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Red squirrels use territorial vocalizations for kin discrimination

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The ability to discriminate among individuals, or among classes of individuals, can provide animals with important fitness benefits. Although several mechanisms for discrimination are possible, most require animals to show stable phenotypic variation that reflects their identity or their membership in a particular class (e.g. sex, mate, kin). For territorial animals that rarely interact physically, vocalizations could serve as long-distance signals that facilitate discrimination. In this study, we tested whether the territorial rattle vocalizations of North American red squirrels, *Tamiasciurus hudsonicus*, are repeatable, and whether they could hence provide the basis for multiple types of discrimination. We measured four structural features from two rattles from each of 76 marked squirrels. All four features were repeatable, which is consistent with territorial rattles being individually distinctive. We then conducted a playback experiment to determine whether squirrels use rattles for discrimination. Specifically, we tested whether squirrels discriminate between the rattles of neighbours and non-neighbours, and kin (coefficient of relatedness, $r \geq 0.25$) and non-kin ($r < 0.125$). Following a 2 x 2 factorial design, we broadcast a rattle from a non-neighbouring nonkin individual to 15 subjects, from a neighbouring nonkin individual to 14 subjects, from a non-neighbouring kin individual to 11 subjects, and from a neighbouring kin individual to 13 subjects. Subjects did not discriminate between the rattles of neighbours and non-neighbours, but did respond differently to the rattles of kin and nonkin. Specifically, squirrels were significantly more likely to produce a rattle of their own in response to the broadcasted rattles of nonkin versus the broadcasted rattles of kin. This result demonstrates that red squirrels can use territorial vocalizations for kin discrimination. It also suggests that they are more tolerant of territorial intrusions by kin.

Keywords:

individually distinctive vocalization

kin recognition

Tamiasciurus hudsonicus

territorial vocalization

territoriality

The ability to discriminate among individuals, or among classes of individuals, can allow animals to avoid inbreeding (Pusey & Wolf, 1996), allocate resources preferentially towards mates and kin (Hamilton, 1964) and focus aggressive behaviours towards threatening individuals (Fisher, 1954; Temeles, 1994). As an example, in many territorial species, individuals are more aggressive to non-neighbours, which tend to be unfamiliar, than they are to neighbours, which tend to be familiar. Known as the 'dear enemy effect', this form of discrimination allows territory holders to direct their aggression towards floaters that may be seeking to usurp their territory. It also allows them to avoid repeated and potentially costly interactions with neighbours that already hold a territory, and which therefore pose less risk of a territory take-over (Fisher, 1954; Temeles, 1994).

Animals can use several different mechanisms for discrimination (Blaustein, 1983; Mateo, 2003; Waldman, Frumhoff, & Sherman, 1988). If individuals, or classes of individuals, are distributed predictably in space, then a simple rule of thumb based on location may suffice. For example, if a species has limited dispersal, then spatial proximity among individuals can provide an efficient mechanism for investing selectively in kin (Kümmerli, Gardner, West, & Griffin, 2009). Where spatial references are unreliable, a mechanism based on phenotypic traits may afford the necessary flexibility. Such mechanisms require animals to show stable phenotypic variation that reflects their identity or their membership in a particular class (e.g. sex, mate, kin; Mateo, 2003; Waldman et al., 1988). If that condition is satisfied, then three additional mechanisms of discrimination are possible (Blaustein, 1983; Mateo, 2003). The first is familiarity-based discrimination. Here, animals learn to recognize specific individuals, such as mates and neighbours, and to then behave differently towards them (Blaustein, 1983; Mateo, 2003; Waldman et al., 1988). This type of discrimination is effective when animals interact repeatedly over time, but it may preclude animals from learning the identities of kin that are encountered only rarely, or in conjunction with nonkin, during the learning period (Komdeur & Hatchwell, 1999; Waldman et al., 1988). Alternatively, kin discrimination may be based on phenotype matching or recognition alleles. In phenotype matching, an animal uses experience with itself (i.e. self-referent phenotype matching) or a known relative (e.g. its mother) to form a generalized template that can then be used to recognize unfamiliar kin (Blaustein, 1983; Mateo, 2003; Waldman et al., 1988). With recognition alleles, a genetic mechanism causes individuals to show a familial form of a phenotypic trait, and to then recognize that form of the trait in others (Blaustein, 1983; Mateo, 2003; Waldman et al., 1988).

