

**Ultrasonic alarm signals of Richardson's ground squirrels
(*Spermophilus richardsonii*).**

by

David R. Wilson

A thesis submitted to the Faculty of Graduate Studies in
partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

University of Manitoba

Winnipeg, Manitoba

© Copyright by David R. Wilson March 2005

THE UNIVERSITY OF MANITOBA
FACULTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

**Ultrasonic alarm signals of Richardson's ground squirrels
(*Spermophilus richardsonii*).**

BY

David R. Wilson

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of
Manitoba in partial fulfillment of the requirement of the degree
Master Of Science**

David R. Wilson © 2005

Permission has been granted to the Library of the University of Manitoba to lend or sell copies of this thesis/practicum, to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film, and to University Microfilms Inc. to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

ABSTRACT

The evolution and maintenance of anti-predator alarm calling are subject to tradeoffs between warning conspecifics and minimizing the risk of predation. Although ultrasonic alarm signals (frequencies > 15 kHz) have not previously been detected in any animal group, their rapid attenuation and highly directional nature could allow callers to warn nearby conspecifics without attracting distant predator attention. I recorded ultrasonic signals from free-living Richardson's ground squirrels (*Spermophilus richardsonii*); these 'whisper calls' were of short duration (225 ± 8 ms, mean \pm SE), loud (66.8 ± 2.1 dB SPL at 1 m from source), and had a dominant frequency of 48.0 ± 2.3 kHz (mean \pm SE). In response to playback of whisper calls, squirrels exhibited more vigilant behaviours than they did in response to playback of background noise, demonstrating unambiguously that whisper calls warn conspecifics of danger.

Squirrels' responses to whisper calls and to pure tones matching the dominant frequency of those calls were not significantly different, suggesting that much of the communicatory value of whisper calls is contained in the dominant frequency. To further elucidate their perceptual capacity, I used a classical conditioning paradigm to train captive squirrels to respond to tones of increasing frequency. Though several problems precluded extensive testing at higher frequencies, it was clear that squirrels could hear up to at least 40 kHz. As cues for localizing sound are greater at higher frequencies, the ability to detect 40 kHz may prove advantageous by allowing recipients to accurately locate the signaler.

Whisper calls elicited less overt behavioural responses from squirrels than did audible calls, suggesting either that whisper calls convey less urgency, or, consistent with the furtive nature of ultrasound, that recipients respond to whisper calls in a less conspicuous fashion. In support of that 'covert signaling hypothesis,' data gleaned from previous alarm calling studies revealed that squirrels produce whisper calls most often when predators are located farther away. Given the short range of ultrasound, however, it is also possible that whisper calls serve to warn nearby kin. Indeed, one of the two contexts in which I confirmed that whisper calls function is during juvenile emergence, when kin are abundant, spatially clustered, and vulnerable to predation.

ACKNOWLEDGMENTS

I am most grateful to Jim Hare, who has provided friendship and invaluable support throughout the past two years. His knowledge and advice with respect to animal behaviour, data collection, and the communication of my results have greatly improved my research. More importantly, however, he has contributed to my overall appreciation of science by allowing me to pursue my own research interests and by always providing me with a stimulating environment, whether in the field, at school, or on our various excursions. I also thank my supervisory committee, including Sue Cosens, Darren Gillis, and Bob MacArthur, for providing guidance throughout my research and helpful comments on earlier versions of my thesis. In that regard, I also thank Dan Blumstein, Brock Fenton, Nathan Lovejoy, and Jan Murie for their insightful comments on how to improve the "Ground squirrel uses ultrasonic alarms" manuscript.

I thank the Sorenson family of Warren, Manitoba and Bob Wrigley of the Assiniboine Park Zoo for allowing me access to the squirrels and for protecting those squirrels from extermination. I also thank Lorna Jakobson for providing a sound-proof chamber and Bob MacArthur for accommodating the squirrels during the auditory response study. John Page helped maintain the equipment necessary for recording ultrasonic whisper calls and Glen Morris provided the equipment and expertise necessary for measuring the amplitude of those calls. Glenda Todd provided valuable assistance in the field during the 2003 playback study.

I thank Brenda Hann for her unwavering support, her guidance throughout my academic career, and her encouragement to pursue my future goals. Jennifer Sloan has been an excellent colleague, lab mate, and friend. I also thank my parents, Bob and Kathy, who have always supported me and encouraged me to pursue the career that I love. Finally, I am especially grateful to my wife Leanne, who has supported, tolerated, and encouraged me over the past two years. She has been a wonderful friend who, in the face of her own research commitments, has always taken an interest in my career aspirations.

The Natural Sciences and Engineering Research Council of Canada provided funding for this research in the form of a postgraduate research scholarship to myself and an operating grant to Jim Hare. Additional funding was provided by a Sigma Xi Grant-in-Aid of Research and the Roger Evans Memorial Scholarship. All research presented herein complies with the guidelines set forth by the Canadian Council on Animal Care.

TABLE OF CONTENTS

CHAPTER	PAGE
1. GENERAL INTRODUCTION	1
REFERENCES	7
2. RICHARDSON'S GROUND SQUIRRELS (<i>SPERMOPHILUS RICHARDSONII</i>) USE ULTRASONIC ALARM SIGNALS	13
METHODS	16
Recording Ultrasonic Alarm Calls	16
Describing Ultrasonic Alarm Calls	19
Construction of Signals for Playback	21
Playback Trials	22
Evaluating Behavioural Responses to Playbacks	25
RESULTS	27
Description of Ultrasonic Alarm Calls	27
Behavioural Responses to Playbacks	28
DISCUSSION	29
REFERENCES	32
3. AUDITORY FREQUENCY RESPONSE OF RICHARDSON'S GROUND SQUIRRELS (<i>SPERMOPHILUS RICHARDSONII</i>)	42
METHODS	45
Subjects	45

Classical Conditioning Paradigm	45
Apparatus	46
Procedure	48
Data Coding and Analyses	49
RESULTS	50
DISCUSSION	50
REFERENCES	53
4. ECOLOGICAL CONTEXT SURROUNDING RICHARDSON'S GROUND SQUIRREL (<i>SPERMOPHILUS RICHARDSONII</i>) ULTRASONIC ALARM CALLS	59
METHODS	63
Playbacks	63
Evaluating Behavioural Responses to Playbacks	66
Context Associated with Call Production	68
RESULTS	69
Behavioural Responses of Litters to Playbacks	69
Context Surrounding Call Production	70
DISCUSSION	71
REFERENCES	73
5. CONCLUDING DISCUSSION	85
REFERENCES	87

APPENDIX

PAGE

A. WHISPER CALL PARAMETERS AND MISCELLANEOUS AND ENVIRONMENTAL GROUPING FACTORS AFFECTING RESPONSES OF GROUND SQUIRRELS TO ULTRASONIC SIGNALS 89

B. GROUND SQUIRREL USES ULTRASONIC ALARMS
Originally published as:
Wilson, D.R. & Hare, J.F. 2004. Ground squirrel uses ultrasonic alarms. *Nature*, **430**, 523 100

LIST OF TABLES

TABLE		PAGE
2.1	Description of ultrasonic alarm calls produced by 15 Richardson's ground squirrels	38
2.2	Squirrel's responses to playback of whisper calls and 3 control calls	39
3.1	Frequency response of 12 juvenile Richardson's ground squirrels that were each subjected to training and testing at two different frequencies (series 1 and series 2)	57
4.1	Number of Richardson's ground squirrel littermates (from 11 different litters) visible before, during, and after the playback of ultrasonic whisper calls and background noise controls	81
4.2	Behavioural responses of 11 Richardson's ground squirrel litters to playback of whisper calls and background noise controls	82
4.3	Influence of contextual variables on the tendency of Richardson's ground squirrels to produce audible vs. ultrasonic alarm calls (mixed calls excluded from analysis)	83
4.4	Influence of contextual variables on the tendency of Richardson's ground squirrels to produce audible vs. ultrasonic alarm calls (mixed calls included in analysis)	84
A1	The influence of the fundamental frequency of whisper calls on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study ..	92

A2	The influence of the sound pressure level of the original whisper call recordings (mean distance between subject and microphone (\pm SE): 0.49 ± 0.02 m) on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study	93
A3	The influence of the playback sound pressure level of whisper calls (dB SPL measured at 1 m from source) on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study	94
A4	The influence of caller sex on the responses of juvenile Richardson's ground squirrels (mean \pm SE) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study	95
A5	The influence of caller age on the responses of juvenile Richardson's ground squirrels (mean \pm SE) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study	96
A6	Autocorrelation of the environmental and miscellaneous grouping factors during playbacks (all treatments combined, $N = 75$) in the 'ground squirrels use ultrasonic alarm signals' study	97
A7	The influence of the distance between subject and speaker on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study	98

A8 Distribution of the environmental and miscellaneous grouping factors among treatments (mean \pm SE) in the playback component of the 'ground squirrels use ultrasonic alarm signals' study 99

LIST OF FIGURES

FIGURE	PAGE
<p>2.1 Ultrasonic alarm call produced by Richardson's ground squirrels.</p> <p>The spectrograph (bottom) shows call duration (238 ms) and dominant frequency (51.6 kHz) of the primary syllable, (+1) and (-1) bands, and a brief chuck trailing the offset of the primary syllable.</p> <p>The signal amplitude is represented by the density of the grayscale.</p> <p>The power spectrum (left) shows the relative amplitude (x-axis) of individual frequencies (y-axis), averaged across the entire signal.</p> <p>The time-amplitude window (top) shows the overall signal amplitude relative to background noise</p>	40
<p>2.2 Mean (+SE) percentage of time devoted to vigilant behaviour before, during, and after the playback of background noise, whisper calls, pure tones, and audible calls</p>	41
<p>3.1 Experimental apparatus for determining the maximum frequency response of the hearing of juvenile Richardson's ground squirrels</p>	58

Chapter 1: General introduction

Many group-living species produce and respond to alarm signals in threatening situations (Macedonia & Evans 1993). By attending to alarm signals, recipients benefit from the enhanced detection of predators afforded by a group (Pulliam 1973; Bertram 1978; Hoogland 1979) and can replace time spent vigilant with time spent foraging, resting, or caring for young (Siegfried & Underhill 1975; Caraco et al. 1980; Abrams 1983). Callers may ultimately benefit through reciprocity (Trivers 1971; Axelrod & Hamilton 1981; Hare 1998a) and/or kinship (Hamilton 1964; Maynard Smith 1965; Dunford 1977), but are immediately burdened with the energetic costs (Eberhardt 1994; Bradbury & Vehrencamp 1998) and increased risk of predation (Sherman 1977; Yasukawa 1989) associated with calling. Selection is thus expected to enhance a caller's fitness by refining the structure (Marler 1955; Klump & Shalter 1984) and efficacy (Marler et al. 1992; Macedonia & Evans 1993; Bradbury & Vehrencamp 1998) of alarm calls.

The ability of a receiver to respond appropriately to an alarm call during a predator encounter depends greatly upon the information content (Marler et al. 1992; Macedonia & Evans 1993; Bradbury & Vehrencamp 1998) and the reliability (Cheney & Seyfarth 1988; Koops & Abrahams 1998; Hare & Atkins 2001; Blumstein et al. 2004) of the signal. Variation in alarm calls may encode semantic information (referential signaling: Seyfarth et al. 1980; Cheney & Seyfarth 1988; Pereira & Macedonia 1991) or the degree of urgency (response

urgency: Blumstein 1995; Blumstein 1999; Warkentin et al. 2001) associated with a threat. Vervet monkeys (*Cercopithecus aethiops*) and ring-tailed lemurs (*Lemur catta*) issue disparate alarm calls to aerial and terrestrial predators, and recipients of these two call-types respond appropriately by hiding in thick bush or climbing high into the trees, respectively (Seyfarth et al. 1980; Pereira & Macedonia 1991). These alarm calls are referential in nature because they communicate specific information about a potential threat with little ambiguity, allowing receivers to respond appropriately to calls even when a predator is not visible.

Macedonia & Evans (1993) and Pereira & Macedonia (1991) noted that, in contrast to vervets and ring-tails, most ground-dwelling animals flee from all types of predator in a similar fashion (e.g. entering burrows). Thus, information regarding the immediacy of a threat (e.g. approach speed or proximity of predator) is potentially more valuable to ground-dwellers than is information pertaining to predator type and/or how to flee (Macedonia & Evans 1993). Warkentin et al. (2001) demonstrated that the rate of repetitive calling by Richardson's ground squirrels (*Spermophilus richardsonii*) varies directly with the degree of risk perceived by the caller. Further, call recipients are more likely to assume the highly vigilant alert posture in response to calls broadcast at a higher call rate, indicating that call rate can be graded to convey the extent of threat imposed by a predator (Marler et al. 1992; Warkentin et al. 2001). Although most studies of ground squirrel communication tend to support only the response urgency hypothesis (Macedonia & Evans 1993; Blumstein 1995, 1999; Warkentin

et al. 2001), it remains possible that a single alarm signaling system may convey both response urgency and reference to external stimuli (Marler et al. 1992).

The reliability of a signal can also affect a receiver's response, as responding to unreliable signals can result in time lost from foraging, resting, or caring for young (Ydenberg & Dill 1986; Koops & Abrahams 1998; Hare & Atkins 2001). Consequently, several species recognize the vocalizations of unreliable individuals and show reduced responsiveness to those callers (Cheney & Seyfarth 1988; Hare & Atkins 2001; Blumstein et al. 2004). Among Richardson's ground squirrels, receivers can further discriminate among different calls issued by the same caller, showing enhanced responsiveness to calls that encode the proximity of a predator with greater certainty (Sloan & Hare 2004). By integrating information about the spatial locations of the caller (e.g. neighbour vs. non-neighbour: Hare 1998b) and predator (e.g. via call rate, Warkentin et al. 2001), a receiver could potentially assess a risk in relation to the location and identity of the caller and tailor its response according to the expected costs and benefits of the situation (Hare 1998a; Koops & Abrahams 1998; Hare & Atkins 2001).

In addition to the benefits provided by enhanced signal information content, aspects of signal structure may also enrich alarm calls by reducing the costs of predation associated with calling. Signals that are short in duration, contain few frequency bands, and lack frequency modulation are difficult for predators to localize (Marler 1955; Brown et al. 1978a and b; Klump & Shalter 1984). Such 'ventriloquial' signals remain highly detectable by conspecifics,

however, because their energy is concentrated in the frequency domain, which maximizes the sound pressure level and overall detectability of the signal to conspecifics (Klump & Shalter 1984). Furthermore, alarm calls are often repeated, which facilitates signal detection in the face of fluctuating environmental noise (Endler 1993; Bradbury & Vehrencamp 1998).

Although callers may be capable of producing ventriloquial alarm signals that reduce their risk of predation by nearby predators, the high detectability of such signals may actually attract more distant predators that would not otherwise have noticed the group, placing the entire group at greater risk of predation (Klump & Shalter 1984; Endler 1993). Furthermore, the intended receivers share with predators the difficulty of locating ventriloquial alarm signals, which may compromise their ability to extract and integrate information regarding spatial relationships with and between the predator and caller (Marler 1955; Klump & Shalter 1984). It is therefore reasonable to assume that the refinement of a species' alarm signals is constrained by the tradeoffs between the degree of conspecific and predator dependence on signal detection and localization. An exception to this evolutionary constraint, however, is when predators are incapable of detecting the signal altogether, either because they lack the physiological capacity or because signalers only signal when the predator is beyond the signal's active space (Endler 1993).

Many small passerine birds are more sensitive to their own alarm calls than are the large birds of prey that feed upon them (Marler 1955). The great tit (*Parus major*) produces an 8 kHz pure tone 'seet' call that warns the entire tit

flock of its primary predator, the European sparrowhawk (*Accipiter nisus*, Latimer 1977). Because the tit is more sensitive than the sparrowhawk to high frequencies (31 vs. 7 m perceptual range at 8 kHz, respectively), and because a tit will only alarm call if a sparrowhawk is over 7 m away, a tit can alert the entire tit flock without drawing the sparrowhawk's attention (Klump & Shalter 1984). However, mammalian predators are generally more sensitive than predatory birds to high-frequency sounds and are thus more likely to detect alarm signals (Masterton et al. 1969; Brown & Pye 1974). Among prey species with mammalian predators then, selection should favour highly specialized signaling systems that prevent eavesdropping by sensitive mammalian predators (Endler 1993).

Very little is known about the use of ultrasound (frequencies > 15 kHz) for alarm communication, though its unique properties - high directionality and rapid environmental attenuation (Sales & Pye 1974; Pye & Langbauer 1998) - have been well studied in the context of echolocation and the pursuit of prey by chiropteran bats and odontocete whales (Norris 1969; Simmons et al. 1979). An alarm signal could presumably exploit these same properties, limiting the audible range of alarm calls to nearby conspecifics (Smith 1979) and allowing callers to direct calls away from predators or at specific intended receivers (Witkin 1977). Sales & Pye (1974) argue that ultrasonic signals did not evolve because they exceed the frequency response of predators, however, as many animals, including several carnivores, can detect ultrasound (Brown & Pye 1974). Many predatory birds, however, are unable to detect ultrasound (Marler 1955; Sales &

Pye 1974; Klump & Shalter 1984) and, thus, it remains possible that ultrasonic signals do function in this capacity among prey with avian predators.

Richardson's ground squirrels are semi-fossorial Sciurid rodents that live in large colonies on the open prairies (Michener & Koepl 1985). In response to predators, squirrels warn conspecifics by producing single or repeated alarm calls that consist of 'chirps,' 'churrs,' 'whistles,' or 'squeals' (Koepl et al. 1978; Davis 1984; Sloan et al. in press). Syllables are short in duration (< 0.5 s), have a fundamental frequency near 8 kHz, are narrow-band or pure tone, and generally lack frequency modulation (see Koepl et al. 1978; Davis 1984); they are loud (84 - 91 dB SPL at 1 m from source: Hare 1998a), easily detected over long distances, and are difficult for humans to localize.

