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SIGNATURE SIGNALS IN THE TERRITORIAL VOCALIZATIONS
OF RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS)* AND
THEIR USE IN KIN RECOGNITION.

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ABSTRACT

SIGNATURE SIGNALS IN THE TERRITORIALVOCALIZATIONS OF RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*) AND THEIR USE IN KIN RECOGNITION.

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Current work on kin recognition in rodents has shown that the dominant modality for recognition is through olfactory mechanisms. In a territorial rodent, such as the red squirrel (*Tamiasciurus hudsonicus*), olfactory mechanisms are may not be the prime modality of recognition compared to vocal mechanisms. The focus of this thesis was to examine the territorial vocalizations of red squirrels for the presence of signature signals and to determine if they are used in kin recognition. Time and frequency elements of the fundamental frequency were extracted from 319 rattles of 149 unique individuals. These data were analyzed using first principle component analysis and then general linear models of the first four principle components, revealing that individual identity and age significantly influenced the time and frequency components of rattles. Juvenile rattles had lower fundamental frequencies and faster syllable rates than adults. It was also found that context did not influence the structure of rattles. Recognition through rattle vocalization was investigated using playback experiments. When rattles were played on the territories of individuals, the territory owners were more likely to rattle in response when they were unrelated to the caller; focal individuals were also more likely to bark in response to neighbors showing neighbor recognition. This study is the first to demonstrate the presence of signature signals in red squirrel rattles and shows acoustical kin and neighbor recognition in a territorial rodent.

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CHAPTER ONE: GENERAL INTRODUCTION

Communication occurs when a signal from one individual influences the behavior of another individual that received the signal (Wiley, 1981; Endler, 1993; Johnstone, 1996). These signals are conveyed in a variety of modalities including visual displays, scents, and vocalizations (Marler, 1967). In order for these signals to be effective they must be detectable by a recipient (Johnstone, 1996), and each of these three modalities allows for effective communication in different situations and habitats.

Visual displays function most effectively when two individuals are in close proximity of one another, occupying the same space and time. The physical environment impacts the effectiveness of visual displays by limiting their range through light scattering. An example of physical influences on visual signals occurs in the comparison of air and water environments; light cannot travel as far in water as it does in air, so the range of the signal is much shorter (Lythgoe, 1988; Wehner, 1997). Visual displays are most commonly found in diurnal social animals, such as many species of birds that live in open environments containing little vegetation or other obstructions (Marler, 1967).

Olfactory communication requires some degree of spatial overlap, but allows for temporal differences in the interactions. Organisms that use olfactory signals, such as beavers (*Castor Canadensis*; Hodgdon and Lancia, 1983) can use scent marks on territories to orient themselves (Müller-Schwarze and Heckman, 1980), assist in finding mates or for identifying kin (Butler and Butler, 1979). Beavers utilize these olfactory cues during times where there is low light, or when communicating with other individuals that have a temporal difference in space use. In addition to being used when individuals visit the same location at different times, scent also plays a role in communication during episodes of physical contact (Marler, 1967). However, the key feature of scent that sets it apart from visual and vocal modalities is its ability to persist in the environment. Olfactory signals are well suited for communication among organisms that occupy stable spatial locations over time, such as carnivores, rodents and primates because scent marks can persist for extended periods of time (Marler, 1967).

Vocal communication, like visual communication, relies on spatial and temporal overlap between the signaler and the receiver to be effective. Vocal signals exist for short periods of time compared to olfactory signals and dissipate into the environment quickly after they have been emitted. This attribute of vocal recognition is both a boon and a hindrance: if an individual hears a vocal signal, it can be aware of the caller's presence in the area immediately, and respond accordingly. Because vocal signals exist for such a short time, temporal differences between presence of the caller and recipient would mean the signal goes unheard. The effectiveness of a vocal signal depends on a variety of factors including habitat (Morton, 1975; Wehner, 1997), background noise (Schwartz and Wells, 1983) and the recipient's ability to interpret the signal (Marler, 1967). Vegetation can scatter vocal signals making it harder for a recipient to extract information from them, or make the signaler hard to localize because of reflection and echo (Wehner, 1997). The broadcast potential of a signal based on habitat has been documented by Morton (1975) who found that different habitats allowed certain frequencies to propagate stronger and farther. For example, frequencies for vocalizations used in grasslands were in the 800 – 1585 Hz range and the frequencies best suited to forest habitats were in the 1585 – 2500 Hz range. Morton (1975) compared the results of his study to a selection of avian vocalizations, and concluded that possessing calls in these frequency ranges boosts the effectiveness and audible distance of a call. Other environmental components such as air pressure, temperature, background noise or even altitude influence can also affect call transmission. While the effectiveness of vocal communication is dependant upon many physical, temporal, and spatial variables, it allows a type of interaction that is not permitted by either visual or scent communication – immediate, direct long-range communication. Because of their quick production and short persistence in the environment, vocal signals are most suited to species that are mobile and depend on quick, accurate exchanges of information during interactions (Marler, 1967).

A typical vocalization includes a fundamental frequency and its corresponding harmonics broken into syllables. The fundamental frequency is the lowest frequency in a set of harmonics. The harmonics are regularly spaced multiples of the fundamental frequency (Halliday et al. 1997). Individual syllables in a vocalization can be distinguished by discontinuities, or breaks, in the fundamental frequency. Common measurements of the fundamental frequency of a vocalization can be described by the minimal and maximum frequencies of each syllable, the modulation of a syllable, the length of the syllables, and the spacing between syllables (e.g., Cheney and Seyfarth, 1982; Rukstalis et al., 2003).

Variation in the structure of fundamental frequencies and syllables of vocalizations is extensively documented and has been shown to contain a variety of information about the caller, including the caller's current behavioral context in many birds and mammals (Norcross and Newman 1993; Rendall et al. 1999; Crockford and Boesch 2003; Wich et al. 2003). Morton (1977) proposed the motivation-structural rule that aggressive vocalizations would have lower fundamental frequencies (low pitch), decreased time between syllables, and wider frequency (noisy) bandwidths in their vocalizations. In contrast to aggressive vocalizations, submissive vocalizations would have higher fundamental frequencies (higher pitch), increased time between syllables, and narrower bandwidths (tonal). Age effects on vocalizations tend to follow the same trend, such that calls of juveniles are of higher pitch, with a tonal sound to the calls, whereas adults tend to have calls of lower fundamental frequency and higher modulation (Morton, 1977). Researchers dating back to Darwin (1872) have studied and discussed the similarity of the vocalizations of submissive adults and juveniles.

Morton (1977) performed an experimental test showing that the calls of several avian species followed the MS-Rule. The MS-Rule has also been shown to apply to the vocalizations of two different mammalian species: white-nosed coatis (*Nasua narica*; Compton et al., 2001) and elk (*Cervus Canadensis;* Feighny et al. 2006). Morton (1977) initially proposed and provided evidence for the MS-Rule in close range vocalizations – those that are typically used in hostile/submissive interactions, but mentioned that the MS-Rule could possibly hold true for long range communications as well. In a study involving bull elk bugles, Feighny et al. (2006) showed that bugles emitted under hostile circumstances showed all the characteristics of an aggressive call as predicted by the motivation-structural rule. This indicated that the MS-Rule can be applied to long-range communication, but to date, there have been few tests of the motivation-structural rule and how it pertains to long distance vocalizations.

In addition to contextual information about the caller, vocalizations can also contain non-contextual information, such as size (e.g. Davis and Halliday 1978; Clutton-Brock and Albon, 1979), sex (Tomaszycki et al., 2001), age (e.g., Cheney and Seyfarth

1990; Nestrova 1990; Blumstein and Daniels 2004; Blumstein and Munos 2005), or individual identity (e.g. Tooze et al, 1990; Rukstalis et al 2003; Puglisi and Adamo 2004; Palacios et al 2007). The identity of a caller can be established when vocalizations contain individual-specific information that is not influenced by the size, age, sex, and behavioral context of the caller. These individual-specific signal components can exist in the frequency, temporal, or rate characteristics of a vocalization, and are collectively called signature signals. Signature signals have been shown to exist in the vocalizations of several mammalian and avian species (e.g. Tooze et al. 1990, Rukstalis et al 2003; Puglisi and Adamo 2004; Palacios et al 2007). These signature signals exist in calls that maintain social order and spacing, such as contact-calls (howls) of wolves (Tooze et al. 1990) and "phee" calls of marmosets (Rukstalis, et al., 2003) and also in anti-predator calls, such as those produced by vervet monkeys (Cheney and Seyfarth, 1988) or Belding's ground squirrels (Leger et al. 1984). The presence of signature signals is a necessary requirement for individual recognition; without individual variation in a signal, there would be no possibility for distinguishing specific individuals, and thus impossible to distinguish specific kin, neighbors, mates, or rivals.

1.1 Recognition Systems

Recognition is the ability of an individual to differentiate between kin, mates, rivals, allies, predators, or prey (Sherman et al., 1997). Individual recognition occurs when an individual-specific trait or signature signal is used to identify an individual during an interaction (Tibbits and Dale, 2007). Individual recognition has been shown to exist in many species of birds (e.g., Leonard et al, 1997; Aubin et al, 2000) and mammals (Johnston, 2003; Mateo, 2003; Searby and Jouventin 2003). Three contexts in which individual recognition has been extensively studied include territoriality, competition, and parent/offspring recognition (reviewed in Tibbits and Dale, 2007). Recognition of neighbors in a territorial system can allow owners to direct their aggressiveness toward potentially threatening strangers instead of their established neighbors. Being able to identify ones established neighbors allows a territory owner to focus energy on showing new individuals where territory boundaries exist instead of reinforcing established boundaries with long-time neighbors (Telemes, 1994). The use and function of individual recognition in territoriality has been documented in both avian (e.g., Falls, 1982; Whitfield, 1986) and mammalian species (e.g., Johnston, 2003; Mateo 2003).

When individual recognition allows for the identification of genetically related individuals it is called kin recognition (Hepper, 1991). Kin recognition can lead to kin discrimination - differential behavioral responses to kin, based on an acoustical, olfactory, or visual cue (Beecher, 1982; Hepper 1991). Three different mechanisms of kin recognition have been proposed: site-specific spatial reference, prior association, and phenotype matching. Site-specific kin recognition occurs when individuals provide preferential treatment to individuals with whom they share a common space, such as a nest or den. (e.g., Holmes and Sherman, 1982; Waldman, 1987; Waldman, 1988; Mateo, 2003). Kin recognition via prior association occurs when individuals interact with one another in situations exclusive to kin, and in doing so learn the unique traits of each other. The most common modality of kin recognition through prior association in mammals is scent, but visual and vocal cues can play an important role as well (Waldman, 1987; Waldman, 1988; Mateo, 2003). Kin recognition through phenotype matching occurs when an individual can determine whether an individual is related to them based on the similarity of the interacting individual's signal to their own or that of their established kin (Waldman, 1987; Waldman, 1988). This uniquely allows for the identification of previously unknown kin (Waldman, 1988). Common modalities of phenotype matching recognition are through olfactory and vocal mechanisms (Waldman, 1987; Waldman, 1988; Mateo, 2003). Kin recognition can influence many behaviors of animals including responses to anti-predator calls (e.g., Hepper 1991, Hare, 1994), cooperation between groups of individuals (e.g., Cheney and Seyfarth, 1982; Hepper 1991), and the location and identification of juveniles by adults (e.g., Cheney and Seyfarth, 1980; Waldman, 1988; Balcombe 1990; Hepper, 1991; Holekamp et al. 1999; Insley 2000; Searby and Jouventin, 2003). In all these examples, the responses of recipients were strongly dictated by the relation of the recipient to the caller. For example, the probability of an individual Columbian ground squirrel (*Spermophilus columbianus)* fleeing in response to the anti-predator call of a related individual was significantly greater than a call from an unrelated individual (Hare, 1994). In vervet monkeys (*Chlorocebus pygerythrus*), the interactions between troops of individuals are influenced by the patterns of dispersal and recognition of vocalizations from dispersed individuals (Cheney and Seyfarth 1982). Troops that shared a genetic link via dispersed individuals were more likely to engage in cooperative behaviors with one another than troops that did not.