North American red squirrels, *Tamiasciurus hudsonicus*, are solitary, diurnal rodents that maintain exclusive year-round territories (Smith, 1968), suggesting that they may benefit from an ability to discriminate between familiar neighbours and less familiar or unfamiliar non-neighbours (Fisher, 1954; Temeles, 1994). Red squirrels are also known to bequeath their territories to recently emerged young (Berteaux & Boutin, 2000; Price & Boutin, 1993), to occasionally nest communally with kin during the winter (Williams et al., 2013) and to show kin-based adoption of orphaned juveniles (Gorrell, McAdam, Coltman, Humphries, & Boutin, 2010). These nepotistic behaviours show that red squirrels have the ability to discriminate between kin and nonkin in certain circumstances. However, the proximate mechanisms underlying this ability remain unknown. In her review of kin discrimination in rodents, Mateo (2003) found that familiarity-based discrimination and phenotype matching are both widespread among rodents. Furthermore, she found that most studies of kin discrimination in rodents focused on olfactory cues, although she noted that other modalities could also be important (Mateo, 2003).

When defending their territories, adult red squirrels sometimes engage in chases and fights that increase their risk of predation (Price, Boutin, & Ydenberg, 1990). Yet, such physical altercations are rare, with most territorial disputes instead involving the production of territorial vocalizations known as ‘rattles’ (Dantzer, Boutin, Humphries, & McAdam, 2012; Gorrell et al., 2010; Lair, 1990; Fig. 1). Rattles are a series of pulses produced in rapid succession (Fig. 1; Smith, 1978). They are produced by both sexes and announce the owner's presence on the territory (Donald & Boutin, 2011; Lair, 1990; Smith, 1978). Rattles are also individually distinctive in at least one population (Digweed, Rendall, & Imbeau, 2012) and are known to be involved in discrimination at some level. Indeed, a playback study showed that red squirrels distinguish between the rattles of neighbours and those of squirrels recorded 10 km away (Price et al., 1990). However, because neighbours tend to have greater relatedness than non-neighbours (Berteaux & Boutin, 2000; Price & Boutin, 1993), it is unclear whether squirrels in that study were discriminating between neighbours and non-neighbours, kin and nonkin, or both (Price et al., 1990).

In the current study, we tested whether red squirrels in southwest Yukon, Canada produce rattles with repeatable acoustic structure, which could provide a basis for discrimination. We then conducted an audio playback experiment that simulated territorial intrusions by broadcasting territorial rattles of neighbouring kin, neighbouring nonkin, non-neighbouring kin and non-neighbouring nonkin inside subjects' territories. Based on the dear enemy hypothesis (Fisher, 1954; Temeles, 1994), we predicted that subjects would respond more aggressively to the rattles of non-neighbours than to the rattles of neighbours. Furthermore, based on the kin selection hypothesis, we predicted that subjects would respond more aggressively to the rattles of nonkin than to the rattles of kin (Hamilton, 1964).

METHODS

Subjects

Subjects were derived from a marked population of North American red squirrels (*T. hudsonicus*; Erxleben, 1777) that has been studied annually in the southwest Yukon Territory of Canada (61°N, 138°W) since 1989 (McAdam, Boutin, Sykes, & Humphries, 2007). All individuals in the population were marked with numbered metal eartags when first captured (usually just after birth when in the natal nest) and were then live-trapped each year throughout their lifetime. We also attached a unique combination of coloured wires or pipe cleaners to their eartags each year to facilitate identification from afar.

Male and female red squirrels reside on individual territories that they defend throughout the year (Smith, 1968). Territories are nonoverlapping, are often contiguous and tend to be stable throughout the year. However, their size varies among populations and years and tends to be larger among females than among males (Dantzer et al., 2012; Price, Broughton, Boutin, & Sinclair, 1986; LaMontagne et al., 2013). Each territory is defended by a single individual, but females will share their territories with their young-of-the-year, as well as with adult males during the 1 day of the year when the female is sexually receptive (Smith, 1968).

Audio Recording and Acoustic Analysis

We recorded territorial rattles from 172 squirrels between April and August of 2005, 2006, 2009 and 2011. Our sample included seven juvenile females, eight juvenile males, 75 adult females and 82 adult males. We had additional recordings from other individuals but these were excluded from the analyses because part of the rattle was either missing or clipped. For a subset of 76 individuals (1 juvenile female, 1 juvenile male, 36 adult females, 38 adult males), we obtained a second recording at a later date (mean ± SD = 114 ± 304 days later; minimum = 2 days; maximum = 1111 days), which allowed us to assess the repeatability of rattle acoustic structure. Each of these 76 individuals was of the same age class for both of its recordings (i.e. juvenile or adult).

