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Predator-Specificity of Multimodal Alarm Signals in the Eastern Gray Squirrel (Sciurus carolinensis)

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UNIVERSITY OF MIAMI

PREDATOR-SPECIFICITY OF MULTIMODAL ALARM SIGNALS IN THE EASTERN GRAY SQUIRREL (SCIURUS CAROLINENSIS)

By

Thaddeus R. M$^{\underline{c}}$Rae

A DISSERTATION

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Predator-Specificity of Multimodal Alarm Signals
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Communication is a fundamental biological phenomenon, necessary in many species for successful reproduction, social interactions, and predator avoidance. Understanding animal communication systems is therefore vital to our understanding of biology. One of the core attributes of any signaling system is the information contained in each signal. In this dissertation I first review the literature on the information contained in sciurid alarm calls, and note the dearth of knowledge about the presence or absence of predator-specific alarm calls in arboreal squirrels. Arboreal squirrels are closely related to ground squirrels, which have a variety of alarm calling systems, but seek refuge in trees like many primates that have been shown to use predator-specific calls.

I then examine the alarm-signaling system of the eastern gray squirrel. Using field experiments I show that some, but not all, of their alarm signals contain information about predator type, specifically, whether a predator is approaching from the air or approaching on the ground. Unlike most studies of alarm signaling, which consider only alarm calls, I considered two signaling modalities, vocal signals and tail signals. When examining the entire alarm-signaling bout, the use of vocal and tail signals is associated with threat type. Of the three vocal alarms (kuks, quaas, and moans), only moans show predator specificity, being highly associated with aerial threats. Of the two tail signals

used as alarms, twitches and flicks, only flicks show predator specificity, being associated with terrestrial threats. This is a unique case of two modalities being used to specify two threat types.

When gray squirrels use alarm vocalizations, their alarm calling bouts can consist of kuks, quaas, and moans. Each calling bout could consist of one, two or all three vocal alarms. To examine the information contained in alarm calls about whether a threat is approaching aerially or terrestrially, I tested alarm calling bouts for an association of each signal type with threat type (aerial or terrestrial). If alarm calls function to communicate with conspecifics, the initial period of calling should be most relevant to squirrels seeking safety, so I focused on the initial 60s of calling. In this initial period the presence of kuks is associated with terrestrial threats, as is the presence of quaas. Moans are exclusively used in response to aerial threats. Initial rates of kuks, moans, and calls in general are also associated with threat type, but rate of quaas is not. Kuks and quaas are usually mixed within calling bouts and are both associated with terrestrial threats.

I then examined squirrels' responses to playbacks of modified calling bouts to test whether kuks and quaas elicit different degrees of antipredator behavior. Kuks and quaas appear to have a similar effect on conspecific behavior, although rate of calling may have a strong impact on response. Additionally, white noise bursts of equal duration to alarm vocalizations appear to be functionally equivalent to kuks or quaas; any sudden, broadband noise may be sufficient to increase alert behavior. This broad acoustic criterion for eliciting a response may facilitate eavesdropping on heterospecific alarm calls, which are often abrupt, broadband sounds.

In combination, this work demonstrates an alarm-signaling system in which signalers use varimodal (either unimodal or multimodal) signals, with the amount of information about predator type contained in their signal varying from general alarms to highly specific alarms. This study is the first to test for predator specificity in multiple signaling modalities; the differential use of tail signals as terrestrial threat alarms and vocal signals as aerial threat alarms highlights the importance of examining multiple signaling modalities in other species.

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CHAPTER 1

INTRODUCTION: DEFINITIONS AND THE ROLE OF INFORMATION IN SIGNALING

COMMUNICATION, SIGNALS, INFORMATION, AND COGNITION

Communication can be defined as the transfer of information by means of signals (Shannon 1948; Smith 1977; Green & Marler 1979; Bradbury & Vehrencamp 2011). When speaking of animal communication, a signal is a behavior or trait that has evolved to transfer information (Otte 1974). Signals in this sense include everything from simple threshold density signals that coordinate bioluminescence in bacteria (Nealson et al. 1970; Bassler 1999) to the complexities of human speech.

Although signals always transfer information, this definition does not require the information to be cognitively processed. For a signal to provide a selective advantage, evolution need not select for the understanding of the information transferred but only a response to the information that benefits the signaler. For example, in the case of quorum sensing in the bioluminescent bacteria *Vibrio harveyi*, the bacterium only bioluminesce when they are in high densities (Nealson et al. 1970). To coordinate their bioluminescence and only glow when they are in sufficient density, each bacterium secretes autoinducers into the environment. As density of bacteria increases, the concentration of these autoinducers in the immediate environment also increases. Levels of autoinducers in the environment act as a signal of conspecific density. When autoinducers reach a particular level in the environment, bacteria density is high enough that each bacterium glows. Although the benefit of coordinated bioluminescence is not yet known, it may be related to mutualisms, as many other *Vibrio* species colonize photophores of marine fish (Herring 1982).

An individual bacterium is surely not conscious of the fact that there are additional individuals surrounding it. In responding to the signal, the bacterium need not make a mental decision (which requires a nervous system) to produce more luciferase. Instead, the signal that carries the information of conspecific density—in this case autoinducers secreted by neighboring bacteria—acts directly on a molecular signaling pathway (Nealson et al. 1970; Bassler 1999). Above a threshold concentration, the autoinducers trigger a cascade that results in the production of luciferase, causing the bacterial assemblage to glow.

This consistent bioluminescence in response to high conspecific density is only possible because information is transferred. Specific environmental conditions (high conspecific density) are associated with the production of the signal (autoinducers) so the signal carries information about the environment. In this case, a simple threshold detector can successfully evaluate the signal. I consider quorum sensing to be a signaling system in the animal communication sense because information is transferred between individuals, using a system specific to that task.

On the opposite end of the signaling complexity spectrum, human language can be used to transfer almost any information the signaler desires. By arranging the smallest linguistic signaling units, words, from the language's vocabulary into combinations that follow the grammar of the language, a person can produce novel combinations that carry the intended information to receivers, as long as the receivers also know the grammar and vocabulary used. Additional information can be communicated, and the information content of particular word combinations is further modified, by intonation, speed, amplitude, and other forms of prosody that may not be strictly part of the language. In contrast to the quorum signaling of bacteria, human language does require cognition to use the information contained in the signals. In both the quorum sensing of bacteria and human language, information is transferred. When studying animal communication, identifying the information contained in particular signals is one of the foundational tasks.

TWO METHODS TO IDENTIFY INFORMATION IN A SIGNAL

In the study of animal communication the information transferred by a signal can be deduced by two methods, the first focusing on signalers, the second focusing on receivers of signals. In the first method, information in a signal is identified by looking for relationships between context and production of a signal, whereas in the second method, information in a signal is identified by looking for relationships between a signal and receivers' responses. Both methods together provide a complete picture of how the signaling system functions.

In the first method, the information contained in a signal can be revealed by looking for relationships between components of the signal and the context in which the signal is given. The signaling context of relevance could be external or internal, i.e. the relevant context could be traits of the signaler itself. For example, male cricket frogs (*Acris crepitans*) of large body size produce calls with a low dominant frequency (Smith & Burmeister 2002). Because dominant frequency and body size are inversely correlated, the dominant frequency of a call contains information about the caller's body size, at least relative to other male frogs calling in the area. Male cricket frogs also change temporal characteristics of their calls, thereby conveying information about likelihood of attacking or tolerating opponents. Both the caller's body size and likelihood of attack are features associated with components of a signal, therefore the signal contains information about those features. In these cricket frog examples, the information in the signal was discovered by looking for relationships between call parameters and context. In this case the relevant context is internal, i.e. features of the caller itself. The context of relevance could also be something external to the signaler. If a ground squirrel gives a particular vocalization whenever a predator comes near (Owings & Leger 1980; Davis 1984; Sloan et al. 2005), then that vocalization carries information about the presence of a threat. But identifying information in a signal based on association with a particular context does not demonstrate whether that information is used, or even perceived, by receivers, although it would be unlikely if such were not the case.

The second method to deduce the information transferred by a signal focuses on the response of receivers to the signal and also shows that the information is part of a functional signaling system (Marler 1961; Searcy & Nowicki 2005). If receivers respond differently to different signals, there are two possible explanations. It could be that the information content of those signals must differ, thus eliciting different responses. Alternatively, the information in the signals could be the same, but contextual differences could change which response is most beneficial for receivers of the same information. By controlling for signaler identity and other relevant aspects of context, playback experiments can be useful to test if differences in response can be ascribed to differences in information content of a signal. Returning to the cricket frog example (Smith & Burmeister 2002), if playbacks of calls with high dominant frequencies are equally as perceptible as calls with low dominant frequencies and females can only perceive the calls and not any other attribute of the males, then we can determine not only whether calls contain information but also whether that information is used by females.

For example, if females are more likely to approach the calls with low dominant frequencies, then we can again conclude that dominant frequency carries information about the caller. If it did not carry information, then there would be no basis for females to make a consistent choice. Again, this use of information does not require any presumption of conscious processing. Whether or not the female is conscious of the difference or what it means, we can conclude that the signal serves to carry information about the caller, and that the female does or does not approach the caller based on that information. If the female showed no preference to approach based on dominant frequency, the interpretation is not as clear. It could be that the signal contains information, but it is not perceived by the female, or is perceived but ignored.

As researchers study animal communication through observing the context of signals and observing receivers' responses to signals, we essentially seek to determine the function of a signal, not what a receiver thinks it means. I define the function of a signal as the information communicated to receivers and how that information changes their behavior.

FUNCTION OF SIGNALS

Animals use signals in many different contexts, and their functions differ accordingly, but the signal usually has some effect on a receiver's behavior unless the signal is ignored. If a putative signal does not influence the behavior of any receivers, then it is not functioning as a signal, even if it is consistently given in a particular context. In Seyfarth and Cheney's experimental study of vervet monkey alarm calls (Seyfarth et al. 1980a), first they demonstrated that vervets produced specific calls in response to specific threats; the monkeys produce three different alarm calls in response to three different predators (leopard, eagle, and snake). By demonstrating this production specificity, Seyfarth and Cheney (Seyfarth et al. 1980a) showed that vervet alarm calls contain information about predator type because they are context-specific. They then used audio playback of alarm calls to see whether vervets responded differently to the three alarm calls in the absence of any information about the type of threat. The monkeys did respond differently to each call type, showing that the alarm calls are functioning as a predator-specific signaling system (Seyfarth et al. 1980b).

In testing for the presence of context-specific and functionally referential signals, alarm calls provide a unique opportunity to focus on signals that have a clear external stimulus that is not a conspecific but that is clearly relevant to the signaler. In order to function, alarm signals must contain information about the presence of a threat. It is, however, possible that many signals contain additional information that is not the primary message, i.e. not relevant to the function for which the signal evolved, and that some of the information in a given signal is not used by receivers. For example, people's shirt color influences call characteristics of black-tailed prairie dogs (*Cynomys ludovicianus*) (Slobodchikoff et al. 2009), but it is not evident that other prairie dogs in the colony respond differently based on these call differences or even based on direct observation of different shirt colors. If receivers' responses do not differ based on call characteristics that contain shirt-color information, we cannot determine whether those signal characteristics have any signaling function, and thus we cannot determine whether prairie dogs perceive the information about shirt color contained in the alarm calls.

So, to understand an animal's signaling system, we must first determine what information is in various signals, and then determine what information is used by receivers. Although signals may contain some information that is not used, in order to have a communication system at least some of the information must be used.

FUNCTIONALLY REFERENTIAL SIGNALS

Most studies of animal communication focus on communication within a species. Specialized signals are often used in courtship, pair bonding, mate guarding, territorial defense, group coordination, maintenance or change of social-hierarchies and many other facets of an organism's social life. In many cases, signals communicate information primarily about the signaler, with little or no information about the external context. Birdsong, for example, may signal a singing male's readiness to attack intruders (Searcy & Beecher 2009). In contrast, people regularly use vocal signals to refer to our environment. These referential signals involve a human signaler perceiving an environmental attribute, encoding that mental perception into a signal, and broadcasting that signal with the intent to communicate information about that attribute. The signal is then perceived by another person and decoded to extract the intended information about the environmental attribute thereby permitting the receiver to respond accordingly (Shannon 1948).

In studies of nonhuman communication, certain signals are consistently associated with some attribute of the signalers' external environment (Manser 2001; Scarantino 2010). This close association of a signal with an environmental attribute looks much like the human process of referential signaling described above; when investigating animal communication, however, we cannot know the intent of the signaler nor the level of conscious perception of meaning (if any) by either signaler or receiver. This black box of the nonhuman mind has led some to contend that animal signaling was being discussed in unnecessarily linguistic terms that encouraged anthropomorphic interpretation of behavior, and thus researchers should abandon the idea of meaning or information in animal signals (Rendall et al. 2009). In my own work on animal communication, I continue to talk about information transfer with no presupposition of human-like linguistic processes (remember the quorum-sensing bacteria). I describe any signal that is consistently associated with some attribute of a signaler's external environment as being *context-specific*, i.e. carrying information about the context. When alarm signals differ by predator type, I use the term *predator-specific*.

When receivers respond in different ways to different context-specific signals, I adopt the term *functionally referential signal* (Seyfarth et al. 1980a; Marler et al. 1992), to describe any signal that is both consistently associated with some attribute of a signaler's environment and that consistently elicits a different response than signals that are not associated with that environmental attribute.

FUNCTIONALLY REFERENTIAL ALARM CALLS

Following Struhsaker's (1967) discovery and Seyfarth et al.'s (1980b) experimental investigation of functionally referential signals in vervet monkey alarm calls, functionally referential signaling has been studied primarily in alarm calls. Struhsaker (1967) and Seyfarth et al. (1980b) showed that vervets in Kenya use three distinct alarm calls in response to leopards, eagles, and snakes, and Struhsaker (1967) observed additional calls that might refer to other predator types. Experiments in which each type of alarm call was played back to groups of vervets in the absence of a threat showed that not only did they respond in different ways to each call type, but their responses were also particularly suited for the predator type that usually elicited the call. Vervets scanned the sky and sought shelter in response to the alarm for eagles, ran up trees and scanned the surrounding area in response to the alarm for leopards, and approached the speaker in response to the alarm for snakes, a response consistent with vervets' usual snake-mobbing behavior.

Other researchers began testing for functionally referential alarm calls in other primates and found various forms of predator-specific alarm calls in Diana monkeys (Zuberbühler 2000), blue monkeys (Papworth et al. 2008), black-and-white colobus (Schel et al. 2009), Campbell's monkeys (Zuberbühler 2001), as well as ring-tailed lemurs (Macedonia 1990), ruffed lemurs (Macedonia 1990), Verreaux's sifaka (Fichtel & Kappeler 2002), and several other arboreal and semi-arboreal primates. Some of these species have also been shown to respond differently to different alarm calls, often in ways clearly appropriate to the predator type associated with the call (see Table 2.1).

Testing for functionally referential alarm calls continues in primates, but some researchers have turned to other taxa. Manser (2001) demonstrated a unique system in meerkats (*Suricata suricatta*), in which information on both predator type (terrestrial, aerial, or snake) and urgency is contained in their alarm calls. Although not in an alarm context, Bugnyar et al. (2001) suggested that ravens may use referential calls in the context of food (but see Heinrich 1988; Clay et al. 2012). Aside from these and a few other examples, most research on referential signaling outside primates has focused on ground squirrel alarm calling systems, which are further reviewed in Chapter 2. Many ground squirrels use different calls for terrestrial and aerial predators, although some seem to encode information about urgency rather than predator type, and some use a single generic alarm call. Regardless of taxa, almost all studies of functionally referential signals focus on vocal signals given in response to predators.

FUNCTIONALLY REFERENTIAL AND MOTIVATIONAL SIGNALS

Marler et al. (1992) proposed a continuum from motivational signals, which express only a signaler's internal motivational state, to signals that functionally refer to external stimuli. They suggest that most, perhaps all, animal signals contain both motivational and functionally referential attributes. While I agree that motivational and referential signals do not necessarily comprise two exclusive categories, I do not agree that a continuum is the right conception. As Scarantino (2010) points out, Marler et al.'s (1992) motivational-referential continuum implies a tradeoff between whether a signal's structure is dependent on motivational state or dependent on the characteristics of an external stimulus. The trouble with this conception is that no such tradeoff is necessary. Rather, the functionally referential and the motivational natures of a signal can vary independently. Hypothetically, a signal's structure could be completely predicted by characteristics of an external stimulus and also be completely predicted by the caller's motivational state. So, when testing for functionally referential or even simply context-specific signals, the potential for motivational differences to exist between signal types does not limit the potential for differences in signal structure to be associated with different external stimuli.

THE SELECTIVE ADVANTAGE OF USING PREDATOR-SPECIFIC ALARMS

SOCIALITY AND THE UTILITY OF PREDATOR-SPECIFIC ALARMS

The utility of predator-specific alarms that correspond to different escape strategies may be affected by the sociality of a prey species. There are several reasons why an individual might benefit from using predator-specific alarm calls, which vary in likelihood depending on the caller's level of sociality. In all but the most solitary species, it may be that calling increases the caller's risk of being attacked but increases the likelihood that the caller's offspring or other kin will survive the encounter with a predator. In this case the call is altruistic (defining altruism based on the immediate risk to the caller, and not potential benefits to its offspring or other kin) and is probably either a form of costly parental care or kin selection (Hamilton 1964; Sherman 1977; Shields 1980; Blumstein et al. 1997). Increasing fitness of related receivers, whether direct selection or kin selection, could explain the use of predator-specific alarm calls when multiple escape strategies are available. Using a general alarm may be ineffective in securing the survival of receivers when multiple escape strategies are available because receivers may need to take time to identify the threat themselves before choosing an appropriate response, thus increasing the time they are vulnerable. By enabling receivers to take the most effective escape strategy for the type of threat present, the fitness benefit to the caller is maximized by using predator-specific alarms. Note also that the same explanation holds if calling is not costly (and therefore not altruistic), as long as calling does not directly increase the caller's chances of survival. Increasing fitness of conspecific receivers may explain predator-specific alarms in any species with predator-specific escape strategies where related conspecifics are likely to be in hearing distance of a caller, which includes most sciurids, even nonsocial species and those with individual territories (but see Digweed & Rendall 2009a). In colonial or highly social ground squirrel species, squirrels more frequently alarm call when kin are present (Dunford 1977; Sherman 1977) and in at least some species direct fitness explains more of the variation in calling than inclusive fitness (Sherman 1977; Blumstein et al. 1997). These and other studies suggest that in many ground squirrel species alarm calling does not increase the caller's survival, because in that case individuals should call regardless of the relatedness of nearby conspecifics. It is unknown if similar patterns hold in tree squirrels or other less social species.

Alternatively, rather than increasing their fitness through receiver survival, alarm callers may increase their fitness by increasing their own chances of survival. This fitness benefit could occur through two types of mechanisms. First, in highly social species, calls may be directed at conspecifics and manipulate conspecifics into behavior that increases the caller's chances of survival, without necessarily benefitting the recipients as much as the caller (Charnov & Krebs 1975). Second, and unrelated to the caller's level of sociality, the calls may be directed at the predator itself and decrease the probability that the predator will harm the caller (Caro 1986b; Zuberbühler et al. 1999; Digweed & Rendall 2009a; Schel et al. 2010). The first possibility, manipulation of conspecific receivers, seems unlikely to favor predator-specific alarm calls. Such manipulative alarm calls that primarily increase caller fitness have been proposed in highly social animals such as flocking birds where such a call could create pandemonium that allows a caller to escape by confounding predators' attempts to single out any single individual in the chaos (Hamilton 1971; Charnov & Krebs 1975). In order for such a mechanism to explain predator-specific alarms, there would need to be different types of pandemonium that could each be elicited by different types of alarm calls and that each best suited a caller's ability to avoid a particular type of predator. It is not clear that such a system exists in any species. Additionally, this pandemonium explanation for manipulative alarm calling requires a significant concentration of individuals in one area to be effective, because if receivers are not in the predator's immediate detection range the effect is lost. This requirement limits the utility of such a system to highly social species with large groups, which may include some, but not most, sciurids.

Manipulation of receivers using predator-specific alarm calls could also be deceptive. If there are multiple incompatible escape strategies, a caller could give a call associated with a predator type other than the actual predator present. This deception could manipulate others into positions of danger, thus decreasing the caller's own likelihood of being attacked. Such deception could exist only at a low level in a stable alarm calling system and presupposes the existence of a predator-specific alarm calling system that usually elicits adaptive responses in receivers, thus deceptive manipulation cannot explain the evolution of predator-specific alarm calls or their general utility.

PREDATOR-SPECIFIC CALLS AS PREDATOR-DIRECTED CALLS

Aside from manipulation of conspecifics, the other mechanisms for callers to increase their own chances of survival via predator-specific alarm calls involve communication with the predator. These mechanisms could evolve regardless of the prey species' sociality. Both predators and other prey species may also eavesdrop on alarm signals that primarily evolved to communicate with conspecifics, modifying their own behavior based on the information in the signal.

The idea of alarm signals directed at predators was first proposed in the form of pursuit-invitation signals in ungulates by Smythe (1970). The notion was criticized, but a later study by Woodland and Jaafar (1980) proposed predator-directed signals with a different function, pursuit deterrence, which is more clearly an adaptive function. The pursuit deterrence hypothesis has been tested in several species with mixed results (Woodland & Jaafar 1980; Tilson & Norton 1981; Caro 1986b). Predator-directed signals may also evolve as a form of harassment, a special case of pursuit deterrence. Clark (2005) found that timber rattlesnakes (*Crotalus horridus*) responded to visual alarms and close approaches by three prey species: eastern chipmunks (*Tamias striatus*), eastern gray squirrels (*Sciurus carolinensis*), and a wood thrush (*Hylocichla mustelina*). Snakes responded by leaving the area and traveling long distances before settling into a new ambush site (Clark 2005).

In addition to pursuit invitation, pursuit deterrence, and harassment, several other predator-directed functions have been proposed for signals given in the presence of predators (Caro 1986a), but hypotheses considering predators as receivers have been tested far less frequently than hypotheses that focus on prey species as receivers. The audience of alarm signals may include conspecifics and other prey species, but by definition it also includes predators, which are the eliciting stimulus for alarm calls and may be near enough to perceive alarm signals. When attempting to understand an animal communication system, it is important to consider all potential receivers, including predators, and any responses to the information contained in signals. Regardless of which species perceive alarm signals or which audience drives the evolution of the signal, the same process is used to detect information contained in the signal, namely testing for associations between context and production of an alarm signal.

There are thus several ways that predator-directed calls could enhance a caller's chances of survival, including pursuit-deterrence signals and harassment (Struhsaker 1967; Woodland & Jaafar 1980; Loughry 1987; Caro 1995; Clark 2005). Some ground squirrels are known to harass rattlesnakes with kicked sand while vocalizing (Clark 2005), vervet monkeys escort snakes while calling (Struhsaker 1967), and some *Callosciurus* species bit snakes while calling (Tamura 1989). In all these cases, the harassed predators are snakes, which are probably ignorant of the vocalizations used by the harassing species. In arboreal squirrels, however, many alarm signals are good candidates for harassment signals, with sharp, loud, repetitive calls used in conjunction with tail signals that make the calling squirrels' locations evident. They can certainly irritate domestic dogs, as many pet owners can attest, although the predator-deterrent function of such signals is unclear and has not been tested.

If predator-specific alarm calls are predator-directed signals, then it may be that different alarm calls evolve to best communicate with different predators. In this case, sociality of the calling species should have little effect in the evolution of the calls. Rather, the acoustic structure of such antipredator calls should be shaped by their effectiveness at removing a predator from the area in the case of harassment calls, or in discouraging pursuit in the case of pursuit-deterrence calls. In both cases, the physiology of the predator's hearing might affect the frequencies used in alarm calls, and if advertising the caller's safe location is part of the effectiveness of the call, then the calls should be selected for their localizability by the predator. If different predators respond more strongly or consistently to different types of calls, this difference in effectiveness could cause the evolution of predator-specific calls even in the absence of other predator-specific escape responses. Predator-directed signaling has been understudied, so it is not clear how common an occurrence it is, or to what degree known alarm calling systems also affect predator behavior.

So, predator-specific calls could evolve: (1) in all but the least social species as mechanisms to increase receivers' fitness when different predators are best avoided by different strategies; (2) regardless of sociality, as mechanisms to increase a caller's chances of survival if calls are predator-directed and different predators require different calls for most effective communication. In systems where callers' survival is increased by manipulation of conspecifics, predator-specific calls are unlikely to evolve without additional selective forces, regardless of whether predator-specific escape strategies are used.

It may be, however, that predator-specific calls could evolve due to a combination of alarm calling strategies that differ with predator type, which offers a third way for predator-specific alarms to evolve. For example, a prey species may respond to one type of predator with an alarm call that manipulates conspecifics, and to another type of predator with harassment calls. Similarly, in response to one predator type a caller may maximize its fitness by informing conspecifics with an alarm, while in response to another predator type a caller may maximize its fitness by giving a predator-directed call. If calls have been selected to communicate with different audiences depending on predator type, this could result in predator-specific calls, which would carry information about predator type. Having different selected audiences, and the associated different signals, in response to different predators is essentially a variation on having different escape strategies. In this case, the best escape strategy is defined by the call type given rather than the location of refuge sought or vigilance behavior used.

There are thus three potential evolutionary pathways for predator-specific calls to arise: (1) conspecific receivers benefitting from predator-specific information; (2) callers increasing their own survival through predator-directed signals that vary with predator type; and (3) a mixed pathway with differences in the audience to which each signal is directed (conspecific or predator) based on predator type. Because transmitting information about the appropriate escape strategy is only useful in social species (either by increasing offspring and kin survival or through reciprocity among callers), social species may be more likely to use predator-specific signals if the signals are related to multiple escape strategies, whereas less social or solitary species may be more likely to use predator-specific signals to increase the efficacy of predator-directed calls regardless of whether predators are best evaded with different escape strategies.

MULTIMODAL SIGNALING

Studies of alarm signals have focused on vocalizations, perhaps because other modes of communication often co-opt anatomy that evolved for other purposes. Although feather color and wing waving in songbirds are clearly signals that function in communication, no one has yet argued that birds' feathers or wings originally evolved primarily for signaling functions. Still, these and other anatomical structures have often been modified to improve their ability as signals, as a brief perusal through any bird guide will show.

While vocalizations, closely followed by color patterns, are perhaps the most studied vertebrate signals to date, communication can happen in any sensory modality in which an organism is capable of both producing and perceiving a signal. Auditory, visual, chemical, tactile, and even electrical signals (Hopkins 1988) have all been identified in animal communication systems.

Recently, researchers in animal communication have escalated their attention to signals in multiple sensory modalities that work together, functioning as multimodal signals (Otovic & Partan 2009). When signals are given simultaneously in multiple sensory modalities, like leg waving (visual) and seismic signaling (tactile) in jumping spider displays (Uhl & Elias 2011), there are several ways the signals can interact. Partan and Marler (2005) have offered a framework for classifying multimodal signals. Their classification system is based on the response of receivers and primarily considers whether the signals are redundant across modalities. If signals in different modalities elicit the same response, they are considered redundant; if signals in different modalities elicit a unique response, they are considered nonredundant. They further classify multimodal signals within each redundancy category based on the combined effect of the

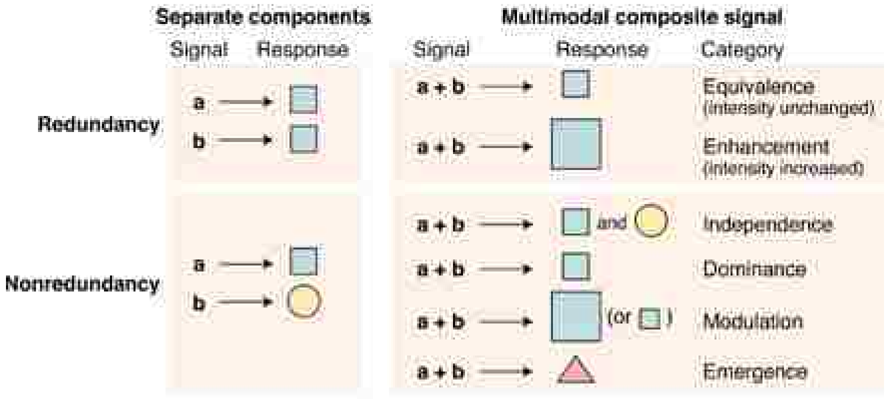

Figure 1.1. Classification of multimodal signals. From Partan and Marler (2005), their classification system for multimodal signals, based on receiver response to the multimodal signal and each of its unimodal components.

signals in different modalities on receivers (Figure 1.1). There are a few ways in which the classification system could be made more comprehensive.

First, the proposed system considers multimodal signals only in the context of a single audience, whereas another possibility is that signals in different modalities may be directed to different receivers. Therefore signals that are apparently redundant when considering a single audience may in fact not be redundant when considering multiple audiences. That complication may be avoided if the classification system is applied explicitly to signal function in single audiences, but Partan and Marler (2005) still omit several possibilities from their classification system.

In Partan and Marler's (2005) system, based on the response of receivers, there are two types of redundant multimodal signals: (1) equivalence, where responses to a multimodal signal are the same as responses to each of the unimodal signals composing the multimodal signal; and (2) enhancement, where responses are qualitatively the same but greater in magnitude when compared with the unimodal signals (Partan & Marler 2005). They classify nonredundant multimodal signals into four categories, again based on receiver response as a measure of meaning: (1) independence, where the multimodal signal elicits both of the responses elicited by the unimodal signals; (2) dominance, where the multimodal signal elicits only one of the two responses elicited by the unimodal signals separately; (3) modulation, similar to dominance, except that the magnitude of the response is changed; and (4) emergence, where a qualitatively different response is elicited by the multimodal signal than that elicited by the unimodal signals. Six classes of multimodal signals are therefore identified in the context of a single audience.

I propose two modifications to make their classification system more complete while maintaining its focus on single-audience signaling systems. First, there should be a third category of redundant signal, *reduction*, because it is entirely possible, even if less likely, that multimodal signals elicit weaker responses than their unimodal component signals. Alternatively, reduction and enhancement could be combined into modulation, which they currently list only under nonredundant signals. Second, it seems quite possible that emergence, which they list only under nonredundant signals, could also occur with redundant signals. For example, perhaps there are two unimodal signals, each in a different sensory modality, that each cause increased vigilance when received separately, but when received together as a multimodal signal, they cause the receiver to flee. Depending on the behavioral categories used to classify response behaviors, this phenomenon could be considered emergence, despite resulting from redundant signals. This example points out another difficulty, namely that depending on the behaviors involved, the line between emergence and enhancement may become blurry. Is a fleeing squirrel exhibiting a qualitatively different response than a vigilant squirrel or simply increasing its level of predator avoidance? Clear definitions of behavioral responses that are based on biological function rather than people's perceptions should improve any classification system's usefulness when applying it to particular signaling systems. While Partan and Marler's (2005) list of nonredundant signal types seems complete, they could strengthen the system for redundant signals by replacing enhancement with modulation and adding a category for emergence.

Another, more extensive classification of the function of multimodal signals comes from Hebets and Papaj (2005). They set out not to classify signals but to propose a theoretical framework for the study of complex signals, in which they include multimodal signals. They list three types of hypotheses, not all mutually exclusive, to explain the function of multimodal signals: (1) content-based hypotheses, where the function depends on the information in a multimodal signal, which can either carry multiple messages or be redundant; (2) efficacy-based hypotheses, where components of a multimodal signal function to increase efficacy either in multiple sensory environments or to overcome inherent sensory constraints of receivers; (3) intersignal interaction hypotheses, where one component of a multimodal signal modifies either the production of, detection of, or response to a second signal component.

To date, most studies of multimodal communication have examined courtship signals, particularly bird song and plumage (Bro-Jørgensen 2010). Multimodal alarm signals are known to exist in a number of species, but there are few studies examining alarms in multiple modalities simultaneously. Partan et al. (2010) are among the few, testing the function of tail signals and vocal signals as multimodal alarms in the eastern gray squirrel. (Their work is discussed further in Chapter 2.) When considering multimodal alarms, any of the above hypotheses could explain their use. Because signals in different modalities could contain different information, studying a single modality will probably not give an accurate picture of the information in multimodal alarm signals.

In order to understand unimodal signals in one sensory modality that are used in conjunction with signals in a different sensory modality, we must look at the information contained in both unimodal components as well as the information contained in the multimodal signal they compose. Most studies of alarm signals have focused on vocalizations, but in this study I investigate both the vocal and tail signals used as alarms by eastern gray squirrels. I test the hypothesis that vocal and tail signals as a multimodal system contain information about predator type and also evaluate the information content of vocal and tail signals as separate unimodal signals.

CHAPTER 2

ALARM CALLING SYSTEMS IN SCIURIDAE AND OTHER MAMMALS

SUMMARY

Many arboreal primates have been shown to use predator-specific calls, and some of these calls have also been shown to be functionally referential, eliciting unique predator-avoidance responses in the absence of a predator. Aside from arboreal primates, most studies of alarm signaling have focused on the alarm calls of ground squirrels. I review sciurid alarm calling systems, including a list of all species studied to date and the type of information contained in their alarm calls, and compare sciurid alarm calls to several nonsciurid mammals. Ground squirrels tend to use urgency-based alarm systems, although even their urgency-based alarms may also contain information on terrestrial versus aerial predator-classes. Arboreal squirrels are understudied, and the few studies of the information in their alarm calls are mostly limited to observations rather than experiments. Some arboreal squirrels appear to use predator-specific alarms, most notably the beautiful squirrels (*Callosciurus*) of Asia and perhaps some North American red squirrels (*Tamiasciurus hudsonicus*). There are no experimental studies of predator-specificity in *Callosciurus*, however, and the experimental evidence in North American red squirrels is mixed. One population in Montana clearly uses different alarm calls in different contexts, although whether it is based on predator type, distance, or another contextual cue is unclear. In contrast, a Canadian population uses alarm calls similarly in multiple contexts, including encounters with nonpredatory species. African forest squirrels of several genera (*Epixerus*, *Funisciurus*, *Heliosciurus*, *Myosciurus*, *Paraxerus*, and *Protoxerus*) have been shown to use multiple alarm-call types, but the function of the

various calls is not clear. Tail signals may also differ between species in African forest squirrels. Arboreal squirrels occupy habitat that is likely to provide multiple escape strategies, a hypothetical prerequisite for functionally referential alarms. Additional studies of the information contained in arboreal squirrel alarm calls are needed. Comparisons of arboreal squirrel alarm-signaling systems to alarm systems of the better-known arboreal primates and ground squirrels will provide key information to clarify the forces that drive the evolution of predator-specific alarms and other alarm systems. I also highlight the need to examine the information contained in alarm signals of multiple modalities, particularly in tree squirrels, which often use tail signals in conjunction with, or in place of, vocal alarms.

BACKGROUND

Most tests for functionally referential alarm systems have looked at mammals, though there have been several studies of birds as well. Although alarm-signaling systems of many animals may include multimodal signals, most studies of alarms consider only vocal alarms. In this chapter I review the current understanding of alarm calling systems in mammals, particularly sciurids. While I focus on the alarm calls of sciurids, I have included several arboreal and semi-arboreal primates for comparison.

The initial discovery of a functionally referential alarm system was in a terrestrial primate, the vervet monkey (Struhsaker 1967), and subsequent studies of alarm systems testing for functionally referential alarms, or simply context-specific alarms, have focused on primates and ground squirrels, with the occasional study of suricates (Manser et al. 2001), birds (Templeton et al. 2005; Soard & Ritchison 2009; Bartmess-LeVasseur et al. 2010), or other taxa.

It has been hypothesized that functionally referential alarm systems should only arise in species that have different escape strategies for different predators, particularly if those strategies are mutually exclusive (Cheney & Seyfarth 1992; Macedonia & Evans 1993). Vervets clearly meet these criteria, as they respond in different ways to leopards, eagles, and snakes, and their three responses are mutually exclusive (Seyfarth et al. 1980b). Habitat may play a role in the diversity of escape responses. Vervets live in a savannah habitat, moving regularly between the ground and trees, which means they can respond to a threat by leaving or entering a tree, switching locations within a tree, etc. Ground squirrels, in contrast, do not typically retreat to trees but to burrows. Some ground squirrel species have a universal response regardless of predator type—run to a burrow (Blumstein & Armitage 1997a). Having a single predator-escape strategy may explain the simpler alarm calling systems in some ground squirrel species. If you have only one possible response, who cares what the threat is? Below I review the calling systems used by several species of sciurid and compare them to other mammals.

SCIURID ALARM CALLS – GROUND SQUIRRELS

California ground squirrels (*Spermophilus beecheyi*), Belding's ground squirrels (*Spermophilus beldingi*), eastern chipmunks (*Tamias striatus*), and some other burrowing sciurids have been shown to use different alarm calls for terrestrial versus aerial predators (Table 2.1). In at least some of these species, however, the difference in use is interpreted as due to differences in urgency, usually defined as distance of threat from caller or suddenness of its approach, rather than categorical classification of threats as terrestrial or aerial. In Table 2.1, I have listed the specificity of alarm calls in each species, based solely on whether a relationship exists between call type or some acoustic parameter and predator type or urgency. In several cases, alarm calls differ in a single parameter based on both predator type and urgency. Although alarm calls in these species may function to signal only one of these factors, that function has not been directly tested in most species, so I list both possibilities because specific information about both factors exists in the calls. Experimental presentations of aerial and terrestrial stimuli that also differ in approach distance or angle could tease apart the roles of predator type and predator distance, speed, or other indicator of urgency.

YELLOW-BELLIED MARMOTS (*MARMOTA FLAVIVENTRIS*)

Yellow-bellied marmots are described as using three call types: whistles, trills, and chucks; 96% of calling bouts, however, contained only whistles (Blumstein & Armitage 1997a). In a study testing for context-specific alarm calls, Blumstein and Armitage (1997a) observed chucks in only five of 537 calling bouts, and they were always followed by whistles. Trills were observed only 14 times and were preceded by whistles. Blumstein and Armitage (1997a) focus their analysis on whistles in response to five stimuli: people, dogs, RoboBadger, eagles, and a model eagle. The acoustic structure of whistles differed based on predator type; specifically, several acoustic variables of whistles in response to the dog differed from whistles in response to the other four stimuli. Blumstein and Armitage (1997a) conclude that the variation is primarily a function of risk, with dogs representing a higher risk than any other stimulus used in the study.

Although clear analysis is not possible from the summary data in the report of Blumstein and Armitage (1997a), it appears that dogs elicited trills 19% of the time, compared with about 3% of the time in response to RoboBadger, 3% of the time to people, and never to the model eagle. It is clear that of the 13 calling bouts with trills that also had a known stimulus, all were in response to terrestrial threats, but those threats included real and model predators as well as aggressive conspecifics. Trills were never used in response to an actual eagle or model eagle or other aerial stimuli (Blumstein & Armitage 1997a). Trills may be a call expressing extreme urgency or risk, but the possible specificity of trills to terrestrial threats deserves further study.

Although the function of trills and chucks remains unclear, playback of recorded yellow-bellied marmot whistles elicited antipredator behavior in conspecifics, and response differed based on the number of calls (single versus four), but not rate (number of calls/total bout length). Whistles also elicited more intense responses than chucks. It may be that yellow-bellied marmots do not have multiple predator escape strategies, and so they signal only degree of risk, not predator type. Based on observed behaviors in response to alarm calls, potential differences in predator-avoidance strategies seem limited to the posture while scanning (all fours or upright on hind legs only) and whether they descend into a burrow or stay at the surface. Additional studies manipulating risk (distance, speed of approach, etc.) within stimulus type could verify whether whistles are functioning as signals of risk.

