Familiarity with neighbours, but not relatedness, is linked to enhanced fitness in a territorial mammal

Authors: Erin R. Siracusa^{1,2*}, Stan Boutin³, Ben Dantzer^{4,5}, Jeffrey E. Lane⁶, David W. Coltman³, Andrew G. McAdam^{1,7}

Affiliations:

¹Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

²Centre for Research in Animal Behaviour, School of Psychology, University of Exeter, Exeter, UK

³Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

⁴Department of Psychology, University of Michigan, Ann Arbor, MI, USA

⁵Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

⁶Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada

⁷Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA

*Correspondence: E. R. Siracusa, School of Psychology, University of Exeter, Exeter, EX4 4QG, UK | erinsiracusa@gmail.com | 585-245-3510

Keywords: dear-enemy, fitness, kin selection, reciprocity, red squirrel, reproductive success, senescence, social behaviour, survival, territoriality

Author Contributions: E.R.S. and A.G.M. conceived of and designed the study. E.R.S. conducted statistical analyses and wrote the manuscript with support from A.G.M. All authors contributed to field logistics, data collection and maintenance, and assisted with review and editing of the manuscript.

Abstract

Competition for limited resources is a defining feature of natural systems. Understanding the relative role of mutually beneficial behaviours versus kin selection in resolving conflict and facilitating cooperation remains a persistent enigma in evolutionary biology. Using 22 years of data from North American red squirrels, we assessed how kinship and familiarity with neighbours affected fitness in a solitary, territorial species. While living near kin did not enhance fitness, familiarity with neighbours was associated with both increased survival and annual reproductive success. These fitness benefits were strong enough to compensate for the effects of aging later in life, with potential consequences for the evolution of senescence. We suggest that such substantial fitness benefits provide the opportunity for the evolution of cooperation between otherwise adversarial neighbours, offering insight into the role that mutually beneficial behaviours might play in facilitating and stabilizing social systems.

Introduction

One of the outstanding questions in evolutionary biology is the extent to which mutualistic benefits and kin-selection can facilitate the evolution of cooperation through mitigation of conflict between interacting organisms (Clutton-Brock 2002). The indirect fitness benefits gained from associating with kin are well established as an important pathway to conflict resolution (Hamilton 1964), however, other pathways to the evolution of cooperation may be more common than previously thought (Clutton-Brock 2002). It has been widely demonstrated that mutually beneficial interactions in the form of stable social bonds among conspecifics can play an important role in mitigating intragroup conflict and enhancing fitness in group-living organisms. For example, stable social relationships are known to be associated with reduced stress levels (Engh *et al.* 2006; Wittig *et al.* 2008), enhanced longevity (Silk *et al.* 2010; Brent *et*

al. 2017), and increased offspring production and survival (Silk 2003; Cameron *et al.* 2009; Vander Wal *et al.* 2015). Social connectedness is also positively associated with human health and longevity (Holt-Lunstad *et al.* 2010), and, in particular, may be important in later life for mitigating effects of aging (Steptoe *et al.* 2013).

While the majority of research has focused on group-living species, where much conflict resolution has already taken place, the question of how cooperation can first be favored in solitary species where interactions are primarily agonistic, remains a key question. Kin can help to mitigate territorial aggression and enhance fitness (Viblanco *et al.* 2010), but there is also potential for stable interactions between neighbours to play an important role in helping to resolve conflict over territorial space (Grabowska-Zhang *et al.* 2012; Bebbington *et al.* 2017). Although physical contact with conspecifics is typically limited, territorial individuals, like their group-living counterparts, engage in regular recurring interactions with neighbouring individuals, often through auditory signaling or olfactory signatures, which are central to the establishment of territorial boundaries (Nowicki *et al.* 1998; Siracusa *et al.* 2017b). It is well understood that familiarity among long-term neighbours allows for the formation of stable social relationships that can facilitate reduced aggression and territory defence, a phenomenon known as the ‘dear enemy’ effect (Temeles 1994). Given that these stable social relationships help to minimize negotiation of territory boundaries and alleviate costs of territoriality for both individuals, social familiarity is illustrative of a mutualism-based pathway to conflict resolution. Familiarity with neighbours is known to have benefits for a variety of individual traits including faster growth rates (Liebgold & Cabe 2008), enhanced body condition (Seppä *et al.* 2001) and increased reproductive success (Beletsky & Orians 1989; Grabowska-Zhang *et al.* 2012).

Owing to the kin-structured nature of many animal societies, it has been difficult for previous research to distinguish between the relative roles of kin selection and mutually beneficial interactions in reducing conflict and facilitating cooperation. In many study systems where the importance of social relationships have been investigated, interactions typically take place between related individuals (Silk 2003; Brent *et al.* 2017). Similarly, studies that look at the benefits of relatedness within a social group (Silk 2007) may inherently be confounded by affiliative relationships among stable social partners. As such, the benefits of kinship and stable social relationships have rarely been parsed in the same system and so the relative importance of each mechanism is difficult to assess (but see Bebbington *et al.* 2017).

Here we used a natural population of North American red squirrels (*Tamiasciurus hudsonicus*) to assess the importance of familiarity with neighbours for resolving conflict over territorial space and thereby enhancing reproductive success and survival, while simultaneously accounting for effects of kinship. Red squirrels are arboreal rodents in which both sexes defend exclusive territories (Smith 1968). Territories are important for overwinter survival (LaMontagne *et al.* 2013) and are defended through vocalizations called ‘rattles’ (Siracusa *et al.* 2017b), which are uniquely identifiable (Wilson *et al.* 2015). Juveniles typically disperse 100 m on average and females are known to bequeath all or part of their territory to offspring (Berteaux & Boutin 2000). As a result, neighbours are sometimes closely related and have been shown to exhibit affiliative behaviour with kin. For example, red squirrels communally nest preferentially with kin (Williams *et al.* 2013) and adopt orphaned pups from related neighbours (Gorrell *et al.* 2010). Red squirrels also have potential to establish long-term familiarity with neighbours since individuals rarely relocate to a vacant territory following natal dispersal (Larsen & Boutin 1995). Squirrels regularly interact with these territory neighbours through auditory signals (Dantzer *et al.*

2012; 2013). Such reoccurring interactions mean that red squirrels establish social relationships with nearby conspecifics that have the potential to shape behaviour and fitness. Previous research has demonstrated, for instance, that neighbours can recognize one another's calls (Robertson *et al.* 2019) and that the risk of territory intrusion (Siracusa *et al.* 2017a) and the time spent on territory defence (Siracusa *et al.* 2019) is reduced among long-term (familiar) neighbours.