Recordings were made opportunistically and without the use of playback. When a squirrel was observed, it was identified and followed at a distance greater than 5 m until it produced at least one rattle. If it produced multiple rattles during a single recording session, we retained only the first for our analyses. The stimulus eliciting the rattle was generally unknown, although red squirrels are known to rattle spontaneously and in response to detection of conspecifics (Smith, 1978). We cannot exclude the possibility that the recordist's presence elicited the rattles. We note, however, that squirrels were habituated to human observers and that they often rattled only after being followed for several minutes. Rattles were acquired with a Marantz digital recorder (model PMD 660; 44.1 kHz sampling rate; 16-bit accuracy; WAVE format) and a shotgun microphone (Sennheiser, model ME66 with K6 power supply; 40–20 000 Hz frequency response (± 2.5 dB); supercardioid polar pattern) that was pointed at the focal squirrel.

We measured the structure of each recorded rattle using Raven Pro software (version 1.5; Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, U.S.A.). Prior to analysis, we filtered rattles with a

200 Hz high-pass filter that removed low-frequency background noise without affecting rattle structure. We then measured four structural features of the rattle (Fig. 1), including (1) duration, (2) call rate, (3) dominant frequency and (4) entropy. Duration is the period of time between the beginning of the first pulse and the end of the last. Call rate is the number of pulses in the rattle minus one, divided by the period of time between the beginning of the first pulse and the beginning of the last. Dominant frequency is the frequency of maximum amplitude from within the first spectral peak (Fig. 1). Entropy is a measure of energy distribution in the frequency domain. Tonal sounds have low entropy values, whereas broadband sounds, such as white noise, have high entropy values. Duration and call rate were measured from the waveform, whereas dominant frequency and entropy were measured from an averaged power spectrum of the entire rattle (512-point fast Fourier transform, 87.5% overlap, Hamming window, 1.5 ms temporal resolution, 86 Hz frequency resolution). Although some rattles included distinct introductory or trailing elements (Fig. 1), these were not included in our measurements.

We used the larger data set (i.e. N = 172 rattles, each from a different individual) to conduct descriptive analyses and to test for the effects of sex, age, year and date on rattle acoustic structure. We used the smaller data set (i.e. N = 76 individuals, with two rattles per individual) to assess the repeatability of rattle acoustic structure. Specifically, we used the R package "ICC" (Wolak, 2013) to calculate the intraclass correlation coefficient (ICC) for each of the four structural features (Wolak, Fairbairn, & Paulsen, 2012). We considered a structural feature to be repeatable if its ICC's 95% confidence interval excluded zero. Finally, we conducted linear regression analyses to test whether repeatability changed over time. For each structural feature, we included the measurement from the second recording as a dependent variable, the measurement from the first recording as an independent variable and the number of days between the two recordings as a second independent variable. A significant interaction between the two independent variables would indicate that the degree of repeatability changes over time.

Neighbour and Kin Discrimination

We conducted a playback experiment to determine whether adult squirrels respond differentially to the rattles of neighbours versus non-neighbours and kin versus nonkin. Following a between-subjects 2 x 2 factorial design, we broadcast a rattle from a non-neighbouring nonkin individual to 15 subjects (7 females, 8 males), from a neighbouring nonkin individual to 14 subjects (2 females, 12 males), from a non-neighbouring kin individual to 11 subjects (4 females, 7 males) and from a neighbouring kin individual to 13 subjects (11 females, 2 males). We determined neighbour status by mapping the territories of all individuals at our study site. For each individual, we located its primary midden, and then determined its territory boundary by noting its location and behaviour relative to a grid that was superimposed on the study site with stakes placed at 30 m intervals (details in McAdam et al., 2007). We determined genetic relatedness among squirrels using a multigenerational pedigree that was developed for this population. Maternal linkages were based on enumeration and permanent marking of juveniles within their natal nest, whereas paternal linkages were based on a microsatellite paternity analysis (Lane, Boutin, Gunn, Slate, & Coltman, 2008; McAdam et al., 2007; McFarlane et al. 2014).

We considered squirrels to be neighbours when they concurrently held spatially adjacent territories (i.e. abutting territory boundaries) and to be non-neighbours when their primary middens were more than 150 m apart. Rattles have been reported to be audible for a distance of 130 m (Smith, 1978), so squirrels that we defined as non-neighbours were likely outside the acoustical range of one another. Also, in this population, the average diameter of an adult's territory is estimated to be between 43 m (after Price & Boutin, 1993) and 68 m (after Price et al., 1986). Territories also tend to be contiguous (Price & Boutin, 1993; Price et al., 1986), suggesting that non-neighbours in our study were separated by at least one other territory. We assume that neighbours were familiar with each other and that non-neighbours were less familiar or unfamiliar with each other. We therefore do not attempt to distinguish between the effects of neighbour status and familiarity, since we assume that these are concordant in this species. We note, however, that non-neighbouring kin may be familiar based on social interactions prior to dispersal.

