact, the case (Figure 5c; paired *t* test, *t*=2.464, *df*=30, *P*=0.02). The duration of the lagging advertisement call may have been responsible for leading males switching to aggressive calls: Lagging advertisement calls that elicited an aggressive call from the other male averaged more click notes than those that did not elicit an aggressive call (Figure 5d; paired *t* test, *t*=4.926, *df*=28, *P*<0.0005)

Female choice tests

Twenty-two females responded to both stimuli. When the advertisement call was presented in alternation with the aggressive call, females strongly preferred the advertisement call (Figure 6; binomial test, *P*=0.004). When the same stimuli were presented in overlap such that the advertisement call began after the aggressive call, this preference disappeared: More females moved toward the speaker broadcasting the aggressive call, although there was no statistically significant preference for either call (Figure 6; binomial test, *P*=0.52). Females responded to these 2 stimuli differently (McNemar’s test, *P*=0.035).

DISCUSSION

A number of factors appear to be at play in acoustic competition in *D. ebraccatus*. My results indicate that both nonrandom call timing and the production of aggressive calls are significant factors. Moreover, these 2 forms of signal competition are related: Because aggressive calls tended to be longer than advertisement calls, males in the normally unattractive leading position may mitigate their disadvantage by giving aggressive calls. Thus, competitive call timing interactions in *D. ebraccatus* are not as simple as the jockeying for leading or lagging positions that have been described in other species (Greenfield 1994a, b). Rather, they appear to involve the complex interplay of female preferences, use of multiple call types and the ability to adjust call complexity.

Call timing interactions were independent of other measures of acoustic competition. Differences in LI between males did not correspond in any consistent manner to differences in other call characters. However, call timing interactions did appear to be affected by distance. Interestingly, the closer together 2 males were, the more lopsided was the call timing interaction in favor of one male. At greater distances, calls would be less easily detected by other males (e.g., Penna et al. 2005), thus it may be difficult for one male to consistently respond rapidly to the calls of another. This result suggests that certain males may pay much higher prices for calling in dense choruses than others. Although masking of call features by chorus noise is a serious cost for any signaler in a dense aggregation (Gerhardt and Klump 1988, Wollerman and

Wiley 2002a, Bee 2008, Bee and Micheyl 2008), males that are worse at timing their calls with respect to other males may be in an especially poor position in the densest choruses. Certain other call characters varied with distance as well, but call timing was the only character for which the disparity between males increased the closer together they were. Other temporal call characters were highly correlated between males, suggesting that although males may alter these characters in competition with other males, the level of response by both males is similar. In contrast to the results presented in this study, a previous study of this species showed strong relationships between distance and some aggressive call characteristics (Wells and Schwartz 1984b). This work was conducted with males that tended to be much closer to one another than those in the current study. Perhaps distance has a stronger effect on call characteristics at higher densities and closer intermale distances. It would be interesting to determine if distance also affects call timing interactions at close range.

I obtained evidence that the production of aggressive calls by leading males may be an important part of competitive call timing interactions in *D. ebraccatus*. Most importantly, I showed that a strong female preference for advertisement calls over aggressive calls when presented in alternation was abolished by positioning these calls in overlap with one another such that the aggressive call led, but also finished after, the advertisement call. This arrangement mimicked the average advertisement call timing that males gave in response to playbacks of aggressive call stimuli (Chapter 4). It is essential to note that not only did females prefer advertisement to aggressive calls when presented in alternation but also they strongly preferred lagging to leading advertisement calls when these were presented with a similar timing relationship to that used in this study between overlapping aggressive and advertisement calls (Wells and Schwartz 1984a). Thus, it was the production of aggressive calls by leading males that enhanced their attractiveness to females. The mechanism responsible for the abolition of preference for advertisement calls when they overlap with aggressive calls must be determined in future studies. One possibility is that females prefer lagging calls when the degree of overlap is low (as has been demonstrated for typical overlapping advertisement calls; Wells and Schwartz 1984a), but this preference is abolished when the degree of overlap is high (as in the overlapping aggressive and advertisement call stimulus used in this study). A similar switch from preference for leading or lagging calls depending on the degree of overlap has been demonstrated in the running frog, *Kassina fusca* (Grafe 1999). Alternatively, female preferences may generally favor the male whose call ends last. This study demonstrated only that females did not discriminate strongly between overlapping advertisement and aggressive calls and thus does not provide strong support for this hypothesis. However, the aggressive call ended only 17 ms after the advertisement call; this interval may be too small for females to detect reliably which male's call ended last. Furthermore, aggressive calls are generally less attractive than advertisement calls in other situations (Wells and Bard 1987), thus a female preference for the last call heard may have conflicted with female preferences for advertisement calls.

There was evidence that males did employ the aggressive call in call timing interactions as I hypothesized. Aggressive calls were longer in duration than advertisement calls and this made them far more likely than advertisement calls to end last when there was overlap, even when given in the leading position. The aggressive calls of leading males on average were longer in duration and contained more click notes than did those of lagging males. The increased duration of aggressive calls was achieved by the addition of a greater number of click notes in these calls; introductory note durations of aggressive calls actually tended to be lower than those of advertisement calls. It is unclear why males, particularly those in the leading position, do not add more clicks to their advertisement calls rather than switch to aggressive calls. In fact, there were no differences in advertisement call duration between leading and lagging males. It may be that the structure of advertisement calls constrains click note production more than does that of aggressive calls. This is a subject for future studies. The important point here is that aggressive calls are indeed longer than advertisement calls and thus influence call timing interactions.

Two predictions that were not supported by these data were that males should spend a greater amount of time giving aggressive calls under 2 conditions: 1) when the overall proportion of overlap in the interaction was higher and 2) when they spent more time in the leading position. These predictions may not have been met because the overall proportion of aggressive calls given during the interaction may be a relatively insensitive measure. Once males switched to aggressive calling, they tended to give fairly long bouts of aggressive calls before switching back to advertisement calls. Thus, the more relevant measure may be the event that triggered the bout of aggressive calling to begin with. Here, in fact, I found further evidence in support of my hypothesis.

Males were far more likely to begin giving aggressive calls after a bout of advertisement call overlap when they were in the leading rather than the lagging position. Furthermore, switches by leading males to bouts of aggressive calling were more likely to be brought on when the lagging male added more click notes to its advertisement call. Portions of a lagging call may be imperceptible to the leading male while it is actually calling (Schwartz and Rand 1991, Narins 1992), precluding its ability to respond to these calls. If the lagging male adds too many click notes, however, this may extend the duration of the call such that the leading male is able to respond.

Given the evidence above, I summarize my proposed mechanism for call timing in *D. ebraccatus* as follows. Individuals respond rapidly to the production of advertisement calls of a nearby male with advertisement calls that partially overlap those of the leading male (Chapter 4). These lagging advertisement calls are more attractive to females (Wells and Schwartz 1984a). When the leading male detects that its calls are being overlapped, it switches to the production of longer aggressive calls. As lagging males must respond extremely rapidly in order to synchronize their own call response to that of their neighbors, signal detection errors in which the lagging male responds with an advertisement call to an aggressive call are likely. Lagging males do not adjust the timing of their advertisement calls in such circumstances (Chapter 4) and thus more of their advertisement call is overlapped, and the male loses its advantage in attracting females. After a few bouts, the lagging male usually switches to aggressive calls as well. These alternate with each other (Wells and Schwartz 1984b; Chapter 4), so neither male necessarily has an advantage. However, females are less attracted to isolated aggressive calls than they are to advertisement calls (Wells and Bard 1987). Thus, there are pressures preventing males from giving only aggressive calls. Usually after a bout of aggressive calling, there is a period of silence, upon which advertisement calling resumes again (personal observation). Bout leadership is not entirely consistent within pairs, further reducing the disadvantage of calling in the leading position for any given bout.

I only tested female preferences for a very limited set of possible call timing arrangements. Further study is necessary to determine the range in female preference space for which leading or lagging calls are more attractive. First, for any given pair of overlapping calls, female preferences are likely to vary with changes in the delay of the lagging call relative to the leading call. Second, there are multiple call characteristics that can affect the full duration of the call, and the interrelationships between these characteristics and female choice of overlapping calls are unknown. For example, might the results of this study have been different if the duration of the aggressive call had been the same, but the call had fewer click notes and a longer introductory note? This is particularly important given the graded nature of aggressive calls. As the duration of the aggressive call introductory note increases, the number of click notes decreases. Hypothetically, several different combinations of introductory note duration and number of click notes could yield a call with the same full duration, yet female choice likely is not based on duration alone (Wells and Schwartz 1984b, Wells and Bard 1987). Thus, further study is required to determine whether the mechanism that I have proposed works for all levels of gradation of *D. ebraccatus* aggressive calls. Finally, female preferences for all these factors are likely to be affected by the type of call that is in each position. I was unable to address these possibilities with the experiments I performed in this study, and I suggest that further study be done in order to relate competitive call timing behavior to female choice in *D. ebraccatus*.

This study highlights the need for more intensive study into the function of aggressive calls. Aggressive calls have been examined in detail in very few species (e.g., Schwartz and Wells 1984, Schwartz 1989) and in no case has the signal value of the aggressive call been adequately described (Wells 2007). Nonetheless, it is clear that in most species that have been studied, aggressive calls are used in the traditional sense of a signal given during close-range agonistic interactions between males (Wells 1977). My hypothesis proposes a novel function for the aggressive call in *D. ebraccatus*. I do not question the aggressive call's role in agonistic interactions in this species, but I suggest that this is not the context in which the majority of aggressive calls are given. Under my hypothesis, it may be more beneficial for the following caller to respond to an aggressive call with an aggressive call. This could lead easily to the high levels of aggressive calling and entire choruses giving aggressive calls that have been observed in the populations in which this study took place (Reichert 2010). Further study of aggressive calling in other species, particularly those in which aggressive calling is relatively common, is likely to unveil additional, novel functions of complex vocal repertoires.

Table 1. Call duration statistics

Call type		Mean	SD	Range
Advertisement	Number of calls	186	95.5	52-397
	Full call duration (ms)	252	49	160-360
	Introductory note duration (ms)	172	19	130-200
	Number of click notes	0.62	0.27	0.01-1.17
Aggressive	Number of calls	20.3	17.4	0-82
	Full call duration (ms)	430	102	260-610
	Introductory note duration (ms)	154	26	110-210
	Number of click notes	2.17	0.59	1.0-3.5

Descriptive statistics of advertisement and aggressive call characteristics along with the number of calls of each type given during 30-min pair recordings. Mean, standard deviation (SD) and range were calculated from the individual averages of each male. *N*=32 males; 3 males gave one or fewer aggressive calls and were excluded from the calculations of aggressive call temporal characteristics.

Table 2. Correlation matrix of advertisement call characters

	TD 1	ID 1	CP 1	DC 1	CN 1	TD 2	ID 2	CP 2	DC 2
ID 1	0.768**								
CP 1	-0.569*	-0.761**							
DC 1	0.082	0.458	-0.686*						
CN 1	0.799**	0.247	-0.210	-0.288					
TD 2	0.311	0.697**	-0.652**	0.474	-0.187				
ID 2	0.522*	0.625*	-0.705**	0.221	0.222	0.638**			
CP 2	-0.517*	-0.548*	0.747*	-0.512*	-0.279	-0.641**	-0.610*		
DC 2	0.570*	0.408	-0.417	0.302	0.426	0.425	0.490	-0.702**	
CN 2	0.075	0.502*	-0.487	0.520*	-0.358	0.907**	0.277	-0.509*	0.254

TD, total duration; ID, introductory note duration; CP, call period; DC, duty cycle; CN, number of click notes. Correlation coefficients represent the correspondence of mean call characters of each male (arbitrarily designated as males 1 and 2 based on their recording channel) in the pair (*N*=16 pairs). * *P*<0.05; ** *P*<0.01.

Figure 1. Waveform displays of synthetic stimuli used in female choice tests. **a** Alternating advertisement call (upper trace) and aggressive call (lower trace). **b** The same calls but arranged so that the advertisement call (upper trace) overlaps with the aggressive call (lower trace). This is a typical arrangement for an advertisement call response to an aggressive call, with the aggressive call leading but also finishing after the advertisement call. Each stimulus was repeated with a period of 6 s; this silent time is not depicted here to allow for an enlarged view of the calls themselves.

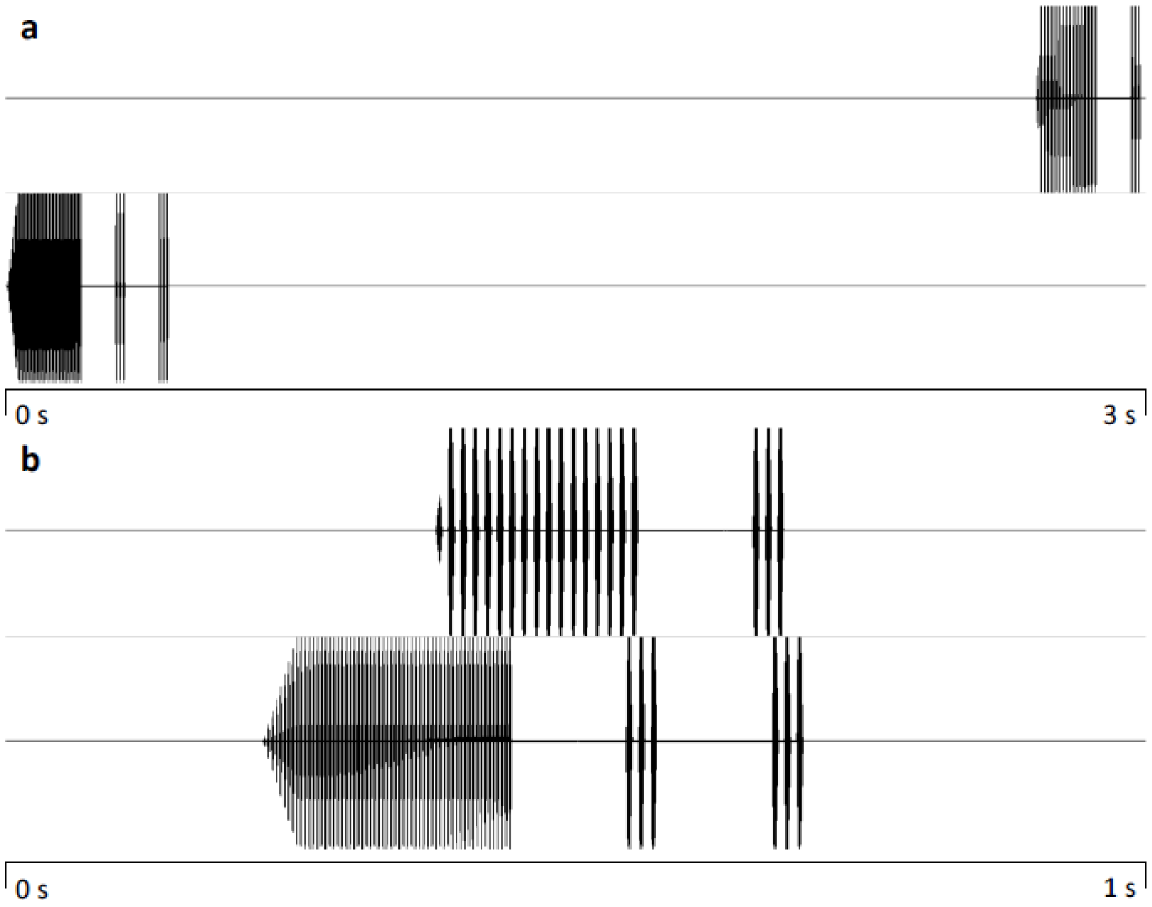


Figure 2. Boxplots of several variables measured during natural calling interactions. The long horizontal line denotes the median, the box contains the upper and lower quartiles of the data, and the whiskers denote data within 1.5 times the interquartile range. Individual data points beyond this range are shown as circles. The proportion of aggressive calls (*N*=32) was obtained by dividing the number of aggressive calls by the total number of calls given for each male. Proportion overlapped (*N*=32) is the proportion of each male's advertisement calls that were given in physical overlap (either in the leading or in the lagging position) with its neighbor's calls. LI (*N*=16) is only shown for the lagging male of each pair; it represents the proportion of the time in which its advertisement calls were in the lagging position when they overlapped with its neighbor's calls.

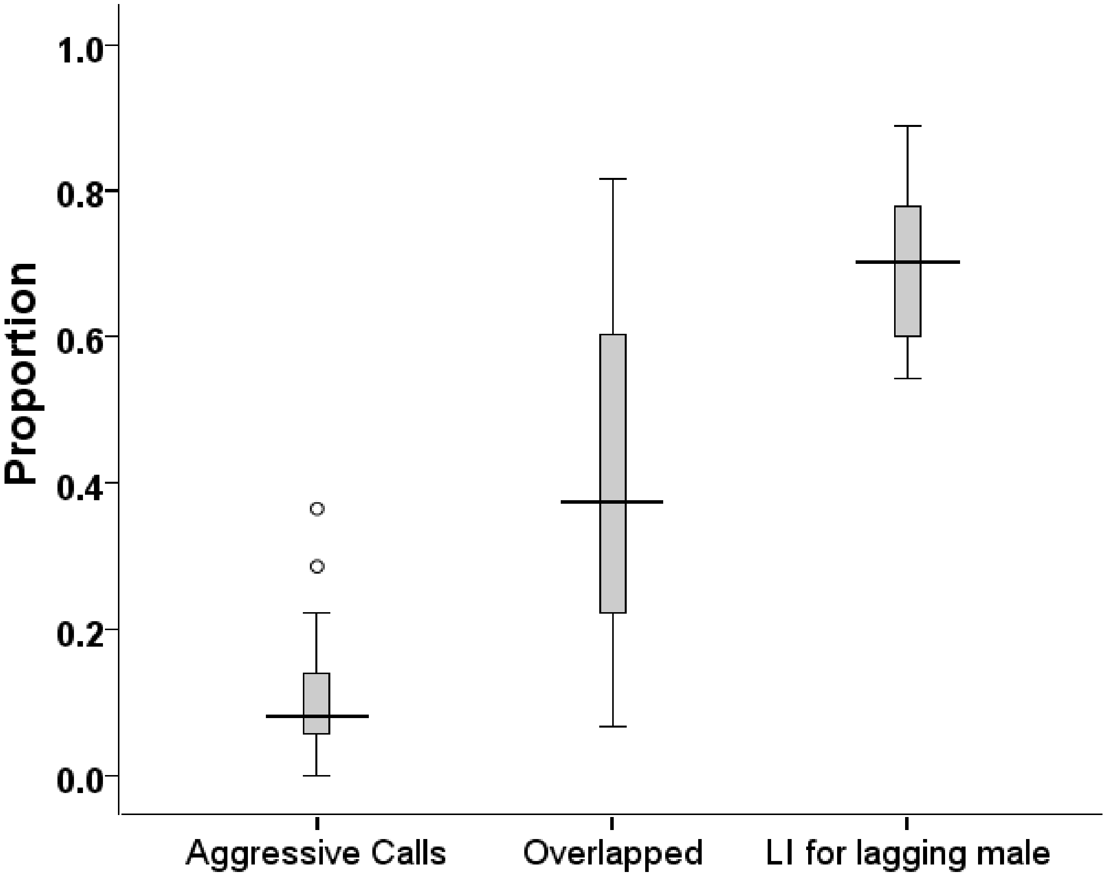


Figure 3. Scatterplots depicting the relationship between intermale distance and **a**, the proportion of calls that overlapped in time (calculated as an average across the 2 males in the pair that includes both leading and lagging calls for each male), and **b**, the difference in LI between lagging and leading males. Lines represent best fit lines from a linear least squares regression. The regression in **b** did not achieve significance (R^2=0.203, P=0.09) but becomes significant if the outlier point in the bottom left of the graph is removed (R^2=0.457, P=0.008).

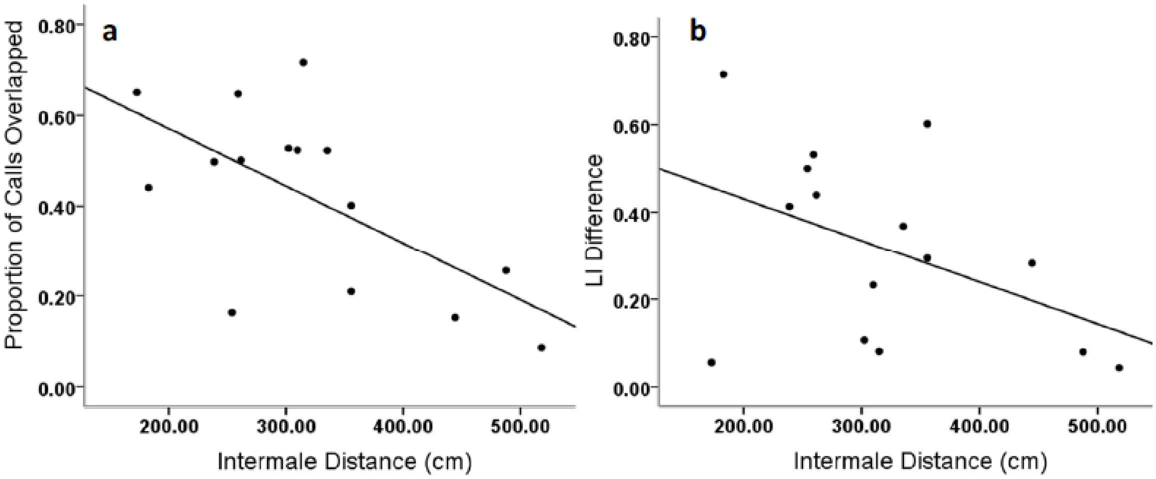


Figure 4. Total call duration of advertisement calls in different timing arrangements. Bars depict mean ± standard error of averages for each male. Left: Duration of advertisement calls that overlapped (this includes both leading and lagging calls) versus duration of advertisement calls that did not overlap. Right: Among calls that did overlap, duration of calls in the leading position versus duration of calls in the lagging position. Asterisk denotes a significant difference in duration between the 2 adjoining bars. For each bar, *N*=32.

