

**Individual variation in the dear enemy phenomenon via territorial vocalizations in red  
squirrels**

by

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## ABSTRACT

# INDIVIDUAL VARIATION IN THE DEAR ENEMY PHENOMENON VIA TERRITORIAL VOCALIZATIONS IN RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*)

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Territoriality arises when the benefits of exclusive access to resources exceed the costs of defense. Behavioural plasticity increases the net benefits of territoriality by reducing defensive effort, often through the dear enemy phenomenon where familiarity reduces intrusion risk. Red squirrels (*Tamiasciurus hudsonicus*) follow the dear enemy phenomenon, but the mechanism by which red squirrels recognize conspecifics is unknown. I hypothesized that they use territorial calls, and predicted that familiar calls would better deter intruders than unfamiliar calls. I temporarily removed squirrels from their territories and replaced them with a speaker broadcasting the owner's call, an unfamiliar call, or silence. Owner calls did not more effectively repel intruders than unfamiliar calls. However, intruder identity varied: unfamiliar neighbours intruded during owner playback, but familiarity did not affect intrusions during unfamiliar playback. Individual variation in familiarity and changes in population density and composition can affect the strength and detectability of dear enemy effects.

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## 1.0 INTRODUCTION

### *1.1 Territoriality and the dear enemy phenomenon*

In populations with intraspecific competition for resources, territoriality as a social structure can arise when individuals benefit from exclusive access to a resource. Resource defense can constitute a substantial portion of a territory owner's time and energy budget (Puckett and Dill 1985, Heinemann 1992), but territoriality is only adaptive when the fitness benefit from the defended resource exceeds the costs of defending said resource from conspecifics (Carpenter and MacMillen 1976). When the resource value of a territory is relatively stable, net benefits can be maximized by reducing defensive costs where possible (Rosell et al. 2008).

Plasticity in territorial behaviour allows for reduction of unnecessary defensive costs. Territory owners across a broad range of species have been shown to discriminate between conspecifics based on familiarity, known as the dear enemy phenomenon (Fisher 1954). The dear enemy phenomenon refers to a reciprocally beneficial relationship between territorial neighbours, where each refrains from intruding on the other, and by virtue of this reduced risk of intrusion both can reduce their defensive effort (Temeles 1994). This phenomenon is thought to arise between neighbours as a form of conditional reciprocity, whereby territory owners form 'agreements' to tolerate one another and both benefit through a decreased defensive effort (Axelrod and Hamilton 1981). Individuals that break this relationship and intrude on familiar neighbours incur a cost through increased intrusions on their own territory and subsequent need to devote more time and energy to territory defense (Akçay et al. 2009). The dear enemy phenomenon has been demonstrated across taxa, in birds (eg. Wei et al. 2011), mammals (Rosell

and Bjørkøyli 2002), amphibians (Jaeger 1981), fish (Leiser and Itzkowitz 1999), reptiles (Qualls and Jaeger 1991), and crustaceans (Booksmythe et al. 2010).

The central predictions of the dear enemy phenomenon are that familiar individuals are less likely than unfamiliar individuals to intrude on or pilfer from one another, and that individuals devote more time and energy to defending against unfamiliar individuals than familiar neighbours. Most studies of the dear enemy phenomenon test these predictions by exposing a territory owner to a cue from a neighbouring individual or a stranger and comparing the intensity of the owner's responses. These experiments are simple to perform and, if territory owners respond more intensely to an unfamiliar cue, provide evidence that individuals can discriminate based on the experimental cue. However, these studies are more directly testing the ability of territory owners to recognize individuals, rather than the territorial plasticity that forms the foundation of the dear enemy phenomenon. There may be a separate cue than that used in the experiment to which territory owners respond when interacting outside of the experimental context. Studies such as these also cannot test whether there is a lower risk of intrusion among familiar individuals, a key component of the dear enemy phenomenon.

### *1.2 Individual recognition*

The dear enemy phenomenon depends on the ability of individuals to recognize the familiarity of conspecifics in some way. For this to occur, there must be a detectable signal of familiarity between individuals. The nature of this signal varies by species: most avian studies use calls or song as the cue of individual identity (eg. Briefer et al. 2008), while most studies of territorial mammals use scent cues (eg. Müller and Manser 2007). In experimental tests of the dear enemy phenomenon, the choice of cue is based on the researcher's hypothesis of how individuals of that species recognize one another. Positive results provide evidence that

individuals can discriminate one another, but a finding of no difference between familiar and unfamiliar cues does not necessarily mean the population does not demonstrate the dear enemy phenomenon. Rather, territory owners might be using different information than that provided during the experiment to adjust their behaviour. Our understanding of the dear enemy phenomenon could be improved by testing alternative modes of recognition within species to determine whether previous findings are biologically meaningful or artifacts of experimental design.

### *1.3 Red squirrels*

North American red squirrels (*Tamiasciurus hudsonicus*) are solitary rodents that defend exclusive territories. In the northern boreal forest, these territories are centred on hoards of their primary food source, white spruce (*Picea glauca*) cones, known as middens (Boutin and Schweiger 1988). A territory with cached resources is required for overwinter survival (Larsen and Boutin 1995). Juveniles generally acquire a territory before their first winter and remain on the same territory throughout their life. Adult dispersal to new territories is rare, but breeding females may move to another territory and bequeath their own midden to one of their juveniles (Berteaux and Boutin 2000). Red squirrels primarily defend their territories through vocalizations known as rattles, and direct physical encounters are rare (Smith 1968, Dantzer et al. 2012). The bioacoustic measures of these calls, such as fundamental frequency and pulse rate, are consistent within individuals (Wilson et al. 2015). Rattles thus provide social information that could be used for individual recognition and discrimination, as red squirrel rattles are individually distinctive (Digweed et al. 2012, Wilson et al. 2015). The other common red squirrel vocalizations are alarm calls known as barks, but these are highly variable in structure and produced in a wide range of situations in response to both predators and conspecifics (Digweed

and Rendall 2009), and as such are not likely to be a relevant cue for discrimination of familiarity.

Red squirrel territorial defense is plastic in response to local density (Dantzer et al. 2012), and to the identity of the individuals in their local neighbourhood (Siracusa et al. in review). The dear enemy phenomenon is well demonstrated for this species: squirrels that have been territorial neighbours for longer periods of time are less likely to intrude on each others' territories (Siracusa et al. 2017a), and red squirrels increase their territorial defence behaviour toward unfamiliar individuals (Siracusa et al., in review). Increased territorial defense can be costly in both time and energy in this species (Stuart-Smith and Boutin 1994), so squirrels in relatively familiar neighbourhoods benefit by reducing defensive effort in response to the lower risk of intrusion in these neighbourhoods.

The territorial function of rattles has been empirically demonstrated with a speaker replacement experiment where broadcasting the owner's rattle reduced the risk of intrusion compared to silence (Siracusa et al. 2017b). Behavioural observations of squirrels found that the scale at which the social environment best predicts squirrel behaviour is 150m around the focal territory (Dantzer et al. 2012), similar to the reported maximum audible distance of a rattle, 130m (Smith 1978). Red squirrels are sensitive to the acoustic environment around their territory, but rattles may convey more information than just the presence or number of neighbouring conspecifics.