Although yellow-bellied marmots seem to have a risk-based alarm calling system with one main call type, a later study of Olympic (*M. olympus*), hoary (*M. caligata*), and Vancouver Island marmots (*M. vancouverensis*) found that they all used at least four acoustically distinct vocalizations during alarm calling bouts (Blumstein 1999a). Again, they appear to primarily communicate risk, mainly reflecting distance, but hoary and Vancouver Island marmot alarms also contained information about terrestrial versus aerial threat type. Playback experiments found no difference in response when testing call type, suggesting that they do not make use of any information about predator type. Interestingly, risk is coded using different acoustic features in each species.

CALIFORNIA GROUND SQUIRRELS (*OTOSPERMOPHILUS BEECHEYI*)

California ground squirrels use three acoustically distinct alarm calls in response to predators. Whistles are commonly used in response to aerial predators, whereas chatters and chats are used in response to large mammalian predators (Owings & Virginia 1978). Comparing responses to whistles versus chatters and chats, Owings and Leger (1980) found that whistles were less likely to elicit upright scanning, and squirrels more quickly resumed normal feeding behavior, whereas chatters and chats elicited upright scanning with antipredator behaviors continuing for a longer period. These different responses could be adaptations to predator type. For example, low-flying raptors quickly pass through an area, and keeping a low profile may help ground squirrels avoid predation (Leger & Owings 1978). So the delayed upright scanning and the short duration of response to whistles may be the most adaptive responses to aerial predators. Similarly, immediate upright scanning that persists for a longer time may be well suited to avoid terrestrial predators, which are more likely than aerial predators to be hidden by objects on the ground, to move more slowly, and to linger in the area (Leger & Owings 1978).

Although there is a pattern of production and response based on predator type, the use of whistles versus chatters and chats can also be interpreted as a risk- or urgency-based alarm system. In this view, whistles are less intense alarms that elicit low-intensity antipredator behavior that quickly fades away, whereas chatters and chats are high-intensity alarms that elicit stronger antipredator behavior that persists.

A later study examining chatter vocalizations in more detail showed that acoustic parameters of chatters differed among aerial predators, terrestrial predators, and aggressive conspecifics (Owings & Leger 1980). Within terrestrial predators, chatters by California ground squirrels also differed in response to snakes versus mammalian predators (dog, badger, and coyote). The alarm calls of California ground squirrels contain some information on predator type, with some overlap of chatter types between stimulus categories, but it is not clear whether the primary function of the alarm calls is to communicate urgency or type of threat. It is also likely that urgency and type of threat covary, and California ground squirrels may categorize threat types based on urgency rather than a predator's physical appearance or terrestrial versus aerial approach.

RICHARDSON'S GROUND SQUIRRELS (*UROCITELLUS RICHARDSONII*)

Richardson's ground squirrels use two primary vocalizations, whistles and chirps. Initially, their alarms were interpreted as predator-specific, with whistles used in response to terrestrial predators and chirps to aerial predators (Davis 1984). The level of production specificity was extreme, with no apparent crossing of alarm calls between predator types. Receivers also appeared to respond in unique ways to each type of alarm call, suggesting a functionally referential system that allowed receivers to use the appropriate strategy for terrestrial or aerial predators simply by hearing the alarm call. Ninety percent of the time, Richardson's ground squirrels ran to their burrow in response to chirps, the putative aerial predator alarm. In response to whistles, the putative terrestrial predator alarm, squirrels stood erect in place and fled only if the threat approached them closely (Davis 1984). Despite the simplicity of their habitat, this result suggested that the ground squirrels used two incompatible strategies in response to terrestrial versus aerial predators.

Davis' (1984) study seemed to provide clear evidence for predator-specific alarms; later studies, however, revealed exceptions to the pattern that change the interpretation. Sloan et al. (2005) presented Richardson's ground squirrels with a hat thrown through the air, and the hat elicited both chirps and whistles. They interpret the alarms as carrying information about the immediacy and ephemerality of a threat; immediate and ephemeral threats may elicit chirps, whereas sustained threats may elicit whistles. Because there was only one stimulus object used in the experiment, which may not accurately portray either an aerial predator or a terrestrial predator, it is difficult to compare these results to those of Davis (1984).

Warkentin et al. (2001) had previously used the same model of hat and found that the alarm calling rate of individual squirrels was significantly negatively correlated with the distance of the caller to the threat, apparently a clear case of calling rate carrying information about distance, possibly indicating risk or urgency in the perception of receivers. Warkentin et al. (2001) also observed that 92% of callers began their repetitive calls with whistles, which fits with Davis' interpretation of whistles as a response to aerial threats, if the hat is perceived as an aerial threat (Warkentin et al. 2001). Certainly, there is information about the aerial or terrestrial nature of the threat in call type, and call rate of repeated calls carries information about distance of threat. The alarm calls of Richardson's ground squirrels seem to comprise a system where both predator type and urgency are encoded in different call attributes, although it is not yet clear how differences in response should be interpreted. Future studies using aerial and terrestrial stimuli approaching in the same manner (speed, distance, angle of approach relative to caller) could clarify the roles of risk and predator type in eliciting whistles and chirps. Further, varying the manner of approach within terrestrial and aerial presentations could also tease apart the role of risk in combination with predator type.

Richardson's ground squirrels are the only ground squirrels known to use ultrasonic alarm calls; they produce a flat narrow-band call at around 50kHz (Wilson & Hare 2004). This "whisper" call is used more frequently to distant than to nearby stimuli (Wilson & Hare 2006), but its function relative to the lower-frequency whistles and chirps is not clear.

GUNNISON'S PRAIRIE DOGS (*CYNOMYS GUNNISONI*)

When it comes to alarms that contain information about specific attributes of a threat, the alarms of Gunnison's prairie dogs carry more detailed information than any other mammals examined to date. Although there are no tests comparing use of categorical call types, Gunnison's prairie dogs have been shown to vary acoustic attributes of alarm calls elicited by different stimuli, even to the extent of changing their alarms in response to individual people wearing differently colored shirts (Slobodchikoff et al. 1991; Placer & Slobodchikoff 2000).

A later study by Kiriazis and Slobodchikoff (2006) showed that Gunnison's prairie dogs used different escape responses to different predators. The prairie dogs descended into their burrows in response to people and directly approaching hawks but stood outside the entrance to the burrow in response to coyotes and dogs (Kiriazis & Slobodchikoff 2006). Playbacks of alarm calls elicited by hawks, people, coyotes, and dogs elicited these same responses, suggesting that their alarms are functionally referential. No study to date has tested for effects of urgency in Gunnison's prairie dog alarm calls.

I have described only a few of the ground squirrel species for which data on alarm call use are available, but they represent the spectrum of ground squirrel alarm systems, from those containing the least to the most information about predator type and characteristics. As Table 2.1 shows, most ground squirrel alarms contain at least some information on whether a threat is aerial or terrestrial, although it is not always clear whether this information content is due to classification by type of predator or urgency of danger.

SCIURID ALARM CALLS – ARBOREAL SQUIRRELS

Alarm calls in arboreal squirrels have not been studied to the same degree as in ground squirrels. Most work is limited to description of alarm-call repertoires. Studies examining whether arboreal tree squirrel alarm calls contain information on predator type are limited to several observational studies and only a handful of experimental studies (Greene & Meagher 1998; Digweed & Rendall 2009b; Digweed & Rendall 2010). The experimental studies are all in a single species, the North American red squirrel (*Tamiasciurus hudsonicus*). Multimodal signaling in sciurid alarms has been looked at only by Partan et al. (2009; 2010), who examined the function of gray squirrels' tail and vocal signals in eliciting alarm behavior in conspecifics. Below I review the research to date on specificity of tree squirrel alarm calls. There is no previous work on specificity of tail-signal alarms.

Table 2.1. Specificity of alarm calls in sciurids and other mammalian species. This table, after Fichtel and Kappeler (2002), shows the information known to be contained in mammalian alarm calling systems, with a focus on sciurids. It is debatable whether some species code information based on predator type (*i.e.*, aerial/terrestrial or cat/dog) or urgency (*i.e.* near/far, sudden/slow approach). This table does not show differences in how researchers identify call types, which varies greatly. Differences in response based on call type recorded here may be either quantitative or qualitative. Based on levels of production specificity and playback experiments to date, it appears that ground squirrel alarms primarily refer to urgency of the threat, which may at times correspond to predator type as well (Macedonia & Evans 1993). Despite the various methods of classifying signals, threats, and responses, some perhaps more appropriate than others, the overall pattern does not seem entirely due to differences in method.

Species & Sociality 1=individual territories 2=overlapping home ranges 3=stable groups	**Specificity of alarm calls & type of study** O=observational study [a] E=experimental test	**Conspecifics' responses differ based on call type?**	**Refuge** A=arboreal B=burrow	**Source**
Rodentia				
Sciuridae				
Callosciurini				
Callosciurus caniceps, 2	terrestrial/aerial, O	?	A	1
Callosciurus erythraeus, 2	bird/cat/snake, O	?	A	2
	snake mobbing calls, O	?		
Callosciurus nigrovittatus, 2	terrestrial/aerial calls, O	?	A	1
Callosciurus notatus, 2	terrestrial/aerial calls, O	?	A	1
Marmotini				
Callospermophilus lateralis, 3	? [e]	?	B	13
Cynomys gunnisoni, 3	person/dog/hawk/coyote [b], E	? [c]	B	3
	person's shirt color, E	?		
Cynomys ludovicianus, 3	person's shirt color, E	?	B	4
Ictidomys tridecemlineatus, 3	urgency [f], E	yes	B	16
Marmota caligata, 3	terrestrial/aerial, urgency, E	yes	B	5
Marmota caudata, 3	urgency, E	no	B	6
Marmota flaviventris, 3	urgency [d], E	yes	B	7
Marmota marmota, 3	terrestrial/aerial, urgency, E	no	B	8
Marmota olympus, 3	urgency, E	yes	B	5
Marmota vancouverensis, 3	terrestrial/aerial, or urgency, E	yes	B	5
Otospermophilus beecheyi, 3	terrestrial/aerial, or urgency, E	yes	B	10
Urocitellus armatus, 3	terrestrial/aerial, or urgency, O	?	B	9
Urocitellus beldingi, 3	terrestrial/aerial, or urgency, E	?	B	11
Urocitellus columbianus, 3	urgency, E	yes	B	12
Urocitellus undulatus, 3	terrestrial/aerial, O	?	B	14
Urocitellus richardsonii, 3	urgency, E	yes	B	15
Tamias striatus, 1, 2	terrestrial/aerial	yes	B	17
Tamiasciurini				
Tamiasciurus hudsonicus, 1	no specificity?, E	no	A	18
Carnivora				
Viverridae				
Suricata suricatta, 3	terrestrial/aerial/snake, plus urgency for each	yes	B	19
Primates				
Lemuridae				
Lemur catta, 3	terrestrial/aerial, E	yes	A	20
Varecia variegata, 3	urgency, E	yes	A	20

Eulemur fulvus rufus, 3	terrestrial/aerial[g], E	yes	A	21
Propithecus verreauxi, 3	terrestrial/aerial[g], E	yes	A	21
Cercopithecidae				
Cercopithecus aethiops, 3	leopard/eagle/snake, E	yes	A[h]	22
Cercopithecus campbelli, 3	leopard/eagle, E	yes	A	23
Cercopithecus diana, 3	leopard/eagle, E	yes	A	24
Colobus guereza, 3	leopard/eagle, E	yes	A	29
Colobus polykomos, 3	leopard/eagle, E	?	A	29
Macaca sylvanus, 3	person/dog[i], E	?	A	25
Macaca radiata, 2, 3	leopard/python[i], E	?	A	26
Papio cyanocephalus, 3	carnivore/crocodile, O	?	A[h]	30
Cebidae				
Cebus apella nigritus, 3	aerial/terrestrial/urgency, E	yes	A	27
Saguinus fuscicollis, 3	terrestrial/aerial, E	yes	A	28
Saguinus mystax, 3	terrestrial/aerial, E	yes	A	28

[a] Observational, as used here, refers to purely descriptive data with no statistical test of alarm call production specificity, regardless of sample size. Experimental refers to natural observations or experimental manipulations that produce data that are analyzed statistically for the presence of threat-specific alarm calls.

[b] Specificity is based on subtle changes in harmonic structure within a single acoustic class of alarm call.

[c] They demonstrate that alarms differ by predator type, and response differs by predator type, but they do not test whether response differs by alarm call type.

[d] Urgency is coded in call rate, not call type; 96.46% of calling bouts consisted solely of "whistles."

[e] *Callospermophilus lateralis* included here after Fichtel and Kappeler (2002), but I do not see evidence for urgency or any other specificity of alarm calls, either in the cited study or elsewhere.

[f] Alarm calls were frequently given to terrestrial predators, but rarely toward aerial predators.

[g] These species showed mixed specificity, with specific calls for aerial threats, but the call given to terrestrial threats was also used in some aerial predator and non-predator contexts. They responded differently to the call types.

[h] Vervets and baboons may retreat to trees or rocky outcroppings, but are not arboreal. Fleeing up a tree is not always the expected escape response, especially in baboons.

[i] No aerial threats were tested.

Sources: **1** (Tamura & Yong 1993) **2** (Tamura et al. 1989; Tamura 1989; Tamura 1995) **3** (Placer & Slobodchikoff 2000; Furrer & Manser 2009; Slobodchikoff et al. 2009) **4** (Frederiksen & Slobodchikoff 2007) **5** (Blumstein 1999a) **6** (Blumstein & Arnold 1995) **7** (Blumstein & Armitage 1997a) **8** (Boero 1992; Blumstein & Arnold 1995) **9** (Balph & Balph 1966) **10** (Leger & Owings 1978; Owings & Leger 1980; Boellstorff & Owings 1995) **11** (Robinson 1980; Leger et al. 1984) **12** (MacWhirter 1992; Harris et al. 2010) **13** (Shriner 1998) **14** (Melchior 1971) **15** (Davis 1984; Warkentin et al. 2001; Sloan et al. 2005) **16** (Schwagmeyer 1980; Schwagmeyer & Brown 1981) **17** (Getty 1981; Burke da Silva et al. 1994; Weary & Kramer 1995) **18** (Greene & Meagher 1998; Digweed & Rendall 2009b; Digweed & Rendall 2010) **19** (Manser 2001; Manser et al. 2001) **20** (Macedonia 1990) **21** (Fichtel & Kappeler 2002) **22** (Seyfarth et al. 1980a; Seyfarth et al. 1980b) **23** (Zuberbühler 2001) **24** (Zuberbühler 2000) **25** (Fischer et al. 1995; Fischer 1998) **26** (Coss et al. 2007) **27** (Wheeler 2010) **28** (Kirchhof & Hammerschmidt 2006) **29** (Schel et al. 2009; Schel et al. 2010) **30** (Fischer et al. 2001)

AFRICAN FOREST SQUIRRELS

There are no experimental studies of alarm calling in any arboreal African squirrels. There are several descriptive studies of communication, including some information on the alarm calls of arboreal African rainforest squirrels from six genera: *Epixerus*, *Funisciurus*, *Heliosciurus*, *Myosciurus*, *Paraxerus*, and *Protoxerus*. Descriptions exist for alarm calls of some species in these genera, but there are no quantitative data on calls used in various predator contexts to examine predator-specificity or whether risk is encoded. Most species described use multiple, acoustically distinct alarm calls, and Emmons (1978) classified them into low- and high-intensity alarms, with most species having two low-intensity alarms that were acoustically convergent with other species and a high-intensity alarm that was more species specific. I will briefly describe the alarm calls of each species and then summarize what we do and do not know about overall patterns in alarm calls of African forest squirrels.

PALM SQUIRRELS (*EPIXERUS*) – Ebian's palm squirrel (*E. ebii*) uses at least three alarm sounds: (1) soft tooth-chatters audible to people only within a few meters; (2) loud chucks, used infrequently; and (3) very loud, short, broadband staccato barks (Emmons 1978). Alarm calling bouts may contain a single vocalization or several repeated calls. Ebian's palm squirrel uses tail signals at the onset of alarm calling, with its tail first hanging straight, then waving laterally a few times (Emmons 1978).

ROPE SQUIRRELS (*FUNISCIURUS*) – Rope squirrel alarm-call repertoires seem to vary in size across species, but this variation may be an artifact of how they are classified. Thomas's Rope Squirrel (*F. anerythrus*) uses two types of alarm calls: (1) chucks, which are short and have several clear harmonics but vary in length and shape; and (2) whistles and soft notes, which are used together in a bout (Emmons 1978). Whistles are loud, with only a few harmonics, and are longer than chucks. Soft notes are quiet sounds that seemed to be made during inhalations between whistles (Emmons 1978).

Fire-footed rope squirrels (*F. pyrrhopus*) used three alarm-call types: (1) chucks, similar to those used by Thomas' rope squirrel but noisier and spanning more frequencies; (2) double chucks, which are quieter chucks in rapid pairs; and (3) staccato calls, which have narrow harmonic bands but are short and repeated rapidly (Emmons 1978).

Ribboned rope squirrels (*F. lemniscatus*) use three call types: (1) chucks, similar to those of Thomas' rope squirrel; (2) double chucks, as above; and (3) pulsed calls of 0.2–0.4s, highly tonal vocalizations that descend in frequency between pulses (Emmons 1978).

Lady Burton's rope squirrels (*F. isabella*) use at least two alarm calls: (1) chucks, which can be indistinguishable from those of ribboned rope squirrels; and (2) warbles, a unique call of undulating frequency, resembling linked chucks with less frequency modulation (Emmons 1978).

Captive fire-footed, ribboned, and Lady Burton's rope squirrels used chucks (and perhaps double chucks) in response to a preserved snake, which they closely approached and inspected from multiple angles (Emmons 1975). The same captive species used chucks and tail flicks in response to a perched raptor on the ground, but fled when the raptor was moved to a 1m perch, and a Lady Burton's rope squirrel began a warble call (Emmons 1975). A captive Thomas' rope squirrel presented with the same raptor gave a warbling version of its whistle/soft-call vocalizations (Emmons 1975).

SUN SQUIRRELS (*HELIOSCIURUS*) – The red-legged sun squirrel (*Heliosciurus rufobrachium*) uses two alarm calls: (1) barks, which are variable in frequency but are always very short, broadband calls; and (2) coos, which are longer, narrowband calls that descend in frequency and become pulsed (Emmons 1978). Emmons (1978) reports that barks were used in response to predators, loud noises, and conspecifics in aggressive interactions. Barks were used in response to a hawk placed outside a red-legged sun squirrel's cage and also in response to a caged genet (Emmons 1975). Coos were used in response to conspecifics placed in the same cage and by free squirrels in response to people (Emmons 1978). Tail signals were used during alarm calling with the tail bent upwards, and then as each call ended, raised over the back while the squirrel gave a small hop with its hind feet—while keeping its front feet planted (Emmons 1978).

AFRICAN PYGMY SQUIRRELS (*MYOSCIURUS*) – The African pygmy squirrel (*Myosciurus pumilio*) has only been observed to use a single alarm call, a soft, repeated "pip," which could not be heard by people beyond a few meters (Emmons 1978). The pygmy squirrels called while continuing to forage vegetation, often positioned on a vertical tree trunk and laterally waving their tails. The alarm call was elicited in wild squirrels by people and not observed otherwise (Emmons 1978).

BUSH SQUIRRELS (*PARAXERUS*) – The alarms of the red bush squirrels (*P. palliatus ornatus* and *P. p. tongensis*) include three alarm calls: (1) clicks, which are soft, short, broadband calls usually repeated several times; (2) trills, which are rapid, repetitive narrowband calls; and (3) alarm barks, which are loud, short, broadband calls (Viljoen 1983). Clicks and trills are also used in nonpredator contexts and seem to play a role in advertising presence to conspecifics (Viljoen 1983). Clicks are used in association with tail flicking while mobbing people, mongooses, and snakes, including snakes in trees. Viljoen (1983) only observed red bush squirrels use barks in predator contexts, usually to raptors and sometimes when a person appeared suddenly. Playbacks of the alarm bark caused squirrels to alarm bark and flee. Red bush squirrels twitched their tails sporadically while moving and when alarm calling moved their tails in synchrony with clicks. Tail flicks were largest during mobbing behavior (Viljoen 1983). Interestingly, a related species, Smith's bush squirrels (*P. c. cepapi*), that lives in savannah rather than forest, usually limits tail signaling to alarm contexts. Although Smith's bush squirrels use similar alarm calls to red bush squirrels, their clicks and barks are higher in pitch, and instead of a trill they use a harsh rattle that extends into higher frequencies than the trill of red bush squirrels (Viljoen 1983). The lower frequencies used by forest species may be an adaptation to enhance transmission distance in an environment with numerous obstacles (Viljoen 1983; Blumstein 2007).

The green bush squirrel (*Paraxerus poensis*), a rainforest species, used two alarm calls in Emmons' (1978) study. They usually used only one alarm call, a buzz, but twice were heard using a bark similar to the other *Paraxerus* species. Buzzes slowed over the course of the calling bout and had high dominant frequencies (5.5kHz) relative to alarm calls of other forest-dwelling *Paraxerus* species. Buzzes were elicited by people (Emmons 1978). Emmons (1975; 1978) saw no evidence for predator-specific production of alarm calls in the green bush squirrel.

GIANT FOREST SQUIRRELS (*PROTOXERUS*) – The giant forest squirrel (*Protoxerus stangeri*) uses three alarm calls: (1) a nonvocal, explosive "chuff"; (2) a loud, broadband, vocal bark with lower frequencies than the chuff; and (3) a loud whinny, which is actually a rapid series of short, narrowband, frequency modulated pulses (Emmons 1978). There is no apparent predator specificity, but the data are sparse. Whinnies were elicited by raptors and a person in Emmons' (1978) study. Alarm calling bouts can last for several minutes, longer than most African forest squirrel species. Giant forest squirrels typically use no distinct tail signals while alarm calling but occasionally twitch the tail upwards while moving from place to place during a calling bout (Emmons 1978).

SUMMARY OF AFRICAN FOREST SQUIRREL ALARMS – The fact that most species of African forest squirrels use multiple alarm-call types, with production of some alarms being very specific to the presence of a predator while other alarms are also used in social or other contexts, suggests that different alarms may carry different types of information. Based on anecdotal observations of predator presentations to captive squirrels and some observations of wild squirrels responding to predators, Emmons (1975; 1978) suggests that alarm calls are not predator-specific but differ based on intensity of an internal state of agitation or alarm. Call types used in both social and predator contexts probably signal agitation level, and in an alarm capacity may signal degree of risk or similar information. The calls used only in the context of a predator could be general alarms or carry more specific information about the nature or urgency of a threat. It is also not certain whether the entire repertoire of alarm calls has been described in some of these species.

Several of the calls of African forest squirrels are structurally similar to alarm calls of North American tree squirrels. African arboreal squirrels are more closely related to New World marmots than to New World tree squirrels (Steppan et al. 2004), but the initial descriptions of African tree squirrels' alarms suggests a convergence of some alarm-call types (Emmons 1978). Future studies clarifying the production specificity and function of African forest squirrel alarm calls will enable comparisons of divergence and convergence of alarm calling systems between two putatively monophyletic groups, the arboreal squirrels of Africa and the Americas (excluding the neotropical pygmy squirrel (*Sciurillus pusillus*)).

A larger monophyletic group contains all ground squirrels (Marmotini and Xerini) and African arboreal squirrels (Steppan et al. 2004; Herron et al. 2004). By comparing alarm calls of ground squirrels, African arboreal squirrels, and New World arboreal squirrels, it may become clear how differences in terrestrial versus arboreal lifestyle and differences in habitat affect the evolution of alarm calling systems.

ASIAN FOREST SQUIRRELS (*CALLOSCIURUS*)

Asian forest squirrels fall into two monophyletic groups, the beautiful squirrels (*Callosciurus*) and the Asian giant squirrels (*Ratufa*). The context of *Ratufa* alarm calling has not been directly studied. The Formosan squirrel (*Callosciurus erythraeus thaiwanensis*) has at least some degree of production specificity in its alarm calls (Tamura 1989; Tamura & Yong 1993; Tamura 1995). The Formosan squirrel used acoustically distinct calls for feral cats, large flying birds, and snakes. The call for feral cats (and rarely other terrestrial animals) began with short, rapidly repeating, broadband barks that slowed down and became longer and more narrowband with several clear harmonics as the call progressed. This pattern resembles that described in eastern gray squirrels (*Sciurus carolinensis*) as a general alarm call and frequently observed in response to feral cats in my present study, except that the later calls do not become as tonal in the gray squirrel (Lishak 1984; present study). In the Formosan squirrel, the call for large flying birds was a single vocalization that differed from those given to feral cats

(Tamura 1995). The snake alarm call was a mobbing call, a high-pitched scream given when a snake was encountered in a tree, which attracted conspecifics to join the mobbing (Tamura 1989).

Playbacks of alarm calls elicited by cats caused squirrels to run up trees and remain immobile. Longer alarms resulted in longer immobility (Tamura 1995). A separate study elicited mobbing calls using a stuffed snake placed in trees but did not play back mobbing calls to test effects on conspecifics in the absence of a visible threat. It seems that Formosan squirrels produce predator-specific calls, but it is not clear how these calls affect conspecifics' behavior, especially in the absence of a predator or other visual cues to threat type. Experimental studies directly testing predator specificity are also needed.

Other *Callosciurus* species may use a similar system to Formosan squirrels. In response to terrestrial threats, the gray-bellied squirrel (*Callosciurus caniceps*) also uses rapid, staccato, noisy barks that develop clear harmonics as the call continues (Tamura & Yong 1993). The plantain squirrel (*Callosciurus notatus*) and black-banded squirrel (*Callosciurus nigrovittatus*) also responded to terrestrial threats with staccato barks, but unlike the Formosan and gray-bellied squirrels, their barks in response to terrestrial threats do not change in harmonic structure over the calling bout (Tamura & Yong 1993).

Similar to the Formosan squirrel's alarm system, gray-bellied, plantain, and black-banded squirrels all used different vocalizations in response to aerial predators than they used for terrestrial predators, but the call type differed across species. Gray-bellied squirrels use a single low-frequency bark like the Formosan squirrel, whereas plantain squirrels and black-banded squirrels use a low “chuckle”—a pulsed bark with some harmonic structure (Tamura & Yong 1993).

Terrestrial and aerial threats elicited alarm calls and avoidance behavior, but snakes were mobbed. Squirrels of all four *Callosciurus* species that have been studied gave high-pitched squeaks during mobbing, although chuckles and buzzes were sometimes used when the snake moved (Tamura 1989; Tamura & Yong 1993). Additional squirrels would approach and join the mobbing and calling, which may or may not be due to the squeak calls of mobbing squirrels. Playback experiments could confirm whether squeaks alone are sufficient to attract conspecifics.

The Asian *Callosciurus* seem to have the most predator-specific alarm vocalizations observed in an arboreal squirrel based on data available to date, but there are no direct tests for association between call types used and stimulus type, although such tests may be possible using data already collected for published studies. Experimental studies to test for predator specificity while controlling other aspects of predator encounters such as distance or speed remain to be done, as well as playbacks comparing response of conspecifics across call types.

NORTH AMERICAN RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*)

Greene and Meager (1998), working in Montana, conducted the first experimental test for predator-specific alarm calls in an arboreal squirrel, the North American red squirrel (*Tamiasciurus hudsonicus*). They observed three distinct alarm calls used in response to natural predators, experimental presentations of both a model bird and a live dog: (1) seets, which are low-amplitude, high-frequency sounds similar to alarm calls used by many small birds; (2) barks, which are loud, explosive calls with broad overtones; and (3) seet-barks, which are acoustically intermediate to seets and barks. Seet-barks start like a seet, add multiple harmonics, and then end with a noisier segment much like a bark (Greene & Meagher 1998).

Green and Meagher (1998) observed seets and seet-barks, but never barks, in response to raptors and ravens. In contrast, barks were the only vocalization used in response to people and dogs, except for one squirrel that produced a seet. They also presented squirrels with a small, colorful, wind-up flying bird model, which elicited proportions of seets and seet-barks similar to those used in response to natural encounters with large birds.

Initially, this result seems to be a clear case of predator specificity. Although ravens are not typically considered predators of red squirrels, they are of similar size and build to hawks that do feed on squirrels. The small, colorful, wind-up flying bird (Amazing TIM model bird, De Ruymbeke Co., Marseille, France) elicited similar responses, however, despite not resembling any predator at all. Although Greene and Meagher (1998) claim that the model is “the general size and shape of a Cooper’s hawk,” it has only a 12" wingspan, which is from a half to a third that of an adult Cooper’s hawk’s wingspan (Society 1999; Clark & Wheeler 2001; Vuilleumeir 2009). It is similar in size to a thrush (Vuilleumeir 2009), and most models of the toy have color patterns unprecedented in Montana birds. It may be a good object to test squirrels’ responses to a novel flying object, but it is not particularly suited to imitating a raptor. The fact that it elicited similar responses to raptors at a distance could mean, as Greene and Meagher (1998) propose, that red squirrels classify threats into broad aerial and terrestrial categories.

Alternatively, red squirrels may use different calls to reflect different levels of alarm. This explanation also fits Greene and Meagher's (1998) data. At similar distances, the small model bird may not elicit the same level of alarm as a large, living, terrestrial predator. It is also not clear that distances to terrestrial and aerial predators are similar in their study. The encounters with raptors were rarely closer than 30m. Of all the large birds, only ravens came within 20m of the responding squirrel, and they did so while flying through the area. If all these birds represent only distant and often passing threats, they may elicit lower levels of alarm. It would be interesting to know whether use of seets and seet barks is associated with either distance to the large birds or flight status (perched or flying).

Similarly, the consistent use of barks in response to terrestrial predators could reflect a higher level of alarm. The terrestrial threats in the study differed from aerial stimuli not only in being terrestrial but also in their closer approach, and perhaps in having their attention focused on the squirrel or their persistence in the area. If these factors do increase level of alarm in red squirrels, then their vocalizations may reflect a gradation from mild (seet) to moderate (seet-bark) to intense (bark) alarm. It is clear that the squirrels in Montana used different calls in different contexts, but the relevant difference in context is not yet clear.

The case of the red squirrel is further complicated by the results of Digweed and Rendall's (2009) work, which did not find any predator specificity of alarm-call type. In a Canadian population, Digweed and Rendall (2009) observed only two alarm calls in response to predators, the seet and seet-bark, although barks were heard in other contexts. Because the bark was the terrestrial-predator call in Green and Meagher's (1998) study, the lack of barks in the observations of Digweed and Rendall (2009) makes direct comparison difficult and suggests the two populations use different alarm calling systems.

The red squirrels in Alberta, Canada, used both seets and seet-barks in mixed bouts when responding to terrestrial predators, aerial predators, and martens, which are squirrel-preying specialists capable of pursuing squirrels either terrestrially or arboreally. They also used both call types for nonpredatory species, although less often (Digweed & Rendall 2009b). During a bout, the most frequent call type shifted from seets to seet-barks. Coyotes elicited a higher proportion of seet-barks and longer calling bouts; these variables are related because the proportion of seet-barks increases as a call continues (Digweed & Rendall 2009b).

Digweed and Rendall (2010) also conducted playbacks, presenting squirrels with repeated seets, seet-barks, or a natural combination of both calls. They found no effect of call type or call speed (each exemplar was manipulated into a fast and slow version of the original call series) on squirrels' orientation to the speaker, time spent vigilant, frequency of vigilance, or squirrels' tendency to "move a short distance up or down a tree."

Studies are needed to clarify the information content and function of red squirrel alarm calls and, given the difference in call repertoire between the Montana and Canada studies, perhaps to test for regional variation in function. Playbacks including pure barks would be enlightening as barks are not produced under the same conditions as seet-barks (Greene & Meagher 1998; Digweed & Rendall 2009b). Red squirrels are highly territorial and their alarm calls are interpreted by Digweed and Rendall (2009a) as primarily "intruder" calls given to both conspecifics and predators entering their territory and primarily directed at the intruder. If this interpretation is correct, then the calls carry information about the presence of an intruder and may still serve an alarm-call function, as Digweed and Rendall (2010) previously showed that playbacks of seets and seet-barks increased vigilance relative to control sounds. Whether or not this vigilance functions primarily to detect conspecifics or predators is an open question. Playbacks of red squirrel alarm calls to predators to test whether the alarm calls of the red squirrel have a predator-deterrence function would be helpful.

EASTERN GRAY SQUIRREL (*SCIURUS CAROLINENSIS*)

The eastern gray squirrel is among the most studied of arboreal squirrels. In addition to being common in its native range throughout the eastern United States, where it is managed and hunted as a small game animal, it has established populations in the western U.S. where it competes with the native western gray squirrel (*Sciurus griseus*) and has been introduced to Australia, Britain, Italy, and South Africa (Huynh et al. 2011). As a result of these introductions, there are many ecological studies examining its interactions with and effects on native species. Despite the extensive literature on the species, until recently there have been few experimental studies of communication behavior in the eastern gray squirrel.

Eastern gray squirrel alarm signal repertoire

The eastern gray squirrel uses several alarm-call types. Lishak's (1984) descriptive work describes six main alarm calls: (1) buzzes, which are low-amplitude buzzing noises audible only within a few meters; (2) kuks, which are broadband barks with sudden onset and ending, often rapidly repeated, with a duration of about 0.1s; (3)

quaas, which have a similar frequency structure to kuks but significantly differ in length (quaas are defined as over 0.15s); (4) moans, which are highly tonal calls that have distinct harmonics and fade gradually in amplitude over time; (5) buzz-quaas, which are compound calls of buzzes and quaas; and (6) modulated quaas and quaa-moans, which are broadband calls resembling quaas, but with distinct harmonics during part of the call.

In my study population in Coral Gables, Florida, I observed only three types of vocalizations in predator contexts: kuks, quaas, and moans. Lishak (1984) describes the kuk and quaa as broadband calls differing mainly in duration (Figure 2.1). He categorized them as separate call types based on a bimodal distribution in the pooled durations of kuks and quaas, with a sharp valley at 0.15s dividing the shorter kuks from the longer quaas. The kuks I observed are very short (<0.1s). A few equally short vocalizations have clear harmonics, but these are usually used in association with moans. Lishak (1984) described kuks as having most of their energy between 2-5 kHz, with additional frequencies between 0.5 and 16kHz. In the calling bouts I observed, overall frequency structure was similar between kuks and quaas in the same bout, but there was often a slight change in dominant frequency, and quaas were often modulated.

Quaas were highly variable in length, frequency structure, and modulation but were always broadband calls over 0.15s with either no clear harmonics or numerous closely spaced harmonics (see Figure 2.1). Many were so noisy that no harmonic bands were visible in spectrograms, regardless of whether a wide or narrow sampling window was used to make the spectrograms. When quaas were modulated, the call would typically rise and fall in frequency over each call, rarely exceeding 1kHz of modulation, often much less. Occasionally, the number of harmonics changed over the course of a

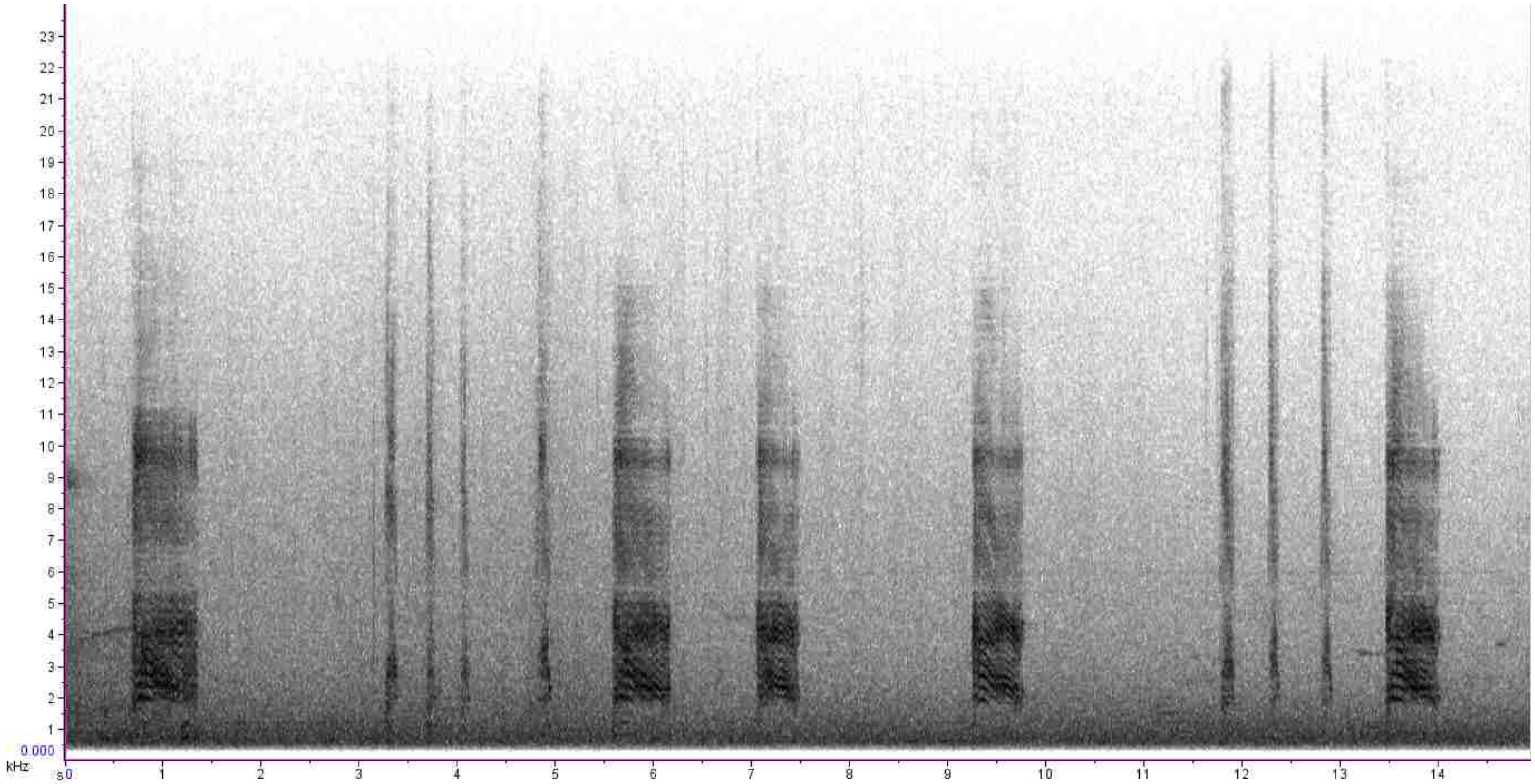

Figure 2.1. Spectrogram of kuks and quaas. This spectrogram shows an eastern gray squirrel (*Sciurus carolinensis*) alarm-calling bout segment showing kuks (short duration) and quaas (long duration). Kuks and quaas have similar frequency structure, although, within a bout, kuks sound slightly different from quaas due to subtle shifts in amplitude across frequencies.

single quaa, with 2–5 abrupt changes in the harmonics present and therefore in dominant frequency (Lishak's "modulated quaa"). Many of these changes consisted of harmonics that were initially present abruptly disappearing, often in a pattern resembling an octave jump in fundamental frequency. I classified all quaa variations as quaas and followed Lishak (1984) in discriminating kuks, quaas, and moans.