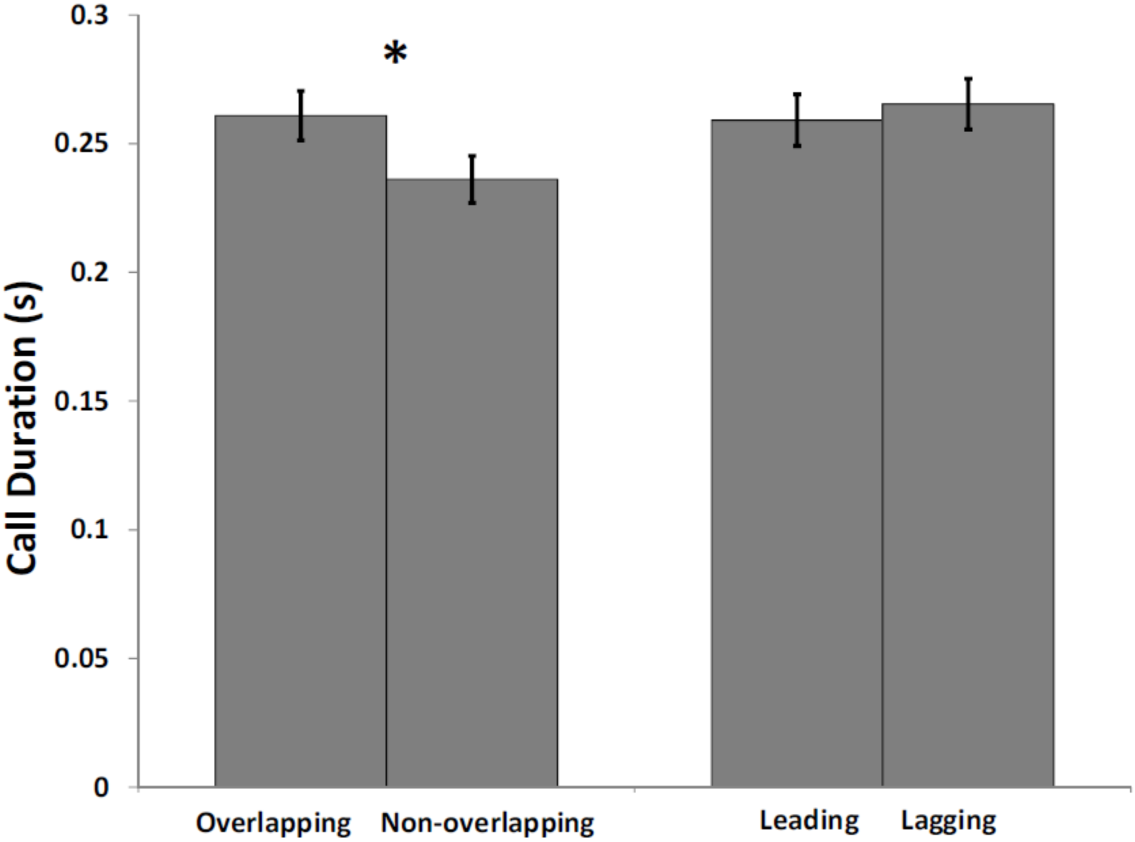


Figure 5. Bar charts of comparisons used in testing predictions of the hypothesis that aggressive calls are used by leading males in call timing competition. Bars represent means ± standard errors of the mean value for each male. **a** The proportion of leading advertisement calls (*N*=32) and aggressive calls (*N*=30) that finished after (were terminal with respect to) the lagging call. **b** The proportion of calls of leading (*N*=16) and lagging (*N*=16) males given during the entire interaction that were aggressive. **c** The proportion of switches to bouts of aggressive calling from advertisement calling initiated by the male that was the leading (*N*=31) or lagging (*N*=31) caller in an overlapping bout of advertisement calls immediately prior to the switch to aggressive calls. Proportion is based on the total number of switches to aggressive calling, which includes other situations not depicted (e.g., aggressive calling after the neighbor giving aggressive calls); thus these proportions do not add up to 1. **d** The number of click notes in overlapping, lagging advertisement calls that were followed by an aggressive call from the leading individual (*N*=28) versus those that were not followed by an aggressive call from the leading male (*N*=32). Asterisks denote significant differences.

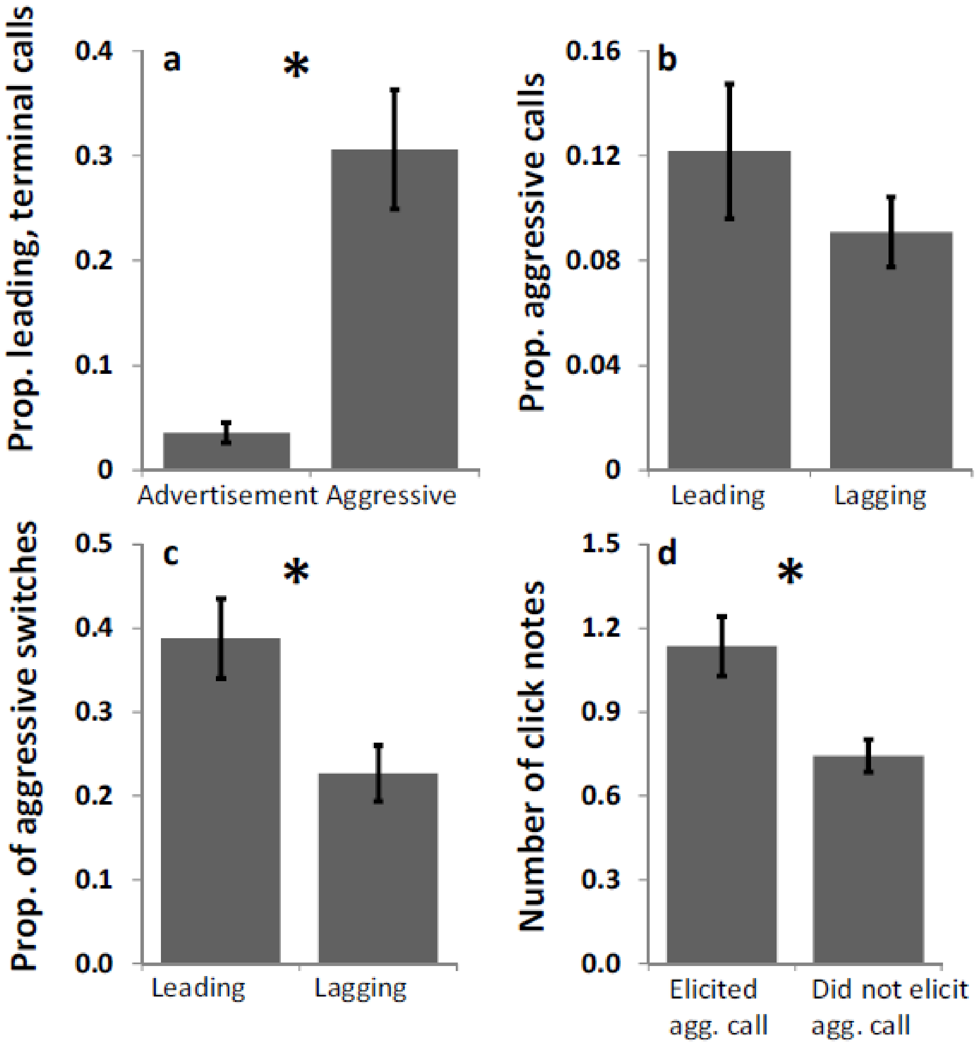
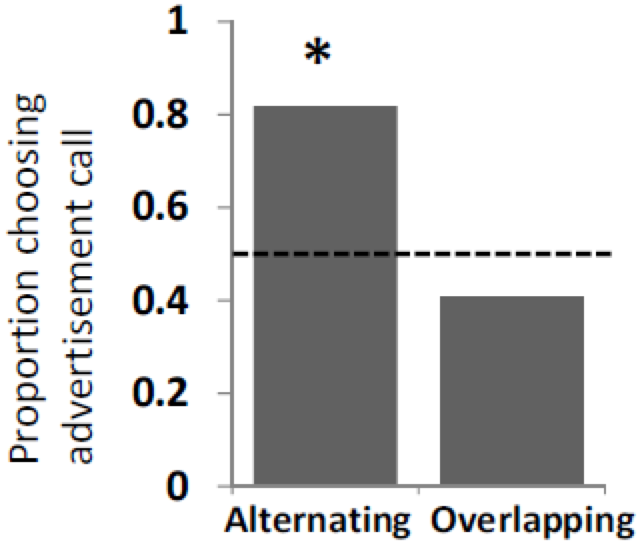


Figure 6. Proportion of females (*N*=22) choosing the advertisement call when given the choice of an advertisement call and an aggressive call that either alternated in time or overlapped such that the aggressive call led, but finished after, the advertisement call. Dotted line depicts the null hypothesis of no preference for either call type. Asterisk denotes a significant preference.



CHAPTER 6

The role of body size on the outcome, escalation and duration of contests in the grey treefrog, *Hyla versicolor*

ABSTRACT

Aggressive interactions in animals are often resolved in favor of the individual with superior fighting ability, or resource-holding potential (RHP). A recent revival of studies of aggressive behavior has focused on the assessment strategies used in animal contests. Strategies of dispute resolution through mutual or self-assessment of RHP differ in the predicted relationship between interaction duration and each competitor’s relative and absolute RHP. We studied potential components of RHP (mass, length, body condition) and their relationship to contest duration and the level of escalation in the grey treefrog, *Hyla versicolor*, using a novel method to stage aggressive interactions in the laboratory. Overall, large males were more likely to win than small males, but they only had an advantage in less escalated interactions and were not more successful in physical fights. There was limited evidence for an effect of body size on interaction duration or the level of escalation. Specifically, the body condition of both the smaller and larger contestant was weakly negatively related to the duration and level of escalation of contests. This relationship is the opposite of what would be expected under any assessment strategy. Given these data, coupled with the lack of relationships between other size measures and interaction duration, we conclude that assessment of

body size does not occur in contests in *H. versicolor*. Other unmeasured components of RHP may play a role in determining interaction duration, and the relatively weak and ineffective fighting abilities of this species may limit the dominance of larger individuals. Relatively little is known about aggressive behavior in frogs. Our method for staging aggressive interactions allows us to address predictions of game theory models in an important group for studies of animal communication.

INTRODUCTION

Contests and the means by which they are resolved show great diversity among animals. Game theory models have successfully characterized not only variation in individual success in aggressive interactions but also how assessment takes place within contests and how assessment tactics interact with characteristics of the contestants to determine the duration and escalation of aggressive disputes (e.g., Parker 1974, Enquist and Leimar 1983, Payne and Pagel 1996, Payne 1998). These models share the common feature that signaling in aggressive interactions can be explained as a means to reveal asymmetries between competitors in overall fighting ability, or resource-holding potential (RHP; Parker 1974). Relationships between aggressive signal characteristics and RHP have been noted in a number of species (e.g., Briffa and Elwood 2000, Brown et al. 2006, Lyons and Morris 2008, Fugère et al. 2011). Several models examine the means by which the assessment of RHP and the gathering of information may take place in aggressive interactions (e.g., Parker 1974, Maynard Smith and Parker 1976, Enquist and Leimar 1983, Payne and Pagel 1996, Payne 1998). However, a recent simulation of assessment strategies during contests suggested that many empirical studies have not adequately distinguished between the different possible means of assessment (Taylor and Elwood 2003, reviewed by Arnott and Elwood 2009). An understanding of the mechanisms by which assessment takes place is crucial to the larger goals of understanding the role of signals in aggressive disputes and the costs and benefits of aggressive interactions.

RHP is often related to competitors’ body sizes because larger individuals are usually more likely to win escalated physical fights (e.g., Clutton-Brock et al. 1979, Austad 1983, Wells 1988b, Briffa 2008). During an aggressive dispute, asymmetries in RHP may determine the likely winner of the contest (Parker 1974, Riechert 1978, Clutton-Brock et al. 1979). Understanding how RHP itself could be assessed is important for a general understanding of aggressive behavior. One possibility is mutual assessment (Enquist and Leimar 1983), in which competitors use aggressive signals to signal RHP and then make the decision to continue or withdraw based on an assessment of the opponent’s RHP relative to their own RHP. This assessment strategy has been invoked in a number of studies in which contest duration or escalation is greater between individuals of similar RHP (e.g., Enquist et al. 1990, Leimar et al. 1991, Marden and Rollins 1994, Hack 1997a), which is one of the key predictions of the original models of mutual assessment (Enquist and Leimar 1983). This prediction is, however, also consistent with other means of assessment (Taylor and Elwood 2003). For example, in ‘war of attrition’ models, an individual involved in an aggressive dispute does not directly assess the RHP of its competitor, but instead persists until the costs it has incurred in the interaction reach a threshold limit based on its own RHP (e.g., Bishop and Cannings 1978, Parker and Rubenstein 1981, Hammerstein and Parker 1982, Mesterton-Gibbons et al. 1996). Such strategies (more generally termed self-assessment strategies) have also received empirical support (e.g., Bridge et al. 2000, Taylor et al. 2001, Prenter et al. 2006, Stuart-Fox 2006, Moore et al. 2008, Elias et al. 2010) and can be divided into those in which persistence in an aggressive interaction is based solely on an individual's RHP (pure self-assessment, sensu Arnott and Elwood 2009), and those based on a combination of individual RHP and costs inflicted by the competitor (cumulative assessment, Payne 1998).

Mutual assessment and self-assessment strategies cannot be distinguished if the only comparison that is made is between RHP differences and contest duration; instead, it is necessary to examine the relationship between individual measures of RHP and contest duration (Taylor and Elwood 2003). Specifically, if contestants engage in cumulative or mutual assessment of RHP, there should be a strong positive relationship between the RHP of the lower-RHP individual and contest duration, and a strong negative relationship between the RHP of the higher-RHP individual and contest duration (Taylor and Elwood 2003). These strategies can then be differentiated by examining the relationship between RHP and contest duration for size-matched pairings of different absolute RHP; no relationship is expected under mutual assessment, while there should be a positive relationship under cumulative assessment (Taylor and Elwood 2003). Under pure self-assessment, there will be a positive relationship between the

RHP of both the lower- and higher-RHP individual and contest duration, but the relationship will be much weaker for the higher-RHP individual (Taylor and Elwood 2003). Only recently have studies made the comparisons necessary to distinguish between these strategies (e.g., Leiser et al. 2004, Morrell et al. 2005a, Prenter et al. 2006, Elias et al. 2008, Elias et al. 2010, Kasumovic et al. 2011).

Although studies of anurans provided some of the earliest positive support for mutual assessment in aggressive contests (e.g., Davies and Halliday 1978), aggressive behavior remains understudied in most frogs, where there has been a heavy focus on studies of sexual selection by female choice (reviewed by Gerhardt and Huber 2002). Aggressive interactions, particularly in non-territorial species, tend to be relatively brief and infrequent (Fellers 1979) and are thus challenging to observe in the field. An alternative is to stage aggressive contests between two live competitors. This method has been used to study aggressive behavior in other taxa (e.g., Hack 1997a, Taylor et al. 2001, Jenssen et al. 2005, Morrell et al. 2005a), but has received little application in studies of aggressive interactions in anurans (but see Davies and Halliday 1978, Fellers 1979, Crump 1988, Forester et al. 1993, Baugh and Forester 1994, Semsar et al. 1998, Caldwell et al. 2010). Staging aggressive interactions in frogs is challenging because aggression in frogs is usually a response to the acoustic presence of another individual at very close proximity (e.g., Brenowitz 1989, Rose and Brenowitz 1991, Marshall et al. 2003), and male frogs do not predictably approach one another when calling. We developed a novel method to stage interactions in the laboratory between male grey treefrogs, *Hyla versicolor*, thus overcoming the primary difficulties associated with studying aggressive behavior in anurans.

Like many frogs, *H. versicolor* is a lekking species in which males gather in choruses and vocalize with conspicuous advertisement calls to attract females to mate (Wells 1977). Aggressive disputes occasionally occur over calling spaces, which in *H. versicolor* are not well-defined territories but rather are temporarily defended areas that vary in size and location both within and between nights (Fellers 1979, Ritke et al. 1990). Aggressive disputes are likely to be related to defense of calling space from rival males in order to maintain a relatively clear acoustic channel for the broadcast (transmission) of advertisement calls (e.g., Schwartz and Gerhardt 1989, 1995). In *H. versicolor* and many other anurans, aggressive disputes consist of the production of acoustically distinctive aggressive calls and in some cases, physical combat (Fellers 1979, Wells and Schwartz 2006).

In this paper we describe our methodology for staging aggressive interactions in *H. versicolor*. We used this technique to stage contests to explore the effects of different body-size variables on success in aggressive interactions, and thus to determine whether body size is related to RHP. In addition, we used the methods suggested by Taylor & Elwood (2003) to attempt to discriminate among different possible means of RHP assessment during aggressive disputes. We made comparisons of the level of escalation and duration of interactions with different body-size variables of individuals to test the predictions of self-assessment and mutual assessment strategies.

METHODS

Staged aggressive interactions

We staged aggressive interactions between pairs of male *H. versicolor*. Testing took place during May–July 2008, May–June 2009, and April–June 2010. Subjects were captured from local populations in various localities in Boone County, Missouri (USA) and returned to the laboratory for testing. Males were temporarily housed in approved animal-care facilities in the laboratory for up to 1 week before being returned to their source population. On testing days, we released 20–80 of these males into an indoor artificial pond, an octagonal enclosure with sides of 2 m in length located within a greenhouse (further details are given in Schwartz et al. 2001), at approximately 1600 hours. To stimulate males to call, we simulated an afternoon rainstorm for 1 h starting at 1800 hours via a timed sprinkler suspended above the pond. At 2000 hours, we began broadcast of a synthetic artificial *H. versicolor* chorus from a speaker located directly above the centre of the pond. On most nights of testing, vigorous and sustained choruses ensued. We staged aggressive interactions at night during the peak of the chorus, from 2100 to 0200 hours.

To select subjects for staged interactions, we searched the artificial pond for males that were calling vigorously. These males were placed individually in wire-mesh cages on one of 20 Styrofoam platforms (approximately 30 x 15 cm) scattered throughout the pond. Males that continued to call on the platforms were selected for testing. We transported males on their platforms to an arena (a wooden runway, length

= 1.8 m, width = 0.3 m) where interactions were staged. Preliminary tests in an acoustically isolated room demonstrated that males rarely called in this situation. Thus, we placed the arenas approximately 3 m outside of the artificial pond. The audible background chorus appeared to stimulate males to continue their natural calling behavior, but was far enough away that males in the arena were much closer to each other than to any male in the pond. To improve our sample size, in 2009 and 2010 we staged interactions simultaneously on two separate arenas located at opposite ends of the room. Pairs of calling males were selected haphazardly and placed in the arena on wheeled platforms on opposite ends of the runway. The runway was surrounded by mesh walls to discourage frogs from escaping and to reduce any visual distractions caused by our movements.

Once both males resumed calling, we removed the cages so that frogs would be free to move about on the platforms. We allowed each male to give 10 calls at this initial position and then pulled the males halfway towards each other (approximately 0.9 m apart) by means of ropes attached to the wheeled platforms. We allowed each male to give 10 calls at the halfway position and then pulled the platforms to the center of the arena so that they abutted one another. At this point, males were very close to each other (mean ± SD intermale distance measured for a subset (*N*=62) of interactions was 13.4±3.5 cm); aggressive interactions only occur when males are in close proximity (Reichert & Gerhardt, personal observation). Once both platforms were pulled to the arena's center, we made digital video recordings for the duration of the interaction with a Sony DCR-SR85 camcorder. Video recordings were made under ambient light conditions (the pond and arena are located in a greenhouse facility; the pond is surrounded by cloth to minimize external light, but the arena tests were performed at night under the glass roof of the greenhouse) using the night vision feature of the camcorder.

We included a trial in the data set if an interaction occurred in the center position; that is, if each male called at least once in the center. The process of pulling the platforms towards one another occasionally disturbed one of the males and caused it to either jump off of the platform or to cease calling. We discarded such trials, along with those in which at least one male did not call in 10 min in the arena's center. Males from failed trials were returned to the pond and were sometimes used in subsequent attempts. Males that engaged in an interaction, however, were not used in subsequent trials. We allowed interactions to proceed until a clear winner and loser could be determined. We defined losers as individuals that either retreated (directed movement at least the length of the platform away from the opponent) or remained in place but ceased calling for at least 5 min while the opponent continued to call. We noted the time of night at the end of the interaction and measured each male's body mass using an electronic balance (Ohaus sp202) and snout–vent length (a standard measure of size in anurans; e.g., Davies and Halliday 1978) with calipers. We used mass and length data to calculate an index of body condition as the residuals of a regression of the cube root of body mass on body length, divided by body length. This measure of body condition was developed by Baker (1992) and was shown to have explanatory power in some previous studies of anurans (e.g., Bee 2001, Baugh and Ryan 2009). We compared these body size variables of competitors to the level of escalation and duration of interactions. All experimental procedures were approved by the University of Missouri Animal Care and Use Committee (protocol numbers 1910 and 6546).