Hare (unpublished data) observed an adult squirrel performing motions consistent with alarm calling (suddenly expanding the thoracic cavity and opening the mouth widely), but without the production of an audible sound. He obtained a preliminary recording of this behaviour with an ultrasound recorder, which revealed that this undocumented 'whisper call' contained ultrasonic frequencies near 50 kHz. Although the production of ultrasound has not been detected in any Sciurid rodent in any context and, although whisper calling is relatively rare, numerous squirrels from every population we have subsequently studied have been observed whisper calling. The whisper call thus constitutes an undescribed vocalization of Richardson's ground squirrels.

No previous study has successfully described whisper calls, established the context in which they are produced, or addressed the possible functions of

these potentially valuable calls. This study thus had four goals: to 1) record and parameterize several whisper calls produced by free-living juvenile and adult Richardson's ground squirrels (production component), 2) determine the maximum auditory frequency response of squirrels (detection component), 3) broadcast whisper calls back to free-living squirrels to test if calls elicit an anti-predator response and thus serve as alarm signals (perception, or functional component), and 4) describe the natural context surrounding this potentially valuable signaling system. I discuss the ecological and evolutionary significance of my results in the context of the unique opportunities that ultrasound provides and the selective pressures that drive alarm communication.

REFERENCES

- Abrams, P.** 1983. Life-history strategies of optimal foragers. *Theoretical and Population Biology*, **24**, 22-38.
- Axelrod, R. & Hamilton, W.D.** 1981. The evolution of cooperation. *Science*, **211**, 1390-1396.
- Bertram, B.C.R.** 1978. Living in groups: predators and prey. In: *Behavioural Ecology, an Evolutionary Approach* (Ed. by J.R. Krebs and N.B. Davies), pp. 64-96, Sinauer Associates Inc., Sunderland, MA.
- Blumstein, D.T.** 1995. Golden-marmot alarm calls. I. The production of situationally specific vocalizations. *Ethology*, **100**, 113-125.
- Blumstein, D.T.** 1999. Alarm calling in three species of marmots. *Behavior*, **136**, 731-757.

- Blumstein, D.T., Verneyre, L. & Daniel, J.C.** 2004. Reliability and the adaptive utility of discrimination among alarm callers. *Proceedings in the Royal Society of London B*, **271**, 1851-1857.
- Bradbury, J.W. & Vehrencamp, S.L.** 1998. *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Brown, C.H., Beecher, M.D., Moody, D.B. & Stebbins, W.C.** 1978a. Localization of primate calls by Old World monkeys. *Science*, **201**, 753-754.
- Brown, C.H., Beecher, M.D., Moody, D.B. & Stebbins, W.C.** 1978b. Localization of pure tones by Old World monkeys. *Journal of the Acoustical Society of America*, **63**, 1484-1492.
- Brown, A.M. & Pye, J.D.** 1974. Auditory sensitivity at high frequencies in mammals. In: *Advances in Comparative Physiology and Biochemistry* (Ed. by O.E. Lowenstein), pp. 1-73, Academic Press, New York.
- Caraco, T., Martindale, S. & Pulliam, H.R.** 1980. Avian flocking in the presence of a predator. *Nature*, **285**, 400-401.
- Cheney, D.L. & Seyfarth, R.M.** 1988. Assessment of meaning and the detection of unreliable signals by vervet monkeys. *Animal Behaviour*, **36**, 477-486.
- Davis, L.S.** 1984. Alarm calling in Richardson's ground squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152-164.
- Dunford, C.** 1977. Kin selection for ground squirrel alarm calls. *American Naturalist*, **111**, 782-785.

- Eberhardt, L.S.** 1994. Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *Auk*, **111**, 124-130.
- Endler, J.A.** 1993. Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London B*, **340**, 215-225.
- Hamilton, W.D.** 1964. The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology*, **7**, 1-52.
- Hare, J.F.** 1998a. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, discriminate among individual alarm callers. *Animal Behaviour*, **55**, 451-460.
- Hare, J.F.** 1998b. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, manifest both littermate and neighbour/stranger discrimination. *Ethology*, **104**, 991-1002.
- Hare, J.F. & Atkins, B.A.** 2001. The squirrel that cried wolf: reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behavioral Ecology and Sociobiology*, **51**, 108-112.
- Hoogland, J.L.** 1979. The effects of colony size on individual alertness of prairie dogs (Sciuridae: *Cynomys* spp.). *Animal Behaviour*, **27**, 394-407.
- Klump, G.M. & Shalter, M.D.** 1984. Acoustic behaviour of birds and mammals in the predator context. I. Factors affecting the structure of alarm signals. II. The functional significance and evolution of alarm signals. *Zeitschrift für Tierpsychologie*, **66**, 189-226.

- Koepl, J.W., Hoffmann, R.S. & Nadler, C.F.** 1978. Pattern analysis of acoustical behavior in four species of ground squirrels. *Journal of Mammalogy*, **59**, 677-696.
- Koops, M.A. & Abrahams, M.V.** 1998. Life history and the fitness consequences of imperfect information. *Evolutionary Ecology*, **12**, 601-613.
- Latimer, W.** 1977. A comparative study of the songs and alarm calls of some *Parus* species. *Zeitschrift für Tierpsychologie*, **45**, 414-433.
- Macedonia, J.M. & Evans, C.S.** 1993. Variation among mammalian alarm call systems and the problem of meaning in animal signals. *Ethology*, **93**, 177-197.
- Marler, P.** 1955. Characteristics of some animal calls. *Nature*, **176**, 6-8.
- Marler, P., Evans, C.S. & Hauser, M.D.** 1992. Animal signals: motivational, referential, or both? In: *Nonverbal Vocal Communication: Comparative and Developmental Approaches* (Ed. by H. Papoušek, U. Jürgens, and M. Papoušek), pp. 66-86, Cambridge University Press, Cambridge.
- Masterton, B., Heffner, H. & Ravizza, R.** 1969. The evolution of human hearing. *Journal of the Acoustical Society of America*, **45**, 966-985.
- Maynard Smith, J.** 1965. The evolution of alarm calls. *American Naturalist*, **99**, 59-63.
- Michener, G.R. & Koepl, J.W.** 1985. *Spermophilus richardsonii*. *Mammalian Species*, **243**, 1-8.

- Norris, K.S.** 1969. The echolocation of marine mammals. In: *The Biology of Marine Mammals* (Ed. by H.T. Andersen), pp. 391-424, Academic Press, New York.
- Pereira, M.E. & Macedonia, J.M.** 1991. Ringtailed lemur anti-predator calls denote predator class, not response urgency. *Animal Behaviour*, **41**, 543-544.
- Pulliam, H.R.** 1973. On the advantage of flocking. *Journal of Theoretical Biology*, **38**, 419-422.
- Pye, J.D. & Langbauer, W.R.** 1998. Ultrasound and infrasound. In: *Animal Acoustic Communication* (Ed. by S.L. Hopp, M.J. Owren, and C.S. Evans), pp. 221-250, Springer-Verlag, New York.
- Sales, G. & Pye, D.** 1974. *Ultrasonic Communication by Animals*. Chapman and Hall, London.
- Seyfarth, R.M., Cheney, D.L. & Marler, P.** 1980. Vervet monkey alarm calls: semantic communication in a free-ranging primate. *Animal Behaviour*, **28**, 1070-1094.
- Sherman, P.W.** 1977. Nepotism and the evolution of alarm calls. *Science*, **197**, 1246-1253.
- Siegfried, W.R. & Underhill, L.G.** 1975. Flocking as an anti-predator strategy in doves. *Animal Behaviour*, **23**, 504-508.
- Simmons, J.A., Fenton, M.B. & O'Farrell, M.J.** 1979. Echolocation and pursuit of prey by bats. *Science*, **203**, 16-21.

- Sloan, J. L. & Hare, J. F.** 2004. Monotony and the information content of Richardson's ground squirrel (*Spermophilus richardsonii*) repeated calls: tonic communication or signal certainty? *Ethology*, **110**, 147-156.
- Smith, W.J.** 1979. The study of ultrasonic communication. *American Zoologist*, **19**, 531-538.
- Trivers, R.L.** 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology*, **46**, 35-57.
- Warkentin, K.J., Keeley, A.T.H. & Hare, J.F.** 2001. Repetitive calls of juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) communicate response urgency. *Canadian Journal of Zoology*, **79**, 569-573.
- Witkin, S.R.** 1977. The importance of directional sound radiation in avian vocalization. *Condor*, **79**, 490-493.
- Yasukawa, K.** 1989. Costs and benefits of a vocal signal: The nest-associated 'Chit' of the female red-winged blackbird, *Agelaius phoeniceus*. *Animal Behaviour*, **38**, 866-874.
- Ydenberg, R.C. & Dill, L.M.** 1986. The economics of fleeing from predators. *Advances in the Study of Behavior*, **16**, 229-249.

Chapter 2: Richardson's ground squirrels (*Spermophilus richardsonii*) use ultrasonic alarm signals.¹

The use of ultrasound is well understood in the context of echolocation and the pursuit of prey by chiropteran bats and odontocete whales (Norris 1969; Simmons et al. 1979), but its function in other animal groups, such as rodents, remains unclear (Smith 1979). Among rodents, purely ultrasonic signals have been described only for the family Muridae (Sales & Pye 1974; Nowak 1999), though ultrasonic harmonic components are present in the audible calls of other rodent taxa (Eiler & Banack 2004). The context for ultrasound production in Murids is highly variable, ranging from infant isolation and distress (Sewell 1968; Sewell 1970) to sexual (Pierce et al. 1989) and predator encounters (Blanchard et al. 1991) in adults. Few studies have focused on how, or if, rodents respond to ultrasound, but those that have suggest that it plays a role in maternal retrieval of infants (Sewell 1970) and prolonging lordosis (Cherry 1989). Most studies of ultrasonic communication, however, have been conducted in a laboratory where normal social behaviour could be compromised and signal function obfuscated (Smith 1979).

Ultrasound includes frequencies that exceed the normal hearing range of humans (Pye & Langbauer 1998: > 15 kHz) and most avian predators (Sales & Pye 1974). Owing to their high frequencies, ultrasonic signals attenuate far more rapidly in the atmosphere than audible signals of the same initial intensity,

¹ A version of this manuscript has been published (see appendix B). **Wilson, D.R. & Hare, J.F.** 2004. Ground squirrel uses ultrasonic alarms. *Nature*, **430**, 523.

making ultrasound detectable only within a short range of its source (Evans et al. 1972; Lawrence & Simmons 1982; Pye & Langbauer 1998). Further, the short wavelengths associated with high frequencies cause ultrasonic signals to be highly directional and to reflect strongly from most objects (Thiessen et al. 1978; Pye & Langbauer 1998). While these unique properties facilitate echolocation (Griffin 1971; Lawrence & Simmons 1982), they limit the use of ultrasound in intraspecific communication to certain highly specific circumstances.

Ultrasound is inaudible to key predators and has a very limited active space, so it could prove ecologically valuable as a furtive alarm signal (Smith 1979; Blanchard & Blanchard 1980; Blanchard et al. 1991). Callers capable of producing ultrasound could potentially warn conspecifics without drawing predator attention (Endler 1983, 1987; Bradbury & Vehrencamp 1998). Even among prey that are depredated by species capable of detecting ultrasound (e.g. Sales & Pye 1974), the short broadcast range of ultrasound could allow callers to warn nearby conspecifics without alerting more distant predators (Smith 1979; Blanchard et al. 1991). Further, the highly directional nature of ultrasound could even allow callers to selectively 'beam' their signals towards intended receivers and away from nearby predators (Witkin 1977). Thus, signalers could maximize their net fitness payoff by utilizing ultrasonic instead of audible alarm calls in certain situations, adding evolutionary stability to the overall signaling system (see Endler 1983, 1987).

In addition to reducing signaling costs, signalers capable of producing ultrasonic alarm calls could also enhance benefits that accrue via kin selection

(Hamilton 1964; Smith 1979). Many ground-dwelling squirrels, including Richardson's ground squirrels (*Spermophilus richardsonii*), reside in kin clusters consisting of young of the year and philopatric adult females (Michener & Michener 1973; Michener & Michener 1977; Michener 1983). By issuing short-range, ultrasonic alarm calls in certain situations, signalers could preferentially warn related individuals, thus providing their kin with a relative survival advantage favoured by natural selection (Hamilton 1964).

In response to predators, laboratory rats (*Rattus norvegicus*) produce an 18 - 24 kHz vocalization that, because it is produced more readily in the presence of familiar conspecifics, is thought to serve an alarm function (Blanchard et al. 1991; Blanchard et al. 1992). This vocalization is not situationally specific, however (see Macedonia & Evans 1993), as it is also produced in a variety of situations that are devoid of predators (Smith 1979). Further, these studies lack a natural context and fail to examine the possible anti-predator responses of recipients, together rendering the ecological value of this potential alarm signal unknown (Macedonia & Evans 1993). Despite its potential ecological and evolutionary value, alarm calling has never been described as a certain function of ultrasonic signaling in any animal group.

Communities of ground-dwelling squirrels (family Sciuridae) have been well-studied for their utilization of audible (ca. 8 kHz) alarm signals (Macedonia & Evans 1993). In this study, I report and describe the first ultrasonic signal detected in any Sciurid rodent, the Richardson's ground squirrel, and

demonstrate for the first time in any animal group that this ultrasonic signal, or 'whisper call,' serves as an anti-predator alarm signal in the natural environment.

METHODS

Recording Ultrasonic Alarm Calls

Research was conducted between 12 April and 13 July 2003 on a free-living population of Richardson's ground squirrels at Assiniboine Park Zoo, Winnipeg, Manitoba (49.874° N, 97.243° W). This site proved ideal for recording ground squirrel ultrasonic vocalizations, given the wind relief provided by surrounding trees and buildings. Squirrels typically occupied raised, grassy berms that surround various zoo enclosures and which further minimized wind interference. Grass was mowed regularly by Parks staff, which facilitated Richardson's ground squirrel observations. All research was limited to early mornings (0530 - 1030 h CST) when wind speed was less than approximately 10 km/h, squirrels were above ground, and public interference was minimal.

Squirrels were live-trapped within one week of their emergence from hibernation (55 adults) or emergence from natal burrows (91 juveniles) using Tomahawk single-door traps (14 X 14 X 40 cm) baited with no-name® smooth peanut butter. Clothespins were attached to the bottoms of trap doors when trapping juveniles to reduce juvenile trapping mortality. Numbered metal ear tags (1005 Monel size 1, National Band and Tag Co.) were attached to the left ear of all individuals for permanent identification and unique marks for identification during trials were applied with a paintbrush to the dorsal pelage using hair dye

(Clairol Hydrience, #52 black pearl). Squirrels were re-trapped periodically to refurbish dye marks that had faded or that had been lost via moulting.

Reproductive status and breeding dates of adult squirrels and the assignment of juveniles to their respective dams were not obtained due to the daily time constraints imposed by working at the zoo.

Recording sessions began when a squirrel was identified and deemed accessible. Individuals that were seen whisper calling or that were known from previous observations to reliably produce whisper calls were preferentially selected. This bias in choice of subjects was considered acceptable because the purpose of the recording component of this study was to collect and parameterize whisper calls, not to establish the context in which calls are produced. The subject was given 1 - 3 min to habituate to me before I approached it and chased it into an escape burrow. I immediately set-up the recording apparatus and waited silently for the squirrel to re-emerge, crouching 3 - 6 m away from the burrow's entrance. If the squirrel emerged, it always remained partially within the burrow's entrance and often initiated a repetitive call while facing the microphone or myself. Although squirrels at the zoo were well-habituated to humans, I wore the same outer clothes each day to promote habituation of squirrels to my presence and to minimize inconsistencies among recording trials.

Calling was recorded with a bat detector (UltraSound Advice, model U30), which was used as a microphone, and a portable ultrasound processor (PUSP: UltraSound Advice). A 6 m cable connected the high-frequency output of the bat

detector to the PUSP's input terminal. The microphone, which rested on a Cansel™ field notebook to prevent contact with wet grass, was placed approximately 25 cm from the burrow (a line connecting the bat detector and the burrow formed a 15° angle with a line between the burrow and myself) and was pointed directly at the burrow's entrance. The PUSP and a Sony minidisc recorder (Model MZ-N707, Type R) used to archive recorded calls were placed in a plastic tray that was secured to my waist with a belt and a strap around my neck. The PUSP was set to a sampling rate of 224 kHz, was manually triggered, and was capable of recording and storing one 16-s sample containing frequencies up to 112 kHz.

Due to the PUSP's limited storage capacity, recordings were continually recycled during a calling bout until a recording with at least one whisper call was obtained or the squirrel stopped calling. Recordings containing whisper calls were transferred from the PUSP to minidisc in time-expanded form. Because the PUSP was not capable of recording while transferring, a 1:10 expansion ratio was typically used to minimize transfer time (2 min 40 s required to transfer a 16-s real-time recording). In several instances, multiple recordings containing whisper calls were obtained from the same calling bout. To ensure that whisper calls did not contain frequencies greater than 112 kHz, two maximum resolution recordings containing whisper calls were also obtained (sampling rate: 448 kHz; recording time: 8 s; expansion rate: 1:20), though no frequency above 97 kHz was recorded from these, or any other recording. After each trial, all distances between the subject, microphone, and myself were measured and the subject's

orientation during calling, relative to myself, was noted. The subject's posture was rarely ascertained, however, because generally only its head protruded from the burrow during calling.

