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The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals.

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The adaptive utility of Richardson's ground squirrel (*Spermophilus richardsonii*) short-range ultrasonic alarm signals.

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Abstract: Richardson's ground squirrels (*Spermophilus richardsonii*, Sabine, 1822) produce audible (ca. 8 kHz) and/or ultrasonic (ca. 48 kHz) alarm vocalizations that warn conspecifics of impending danger. Audible calls have a larger active space than ultrasonic calls because they travel farther, are louder, and they contain frequencies to which conspecific and allospecific recipients are more sensitive. In our first experiment, we presented an alarming stimulus to 103 squirrels to examine the effect of threat proximity on signal type. The ratio of ultrasonic to audible alarm calls increased with increasing distance from the stimulus. We conclude that the size of the active space influences signalling strategy and that squirrels emitting ultrasonic calls can signal conspecifics to the exclusion of distant predators. As recipients of ultrasonic calls must be close to the signaler, one context in which ultrasonic calling may be most adaptive is during natal emergence, when juveniles are particularly abundant, highly vulnerable to predation, and clustered in space. In our second experiment, we broadcast ultrasonic alarm signals to emerging juveniles and found that they, like older individuals, respond to calls by increasing vigilance. We discuss the adaptive utility of multiple signalling strategies in light of our findings.

Introduction

Among species that produce antipredator alarm signals, natural selection should favour signals that better inform receivers (but see Charnov and Krebs 1975) and, unless sexual selection underlies signalling (Zahavi 1975), reduce the associated risk of predation (Klump and Shalter 1984). Consistent with this, many signalling systems have been demonstrated not to be reflexive, but rather, sensitive to intrinsic and extrinsic factors, such as the signaler's life history (Abrams 1983), the presence or absence of a suitable audience (Karakashian et al. 1988), or the location of a given predator (Warkentin et al. 2001). The great tit (*Parus major*, Linnaeus, 1758), for example, produces an 8-kHz 'seet' alarm call when its primary predator, the European sparrowhawk (*Accipiter nisus*, Linnaeus, 1758), is distant, but halts signal production when that predator approaches the signal's audible range (Klump and Shalter 1984). Because tits are more sensitive than sparrowhawks at 8 kHz, the audible range of 'seet' calls is much greater for tits than it is for sparrowhawks (31 versus 7 m, respectively), thereby expanding the range over which cryptic alarm communication among tits is possible (Klump et al. 1986).

Richardson’s ground squirrels (*Spermophilus richardsonii*, Sabine, 1822) produce alarm vocalizations that are audible to humans (fundamental frequency: ca. 8 kHz), often repeated for several minutes, and capable of propagating throughout an entire colony (Koeppl et al. 1978; Hare 1998a). Calls convey to conspecifics valuable information (Pulliam 1973) about the identity (Hare 1998a) and reliability (Hare and Atkins 2001) of the signaler, as well as the proximity of the predator (Warkentin et al. 2001; Sloan et al. 2005). In addition to 'audible' calls, squirrels also produce and respond to 'ultrasonic' (fundamental frequency: ca. 48 kHz) alarm signals, though the apparent incidence of ultrasonic signalling, perhaps owing to humans' inability to detect ultrasound (i.e. frequencies > 15 kHz), is lower than that of its audible counterpart (Wilson and Hare 2004). We use the terms 'audible' and 'ultrasonic' to refer only to humans' ability and inability to detect the associated frequencies; we do not imply that squirrels or their predators are capable of hearing audible, but not ultrasonic, signals. Indeed, some Richardson's ground squirrel predators (reviewed in Michener and Koeppl 1985), such as the coyote (*Canis latrans*, Say, 1823), domestic dog (*C. familiaris*, Linnaeus, 1758), red fox (*Vulpes vulpes*, Linnaeus, 1758), and domestic cat (*Felis catus*, Linnaeus, 1758), are capable of detecting frequencies at least as high as 48 kHz, while others, such as humans, buteos, harriers, falcons, eagles, and owls, are insensitive to frequencies exceeding 15 kHz (Peterson et al. 1969, Klump et al. 1986, Sales and Pye 1974).