Measures of escalation

We first defined levels of escalation by dividing the interactions into four discrete categories based on the presence or absence of specific behaviors. These non-overlapping categories were, in increasing order of escalation: both males only gave advertisement calls (ADV), only one male gave aggressive calls (AG1), both males gave aggressive calls (AG2) and, males physically fought (PF). A physical fight was defined as any physical contact that happened during the course of an aggressive interaction. We included interactions that only involved advertisement calls because, although perhaps not strictly an aggressive behavior, advertisement calling is used in male–male competition as well as mate attraction (Wells 1977), and advertisement calling is a necessary prerequisite to escalation to aggressive calling and physical fighting (see Results, below). Next, we measured the duration of various components of the interaction. Durations were obtained by replaying videos of the interactions and noting the times at which various behaviors occurred. The start of the interaction was considered to be the earliest time in which each male had initiated at least one call in the center position. We measured the following durations: (1) interaction duration, the difference in time between the loser's last call and the start of the interaction, (2) aggressive calling duration, the difference in time between the first and last aggressive calls given by either male, (3) aggressive call interaction duration, the difference in time between the beginning of the aggressive call interaction (i.e., the first moment in time in which both males had given aggressive calls) and the last aggressive call, (4) physical fight duration, the amount of time in which males were in physical contact with one another. Occasionally, fights involved bouts of physical contact separated by periods in which the males were not in contact with one another. We considered fights to last from the first moment of contact to the final moment of separation, including any periods of separation in between.

Statistical analyses

We checked all continuous variables for normality prior to executing statistical tests using a combination of Q-Q plots and normality statistics (Shapiro–Wilk tests). Variables that did not achieve normality were ln transformed prior to use in parametric statistical tests. We used parametric tests on all continuous variables, with the exception of the non-parametric Jonckheere–Terpstra test, which was used in some analyses to take advantage of the ordinal nature of the levels of escalation. We first tested whether success in aggressive interactions could be attributable to body size variables. We used binomial tests to test whether interactions tended to be won by the male that was heavier, longer, or in better condition. We also examined whether individuals that were larger for all three size variables were especially likely to win. We performed these tests both on the entire data set and for each of the four levels of escalation.

We examined whether any of the body size variables were related to the level of escalation or duration of contests in order to test the predictions of different assessment strategies. Each individual body size variable (mass, length and condition) was tested separately in the following analyses. We used multinomial logistic regressions of the individual body size measurements of both the larger- and smaller-sized individual to determine whether body size affected the level of escalation of contests. To determine the effects of these size variables on interaction duration, we used linear least-squares regression analyses that tested whether any of the measures of interaction duration could be predicted by the size of the larger or smaller contestant. We ran separate regressions for the larger and smaller individual. Interaction durations were ln transformed prior to analyses to achieve normality. In addition, we analyzed the effects of differences in body size between the larger and smaller competitor on the level of escalation and duration of interactions. As described above, this latter set of analyses is considered insufficient to discriminate between different assessment strategies (Taylor and Elwood 2003). Nevertheless, since some of these strategies are driven by size differences we felt these analyses could be illustrative.

Preliminary analyses suggested that the magnitudes of the response variables were similar from year to year; thus, we combined values across the 3 years of the study for statistical analyses. Of 185 total interactions, we excluded from analyses those interactions in which we were unable to determine a clear winner and loser (*N*=3), were unable to measure interaction duration because one male ceased calling immediately before the other gave its first call (*N*=8), or in which we discarded an inaccurate measure of length (*N*=1). Thus, statistical analyses were performed on a total sample of 173 interactions. All analyses were two-tailed tests performed at α=0.05 in SPSS 16.0.1 software (SPSS Inc., Chicago, IL, U.S.A., 2007).

RESULTS

Description of aggressive interactions

The staged interactions divided relatively evenly among the different levels of escalation; physical fights were somewhat more common than the other escalation levels (Figure 1). The initial bout of aggressive calling was always given in response to the advertisement calls of the other male. Interactions that escalated to physical fighting involved mild grappling with the forelimbs, occasional thrusts of the head and generally ineffective attempts to grab the other male. Physical fighting never resulted in injury, which is unlikely because of the relative weakness with which males attacked one another and the lack of body parts that could be considered weaponry. Interaction duration was highly variable. The total duration of interactions increased with the level of escalation; physical fights tended to be the longest in duration, while ADV interactions tended to be the shortest (Figure 2; Jonckheere–Terpstra test: *N*=173, *P*<0.001). The amount of time spent aggressive calling by one or both males also increased with level of escalation (Figure 2; Jonckheere–Terpstra: *N*=128, *P*<0.001; *N*=86, *P*=0.003, respectively). There was no effect of the time of night on the level of escalation of interactions (ANOVA: $F_{3,167}$=0.35, P=0.79) or on the total duration of interactions (linear regression: $F_{1,169}$=1.05, β=4 X 10^{-5}, R^2=0.006, P=0.31).

In interactions not involving a physical fight, the loser was more likely to cease calling but not move away from the winner (Table 1; binomial test: $P<0.0001$). In physical fights, the loser was significantly more likely to retreat than to remain in place but cease calling (Table 1; binomial test: P=0.04).

Influence of body size on interaction success

When all levels of escalation were combined, larger males were significantly more likely to win, although the effect was not strong (Table 2). As the difference in mass between the larger and smaller competitor increased, the heavier male was more likely to win (logistic regression: χ^2_1=4.0, P=0.046). There was, however, no relationship between the difference in length or condition and the likelihood of the winner being longer (logistic regression: χ^2_1=0.59, P=0.44) or in better condition (logistic regression: χ^2_1=0.14, P=0.71), respectively. The importance of body size in interaction success depended on the level of escalation of the interaction (Table 2). Specifically, individuals that were heavier or in better condition won a significantly greater proportion of interactions only for AG1 interactions. Longer males did not win a significantly greater proportion of interactions for any of the levels of escalation. Both length (Pearson correlation: r=0.84, N=346, $P<0.001$) and condition (Pearson correlation: r=0.54, N=346, $P<0.001$) were highly correlated with mass, but in many interactions males were larger for one variable but smaller for another. Ninety-one of 173 interactions involved an individual that was larger than its competitor for all three size variables. Of these, 66 were won by the larger individual (Table 2; binomial test: $P<0.0001$). Individuals that were larger in all three size variables were significantly more successful within the less escalated ADV and AG1 interactions, but were not significantly more likely to win for more escalated AG2 interactions and physical fights (Table 2).

Determinants of the level of escalation

Logistic regressions revealed no relationship between the mass of either the larger ($\chi^2_3=4.8$, $P=0.18$) or the smaller ($\chi^2_3=0.9$, $P=0.83$) contestant on the level of escalation (Figure 3a). There was also no significant relationship overall between the level of escalation and the difference in mass between the heavier and lighter competitor ($\chi^2_3=5.23$, $P=0.156$). However, interactions that did not escalate to aggressive calling involved significantly larger mass asymmetries than those that did (Figure 4; logistic regression of mass difference on escalated versus nonescalated interactions: $\chi^2_1=4.56$, $P=0.033$). In fact, further analyses showed that this difference was mainly attributable to the larger individual's mass being higher in interactions that did not escalate to aggressive calling than those that did (*t* test: $t_{171}=2.06$, $P=0.041$). There was no difference in the mass of the smaller male between these different levels of escalation (*t* test: $t_{171}=0.18$, $P=0.86$).

Although there was no effect of the difference in the competitors' body conditions on the level of escalation ($\chi^2_3=3.59$, $P=0.31$), we found that the level of escalation was affected by individual condition. The body condition of both the individual in better (χ^2_3=9.89, P=0.02) and worse (χ^2_3=11.95, P=0.008) condition was negatively related to the level of escalation of interactions (Figure 3b). Interactions that did not escalate to aggressive calling tended to involve competitors with higher body conditions than those that did involve aggressive calling (Figure 3b; *t* test of average competitor body condition for ADV interactions versus all other levels of escalation: t_{171}=3.41, P=0.001).

There was no relationship between the length of either the longer (χ^2_3=1.52, P=0.68) or the shorter (χ^2_3=3.44, P=0.33) contestant on the level of escalation (Figure 3c). There was also no relationship between the difference in length between the longer and shorter competitor and the level of escalation (χ^2_3=4.80, P=0.19).

Determinants of interaction duration

Regressions of the mass of the larger and smaller individual on the total duration of interactions were not significant when interaction durations were combined across levels of escalation (Figure 5; linear regression: larger mass, $F_{1,171}$=2.57, β=-0.23, R^2=0.02, P=0.11; smaller mass, $F_{1,171}$=0.16, β=-0.06, R^2=0.001, P=0.69). This remained the case when separate regressions were run for each level of escalation individually (Table 3), with the exception of the mass of the larger male in AG2 interactions ($F_{1,31}$=4.73, β=-0.43, R^2=0.13, P=0.04). Likewise, there were no significant relationships between the mass of the heavier or lighter individual and any of the other measures of duration (Table 3). Furthermore, there was no significant relationship between the difference in mass between larger and smaller individuals and the total interaction duration (linear regression: $F_{1,171}$=2.03, β=-0.24, R^2=0.01, P=0.16).

In contrast to the lack of an effect of body mass, we found evidence that interaction duration was related to body condition. When all levels of escalation were combined, the condition of the individual with both higher (linear regression: $F_{1,171}$=6.99, β=-333.1, R^2=0.04, P=0.01) and lower ($F_{1,171}$=4.41, β=-273.9 R^2=0.03, P=0.04) condition was weakly, but significantly, negatively related to the total interaction duration (Figure 6). Within the individual levels of escalation, this relationship remained significant only for the body condition of the larger competitor in AG2 interactions ($F_{1,31}$=5.38, β=-434.9, R^2=0.15, P=0.03; Table 3). The individual body conditions of the higher- and lower-condition individuals were unrelated to any of the other measures of duration (Table 3). There was no significant relationship between the difference in body condition of the larger and smaller individuals and the total interaction duration (linear regression: $F_{1,171}$=0.65, β=-145.3, R^2=0.004, P=0.42).

The individual body lengths of the longer and shorter competitor were unrelated to interaction duration (Figure 7; linear regression: longer individual, $F_{1,171}$=0.13, β=0.02, R^2=0.001, P=0.72; shorter individual, $F_{1,171}$=0.34, β=0.03, R^2=0.002, P=0.56). Likewise, there were no significant relationships between individual length and interaction duration within each individual level of escalation (Table 3). The individual lengths of the longer and shorter competitor were unrelated to any of the other measures of duration (Table 3). There was no significant relationship between the difference in length between the longer and shorter competitor and the total interaction duration (linear regression: $F_{1,171}$=0.107, β=-0.023, R^2=0.001, P=0.74).

DISCUSSION

Body size and RHP

We found a weak but statistically significant relationship between body size and success in aggressive interactions in *H. versicolor*. In general, body size is related to fighting success in a wide range of taxa (reviewed by Archer 1988). A relationship between body size and success in aggressive interactions has been demonstrated in many other frog species (e.g., Davies and Halliday 1978, Howard 1978, Wells 1978b, Arak 1983, Robertson 1986), although in many cases the effect of size was much stronger than that observed in this study. When we restricted our analyses only to those interactions that involved physical fighting, however, we found that larger male *H. versicolor* did not have an advantage in these interactions. RHP is typically defined as a measure of fighting ability (Parker 1974), yet the only significant larger-male advantage we detected occurred in less escalated AG1 interactions. The limited explanatory power of body size in determining the outcome of interactions suggests that it is, at best, a minor component of RHP in *H. versicolor*. The fact that body size does not play a large role in determining the outcome of fights in this species is not particularly surprising given their lack of weaponry and the relatively ineffectual nature of combat. In other words, given the structure of contests, it is not clear how large body size could give males an advantage in physical fights. Studies of other non-weaponized species also showed that body size is a less important predictor of fight outcome than other variables such as energetic reserves (Marden and Waage 1990, Kemp and Wiklund 2001). It is therefore puzzling that size appears to play a role in less escalated interactions. Why should smaller males be more likely to give up in nonphysical interactions when, if they escalate to physical fighting, they are not necessarily at a disadvantage? Below, we speculate on other variables that may be more closely tied to RHP that could have generated this result.

We found some significant relationships between the body size variables we measured and the level of escalation and duration of interactions, but, taken together, these were not concordant with the predictions of the different assessment strategies. In particular, the body condition of both the higher- and lower-condition individual explained a significant amount of variance in both the level of escalation and the duration of interactions, but the latter relationship is the opposite of what is expected under any of the assessment strategies we considered. Individuals of higher body condition tended to be involved in shorter, and less escalated, interactions. Thus, although males of higher body condition may have had an advantage at certain levels of escalation, increased body condition was negatively, albeit weakly, related to individual persistence in contests for both contestants. It is unclear why this should be the case. Perhaps males in especially good condition are less motivated to enter into escalated interactions involving aggressive calling because they are superior advertisement callers and would have less to lose if forced to move to a different calling space than individuals in poor condition. Indeed, body condition correlates positively with several advertisement call characteristics known to be important in female choice in *H. versicolor* (Reichert & Gerhardt, unpublished data). Another possibility is that individuals in especially poor condition are particularly motivated to defend a calling space once they have secured one. Some models have examined situations in which smaller individuals are expected to be more aggressive (Grafen 1987, Just and Morris 2003, Morrell et al. 2005b), particularly when they may have difficulty obtaining another resource. Further study is necessary to determine how body condition influences persistence in contests.

Neither the mass nor the length of individual contestants played any role in determining the level of escalation or duration of interactions. Despite this, the difference between competitors in mass was related to the level of escalation of the interaction. This result was mainly driven by a large difference between the larger and smaller male in ADV interactions; there was little variation in mass difference across the remaining interaction types. Again, these results are not consistent with what would be expected under any of the assessment strategies because there were no significant relationships between individual mass or length and the escalation or duration of contests. These results are, however, consistent with our observations on the effects of body size on the likelihood of winning. Namely, if large body size does not confer a strong advantage at most levels of escalation, we should not expect that assessment of body size, by whatever strategy, should take place.

In summary, we conclude that assessment during contests is only weakly related to body size. This does not mean that assessment of RHP does not take place during contests in *H. versicolor*. Indeed, we suspect that assessment is taking place during these contests, but we were unable to detect how this happens because we operated under the assumptions of models that consider body size to be a major component of RHP. Our observations of aggressive interactions in *H. versicolor* raise doubts about whether this behavior can be described adequately by any current model of aggressive signaling of size-related RHP. Classes of models that consider animals that are capable of intense and injurious fighting (e.g., Maynard Smith and Price 1973, Parker 1974, Adams and Mesterton-Gibbons 1995, Hurd 1997) clearly do not apply because the costs of fighting in terms of both injury risk and energetic expenditure appear to be very low in *H. versicolor*. At the same time, war of attrition models (e.g., Maynard Smith 1974, Parker and Rubenstein 1981, Hammerstein and Parker 1982, Mesterton-Gibbons et al. 1996, Payne and Pagel 1996), in which contest outcome is determined by which individual is capable of persisting the longest, also do not seem applicable. The interactions we observed were very brief, particularly when considering that they took place within a 3- to 4-h-long bout of nightly advertisement calling. In addition, war of attrition models generally do not consider situations in which physical combat sometimes resolves the contest. Aggressive interactions in *H. versicolor* show some characteristics of each type of model. Males often escalated to physical combat, but this combat was relatively brief and noninjurious. The predicted effects of body size on contest outcome and duration are unknown in this situation, and thus it may not be surprising that we found only limited evidence for the importance of body size in *H. versicolor* aggressive contests.

Other measures of RHP

Other measures of RHP, such as energetic state, might be more related to contest outcome than the bodysize variables that we measured (e.g., Smith and Taylor 1993, Hack 1997b, Briffa and Elwood 2004, Prenter et al. 2006, Briffa and Sneddon 2007). Although often considered to be a proxy for energetic state, body condition is at best a crude measure that has had only limited explanatory power in empirical studies (Peig and Green 2009). The energetic costs of physical fighting are unknown in this species, although the behaviors shown during fights do not appear to be particularly strenuous. Calling in these competitive situations is undoubtedly energetically expensive, however. Indeed, signaling during competitive male male interactions is known to be related to energetic expenditure in a number of taxa (e.g., Clutton-Brock and Albon 1979, Wells and Taigen 1986, Hack 1997b, Briffa and Elwood 2001). In *H. versicolor*, the energetic cost of advertisement calling is high (Taigen and Wells 1985) and, importantly, costs probably increase as males increase advertisement-call effort in close-range competition (Reichert & Gerhardt, unpublished data). The energetic cost of aggressive calling in *H. versicolor* is unknown but likely to be high as well. If the ability to produce effective signals in competitive acoustic interactions is tied to the energetic state of the competitors, then energetic state may be a major component of RHP. Our method to stage aggressive interactions in *H. versicolor* could be expanded to test whether energy reserves play a role in determining the outcome of contests. For example, contests could be staged between competitors that had been given access to either low or high levels of food, or between competitors that had spent more or less time within a night giving energetically expensive advertisement calls prior to the staged interaction.

Previous studies of aggressive behavior in some frog species, including *H. versicolor*, suggested that residents have an advantage over intruders in aggressive interactions (Wells 1978b, Fellers 1979, Sullivan 1982, Crump 1988, Given 1988, Semsar et al. 1998, Pröhl and Hödl 1999). We argue that our study was neutral with regard to residency because both males simultaneously defended a calling space in which they were the established resident. Males were pulled forward while passively sitting atop a platform (their calling space), and neither frog behaved as an intruder in the sense that it actively moved into the calling space of another individual. Interactions are not always decided by only one trait; when interactants are symmetric in one trait, asymmetries in other traits may be used to decide interaction outcome (Dugatkin and Ohlsen 1990, Beaugrand et al. 1991, Dugatkin and Biederman 1991, Beaugrand et al. 1996, Eshel and Sansone 2001, Kokko et al. 2006). Thus, our study protocol should not have affected our ability to determine whether assessment of size-based RHP takes place, but may have influenced the average level of escalation we observed. Because competitors were symmetric with regards to residency status, our staged interactions may have been even more likely to escalate compared to those in nature. Indeed, in a study of damselflies in which each competitor was given residency status on the same territory, escalated interactions were unusually common (Marden and Waage 1990).

Costs and benefits of aggression

Following physical fights, losers were more likely to move away from the winner, while in less escalated interactions, losers went silent but tended to remain in place. This difference in behavior between more and less escalated interactions was also observed by Fellers (1979) for natural interactions in *H. versicolor*. We have no reason to believe that silent males adopted a satellite mating strategy because they did not show changes in posture and other behaviors associated with satellite strategies (Forester and Lykens 1986), and because satellite behavior is not a prominent tactic in *H. versicolor* (Reichert & Gerhardt, personal observation). Nevertheless, by remaining in an inconspicuous position close to a calling male, they may have been functionally satellites. That is, if a female approached the calling male, it is entirely possible that she would cross the path of the silent male first. Thus, there may be some benefit to resolving interactions through physical fights rather than through less escalated calling interactions. Physical fights tended to be decisive in that the loser moved far from the winner, allowing the winner a calling space free not only from the acoustic interference of the loser’s calls, but also from any threat posed to its mating success by the loser’s presence. Males may therefore benefit by escalating to physical fighting. The downside is that the results of fights are unpredictable, at least from the size variables we measured. Thus, at least for a larger male, there may be a trade-off between the likelihood of winning and the benefits of victory. Winning physical fights may be more beneficial for large males because their opponents move further away, but they have a greater likelihood of winning calling interactions. In this situation, sufficiently motivated small males may have little to lose by escalating to a physical fight (Just and Morris 2003, Just et al. 2007).

A more general explanation for our results may lie in the possibility that selection is not particularly strong for traits involved in assessment or the enhancement of fighting ability in *H. versicolor* and other frog species with a similar breeding system. Although calling spaces are an essential resource to attract females, any particular calling space may not be that valuable because alternative spaces are available even at the highest male densities (Reichert & Gerhardt, personal observation). Thus, males may not defend their calling spaces with maximum effort and this may consequently reduce the consistency with which larger males win interactions and increase the variability of the duration of interactions. By contrast, in frog species that defend more limited resources such as oviposition sites or females themselves, fighting behaviors can be much more intense and prolonged than those we observed in *H. versicolor* (e.g., Davies and Halliday 1978, Kluge 1981, Townsend et al. 1984, Channing et al. 1994, Martins et al. 1998), and there appears to be a stronger role of body size on contest outcome (Wells 1978b, Robertson 1986, Given 1988). Thus, an interesting extension of our experiment could be to limit the availability of the calling space resources, for example, by manipulating the number of perches in our artificial pond, and examining whether this leads not only to increased aggression, but also to a greater large-male advantage.

More research into the determinants of contest outcomes is necessary to uncover additional components of RHP that may be involved in assessment during contests. For example, we are performing analyses to determine whether the aggressive vocal signals themselves may indicate success in aggressive interactions and thus be related to RHP (Chapter 7). In addition, because behavioral manipulations are straightforward in *H. versicolor*, future studies involving the manipulation of other potential components of RHP such as hormonal and energetic state are feasible. These additional comparisons will be facilitated by the novel method described in this paper to stage aggressive interactions. Males readily displayed the entire range of aggressive behaviors that have been noted in natural interactions, and we were able to generate large sample sizes. Our methodology is likely to work well with other anurans with similar calling patterns, facilitating comparative studies of contest behavior across species. The study described here should lay the groundwork for expanding our currently limited knowledge of aggressive behavior in this and other frog species.