Kin recognition is a commonly studied behavior in rodents. In a review, Mateo (2003) found that many rodents for which kin recognition has been extensively studied exhibited kin recognition through olfactory mechanisms. Mateo identified 33 species in which olfactory kin recognition occurred and 12 of these species were capable of phenotype matching. Even though the majority of rodents studied use olfactory mechanisms for kin recognition, olfactory means may not be the most efficient modality for every rodent species: highly territorial diurnal rodents that minimize direct contact could utilize vocal signals for recognition more easily than olfactory modalities.

1.2 Vocalizations of Red Squirrels

One such species in which olfactory kin recognition would be inefficient compared to that of acoustical kin recognition is the North American red squirrel (*Tamiasciurus hudsonicus*). Red squirrels are territorial tree squirrels whose social structure consists of solitary males and females defending exclusive territories year-round (Smith, 1968). These exclusive territories are based around middens, large piles of cone bracts in which new cones are cached each fall (Smith, 1968; Gurnell, 1984).

Red squirrels use a variety of vocalizations during defense of their territories and interactions with conspecifics. The screech, also referred to as the squeak (Embry, 1970) is a loud, high pitched squeal that is used as an offensive threat call against territory intruders (Nodler, 1973) and sounds like a "tsew" (Lair, 1990). The growl vocalization used by red squirrels is a low-pitched, defensively oriented call that has a short audible range (Smith, 1978). The screech and growl vocalizations are used in direct interactions between conspecifics and typically indicate forthcoming aggression from a territory owner to an intruder (Lair, 1990).

Another vocalization used by red squirrels is the buzz, or appeasing call, that are typically emitted as a submissive attempt to prevent aggression from the territory owner (Smith, 1978; Lair, 1990). Buzz vocalizations are also emitted during interactions between young individuals and their mothers (Lair, 1990) and during mating chases during which males often buzz when attempting to mate with females (Smith, 1968; Lair, 1990).

The bark, or alarm call, was once thought to be a singular generic vocalization that signaled forthcoming aggressive behavior, such as charging from a territory owner to an intruder, or the presence of a predator (Smith, 1968; Searing, 1977, Smith, 1978, Lair, 1990). However more recent work by Green and Meagher (1998) showed that the classical "bark" vocalization was potentially the lumping of three distinct anti-predator calls. These calls were the bark, the seet, and seet-bark. Bark calls are audibly different than a screech and seets are sometimes considered squeaks. While bark vocalizations sound similar, they produce distinctly different sonograms when digitally analyzed, and they are used in response to different predators. Barks were found to be used in the presence of terrestrial predators while the seets and seet-barks were used in the presence of aerial predators (Greene and Meagher, 1998).

1.3 Rattle Vocalizations

The rattle is the territorial call of red squirrels. It is used to claim ownership of a territory and declare the owner's presence on the territory (Smith 1968; Smith 1978). Rattles are composed of a series of individual syllables emitted in rapid succession in a bout that can last from a fraction of a second to more than five seconds. The fundamental frequency of a rattle is between 0.5 and 2.0 kHz (Smith, 1978) and is associated with a variable number of harmonics (Figure 2.1, also in Smith, 1978). The numerous harmonics typically found in rattles result in a wide total bandwidth of the vocalizations that can exceed 20 kHz (Figure 2.1, also see Smith, 1978). Smith (1978) documented that the rattle was an easily localizable call that could be heard from up to 130m away. Rattles are easily localizable due to the repetitive use of similar syllables and the wide range of frequencies the call contains (Marler, 1955; Marler 1967) The rattle vocalization is commonly used in response to a neighboring individual's calls (Smith, 1978) or a territory intrusion (Smith, 1978). The rattle has also been documented to be broadcasted in what appears to simply be an unelicited broadcast (Smith, 1978; Lair, 1990). Finally, rattles are also emitted during mating chases (Smith, 1968; Smith, 1978; Lair, 1990), an event that occurs once per breeding season per female, where males pursue an estrous female and attempt to mate with her (Smith, 1968). Both the intruding males and estrous females rattle frequently during this temporary breakdown of the territorial boundaries during the event (Smith, 1968). Rattle vocalizations might allow individuals to know who is present during mating chases and where the female is located during the chase, even if she moves off of her territory and vocalizes, but this has not yet been investigated.

Even though barks, squeaks, growls, and buzzes are used frequently in different interactions, they all contain a common trait: they are short range vocalizations that are associated with direct interactions with other individuals, intruders, or predators. The rattle, while used in some close-range interactions, such as mating chases, is most often used in long-range communication where additional visual or scent cues would be minimized. Based on the variety of situations and contexts in which rattles are emitted, their use in defense of territories, and the ability to be heard over long distances, the rattle is the most likely vocalization to exhibit signature signals and thus allowing for vocal kin recognition in red squirrels.

Kin recognition in red squirrels could mediate several common interactions, including their territoriality. If the behaviors of red squirrels are considered within the scope of kin-selection theory (Hamiltion, 1964) a territory owner may consider a related neighbor no threat to their ownership of their midden, or could incline an individual to attempt to usurp an unrelated neighbor from their territory. Hamilton (1964) stated that an altruistic act would occur when $rB > C$ where r is the relatedness of two individuals, B is the benefit of kin altruism (altruistic acts to benefit a genetically similar individual) and C is the cost of the altruistic act. For red squirrels, benefits of having larger territories could potentially increase survivability or the survivability of offspring, while costs could include finding a new territory, loss of offspring, or death. In the scope of territorially, an altruistic act would be considered not attempting to take a territory from a related individual, and cost would be the penalty one could incur from not acquiring the resources on a neighbors territory. Based on Hamilton's Rule, an individual could lower its inclusive fitness by taking all or part of a territory away from a relative; however, it is also possible that usurping the territory would benefit an individual's inclusive fitness because of the increase in direct fitness (how many offspring they produce). If an individual's inclusive fitness would be lowered more by usurping a related neighbor's territory than the direct fitness gains that might occur from occupying the territory there would be no net fitness gain associated with taking a related individual's territory. Additionally, it would benefit an individual's inclusive fitness to defend the territory boundaries shared with unrelated individuals and to direct any potential territory expansion in the direction of these unrelated neighbors. An example of kin mediated territorially is exhibited in Belding's ground squirrels (*Spermophilus beldingi)*, which are more likely to chase unrelated individuals than related individuals away from potential nesting sites (Sherman, 1980; Sherman, 1981).

1.4 Hypotheses and Predictions

Through the analysis of digitally recorded vocalizations, I examined the calls of individual red squirrels in the study population. I then used these recorded vocalizations to perform a second study involving vocal playback experiments to determine if red squirrels were capable of kin recognition through vocalizations. The following chapters document my analysis of the recorded call components and playback experiment.

For my first study I hypothesized that the vocalizations of red squirrels contain contextual and non-contextual information. If the rattles of red squirrels contain signature signals, I predicted that the effects of identity on the acoustical structure would account for a significant amount of the variation in call components that cannot be explained by physical or environmental variables. If red squirrels follow the motivation-structure rule proposed by Morton (1977), I predicted that rattles elicited in more aggressive contexts would have lower fundamental frequencies and higher modulations. I also predicted juvenile squirrels would have vocalizations with higher fundamental frequencies and lower modulation compared to adult squirrels. The results of this study are documented in chapter two of my thesis.

In my second study, I hypothesized that red squirrels are capable of recognizing kin based on rattle vocalizations. If red squirrels are capable of recognizing kin, I predicted that squirrels would respond to rattles from related individuals with less rattles and barks, as well as exhibit a lower tendency to approach the speakers, than if the rattle came from an unrelated individual. I also examined the potential for individual recognition in red squirrels by looking at the differential response in probability of rattling, barking, and approaching the speaker toward neighbors and non-neighbors. The results of this playback study are documented in chapter three of my thesis. By examining the differences in the probability of barking in response to playbacks, I sought to determine the function of the bark call. If barks are a precursor to aggression (Lair 1990) then I would expect playbacks to elicit barks in focal animals. In contrast, if barks serve only as alarm calls (Greene and Meagher, 1998) then on-territory rattle playbacks should not influence the probability of an individual barking.

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CHAPTER TWO
SIGNATURE SIGNALS IN THE RATTLES OF RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*).

2.1 Introduction:

Vocalizations are one of the modalities by which animals communicate with one another. Vocal communication allows animals to communicate over long distances without engaging in direct contact with another individual (Marler, 1967). Variation in the structure of vocalizations has been shown to convey information about the caller's size (Davis and Halliday 1978; Clutton-Brock and Albon 1979) sex (Tomaszycki et al 2001), age (Cheney and Seyfarth 1990; Nestrova 1990; Blumstein and Daniels 2004; Blumstein and Munos 2005) current circumstances (Norcross and Newman 1993; Rendall et al. 1999; Crockford and Boesch 2003; Wich et al. 2003, Slocombe and Zuberbuhler, 2005a,b) and identity (Holekamp et al. 1999; Rukstalis et al 2003; Puglisi and Adamo 2004; Palacios et al 2007).

Vocalizations of animals can also vary based on the contextual state of the caller and can be explained using the MS-Rule (Morton, 1977). Morton (1977) proposed that aggressive vocalizations would have lower fundamental frequencies (low pitch), decreased time between syllables, and wider frequency (noisy) bandwidths in their vocalizations because larger animals would be able to produce lower frequencies due to the increased size of their vocal cords. These larger animals also tend to win aggressive interactions due to their size, so the lower frequencies are a reliable signal of which individuals to avoid (Morton, 1977). In contrast to aggressive vocalizations, submissive vocalizations tend to have higher fundamental frequencies (higher pitch), increased time between syllables, and narrower bandwidths (tonal; see Compton et al., 2001, Feighney et al., 2006, and Theis et al., 2007). Age effects on vocalizations were first described by Darwin (1872) and many researchers have confirmed his ideas on the similarity of the vocalizations of submissive adults and juveniles. Darwin concluded that adults who have been defeated or are in a submissive role use vocalizations structurally similar to juvenile vocalizations to prevent or stop aggression from other individuals. Morton (1977) included an empirical test of the motivation-structural rule in birds in which he showed that the calls of several avian species followed the MS-Rule. In addition to the initial work in avian species, the MS-Rule has been shown to apply to the vocalizations of two different mammalian species: white-nosed coatis (*Nasua narica;* Compton et al., 2001) and elk (*Cervus canadensis;* Feighny et al. 2006).

In addition to being influenced by context, age, sex, or environmental factors calls can also contain individual-specific components. These individual-specific signal components can exist in the frequency, time, or rate aspects of a vocalization and are collectively called signature signals. Signature signals have been shown to exist in the vocalizations of several mammalian and avian species (e.g. Tooze et al. 1990, Rukstalis et al 2003; Puglisi and Adamo 2004; Palacios et al 2007). These signature signals are present in vocalizations that maintain social order and spacing, such as contact-calls (howls) of wolves (*Canis lupus;* Tooze et al. 1990) and "phee" calls of marmosets (*Callithrix jacchus;* Rukstalis, et al., 2003) and also in anti-predator calls, such as those produced by vervet monkeys (Cheney and Seyfarth, 1988) or Belding's ground squirrels (Leger et al. 1984).