Moans in my present study seemed to take two distinct acoustic forms that were not separated by Lishak (1984) (Figure 2.2). The first form of moans was highly stereotyped and changed little over the course of a calling bout. Moans of this first form contained multiple harmonics at the onset, but most harmonics quickly disappeared, forming a long, clear, descending tone with most of the energy contained in 2–8 widely spaced harmonics. Moans of the second form did not always have the long descending tone but were variable in modulation and tended to be shorter than the first form, similar to quaas in duration but with fewer harmonic bands than quaas (Figure 2.2C). Both forms of moans had far fewer harmonics than quaas and each harmonic band was clearly distinct, in sharp contrast to the indistinct harmonics of quaas (Figures 2.1, 2.2).

The acoustic properties of kuks make them easy to localize; their broad frequency range and abrupt onsets enhance the detection of differing arrival times to receivers' ears, making it easier for receivers to locate the direction of origin (Klump & Shalter 1984). Kuks' short durations and abrupt endings enable distinct, rapid repetitions. These rapid repetitions enable receivers to rapidly fine-tune their estimates of the direction from which the kuks are arriving (Klump & Shalter 1984). Quaas are also easily localizable for similar reasons, although they are not repeated at the same rate as the initial rapid kuks

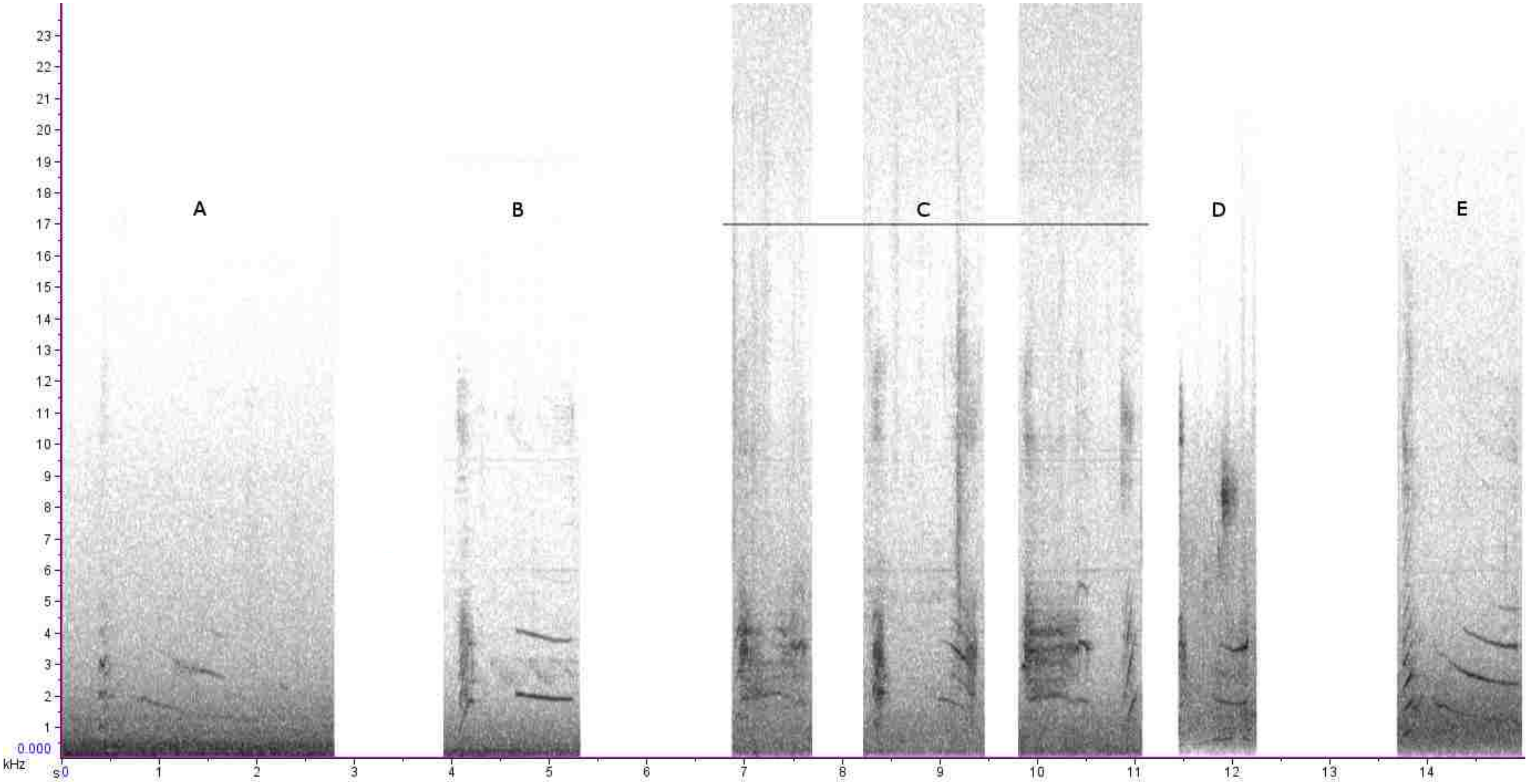

Figure 2.2. Spectrogram of moans. Examples of moans from five different calling bouts (A-E). A, B, and D demonstrate the typical moan, which initially has a sharp frequency increase with many harmonic bands that fade in amplitude, only to increase in amplitude again with fewer harmonic bands in a squeaky, descending tone. Some calling bouts have extremely uniform moans, while others are highly variable. C shows four exemplars from one bout that are highly variable. The third exemplar from C demonstrates the second form of moan, which resembles a quaa with fewer harmonics. In this case it is followed by a short, highly tonal, rising squeak, an uncommon vocalization. E demonstrates a typical moan, except that the trailing side has a similar frequency structure to the start of the call.

that often start an alarm calling bout. To the human ear, moans are fainter than kuks or quaas and usually of higher pitch, although due to their narrow range of frequencies in tight harmonic bands, they actually have less energy in high frequencies than either kuks or quaas. This concentration of energy into a narrow frequency band makes them harder to localize (Klump & Shalter 1984).

In addition to their vocal alarms, gray squirrels use tail motions as alarm signals (Partan et al. 2009). Tail alarms and vocal alarms are used both separately and in combination as multimodal signals (Partan et al. 2009; present study). Partan et al. (2009) found that both signals together elicited a stronger response from conspecifics than either signal alone. They further found that there were differences in response between urban and rural populations, with urban populations responding more to tail flagging than did rural populations (Partan et al. 2010).

Predator encounters of eastern gray squirrels observed in this study

In the present study I observed regular encounters between gray squirrels and feral cats (*Felis catus*). Squirrels on the ground nearly universally responded by fleeing to a tree and once in a tree ascending to at least three meters, turning, and orienting* toward the cat while flicking the tail. I defined two levels of amplitude of motion in tail signals, small twitches and large flicks, which are further detailed in Chapter 3. Squirrels in this population typically begin signaling by silently twitching the tail and then escalating to large flicks. If the cat is walking, the squirrels often move through the branches in the same direction, tail flicking at every pause. If squirrels vocalize in response to a cat, they universally begin with a rapid bout of kuks while flicking their tails. From that point, tail signaling usually decreases while vocalizations continue. The initial rapid burst of kuks slows within several seconds. If calling continues, quaas are typically interspersed with kuks, with quaas becoming more numerous and kuks less so as the calling bout progresses. Many times this alarm call would alert me to the presence of a feral cat hidden in the bushes. Squirrels often, but not always, alarm signal (tail and/or vocal signals) to passing cats. It is unknown why in some encounters squirrels apparently ignored cats while in other encounters squirrels persisted in tail flicking and calling for extended periods. Alarm bouts in response to cats, from first tail signal to last vocalization, typically lasted 2–5 minutes, but occasionally extended over 10 minutes.

Few encounters with raptors were observed. Once, when a red-tailed hawk (*Buteo jamaicensis*) flew into a tree, a few sharp, tonal barks from a gray squirrel were heard from the same tree and the hawk flew off several seconds later. Several encounters with red-shouldered (*Buteo lineatus*) and Cooper's hawks (*Accipiter coope*rii) were observed. A Cooper's hawk once repeatedly dived at a squirrel on the ground in a parking lot. The squirrel was never seen to tail signal beyond a few small tail twitches and never vocalized during the half hour it was repeatedly approached by the hawk. The squirrel remained on the ground throughout that period, apparently watching the hawk and dodging every time the hawk dived. The squirrel occasionally sought refuge under parked vehicles, but spent most of the time on all fours in open areas of asphalt, with head up and feet spread widely. During the entire encounter the squirrel was never more than 15m from a tree and several times could have reached the tree safely. It remained on the ground, evading the hawk until the hawk was panting with apparent exhaustion on the roof of a parked truck. Once the hawk flew off (to a tree about 30m distant) the squirrel returned to the nearby tree. This encounter was exceptional, but noteworthy for the way the squirrel avoided the hawk by remaining on the open ground.

Other encounters with hawks were between a squirrel already in a tree and a hawk perched nearby or flying through the area. When a hawk flew into a tree with a squirrel, or a nearby tree, the squirrel would typically freeze and several times was seen to extend all four legs in an elevated posture. Despite these clear alert responses to a hawk's presence, three other times squirrels were seen foraging on the ground under oaks where a red-shouldered hawk was perched and returning to limbs in that same tree to eat acorns. It is possible that they were unaware of the hawk, but this seems unlikely as in all cases the hawk was in plain sight and occasionally moving to groom its feathers. In one case, two squirrels that were foraging and eating also ascended to the limb where the hawk was perched and approached within a meter of it, causing the hawk to shy away before the squirrels leaped away, descended the trunk, and resumed foraging. This behavior repeated intermittently until the hawk flew off after about a quarter of an hour. Immediately upon the hawk's departure, a squirrel ran from the ground to the limb where the hawk had been perched and put its nose to the spot where the hawk had perched and began intensely moving its nose around that area of the limb. The squirrel was closely followed by a second, who also behaved as if smelling the perch. The first squirrel then turned in the direction the hawk had flown and began an extended calling bout. The calls started slower than was typical in response to cats, but consisted of kuks and quaas, which were the call types consistently heard during encounters between squirrels and cats.

COMPARISON AND CONCLUSION

HABITAT EFFECTS ON PREDATOR SPECIFICITY OF ALARMS

Ground squirrels use a variety of alarm calling systems, but in many cases it seems that alarm calls contain information on urgency rather than predator type directly, although different predator types may tend to present different levels of urgency. An urgency-based alarm system would fit with the hypothesis that ground squirrels have essentially one escape response, so what they are responding to is irrelevant. Instead, knowing how intensely to respond may be more important than knowing the type of predator. Although it is not immediately apparent that multiple, incompatible escape strategies are available to ground squirrels, it is possible that they do face incompatible options when attempting to avoid predation. For example, a ground squirrel that runs to a burrow opening must decide whether to retreat down the burrow or stand at the opening. Similarly, any ground squirrel hearing an alarm could stand up on its hind legs and increase its field of view or reduce its profile by staying on all fours. Future studies directly manipulating level of urgency within terrestrial and aerial predator classes could reveal whether these behaviors are truly predator-specific strategies or simply different levels of a single response.

Arboreal squirrels usually retreat to a tree when threatened. This behavior may initially look like a single escape-response, but trees offer more predator-avoidance strategies than most burrows. Retreating down a burrow blocks an occupant’s view of anything surrounding the burrow, whereas an animal retreating to a tree may have improved its view of the surrounding area by elevating itself above ground-level visual obstacles. So a tree could be used as a vantage point as well as being used to block a predator’s view of the prey individual (depending on the relative size of the prey species and structure of the tree).

Trees also differ from burrows in their ability to block terrestrial and aerial predators. A burrow effectively blocks any aerial threat, and only rapidly digging species like badgers pose significant threats to a ground squirrel in a burrow, particularly if the burrow is connected to others or includes an escape tunnel. But if a ground squirrel is forced to use an escape tunnel and return to the surface, it once again faces a foot race with death to find another shelter. In contrast, a tree squirrel that flees up a tree from a terrestrial predator is usually safe. With few exceptions, notably martens and related arboreal mustelids (*Martes* spp.), most mammalian predators have no chance of capturing a tree squirrel in a tree. Further, a squirrel in a tree can often flee through the canopy to neighboring trees without returning to the surface of the ground. Trees thus offer near certain escape from terrestrial threats, at least as much as burrows and perhaps even more so.

When facing aerial threats, the situation is different for ground squirrels and tree squirrels. A raptor cannot hope to pursue a squirrel down a burrow, but many woodland raptors are adept at chasing prey through the canopy and regularly take prey from tree branches (e.g., red-tailed hawks (Barkalow & Shorten 1973), goshawks (Widén 1987; Salafsky & Reynolds 2005), Cooper’s hawks (Meng 1959; Bielefeldt et al. 1992)). I have seen both red-tailed hawks and Cooper’s hawks perform remarkable acrobatics in pursuit of gray squirrels. A ground squirrel in a burrow may be losing foraging time, but it is relatively safe from both aerial and terrestrial predators. A tree squirrel in a tree is safe from terrestrial predators but not necessarily from aerial predators. If tree squirrels’ escape behaviors differ in their relative effectiveness against aerial and terrestrial threats, and ground squirrels' escape behaviors fall into a single strategy that is similarly effective to all predators, then tree squirrels may benefit more than ground squirrels from explicitly communicating predator type if predator-specific alarms enable conspecifics to take the appropriate escape strategy (see Chapter 1).

In addition to its role in shaping escape responses, habitat may also play a role in the evolution of alarm calling systems due to the different acoustic properties of different habitats. Differing acoustic properties have been suggested to explain differences in dominant frequency between congeneric savannah and forest squirrels (Viljoen 1983). Similarly, the small alarm-call repertoire of some marmots may be due to habitat structure that degrades details of alarm calls, making call rate a more effective carrier of information than changes in frequency structure (Blumstein & Daniel 1997).

Comparisons of alarm calling systems between mammals with terrestrial and arboreal escape-strategies are confounded by the taxa that have been investigated. Most studies of mammals that can seek refuge in trees examine primates, and most studies of mammals that seek refuge on or under the ground examine squirrels. It is thus unclear whether any differences observed are due to habitat type or due to the many other differences between primate and squirrel taxa (social structure, body size, anatomy, diet, etc.). Tree squirrels offer the opportunity to study multiple arboreal species that are closely related to ground squirrels, but occupy similar habitat to the many primates that have already been investigated. Tree squirrels thus isolate effects of phylogeny and habitat when comparing alarm-call systems with arboreal primates and ground squirrels, respectively.

SOCIALITY EFFECTS ON PREDATOR SPECIFICITY OF ALARMS

In addition to the role of habitat and associated escape strategies, sociality may affect the type of alarm calling system used. Blumstein and Armitage (1997b) found that social complexity was positively correlated with ground squirrels' alarm-call repertoire size (defined as the number of categorical alarm-call types that are distinct to human observers), even after controlling for effects of phylogenetic similarity. Their definition of social complexity uses the formula

$$SC_{\mathrm{d}} = \log[(H(X)_{\mathrm{t}})T_{\mathrm{nd}}]. \qquad (1)$$

In this formula (1), T_{nd} is the time to natal dispersal and $H(X)$ comes from the Shannon-Weiner formula,

$$H(X) = -\sum p(i)\log_2 p(i), \qquad (2)$$

which in this case considers the proportion of all individuals belonging to various social roles (breeding female, nonbreeding female, dependent offspring, etc.) in a typical group (Blumstein & Armitage 1997b). $H(X)$ increases as the number of social roles in a group increases and as the individuals in a group are more evenly distributed across social roles. The simplest sociality in this system is a species that is always solitary. Blumstein and Armitage's (1997b) method is a vast step toward objectively quantifying sociality, but their use of alarm-call repertoire size is probably too simple a measure of alarm-system complexity, as they acknowledge. Alarm-call repertoire size may loosely relate to the amount of information available from alarm calls, but a more objective measure of the information about predators that is contained in species' alarm calls is needed. Such a measure of alarm-system complexity would require playbacks to examine which information in calls is actually used by receivers. Ideally, both categorical differences and continuous variation in response to various alarm-call characteristics should be incorporated into future measures of alarm-system complexity.

Ground squirrels span the range of sociality from solitary to highly social, and there are many studies examining the information content of alarm calls and receivers' responses to differing alarm calls. Tree squirrels also span the range of sociality, but there are very few studies of the information contained in their alarm calls. Before it can be determined whether the trend of social complexity correlating with alarm-system complexity is a general rule across taxa, we need additional studies describing the information contained in the alarm calls of species other than ground squirrels. Biologically, the most important information for an individual encountering a predator is any information that enables it to select the response that maximizes its fitness. Given the distinct hunting strategies of terrestrial and aerial predators, information on the terrestrial or aerial nature of a threat may be important to individuals deciding on the most appropriate response to a predator's presence. Testing for predator specificity of alarm calls is thus a solid first step toward identifying the information content of alarm-call systems.

THE NEED FOR STUDIES OF PREDATOR SPECIFICITY IN TREE SQUIRRELS

Studies of the predator specificity and other information in mammalian alarm calls have primarily examined primates and ground squirrels. Arboreal tree squirrels provide an ideal complementary system to test for effects of habitat type, multiple escape responses, and social complexity on the evolution of predator-specific alarm calls. Comparing the alarm calling systems among multiple species of tree squirrels with differing social complexity will ultimately allow a test for correlation of alarm-system complexity with social complexity. Differences from the correlation seen in ground squirrels may mean that, in arboreal habitats, sociality is less important than the existence of multiple escape strategies in driving alarm-system complexity. Tree squirrels could thus provide a test of whether social complexity is correlated with alarm-call repertoire in related taxa that differ from ground squirrels in habitat type.

Comparing the alarm calling systems of tree squirrels to those of arboreal primates provides another interesting comparison, because they share habitat type and the associated variety of predator-escape strategies, but primates tend to form more stable and complex social groups than tree squirrels. Arboreal primates also differ from tree squirrels in diet, external anatomy, perhaps cognitive ability, and often size. Additional studies of tree squirrel alarm systems will provide comparisons useful for examining the effect of these differences on alarm-call systems. Researchers have made significant progress toward understanding the information contained in alarm signals of terrestrial ground squirrels and arboreal primates. To increase our overall understanding of alarm calling systems, and of the principles governing animal communication systems in general, studies of the information contained in the alarms of arboreal squirrels are a logical next step.

Given the need to expand experimental studies of the information in alarm systems of tree squirrels, particularly predator specificity, eastern gray squirrels are an ideal starting point. They face significant predation risk from terrestrial and aerial predators, which may require different escape strategies. They also demonstrate a typical tree squirrel social system—they are neither territorial nor do they form stable groups. Logistically, gray squirrels are widespread and abundant, making experimental field studies feasible. As a step toward identifying the information content of gray squirrel alarm calls, in the following chapters I present results of an examination of eastern gray squirrels' use of tail signals and vocal signals in response to terrestrial and aerial threats.

CHAPTER 3

EASTERN GRAY SQUIRRELS USE MULTIMODAL ALARM SIGNALS AS GENERAL ALARMS AND PREDATOR-SPECIFIC ALARMS

SUMMARY

Eastern gray squirrels are known to use multiple vocal alarm signals and sometimes to use them in conjunction with tail signals. In an experiment to determine predator- specificity of alarm signals, I presented individually identified squirrels with a model cat and a model hawk to simulate attacks by aerial and terrestrial predators. In response to the stimuli, squirrels used two tail signals (small twitches and large flicks) and three acoustically distinct alarm calls (kuks, quaas, and moans). Squirrels used twitches, kuks, and quaas independently of stimulus type when presented with aerial or terrestrial stimuli. In contrast, flicks and moans were associated with stimulus type, with moans used almost exclusively in response to the model hawk. Flicks were used more often in response to the model cat, but did not have the same level of production specificity as moans.

In some studies demonstrating predator-specific alarm calling, it has been unclear whether predator type (raptor, carnivore, etc.) or the predator's manner of approach was driving the types of alarm signals used. To test whether squirrels use specific signals based on manner of approach, I presented squirrels with a 30cm red ball, that approached either aerially, like the model hawk, or terrestrially, like the model cat. Squirrels again showed specificity of some signals and not of others, but the pattern of signal use in response to the ball approaching aerially versus terrestrially did not match the pattern seen in response to the two model predators. Kuks, quaas, and vocalizing in general were each associated with the ball's manner of approach, with the aerial ball eliciting fewer

vocalizations in each case. Twitches, flicks, and tail signaling in general did not show any association with the ball's manner of approach. A control for the effects of the base used in terrestrial presentations examined signal use elicited by the base alone and the base with the model cat. This test showed that tail and vocal signals in response to the base approaching alone versus the model cat on the base were independent of terrestrially approaching object type.

It appears that the nature of terrestrially approaching objects does not have a major effect on focal squirrels' use of vocal and tail signals, but that the type of aerially approaching objects does significantly affect response. To directly test the effects of the approaching object's physical appearance, I compared responses to the model predator versus the ball when they are presented in the same manner. Alarm signals were used independently of whether the terrestrially approaching stimulus was a model cat or the terrestrial ball. When comparing the aerially approaching stimuli, whether a squirrel vocalized was associated with type of aerially approaching object. The overall pattern of more frequent vocal signal use in response to the hawk was reflected in the specific analysis. Quaa use was associated with aerial object type, with quaas used more often to the hawk. When examining tail signal use, twitches, flicks, and tail signaling in general did not show any association with whether the aerial object was the model hawk or the ball. It appears that the physical appearance of an approaching object has a significant effect on squirrels' responses to aerially approaching objects, but not on their responses to terrestrially approaching objects. I conclude that eastern gray squirrels use a multimodal alarm-signaling system where particular signal types in each modality can specify predator type or be used as general alarms. This is the first such multimodal alarm-signaling system to be described.

BACKGROUND

FUNCTIONALLY REFERENTIAL SIGNALS

Functionally referential signals are signals that function as if they refer to an external object. Aside from studies of human communication, most experimental studies of putative functionally referential signals have focused on alarm calls, beginning with Seyfarth et al.'s (1980) seminal work verifying that vervet monkeys (*Cercopithecus aethiops*) in Kenya use acoustically distinct alarm call types for leopards, eagles, and snakes. Further, they demonstrated that vervets hearing these calls in the absence of a threat respond in a manner appropriate to the threat that normally elicits that call type. Vervets are primarily terrestrial but usually seek refuge in trees when threatened. Subsequent studies by researchers around the world have revealed similar functionally referential alarm signals in Barbary macaques (*Macaca sylvanus*) (Fischer & Hammerschmidt 2001), as well as several semiarboreal or arboreal primates: Diana monkeys (*Cercopithecus diana*) (Zuberbühler 2000), blue monkeys (*Cercopithecus mitis stuhlmanni*) (Papworth et al. 2008), putty-nosed monkeys (*Cercopithecus nictitans martini*) (Arnold & Zuberbühler 2006), and ring-tailed lemurs (*Lemur catta*) (Macedonia 1990), among others.

The terrestrial suricates (*Suricata suricatta*) use a complex alarm-call system that includes both general alarms and predator-class specific alarms and clearly codes level of urgency in the same vocalizations (Manser 2001). Many studies have also tested for functionally referential alarm systems in ground squirrels: California ground squirrels

(*Spermophilus beecheyi*) (Van Lanen 2010), eastern chipmunks (*Tamas striatus*) (Weary & Kramer 1995), Richardson's ground squirrels (*Spermophilus richardsonii*) (Davis 1984), black-tailed prairie dogs (*Cynomys ludovicianus*) (Frederiksen & Slobodchikoff 2007), and yellow-bellied marmots (*Marmota flaviventris*) (Blumstein & Armitage 1997a). Rather than referring to type of predator, ground squirrel alarms primarily refer to urgency of the threat, which may at times correspond to predator type as well (Macedonia & Evans 1993).

It has been hypothesized (Furrer & Manser 2009) that functionally referential alarm systems should evolve only when the prey have multiple strategies to respond to a threat, and different threats are best evaded by different strategies. Predator-specific calls can then inform receivers about the type of threat, enabling them to employ the appropriate escape strategy without waiting to identify the predator type through direct observation. Among the various threats experienced by small mammals when on the ground, aerial and terrestrial predators are among the most divergent in their manner of hunting, with true terrestrial predators limited to attacking from the ground, and aerial predators consistently striking from above. If a prey species does have different escape strategies, its escape strategies are likely to differ in response to aerial versus terrestrial threats, which explains why referential systems often have different alarm calls for terrestrial versus aerial threats.

Despite the multitude of studies testing for referential signaling in terrestrial ground squirrels, only one species of tree squirrel, the North American red squirrel (*Tamisciurus hudsonicus*), has been tested experimentally for the presence of functionally referential signals. Results in the red squirrel are mixed (Greene & Meagher 1998;

Digweed & Rendall 2010) but seem to show that different alarm calls or different calling patterns are not used for different classes of predator. The red squirrel is unusual among tree squirrels for its extreme territoriality, and Digweed and Rendall (2010) interpret calls in response to predators as general intruder calls. The red squirrels' territoriality is likely to influence their alarm calling system, making them useful for comparisons of alarm systems between sciurids of differing sociality. When comparing effects of habitat complexity on the use of predator-specific alarm systems, red squirrels' territoriality becomes a confounding factor. The role of sociality in alarm calling is not well understood, but in ground squirrels Blumstein and Armitage (1997) found a significant role of social complexity on alarm-signal repertoire.

Unlike red squirrels, most other tree squirrel species do not defend territories. Instead, most tree squirrels have undefended, extensively overlapping home ranges and freely associate with conspecifics, even occupying the same tree, although most species do not form stable groups as do colonial ground squirrels and most arboreal primates. Tree squirrels are closer phylogenetically to ground squirrels with urgency-based alarm systems, but closer in habitat type (and thus potential escape strategies) to arboreal primates with referential alarm systems using predator-specific calls. Nonterritorial arboreal squirrels thus provide the opportunity to test whether habitat complexity is sufficient to drive the evolution of predator-specific alarms.

If the evolution of functionally referential alarm systems is driven by having different escape strategies for different predators, then nonterritorial arboreal squirrels, which are preyed on by terrestrial and aerial predators and live in a complex habitat, should use referential alarms much like those of many arboreal primates. If nonterritorial arboreal squirrels do not use predator-specific alarms, it suggests that habitat complexity is not sufficient for the evolution of functionally referential alarm signaling.

MULTIMODAL SIGNALS

Almost all studies of alarm signals focus on vocal signals, which may be appropriate in some species; research on courtship signaling, however, shows that signals in multiple sensory modalities are often working in concert to communicate more information, or communicate more effectively, than signals in one modality alone (Otovic & Partan 2009; Uhl & Elias 2011).

Arboreal squirrels frequently use tail signals as alarms, as Partan et al. (2009) confirmed by presenting wild squirrels with a robotic squirrel. The robo-squirrel “Rocky” mimicked the alarm signals of eastern gray squirrels (*Sciurus carolinensis*) using a tail movement, a vocalization, or both. Whether used together or separately, both the tail signal and the vocal signal elicited alarm calls and antipredator behavior in wild conspecifics, although the response was strongest to the combined visual and auditory stimuli of the tail and vocal signals together. This enhanced response to multimodal signals highlights the need to consider signals in multiple modalities when investigating alarm calling systems. Playback experiments confirm that eastern gray squirrels respond to conspecific alarm calls by increasing time spent scanning and decreasing time spent foraging (present study), as further discussed in Chapter 4.

In the present study, I tested eastern gray squirrels for the presence of predator-specific alarm signals, which are a prerequisite for a functionally referential alarm-signaling system. Eastern gray squirrels are a non-territorial, arboreal species that frequently forage on the ground, providing an interesting comparison to previous studies of arboreal primates and terrestrial ground squirrels as well as the arboreal, but territorial, red squirrel. The eastern gray squirrels (*Sciurus carolinensis*) examined in this study occupy habitat that is structurally much like that of vervets, consisting of grassy areas with scattered trees in a subtropical area where there is foliage year-round.

Based on a search of work published to date, this study is the first in any species to consider alarms in more than one modality while testing for predator-specific alarm signals. I first tested for predator-specific alarm signals by testing whether each individual alarm signal type is associated with whether the threat is a model cat approaching terrestrially or a model hawk approaching aerially. If a signal is predator-specific in this experiment, the specificity could be due to differences in predator type based on physical appearance (colors, shape, and size) of the stimulus object, or based on of an object's manner of approach (terrestrially or aerially). To control for physical resemblance to any particular predator and directly test for effects based on manner of approach, I then examined whether the presence of each individual alarm signal type in a squirrel's response is associated with whether a novel object approached either terrestrially or aerially. Finally, I controlled manner of approach and directly tested for effects of physical resemblance to a predator by running two comparisons. I first compared the model cat to the terrestrial ball, and then compared the model hawk to the aerial ball. I thus examine: (1) the effects of the combination of physical appearance and manner of approach; (2) the effects of manner of approach while controlling physical appearance; and (3) the effects of physical appearance while controlling manner of approach.

METHODS AND MATERIALS

LOCATION

The presentations were carried out on the University of Miami campus in Coral Gables, Florida (80° 16.732'W, 25° 43.393'N), between January 29, 2010, and July 13, 2011. Presentations were conducted in grassy lawns with mature trees consisting primarily of live oaks (*Quercus virginiana*) and several palm species, all of which produced fruits that were regularly eaten and cached by gray squirrels. Squirrels were also seen foraging on oak flowers and licking palm flowers.

SUBJECTS

Gray squirrels are ubiquitous in the urban areas of the eastern United States and have colonized cities around the world, often becoming invasive. I examined an urban population of gray squirrels in Miami, Florida, within their native range. Forty-eight squirrels were trapped on the Coral Gables campus of the University of Miami with Havahart live traps, model 1030, baited with roasted peanuts in the shell. Each captured squirrel was fitted with numbered Monel ear tags (style 1005-1 from National Band and Tag Company, color-coded to enhance individual identifiability), weighed, sexed, and released at the location of capture.

By the study's end, the proportion of tagged squirrels was over 90% of all squirrels sighted in the main study area, a quadrangle located at 25° 43.393′ N 80° 16.732′. Not counting the areas covered by buildings, the entire study area was about 1.35ha. In a study of gray squirrel population in urban parks similar in habitat structure to the present site, Parker and Nilon (2008) measured typical urban gray squirrel densities that ranged from 2.1/ha to 49.1/ha between sites and season. In combination with the low

Figure 3.1. Canon T2i showing the utility of photoscoping in reading ear tags and seeing other detail in the field. Both photos show the entire squirrel when zoomed out. **(A)** The squirrel's ear tag color code (yellow, white, green) is readily visible when fully zoomed in on the photo just taken, in this case from a distance of approximately 13m. **(B)** This photo was taken from about 8m away, and shows even more detail.

encounter rates of untagged squirrels in my study, these urban gray squirrel densities suggest that I marked the majority of squirrels present in my study area (48 individuals in 1.35ha).

INDIVIDUAL IDENTIFICATION

In order to avoid oversampling the responses of any individual squirrel and to control the order of model presentation, it was necessary to identify individual squirrels in the field without recapturing them. The only other experimental studies of predator-specific alarm calls in a tree squirrel species are of the red squirrel, which is highly territorial, making individual identification possible simply by the proximity of the focal squirrel to the midden at the center of its territory (Digweed & Rendall 2009a; Digweed pers. comm.) Previous behavioral studies of gray squirrels in the field have used hair dye (Flyger 1960; Leaver et al. 2007), but dye must be reapplied after several months. Given the duration of the project, I elected to use color-coded ear tags, which only need to be applied once. Each tag was painted with a color code consisting of three different colors of Testors enamel paint to facilitate individual identification from a distance. I used six colors of paint, which provided 120 possible combinations of three non-repeating colors. The enamel color codes endured for 10 to 15 months or longer before wearing off. The unique sequential numbers stamped on the back of each ear tag remained indefinitely, with squirrel number one being the first squirrel captured.

The color-coded side of each ear tag is only 6 by 2.5mm, and squirrels were usually identified from a distance of 10m or more. Initially, a spotting scope was used to read color codes (Tele Vue-76, with a Baader Planetarium Amici Prism Star Diagonal and Tele Vue 8–24mm zoom eyepiece), a method that proved effective when the squirrels were stationary for extended periods. Stationary squirrels are usually in trees, either eating, resting, or sleeping (or dead) (pers. obs.). Squirrels rarely were seen sleeping outside their nests, and a resting squirrel could remain motionless on a tree branch for hours at a time. Because my experimental design required each focal squirrel to be on the ground so I could gauge responses when they are equally susceptible to both aerial and terrestrial threats, I focused on identifying more active squirrels, which frequently descended to the ground to forage or cache food.

Although active squirrels were regularly seen coming to the ground, their near-constant movement made using a spotting scope for identification difficult. To clearly observe ear tags on active squirrels, I used digital SLR cameras (Canon T2i and XSi) in conjunction with telephoto lenses (Canon EF 100-400mm f/4.5-5.6 L IS USM and Canon EF 70-200mm f/4-5.6 IS USM). While photography has long been used to produce photographs that can be carried into the field to aid individual identification in large species such as sperm whales (Dufault & Whitehead 1995) and elephants (Douglas-Hamilton 1972), digital photography offers a new tool for wildlife biologists seeking to identify individuals. All digital SLR cameras (DSLRs) include a review screen on the camera body that can be used to review photos as soon as a picture is taken. Wildlife observers can use the review screen to zoom in and view any portion of a photo at full resolution. The combination of high-quality telephoto lenses and DSLRs enables an observer to capture a stationary image of a moving organism and then, while still in the field, view detail in that stationary image that would not be visible even with a traditional spotting scope trained on a moving subject (Figure 3.1). This method of observation,

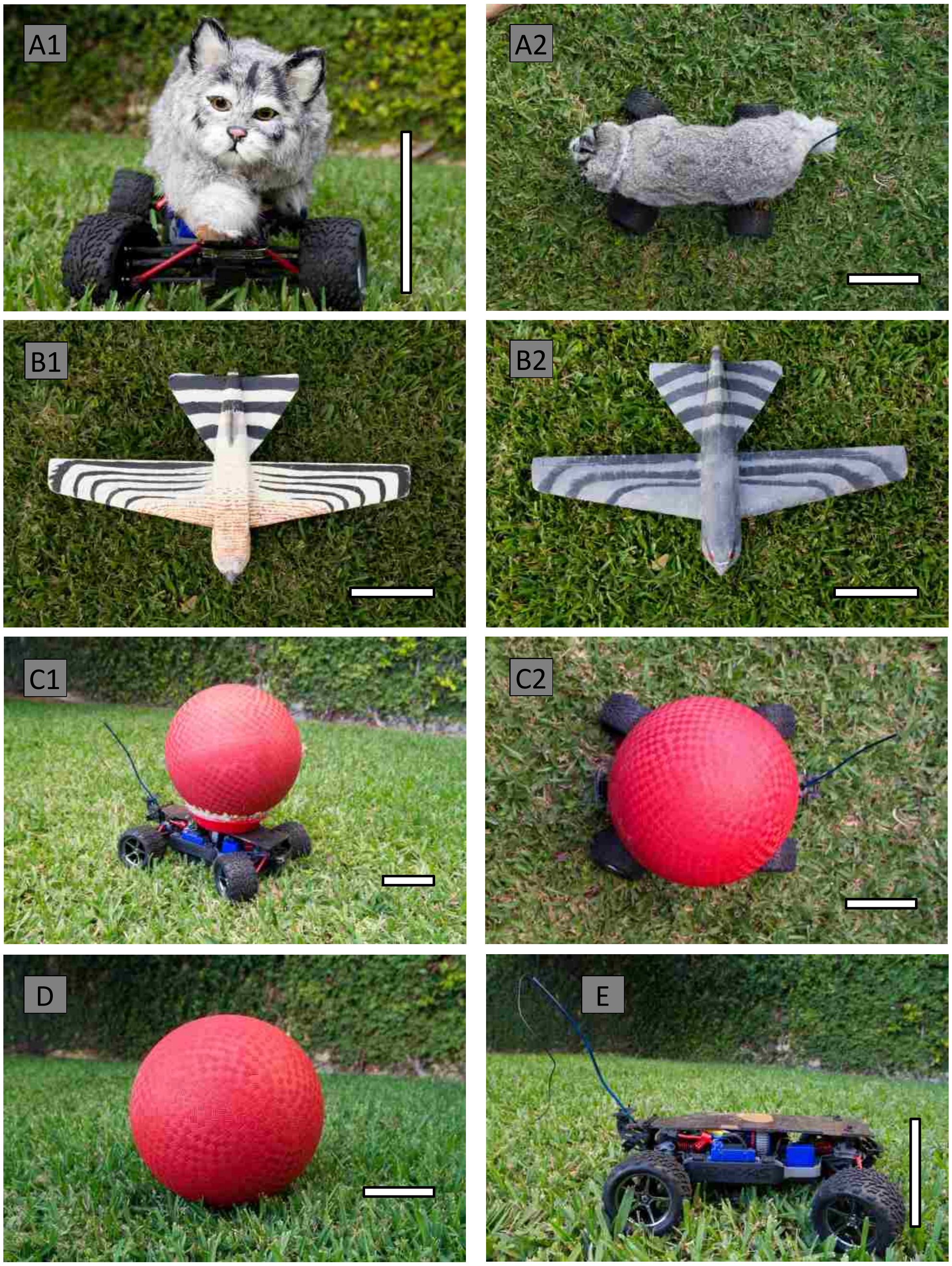

Figure 3.2. The five objects (A-E) used as moving stimuli. All scale bars represent 10cm. **A and B** show the two stimuli designed to resemble real predators and used to simulate predator attacks. **A1 and A2** show the model cat mounted on the radio-controlled motorized base, viewed from the front (A1) and above (A2). **B1 and B2** show the model hawk glider, viewed from below (B1) and above (B2). **C and D** show the red ball used to simulate attack by a novel object, where the same object can approach either terrestrially or aerially. **C1 and C2** show the ball mounted on the base, viewed from a 3/4 front view (C1) and from above (C2). **D** shows the ball alone, which was thrown by hand in the same manner as the hawk glider to simulate attack by an aerially-approaching novel object. **E** shows the base alone, which was included to test for specific effects of the red ball and cat model.

using telephoto lenses and DSLRs to make photographs and then zooming in on digital images to observe details while still in the field, is a novel use of photographic equipment that could prove very useful to wildlife researchers investigating questions that require them to observe details of moving subjects from a distance. This method, which I term "photoscoping[1]" has the additional benefit of providing a permanent record of each observation in the form of a digital photograph.

EXPERIMENTAL STIMULI

To simulate encounters with terrestrial and aerial threats, five moving stimulus objects were presented to individual squirrels—three approaching terrestrially and two approaching aerially (Table 3.1, Figure 3.2). The three terrestrially approaching stimuli consisted of a radio-controlled, motorized, wheeled base (a modified Traxxas eRevo mini) under three conditions. The base was driven toward the focal squirrel while carrying a model cat resembling the domestic cat (Felis catus), carrying a red ball approximately 30cm in diameter attached to the motorized base (to control for cat-sized object versus cat), or carrying nothing. The base approaching alone was a control to distinguish effects of terrestrial motion alone from effects specific to the model cat or red ball. The two aerially approaching stimuli consisted of a customized, Styrofoam glider painted and shaped to resemble an adult Cooper's hawk (Accipiter cooperii) and a ball approximately 30cm in diameter, identical to that used on the motorized base but thrown through the air.