Table 1. Behaviour of losers at the end of the interaction at each level of escalation

	Level of escalation				
Loser behavior	ADV	AG1	AG2	PF	Total
Silent; no retreat	36**	38**	24*	19	117**
Retreat	5	7	9	35*	56

Entries in the table denote the number of individuals that performed each behavior at a given level of escalation (ADV, both males only gave advertisement calls; AG1, one of the two males gave aggressive calls; AG2, both males gave aggressive calls; PF, physical fight). Asterisks denote the level of significance of two-tailed binomial tests for behaviors that were observed significantly more often than the alternative within each level of escalation. *$P<0.05$; **$P<0.0001$.

Table 2. Proportion of interactions won by the larger-sized male at each level of escalation

Proportion won by male larger in:	Level of escalation				
	ADV	AG1	AG2	PF	Total
Mass	23/41	33/45**	20/33	28/54	104/173**
Condition	25/41	35/45***	21/33	28/54	109/173***
Length	27/41	28/45	20/33	30/54	105/173**
All categories	17/23*	21/24***	12/16	16/28	66/91***

Proportions are shown as the number won by the larger male over the total number of interactions at that level of escalation. In addition to the three main size variables, we calculated the proportion of interactions won by individuals that were larger for all three size categories (‘All Categories’). Asterisks denote significant *P* values for two-tailed binomial tests of the proportion of interactions won by the larger male versus the null expectation of 50%. **P*<0.05; ***P*<0.01; ****P*<0.001.

Table 3. Results of linear least-squares regression analyses of individual size variables for the larger or smaller individual against the different measures of interaction duration at each level of escalation

Size measure	Duration measure	Level of escalation	*N*	*F*	*β*	R^2	*P*
Larger mass	TD	Combined	173	2.6	-0.23	0.02	0.111
		ADV	41	0.573	-0.201	0.01	0.454
		AG1	45	0.05	-0.06	0.001	0.824
		AG2	33	4.732	-0.428	0.13	**0.037**
		PF	54	0.02	0.018	0.0003	0.889
	ACD	Combined	126	0.134	0.063	0.001	0.715
		AG1	39	0.789	0.299	0.021	0.38
		AG2	33	3.904	-0.357	0.112	0.057
		PF	54	0.43	0.058	0.008	0.515
	AID	Combined	86	0.566	-0.096	0.007	0.454
		AG2	33	1.861	-0.322	0.057	0.182
		PF	53	0.955	0.09	0.018	0.333
	PFD	PF	54	2.241	0.335	0.041	0.14
Larger condition	TD	Combined	173	6.985	-333.123	0.039	**0.009**
		ADV	41	1.736	-377.521	0.043	0.195
		AG1	45	0.057	-55.585	0.001	0.813
		AG2	33	5.376	-434.884	0.148	**0.027**
		PF	54	0.004	-6.646	0.00008	0.947
	ACD	Combined	126	0.15	58.166	0.001	0.699
		AG1	39	1.971	413.656	0.051	0.169
		AG2	33	1.876	-245.038	0.057	0.181
		PF	54	0.088	-21.16	0.002	0.767
	AID	Combined	86	0.258	-55.522	0.003	0.613
		AG2	33	0.252	-116.712	0.008	0.62
		PF	53	0.024	11.277	0.0004	0.878
	PFD	PF	54	1.379	-211.4	0.026	0.246
Larger length	TD	Combined	173	0.125	0.021	0.001	0.724
		ADV	41	0.484	-0.075	0.012	0.491
		AG1	45	0.466	0.081	0.011	0.499
		AG2	33	0.514	-0.067	0.016	0.479
		PF	54	0.007	-0.004	0.0001	0.933
	ACD	Combined	126	0.409	0.045	0.003	0.524
		AG1	39	0.144	0.058	0.004	0.707
		AG2	33	0.778	-0.075	0.024	0.385
		PF	54	0.968	0.031	0.018	0.33
	AID	Combined	86	0.01	0.005	0.0001	0.919
		AG2	33	0.747	-0.093	0.024	0.394
		PF	53	0.95	0.033	0.018	0.334
	PFD	PF	54	3.752	0.152	0.067	0.058
Smaller mass	TD	Combined	173	0.158	-0.06	0.001	0.691
		ADV	41	2.143	-0.366	0.052	0.151
		AG1	45	0.916	0.277	0.021	0.344

		AG2	33	2.234	-0.359	0.067	0.145
		PF	54	0.828	-0.105	0.016	0.367
	ACD	Combined	126	0.827	0.162	0.007	0.365
		AG1	39	1.04	0.364	0.027	0.314
		AG2	33	0.677	-0.183	0.021	0.417
		PF	54	0.123	0.029	0.002	0.728
	AID	Combined	86	0.209	-0.06	0.002	0.649
		AG2	33	0.366	-0.172	0.012	0.55
		PF	53	0.17	0.036	0.003	0.682
	PFD	PF	54	0.011	0.022	0.0002	0.919
Smaller condition	TD	Combined	173	4.41	-273.863	0.025	**0.037**
		ADV	41	0.234	114.635	0.006	0.632
		AG1	45	0.947	-250.367	0.022	0.336
		AG2	33	2.587	-329.6	0.077	0.118
		PF	54	0.084	-32.238	0.002	0.773
	ACD	Combined	126	0.816	149.944	0.007	0.368
		AG1	39	2.458	537.177	0.062	0.125
		AG2	33	0.259	-98.109	0.008	0.614
		PF	54	0.105	-25.692	0.002	0.747
	AID	Combined	86	0.322	-66.044	0.004	0.572
		AG2	33	0.001	9.378	0.00004	0.97
		PF	53	0.016	10.038	0.0003	0.901
	PFD	PF	54	1.258	-224.842	0.024	0.267
Smaller length	TD	Combined	173	0.341	0.032	0.002	0.56
		ADV	41	2.705	-0.172	0.065	0.108
		AG1	45	0.84	0.095	0.019	0.365
		AG2	33	1.398	-0.108	0.043	0.246
		PF	54	0.64	-0.032	0.012	0.427
	ACD	Combined	126	0.165	0.026	0.001	0.685
		AG1	39	0.028	0.022	0.001	0.867
		AG2	33	1.271	-0.094	0.039	0.268
		PF	54	0.145	0.011	0.003	0.705
	AID	Combined	86	0.499	-0.033	0.006	0.482
		AG2	33	1.519	-0.13	0.047	0.227
		PF	53	0.035	0.006	0.001	0.853
	PFD	PF	54	1.039	0.073	0.02	0.313

Statistically significant *P* values (*P*<0.05) are shown in bold. Combined: regression combining results across the different levels of escalation. Some durations are not applicable for some levels of escalation. Definitions of duration and level of escalation are given in the text. Duration measures abbreviated as follows: TD: total interaction duration; ACD, aggressive calling duration; AID, aggressive call interaction duration; PFD, physical fight duration.

Figure 1. The percentage of interactions (*N*=173 total interactions) at each level of escalation. Levels of escalation are mutually exclusive categories and are ordered on the *x*-axis from least to most escalated (ADV, both males only gave advertisement calls; AG1, one of the two males gave aggressive calls; AG2, both males gave aggressive calls; PF, physical fight).

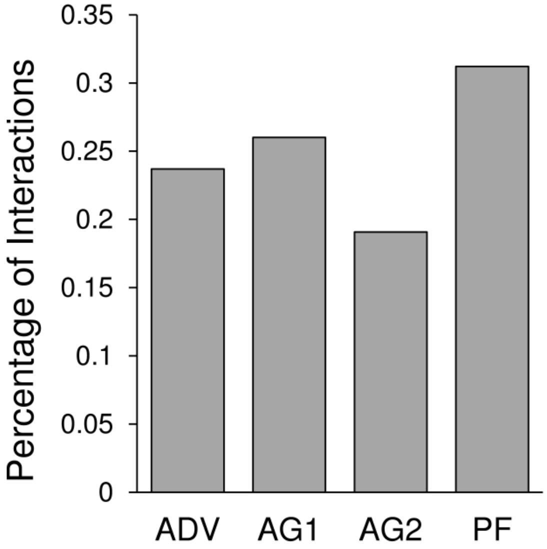


Figure 2. Mean ± SE duration of various components of male–male interactions in *H. versicolor*. Interaction durations are defined in the methods. **a** Total interaction duration. **b** Aggressive calling duration. **c** Aggressive-call interaction duration. Not all durations apply to all levels of escalation, thus **b** and **c** have fewer bars than **a**. *N*=41 for ADV, 45 for AG1 in **a**, 41 for AG1 in **b**, 33 for AG2 and 54 for PF. Sample sizes differ for AG1 in **b** because we excluded from the calculations four interactions in which only one aggressive call was given.

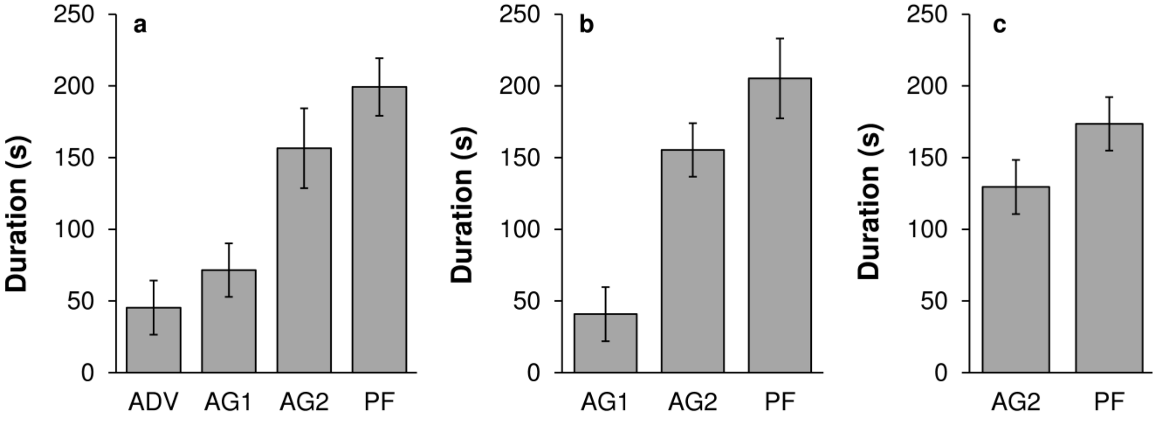


Figure 3. Averages (±1 SD) of the body size variables for the larger (*grey bars*) and smaller (*white bars*) competitor for each level of escalation: **a** body mass; **b** body condition; **c** snout–vent length. *N*=41 for ADV, 45 for AG1, 33 for AG2 and 54 for PF.

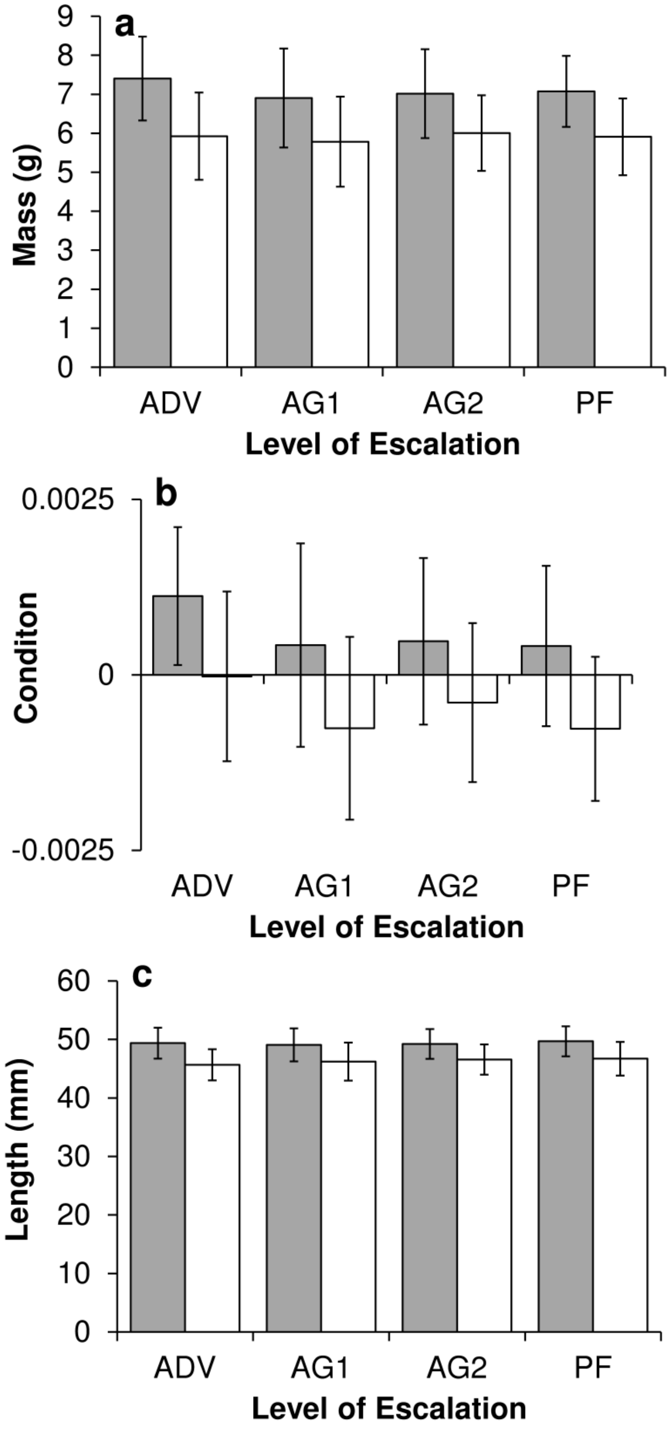


Figure 4. The mean difference in the mass of competitors (larger male mass – smaller male mass) for the different levels of escalation. Error bars show ±1 SE. Sample sizes as in Figure 3.

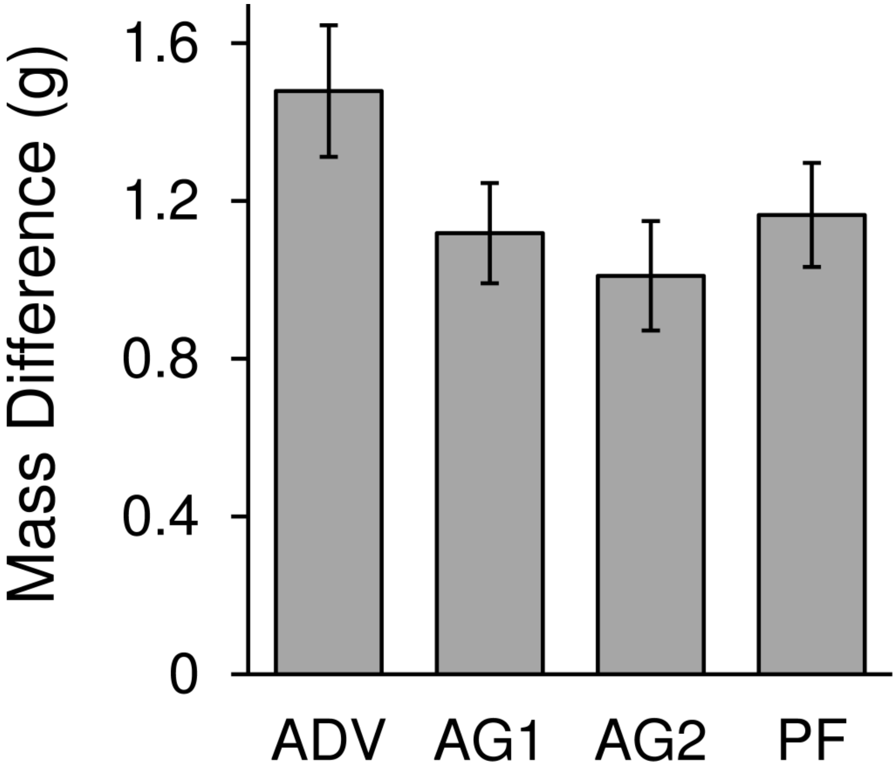


Figure 5. Relationship between body mass of the **a** larger and **b** smaller contestant on ln-transformed interaction duration. Trend lines were calculated from a linear least-squares regression. *N*=173 for each graph.

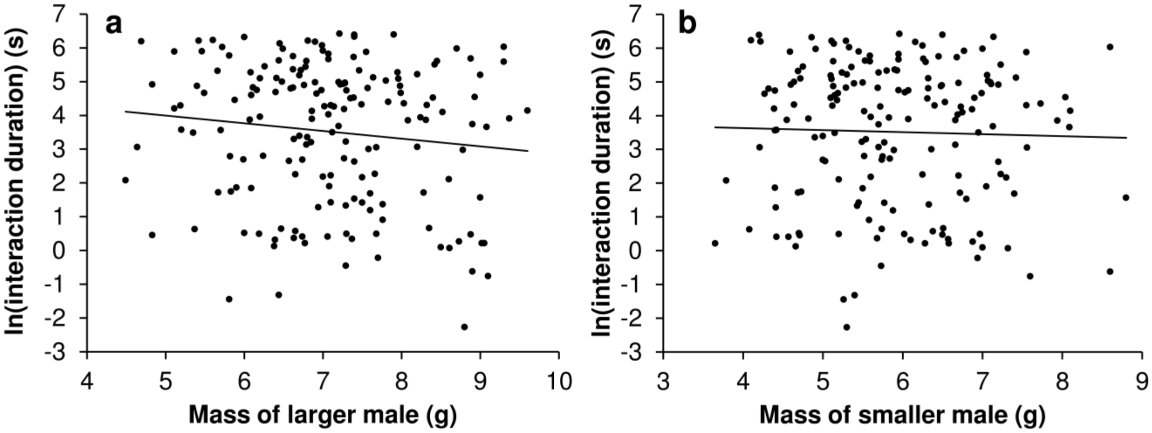


Figure 6. Relationship between the body condition of the **a** higher-condition and **b** lower-condition contestant on ln-transformed interaction duration. Trend lines were calculated from a linear least-squares regression. *N*=173 for each graph.

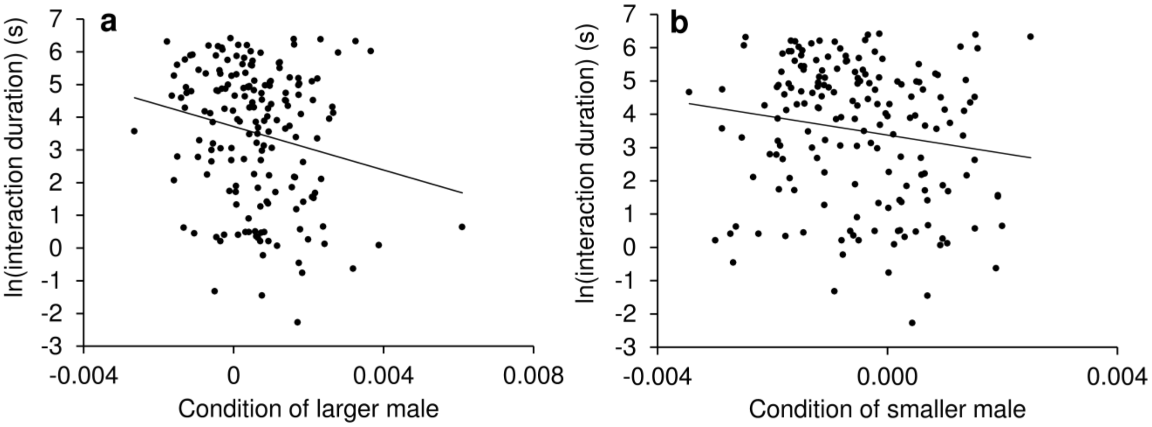
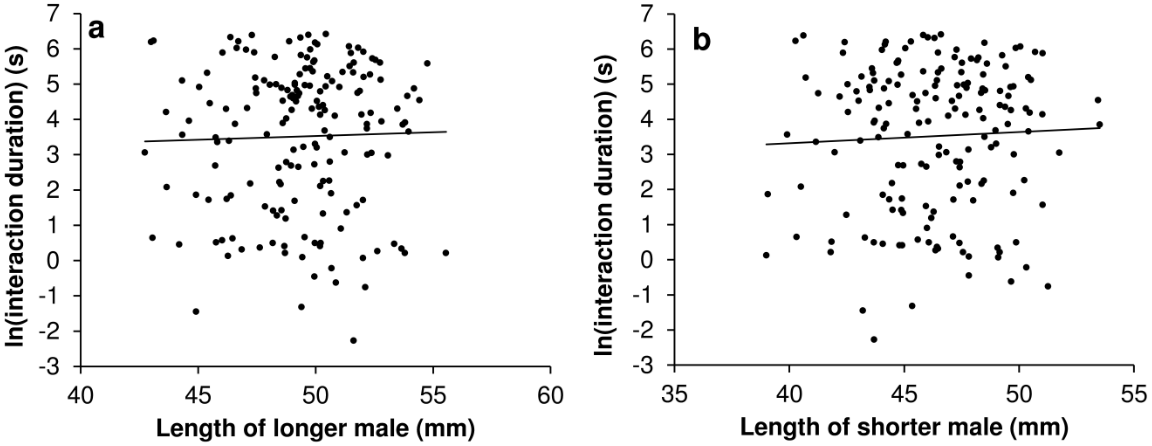


Figure 7. Relationship between the snout–vent length of the **a** longer and **b** shorter contestant on ln-transformed interaction duration. Trend lines were calculated from a linear least-squares regression. *N*=173 for each graph.