Although the dear enemy phenomenon has been clearly shown for this species, the cue used to recognize the familiarity of conspecifics and adjust behaviour accordingly is unknown. Tests of the dear enemy phenomenon in mammals commonly use scent cues (Rosell and Bjørkøyli 2002, Raynaud and Dobson 2011, Monclús et al. 2014). Red squirrels are able to

discriminate conspecifics by scent in captivity (Vaché et al. 2001), but whether this applies to territorial interactions in nature is unknown. Given the importance of rattles in territoriality (Dantzer et al. 2012) and the individual specificity of these calls (Wilson et al. 2015), an acoustic mode of familiarity discrimination in red squirrels seems probable.

#### *1.4 Hypotheses and predictions*

My project tested whether red squirrels recognize familiarity via vocalizations, facilitating the dear enemy phenomenon in this species. I hypothesized that the mechanism by which squirrels discriminate between familiar and unfamiliar individuals and adjust their territorial behaviour is via rattles. I used an experimental design involving removal of the territory holder and speaker playback of a familiar rattle, unfamiliar rattle, or silence to test two key predictions.

First, I predicted that if rattles are the cue used by squirrels in adjusting their territorial behaviour, then neighbouring squirrels would intrude at a higher rate in response to an unfamiliar rattle than to playback of that territory owner's rattle. Second, as per the dear enemy phenomenon, simulating an unfamiliar territory owner should lead to higher defensive effort by neighbouring squirrels. I predicted that rattling rates in the surrounding acoustic neighbourhood would be higher when broadcasting an unfamiliar rattle than when broadcasting the owner's rattle during these temporary removals. As well, the rate of intrusion would also be higher in response to silence than to the owner's rattle, given the defensive function of rattles as shown by Siracusa et al. (2017b).

## 2.0 METHODS

### 2.1 Study system

This study was part of the Kluane Red Squirrel Project, a long term study of a wild population of red squirrels on Champagne and Aishihik First Nations land along the Alaska Highway in the southwest Yukon (61° N, 138° W), which has been running since 1987 (McAdam et al. 2007). The study site is boreal forest dominated by white spruce (*Picea glauca*), the cones of which are cached in middens near the centre of each squirrel's territory.

As part of this project, every red squirrel living within several ~40 ha study grids was tagged with unique alphanumeric metal ear tags and monitored throughout its life. Coloured wires and pipe cleaners were threaded through the metal ear tags to allow identification of individuals from a distance. Territory ownership was determined through biannual censuses of the population every spring and fall based on live trapping and behavioural observations, so that the location of every individual's territory was recorded twice a year. As ownership was known for every individual in the population, familiarity could be measured between neighbours as the length of time from when those two squirrels first occupied neighbouring territories to the present day.

I selected an arbitrary sample of adult male squirrels ( $n = 42$ ) from two study grids (KL and SU) as focal individuals. Focal territories were separated by  $> 60\text{m}$  (mean  $\pm$  SE:  $82.9 \pm 4.1$  m), to reduce confounding effects of neighbours between trials; separation by  $> 130\text{m}$  (the audible range of the speaker) would preclude any interference of trials, but this radius prevented me from obtaining enough suitable focal individuals. I avoided using female squirrels both due to the ethical concerns of removing a mother from her pups during lactation, as well as the

potential for maternal protection to affect the intensity of territory defense independent of the social information of interest.

## *2.2 Rattle collection and processing*

I recorded rattles from each focal squirrel to use in speaker replacement trials. My speaker replacement experiment had a repeated measures design where each of the 42 individuals were temporarily removed from their territory three times, and replaced with a speaker broadcasting their own rattle, an unfamiliar rattle, or silence in a randomized order. Each individual's recorded rattles were used twice, once at their own territory as the owner treatment and once at the territory of an individual on the other study grid as the unfamiliar treatment.

I deployed an audio recorder (Zoom Corporation® H2N audio recorder, Tokyo, Japan) on the midden of each focal squirrel and recorded for 24 hours. I then went through waveforms of these recordings and selected the three clearest and highest quality recordings of rattles from each individual, defined as those with minimal background noise and no interference from wind or other vocalizations. Owner rattles were distinguished from neighbours by amplitude, as neighbouring squirrels would only be rattling far from the owner's midden and thus would be at a much lower amplitude. This approach has been previously shown to reliably identify the calls of owners (Siracusa et al. in review). These three rattles were extracted and normalized to the same amplitude for every individual using Avisoft-SAS Pro software (Avisoft Bioacoustics), but were otherwise left unmanipulated. The three rattles were combined in a 21-minute audio file with seven minutes of silence separating each rattle; one rattle every seven minutes is the average natural rate for this population (Dantzer et al. 2012).

### *2.3 Speaker replacement experiment*

Focal individuals were trapped using Tomahawk traps (Tomahawk Live Trap, Tomahawk, WI, USA), and temporarily removed from their midden. Squirrels were placed in a modified box ( $41 \times 17.5 \times 19$  cm) and provided with a small amount of peanut butter and a slice of apple for food and hydration (Donald and Boutin 2011). The box was placed in a sheltered location away from other squirrel middens, and disinfected with alcohol between removals. All trials were conducted from May to August between 7am – 12pm, the main activity period of squirrels during the summer months (Studd et al. 2016). Removals were not conducted during precipitation or high winds, as squirrels tend to be less active during these weather conditions (Williams et al. 2014).

Once the owner was removed from its territory, I set up an SME-AFS field speaker (playback range 0.1 – 22.5 kHz, Saul Mineroff Electronics) on the centre of the midden. The speaker played one of three treatments: the owner's rattle, an unfamiliar rattle, or silence. Rattles were broadcast from this speaker at natural levels of 65–75 dB (Shonfield et al. 2017) measured 2m from the speaker using a digital sound level metre (RadioShack model 33-2055A). I also deployed a Zoom recorder on the focal midden, to record the rattling behaviour of neighbouring individuals throughout the removal. I also manually noted the time and approximate location of any audible neighbour rattles during each trial, as the Zoom recorder could not identify from which direction each rattle was emitted.

Each squirrel was temporarily removed three times, with the treatments performed in randomized order for each individual, separated by 21 - 48 days (median = 28). As dispersal between the two study grids is low, and no squirrels were within 130m of a squirrel on the other study grid, squirrels were paired so that each playback file was used twice, once at their own

territory and once as the unfamiliar treatment at a territory on the other study area. The playback audio files were given 5-digit numerical names so that the identity of the rattle was not known when playing the files and collecting data in the field. The audio treatments during temporary removals were thus partially blind, because the researcher performing the removals did not know if it was the owner or an unfamiliar rattle playing, but the silent trials were identifiable.