The presence of signature signals in vocalizations provides the potential for individual kin recognition. Without individual variation it is impossible to identify specific individuals, and thus identify specific kin. Most previous investigations of kin recognition in small mammal systems, however, have been based on olfactory cues (Mateo 2003), which may not be the primary modality for communication when there is limited or no direct contact between individuals such as in territorial species.

One such system where acoustical cues may be used in place of olfactory cues for kin recognition is the North American red squirrel (*Tamiasciurus hudsonicus*). North American red squirrels are solitary, territorial animals that communicate primarily through vocalizations (Smith, 1968, Smith, 1978). Territories of red squirrels in white spruce (*Picea glauca*) forests are approximately 0.28 to 0.46 hectares (Price et al., 1986), and are centered on a midden, or cache, where spruce cones are stored as a food source for the winter and subsequent years (Smith, 1968; Price et al. 1986). Both male and female red squirrels are highly territorial, defending year round territories from all conspecifics with the exception of the one day per breeding season when a female is in estrus and allows males onto her territory to mate (Smith, 1968; Gurnell, 1984; Price et al. 1986; Boutin and Schweiger, 1988). This strict territoriality and limited direct interactions in red squirrels suggests that acoustical cues may be used for kin recognition.

In the defense and maintenance of their territories, red squirrels use a vocalization known as a rattle to proclaim ownership of a territory. This vocalization is the most common vocalization used by red squirrels (Smith, 1968, Smith 1978, Lair, 1990). The rattle is used as an advertisement by a territory owner to proclaim its presence on and willingness to defend a territory (Smith, 1968). The rattle vocalization is composed of a series of rapidly repeating syllables that are emitted in calling events. One calling event can be composed of a short rattle lasting approximately one second to a series of successive rattles lasting over five minutes (Smith, 1978). These syllables include a well defined fundamental frequency and the corresponding harmonics. The harmonics are tightly spaced and the individual syllables appear as dark lines on a sonogram (Figure 1; see also Smith 1978). Rattles can be emitted in response to another call, a territory intrusion, or used spontaneously to proclaim ownership of a territory (Smith, 1968; Embry, 1970; Lair, 1990). Rattles are always broadcast from within an individual's territory with one exception; males and females rattle outside of their territories during a mating chase (Smith, 1978; Price et al. 1986), when the territorial system temporarily breaks down. Initial studies of the rattle by Smith (1978) were inconclusive regarding whether the rattles of red squirrels carried information related to the identity of the caller. Smith was unable to detect a significant difference in his small number of replicated samples, but believed there were sufficient differences in the calls to allow for limited identification of individuals.

An individual's rattle has been reported to be heard up to 130m away in western hemlock (*Tsuga heterophylla*) and lodgepole pine (*Pinus contorta*) forests (Smith, 1978), which would correspond to an audible area of 5.3 hectares. Densities of red squirrels in the southwest Yukon Territory of Canada varied from 1.3 individuals per hectare to 2.3 individuals per hectare during the 2005 and 2006 summers (Boutin, Humphries and McAdam, unpublished data). Therefore, a single red squirrel could potentially hear vocalizations from 7 to 13 surrounding conspecifics (Figure 2). The direction of a vocalization alone might, therefore, be insufficient to determine identity along a single bearing. Because directionality alone would not allow for accurate identification of neighboring individuals through rattles, I hypothesized that red squirrel rattles contain unique signature signals. I predicted that the identity of the rattling individual would explain a significant amount of variance in the rattles from different individuals. I also hypothesized that the rattles of red squirrels would contain information regarding physical and contextual states of the caller. I predicted that the vocalizations recorded in aggressive contexts would be of lower frequency and higher modulation than the rattles recorded in non-aggressive contexts based on the motivation-structural rule (Morton, 1977). I predicted juvenile calls would be of lower fundamental frequencies and have higher rates than adults. Finally, because there is no physical sexual dimorphism (Larsen and Boutin, 1993) or behavioral sexual dimorphism (males and females both defend exclusive territories and use the same vocalizations) of red squirrels (Smith, 1968); I predicted that male and female squirrels would no exhibit sexual dimorphism in their rattles

2.2 Methods

2.2.1 Study Site and Location

Recordings of red squirrel rattles were made in a natural population in the southwestern Yukon Territory, Canada (61°N, 138°W) during the months of June, July, and August in 2005 and 2006. A detailed description of the area can be found in McAdam et al. (2007). A total of 291 adult recordings and 21 juvenile recordings were made from 149 unique individuals. All individuals sampled were part of a long-term study. Individuals were identified using numbered metal ear tags, but for behavioral observation identification was established using unique combinations of colored wires passed through the ear tags of the individuals.

2.2.2 Recording of Rattles

Rattles were recorded between 0600 and 1100 hours by waiting at the midden of the focal individual until a call was recorded opportunistically or by eliciting a call via a playback. Opportunistic recordings were made by placing the recording equipment on or near the focal squirrel's midden until that individual emitted a call. If an individual did not rattle within approximately five minutes of my arrival at the midden, a playback of a conspecific's rattle was used in an attempt to elicit a response from the focal individual. Playbacks were performed using a portable CD player with built-in speakers that was placed on the focal individual's midden. A conspecific's rattle was arbitrarily chosen as the stimulus from a catalogue of rattles recorded from the same study area. During a playback, the recording equipment was placed underneath or near the focal individual to record any responses. Rattles were recorded using a Marantz Professional Solid State Recorder PMD660 that was able to record signals in the 0.01 kHz to 22.5 kHz range. The sound file was stored as an uncompressed 16 bit .wav file to preserve call characteristics. A foam windshield was employed to reduce interference and a low-noise cord connected the multi-directional microphone to the recorder

During each rattle recording, the identity of the focal individual was confirmed and the squirrel's location in the tree and behavior were noted. The location of the individual was classified as being within 3m of the ground or greater than 3m from the ground.

Recorded rattles were grouped into three categories based on the behavioral context of the call: unelicited, responsive, and provoked. Unelicited and responsive rattles were recorded by waiting at the midden of the focal individual until they emitted a spontaneous rattle. Unelicited rattles were rattles emitted with no apparent stimulus and responsive rattles were rattles emitted in response to a vocalization of a nearby neighbor. A provoked call was a rattle recorded in response to a playback on the focal individual's territory or a rattle recorded during a chase or other direct confrontation between two individuals. A provoked call emitted in response to a playback was defined to be any call that occurred within three minutes of the start of the playback call. In most cases (>90%) the provoked rattle occurred within thirty seconds of the playback rattle ending.

2.2.4 Data Extraction

The first data extracted were for total call length, which was measured as the length of time from the first rattle syllable to last rattle syllable. Secondly, a subset of 10 syllables was used to characterize each recorded call. Because the calls' fundamental frequencies tended to change rapidly in the beginning and end of the calls, a sub-sample of syllables was taken between the 11th and 21st syllable, which is approximately the middle of most calls. If the call was shorter than 21 syllables, the mid-rattle syllable was used as the midpoint for the sub-sample and the four syllables before and after it were used to create the sub-sample. All calls with less then 11 syllables ($n = 2$) were omitted from this study. Only the fundamental frequency of this subset of syllables was analyzed and the following frequency measurements were taken: maximum fundamental frequency, minimum fundamental frequency, bandwidth (maximum fundamental frequency – minimum fundamental frequency), and modulation (bandwidth/syllable length). Time measurements taken from a vocalization included the average syllable length, and call rate (syllables per second). The measurements are shown in Figure 3. Calls were analyzed using RAVEN 1.2.1 with a 256 sample fast fourier transformation using a Hanning Window 4096 sample DFT with a 10.8 hertz grid resolution (Charif et al. 2004).

2.2.5 Analyses

Two principal component analyses (PCA) were used to reduce the multivariate dataset into a smaller set of variables. The first PCA analysis incorporated only rattles of adult squirrels while the second PCA included both adult and juvenile squirrels. The second analysis was preformed to determine the effects of age on the structure of rattles. Principal components were calculated using an eigenvector analysis on the correlation matrix of the extracted data variables. The principal component scores (PC scores) for each call were calculated by multiplying the vector of standardized values of the eight acoustical variables by the eigenvector for each principal component for each call.

General linear models and linear mixed-effect models were used to analyze variation in the four principal component scores for both the adult PCA and the combined adult and juvenile PCA. Each principal component was modeled using two individual variables (sex and age), three environmental variables (time of day, Julian date, location: < 3m or > 3m from the ground), and the behavioral context (unelicited, elicited, or provoked). Individual identity was also included as a random effect in mixed-effects models for the PCA incorporating both adults and juveniles. Mass was not included in the final models because mass data were available for only 200 of 320 recordings; models based on a smaller data set that included lowest seasonal mass of individuals gave similar results as the models that excluded mass. Significance of the variables was assessed using the complete model. Analyses were performed using R (R Development Core Team, 2007). The presence of signature signals was assessed by comparing the linear mixed-effects models and their corresponding nested general linear model with a likelihood ratio test to assess the significance of the identity random effect in each principal component (Edwards, 1972).

2.3 Results

The seven measurements taken on the recorded rattles (Table 2.1) were reduced into four principal components for both principal component analyses. For the data set including only adults, the first four principal components account 91% of the variation (Table 2.2) while the four principal components from the combined adult and juvenile PCA accounted for 94% of the variance in rattle vocalizations (Table 2.3). Principal component one described the frequency and time elements in the fundamental frequency of calls. Larger scores of principal component one corresponded to rattles with narrow bandwidths, lower modulation and shorter syllable lengths. The second principal component also described frequency aspects and syllable length of the rattles. Larger scores for principal component two corresponded to rattles with higher fundamental frequencies, shorter syllable lengths and higher modulations. Principal component three described mostly the time elements of the fundamental frequencies of the rattles. Larger scores for principal component three corresponded to calls with higher rates (syllables/second) and shorter syllable lengths. Principal component four scores were heavily based on the length of the rattles. Since the call length loaded in negatively with the principal component score, larger scores were indicative of shorter calls.

The results of the models for the PCA including only adults showed there were no differences between male and females ($|t|_{277} \leq 1.1$, $p > 0.3$ for all models), and there were no differences in the structure of rattles in regards to context (i.e., unelicited, responsive, or provoked call; $|t|_{277} \leq 1.8$, $p > 0.07$ for all models) for any of the principal components. Environmental factors (time of day, Julian date, and location) were shown to not influence the structure of calls ($|t|_{277} \leq 0.9$ $p > 0.5$ for all models) except for principal component two for which recordings made toward the end of the field season tended to have higher scores for principal component two than those recorded earlier in the season ($\beta \pm SE$, 0.009 ± 0.005, $t_{155} = 2.05$, $p = 0.04$). Higher scores of PC2 were associated with generally higher fundamental frequencies.