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[1] Not to be confused with digiscoping, which is any method of making telephoto photographs using a digital camera (often a point-and shoot with no removable lens) attached to a spotting scope rather than using a digital SLR with a telephoto lens. Digiscoping is occasionally referred to as photoscoping, but digiscoping is the usual term.

These stimuli were chosen to distinguish between potential effects of predator type and manner of approach. The domestic cat and Cooper's hawk are both known to prey on adult gray squirrels in Miami and both are present in the study area. The red ball is a novel object to the squirrels. So squirrels were presented with a model predator approaching in the natural way, either aerially or terrestrially, and a red ball approaching in the same manner as the model predator, either aerially or terrestrially. Comparing responses to predator models and a novel object allowed me to distinguish whether squirrels respond similarly to any object approaching in a particular manner or respond uniquely to objects resembling known predators.

Table 3.1. Stimuli used in presentation trials. The cat and hawk models were designed to mimic natural predators, while the ball was intended to be an object that could approach either terrestrially or aerially and not resemble a known threat. All terrestrial stimuli were either mounted on or consisted of a radio-controlled (RC) motorized base. The aerial objects were each thrown by hand in the direction of the focal squirrel.

stimulus name	manner of approach	type of object
model cat	terrestrial (on RC base)	simulated predator
terrestrial ball	terrestrial (on RC base)	novel object
base alone	terrestrial (it is the RC base)	control object/novel object
model hawk	aerial (hand tossed)	simulated predator
aerial ball	aerial (hand tossed)	novel object

EXPERIMENTAL DESIGN

To eliminate any order effects a balanced order design was used where each individual squirrel was assigned to a unique, predetermined sequence of the five stimulus presentations based on the order of initial capture (and thus tag number). These sequences were ordered so that the first stimulus received by each consecutive squirrel rotated through the five stimuli.

Once a squirrel was identified in the field and was on the ground exhibiting calm behavior (Table 3.2), audio and video recording began while the next stimulus in its sequence was prepared and sent toward the squirrel from an initial distance of 10 meters. All stimuli were kept together in a large opaque plastic bin (Rubbermaid Roughneck Storage Box, 18 gallon) when not in use, and the bin was used to screen the stimulus object from the focal squirrel's view until the moment the stimulus was sent toward the squirrel. Each presentation trial consisted of one stimulus presentation and lasted from the initial presentation of the stimulus until two minutes after the squirrel was silent and had resumed calm behavior or until the squirrel left the area if it did so before it resumed calm behavior for two minutes. No squirrel received more than two presentation trials in one day, and if it received two trials in a day they were separated by at least 45 minutes.

Table 3.2. Behaviors used to define calm behavior in determining trial length. Intermittent scanning while on all four feet was normal behavior for foraging squirrels and so brief (<3s) periods of scanning on all fours were considered neutral behavior and did not extend the length of the trial. For more extensive definitions, see table 5.2.

behavior code	behavior name	calm
FC	foraging/caching	calm
ET	eating	calm
GR	grooming	calm
CH	chasing	calm
WC	walking, climbing or single jumps	calm
AR	resting	
SL	sleeping	
ON	other behavior (not predator associated)	calm
S4	scanning on all fours	neutral/antipredator
S2	scanning upright	antipredator
RN	running (not in chase with squirrel)	antipredator
HD	hiding (head and belly against surface, not resting)	antipredator
OP	other predator-associated behavior	antipredator

ANALYSIS OF VOCALIZATIONS

Audio recordings of every presentation trial were made in either 16-bit linear PCM format at a 48kHz sampling rate using a Marantz PMD660, or in 24-bit linear PCM format at a 96.7kHz sampling rate using a Marantz PMD661. A pair of Sennheiser ME67 microphones was used with black WindCutter windscreens.

Background noise in urban areas has the potential to change vocal behaviors of urban populations relative to their rural counterparts in birds (Rabin & McCowan 2003) and perhaps eastern gray squirrels (Partan et al. 2010). My study site has continuous noise from surrounding buildings, primarily produced by their HVAC systems. Sound-pressure levels were measured during and after the study at several spots where recordings were made. Ambient sound-pressure levels, with no wind, in the absence of people walking or talking and with no vehicles running within earshot varied from 49 to 56 decibels A-weighted and 65 to 70 decibels C-weighted. Aside from wind noise, background noise was very similar in sound pressure level and frequency composition throughout the study site, with low frequencies being quite loud and the noise levels tapering to near silence at higher frequencies (Figure 3.3). Regular disturbances (leaf blowers, lawn mowers, buses, people, etc.) would occasionally raise the C-weighted and A-weighted sound pressure levels to over 80dB. Figure 3.3 shows a sound spectrum representative of the sound spectra of background noise throughout the study site year-round. Sound pressure measurements were made with an American Recorder Technologies SPL-8810 sound-pressure level meter at 1m above ground level.

All squirrel vocalizations were analyzed using Raven Pro 1.4 software (Bioacoustics Research Program 2011). Spectrograms were produced using a short-time Fourier transform (STFT) with a Hanning window of 1024 samples and each audio file was scanned visually on the computer monitor and by ear for squirrel vocalizations. Each vocalization was classified as either a kuk, quaa, or moan after Lishak (1984), and no

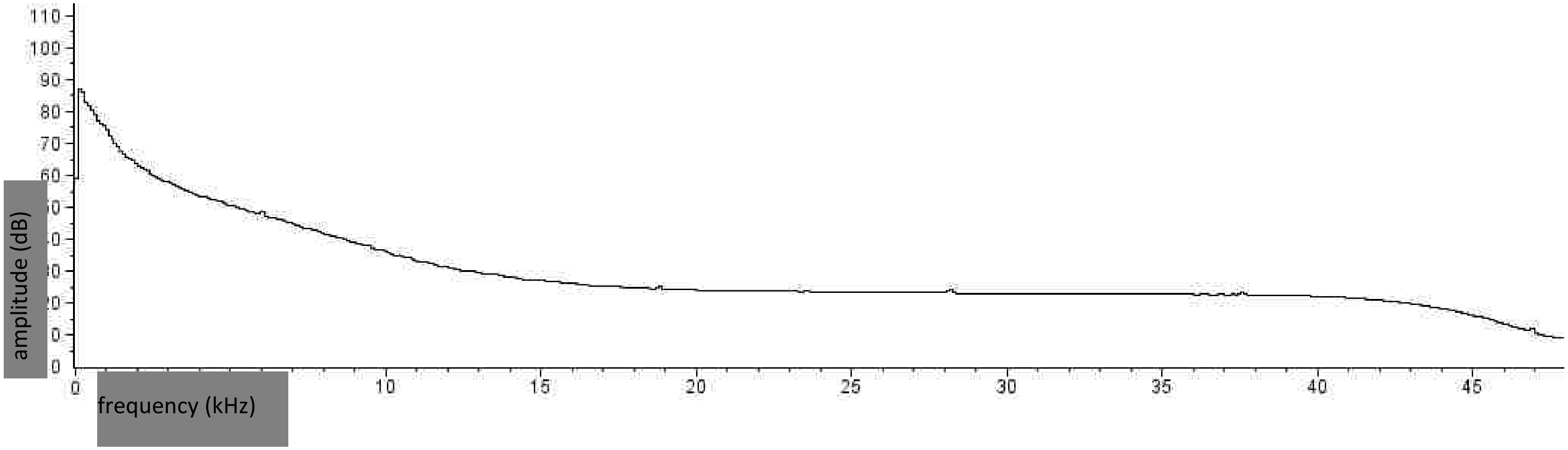

Figure 3.3. Sound spectrum of background noise. Produced in Raven Pro 1.4 from 120s of background noise recorded July 8, 2011, at the University of Miami, Coral Gables, Florida. The high amplitude below 5kHz overlaps the fundamental and dominant frequencies of most eastern gray squirrel vocalizations.

other vocalizations were found. While kuks are of very short duration, quaas and moans are longer and are separated from kuks by length (Lishak 1984). Quaas are broadband vocalizations, usually with indistinct harmonics. When harmonic bands of quaa vocalizations were visible in the spectrogram, there were eight or more harmonics visible, and the harmonics were very closely spaced (Figure 2.2, 3.4). To a human listener, quaas sound harsher, more noisy, and usually louder than moans. Moans, in contrast to quaas, were defined by the presence of clear harmonics and had fewer than eight visible harmonic bands, often only two. See Chapter 2 of this study for details of call categorization. In determining which vocal signals were used in each trial, I considered a signal to be present if it was produced at least once between the time the stimulus object was presented and the time the squirrel resumed normal behavior for two minutes or left the area.

ANALYSIS OF TAIL SIGNALS

In addition to audio recordings, video recordings of every presentation trial were made using a Panasonic HDC-SD10 camcorder at 1080p 30fps AVCHD. Focal squirrels' use of tail signals was scored using Sony Vegas 9 or 11 to play back the video recordings (Sony 2009; Sony 2011). Sony Vegas enables video to be slowed or viewed frame-by-frame while showing the cumulative duration of a video to the nearest millisecond, therefore permitting accurate identification of tail signals and noting the time to the nearest frame (0.033s resolution). In determining which tail signals were used in each trial, I considered a signal to be present if it was produced at least once between the time the stimulus object was presented and the time the squirrel resumed normal behavior for two minutes or left the area.

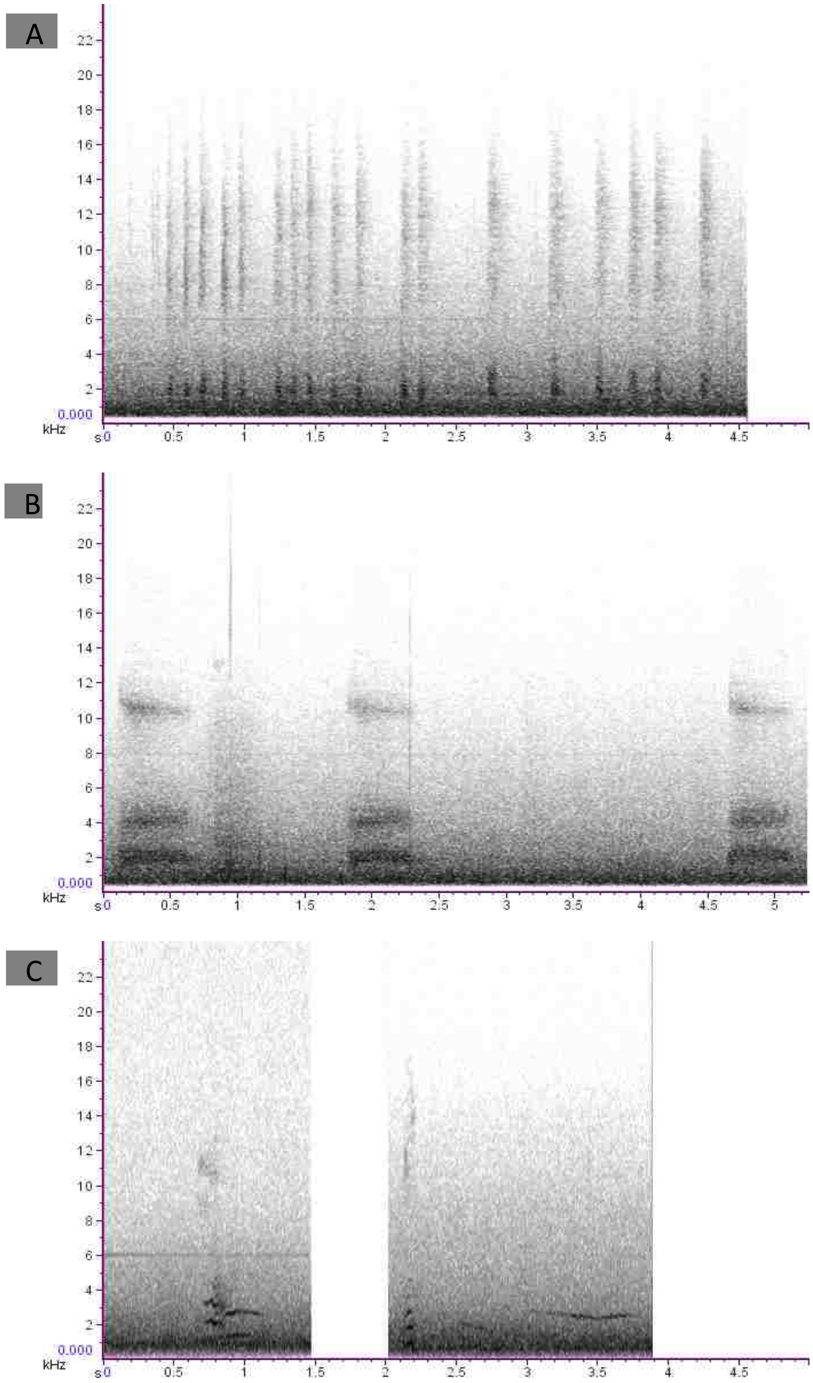

Figure 3.4. Spectrograms of alarm vocalizations observed in eastern gray squirrels. All spectrograms were created in Raven Pro 1.4, using a 1024 sample Hanning window on digital recordings originally recorded with a 96kHz sample rate. The dark background, especially at frequencies below 2kHz, represents the constant background noise of the urban setting; further lightening or increasing the contrast in the image results in significant loss of detail. **(A)** Kuks, in this case a rapid sequence from the start of an alarm calling bout. **(B)** Quaas, which are usually used in the same bouts with kuks. **(C)** Moans, from two different recordings, showing the narrow harmonic bands that tend to gradually trail off in each vocalization.

STATISTICAL ANALYSES

Each trial was thus scored for the presence or absence of each type of vocal or tail signal element (three vocal elements: kuks, quaas, and moans; and two tail elements: twitches and flicks). Contingency tables were constructed from the presence and absence data for each individual signal type. Fisher's exact test probabilities were calculated for 2 x 2 contingency tables and larger tables were analyzed using Fisher-Freeman-Halton exact tests for r x c contingency tables. When significant associations ($p<0.05$)were found, the asymmetrical index of predictive association (asymmetric lambda) was also calculated to measure the overall decrease in uncertainty of predicting the stimulus type given knowledge of a particular signal type's presence in a squirrel's response. Because all tests were of a priori hypotheses predicated on the experimental design, no Bonferroni corrections are employed. All statistical analyses were calculated using JMP Pro, Version 9 (SAS Institute Inc., Cary, NC, 1989-2011).

RESULTS

ALARM SIGNALS OBSERVED

VOCAL ALARM SIGNALS OBSERVED

Overall, gray squirrels vocalized in response to only 15% of the presentations. During stimulus presentations, the only vocalizations used by squirrels were kuks, quaas, and moans. Similarly, during incidental observations of natural encounters with predators only kuks, quaas, and moans were used. Squirrels used other kinds of call elements in mating chases, aggressive interactions, and other social contexts. Except in response to experimental trials and natural encounters with predators, squirrels were never observed to vocalize unless they were directly interacting with another squirrel.

TAIL ALARM SIGNALS OBSERVED

Squirrels used tail signals more frequently than vocalizations, tail signaling occurs in response to 69% of the presentations. There is no published ethogram of gray squirrel tail signals, so I defined tail signals as any cyclical movement of the tail while a squirrel's feet were in the same location (i.e. the squirrel was not shifting its body, walking, jumping, or running). Raising and then immediately lowering the tail while standing still was considered to be one tail signal. Simply shifting the tail without a return movement was not considered a tail signal.

I further classified tail signals into qualitative categories based on the magnitude of the angle of movement by the anterior half of the tail during one cycle of movement and return. The angle was estimated relative to the starting position of the tail, so the tail's starting position did not affect classification of tail signals. The tail moving at least 45 degrees was used as the classification criterion because most tail signals fell clearly on one side or the other of 45 degrees and the overall shape of movement differed qualitatively between signals greater or less than 45 degrees in amplitude. Movements less than 45 degrees are labeled "twitches" and movements greater than 45 degrees "flicks." Twitches looked like a wave running through the tail, which remained mostly parallel to either the substrate or the squirrel's body with most of the movement along the dorso-ventral axis. Twitches were occasionally used with the tail raised over the back while the squirrel was standing on all fours or sitting on its haunches. More often, the tail was held parallel to the surface of the tree while the squirrel was on the trunk or a large branch.

Flicks looked qualitatively different from twitches. Rather than remaining generally parallel to the substrate or squirrel's body, as with twitches, tail flicks caused the tail to make a whipping motion, where the tip of the tail curved back toward the base of the tail as the tail changed direction. Flicks also frequently included movement in the dorso-ventral and lateral axes simultaneously, which in conjunction with their greater amplitude produced large, conspicuous movements. While flicking, the path of the tail's tip varied widely, following arcs, figure eights, circles, and various squiggles. In contrast, twitches were more controlled, so the tail tip usually followed a simple short arc.

In addition to their use by squirrels responding to the experimental stimuli, twitches were occasionally observed in foraging squirrels, especially when descending a tree trunk, despite the absence of any apparent source of danger. Flicks were never observed except in response to experimental stimuli or natural encounters with terrestrial predators.

EXPERIMENTAL RESULTS

MODEL CAT VS. MODEL HAWK

Comparing squirrels' vocal signal use in response to the model predator stimuli (model cat, $N = 34$; model hawk, $N = 29$) showed that squirrels did not often vocalize, regardless of predator type (Table 3.3, Figure 3.5). Kuks were used in 11.8% of encounters with the model cat and 17.2% of encounters with the model hawk. Quaas were used in 8.8% of encounters with the model cat but 20.7% of encounters with the model hawk. Kuk and quaa vocalizations were used independently of predator type, with squirrels responding to approach of the model cat and model hawk similarly (Fisher's exact tests: kuk use, $P = 0.721$; quaa use, $P = 0.280$). Moans were used in only 2.9% of encounters with the model cat and 24.1% of encounters with the model hawk. There was, therefore, an effect of predator type on whether the focal squirrel used moans (Fisher's exact tests, $P = 0.0195$), with the model hawk eliciting moans more often than the model cat.

Table 3.3. Vocal signal use in response to model predator type. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each vocal signal (kuk, quaa, and moan) and vocal signals in general (any vocal signal), in response to the model hawk and model cat.

	kuk	no kuk	quaa	no quaa	moan	no moan	any vocal signal	no vocal signal
model cat (*N* = 34)	4	30	3	31	1	33	4	30
model hawk (*N* = 29)	5	24	6	23	7	22	9	20
Fisher's exact	*P* = 0.721		*P* = 0.280		*P* = **0.0195**		*P* = 0.0705	

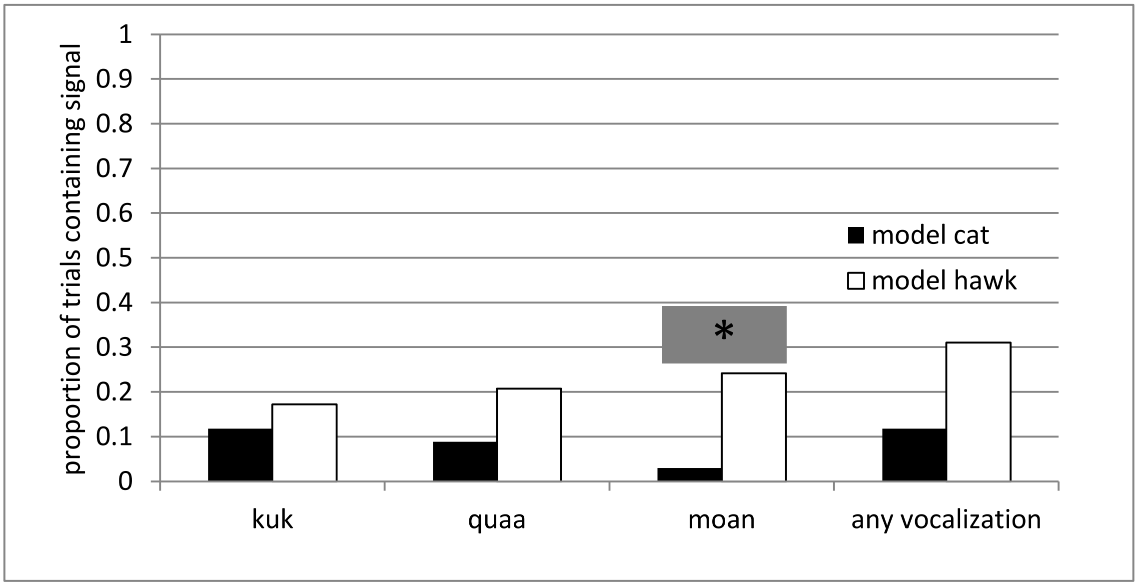

Figure 3.5. Proportion of all model cat (*N* = 34) and model hawk (*N* = 29) trials that elicited kuks, quaas, or moans from the focal squirrel, and proportion of trials with any vocalization. Some calling bouts contained multiple call types and thus contributed to more than one category of call. Kuks and quaas were used as general alarms, independent of threat type, while moans were strongly associated with threat type (Fisher's exact test, *P* = 0.0195).

Squirrels were more likely to use moans in response to the model hawk than the model cat, so moans carry information about the presence of an aerial predator. The presence or absence of a moan provides enough information to reduce the error in correctly predicting predator type by about 21% (asymmetric lamba = 0.207). There was no association of predator type with whether or not a squirrel vocalized (Fisher's exact test, *P* = 0.0705).

Table 3.4. Tail signal use in response to model predator type. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each tail signal (twitch and flick) and tail signals in general (twitch or flick), in response to the model cat and model hawk.

	twitch	no twitch	flick	no flick	any tail signal	no tail signal
model cat (*N* = 34)	24	10	15	19	25	9
model hawk (*N* = 29)	19	10	5	24	19	10
Fisher's exact		*P* = 0.788		*P* = **0.031**		*P* = 0.586

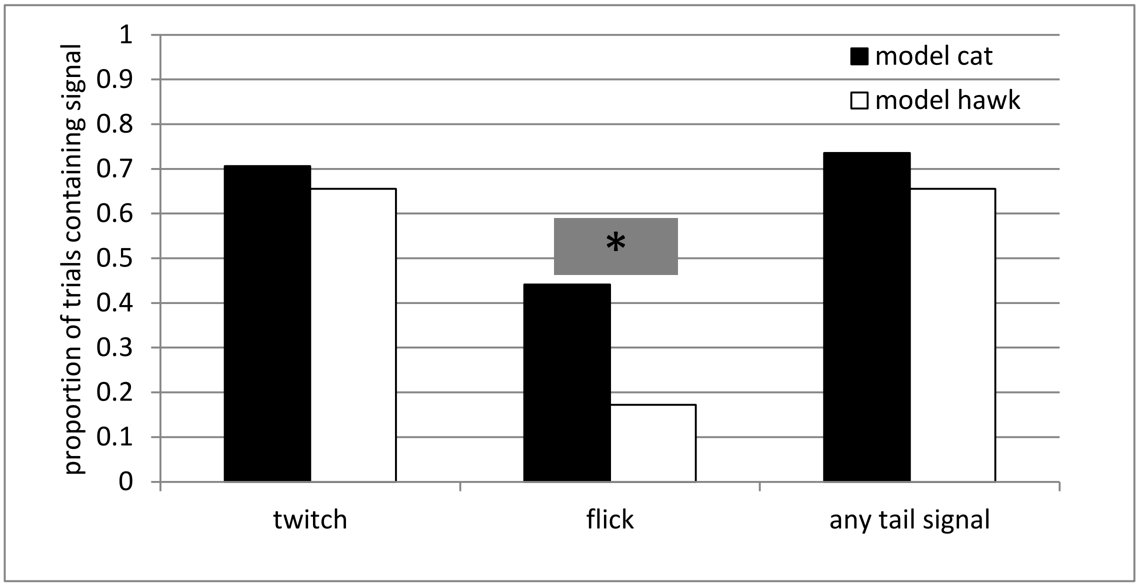

Figure 3.6. Proportion of all model cat (*N* = 34) and model hawk (*N* = 29) trials that elicited twitches or flicks from the focal squirrel, and the proportion of trials with any tail signal. Some signaling bouts contained multiple tail signal types and thus contributed to more than one category. Twitches were used as general alarms, independent of threat type, while flicks were associated with threat type (Fisher's exact test, *P* = 0.031).

Comparing squirrels' tail signal use in response to model predator stimuli (model cat, *N* = 34; model hawk, *N* = 29) showed that squirrels used tail signals as either general or specific alarms (Table 3.4, Figure 3.6). Tail twitches were used in 70.6% of encounters with the model cat and 65.5% of encounters with the model hawk; their usage is therefore independent of predator type (Fisher's exact tests, $P = 0.788$). Flicks, however, were used in 44.1% of encounters with the model cat but only 17.2% of encounters with the model hawk. There was, therefore, an association of predator type with whether the focal squirrel used flicks (Fisher's exact tests, $P = 0.031$). Squirrels were more likely to use flicks in response to the model cat than the model hawk, so flicks carry information about the presence of a terrestrial threat. The presence or absence of a flick provides enough information to reduce the error in correctly predicting predator type by about 17.2% (asymmetric lamba = 0.172). There was no association of predator type with whether or not a squirrel used tail signals (Fisher's exact test, $P = 0.586$). Squirrels were no more likely to use tail signals in reponse to one type of predator than the other.

TERRESTRIAL VS. AERIAL APPROACH OF A NOVEL OBJECT

To determine whether kuks, quaas, and moans are associated with whether a threat approaches terrestrially versus aerially, thus specifying the type of threat based on mode of approach, I compared signal use in response to a red ball approaching either terrestrially ($N = 29$) or aerially ($N = 33$) (Table 3.5, Figure 3.7). When comparing vocal signals used in response to the terrestrial ball and aerial ball, a clear pattern emerges (Table 3.5, Figure 3.7). Kuks were used in 20.6% of encounters with the terrestrial ball and 3.4% of encounters with the aerial ball, showing an association with the manner of approach (Fisher's exact test, $P = 0.0206$). Similarly, quaas were used in 14.7% of encounters with the terrestrial ball and never in encounters with the aerial ball and thus also show an association with the manner of approach (Fisher's exact test, $P = 0.0184$). Although they are not predator-specific in response to model predators (Table 3.5), both kuks and quaas carry information about whether a threat is terrestrially or aerially approaching when considering the same novel object approaching in either manner. The presence or absence of a kuk provides enough information to reduce the error in correctly predicting the manner of approach by about 21% (asymmetric lamba = 0.207). The presence or absence of a quaa provides enough information to reduce the error in correctly predicting the manner of approach by about 17.2% (asymmetric lamba = 0.172).

Table 3.5. Vocal signal use in response to terrestrial ball versus aerial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each vocal signal (kuk, quaa, and moan) and vocal signals in general (any vocal signal), between a red ball approaching aerially and terrestrially .

	kuk	no kuk	quaa	no quaa	moan	no moan	any vocal signal	no vocal signal
terrestrial ball (*N* = 29)	7	22	5	24	0	29	8	21
aerial ball (*N* = 33)	1	32	0	33	2	31	2	31
Fisher's exact		*P* = **0.0206**		*P* = **0.0184**		*P* = 0.494		*P* = **0.0356**

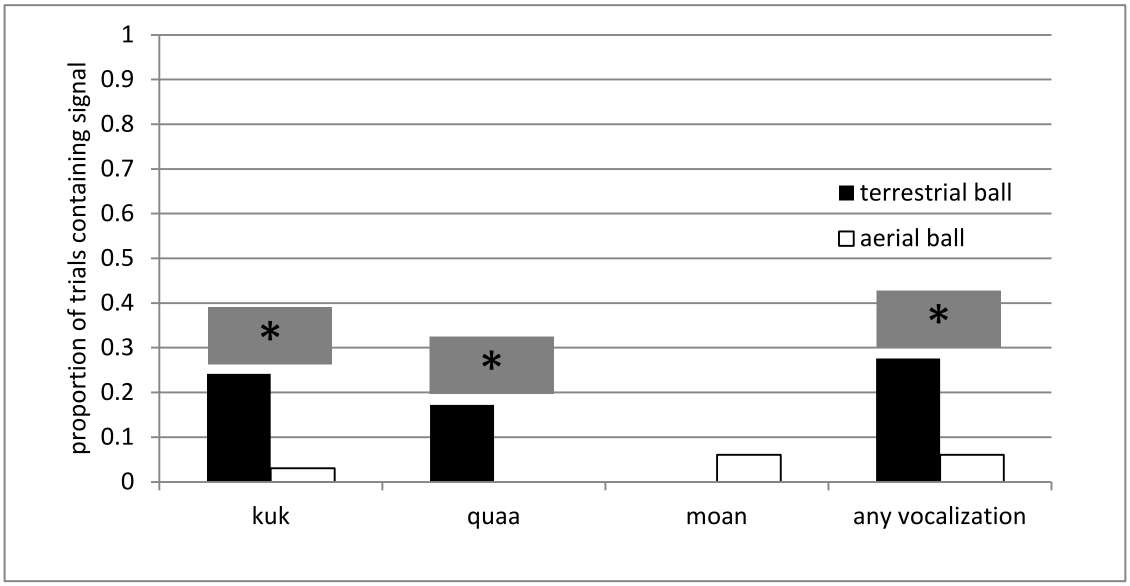

Figure 3.7. Proportion of all terrestrial ball (*N* = 29) and aerial ball (*N* = 33) trials that elicited kuks, quaas, or moans from the focal squirrel, and proportion of trials with any vocalization. Some calling bouts contained multiple call types and thus contributed to more than one category of call. Kuks and quaas were used more often in response to the terrestrially-approaching ball, while moans were not associated with threat type. Squirrels were also more likely to vocalize, regardless of signal type, in response to the ball when it was approaching terrestrially.

Moans were used in only two trials, both times in response to the aerial ball, and therefore show no association with manner of approach (Fisher's exact test, $P = 0.494$). Overall, squirrels vocalized in 23.5% of the terrestrial ball trials but only 6.9% of the aerial ball trials, showing an association between vocalizing and manner of approach (Fisher's exact test, $P = 0.0356$). Whether or not squirrels vocalized thus also carries information on the manner of the ball's approach. If this information is used it would reduce the error in correctly predicting the manner of approach by about 21% (asymmetric lamba = 0.207).

To determine whether tail twitches and flicks are associated with whether a threat approaches terrestrially versus aerially, thus specifying the type of threat based on mode of approach, I compared tail signal use in response to a red ball approaching either terrestrially ($N = 29$) or aerially ($N = 33$) (Table 3.6, Figure 3.8). In contrast to the pattern seen with vocal signals, tail signals used in response to the terrestrial versus aerial ball show no clear pattern (Table 3.6, Figure 3.8). Twitches were used in 67.6% of encounters with the terrestrial ball and in 69.0% of encounters with the aerial ball. As with the model predators, squirrels used twitches independently of whether the ball approached terrestrially or aerially (Fisher's exact test, $P = 0.168$). Flicks were used in 52.9% of encounters with the terrestrial ball and 44.8% of encounters with the aerial ball. Flicks were therefore not associated with the manner of the ball's approach, showing no association with whether the ball approached terrestrially or aerially (Fisher's exact test, $P = 0.126$). The overall likelihood of tail signaling also shows no association with manner of the ball's approach (Fisher's exact test, $P = 0.091$).

Table 3.6. Tail signal use in response to terrestrial ball versus aerial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each tail signal (twitch and flick) and tail signals in general (twitch or flick), between a red ball approaching aerially and terrestrially.

	twitch	no twitch	flick	no flick	any tail signal	no tail signal
terrestrial ball (*N* = 29)	23	6	18	11	24	5
aerial ball (*N* = 33)	20	13	13	20	20	13
Fisher's exact		*P* = 0.168		*P* = 0.126		*P* = 0.091

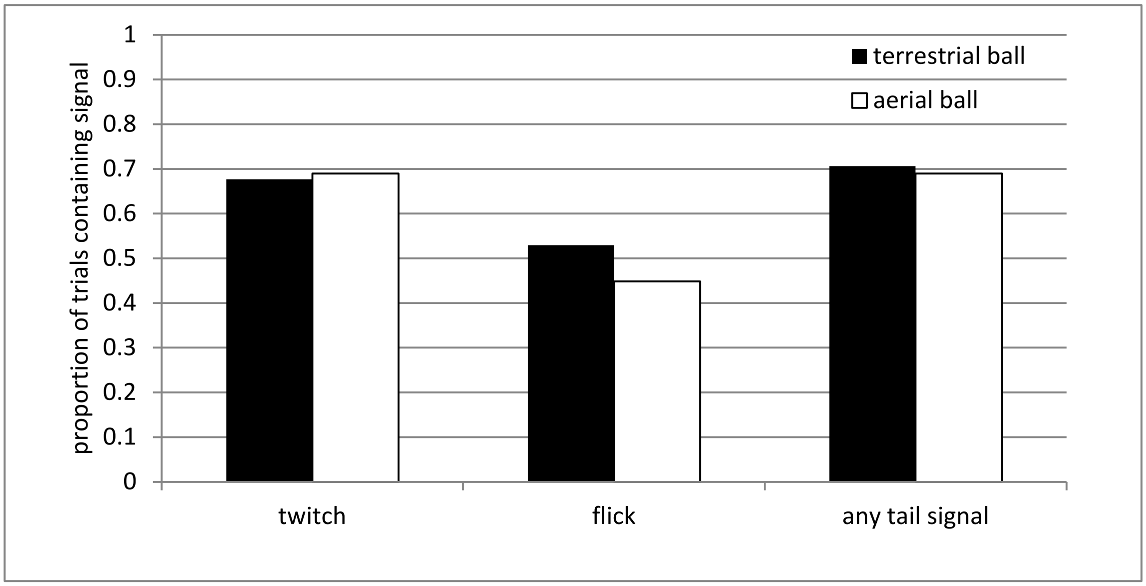

Figure 3.8. Proportion of all terrestrial ball (*N* = 29) and aerial ball (*N* = 33) trials that elicited twitches or flicks from the focal squirrel, and the proportion of trials with any tail signal. Some signaling bouts contained multiple tail signal types and thus contributed to more than one category. Twitches and flicks were used as general alarms, independent of threat type.

Tail signals thus differ between model predators and a novel object in whether they carry information about whether a threat is aerial or terrestrial. Twitches are general alarms, not specifying manner of approach, regardless of whether the object resembles a predator. Flicks, in contrast, carry information about the presence of a terrestrial threat only in the case of predator models and do not specify manner of approach in the case of the ball.

NULL CONTROL – BASE ALONE VS. MODEL CAT ON BASE

While it is possible to present either the hawk model or the aerial ball without any additional experimental paraphernalia, the model cat and the terrestrial ball were each mounted on a RC base to enable their terrestrial approach. To test for effects of the base itself, I compared the use of vocal (Table 3.7) and tail (Table 3.8) signals in response to the model cat versus the base with nothing mounted on it. Kuks were used in 11.8% of encounters with the model cat and 3.2% of encounters with the base alone, quaas were used in 8.8% of encounters with the model cat and 3.2% of encounters with the base alone, and moans were used in11.8% of encounters with the model cat and 3.2% of encounters with the base alone. When examining each vocal signal (Table 3.7), a lack of association with stimulus type is observed (Fisher's exact test, kuks, *P* = 0.358; quaas, *P* = 0.615; moans, *P* = 1). Whether squirrels used vocal signals was also independent of whether the terrestrially approaching object was the model cat or base alone (Fisher's exact test, *P* = 0.358). Although in only a single trial did the focal squirrel vocalize in response to the base alone, it appears that whether or not the base has the model cat mounted does not have a large effect on focal squirrels' use of vocal signals.

Table 3.7. Vocal signal use in response to model cat versus RC base alone. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each vocal signal (kuk, quaa, and moan) and vocal signals in general (any vocal signal), in response to the terrestrial approach of either the model cat or the RC base with nothing mounted on it.

	kuk	no kuk	quaa	no quaa	moan	no moan	any vocal signal	no vocal signal
model cat (*N* = 34)	4	30	3	31	1	33	4	30
RC base alone (*N* = 31)	1	30	1	30	0	31	1	30
Fisher's exact	*P* = 0.358		*P* = 0.615		*P* = 1		*P* = 0.358	

Table 3.8. Tail signal use in response to model cat versus RC base alone. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each tail signal (twitch and flick) and tail signals in general (twitch or flick), in response to the terrestrial approach of either the model cat or the RC base with nothing mounted on it.

	twitch	no twitch	flick	no flick	any tail signal	no tail signal
model cat (*N* = 34)	24	10	15	19	25	9
RC base alone (*N* = 31)	19	12	11	20	19	12
Fisher's exact		*P* = 0.446		*P* = 0.613		*P* = 0.426

When examining tail signals (Table 3.8), twitches were used in 70.6% of encounters with the model cat and 61.3% of encounters with the base alone. Flicks were used in 44.1% of encounters with the model cat and 34.5% of encounters with the base alone. Twitches and flicks were thus independent of whether the terrestrially approaching object was the cat on the base or the base alone (Fisher's exact test, twitches, $P = 0.446$; flicks, $P = 0.613$). As with vocal signals, whether squirrels used tail signals was independent of whether the terrestrially approaching object was the model cat or base alone (Fisher's exact test, $P = 0.426$). As with vocal signals, it appears that whether or not the base is carrying the model cat does not have a large effect on focal squirrels' use of tail signals.

PREDATOR MODEL VS. NOVEL OBJECT

The previous comparison of response to the model cat versus the base alone suggests that the type of approaching object does not have a great effect on squirrels' responses to terrestrially approaching objects. To directly test the hypothesis that response to stimuli of about the same size approaching in the same manner is independent of the their exact physical appearance I compared the response of squirrels to either a model predator or the ball, each approaching in the same manner. I first compare the model cat to the terrestrial ball to test for effects of stimulus appearance on squirrels' use of each signal in response to terrestrial threats, and then compare the model hawk to the aerial ball to test for effects of appearance on squirrels' use of each signal in response to aerial threats.

MODEL CAT VS. TERRESTRIAL BALL – When comparing vocal signals used in response to the model cat versus terrestrial ball no clear association is seen (Table 3.9, Figure 3.9). Kuks were used in 11.8% of encounters with the model cat and 24.1% of encounters with the terrestrial ball, showing no association with the type of terrestrial object (Fisher's exact test, $P = 0.319$). Similarly, quaas were used in 8.8% of encounters with the model cat and in 17.2% of encounters with the terrestrial ball and show no association with the type of terrestrial object (Fisher's exact test, $P = 0.098$). Moans were used in only a single alarm calling bout, and so show no association with the type of terrestrial object (Fisher's exact test, $P = 1$). Whether or not squirrels used vocal signals also showed no association with the type of terrestrial object (Fisher's exact test, $P = 0.358$).

When comparing tail signals used in response to the model cat versus terrestrial ball no clear association is seen (Table 3.10, Figure 3.10). Twitches were used in 70.6% of encounters with the model cat and 79.3% of encounters with the terrestrial ball, showing no association with the type of terrestrial object (Fisher's exact test, $P = 0.564$). Similarly, flicks were used in 44.1% of encounters with the model cat and in 62.1% of encounters with the terrestrial ball and show no association with the type of terrestrial object (Fisher's exact test, $P = 0.208$). Whether or not squirrels used vocal signals also showed no association with the type of terrestrial object (Fisher's exact test, $P = 0.197$).