Describing Ultrasonic Alarm Calls

Time-expanded recordings were transferred from minidisc to Avisoft SASLab Plus where they were compressed to the original time base using the appropriate time-compression ratio. Only one recording, selected for its high signal-to-noise ratio, was analyzed per individual, eliminating pseudoreplication (Machlis et al. 1985). If that recording contained multiple syllables (range: 1 - 5), only mean values were reported for that individual (Machlis et al. 1985). Calls were analyzed using a time-amplitude window, a 256-point fast Fourier transform (FFT) spectrograph with Hamming window, and a power spectrum (see Fig. 2.1). The measured parameters include: 1) duration of the primary syllable, 2) duration of a relatively short pulse of sound occasionally trailing the primary syllable, described for audible calls as a 'chuck,' (Sloan et al. in press) 3) latency of the chuck following the offset of the primary syllable, 4) dominant, or most intense, frequency of the primary syllable, 5) minimum/maximum frequency and bandwidth of the dominant frequency, 6) frequencies of the bands found immediately above (+1) and below (-1) the dominant band of the primary syllable, as well as an additional band (+2) occasionally found above the (+1) band, and 7) the dominant frequency of the chuck.

Endpoints for time measurements were considered to be where the amplitude of the signal component (i.e. primary syllable or chuck) became consistently greater than the surrounding background noise. Endpoints were extracted either from the FFT (relative signal amplitude denoted by the intensity of grayscale) in conjunction with the adjacent power spectrum or from the time-amplitude display, depending on which display provided greater resolution. Frequency measurements of the various bands were derived from the FFT and represent the mean frequency of that band across the entire duration of the signal component (i.e. primary syllable or chuck).

The amplitude of whisper calls was measured indirectly in a sound-proof chamber by replaying the signal into a Brüel and Kjær 0.25" microphone (distance from source matched to recording conditions in field) attached to a type 2204 impulse precision sound level meter (response: hold). Whisper calls were broadcast from a Racal Store 4DS instrumentation recorder through an ultrasound amplifier (model S55) and loudspeaker (model S56) from UltraSound Advice. I ensured that no artifacts were added to the signals when transferring them from the PUSP to the instrumentation recorder by repeatedly transferring a test signal between the two devices. After cycling the test signal across the two devices five times, the signal remained spectrally identical to its original printout. For each whisper call measured, the playback volume was first calibrated by repeatedly re-recording the signal with the PUSP and bat detector (distance from source matched to recording conditions in field). The playback volume was adjusted so that the signal amplitude on the PUSP (represented by the number

of pixels on the spectrogram) matched the printout from the original field recording. This estimate of amplitude is conservative because ultrasound is highly directional and squirrels often did not call directly into the microphone.

Construction of Signals for Playback

A total of 23 time-expanded recordings from 15 different callers were transferred from minidisc to Canary™ on a Macintosh computer. For each caller, I used the syllable with the highest signal-to-noise ratio to construct one repeated whisper call containing three identical syllables separated by 4-s intersyllable silences (mean \pm SE intersyllable length during natural calling bouts: 4.15 ± 0.40 s, Sloan & Hare 2004). Temporal endpoints of the syllable were determined using the same method described above. All upper and lower frequencies of the signal that were no more intense than comparable frequencies recorded immediately prior to the signal were deemed background noise and were filtered out on Canary™.

For each of the 15 callers, three additional calls were constructed on Canary™ that, in place of the three syllables, contained either: 1) background noise recorded immediately prior to the alarm call, edited to have the same frequency limits as the alarm call, 2) a pure tone produced by a signal generator (Lafayette Instrument Co., Model A1421) that matched the amplitude and frequency of the whisper call's fundamental frequency, or 3) a unique audible call that was recorded from a caller of the same sex at a spatially remote site between 1994 and 1998 (see Hare 1998a). The duration of the background noise

and the pure tone matched their corresponding whisper call; audible calls, however, were natural and thus did not share an identical duration with their corresponding whisper call, though they were matched as closely as possible during syllable selection.

Because ultrasonic signals are highly directional, and because the squirrel's angle with the microphone often deviated from normal in the field, the amplitude of recordings and the variation in amplitude among recordings are not necessarily representative of calls produced in the natural context. To reduce any potential confound imposed by the amplitude of playbacks, all whisper calls and their associated pure tones were amplified to match the relative amplitude of the whisper call with the highest signal-to-noise ratio; background noise was amplified by the same factor as its corresponding whisper call. Amplifications were performed on Canary™ and were calculated using the average intensity of the entire signal, rather than the maximum intensity found within the signal. A total of 60 calls representing four treatments and 15 distinct whisper callers were thus constructed and transferred back to minidisc for playback via the PUSP.

Playback Trials

Playbacks were broadcast to juvenile squirrels from a marked population near Warren, Manitoba (50.170 °N, 97.694 °W) between 17 July and 17 August 2003 to test if squirrels detect and respond to whisper calls in situ. This population was studied continuously since juvenile emergence and was well-habituated to human observers. However, I wore the same outer clothing

each day to promote further habituation of squirrels to my presence and to minimize inconsistencies among playback trials. Individual squirrels were distinctly marked with black hair dye and ear tags using the same methods described above.

A Sony XM-2025 audio amplifier connected to the Sony minidisc player and a Genexxa Pro LX5 loudspeaker was used to broadcast audible calls at a natural amplitude (84 - 91 dB SPL at 1 m: see Hare 1998a). The ultrasound amplifier, connected to the PUSP and the ultrasound loudspeaker, was used to broadcast ultrasonic calls at a natural amplitude (range: 74 - 82 dB SPL at 1 m). Preliminary testing of the ultrasound playback apparatus with an oscilloscope, however, revealed a spurious 5.5 kHz tone produced presumably by the PUSP's output circuit. After two failed attempts by the manufacturer (UltraSound Advice) to remove this tone, and despite the tone's extremely low amplitude (detectable to humans only within approximately 5 cm of the speaker), we placed an inline high-pass filter (General Radio Co., Type 1952) between the PUSP and the amplifier, which attenuated frequencies below 20 kHz without affecting the resolution or the amplitude of the original signal. The filter's weight (approximately 15 kg), however, required that the playback apparatus be carried in a Little Tikes™ red wagon. The 2 speakers (Genexxa Pro LX5 for audible and UltraSound Advice S56 for ultrasound) were concealed interchangeably within an empty speaker box (19 X 15 X 33 cm) covered with speaker cloth to provide them with a common height and visual appearance during trials.

Trials began by identifying through binoculars a suitable squirrel, which included any completely visible individual that could be approached to within 10 m. Preference was given to individuals that resided at least 10 m away from any visual obstruction, such as trees or derelict buildings. The sex of the receivers was balanced across male, female, juvenile and adult callers to avoid biases related to those factors, and no more than two recipients, spatially separated by at least 100 m, received calls from a single caller. The presentation order of the four treatments to a single recipient was randomized by tossing a coin, and no more than two treatments, separated by at least one hour, were presented to that individual in a single day. Consecutive playbacks of different callers were performed at least one hour apart, or if within the same hour, were spatially separated by at least 50 m with respect to the playback equipment.

After locating a potential subject, a predetermined call, unless it was audible, was transferred from minidisc to the PUSP using the appropriate time-compression ratio (i.e. 10:1 or 20:1). I pulled the wagon to within 10 m of the subject and placed the speaker 4 to 8 m away from and facing the subject. Meanwhile, a field assistant erected a Sony DCR-TRV120 digital video camera atop a Manfrotto tripod immediately beside the wagon. When the squirrel began foraging, it was videotaped for 1 min before and after the playback was broadcast. For ultrasonic calls, the exact time of playback was noted on the camera's time code to facilitate data coding. Due to the rapid attenuation and high directionality of ultrasound, ultrasonic trials were aborted if the subject did not remain within 8 m of the front of the speaker and 15° of a line perpendicular

to the front of the speaker during the pre-playback period. Immediately following the trial, I estimated the speaker angle relative to the subject, measured all distances between the camera, speaker, and subject at the time of initial playback, and recorded environmental data using a hand-held weather meter (Kestrel[®], model 3000) held at shoulder level. Before the playback apparatus was moved, the call was replayed with the U30 bat detector held in place of the squirrel's head to test if it could detect the call under the playback conditions. If the playback was audible, the signal's amplitude was measured by replaying the signal into a sound level meter (Audio Dynamics Corp., model SLM-2) held in place of the squirrel's head. Trials involving ultrasonic playbacks were discontinued when wind speed reached 10 km/h (mean wind speed \pm SE: 3.88 ± 0.29 km/h). Audible playbacks, however, were permitted in wind speeds up to 15 km/h (mean wind speed \pm SE: 6.64 ± 0.77 km/h) because calm periods (wind < 10 km/h) were extremely rare and winds up to 15 km/h did not noticeably attenuate audible calls.

Evaluating Behavioural Responses to Playbacks

Squirrels' responses to the four playback treatments were compared to determine if whisper calls serve an alarm function in situ. Hare (1998a) described a positive relationship between the height of a squirrel's head and that squirrel's level of alertness. That positive relationship is manifested in squirrels' postures, which include in order of increasing vigilance: non-vigilant behaviour (standing on four feet with the head below the horizontal plane), low vigilance (standing on

four feet with the head elevated above the horizontal plane), slouch, and alert (sensu Hare and Atkins 2001). Escape behaviour, including running and entering burrows, is considered the most extreme response. If whisper calls serve as cryptic warning signals to nearby receivers, however, those receivers should replace conspicuous anti-predator responses, such as slouch, alert, and running, with less conspicuous responses, such as low vigilance monitoring of the predator, to avoid attracting predators to the vicinity and conflicting with the signaler (Axelrod & Hamilton 1981). Thus, all vigilant behaviours (low vigilance, slouch, alert, escape behaviour) were incorporated into a single total vigilance score that weighted each vigilance posture equally. Vigilance was coded from videotape during the pre-playback (30 s), playback (14 +/- 1 s) and post-playback (30 s) periods using a stopwatch and the time code (accurate to nearest 0.25 s) from the videotape.

Because the end of an alarm call may or may not indicate the diminishment of a threat, I used paired-sample *t* tests to compare across treatments the change in a receiver's vigilance from the pre-treatment period to both the treatment and post-treatment periods. Squirrels' qualitative responses to playbacks, including the initial vigilance response and the duration of that response, were compared across treatments using Wilcoxon signed-ranks tests instead of paired-sample *t* tests because those data consistently violated the parametric assumptions of normality and homoscedasticity. The initial vigilance posture was considered the most pronounced vigilance posture (low vigilance = 1, slouch = 2, alert = 3, escape behaviour = 4) assumed within 1 s of

the onset of the first syllable. The duration of the initial posture ended with the first observable reduction in vigilance or the end of the post-treatment period. Appendix A examines the influence of call structure (dominant frequency, amplitude) and the environmental (cloud cover, temperature, wind speed, relative humidity) and miscellaneous (speaker angle, distance between subject and speaker) grouping factors surrounding playbacks on squirrels' behavioural responses. All statistical analyses were performed on Statview[®] 5.0.1 on a Macintosh computer and results were considered statistically significant where $P \leq 0.05$.

RESULTS

Description of Ultrasonic Alarm Calls

Whisper calls were recorded from 15 different squirrels, including five adults (three males, two females) and nine juveniles (three males, five females, one unknown sex) in 2003, and one individual of unknown age and sex in 2000. Table 2.1 summarizes the characteristics of the whisper calls recorded. Caller sex and caller age (juvenile/adult) had no apparent effect on any of the call parameters examined (unpaired t tests, all $P > 0.05$). The mean amplitude of whisper calls (\pm SE) was 66.8 ± 2.1 dB SPL at a mean distance between the caller and the microphone of 0.49 ± 0.02 m. However, this value likely underestimates the amplitude, given the highly directional nature of ultrasound and the tendency for callers to not call directly into the microphone. Because there is little variation in the amplitude of audible calls (range: 84 - 91 dB SPL at

1.0 m from squirrel, Hare 1998a), it is likely that the loudest call (83.7 dB SPL at 0.55 m from squirrel) is more representative of the realized sound pressure level of whisper calls. Distance of the microphone to the calling squirrel (range: 0.28 - 0.65 m) apparently did not influence the recorded sound pressure level (least squares regression: $y = 59.38 + 14.08x$, $R^2 = 0.074$, $P = 0.33$).

The mean (\pm SE) duration and dominant frequency of the primary syllable were 225 ± 8 ms and 48.0 ± 2.3 kHz, respectively (Table 2.1). While a single dominant frequency was always obvious from the power spectrum, the bandwidth of the dominant frequency was highly variable, ranging from nearly a pure tone (Fig. 2.1) to 15 kHz. Nine of the 15 callers produced an additional non-harmonic frequency band both above (+1) and below (-1) the dominant frequency band (Fig. 2.1), and two of those nine callers produced yet another non-harmonic band (+2) above the first (+1). Frequency and amplitude modulation were not observed in any of the bands from any of the calls. Chucks followed three of the 15 whisper calls analyzed, were brief (28 ± 1 ms), and always contained a lower dominant frequency (31.6 ± 6 kHz) than their preceding primary syllable (Fig. 2.1, Table 2.1). It was not possible to describe chucks in finer detail, however, as their relatively low amplitude rendered them difficult to resolve from background noise.

Behavioural Responses to Playbacks

In response to the playback of whisper calls in the field, juvenile squirrels devoted more of their time to vigilant behaviour and assumed a more vigilant

initial posture than they did in response to background noise alone (Table 2.2, Fig. 2.2). Squirrels also spent more time vigilant in response to the pure tone than in response to background noise (playback: $t_{17} = 3.10$, $P = 0.01$; post-playback: $t_{17} = 3.93$, $P < 0.01$), though behavioural responses to pure tones were not significantly different from those elicited by whisper calls (Table 2.2). The sex of recipient squirrels did not influence the recipient's behavioural responses to the playback of whisper calls (unpaired t tests, all $P > 0.25$). Finally, when played audible alarm calls, squirrels spent significantly more of their time engaged in vigilant behaviour than they did in response to whisper calls (Table 2.2, Fig. 2.2). Given our inability to detect ultrasound in situ, however, we cannot be certain that the magnitude of this difference is not merely due to squirrels' failure to detect whisper calls, but not audible calls, during certain trials. However, the initial response to the playback of audible calls was also qualitatively different from responses to whisper calls, as squirrels assumed more alert postures and remained in those postures for significantly longer following the playback of audible calls (Table 2.2).

DISCUSSION

Results demonstrate that whisper calls produced in the field by Richardson's ground squirrels act as anti-predator alarm signals to nearby conspecifics and, given the similarity of responses to whisper calls and pure tones, that the dominant frequency (48 kHz) of those alarm calls plays an important role in eliciting vigilant behaviour in call recipients. Consistent with audible calling

(Koepl et al. 1978), the sex and age of callers and the sex of respondents had no effect on call parameters or behavioural responses, respectively. Taken together, these results suggest a common function of whisper calls among all individuals.

Whisper calls are short in duration, contain narrow or pure tone frequency bands, and lack frequency modulation (see Table 2.1), suggesting that they would be difficult for receivers to localize (Marler 1955; Klump & Shalter 1984; Endler 1993). Whisper calls are also potentially highly detectable in the face of fluctuating environmental noise, given their loud initial amplitude (exceeding 80 dB SPL) and repeated production (Klump & Shalter 1984; Endler 1993; Bradbury & Vehrencamp 1998). Further, the high dominant frequency (ca. 48 kHz) of whisper calls is subject to rapid environmental attenuation and narrow angular propagation, together limiting their potential signaling range (Evans et al. 1972; Smith 1979; Lawrence & Simmons 1982; Pye & Langbauer 1998). Although I did not examine potential visual conspicuousness associated with signaling (e.g. Hersek & Owings 1994), it is clear that the signal proper is highly furtive and capable of being detected by only a very limited audience.

Responses to whisper calls were less pronounced than responses to audible calls (see Table 2.2), suggesting that whisper calls either convey a less urgent message than audible calls or that respondents react in a less conspicuous fashion (i.e. elevating only the head as opposed to standing upright on the hind feet, see Hare 1998a). It is unlikely, however, that such a structurally disparate alarm signal would evolve as a redundant mechanism for conveying

reduced response urgency, given the specialized physiological adaptations necessary for producing and detecting such high frequency signals (see chapter 3). Indeed, Richardson's ground squirrels are highly capable of conveying various degrees of response urgency associated with audible alarm calls simply by varying the rate of syllable production (Warkentin et al. 2001), modulating the signal frequency (Davis 1984; Sloan et al. in press), or appending/removing brief pulses of sound known as 'chucks' to the end of the primary syllables (Sloan et al. in press). Rather, because respondents to short-range alarm calls are necessarily located in close proximity to the signaler, it is more likely that receivers respond to inconspicuous signals in an inconspicuous fashion to avoid attracting predators to the vicinity and conflicting with the signaler (Axelrod & Hamilton 1981). That is not to say that whisper calls do not convey varying degrees of response urgency, however, as signal variation similar to that found in audible calls is also apparent in whisper calls (e.g. ultrasonic chucks, Table 2.1).

While the complete function of whisper calling cannot be ascertained from this study alone, it is reasonable to assume that selection would favour the use of whisper instead of audible alarm calls in certain circumstances. However, this study lacks the contextual data necessary for determining whether whisper calling serves as a context-dependent alternative signaling strategy (Maynard Smith 1978; Abrams 1983; Hasson 1994). The prevalence of above-ground kin located near the signaler (Hare 1998b, Hare 2004), or the likelihood that a predator has noticed a potential caller (e.g. by assessing predator distance and applying simple rules of thumb: Bouskila & Blumstein 1992; Warkentin et al.