Although the structure of audible and ultrasonic alarm signals appears very similar (see figures 1 and 2 in Sloan et al. 2005 and Wilson and Hare 2004, respectively), their fundamental frequencies are highly disparate (8 versus 48 kHz). Atmospheric attenuation of sound is frequency-dependent and the initial amplitude of audible calls (84 - 91 dB SPL at 1 m from source; Hare 1998a) is higher than that of ultrasonic calls (58 - 84 dB SPL at 0.5 m from source; Wilson and Hare 2004). Thus, audible calls should always be louder than ultrasonic calls and that difference should be most pronounced at greater distances from the caller (Smith 1979a; Lawrence and Simmons 1982). Furthermore, hearing studies conducted on ground squirrels and ground squirrel predators have consistently demonstrated that those species sensitive to 48 kHz are even more sensitive to 8 kHz (Peterson et al. 1969, 1974; Hamill et al. 1989). The differences in the physical properties of the two signals and in the capacities of receivers to detect those signals together suggest that the ecologically-relevant space, or active space, of audible calls is larger than that of ultrasonic calls. In this study, we conducted two experiments that examine the adaptive utility of short-range ultrasonic alarm signals: the first, addressing the production of ultrasonic versus audible calls, and the second, the perception of ultrasonic calls by newly emerged juveniles.

The production of short-range ultrasonic as opposed to long-range audible alarm calls may depend on several non-mutually-exclusive factors. The presence or absence of offspring, non-descendent kin, or unrelated conspecifics within the active space of each potential signal may affect which signal type is ultimately produced, particularly if callers accrue benefits via parental investment (Blumstein et al. 1997), kin selection (Hamilton 1964), or reciprocal altruism (Trivers 1971). Although referential signalling (Seyfarth et al. 1980) has not previously been demonstrated in Richardson's ground squirrels (Sloan et al. 2005), it is possible that predator type, categorized according to the predator's sensitivity to high frequency sound, may also affect signal type. Issuing calls to which a nearby predator is insensitive reduces the probability of attracting that predator's attention (Yasukawa 1989). The probability that a predator has detected a potential caller may also influence signalling behaviour. Remaining cryptic by emitting short-range ultrasonic calls when the probability of having been detected is low (Klump et al. 1986) or pronouncing one's vigilance by producing conspicuous long-range audible calls when the probability of having been detected is high (i.e. pursuit-deterrent signalling: Woodland et al. 1980; Shelley and Blumstein 2005) may be optimal strategies in their respective situations. As squirrels are capable of gauging the proximity of a threat (Warkentin et al. 2001), it is also possible that signal type depends on the location of the predator relative to the caller. By issuing short-range ultrasonic calls when a predator is beyond the active space of ultrasonic, but not audible, alarm calls, squirrels may be able to signal nearby conspecifics to the exclusion of the presumptive predator. In our first experiment, we explored the relationship between threat proximity and a caller's tendency to produce audible versus ultrasonic alarm signals.

Studies of parental investment have demonstrated that parents are most likely to produce alarm signals when offspring are very young and 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). Among ground squirrels, descendent kin are most abundant, most vulnerable to predation, and have the most to gain from being warned immediately following their initial emergence from the natal burrow (Michener and Koeppl 1985; Mateo 1996; Blumstein et al. 1997). As ultrasonic calls have a limited active space (Lawrence and Simmons 1982), this critical period when kin are clustered in space may also be one of the few situations in which short-range ultrasonic signals reliably reach conspecifics. Although the period in which adults produce ultrasonic alarm signals includes natal emergence (Wilson and Hare 2004), it remains unknown whether newly emergent juveniles that have yet to disperse are capable of responding appropriately to those calls. Mateo (1996) demonstrated that juvenile Belding's ground squirrels (*S. beldingi*, Merriam, 1888) emerge from the natal burrow incapable of discriminating alarm signals from non-threatening environmental noise, and only develop the capacity to recognize alarm calls by their fifth day above ground when they are beginning to venture farther from the natal burrow. In our second experiment, we tested whether spatially clustered juvenile ground squirrels during their initial five days above ground are capable of responding appropriately and selectively to the playback of short-range ultrasonic alarm signals.

Materials and methods

Effect of threat proximity on call production

We examined the effect of threat proximity on the production of alarm calls by 103 free-living juvenile (*N* = 92; ranging between 22 and 70 days post-emergence) and adult (*N* = 11) Richardson's ground squirrels (for detailed methods see Hare 1998a; Hare and Atkins 2001; Warkentin et al. 2001). Subjects occupied cattle pastures near Oak Lake Provincial Recreation Park, Manitoba (49º41' N, 100º43' W), between 1994 and 1996, near Brandon, Manitoba (49º47' N, 99º59' W), in 1997, and Delta Marsh, Manitoba (50º03' N, 98º20' W), in 1998. All squirrels were given unique dye marks (Clairol Hydrience 52, Black Pearl, Stamford, Connecticut, U.S.A.) on their dorsal pelage to facilitate identification during trials, as well as metal ear tags (National Band and Tag Company no. 1005, Newport, Kentucky, U.S.A.) to allow identification of individuals from one year to the next.