Given these benefits of established social relationships with familiar neighbours, in this study we tested whether red squirrels that were more familiar with neighbouring conspecifics had increased annual reproductive success (ARS) and a higher probability of surviving to the following year, than squirrels that were less familiar with their social neighbourhood (i.e. squirrels within a 130 m radius; Fig. 3). Given that in humans, strong social connections in old age have been demonstrated to reduce mortality risk and enhance longevity (Steptoe *et al.* 2013), we also tested whether familiar social relationships might help to buffer organisms against the effects of senescence. To do this, we looked for effects of familiarity on survival and reproductive success in red squirrels specifically during the 'senescent' period (squirrels ≥ 4 years old; McAdam *et al.* 2007; Descamps *et al.* 2008). We tested these hypotheses using 22 years of data from a natural population of red squirrels in the Yukon, Canada. Through genetic analysis and monitoring of red squirrel pups in the natal nest we were able to establish a long-term pedigree, allowing us to assess effects of social familiarity while simultaneously accounting for relatedness. Our findings demonstrate that, even in the absence of kin benefits, stable social relationships with territory neighbours are associated with substantial fitness benefits that may offset the negative effects of aging.

Materials and Methods

Study population

We studied a wild population of North American red squirrels located in the southwest Yukon, Canada (61° N, 138° W). This population has been followed since 1987 on two 40-ha study grids separated by the Alaska Highway (‘Kloo’ and ‘Sulphur’). We monitored squirrels annually from March to August and used a combination of live-trapping and behavioural observations to assess territory ownership, track reproduction and survival, and determine offspring recruitment (Berteaux & Boutin 2000; McAdam *et al.* 2007). We trapped squirrels using Tomahawk live-traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) baited with peanut butter. During their single-day oestrous, female red squirrels mate with multiple males and produce multiply-sired litters (Lane *et al.* 2008), with an average of three pups per litter (Humphries & Boutin 2000). We monitored the reproductive statuses and parturition dates of females through palpation, mass changes, and evidence of lactation. After parturition, we used radio telemetry and/or behavioural observations to follow females to their nests. We fitted pups with unique alphanumeric metal ear tags (Monel #1; National Band and Tag, Newport, KY, USA) at 25 days old, allowing us to follow individuals throughout their lifetimes.

Measuring familiarity

We completed a full census of the population twice annually in May and August and determined territory ownership using the aforementioned methods. We defined each squirrel’s social neighbourhood to be all conspecifics whose middens (i.e. the center of the territory) were within a 130 m radius of the focal squirrel’s midden (Fig. 3). One hundred and thirty meters is the farthest distance that red squirrel territorial vocalizations (‘rattles’) are known to be detectable to the human ear (Smith 1978), and is similar to the distance at which red squirrels have been demonstrated to be most responsive to changes in local density (150 m; Dantzer *et al.* 2012). This therefore suggests that 130 m is a reasonable measure of the distance at which squirrels can

receive and respond to information about their social environment. We measured the pairwise familiarity between the territory owner and each neighbour as the number of days that both individuals had occupied their current territories within 130 m of each other. We then averaged all pairwise measures of familiarity to obtain a measure of each individual's average familiarity with its neighbours. The timing of our semi-annual censuses meant that we could only update each squirrel's familiarity with its neighbours twice per year. Therefore, an individual's average familiarity could increase by 90 days if all neighbours were maintained from the May census to the August census, and by 270 days if all neighbours were maintained from August to May of the following year.

Measuring relatedness

We temporarily removed juveniles from their natal nest immediately following parturition to weigh, sex, and take ear tissue samples. Observing pups in the natal nest also allowed us to assign maternity with certainty. Starting in 2003, we determined paternity by genotyping all individuals at 16 microsatellite loci (Gunn *et al.* 2005) and assigned paternity with 99% confidence using CERVUS 3.0 (Kalinowski *et al.* 2007). Genotyping error based on known mother-offspring pairs was 2%. Additional details on the genetic methods can be found in Lane *et al.* (2008). Using the established multigenerational pedigree, we calculated the coefficient of relatedness between the territory owner and each neighbour. We averaged all pairwise measures of relatedness to provide a measure of the average relatedness between each squirrel and all of its neighbours.

Fitness measures

For individuals alive between 1994 and 2015, we measured annual survival as the probability of surviving to the following year ($N = 2346$ records over 1009 individuals). Given that long-term monitoring of individuals in this population first began in 1987, we excluded data prior to 1994 to ensure accurate measurement of familiarity between neighbours. Our ability to successfully redetect adults in the population each year is estimated to be 1.0 (Descamps *et al.* 2009).

We measured female ARS as the number of pups recruited each year (i.e. surviving overwinter). We included data from 450 breeding females between 1994 and 2015 ($N = 981$ records). All females with a known parturition date were considered to have bred; all other non-breeding females were excluded from analysis. Since red squirrel males do not contribute to the raising of pups and the male's social environment is, therefore, unlikely to affect pup recruitment, we defined male ARS as the number of pups sired each year. We used data from 207 males between 2003 and 2014 ($N = 412$ records). We excluded male reproductive success outside of this timeframe because paternity data was not available prior to 2003 and after 2014. For all analyses we only used data for adults (≥ 1 year old) whose age could be assigned with certainty (i.e. individuals tagged in nest as juveniles).