I observed the focal territory from >10m away and recorded the time and identity of the first squirrel to step over the edge of the midden, or travel through trees to cross this same threshold. The edge of the midden was defined by the extent of visible cone bracts on the ground. Removals ended after the first intrusion or, if no intrusion occurred, after a maximum latency of two hours as for previous speaker replacement experiments in this population (Donald and Boutin 2011, Siracusa et al. 2017a). Upon completion of the trial, the owner was returned to the midden and released, and the speaker was removed. This research was approved by the University of Guelph Animal Care Committee (AUP 1807).

When performing a temporary removal experiment, the owner is prevented from calling to defend its territory; the treatment is effectively removing their territorial vocalizations from the acoustic environment, and replacing these vocalizations at a hypothesized rate with a speaker. To know the effective magnitude of this treatment, it is important to know actual vocalization rates in the study population during the experiment. To characterize the social context of the population in which I conducted my study, I deployed Zoom recorders as described above in *Rattle collection and processing* on the middens of other squirrels (n = 42) throughout the summer. These audio recordings were analyzed as described in Siracusa et al (in review) (modified to include the hours of 08:00 – 13:00 rather than 07:00 – 13:00 due to reduced

temporal coverage in 2017) to obtain an average number of rattles per individual throughout the morning active period during which I conducted my speaker replacements.

#### *2.4 Long term data*

As part of the Kluane Red Squirrel Project, the age, sex, and history of territory ownership for every tagged individual in the population was known. I identified every squirrel living within acoustic range of the focal individuals during the experiment, based on the 130m range of red squirrel rattles. By identifying the earliest census in which the focal squirrel and a neighbour occupied their respective territories, I calculated pairwise familiarity with each neighbour as the number of days that those two squirrels occupied neighbouring territories. Neighbourhood density, age and sex of neighbours, and the distance of each neighbour from the focal midden were also obtained from the existing long-term KRSP data.

#### *2.5 Trial selection*

A total of 130 temporary removals were performed among 42 male squirrels. Some trials had to be discarded due to weather (rain during the trial;  $n = 6$ ) or speaker malfunctions ( $n = 9$ ); excluding these, there were 115 removals across the three playback types. Of these 115 trials, there were four (one silent, one owner playback, two unfamiliar playback) in which an intruder appeared within three minutes of the removal starting. In all stimulus tracks, the first rattle in the audio track played after three minutes of silence, so in these removals an intrusion occurred prior to the first possible rattle in the recording. These trials cannot be considered as either playback, and rather than reclassifying them as silent trials – which would substantially increase the overall risk of intrusion for the silent treatment – they were removed from the analysis. Thus, the models described below include 111 temporary removals among 41 individuals: 35 owner playback, 34 unfamiliar playback, and 42 silent trials.

There were 19 trials in which an intrusion occurred, but the intruding individual was identified as a squirrel living outside the 130m radius around the removal territory. These squirrels cannot be considered to have experienced the speaker treatment equivalently to neighbouring squirrels to the removal territory. The dear enemy phenomenon would only predict differing risks of intrusion when playing an owner's call compared to an unfamiliar call if all individuals hearing these rattles broadcast were familiar with the territory owner. For squirrels outside the social acoustic neighbourhood, there is no reason to predict discrimination between these rattles because both calls are unfamiliar. Additionally, it is unclear to what extent these squirrels would have heard any rattles being broadcast as their territories lie outside the acoustic range of the speaker. Thus, all results presented below include these 19 trials redefined as not having an intrusion but with a maximum latency of when the non-neighbour intrusion occurred.

All trials were conducted between May and August 2017. Local density and familiarity for my focal squirrels ( $n = 41$ ) during this period were  $2.65 \pm 0.12$  squirrels/ha and  $495 \pm 31$  days, respectively. Individual rattling rates differed between the two study grids, with squirrels on KL rattling more than those on SU ( $32.1 \pm 9.2$  vs.  $13.5 \pm 2.1$  rattles/5 h;  $t = 2.25$ ,  $df = 40$ ,  $p = 0.03$ ). The overall average was 21.5 rattles over five hours, corresponding to one rattle every 14 minutes.

## *2.6 Statistical analysis*

To test whether the rattle playback affected the risk of intrusion, I used a Cox proportional hazard mixed effects model with the *coxme* package (version 2.2-5, Therneau 2015). This model works well with censored data, where the time to an event is recorded but in some trials the event never occurs. The binary response (did an intrusion occur?), and the latency to the event (how long did it take for the intruder to appear?), which has a maximum value of

120 minutes, are incorporated together as a single response known as a hazard function. A short latency to the event, and high probability of the event occurring, is a high hazard, whereas a low hazard corresponds to a longer latency and lower probability of the event occurring. The audio treatment was included as a three-level categorical predictor, and because there were repeated measures for the same individual, a random effect of owner ID was included to account for variation in intrusion risk among neighbourhoods.

To test whether neighbours changed their defensive behaviour in response to the different rattle playbacks, the neighbourhood rattling rate (number of rattles/min from all neighbours) during each removal was analyzed using a mixed effects linear model, testing for an effect of audio treatment while also incorporating day of year, local neighbourhood density and familiarity, and a random effect of owner ID. The number of rattles/min heard by observers during the removals was highly right skewed and was  $\log_{10}(x + 0.1)$  adjusted prior to analysis (+0.1 as three trials had zero rattles heard).

### *2.7 Post hoc analyses*

As initial neighbourhood-level analyses found no differences between owner and unfamiliar rattle playbacks on intrusion risk (see *Overall intrusion risk* results below), two sets of post hoc analyses were performed. First, several other neighbourhood covariates that are known to affect intrusion probability and intensity of defense (Siracusa et al. 2017a) were included in the models of hazard of intrusion and rattling rate. Local neighbourhood density, average neighbourhood familiarity, study grid, and day of year were added to each model, and an interaction term between each of these and the playback treatment was included to test whether responses to different playback types were dependent on one of these other factors.

The second stage of post hoc analyses considered which individual squirrels within the acoustic neighbourhood were more likely to intrude during a given trial. Red squirrel neighbourhoods are not homogenous, and neighbours have varying degrees of familiarity with each focal individual. My initial prediction that there would be more intrusions during unfamiliar rattle playback presumed that all neighbours were uniformly familiar with the removed squirrel and that all neighbours would exhibit the same dear enemy relationships. However, new neighbours or squirrels that have just joined the social neighbourhood would not have as much familiarity with the focal squirrel being removed, and so might not be expected to discriminate as strongly between the two playback types, leading to less difference between the audio treatments. Based on the study of Siracusa et al. (2017a) which found that unfamiliar neighbours were more likely to intrude than familiar neighbours within the same neighbourhood, I predicted that relatively familiar and unfamiliar neighbours would differ in their responses to the owner and unfamiliar rattle playbacks. I tested this using a Cox proportional hazard model, but rather than considering whether any squirrel intruded during the trial as above ( $n = 29$  intrusions among 111 trials), I considered every squirrel living within 130 m of the focal territory as a potential intruder and tested which of these intruded during each trial ( $n = 29$  intruders among 1559 neighbours). For each speaker replacement trial, all neighbours were considered potential intruders and coded as intruding (1) or not intruding (0) at each temporary removal. I used a Cox proportional hazard model as for the neighbourhood-level model above, but now with the “event” corresponding to a single individual intruding, rather than the trial. As removals were censored after the first intruder appeared, other neighbours for a given trial had less opportunity to intrude if another neighbour intruded first; the maximum (censored) latency for each trial was thus set as the time for the first intruder to appear, if one did. Thus, if no squirrel intruded, all

neighbours were scored as 0 for the event and 120 minutes as the maximum latency; if a neighbour intruded after 45 minutes, this neighbour was scored as a 1 for the event while all other neighbours were 0, and all squirrels had the same latency of 45 minutes. For trials with non-neighbour intruders, the maximum censored latency for all neighbours was the time to this intrusion, but all individuals were scored as 0 (not intruding).