The results of the models of the combined adult and juvenile principal components also showed no differences between male and female squirrel rattles in any of the four principal components ($|t|_{155} < 1.45$, df = 155 $p > 0.1$ for all models). Juveniles had lower values for principal component two ($\beta \pm SE$, -1.05 ± 0.29, $t_{155} = 3.63$, $p = 0.011$; Figure 2.4) and principal component three ($\beta \pm SE$, -0.83 ± 0.25, $t_{155} = 3.267$, $p = 0.015$; Figure 2.5). On average, the fundamental frequency of a rattle syllable produced by adults (age > 1 year) started at 1280 Hz and ended at 870 Hz while rattle syllables produced by juveniles (age < 1 year) started at 1180 Hz and ended at 783 Hz. The average rate of syllables for juveniles was 25 syllables per second with a total average call length of 3.4 seconds while adults produced on average 23 syllables per second with an overall average call length of 3.0 seconds (Table 1.1)

The analysis of principal component four showed no differences between unelicited calls and responsive calls ($\beta \pm SE$, 0.04 ± 0.16 $t_{155} = 0.08$, $p > 0.5$), however, provoked rattles had significantly lower scores for principal component four than unelicited calls ($\beta \pm SE$, -0.28 ± 0.14, $t_{155} = 2.11$, $p = 0.036$; Figure 6). Provoked calls were on average 3.42 seconds long (n= 88) whereas unprovoked calls were 2.91 seconds long ($n = 231$). Provoked calls were on average 10 Hz lower in frequency and contained 1 additional syllable per second than unprovoked calls. No environmental factors were shown to influence the structure of rattles: Julian date, time of day, and location showed no significant influence on the structure of the rattles ($|t|_{155} < 1.87$, $p > 0.05$ for all models).

The influence of identity on rattles structure was assessed using the adult and juvenile data set. The random effect for individual identity was shown to significantly improve the models relative to models of PC scores that included only fixed effects (i.e., sex, age, time of day, Julian day, location, and context) for components one ($\chi^2 = 15.16$, df = 1, $p < 0.0001$), two ($\chi^2 = 43.02$, df = 1, $p < 0.0001$), three ($\chi^2 = 71.44$, df = 1, $p < 0.0001$), and four ($\chi^2 = 39.52$, df = 1, $p < 0.0001$), which is indicative of signature signals in each principal component. The random effect for identity was calculated prior to fixed effects and explained 38.8% of the variance for PC1, 50.6% of the variance for PC2, 54.7% of the variance for PC3, and 48.3% of the variance for PC4.

2.4 Discussion

The presence of a signature signal in a vocalization is a fundamental requirement for individual recognition through vocalizations (Sherman et al., 1997). Individual recognition allows an organism to identify their kin, neighbors, rivals, friends, or mates based on prior interactions (Sherman et al., 1997). Individual recognition has been shown to exist in many avian (e.g. Leonard et al, 1997; Aubin et al, 2000) and mammalian species (e.g. Johnston, 2003; Mateo, 2003; Searby and Jouventin 2003) and this study has shown another mammalian system with signature signals, the North American red squirrel (*Tamiasciurus hudsonicus*). Individual variation accounted for between approximately 39% and 55% of variation in the acoustical components of rattles, meaning that approximately half of the rattle structure is a signature signal based on the identity of the caller.

The initial studies of the rattle by Smith (1978) were inconclusive as to whether the vocalizations of red squirrels carried information related to identity of the caller. Smith was unable to detect a significant difference in his replicated samples, but believed there were sufficient differences in the calls to allow for limited identification of individuals. The normal response to a territory owner's rattle is for the intruder to leave the caller's territory (Smith, 1968), but because of the use of rattles in mating chases, where territoriality breaks down, Lair (1990) suggested that the rattle might also serve as a signature signal for individual recognition in red squirrels. The results of the current study confirm that there are signature signals in the rattles of red squirrels and that they possess the fundamental requirements for individual recognition. The rattle vocalization is used extensively in mating chases (Smith, 1968; Smith 1978; Lair 1990) and could be used to assist males trying to find estrus females during the chases, but I found no differences between the structure of male and female rattles. It is likely, therefore, that red squirrels involved in mating chases identify the female based on her unique call components and not on a generalized "female" call.

Smith (1978) and Lair (1990) had differing views on the purpose of the rattles. Smith (1978) considered the rattle a "keep-out" call while Lair (1990) proposed that the rattle vocalization was used to convey multiple meanings. Lair's ideas would be supported if there was a difference in the frequency and time elements of rattles under different contexts. The results of the analysis of principal component four shows there were slight differences between the unprovoked and provoked rattles when I included juveniles in the data set – the provoked rattles were ~10 Hz lower in frequency on average and ~0.5 seconds longer. Even though there was only a slight frequency difference, we did see a significant difference in the length of rattles. This trend was not present when I examined the data set excluding juveniles. This is most likely due to the juvenile recordings being taken in a provoked state (n = 4). The eigenvectors for principal component four showed that call length was the dominant component in PC4, so removing the juveniles from the data set did not radically change the eigenvectors for PC4. Because the effect of context on PC4 disappears when we remove juveniles from the data set I can conclude that behavioral context does not influence the structure of adult rattles.

My results show that red squirrels do not adhere to the motivation-structural rule (Morton, 1977). If red squirrels followed the MS-Rule I would have expected the fundamental frequency of the provoked rattles to be significantly lower than those of unprovoked rattles, which was not the case in this study as we saw only a slight difference. In retrospect, it is perhaps unreasonable to expect the MS-Rule to apply to red squirrel territorial vocalizations. If an individual emitted passive or submissive rattles, they would identify themselves as a prime target for territory intrusion or cone theft. The purpose of emitting a submissive, long range call is to avoid aggression from other individuals. However, in a system where displacement from a territory reduces survivability dramatically (Larsen and Boutin, 1993), the benefit of being submissive towards potential territory usurpers is minimal. While the motivation-structural rule exists in close contact vocalizations (Compton et al., 2001) and some long range vocalizations (Feighney et al., 2006), the motivation-structural rule is not followed in the rattles of red squirrels

Differences in vocalizations based on age have been documented in many different studies (e.g. Darwin, 1872; Morton, 1977; Sousa-Lima et al. 2002, Theis et al. 2007). To date, many papers have discussed the relationship between lower fundamental frequencies and larger body size associated with adulthood (Darwin, 1872; Morton, 1977; Hauser, 1993; Thesis et al. 2007). This study found the opposite of previous literature. Principal component two was explained by fundamental frequency and modulation of the calls in the opposite direction to what would be predicted based on previous literature. Larger scores for principal component two were indicative of calls with higher modulation and higher frequencies. While principal component three is not as heavily influenced by the frequencies of the rattles as it is the rate of the rattles, the eigenvectors associate larger scores in principal component three with lower frequencies, reinforcing the difference between adults and juveniles found for principal component two. Higher scores for principal component three, which are indicative of higher rattle rates (syllables/sec), indicate that juveniles vocalized more rapidly than adults when they rattle.

I propose two possible explanations for the occurrence of lower fundamental frequencies in juvenile red squirrel rattles compared to adult red squirrels. The first possibility is that the emphasis harmonics of the juvenile rattles are higher than those of adults. A harmonic series is comprised of a fundamental frequency and its associated harmonics that are linearly spaced frequency intervals above the fundamental frequency; the emphasis harmonic is the harmonic with the highest amplitude in the series (Halliday et al. 1997). If the emphasis harmonics of juveniles are higher than those of adults, then the juvenile vocalizations would generally sound higher pitched than adults, as has been documented previously (e.g. Darwin, 1872; Morton, 1977; Hauser, 1993; Sousa-Lima et al. 2002; Thesis et al. 2007). This study did not empirically examine the differences in the harmonic structure of red squirrel rattles, only the differences in the structure of the fundamental frequency.

The second possibility is that the variance we saw was due to low sample size for individual juveniles. Due to the large amounts of variation based on identity we found in the initial modeling, it is possible that these juveniles sampled have lower frequency calls in general, which would cause the juvenile data set to be skewed toward lower frequencies. Only seven juveniles were sampled and the recordings (n = 21) were made after these individuals had dispersed and claimed their own territories. Collecting recordings of juveniles is difficult due to high mortality rates and difficulty in tracking their dispersal. During the summer in which juvenile recordings were made, few vacant territories in the monitored research area were available. While both of these ideas are potential answers to the differences we see between adults and juveniles in principal components two and three, none can fully explain them.

The results of this study provide the opportunity for much future research on a system that goes against the grain when compared to classic communication literature. Future work could focus on differences between adult and juvenile rattles to understand the ontogeny of rattle development. Finally, my study has shown that identity has only provided us with an explanation for 39% of the variation we see in principal component one; the other 61% was not thoroughly explained by any of my included physical or environmental variables. An expansion of physical (reproductive status, actual age) and environmental predictors (weather, temperature, density, habitat, and seasons) could yield additional information on what influences the acoustical structure of rattles.

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Table 1. Summary statistics for the fundamental frequency (F_0) measurements of the 319 recorded rattles of red squirrels (*Tamiasciurus hudsonicus*). The data were extracted using Raven 1.2.1.

	Adults		Juveniles	
Rattle Component	Mean	±SE	Mean	±SE
Peak F_0 (Hz)	1287.9	6.37	1181.0	38.29
Minimum F_0 (Hz)	869.3	6.59	783.5	29.50
Bandwidth (Hz)	418.5	6.73	397.1	27.26
Modulation (kHz/second)	37.8	0.05	36.0	2.39
Syllable Length (seconds)	0.011	0.01	0.011	0.003
Call Length (seconds)	2.96	0.09	3.47	0.46
Syllables/second	22.83	0.16	24.90	0.57

Table 2.2: Principal component eigenvectors and the proportion of the variance explained by each component for red squirrel rattles using the adult dataset. PC1 represents bandwidth, PC2 represents frequency elements of the fundamental frequency, PC3 represents the time elements of the fundamental frequency and PC4 represents the fundamental frequency, modulation, and length of the rattles.

Call Component	PC1	PC2	PC3	PC4
Peak Fundamental	-0.32	0.53	-0.15	-0.22
Min Fundamental	0.33	0.60	-0.26	-0.20
Bandwidth	-0.63	-0.01	0.11	-0.01
Syllable Length	-0.44	-0.33	0.34	-0.26
Call Length	0.02	-0.09	-0.34	-0.88
Modulation	-0.40	0.35	-0.46	0.25
Syllables/Sec	0.21	0.00	-0.67	-0.01
Proportion of the Variance Explained	0.35	0.23	0.19	0.14

Table 2.3: Principal component eigenvectors and the proportion of the variance explained by each component for red squirrel rattles using adult and juvenile datasets. PC1 represents bandwidth, PC2 represents frequency elements of the fundamental frequency, PC3 represents the time elements of the fundamental frequency and PC4 represents fundamental frequency, modulation, and length of the rattles.

Call Component	PC1	PC2	PC3	PC4
Peak Fundamental	-0.36	0.58	-0.16	-0.16
Min Fundamental	0.27	0.62	-0.28	-0.14
Bandwidth	-0.64	-0.03	0.11	-0.02
Syllable Length	-0.39	-0.40	-0.37	-0.19
Call Length	0.02	-0.05	0.20	-0.94
Modulation	-0.43	0.31	0.45	0.16
Syllables/Sec	0.19	-0.01	0.69	-0.03
Proportion of the Variance Explained	0.33	0.25	0.19	0.16

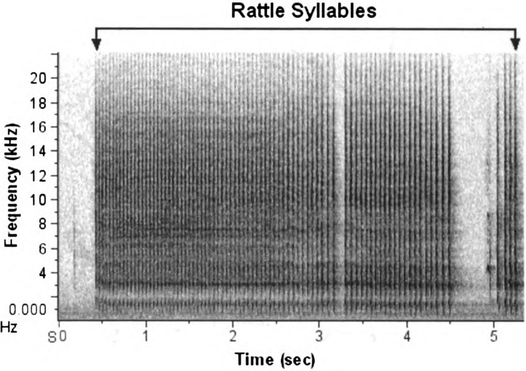

Figure 2.1: Sonogram of the rattle vocalization of a North American red squirrel (*Tamiasciurus hudsonicus*). The dark vertical lines represent individual syllables. The total length of the rattle portion of this call is four seconds.