Previously untested squirrels were approached to a distance of approximately 15 m and the recording apparatus, including a Sony TCD-D7 recorder (48 kHz sampling rate) and either a Dan Gibson EPM P-650 parabolic microphone (1994 - 1996; frequency response: 150 - 20,000 Hz) or an Audio-Technica AT815b condenser microphone (1997 - 1998; frequency response: 40 - 20,000 Hz) mounted atop a Vivitar tripod, was set up. During this time, subjects often ran to nearby burrows, thereby expanding the range of subject-observer distances to between 3 and 17 m. 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 call-eliciting stimulus was tossed like a Frisbee^{TM} from hip level to within 0.4 - 14.4 m of the subject. The hat was thrown 0 - 30º from a line connecting the subject and the observer, but was never thrown directly above the subject. This method allowed us to control and accurately measure the distance between the subject and the stimulus because in all cases calling did not commence until after the hat had settled on the ground. Furthermore, the hat method reliably evoked antipredator responses that were qualitatively similar to those observed in response to natural predators, including heightened vigilance, escape to burrows, and the production of alarm calls, but without the potentially confounding effects associated with uncontrolled natural predator encounters (MacWhirter 1992). Only one subject was recorded during each hat presentation to avoid potential problems associated with the possible non-independence among individuals' responses (Machlis et al. 1985). All recordings were made by J.F.H. while wearing the same outer clothing and throwing style was kept constant across trials and years, thereby minimizing any potentially confounding effects imposed by the observer (Slobodchikoff et al. 1991). Recording sessions were discontinued when wind speed reached approximately 10 km/h.

Following each hat presentation, the subject's response was categorized as 'audible,' 'ultrasonic,' or, if it contained a mixture of audible and ultrasonic syllables, 'mixed.' Calling was defined visually as the sudden expansion of the thoracic cavity concurrent with opening of the mouth, while call type was defined acoustically by the presence and/or absence of audible sound (confirmed by later inspecting the audio recordings) associated with calling (excluding the faint sounds of rushing air associated with ultrasonic calling, Wilson and Hare 2004). The locations (accurate to nearest 0.5 m) of the subject, hat, and observer at the onset of calling were recorded relative to a 10 x 10 m Cartesian coordinate grid constructed on the site with wire-pin flags, and distances of the subject to both the hat and observer were calculated using the Pythagorean theorem.

A multinomial (three-state dependent variable, coded using 'reference cell' coding as: audible call = 0, mixed call = 1, ultrasonic call = 2) logistic regression model (Hosmer and Lemeshow 2000) was used to test for the possible effect of stimulus distance (independent variable) on the tendency of callers to produce each call-type. However, because the distance between the caller and hat was highly correlated with the distance between the caller and observer (Spearman rank correlation:: $r_s = 0.474$, $N = 103$, $p < 0.0001$), we limited our definition of the call-eliciting stimulus to the combined effects of the hat and observer. The effect of the combined call-eliciting stimulus on call-type was then examined using a multivariable (independent variables: subject-hat and residuals of subject-observer when regressed against subject-hat) multinomial logistic regression model, which accounts for all variation contained within the combined stimulus and avoids problems associated with collinearity (Hosmer and Lemeshow 2000). Miscellaneous variables, including time of day (0735 - 1410 hours CST), age (coded as: adult = 0, juvenile = 1), juvenile age (22 - 70 days; measured as the number of days following the intial emergence from the natal burrow), and sex (coded as: female = 0, male = 1) of the subject were also tested for any possible effect on call-type using a univariable multinomial logistic regression model. For all analyses, overall model fit was tested for significance using the logistic likelihood ratio test (Hosmer and Lemeshow 2000), which tests the null hypothesis that no linear relationship exists between the logit and the independent variables. The test statistic (*G*) is approximately distributed as chi-square and is calculated by subtracting the log-likelihood of the final model from that of the model containing only the intercept, and then multiplying the resulting value by -2. Odds ratios are calculated for mixed and ultrasonic calls relative to audible calls (reference group), and are considered statistically significant where their 95% confidence intervals exclude the value one (see Hosmer and Lemeshow 2000). Analyses were performed on StatView® 5.0.1 on a Macintosh computer and were considered statistically significant where $p \leq 0.05$ (all tests were two-tailed).

Playbacks

We conducted a playback experiment on 13 emerging litters of free-living juvenile Richardson's ground squirrels at Assiniboine Park, Winnipeg, Manitoba (49.874º N, 97.243º W) between 21 and 28 May 2004. This location proved ideal for broadcasting ultrasound because of the wind relief provided by the surrounding trees and buildings. Litters emerging from their natal burrows were located by scanning burrow entrances, which was facilitated by periodic mowing of the grass by Park staff. Squirrels were not trapped and marked for identification because of daily time constraints imposed by working at the park and because trials needed to be completed within five days of initial juvenile emergence. Litters remained individually distinct, however, because emerging squirrels remain spatially clustered around their natal burrow and do not begin interacting with other litters until approximately two weeks post-emergence (Michener and Koeppl 1985). We wore the same outer clothes each day to promote habituation of squirrels to our presence and to minimize inconsistencies among trials (Slobodchikoff et al. 1991).