It is possible that our ability to accurately assess recruitment is somewhat confounded by dispersal beyond the borders of our study grids (Martinig *et al.* 2020). On average, juveniles typically disperse less than 100 m (Berteaux & Boutin 2000; Cooper *et al.* 2017), and estimates of juvenile survival do not differ between the center and edge of our study grids, which might be expected if dispersing juveniles were mistakenly considered to have died (McAdam *et al.* 2007). However, 37% of our population is comprised of immigrants from outside our study grids (Martinig *et al.* 2020), which suggests that some juvenile recruitment outside the study area is likely to have been missed by our methods.

Ethical note

This study required trapping individuals using Tomahawk live traps to determine territory ownership and assess reproductive status. Traps were checked every 60-90 min and squirrels were never left in a trap for longer than 120 min. We also entered the natal nest when pups were 1-2 days old and 25 days old to collect DNA, measure pup growth, and tag individuals. We returned the pups to the natal nest immediately after processing to minimize time spent away from the dam. These procedures had no detectable negative effects on the survival or welfare of the study animals. This research was approved by the University of Guelph Animal Care Committee (AUP number 1807).

Statistical analyses

Annual survival and ARS

To assess the effects of familiarity on ARS and survival, we used generalized linear mixed effects models (GLMMs) with a BOBYQA optimizer. We used a binomial distribution (logit-link) to assess probability of annual survival and a Poisson distribution (log-link) for male and female ARS. For all models we fitted average familiarity, a linear and quadratic term for age, grid density, and average relatedness as continuous predictors and included study grid as a categorical fixed effect (Table 1). For the survival models we checked for an effect of sex, but sex was not significant and so was not considered further. We included a random effect of squirrel ID and year to account for repeated measures of individuals and temporal variation in resource availability, respectively (Table S1). To account for inherent spatial structure in our data, we grouped squirrels into 150 m squares (within each year) based on their known spatial locations and included this ‘spatial ID’ as a random effect in all of our models. Models for the

senescent period included the same covariates as the full models described above, but only included a linear effect of age.

Mechanisms underlying male reproductive success

We conducted a post-hoc analysis to reveal potential mechanisms by which familiarity with neighbours might lead to increased male reproductive success. We had two hypotheses: 1) that familiarity with neighbours leads to increased energetic resources, allowing males to travel farther to mate or 2) that familiarity with females provides males with more mating opportunities within the males' social neighbourhood. To test hypothesis one, we assessed whether familiarity with neighbours affected the farthest distance that a male traveled to mate using a linear mixed effect model (LMM) with average familiarity, average relatedness, age, and grid density as continuous fixed effects and squirrel ID and year as random effects (Table S2, S3). To test hypothesis two, we measured whether average familiarity affected the number of pups that a male sired inside or outside his neighbourhood. We fitted both these models using a Poisson GLMM with the same fixed and random effects structure as above.

Data analysis

We conducted statistical analyses using R version 3.4.1 (R Core Team 2017) and fitted models using the lme4 package (version 1.1-13; Bates *et al.* 2015). We obtained *P* values for the LMMs using the package lmerTest (version 3.0-1; Kuznetsova *et al.* 2017). For all models, we checked for significant non-linearities between the predictor and response variables by fitting generalized additive models. For GLMMs we checked for overdispersion by assessing whether the sum of squared Pearson residuals approximated a Chi-squared distribution with *N-P* degrees of freedom (Bolker *et al.* 2009). Of the 1009 squirrels used in this analysis 12 individuals were not in the

pedigree, meaning there were 25 neighbourhoods for which we could not calculate relatedness with neighbours. We assigned these neighbourhoods the mean average neighbourhood relatedness ($r = 0.05$). To facilitate direct comparison of effect sizes (Schielzeth 2010) we standardized all continuous predictors to a mean of zero and unit variance. We estimated all effect sizes by using first and third quartile familiarity values while holding all other variables constant at their mean and using ‘Kloo’ as the study grid. We present all means $\pm$ SE, unless otherwise stated, and consider all differences statistically significant at $\alpha < 0.05$.

Results

Survival

We analyzed 2346 records of annual survival from 1009 squirrels. Over the 22-year period in which we analyzed survival (1994-2015), average familiarity ranged between 0 and 1186 days (mean = 191 ± 4 days) and average relatedness ranged between 0 and 0.5 (mean = 0.05 ± 0.001). We found no effect of average relatedness of the social neighbourhood on male or female survival in either the full models or senescent models (all $|z| < 1.21$, all $P > 0.22$; Table 1). Based on the full models, increased familiarity with neighbouring individuals was associated with a 6% increase in male and female survival ($\beta = 0.18 \pm 0.06$, $z = 2.78$, $P = 0.005$; Table 1). In the senescent period (≥ 4 years old; $N = 512$ records over 288 individuals), living near familiar neighbours was associated with a 17% increase in the probability of annual survival for both sexes ($\beta = 0.45 \pm 0.11$, $z = 3.40$, $P < 0.001$; Fig. 1; Table 1). For example, squirrels with high familiarity with their neighbours (500 days; third quartile) had a 69% chance of surviving to the next year, while squirrels with low familiarity with their neighbours (130 days; first quartile) had a 52% chance of surviving to the next year.

Male ARS

We analyzed 412 records of 207 males between 2003 and 2014. Over this timespan, average familiarity ranged between 0 and 1186 days (mean = 167 ± 9 days) and average relatedness ranged between 0 and 0.33 (mean = 0.04 ± 0.003). There was no effect of average relatedness of the social neighbourhood on number of pups sired in either the full models or senescent models (all $|z| < 1.48$, all $P > 0.13$; Table 1). In contrast, in full models including all data increased familiarity with neighbouring individuals was associated with a 40% increase in the number of pups sired ($\beta = 0.18 \pm 0.09$, $z = 2.04$, $P = 0.04$; Table 1). In the senescent period alone, the fitness benefits associated with increased social familiarity were even more substantial. For squirrels aged 4 and older ($N = 78$ records over 60 individuals), living near familiar neighbours was associated with an 89% increase in the number of pups sired ($\beta = 0.39 \pm 0.15$, $z = 2.67$, $P = 0.008$; Fig. 1; Table 1). Males with high familiarity with their neighbours (500 days, third quartile) sired 1.7 pups annually while males with low familiarity with their neighbours (130 days, first quartile) sired 0.9 pups annually.