CHAPTER 7

Aggressive calling and assessment in contests of the grey treefrog *Hyla versicolor*

ABSTRACT

Aggressive signaling is used to resolve disputes in many animals, yet little is known about the function of aggressive vocalizations in what is otherwise one of the most well studied systems in communication research, anuran amphibians. We staged aggressive interactions between male grey treefrogs, *Hyla versicolor*, and analyzed the advertisement and aggressive calls of winners and losers to address the following questions: (1) Is there a relationship between advertisement and aggressive call characteristics and the outcome of contests? (2) Do the characteristics of winners' calls suggest a relationship between call characteristics and underlying resource-holding potential (RHP)?, and (3) Are calls, particularly aggressive calls, involved in assessment during contests, and if so, what assessment strategy is used? In less escalated interactions, winners and losers differed in advertisement call characteristics that are likely related to body size and energetic capacity. In highly escalated aggressive calling interactions, however, there were no differences between winners and losers in advertisement call characteristics, but there were differences in aggressive call effort and the decrease in frequency from advertisement to aggressive calls. Finally, in physical fights, there were no differences between winners and losers for any call characteristics.

Aggressive interactions in *H. versicolor* apparently proceed via mutual assessment. Males that produced calls that are known or likely to be more energetically demanding were more successful in staged interactions. Because body size has relatively little influence on the outcome of aggressive interactions in this species, we suggest that males assess an energetically based RHP.

INTRODUCTION

Many animal contests are characterized by the use of signals rather than overt physical fighting to determine the outcome of disputes (Maynard Smith and Price 1973). The logic behind the use of aggressive signals was established by early game theory models. One common conclusion is that both winners and losers benefit by using asymmetries in aggressive signals as a proxy for direct physical confrontation, particularly when the costs of fighting are high or the value of the contested resource is low (Maynard Smith and Price 1973, Maynard Smith 1974, Parker 1974, Maynard Smith and Parker 1976, Parker and Rubenstein 1981). In many cases, aggressive signals may provide contestants with a direct indication of their individual and relative resource holding potentials (RHP; a measure of fighting ability), thus indicating the likely winner were a fight to take place (e,g, Parker 1974, Riechert 1978, Clutton-Brock and Albon 1979). When signals are used for assessment, the capacity for the signal to provide reliable information concerning the signaler’s RHP is often assumed to be maintained by properties of the signal being directly constrained by RHP (Enquist 1985, Grafen 1990).

Various strategies for the assessment of RHP in contests have been described (e.g., Hammerstein and Parker 1982, Enquist and Leimar 1983, Enquist 1985, Enquist et al. 1990, Payne and Pagel 1996, Payne 1998), and much recent research has focused on documenting the use of these strategies in animal contests (e.g., Bridge et al. 2000, Morrell et al. 2005a, Prenter et al. 2006, Stuart-Fox 2006, Elias et al. 2008, Moore et al. 2008, Elias et al. 2010). Nonetheless, how RHP assessment takes place during contests remains a controversial topic because experimental techniques to differentiate between the various strategies of RHP assessment have been described only recently. Most of these techniques infer the assessment strategy indirectly through relationships between RHP and contest duration (Taylor and Elwood 2003, Arnott and Elwood 2009, Briffa and Elwood 2009).

Assessment strategies vary in the degree to which the opponent influences an individual’s persistence in the contest (Taylor and Elwood 2003). The particular strategy adopted by a species will depend on individuals’ perceptual abilities to assess their opponents’ signals, the strength of the relationship between signal characteristics and RHP, and the consequences of physical fighting (Arnott and Elwood 2009, Briffa and Elwood 2009). In contests involving mutual assessment, each individual gauges its RHP relative to that of its competitor. Whether to persist or flee is then influenced by the certainty of its assessment of relative RHP (Enquist and Leimar 1983, Enquist et al. 1990). Such contests are predicted to be more escalated, or longer in duration, when individuals are similar to one another in RHP (Enquist and Leimar 1983, Enquist et al. 1990). Furthermore, a positive relationship is expected between contest duration and loser RHP, and a negative relationship between contest duration and winner RHP (Taylor and Elwood 2003). Under pure self-assessment strategies, each individual persists only up to a threshold based on its individual RHP and does not assess the RHP of its competitor (Mesterton-Gibbons et al. 1996, Payne and Pagel 1996, Arnott and Elwood 2009). These contests are not resolved directly by RHP asymmetries; instead, the absolute RHP of the loser determines contest duration (Taylor and Elwood 2003). Unlike in mutual assessment, winner absolute RHP should show an incidental weakly positive relationship with contest duration (Taylor and Elwood 2003). Finally, in cumulative assessment, individuals persist according to their own RHP but may suffer damage from attacks by the opponent (Payne 1998). These interactions are expected to show similar relationships between contestant RHP and duration as in mutual assessment but can be distinguished by examining contest structure in more detail to determine whether individuals are capable of inflicting costs on one another (Payne 1998, Arnott and Elwood 2009, Briffa and Elwood 2009).

Experimental techniques to differentiate between different assessment strategies have usually been described in terms of comparisons of contest structure and duration with direct measures of RHP, usually body size (Taylor and Elwood 2003, Arnott and Elwood 2009, Briffa and Elwood 2009). In some cases, however, it may be difficult to measure RHP directly, particularly if RHP is determined by less detectable physiological mechanisms (Briffa and Sneddon 2007). In these cases, insights about the assessment strategy may be gained by analyzing the characteristics of aggressive signals as a proxy for RHP. This approach can be particularly useful if a comparison of aggressive signal characteristics between winners and losers demonstrates that signaling asymmetries determine the outcome of contests. The magnitude of these asymmetries, and of the individual winner and loser signal characteristics themselves, can then be related to contest duration and the level of escalation in order to test the predictions of various assessment strategies. Finally, á priori knowledge of physical and physiological constraints on signal characteristics can be combined with knowledge of the importance of those signal characteristics in determining contest outcome and duration to suggest the potential underlying relationship between signal structure and RHP. In this study we utilized this approach to examine the use of aggressive calls in disputes over calling space in the grey treefrog, *Hyla versicolor*.

Anurans offer many advantages to the study of communication, and there is a strong general understanding of both proximate and ultimate forces involved in anuran vocalization behavior (Gerhardt and Huber 2002). The context of communication in many of these species is the chorus, in which males gather during the breeding season and produce conspicuous vocalizations (advertisement calls) to attract mates (Wells 1977). Particularly damaging to a male’s attempts to attract females is the intrusion upon his calling space by another calling male; acoustic interference is likely to decrease both the localizability and the attractiveness of a male’s calls (e.g., Schwartz and Gerhardt 1989, 1995). Thus, even in species in which males do not have fixed territories, males defend their calling space from rivals to maintain a relatively clear acoustic channel for transmission of their own advertisement calls. In many species, males give a different type of call, generally termed an aggressive call, in close-range agonistic interactions (reviewed by Wells and Schwartz 2006). Although the precise communication significance of aggressive calls is unknown for any species of frog (Wells 2007), such signals are usually produced in the context of defending a calling space and therefore are assumed to repel rival males (e.g., Fellers 1979, but see Reichert 2011b).

In a previous study of aggressive behavior in *H. versicolor* (Chapter 6), we staged aggressive interactions between males in order to determine if *H. versicolor* males assess RHP based on body size. We found limited evidence for an effect of body size on the outcomes of interactions at most levels of escalation. Not surprisingly, we were unable to show how contestants might have used any of the body size variables we measured in order to assess RHP (Chapter 6). Indeed, larger males did not have an advantage in physical fights, which tended to be brief and noninjurious (Chapter 6). In this paper, we present evidence to support the hypothesis that there is an underlying RHP that determines the outcome of contests and that males assess RHP via aggressive calling competition. RHP may be more related to underlying physiological constraints on the ability to defend a calling space vocally, particularly energetic reserves. Advertisement calling in frogs, including *H. versicolor*, is extremely energetically costly (Taigen and Wells 1985, Prestwich et al. 1989, Wells and Taigen 1989, Prestwich 1994, Wells 2001). If aggressive calls are similarly costly, then RHP could be assessed indirectly by variation in those aggressive call characteristics that are the most energy constrained. We used analyses of staged interactions in *H. versicolor* to examine the structure and use of aggressive calls. We will show that: (1) Assessment of aggressive calls occurs; (2) Certain characteristics of aggressive calls predict the outcome of contests; and (3) Such characteristics of aggressive calls are likely to be constrained by physiological costs of calling.

METHODS

Study species

We studied aggressive calling in *H. versicolor*, a common North American treefrog. The breeding season of *H. versicolor* occurs from late spring to mid-summer. Males gather nightly in choruses along the shores of small ponds. Competition for mates is intense, and the operational sex ratio is strongly male biased (Sullivan and Hinshaw 1992). Females select mates largely, if not entirely, on acoustic characteristics of the males' calls. Acoustic interactions between males involving advertisement calls, and the criteria used by females in mate choice have been well documented (Gerhardt 2001). Aggressive calls are given occasionally by males, usually when neighboring males begin calling at close range (Pierce and Ralin 1972, Fellers 1979). Males are nonterritorial, and most aggressive calling occurs early in nightly chorus formation before calling spaces have become relatively well defined and stable (Wells 1988a). In some cases aggressive calling interactions escalate to brief physical fights.

Staged aggressive interactions

We observed staged interactions between 186 pairs of male *H. versicolor* in April to July of 2008 to 2010. Full details of the protocol used to stage aggressive interactions are given elsewhere (Chapter 6). Briefly, we stimulated males captured from nearby natural populations to call in our testing facility, an indoor artificial pond (details in Schwartz et al. 2001). Males chosen for testing were selected haphazardly and placed in an arena on platforms that could be wheeled towards one another. We recorded ten calls from each male at the initial position, in which the competitors were 1.8 m apart. We then wheeled the competitors to the halfway position of 0.9 m apart. We recorded an additional ten calls from each male at this position and then wheeled the competitors together until their respective platforms were adjacent to one another (“center position”). Aggressive interactions only took place at the center position. We defined an interaction as having taken place whenever both males called at least once in the center position and there was a clear winner and loser. We excluded from the analyses any attempts in which one or both males stopped calling or attempted to escape prior to reaching the center position. Losers were defined as individuals that, in the course of the interaction, either stopped calling for at least 5 min while its opponent (the winner) remained calling or retreated from the interaction by moving at least one platform length away from its opponent (the winner). Once an individual participated in an interaction, we returned it to the place of capture and did not use it in any subsequent interactions.

We used directional microphones (Sennheiser ME-66, ME-67 & ME-80) to continuously record each male’s calls onto separate audio tracks of a digital audio recorder (Marantz PMD-660 & PMD-661; 44.1 KHz sampling rate, 16-bit PCM files) for all positions throughout the staged interaction. In the center position we also recorded digital video of the interaction using the night vision feature of a Sony DCR-SR85 camcorder. Interactions were allowed to continue until one of the males met our criteria for losing the interaction, at which time we ceased recording video and audio. We noted the winner and loser of the interaction, the time of night at which the interaction took place, and the body mass, snout–vent length (SVL) and cloacal temperature of each competitor. We derived an index of body condition from these data as the residuals of a regression of the cube root of body mass on SVL, divided by SVL (Baker 1992). From the video recordings we also noted which male first produced advertisement calls, aggressive calls, and initiated physical contact; this male was labeled the 'instigator' for each respective behavior.

Call analyses

We analyzed temporal and spectral characteristics of every advertisement call and aggressive call given by each male throughout the interaction using the Raven Pro 1.3 software package (Cornell Laboratory of Ornithology). For advertisement calls, we measured call duration, the number of pulses per call, and the frequency of each call's two frequency peaks (measured to the nearest 10 Hz as the frequency of maximum amplitude for each peak from the spectrogram; Hamming window, discrete Fourier transform size=4096 samples). From these measurements we determined the pulse rate as the number of pulses divided by the duration of the call excluding the final pulse, the call period as the time between the onsets of consecutive calls of the same male, and the duty cycle as the call duration divided by the call period. Call periods longer than 30 s were excluded from analyses. Advertisement call characteristics were averaged separately for each male at each position. We will give a more detailed description of alterations of advertisement calls with proximity to the opponent elsewhere. For the purposes of this study, advertisement calls were analyzed primarily to facilitate comparisons of advertisement and aggressive call characteristics and to test predictions of contest models based on the relationship between call characteristics and interaction duration.

For aggressive calls, we measured call duration, frequency, call period, and duty cycle as above. We did not measure pulse number or pulse rate for aggressive calls because the aggressive calls of *H. versicolor* are not strongly amplitude modulated (see results below). Preliminary analyses suggested that males tend to group their aggressive calls into somewhat discrete bouts. We defined a bout as a string of aggressive calls in which the call periods of consecutive calls were less than 1 s. We then counted each male's number of bouts of aggressive calls. We also measured its number of aggressive calls per bout and the within-bout duty cycle, defined as the average duty cycle calculated only over those calls included in the bout, which by definition consisted of at least 2 calls. Previous studies (Pierce and Ralin 1972, Littlejohn 2001) and our preliminary analyses indicated that the frequency peaks of aggressive calls were lower than those of advertisement calls (see results below). Thus, we calculated the frequency decrease for each frequency peak as the difference between each male's average advertisement and aggressive call frequency for that peak. The advertisement call frequency used for this calculation was the average call frequency at the initial position as not all males gave advertisement calls in the center position. Our use of initial position advertisement call frequencies is suitable because this characteristic is known to be highly static within males (Gerhardt 1991), and we had no evidence that it changed with position (Reichert & Gerhardt, unpublished data). Finally, we analyzed the relative amplitude of advertisement and aggressive calls. We did not measure call amplitude directly during the experiments, so we extracted relative root-mean-squared amplitudes from waveform displays in the Raven software. Only males that gave at least five advertisement calls and five aggressive calls without changing position were included in these analyses. We averaged amplitudes for advertisement and aggressive calls separately for each male and then calculated its relative amplitude in dB as $20*\log_{10}$(aggressive call amplitude/advertisement call amplitude). All analyzed aggressive calls were given in the center position, thus we present these data as average call properties for each male at this position. We were not always able to measure all aggressive call characteristics for all males because we only included recordings in which at least three aggressive calls were given, and in some cases it was difficult to measure certain call parameters due to temporal overlap between the two males' calls. In addition, we were unable to measure temperature for some interactions, and therefore did not include these data in analyses of temperature corrected call characteristics. Thus, the sample sizes for call characteristics are variable.

Relationships with size and temperature

Many characteristics of anuran vocalizations vary with temperature (Gayou 1984). Thus, we ran linear regression analyses with each call characteristic against body temperature in order to temperature correct our measurements. Although winner and loser call characteristics are not strictly independent within a dyad, we included both in the regression analyses because we wished to use uniform criteria to perform the temperature correction. Whenever temperature was significantly correlated with a call characteristic, we used the slope of the regression and the mean temperature (23.4°C) to adjust values of each individual's measure for that call characteristic to what it would be expected to be at the mean temperature. We then used correlation analyses to determine the relationships between individual body size variables and each of the advertisement and aggressive call characteristics. We also used correlation analyses to determine whether different advertisement and aggressive call characteristics were correlated with one another.

Relationships with interaction escalation and outcome

Interactions were divided into four discrete, nonoverlapping levels of escalation depending on which aggressive behaviors were performed by each male. These were, in order of increasing escalation: neither male gave aggressive calls (ADV), one of the two males gave at least one aggressive call (AG1), both males gave at least one aggressive call (AG2), and males physically fought (PF). We used paired *t* tests to determine whether winners and losers differed for each aggressive call characteristic for AG2 and

PF interactions. For AG1 interactions, only one male gave aggressive calls, so for this level of escalation we used independent samples *t* tests to compare winners' and losers' aggressive calls. Paired *t* tests were used for comparisons of advertisement call characteristics at all levels of escalation. To test the global hypothesis that winners differed from losers in call characteristics and to avoid the problems associated with the non-independence of multiple comparisons of call characteristics that are correlated with one another, we used a combined probability test for dependent variables (Brown 1975). This test combines the *P* values from the multiple *t* tests for different call characteristics to give a single test statistic for the overall differences between winner and loser call characteristics at each level of escalation.

We determined the assessment strategy used by males by comparing call characteristics to the level of escalation and duration of interactions. We used general linear models to determine if either winner or loser call characteristics, analyzed separately, changed with increasing level of escalation. We used correlation analyses to determine the strength and direction of any relationship between winner or loser call characteristics and the duration of interactions. We considered that an interaction had been initiated when both males had given at least one call in the center position. We measured interaction duration directly from the video recordings as the difference in time between the loser's last call and the start of the interaction. Interaction durations were ln-transformed to achieve normality for statistical testing.

Several assessment strategies are predicted to involve relationships between escalation or duration and the magnitude of the difference between competitors in some trait. In particular, a major tenet of the mutual assessment strategy is that contest duration is driven directly by asymmetries rather than absolute values of call characteristics (Enquist 1985). Thus, we calculated absolute values of the differences between winners and losers for each aggressive call characteristic and used the same analyses as we used for individual call characteristics to determine if asymmetries in calling between winners and losers were related to either the level of escalation or the duration of interactions.

We tested whether instigators of various behaviors were more successful in aggressive interactions. Thus, we compared whether the winner or loser was the instigator of advertisement calling, aggressive calling, or physical contact for all interactions combined and for each separate applicable level of escalation. We compared aggressive call characteristics between instigators and non-instigators using *t* tests. We also compared the duration and level of escalation of interactions that were instigated by either the ultimate winner or the ultimate loser of the contest. Finally, we determined if an individual's status as an instigator was related to his status as the larger or smaller sized individual in the interaction. All statistical tests were two-tailed and were calculated by hand or with SPSS 16.0.1 software (SPSS Inc., 2007) at alpha=0.05.

Ethical note

We never observed any injuries or indications of undue stress during these experiments. Our experimental protocols were approved by the University of Missouri Animal Care and Use Committee (protocol numbers 1910 & 6546).

RESULTS

Description of aggressive calls

Aggressive calls differed both temporally and spectrally from advertisement calls (Figure 1, Table 1). Aggressive calls are much shorter, their frequencies are lower and they generally lack pronounced amplitude modulation. Patterns of variability of call characteristics between males were mostly concordant between advertisement and aggressive calls. Call period, call duration and duty cycle tended to be highly variable, while there was less variation in frequency (Table 1). However, the amount by which males decreased the frequency of their aggressive calls compared to their advertisement calls was highly variable. The amplitudes of aggressive calls were lower than those of advertisement calls for 64 of the 67 individuals for which we were able to measure relative amplitude. The median amplitude of aggressive calls relative to advertisement calls was -5.3 dB. The relative amplitude of aggressive calls was negatively correlated with the magnitude by which males decreased the frequency of their aggressive calls compared to advertisement calls (low frequency peak: r=-0.27, N=64 males, P=0.03; high frequency peak: r=-0.49, N=64 males, P<0.001). Although these call types differed greatly, many properties of advertisement calls were correlated with properties of aggressive calls (Table 2). For example, males with high advertisement call duty cycles also tended to have high aggressive call duty cycles. Unlike advertisement calls, most properties of aggressive calls were uncorrelated with male body temperature (Table 3). The exceptions were aggressive call duration and the decreases in frequency from advertisement to aggressive calls. The absolute frequency of aggressive calls, however, was uncorrelated with body temperature.

Several characteristics of both advertisement and aggressive calls were correlated with male body size (Table 4). As expected, the frequencies of both call types were negatively correlated with all three measures of size. For advertisement calls, body mass and condition were negatively correlated with call period and positively correlated with duty cycle (Table 4). For aggressive calls, both call duration and the within-bout duty cycle were positively correlated with body mass and condition. The magnitude of the decrease in frequency from advertisement calls to aggressive calls was negatively correlated with body mass for the high frequency peak, but not for the low frequency peak (Table 4).

Calling and interaction outcome

In less escalated interactions (ADV & AG1), winners and losers tended to differ in several advertisement call characteristics, most notably duty cycle and call frequency (Figure 2). Combined probability tests revealed a significant overall difference between winners and losers for advertisement call characteristics in both ADV ($\chi^2_{4.1}$=10.7, P=0.03) and AG1 interactions ($\chi^2_{6.1}$=17.9, *P*=0.006). In more escalated AG2 interactions and physical fights, however, there were no differences in competitors' advertisement calls (Figure 2; $\chi^2_{4.9}$=4.7, *P*=0.45 for AG2; $\chi^2_{5.3}$=7.5, *P*=0.19 for PF). In AG2 interactions there was a significant overall difference between winners and losers in their aggressive call characteristics (Figure 3; $\chi^2_{9.0}$=24.6, *P*=0.004). Winners of AG2 interactions tended to have a greater decrease in frequency from advertisement calls to aggressive calls and a higher within-bout duty cycle. In less escalated AG1 interactions, only the magnitude of the low frequency peak of winners' aggressive calls differed between winners and losers, and a global analysis revealed no overall differences between winners' and losers' aggressive calls (Figure 3; $\chi^2_{10.3}$=14.1, *P*=0.17). In the most escalated interaction type, physical fights, there were no differences in aggressive call characteristics between winners and losers (Figure 3; $\chi^2_{8.3}$=4.5, *P*=0.81).