Upon identification of a newly emerging litter that had not previously been tested, we observed the litter and the surrounding area through binoculars for approximately 30 minutes. Litters were only considered suitable for testing 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 us to discriminate among litters. During this time, we also counted the minimum number of unique individuals observed, approximating the number of individuals comprising the litter. We then chased the litter into its burrow and set-up the playback apparatus, which included an ultrasound amplifier (Ultra Sound Advice, model S55) and a Portable Ultrasound Processor (PUSP: Ultra Sound Advice), 7 - 11 m from the burrow's entrance. An ultrasound loudspeaker (Ultra Sound Advice, model S56; frequency response: 18 - 200 kHz) concealed within an empty speaker box (19 x 15 x 33 cm) was connected to the amplifier and placed on the ground facing the burrow 3 - 5 m from 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, we selected the playback treatment by tossing a coin and transferred an appropriate time-expanded call from minidisc to the PUSP using the appropriate time-compression ratio.

A total of 26 playbacks representing two treatments were constructed on Canary™ using ultrasonic alarm calls recorded from 13 different ground squirrels (four adult males; three adult females; three juvenile males; three juvenile females) at Assiniboine Park in 2003 (for detailed recording methods see Wilson and Hare 2004). Calls were recorded using the PUSP (224 kHz sampling rate, manual triggering) and a bat detector (model U30; frequency response:

15 - 180,000 Hz) from Ultra Sound Advice. From each of those 13 callers, we selected the syllable with the highest signal-to-noise ratio (see signal parameters in Table 1) and used it to construct one test playback, which contained three repetitions of the selected syllable separated by 4-s intersyllable silences. This bout structure is typical of Richardson's ground squirrel alarm calls (Sloan and Hare 2004). For each test playback, we also constructed a matching control playback that contained a noise pulse in place of the three alarm syllables. Noise pulses were derived from the 13 original recordings and consisted of the white and environmental noise recorded immediately prior to the alarm syllable they were replacing, edited to have the same frequency and temporal limits as that alarm call. Each litter was then assigned a different matched pair of playbacks to be broadcast in random order, at a natural amplitude (74 - 82 dB SPL at 1 m from the speaker). Calls were assigned such that the 2003 callers (identified by permanent metal ear tags, National Band and Tag Company no. 1005, Newport, Kentucky, U.S.A.) were not located in the same general vicinity as their 2004 call recipients, and, in the case of female callers, that calls were not broadcast to their offspring.

We ensured that our recording and playback equipment introduced no artifacts into the 26 playback stimuli by: 1) broadcasting calls from our playback system, 2) recording those calls with a Racal Store 4DS high-frequency tape recorder (tape speed: 76.2 cm/s) and the bat detector, 3) re-broadcasting those calls from the Racal using the ultrasound amplifier and loudspeaker, and 4) re-recording those calls using the original recording apparatus (i.e. PUSP and bat detector). This procedure was repeated five times on the resulting recordings to amplify potential artifacts. The final recordings were then compared to the original recordings using 256-pt spectrograms (Hamming windowing) generated by Avisoft SASLab Plus and no differences were detected (Wilson 2005).

When the first juvenile emerged, DRW 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 of 20 min to emerge, though at least two squirrels were required above ground for videotaping to commence. When the majority (> 50%) of squirrels above ground began foraging, they were videotaped 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, we noted the trial order, time of day (0830 - 1400 hours CST), day within year (142 - 149), angle of the speaker relative to the litter (0 - 10º), and the distance (accurate to the nearest 10 cm) from the litter to both the speaker (3.2 - 4.9 m) and observer (6.9 - 10.6 m). We also noted the percent cloud cover (0 - 100 %) and, using a Kestrel^{TM} 3000 pocket weather meter held 1.9 m above ground level, measured wind speed (2.3 - 6.9 km/h), temperature (8.3 - 18.5 ºC), and relative humidity (26 - 74 %). 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 and that the playback apparatus was indeed functioning. All research was conducted in accordance with the Guidelines for the Treatment of Animals in Behavioural Research and Teaching, as well as with the guidelines set forth by the Canadian Council on Animal Care, as outlined and approved under protocol number F99-041 of the University of Manitoba's Fort Garry Campus Protocol Management and Review Committee.