We considered a subject to be kin with the individual that provided the stimulus rattle if the two squirrels had a coefficient of relatedness (*r*) that was greater than or equal to 0.25, as determined by our multigenerational pedigree (Lane et al., 2008; McAdam et al., 2007; McFarlane et al., 2014). For example, kin could consist of a parent and offspring ($r = 0.5$), two siblings ($r = 0.5$), two half-siblings ($r = 0.25$), a grandparent and grandchild ($r = 0.25$), or an aunt/uncle and niece/nephew ($r = 0.25$). Squirrels were considered to be nonkin if their coefficient of relatedness was less than 0.125, as would be the case for first cousins once removed ($r = 0.06$), second cousins ($r = 0.03$), third cousins ($r < 0.01$), or any other more distantly related individuals.

We began each playback trial when we identified a previously untested squirrel within its own territory. We placed a portable stereo (GPX, model BCDW9815CNP) approximately 10 m away from the squirrel, oriented it so that its two speakers faced the squirrel, and camouflaged it with foliage. We then observed the subject for a 3 min pre-playback period to allow it to habituate to our presence and to ensure that it was not displaying any overt responses to the observer or the playback apparatus. Immediately following this, we began a 3 min playback period, which commenced with the broadcast of a single rattle. To differentiate between responses to the speaker and responses to the observer, the observer stood 10 m away from the stereo, such that the line between the stereo and observer was perpendicular to the line between the stereo and subject. Only one subject faced the speaker during the pre-playback observation period, whereas all individuals except one faced the speaker during the playback period, thereby suggesting that squirrels heard and responded to the playback stimulus.

Playback stimuli were selected from our library of rattle recordings on the basis of high signal-to-noise ratio and typical length (2–5 s). They were not filtered prior to playback. We also selected stimuli such that they satisfied the criteria for our four playback treatments (i.e. all combinations of kinship and neighbour status). We did not have playback stimuli representing all four treatments for every subject in the experiment, so we assigned treatments to subjects in a semirandom order. Only one stimulus was broadcast in each trial, but each stimulus could be used in up to four trials if those trials were from different treatments.

For each trial, we scored two dependent variables during the 3 min playback period. These included (1) whether or not the subject produced a rattle and (2) whether or not the subject approached the stereo. We defined 'approach' as one or more steps directly towards the stereo. We considered the production of a rattle and approach towards the speaker as aggressive responses, since these behaviours precede chases and fights in the context of territory defence (Price et al., 1990). Each dependent variable was analysed using logistic regression. Sex of the subject, kin status and neighbour status of the stimulus, and all two-way and three-way interactions were entered into the models as independent variables using a forward stepwise selection procedure (probability for stepwise entry into the model: $P = 0.05$).

During the pre-playback period, subjects never approached the stereo. However, 11 of the 53 subjects did produce a rattle during the pre-playback period. These rattles were likely the result of normal territorial defence, in which squirrels rattle approximately once every 8 min (Dantzer et al., 2012). However, these rattles could also indicate that the focal squirrel was responding to the observer or playback apparatus, as opposed to the playback stimulus. We therefore analysed our data with and without these 11 trials. The results with respect to statistical significance were identical, so we report only those results derived from the more inclusive data set (i.e. $N = 53$). Statistical analyses were performed using PASW (version 18 for Mac; IBM Corporation, Armonk, NY, U.S.A.).

Ethics Statement

The Institutional Animal Care and Use Committee at Michigan State University approved all procedures used in this study (no. 04/08-046-00).

RESULTS

Acoustic Analysis

We recorded one unsolicited territorial rattle from each of 172 individually marked red squirrels. Rattles had an average ± SD duration of 3.0 ± 1.4 s (range 0.4–10.0 s), an average call rate of 19.1 ± 2.0 pulses/s (range 12.6–25.2 pulses/s), an average dominant frequency of 1124 ± 152 Hz (range 770–1460 Hz) and an average entropy of 6.5 ± 0.3 bits (range 5.6–7.1 bits). None of the acoustic features differed significantly between females and males (unpaired *t* tests: all $|t_{171}| < 1.64$, all $P \geq 0.10$), between adults and juveniles (all $|t_{170}| < 1.60$, all $P \geq 0.11$), among the four recording years (ANOVA: all $F_{3,168} \leq 1.19$, all $P \geq 0.31$), or in relation to the Julian day on which the rattle was recorded (simple linear regression: all $F_{1,170} \leq 2.95$, $P \geq 0.09$). The four acoustic features were generally independent of each other, although call rate was inversely correlated with duration (Pearson correlation: $r_{170} = -0.29$, $P < 0.001$) and dominant frequency ($r_{170} = -0.19$, $P = 0.012$).