2001), could potentially influence the signaling strategy adopted by the signaler in a given situation (Maynard Smith 1978; Abrams 1983). It is clear, however, that these calls function as anti-predator vocalizations and, given their spectral characteristics, both limit the audience and reduce the probability that a predator will detect the signaler.

REFERENCES

- Abrams, P.** 1983. Life-history strategies of optimal foragers. *Theoretical and Population Biology*, **24**, 22-38.
- Axelrod, R. & Hamilton, W.D.** 1981. The evolution of cooperation. *Science*, **211**, 1390-1396.
- Blanchard, R.J. & Blanchard, D.C.** 1980. The colony model: experience counts. *Behavioral and Neural Biology*, **30**, 109-112.
- Blanchard, R.J., Blanchard, D.C., Agullana, R. & Weiss, S.M.** 1991. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology and Behavior*, **50**, 967-972.
- Blanchard, R.J., Agullana, R., McGee, L., Weiss, S. & Blanchard, D.C.** 1992. Sex differences in the incidence and sonographic characteristics of antipredator ultrasonic cries in the laboratory rat (*Rattus norvegicus*). *Journal of Comparative Psychology*, **106**, 270-277.

- Bouskila, A. & Blumstein, D.T.** 1992. Rules of thumb for predation hazard assessment: Predictions from a dynamic model. *American Naturalist*, **139**, 161-176.
- Bradbury, J.W. & Vehrencamp, S.L.** 1998. *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Cherry, J.A.** 1989. Ultrasonic vocalizations by male hamsters: parameters of calling and effects of playbacks on female behaviour. *Animal Behaviour*, **38**, 138-153.
- Davis, L.S.** 1984. Alarm calling in Richardson's ground squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152-164.
- Eiler, K.C. & Banack, S.A.** 2004. Variability in the alarm call of golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *Journal of Mammalogy*, **85**, 43-50.
- Endler, J.A.** 1983. Natural and sexual selection on color patterns in Poeciliid fishes. *Environmental Biology of Fishes*, **9**, 173-190.
- Endler, J.A.** 1987. Predation, light intensity, and courtship behaviour in *Poecilia reticulata*. *Animal Behaviour*, **35**, 1376-1385.
- Endler, J.A.** 1993. Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London B*, **340**, 215-225.
- Evans, L.B., Bass, H.E. & Sutherland, L.C.** 1972. Atmospheric absorption of sound: theoretical predictions. *Journal of the Acoustical Society of America*, **51**, 1565-1575.

- Griffin, D.R.** 1971. The importance of atmospheric attenuation for the echolocation of bats (Chiroptera). *Animal Behaviour*, **19**, 55-61.
- Hamilton, W.D.** 1964. The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology*, **7**, 1-52.
- Hare, J.F.** 1998a. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, discriminate among individual alarm callers. *Animal Behaviour*, **55**, 451-460.
- Hare, J.F.** 1998b. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, manifest both littermate and neighbour/stranger discrimination. *Ethology*, **104**, 991-1002.
- Hare, J.F.** 2004. Kin discrimination by asocial Franklin's ground squirrels (*Spermophilus franklinii*): Is there a relationship between kin discrimination and ground squirrel sociality? *Ethology, Ecology & Evolution*, **16**, 157-169.
- Hare, J.F. & Atkins, B.A.** 2001. The squirrel that cried wolf: reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behavioral Ecology and Sociobiology*, **51**, 108-112.
- Hasson, O.** 1994. Cheating signals. *Journal of Theoretical Biology*, **167**, 223-238.
- Hersek, M.J. & Owings, D.H.** 1994. Tail flagging by young California ground squirrels, *Spermophilus beecheyi*: age-specific participation in a tonic communication system. *Animal Behaviour*, **48**, 803-811.
- Klump, G.M. & Shalter, M.D.** 1984. Acoustic behaviour of birds and mammals in the predator context. I. Factors affecting the structure of alarm signals. II.

The functional significance and evolution of alarm signals. *Zeitschrift für Tierpsychologie*, **66**, 189-226.

Koepl, J.W., Hoffmann, R.S. & Nadler, C.F. 1978. Pattern analysis of acoustical behavior in four species of ground squirrels. *Journal of Mammalogy*, **59**, 677-696.

Lawrence, B.D. & Simmons, J.A. 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *Journal of the Acoustical Society of America*, **71**, 585-590.

Macedonia, J.M. & Evans, C.S. 1993. Variation among mammalian alarm call systems and the problem of meaning in animal signals. *Ethology*, **93**, 177-197.

Machlis, L., Dodd, P.W.D. & Fentress, J.C. 1985. The pooling fallacy: problems arising when individuals contribute more than one observation to the data set. *Zeitschrift für Tierpsychologie*, **68**, 201-214.

Marler, P. 1955. Characteristics of some animal calls. *Nature*, **176**, 6-8.

Maynard Smith, J. 1978. Optimization theory in evolution. *Annual Review of Ecology and Systematics*, **9**, 31-56.

Michener, G.R. 1983. Kin identification, matriarchies, and the evolution of sociality in ground-dwelling Sciurids. In: *Advances in the Study of Mammalian Behavior* (Ed. by J.F. Eisenberg and D.G. Kleiman), pp. 528-572, The American Society of Mammalogists, Pennsylvania.

Michener, G.R. & Michener, D.R. 1973. Spatial distribution of yearlings in a Richardson's ground squirrel population. *Ecology*, **54**, 1138-1142.

- Michener, G.R. & Michener, D.R.** 1977. Population structure and dispersal in Richardson's ground squirrels. *Ecology*, **58**, 359-368.
- Norris, K.S.** 1969. The echolocation of marine mammals. In: *The Biology of Marine Mammals* (Ed. by H.T. Andersen), pp. 391-424, Academic Press, New York.
- Nowak, R.M.** 1999. *Walker's Mammals of the World*. The John Hopkins University Press, Baltimore.
- Pierce, J.D., Sawrey, D.K. & Dewsbury, D.A.** 1989. A comparative study of ultrasonic vocalizations during copulation. *Behavioral and Neural Biology*, **51**, 211-221.
- Pye, J.D. & Langbauer, W.R.** 1998. Ultrasound and infrasound. In: *Animal Acoustic Communication* (Ed. by S.L. Hopp, M.J. Owren, and C.S. Evans), pp. 221-250, Springer-Verlag, New York.
- Sales, G. & Pye, D.** 1974. *Ultrasonic Communication by Animals*. Chapman and Hall, London.
- Sewell, G.D.** 1968. Ultrasound in rodents. *Nature*, **217**, 682-683.
- Sewell, G.D.** 1970. Ultrasonic communication in rodents. *Nature*, **227**, 410.
- Simmons, J.A., Fenton, M.B. & O'Farrell, M.J.** 1979. Echolocation and pursuit of prey by bats. *Science*, **203**, 16-21.
- Sloan, J.L., Wilson, D.R. & Hare, J.F.** In press. Functional morphology of Richardson's ground squirrel (*Spermophilus richardsonii*) alarm calls: the meaning of chirps, whistles and chucks. *Animal Behaviour*.

- Smith, W.J.** 1979. The study of ultrasonic communication. *American Zoologist*, **19**, 531-538.
- Thiessen, D.D., Graham, M. & Davenport, R.** 1978. Ultrasonic signaling in the gerbil (*Meriones unguiculatus*): social interaction and olfaction. *Journal of Comparative and Physiological Psychology*, **92**, 1041-1049.
- Warkentin, K.J., Keeley, A.T.H. & Hare, J.F.** 2001. Repetitive calls of juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) communicate response urgency. *Canadian Journal of Zoology*, **79**, 569-573.
- Witkin, S.R.** 1977. The importance of directional sound radiation in avian vocalization. *Condor*, **79**, 490-493.

Table 2.1. Description of ultrasonic alarm calls produced by 15 Richardson's ground squirrels.

Variable	$\bar{X} (\pm SE)$	Range	N
Duration of 1 ^o syllable (ms)	225 ± 8	174 - 290	15
Dominant frequency (kHz)	48.0 ± 2.3	27.2 - 62.8	15
Minimum (kHz)	43.5 ± 2.5	25.3 - 54.4	12*
Maximum (kHz)	51.4 ± 2.7	29.1 - 64.2	12*
Bandwidth (kHz)	7.9 ± 1.4	2.6 - 15.0	12*
Frequency (+1)	63.8 ± 3.6	51.6 - 83.4	9
Frequency (-1)	31.7 ± 3.3	16.9 - 45.9	9
Frequency (+2)	77.8 ± 18.8	59.1 - 96.6	2
Amplitude (dB SPL)	66.8 ± 2.1	57.7 - 83.7	15
Chuck latency (ms)	20 ± 2	18 - 23	3
Chuck duration (ms)	28 ± 1	26 - 30	3
Chuck frequency (kHz)	31.6 ± 6.0	22.7 - 43.1	3

* Three individuals were excluded from the analysis because the limits of the dominant frequency band were not discernable.

Table 2.2. Squirrel's responses to playback of whisper calls and 3 control calls.

Variable	Whisper	Control	Difference (SE)	<i>P</i>
Background Noise (N = 18*)				
Playback vigilance (%)	21.6	-3.5	25.1 (10.5)	0.04**
Post-playback vigilance (%)	18.9	-11.6	30.5 (10.6)	0.01
Initial response (1 - 4)	0.61	0.06	0.55 (0.19)	0.01
Duration of initial (s)	10.08	4.07	6.01 (2.86)	0.18
Tone (N = 19)				
Playback vigilance (%)	22.5	19.3	3.2 (10.9)	0.77
Post-playback vigilance (%)	21.7	12.0	9.7 (9.6)	0.33
Initial response (1 - 4)	0.63	0.32	0.31 (0.22)	0.17
Duration of initial (s)	9.59	5.50	4.09 (3.99)	0.33
Audible (N = 19)				
Playback vigilance (%)	22.5	50.4	-27.9 (6.4)	<0.01
Post-playback vigilance (%)	21.7	35.4	-13.7 (5.6)	0.03
Initial response (1 - 4)	0.63	2.37	-1.74 (0.40)	<0.01
Duration of initial (s)	9.59	22.92	-13.33 (4.70)	<0.01

Bold subheadings refer to which control the whisper call is being compared. Mean percentages for vigilance are corrected for pre-playback behaviour (i.e. negative values indicate greater vigilance in the pre-playback period).

* indicates that 1 trial was removed from the analysis due to nearby audible calling during playback of the background noise control.

** indicates that data were log transformed before analysis to meet the parametric assumption of homoscedasticity.

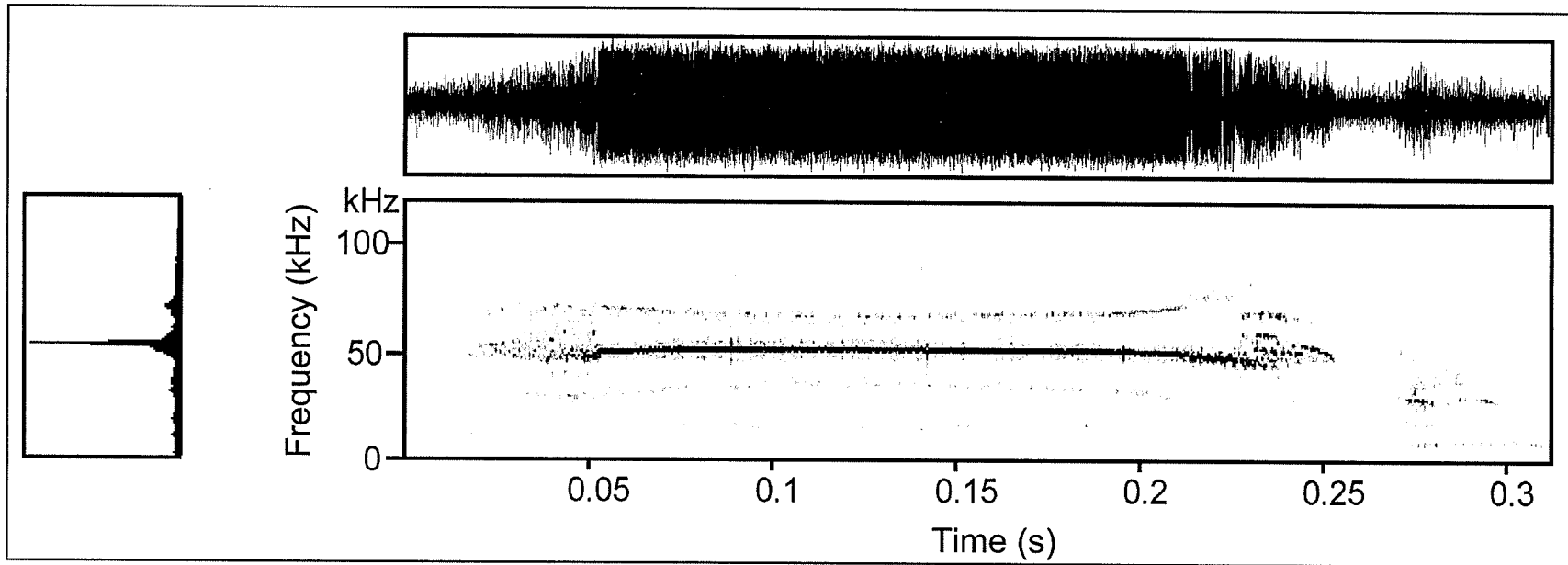


Figure 2.1. Ultrasonic alarm call produced by Richardson's ground squirrels. The spectrograph (bottom) shows call duration (238 ms) and dominant frequency (51.6 kHz) of the primary syllable, (+1) and (-1) bands, and a brief chuck trailing the offset of the primary syllable. The signal amplitude is represented by the density of the grayscale. The power spectrum (left) shows the relative amplitude (x-axis) of individual frequencies (y-axis), averaged across the entire signal. The time-amplitude window (top) shows the overall signal amplitude relative to background noise.

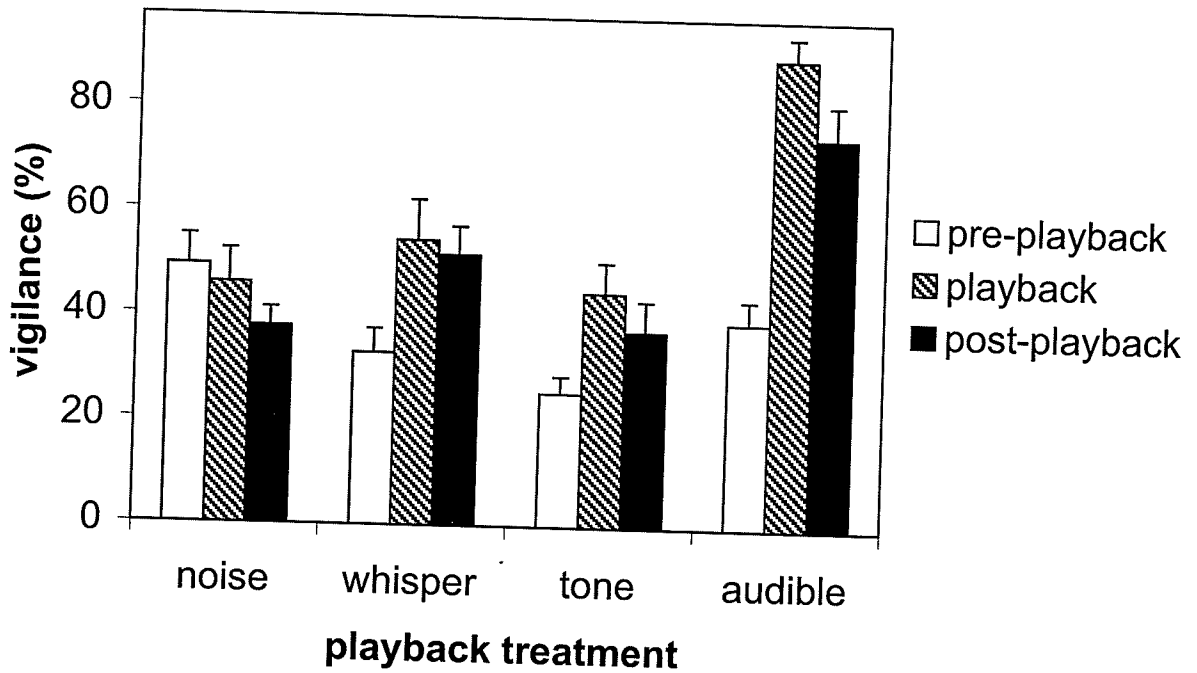


Figure 2.2. Mean (+SE) percentage of time devoted to vigilant behaviour before, during, and after the playback of background noise, whisper calls, pure tones, and audible calls.

Chapter 3: Auditory frequency response of Richardson's ground squirrels (*Spermophilus richardsonii*).

With the exception of certain insects (Pierce 1948; Sales & Pye 1974), the use of ultrasound (frequencies > 15 kHz) in animal communication appears to be unique to mammals (Masterton et al. 1969) and, since its initial discovery in echolocating bats (Pierce & Griffin 1938), has been reported in rodents, chiropterans, cetaceans, pinnipeds, insectivores, marsupials, primates, xenarthrans, artiodactyls, and carnivores (reviewed in Sales & Pye 1974; Brown & Pye 1975). The ability of mammals to hear ultrasound is associated with morphological specialization of the auditory tract. In the outer ear, large pinnae (Manley 1971; Manley et al. 1972) and small tympanic membranes (Manley et al. 1972) facilitate the reception of ultrasound. Transfer of those ultrasonic frequencies through the middle ear is most effective when the ossicles are small and are held rigidly in place by stiff inter-ossicular joints, tense middle ear muscles, and an ossified connection between the malleus and the auditory bulla (i.e. 'microtype' middle ear morphology: Brown & Pye 1975; Fleischer 1978; Mason 2001). Finally, within the inner ear, an elongated basilar membrane (Manley 1971) and enlarged basilar turn (site of neural reception of high-frequency signals, Pye 1970) allow for maximum neuronal reception of the physical stimuli.