Evaluating behavioural responses to playbacks

Vigilant Richardson’s ground squirrels elevate their heads (Hare 1998a) and thus, vigilant behaviour was collectively considered as any posture where the squirrel’s head was elevated above the horizontal plane. The time that each squirrel devoted to vigilant behaviour was coded from videotape before (30 s), during (12.5 - 13.0 s), and after (30 s) the playback of each call-type using a stopwatch and the video’s time code. Because squirrels in this playback experiment lacked unique identification marks, any individual that was not visible continuously 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 vigilance values were reported for each period to avoid problems associated with the possible non-independence of responses among littermates (Machlis et al. 1985). We present descriptive statistics, however, for the initial postures assumed by individual squirrels at the onset of the playback, categorized as non-vigilant (standing on four feet with the head held below the horizontal plane), low vigilance (standing on four feet with the head elevated above the horizontal plane), slouch (posterior only on ground with head elevated above an arched back), or alert (posterior only on ground with head elevated above an erect back, sensu Hare and Atkins 2001). All behaviours were scored from videotape by an observer who was blind to the playback treatments.

Durations of vigilant behaviour within each period were converted to percentages of their respective periods' total durations to facilitate comparisons across periods of unequal duration. The changes 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. Data conformed to the parametric assumptions of normality (D'Agostino's D-test, all $p > 0.1$) and homoscedasticity (F-test, all $p > 0.25$) and thus did not require transformation. Trial order was balanced across treatments (seven of 13 litters received the alarm call before the control call) and thus does not confound our interpretation of vigilant behaviour. Because high-frequency sound attenuates rapidly, we also used linear regression to test if the distance between the speaker and the litter (3.2 - 4.9 m) affected vigilant behaviour. All analyses were performed on StatView® 5.0.1 on a Macintosh computer and results were considered statistically significant where $p \leq 0.05$ (all tests were two-tailed).

Results

Effect of threat proximity on call production

A total of 103 Richardson's ground squirrel alarm calls, including 87 audible calls, 10 ultrasonic calls, and six calls containing both audible and ultrasonic syllables (i.e. mixed calls), were included in the analysis. Each call was elicited by a different hat presentation and no caller contributed more than one alarm call to the dataset. We note, however, that for one mixed call the distance between the subject and hat was 5.8 m greater than for any other observation. We ran all analyses with and without this extreme observation and found that the results with respect to significance ($\alpha = 0.05$) remained identical across all variables. For lack of a suitable reason to exclude this observation it has been included in all analyses presented herein (i.e. $N = 103$).

The distance between the caller and the combined call-eliciting stimulus (independent variables: subject-hat distance and residuals of subject-observer distance when regressed against subject-hat distance) accounted for a significant amount of the variation observed in call-type (multivariable multinomial logistic regression, whole model likelihood ratio test: $G_4 = 16.983$, $p = 0.0019$, $R^2 = 0.155$), such that the odds of producing mixed and ultrasonic calls were significantly greater when the stimulus was farther away (Table 2; Fig. 1). The mean +/- SE distances between the subject and hat were 2.94 +/- 0.17 m for audible calls, 6.35 +/- 1.70 m for mixed calls, and 4.03 +/- 0.67 m for ultrasonic calls. The mean +/- SE distances between the subject and observer were 6.99 +/- 0.24 m for audible calls, 9.74 +/- 1.37 m for mixed calls, and 9.10 +/- 1.09 m for ultrasonic calls. Of the four miscellaneous variables examined, time of day, caller sex, and caller age (juvenile versus adult) had no effect on call-type (univariable multinomial logistic regression, whole model likelihood ratio test: all $p > 0.1$; see Table 3). Juvenile age did account for a significant amount of the variation in call-type (univariable multinomial logistic regression, whole model likelihood ratio: $G_2 = 12.288$, $p = 0.0021$, $R^2 = 0.132$), such that the odds of producing mixed calls were greater for older individuals

(Table 4). The mean +/- SE juvenile age was 35.70 +/- 1.41 days post-emergence for audible calls, 53.67 +/- 3.28 days post-emergence for mixed calls, and 38.00 +/- 3.25 days post-emergence for ultrasonic calls.

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 squirrels were not visible during the playback period. In response to the playback of ultrasonic alarm calls, the remaining 11 litters devoted significantly more time to vigilant behaviour than they did in response to the control treatment (Table 5). Despite the rapid attenuation of ultrasound, the distance between the litter and the speaker during alarm call playbacks did not affect the amount of time that litters devoted to vigilant behaviour during either the playback or post-playback periods (linear regression: both $p > 0.45$). Although we did not statistically analyze individuals' initial postural responses to calls, the majority of individual squirrels responded to the playback of both the alarm and control calls by immediately assuming the low vigilance posture (Table 6).