For this within-neighbourhood analysis of intrusion risk, several individual-level factors were included in the model to investigate which characteristics made neighbours more likely to intrude than others, and whether these factors interacted with the playback treatment. I included day of year, neighbour sex, neighbour age, pairwise familiarity with the removed owner, and distance from focal midden as fixed effects, and a random effect of trial ID. Neighbour age, familiarity, and distance were all standardized as  $z$ -scores within each trial relative to the neighbourhood average. Playback type was interacted with each of these in turn.

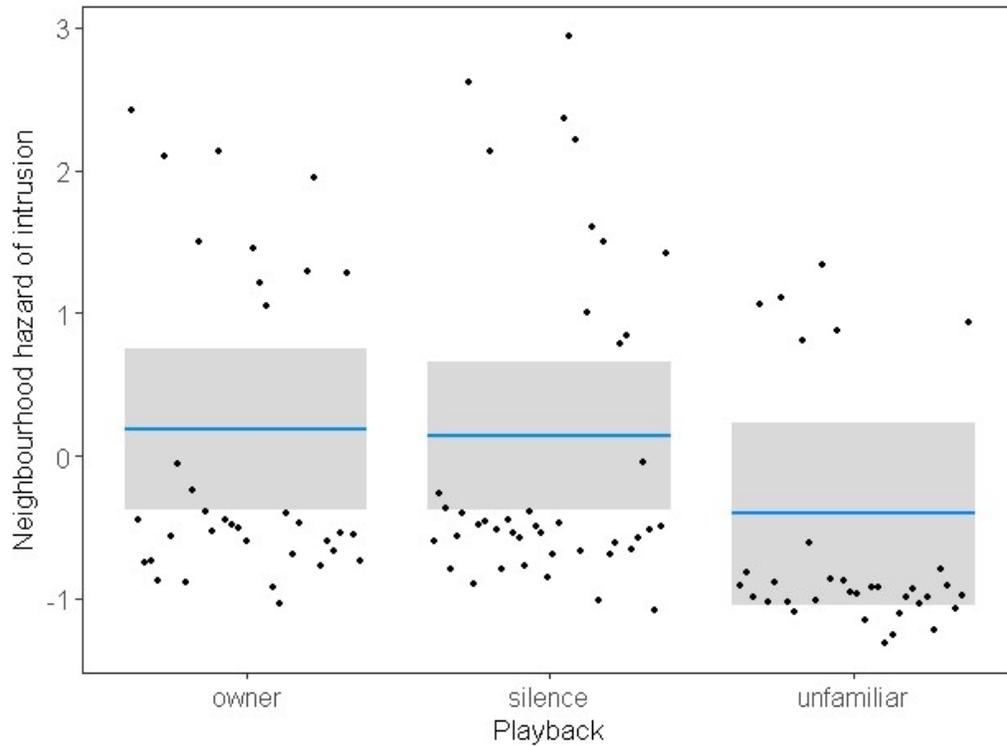
All statistical analyses were conducted using R version 3.3.3 (R Core Team 2017), using the packages *coxme* (version 2.2-5, Therneau 2015), *lme4* (Bates et al. 2015), and *visreg* (version 2.4-1, Breheny and Woodrow 2017). Reported estimates are means  $\pm$  SE.

## **3.0 RESULTS**

### *3.1 Overall intrusion risk*

There were no differences in intrusion hazard between the owner and unfamiliar rattle playbacks, or between either of these and the silent trials (Figure 1; Table 1). The overall probability of intrusion was 24%, and the proportion of trials with an intrusion did not vary between treatments (Figure 2), nor did the average latency (min) to an intruder (owner =  $43.7 \pm 7.7$ , silence =  $46.1 \pm 7.7$ , unfamiliar =  $59.5 \pm 7.9$ ; overall =  $49.1 \pm 4.6$ ). Including all intrusions,

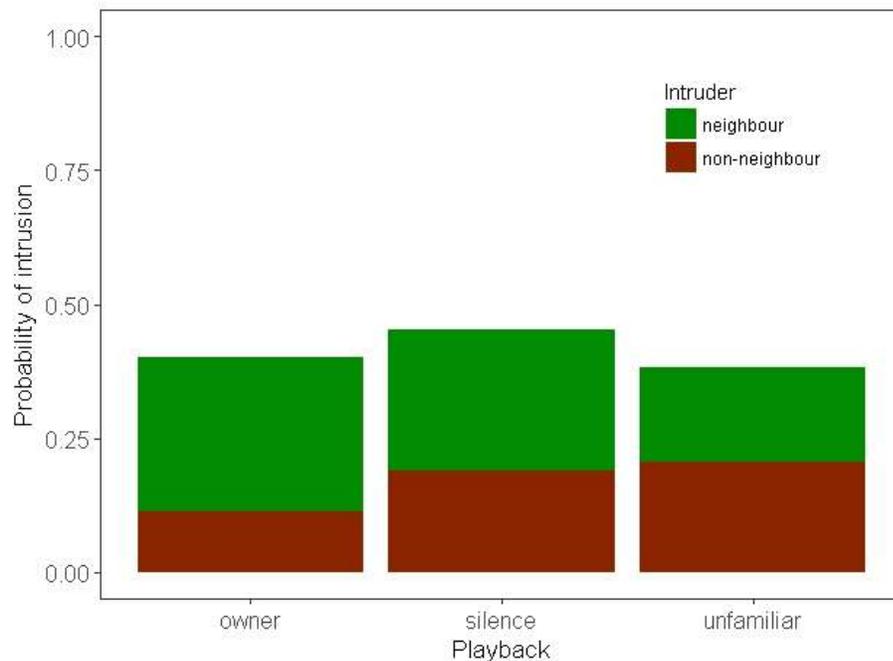
or only those from within the social neighbourhood, did not change the interpretation of the playback effect.



**Figure 1.** Hazard of intrusion from neighbouring squirrels during a temporary removal under three playback treatments. A higher hazard corresponds to a higher probability of an intrusion occurring, and a shorter latency to this event within the maximum duration of 2 hours. There were no differences in intrusion hazard between owner playback and silence ( $z = -0.15, p = 0.88$ ) or owner and unfamiliar playbacks ( $z = -1.48, p = 0.14$ ).