These specializations represent the 'general' condition found among species capable of hearing ultrasound and are in no way an exhaustive account of all high-frequency hearing adaptations, nor is the presence of these

specializations necessarily synonymous with hearing ultrasound. The frequency response of a species can also be limited by the capacity of the peripheral and central nervous systems to transduce (Wever et al. 1972) and integrate (Brown 1970) those signals, respectively.

Our understanding of the frequencies detected by mammals has significantly improved with the refinement of electrophysiological techniques (reviewed by Brown & Pye 1975). Measuring neuronal activity associated with the detection of acoustic stimuli provides reliable, quantitative data without relying on the cooperation of the subject (for examples see Brown 1970; Hamill et al. 1989). However, frequencies that excite neurons in the peripheral or central nervous system can only be said to be consciously heard by the subject if they are capable of eliciting a behavioural response (Brown 1970). Only when an animal is capable of responding to a frequency can a signal exploiting that frequency have any ecological value. Thus, while electrophysiological techniques remain useful for comparing frequency responses within and among taxa (see Sales & Pye 1974), determining the frequency threshold that is ecologically important to a species requires conditioning experiments that demonstrate signal integration and behavioural responses of the whole organism (Cynx & Clark 1998).

Research on high-frequency hearing in rodents has been largely confined to the family Muridae (but see Peterson et al. 1974), which is the only rodent group known to produce purely ultrasonic vocalizations (Sales & Pye 1974; Nowak 1999). Mice (species of *Mus* and *Peromyscus*), for example, produce and

respond to frequencies exceeding 70 kHz (Ralls 1967). Eiler & Banack (2004), however, have demonstrated that the audible (ca. 9 kHz) alarm calls of golden-mantled ground squirrels (family Sciuridae, *Spermophilus lateralis*) also contain ultrasonic harmonic components. Further, Hamill et al. (1989) demonstrated that 32 kHz tones (higher frequencies were not tested) are capable of eliciting auditory brainstem responses in these squirrels, while frequencies as high as 100 kHz are capable of eliciting cochlear microphonic responses (Peterson et al. 1974). Taken together, these results suggest that the ultrasonic harmonic components contained in golden-mantled ground squirrel vocalizations could encode valuable information, though auditory brainstem responses and, particularly, cochlear microphonic responses do not reliably indicate hearing at the conscious level (Brown & Pye 1975).

Recent work by Wilson & Hare (2004) demonstrated that Richardson's ground squirrels (*Spermophilus richardsonii*) produce and respond to purely ultrasonic (dominant frequency: 48.0 ± 2.3 kHz, mean \pm SE) alarm signals, or whisper calls, in their natural environment. The maximum frequency response of these squirrels could not be ascertained from that study, however, as the alarm signals used to elicit behavioural responses included frequencies ranging from 25 - 97 kHz. I therefore conducted a controlled experiment to determine the maximum frequency response of this species and to elucidate the whisper call parameters that are most salient to listening ground squirrels. Further, because considerable disparity with respect to environmental attenuation, directionality, and localizability exists between 25 and 97 kHz signals (Masterton et al. 1969;

Lawrence & Simmons 1982; Pye & Langbauer 1998), my results provide insight into the potential function of these signals.

METHODS

Subjects

A total of six male and six female juvenile Richardson's ground squirrels were live-trapped from the Assiniboine Park Zoo (49.874° N, 97.243° W) between 20 and 25 September 2003. Squirrels were kept within the animal holding facility in the Department of Zoology, University of Manitoba, in polycarbonate holding cages (25 X 45 X 20 cm). Wood shavings and torn paper towels were provided for nest material and were changed bi-weekly. Squirrels were provided food (Purina Rodent Blocks) and water ad lib, received habitrail® Hamster Puffs on a weekly basis, and were maintained under a 12:12 h day/night light cycle (light onset: 0700 CST) at 21°C. During the 2004 spring emergence (8 April), all surviving individuals (five males, four females) were released at their original point of capture.

Classical Conditioning Paradigm

I used classical conditioning (see Pavlov 1927; Rescorla 1968; Cynx & Clark 1998) to train each of the 12 squirrels to respond to a tone. Training trials involved a 2-s presentation of a sine wave at a certain test frequency (see below) as a conditioned stimulus (CS) immediately before a burst of air (unconditioned stimulus: UCS) was delivered to the subject. The UCS produced an observable

'flinch' (unconditioned response: UCR) on the part of the subject, and, after five pairings with the CS (training trials), subjects typically responded with a flinch (conditioned response: CR) to the CS alone (test trial). If, however, the CR was not apparent during that first test trial, an additional five training trials were conducted. A total of four test trials were conducted after the first trial in which the CS alone elicited a response to ensure that the observed flinch was not merely a random event. An additional training trial was conducted to re-establish the CR if it became noticeably less pronounced after multiple test trials. While an operant conditioning paradigm may have been more effective than classical conditioning for examining the frequency response of squirrels, there was insufficient time available to operantly condition each of the 12 squirrels before they entered hibernation.

Apparatus

Squirrels were trained and tested between 27 September and 22 October 2003 in a round Plexiglas™ arena (0.6 m diameter, 0.65 m height) in an Industrial Acoustics, Inc. soundproof chamber (dimensions: 2.5 X 3.0 X 2.5 m, incandescent lighting: 122.1 lux at the arena floor, temperature: 23.0 °C) within the Department of Psychology, University of Manitoba (Fig. 3.1). The arena had six air nozzles that were fastened 2 cm above the arena floor, spaced equidistantly around the outer wall, and pointed towards the center of the arena. Each nozzle was connected via equal-length 0.25" diameter air hose to one common 0.25" diameter hose, which passed through the wall and was connected

to a 42.5-L air tank in the adjacent observation room. The air tank was maintained at 90 - 95 psi and an in-line blaster valve allowed me to deliver sudden bursts (approximately 1 s duration) of air to the squirrel. A video camera placed on a tripod beside the arena was connected through a conduit panel to a Sony High-8 editing deck (model EV-S7000) and a 13" Sylvania monitor (model 6413CTC) in the observation room, which allowed me to monitor and videotape the squirrel's response without entering the soundproof chamber.

Conditioned stimuli were produced with a signal generator (Lafayette Instrument Co., Model A1421) and were delivered by pressing an in-line switch. Signals were relayed through a digital signal analyzer (Scientific-Atlanta, model 13359) to confirm their frequency, amplified with the appropriate amplifier, passed through the conduit panel, and broadcast through the appropriate speaker, which was placed face down on the clear perforated Plexiglas™ roof of the arena and concealed from the squirrel with black speaker cloth. A Sony XM-2025 audio amplifier and Genexxa Pro LX5 loudspeaker (frequency response: 0.085 - 25 kHz) were used to broadcast signals below 20 kHz, while an ultrasound amplifier (UltraSound Advice, model S55) and ultrasound loudspeaker (UltraSound Advice, model S56; frequency response: 10 - 150 kHz) were used to broadcast signals exceeding 20 kHz.

Procedure

Before a squirrel was tested, it was placed in the arena for three 5-min periods separated by one hour each to familiarize it with the testing apparatus.

During trials, stimuli were not presented until the squirrel remained motionless for at least 30 s, which increased the probability that any response by the squirrel could be attributed to the signal proper. The precise time of signal delivery was noted on the camera's time code (accurate to nearest 0.25 s) to facilitate data coding. All training and test trials were separated by at least 5 min and the arena was cleaned with a 50:50 water-vinegar solution between subjects.

I intended to train and test each squirrel at multiple frequency intervals to determine the rough limits of each individual's maximum frequency response. However, squirrels failed to respond reliably to both the CS and the UCS beyond the first training/testing series, perhaps owing to habituation to the UCS and/or learned helplessness (Winston et al. 2001). Consequently, only one randomly selected male and female squirrel could be trained and tested at each frequency increment (8, 18, 28, 38 or 48 kHz). If one or both squirrels failed to respond to a given frequency, the subsequent pair was tested at the highest frequency responded to by at least two squirrels to ensure that the conditioning paradigm remained effective.

Fifteen days after all of the squirrels were originally tested, I re-tested one randomly-selected male and female at 38 kHz and found that the female, but not the male, was again capable of producing a CR. The remaining 11 squirrels (one female squirrel died before testing commenced) were thus subjected in random order to a second series of training and test trials to further resolve the limits of their maximum frequency response. Any individual that failed to respond during the first test series was trained and tested in the second series at the highest

frequency responded to by others in their first series. This tested whether a squirrel that failed to respond in series one was indeed capable of being conditioned. Thus, at least one male and one female in the second series were tested at each of the following frequencies: 38, 39, and 40 kHz.

Data Coding and Analyses

The auditory threshold experiment was intended to determine whether or not Richardson's ground squirrels can detect various ultrasonic frequencies and, thus, details of their behavioural responses to each frequency were not quantified. Rather, each test trial was considered 'positive' if the subject's behaviour changed (i.e. 'flinch') at the onset or offset of the 2-s tone and 'negative' if the subject's behaviour remained unaltered in response to the tone. Because squirrels remained motionless for 30 s before the signal, any response to the brief tone was unlikely to be a chance occurrence. To further reduce the probability of committing a Type I error, however, a squirrel was only deemed responsive to a given frequency if it responded positively to at least three of five test trials. Although trials were deemed positive or negative in situ, I confirmed those responses from the video archive at a later time. The maximum frequency response of juvenile Richardson's ground squirrels was considered the maximum frequency detected by at least two squirrels (one male and one female).

RESULTS

During the first test series, a different pair of juvenile Richardson's ground squirrels was found to be responsive to each frequency (8, 18, 28, and 38 kHz, in that order, Table 3.1). Neither of the two squirrels tested at 48 kHz, however, could be trained to produce a CR, though both continually produced an UCR during training. The final pair of squirrels was tested at, and responsive to, 38 kHz, indicating that failure of the previous pair to respond to 48 kHz was probably not a result of trial order. When tested in the second series, however, the two squirrels that failed to respond to 48 kHz in the first series also failed to respond to 38 kHz, suggesting that those two individuals may have been incapable of being conditioned. On the other hand, only one of the two squirrels initially tested in the second series at 38 kHz proved responsive, suggesting that conditioning in the second series was unreliable. Among the remaining seven squirrels tested in series two, one of two individuals tested at 39 kHz and four of five individuals tested at 40 kHz were deemed responsive to those frequencies (Table 3.1). While failure to respond to a given frequency does not necessarily represent failure to detect that frequency, it is clear that juvenile Richardson's ground squirrels detect ultrasonic frequencies as high as 40 kHz.

DISCUSSION

My results demonstrate that juvenile Richardson's ground squirrels are generally capable of hearing ultrasonic frequencies as high as 40 kHz. To my knowledge, this represents the highest known frequency capable of eliciting a behavioural

response in any non-Murid rodent and is consistent with the behavioural responses of squirrels to ultrasonic alarm calls observed in field experiments (see chapter 2). Indeed, only two of the 15 whisper calls recorded and used for playback in that study contained minimum frequencies exceeding 40 kHz. It is unusual, however, that a species' maximum frequency response would be less than the mean dominant frequency of its own vocalization (48.0 ± 2.3 kHz, mean \pm SE), as selection should favour compatibility between the signaler and receiver, thus enhancing the efficiency of the communication system (Bradbury & Vehrencamp 1998). Indeed, most species exhibit pronounced sensitivity to the mean dominant frequency of their own vocalizations (Grinnel 1963; Brown 1970).

It is possible that the conditioning study, owing to a combination of factors, underestimated the maximum frequency response of Richardson's ground squirrels. The limited sample size and inability to reliably condition subjects to multiple frequencies precluded further testing at higher frequencies. Furthermore, the highly directional propagation of high-frequency sound may have contributed to the unreliable conditioning of subjects to frequencies between 38 and 40 kHz; acoustic stimuli were probably less intense at the edge of the arena (25° from normal relative to speaker) than in the center (see Pye & Langbauer 1998). The problem imposed by spatial variation in signal intensity may have been compounded by the fact that species that produce both audible and ultrasonic vocalizations exhibit significantly reduced auditory sensitivity to frequencies between those vocalizations (Grinnell 1963; Brown 1970), which would include 38 - 40 kHz in Richardson's ground squirrels (see chapter 2). Thus, subjects may

have been more reliably conditioned to high frequencies if they were confined to the space immediately below the loudspeaker, as suggested by Brown & Pye (1975).

Sensitivity to 40 kHz alarm calls may enhance a signal recipient's ability to accurately localize the caller (Masterton et al. 1969; Bradbury & Vehrencamp 1998), which may provide valuable contextual information about the predator encounter eliciting the alarm call (see Sloan et al. in press). In species that have close-set ears and produce audible vocalizations, the long wavelengths associated with those audible signals provide an undetectable interaural amplitude difference (Masterton et al. 1969; Bradbury & Vehrencamp 1998) that, unless augmented by some other cue (e.g. frequency-modulated or temporally segregated signal components), renders receivers inefficient at locating the caller (see Sloan et al. in press). Because high-frequency sound attenuates rapidly in the environment and reflects strongly from most objects (Evans et al. 1972; Lawrence & Simmons 1982; Pye & Langbauer 1998), however, the interaural amplitude difference increases significantly at high frequencies, permitting receivers with small heads to locate the caller with greater accuracy (Masterton et al. 1969). Thus, this study confirms that Richardson's ground squirrels are capable of hearing ultrasonic whisper calls and, given the squirrels' ability to detect 40 kHz, suggests that these signals may provide the receiver with valuable cues regarding the signaler's location.

REFERENCES

- Bradbury, J.W. & Vehrencamp, S.L.** 1998. *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Brown, A.M.** 1970. Bimodal cochlear response curves in rodents. *Nature*, **228**, 576-577.
- Brown, A.M. & Pye, J.D.** 1975. Auditory sensitivity at high frequencies in mammals. In: *Advances in Comparative Physiology and Biochemistry* (Ed. by O. Lowenstein), pp. 1-73, Academic Press, Inc., New York.
- Cynx, J. & Clark, S.J.** 1998. The laboratory use of conditional and natural responses in the study of avian auditory perception. In: *Animal Acoustic Communication* (Ed. by S.L. Hopp, M.J. Owren, and C.S. Evans), pp. 353-377, Springer-Verlag, New York.
- Eiler, K.C. & Banack, S.A.** 2004. Variability in the alarm call of golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *Journal of Mammalogy*, **85**, 43-50.
- Evans, L.B., Bass, H.E. & Sutherland, L.C.** 1972. Atmospheric absorption of sound: theoretical predictions. *Journal of the Acoustical Society of America*, **51**, 1565-1575.
- Fleischer, G.** 1978. Evolutionary principles of the mammalian middle ear. *Advances in Anatomy, Embryology, and Cell Biology*, **55**, 1-70.
- Grinnell, A.D.** 1963. The neurophysiology of audition in bats: intensity and frequency parameters. *Journal of Physiology*, **167**, 38-66.

- Hamill, N.J., McGinn, M.D. & Horowitz, J.M.** 1989. Characteristics of auditory brainstem responses in ground squirrels. *Journal of Comparative Physiology B*, **159**, 159-165.
- Lawrence, B.D. & Simmons, J.A.** 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *Journal of the Acoustical Society of America*, **71**, 585-590.
- Manley, G.A.** 1971. Some aspects of the evolution of hearing in vertebrates. *Nature*, **230**, 506-509.
- Manley, G.A., Irvine, D.R.F. & Johnstone, B.M.** 1972. Frequency response of bat tympanic membrane. *Nature*, **237**, 112-113.
- Mason, M.J.** 2001. Middle ear structures in fossorial mammals: a comparison with non-fossorial species. *Journal of Zoology*, **255**, 467-486.
- Masterton, B., Heffner, H. & Ravizza, R.** 1969. The evolution of hearing. *Journal of the Acoustical Society of America*, **45**, 966-985.
- Nowak, R.M.** 1999. *Walker's Mammals of the World*. The John Hopkins University Press, Baltimore.
- Pavlov, I.P.** 1927. *Conditioned Reflexes: an Investigation of Physiological Activity of the Cerebral Cortex*. Oxford University Press, London.
- Peterson, E.A., Levison, M., Lovett, S., Feng, A. & Dunn, S.H.** 1974. The relation between middle ear morphology and peripheral auditory function in rodents, 1: Sciuridae. *Journal of Auditory Research*, **14**, 227-242.
- Pierce, G.W.** 1948. *The Songs of Insects*. Harvard University Press, Cambridge, Massachusetts.

- Pierce, G.W. & Griffin, D.R.** 1938. Experimental determination of supersonic notes emitted by bats. *Journal of Mammalogy*, **19**, 454-455.
- Pye, A.** 1970. The structure of the cochlea in Chiroptera. A selection of Microchiroptera from Africa. *Journal of Zoology*, **162**, 335-343.
- Pye, J.D. & Langbauer, W.R.** 1998. Ultrasound and infrasound. In: *Animal Acoustic Communication* (Ed. by S.L. Hopp, M.J. Owren, and C.S. Evans), pp. 221-250, Springer-Verlag, New York.
- Ralls, K.** 1967. Auditory sensitivity in mice, *Peromyscus* and *Mus musculus*. *Animal Behaviour*, **15**, 123-128.
- Rescorla, R.A.** 1968. Probability of shock in the presence and absence of cs in fear conditioning. *Journal of Comparative and Physiological Psychology*, **66**, 1-5.
- Sales, G. & Pye, D.** 1974. *Ultrasonic Communication by Animals*. Chapman and Hall, London.
- Sloan, J.L., Wilson, D.R. & Hare, J.F.** In press. Functional morphology of Richardson's ground squirrel (*Spermophilus richardsonii*) alarm calls: the meaning of chirps, whistles and chucks. *Animal Behaviour*.
- Wever, E.G., McCormick, J.G., Palin, J. & Ridgway, S.H.** 1971. The cochlea of the dolphin, *Tursiops truncatus*: hair cells and ganglion cells. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 2908-2912.
- Wilson, D.R. & Hare, J.F.** 2004. Ground squirrel uses ultrasonic alarms. *Nature*, **430**, 523.