Aggressive calling and the level of escalation and duration of interactions

We analyzed the relationship between aggressive call characteristics and the level of escalation separately for winners and losers in order to test the predictions of different assessment strategies. These data provided mixed evidence for any of the assessment strategies. For aggressive call frequency characteristics, we observed a significant change in these calls with the level of escalation for losers but not for winners. Losers tended to give lower frequency calls, and to reduce the frequency of their aggressive calls more, in highly escalated physical fights than in less escalated AG1 and AG2 interactions (Table 5; see also Figure 3). There were no differences between the different levels of escalation for the frequencies of winners' aggressive calls. For within-bout duty cycle and call duration, however, there was a relationship between the magnitude of these call characteristics and the level of escalation for winners (Table 5). The longest call durations and greatest duty cycles were associated with intermediate AG2 interactions (Table 5, Figure 3). Losers showed a similar pattern for call duration, but there was no relationship between within-bout duty cycle and the level of escalation for losers (Figure 3). These results are not consistent with either mutual assessment or self-assessment strategies of contest behavior. Furthermore, there were no correlations between aggressive call characteristics and interaction duration when we analyzed these relationships separately for winners and losers (data not shown, all *P*'s>0.05).

Aggressive call asymmetries and the level of escalation and duration of interactions

More escalated interactions involved competitors that were more evenly matched for some aggressive call characteristics than less escalated interactions. This conclusion is based on analyses that were restricted to those aggressive call characteristics we previously identified as probably related both to RHP and the outcome of interactions: call duration, within-bout duty cycle, absolute frequency and magnitude of frequency decrease. In physical fights, competitors tended to be more evenly matched than in AG2 interactions for both the within-bout duty cycle and the magnitude of the high frequency peak (Figure 4). Other differences between competitors' aggressive calls also tended to be lower for physical fights than for AG2 interactions, but the difference was not statistically significant. When all call characteristics were combined, there was a significant difference in aggressive call asymmetries between AG2 interactions and physical fights ($\chi^2_{5.6}$=12.3, *P*=0.03). Likewise, the duration of the interaction was negatively correlated with the magnitude of the difference between winners and losers for both the within-bout duty cycle (*r*=-0.25, *N*=80, *P*=0.03) and the frequency decrease from advertisement calls to aggressive calls for the low frequency peak (*r*=-0.289, *N*=75, *P*=0.01). These differences remained significant, or nearly so, when we analyzed the correlations separately for AG2 interactions (within-bout DC, *r*=-0.37, *N*=28, *P*=0.06; low frequency decrease, *r*=-0.389, *N*=30, *P*=0.03). We argue therefore that these correlations were not merely attributable to the greater duration of physical fights compared to AG2 interactions (Chapter 6). For physical fights, however, there were no correlations between aggressive call asymmetries and interaction duration (within-bout DC, *r*=-0.02, *N*=52, *P*=0.89; low frequency decrease, *r*=-0.14, *N*=45, *P*=0.36). We also found no correlations between asymmetries in advertisement call characteristics and interaction duration (data not shown, all *P*'s>0.05).

Instigators and aggressive calling

Overall, instigators of advertisement calling, aggressive calling and fights were not more likely to be winners than non-instigators (binomial test: advertisement calling, 99/180 trials won by the male that advertisement called first, *P*=0.21 aggressive calling, 63/137 trials won by the male that aggressive called first, *P*=0.39; physical fights, 34/56 trials won by male that initiated physical contact, *P*=0.14). However, the success of instigators of advertisement calling and aggressive calling depended on the level of escalation that the interaction reached (Figure 5). Instigators of aggressive calling in physical fights were ultimately more likely win those interactions when compared to instigators of less escalated interactions (χ^2 comparison of PF to AG1 & AG2 combined, χ^2_1=7.3, P=0.007). Aggressive call instigators tended to have a lower SVL than non-instigators (paired *t* test, mean difference=0.99 mm, t_{134}=3.45, P=0.001). Instigators of advertisement calling or fighting did not differ in size from non-instigators.

For instigators of aggressive calling, we found several differences between the properties of instigators' and non-instigators' aggressive calls. Aggressive call instigators' calls had higher low frequency peaks (t_{83} =2 .24, P = 0.027) and a smaller decrease in frequency in the low frequency peak (t_{74} =2.01, P = 0.048). There was also a trend for aggressive call instigators to have lower duration aggressive calls (t_{75} = 1.85, P = 0.068) compared to non-instigators. We explored these differences further by examining whether winner and loser aggressive call characteristics differed when either the winner or loser of the interaction was the aggressive call instigator, respectively. We observed no differences between winners' and losers' call characteristics when the winner instigated aggressive calling (Figure 6; combined probability test: $\chi^2_{10.0}$=4.6, P=0.92). There were, however, differences between winners and losers in call duration and most frequency characteristics of aggressive calls when the loser instigated aggressive calling (Figure 6; combined probability test: $\chi^2_{7.6}$=25.9, P=0.0005). Interactions in which the loser instigated aggressive calling or physical contact were shorter in duration than those in which the winner instigated those behaviors (Figure 7; aggressive calling: t_{132}=2.85, P=0.005; physical contact: t_{53}=2.34, P=0.02).

DISCUSSION

Assessment strategies in H. versicolor

We obtained strong evidence that aggressive calls are used to mediate contests in *H. versicolor*. Furthermore, males appear to be responsive to variation in those aggressive call characteristics that are likely to be related to the energetic costs of calling or its efficiency. Below, we discuss the evidence that contests involving aggressive calling are determined by mutual assessment of aggressive call characteristics, with victory likely to go to the male that produces the most energetically costly display. Thus, although fights in *H. versicolor* are relatively benign compared to those of most other animals (e.g., Austad 1983, Waas 1991, Haley 1994, Huntingford et al. 1995, Neat et al. 1998b, Lappin and Husak 2005), aggressive calling appears to play a similar role in resolving contest outcome as aggressive signaling displays in many other animal species (e.g., Maynard Smith and Price 1973, Clutton-Brock and Albon 1979, Archer 1988, Kemp and Alcock 2003).

Our data on aggressive calling provide only partial support for various established models of assessment during contests. Some analyses were consistent with self-assessment strategies. Specifically, losers but not winners tended to show differences in some of the characteristics of their aggressive calls, presumably in the direction of increased cost, with the level of escalation. For other characteristics, however, only winners showed differences in call characteristics at different levels of escalation. Furthermore, the relationship between call characteristics and the level of escalation was not monotonic for call duration and within-bout duty cycle. Some of our data were also consistent with mutual assessment strategies. We observed that longer, more escalated interactions tended to involve decreased asymmetries between competitors' aggressive call characteristics. However, both mutual assessment and cumulative assessment strategies are predicted to show a positive relationship between RHP (here, interpreted as signal quality) and interaction duration for losers, and an equally strong but negative relationship for winners (Taylor and Elwood 2003). We observed the former in some of our analyses, but never the latter.

We nevertheless conclude that our data best support a strategy mutual assessment of aggressive call characteristics in *H. versicolor*. In support of this argument, we first point out that mutual assessment itself is merely the concept that competitors assess one another's signals during aggressive interactions (Enquist and Leimar 1983). Thus, the best support for this mode of assessment is gained not through indirect predictions of the relationships between RHP and interaction duration, but rather directly, through evidence that individuals perceive and are responsive to variation in their competitor's signals of RHP during contests. This approach may be more applicable to many animal species whose contests do not necessarily meet the specific assumptions of models of animal aggressive behavior.

Our evidence that individual *H. versicolor* assessed the signals of their competitors during contests is fourfold. First, although insufficient to rule out other competing hypotheses (Taylor and Elwood 2003, Arnott and Elwood 2009, Briffa and Elwood 2009), we found a negative relationship between aggressive call asymmetries and both the level of escalation and the duration of interactions. If mutual assessment can be confirmed by other means, this result implies that contests will be longer when it is more difficult for contestants to determine which is the superior RHP individual. Second, the structure of aggressive calling interactions facilitates mutual assessment of call characteristics. One individual's bouts of aggressive calling rarely overlapped with those of its competitor (Reichert, personal observation). Alternating aggressive calls may allow males to assess more effectively one another's signals (Schwartz 1987, Greenfield 1994a); there is no reason to expect such call alternation under self-assessment. Third, the differences in calling and behavior between instigators of aggressive calling that either won or lost the interaction imply mutual assessment. Unlike other animal species (Jackson 1991, Hack 1997a, Taylor et al. 2001), instigators of aggressive calling in *H. versicolor* were not more likely to win. However, interactions in which the ultimate loser instigated aggressive calling were much shorter than those in which the ultimate winner instigated aggressive calling; these interactions were also characterized by greater differences between winners' and losers' aggressive calls. This asymmetry was sometimes driven by superior call characteristics of the winner, while for other call characteristics the asymmetry was reflected in the inferior call characteristics of the loser. Thus, the decreased duration of loser instigated interactions cannot be attributed to losers being absolutely inferior callers; rather, the relevant measure was the relative qualities of their opponents' aggressive calls. We propose that when an individual instigates aggressive calling, it assesses any aggressive call responses of its rival relative to its own RHP. If its rival is clearly superior, the instigator quickly backs down. If the two competitors are relatively well matched, the interaction escalates and takes much longer to resolve. Fourth and finally, preliminary results of playback tests show that males appear to be responsive to variation in aggressive call characteristics, suggesting that males evaluate the aggressive calls of their opponents when making the behavioral decision to persist, escalate or retreat during contests (Reichert & Gerhardt, unpublished data). Studies of acoustic competition in several other anuran species have also shown evidence for mutual assessment of call characteristics (Davies and Halliday 1978, Wagner 1989a, Wells 1989, Wagner 1992, Schwartz 1994, Burmeister et al. 2002).

Signals of RHP and energetic state

In a previous study of aggressive interactions in *H. versicolor* (Chapter 6), we concluded that body size and condition are not major components of RHP. Given the results of the current study, we hypothesize that victory in aggressive calling interactions is based on mutual assessment of aggressive call characteristics related to the energetic cost of calling. Thus, we propose that RHP is based on the individual's underlying physiological state. Similar hypotheses have been proposed for aggressive communication both in anurans and in other animal species (e.g., Wells and Schwartz

1984b, Robertson 1986, Wagner 1992, Marden and Rollins 1994, Schwartz 1994, Hack 1997b, Neat et al. 1998a, Neat et al. 1998b, ten Cate et al. 2002).Further study is necessary to confirm energy based RHP differences between winners and losers. For now we discuss the evidence obtained from analyses of advertisement and aggressive calls that energetic state is a determinant of RHP in this species.

Although certain characteristics of advertisement calls are related to body size and energy expenditure (Taigen and Wells 1985, Wells and Taigen 1986; this study), there were no differences in advertisement call characteristics between competitors in highly escalated interactions. However, in less escalated ADV and AG1 interactions, winners' advertisement calls tended to have lower frequency peaks and to be given at a higher effort than those of losers. The latter characteristic in particular is known to vary positively with energy expenditure (Taigen and Wells 1985). In AG2 interactions, competitors did not differ in advertisement call characteristics, but did show differences in aggressive calling. Finally, in physical fights, males did not differ in either advertisement or aggressive call characteristics. This pattern of call asymmetries across different levels of escalation fits one of the predictions of the sequential assessment model of aggressive contests (Enquist et al. 1990). We hypothesize that in close-range interactions, contestants mutually assess each other's advertisement calls. If there are large differences, the contest is generally resolved in favor of the more vigorous caller (Figure 2). If, however, males' advertisement calling performances are similar, they escalate to aggressive calls. Mutual assessment of aggressive calls takes place, and if large differences exist between males, then an AG2 interaction takes place, and the male that produces the most vigorous aggressive calls is the likely victor (Figure 3). Finally, if neither advertisement calls nor aggressive calls are sufficient to resolve the interaction, males engage in physical combat. It is unclear what factors influence success in physical fights in *H. versicolor*, although neither body size (Chapter 6) nor call characteristics (this study) appear to play a role.

No study has measured the energetic cost of aggressive vocalizations in any anuran species. Nonetheless, based on general principles of sound production and previous studies of the energetic costs of advertisement calling (Ryan 1988b, Prestwich 1994, Wells 2001), there is almost certainly a positive relationship between variation in key aggressive call characteristics and energetic costs or efficiency (see below: role of call frequency). It should also be possible to estimate the absolute magnitude of those costs. With regard to gross temporal properties of the calls, winners had higher within-bout duty cycles than losers for AG2 interactions. Duty cycle is a measure of calling effort and thus should be directly related to increased energetic expenditure. Indeed, several studies of advertisement calling in frogs have shown that energy costs increase with increasing advertisement call duty cycle (Bucher 1982, Taigen and Wells 1985, Wells and Taigen 1986, Wells and Taigen 1989, Wells et al. 1996). Interestingly, aggressive call duty cycles were substantially higher than advertisement call duty cycles (Table 1). Thus it is possible that aggressive calling is more energetically demanding than advertisement calling, and may perhaps reach the upper limits of individual vocal performance. If aggressive calls are indeed more challenging to produce, this may allow differences between individuals in energetic state to be revealed more readily by aggressive calls than by advertisement calls. This could thus be a large part of the explanation for why aggressive calls are used in contests in the first place.

The role of call frequency

Frequency is expected to be an important signal characteristic in aggressive interactions in frogs because it is usually negatively correlated with body size (Davies and Halliday 1978, Arak 1983, Robertson 1986, Given 1987, Ryan 1988a, but see Bee 2002). We observed that males decreased the frequency of their calls when switching from advertisement to aggressive calls. Frequency decreases associated with aggressive vocalizations have been noted in several other species of anurans (Wagner 1989a, 1992, Grafe 1995, Bee and Perrill 1996, Howard and Young 1998, Given 1999, Bee et al. 2000, Bee and Bowling 2002, Burmeister et al. 2002). Call frequency is highly constrained by the size and resonant properties of the vocal production apparatus (Martin 1972, Ryan 1986a). Any deviations from the natural frequency will reduce the efficiency of vocalization. Thus, it is not surprising that the relative amplitude of aggressive calls had a negative relationship with the decrease in frequency from advertisement calls to aggressive calls. Males appear to face a tradeoff between the production of lower frequency and louder aggressive calls. Indeed, we observed that the largest frequency drops took place in the most escalated interactions, suggesting that males maintain some control over their calling efficiency in contests. In addition to decreasing the efficiency of calling, the continued production of low frequency aggressive calls may be energetically costly.

Wagner (1992) proposed several hypotheses for the function of frequency decreases during aggressive calling. According to the “signal of size” hypothesis, either the lowered aggressive call frequency or the magnitude of the frequency decrease itself is a stronger predictor of body size, and thus RHP, than the initial call frequency (Wagner 1992). We can reject this hypothesis for *H. versicolor* for several reasons. First, size plays only a limited role in the outcome of aggressive interactions in this species (Chapter 6). Second, although aggressive call frequency is strongly correlated with body size, advertisement call frequency is correlated just as strongly (Table 4). Finally, the magnitude of the frequency decrease was only weakly correlated with body size, and this relationship was negative. The “dishonest signal of size” hypothesis poses that the decrease in frequency in aggressive calls is primarily due to an attempt by inferior individuals to bluff and appear larger than they actually are (Wagner 1992, Bee et al. 2000). Although we observed a slight negative correlation between body size and the magnitude of the frequency decrease, we can discount this hypothesis as well because aggressive call frequency remains tightly correlated with body size and because assessment of body size does not appear to be an important component of contest success. Finally, a decrease in frequency could be a “signal of size-independent fighting ability” (Wagner 1992). In this scenario, the frequency decrease is related to some aspect of contestant RHP other than body size, as well as the likelihood of escalation. Our results suggest that assessment of aggressive calling in *H. versicolor* is best explained by this hypothesis. For AG2 interactions, although there were no size differences between winners and losers (Chapter 6), winners had larger frequency decreases than losers. The ability to decrease frequency to a greater extent can plausibly be linked to superior energetic status, a potential determinant of RHP that may not be as easily assessed for the relatively static frequency peaks themselves. Wagner (1992) came to a similar conclusion for the function of frequency decreases during aggressive interactions in *Acris crepitans*. Preliminary results from playback tests suggest that male *H. versicolor* are responsive to variation in frequency decreases in aggressive calls (Reichert & Gerhardt, unpublished data).

Conclusions

Anurans have much to offer for the study of aggressive signaling, yet relatively little work has been performed on this topic. This study is one of the few studies of aggressive interactions in anurans that has simultaneously measured body size, interaction duration and call characteristics. The method we have developed to stage aggressive interactions (Chapter 6) should facilitate similar studies in other species so that the predictions of models of aggressive signaling can be addressed in a comparative framework. Future work should focus on the relationships between RHP and variation in aggressive call characteristics, with a particular emphasis on the energetic costs of aggressive calling. Further studies that examine the determinants of fight outcome in non-weaponized anuran species would also be useful. Anurans are highly responsive, and their signals are easily synthesized and manipulated. With a basic knowledge of anuran contest structure, a wide variety of playback tests can be constructed to test general models of animal aggressive signaling.

Table 1: Descriptive statistics of advertisement and aggressive calls

	Advertisement calls	*N*	Aggressive calls	*N*
Pulse number	18.8 (4.5)	321	-	-
Call duration	0.814 (0.195)	321	0.162 (0.043)	189
Call period	6.02 (2.47)	321	2.22 (1.71)	211
Duty cycle	0.162 (0.041)	320	0.295 (0.089)	206
Pulse rate	23.5 (1.62)	321	-	-
Low frequency peak	1.17 (0.07)	321	1.02 (0.07)	211
High frequency peak	2.29 (0.14)	321	2.02 (0.14)	211
Calls per bout	-	-	2.78 (1.45)	222
Bout duty cycle	-	-	0.439 (0.080)	201
Low frequency decrease	-	-	0.16 (0.05)	187
High frequency decrease	-	-	0.29 (0.09)	187

Statistics are presented as means (standard deviation). All advertisement call characteristics, along with the call duration and frequency decreases of aggressive calls, are corrected to the mean body temperature of 23.4°C. Advertisement call means were calculated from the initial position recordings because not all males gave advertisement calls in the center position. Some characteristics only applied to one or the other call type.

Table 2: Correlation matrix of advertisement and aggressive call characteristics

	Ad PN	Ad CD	Ad DC	Ad LF	Ad HF	Ag CD	Ag BoutDC	Ag LF	Ag DF	AgLF drop
AdCD	0.95**									
AdDC	0.24**	0.21**								
AdLF	-0.07	-0.05	-0.26**							
AdHF	0.08	0.10	-0.24**	0.83**						
AgCD	0.11	0.08	0.24**	-0.27**	-0.27**					
AgBoutDC	0.24**	0.18*	0.31**	-0.21**	-0.20**	0.64**				
AgLF	-0.12	-0.08	-0.23**	0.71**	0.77**	-0.25**	-0.32**			
AgDF	-0.07	-0.05	-0.22**	0.68**	0.74**	-0.24**	-0.29**	0.93**		
AgLFdrop	0.06	0.03	0.00	0.40**	0.13	-0.04	0.12	-0.36**	-0.29**	
AgHFdrop	0.17*	0.18*	-0.02	0.29**	0.37**	-0.09	0.16*	-0.16*	-0.32**	0.60**

Values are Pearson correlation coefficients. Sample sizes vary for each characteristic because we were unable to measure some characteristics in all males, and not all males gave aggressive calls. Sample sizes range from 177-321 males. Advertisement call values were measured from males calling in the initial position. Ad, advertisement call; Ag, aggressive call. PN, pulse number; CD, call duration; DC, duty cycle; LF, low frequency peak; HF, high frequency peak; BoutDC, within-bout duty cycle for aggressive calls; LFdrop, difference in the low frequency peak between advertisement and aggressive calls; HFdrop, difference in the high frequency peak between advertisement and aggressive calls. Call characteristics that were correlated for temperature were temperature-corrected to 23.4°C for this analysis. Correlations are not strictly independent as we included calls of both males in the interacting pair. Preliminary analyses with only one of the two males' calls included gave similar results. * *P*<0.05; ** *P*<0.01.

Table 3: Correlations between call characteristics and temperature

	Advertisement calls	*N*	Aggressive calls	*N*
PN	-0.183**	321	-	
CD	-0.495**	321	-0.301**	189
CP	-0.141*	321	-0.079	184
DC	-0.297**	321	-0.046	183
PR	0.821**	321	-	
LF	0.196**	321	-0.026	189
HF	0.127*	321	-0.029	189
Calls per bout	-		0.079	197
Within-bout DC	-		-0.035	179
LF decrease	-		0.289**	187
HF decrease	-		0.183*	187

Values are Pearson correlation coefficients for the relationship between the given call characteristic and male body temperature. Advertisement call values were measured from males calling in the initial position. Abbreviations as in Table 2. * *P*<0.05; ** *P*<0.01.

Table 4: Correlations of body size and call characteristics

	Advertisement calls			Aggressive calls		
	Mass	SVL	Condition	Mass	SVL	Condition
PN	-0.040	0.004	-0.071	-	-	-
CD	-0.071	-0.022	-0.101	0.303**	0.179*	0.310**
CP	-0.155**	-0.059	-0.223**	0.004	-0.068	0.101
DC	0.222**	0.076	0.328**	0.065	0.023	0.074
PR	0.102	0.089	0.077	-	-	-
LF	-0.678**	-0.561**	-0.448**	-0.746**	-0.604**	-0.458**
HF	-0.810**	-0.652**	-0.560**	-0.691**	-0.561**	-0.417**
Calls per bout	-	-	-	-0.087	-0.032	-0.102
Within-bout DC	-	-	-	0.222**	0.045	0.321**
LF decrease	-	-	-	0.085	0.085	0.052
HF decrease	-	-	-	-0.187*	-0.132	-0.141

Values are Pearson correlation coefficients for the relationship between the given call characteristic and male body size variable. Abbreviations as in Table 2. Advertisement call values were measured from males calling in the initial position. Sample sizes range from 320-321 for advertisement calls and 186-221 for aggressive calls. * *P*<0.05; ** *P*<0.01.