**Table 1.** Mixed effects Cox proportional hazard model of hazard of territory intrusion by neighbouring red squirrels testing the effect of a speaker replacement experiment. A random effect of territory owner did not improve the model beyond that including only fixed effects ( $X^2_1 = 1.64$ ,  $p = 0.20$ ), but was included to account for the repeated measures design of multiple trials at each territory ( $n = 111$  trials at 41 territories).

Fixed effects			
<i>Parameter</i>	$\beta \pm SE$	<i>z</i>	<i>p</i>
Playback: silence	$-0.068 \pm 0.446$	-0.15	0.88
Playback: unfamiliar	$-0.783 \pm 0.529$	-1.48	0.14
Random effects			
<i>Group</i>	<i>SD</i>	$\sigma^2$	
Territory	0.766	0.586	



**Figure 2.** Proportion of trials where a neighbouring (green) or non-neighbouring (brown) squirrel intruded during a 2-hour temporary removal during owner rattle playback ( $n = 35$ ), silence ( $n = 42$ ), or unfamiliar rattle playback ( $n = 34$ ).

### 3.2 Neighbourhood rattling rates

Contrary to my second prediction, the speaker playback did not affect the overall rattling rate of the neighbourhood ( $F_{2,76} = 0.267, p = 0.77$ ; Table 2). Although trials varied in length, with shorter trials when intrusions occurred, testing whether duration of the trial affected the observed rattling rate per min did not find an effect ( $t = -0.27, df = 109, p = 0.79$ ).

**Table 2.** Mixed effects linear model of neighbourhood rattling rate during a speaker replacement experiment of red squirrels. Rattling rate (rattles/min) was log adjusted prior to analysis. A random effect of territory owner did not improve the model beyond that including only fixed effects ( $X^2_1 = 1.67, p = 0.20$ ), but was included to account for the repeated measures design of multiple trials at each territory (n = 111 trials at 41 territories).

Fixed effects				
<i>Parameter</i>	$\beta \pm SE$	<i>t</i>	<i>df</i>	<i>p</i>
Playback: silence	$-0.093 \pm 0.129$	-0.19	75.5	0.47
Playback: unfamiliar	$-0.041 \pm 0.136$	-0.31	77.7	0.76
Random effects				
<i>Group</i>	<i>SD</i>	$\sigma^2$		
Territory	0.213	0.045		

### 3.3 Post hoc analyses

My post hoc prediction that neighbourhood characteristics such as average familiarity or local density would influence the risk of intrusion at a territory was not supported. None of the other neighbourhood factors had a significant effect when included in the Cox proportional hazard model (Table 3). Examination of statistical interactions between playback type and each of these covariates in turn also provided no evidence that the effect of the speaker on intrusion

hazard was dependent on any of these factors (familiarity:  $X^2 = 0.03$ ,  $df = 2$ ,  $p = 0.99$ ; date:  $X^2 = 2.76$ ,  $df = 2$ ,  $p = 0.25$ ; grid:  $X^2 = 0.87$ ,  $df = 2$ ,  $p = 0.65$ ; density:  $X^2 = 1.26$ ,  $df = 2$ ,  $p = 0.53$ ).

**Table 3.** Mixed effects Cox proportional hazard model of hazard of territory intrusion by neighbouring red squirrels testing the effect of a speaker replacement experiment along with several other covariates. Interactions between playback and each covariate were tested sequentially but are not shown as none were significant. A random effect of territory owner did not improve the model beyond that including only fixed effects ( $X^2_1 = 0.57$ ,  $p = 0.45$ ), but was included to account for the repeated measures design of multiple trials at each territory ( $n = 111$  trials at 41 territories).

Fixed effects			
<i>Parameter</i>	$\beta \pm SE$	<i>z</i>	<i>p</i>
Playback: silence	$-0.088 \pm 0.451$	-0.19	0.85
Playback: unfamiliar	$-0.773 \pm 0.529$	-1.46	0.14
Day of year	$0.002 \pm 0.008$	0.27	0.79
Local density	$0.193 \pm 0.357$	0.54	0.59
Study grid: SU	$-0.666 \pm 0.574$	-1.16	0.25
Local familiarity	$0.002 \pm 0.001$	1.21	0.23
Random effects			
<i>Group</i>	<i>SD</i>	$\sigma^2$	
Territory	0.578	0.334	

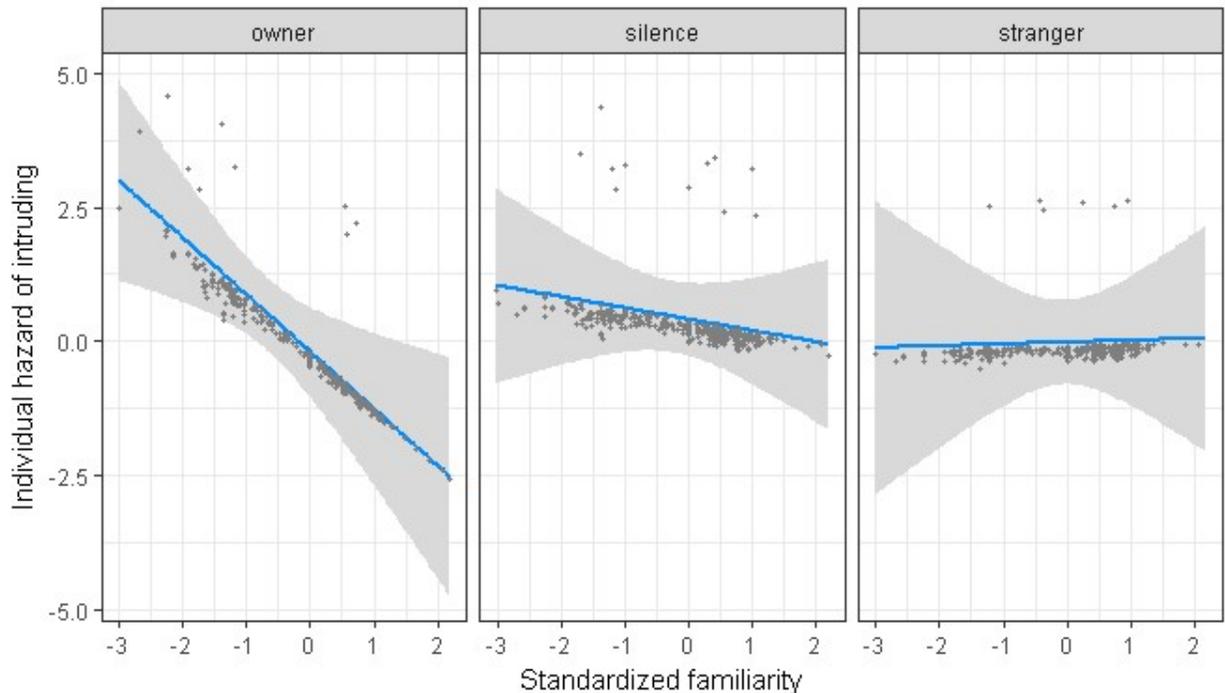
Although these neighbourhood characteristics did not predict the risk of intrusion, my post hoc prediction that these factors would influence neighbourhood rattling rates was partly supported (Table 4). Local density had a positive effect on neighbourhood rattling rate; with more neighbours around the removal midden, there were more audible neighbour rattles. After accounting for the effect of local density, there were also more rattles on KL grid than on SU, suggesting that there are underlying differences in the behaviour of the squirrels in these areas