Winston, C.R., Leavell, B.J., Ardayfio, P.A., Beard, C. & Commissaris, R.L.

2001. A nonextinction procedure for long-term studies of classically conditioned enhancement of acoustic startle in the rat. *Physiology and Behavior*, **73**, 9-17.

Table 3.1. Frequency response of 12 juvenile Richardson's ground squirrels that were each subjected to training and testing at two different frequencies (series 1 and series 2).

Conditioned stimulus (kHz)	# responding in series 1	# responding in series 2	Total # responding
8.0	2 / 2	-----	2 / 2
18.0	2 / 2	-----	2 / 2
28.0	2 / 2	-----	2 / 2
38.0	4 / 4	1 / 4*	5 / 8*
39.0	-----	1 / 2	1 / 2
40.0	-----	4 / 5**	4 / 5**
48.0	0 / 2*	-----	0 / 2*

* includes two individuals that were not capable of being trained in either series.

** one individual died prior to testing in series 2.

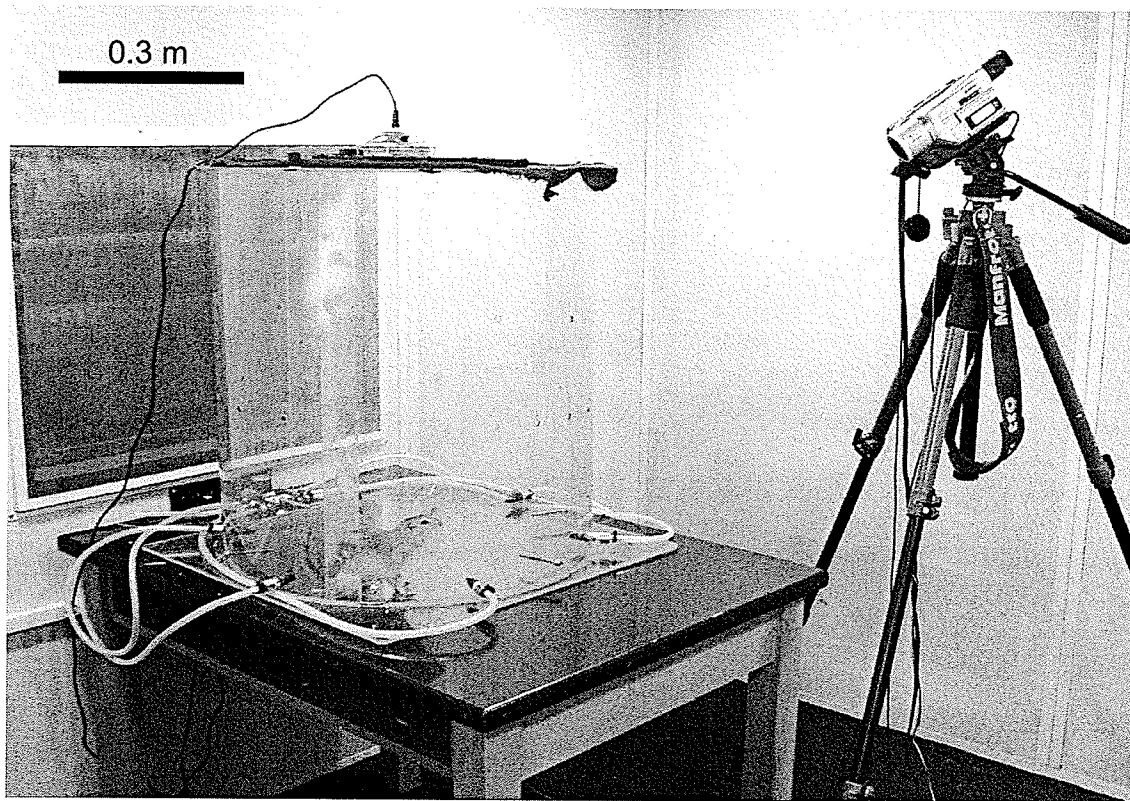


Figure 3.1. Experimental apparatus for determining the maximum frequency response of the hearing of juvenile Richardson's ground squirrels.

**Chapter 4: Ecological context surrounding Richardson's ground squirrel
(*Spermophilus richardsonii*) ultrasonic alarm calls.**

The evolution of alarm calling in non-human species is fascinating in that calling appears altruistic; that is, call recipients benefit from the early detection of predators (Pulliam 1973), while callers incur energetic costs (Eberhardt 1994), time lost from foraging, resting, and caring for young (Abrams 1983; Ydenberg & Dill 1986), and no readily apparent benefits. Smythe (1974) suggested that alarm signals persist because they provoke ambush predators to attack prematurely, thus reducing the likelihood of that attack being successful. However, Sherman (1977) found that three of the six adult Belding's ground squirrels (*Spermophilus beldingi*) that were successfully killed by predators in his study had alarm called immediately prior to the attack, suggesting that alarm calling does not enhance the probability of surviving an attack. Other studies (Woodland et al. 1980; Caro 1995; Shelley & Blumstein 2005) suggest that alarm calling reduces the probability of a predator attack in the first place (i.e. pursuit-deterrence) by indicating to the predator that it has been detected, that any element of surprise has expired, and that any further investment in pursuing the caller would be futile. Contrary to the pursuit-deterrence hypothesis, however, studies have demonstrated that predators do not preferentially attack non-vigilant prey (Cresswell et al. 2003), but, rather, that predators are most likely to attack conspicuous callers (Sherman 1977; Yasukawa 1989). Because alarm calls are apparently costly, it is also theoretically possible that alarm calling is maintained

via sexual selection. Individuals that produce alarm calls may be demonstrating to potential mates their underlying resistance to predators (Zahavi 1975).

Because the immediate benefits of alarm calling are not readily apparent, Hamilton's (1964) kin selection theory has often been invoked to explain such apparently altruistic behaviour (Dunford 1977; Sherman 1977; Davis 1984; Hauber & Sherman 1998), suggesting that callers benefit by enhancing the survival of descendent (direct fitness) and non-descendent (indirect fitness) kin, which ultimately carry like copies of the caller's genes into future generations. However, alarm calling can only be maintained via kin selection if a caller's kin are alive, vulnerable to predation, and within earshot of the call (Hamilton 1964; Maynard Smith 1965). Among the many contexts in which kin may stand to benefit from alarm calling, the preponderance of vulnerable young at juvenile emergence may render kin-biased alarm signaling particularly adaptive at that time (Maynard Smith 1965).

Among most alarm systems, natural selection has shaped signals in a fashion that maximizes their detection by related and unrelated group members alike (Marler 1955; Klump & Shalter 1984), consequently diminishing the selective advantage associated with warning kin (Wilson 2004). Thus, Trivers' (1971) notion of reciprocal altruism, whereby callers incur the cost of warning others on the condition that those warned will ultimately 'return the favour,' may also contribute to the evolution of alarm calling (Axelrod & Hamilton 1981; Wilson 1997, 2001; Hare 1998a; Tarpay et al. 2004). Indeed, selection at the broader group level is possible among species that can discriminate among individuals

(e.g. Hare 1998a; Blumstein & Daniel 2004) and adjust their behaviour according to an individual's past performances (Axelrod & Hamilton 1981; Cheney & Seyfarth 1988; Hare & Atkins 2001; Blumstein et al. 2004).

Regardless of the evolutionary impetus for calling, selection should favour alarm signals that better inform receivers (but see Charnov & Krebs 1975) and, unless sexual selection underlies calling (Zahavi 1975), reduce the risk of predation associated with signaling (Macedonia & Evans 1993). Richardson's ground squirrels (*Spermophilus richardsonii*) produce audible (ca. 8 kHz) alarm vocalizations that are loud (84 - 91 dB SPL at 1 m from source) and capable of alerting an entire colony of danger (Davis 1984; Hare 1998a). These signals convey valuable information about the location (Warkentin et al. 2001) and nature (Sloan et al. in press) of threat, allowing receivers to respond to a situation in the most appropriate fashion (Abrams 1983; Ydenberg & Dill 1986). Given that alarm calls are highly detectable and are often repeated for several minutes (Davis 1984), however, calls may actually endanger the group in certain circumstances by attracting predators that would otherwise have overlooked the group (Smythe 1970; Klump & Shalter 1984). Under these circumstances, the costs may actually outweigh the benefits of signaling, suggesting that a potential caller's fitness may best be served by remaining silent (Maynard Smith 1965; Smythe 1970; Klump & Shalter 1984).

Wilson & Hare (2004) have demonstrated that Richardson's ground squirrels also produce ultrasonic alarm signals, or whisper calls. The high frequency (ca. 48 kHz) and rapid environmental attenuation of ultrasound (Evans

et al. 1972; Smith 1979a; Lawrence & Simmons 1982) prevents most predators from detecting whisper calls (Sales & Pye 1974; Smith 1979b), but also limits the range over which whisper calls effectively warn conspecifics. Despite their limited potential to warn others, however, whisper calls may serve as an alternative, covert signaling strategy in situations where predators are likely to overlook members of the group.

This study examines the ecological context associated with the production and perception of whisper calls to gain insight into the evolution and adaptive utility of whisper calling as an alternative signaling strategy. Studies of parental investment have demonstrated that parents are most likely to produce alarm calls when offspring are very young and are most vulnerable to predation, suggesting that the developmental stage when juveniles are first exposed to predators is critical to a parent's reproductive success (East 1981; Blumstein et al. 1997). Although Wilson (see chapter 2) recorded whisper calls from adult Richardson's ground squirrels between 1 May and 10 June 2003, which includes the time when juveniles first emerge (first juvenile appeared aboveground on 23 May), it was unknown if emerging juveniles were capable of responding to whisper calls in an appropriate fashion (see chapter 2). I thus tested if recently emerged juvenile squirrels that have yet to disperse into the broader population respond with anti-predator behaviour to the playback of whisper calls, which would be consistent with whisper call production as a form of parental investment. Indeed, descendent kin are most abundant, most concentrated in space, most vulnerable to predation, and have the most to gain from being warned, immediately following

their initial emergence (Michener & Michener 1973; Michener 1983; Michener & Koepl 1985; Mateo 1996; Blumstein et al. 1997).

I also tested if the distance between a predator model and a caller affects that caller's signaling strategy (i.e. audible versus ultrasonic). Warkentin et al. (2001) demonstrated that Richardson's ground squirrels assess the distance between themselves and a potential predator prior to alarm calling. Because a predator's ability to visually detect prey declines with increasing distance from the prey (Maynard Smith 1965; Klump & Shalter 1984), I predicted that potential callers assess a situation prior to calling and favour the stealthy whisper call when predators are located farther away. Individuals should favour short-range whisper calls when predators are likely to overlook them, but use audible calls that better warn the entire group when predators are close enough to detect whisper calls.

METHODS

Playbacks

I conducted a playback experiment on emerging litters of free-living juvenile Richardson's ground squirrels at the Assiniboine Park Zoo, Winnipeg, Manitoba (49.874° N, 97.243° W) between 21 and 28 May 2004. This location proved ideal for broadcasting ultrasound because of the wind relief provided by the surrounding trees, buildings, and raised berms. Litters emerging from their natal burrows were located by scanning burrow entrances, which was facilitated by periodic mowing of the grass by Parks staff. Squirrels were not trapped and

marked for identification because of time constraints imposed by working at the zoo and because trials needed to be completed immediately following initial juvenile emergence. Litters remained individually distinct, however, because emerging squirrels remain spatially clustered around their natal burrow (Michener and Koepl 1985). I wore the same outer clothes each day to promote habituation of squirrels to my presence and to minimize inconsistencies among trials.

Upon identification of a newly emerging litter that had not previously been tested, I observed the litter and the surrounding area through binoculars for approximately 30 minutes. Litters were only considered suitable if they were located at least 15 m away from another litter and the juveniles remained within 1 m of their natal burrow, thus allowing me to discriminate among litters. During this time, I also counted the minimum number of unique individuals observed, approximating the number of individuals comprising the litter. I then chased the litter into its burrow and set-up the playback apparatus, which included an ultrasound amplifier (UltraSound Advice, model S55) and a Portable Ultrasound Processor (PUSP: UltraSound Advice), 7 - 11 m from the burrow's entrance. An ultrasound loudspeaker (UltraSound Advice, model S56) connected to the amplifier was placed 3 - 5 m from the burrow's entrance and was used to broadcast calls at a natural amplitude (74 - 82 dB SPL at 1 m). The spurious 5.5 kHz tone produced by the PUSP's output circuit was not removed in situ, given its extremely low amplitude (audible to humans only within 5 cm of the speaker), its consistency across treatments, and the need for a heavy (ca. 15 kg) bandpass

filter to remove it (see chapter 2). The speaker, which was concealed in an empty speaker box (19 X 15 X 33 cm), was placed on the ground 3 - 5 m from the burrow's entrance and oriented directly toward the burrow's entrance. A Sony DCR-TRV120 digital video camera was mounted atop a Velbon tripod and erected directly above the PUSP to the maximum height (1.3 m above ground level) operable from a kneeling position. While waiting for the litter to re-emerge, I selected the playback type by tossing a coin and loaded the call onto the PUSP using the appropriate time-compression ratio. Playbacks included broadcast of either a whisper call or a control call containing background noise, but no other discernable signal.

Calls for playbacks were constructed on Canary™ in the context of a previous study (see chapter 2) and were archived on minidisc in time-expanded form. Each whisper call was formed via repetition of a single syllable (separated by 4-s intersyllable silences) derived from a unique caller at the Assiniboine Park Zoo in 2003 and selected for its high signal-to-noise ratio. Control calls contained background noise that was recorded immediately prior to the whisper call and was edited to have the same frequency and temporal limits as the whisper call.

When the first juvenile emerged, I remained motionless and began viewing it through the video camera. The remaining squirrels counted prior to their retreat into the burrow were given a maximum 20 min to emerge, though at least two squirrels were required above ground for filming to commence. When the majority (> 50%) of squirrels above ground began foraging, they were filmed for 30 s prior to and following the playback. The exact time of playback was

noted on the camera's time code (accurate to nearest 0.25 s) to facilitate data coding. Following the initial playback, the apparatus was not moved and the remaining call-type was loaded onto the PUSP for playback, thus minimizing contextual and environmental variation between the two treatments. Because squirrels typically remained above ground between the two trials and thus did not require time to re-emerge, playbacks to a given litter were separated by a minimum of 20 min. Playbacks to one of the 13 litters tested, however, were separated by one day due to the onset of precipitation immediately following the first trial.

Following each set of playbacks, I noted the time of day and measured all distances (accurate to nearest 10 cm) between the burrow entrance, the speaker, and myself. I also noted the percent cloud cover and, using a Kestrel[®] 3000 pocket weather meter held at shoulder level (1.9 m above ground level), measured wind speed, temperature, and relative humidity. Trials were discontinued when wind speed reached 7 km/h. Finally, before the apparatus was moved, both call-types were replayed into a U30 bat detector held at the burrow's entrance to confirm that it could detect the signal under the playback conditions.

Evaluating Behavioural Responses to Playbacks

The percentage of time that each squirrel devoted to vigilant behaviour was coded from videotape before (30 s), during, and after (30 s) the playback of each call using a stopwatch and the video's time code (accurate to nearest 0.25

s). Vigilant Richardson's ground squirrels elevate their heads (Hare 1998a) and thus, vigilant behaviour was considered as any posture where the squirrel's head was elevated above the horizontal plane. I also measured the initial vigilance posture assumed following the onset of the playback, coded as 1 = low vigilance (standing on 4 feet with the head elevated above the horizontal plane), 2 = slouch, or 3 = alert (sensu Hare and Atkins 2001), and the duration of that initial posture, which terminated with any observable decrease in vigilance or the end of the post-playback period. Because squirrels lacked unique identification marks, any individual that was not continuously visible throughout the final 20 s of the pre-playback period and the entire playback period was not included in the analysis. Any squirrel that disappeared from view within the first 20 s of the post-playback period was also excluded from the analysis of that period. For each litter, only mean values (see Table 4.1) were reported for each period to avoid problems associated with pseudoreplication (Machlis et al. 1985).

The change in the percentage of time spent vigilant from the pre-playback period to both the playback and post-playback periods were calculated and compared between the two treatments with paired-sample *t* tests. The initial posture and the duration of the initial posture were also compared between treatments with paired-sample *t* tests. All data conformed to the parametric assumptions of normality (D'Agostino's D-test, all $P > 0.1$) and homoscedasticity (F-test, all $P > 0.25$). Miscellaneous and environmental grouping factors, including distance between the litter and the speaker (3.2 - 4.9 m), angle of the speaker relative to the litter (0 - 10°), distance between the litter and observer

(6.9 - 10.6 m), trial order (1 - 2), time (0830 - 1400 h), day within year (142 - 149), wind speed (2.3 - 6.9 km/h), temperature (8.3 - 18.5 °C), relative humidity (26 - 74%), and cloud cover (0 - 100%) were balanced between the two treatments (unpaired *t* tests, all $P > 0.3$) and thus do not confound my interpretation of the dependent variables of interest. Analyses were performed on StatView® 5.0.1 on a Macintosh computer and results were considered statistically significant where $P \leq 0.05$.