Discussion

The persistence of multiple signalling strategies can best be explained if the fitness payoff of each strategy is context-dependent (Maynard Smith 1976, 1978; Ydenberg and Dill 1986); if the costs and benefits of signalling were fixed, the superior strategy would always replace the inferior strategy over time. Our results demonstrate that the probability of Richardson's ground squirrels producing ultrasonic instead of audible alarm calls increases with increasing distance from the call-eliciting stimulus. Because sound attenuation is frequency-dependent (Lawrence and Simmons 1982), distant predators are more likely to detect audible as opposed to ultrasonic alarm calls. As ultrasonic alarm signals are inaudible to humans and are produced predominantly when we are distant from the signaler, our results also help to explain the apparent paucity of these interesting signals (first documented in Wilson and Hare 2004). Although we have observed squirrels engaged in ultrasonic alarm signalling in response to both natural (e.g. badger, *Taxidea taxus*, Schreber, 1777; bird of prey) and simulated (e.g. taxidermically-prepared badger and bird of prey, *Circus cyaneus*, Linnaeus, 1766) predator encounters in every population that we have studied, the use of a dispersed array of remote microphones sensitive to high-frequency sound (e.g. Hiryu et al. 2005) would be necessary to overcome our perceptual limitations and properly quantify the natural incidence of ultrasonic alarm signalling during all stages of a predator encounter. Our finding that juvenile squirrels were more likely to produce mixed calls when they were older could be explained either by seasonal changes in a caller's vulnerability or by developmental factors related to call production. However, further investigation into the development of antipredator behaviour in juveniles is necessary.

That ultrasonic alarm calls were produced rarely when the stimulus was nearby could reflect the fact that many of the mammalian predators that feed upon ground squirrels are capable of detecting frequencies in excess of 48 kHz (Peterson et al. 1969). Producing ultrasonic alarm calls when predators are nearby may thus be an unreliable mechanism for avoiding predator detection, particularly given the wide range of predators that feed upon ground squirrels (reviewed in Michener and Koeppl 1985). Exploiting the differential attenuation of audible and ultrasonic alarm signals (Smith 1979b) may be a more reliable mechanism by which squirrels can signal conspecifics to the exclusion of their predators (Klump et al. 1986). Differential sensitivity between squirrels and at least some of their predators to 48-kHz sound (Peterson et al. 1969) may then serve to expand the space in which cryptic alarm communication among squirrels is possible. Unfortunately, as Richardson's ground squirrels (family Sciuridae) are the only non-murid rodents known to produce purely ultrasonic vocalizations (Sales and Pye 1974), few studies have investigated their sensitivity to high-frequency sound. Hamill et al. (1989) demonstrated in golden-mantled ground squirrels that 32-kHz tones (higher frequencies were not tested) are capable of eliciting auditory brainstem responses (*S. lateralis*, Say, 1823). Further, frequencies as high as 100 kHz can elicit cochlear microphonic responses (Peterson et al. 1974). Although such electrophysiological techniques are useful for comparing frequency responses within and among taxa, they do not demonstrate signal integration and behavioural responses, and thus fail to determine the frequency threshold that is ecologically important to the species (Cynx and Clark 1998). Wilson (2005) demonstrated that Richardson's ground squirrels can detect and respond to frequencies at least as high as 40 kHz using a classical conditioning paradigm, but habituation in that study prevented testing beyond 40 kHz. Among ground squirrel predators, several fissiped carnivores exhibit cochlear microphonic responses to frequencies in excess of 65 kHz, though, again, the relevant auditory threshold curves are lacking (Peterson et al. 1969). In contrast, auditory threshold curves have been calculated for the European sparrowhawk and the American kestrel (*Falco sparverius*, Linnaeus, 1758), and suggest that many birds of prey are insensitive to frequencies exceeding 8 kHz (Sales and Pye 1974; Klump et al. 1986). An exciting future direction would be to map the functional space of ultrasonic alarm calls by integrating the rate of signal attenuation in a variety of natural habitats with the amplitude and frequency response thresholds of both conspecifics and a variety of eavesdropping predators.