Table 5: Winner and loser aggressive call characteristics and escalation

	Winners				Losers			
	F	*N*	*P*	Post hoc	*F*	*N*	*P*	Post hoc
Within-bout DC	3.72	97	**0.03**	AG2>PF	0.42	106	0.66	
Call duration	8.92	90	**0.001**	AG2>PF	6.31	99	**0.003**	AG2>PF
Low frequency peak	0.12	100	0.89		3.32	111	**0.04**	AG1>PF
High frequency peak	0.13	100	0.88		3.76	111	**0.03**	AG1>PF
Low frequency drop	0.77	90	0.47		4.19	97	**0.02**	PF>AG2
High frequency drop	1.78	90	0.18		9.21	97	**0.001**	PF>AG2

Results of general linear models testing variation in aggressive call characteristics at three different levels of escalation. Models were run separately for winners and losers. Post hoc tests were run with a Bonferroni correction for models that returned a significant main effect. Significant post hoc comparisons, along with the direction of the difference, are shown in the post hoc columns.

Figure 1: Typical advertisement and aggressive calls. **a** Waveform display of a typical advertisement call. **b** Waveform display of a typical aggressive call. **a** and **b** were given three seconds apart by the same male. **c** Two bouts of aggressive calling. The first bout contains two aggressive calls and the second bout contains four aggressive calls. **d** Power spectrum of the advertisement call in **a** illustrating the two major frequency peaks. **e** Power spectrum of the aggressive call in **b**. The two thin vertical lines in **d** and **e** illustrate the frequencies of aggressive calls compared to advertisement calls. The vertical lines pass directly through the frequency peaks of the aggressive call and show that the aggressive call frequency peaks are lower than the advertisement call frequency peaks.

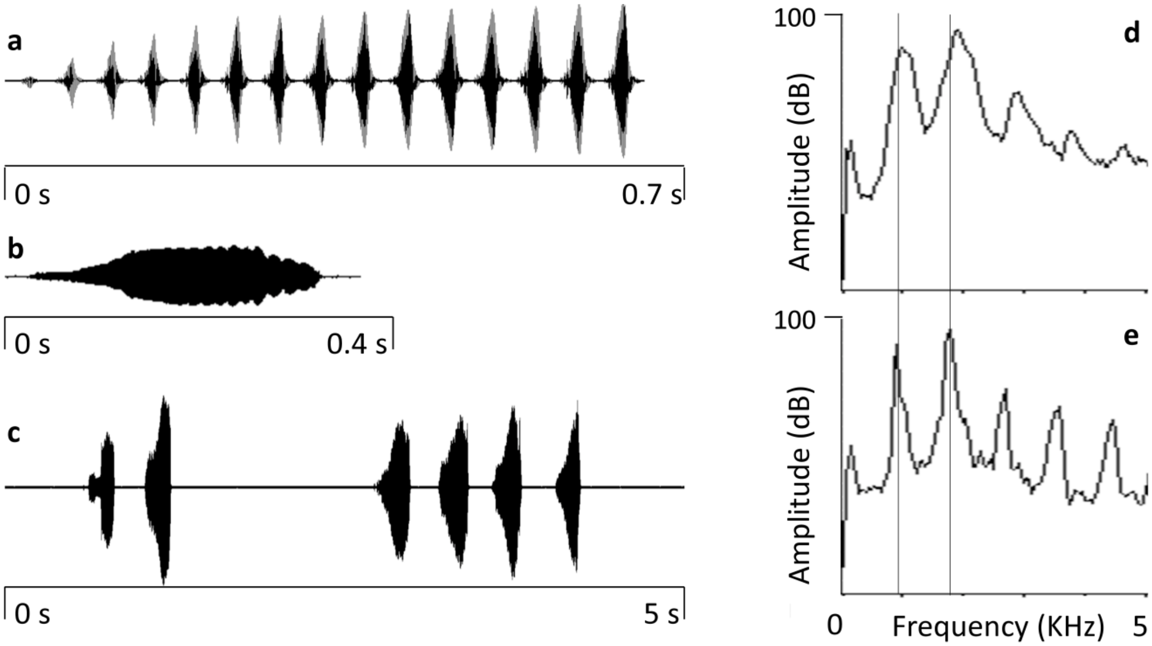


Figure 2: Average (±SE) temperature corrected advertisement call characteristics for losers (grey bars) and winners (white bars) at different levels of escalation (abbreviations defined in the methods). **a** Call duration, **b** duty cycle, **c** call period, **d** high frequency peak. Asterisks indicate significant results ($P<0.05$) of paired *t* tests for comparisons of winner and loser advertisement call characteristics at the given level of escalation. Averages presented here were measured at the halfway position, as not all males gave sufficient numbers of advertisement calls in the center position for analysis. *N*=41 pairs for ADV, 38 pairs for AG1, 35 pairs for AG2 and 46 pairs for PF.

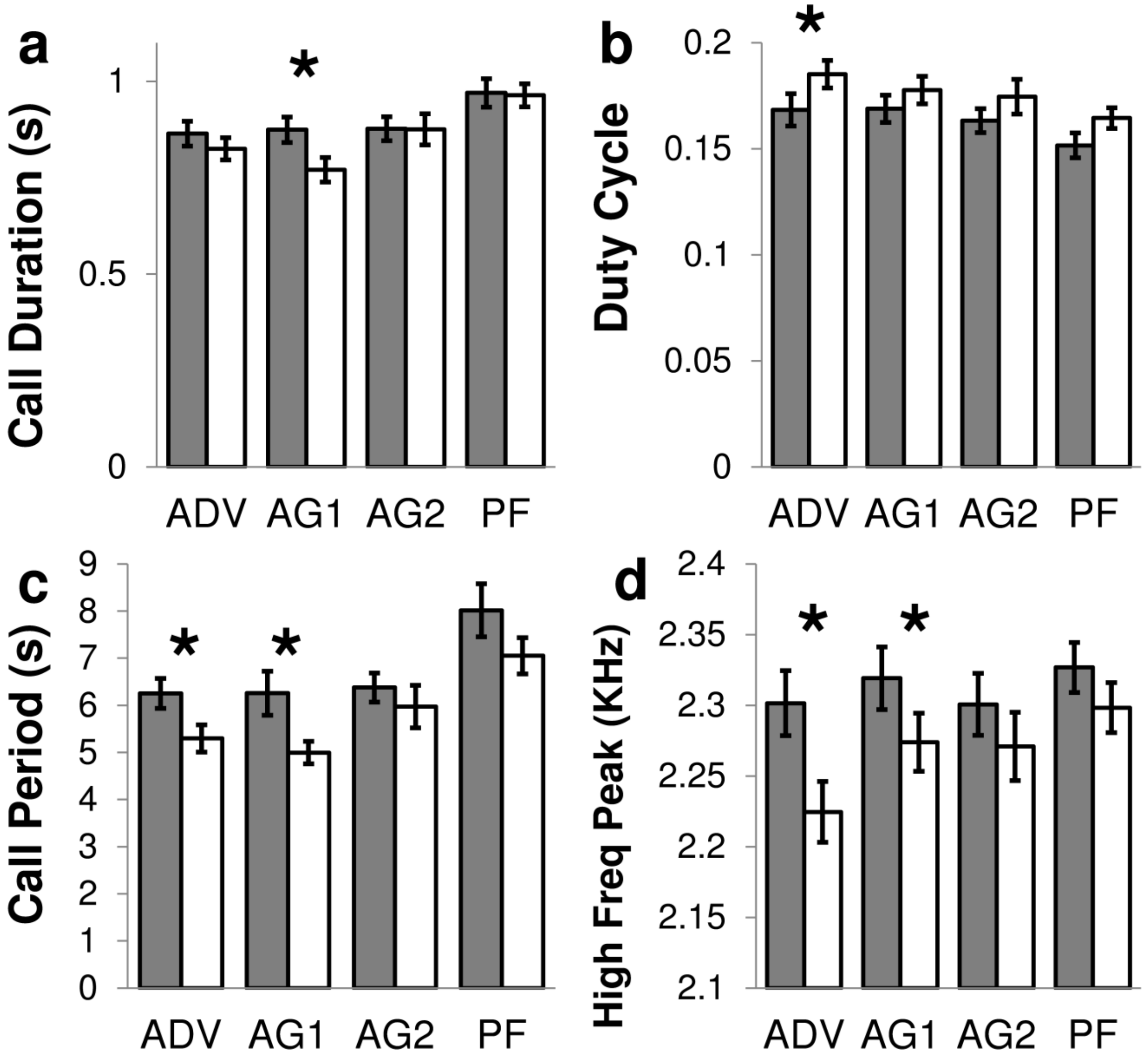


Figure 3: Average (± SE) aggressive call characteristics for losers (grey bars) and winners (white bars) at different levels of escalation. **a** Within-bout duty cycle, **b** temperature corrected call duration, **c** low frequency peak, **d** high frequency peak, **e** temperature corrected magnitude of the decrease in frequency between advertisement and aggressive calls of the low frequency peak, **f** temperature corrected magnitude of the decrease in frequency between advertisement and aggressive calls of the high frequency peak. Asterisks indicate significant results (*P*<0.05) of independent samples (in the case of AG1) or paired *t* tests (AG2 and PF) for comparisons of winner and loser aggressive call characteristics at the given level of escalation. Sample sizes varied slightly depending on the characteristic as not all characteristics could be measured from all males' recordings. For losers, *N*=19-24 males for AG1, 33-34 males for AG2, and 45-53 males for PF. For winners, *N*=11-14 males for AG1, 31-32 males for AG2, and 46-54 males for PF.

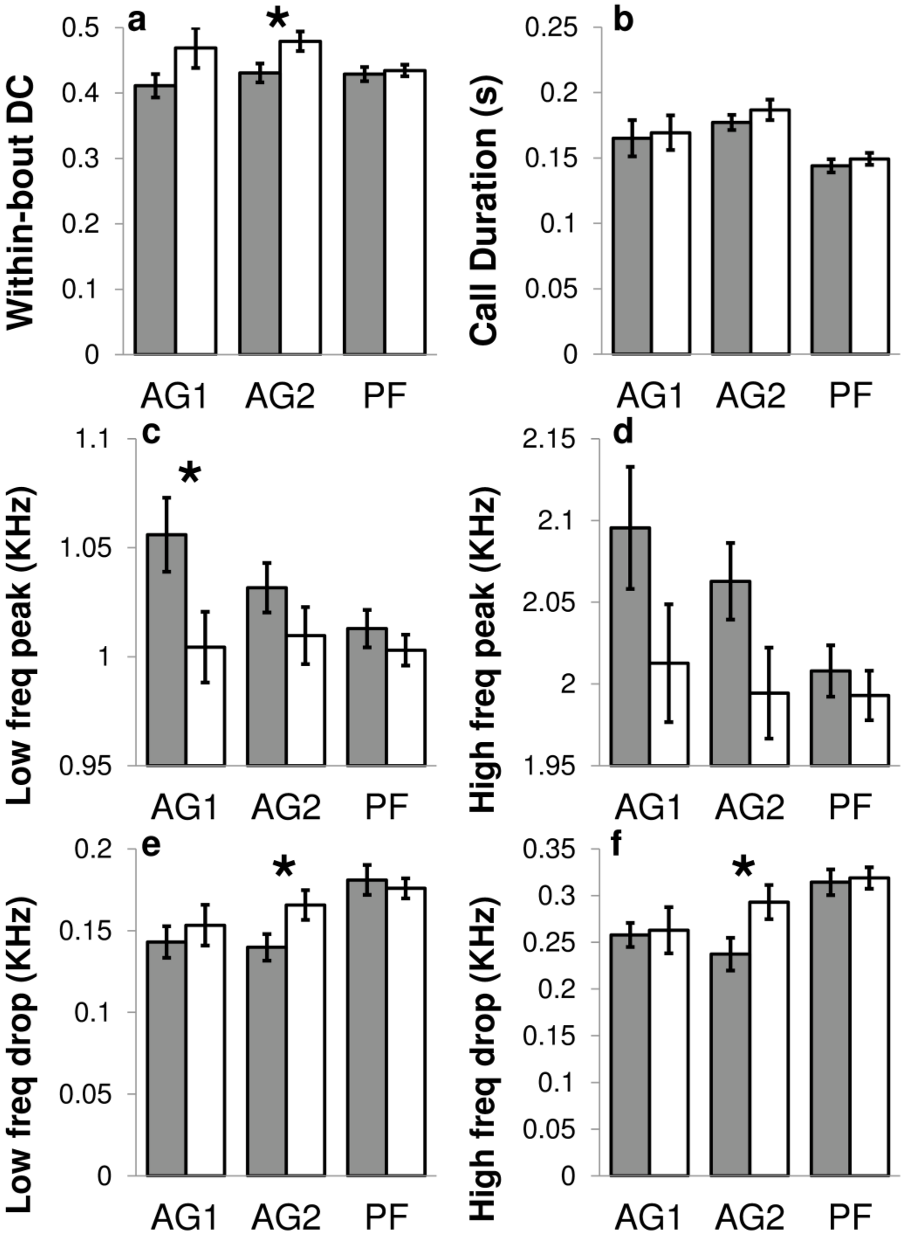


Figure 4: Aggressive call asymmetries at different levels of escalation. Bars represent the average (±SE) of the absolute values of the differences between winners and losers for each aggressive call characteristic in less escalated AG2 interactions and more escalated physical fights. For both within-bout DC (**a**) and the high frequency peak (**d**), there was a greater difference between competitors in AG2 interactions than in physical fights based on an independent samples *t* test. *N*=30-31 pairs for AG2, 45-53 pairs for PF. **P*<0.05.

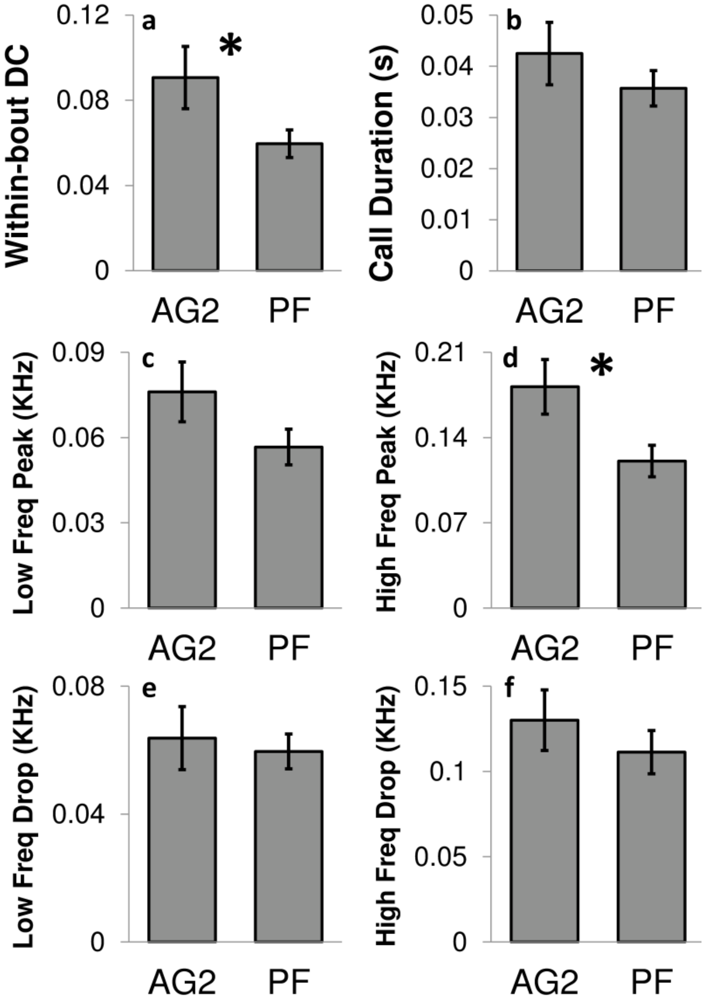


Figure 5: Success of instigators by level of escalation. Bars show the percentage of interactions won at each level of escalation by the individual that instigated **a**, advertisement calling (ADV, *N*=43; AG1, *N*=47; AG2, *N*=34; PF, *N*=56) and **b**, aggressive calling (AG1, *N*=46; AG2, *N*=35; PF, *N*=56). In **b**, instigators were more likely to win physical fights than the other two interaction types combined (*χ^2 test, $P<0.05$).

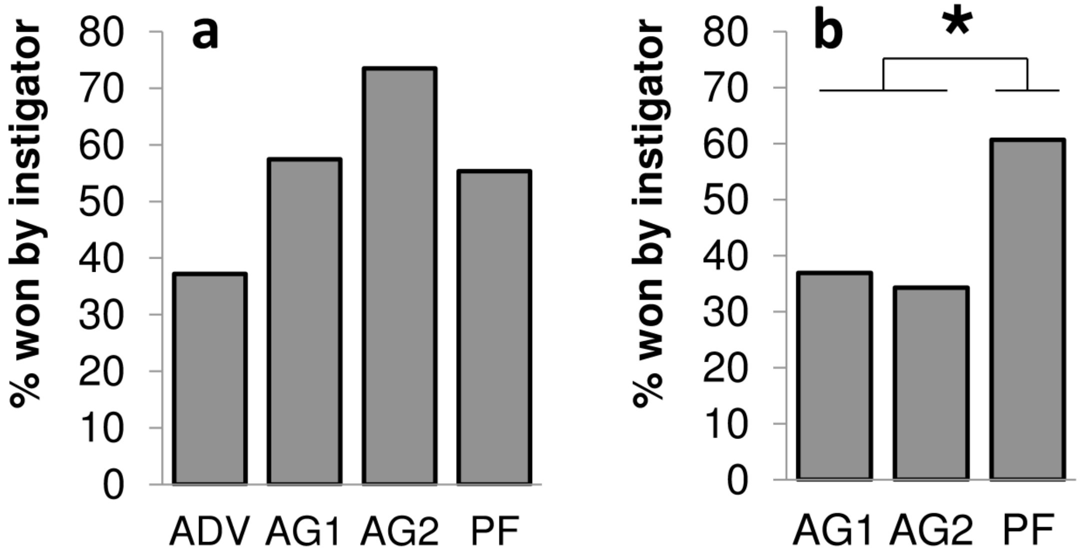


Figure 6: Average aggressive call characteristics (± SE) for losers (grey bars) and winners (white bars) when either the winner or the loser was the instigator of aggressive calling. Asterisks indicate significant results ($P<0.05$) of paired *t* tests for comparisons of mean call characteristics between winners and losers. *N*=37-42 males per bar.

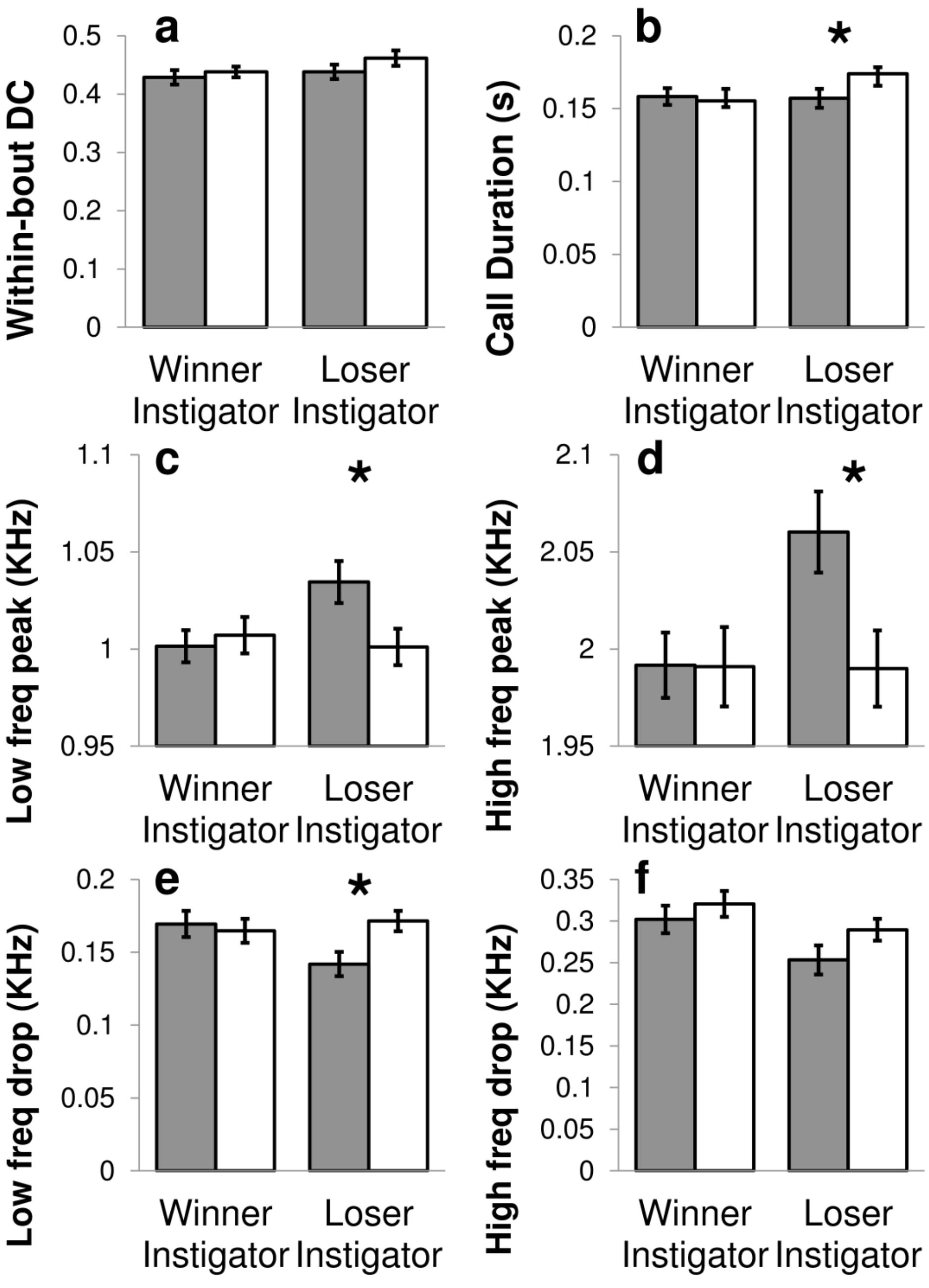
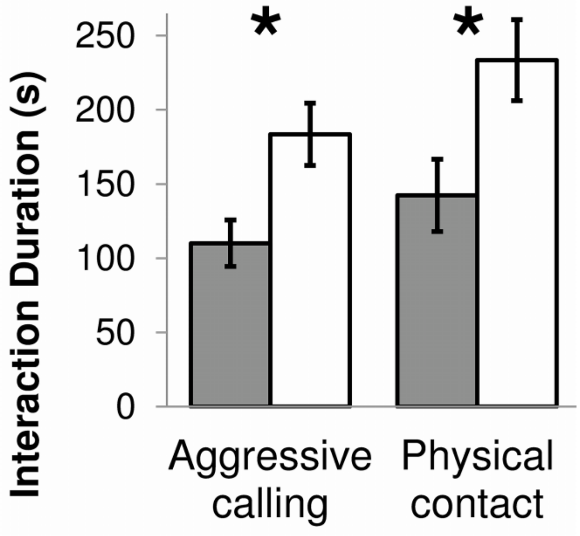


Figure 7. Total interaction duration by whether the loser (grey bars) or the winner (white bars) instigated aggressive calling and physical contact. Asterisks denote a significant difference in interaction duration in an independent samples *t* test between interactions in which the given behavior was instigated by winners compared to those instigated by losers. *$P<0.05$. $N=73$ interactions in which the loser instigated aggressive calling, $N=61$ interactions in which the winner instigated aggressive calling, $N=22$ interactions in which the loser instigated physical contact, $N=33$ interactions in which the winner instigated physical contact.