beyond the differences in population density. Neighbourhood rattling rate also increased with day of year, with more rattles later in the summer. However, average familiarity around the removal territory did not affect neighbourhood rattling rates. The average of each neighbour's own neighbourhood familiarity ( $t = 0.67$ ,  $df = 109$ ,  $p = 0.50$ ) and density ( $t = 0.68$ ,  $df = 109$ ,  $p = 0.50$ ) also did not affect rattling rate during the speaker replacement. There was no significant effect of playback with the inclusion of these covariates in the model ( $F_{2,104} = 0.37$ ,  $p = 0.69$ ), and none of the interactions between the playback type and each of these factors were significant.

**Table 4.** Linear mixed effects model of log adjusted neighbourhood rattling rate, considering other neighbourhood characteristics in addition to speaker playback type. Interactions between playback and each covariate were tested sequentially but are not shown as none were significant. A random effect of territory owner did not improve the model beyond that including only fixed effects ( $X^2_1 = 0$ ,  $p = 1$ ;  $\Delta AIC$  of mixed effect vs. fixed = 54.9), but was included to account for the repeated measures design of multiple trials at each territory ( $n = 111$  trials at 41 territories).

Fixed effects				
<i>Parameter</i>	$\beta \pm SE$	<i>t</i>	<i>df</i>	<i>p</i>
Playback: silence	$-0.065 \pm 0.116$	-0.56	103	0.58
Playback: unfamiliar	$-0.110 \pm 0.123$	-0.90	103	0.37
Day of year	$0.005 \pm 0.002$	2.74	103	0.007
Local density	$0.214 \pm 0.084$	2.53	103	0.013
Study grid: SU	$-0.366 \pm 0.126$	-2.89	103	0.005
Local familiarity	$0.0003 \pm 0.0003$	1.12	103	0.26
Trial duration	$0.0009 \pm 0.001$	0.69	103	0.49
Random effects				
<i>Group</i>	<i>SD</i>	<i>Variance</i>		
Territory	0.000	0.000		

Overall, closer neighbours had a higher hazard of intrusion than more distant neighbours, and there was a trend for younger neighbours to intrude more than older squirrels (Table 5). Male and female neighbours had the same risk of intruding, and day of year had no effect on hazard of intruding by individual neighbours. Notably, the response of neighbours to each playback type depended on their familiarity with the removed owner: more and less familiar neighbours responded differently to the playback treatments (Figure 3). Neighbours of any familiarity were equally likely to intrude during silent removals (slope =  $-0.16 \pm 0.34$ ,  $p = 0.63$ ) and unfamiliar rattle playbacks (slope =  $-0.07 \pm 0.49$ ,  $p = 0.88$ ). However, familiar and unfamiliar neighbours had differing responses to the owner playback: familiar neighbours refrained from intruding when broadcasting the owner's call, but unfamiliar neighbours were more likely to intrude (slope =  $-1.12 \pm 0.39$ ,  $p = 0.004$ ).



**Figure 3.** Relative hazard of intrusion by individual neighbouring squirrels during a temporary removal, under three audio treatments. Intrusion hazard is modelled using a mixed effects Cox proportional hazard model, accounting for the latency to an intrusion event and whether or not

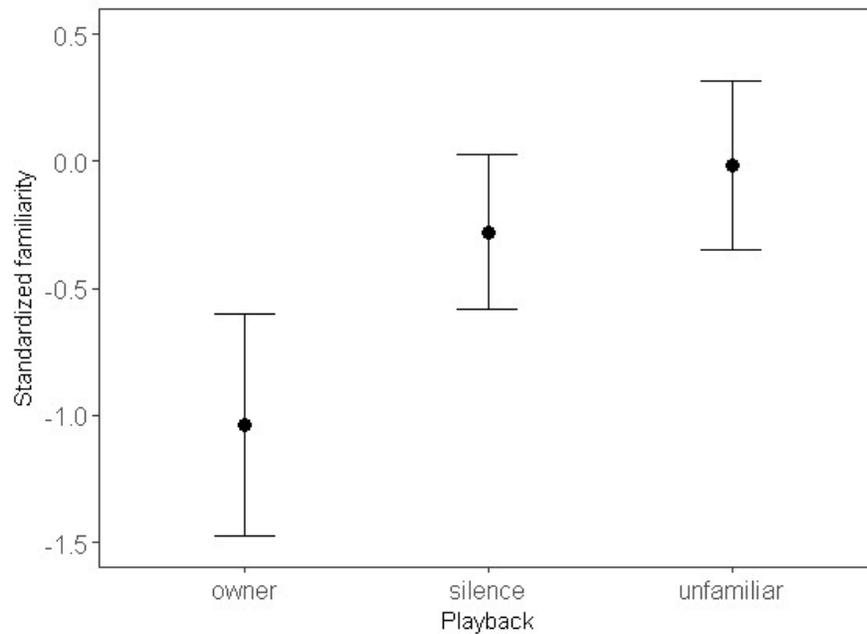
one occurred within the two-hour temporary removal. There was a higher hazard of intrusion from relatively unfamiliar neighbours when broadcasting the owner's rattle ( $\beta = -1.05$ ,  $z = -2.82$ ,  $p = 0.005$ ), whereas relative familiarity did not affect the hazard of intrusion during silence ( $\beta = -0.16$ ,  $z = 0.49$ ,  $p = 0.63$ ) or unfamiliar rattle playbacks ( $\beta = 0.07$ ,  $z = 0.15$ ,  $p = 0.88$ ).

**Table 5.** Mixed effects Cox proportional hazard model testing individual hazard of intrusion among neighbouring red squirrels during a speaker replacement experiment ( $n = 1513$ ). A random effect of territory owner did not improve the model beyond that including only fixed effects ( $X^2_1 = 0.7$ ,  $p = 0.40$ ), but was included to account for the repeated measures design of multiple trials at each territory.