Context Associated with Call Production

I examined the context associated with the production of alarm calls recorded during previous alarm communication studies (for details see Hare 1998a; Hare & Atkins 2001; Warkentin et al. 2001). Calls and contextual notes were recorded by Hare between 1994 and 1998 from marked free-living juvenile Richardson's ground squirrels (juveniles ranged between 22 and 70 days post-emergence) occupying cattle pastures near Oak Lake Provincial Recreation Park, Manitoba (49°41' N, 100°43' W), between 1994 and 1996, near Brandon, Manitoba (49°47' N, 99°59' W), in 1997, and Delta Marsh, Manitoba (50°03' N, 98°20' W), in 1998. Upon identification of a previously untested squirrel, Hare approached it to within 15 m and set-up the recording apparatus, which included a Sony TCD-D7 recorder set to a sampling rate of 48 kHz and either a Dan Gibson EPM P-650 parabolic microphone (1994 - 1996) or an Audio-Technica AT815b condenser microphone (1997 - 1998) mounted atop a Vivitar tripod. When the subject emerged, recording commenced and a tan Biltmore hat

(32.5 x 19.5-cm brim x 13 cm high) used as a predator model was tossed from hip level to within 1 - 8 m of the subject. The hat was thrown 0 - 30° from a line connecting the subject and the observer, but was never thrown directly above the subject. Trials were discontinued when wind speed reached approximately 10 km/h.

While the objective of these recording sessions was to create a library of audible alarm calls, detailed notes were made following the production of all audible and ultrasonic alarm calls. Contextual notes included time of day (0735 - 1410 h), day within year (162 - 215), and locations (accurate to nearest 0.5 m) of the subject, model and observer at the onset of calling relative to a 10 x 10 m Cartesian coordinate grid constructed on the site with wire-pin flags. Distances of the subject to both the hat (0.4 - 14.4 m) and observer (3.0 - 17.0 m) were calculated using the Pythagorean theorem. Logistic regression was used to test for the possible effect of each contextual variable on the tendency of callers to produce audible vs. ultrasonic alarm calls. Chi-square tests were used to examine the possible influence of caller sex (male/female) and age class (juvenile/adult) on the production of audible versus ultrasonic alarm calls. Analyses were performed on StatView[®] 5.0.1 on a Macintosh computer and were considered statistically significant where $P \leq 0.05$.

RESULTS

Behavioural Responses of Litters to Playbacks

Playbacks were broadcast to 13 litters of juvenile Richardson's ground squirrels, though two litters were excluded from the analysis because less than two squirrels were visible during the playback period. In response to playback of ultrasonic alarm calls, litters devoted significantly more time to vigilant behaviour than in response to background noise (Table 4.2). The initial vigilance posture was also greater following the onset of the alarm call playback, though the duration of that elevated vigilance posture did not differ significantly between the two treatments (Table 4.2).

Context Surrounding Call Production

I documented the context surrounding 104 Richardson's ground squirrel alarm calls, including 87 pure audible calls, 10 pure whisper calls, and seven calls that began as audible but became ultrasonic during the call (mixed call). When mixed calls were excluded from the analysis, squirrels were significantly more likely to produce ultrasonic calls than audible calls at greater distances from the observer (9.10 ± 1.09 m versus 6.99 ± 0.24 m, mean \pm SE, Table 4.3). When mixed calls were included as ultrasonic calls in the analysis, greater distances of both the observer (9.02 ± 0.84 m versus 6.99 ± 0.24 m, mean \pm SE) and the hat (4.70 ± 0.75 m versus 2.93 ± 0.17 m) from the subject significantly increased the probability of that subject producing an ultrasonic alarm call (Table 4.4). Time of day (Tables 4.3 and 4.4, all $P > 0.5$) and sex and age of the receiver (Fisher's

exact test, all $P > 0.05$) had no effect on call-type in either analysis. When mixed calls were included in the analysis, however, the day within year had a significant influence on the type of call produced (audible call: 185.2 ± 1.6 days, mean \pm SE; whisper call: 192.6 ± 2.4 days; Table 4.4).

DISCUSSION

The results of my playback experiment demonstrate that juvenile Richardson's ground squirrels that have recently emerged from their natal burrow, but which have not yet dispersed into the broader population, respond with increased vigilance to ultrasonic alarm calls. This, together with my earlier finding that the period in which adult squirrels produce whisper calls includes juvenile emergence (see chapter 2), is consistent with parental investment and, in the broader sense, kin selection as the evolutionary impetus for producing whisper calls (Hamilton 1964; Maynard Smith 1965). Newly emerging juvenile squirrels, which are numerous, highly susceptible to predation, and densely concentrated around the natal burrow, represent the reproductive success of parents and siblings (Drickamer et al. 1996). By issuing short-range whisper calls instead of long-range audible calls, it is possible for signalers to both reduce the probability of attracting predators to the natal area (Smith 1979b) and provide kin with a relative survival advantage over non-kin (Wilson 2004).

These results do not, however, refute the potential contribution of reciprocal altruism to the evolution of alarm signals in general, nor do they imply that whisper calls are produced only in the presence of kin. Rather, I suggest that

reciprocal altruism contributes more substantially to the evolution and maintenance of audible calls than whisper calls. If the benefits of alarm calling are provided strictly via kin selection, the production of audible calls would be unnecessary, as a caller could selectively warn the majority of its kin with low-risk whisper calls. Yet, of the 104 alarm calls examined in the production component of this study, only 10 were ultrasonic, suggesting that despite the associated risk of predation (Yasukawa 1989), audible calling yields a greater net fitness payoff in most of the situations examined in this study. Given that the cost associated with whisper calling is almost certainly less than that of audible calling (Smith 1979b), the benefits of audible calling must typically exceed those provided by whisper calling. Since both call-types presumably warn kin, it is likely that the additional benefits of audible calling are derived via reciprocal altruism (Trivers 1971) or, perhaps, sexual selection (Zahavi 1975) or even social status (Zahavi 1995).

The persistence of multiple signaling strategies can best be explained if the fitness payoff of each strategy is context-dependent (Maynard Smith 1976, 1978; Abrams 1983); if the costs and benefits of signaling were fixed, the superior strategy would always replace the inferior strategy over time. My results demonstrate that the tendency of Richardson's ground squirrels to produce whisper calls is directly related to the distance between the caller and predator. This supports my hypothesis that whisper calling serves as an alternative signaling strategy in situations where predators are likely to overlook silent individuals (see Maynard Smith 1965; Klump & Shalter 1984). The effect of day

within year on the type of call produced in this study, however, may better reflect developmental delays in juvenile whisper call production than context-dependent signaling, as the risk of predation associated with calling should not increase throughout the year (Sherman 1977). However, further investigation into the development of call production in juveniles is necessary. Another potential factor affecting strategy selection is the presence or absence of kin within the active space of a whisper call. Richardson's ground squirrels are known to discriminate kin from non-kin (Hare 1998b) and audience effects on alarm calling have been documented in several species (Evans & Marler 1991; Striedter et al. 2003). However, I did not examine the potential effects of kin presence in this study.

By integrating information about predator distance (Warkentin et al. 2001) and the presence of nearby kin (Hare 1998b), callers could use exact information (but see Koops & Abrahams 1998) or simple rules of thumb (Bouskila & Blumstein 1992) to select the optimal strategy for a given situation (Maynard Smith 1978; Abrams 1983). A signaler's strategy could even be adaptable to situational changes, such as shifts in predator location, if the caller continually assesses the situation (e.g. Wilson & Hare 2003) and applies some form of information updating (Owings & Hennessy 1984; Bradbury & Vehrencamp 1998). Although this study does not ascertain the complete ecological context associated with whisper calling, it appears that whisper calls enhance the survival of vulnerable young and reduce the probability that distant predators will be attracted to the natal area.

REFERENCES

- Abrams, P.** 1983. Life-history strategies of optimal foragers. *Theoretical and Population Biology*, **24**, 22-38.
- Axelrod, R. & Hamilton, W.D.** 1981. The evolution of cooperation. *Science*, **211**, 1390-1396.
- Blumstein, D.T. & Daniel, J.C.** 2004. Yellow-bellied marmots discriminate between the alarm calls of individuals and are more responsive to calls from juveniles. *Animal Behaviour*, **68**, 1257-1265.
- Blumstein, D.T., Steinmetz, J., Armitage, K.B. & Daniel, J.C.** 1997. Alarm calling in yellow-bellied marmots: II. The importance of direct fitness. *Animal Behaviour*, **53**, 173-184.
- Blumstein, D.T., Verneyre, L. & Daniel, J.C.** 2004. Reliability and the adaptive utility of discrimination among alarm callers. *Proceedings in the Royal Society of London B*, **271**, 1851-1857.
- Bouskila, A. & Blumstein, D.T.** 1992. Rules of thumb for predation hazard assessment: Predictions from a dynamic model. *American Naturalist*, **139**, 161-176.
- Bradbury, J.W. & Vehrencamp, S.L.** 1998. *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Caro, T.M.** 1995. Pursuit-deterrence revisited. *Trends in Ecology and Evolution*, **10**, 500-503.
- Charnov, E.L. & Krebs, J.R.** 1975. The evolution of alarm calls: altruism or manipulation? *American Naturalist*, **109**, 107-112.

- Cheney, D.L. & Seyfarth, R.M.** 1988. Assessment of meaning and the detection of unreliable signals by vervet monkeys. *Animal Behaviour*, **36**, 477-486.
- Cresswell, W., Lind, J., Kaby, U., Quinn, L. & Jakobsson, S.** 2003. Does an opportunistic predator preferentially attack nonvigilant prey? *Animal Behaviour*, **66**, 643-648.
- Davis, L.S.** 1984. Alarm calling in Richardson's ground squirrels (*Spermophilus richardsonii*). *Zeitschrift für Tierpsychologie*, **66**, 152-164.
- Drickamer, L.C., Vessey, S.H. & Meikle, D.** 1996. *Animal Behaviour*, fourth edition. Wm. C. Brown Publishers, Chicago.
- Dunford, C.** 1977. Kin selection for ground squirrel alarm calls. *American Naturalist*, **111**, 782-785.
- East, M.** 1981. Alarm calling and parental investment in the robin *Erithacus rubecula*. *IBIS*, **123**, 223-230.
- Eberhardt, L.S.** 1994. Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *Auk*, **111**, 124-130.
- Evans, L.B., Bass, H.E. & Sutherland, L.C.** 1972. Atmospheric absorption of sound: theoretical predictions. *Journal of the Acoustical Society of America*, **51**, 1565-1575.
- Evans, C.S. & Marler, P.** 1991. On the use of video images as social stimuli in birds: audience effects on alarm calling. *Animal Behaviour*, **41**, 17-26.
- Hamilton, W.D.** 1964. The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology*, **7**, 1-52.

- Hare, J.F.** 1998a. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, discriminate among individual alarm callers. *Animal Behaviour*, **55**, 451-460.
- Hare, J.F.** 1998b. Juvenile Richardson's ground squirrels, *Spermophilus richardsonii*, manifest both littermate and neighbour/stranger discrimination. *Ethology*, **104**, 991-1002.
- Hare, J.F. & Atkins, B.A.** 2001. The squirrel that cried wolf: reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behavioral Ecology and Sociobiology*, **51**, 108-112.
- Hauber, M.E. & Sherman, P.W.** 1998. Nepotism and marmot alarm calling. *Animal Behaviour*, **56**, 1049-1052.
- Klump, G.M. & Shalter, M.D.** 1984. Acoustic behaviour of birds and mammals in the predator context. I. Factors affecting the structure of alarm signals. II. The functional significance and evolution of alarm signals. *Zeitschrift für Tierpsychologie*, **66**, 189-226.
- Koops, M.A. & Abrahams, M.V.** 1998. Life history and the fitness consequences of imperfect information. *Evolutionary Ecology*, **12**, 601-613.
- Lawrence, B.D. & Simmons, J.A.** 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *Journal of the Acoustical Society of America*, **71**, 585-590.
- Macedonia, J.M. & Evans, C.S.** 1993. Variation among mammalian alarm call systems and the problem of meaning in animal signals. *Ethology*, **93**, 177-197.

- Machlis, L., Dodd, P.W.D. & Fentress, J.C.** 1985. The pooling fallacy: problems arising when individuals contribute more than one observation to the data set. *Zeitschrift für Tierpsychologie*, **68**, 201-214.
- Marler, P.** 1955. Characteristics of some animal calls. *Nature*, **176**, 6-8.
- Mateo, J.M.** 1996. The development of alarm-call response behaviour in free-living juvenile Belding's ground squirrels. *Animal Behaviour*, **52**, 489-505.
- Maynard Smith, J.** 1965. The evolution of alarm calls. *American Naturalist*, **99**, 59-63.
- Maynard Smith, J.** 1976. Evolution and the theory of games. *American Scientist*, **64**, 41-45.
- Maynard Smith, J.** 1978. Optimization theory in evolution. *Annual Review of Ecology and Systematics*, **9**, 31-56.
- Michener, G.R.** 1983. Kin identification, matriarchies, and the evolution of sociality in ground-dwelling Sciurids. In: *Advances in the Study of Mammalian Behavior* (Ed. by J.F. Eisenberg and D.G. Kleiman), pp. 528-572, The American Society of Mammalogists, Pennsylvania.
- Michener, G.R. & Koepl, J.W.** 1985. *Spermophilus richardsonii*. *Mammalian Species*, **243**, 1-8.
- Michener, G.R. & Michener, D.R.** 1973. Spatial distribution of yearlings in a Richardson's ground squirrel population. *Ecology*, **54**, 1138-1142.
- Owings, D.H. & Hennessy, D.F.** 1984. The importance of variation in Sciurid visual and vocal communication. In: *The Biology of Ground-dwelling*

Squirrels (Ed. by J.O. Murie and G.R. Michener), pp. 169-200, University of Nebraska Press, Nebraska.

Pulliam, H.R. 1973. On the advantage of flocking. *Journal of Theoretical Biology*, **38**, 419-422.

Sales, G. & Pye, D. 1974. *Ultrasonic Communication by Animals*. Chapman and Hall, London.

Shelley, E.L. & Blumstein, D.T. 2005. The evolution of vocal alarm communication in rodents. *Behavioral Ecology*, **16**, 169-177.

Sherman, P.W. 1977. Nepotism and the evolution of alarm calls. *Science*, **197**, 1246-1253.

Sloan, J.L., Wilson, D.R. & Hare, J.F. In press. Functional morphology of Richardson's ground squirrel (*Spermophilus richardsonii*) alarm calls: the meaning of chirps, whistles and chucks. *Animal Behaviour*.

Smith, J.C. 1979a. Factors affecting the transmission of rodent ultrasounds in natural environments. *American Zoologist*, **19**, 432-442.

Smith, W.J. 1979b. The study of ultrasonic communication. *American Zoologist*, **19**, 531-538.

Smythe, N. 1970. On the existence of "pursuit invitation" signals in mammals. *American Naturalist*, **104**, 491-494.

Striedter, G.F., Freibott, L., Hile, A.G. & Burley, N.T. 2003. For whom the male calls: an effect of audience on contact call rate and repertoire in budgerigars, *Melopsittacus undulatus*. *Animal Behaviour*, **65**, 875-882.

- Tarpy, D.R., Gilley, D.C. & Seeley, T.D.** 2004. Levels of selection in a social insect: a review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement. *Behavioral Ecology and Sociobiology*, **55**, 513-523.
- Trivers, R.L.** 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology*, **46**, 35-57.
- Warkentin, K.J., Keeley, A.T.H. & Hare, J.F.** 2001. Repetitive calls of juvenile Richardson's ground squirrels (*Spermophilus richardsonii*) communicate response urgency. *Canadian Journal of Zoology*, **79**, 569-573.
- Wilson, D.S.** 1997. Introduction: multilevel selection theory comes of age. *American Naturalist*, **150** (supplement), 1-4.
- Wilson, D.S.** 2001. Evolutionary biology: struggling to escape exclusively individual selection. *Quarterly Review of Biology*, **76**, 199-205.
- Wilson, D.S.** 2004. What is wrong with absolute individual fitness? *Trends in Ecology and Evolution*, **19**, 245-248.
- Wilson, D.R. & Hare, J.F.** 2003. Richardson's ground squirrels (*Spermophilus richardsonii*) do not communicate predator movements via changes in call rate. *Canadian Journal of Zoology*, **81**, 2026-2031.
- Wilson, D.R. & Hare, J.F.** 2004. Ground squirrel uses ultrasonic alarms. *Nature*, **430**, 523.
- Woodland, D.J., Jaafar, Z. & Knight, M.** 1980 The "pursuit deterrent" function of alarm signals. *American Naturalist*, **115**, 748-753.

- Yasukawa, K.** 1989. Costs and benefits of a vocal signal: The nest-associated 'Chit' of the female red-winged blackbird, *Agelaius phoeniceus*. *Animal Behaviour*, **38**, 866-874.
- Ydenberg, R.C. & Dill, L.M.** 1986. The economics of fleeing from predators. *Advances in the Study of Behavior*, **16**, 229-249.
- Zahavi, A.** 1975. Mate selection - a selection for a handicap. *Journal of Theoretical Biology*, **53**, 205-214.
- Zahavi, A.** 1995. Altruism as a handicap - the limitations of kin selection and reciprocity. *Journal of Avian Biology*, **26**, 1-3.

- ∞ Table 4.1. Number of Richardson's ground squirrel littermates (from 11 different litters) visible before, during, and after the playback of ultrasonic whisper calls and background noise controls.