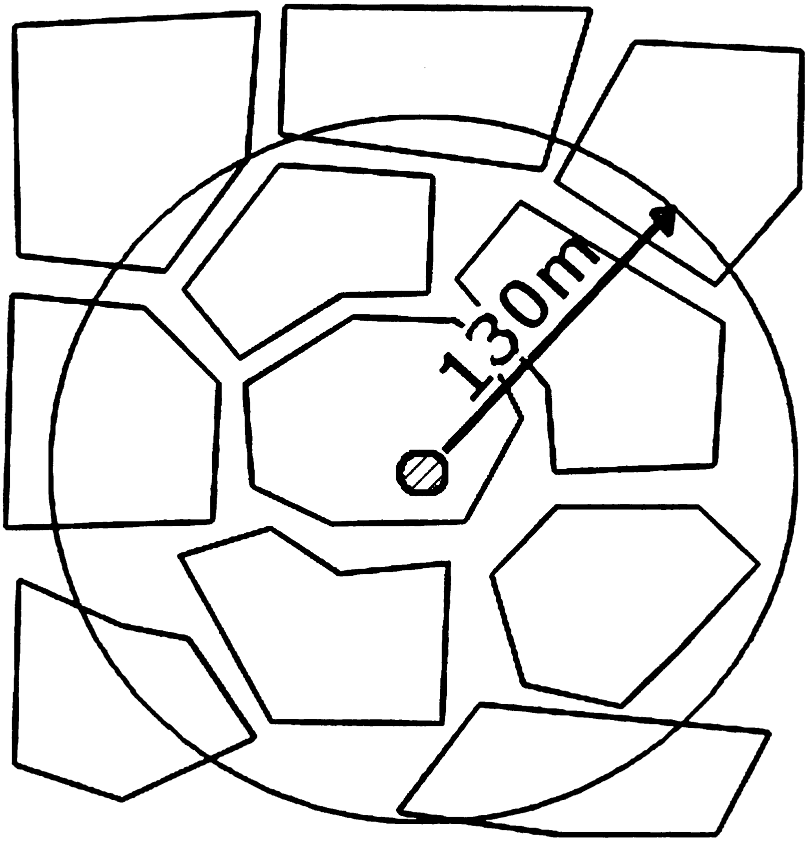

Figure 2.2: Polygons indicate hypothetical territories of red squirrels, the shaded circle indicates the focal squirrel and the large circle indicates the 130m radius that a rattle can be heard from (Smith, 1978).

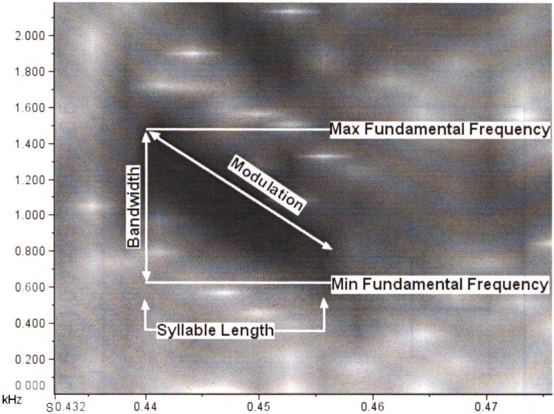

Figure 2.3. Spectrograph of measurements taken on the fundamental frequency of a rattle syllable. The fundamental frequency of the syllable is indicated by the shaded dark bar in the center of the spectrograph. Seconds are represented on the x-axis while frequency (Hz) is represented on the y-axis. The relative power of the signal (db) is indicated by darker shading. This particular syllable has a maximum fundamental frequency of 1500 Hz, a minimum fundamental frequency of 600 Hz and is 0.015 seconds long.

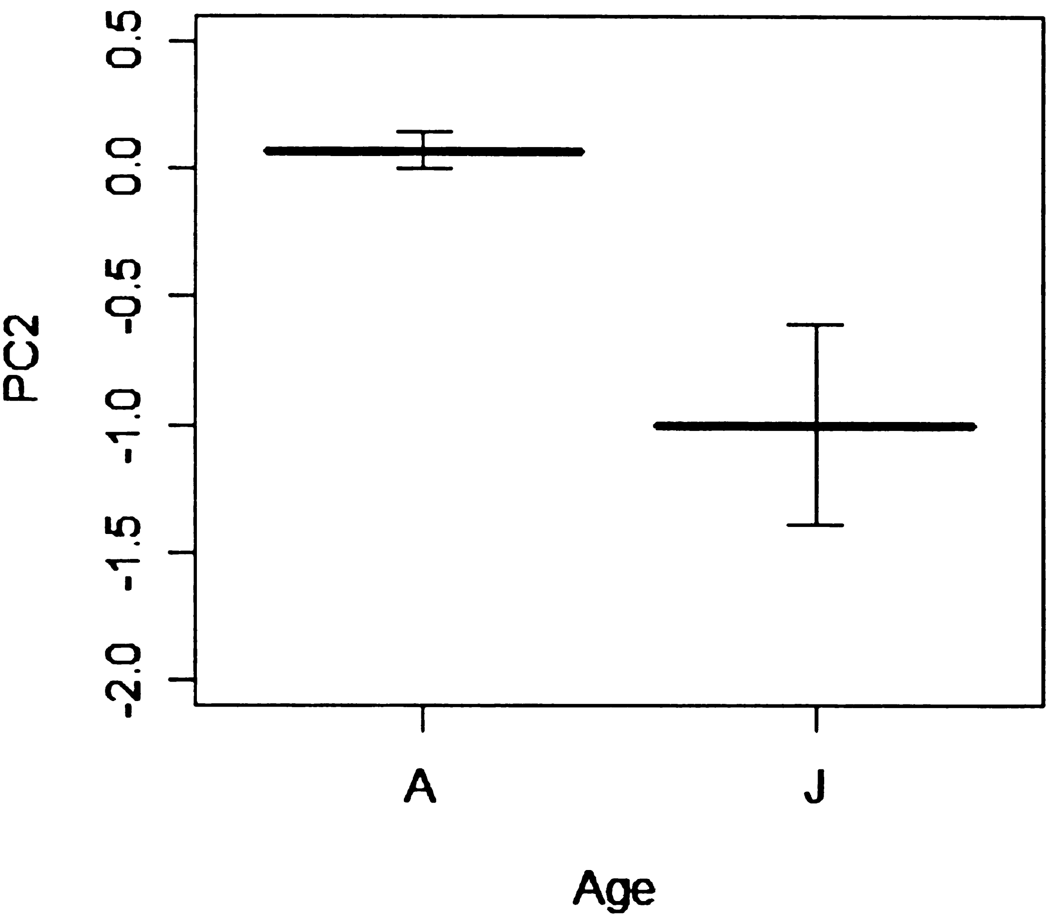

Figure 2.4: The effects of age of an individual on principal component two of red squirrel rattles. Larger PC scores are indicative of higher fundamental frequencies, low bandwidth of the fundamental frequency and low amounts of frequency modulation in the rattle. Data are represented by mean +/- SE. Rattles recorded from adults are represented by "A" and rattle recorded from juveniles (< 1 year old) are represented by "J."

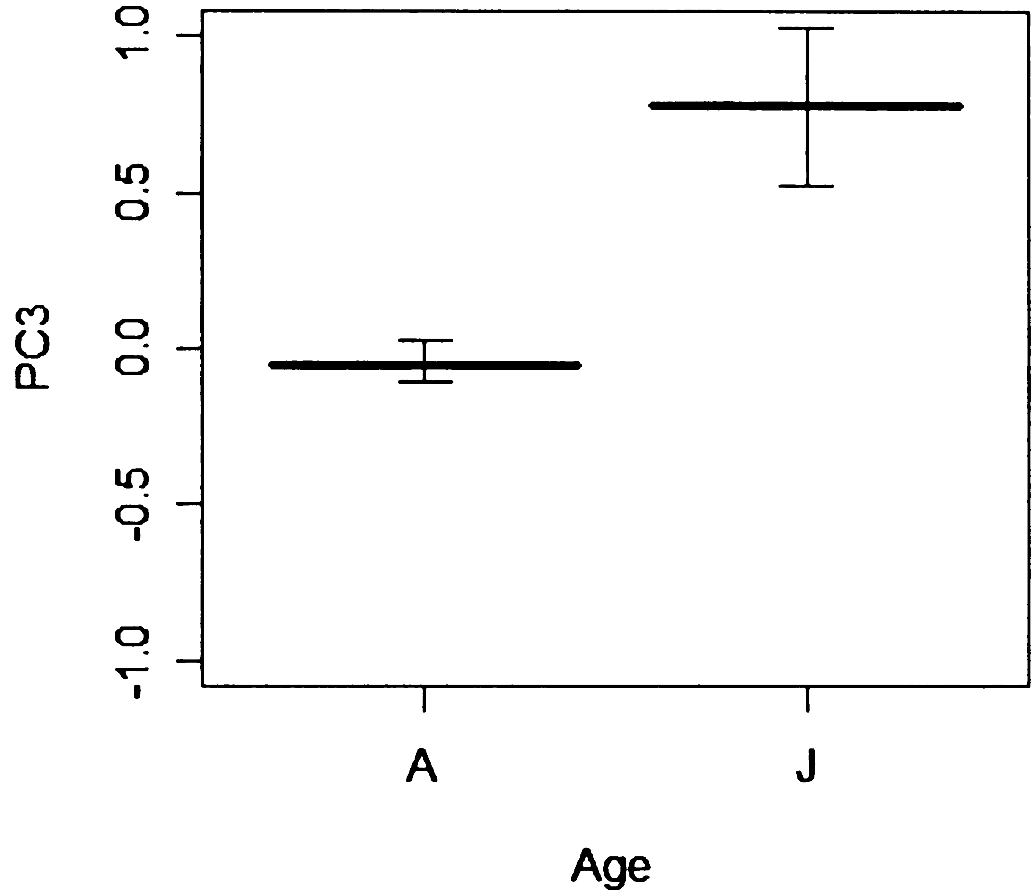

Figure 5: The effects of the age of an individual on principal component three of red squirrel rattles. Larger PC scores are indicative of increased rate in the syllables per second and shorter syllable length in the rattle. Dare are represented by mean +/- SE. Rattles recorded from adults are represented by "A" and rattle recorded from juveniles (< 1 year old) are represented by "J."