Fixed effects			
<i>Parameter</i>	$\beta \pm SE$	<i>z</i>	<i>p</i>
Playback: silence	0.604 $\pm$ 0.600	1.01	0.31
Playback: unfamiliar	-0.005 $\pm$ 0.656	-0.01	0.99
Neighbour age	-0.311 $\pm$ 0.224	-1.39	0.17
Neighbour distance	-0.025 $\pm$ 0.007	-3.50	0.0005
Neighbour sex: male	-0.224 $\pm$ 0.415	-0.54	0.59
Day of year	0.003 $\pm$ 0.007	0.34	0.74
Neighbour familiarity	-1.054 $\pm$ 0.374	-2.82	0.005
Playback:silence * Neighbour familiarity	0.837 $\pm$ 0.468	1.79	0.074
Playback:unfamiliar * Neighbour familiarity	1.079 $\pm$ 0.573	1.88	0.06
Random effects			
<i>Group</i>	<i>SD</i>	$\sigma^2$	
Territory	0.607	0.368	

The differences in response among individual neighbours between the three treatments can be elucidated by comparing the relative familiarity of intruders under the three playback types (Figure 4). When the owner's rattle was broadcast, intruders ( $n = 9$ , mean familiarity  $z$ -score =  $-1.04 \pm 0.44$ ) were relatively unfamiliar compared to other neighbours ( $t = -2.85$ ,  $df = 23$ ,

$p = 0.009$ ), while intruders during silent trials ( $n = 14$ ,  $z\text{-score} = -0.22 \pm 0.27$ ) and trials playing an unfamiliar call ( $n = 6$ ,  $z\text{-score} = -0.02 \pm 0.33$ ) were not different from the average familiarity within the neighbourhood (silence  $t = -0.84$ ,  $df = 23$ ,  $p = 0.41$ ; unfamiliar  $t = -0.03$ ,  $df = 23$ ,  $p = 0.97$ ). Squirrels intruding when the owner's rattle was broadcast were marginally less familiar than intruders during the unfamiliar playback ( $t = -1.8$ ,  $df = 23$ ,  $p = 0.08$ ).



**Figure 4.** Standardized familiarity (z-scores, relative to neighbourhood) of intruding neighbours during temporary removals when broadcasting the owner's call ( $n = 9$ ), silence ( $n = 14$ ), or an unfamiliar call ( $n = 6$ ). Standardized familiarity for intruders on the owner's rattle was different from zero ( $t = -2.85$ ,  $df = 23$ ,  $p = 0.009$ ), but this was not the case for either of the other two groups (silence:  $t = -0.84$ ,  $df = 23$ ,  $p = 0.41$ ; unfamiliar:  $t = -0.03$ ,  $df = 23$ ,  $p = 0.97$ ).

#### 4.0 DISCUSSION

Analyses at the level of individual neighbours found that red squirrels are capable of discriminating between the rattles of familiar and unfamiliar individuals, and that squirrels adjust

their behaviour in response to these social cues. However, variation among individuals based on their familiarity within the social environment, as well as annual differences in population density and composition, affected the overall strength of the dear enemy effects observed in this population and made this behavioural plasticity more difficult to detect.

My initial prediction was that broadcasting a red squirrel's rattle from its territory would reduce intrusions by neighbouring squirrels compared to either playback of an unfamiliar rattle or silence. This prediction was not supported by these analyses, nor did I find that rattling rate from surrounding territories was affected by the identity of the rattle played. However, post hoc analyses revealed that neighbouring squirrels differed in their responses to the owner and unfamiliar rattles based on their pre-existing familiarity with the owner. Familiar neighbours discriminated between the playback treatments as expected from the dear enemy phenomenon, whereas unfamiliar neighbours responded similarly to both rattles. Differing responses of familiar and unfamiliar neighbours masked any differences in intrusion risk overall at the neighbourhood level, but the identity of intruders differed between playback types (Figure 4).

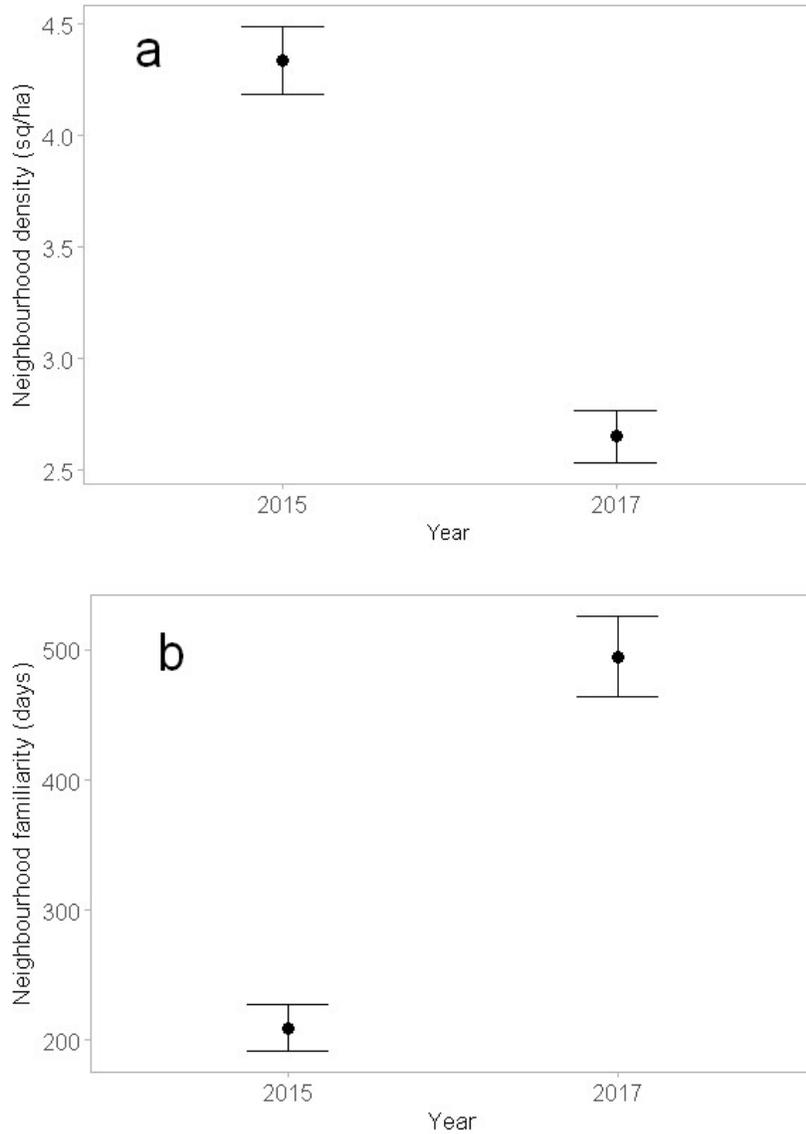
It is unlikely that censoring trials upon the first intrusion affected these relationships. If either non-neighbour intrusions or those from within the social neighbourhood happened earlier, it is possible that detecting intruders from a different source was prevented by ending the trial upon seeing the first squirrel. However, in about 60% of all trials, there were no intruders at all; if there were a masking effect preventing detection of both types of intruders at a given territory, there should be fewer removals with no intruder at all. Additionally, there was no difference between the average latencies of intrusions coming from within the neighbourhood ( $45.9 \pm 5.8$  min) and those from non-neighbours ( $53.8 \pm 7.4$  min) ( $t = 0.85$ ,  $df = 44$ ,  $p = 0.40$ ), so it is

unlikely that censoring trials when the first intruder appeared prevented detection of a later intrusion.