The acoustic structure of rattles was repeatable among the 76 squirrels that were recorded on 2 separate days (Fig. 2). Intraclass correlation coefficients were significantly greater than zero for each of the four acoustic features, including duration (ICC = 0.26; 95% CI: 0.05, 0.47), call rate (ICC = 0.66; 95% CI: 0.53, 0.78), dominant frequency (ICC = 0.47; 95% CI: 0.29, 0.65) and entropy (ICC = 0.34; 95% CI: 0.14, 0.54). Furthermore, the strength of these relationships was unaffected by the number of days separating the two recordings. Multiple linear regression analyses revealed no significant interactions between the number of days between the two recordings and the initial measurements of duration (overall model: $F_{3,72} = 2.65$, $P = 0.056$; initial measurement: $F_{1,72} = 6.79$, $P = 0.011$; days between recordings: $F_{1,72} = 2.28$, $P = 0.135$; interaction: $F_{1,72} = 1.78$, $P = 0.186$), call rate (overall model: $F_{3,72} = 23.11$, $P < 0.001$; initial measurement: $F_{1,72} = 62.78$, $P < 0.001$; days between recordings: $F_{1,72} = 0.04$, $P = 0.834$; interaction: $F_{1,72} = 0.10$, $P = 0.749$), dominant frequency (overall model: $F_{3,72} = 7.022$, $P < 0.001$; initial measurement: $F_{1,72} = 18.51$, $P < 0.001$; days between recordings: $F_{1,72} = 0.19$, $P = 0.667$; interaction: $F_{1,72} = 0.17$, $P = 0.684$) and entropy (overall model: $F_{3,72} = 3.18$, $P = 0.029$; initial measurement: $F_{1,72} = 7.33$, $P = 0.008$; days between recordings: $F_{1,72} = 0.24$, $P = 0.627$; interaction: $F_{1,72} = 0.25$, $P = 0.620$).

Neighbour and Kin Discrimination

We broadcast a territorial rattle to 53 adult squirrels. In response, 23 individuals produced a rattle of their own. Kin status was the only predictor of rattle production, with subjects being significantly more likely to call in response to nonkin than in response to kin (overall model: $N = 53$, $\chi^2_1 = 6.22$, $P = 0.013$, Nagelkerke pseudo $R^2 = 0.15$; kinship: Wald $\chi^2_1 = 5.75$, $P = 0.017$, odds ratio = 4.25; variables not included in model: all $P \geq 0.237$; Fig. 3). In total, 59% of subjects rattled in response to a nonkin playback and 25% rattled in response to a kin playback (Fig. 3). This 25% frequency of rattle response to kin was similar to the incidence of rattles in the pre-playback period (i.e. 11 of 53 subjects, or 21%). In contrast to kin status, the frequencies of rattle responses to neighbours (i.e. 10 of 27 subjects, or 37%) and non-neighbours (13 of 26 subjects, or 50%) were statistically indistinguishable. Overall, our final model correctly predicted 66% of all responses, including 60% of the individuals that did not produce a rattle and 74% of the individuals that did. In contrast to the production of rattles, none of the independent variables predicted whether or not the subject approached the stereo (variables not included in model: all $P \geq 0.217$), although 27 of the 53 individuals did approach during the playback period.

DISCUSSION

Red squirrels produced territorial vocalizations with repeatable acoustic structure, and they used these vocalizations to discriminate between kin and nonkin. We found no evidence that squirrels use rattles to discriminate between neighbours and non-neighbours.

Our acoustic analysis showed that red squirrels produce territorial rattles that reflect the signaller's identity. The ICCs of the four structural features that we measured were each significantly greater than zero, which is consistent with rattles in this population being individually distinctive. Call rate had the highest degree of repeatability, with variance among individuals accounting for 66% of the variance observed in this feature (Fig. 2). Dominant frequency had an intermediate level of repeatability (47%), while entropy and duration had relatively low levels (34% and 26%, respectively; Fig. 2). Our results are similar to those of a previous study, which documented individual distinctiveness in the rattles of a population of red squirrels in Alberta, Canada (Digweed et al., 2012). In that study, the features contributing the most to individual distinctiveness were the length of individual pulses and, as in our own study, the frequencies at which spectral energy was concentrated (Digweed et al., 2012). Although we did not measure pulse length in our study, we suspect that it would be strongly and inversely correlated with our measure of call rate, since high call rates can only be achieved by shortening the individual pulses or shortening the silent intervals between pulses, or both. Together with Digweed et al. (2012), our findings suggest that repeatable acoustic structure is a widespread feature of the rattle vocalizations of North American red squirrels. Our findings also contribute to a growing literature that suggests that repeatable signals are widespread among taxa (e.g. Bee & Gerhardt, 2001; Boughman & Moss, 2003; Martins, 1991; McGregor & Westby, 1992; Rukstalis, Fite, & French, 2003; Stoddard, 1996; Tooze, Harrington, & Fentress, 1990).