CHAPTER 8

General Discussion

The experiments described in this dissertation show that it is possible to obtain evidence for the communicative significance of aggressive calls in anurans. The questions I have addressed and the techniques I have developed should be applicable to a wide range of anuran species. Here, I give a non-exhaustive list of the major questions that must be addressed to gain a better understanding of aggressive calling behavior in anurans.

1. What is the function of aggressive calling?

As I argued in the introduction, a general understanding of the function and communicative significance of aggressive calls is largely lacking in anurans. My studies with *Dendropsophus ebraccatus* and *Hyla versicolor* underscore the necessity of detailed behavioral investigations of aggressive calling before inferences can be made regarding the presumed function of these calls. In particular, for *D. ebraccatus*, I argued that aggressive calls are not, in fact, primarily used in an agonistic context but instead are part of a male strategy to maintain attractiveness to females in competitive call timing interactions (Reichert 2011b). In *H. versicolor*, males appeared to use aggressive calls in the more traditional sense, although the relationship between aggressive calling and typical measures of RHP was not straightforward (Chapter 6 & 7).

Future studies must address these questions in many other anuran species so that broad patterns can emerge. Ideally, studies should incorporate field observations of aggressive behavior, staged interactions between males (including interactions that manipulate variables likely to be important in determining contest outcome such as residency and hormonal status, see below), and playback tests with individual males. Field observations can determine, among other things, the frequency with which aggressive behaviors are expressed naturally, the relationship between opponent proximity and aggression, and the effects of chorus density and environmental variables on aggression. Staged interactions will be crucial to examine closely all stages of agonistic encounters. In many species, aggressive interactions are brief and unpredictable, and it is difficult to obtain meaningful sample sizes for analyses. In addition, it is difficult to simultaneously monitor the calling and movements of both males in the field, while this is relatively straightforward in a more controlled setting. Staged interactions also allow for powerful experimental manipulations to one or both competitors in order to single out those variables that are most important in determining the structure and outcome of contests. Thus, staged interactions will be a valuable tool in future studies of anuran aggression. However, staged interactions cannot replicate easily some variables that may be important in determining contest outcome such as local environmental conditions and residency/intruder asymmetries. In addition, it is likely that many species will not express natural calling and aggressive behavior in the more confined settings required to stage aggressive interactions. Territorial species may not call if removed from their territories, and many species are simply too skittish to tolerate calling in an experimental arena. Finally, playback tests will be essential to determine whether males are responsive to aggressive calls and to individual aggressive call characteristics. If aggressive calls are indeed assessed by males during disputes, it is important to know which specific characteristics are assessed by males. With this information, it will be possible to relate aggressive call characteristics to RHP. In addition, this method may elicit the full range of aggressive behaviors, and thus simulate an aggressive interaction in species that are not amenable to staged encounters. Interactive playbacks (e.g., Schwartz 1994) may be especially useful to replicate the mutual assessment that likely takes place in anuran aggressive interactions.

2. *Variation in aggressive calling across species*

Different species vary in life history traits that are likely to affect if, how and when aggressive calls are used to resolve disputes. Thus, comparative studies of aggressive calling across species would be especially useful not only to gain a general understanding of the function and evolution of anuran aggressive calls but also to test the predictions of game theory models that predict different levels of aggressive signaling depending on the costs and benefits of such signals. Few studies have made comparisons across different species' aggressive calls; those that have done so have mostly examined only the structure of the aggressive calls themselves (Schwartz and Wells 1984, 1985, Littlejohn 2001, Owen 2003). These studies do not address differences in what is communicated during aggressive disputes, however. To address these differences, experiments involving staged aggressive interactions could be carried out for multiple anuran species. Phylogenetically controlled comparisons of aggressive behavior between species could then be made. For example, species may differ in the relationship between body size and aggressive call characteristics, the relationship between aggressive call characteristics and the outcome and level of escalation of interactions, the particular aggressive call characteristics that are important in resolving aggressive disputes, responsiveness to aggressive calls, likelihood of habituating to aggressive calls, and the amount of time spent giving aggressive calls. These differences can then be mapped along with known differences in life history traits to test whether those life history traits may be driving differences in the use of aggressive calls between species. Relevant life history traits include the development of weaponry and general fighting ability, length of the breeding season, relative energetic costs of advertisement and aggressive signals, degree of territoriality, typical chorus density, and the attractiveness of advertisement and aggressive calls to females.

3. *Proximate mechanisms of aggressive calling*

Very little is known about proximate factors involved in aggressive signaling in frogs. Such studies are necessary in order to estimate the costs of engaging in aggressive disputes and the potential relationships between RHP and aggressive call characteristics. Other than a few studies that have examined the influence of hormones on aggressive behavior (Marler et al. 1995, Chu et al. 1998, Semsar et al. 1998, Trainor 2003), this topic has been almost completely ignored in frogs. An especially important piece of information is the energetic costs of aggressive calling and fighting. Advertisement calling in frogs is known to be highly energetically costly (Wells 2001). Aggressive calls are likely to be energetically costly as well, but until experiments measuring the energetic cost of aggressive calls are performed, it is unknown whether they are more or less costly on a call-per-call basis than advertisement calls. If aggressive calls are indeed more costly than advertisement calls, a male's engagement in aggressive interactions could limit its energy available to give advertisement calls and attract females. This knowledge would also be useful in evaluating links between aggressive call characteristics and RHP. Variations on the method I used to stage aggressive interactions between males could address the importance of energetic constraints on aggressive calling and success in aggressive interactions. For example, males could be divided into fed or unfed treatments and interactions staged between males of these two groups. If energy constraints are important, then the fed males would be predicted to win more of their interactions. Likewise, contests could be staged between males, who are afterwards assayed for lipid and glycogen levels in the muscle, levels of aerobic enzymes and the mass of the call producing muscles (similar studies on advertisement call energetics have been performed by Taigen et al. 1985, Wells and Taigen 1989, Bevier 1995, Schwartz et al. 1995, Wells et al. 1995, Bevier 1997). Winners and losers may differ in these physiological characteristics. In addition, comparisons of energetics could be made between individuals that either did or did not engage in an aggressive interaction. Individuals may call for less time after an escalated aggressive interaction than those that were not involved in an aggressive interaction. Finally, since many species do not have weaponry, the costs of fighting, if any, are likely to be related to energetic constraints. Fighting involves use of musculature that is probably under anaerobic control (Wells 1978b). Although fights in many anuran species do not appear particularly vigorous (personal observation), the real costs are unknown. Anaerobic metabolism is limited to short-burst activities and can require an extensive recovery period that limits performance of other activities (Bennett and Licht 1973, Gatten 1985). Might such costs be associated with anuran fighting behavior?

Another ripe area of research is the effects of hormones on aggressive behavior in anurans. Hormones are known to modulate aggression in other animals (e.g., Mougeot et al. 2005), including frogs (Marler et al. 1995, Chu et al. 1998, Semsar et al. 1998, Trainor 2003). Agonistic experience may alter levels of circulating hormones (Wingfield et al. 1990, Schuett and Grober 2000), thus comparisons could be made between individuals that have engaged in aggressive interactions and those that have not. Furthermore, the levels of hormones may be correlated with the intensity or duration of the aggressive interaction (Winberg and Lepage 1998, Elofsson et al. 2000, Sloman et al. 2001), thus comparisons could be made between males that were involved in aggressive interactions of either high or low intensity. Finally, hormone expression may differ between winners and losers of aggressive interactions (reviewed by Hsu et al. 2006). Such differential responses to winning and losing may be a proximate factor responsible for the so-called winner and loser effects (Oyegbile and Marler 2005, Hsu et al. 2006). To confirm the role of hormones in the outcome of interactions, the hormone levels of contestants in staged interactions can be manipulated by injecting them with different dosages. If hormone levels play a role in, for example, persistence due to aggressive motivation, this could easily be confirmed by noting the relationship between hormone treatment and likelihood of success in aggressive interactions. Manipulating hormone levels may also lead to changes in the likelihood of escalation and the duration of interactions.

Finally, relatively little is known about how aggressive calls are produced and processed by the anuran auditory system. Aggressive calls in many species differ substantially in temporal and spectral characteristics from advertisement calls, yet they are presumably produced by the same neuromuscular pathways. Characteristics of advertisement calls are likely to be constrained by strong selection due to female choice (Gerhardt 1991). Does this selection influence the structure of aggressive calls as well? Presumably, strong selection on advertisement call characteristics would carry over to aggressive calls as well because the two traits are linked by a common sound production mechanism. Is there evidence for such covariance (i.e., does variation between species in advertisement call characteristics map onto variation in aggressive call characteristics?)? Or, perhaps frogs are more versatile in their calling abilities and selection on one call type does not constrain severely the ability to maintain the characteristics of another call type (e.g., Castellano et al. 2002). Owen's (2003) study of aggressive call evolution in chorus frogs of the genus *Pseudacris* dealt with many of these questions. Further study of other groups is necessary for general conclusions to be reached. Similar questions can be asked regarding the perception of aggressive calls by males and females as both call types reach the same peripheral auditory system. How does the brain process aggressive calls and does it differentiate them from advertisement calls? The two call types could be categorized differently via processing in discrete neural channels (e.g., Rose and Brenowitz 2002) or could be processed along a continuum with certain call types providing more stimulation than others. Such processing may differ between the sexes. Aggressive calls in many species are unlikely to be salient to females, and females are generally unresponsive to aggressive calls (Oldham and Gerhardt 1975, Schwartz and Wells 1985, Wells and Bard 1987, Backwell 1988, Grafe 1995, Brenowitz and Rose 1999, Gerhardt et al. 2007). Males, however, are much more responsive to aggressive calls (e.g., Wells and Schwartz 1984b, Schwartz 1989) and are expected to assess aggressive call characteristics in order to determine their behavioral response to an opponent. On the one hand, females may have little need for complex processing of aggressive call characteristics, and such calls may simply be filtered out in the early stages of neural processing. On the other hand, it seems unlikely that such large differences between males' and females' auditory systems could evolve. Indeed, many studies have shown that male anurans have similar biases and preferences for advertisement call characteristics as females (Arak 1988, Ryan and Rand 1998, Humfeld 2008, Bernal et al. 2009).

4. Geographic variation in aggressive calls

Not only might there be differences between species in aggressive calling but also there may be differences within species. In particular, aggressive calls may vary across a species' range. For example, population density may be greater in some areas of the species' range than others. This could lead to a higher level of aggressive disputes over calling spaces in regions with high population densities. The aggressive calls from populations with high levels of aggressive disputes could vary in several ways from aggressive calls from less dense populations. For example, since assessment takes place more often in the more dense population, the relationship between RHP and aggressive call characteristics may be tighter in the denser population. Aggressive call characteristics from dense populations may be less variable since they are presumably under stronger selection. However, several studies have shown that, within a single population, the likelihood of responding to aggressive calls decreases with increased population density (Brenowitz 1989, Rose and Brenowitz 1991, Brenowitz and Rose 1994, Rose and Brenowitz 1997, Marshall et al. 2003). Perhaps this effect could be seen between populations as well. In this case, males from high density populations would have higher baseline aggressive thresholds (i.e., thresholds prior to habituation) than males from low density populations since the latter are less likely to be exposed to loudly calling males. Both populations may show habituation to aggressive calls, but since the calling spaces of the less dense populations tend to be larger, males from those populations would be expected to be more likely to initiate aggressive calling to lower amplitude intruders.

Geographic variation in aggressive call characteristics may also be shaped by the presence and absence of other species. Many anurans engage in interspecific aggressive calling (reviewed by Gerhardt and Schwartz 1995), and such interspecific aggression would be expected when multiple species compete for the same resources (e.g., Orians and Willson 1964). In anurans, the contested resource in interspecific interactions is acoustic space. If the characteristics of two species' advertisement calls overlap, and if these species call in the same place and time, then each may be a source of interference upon the other's calls. Thus, individuals may defend calling spaces from both conspecifics and heterospecifics with aggressive calls. If species communicate mutually with one another through aggressive signals, then there may be selection for convergence of aggressive call characteristics. Such convergence will make aggressive calls more easily perceived and assessed by heterospecific rivals, and thus improve individuals' abilities to repel competitors. Populations in allopatry, however, are free to diverge, and the most effective aggressive call characteristics in this situation may differ. Thus, comparisons of aggressive call characteristics in sympatry and allopatry between species with similar advertisement calls may reveal convergence, or greater similarity in aggressive call structure, in sympatry (Cody 1969, 1973). An alternative hypothesis is conservation of aggressive call structure. There is likely to be strong selection for advertisement calls to diverge in sympatry to avoid heterospecific matings (e.g., Moriarty Lemmon 2009). Selection pressures for aggressive call divergence are likely to be much weaker, as the consequences of heterospecific aggression are much less severe. Thus, two closely related species may have similar aggressive calls due to common ancestry, while advertisement calls may have diverged more due to reproductive character displacement (Gerhardt and Schwartz 1995).

5. Other determinants of contest success

Success in animal contests is often, but not always, determined by RHP (Parker 1974). Many other factors may be important including prior residency, previous experience, motivation and subjective resource value (e.g., Riechert 1984, Baugh and Forester 1994, Beaugrand et al. 1996, Hoefler 2002, Stuart-Fox and Johnston 2005, Brown et al. 2007, Arnott and Elwood 2008, 2009, Hoefler et al. 2009, Kasumovic et al. 2009, Elias et al. 2010, Kasumovic et al. 2010, Kasumovic et al. 2011). All of these variables could be addressed through a combination of field manipulations and staged aggressive contests. For example, effects of residency could be studied in territorial species by comparing aggressive responses to intruders by males that had been given different amounts of time to establish their territories. It may be possible to manipulate both males into claiming ownership of the same territory, thus removing resident/intruder asymmetries while at the same time testing the prediction that disputes will be more escalated in this more symmetric situation (e.g., Marden and Waage 1990). Previous experience effects on aggressive behavior are important in many taxa but have received little attention in anurans (but see, e.g., Burmeister et al. 1999). In particular, little is known about the effects of previous winning and losing experiences on the outcome of future aggressive interactions. Such winner and loser effects are common in other taxa (reviewed by Hsu et al. 2006, Rutte et al. 2006), and may be important in some anuran species as well. Staged aggressive interactions could be implemented to test whether previous winners and losers maintained their status in subsequent interactions. In addition, studies of territorial species capable of some learning and individual recognition (Bee and Gerhardt 2001a, e.g., Bee and Gerhardt 2001b, Bee et al. 2001, Bee and Gerhardt 2002, Bee 2004) may reveal an additional role of previous experience. Specifically, males that had previously won or lost an encounter may behave differently when approached by that individual again (e.g., Morris et al. 1995). Finally, motivation and resource value may play a role in determining the outcome of contests. Motivation could be manipulated by feeding experiments or hormonal treatments, as described above. Resource value will depend on the breeding system of the species under study. The territories of territorial species could be manipulated to be high or low quality. These manipulations may change the expression of aggressive behaviors by the territory holders, and may influence their likelihood of winning aggressive interactions. In lekking species, resources are less obvious. One possibility is to manipulate cues of the presence of females. A calling space should be more valuable to a male on nights when more females are present. Thus, for example, one could carry out staged interactions between a male removed from amplexus vs. a male that had not yet mated and predict that the male that had been exposed to a female would perceive his calling space as more valuable (or have a higher motivation), and thus be more likely to win.

6. The big question: aggressive calls and fitness

A major assumption of my study of aggressive calling in anurans remains untested. That is, are there links between variation in aggressive calls and success in aggressive interactions and fitness? Models that examine the costs and benefits of communication, aggressive or otherwise, usually assume that communication can only be maintained (i.e., is evolutionarily stable) if it is adaptive on average (e.g., Enquist 1985, Grafen 1990, Hurd 1995, Johnstone 1997). Alternatively, aggressive signaling in some species may be a non-adaptive remnant of an ancestral trait maintained by correlated selection on other traits (Arnold 1992). Given the elaborate behaviors involved, it is unlikely that such traits would be maintained unless they provided some benefit to individuals that use aggressive signals. Nonetheless, it is unclear if *variation* in aggressive call characteristics is adaptive. Indeed, it has been difficult to demonstrate clear fitness consequences for variation in advertisement call characteristics in the field (e.g., Sullivan and Hinshaw 1992, Schwartz et al. 2001). This information would provide much stronger support to hypotheses of the importance of aggressive calling in resolving aggressive disputes. If the outcome of aggressive disputes does not ultimately affect a male's fitness, then it is unclear why males should engage in such disputes in the first place. Do males that have more success in aggressive interactions have higher reproductive success? In addition, little is known about the heritability of anuran call traits, much less aggressive call characteristics (for similar studies in other taxa see, e.g., Hedrick 1988, David et al. 2000, Jia et al. 2000). Variation in aggressive calling can lead to variation in fitness only to the extent that aggressive call characteristics (or the underlying variables that influence aggressive call characteristics) are heritable (Boake 1989). If males that have more success in aggressive interactions have higher reproductive success, do they pass on these characteristics to their offspring?

These questions will be difficult to address, as they have been for advertisement calls. However, some indirect measures should be relatively straightforward. If success in aggressive interactions influences fitness, it is likely to do so through an increased ability to attract mates. Thus, observations of the mating success of individuals that won, lost or did not participate in aggressive interactions could give clues into the relative fitness of males in each of those groups. Such observations could be facilitated by releasing females into an artificial pond in which the behaviors of every male can be tracked and winners and losers of interactions noted. Measurements of the repeatability of aggressive call characteristics would allow for estimates of the heritability of those call characteristics to be made. Repeatability sets an upper limit on heritability, which in turn determines the amount of variance in a trait that can influence fitness (Boake 1989). If aggressive call characteristics are highly heritable, yet variable between males, then there is the potential for natural selection to act on aggressive calls. Finally, success in aggressive interactions may be an indicator of superior genetic quality. If females can identify these males (perhaps through correlations with advertisement call characteristics), then good genes selection would favor female choice of superior competitors. Breeding experiments along the lines of Welch et al. (1998) in which females were mated with males that either consistently won or lost aggressive interactions could indicate if offspring of superior competitors had superior growth or survival. This is a challenging, long term goal, but one that is essential to address the evolutionary significance of aggressive calling in frogs.

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VITA

Michael Stewart Reichert was born September 28, 1982, in Dayton, Ohio. His family moved to Louisville, Kentucky in his early life and he graduated from Saint Xavier High School in May, 2000. He then moved to Chapel Hill, North Carolina to attend the University of North Carolina, where he received his B.S. degree with honors in 2004 as a Biology major and Chemistry minor. While at the University of North Carolina, he was an undergraduate research assistant with Dr. Allison Welch. Dr. Welch introduced him to research on frog communication and helped him develop a project that was formative to his dissertation research. He also spent a semester studying natural resource management and human ecology in the Brazilian Amazon. After graduation, Michael moved back to Louisville where he spent a year working as a laboratory technician at the University of Louisville with Dr. Cynthia Corbitt. In 2005, he enrolled in the graduate program in Ecology, Evolution and Behavior at the University of Texas, where he spent two years working under Dr. Michael Singer. His decision to continue research on frog communication led him to leave the University of Texas. He enrolled in the Ph.D. program in Biological Sciences at the University of Missouri in 2007 and worked in the laboratory of Dr. Carl Gerhardt. He earned his Ph.D. in December of 2011. He will begin a postdoc at the University of Wisconsin-Milwaukee in the laboratory of Dr. Gerlinde Höbel in 2012.