Female ARS

We analyzed 981 records of 450 breeding females between 1994 and 2015. Over this period, average familiarity ranged between 0 and 892 days (mean = 213 ± 6 days) and average relatedness ranged between 0 and 0.5 (mean = 0.07 ± 0.002). We found no effect of average relatedness of the social neighbourhood on number of pups recruited in either the full models or senescent models (all $|z| < 0.80$, all $P > 0.42$; Table 1). In the full model, there was also no effect of familiarity on number of pups recruited ($\beta = 0.06 \pm 0.05$, $z = 1.38$, $P = 0.17$; Fig. 4.1). However, in the senescent period (≥ 4 years old; $N = 268$ over 170 individuals), familiarity with neighbours was associated with a 40% increase in the number of pups recruited ($\beta = 0.23 \pm 0.09$, $z = 2.52$, $P = 0.01$; Fig. 1; Table 1). Females with high familiarity with their neighbours (500

days; third quartile) recruited 0.7 pups annually compared to females with low average familiarity with their neighbours (130 days; first quartile) that recruited 0.5 pups annually.

Mechanisms underlying male reproductive success

Analysis of the spatial locations of male paternity success revealed that males traveled farther to mate as familiarity with neighbours increased ($\beta = 19.93 \pm 8.83$, $t = 2.26$, $P = 0.03$; Fig. 2). This does not appear to be due to inbreeding avoidance (see Table S2). Additionally, while the number of pups sired outside the social neighbourhood (130 m radius) was positively associated with social familiarity ($\beta = 0.39 \pm 0.10$, $z = 3.79$, $P < 0.001$; Fig. 2; Table S2), there was no association between familiarity and siring success within a male's social neighbourhood ($\beta = 0.12 \pm 0.10$, $t = 1.25$, $P = 0.21$; Table S2).

Discussion

Stable social relationships among group-living species are known to play an important role in mediating intragroup conflict and enhancing fitness (Silk 2003; Cameron *et al.* 2009; Vander Wal *et al.* 2015). For territorial species, maintaining territorial boundaries can be a costly endeavor and long-term associations among neighbours may serve an equally important role in helping to alleviate the negative effects of competition (Fisher 1954; Temeles 1994). Here we used 22 years of data from a population of territorial red squirrels to test the direct fitness effects of long-term familiarity with neighbouring conspecifics (Fig. 3). We tested for these effects while controlling for kinship to help elucidate the importance of mutualistic interactions relative to inclusive fitness benefits, the relative importance of which has previously been difficult to disentangle (c.f. Cameron *et al.* 2009; Bebbington *et al.* 2017). Our findings demonstrate that stable social relationships with neighbouring competitors are associated with increased survival

and reproductive success in solitary red squirrels, particularly in the senescent period, while relatedness with neighbours is not (Fig. 3).

While we did not find evidence that kin provide fitness benefits for red squirrels, we acknowledge that low average relatedness among neighbours (average neighbourhood relatedness = 0.05 ± 0.001) could have constrained the potential for kin selection to act in this system. We recognize that our findings do not mean that neighbour relatedness has no biologically meaningful effects on red squirrel fitness, but simply that the scale at which we attempted to measure these effects did not detect any influence of kinship. If, for instance, the primary benefits of kinship come from sharing food (Siracusa *et al.* 2017a), relatedness of nearest neighbours may be a more relevant measure to assess whether red squirrels obtain benefits from kin. Future work should attempt to use other measures of the social environment to offer additional insight as to the relative importance of kin-selection in this system. Regardless, our results demonstrate that stable social relationships are associated with increased fitness in red squirrels, independent of kinship, suggesting that inclusive fitness benefits are not a necessary prerequisite to facilitate cooperation between social partners.

Although we do not have direct evidence for the mechanism by which familiarity with neighbours might lead to increased reproductive success, previous work in this study system has shown that social familiarity results in reduced risk of pilfering (Siracusa *et al.* 2017a) and reduced time spent on territory defence (Siracusa *et al.* 2019). Conceivably then, these direct fitness benefits may result from cooperative agreements of non-intrusion among long-term neighbours, which might increase food stores or reduce time and energy allocated to territory defence. We sought to provide some mechanistic understanding of how familiarity contributes to fitness by exploring two hypotheses in male red squirrels. We first hypothesized that if

familiarity is providing an increase in energetic resources (Siracusa *et al.* 2017a; 2019) this might allow male squirrels in familiar social neighbourhoods to travel farther to obtain mating opportunities (Lane *et al.* 2009; 2010), which might explain the large observed increase in siring success associated with social familiarity. Alternatively, we hypothesized that social familiarity might directly benefit males through increased mating success within their more familiar social neighbourhoods. Our analyses revealed that males traveled farther to mate and sired more pups outside their social neighbourhood (>130 m away) when more familiar with their neighbours (those within 130 m; Fig. 2). However, we found no association between familiarity and a male's siring success within his social neighbourhood. Together, these results suggest that stable social relationships with neighbours enhance male fitness by allowing them to travel farther and increase their siring success (Lane *et al.* 2009). This might result from familiarity with neighbours increasing the energetic resources available to males through reduced territory defence (Siracusa *et al.* 2019) or reduced cache pilfering by neighbours (Siracusa *et al.* 2017a).

Our findings also demonstrate a previously unappreciated benefit of maintaining social relationships into later life. The magnitudes of the benefits of social familiarity in the senescent period were sufficient to offset age-related declines in survival and reproductive success (Nussey *et al.* 2013). Specifically, for an average 4-year old squirrel, aging by one year decreases the probability of survival from 68% to 59%. But if that individual were to maintain all of its neighbours, such that average familiarity increased by one year (from the mean familiarity of 318 days to 679 days), the maintenance of those stable social relationships would more than compensate for the change in age, resulting in an increase in survival probability from 68% to 74%. However, despite the large individual benefits of increased social familiarity late in life, few squirrels currently enjoy this fitness advantage. For example, only 4% of 4-year olds

surviving to age 5 maintain all of their neighbours, and mean familiarity does not continue to increase with age through the senescent period (Fig. S1). As a result, our observed fitness increases in later life associated with social stability are not currently widespread enough to affect the decline in the force of natural selection with age and the arrival of the mutational ‘wall of death’ (Hamilton 1966; see Supplementary Materials for methods and a more in-depth discussion of these results; Fig. S2).