Our playback study provided no evidence that red squirrels discriminate between the territorial rattles of neighbours and non-neighbours, despite the potential benefits that such discrimination could afford (Fisher, 1954; Temeles, 1994). We note that, among unrelated squirrels, neighbour status and familiarity are probably highly concordant. Thus, it is unclear whether subjects failed to respond to differences in neighbour status or to differences in familiarity, although we are unaware of any biologically meaningful distinction between these in this species. Our findings contrast with a previous playback experiment, which found that squirrels were more likely to respond to rattles from non-neighbouring individuals (Price et al., 1990). However, that study did not consider kinship in their playback design. Since neighbours tend to be more closely related than non-neighbours (Berteaux & Boutin, 2000; Price & Boutin, 1993), it is possible that the differential response in that study was due to correlated differences in the squirrels' relatedness, as opposed to differences in their neighbour status per se. It is also possible that discrimination is context dependent, and that squirrels in our study did not discriminate, whereas those in the previous study did. An alternative explanation is that squirrels in our study responded differentially to neighbours and non-neighbours, but these differences were not reflected by the variables that we measured. We believe that this explanation is unlikely, however, since the two studies used similar response variables, a similar playback design and the same population of red squirrels (Price et al., 1990).

Our playback study provides experimental evidence that red squirrels use territorial rattles to discriminate between kin and nonkin. Specifically, focal squirrels were more likely to produce a territorial rattle in response to the simulated intrusion of an unrelated squirrel (coefficient of relatedness, $r < 0.125$) than they were to the simulated intrusion of a related squirrel ($r \geq 0.25$). In past research on this squirrel population, we have found a mix of kin discriminatory and kin nondiscriminatory behaviour. In two cross-fostering experiments, mothers did not discriminate between their own young and those of another female, as evidenced by similar mass gain and survival between fostered and nonfostered juveniles (Humphries & Boutin, 1996; McAdam, Boutin, Réale, & Berteaux, 2002). Yet other studies show that red squirrels nest communally with kin during winter (Williams et al., 2013), bequeath territories to recently emerged offspring (Berteaux & Boutin, 2000; Price & Boutin, 1993) and show kin-biased adoption of orphaned juveniles (Gorrell et al., 2010). These findings suggest that kin discrimination may depend on several factors, including the life history stage of the individuals involved, the context in which discrimination occurs and the signals and cues that are available for assessment (McAdam et al., 2002).

A limitation of our study is that it could not identify the structural features of rattles that are used in kin discrimination. Nevertheless, we suggest that call rate and dominant frequency may be important, given their high levels of repeatability. Another limitation of our study is that it did not reveal the specific mechanism underlying acoustically based kin discrimination. Specifically, we cannot distinguish between familiarity-based discrimination, phenotype matching and recognition alleles, since subjects in our "kin" treatments may have had prior experience with the individuals that provided the playback stimuli (Komdeur & Hatchwell, 1999; Waldman et al., 1988). Nevertheless, the natural history of red squirrels provides some insight into which mechanism red squirrels might use. Juvenile red squirrels remain on their mother's territory until approximately 70 days of age. Many juveniles then establish territories near or, in some cases, within their natal territory (Berteaux & Boutin, 2000; Larsen & Boutin, 1994; Price & Boutin, 1993). The often-prolonged proximity among mothers and offspring suggests that a familiarity-based mechanism could facilitate kin discrimination among these family members (Komdeur & Hatchwell, 1999). Note, however, that male red squirrels provide no paternal care and interact with their mates only during copulation (Smith, 1968). Consequently, a familiarity-based mechanism would not allow red squirrels to discriminate between their fathers (and paternally related kin such as paternal half-sibs) and unrelated individuals.

The ability to use territorial vocalizations to discriminate between kin and nonkin could provide red squirrels with several fitness benefits. For example, red squirrels are known to increase their inclusive fitness by selectively adopting orphaned relatives (Gorrell et al., 2010). Rattles could therefore provide the basis for the expression of this adaptive behaviour. As a species that defends a central cache of food from pilfering (Donald & Boutin, 2011; Gerhardt, 2005), the ability to discriminate between kin and nonkin could also allow red squirrels to focus defensive behaviours on unrelated neighbours. At the same time, it could allow them to be more tolerant of intrusions from related neighbours, since this could increase the neighbour's survival and, thus, the resident's inclusive fitness (Hamilton, 1964). Regardless of any fitness benefits involved, our study shows that red squirrels produce territorial vocalizations with repeatable acoustic structure, and that they use these vocalizations to discriminate between kin and nonkin individuals.