At first the initial tests of my predictions appeared to be contrary to previous studies documenting the territorial defense function of rattles (Siracusa et al. 2017b) and the effects of familiarity on intrusion risk (Siracusa et al. 2017a) in this system. Overall, intrusion rates in this study were lower than those observed in a previous speaker replacement study conducted in 2015 (Siracusa et al. 2017b). This previous study was conducted in fall 2015, a year immediately following a white spruce mast year in the study area. In a mast year, trees coordinate their production of cones to create a superabundance of resources, followed by several years of very low or zero cones produced so that cone availability varies by several orders of magnitude (Lamontagne and Boutin 2007). Squirrel populations track this variation, with densities ranging from 0.4 – 3.0 squirrels/ha (Shonfield et al. 2012). Recruitment of juvenile red squirrels is much higher than usual following a mast year, resulting in high densities and low familiarities in the subsequent year. Density typically declines and average familiarity increases in the years following the last mast event. The average local density and familiarity in this study were significantly lower ( $t = -8.41$ ,  $df = 94$ ,  $p = 4.5 \times 10^{-13}$ ) and higher ( $t = 8.48$ ,  $df = 94$ ,  $p = 3.1 \times 10^{-13}$ ) respectively than those in 2015 (Figure 5). These interannual differences could explain the reduction in overall intrusion risk between years, but how these factors might affect the strength of the dear enemy phenomenon in this population is less clear. The primary driver of discrimination between familiar and unfamiliar individuals is to reduce the time and energy spent on territory defense (Ydenberg et al. 1988). Red squirrels devote less effort to territory defense in neighbourhoods with fewer and more familiar neighbours (Siracusa et al, in review); when

overall defense is lower, the pressure to reduce defense is not as strong, potentially leading to weaker discrimination between familiar and unfamiliar individuals.

Most tests of the dear enemy phenomenon define familiarity as binary categories of neighbours and strangers. However, familiarity with territorial neighbours is a continuum, and in longer-lived year-round territorial species familiarity can accrue substantially. Average lifespan in this system for red squirrels that recruit into the population as adults is 3.5 years (McAdam et al. 2007), but the highest pairwise familiarity between two red squirrels in this study was 6.2 years. Other squirrels had been neighbours for less than a week prior to the temporary removal: these situations are evidently not equivalent, but both would be categorized as neighbours in a binary model. The lack of overall difference in intrusion risk between the familiar and unfamiliar rattle playback, but the clear difference in response to these treatments based on pre-existing familiarity, corroborates the necessity of considering familiarity in territorial populations as a continuous measure. The available data for territory occupancy from this long-term project allowed me to identify these individually variable responses that were masked when considering entire neighbourhoods. These more nuanced effects of individual familiarity in response to cues from neighbours might similarly obscure dear enemy relationships in other systems where familiarity has been classified as neighbour or non-neighbour. Further studies of the dear enemy phenomenon would benefit from considering individual variation within territorial populations, and the effects this variation can have on observed overall trends.



**Figure 5.** Comparison of (a) local density and (b) average familiarity (means  $\pm$  SE) within 130m of focal squirrels used for temporary removal experiments in 2015 (n = 55) and 2017 (n = 41). With two further years of very low cone production since the 2014 mast event, average familiarity in 2017 has accrued as there was minimal recruitment of juveniles during this period, while population density has fallen with the lack of new resources.

The central anomalous finding of this study, in light of the results from Siracusa et al. (2017a), is that unfamiliar neighbours failed to intrude during silent trials, but did intrude on the owner's rattle. Why these unfamiliar squirrels refrained from intruding during silence, but were

seemingly attracted by the owner's rattle, is unclear. Potentially, in a less densely populated social environment with scarce acoustic information, the presence of the owner rattle signaled to unfamiliar neighbours to the existence of a nearby midden from which they could pilfer, whereas the lack of information during silence created uncertainty that discouraged these squirrels from intruding. The motivation to intrude may also have differed, with neighbours investigating rattle playbacks not to pilfer cones from the midden, but to acquire information about the territory owner. This raises questions of the importance of silence in populations where the primary territorial defense is acoustic.

Siracusa et al. (2017b) documented a strong effect of broadcasting the owner's rattle relative to silence, reducing the risk of intrusion at the same territory by 2.5 times. This is a much stronger effect than that observed in this study, and this discrepancy can likely be explained by year to year variation in both intrusion pressure and ambient rattling rate. In addition to differences between years, these speaker replacement experiments were also conducted during different seasons, which could also affect the strength of the treatment. Siracusa et al. (2017b) conducted their trials in the fall, when neighbourhood rattling rates during removals in this study were highest ( $t = 2.71$ ,  $df = 109$ ,  $p = 0.007$ ). Under conditions where ambient rattling rate is high, such as low familiarity and high density (as in 2015), the absence of rattles from a territory becomes a meaningful source of information to neighbours that the owner is not actively defending their midden. However, squirrels on the same study grid (KL) rattled 45% less in 2017 than they did in 2015, with  $46.44 \pm 1.8$  ( $n = 85$ ) rattles per morning in 2015 vs.  $32.1 \pm 9.2$  ( $n = 18$ ) in 2017 ( $t = 2.52$ ,  $df = 101$ ,  $p = 0.013$ ). My speaker replacement used the previously reported average rattling rate of 1 call per 7 minutes (Dantzer et al. 2012), whereas the average time between rattles across both grids in 2017 was 14 minutes. A silent 2-hour removal thus

corresponds to only 8 or 9 missing rattles “removed” from the acoustic environment, rather than 17 rattles based on the playback rate. Two hours of silence from the removal territory is thus not as clear a signal of the owner’s absence, because neighbours are not expecting as much defensive effort even when the owner is present.

Speaker replacement or targeted playback experiments are often used to test the dear enemy phenomenon or the value of territorial vocalizations more generally. An important component of these studies is the temporal rate at which calls are broadcast from the speaker; this is generally based on reported average vocalization rates for the study species, but as demonstrated in this study, these rates are not fixed values and the speaker may not be simulating typical defensive behaviour in that population. Comparisons of defensive calls relative to silence on a territory are implicitly testing how neighbours will respond to silence, which is dependent on the expected amount of acoustic information present in the environment.

This speaker replacement experiment demonstrated that red squirrels are capable of discriminating familiarity in vocalizations, but did not respond behaviourally as I had predicted. My results differed from previous temporary removal and speaker replacement studies in the population, likely due to interannual differences in social composition and defensive effort. The extensive within-neighbourhood variation in familiarity documented in this system, and the individually variable responses of neighbours to familiar and unfamiliar vocalizations, reinforces the need to consider territorial familiarity as a continuous and variable trait within populations, rather than as a binary of neighbours and strangers. I also highlight the importance of silence as information in acoustically territorial populations, and how the relative value of silence depends on expected rates of vocalization.

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