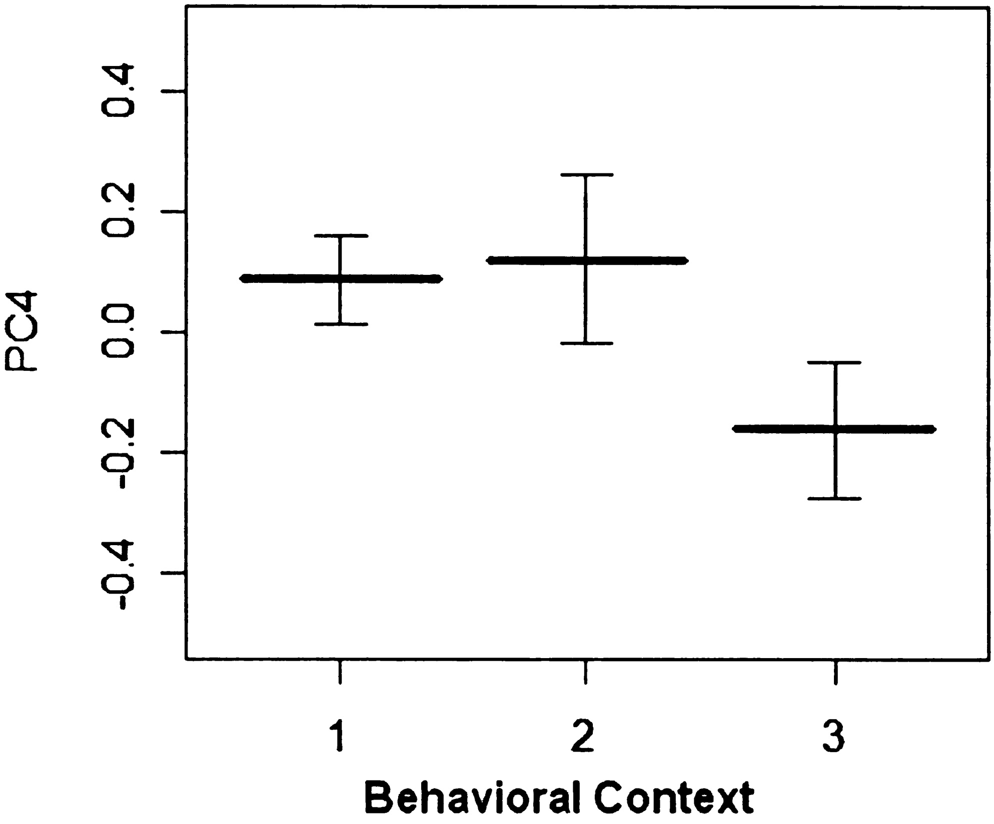

Figure 6: The effects of behavioral context on principal component four for red squirrel rattles. Larger PC scores are indicative of shorter overall call durations. Data are represented by mean +/- SE. Behavioral contexts were classified as unelicited (1), responsive (2) and provoked (3). These data were generated from the adult and juvenile combined data set.

Chapter Three

Kin recognition in the Territorial Vocalizations of Red Squirrels *(Tamiasciurus hudsonicus)*

3.1 Introduction:

Kin recognition is the ability of an individual to assess the genetic relatedness of another individual based on acoustical, olfactory, or visual cues (Hepper, 1991; Beecher, 1992). The study of kin recognition can provide insights into many aspects of animal behavior, including dispersal, mate choice, social cooperation and even territoriality (Blaustein et al. 1987; Hepper 1991; Pusey and Wolf 1996; Sherman et al. 1997, Tibbits and Dale, 2007). Kin recognition has been shown to mediate territoriality (e.g. Falls, 1982; Whitfield, 1986; Johnston, 2003; Mateo 2003) by helping establish territory borders and focus energy on defending territories from new neighbors rather than kin or established neighbors (Telemes, 1994).

The ability to differentiate kin from non-kin allows the individual to alter its behavior based on how closely it is related to the individual it is interacting with. Recognition of kin has been shown to influence responses to anti-predator calls (e.g., Hepper 1991, Hare, 1994), cooperation between groups of individuals (e.g., Cheney and Seyfarth, 1982; Hepper 1991), and the location and identification of juveniles by adults (Waldman, 1988; Balcombe 1990; Hepper, 1991; Holekamp et al. 1999; Insley 2000; Searby and Jouventin, 2003). In all the examples given above, the response of the recipient was strongly dictated by the recipient's relation to the signaler. For example, in Columbian ground squirrels (*Spermophilus columbianus*), the probability of an individual fleeing in response to the anti-predator call of a related individual was significantly greater than a call from an unrelated individual (Hare, 1994). In vervet monkeys, (*Cercopithecus aethiops*), interactions between troops are influenced by the patterns of dispersal and prior knowledge of related individuals' vocalizations (Cheney and Seyfarth 1982). The behavior of the monkey troops documented by Cheney and Seyfarth (1982) is an example of kin selection theory (Hamilton, 1964). Kin selection (Hamilton, 1964) predicts that genetic relatedness plays an important part in how animals will interact. Individuals should be more inclined to associate with and assist related individuals because it will increase their inclusive fitness and kin could potentially be lower threat than non-kin because usurping one's kin can negatively impact an individual's inclusive fitness.

Possible mechanisms of kin recognition include site-specific spatial reference (Beecher, 1981; Holmes and Sherman, 1982), prior association (Holmes and Sherman, 1982; Waldman, 1988), and phenotype matching (Holmes and Sherman, 1982, Waldman, 1988). Site-specific kin recognition is considered an indirect form of kin recognition where individuals assume that conspecifics they share a common space with, such as a nest or den, are kin. Kin recognition by prior association occurs when individuals interact with conspecifics in a fashion only kin would, such as juvenile play fighting or nursing, and in doing so learn the unique traits of that individual. The unique traits learned through these interactions are commonly olfactory in mammals, but the possibility for learning visual and acoustical traits of relatives exists as well (Waldman, 1988; Mateo, 2003). The third mechanism is phenotype matching, which occurs when an individual receives a signal from an unknown individual and can determine if the caller is related to them based on the signal similarity to the recipient's own signal or a signal from their established kin (Waldman, 1988).

Recognition through these mechanisms does not allow for positive identification in every situation and can be misleading. For example, cross-fostered offspring exposed to foster parents calling during a key point in vocal development might begin to structure their calls after the foster parents (Sharp et al., 2005). By structuring their vocalizations after the foster parents, the juveniles gained a vocal signal that would allow for misrecognition by related individuals. Misrecognition of foster offspring has been shown experimentally (e.g., Sharp et al., 2005) and naturally (e.g., Brown, 1984; Lyon, 1993) in birds. In order for phenotype matching to be a reliable mechanism for kin recognition some portion of the signals must also be genetically influenced.

Kin recognition is a commonly studied phenomenon in rodents. In a review, Mateo (2003) found that many rodent species recognize kin through olfactory mechanisms. Based on a comprehensive review of rodent recognition literature, Mateo (2003) identified 33 species in which olfactory kin recognition occurred. Twelve of the species reviewed were capable of phenotype matching based on these olfactory cues because individuals were able to recognize kin without prior association. Even though the majority of rodents studied use olfactory mechanisms as a primary modality for kin recognition, the potential for recognition through other modalities exists. In highly territorial rodents that minimize direct contact, the primary modality for kin recognition might instead be vocalizations.

One such species in which vocalizations could provide a basis for kin recognition is the North American red squirrel (*Tamiasciurus hudsonicus*). North American red squirrels are solitary, territorial animals that communicate territoriality primarily through a vocalization called a rattle (Smith, 1968, Smith, 1978; Lair 1990). The rattle is used as an advertisement by a territory owner to proclaim its presence on, and willingness to defend a territory (Smith, 1968). The rattle call is a series of rapidly repeating syllables that are emitted in bouts that can be as short as a single rattle lasting one second to a series of rattles used in succession, which can exceed five minutes (Smith, 1978). These syllables include a well defined fundamental frequency and the corresponding harmonics. The harmonics are tightly spaced and the individual syllables appear as dark lines on a sonogram (Figure 2.1; see also Smith 1978). This call is emitted as a response to a neighboring individual's calls, a territory intrusion, or as an unelicited broadcast. Red squirrels have also been thought to use a "bark" alarm call, in response to different predators (Green and Meagher, 1998) and in interactions with conspecifics (Lair, 1990).

Red squirrel rattles have been shown to possess individual-specific components (i.e., signature signals; Chapter Two). The presence of signature signals provides the opportunity for identification of specific individuals in the population by other individuals. Territories of red squirrels in white spruce (*Picea glauca*) forests are approximately 0.28 to 0.46 hectares (Price et al., 1986) and are centered on a midden, or cache, where spruce cones are stored for future consumption (Smith, 1968; Price et al. 1986). Both male and female red squirrels are highly territorial, defending year-round territories from all conspecifics with the only exceptions being juveniles on their natal territory prior to dispersal and during mating chases when the territorial system temporarily breaks down (Smith, 1968; Gurnell, 1984; Price et al. 1986; Boutin and Schweiger, 1988).

Given the territoriality of red squirrels and the established presence of signature signals in their territorial rattle vocalization, I hypothesized that red squirrels are capable of recognizing kin and other conspecifics through rattle vocalizations. I further predict that if red squirrels are capable of individual recognition, I would see a difference in the probabilities of receiving rattles, barks, and differences in the probability of individuals approaching the speaker by individuals involved in interactions with familiar individuals (neighbors) and unfamiliar individuals (non-neighbors). The dear-enemy hypothesis (Telemes, 1994) predicts that an individual will devote more energy toward defending territories from previously unknown individuals than established neighbors; therefore, I predicted focal individuals would have a higher probability of rattling at on-neighbors to affirm their status as territory owners

Finally, I also examined whether or not red squirrels barked in response to playbacks of rattles. If barks serve only as an alarm call (sensu Green and Meagher, 1998), then I would expect no effect of the playback on the probability of barking. However, if barks are used in place of direct (physical) aggressive interactions between individuals (Lair, 1990) then I predicted that focal individuals would have higher probabilities of barking in response to the playback of a rattle compared to a pre-playback observational period.

3.2 Methods:

3.2.1 Study Location

This study was conducted on a natural population of North American red squirrels *(Tamiasciurus hudsonicus)* in the southwestern Yukon Territory, Canada (61°N, 138°W) during the months of June, July, and August in 2006. A detailed description of the area can be found in McAdam et al. (2007). All individuals sampled were part of a long-term study and were uniquely identified using numbered metal ear tags. For behavioral observation, identification was established using unique combinations of colored wires, pipe cleaners, or disks passed through the ear tags of the individuals that allowed for identification from a distance. Females were marked with only colored wires, males were marked with a combination of wires and pipe cleaners, and young-of-the-year (juveniles) were marked with a combination of plastic discs and wires. The individuals involved in this study were pre-tagged for a prior study (McAdam et al. 2007) and were not captured or handled in any manner for this study.

3.2.2 Playback Design

Acoustical playbacks of red squirrel rattles were conducted on focal individuals by placing a GPX portable stereo approximately 10 meters from the focal squirrel with the speakers directed toward the individual and camouflaged with foliage. Each playback trial was comprised of a three-minute pre-observational period, a playback of a recorded rattle, and then a three-minute post-observational period. The post-rattle playback observation period began with the recorded rattle being emitted from the speaker. Only one rattle was played per playback trial and these rattles ranged from two to five seconds in length. In order to differentiate between a response to the speaker and the observer, the observer stood perpendicular to the speaker and the focal squirrel. There were no trials during this study in which the focal individual faced the observer and then vocalized. If the focal individual moved during the playback trial, the observer moved to attempt to keep the focal individual in sight. If visual contact was lost for more then several seconds or the focal individual moved more than 20 meters from the speaker, the trial was restarted. The spatial layout of the speaker, midden, and observer for playback trials is depicted in Figure 3.1.