Nevertheless, such individual fitness benefits of familiarity provide strong incentive for squirrels to reduce turnover in their social neighbourhood. While squirrels compete with territorial neighbours for resources (Donald & Boutin 2011), space (Smith 1968), and reproductive success (Fisher *et al.* 2017), here we have shown that individuals also benefit from the enhanced survival of neighbours. The idea that territorial neighbours might engage in cooperative behaviours to prevent neighbourhood turnover has been theorized (Getty 1987). Here our documented benefits of social familiarity provide the opportunity for the evolution of cooperative behaviour toward otherwise adversarial neighbours. Specifically, while the loss of an unfamiliar neighbour would have no effect on survival probability, the loss of a neighbour with 6 years of familiarity would decrease the owner’s probability of survival by 7%. A squirrel should, therefore, be willing to engage in behaviours that ensure their neighbour’s survival as long as this does not reduce the actor’s survival by more than 7%. This scope for cooperation is comparable to kin selection interactions between first cousins once removed ($r = 0.06$; Hamilton 1964). It is possible then that reduced aggression when interacting with familiar neighbours (i.e. the dear-enemy phenomenon; Temeles 1994) is a mechanism that not only serves to reduce an individual’s own time and energy spent on defence, but also enhances the survival of familiar neighbours, which provides indirect fitness returns through increased social familiarity. In red

squirrels, reduced territory defence toward familiar individuals (Siracusa *et al.* 2019) may convey survival benefits to neighbours through reduced aggression or lowered defense of food resources. Red squirrels have also been known to nest communally during cold temperatures (Williams *et al.* 2013), which might enhance neighbour survival. Other mechanisms by which red squirrels might reduce neighbourhood turnover such as predator alarm calling, or forming defensive coalitions (e.g. Getty 1987) to prevent a territory takeover, have yet to be tested. Ultimately, if cooperative behaviours enhancing the survival of familiar neighbours were to become widespread enough that mean social familiarity increased with age across the population, then this socially driven increase in fitness late in life could influence the force of selection and consequently the evolution of senescence (Fig. S2).

Finally, it is important that we address the potential issues associated with spatial confounds in this study. Individual squirrels are distributed fairly continuously across our study areas, so individually defined neighbourhoods are spatially overlapping. It is possible that our observed fitness effects could be confounded by spatial variation in resource availability or predation risk. In particular, areas with consistently low survival would have high turnover and hence low average familiarity locally. If this kind of spatial autocorrelation in survival were present, low neighbourhood familiarity might be linked to low survival in the absence of a causal relationship. While this is a relevant concern that warrants consideration, there are several reasons why we do not think this is likely.

First, there is no evidence of consistent spatial variation in spruce cone production (the primary food for red squirrels; Boutin *et al.* 2006; Fletcher *et al.* 2010) across years in this study system (LaMontagne *et al.* 2013). Therefore, there should not be locations that are consistently rich or poor in food resources. Second, given this lack of consistency in the spatial distribution of

resource availability, it would have been inappropriate to fit a constant spatial autocorrelation term in our models (Fisher *et al.* 2019). Fitting separate spatial autocorrelation terms for each year would have been ideal, however, it was not possible to obtain convergence from our data using this method. Therefore, as a simpler alternative, we added a spatial random effect (‘spatial ID’) to our models by grouping squirrels into 150 m squares (within each year) based on their known spatial locations (Fisher *et al.* 2019). Fitting this as a random effect allowed us to account for any similarity among squirrels within each 150 m x 150 m area in each year (Table S1). All effects of familiarity we observed were robust to its inclusion (Table 1), and our results remained unchanged even when this spatial grouping term was defined to be a 75 m or 300 m square (see Table S4).

Third, we calculated Moran’s I (ape package, version 5.1; Paradis & Schliep 2019) for each study grid and year to test whether similarity in ARS or probability of survival was related to the spatial proximity of any two individuals. We found only 9 out of 112 year/grid combinations in which the observed Moran’s I value was significantly greater than the expected value, indicating that the trait in question demonstrated positive spatial autocorrelation (Table S5). We then proceeded to remove those year/grid combinations with positive autocorrelation from our analyses and found that our conclusions remained the same when our analyses were rerun with this limited dataset (Table S6).

Ultimately all of these concerns stem from the possibility that our findings might result from among individual differences in familiarity that result from covariation with individuals or locations of high quality. By refitting our ARS models using a within-individual mean centering approach (van de Pol & Wright 2009) we were able to demonstrate why this is unlikely to be the case. We split our familiarity term into an among-individual effect (the mean familiarity score

for an individual across all observations) and a within-individual effect (the deviation in each familiarity observation for each individual from their mean score), and found that associations between familiarity and fitness are primarily driven by within individual changes in familiarity rather than among individual differences in familiarity (Table S7). In other words, it appears that some individuals do not consistently experience high familiarity environments and others low familiarity environments due to some intrinsic (individual quality) or extrinsic (habitat quality) factor. It is instead the within individual changes in familiarity that occur within an individual's lifetime that are associated with variation in fitness, resulting in the patterns observed in our data.

Conclusion

Although interactions among territorial individuals are fundamentally defined by competition for food, mates, or other resources, here we have shown that stable social relationships can limit these competitive interactions and provide important fitness benefits that might feed back to maintain strong affiliative relationships among territorial neighbours. Similar fitness benefits of social familiarity have also been demonstrated in territorial birds such as red-winged blackbirds (Beletsky & Orians 1989) and great tits (Grabowska-Zhang *et al.* 2012). Our findings, in conjunction with these previous studies, demonstrate that the benefits of stable social relationships are not exclusive to group-living organisms but can extend to solitary species, suggesting a shared evolutionary importance of stable relationships across a range of social complexities. The strength of the effects of familiarity, particularly in the senescent period, was surprising. We suggest that by increasing the force of selection later in life, stable social relationships have the potential to lead to the evolution of slower rates of aging, although these late-life social benefits are not currently widespread enough to affect senescence in red squirrels. Importantly, our results indicate that intraspecific conflict can be mitigated through mutualistic

432 pathways, with resulting fitness benefits, independent of kinship effects (see also Cameron *et al.*
433 2009; Bebbington *et al.* 2017). Consequently, stable social relationships with neighbours may be
434 an important and overlooked contributor to fitness in territorial species, and may offer insight
435 into the relative importance of mutualistic behaviours for facilitating conflict resolution.