Table 3.9. Vocal signal use in response to model cat versus terrestrial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each vocal signal (kuk, quaa, and moan) and vocal signals in general (any vocal signal), in response to the model cat or the terrestrial ball.

	kuk	no kuk	quaa	no quaa	moan	no moan	any vocal signal	no vocal signal
model cat (*N* = 34)	4	30	3	31	1	33	4	30
terrestrial ball (*N* = 29)	7	22	5	24	0	29	8	21
Fisher's exact		*P* = 0.319		*P* = 0.098		*P* =1		*P* = 0.197

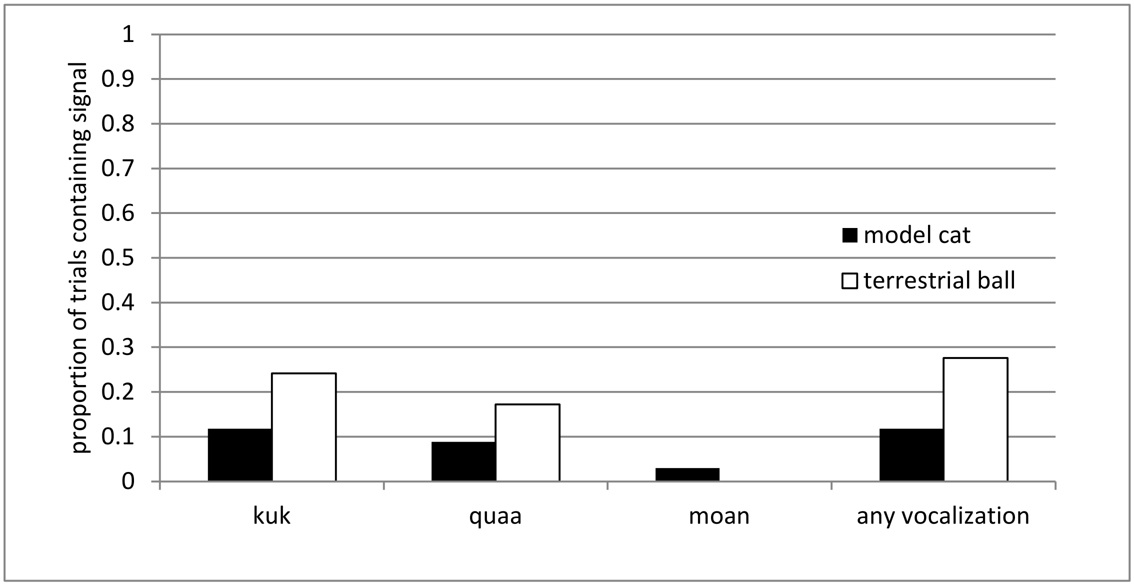

Figure 3.9 Proportion of all model cat (*N* = 34) and terrestrial ball (*N* = 29) trials that elicited kuks, quaas, or moans from the focal squirrel, and proportion of trials with any vocalization. Some calling bouts contained multiple call types and thus contributed to more than one category of call. Kuks, quaas, and moans were not associated with threat type, and neither was whether a squirrel vocalized.

Table 3.10. Tail signal use in response to model cat versus terrestrial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each tail signal (twitch and flick) and tail signals in general (twitch or flick), in response to the model cat or the terrestrial ball.

	twitch	no twitch	flick	no flick	any tail signal	no tail signal
model cat (*N* = 34)	24	10	15	19	25	9
terrestrial ball (*N* = 29)	23	6	18	11	24	5
Fisher's exact		*P* = 0.564		*P* = 0.208		*P* = 0.545

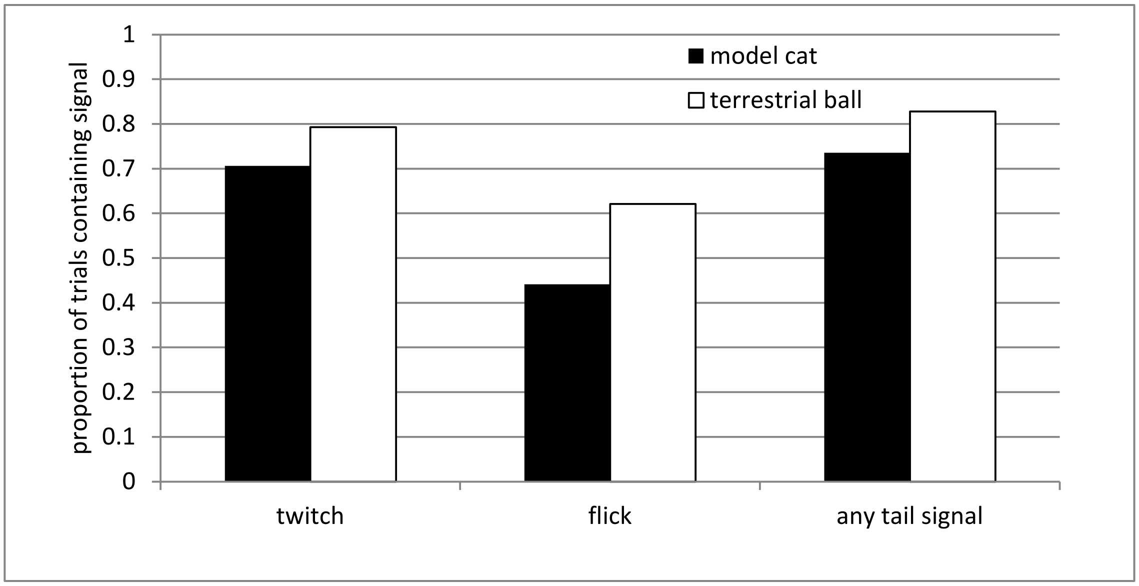

Figure 3.10. Proportion of all model cat (*N* = 34) and terrestrial ball (*N* = 29) trials that elicited twitches or flicks from the focal squirrel, and the proportion of trials with any tail signal. Some signaling bouts contained multiple tail signal types and thus contributed to more than one category. Twitches and flicks were used as general alarms, independent of threat type.

MODEL HAWK VS. AERIAL BALL – In contrast to the lack of association seen when comparing signals used in response to the model cat and the terrestrial ball, when comparing vocal signals used in response to the model hawk versus aerial ball a clear pattern of association is seen (Table 3.11, Figure 3.11). Kuks were used in 17.2% of encounters with the model hawk and 3.0% of encounters with the aerial ball, showing no association with the type of aerial object (Fisher’s exact test, $P = 0.090$). Similarly, quaas were used in 20.7% of encounters with the model hawk and in no encounters with the aerial ball and show an association with the type of aerial object (Fisher’s exact test, $P = 0.008$). Squirrels used quaas exclusively in response to the model hawk, which resembles an actual predator, rather than in response to the aerial ball. Squirrels use quaas in response to aerial objects based not simply on the manner of approach but also on the object’s physical appearance. Moans were used in 20.6% of encounters with the model hawk but only 6.9% of encounters with the aerial ball, but show no association with the type of aerial object

Table 3.11. Vocal signal use in response to model hawk versus aerial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each vocal signal (kuk, quaa, and moan) and vocal signals in general (any vocal signal), in response to the model hawk or the aerial ball.

	kuk	no kuk	quaa	no quaa	moan	no moan	any vocal signal	no vocal signal
model hawk (N = 29)	5	24	6	23	7	22	9	20
aerial ball (N = 33)	1	32	0	33	2	31	2	31
Fisher's exact	P = 0.090		P = **0.008**		P =0.070		P = **0.017**	

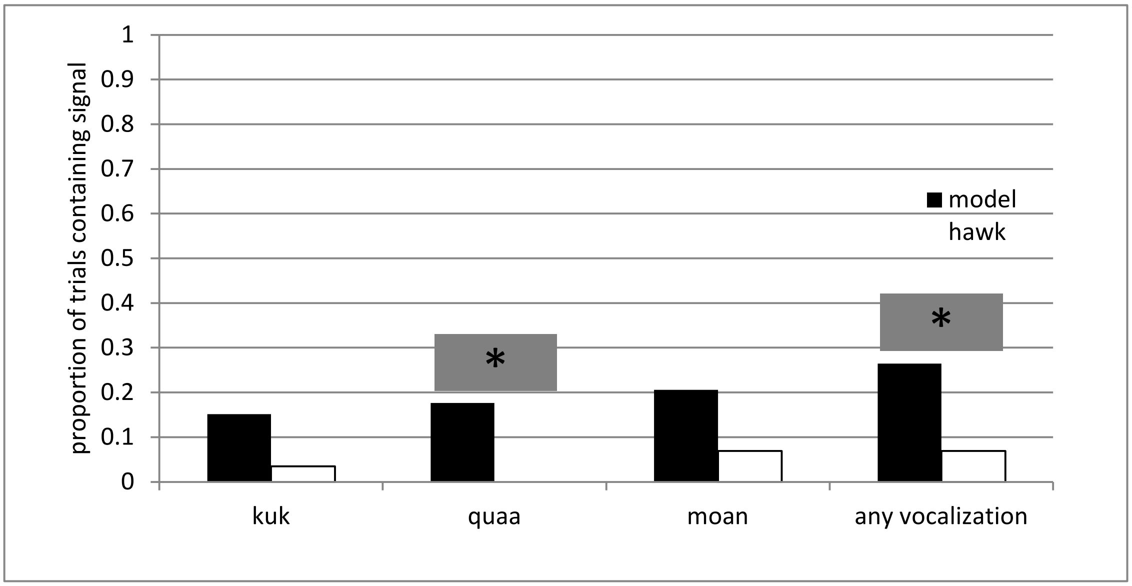

Figure 3.11. Proportion of all model hawk (N = 29) and aerial ball (N = 33) trials that elicited kuks, quaas, or moans from the focal squirrel, and proportion of trials with any vocalization. Some calling bouts contained multiple call types and thus contributed to more than one category of call. Kuks and moans are not associated with threat type. Quaas are associated with type of aerial threat, being used exclusively to the model hawk. Whether a squirrel vocalized at all is also associated with type of aerial object.

(Fisher's exact test, $P = 0.070$). Whether or not squirrels used vocal signals was associated with the type of aerial object (Fisher's exact test, $P = 0.358$), with the model hawk more frequently eliciting vocalizations than the aerial ball.

When comparing tail signals used in response to the model hawk versus aerial ball no clear association is seen (Table 3.12, Figure 3.12). Twitches were used in 55.9% of encounters with the model hawk and 70.0% of encounters with the aerial ball, showing no association with the type of aerial object (Fisher's exact test, $P = 0.564$). Similarly, flicks were used in 14.7% of encounters with the model hawk and in 44.8% of encounters with the aerial ball and show no association with the type of aerial object (Fisher's exact

Table 3.12. Tail signal use in response to model hawk versus aerial ball. Combined 2 x 2 contingency tables comparing the presence and absence in a trial of each tail signal (twitch and flick) and tail signals in general (twitch or flick), in response to the model hawk or the aerial ball.

	twitch	no twitch	flick	no flick	any tail signal	no tail signal
model hawk ($N = 29$)	19	10	5	24	19	10
aerial ball ($N = 33$)	20	13	13	20	20	13
Fisher's exact		$P = 0.794$		$P = 0.091$		$P = 0.794$

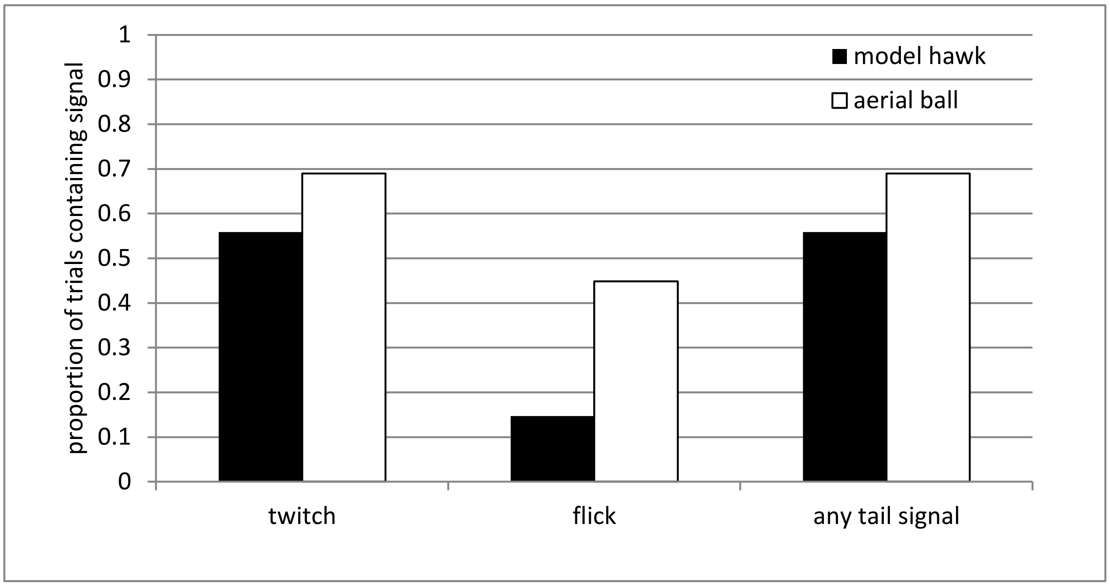

Figure 3.12 Proportion of all model hawk (*N* = 29) and aerial ball (*N* = 33) trials that elicited twitches or flicks from the focal squirrel, and the proportion of trials with any tail signal. Some signaling bouts contained multiple tail signal types and thus contributed to more than one category. Twitches and flicks were used independently of threat type, as was vocalizing in general.

test, *P* = 0.208). Whether or not squirrels used tail signals also showed no association with the type of aerial object (Fisher's exact test, *P* = 0.197).

DISCUSSION

In eastern gray squirrels the association of multimodal alarms in response to terrestrial versus aerial predators is due to one vocal signal and one tail signal that each specify a different type of threat, namely, the moan, associated with aerial threats, and the flick, associated with terrestrial threats. The other two vocal alarms, the kuk and quaa, and the other tail signal alarm, the twitch, do not have information about whether the threat is terrestrial or aerial, but appear to be general alarms. This specificity, however, could be based on the predator's physical appearance (cat or hawk), its manner of approach (aerial or terrestrial), or an interaction of the two.

From my results it appears that squirrels do use predator-specific alarm signals, but that specificity varies between terrestrial and aerial threats. Terrestrially approaching threats elicit flicks more than aerial threats when the aerial threat resembles a known predator (Table 3.4), but not when the aerial threat is a novel object (Table 3.6). The use of all five alarm signals (kuks, quaas, moans, twitches, and flicks), is independent of whether the terrestrially approaching object physically resembles a specific predator (Tables 3.7-3.10). In contrast, use of vocal signals, and quaas in particular, is not independent of whether the aerially approaching object physically resembles a specific predator. In response to the hawk there were more vocalizations.

The availability of signals that do (moans, flicks) or do not (kuks, quaas, and twitches) carry significant information about threat type means that squirrels can choose to specify whether the threat is terrestrial or aerial, or to give a general alarm instead.

Squirrels can also give an alarm in only one modality, either tail signaling or vocalizing, or combine tail signals and vocalizations into a multimodal alarm. With a single modality, gray squirrel alarms can still be general or specific, but a single modality cannot specify both aerial and terrestrial threats simply by the signals present in the signaling bout. While some species use predator-specific alarms and some use general alarms, the alarm system of eastern gray squirrels is apparently the first to be described where the signaler can choose between general and specific alarms in the same scenario.

The criterion for predator-specific vocalizations is usually near-exclusive use of an acoustic call type in the context of a specific predator type (Seyfarth et al. 1980b; Macedonia & Evans 1993). In eastern gray squirrels, moans fit this exclusivity criterion well. Ninety percent of the responses with moans were in response to aerial stimuli. Further, moans were never observed in nonpredator contexts (when including experimental trials among predator contexts).

Using a similar exclusivity criterion for tail signals to be deemed predator-specific tail signals should be used primarily in the context of a specific predator type and not in other circumstances. Despite their association with terrestrial predators and similar frequency of use in response to other terrestrially approaching stimuli, 29% of the trials where squirrels used flicks were in response to aerial stimuli. This association hardly constitutes an exclusive use of flicks in response to terrestrial threats. So although flicks do contain some information about threat type, there is much less certainty than with moans, which are rarely used in response to terrestrial threats. If flicks do function as a referential signal simply specifying any type of terrestrial threat, they would have a 29% error rate. Despite this low specificity, flicks do contain biologically relevant levels of information, providing enough information to reduce the error in correctly predicting the presence of a terrestrial threat (versus an aerial threat) by 38.5% (asymmetric lambda = 0.385).

Alternatively, the use of flicks in response to some aerial stimuli may be due to the fact that all stimuli ended up on the ground, which could cause some squirrels to classify aerially approaching stimuli as terrestrial. By the time squirrels started signaling, the stimuli were usually at rest on the ground. While the broader use of the terrestrial signal may be due to classification of some aerial stimuli as terrestrial, the fact that flicks are sometimes used in nonpredator contexts also supports the idea that they are not primarily functioning to carry information on the presence of a specific predator type.

If alarm signals do not primarily carry information on predator type, an alternative hypothesis is that they carry information about urgency. Urgency-based alarm-signaling systems have been considered as an alternative hypothesis to explain alarms that differ based on distance to predator or the suddenness of predators' appearance (Blumstein 1999a). In these systems, the same calls that carry urgency information sometimes also differ based on predator type (Macedonia & Evans 1993).

Like eastern gray squirrels, California ground squirrels have an alarm system that contains information on aerial versus terrestrial predator type. In California ground squirrels there are two distinct alarm calls, whistles and chatters, with whistles used in response to aerial threats and chatters used in response to terrestrial threats (Leger et al. 1980; Owings & Leger 1980). Despite the high degree of production specificity, especially in response to aerial threats, the calls have been interpreted as carrying information about urgency, because urgency seems to covary with predator type

(Macedonia & Evans 1993). This interpretation is supported by the fact that the terrestrial alarm was occasionally used in response to distant raptors, and the aerial alarm was occasionally used in response to rapidly moving terrestrial threats. Playback experiments, however, showed that responses differed to the two call types in ways that seem suited to the avoidance of each predator (Leger & Owings 1978), supporting the idea that whistles and chatters are functionally referential, despite the reduced production specificity in Owings and Leger's (1980) study. This difference in response could also be interpreted as a difference in vigilance because the primary difference is the latency to stand upright. But this explanation seems less likely because the delayed upright scanning was in response to aerial alarms, and aerial predators are generally considered to be high urgency in ground squirrels that use clear urgency-based systems (Furrer & Manser 2009).

In eastern gray squirrels, tail flicks do not show strong production specificity, although they are associated with terrestrial threats. It is interesting that although Seyfarth et al. (1980) observed very strong production specificity of the three predator-specific calls in vervets, the leopard alarm, given to a terrestrial threat, was occasionally used in response to aerial threats and in agonistic encounters with conspecifics. The snake and eagle calls were more specific and have not been described in other contexts (Seyfarth et al. 1980b; Macedonia & Evans 1993). A similar pattern of lower production-specificity in terrestrial alarms has been observed in ring-tailed lemurs and California ground squirrels (Leger et al. 1979; Macedonia 1990). The reason, if any, for this convergence between vervets, ring-tailed lemurs, California ground squirrels, and eastern gray squirrels is not clear. Certainly, production specificity of flicks, the terrestrial alarm in eastern gray squirrels, is even lower than the terrestrial alarms in the other species. Playbacks of tail signals using a robotic squirrel capable of multiple tail-signal types may be needed to test the function of flicks and determine whether they are functionally referential.

Regardless of whether they are functionally referential, tail signals in the eastern gray squirrel may also signal stress, with twitches signaling moderate levels of stress and flicks signaling extreme stress. An urban population of fox squirrels (*Sciurus niger*) in California used flicks when frustrated by a previously solvable puzzle box (Roblado, pers. comm.), and gray squirrels are known to use tail signals during agonistic interactions with conspecifics (Barkalow & Shorten 1973; Steele & Koprowski 2003; pers. obs.).

If tail signals directly reflect stress in gray squirrels, then flicks should be used equally for equally dangerous predators. Although predation and attack rates are unknown, aerial predators are not apparently less dangerous than terrestrial predators, as both raptors and cats have been observed attacking squirrels in the study population (pers. obs.). If tail signals reflect stress, the signals may be modified in a way that explains the reduced use of flicks in response to aerial stimuli. Terrestrial predators like cats generally give up the chase when a squirrel reaches a tree and they are not capable of pursuing a squirrel through the treetops. A squirrel that reaches a tree is therefore relatively safe from terrestrial threats. Tail flicks are very conspicuous; they readily drew my attention to previously unobserved squirrels. A squirrel flicking in response to a terrestrial threat is visibly announcing its own location. Because it is safe from the predator left on the ground, the squirrel can signal its level of stress with impunity. It is even possible that flicks have been selected to advertise the squirrel's location to terrestrial predators, perhaps discouraging predators from lingering in an area where their prey has spotted them and reached safe refuge.

When the predator is a raptor, a squirrel is not safe simply because it is in a tree. Red-shouldered hawks (*Buteo lineatus*) and Cooper's hawks are common on the study site, and both are capable of taking prey from tree branches, phone lines, and other places that would provide safety from terrestrial threats. It may be that squirrels faced with an aerial threat are no less agitated but suppress tail signaling because it would advertise their location to a visually oriented predator capable of attacking them in the tree. If flicks signal extreme stress, a mechanism to suppress tail signaling in the presence of aerial threats could explain the reduced tail signaling I observed in response to aerial stimuli. Even if the calls in response to raptors are predator-directed, it may behoove squirrels not to visually advertise their location to aerial predators.

The notion that gray squirrels signal stress with flicks and suppress them in the presence of aerial threats also fits well with the low production specificity of flicks relative to moans. Because raptors often attack in a stoop from above, raptors on the ground may not always pose a significant threat relative to raptors in flight or in trees. If so, then an aerial stimulus that ends up on the ground might sometimes be classified by squirrels in trees as low risk, similar to terrestrial stimuli that are always on the ground. If grounded raptors are unlikely to successfully attack, it might free squirrels to use flicks and signal their true stress levels, because the cost of detection is lower.

Even if stress is the proximate cause of tail flicks, their function may not be to signal stress. Rather, they may still function as alarm signals or serve to encourage a predator's departure by advertising that the squirrel has detected the predator. In addition to testing the effect of flicks on conspecifics, future studies using a robotic squirrel could also assess the effect on the latency for predators to leave in response to flicking and nonflicking squirrel robots.

Partan et al. (2009) found that eastern gray squirrels responded more strongly to a robotic squirrel that tail signaled and broadcast alarm calls than to either modality alone, suggesting a reinforcement function for multiple modalities in their alarm signals. In a later study, Partan et al. (2010) showed that urban squirrels responded more strongly than rural squirrels to tail signals, regardless of whether vocal signals were also presented. They suggest that gray squirrels may shift their attention to visual signals in noisy urban environments. The results of my current study showing that alarms without flicks or moans are general alarms would fit well with a signal reinforcement hypothesis. In the case of these general-alarm signals (kuks, quaas, and twitches), it seems that the vocalizations are the primary carriers of information about the presence of a threat, because twitches are used in many other contexts. Twitches may then function to reinforce general vocal alarms, making the signal more noticeable or amplifying its effect.

Eastern gray squirrels apparently encode information about predator type in some, but not all, of their alarm signals, and the amount of information varies. Meerkats (*Suricata suricatta*) have a unimodal alarm calling system that separately encodes both predator type and urgency in the same vocal signals (Manser 2001). Tufted capuchins (*Cebus apella nigritus*) have distinct aerial and terrestrial alarms but also encode risk in terrestrial-alarm call rate and have a second terrestrial-threat alarm call of unknown function. Given the lack of predator specificity of kuks and quaas and the limited specificity of flicks, it may be that gray squirrels encode some urgency information as well. Additional studies to directly test for urgency effects on the multimodal alarms used by gray squirrels as well as effects on vocal and tail signals separately would clarify whether urgency information is contained in alarm signals.

Ruffed lemurs use a semireferential system, where different alarm calls are used in response to aerial predators (abrupt roars) and terrestrial predators (pulsed squawks); however, aerial-predator alarm calls are also used in social contexts and alarms to terrestrial threats seem to be based on stress level, although they may sometimes function in a referential way (Macedonia 1990; Blumstein 1999b). This system contrasts with that of ring-tailed lemurs, which have a clearly functionally-referential alarm calling system (Macedonia 1990). It has been proposed that functionally referential systems will only arise in species with multiple escape strategies, especially if they are mutually exclusive (Seyfarth et al. 1980a; Macedonia & Evans 1993; Blumstein 1999b). It has been proposed that habitat differences may drive the differences in alarm systems in these lemur species (Macedonia & Evans 1993). Ring-tailed lemurs frequently descend to the ground and live in more open habitat, where they could potentially be cut off from escape to a tree. Ruffed lemurs live in dense rainforest and rarely descend to the ground. Given these differences in habitat and habit, Macedonia and Evans (1993) propose that ruffed lemurs have little need for a terrestrial alarm, and so they have a fuzzy system that may communicate some information about the probability of a terrestrial threat being present but that is not clearly referential like the system of the ring-tailed lemurs. Eastern gray squirrels fall somewhere between ruffed and ring-tailed lemurs in their use of arboreal versus terrestrial habitat. Gray squirrels usually remain in trees, and come to the ground primarily to forage and cache food, which they do regularly. They therefore regularly face threats from terrestrial predators in addition to the aerial predators that can attack them either on the ground or in a tree, somewhat like the situation of ring-tailed lemurs. Thus, if gray squirrels' alarm signals are directed to conspecifics, signalers may benefit from using predator-specific alarms that enable receivers to discern the nature of the threat without seeing the predator directly (see Chapter 2). Eastern gray squirrels use different escape strategies for terrestrial and aerial threats (Chapter 4), which is a likely prerequisite for the evolution of functionally referential alarm signals.

In summary, eastern gray squirrels have an alarm system of mixed specificity. Their use of vocal and tail signals in combination clearly specify aerial threats (and differ based on the type of aerial threat) and contain at least some information about the presence of terrestrial threats (regardless of the type of terrestrial threat), but they also have general alarms in each modality that do not specify predator type.

In addition to differences in specificity, alarm signals in eastern gray squirrels differ in the number of sensory modalities they employ. Gray squirrel alarm signals are thus *varimodal*, a term I coin to describe any signaling system where the number of modalities utilized in signaling may vary. In the case of gray squirrels, alarms may be given in either or both modalities, with squirrels using a general or specific alarm as a unimodal signal or combing modalities to give either specific or general multimodal alarms. This mixed specificity in unimodal and multimodal signals is interesting because it enables squirrels to either specify the threat type or give a general alarm regardless of the modalities used, thus controlling the amount of information that they broadcast about threat type. Studies are needed to test the functions of this varimodal alarm signaling system that contains both threat-specific and nonspecific alarms. If threat-specific alarms are functionally referential, it is all the more intriguing that squirrels often use non-specific alarms. If the threat-specific alarms are not functionally referential, it suggests that the differences in signals used may be functioning to reduce risk to the caller based on the attack capabilities of the threat, and that perhaps the signals are predator-directed.

Additional studies directly testing for differences in varimodal alarms used in response to different levels of urgency may clarify the information content of tail signals as well as that of the general alarm calls (kuks and quaas). This study highlights the importance of considering multiple modalities when investigating animal communication. If vocal signals were considered alone, much of the information would be missed. By examining both modalities, this study has uncovered a unique case of alarm signaling where multimodal signals carry information on predator type.

CHAPTER 4

DIFFERENCES IN COMPOSITION OF ALARM CALLING BOUTS AND ESCAPE STRATEGIES USED BY ARBOREAL SQUIRRELS IN RESPONSE TO AERIAL- VERSUS TERRESTRIAL-PREDATOR THREAT STIMULI

SUMMARY

Eastern gray squirrels use kuks, quaas, and moans—three acoustically distinct vocalizations, or alarm call elements—in their alarm calling bouts. Although moans are highly specific to aerial threats, kuks and quaas show no clear association with either terrestrial or aerial predator type when simply examining their presence or absence in a signaling bout (see Chapter 3). Here I examine the relationship between alarm call composition (the presence and rate of kuks, quaas and moans) and the type of stimulus eliciting the alarm calls (aerial or terrestrial). When the initial 30 seconds or 60 seconds of calling is examined, the presence of kuks (only in the 30s period), quaas (only in the 60s period), and moans (both periods) are all associated with stimulus type, with kuks and quaas usually used in response to terrestrial stimuli and moans exclusively used in response to aerial stimuli. Rate of calling also differed by stimulus type. Calling bouts elicited by terrestrial threats averaged twice the number of call elements in the first 30s and 60s of calling than calling bouts elicited by aerial threats, and terrestrial threats elicited more kuks in each period. Bout duration did not differ by stimulus type. Gray squirrels also show different initial escape responses for terrestrial versus aerial threats. In response to aerial stimuli squirrels ran to the opposite side of the tree trunk from the stimulus. In response to terrestrial stimuli squirrels ran to a point midway around the trunk where they could see the stimulus object (and where they could be seen if the threat were a real predator) but could quickly flee to the opposite side. This pattern in escape

response fits with the general pattern of antipredator behavior emerging in gray squirrels—they use less conspicuous behaviors when responding to aerial threats and more conspicuous behaviors in response to terrestrial threats. Alarm signals and escape responses that are threat-specific are prerequisites for a functionally referential alarm system. The alarm calls of gray squirrels are predator-specific and they use incompatible escape responses to terrestrial and aerial threats. Playback studies are underway to test for functional referentiality by testing for response specificity to different alarm calls.

BACKGROUND

Functionally referential alarms have both production specificity and response specificity (Marler et al. 1992). Although usually discussed in terms of categorical signals and categorical threats, production specificity can be thought of as any association of particular alarm signal characteristics with particular threat characteristics. Strong production specificity is a prerequisite for response specificity, the second component of functionally referential alarms (Marler et al. 1992).

Eastern gray squirrels use a multimodal alarm signaling system in which the signaling squirrel does not always vocalize. When it does vocalize, it may use up to three types of alarm call elements (kuks, quaas, and moans) in any combination. When a squirrel hears an alarm calling bout, what information about the eliciting stimulus is contained in the composition of that bout? A gray squirrel hearing a conspecific alarm call can potentially obtain information on the stimulus eliciting the alarm based on the composition of the alarm call elements.

Here I examine the presence and rate of the three alarm call elements (kuks, quaas, and moans) in the initial 30s and 60s of each alarm calling bout to determine if they contain information on whether the eliciting stimulus is an aerial or terrestrial threat. I examine this initial period because that period is most important to receivers evaluating a threat and determining an initial course of action, and I do not choose a shorter period because quaas are often not used until 30-50 seconds into the alarm calling bout.

I tested two separate hypotheses related to alarm call composition for each type of alarm call element (kuks, quaas, and moans). First, I examined whether the presence or absence of each type of alarm element in a calling bout is associated with terrestrial versus aerial stimulus type, the most conservative measure of specificity. Second, I tested whether the rate of each type of alarm element, and rate of alarm elements in general, differs in the initial 30s and 60s of alarm calling bouts elicited by aerial versus terrestrial stimuli.

Functionally referential alarms are only expected to evolve if different threats elicit different escape responses (Furrer & Manser 2009). If prey use predator-specific escape responses, and if they use predator-specific alarm signals, then receivers could respond appropriately based on the information in the call without any need to see the threat themselves. In light of the connection between predator-specific signals and predator-specific escape responses, I also examined the escape responses of focal squirrels presented with aerial and terrestrial stimuli to determine if their responses qualitatively differ in response to terrestrial versus aerial stimuli.

METHODS AND MATERIALS

Individually identified eastern gray squirrels on the campus of the University of Miami in Coral Gables, Florida (80° 16.732'W, 25° 43.393'N), were presented with a series of stimuli that approached either through the air ($N = 62$ trials) or on the ground (N

= 94 trials) to simulate encounters with aerial and terrestrial predators. The order of stimulus presentations to each squirrel was predetermined using a balanced order design. All stimuli were sent directly toward the focal squirrel from an initial distance of approximately ten meters and each trial ended when a squirrel had resumed calm behavior for two minutes or left the area. (See Chapter 3 for details of stimulus objects and presentation method.) These experiments took place between January 29, 2010, and July 13, 2011. Presentations were conducted in grassy lawns dotted with mature trees consisting primarily of live oaks (*Quercus virginiana*) and several palm species.

Vocal alarms of focal squirrels ($N = 22$ alarm calling bouts) were recorded as uncompressed WAV files using Sennhesier ME67 microphones and Marantz 660 and 661 digital recorders as detailed in Chapter 3. Recorded alarm calls were visualized in Audacity 1.3 (Audacity Development Team 2011) as spectrograms using a 1024-sample Hanning window. Individual alarm vocalizations in each alarm bout were identified and classified while both visually inspecting and listening to the sound files. In trials when squirrels vocalized, the number of renditions of each alarm-call element (kuk, quaa, and moan elements) in the first 30s and first 60s (first and second 30s combined) after calling began was counted. I also counted the number of alarm calling bouts by focal squirrels that contained each type of call element within each analysis period (30s and 60s). Occasionally other squirrels in the area would vocalize, but only the focal squirrels' responses are included in these data.

The first 60s of an alarm calling bout was chosen as the period of analysis in order to enable examination of quaa usage, because quaas often did not occur until a squirrel had been vocalizing for 30-50 seconds. If quaas contain any different information than kuks (which were often the first call elements vocalized, and always preceded the first quaa), then a squirrel would have to listen for about a minute to obtain that information. I therefore examined the first minute of each alarm call. Because 60s is a relatively long period, I also looked at usage of each call element in the first 30s of alarm calling bouts.

The small sample sizes of alarm calls in response to some stimuli would make it impossible to detect even a strong effect using the same categories used in Chapter 3. Manner of approach, however, is a trait of biological relevance, shared by all stimuli. Its expression both in these experiments and in nature is in only two phenomenological categories, namely aerially and terrestrially. The manner of approach is a key difference between predator types that might require different escape strategies and therefore perhaps elicit different alarm calls to alert conspecifics whether the threat is aerial or terrestrial. An aerial versus terrestrial comparison also reflects the categories used in other studies of sciurid alarms (where objects used as moving stimuli range from realistic predator models to hats and Frisbee flying discs). The analysis presented here therefore collapses stimulus presentations into these two categories in order to examine the internal composition of gray squirrel alarm calls used in response to aerial versus terrestrial threats.

All statistical tests were calculated using JMP Pro 9 (SAS Institute Inc., Cary, NC, 1989-2011). Due to the non-normal distribution of most of the data (as determined by Shapiro-Wilk tests), Mann-Whitney *U* tests were used for all analyses of the numbers of call elements and bout duration elicited by terrestrial versus aerial stimuli. Fisher's exact tests were used for all tests of association between stimulus type (terrestrial or aerial) and whether or not a particular call type was used in the initial portions of focal squirrels' alarm calls (1-0 scoring).

RESULTS

ALARM CALLS USED IN RESPONSE TO TERRESTRIAL AND AERIAL STIMULI

KUKS

In eastern gray squirrels, initial rate of kuks contains information about whether a threat is aerial or terrestrial. Terrestrial stimuli elicited more kuks in the first 30s than did aerial stimuli, with a median of 3 kuks in response to aerial stimuli and 29 to terrestrial stimuli (difference = 27) (Figure 4.1). A similar pattern occurred in the first 60s of calling, with a median of 4 kuks in response to aerial stimuli and 42 in response to terrestrial stimuli (difference = 40) (Figure 4.2). The differences in the number of kuks used by alarm-calling focal squirrels in response to aerial (*N* = 10) versus terrestrial (*N* =

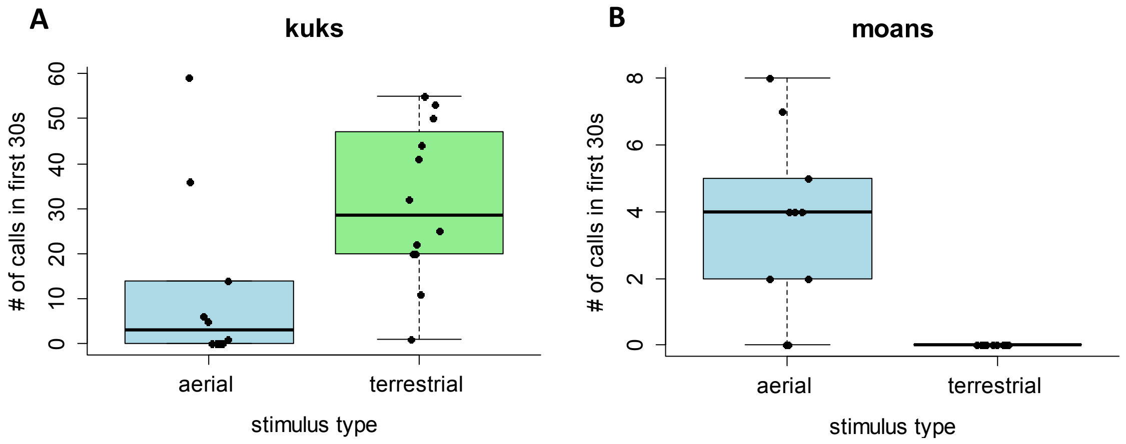

Figure 4.1. Number of kuks and moans in the first 30s of calling to aerial and terrestrial stimuli. (A) The number of kuks in the first 30s of calling differs in responses to aerial (N = 10) and terrestrial (N = 12) stimuli (*U* = 96.5, *P* = 0.0172), with more kuks used in response to terrestrial stimuli. **(B)** The number of moans in the first 30s of calling differs in responses to aerial (N = 10) and terrestrial (N = 12) stimuli (*U* = 12, *P* = 0.00027), with moans only used in response to aerial stimuli.

12) stimuli during the first 30s and 60s of calling were significant (30s period: $U = 96.5$, $P = 0.0172$; 60s period: $U = 100$, $P = 0.009$).

The presence or absence of kuks in the initial portion of a squirrel's alarm call also contains information about the presence of a terrestrial threat. Alarm calls in response to aerial stimuli often did not contain kuks in the initial 30s (4 of 10), whereas calls in response to terrestrial stimuli always contained kuks (12 of 12) (Table 4.1). Whether or not focal squirrels used kuks was associated with stimulus type during the first 30s of calling (Fisher's exact test, $P = 0.0287$). The presence or absence of a kuk in the first 30s of calling provides enough information to reduce the error in correctly predicting the manner of approach by about 40% (asymmetrical lambda = 0.400). When examining the first 60s of calling, however, this association disappeared, with 9 of 10 alarm calls in response to terrestrial stimuli containing kuks in the first 60s (Fisher's exact test, $P = 0.455$).

QUAAS

In contrast to the pattern seen in kuks, the initial rate of quaas does not contain information on whether a threat was aerial or terrestrial. Only a single alarm calling bout contained quaas in the first 30s, so no analyses were performed on numbers of quaas in that period (Figure 4.1, Table 4.1).