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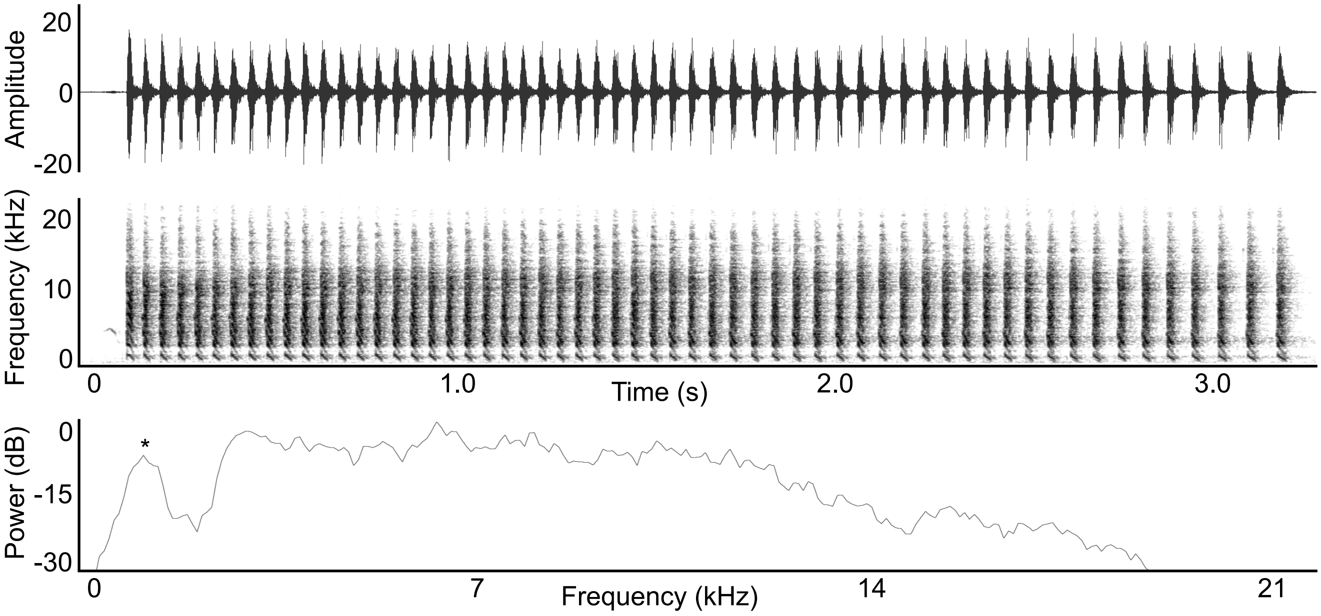

Figure 1. The rattle vocalization of a juvenile female North American red squirrel. It is depicted as (a) a waveform (units on the *Y* axis are arbitrary), (b) a spectrogram and (c) a power spectrum. The spectrogram and power spectrum were generated using a 512-point fast Fourier transform, 87.5% overlap and Hamming window. The spectrogram has a frequency resolution of 86 Hz, a temporal resolution of 1.5 ms and an amplitude range of 50 dB (depicted by the grey scale). The power spectrum is an averaged power spectrum of the entire rattle, excluding the faint introductory note that is visible on the spectrogram. Duration and call rate were measured from the waveform, whereas entropy and the dominant frequency of the first spectral peak (marked with an asterisk) were measured from the power spectrum. The rattle has been filtered with a 200 Hz high-pass filter.