Acknowledgments: We thank the Champagne and Aishihik First Nations for allowing us to conduct our research within their traditional territory and thank Agnes MacDonald and her family for long-term access to her trapline. We are grateful to all of the field technicians who have contributed to the long-term KRSP database over the years, and J. Kapitan at KAP Design for assistance with figure design. This research was supported by Discovery Grants and Northern Research Supplements from the Natural Sciences and Engineering Research Council of Canada (A.G.M.: RGPIN-2015-04707; S.B.: RGPIN-2014-05874; J.E.L.: RGPIN-2014-04093, RGPNS-2014-459038; D.W.C.: RGPIN-2011-312207), as well as funding from the University of Michigan (B.D.), and Grants-in-Aid of research from the American Society of Mammalogists and the Arctic Institute of North America (E.R.S.).

References:

1. Bates, D., Maechler, M., Bolker, B.M. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.
2. Bebbington, K., Kingma, S.A., Fairfield, E.A., Dugdale, H.L., Komdeur, J., Spurgin, L.G., *et al.* (2017). Kinship and familiarity mitigate costs of social conflict between Seychelles warbler neighbors. *PNAS*, 46, 201704350–15.
3. Beletsky, L.D. & Orians, G.H. (1989). Familiar neighbors enhance breeding success in birds. *PNAS*, 86, 7933–7936.
4. Berteaux, D. & Boutin, S. (2000). Breeding dispersal in female North American red squirrels. *Ecology*, 81, 1311–1326.
5. Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., *et al.* (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127–135.
6. Boutin, S., Wauters, L., McAdam, A., Humphries, M., Tosi, G. & Dhondt, A. (2006). Anticipatory reproduction and population growth in seed predators. *Science*, 314, 1928–1930.
7. Brent, L.J.N., Ruiz-Lambides, A. & Platt, M.L. (2017). Family network size and survival across the lifespan of female macaques. *Proceedings of the Royal Society B: Biological Sciences*, 284, 20170515–7.

8. Cameron, E.Z., Setsaas, T.H. & Linklater, W.L. (2009). Social bonds between unrelated females increase reproductive success in feral horses. *PNAS*, 106, 13850–13853.
9. Clutton-Brock, T. (2002). Breeding Together: Kin Selection and Mutualism in Cooperative Vertebrates. *Science*, 296, 69–72.
10. Cooper, E.B., Taylor, R.W., Kelley, A.D., Martinig, A.R., Boutin, S., Humphries, M.M., *et al.* (2017). Personality is correlated with natal dispersal in North American red squirrels (*Tamiasciurus hudsonicus*). *Behaviour*, 154, 939–961.
11. Dantzer, B., Boutin, S., Humphries, M.M. & McAdam, A.G. (2012). Behavioral responses of territorial red squirrels to natural and experimental variation in population density. *Behav Ecol Sociobiol*, 66, 865–878.
12. Dantzer, B., Newman, A.E.M., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M., *et al.* (2013). Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science*, 340, 1215–1217.
13. Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.-M. (2008). Age-specific variation in survival, reproductive success and offspring quality in red squirrels: evidence of senescence. *Oikos*, 117, 1406–1416.
14. Descamps, S., Boutin, S., McAdam, A.G., Berteaux, D. & Gaillard, J.M. (2009). Survival costs of reproduction vary with age in North American red squirrels. *Proceedings of the Royal Society B: Biological Sciences*, 276, 1129–1135.
15. Donald, J.L. & Boutin, S. (2011). Intraspecific cache pilferage by larder-hoarding red squirrels (*Tamiasciurus hudsonicus*), 92, 1013–1020.
16. Engh, A.L., Beehner, J.C., Bergman, T.J., Whitten, P.L., Hoffmeier, R.R., Seyfarth, R.M., *et al.* (2006). Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas ursinus*). *Proceedings of the Royal Society B: Biological Sciences*, 273, 707–712.
17. Fisher, D.N., Boutin, S., Dantzer, B., Humphries, M.M., Lane, J.E. & McAdam, A.G. (2017). Multilevel and sex-specific selection on competitive traits in North American red squirrels. *Evolution*, 71, 1841–1854.
18. Fisher, D.N., Wilson, A.J., Boutin, S., Dantzer, B., Lane, J.E., Coltman, D.W., *et al.* (2019). Social effects of territorial neighbours on the timing of spring breeding in North American red squirrels. *Journal of Evolutionary Biology*, 32, 559–571.
19. Fisher, J. (1954). Evolution and bird sociality. In: *Evolution as a Process* (eds. Huxley, J., Hardy, A.C. & Ford, E.B.). London, U.K., pp. 71–83.
20. Fletcher, Q.E., Boutin, S., Lane, J.E., LaMontagne, J.M., McAdam, A.G., Krebs, C.J., *et al.* (2010). The functional response of a hoarding seed predator to mast seeding. *Ecology*, 91, 2673–