In the first 60s of calling, aerial stimuli elicited a median of 0 quaas, whereas terrestrial stimuli elicited a median of 1 quaa (difference = 1) (Figure 4.2). The difference in the number of quaas used by alarm-calling focal squirrels in response to aerial ($N = 10$) versus terrestrial ($N = 12$) stimuli during the first 60s of calling ($U = 80.5$, $P = 0.149$) was not significant. Interestingly, however, the rate of quaas in the first 60s showed distinctly different distributions between stimulus types in the 60s period. Focal squirrels used either no quaas or at least nine quaas in response to aerial stimuli, whereas

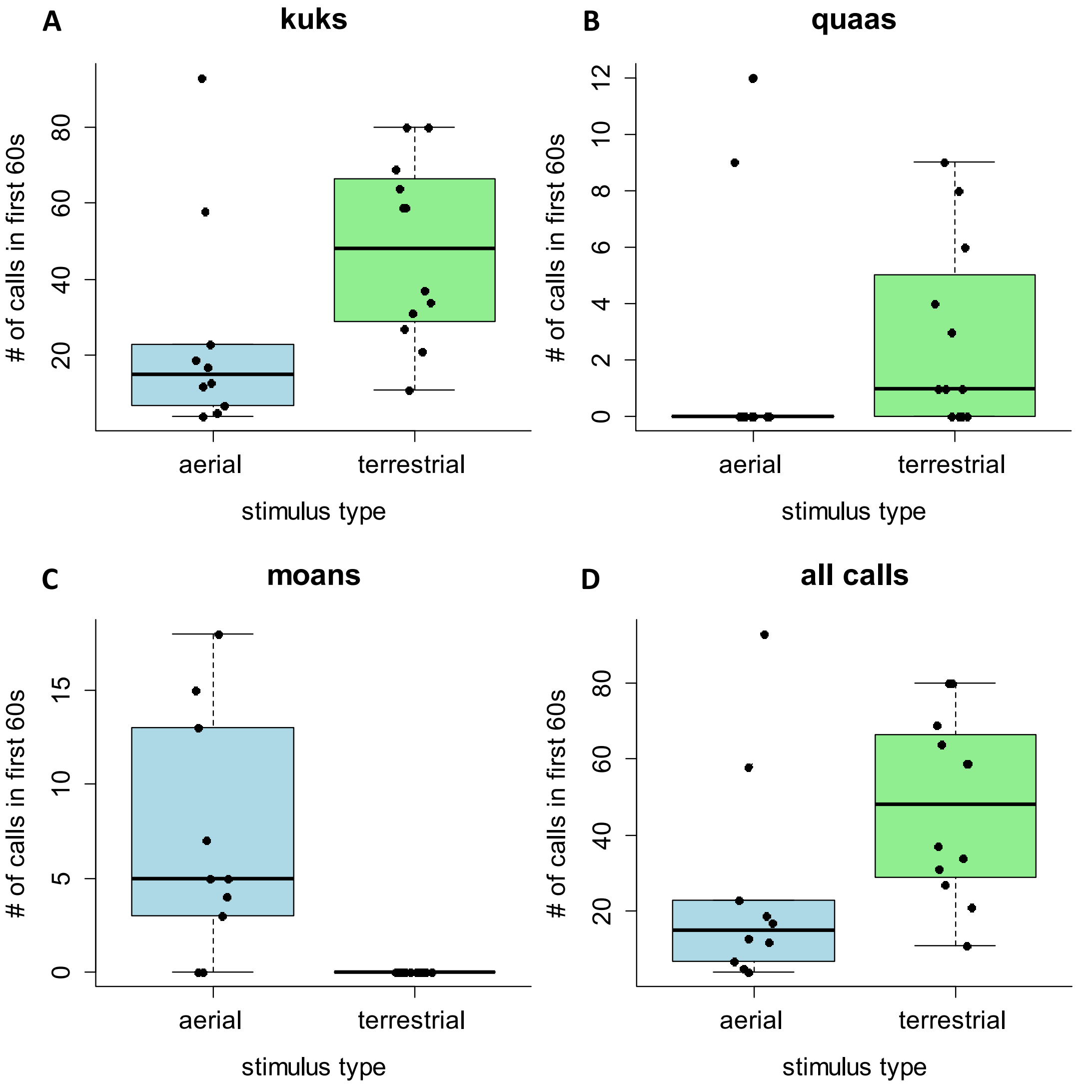

Figure 4.2. Number of calls in the first 60s of calling to aerial and terrestrial stimuli. The numbers of kuks, moans, and vocalizations overall differ in response to aerial (*N* = 10) and terrestrial (*N* = 12) stimuli. The number of quaas used in the same period is not significantly different in response to aerial versus terrestrial stimuli. **(A)** The number of kuks used differs between stimulus type (*U* = 100, *P* = 0.009). More kuks are used in response to terrestrial than aerial stimuli. **(B)** The number of quaas used does not differ between aerial and terrestrial stimuli (*U* = 80.5, *P* = 0.149), but whether squirrels used quaas in the first 60s of calling is associated with stimulus type (Fisher's exact, *P* = 0.0427). Aerial stimuli elicited either no quaas, or over eight quaas, while the number of quaas in response to terrestrial stimuli shows a more even distribution. **(C)** Moans were exclusively used in response to aerial stimuli (*U* = 12, *P* = 0.000278). **(D)** When pooling all call types together, the overall number of calls in the first 60s differed in response to aerial versus terrestrial stimuli (*U* = 96, p = 0.0192), with terrestrial stimuli eliciting more calls.

squirrels' alarm calls to terrestrial stimuli showed a more uniform distribution of quaa rate, ranging from zero to nine quaas in the first 60s (Figure 4.2).

Although initial quaa rate does not contain information on threat type, the presence of quaas in the initial 60s of calling specifies a terrestrial threat. Squirrels used quaas in 2 of 10 calling bouts to aerial stimuli and 8 of 12 calling bouts to terrestrial stimuli. Whether or not focal squirrels used quaas was associated with stimulus type during the first 60s following the start of calling (Fisher's exact test, $P = 0.0427$) with a greater likelihood of use in response to terrestrial stimuli (Table 4.1). The presence or absence of a quaa in the first 60s of calling provides enough information to reduce the error in correctly predicting the manner of approach by about 40% (asymmetrical lambda = 0.400).

MOANS

The initial rate of moans contains information about whether a threat is aerial or terrestrial. In the first 30s of calling, aerial threats elicited a median of four moans, whereas terrestrial threats elicited a median of zero moans (Figure 4.1). The pattern was similar in the first 60s of calling, where aerial threats elicited a median of five moans and terrestrial threats elicited a median of zero moans (difference = 5) (Figure 4.2). There was a significant difference in the number of moans used by alarm-calling focal squirrels in response to aerial ($N = 10$) versus terrestrial ($N = 12$) stimuli during the first 30s and 60s from the start of calling ($U = 12$, $P = 0.00027$ for both periods).

The presence of moans in the initial portion of a squirrel's alarm call also contains information on whether a threat is aerial or terrestrial; moans clearly specify aerial

Table 4.1. Presence or absence of each call type in the first 30s and 60s of calling to terrestrial versus aerial stimuli. Combined 2 x 2 contingency tables showing the number of alarm calls by the focal squirrel in 22 trials that contained each call in the first 30s and first 60s following the start of the calling bout.

	In 1st 30s after call starts						In 1st 60s after call starts					
	no kuk	kuk	no quaa	quaa	no moan	moan	no kuk	kuk	no quaa	quaa	no moan	moan
terrestrial stimulus	0	12	12	0	12	0	0	12	4	8	12	0
aerial stimulus	4	6	9	1	2	8	1	9	8	2	2	8
Fisher’s exact	P = **0.0287**		P = 0.455		P = **0.000141**		P = 0.455		P = **0.0427**		P = **0.000141**	

threats. Moans in the first 30s and 60s of calling were used exclusively in response to aerial stimuli (Table 4.1), so whether or not focal squirrels used moans was strongly associated with stimulus type during the 30s and 60s (Fisher's exact tests, *P* = 0.00014 for both periods) following the start of calling. The presence or absence of a moan in the first 30s of calling provides enough information to reduce the error in correctly predicting the manner of approach by about 80%, as does the presence of a moan in the first 60s of calling (asymmetrical lambda = 0.800 for both periods).

OVERALL CALLING RATE

Initial calling rate, regardless of call type, also contains some information on whether a threat is aerial or terrestrial. In the first 30s of calling aerial stimuli elicited a median of only 8 calls, whereas terrestrial stimuli elicited a median of 29 calls (difference = 21). Similarly, in the first 60s of calling, aerial stimuli elicited a median of only 15 calls, whereas terrestrial stimuli elicited a median of 48 calls (difference = 33) (Figures 4.1, 4.2). There was a significant difference in the overall number of calls (kuks, quaas, and moans) used by alarm-calling focal squirrels in response to aerial (*N* = 10) and terrestrial (*N* = 12) stimuli in the first 30s and 60s of calling (30s period: *U* = 91.5, *P* = 0.0405; 60s period: *U* = 96, *P* = 0.0192). This pattern of more rapid calling to terrestrial stimuli than to aerial stimuli is largely driven by the rapid kuks that usually initiate alarm calls elicited by terrestrial stimuli.

CALLING BOUT DURATION

The duration of calling was similar regardless of stimuli type. Alarm calling lasted for a median of 131s in response to aerial stimuli, and a median of 119s in response to terrestrial stimuli (difference = 12s) (Figure 4.3). The difference in the duration of calling bouts in response to aerial ($N = 10$) versus terrestrial ($N = 12$) stimuli was not significant ($U = 51$, $P = 0.575$).

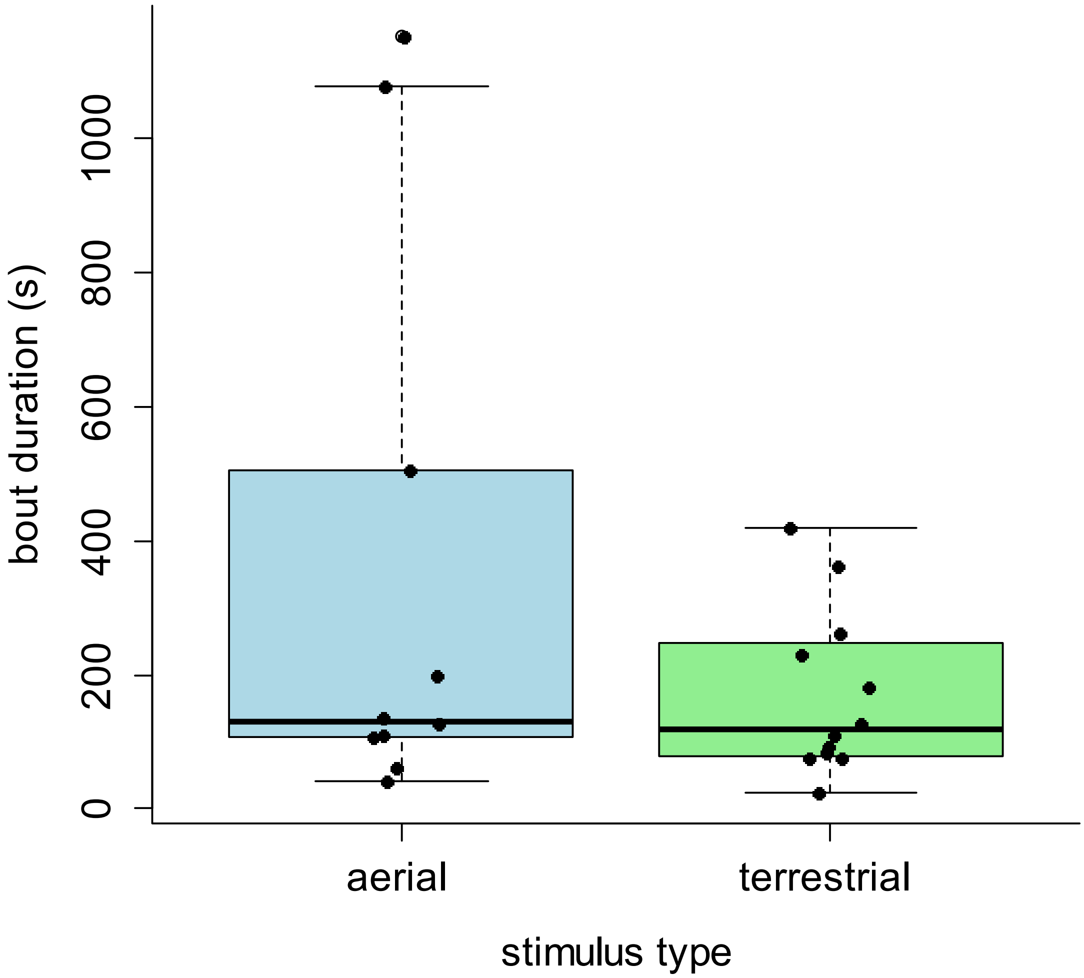

Figure 4.3. Durations of alarm calling bouts to aerial versus terrestrial stimuli. Duration of entire alarm calling bout did not differ in response to aerial ($N = 10$) versus terrestrial ($N = 12$) stimuli ($U = 51$, $P = 0.575$).

ESCAPE RESPONSES TO TERRESTRIAL AND AERIAL STIMULI

Eastern gray squirrels reach the relative safety of a tree trunk sooner in response to aerial threats than in response to terrestrial threats. Focal squirrels consistently responded to all stimuli by fleeing to the trunk of a nearby tree, but the median latency to reach the tree was 5.0s in response to terrestrial stimuli and 1.6s in response to aerial stimuli (difference = 3.4s). Whether a threat was approaching aerially (*N* = 51) or terrestrially (*N* = 81) significantly affected the latency to reach the tree trunk after the stimulus object was in motion ($U = 513$, $P = 2.09 \times 10^{-13}$), with latency to reach the tree trunk lower in response to aerial stimuli than in response to terrestrial stimuli (Figure 4.4). The greater numbers of squirrels used in this analysis of escape behavior relative to

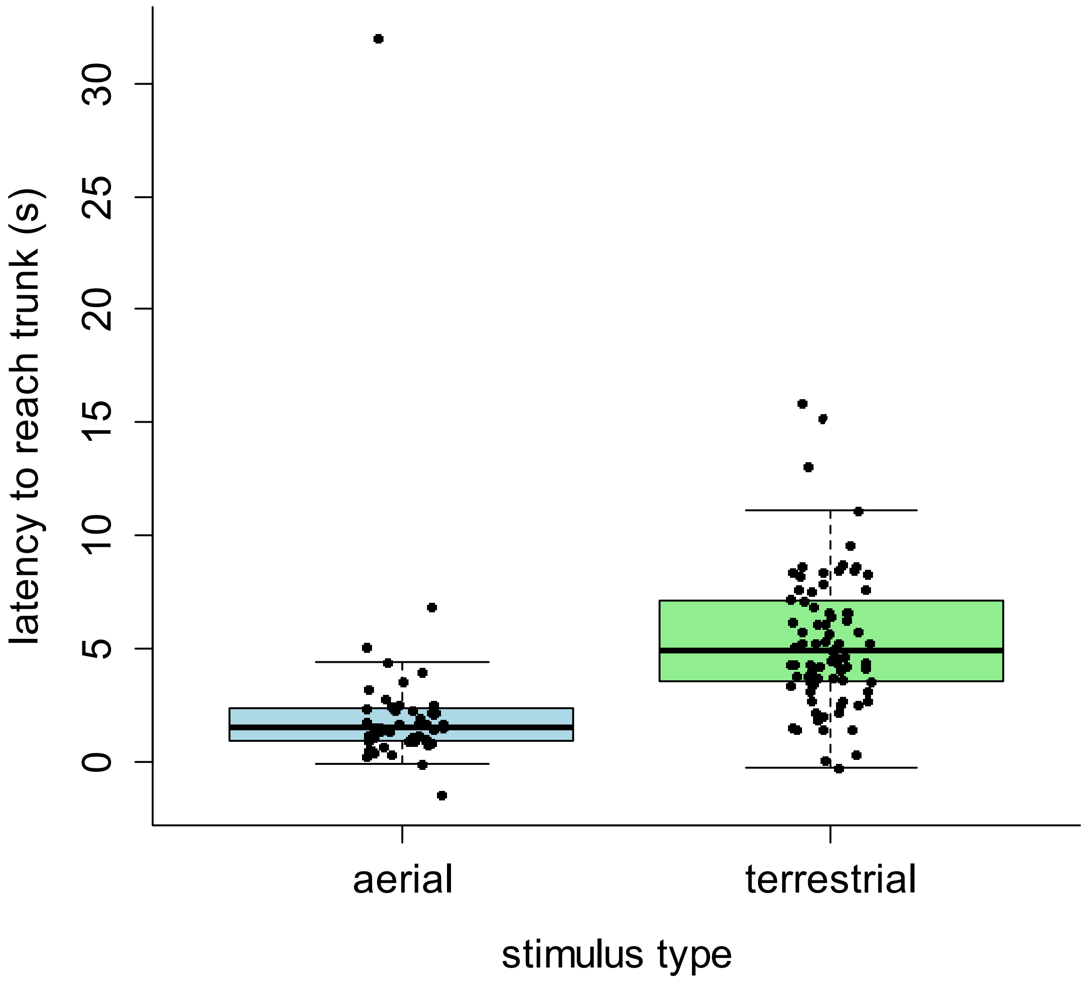

Figure 4.4. Latencies to reach tree trunk in response to aerial versus terrestrial stimuli. Focal squirrels' latencies to reach the trunk after release of the stimulus object differed based on stimulus type ($U = 513$, $P = 2.09\text{x}10^{-13}$). Latencies to reach the trunk were shorter in response to aerial stimuli (*N* = 51) than in response to terrestrial stimuli (*N* = 81).

Table 4.2. Escape strategies in response to aerial versus terrestrial stimuli. Escape responses of eastern gray squirrels differed by stimulus type (Fisher-Freeman-Halton exact test, $P = 0.00397$). Squirrels usually fled to the opposite side of the tree trunk from the stimulus in response to aerial stimuli. In response to terrestrial stimuli, squirrels usually fled to a location midway around the trunk relative to the stimulus, where they could see the stimulus and were at least partially visible from the viewpoint of the stimulus object. Squirrels rarely fled to the same side of the trunk as the stimulus, regardless of stimulus type. See Figure 4.5 for a diagram defining *same*, *midway*, and *opposite*.

	Side of tree trunk at first pause, relative to stimulus		
	same	midway	opposite
Aerial stimulus ($N = 24$)	2	9	13
Terrestrial stimulus ($N = 36$)	1	28	7

the numbers used in analysis of alarm calling reflects that many focal squirrels did not alarm call, and thus could not be used in the prior analysis of alarm call content.

Eastern gray squirrels appear to use different escape strategies in response to aerial and terrestrial threats. Once squirrels reach a tree, their escape responses qualitatively differ in response to aerial and terrestrial threats. In response to terrestrial threats, eastern gray squirrels flee to locations providing a clear line of sight to the threat (29 of 36 trials) and presumably a clear view of the squirrel by the threats when threats are actual predators. In response to aerial threats, squirrels often (13 of 24 trials) flee to sheltered locations that do not provide a line of site to the threat.

The location of a squirrel's first pause after fleeing to a tree was associated with stimulus type (Fisher-Freeman-Halton exact test, $P = 0.00397$) (Table 4.2). A pause was defined as being stationary for at least 0.5s. Squirrels typically paused on the tree trunk, so I classified their location relative to the stimulus into one of three categories: same side of trunk, midway around trunk, and opposite side of trunk (see Figure 4.5). In response to aerial stimuli, focal squirrels usually ran to the opposite side of the tree from the stimulus, placing the main trunk of the tree between themselves and the stimulus object. In response to terrestrial stimuli, squirrels usually ran to a point on the trunk midway between the side facing the stimulus and the side opposite the stimulus. From this location the stimulus was clearly in their field of view, and all or most of their body could be seen from the point of view of the stimulus object. Squirrels rarely ran to the same side of the trunk as the stimulus object in response to either aerial or terrestrial stimuli (Table 4.2).

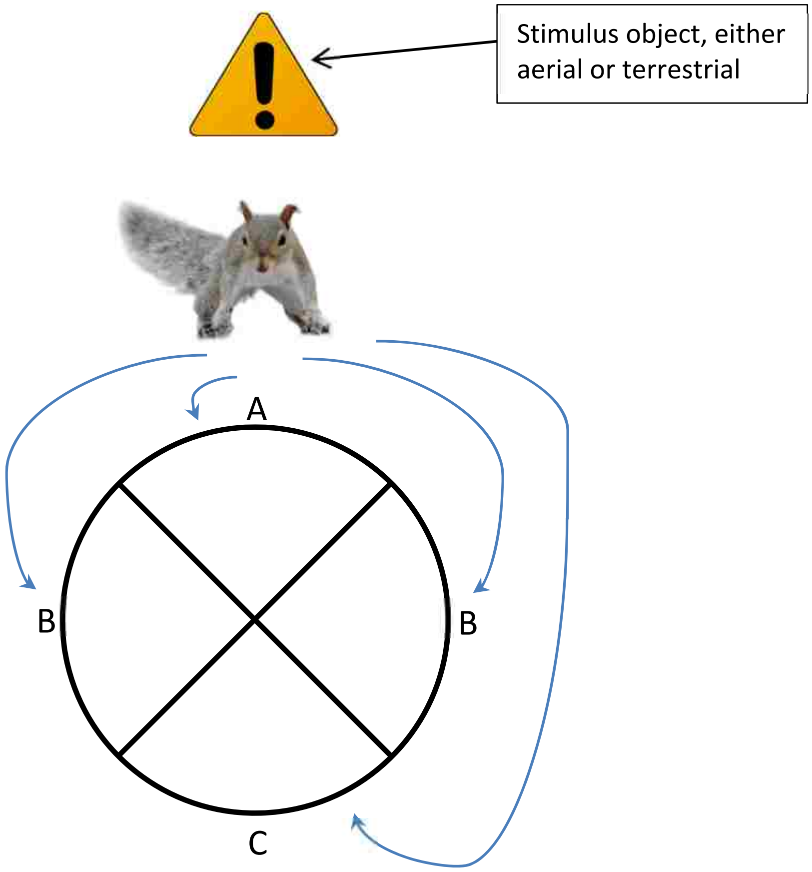

Figure 4.5. Three categories of location on trunk relative to threat. This diagram represents the view from above immediately after a stimulus object has stopped moving. The circle represents the trunk of a tree in cross-section, with the two intersecting lines dividing its surface into four quadrants. A squirrel could flee (blue arrows) to any of the four quadrants on the trunk surface, which I classified into three categories: **(A)** *same*, the quadrant of the tree facing the stimulus; **(B)** *midway*, the two quadrants midway around the tree trunk relative to the stimulus; **(C)** *opposite*, the quadrant farthest from the stimulus object, with the trunk between the squirrel and the stimulus.

DISCUSSION

THE INITIAL PORTION OF AN ALARM CALLING BOUT SPECIFIES THREAT TYPE

The initial portion of calling bouts should be the most salient and relevant for receivers choosing among different initial responses to predators. In a study analyzing the entire alarm calling bout, the alarm calls of eastern gray squirrels showed no association of kuk or quaa use with terrestrial and aerial stimulus types (Chapter 3). In contrast, when examining only the initial portion of calling bouts, kuks and quaas are associated with threat type. Because in the initial portion of alarm-call bouts kuks and quaas are used more often to terrestrial threats, they are not general alarms but carry some information about the presence of a terrestrial threat. Moans are used exclusively in response to aerial stimuli, similar to the pattern when analyzing entire calling bouts.

In the first 30s of alarm calling, there is information on whether the threat is terrestrial or aerial. If the initial 30s of calling contains kuks, then the eliciting threat is terrestrial in 66% of cases. Although this level of specificity is somewhat informative, reducing the error in correctly predicting the threat type by 40%, it still leaves some ambiguity. This ambiguity is lessened by the rate of kuks. The number of kuks in the first 30s is three times higher in response to terrestrial threats than to aerial threats, adding additional information on threat type. The median number of kuks in the first 30s, regardless of threat type, is 20. Whether or not the number of kuks in the first 30s is less than 20 contains enough information to reduce the error in correctly predicting threat type by 60%, so kuk rate contains even more information on threat type than simply the presence or absence of kuks.

Information on the presence of a terrestrial threat is further increased by the use of quaas. Squirrels' use of quaas in the initial 60s of calling is strongly associated with threat type. Specificity of quaas is also higher than that of kuks; 80% of the times when quaas were used the threat was terrestrial. Despite their greater specificity than kuks, quaas' less frequent presence means that the information in quaas in the first 60s reduces the error in correctly predicting the threat type by 40%, just as with kuks in the first 30s. When examining the initial period of an alarm calling bout the combination of information from kuk presence and kuk rate in the first 30s and of the use of quaas the first 60s of calling increase the reliability of information on the presence of a terrestrial threat, with a 70% reduction of error in correctly predicting the threat type (Asymmetrical lambda = 0.700).

The use of alarm calls is even more specific in squirrels' responses to aerial threats. If the first 30s of calling contained moans, the threat was always aerial; the same is true for the first 60s of calling. If the first 60s of calling in response to an aerial stimulus contained kuks, they were used in small number, averaging only 36% the mean number of kuks in response to terrestrial stimuli, so low initial rate of kuks adds another source of information about the presence of an aerial threat. Eighty-three percent of the bouts containing fewer than 20 kuks (the median number of kuks regardless of predator type) in the first 30s were in response to aerial threats (Figure 4.1). So while the presence of moans contains extremely reliable information on the presence of an aerial threat, a slow rate of kuk use also contains reliable information about the presence of an aerial threat, although it does not increase the reliability of the information in the moans.

The alarm-call types and calling rates in the initial period of calling clearly contain information on whether a threat is terrestrial or aerial. In this study, however, all stimuli ended up on the ground, which could have led some squirrels to classify experimental aerial stimuli as terrestrial threats. This type of classification mismatch between the experimental stimulus categories and squirrels' perceptions may have happened in a few trials, because eastern gray squirrels primarily base their alarm signaling responses on the manner of approach, not whether the object physically resembles a terrestrial or aerial predator species (Chapter 3). Classifying threats by location or manner of approach instead of physical appearance could make identification "mistakes" by squirrels more likely in this experiment than in natural encounters where aerial threats do not usually stay on the ground after a failed attack. The two outliers where squirrels used rapid kuks in response to an aerial stimulus (Figure 4.1) are the same trials where quaas (usually a terrestrial alarm) were used in response to aerial stimuli. If these trials are cases of misclassification, it suggests that in natural encounters production specificity may be even higher.

The alarm calls of eastern gray squirrels may be a case of complex signaling where the use of particular call types and the rate of each call type combine to increase the specificity of alarm calls (Hebets & Papaj 2005). The fact that kuks and quaas are usually used in combination is intriguing, because moans and quaas could be sufficient to distinguish aerial and terrestrial predators. The sudden start of each kuk, combined with their rapid repetition, may make them easier to localize than quaas, certainly more so than moans, and suggests that they may advertise the caller's location (Klump & Shalter 1984). There may be information on additional characteristics of a threat aside from whether they approach terrestrially or aerially that makes the use of kuks and quaas important. If kuks primarily signal stress or degree of alarm, as suggested in Chapter 3, it may be that they are suppressed in the presence of aerial threats to avoid advertising location to a predator that could attack them even in the refuge of a tree. This suppression would result in some information on predator type being contained in kuks, but kuks' primary function may be to communicate other characteristics, like distance or speed of approach. Quaas, in contrast, have higher production specificity and seem to be a terrestrial predator alarm call.

TERRESTRIAL AND AERIAL THREATS ELICIT DIFFERENT ESCAPE STRATEGIES

Functionally referential alarms are only expected if different threats are best avoided by different strategies (Furrer & Manser 2009). Eastern gray squirrels use different and mutually exclusive escape responses to aerial versus terrestrial predators. In keeping with the pattern seen in their multimodal alarms (Chapter 3) and vocal alarms, the escape response to aerial threats seems to make squirrels less conspicuous than their response to terrestrial threats. In response to terrestrial threats squirrels placed themselves with the entire tree trunk between them and the stimulus. In the case of a real aerial predator, the trunk would block the raptor's line of sight and provide physical shelter from direct attack. In contrast, in response to terrestrial threats, squirrels positioned themselves where they had a clear line of sight to the stimulus, but could quickly retreat to the opposite side of the tree. It is not possible for a squirrel to both completely shelter itself from view of a predator and maintain its own view of the predator. Given this tradeoff between sheltering and watching, squirrels would benefit from knowing both the location of a predator and whether it is a terrestrial or aerial threat. The alarm calls contain information on whether a threat is terrestrial or aerial, and alarm calls may also advertise the location of the caller, especially kuks and quaas. Receivers could potentially take the appropriate escape response without needing to see the threats themselves, especially if alarm calls also contain information on the proximity of the caller to the threat, which has not yet been tested.

This study shows differences in the location to which eastern gray squirrels initially flee, and differences in how quickly they reach the tree. It is unknown whether latency to reach the tree and location relative to the threat are relevant to squirrels that are not being pursued themselves. These differences in response observed in squirrels pursued by terrestrial and aerial threats make sense for the squirrel that is being pursued, but may or may not be important to squirrels seeking safety that are not being pursued by a threat themselves. Future studies testing for other differences in response would clarify the extent to which escape strategies differ in response to terrestrial and aerial threats, both in pursued squirrels and nonpursued squirrels.

INTERPRETATION AND FUTURE STUDIES

Despite the remaining questions about the diversity of gray squirrels' escape responses, they clearly have predator-specific alarm calls. Playbacks of each alarm-call type are needed to determine the function of each call type and whether kuks, quaas, and moans are functionally referential (Seyfarth et al. 1980; Marler et al. 1992). The specificity seen in rate of calling also deserves further study, because rate could also be functionally referential. The high levels of alarm-call specificity seen in this study fit well with a system where threats are categorized as aerial or terrestrial, but the overlap of information in call type, and call rate, as well as the association of two different call types with terrestrial threats are particularly interesting. As mentioned, it may be that these overlapping sources of information all serve to reinforce one another. An alternative possibility is that some of the overlapping sources of information also contain information about aspects of the encounter other than whether the threat is terrestrial or aerial. The presence of additional types of information in kuks could explain the use of both kuks and quaas in response to terrestrial threats rather than using a single type of call. If kuks are primarily associated with some threat characteristic other than its aerial or terrestrial approach, that could explain the relatively low specificity of kuks to terrestrial threats. Both the approach speed of a threat and distance from the threat to the squirrel could affect focal squirrels' responses, as has been seen in several ground squirrel species (Leger et al. 1980; Blumstein & Armitage 1997a; Warkentin et al. 2001; Furrer & Manser 2009). If alarm calls of eastern gray squirrels also contain information about distance, speed, or other characteristics of the threat, that information could potentially be used by receivers to adjust their responses accordingly. Although there is no reason to think that people and squirrels classify threat characteristics as categorical or continuous in a similar manner, studies manipulating continuous characteristics of a threat could be particularly relevant when studying a continuous response variable like call rate.

Studies manipulating other characteristics of threats are needed. Such studies could clarify whether the multiple characteristics of gray squirrel alarm calls that carry information about whether predators are terrestrial or aerial primarily reinforce each other's information content or primarily carry information about other threat characteristics. Testing for associations with other threat characteristics would be especially helpful in clarifying the use of kuks, instead of only the more specific quaas, in response to terrestrial threats. Given the differences in the rate of kuks in response to aerial versus terrestrial stimuli, it may be that kuk rate contains information on some continuous characteristic of a threat that differs between the aerial and terrestrial stimuli used in this study. If so, then kuk rate, rather than kuk presence, could still be functionally referential, even if the threat characteristic affecting kuk rate is continuous, not categorical.

An alarm calling system that contains information on both threat type (aerial or terrestrial) and a continuous predator trait has already been identified in fowl. Further, the calls are functionally referential for both types of information. Wilson and Evans (2012) showed that male domestic fowl (*Gallus gallus*), which have functionally referential alarms for terrestrial versus aerial predators, also encode information about apparent magnitude (size, speed, and proximity) of aerial threats in several parameters of their aerial alarms, including amplitude. Female fowl respond by adjusting the duration and magnitude of their antipredator behavior (Wilson & Evans 2012). While differences in the male's aerial alarm call may reflect varying levels of fear or other internal states, the calls function as if they refer to magnitude characteristics of aerial threat that may indicate immediacy of risk—their acoustic characteristics change with characteristics of a predator, and females consistently change their response based on the acoustic changes (in this case amplitude). Although Wilson and Evans (2012) do not use the term, the aerial alarm calls are functionally referential in regards to the apparent magnitude of or risk from aerial threats. This example highlights that any kind of information about an external stimulus can be contained in a call and potentially used by receivers (Marler et al. 1992; Evans 1997). In eastern gray squirrels kuks show low specificity to aerial versus terrestrial classes of stimuli, despite a statistically significant association. Given their relatively low specificity, kuks are a likely candidate for a functionally referential signal that carries information about a continuous rather than categorical trait of the environment. Studies are needed to clarify whether kuks' association with terrestrial versus aerial stimuli type is truly due to stimulus type itself or is due to some other characteristic of the encounter that also tends to differ between terrestrial and aerial stimuli.

In gray squirrels' encounters with aerial and terrestrial predators, there may be several characteristics of a predator other than whether it is terrestrial or aerial that affect acoustic parameters of their alarm calls. The fact that gray squirrels use three categories of acoustically distinct alarm calls seems redundant if the primary function of multiple alarm calls is to distinguish two categories of threats. Studies manipulating additional threat characteristics, including continuous parameters like angle of approach or speed, may reveal additional information contained in eastern gray squirrel alarm calls. Conservatively, it can be said that moans and quaas are highly predator-specific, with moans associated with aerial threats and quaas associated with terrestrial threats. In Chapter 5, I present the results of playback experiments to test whether kuks and quaas, which are both associated with terrestrial threats, elicit different responses when played back to squirrels.

CHAPTER 5

KUK AND QUAA ELEMENTS IN ALARM CALLING BOUTS OF EASTERN GRAY SQUIRRELS ELICIT SIMILAR DEGREES OF ANTIPREDATOR BEHAVIOR AND ARE FUNCTIONALLY EQUIVALENT TO WHITE NOISE BURSTS

SUMMARY

Eastern gray squirrels (*Sciurus carolinensis*) use three acoustically distinct vocal elements in alarm calling bouts, but it is not yet clear whether those elements elicit different antipredator behavior in conspecifics. I conducted three playback experiments to compare the relative effects of the kuk and quaa elements contained in mixed calling bouts on gray squirrels' head-down behavior (foraging and caching) and scanning behavior. In the first experiment, squirrels responded similarly to white noise and complete alarm calling bouts consisting of kuks and quaas. In the second experiment, kuks or quaas were isolated from the same original alarm call and presented separately while maintaining their original temporal pattern. In response to kuks, squirrels initiated scanning sooner, did so less often, and spent more time scanning than in response to quaas isolated from a calling bout. This pattern could be caused by either the temporal pattern of calling or call type present, so in a third experiment I restored the temporal pattern of a bout of alarm calling by replacing the missing element type with bursts of white noise of equal duration to the missing elements. Responses no longer differed, suggesting that temporal pattern, rather than element type, is the most salient aspect of alarm calls. Because kuks or quaas can be replaced with white noise and still elicit an increase in antipredator behavior, responses may reflect sensitivity to a generalized alarm call consisting of any broadband sound with an abrupt onset. Responding to any such sound may enhance perception of conspecific alarms by responding to any similar sound

as an alarm, or facilitate eavesdropping of heterospecific alarm calls, because the alarm calls of many mammalian and avian species are characterized by abrupt onset and broadband, often noisy, frequency structure.

BACKGROUND

In testing for functionally referential alarm calls, many studies have tested for production of predator-specific alarm calls (Gyger & Marler 1987; Greene & Meagher 1998; Zuberbühler 2001; Manser 2001; Coss et al. 2007), but fewer have taken the second step of testing for perception specificity by playing back recordings of different alarm calls and examining conspecific responses (Macedonia & Evans 1993; Fichtel & Kappeler 2002; Kirchhof & Hammerschmidt 2006; Digweed & Rendall 2010; Wilson & Evans 2012). Previous work has shown that gray squirrels use three distinct vocalizations as elements of their alarm calls: *kuks*, *quaas*, and *moans* (Lishak 1984; Chapters 2 and 3, present study) and that these three elements are often used in combinations within a single calling bout. Kuks and quaas, in particular, are usually used together (Chapter 3). In the first 60s of alarm calls, the presence of moans is associated with aerial threats and the presence of either kuks or quaas is associated with terrestrial threats, although kuks have lower production specificity than either quaas or moans (Chapters 3 and 4).

Because a single alarm call element could suffice to specify the presence of a terrestrial threat, the use of two acoustically distinct elements (kuks and quaas) within calling bouts to terrestrial threats suggests that kuks and quaas may serve different functions—despite their shared association with terrestrial threats. In the present study I first examined whether alarms consisting of mixed kuk and quaa elements elicit antipredator behavior in conspecifics beyond a simple startle response. I then examined whether kuks and quaas, when presented separately, elicit different responses in conspecifics or elicit similar behavior regardless of which element is present.

METHODS

To determine whether kuk and quaa elements elicit different responses in eastern gray squirrels, I conducted three experiments. I first examined whether the frequency structure and temporal pattern of kuks and quaas in complete alarm calling bouts elicit greater antipredator behavior than unstructured noise. In this first experiment I examined squirrels' responses to playback of audio recordings containing intact alarm calling bouts consisting of mixed kuk and quaa elements and compared squirrels' responses to playback of continuous white noise. In the second and third experiments I excised either kuks or quaas from recordings of mixed bouts and examined squirrels' responses to each alarm call element in the absence of the other.

GENERAL DESIGN AND PROCEDURE OF PLAYBACK EXPERIMENTS

In all experiments individually identified squirrels (see Chapter 3 for marking and identification methods) were each presented with a pair of audio files. Video recordings of squirrels' responses were made using a Panasonic HDC-SD10 camcorder at 1080p 30fps AVCHD. Pairs of audio files were matched for amplitude and duration, as detailed under the following sections describing each experiment. In all experiments, each pair of recordings was used once, with each individual squirrel receiving unique exemplars.

Each trial consisted of playing a single audio file from a netbook (Asus® Eee T91MT) through a small portable amplifier (Philips SBA1500) and speaker (Klipsch ProMedia satellite speaker). The only volume control was on the netbook, which was always set to the highest level in order to consistently replicate amplitude across trials. In all trials the audio was not played until the squirrel was on the ground and was approximately 10 meters from the speaker. Each trial continued until the squirrel left the area (travelled until it was out of sight, at least 20m) or had resumed calm behavior for two minutes, whichever occurred first.

Order effects in each experiment were controlled by alternating which type of acoustic stimulus was presented first to each squirrel. Each squirrel received no more than two trials in one day, and if it received two trials in a day they were separated by at least 45 minutes. Because Experiments 2 and 3 were conducted concurrently, the order of experiments was also alternated when an individual squirrel was used in both experiments. See Table 5.1 for the order of Experiments 2 and 3 and order of trials within each experiment.

Table 5.1. Order of acoustic stimulus presentations in Experiments 2 and 3. This table shows the balanced order design in which the presentation order of the four stimuli within Experiments 2 and 3 was alternated and the order of experiments was also alternated. The order of playback trials repeated after every four squirrels. In this table the name of each trial specifies the experiment number (2 or 3) and the stimulus type within that experiment (A or B), thus Experiment 2, stimulus B is represented here as 2B.

Squirrel #	1st trial	2nd trial	3rd trial	4th trial
Squirrel 1	2A	2B	3A	3B
Squirrel 2	2B	2A	3B	3A
Squirrel 3	3A	3B	2A	2B
Squirrel 4	3B	3A	2B	2A

EXPERIMENT ONE – ALARM CALLS VERSUS WHITE NOISE

In the first experiment I sought to determine whether alarm calls consisting of kuks and quaas elicited antipredator behaviors in conspecifics more than did continuous white noise of the same duration as a calling bout. To test for differences in response, each squirrel was presented with a pair of audio files. One audio file consisted of a complete alarm calling bout consisting of kuk and quaa elements. The other audio file consisted of continuous white noise, matched in duration to the entire alarm calling bout. The amplitude of the white noise and of the first three elements in the alarm calling bout ranged from 65-70dB, but was matched within pairs (+/-2dB). Sound pressure level measurements were made at 1m from the speaker with an American Recorder Technologies SPL-8810 sound pressure level meter A-weighted, and on the fast setting.