Rattles used for the playbacks were recorded from individuals in the study population. These recordings were made opportunistically by recording the spontaneous and responsive rattles of red squirrels (Chapter two). The recorded calls were imported into Raven 1.2.1 and were amplified by a factor of five. Each amplified call was then burned onto a blank CD as an mp3 file and played from a portable stereo. The rattle used for each playback trial was selected based on two conditions: kinship and proximity (neighbor relationship) that were randomly applied in a fully factorial design. For the purpose of this study, proximity was assessed as being neighboring or non-neighboring. Neighbors were considered to be pairs of individuals whose primary middens were less than 150m apart; non-neighbors were considered to be pairs of individuals whose primary middens were more then 150m from one another. Middens closer than 150m were typically found on adjacent territories. The 150m distance was chosen based on the 130m audible distance documented by Smith (1978) with 20m additional buffer zone added to compensate for territory size. Because territory size ranges from 0.28 to 0.43 hectares (~15m radius) it is possible that while the primary middens may be greater then 130m apart, the edge of the territories could be as close as 100m. The distance of 150m was selected to minimize the possibility that the two individuals involved in the non-neighboring playbacks interacted regularly. Kin were classified as individuals with a relatedness value (r-value) $\geq$ 0.25. Relatedness was calculated using a maternal and paternal pedigree for the individuals in the long term study area. The maternal pedigree was constructed using behavioral observations of females and natal young at nests (Lane et al. 2007) and the paternal side of the pedigree was established using microsatellite analysis of tissue samples collected from individuals in the study population (Gunn et al. 2005; Lane et al. 2007).

An r-value of 0.25 could represent grandmother-granddaughter relationships or half-siblings. Sibling pairings were assigned an r-value of 0.25 even if paternity was not known since both full and half-sibs have r-values of at least 0.25. Of the 30 trials conducted involving kin, 20 trials were conducted using mother-offspring pairings, seven involving sibling pairings, and three involving sire-offspring pairings. Non-kin were classified as any individuals with an $r < 0.25$.

No individual was used for the same treatment twice (e.g., a mother would not be used in a neighboring-kin treatment twice if she lived adjacent to two daughters). In addition to not replicating treatments on a focal individual, each recorded rattle was selected and used for three or four different treatments, but only once for a specific treatment.

Trials were nullified if an intruding squirrel entered the focal individual's territory and a direct interaction occurred ($n = 1$) or visual contact with the focal individual was lost ($n = 6$). Playback trials were rescheduled if the rattles of neighboring individuals were heard during the pre-observation period ($n = 3$) or if the focal individual engaged in alarm calling at the researcher ($n = 5$). Any individuals that engaged in alarm calling behavior as the observer approached their midden were left alone and revisited to at a later time to conduct the playback trial.

3.2.3 Data Analysis

During the pre and post-observational periods, the number and type of vocalizations emitted by the focal individual were recorded (rattles, rattles with chews, barks, and squeaks). In addition to the vocalizations being documented in these periods, I also documented whether the focal individual looked at or approached the speaker and whether there were any neighboring conspecifics in the area at the time of the playback trial. The data were analyzed using generalized linear models. Time looking at the speaker was not recorded because it was not possible to video tape the playback trials. The probability of rattling, barking, and approaching the speaker in response to the playback were modeled using separate generalized linear models with a binary response for each behavior. The two experimental factors were included as predictors in the model: proximity (Neighbor, n = 32; Non-Neighbor, n = 32) and kin (Kin, n = 30; Non-Kin, n = 34). Individuals in the pre-observation period were not split based on relatedness while individuals in the post-observational phase were divided into four categories based on the kinship and neighbor relationships.

There was some over-dispersion in the rattle model ($\psi = 1.24$; residual deviance = 75.77 on 61 degrees of freedom) and the bark model ($\psi = 1.36$; residual deviance = 83.03 on 61 degrees of freedom) and there were no signs of data points with heavy influence on the relationships found by the models. The models were initially constructed using factors for sex of the focal individual, time of day (converted to continuous decimal format, 0.0 = 0000h, 0.5 = 1200h, etc.), and an interaction between kinship and proximity, but these factors had no significant influence on the behavioral response frequencies. These factors were removed and the models reassessed. In the one instance in which the focal individual did not look at the speaker, he raised his head following the playback then immediately put it down again. The frequency of rattles and barks in the pre-observational period were compared to the post-observational rates using Fisher's Exact tests (Zar, 1996).

3.3 Results:

During the three-minute pre-observational phase, the focal individual rattled in 22% of the trials and barked in 19% of the trials. There was a trend for the focal individuals chosen for the non-neighboring treatments to rattle more during the pre-observation period than those chosen for the neighboring treatments (z = 1.83, df= 63, p = 0.066). This trend was also evident in barks (z = 1.94, df = 63, p = 0.052). During no pre-observational phase did the focal individual look at or approach the speaker. The probability of the focal individual rattling during the pre-observational phase was not influenced by the sex of the individual (z = -1.02, df = 63, p = 0.308) or the time of day (z = -0.54, df = 63, p = 0.96). The probability of the focal individual barking during the pre-observational phase was also not influenced by the sex of the individual (z = -0.49, df = 63, p = 0.624) or the time of day (z = -0.588, df = 63, p = 0.556).

Focal individuals were less likely to rattle in response to the playback of a related individual (z = 2.95, df = 63, p = 0.003), but there was no influence of neighbor relationship on the probability of rattling (z = 1.419, df = 63, p = 0.156). The probability of hearing a rattle in the post-observational period in trials involving kin (24%) was not significantly different from the frequency of rattles in the pre-observational phase for neighbors (19%; Fisher's Exact, p = 0.22) or non-neighbors (32%; Fisher's Exact, p = 0.34). The frequency of the focal individual responding to a playback of an unrelated individual during the post-observational phase (62%) was significantly higher than the frequency of rattles in the pre-observational phase of the neighboring treatments (12.5%;

Fisher's Exact, $p < 0.001$) and non-neighboring treatments (31.3%; Fisher's Exact, $p = 0.012$, Figure 3.2).

There was no difference in the probability of receiving a bark in response to playbacks based on kinship ($z = 0.954$, $df = 63$, $p = 0.330$) or proximity ($z = -1.339$, $df = 63$, $p = 0.181$). However, focal individuals were three times more likely to emit a bark in response to a playback of their neighbors rattle (Fisher's Exact, $p = 0.015$) when compared to the pre-observational period (Figure 3.3); however, focal individuals were not more likely to bark at the play back of a non-neighbor (Fisher's Exact, $p = 0.41$; Figure 3.3). Individuals tended to approach the speaker more frequently in response to a playback involving a neighboring individual's call than a non-neighboring individual's call ($z = -1.757$, $df = 63$, $p = 0.079$) but there was no effect of relatedness on the probability of approaching the speaker ($z = 0.386$, $df = 63$, $p = 0.7$).

3.4 Discussion:

Evidence from this study confirmed my prediction that red squirrels are capable of kin recognition – focal individuals were more likely to rattle in response to a playback of an unrelated individual's rattle. Focal individuals were no more likely to rattle at a playback of their own kin than they were to spontaneously rattle when there was no stimulus involved during the pre-playback observational period. Behaviorally, this shows that individuals do not alter their vocalization rates in response to simulations of territory intrusions by kin, but they did toward non-kin. This clear ability to alter behavior in response to the relatedness of the individual with which they are interacting shows that red squirrels are capable of kin recognition.

One point of interest in the playback data is the trend for individuals chosen for the non-neighboring treatments to rattle more during the pre-observational phase than those in the neighboring treatments. The individuals for each treatment were randomly chosen, and the trend could be due the personality of the squirrels. Recent work by Boon et al. (2007) showed that red squirrels exhibit different personalities. Given that the squirrels exhibit different personalities, it is not unreasonable to conjecture that some individuals rattle more often than other. It is possible that the individuals chosen in the non-neighboring treatments were generally more vocal than the ones chosen for the neighboring treatments. Beyond this idea, there is no explanation for the difference we see between neighboring and non-neighboring rattle probabilities in the pre-observational phase of the playbacks.

Barks have been shown to be used in place of aggressive behavior, such as charging (Lair, 1990) and they are used as anti-predator calls (Green and Meagher, 1998). If the bark served solely as an anti-predator call we would expect the same probability of barking in the pre- and post-observational phase and this was not the case in my study. I found that focal individuals were more likely to bark after hearing a rattle playback from a neighboring individual. However, the probability of barking in response to a rattle playback was independent of relatedness, so I found no evidence for kin discrimination based on the barking vocalization. If red squirrels bark in response in an aggressive encounter then it is not clear why they would respond differentially towards kin in terms of rattles but not barks.

While this study offers little insight into the function of the bark vocalization, the results of the bark analysis do show that red squirrels are capable of neighbor-class recognition. When we compare the probabilities of barking in the pre and post observational phases, we see a significant increase in the probability of barking at neighboring individuals. These differential responses to neighbors and non-neighbors shows that red squirrel recognize and respond differently to their neighbors. This is opposite of what we would expect based on the dear-enemy hypothesis; I expected higher probabilities of responding to non-neighboring individuals. One discrepancy to the dear-enemy hypothesis is that the playback studies simulated a territory intrusion by the neighbor; this would represent a breakdown in the "dear-enemy" by the focal individual's neighbor. This could result in the focal individuals taking quick and aggressive action against the neighbor that violated the "dear-enemy" status.

We have already shown that red squirrels can recognize kin and neighbors. However, these groups can be recognized without recognizing a specific individual. Kin can be recognized based on lineage specific traits (Waldman, 1988) and neighbors can be recognized because the vocalizations are heard regularly and the recipient learns them as a neighboring call. The current study cannot determine if red squirrels engage in individual recognition, but signature signals and class recognition are present in the red squirrel rattles.

This study of the vocalizations of red squirrels is the first to show vocal communication as a primary modality for kin recognition in a rodent (Mateo, 2003). All previous work regarding kin recognition in rodents has been directed at prior association and phenotype matching via olfactory mechanisms. We can gain some insight into the possible mechanism of kin interaction by examining the results of this study in conjunction with the basic behavior of red squirrels. Site-specific kin recognition would be a poor mechanism of recognition in red squirrels because this mechanism of recognition typically involves social organisms with low rates of dispersal (Sherman and Holmes, 1982). This is quite the opposite of red squirrels who are asocial (Smith 1968) and readily disperse (Berteaux and Boutin, 2000). Even though adults most likely do not use site-specific recognition, there is evidence to support the idea that mother's use site-specific recognition to identify their offspring at early ages. McAdam and Boutin (2003) conducted a cross fostering experiment where unrelated pups were taken from their nest and placed in a nest of an unrelated individual. These pups were not rejected by the foster mother and were raised as her own. Based on this information, we can conclude site-specific kin recognition most likely occurs in the natal nest, but once the offspring emerge from the nest, the territoriality and dispersal patterns of red squirrels remove any possibility for site-specific kin recognition.

Determining whether adult red squirrels are capable of kin recognition based on prior association, phenotype matching, or a combination of both is difficult at the present time. Mothers and juveniles interact on the natal territory for some time after emergence, and there is the possibility that the mother and her offspring learn to recognize one another's rattles in the process. Because most of the playback trials (90%) were done with mother-offspring or nest mate sibiling pairs, we cannot completely tease apart the effects of prior association and phenotype matching in red squirrels. Some possible insight into phenotype matching can be found in the remaining trials that were conducted on sire-offspring pairs (n = 3). One-third of the sire-offspring pairs, which had not previously associated with one another, rattled in response to their relative's rattle. This is similar to the rattle probabilities of mothers and offspring rattling at one another (26%)

and is approximately half the probability of rattling at a non-kin playback (~62%). The possibility is strong that phenotype matching between sires and offspring occurs, but we do not have the sample size to make a definitive conclusion at this time. More playback experiments involving related individuals that have never interacted with one another, such as father-offspring pairs, would provide us with definitive proof for phenotype matching should the results be consistent with this study.