- 2683.
21. Getty, T. (1987). Dear enemies and the prisoner's dilemma: why should territorial neighbors form defensive coalitions? *Integrative and Comparative Biology*, 27, 327–336.
22. Gorrell, J.C., McAdam, A.G., Coltman, D.W., Humphries, M.M. & Boutin, S. (2010). Adopting kin enhances inclusive fitness in asocial red squirrels. *Nature Communications*, 1, 1–4.
23. Grabowska-Zhang, A.M., Wilkin, T.A. & Sheldon, B.C. (2012). Effects of neighbor familiarity on reproductive success in the great tit (*Parus major*). *Behavioral Ecology*, 23, 322–333.
24. Gunn, M.R., Dawson, D.A., Leviston, A., Hartnup, K., Davis, C.S., Strobeck, C., *et al.* (2005). Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their cross-utility in other species. *Mol Ecol Notes*, 5, 650–653.
25. Hamilton, W.D. (1964). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7, 1–16.
26. Hamilton, W.D. (1966). The moulding of senescence by natural selection. *Journal of Theoretical Biology*, 12, 12–45.
27. Holt-Lunstad, J., Smith, T.B. & Layton, J.B. (2010). Social Relationships and Mortality Risk: A Meta-analytic Review. *PLoS Medicine*, 7, e1000316.
28. Humphries, M. & Boutin, S. (2000). The determinants of optimal litter size in free-ranging red squirrels. *Ecology*, 81, 2867–2877.
29. Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007). Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099–1106.
30. Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTestPackage: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1–26.
31. LaMontagne, J.M., Williams, C.T., Donald, J.L., Humphries, M.M., McAdam, A.G. & Boutin, S. (2013). Linking intraspecific variation in territory size, cone supply, and survival of North American red squirrels, 94, 1048–1058.
32. Lane, J., Boutin, S., Gunn, M., Slate, J. & Coltman, D. (2008). Female multiple mating and paternity in free-ranging North American red squirrels. *Animal Behaviour*, 75, 1927–1937.
33. Lane, J.E., Boutin, S., Gunn, M.R. & Coltman, D.W. (2009). Sexually selected behaviour: red squirrel males search for reproductive success. *Journal of Animal Ecology*, 78, 296–304.

- 34.Lane, J.E., Boutin, S., Speakman, J.R. & Humphries, M.M. (2010). Energetic costs of male reproduction in a scramble competition mating system. *Journal of Animal Ecology*, 79, 27–34.
- 35.Larsen, K.W. & Boutin, S. (1995). Exploring territory quality in the North American red squirrel through removal experiments. *Canadian journal of zoology*, 73, 1115–1122.
- 36.Liebgold, E.B. & Cabe, P.R. (2008). Familiarity with adults, but not relatedness, affects the growth of juvenile red-backed salamanders (*Plethodon cinereus*). *Behav Ecol Sociobiol*, 63, 277–284.
- 37.Martinig, A.R., McAdam, A.G., Dantzer, B., Lane, J.E., Coltman, D.W. & Boutin, S. (2020). The new kid on the block: immigrant males win big whereas females pay fitness cost after dispersal. *Ecol Letters*, 23, 430–438.
- 38.McAdam, A., Boutin, S., Sykes, A. & Humphries, M. (2007). Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Ecoscience*, 14, 362–369.
- 39.Nowicki, S., Hughes, M. & Searcy, W. (1998). The Territory Defense Function of Song in Song Sparrows: A Test with the Speaker Occupation Design. *Behaviour*, 135, 615–628.
- 40.Nussey, D.H., Froy, H., Lemaître, J.-F., Gaillard, J.-M. & Austad, S.N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*, 12, 214–225.
41. Paradis, E. & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528. [doi:10.1093/bioinformatics/bty633](https://doi.org/10.1093/bioinformatics/bty633). HAL: [ird-01920132](https://hal.archives-ouvertes.fr/ird-01920132).
42. R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- 43.Robertson, J.G., Boutin, S., Humphries, M.M., Dantzer, B., Lane, J.E. & McAdam, A.G. (2019). Individual variation in the dear enemy phenomenon via territorial vocalizations in red squirrels. *Behaviour*, 155, 1073–1096.
- 44.Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, 1, 103–113.
- 45.Seppä, T., Laurila, A., Peuhkuri, N., Piironen, J. & Lower, N. (2001). Early familiarity has fitness consequences for Arctic char (*Salvelinus alpinus*) juveniles. *Can. J. Fish. Aquat. Sci.*, 58, 1380–1385.
- 46.Silk, J.B. (2003). Social Bonds of Female Baboons Enhance Infant Survival. *Science*, 302, 1231–1234.
- 47.Silk, J.B. (2007). The adaptive value of sociality in mammalian groups. *Philosophical*

- 608 *Transactions of the Royal Society of London B: Biological Sciences*, 362, 539–559.
- 609
- 610 48.Silk, J.B., Beehner, J.C., Bergman, T.J., Crockford, C., Engh, A.L., Moscovice, L.R., *et al.*
- 611 (2010). Strong and Consistent Social Bonds Enhance the Longevity of Female Baboons. *Current*
- 612 *Biology*, 20, 1359–1361.
- 613
- 614 49.Siracusa, E., Boutin, S., Humphries, M.M., Gorrell, J.C., Coltman, D.W., Dantzer, B., *et al.*
- 615 (2017a). Familiarity with neighbours affects intrusion risk in territorial red squirrels. *Animal*
- 616 *Behaviour*, 133, 11–20.
- 617
- 618 50.Siracusa, E., Morandini, M., Boutin, S., Humphries, M.M., Dantzer, B., Lane, J.E., *et al.*
- 619 (2017b). Red squirrel territorial vocalizations deter intrusions by conspecific rivals. *Behaviour*,
- 620 154, 1259–1273.
- 621
- 622 51.Siracusa, E.R., Wilson, D.R., Studd, E.K., Boutin, S., Humphries, M.M., Dantzer, B., *et al.*
- 623 (2019). North American red squirrels mitigate costs of territory defence through social plasticity.
- 624 *Animal Behaviour*, 151, 29–42.
- 625
- 626 52.Smith, C.C. (1968). The Adaptive Nature of Social Organization in the Genus of Three
- 627 Squirrels *Tamiasciurus*. *Ecological Monographs*, 38, 31–64.
- 628
- 629 53.Smith, C.C. (1978). Structure and Function of the Vocalizations of Tree Squirrels
- 630 (*Tamiasciurus*), 59, 793–808.
- 631
- 632 54.Stepto, A., Shankar, A., Demakakos, P. & Wardle, J. (2013). Social isolation, loneliness, and
- 633 all-cause mortality in older men and women. *PNAS*, 110, 5797–5801.
- 634
- 635 55.Temeles, E. (1994). The role of neighbours in territorial systems: when are they'dear
- 636 enemies'? *Animal Behaviour*, 47, 339–350.
- 637
- 638 56.van de Pol, M. & Wright, J. (2009). A simple method for distinguishing within- versus
- 639 between-subject effects using mixed models. *Animal Behaviour*, 77, 753–758.
- 640
- 641 57.Vander Wal, E., Festa-Bianchet, M., Reale, D., Coltman, D. & Pelletier, F. (2015). Sex-based
- 642 differences in the adaptive value of social behavior contrasted against morphology and
- 643 environment. *Ecology*, 96, 631–641.
- 644
- 645 58.Viblan, V.A., Arnaud, C.M., Dobson, F.S. & Murie, J.O. (2010). Kin selection in Columbian
- 646 ground squirrels (*Urocitellus columbianus*): littermate kin provide individual fitness benefits.
- 647 *Proc. Biol. Sci.*, 277, 989–994.
- 648
- 649 59.Williams, C.T., Gorrell, J.C., Lane, J.E., McAdam, A.G., Humphries, M.M. & Boutin, S.
- 650 (2013). Communal nesting in an “asocial” mammal: social thermoregulation among spatially
- 651 dispersed kin. *Behav Ecol Sociobiol*, 67, 757–763.
- 652
- 653 60.Wilson, D.R., Goble, A.R., Boutin, S., Humphries, M.M., Coltman, D.W., Gorrell, J.C., *et al.*