Parameter	<u>Whisper call</u>			<u>Background noise</u>		
	before	during	after	before	during	after
Minimum	3	3	2	3	3	3
Maximum	5	5	5	5	5	5
Mean	3.91	3.91	3.64	3.82	3.82	3.82
Standard error	0.25	0.25	0.31	0.18	0.18	0.18

Table 4.2. Behavioural responses of 11 Richardson's ground squirrel litters to playback of whisper calls and background noise controls.

Variable	Whisper	Noise	Difference (SE)	<i>P</i>
Playback vigilance (%)	18.1	6.6	11.5 (4.8)	0.04
Post-playback vigilance (%)	11.4	-2.7	14.1 (7.6)	0.09
Initial response (1 - 3)	1.12	0.96	0.16 (0.07)	0.04
Duration of initial (s)	10.25	10.54	-0.29 (2.86)	0.92

Mean percentages for vigilance are corrected for pre-playback behaviour (i.e. negative values indicate greater vigilance in the pre-playback period).

∞ Table 4.3. Influence of contextual variables on the tendency of Richardson's ground squirrels to produce audible (zero) vs. ultrasonic (one) alarm calls (mixed calls excluded from analysis).

Variable (x)	Logit(P) =	Chi-Square	<i>P</i>	Correct Predictions	Effect Size (<i>R</i> ²)
Time	-0.95 - 0.13x	0.17	0.68	90%	0.00
Date	-4.12 + 0.01x	0.20	0.66	90%	0.00
Subject-hat	-3.31 + 0.34x	3.31	0.07	90%	0.05
Subject-observer	-4.47 + 0.28x	5.10	0.02	91%	0.09

Sample size = 97, including 87 pure audible calls and 10 pure ultrasonic calls.

Table 4.4. Influence of contextual variables on the tendency of Richardson's ground squirrels to produce audible (zero) vs. ultrasonic (one) alarm calls (mixed calls included in analysis).

Variable (x)	Logit(P) =	Chi-Square	P	Correct Predictions	Effect Size (R^2)
Time	-0.18 - 0.16x	0.37	0.54	84%	0.00
Date	-8.69 + 0.04x	3.89	0.05	84%	0.04
Subject-hat	-3.08 + 0.40x	9.65	0.00	84%	0.10
Subject-observer	-3.82 + 0.27x	7.57	0.01	85%	0.09

Sample size = 104, including 87 pure audible calls and 17 ultrasonic calls (10 pure ultrasonic and seven mixed calls).

Chapter 5: General conclusions and future directions

Richardson's ground squirrels (*Spermophilus richardsonii*) produce ultrasonic (ca. 48 kHz) alarm signals, or whisper calls, that warn conspecifics of potential danger (see chapter 2). Owing to the rapid attenuation and highly directional nature of ultrasound (Smith 1979a), whisper calls have a very limited active space and are thus capable of warning only those individuals located in close proximity to the caller. As this is where a caller's kin typically reside (Michener & Michener 1973), it is possible that whisper calling is maintained, at least in part, via kin selection (Hamilton 1964) or parental investment (Blumstein et al. 1997). Consistent with that notion, my results demonstrate that one of the many possible contexts in which whisper calls function is during juvenile emergence (chapter 4), when kin are numerous, spatially clustered, and highly vulnerable to predation (Michener & Michener 1973; Blumstein et al. 1997).

Given the furtive nature of ultrasound (Smith 1979a, 1979b), however, it is also possible that whisper calling persists as a means by which callers can warn nearby conspecifics of danger without attracting distant predator attention. Consistent with that 'covert signaling hypothesis,' squirrels were more likely to produce ultrasonic as opposed to audible alarm calls when a potential predator was located farther away (chapter 4). Further, squirrels responded in a less conspicuous fashion to whisper as opposed to audible alarm calls (chapter 2), supporting the idea that whisper calling reduces the probability of calling squirrels being noticed by distant predators that are otherwise likely to overlook them.

The ability of small mammals to localize sound improves at higher frequencies and, so, may also contribute to the evolution of ultrasonic signals (Masterton et al. 1969). Whisper calls contain frequencies that extend well into the ultrasonic range (see table 2.1), which, due to the rapid attenuation and highly directional nature of high-frequency sound, provides receivers with binaural disparities in sound pressure level. Because receivers are capable of detecting frequencies at least as high 40 kHz (chapter 3), those disparities may serve as cues about the signaler's location (Masterton et al. 1969; Bradbury & Vehrencamp 1998). By localizing the signaler, listeners could potentially gain valuable information about the call-eliciting stimulus, allowing squirrels to respond to the situation in the most appropriate fashion (Sloan et al. in press).

Although it is clear that the active range of whisper calls is limited, the maximum distance over which conspecific receivers can detect whisper calls, and the minimum distance beyond which various predators cannot detect whisper calls, remains unknown. A logical next step then is to replay whisper calls in the field and measure the rate of signal attenuation at various angular deviations from the source. By integrating information about the auditory sensitivity (with respect to amplitude) of squirrels and their predators to the ultrasonic frequencies contained in whisper calls, it would be possible to construct a spatial representation of the physical limitations of signal propagation. Future research could also focus on elucidating the adaptive utility of this short-range alarm signal. For example, does the presence of kin within the active space of a whisper call, the relative safety of the signaler with respect to its

posture (i.e. conspicuousness) or its distance from an escape burrow, or the type of predator posing the threat (e.g. avian versus terrestrial) influence a caller's signaling strategy? Are signal recipients capable of localizing the caller? Although their complete function remains unclear, my results demonstrate unambiguously that whisper calls serve as anti-predator alarm signals that warn conspecifics of potential predators.

REFERENCES

- Blumstein, D.T., Steinmetz, J., Armitage, K.B. & Daniel, J.C.** 1997. Alarm calling in yellow-bellied marmots: II. The importance of direct fitness. *Animal Behaviour*, **53**, 173-184.
- Bradbury, J.W. & Vehrencamp, S.L.** 1998. *Principles of Animal Communication*. Sinauer Associates Inc., Sunderland, MA.
- Hamilton, W.D.** 1964. The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology*, **7**, 1-52.
- Masterton, B., Heffner, H. & Ravizza, R.** 1969. The evolution of hearing. *Journal of the Acoustical Society of America*, **45**, 966-985.
- Michener, G.R. & Michener, D.R.** 1973. Spatial distribution of yearlings in a Richardson's ground squirrel population. *Ecology*, **54**, 1138-1142.
- Sloan, J.L., Wilson, D.R. & Hare, J.F.** In press. Functional morphology of Richardson's ground squirrel (*Spermophilus richardsonii*) alarm calls: the meaning of chirps, whistles and chucks. *Animal Behaviour*.

Smith, J.C. 1979a. Factors affecting the transmission of rodent ultrasounds in natural environments. *American Zoologist*, **19**, 432-442.

Smith, W.J. 1979b. The study of ultrasonic communication. *American Zoologist*, **19**, 531-538.

Appendix A: Whisper call parameters and miscellaneous and environmental grouping factors affecting responses of ground squirrels to ultrasonic signals.

Influence of Whisper Call Parameters on Behavioural Responses

Whisper call parameters, including the fundamental frequency of the primary syllable (27.2 - 62.8 kHz), amplitude of both the original call recording (57.7 - 83.7 dB SPL at a mean (\pm SE) distance from the microphone of 0.49 ± 0.02 m) and the amplified call playback (74 - 82 dB SPL at 1 m from the speaker), caller sex, and caller age (juvenile/adult), were examined for their potential role as independent variables. Behavioural responses of juvenile ground squirrels were not influenced by any of the structural properties of whisper calls (regression analysis: all $P > 0.1$, Tables A1 - A3), though male callers did elicit a significantly greater initial response from listeners than did female callers (unpaired t test: $t_{15} = 2.18$, $P = 0.05$, Table A4). Caller age had no effect on behavioural responses (unpaired t tests: all $P > 0.25$, Table A5).

Autocorrelation of Miscellaneous and Environmental Grouping Factors

I examined autocorrelations among the environmental and miscellaneous grouping factors associated with playbacks, including day within year (198 - 230), time of day (0715 - 2033 h), wind speed (0 - 14.5 km/hr), relative humidity (26 - 83%), cloud cover (0 - 100%), temperature (13.3 - 30.0 °C), distance between speaker and squirrel (1.9 - 8.6 m), and speaker angle relative to squirrel

(0 - 40°), by constructing a correlation matrix (all treatments combined, Table A6). Correlations were described where $P \leq 0.05$ (Spearman rank correlation: $N = 75$).

The distance between the subject and speaker and the speaker angle relative to the subject were not significantly correlated with any of the environmental grouping factors or with each other (Spearman rank correlation: all $P > 0.1$). Environmental grouping factors were correlated in a predictable fashion: cloud cover and temperature increased with increasing time of day, while humidity decreased with increasing temperature and time of day (Spearman rank correlation: all $P < 0.05$).

Influence of Grouping Factors on Behavioural Responses

Unpaired t tests were used to test if the environmental and miscellaneous grouping factors were balanced across treatments, as potential effects of those factors will not confound the analysis of the dependent variables of interest when balanced. Given the potential role of ultrasound as a short-range signal, however, I also regressed behavioural responses to whisper calls on the distance between the subject and the speaker (1.9 - 8.6 m), though this distance did not significantly influence any of the dependent variables of interest (regression analysis: all $P > 0.15$, Table A7). All environmental and miscellaneous grouping factors examined were equally balanced across the whisper call and background noise treatments (Table A8). Time of day, relative humidity, cloud cover, and temperature, however, were not balanced between the whisper call and pure

tone treatments (all $P < 0.05$, Table A8). Similarly, time of day, wind speed, humidity, and temperature, were not balanced between the whisper call and the audible call treatments (all $P < 0.05$, Table A8).

Where miscellaneous grouping factors were imbalanced across treatments, I examined potential autocorrelations between those factors (whisper call - control call) and the dependent variables of interest (whisper call - control call). An unbiased interpretation of treatment effects is possible when behavioural differences between those treatments are not correlated with any of the unbalanced factors. The only significant correlation found was between relative humidity and total post-playback vigilance in the whisper call vs. audible call comparison (Spearman rank correlation: $r_s = 0.562$, $N = 19$, $P = 0.02$). ANCOVA could not be performed due to the paired nature of the data and, thus, the analysis of treatment effect (whisper call vs. audible call) on post-playback vigilance should be interpreted with caution.

Table A1. The influence of the fundamental frequency of whisper calls on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study.

Dependent Variable (x)	Regression	R^2	t	P
Initial response	$1.97 - 0.03x$	0.13	-1.56	0.14
Duration of initial response	$32.01 - 0.48x$	0.11	-1.42	0.17
Playback vigilance	$-0.23 + 0.01x$	0.07	1.09	0.29
Post-playback vigilance	$-0.053 + 0.01x$	0.04	0.81	0.43

Note: Playback and post-playback vigilance values are corrected for pre-playback vigilance behaviour.

8 Table A2. The influence of the sound pressure level of the original whisper call recordings (mean distance between subject and microphone (\pm SE): 0.49 ± 0.02 m) on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study.

Dependent Variable (x)	Regression	R^2	t	P
Initial response	$0.06 + 0.01x$	0.01	0.39	0.70
Duration of initial response	$-18.72 + 0.42x$	0.07	1.09	0.29
Playback vigilance	$-0.79 + 0.02x$	0.13	1.57	0.13
Post-playback vigilance	$-0.42 + 0.01x$	0.08	1.22	0.24

Note: Playback and post-playback vigilance values are corrected for pre-playback vigilance behaviour.

Table A3. The influence of the playback amplitude of whisper calls (dB SPL measured at 1 m from source) on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) in the 'ground squirrels use ultrasonic alarm signals' study.

Dependent Variable (x)	Regression	R^2	t	P
Initial response	1.11 - 0.01x	0.00	-0.07	0.94
Duration of initial response	151.04 - 1.79x	0.08	-1.25	0.23
Playback vigilance	4.97 - 0.06x	0.14	-1.68	0.11
Post-playback vigilance	2.28 - 0.03x	0.04	-0.88	0.39

Note: Playback and post-playback vigilance values are corrected for pre-playback vigilance behaviour.

Table A4. The influence of caller sex on the responses of juvenile Richardson's ground squirrels (mean \pm SE) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study.

Variable	Male ($N = 9$)	Female ($N = 8$)	t	P
Initial response (1 - 4)	1.00 \pm 0.29	0.25 \pm 0.16	2.18	0.05
Duration of initial response (s)	13.37 \pm 6.04	7.58 \pm 3.07	0.82	0.42
Playback vigilance	0.21 \pm 0.15	0.25 \pm 0.11	-0.21	0.84
Post-playback vigilance	0.16 \pm 0.12	0.23 \pm 0.05	-0.51	0.62

Note: Playback and post-playback vigilance are corrected for pre-playback vigilance behaviour. Two observations were excluded from the analysis due to unknown sex.

Table A5. The influence of caller age on the responses of juvenile Richardson's ground squirrels (mean \pm SE) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study.

Variable	Juveniles (<i>N</i> = 11)	Adults (<i>N</i> = 7)	<i>t</i>	<i>P</i>
Initial response (1 - 4)	0.55 \pm 0.16	0.71 \pm 0.42	0.44	0.67
Duration of initial response (s)	9.79 \pm 4.13	10.54 \pm 6.01	0.11	0.92
Playback vigilance	0.25 \pm 0.11	0.16 \pm 0.15	-0.52	0.61
Post-playback vigilance	0.24 \pm 0.09	0.10 \pm 0.07	-1.13	0.27

Note: Playback and post-playback vigilance are corrected for pre-playback vigilance behaviour. One observation was excluded from the analysis due to unknown age.

57 Table A6: Autocorrelation of the environmental and miscellaneous grouping factors during playbacks (all treatments combined, $N = 75$) in the 'ground squirrels use ultrasonic alarm signals' study.

	Day	Time	Wind	Humidity	Cloud	Temperature	Speaker-squirrel	Speaker angle
Day	1.000							
Time	-0.095	1.000						
Wind	-0.055	-0.039	1.000					
Humidity	0.162	-0.596	0.043	1.000				
Cloud	0.019	0.364	0.004	-0.091	1.000			
Temperature	0.155	0.569	-0.182	-0.734	0.204	1.000		
Speaker-squirrel	0.162	-0.028	0.056	0.021	0.042	-0.054	1.000	
Speaker angle	-0.107	-0.014	0.078	0.041	0.044	0.105	-0.088	1.000

Note: one trial removed from background noise control due to audible calling during playback.

- ∞ Table A7. The influence of the distance between subject and speaker on the behavioural responses of juvenile Richardson's ground squirrels ($N = 19$) to whisper calls in the 'ground squirrels use ultrasonic alarm signals' study.

Dependent Variable	Regression	R^2	t	P
Initial response	$-0.17 + 0.16x$	0.10	1.39	0.18
Duration of initial response	$-0.25 + 2.00x$	0.05	0.91	0.38
Playback vigilance	$0.30 - 0.02x$	0.00	-0.25	0.80
Post-playback vigilance	$0.30 - 0.02x$	0.01	-0.38	0.71

Note: Playback and post-playback vigilance values are corrected for pre-playback vigilance behaviour.

Table A8. Distribution of the environmental and miscellaneous grouping factors among treatments (mean \pm SE) in the playback component of the 'ground squirrels use ultrasonic alarm signals' study.

Variable	Whisper	Control	<i>t</i>	<i>P</i>
Noise (N = 18*)				
Day	207.5 \pm 2.1	209.2 \pm 2.7	0.48	0.63
Time	9.1 \pm 0.7	9.9 \pm 0.9	0.70	0.49
Wind	4.4 \pm 0.5	3.8 \pm 0.6	-0.74	0.47
Humidity	62.8 \pm 2.7	62.8 \pm 3.1	-0.01	0.99
Cloud	9.7 \pm 2.2	18.6 \pm 7.2	1.18	0.25
Temperature	19.6 \pm 0.8	21.5 \pm 1.0	1.46	0.15
Speaker - squirrel	4.9 \pm 0.4	4.7 \pm 0.2	-0.49	0.62
Angle	3.6 \pm 1.4	4.2 \pm 2.4	0.20	0.84
Tone (N = 19)				
Day	207.6 \pm 2.0	207.4 \pm 2.0	-0.07	0.94
Time	9.0 \pm 0.7	11.3 \pm 0.8	2.26	0.03
Wind	4.2 \pm 0.5	3.5 \pm 0.5	-1.14	0.26
Humidity	63.2 \pm 2.6	51.5 \pm 3.2	-2.83	0.01
Cloud	9.2 \pm 2.2	45.5 \pm 10.7	3.32	0.00
Temperature	19.5 \pm 0.8	22.8 \pm 1.0	2.60	0.01
Speaker - squirrel	4.9 \pm 0.3	4.5 \pm 0.4	-0.74	0.46
Angle	3.4 \pm 1.3	3.4 \pm 1.5	0.00	1.00
Audible (N = 19)				
Day	207.6 \pm 2.0	207.7 \pm 2.5	0.03	0.97
Time	9.0 \pm 0.7	12.1 \pm 0.8	3.10	0.00
Wind	4.2 \pm 0.5	6.6 \pm 0.8	2.68	0.01
Humidity	63.2 \pm 2.6	51.6 \pm 2.4	-3.31	0.00
Cloud	9.2 \pm 2.2	27.1 \pm 9.3	1.88	0.07
Temperature	19.5 \pm 0.8	23.4 \pm 0.8	3.59	0.00
Speaker - squirrel	4.9 \pm 0.3	5.6 \pm 0.2	1.57	0.13
Angle	3.4 \pm 1.3	6.3 \pm 2.3	1.08	0.29

Bold subheadings refer to which control the whisper call is being compared.

* indicates that one trial was removed from the analysis due to nearby audible calling during playback of the background noise control.