EXPERIMENT TWO – KUKS VERSUS QUAAS

In the second experiment I sought to determine whether kuks and quaas elicited different responses in conspecifics by eliminating either kuks or quaas from recordings of mixed alarm calling bouts. To control for possible effects of caller or caller's context, each pair of acoustic stimuli was created from a single original alarm calling bout. Kuks and quaas are distinguished not only by their duration and sound frequency patterns, but by their patterns of use throughout a calling bout. An unmanipulated calling bout typically began with rapid kuks that slowed within a few seconds, after which the calls began alternating intermittently between kuks and quaas, with the proportion of quaas increasing as the calling bout progressed (Chapter 2, Figure 2.2).

To create separate kuk and quaa acoustic stimuli, I created two copies of the original audio file and then eliminated either kuks or quaas and replaced them with background noise of equal duration from elsewhere in the original recording. Each acoustic stimulus then consisted of either all the kuk elements from the original bout, or all the quaa elements from the original bout, and maintained the natural timing of each element type within a bout (Figure 5.1). Duration from the first to last alarm call element (kuk or quaa) differed within pairs (Figure 5.1). Amplitude was manipulated uniformly across each audio file until the first three elements (either kuks or quaas) were within 65-

70dB (see Experiment 1 methods for measurement details), and were amplitude-matched within pairs (+/-2dB). Acoustic stimuli within each pair thus differed both in their patterns of amplitude over time and in their patterns of frequency over time, due to differences in the elements (kuks or quaas) they contained. Any differences in response by conspecifics elicited by acoustic stimuli containing kuks versus those containing quaas could be due to either temporal pattern of signaling or the frequency structure of elements (kuks or quaas) or a combination of temporal pattern and frequency structure.

EXPERIMENT THREE – KUKS PLUS WHITE NOISE VERSUS QUAAS PLUS WHITE NOISE

In the second experiment both the time pattern of elements and the elements present differed between the kuk and quaa acoustic stimuli. So in Experiment 3 I sought to determine whether the presence of kuks versus quaas elicited different responses when the temporal pattern of elements was held constant. Toward that end, rather than silence calls by replacing them with background noise, I replaced either kuks or quaas with white noise elements of equal duration to the replaced alarm elements (Figure 5.1). All white noise elements within a single acoustic stimulus were of equal amplitude. I uniformly manipulated amplitude in each audio file until the first three elements were 65-70dB (see Experiment 1 methods for measurement details) and were amplitude-matched (+/-2dB) within pairs. This process produced two files of equal duration, one with kuk and white noise elements, and one with quaa and white noise elements, with identical background noise throughout both files. Each pair of acoustic stimuli thus had nearly equal patterns of amplitude over time, so *kuk plus noise* stimuli differed from *quaa plus noise* stimuli primarily in their patterns of frequency structure over time, because of differences in the type of elements (vocalizations or white noise) each point in time (see Figure 5.1). Any differences in response by conspecifics elicited by acoustic stimuli containing kuks versus those containing quaas would likely be caused by differences in the structure of the elements (kuks, quaas, and white noise) rather than the temporal pattern of elements.

Table 5.2. Definitions of behaviors scored continuously for 60s in each trial. Focal squirrel behavior was scored continuously in the 60s following the start of the first vocalization or white noise in the acoustic stimulus presented to each squirrel using these behavior categories. These behaviors were used to define calm behavior in determining trial length. Intermittent scanning while on all four feet was normal behavior for foraging squirrels, so brief (<3s) periods of scanning on all fours were considered neutral behavior and did not extend the length of the trial. Behaviors marked with an asterisk were not observed during any trial; behaviors marked with a dagger were observed only once across all three experiments.

behavior name	definition of behavior	antipredator?
foraging/caching (head-down behavior)	Nosing around ground or digging	no
eating	Holding object to mouth with forepaws, or chewing or biting it	no
grooming†	Running forepaws through fur, chewing self, or scratching self with hind feet	no
chasing	Focal squirrel either running behind another squirrel or another squirrel running behind focal squirrel	no
walking/climbing	Moving on ground or in tree, either maintaining at least one foot in contact with substrate, or hopping less than the length of the squirrel's body –also includes single large jumps that are not immediately preceded or followed by running	no
resting*	Chin and belly against top of horizontal surface (less than 45° angle)	no
sleeping*	Resting with eyes closed	no
other calm† behavior	Other calm behaviors (not predator associated)	no
scanning on all fours	Head level with back or higher, with at least three feet on the substrate	maybe
scanning upright	Standing with both forefeet off the substrate but not eating	yes
running	In tree or on ground with consecutive jumps at least the length of the squirrel’s body and not in chase with another squirrel	yes
hiding†	Chin and belly against surface, not resting	yes
other antipredator behavior*	Other predator-associated behavior	yes

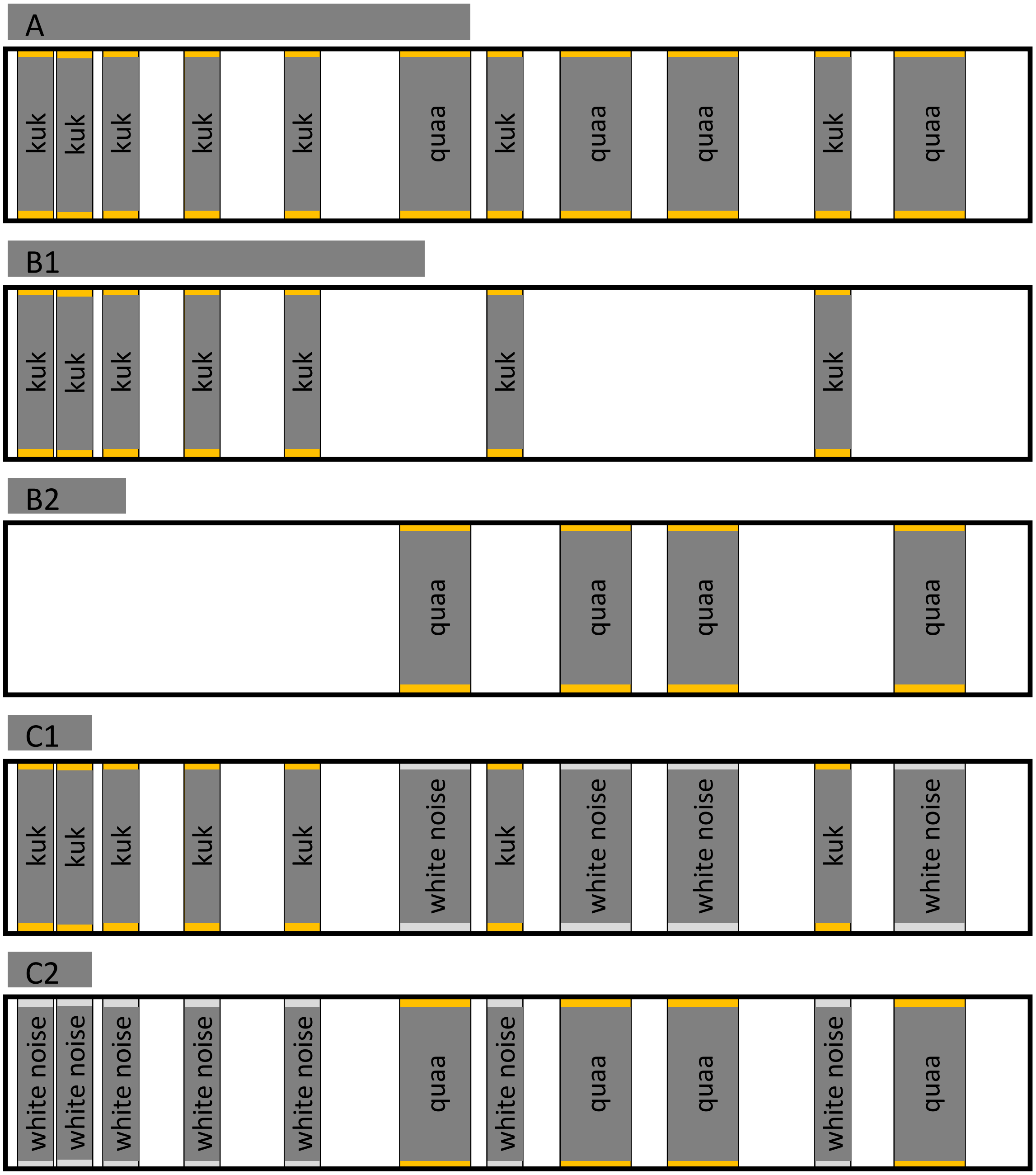

Figure 5.1. The five variations of an alarm call used in Experiments 1, 2, and 3. This diagram represents the temporal arrangement (from left to right representing first to last) of kuk and quaa alarm calls within a bout. **(A)** The original alarm calling bout was played back in Experiment 1 with kuks and quaas intact. The other stimulus in Experiment 1 was continuous white noise, which is not shown in the diagram. **(B)** In Experiment 2, either kuks or quaas were removed from an alarm calling bout, and the remaining calls were left in their natural temporal arrangement, so each stimulus had only kuks (B1) or only quaas (B2), and also varied in temporal pattern of sounds present. **(C)** In Experiment 3, rather than remove kuks or quaas, they were replaced with bursts of white noise that matched the alarm calls in duration. In this experiment, stimuli with kuks plus white noise (C1) and with quaas plus white noise (C2) had identical temporal patterns of sound, and so differed only in which call type was present.

BEHAVIORAL ANALYSIS

Behavior of focal squirrels was scored from video recordings using Sony Vegas 9 or 11 video editing software (Sony Creative Software Development Team 2009, 2011). The frequency of occurrence and total duration of each behavior category were scored for 60s following the start of the first element (either white noise or vocalization) in a playback trial.

Latency to first occurrence was also scored for each behavior observed in a trial. See Table 5.2 for definitions of behaviors used. The majority of behaviors, both in duration and frequency, were foraging/caching, scanning on all fours, scanning upright, eating, and walking, with foraging/caching and scanning being longer in total duration than the others. I focused my analysis on foraging/caching (hereafter referred to as *head-down behavior*) and scanning (which includes both upright scanning and scanning on all fours), because they composed the majority of behaviors (by duration and frequency of occurrence) and represent a clear tradeoff. A squirrel cannot place its head down to the ground, which obscures its vision, while simultaneously scanning its surroundings, so head-down behavior and scanning are useful measures to test for changes in priority of behaviors caused by alarm signals.

When examining latency to initiate scanning or head-down behavior, no measure can be obtained for squirrels that never initiated the behavior of interest. Trials where squirrels did not initiate a behavior are excluded from analysis for latency, but retained when examining rate and total duration of behaviors. In experiments where paired data for latency were not available for all squirrels, only individuals that initiated the behavior in both trials are included in paired statistical analysis, although all trials in which squirrels initiated the behavior are used in calculating mean or median latency.

STATISTICAL ANALYSES

A paired design was used in each experiment, with each individual squirrel in an experiment receiving two matched stimuli, so paired analyses are used throughout. When difference measurements in response variables were normally distributed, as determined by a Shapiro-Wilk normality test, paired t-tests were used to compare responses of squirrels to the two acoustic stimuli within each experiment. When differences across pairs were not normally distributed, I used exact Wilcoxon matched-pair signed-ranks (WMPSR) tests instead of paired t-tests. All statistical analyses were calculated using JMP Pro 9 (SAS Institute Inc., Cary, NC, 1989-2011).

RESULTS

EXPERIMENT ONE – ALARM CALLS VERSUS WHITE NOISE

SCANNING BEHAVIOR

Analysis of scanning suggests that eastern gray squirrels ($N = 10$) did not differ in response to white noise and to complete alarm calls consisting of kuks and quaas (Figure 5.2). The mean latency from the start of the audio playback until squirrels began scanning did not differ in response to alarm calls ($N = 9$, $\bar{x} = 30.65$s, $s = 43.04$s) and in response to white noise ($N = 9$, $\bar{x} = 28.06$, $s = 22.78$), with a difference of only 2.59s, about 9%. Including only squirrels that scanned in both trials, latency to scanning does not differ in response to alarm calls ($N = 8$, $\bar{x} = 30.18$s, $s = 45.98$s) and white noise ($N = 8$, $\bar{x} = 28.52$s, $s = 24.98$s), with a mean difference of only 1.66s, about 6% ($t = -0. 053$, df = 7, $P =$

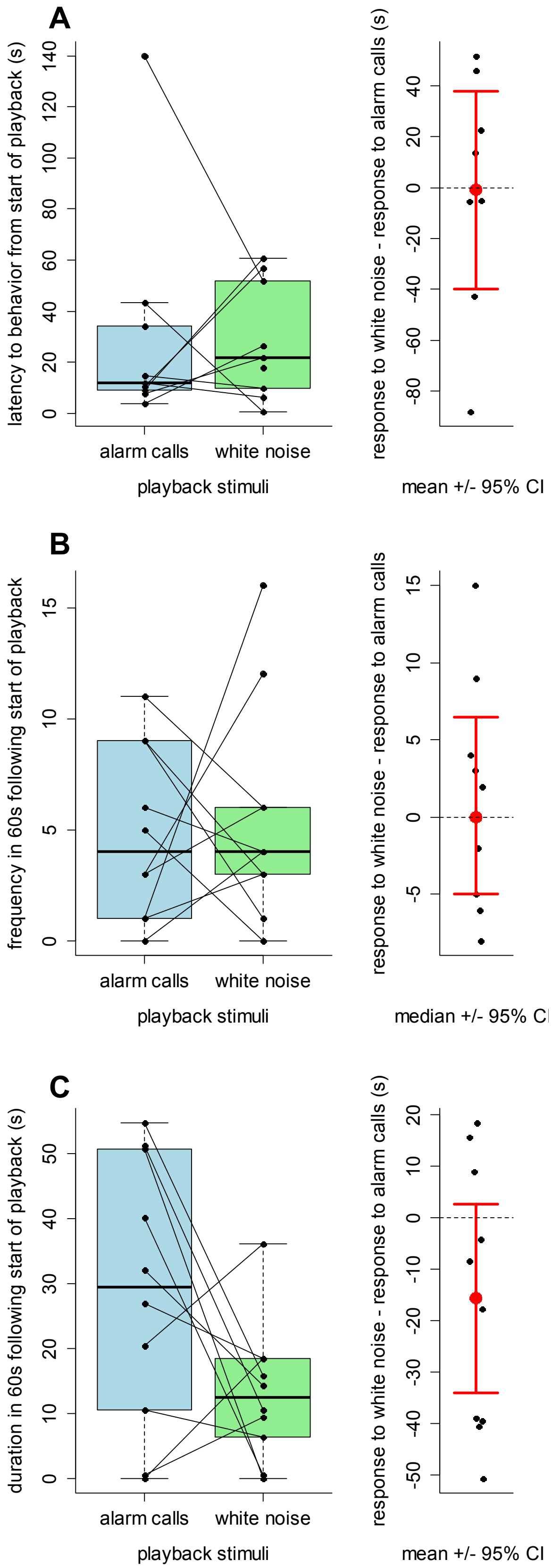

Figure 5.2. Scanning behavior in Experiment 1. In paired trials of the same individual squirrels, scanning behavior of focal squirrels was similar in response to alarm calls and white noise. **(A)** Squirrels' latency to scan after the start of the acoustic stimulus varied widely within and among squirrels and showed no clear pattern. There is no significant difference in mean latencies to scan (*t* = -0. 053, df = 7, *P* = 0.959). **(B)** The number of times squirrels scanned in the 60s following the start of the acoustic stimulus was also quite variable with no clear trend. There is no significant difference in the number of times each squirrel scanned in response to white noise versus alarm calls (exact WMPSR test, T^+ = 27.5, *P* = 1). **(C)** The total duration of scanning by squirrels in the 60s following the start of the acoustic stimulus was lower in response to white noise than in response to alarm calls in 8 of 10 squirrels. There is no significant difference in the mean duration of scanning in response to white noise versus alarm calls (*t* = -1.937, df = 9, *P* = 0. 0848).

0.959) (Figure 5.2A). Frequency of scanning in the 60s following the start of the audio playback was extremely inconsistent (Figure 5.2B). The mean number of times focal squirrels (*N* = 10) initiated scanning was 4.8 (*s* = 3.85) in response to alarm calls and 5.5 (*s* = 4.95) in response to white noise, with a mean difference of 0.7, about 14% (exact WMPSR test, T^+ = 27.5, *P* = 1).

The mean total duration of scanning by focal squirrels (*N* = 10) in the 60s following the start of audio playback of the stimulus did not differ in response to alarm calls ($\bar{x}$ = 28.29s, *s* =20.61s) versus white noise ($\bar{x}$ = 13.01s, *s* = 10.48s), with a mean difference of 15.28s, about 75% (*t* = -1.937, df = 9, *P* = 0.0848), even though 7 of 10 squirrels spent more time scanning in response to alarm calls than to white noise (Figure 5.2C).

HEAD-DOWN BEHAVIOR

Analysis of head-down behavior shows that eastern gray squirrels respond somewhat differently to alarm calls and white noise (Figure 5.3). By design, squirrels were always on the ground when the playback of a stimulus began, which meant that they were usually engaged in head-down behavior, and occasionally walking, with short bouts of scanning interspersed throughout. The mean latency to initiate head-down behavior after the start of the playback was 54.97s (*N* = 6, *s* = 36.41s) after the start of alarm calls, and 31.9s (*N* = 9, *s* = 20.74s) after the start of continuous white noise, with a mean difference of 23.07s, about 53% (*N* = 6, *t* = -3.387, df = 5, *P* = 0.0195). Every squirrel that foraged or cached in both trials (*N* = 6) waited longer to initiate head-down behavior in alarm call trials than in white noise trials (Figure 5.3A).

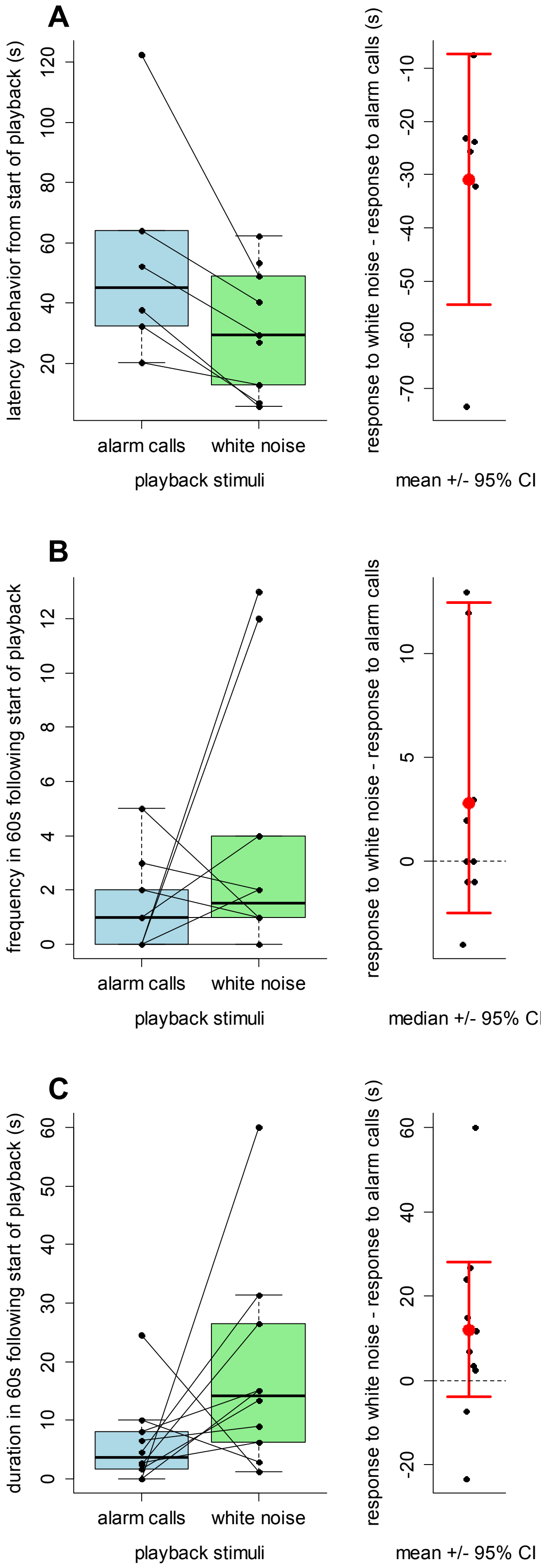

Figure 5.3. Head-down behavior in Experiment 1. In paired trials of the same individual squirrels, head-down behavior of focal squirrels was somewhat different in response to alarm calls and white noise. **(A)** Squirrels delayed head-down behavior after hearing an alarm call more than in response to white noise. Latencies to forage or cache after the start of the acoustic stimulus were consistently shorter in response to white noise than to alarm calls. There is a significant difference in mean latency to scan in response to alarm calls versus continuous white noise (t = -3.387, df = 5, P = 0.0195). **(B)** The number of times squirrels initiated head-down behavior in the 60s following the start of the acoustic stimulus was quite variable with no clear trend. There is no significant difference in the mean number of times each squirrel initiated foraging or caching in trials with alarm calls versus trials with white noise (exact WMPSR test, T^+ = 20, P = 0.344). **(C)** In response to alarm calls 8 of 10 squirrels spent less time in head-down behavior than in response to white noise. There is no significant difference in the mean duration of individual squirrels' head-down behavior in trials with white noise versus trials with alarm calls (t = 1.698, df = 9, P = 0.124).

behavior after the alarm call than it did after the white noise (Figure 5.3A). The mean latency for squirrels (*N* = 6) to initiate head-down behavior is significantly different between trials.

While latency shows a clear effect of stimulus type, frequency of initiating head-down behavior does not. The median number of times focal squirrels (*N* = 10) initiated head-down behavior during the 60s following the start of the acoustic stimulus playback was 1 ($\bar{x}$ = 1.3, *s* = 1.64) in trials with alarm calls and 1.5 ($\bar{x}$ = 3.7, *s* = 4.76) in trials with white noise, with a difference of 0.5, exactly 40% (exact WMPSR test, T^+ = 20, *P* = 0.344) (Figure 5.3B).

The mean total duration of head-down behavior in the 60s following the start of audio playback did not differ in response to alarm calls ($\bar{x}$ = 6.05s, *s* = 7.31s) versus white noise ($\bar{x}$ = 18.03s, *s* = 17.6s), with a difference of 11.98s, about 100% (*t* = 1.698, df = 9, *P* = 0.124), even though 8 of 10 squirrels spent less time in head-down behavior in alarm call trials than in white noise trials (Figure 5.3C).

Analysis of both types of behavior (scanning and head-down behavior) suggests squirrels' responses did not differ overall, with alarm calls and white noise eliciting similar latencies, frequencies, and durations of scanning and head-down behaviors. The exception is latency to initiate head-down behavior, which is longer in trials with alarm calls. In several of the trials, squirrels showed a strong startle response to the onset of the continuous white noise, but habituated over time.

LATENCY TO REACH TREE TRUNK

Comparing the latency for a focal squirrel to reach a tree trunk when presented with complete alarm calls versus white noise shows that squirrels did not reach the tree sooner in response to either stimulus (*t* = 0.428, df = 7, *P* = 0.682) (Figure 5.4). Sometimes a squirrel did not return to the tree during a trial, so of the 10 squirrels in Experiment 1, only 7 could be used in this analysis. Reaching a tree was not necessarily a flight response, as squirrels often ascend a tree to eat food they have found while foraging on the ground, even in the absence of any experimental acoustic stimulus.

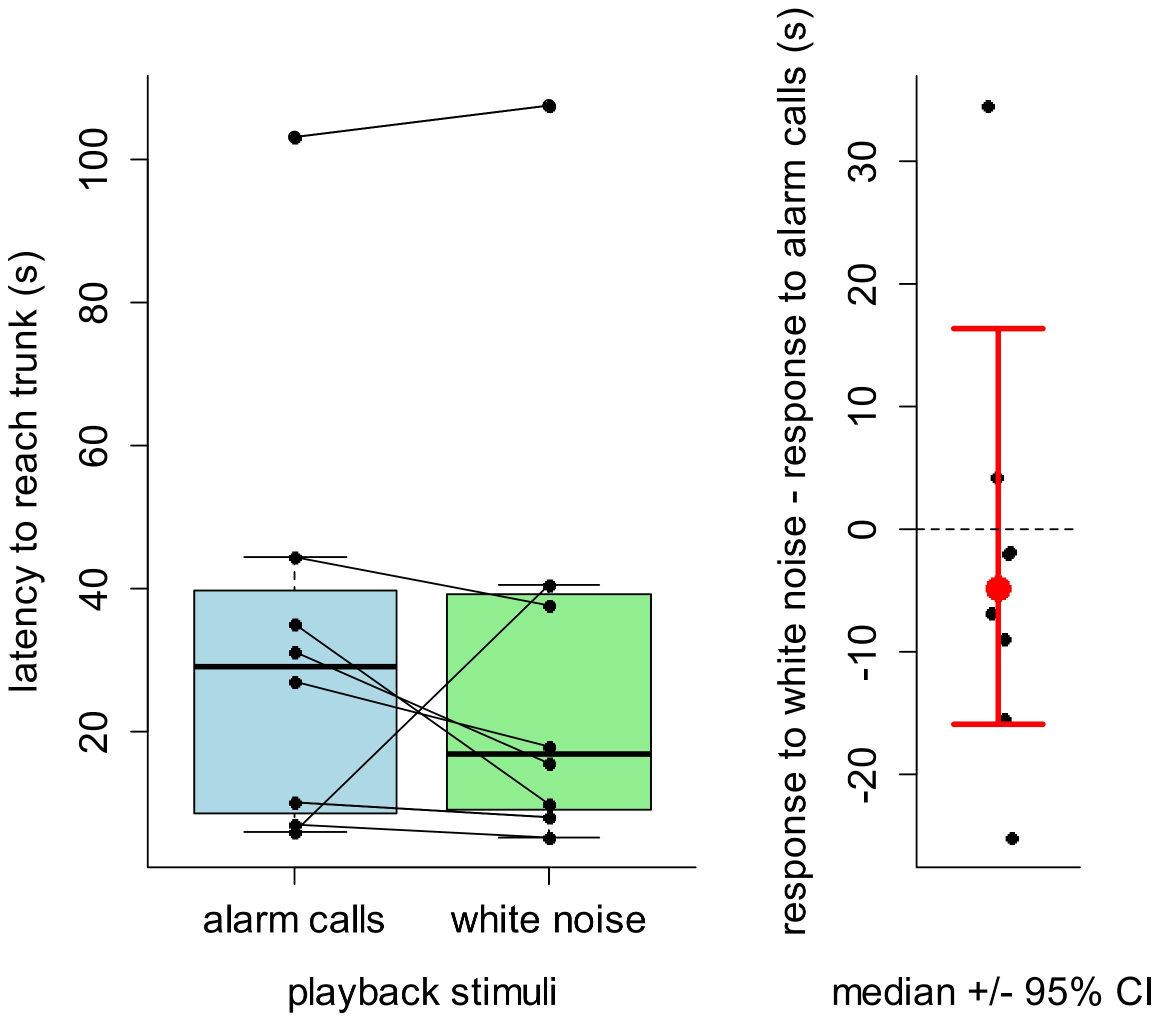

Figure 5.4. Latency to reach tree trunk in Experiment 1. In paired trials to the same individual squirrels (*N* = 8), the time from the start of the acoustic stimulus until the focal squirrel reached the tree did not differ in response to alarm calls and white noise (*t* = 0.428, df = 7, *P* = 0.682).

EXPERIMENT TWO – KUKS VERSUS QUAAS

SCANNING BEHAVIOR

Analysis of scanning in response to kuk versus quaa elements from the same original alarm calls with their original temporal patterns revealed that eastern gray squirrels ($N = 9$) responded differently to the kuks than to the quaas (Figure 5.5). From the start of the first vocalization in the acoustic stimulus, squirrels' latencies to begin scanning were consistently shorter in response to the kuks ($\bar{x} = 22.11$s, $s = 14.33$s) than in response to the quaas ($\bar{x} = 64.51$s, $s = 19.68$s), with a mean difference of 42.11s, about 98% ($t = 5.697$, df = 8, $P = 0.000456$). All squirrels (N = 9) scanned in both trials and scanned sooner in response to kuks than in response to quaas (Figure 5.5A).

Frequency of scanning in the 60s following the start of the first vocalization in an acoustic stimulus also shows a pattern. The mean number of times focal squirrels ($N = 9$) initiated scanning was 7.11 ($s = 5.33$) in response to the kuks and 16.11 ($s = 10.35$) in response to the quaas, with a mean difference of 9, about 78% ($t = 2.5456$, df = 8, $P = 0.0344$), with kuks eliciting less frequent scanning than quaas (Figure 5.5B).

Kuk and quaa elements also elicited different total durations of scanning during the 60s following the start of the first vocalization in an acoustic stimulus. The mean duration of scanning by all nine squirrels was 32.16s ($s = 17.01$s) in response to kuks and 20.38s ($s = 10.71$s) in response to quaas, with a mean difference of 11.78s, about 45% ($t = -2.728$, df = 8, $P = 0.0259$), with the kuks eliciting more scanning than the quaas (Figure 5.5C).

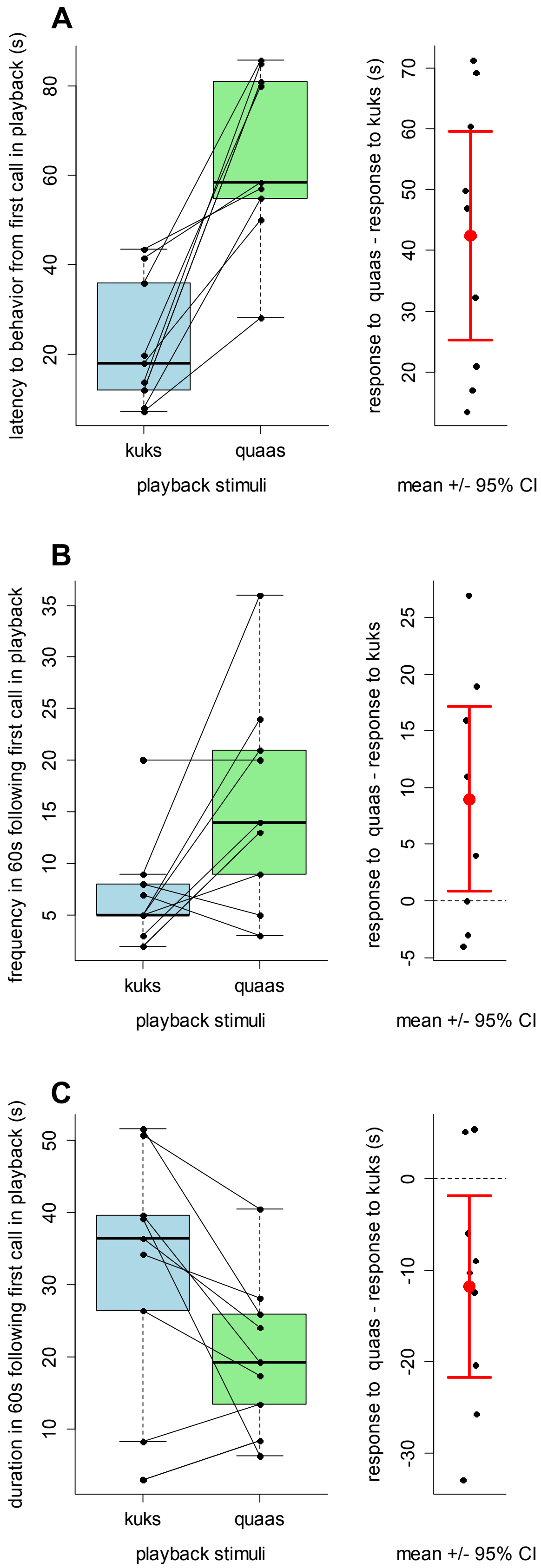

Figure 5.5. Scanning behavior in Experiment 2. In paired trials of the same individual squirrels, scanning behavior of focal squirrels differed in response to acoustic stimuli containing kuk elements versus those containing quaa elements. **(A)** Mean latency to begin scanning after the start of the first element in the audio playback differs between stimuli (t = 5.697, df = 8, P = 0.000456), with squirrels scanning sooner in response to kuk elements from an alarm call than in response to the quaa elements from the same alarm call. **(B)** There is a significant difference in the mean number of times each squirrel scanned in response to kuks versus quaas in the 60s following the start of the first vocalization in the acoustic stimulus (t = 2.5456, df = 8, P = 0.0344), with frequency of scanning higher in response to quaas than in response to kuks. **(C)** There is also a significant difference in the mean total duration of scanning by squirrels in the 60s following the start of the first vocalization (t = -2.7276, df = 8, P = 0.0259) with squirrels spending more time scanning in response to kuks than in response to quaas.

HEAD-DOWN BEHAVIOR

Analysis of head-down behavior suggests that eastern gray squirrels (*N* = 9) behave somewhat differently in trials with kuk elements relative to trials with quaa elements (Figure 5.6). Of the nine eastern gray squirrels presented with both stimuli in Experiment 2, only one initiated head-down behavior in both trials, so no statistical analysis could be calculated or paired data plotted.

The mean latency to initiate head-down behavior after the start of the first element in the acoustic stimulus was 39.92s (*N* = 3, *s* = 15.187s) after the start of kuks, and 64.4s (*N* = 6, *s* = 24.38s) after the start of quaas, with a mean difference of 24.48s, about 47%. It is not apparent whether this difference in means represents a consistent difference in

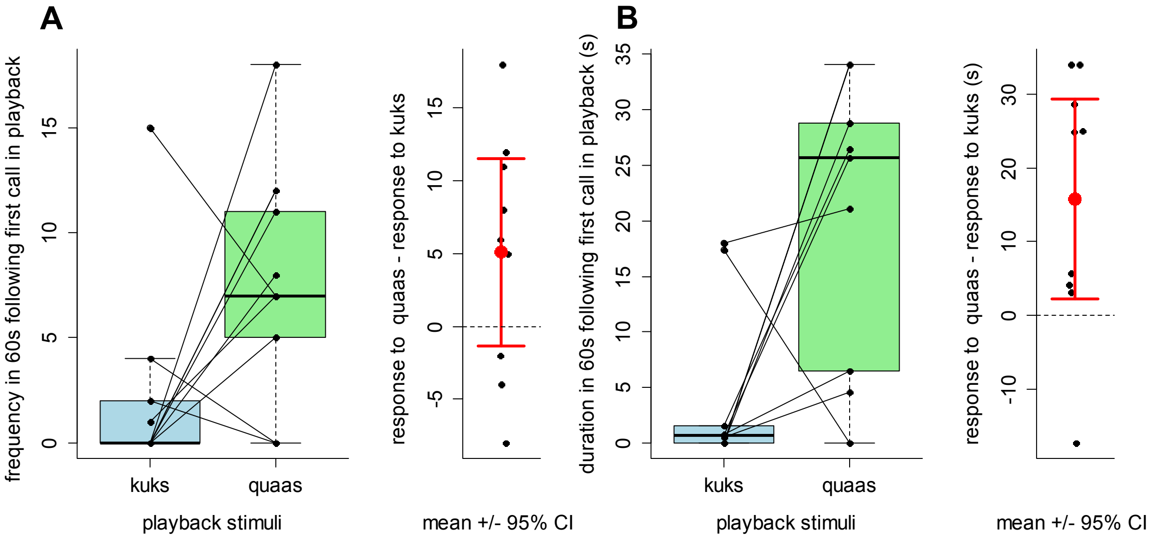

Figure 5.6. Head-down behavior in Experiment 2. In paired trials of the same individual squirrels, head-down behavior of focal squirrels was somewhat different in response to kuk and quaa elements from the same original call presented in their original temporal pattern. **(A)** The number of times squirrels initiated head-down behavior in the 60s following the start of the first alarm element in the acoustic stimulus was quite variable. There is no significant difference in the mean number of times each squirrel initiated foraging or caching in trials with kuks versus trials with quaas (t = 1.828, df = 8, P = 0.105). **(B)** The mean total duration of head-down behavior by squirrels in the 60s following the start of the first vocalization is significantly different in trials with kuks versus trials with quaas (t = 2.6875, df = 8, P = 0.0276), with squirrels spending less time in head-down behavior in response to kuks than in response to quaas.

behavior. Additional data are needed to clarify whether kuks and quaas in a natural temporal pattern have different effects on latency to resume head-down behavior.

Data on frequency of initiating head-down behavior are available for all nine squirrels, and it appears that squirrels initiated head-down behavior irrespective of whether kuk or quaa elements are contained in the acoustic stimulus (Figure 5.6A). The mean number of times focal squirrels (N = 9) initiated head-down behavior during the 60s following the start of the first vocalization in the audio playback was 2.44 ($s = 4.9$) in trials with kuks and 7.56 ($s = 5.73$) in trials with quaas, with a mean difference of 5.12, about 102% ($t = 1.828$, df = 8, $P = 0.105$) (Figure 5.6A).

Total durations of head-down behavior in the 60s following the start of the first element in the audio playback were usually much shorter in trials with kuks ($N = 9$, $\bar{x} =$ 4.31s, $s = 7.58$s) than in trials with quaas ($N = 9$, $\bar{x} = 20.15$s, $s = 13.076$s), with a mean difference of 15.84s, about 130% ($t = 2.6875$, df = 8, $P = 0.0276$) (Figure 5.6B).

Analysis of both scanning and head-down behavior reveals that squirrels forage or cache (head-down) less and scan more in response to the kuk elements than in response to the quaa elements. The kuks in an alarm calling bout thus elicit a greater shift toward antipredator behavior than do the quaas in that calling bout. This difference in the effect of kuks and quaas could be due to differences in temporal pattern of the elements. Kuks were much more rapid than quaas, and there were thus more kuks than quaas in each alarm calling bout. To quantify the difference in calling rate between kuk and quaa acoustic stimuli ($N = 9$) used in Experiment 2, I counted the number of kuks or quaas in 5s and 60s periods from the start of the first vocalization in an acoustic stimulus.