- (2015). Red squirrels use territorial vocalizations for kin discrimination. *Animal Behaviour*, 107, 79–85.
61. Wittig, R.M., Crockford, C., Lehmann, J., Whitten, P.L., Seyfarth, R.M. & Cheney, D.L. (2008). Focused grooming networks and stress alleviation in wild female baboons. *Hormones and Behavior*, 54, 170–177.

Figure 1. Effects of average neighbourhood familiarity on a) annual survival (N = 512), b) male annual reproductive success (ARS; N = 78), and c) female ARS (N = 268) during the senescent period (≥ 4 years old). Shaded grey bars indicate 95% confidence intervals. Values on x-axis are standardized measures of average familiarity. Points indicate raw data with a small amount of jitter introduced to show overlapping points.

Figure 2. Effects of average neighbourhood familiarity on a) the distance that males traveled to mate and b) number of pups sired outside the social neighbourhood (i.e. 130 m radius; N = 199). Shaded grey bars indicate 95% confidence intervals. Values on x-axis are standardized measures of average familiarity. Points indicate raw data.

Figure 3. Schematic depiction of a low and high familiarity red squirrel neighbourhood with associated fitness metrics. Low and high familiarity are based on first and third quartile familiarity values. Neighbourhoods include all squirrels within a 130 m radius of the focal squirrel's midden, this reflects the acoustic social environment as 130 m is the farthest distance that red squirrel rattles (unique territorial vocalizations) carry. The biology of the system is simplified here for sake of space and clarity. On average squirrels have 13 neighbours and multiple spruce trees per territory. Familiarity with neighbours can only be updated twice per

683 year, see methods for details. Effect sizes reported here are based on models from the senescent
684 period. Confidence intervals around the predicted effects of familiarity on these three measures
685 of fitness can be found in Figure 1.

686

Table 1. Fixed effects from annual survival, male annual reproductive success (ARS), and female ARS generalized linear mixed-effects models. Models are based on the full and senescent (≥ 4 years old) datasets are shown with significant effects indicated in bold. Regression coefficients are standardized.

Data set	N	Response	Fixed effect	Parameter $\pm$ SE	<i>z</i>	<i>P</i>
Full data						
	2346	Probability of survival	Familiarity	0.18 ± 0.06	2.78	0.005
			Age	-0.27 ± 0.09	-2.94	0.003
			Age²	-0.11 ± 0.04	-2.57	0.01
			Relatedness	0.008 ± 0.05	0.15	0.88
			Density	0.10 ± 0.11	0.92	0.36
			Grid-SU	0.17 ± 0.10	1.67	0.10
	412	No. pups sired (<i>Male ARS</i>)	Familiarity	0.18 ± 0.09	2.04	0.04
			Age	0.68 ± 0.14	4.75	<0.001
			Age²	-0.41 ± 0.08	-5.32	<0.001
			Relatedness	-0.11 ± 0.08	-1.47	0.14
			Density	0.04 ± 0.12	0.37	0.71
			Grid-SU	-0.07 ± 0.19	-0.38	0.70
	981	No. pups recruited (<i>Female ARS</i>)	Familiarity	0.06 ± 0.05	1.38	0.17
			Age	0.08 ± 0.05	1.42	0.16
			Age²	-0.10 ± 0.04	-2.72	0.007
			Relatedness	0.02 ± 0.04	0.63	0.53
			Density	-0.37 ± 0.13	-2.78	0.005
			Grid-SU	-0.06 ± 0.08	-0.78	0.43
Senescent data						
	512	Probability of survival	Familiarity	0.45 ± 0.11	3.40	<0.001
			Age	-0.36 ± 0.10	-3.42	<0.001
			Relatedness	0.13 ± 0.11	1.20	0.23
			Density	0.15 ± 0.11	1.33	0.18
			Grid-SU	0.14 ± 0.21	0.68	0.49
	78	No. pups sired (<i>Male ARS</i>)	Familiarity	0.39 ± 0.15	2.67	0.008
			Age	-0.39 ± 0.19	-2.01	0.04
			Relatedness	0.05 ± 0.14	0.37	0.72
			Density	0.11 ± 0.19	0.60	0.55
			Grid-SU	0.26 ± 0.32	0.82	0.41
	268	No. pups recruited (<i>Female ARS</i>)	Familiarity	0.23 ± 0.09	2.52	0.01
			Age	-0.29 ± 0.10	-2.79	0.005
			Relatedness	0.06 ± 0.08	0.79	0.43

Density	-0.38 ± 0.17	-2.20	0.03
Grid-SU	-0.32 ± 0.17	-1.85	0.07

691

692

693

694

695

696

697

698