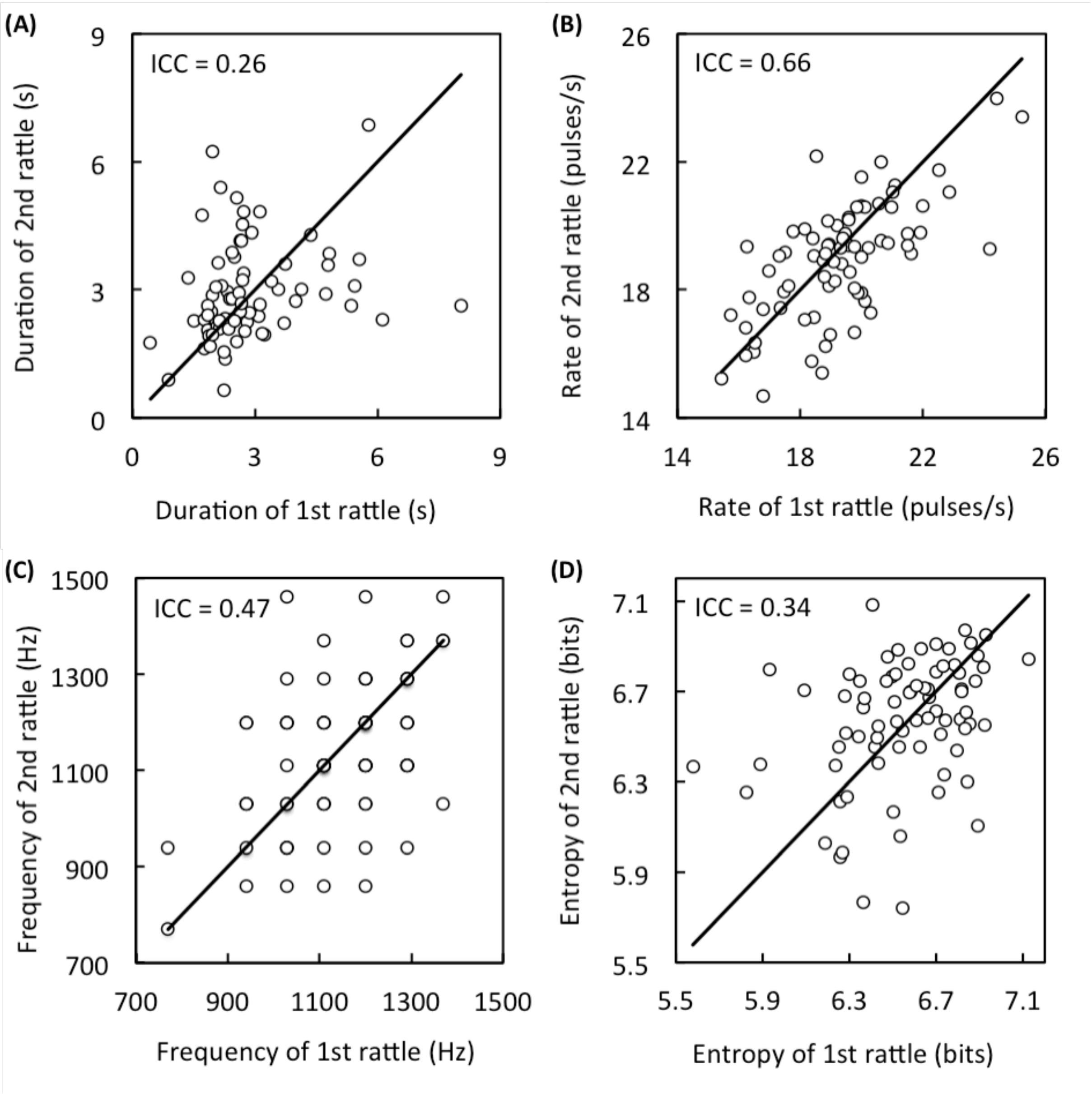

Figure 2. Repeatability of rattle acoustic structure among 76 red squirrels. The *X* axis shows measurements from the first recording of each individual, and the *Y* axis shows measurements from a second recording of the same individuals at a later date (mean ± SD = 114 ± 304 days later; minimum = 2 days; maximum = 1111 days). Measurements include (a) duration, (b) call rate, (c) dominant frequency and (d) entropy. Lines of unity and intraclass correlation coefficients (ICC) are provided for reference.

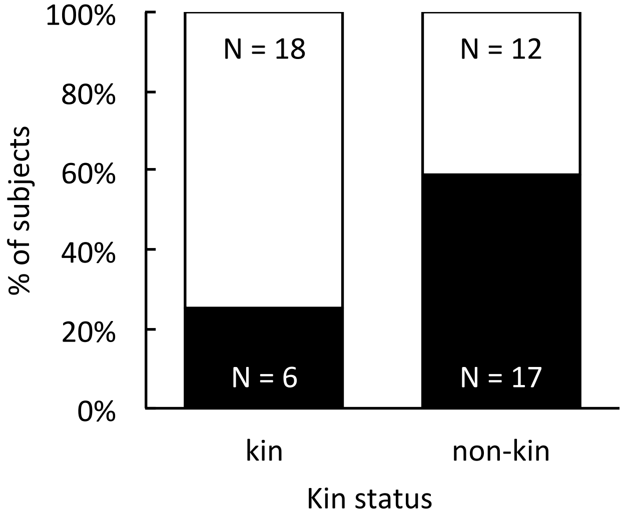

Figure 3. Vocal responses of 53 adult red squirrels to the playback of a territorial rattle. Broadcasted rattles were derived from individuals that were either kin (coefficient of relatedness, $r \geq 0.25$; includes 13 neighbours and 11 non-neighbours) or nonkin ($r < 0.125$; includes 14 neighbours and 15 non-neighbours) to the subject being tested. The *Y* axis shows the percentage of subjects that produced a rattle (black), as well as the percentage that did not (white), during the 3 min playback period. The absolute number of individuals is given on each bar.