Our playback experiment demonstrates that juvenile Richardson’s ground squirrels that have recently emerged from their natal burrow, but which have yet to disperse into the broader population, detect ultrasonic alarm signals and respond to them by increasing vigilance. Responses do not simply represent reaction to a novel stimulus because the change in the percentage of time devoted to vigilant behaviour was significantly greater following the alarm call than the control call (Table 5). It is unlikely that this difference can be attributed to certain individuals failing to detect the control call, but not the alarm call, because all but one of the individuals tested manifested vigilant behaviour at the onset of the 22 playbacks (Table 6). Thus, squirrels detected and responded with increased vigilance to both treatments (Table 6), but maintained that enhanced vigilance for longer following the playback of alarm calls (Table 5). Given the rapid attenuation of ultrasonic alarm signals (Lawrence and Simmons 1982), the clumped spatial distribution of juveniles during natal emergence (Michener and Koeppl 1985), and the capacity of newly emerging juveniles to respond specifically and appropriately to those signals, it is clear that natal emergence is one of the critical contexts in which these short-range ultrasonic alarm signals function. Furthermore, any benefits accrued by the signaler via parental investment (Blumstein et al. 1997), kin selection (Hamilton 1964), or reciprocal altruism (Trivers 1971; Wilson 2004) would also be maximal in this context, as emerging juvenile squirrels are abundant, highly vulnerable to predation, and have much to gain from being warned (Mateo 1996). Since Richardson's ground squirrels can recognize individuals (Hare 1998a) and discriminate kin from non-kin (Hare 1998b), it may even be possible for signalers to further optimize the net payoff of signalling if, in some circumstances, they issue long-range audible alarm calls that serve as a general warning to many group members, and, in other circumstances, issue short-range ultrasonic alarm calls that selectively warn nearby individuals, such as kin or neighbours that have proven to be reliable signalers in the past (Maynard Smith 1976, 1978; Witkin 1977; Axelrod and Hamilton 1981).

By integrating information about predator distance (Warkentin et al. 2001) and the presence or absence of nearby familiar or related conspecifics (Hare 1998b; Hare and Atkins 2001), callers could use exact information (but see Koops and Abrahams 1998) or simple rules of thumb (Bouskila and 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 or changes in the abundance of nearby squirrels, if the caller continually assesses the situation (e.g. Wilson and Hare 2003) and applies some form of information updating (Owings and Hennessy 1984; Bradbury and Vehrencamp 1998). The current study demonstrates that Richardson's ground squirrels issue short-range ultrasonic alarm calls when the eliciting stimulus is distant and long-range audible calls when that stimulus is nearby. Furthermore, vulnerable juvenile squirrels that have recently emerged from their natal burrow are capable of deciphering ultrasonic alarm signals from environmental noise and responding to those calls appropriately by maintaining increased vigilance.

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Table 1. Description of ultrasonic alarm signals used to construct playback stimuli that were broadcast to 11 litters of juvenile Richardson's ground squirrels.

Variable	Mean	SE	Min	Max
Syllable duration (ms)	235.7	9.0	183.5	296.5
Amplitude (dB SPL at 1 m from source)	79.1	0.7	73.9	82.1
Dominant frequency (kHz)	46.2	2.6	27.2	56.3
Lower quartile (kHz)*	32.1	2.5	24.4	52.5
Upper quartile (kHz)**	75.8	3.3	60.0	105.0

Note: Measurements were made with Avisoft-SASLab Plus using a 256-point spectrogram with Hamming window. * below this frequency is 25% of the signal's energy ** below this frequency is 75% of the signal's energy

Table 2. Effect of the combined call-eliciting stimulus distance (independent variable) on the type of alarm call produced by 103 Richardson's ground squirrels, examined using a multivariable multinomial (three-state dependent variable, coded as audible call = 0, mixed call = 1, ultrasonic call = 2) logistic regression model.

Logit	Variable	Coeff.	SE	Odds Ratio	95 % CI
1	Subject-hat distance	0.635	0.212	1.887	1.246, 2.857
	Subject-observer residuals	0.170	0.213	1.185	0.780, 1.800
	Intercept	-5.295	1.122		
2	Subject-hat distance	0.382	0.183	1.465	1.023, 2.097
	Subject-observer residuals	0.254	0.126	1.289	1.007, 1.650
	Intercept	-3.582	0.804		

Log-likelihood = -46.405

Note: Logit 1 represents the logit-transformed ratio of the probabilities of producing mixed and audible alarm calls, which is related linearly to the two independent variables and the intercept by multiple regression. Logit 2 represents the logit-transformed ratio of the probabilities of producing ultrasonic and audible alarm calls. For every one-meter increase in distance between the subject and call-eliciting stimulus, the odds of producing the call-type of interest (i.e. mixed or ultrasonic call) relative to that of the reference call (i.e. audible call) increase by the specified odds ratio. Odds ratios are statistically significant where their 95% confidence intervals (CI) do not contain 1.0. As an example, every one-meter increase in distance from the hat significantly increases the odds of a squirrel producing an ultrasonic as opposed to an audible alarm call by an estimated 1.465 times.

Table 3. Effect of four miscellaneous variables on the tendency of 103 Richardson's ground squirrels to produce three different types of alarm call, examined using a univariable multinomial (three-state dependent variable, coded as audible call = 0, mixed call = 1, ultrasonic call = 2) logistic regression model.