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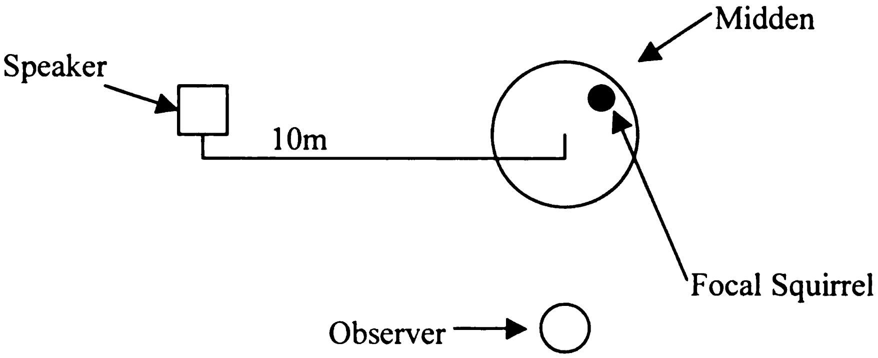

Figure 3.1: Spatial layout of the playback study. The speaker was placed 10m from the center of the focal individual's midden directed toward the individual and the observer maintained between a 60° and 90° angle between the individual and the speaker.

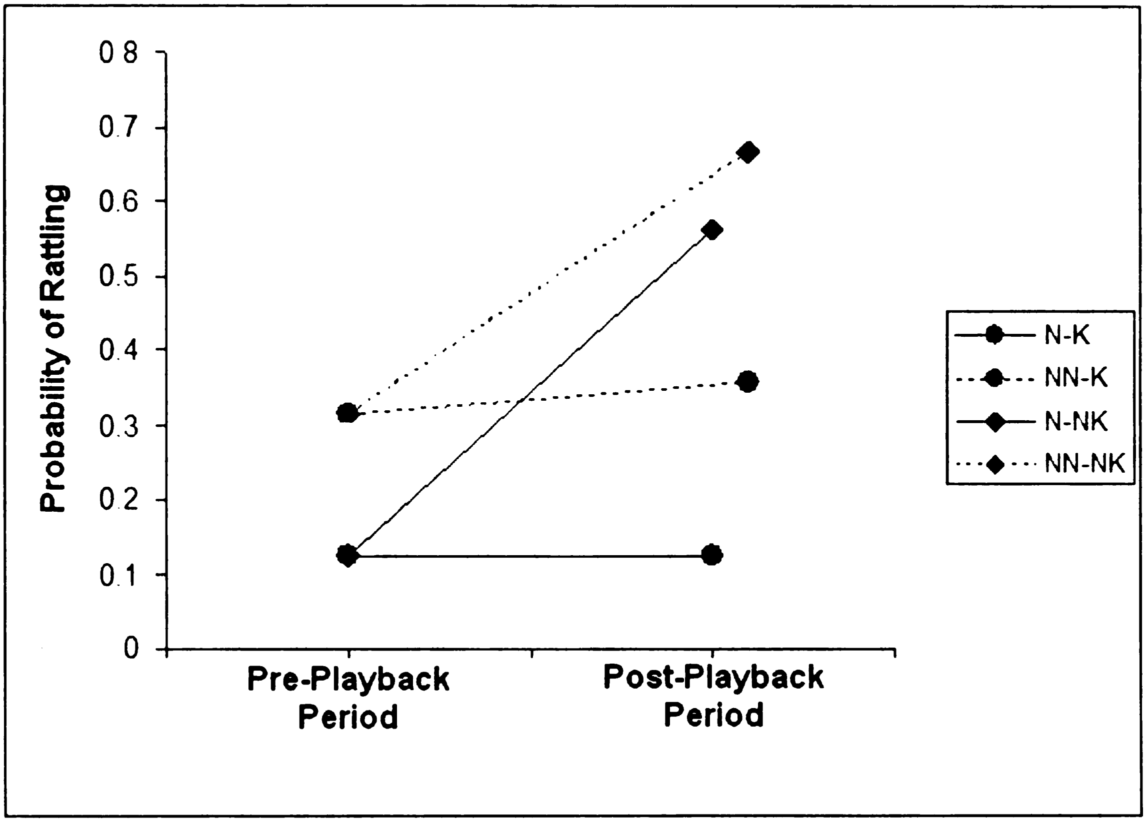

Figure 3.2. Probability of the focal individual rattling during the pre and post playback observational periods of a playback trial. Responses are grouped based on the relationship between the focal individual and the individual used in the playback recording being neighbors (N) or non-neighbors (NN) in the pre-observational phase, and grouped based on being neighbors or non-neighbors and kin (K) or non-kin (NK) in the post-observational phase. Data are presented as mean.

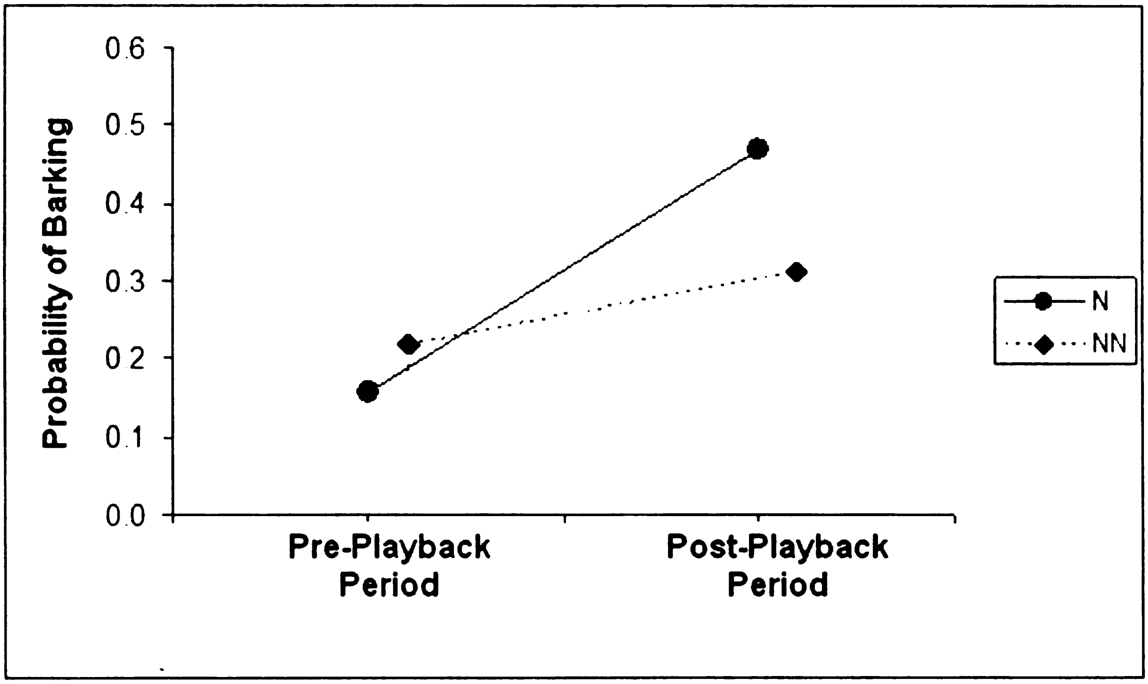

Figure 3.3. Probability of the focal individual barking during the pre and post playback observational periods of a playback trial. Responses are grouped based on the relationship between the focal individual and the individual used in the playback recording being neighbors (N) or non-neighbors (NN). The data are presented as mean.

CHAPTER FOUR: GENERAL CONCLUSION

Early studies of red squirrel vocalizations led researchers (Smith, 1978; Lair, 1990) to believe that the rattles of red squirrels could have signature signals or be used to identify one another. The analysis of our principal components showed that a significant amount of the variation in rattle structure is due to the identity of the rattling squirrel. This result is consistent with my hypothesis that signature signals exist in the rattles of red squirrels. This identity effect accounted for between 39% and 54% of the variation I saw in rattle structure.

The dimensional reduction of the dataset yielded four variables that explained 94% of the variance in rattles. Principal component one explained 33% of the variance, but the principal component was not significantly influenced by any other variable besides identity, and even then it was the lowest of the four principal components (39%). The second and third principal components were significantly influenced by the age of the calling individual. Juvenile squirrels had rattles with syllables per second and lower fundamental frequencies, which is opposite of what has been found previously (e.g. Darwin, 1872; Morton, 1977; Sousa-Lima et al. 2002, Theis et al. 2007). Currently there is no explanation as to why juveniles would have lower fundamental frequencies. Possible explanations could include: 1) red squirrels have an unique ontogeny in their vocalizations or 2) there is more information present in the harmonics of rattles that I did not examine. Only future work on the ontogeny or harmonics in red squirrels will be able to determine this.

The fourth principal component was explained by the behavior context in which the vocalization was emitted in when I looked at the data set including adults and juveniles, but these effects went away when I looked at the adult calls. Even though age was not a significant predictor of PC4 in the combined adult and juvenile data set, the removal of the juvenile rattles altered the model of the principle component score. This could be because the juvenile vocalizations recorded in the provoked state (n = 4) were driving principle component four, therefore leading me to believe that all squirrels rattle longer in a provoked state, when it is possibly only juveniles.

The motivation-structural rule (Morton, 1977) predicts that vocalizations used in aggressive contexts, such as provoked circumstances, would have lower fundamental frequencies and modulations, sounding more tonal, but this was not the case with my data. The current study shows that red squirrels do not follow the MS-Rule. Morton proposed his motivation-structural rule for short range communications, but conjectured that long range vocalizations might also contained signature signals too. Feighny et al. (2006) found that long range vocalizations of elk follows the motivation-structural rule, but the results of my study show this is not true for all mammals. While there are occurrences of the motivation-structural rule in long range mammalian communications, each species will need to be independently evaluated to determine if the vocalizations follow the MS-Rule.

Identity plays a large role in explaining the variation in rattles we see in the study population. Having an individual-specific variation in a signal is a key requirement for individual recognition (Sherman et al., 1997). Individual recognition allows an individual to identify specific kin, neighbors, and mates (Sherman et al., 1997; Tibbits and Dale, 2007). In this study I have shown definitively that red squirrels exhibit kin recognition and that they potentially recognize other classes of individuals (such as neighbors) or even specific individuals. The results of my playback study showed that individuals were significantly less likely to rattle in response to the rattle of a related individual than they were an unrelated individual; this different response in behavior based on relatedness is evidence for kin recognition (Sherman et al., 1997). When I compared our preliminary observation period results to the post-playback observation period results, we can also conclude that an individual is no more likely to rattle at a related individual than it is to spontaneously rattle when there is no stimulus involved (Figure 3.2).

One area of kin recognition that this study was not able to completely address was the mechanism through which red squirrels recognize kin. Based on the success of cross-fostering experiments (McAdam and Boutin, 2003) we can assume that site-specific recognition occurs in the natal nest prior to emergence of the pups, but this most likely breaks down once the pups emerge and disperse. We cannot determine whether prior association or phenotype matching is the primary mechanism for recognition in red squirrels because the majority of my playback trials were done with mother-offspring and maternal sibling pairs. These groups have the potential to use either mechanism for kin recognition. Future work involving father-offspring or paternal siblings could provide insight into kin recognition in red squirrels and will be able to determine if they use prior association, phenotype matching, or both to identify their kin. Should phenotype matching occur in red squirrels, the most likely source of this matching will be in the frequency or harmonic elements of the calls and any future research to investigate the mechanism of kin recognition of red squirrel should begin their search in the fundamental frequencies and harmonics of the rattles.

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