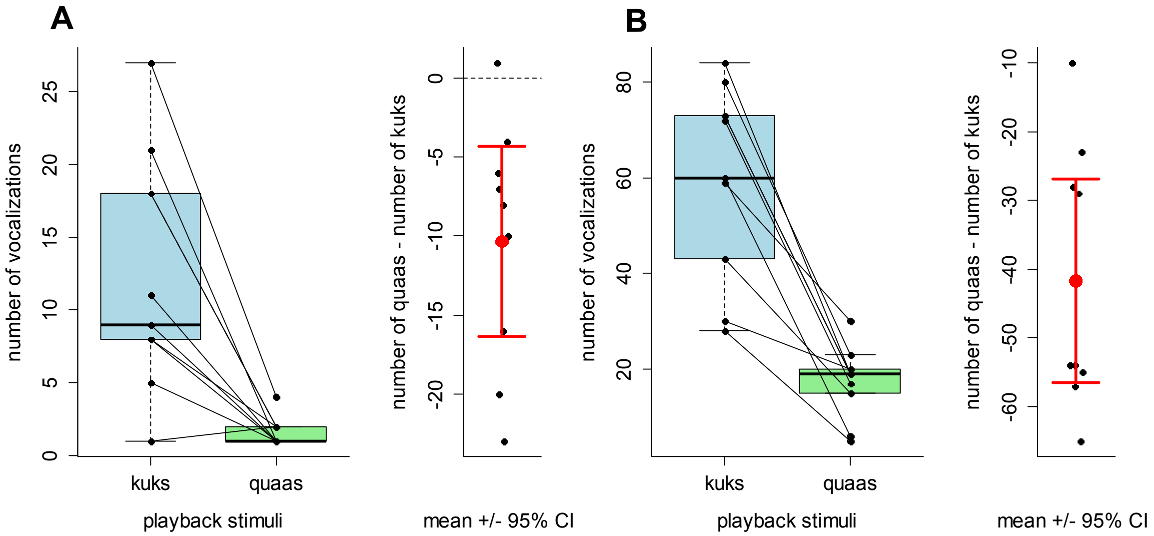

Figure 5.7. Rate of vocal elements in kuk and quaa stimuli used in Experiment 2. Kuks are given more rapidly than quaas, so when isolated into separate audio files to be used as stimuli in Experiment 2, playbacks of kuks inherently had higher calling rates than playbacks of quaas. **(A)** The first 5s of calling in acoustic stimuli consisting of kuks contained a mean of 12 elements (s = 8.32), whereas the first 5s of calling in stimuli consisting of quaas contained a mean of 1.67 elements (σ = 1). **(B)** The first 60s of calling in acoustic stimuli consisting of kuks contained a mean of 58.78 calls (s = 20.89), whereas the first 60s of calling in stimuli consisting of quaas contained a mean of 17.11 calls (s = 7.83).

In Experiment 2, each acoustic stimulus was composed solely of either kuks (N = 9) or quaas (N = 9). For each type of acoustic stimulus, in the first 5s of calling the mean numbers of elements were 12 kuks (s = 8.32) and 1.67 quaas (s = 1), with a mean difference of 10.33, about 151% (t = -3.961, df = 8, P = 0.00417) (Figure 5.7A). In the first 60s of calling, which approached the total duration of some stimuli, the means were 58.78 kuks (s = 20.88) and 17.11 quaas (s = 7.83), with a mean difference of 41.67, about 110% (t = -6.4984, df = 8, P = 0.000188) (Figure 5.7B).

LATENCY TO REACH TREE TRUNK

Comparing the latency for a focal squirrel to reach a tree trunk when presented with complete alarm calls versus white noise shows that squirrels reached the tree sooner in response to kuks than in response to quaas (t = 0.571, df = 5, P = 0.177) (Figure 5.4).

As in Experiment 1, sometimes a squirrel did not reach a tree during a trial, so of the nine squirrels in Experiment 2, only six could be used in this analysis. Five of the six squirrels reached a tree sooner in response to the kuks than in response to the quaas.

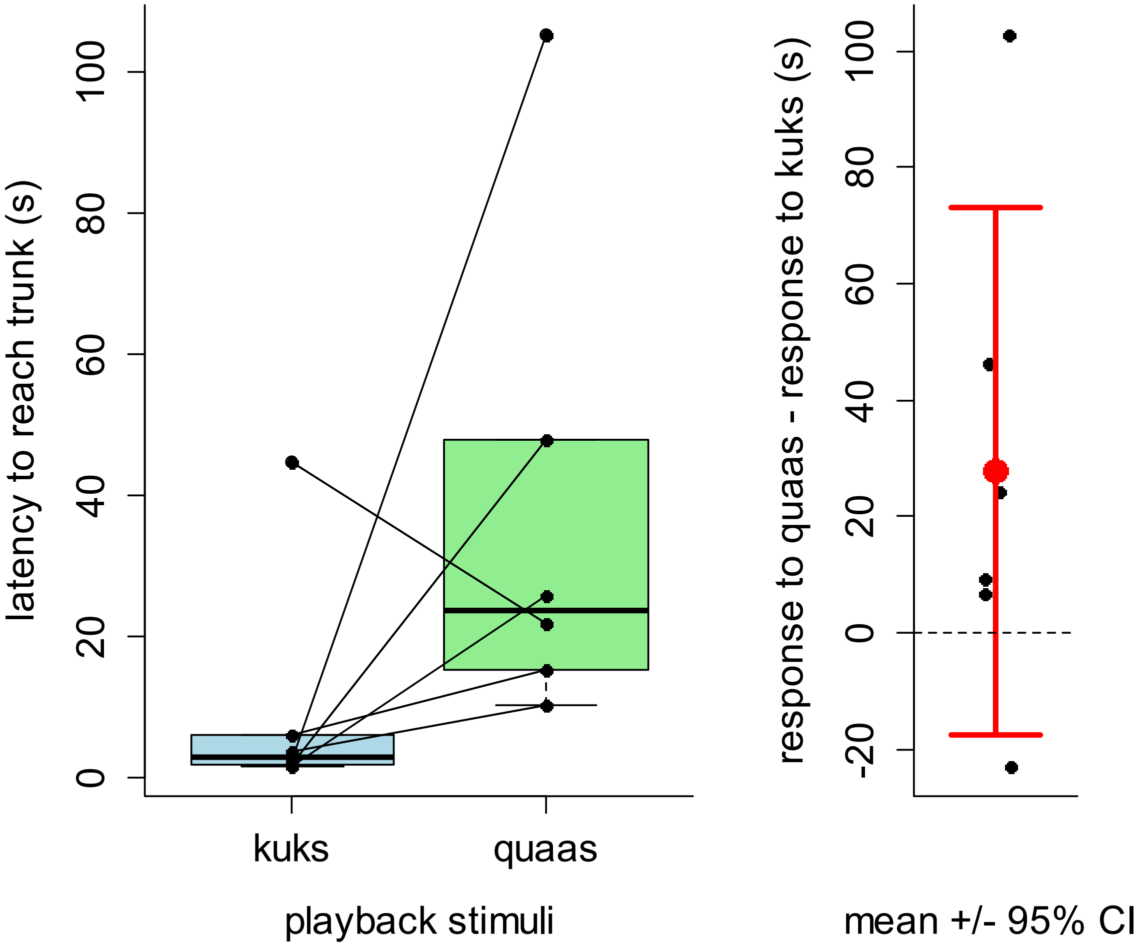

Figure 5.8 Latency to reach tree trunk in Experiment 2. In paired trials to the same individual squirrels (*N* = 6), the time from the start of the acoustic stimulus until the focal squirrel reached the tree was shorter in response to kuk elements than to quaa elements from the same original call presented in their original temporal pattern (*t* = 1.571, df = 5, *P* = 0.177).

In summary, the general pattern observed in Experiment 2 is more antipredator behavior in response to the kuks than in response to the quaas. The differences in antipredator behavior may be caused by the faster calling rate of kuks having a stronger effect than the slower calling rate of quaas. The high frequency of scanning in trials with quaas is not a reflection of elevated antipredator behavior relative to trials with kuks. Rather, the high scanning frequency is because foraging squirrels frequently lift their heads for momentary scans, thus raising scanning frequency but overall spending very little time scanning. In trials with kuks, head-down behavior was reduced and scanning was of longer duration relative to trials with quaas, reflecting a few prolonged scans in trials with kuks and many very brief scans in trials with quaas. Similarly, the rapid retreat of squirrels to trees elicited by kuks suggests that, within a calling bout, kuks have a greater effect than quaas in eliciting retreat behavior in conspecifics.

These differences in scanning and latency to reach a tree suggest a general pattern of increased antipredator behavior in response to the kuks versus the quaas. It thus appears that in a normal gray squirrel alarm calling bout consisting of kuks and quaas, the kuks are more salient than the quaas in elevating conspecifics' antipredator behavior. To further elucidate the relative function of kuks and quaas, I designed another experiment to examine squirrels' responses to kuks and quaas. In this third experiment I again isolated kuk and quaa elements from the same original alarm call, but I held the temporal pattern of elements constant by replacing one element type with bursts of white noise rather than simply eliminating them.

EXPERIMENT THREE – KUKS PLUS WHITE NOISE VERSUS QUAAS PLUS WHITE NOISE

SCANNING BEHAVIOR

Analysis of scanning in response to stimuli that differed in the presence of kuks or quaas but had the same temporal pattern of sound production suggests that eastern gray

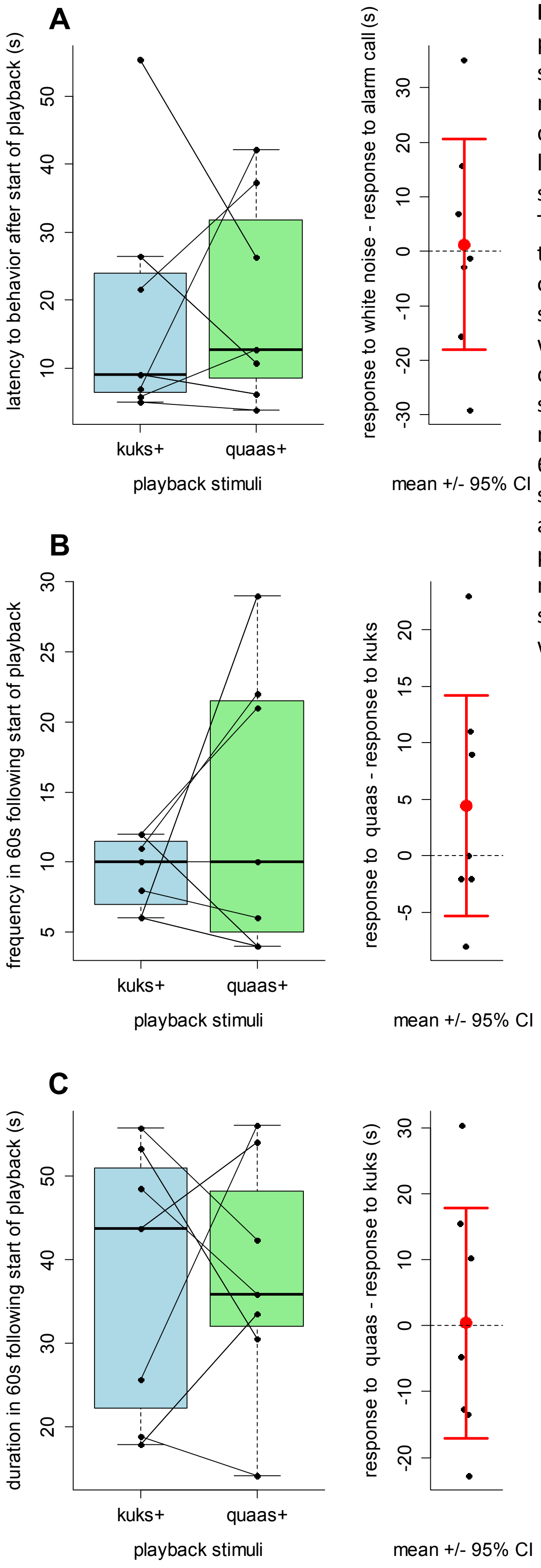

Figure 5.9. Scanning behavior in Experiment 3. In paired trials of the same individual squirrels, scanning behavior of focal squirrels was similar in response to kuks+ (kuks plus white noise) and quaas+ (quaas plus white noise). **(A)** Squirrels' latency to scan after the start of the acoustic stimulus varied widely and showed no clear pattern. There is no significant difference in mean latencies to scan (*t* = 0. 161, df = 6, *P* = 0.877). **(B)** The number of times squirrels scanned in the 60s following the start of the acoustic stimulus was also quite variable with no clear trend. There is no significant difference in the mean number of times each squirrel scanned in response to kuks plus white noise versus quaas plus white noise (*t* = 1.112, df = 6, *P* = 0.309). **(C)** The total duration of scanning by squirrels in the 60s following the start of the acoustic stimulus was similar in response to kuks plus white noise and quaas plus white noise. There is no significant difference in the mean duration of scanning in response to trials with kuks versus trials with quaas (*t* = -0.572, df = 7, *P* = 0.956).

squirrels (*N* = 7) did not differ in response to different types of stimuli (Figure 5.9). The mean latency from the start of the audio playback until squirrels began scanning did not differ in response to kuks plus white noise ($\bar{x}$ = 18.60s, *s* = 18.24s) versus quaas plus white noise ($\bar{x}$ = 19.87s, *s* = 15.38s), with a mean difference of only 1.27s, about 7% (*t* = 0.161, df = 6, *P* = 0.877) (Figure 5.9A).

Frequency of initiation of scanning in the 60s following the start of the audio playback was more variable in response to stimuli with quaas plus white noise than in response to kuks plus white noise. The mean number of times focal squirrels (*N* = 7) initiated scanning was 9.29 (*s* = 2.63) in response to stimuli with kuks and 13.71 (*s* = 10.14) in response to stimuli with quaas, with a mean difference of 4.42, about 39% (*t* = 1.112, df = 6, *P* = 0.309) (Figure 5.9B).

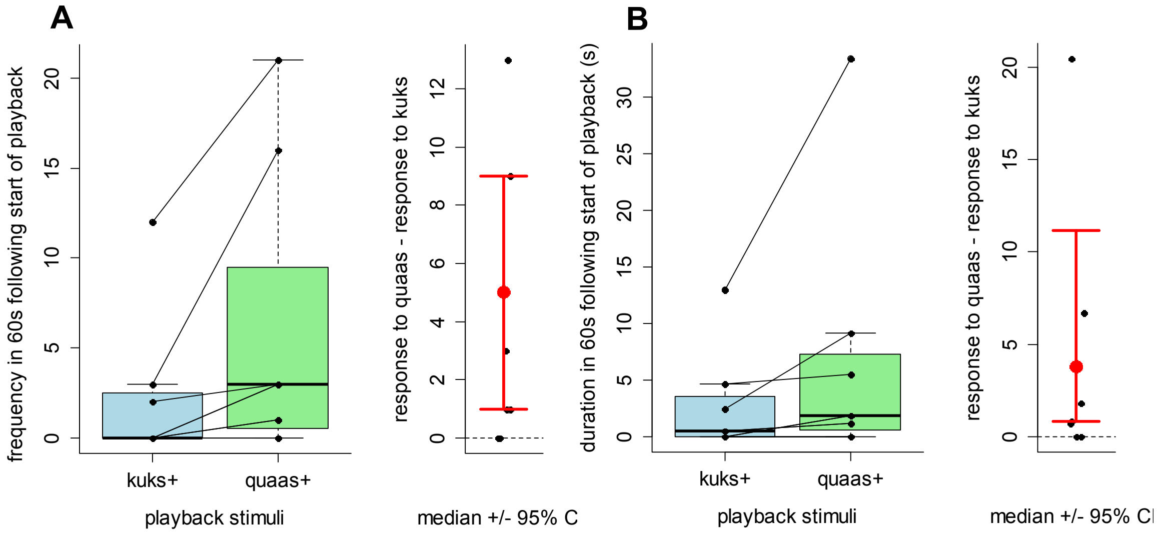

Figure 5.10. Head-down behavior in Experiment 3. In paired trials of the same individual squirrels (*N* = 7), head-down behavior of focal squirrels was similar in response to kuks+ (kuks plus white noise) and quaas+ (quaas plus white noise). **(A)** The number of times squirrels initiated head-down behavior in the 60s following the start of the acoustic stimulus was quite variable, and the difference is not significant (exact WMPSR test, T^+ = 15, *P* = 0.0625). **(B)** The total duration of head-down behavior in the 60s following the start of the playback is not different in trials with kuks versus trials with quaas (exact WMPSR test, T^+ = 15, *P* = 0.0625).

The mean total duration of scanning by focal squirrels (*N* = 7) in the 60s following the start of playback did not differ in response to stimuli with kuks ($\bar{x}$ = 37.70s, *s* = 16.41s) versus stimuli with quaas ($\bar{x}$ = 38.11s, *s* = 14.43s), with a mean difference of 0.41s, about 1% (*t* = -0.0572, df = 7, *P* = 0.956) (Figure 5.9C).

HEAD-DOWN BEHAVIOR

Analysis of head-down behavior in response to stimuli that differ in the presence of kuks or quaas but have the same temporal pattern of sound production suggests that eastern gray squirrels (*N* = 7) did not differ in response to the two types of stimuli (Figure 5.10). Of the seven eastern gray squirrels that were presented with both stimuli in Experiment 3, only one initiated head-down behavior in both trials, so no statistical analysis could be run or figures of paired data made. The mean latency from the start of the audio playback for squirrels to initiate head-down behavior was 21.58s (*N* = 3, *s* = 2.59s) in trials with kuks, and 25.33s (*N* = 4, *s* = 23.45s) in trials with quaas, with a mean difference of 3.75s, about 16%. These results are inconclusive, but suggest latency to initiate head-down behavior did not differ in response to kuk and quaa elements isolated from the same alarm call when temporal pattern of sound was matched by adding white noise elements.

Frequency of head-down behavior in the 60s following the start of audio playback also did not differ in response to the two types of stimuli. Focal squirrels (*N* = 7) initiated head-down behavior during the 60s following the start of the acoustic stimulus playback a median of 0 times ($\bar{x}$ = 2.43, *s* = 4.40) in trials with kuks and 3 times ($\bar{x}$ = 6.29, *s* = 8.56) in trials with quaas, with a difference of 3, or 200% (exact WMPSR test, T^+ = 15, *P* = 0.0625) (Figure 5.10A).

The duration of head-down behavior in the 60s following the start of audio playback shows a similar pattern to that observed in frequency of head-down behavior. In the 60s following the start of audio playback, focal squirrels spent a median of 0.50s ($\bar{x}$ = 2.93, *s* = 4.75) in head-down behavior when presented with stimuli containing kuks. When presented with stimuli containing quaas, squirrels spent a median of 1.83s ($\bar{x}$ = 7.29 *s* = 11.98) in head-down behavior, which gives a difference of 1.33s, about 26% (exact WMPSR test, $T^+ = 15$, $P = 0.0625$) (Figure 5.10B).

LATENCY TO REACH TREE TRUNK

Comparing the latency for a focal squirrel to reach a tree trunk when presented with stimuli that differ in the presence of kuks or quaas but have the same temporal pattern of sound production suggests that focal squirrels (N = 6) did not reach the tree sooner in response to either stimulus (exact WMPSR test, $T^+ = 10$, $P = 1$) (Figure 5.11). One squirrel did not return to the tree during a trial, so of the seven squirrels in Experiment 3, only six could be used in this analysis. Reaching a tree was not necessarily a flight response, but the short latencies in several trials suggest that the movement to the tree was a direct response to the acoustic stimulus (Figure 5.11).

Analysis of scanning, head-down behavior, and latency to reach a tree suggests that temporal pattern of sound elements is more important than whether the sound elements are kuks, quaas, or white noise. In response to pairs of stimuli with the same temporal pattern of sound elements, but differing in whether they contained kuks or quaas, squirrels did not differ in their use of antipredator behaviors (Figures 5.10, 5.11).

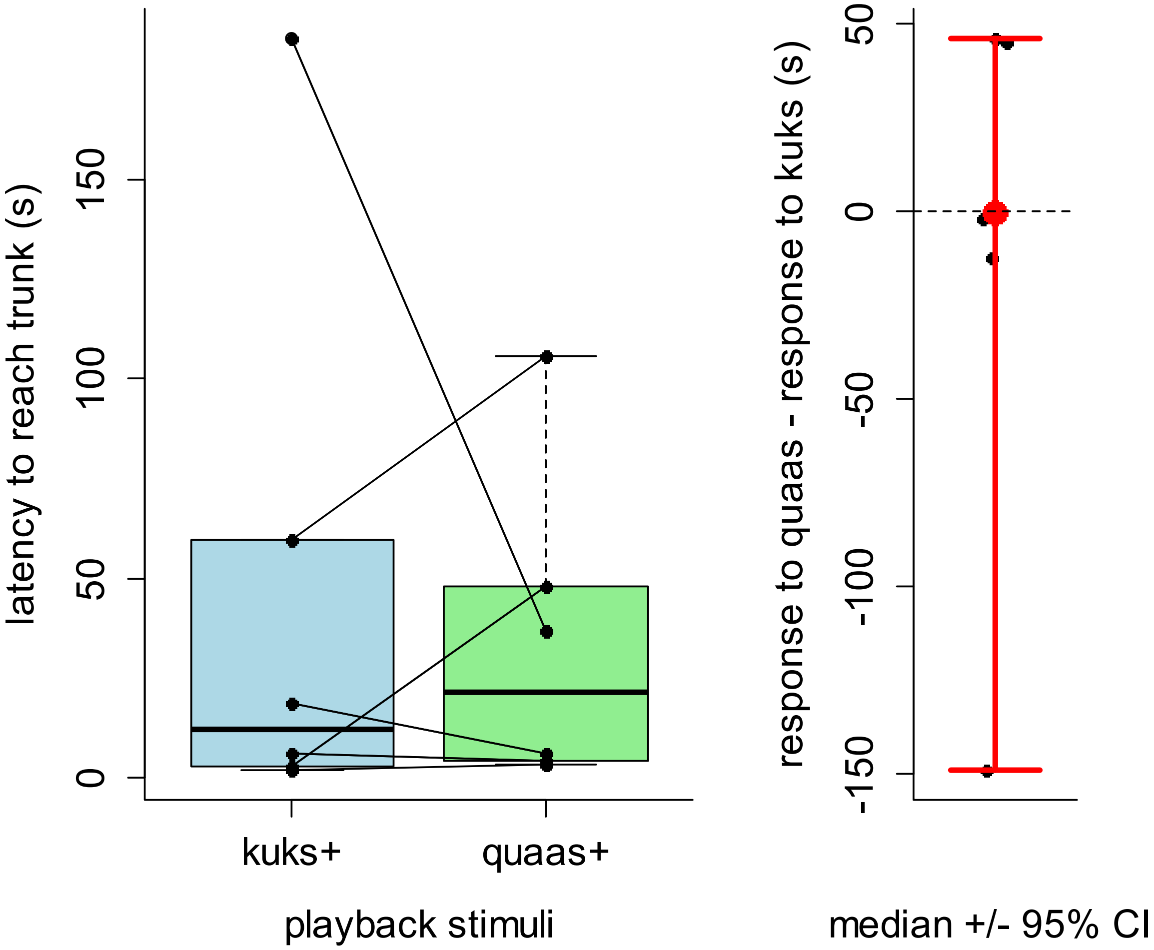

Figure 5.11. Latency to reach tree trunk in Experiment 3. In paired trials of the same individual squirrels (*N* = 6), the time from the start of the acoustic stimulus until the focal squirrel reached the tree did not differ in response to kuks+ (kuks plus white noise) and quaas+ (quaas plus white noise) (exact WMPSR test, T^+ = 10, *P* = 1).

DISCUSSION

RETREAT TO TREE

The results of Experiment 2 suggest that one function of alarm calls, and of kuks in particular, is to elicit retreat behavior in conspecifics. Across all three experiments, the latencies to reach the tree were long. These long latencies suggest that the acoustic stimuli usually did not elicit rapid flight to a tree, but first elicited scanning, and then only in some cases did the squirrel flee to a tree. Squirrels also would sometimes find a food object, place it in their mouth, and immediately retreat to a tree, as I also observed them to do outside experimental trials. It is also possible that some squirrels did not immediately hear the acoustic stimulus when it was broadcast. The levels of background noise in the study site are high, and although the playback of the acoustic stimuli was audible to human listeners, squirrels may or may not have perceived the calls initially. In several trials the focal squirrel would continue foraging and walking with no apparent change in behavior after the playback began, and then suddenly, after one of the vocalizations or white noise bursts, the squirrel would abruptly lift its head, momentarily scanning and then run to a tree. These observations support the interpretation that the long latencies, both to reach a tree and to begin scanning, are sometimes a result of a squirrel that did not initially perceive the acoustic stimulus. If squirrels sometimes did not hear or perceive the initial portion of the acoustic stimulus, it could also explain the extreme variation in responses observed.

IMPORTANCE OF TEMPORAL SOUND PATTERN

In eastern gray squirrels, bursts of white noise are functionally equivalent to kuks and quaas in their effects on conspecifics' behavior. Kuks and quaas are both noisy, broadband vocalizations; it may be that when squirrels hear such noisy vocalizations, they respond primarily to the temporal pattern of elements produced in an alarm calling bout, not to any structure of the alarm elements themselves. A response based solely on temporal pattern, or rhythm, of noisy calls could explain the difference in response to kuks versus quaas in Experiment 2, because the time pattern of elements was distinctly different within pairs of acoustic stimuli. Such a rhythm-based signaling system could also explain the similarity of squirrels' responses to the stimuli in Experiment 3, because the time pattern of elements (vocalizations plus white noise) was identical within each pair of stimuli.

Lishak (1984) suggested that quaas and kuks have no significant differences in acoustic frequency structure, but also showed that when durations of kuks and quaas are pooled, the durations of individual vocalizations form a distinct bimodal distribution, suggesting kuks and quaas are categorically different call types (Lishak 1984). Such a system is reminiscent of the signal structure used in Morse code, where the only parameter of import is duration of each element. In the case of gray squirrels, short elements (kuks) are often used rapidly at the start of alarm calling bouts, while long elements (quaas) are used at slower rates after the initial rapid kukking is past. It is thus unclear whether rate, number, or duration of each element is the primary driver of the differences in response observed in Experiment 2.

Both kuks and quaas are associated with a terrestrial predator's presence, as shown by the results presented in Chapter 4. Because a single alarm element could suffice to designate a threat as terrestrial, the combination of two types of alarm elements in response to a single class of predator suggests that kuks and quaas may serve different functions. Combinatorial calls composed of two element types are typically studied as units, and the separate function of element types within mixed calling bouts has not been well-explored. Most studies of combinatorial calls look at several Paridae species, which also combine two types of individual vocalizations (notes) within alarm calls (Ficken et al. 1978; Clucas et al. 2004; Templeton et al. 2005; Soard & Ritchison 2009), similar to the manner in which gray squirrels combine kuks and quaas. As in gray squirrels, chickadees typically initiate their calling with one type of vocalization and then switch to a second type (Ficken et al. 1978). In black-capped chickadees, the first note type (the "chick") is short and is followed by a longer note type (the "dee") (Templeton et al. 2005). Although the frequency structures of squirrels' calls (kuk and quaa) and chickadees' notes (chick and dee) are different, the temporal pattern is analogous. Both squirrels and chickadees use two types of vocalizations in mixed calling bouts, usually starting with the short and ending with the long (Ficken et al. 1978; Lishak 1984).

In black-capped (Templeton et al. 2005) and Carolina chickadees (Soard & Ritchison 2009), the number of notes in a call varies with the size of a raptor, thus there is information contained in the temporal pattern, or rhythm, of the call. It also has been shown that changing the frequency of switching between call types changes the response in black-capped chickadees, even when number of each call type and overall duration are held constant (Hailman & Ficken 1987). It is not known, however, if each call type serves a different function or if the two combine to form a single signal. In gray squirrels, it appears that kuks and quaas in a call have different effects, but the difference may be caused by either rate of call, call type, duration from first to last call, or some combination of the three, which all differ between kuks and quaas in a bout.

The two-note, repetitive mobbing calls of black-capped chickadees have been shown to recruit conspecifics as well as other small birds (Hurd 1996). Whether the number of repetitions affects the behavior of receivers (whether conspecifics, other small birds, or the perched raptors eliciting the calls) in mobbing contexts is unknown. In the context of recruitment to food, it has been shown in black-capped chickadees that using a greater number of “dee” notes, the second note in the call, reduces latency of conspecifics to arrive at a feeding station (Clucas et al. 2004). In the present study of gray squirrels, Experiments 2 and 3 in combination suggest that rate of calling changes the magnitude of conspecifics’ antipredator responses, but it is not clear whether this effect differs between kuks and quaas. In Experiment 2, the kuks isolated from a calling bout elicit longer scanning than do the quaas, which could be caused by the type of calls present or caused by differences in temporal pattern of calling (either rate or overall duration of calling). In Experiment 3, where duration and rate of sounds are identical, but element types present still differ, kuks plus white noise and quaas plus white noise elicit similar responses, which suggests that temporal pattern of elements, not element structure, is the driving factor in elevating antipredator behavior of conspecific receivers.

GENERALIZED RESPONSE TO SUDDEN NOISE MAY REFLECT ADAPTIVE SENSORY BIAS

An additional implication of Experiments 1, 2, and 3 is that either alarm vocalization (kuk or qua) can be substituted with white noise without affecting the function of the calling bout. While the results of Experiment 1 are difficult to interpret, they suggest that antipredator behavior can be elicited not only by alarm calls, but by any startling auditory stimuli, even a sound that is not associated with an actual threat. Alternatively, it may be that white noise is actually perceived as an alarm call. Given the sudden onset and broad frequency range of white noise, it may sufficiently resemble the abrupt, broadband alarm vocalizations of squirrels to trigger the same responses elicited by actual alarm calls. There are at least two possible explanations for this generalized response to sudden noise.

ADAPTATION FOR EAVESDROPPING ON HETEROSPECIFIC ALARMS

Contrary to the combinatorial calls of chickadees, in gray squirrels it does not seem to matter which element type is repeated. Simply repeating or continuing any broadband sound is sufficient to elevate antipredator behavior. This generalized response to alarm calls and white noise may represent adaptations to respond to alarm calls of other species. European red squirrels (*Sciurus vulgaris*) are known to respond to calls of Eurasian jays (*Garrulus glandarius*) (Randler 2004). Eurasian jay alarm calls have abrupt onsets and endings and are noisy broadband calls without distinct harmonic bands. The "dee" notes used by black-capped chickadees, where the number of "dees" encodes predator size, are also broadband calls, although less noisy than those of most squirrels and jays. Given that small birds and squirrels both are preyed upon by raptors and small terrestrial carnivores, eavesdropping on heterospecific alarm calls may benefit gray squirrels by making them aware of threats to which conspecifics have not yet responded. Such benefits of eavesdropping on heterospecific alarm calls may select for a general pattern of elevating antipredator behavior in response to any sound that starts abruptly and has a broad frequency range, characteristics that are typical of many mammalian and avian alarm calls.

SENSORY BIAS

Alternatively, this generalized response to sudden, broadband noise may reflect the evolutionary history of alarm calls. The acoustic structure of animal vocalizations is often important to facilitate their function (Green & Marler 1979; Klump & Shalter 1984), which may be the case with the easily localizable kuks and quaas which announce the caller's presence and location. But the acoustic structure may also reflect a sensory bias that was exploited in the evolution of the signal. Alarm calls are by definition strongly associated with the presence of a predator, and often function to alert conspecifics to the predator's presence. But there are other cues to a predator's presence as well, including scent, sight, and sound. Many carnivores attack by running at high speed toward prey. The noise created by the sudden movement of a running terrestrial predator would be strongly associated with imminent danger, and individuals that increase their alert behavior when hearing such sounds should have a selective advantage over those that do not.

The sounds of a running terrestrial predator in a wooded environment like the eastern gray squirrel's native habitat would often include the sounds of breaking twigs and small branches, a rapid stirring of fallen leaves, and the sound of living vegetation being pushed aside and springing back into place as the predator passes. These sounds are all very noisy, with little or no acoustic frequency structure. It may be that squirrels have adapted to increase alert behavior when hearing any broadband noise because such sounds are also made by attacking predators.

If the gray squirrels' ancestors associated sudden broadband sounds with predator attack, then vocalizations that acted on that existing sensitivity could be more effective at increasing alert behavior in conspecifics than vocalizations that had other acoustic structures. If so, then broadband calls would be more effective at increasing inclusive fitness, and the calling system would spread. This might be a case of natural selection initially favoring calls that resemble the sound of the threat whose presence they signaled. Once such a signaling system was established, it could easily diversify to carry additional information. Whether or not this hypothetical evolutionary scenario occurred, gray squirrels' increase of alert behavior when hearing sudden broadband sounds could reflect a continuing association of such sounds with a terrestrial predator attack. If broadband sounds do have a unique effect of increasing alert behavior, then experiments presenting squirrels with pure tones versus white noise should result in less alert behavior being elicited by sudden tones than by sudden white noise. Such studies could clarify whether broadband noise is unique in eliciting increased antipredator behavior or whether any sudden sound will do so, regardless of frequency structure.

This study suggests that temporal pattern of calling, not call type, of gray squirrel kuks and quaas is most important in changing the foraging and scanning behaviors of conspecifics. Studies are needed, however, that examine other aspects of conspecific response as well as effects of kuks and quaas on predators. Studies also are needed that control for calling rate and duration of calling bouts consisting of either kuks or quaas in order to clarify whether the differences in response observed in Experiment 2 are due to rate or duration of calling. It does seem that any sudden, broadband sound can elicit alert behavior, which could be an adaptation for heterospecific eavesdropping or perhaps reflect a sensory bias caused by squirrels' sensitivity to the sudden, broadband noise that running carnivores may make as they attack.

CHAPTER 6

CONCLUSIONS

The results of this dissertation research demonstrate that eastern gray squirrels use predator-specific alarm signals in two modalities, i.e. tail signals and vocal signals, because both modalities contain information about the terrestrial versus aerial nature of a threat.

VOCAL AND TAIL SIGNALS USED IN RESPONSE TO AERIAL AND TERRESTRIAL STIMULI

After confirming that gray squirrels at my study site use three alarm calls and two types of tail signals in response to natural encounters with terrestrial and aerial predators, I proceeded to test the hypothesis that vocal and tail signals carry information on whether a predator is terrestrial or aerial. The most conservative test for predator-specific signaling is to examine the presence or absence of a signal in an entire signaling bout. When analyzing the signals present in an entire alarm signaling bout I found that vocal and tail signals differ in the information they contain. Flicks, a tail signal, are associated with terrestrial predators, while moans, a vocal signal, are associated with aerial predators. Gray squirrels thus have two predator-specific signals, one in each modality. Further, the results presented here suggest that physical resemblance to a predator, or even an animal, is not necessary in order for a terrestrial threat to elicit alarm signals. In contrast, an aerial threat that does not resemble an actual predator elicits different alarm signals than an aerial threat that does resemble an actual predator.

INFORMATION CONTENT OF ALARM VOCALIZATIONS

Further examination revealed that the initial portion of an alarm calling bout is more predator-specific than the entire alarm-signaling bout. Although when examining the entire alarm-signaling bout kuks and quaas showed no significant association with aerial versus terrestrial threats, kuks and quaas present in the first 30s or 60s of an alarm calling bout were associated with whether a threat was aerial or terrestrial. As expected, moans remained specific to aerial threats when examining only the initial portion of a calling bout. Kuks and quaas are used more frequently to terrestrial threats, with quaas being more specific in use than kuks.

In addition to the association of threat type with the presence or absence of each call type, the number of kuks and moans used by squirrels also differed between threat types, reinforcing the information contained in the simple presence of a call type. Overall, regardless of call type, squirrels used more calls in response to terrestrial threats than to aerial threats. Calling rate in the initial portion of a calling bout thus also contains information on threat type.

It is possible that squirrels do not perceive threats in the “aerial” and “terrestrial” categories used in this study, but differentiate based on some other criterion that also differs between the terrestrial and aerial stimuli used here. Considering the two main factors hypothesized to explain use of multiple alarm-call types, namely, predator-specificity and urgency, it is possible that squirrels responded based on perceived differences in urgency. This possibility is especially interesting in light of ground squirrels that use “aerial” alarms to rapidly approaching terrestrial threats and “terrestrial” alarms to distant raptors (Macedonia & Evans 1993; Blumstein & Armitage

1997a; Sloan et al. 2005). In this study, however, distance and speed of approach were controlled across terrestrial and aerial stimuli. Although I did not test for effects of approach speed, distance or other measures that might be labeled “urgency,” it is unlikely that they explain the strong associations of moans with aerial threats and of quaas with terrestrial threats. The lesser association of kuks with terrestrial threats could reflect that kuks primarily encode some other type of information about the encounter. Studies directly manipulating aspects of approach within terrestrial and aerial categories of threats may be useful to clarify the primary information content of kuks.

DIFFERING ESCAPE STRATEGIES TO AERIAL VERSUS TERRESTRIAL THREATS

Although this study shows that at least some alarm signals of eastern gray squirrels carry information about whether a threat is terrestrial or aerial, it remains to be seen whether gray squirrels use the available information about predator type when responding to conspecifics’ alarms, in either or both signaling modalities. Predator-specific calls are only expected to evolve if alarms are either predator-directed or if alarms are directed to conspecifics and different predators are best avoided with different escape strategies. In this study, I showed that squirrels pursued by aerial versus terrestrial threats use different escape strategies. Gray squirrels fleeing from aerially-approaching threats often flee to the opposite side of a tree trunk from the threat, while squirrels fleeing from terrestrially approaching threats usually flee to a point midway around the trunk where the threat remains in their view. Fleeing squirrels also reached the tree trunk sooner in response to aerial threats than in response to terrestrial threats, perhaps because of differences in running speed or distance from stimulus at first detection. These differences in response, both speed to reach tree and location on tree trunk relative to threat, may or may not be relevant to squirrels not being directly pursued, and there are a number of other potential differences in escape response that were not tested in this study. Still, the difference in escape responses observed, in combination with squirrels' spatially complex arboreal habitat, suggest that gray squirrels might benefit from functionally referential alarm calls that enable conspecifics to take the appropriate escape strategy for each threat type.

Interestingly, vervet monkeys, which use at least three functionally referential alarms to specify different types of threats (Struhsaker 1967; Seyfarth et al. 1980a), occupy grassy habitat with scattered trees, which is structurally similar to the habitat occupied by gray squirrels in this study. Although vervets are more terrestrial than gray squirrels, both species regularly forage on the ground and retreat to trees when threatened. Studies testing the response of eastern gray squirrels to conspecific alarm signals are needed to determine whether the threat-specific vocal and tail signals are functionally referential or not.

CONSPECIFIC RESPONSE TO KUK VERSUS QUAA ALARM CALL ELEMENTS

As a first step toward understanding the functions of gray squirrels' predator-specific alarm signals, I examined the response of gray squirrels to kuks and quaas, which are both used to terrestrial threats, to test whether they elicit different responses in conspecifics. I found that kuks and quaas isolated from the same original alarm call and presented in a natural temporal pattern produce different responses, with the kuks in a calling bout eliciting more intense antipredator behavior than the quaas in a calling bout. This difference, however, is completely erased when the missing call type is replaced with bursts of white noise with equal duration to the missing vocalizations. Studies are needed to further explore this phenomenon, but it appears that squirrels primarily attend to the rhythm of kuks and quaas, rather than any particular frequency structure contained in the calls. The results suggest that any sudden, broadband sound is sufficient to elicit increased alert behavior in gray squirrels.

In conclusion, this dissertation reveals a multimodal alarm signaling system in an arboreal mammal where alarm signals contain information about predator type. Pursued squirrels also use different escape strategies in response to aerial versus terrestrial threats. This complex alarm signaling system offers a rich field of research opportunities. Studies are needed to reveal the effects of various tail and vocal signal combinations on conspecifics, other prey species, and predators, and studies are also needed to test whether, in addition to aerial versus terrestrial predator type, alarm signals contain other information about predator encounters. Both avenues of inquiry may help explain the use of such a complex set of alarm signals.

Ultimately, combined with studies of alarm signals in other tree squirrels, this study will allow comparisons with arboreal primates and terrestrial squirrels, furthering theoretical work on the factors influencing the evolution of alarm systems with differing amounts of information, including the evolution of functionally referential alarms.

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