	Whole Model Logistic Likelihood Ratio Test				
Variable	Log likelihood	*G*	*DF*	*p*	R^2
Time of day	-54.845	0.444	2	0.8011	0.004
Age of subject*	-52.892	4.350	2	0.1136	0.039
Juvenile age (*N* = 92)	-40.302	12.288	2	0.0021	0.132
Sex of subject**	-53.846	2.441	2	0.2951	0.022

* coded as adult = 0, juvenile = 1

** coded as female = 0, male = 1

Table 4. Effect of juvenile age (independent variable) on the type of alarm call produced by 92 juvenile Richardson's ground squirrels, examined using a univariable multinomial (three-state dependent variable, coded as audible call = 0, mixed call = 1, ultrasonic call = 2) logistic regression model.

Logit	Variable	Coeff.	SE	Odds Ratio	95 % CI
1	Juvenile age	0.145	0.054	1.156	1.040, 1.286
	Intercept	-9.240	2.870		
2	Juvenile age	0.015	0.032	1.016	0.954, 1.081
	Intercept	-2.991	1.274		

Log-likelihood = -40.302

Note: Logit 1 represents the logit-transformed ratio of the probabilities of producing mixed and audible alarm calls, which is related linearly to the two independent variables and the intercept by multiple regression. Logit 2 represents the logit-transformed ratio of the probabilities of producing ultrasonic and audible alarm calls. For every one-day increase in juvenile age, the odds of producing the call-type of interest (i.e. mixed or ultrasonic call) relative to that of the reference call (i.e. audible call) increase by the specified odds ratio. Odds ratios are statistically significant where their 95% confidence intervals (CI) do not contain 1.0. As an example, every additional day following the initial emergence from the natal burrow significantly increases the odds of a juvenile squirrel producing a mixed as opposed to an audible alarm call by an estimated 1.152 times.

Table 5. Behavioural responses of 11 Richardson’s ground squirrel litters to the playback of ultrasonic alarm calls and background noise control calls.

Variable	Alarm	Noise	Difference (SE)	t_{10}	*p*
Playback vigilance (%)	18.1	6.6	11.5 (4.8)	2.364	0.0396
Post-playback vigilance (%)	11.4	-2.7	14.1 (7.6)	1.855	0.0933

Note: Time spent vigilant was measured during three periods (30 s before the playback, the 12.5 - 13 s during the playback, and the 30 s following the playback) and was expressed as a percentage of the respective period's total duration. The percentages of time devoted to vigilant behaviour during the playback and post-playback periods were then corrected for baseline behaviour by subtracting from them the percentage of time devoted to vigilant behaviour during the pre-playback period. Thus, negative values indicate greater vigilance in the pre-playback period.

Table 6. Initial postural responses of individual juvenile Richardson's ground squirrels assumed immediately following the onset of playback of ultrasonic alarms calls (*N* = 32) and background noise control calls (*N* = 31).

	Treatment	
Initial Posture	Alarm Call	Control Call
Non-vigilant	0	1
Low vigilance	28	30
Slouch	4	0
Alert	0	0

Note: All postures are categorized as either non-vigilant (standing on four feet with the head held below the horizontal plane), low vigilance (standing on four feet with the head held above the horizontal plane), slouch (posterior only on ground with head elevated above an arched back), or alert (posterior only on ground with head elevated above an erect back, sensu Hare and Atkins 2001). The duration of these behaviours is not reflected in this table.

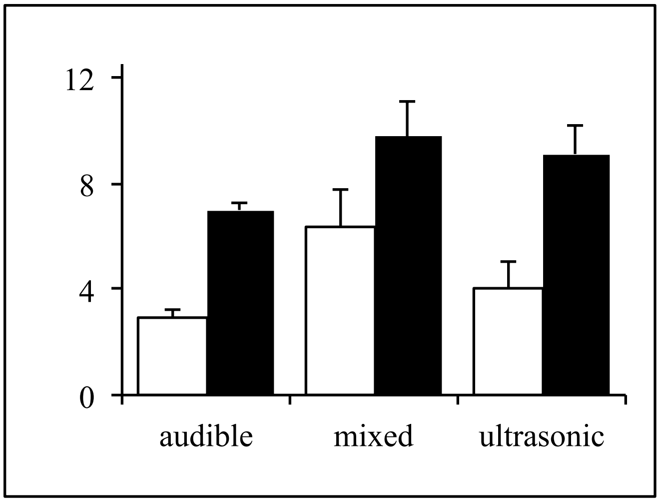

Fig. 1. Mean distance (+ SE) from the two components of the combined call-eliciting stimulus (white bars = hat; black bars = observer) at which 103 Richardson's ground squirrels produced audible (*N* = 87), mixed (*N* = 6), and ultrasonic (*N* = 10) alarm signals.