Familiar neighbours, but not relatives, enhance fitness in a territorial mammal

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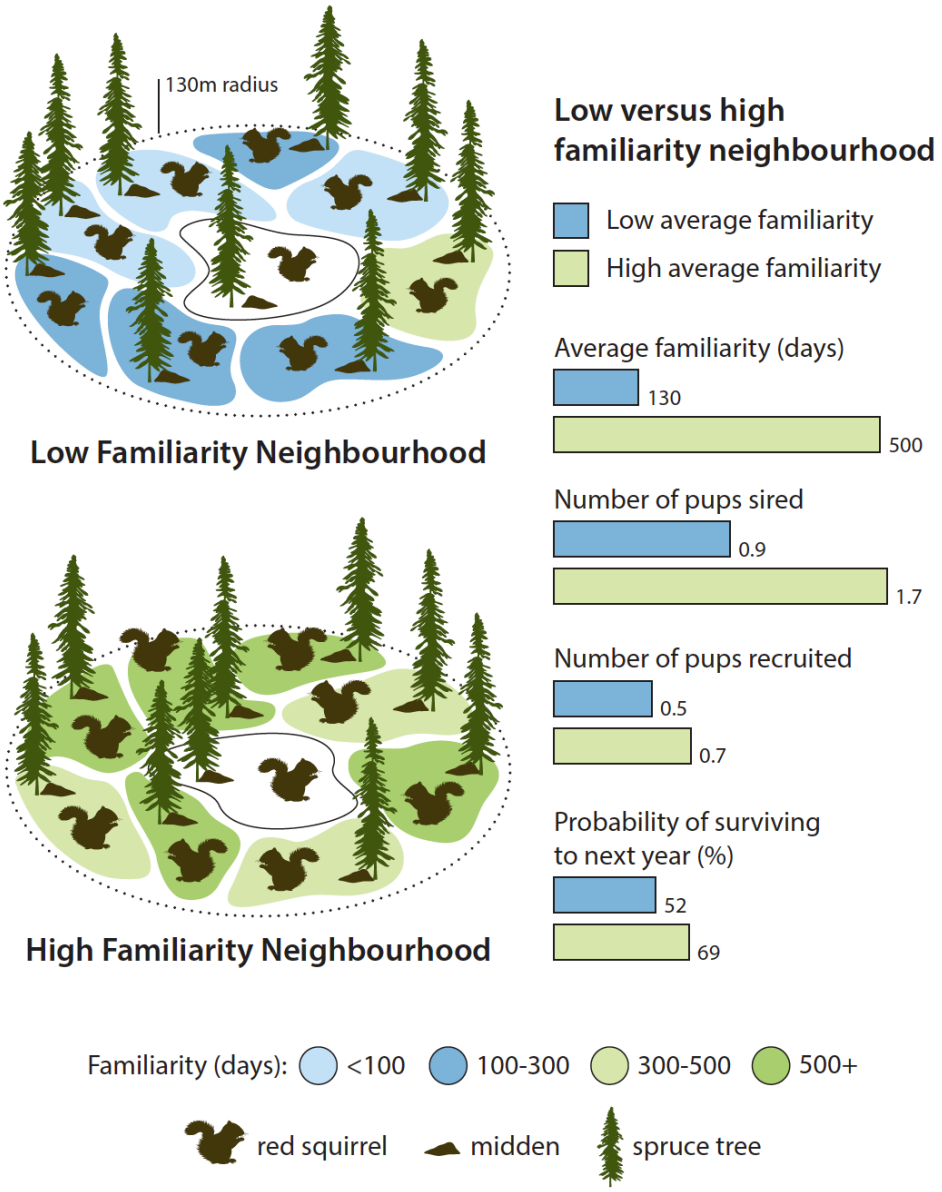
Keywords: dear-enemy, familiarity, fitness, kin selection, mutualism, reciprocity, red squirrel, reproductive success, senescence, social behaviour, survival, territoriality

Graphical Abstract



Summary

One of the outstanding questions in evolutionary biology is the extent to which mutually beneficial interactions and kin-selection can facilitate the evolution of cooperation by mitigating conflict between interacting organisms. The indirect fitness benefits gained from associating with kin are an important pathway to conflict resolution [1], but conflict can also be resolved if individuals gain direct benefits from cooperating with one another (e.g. mutualism or reciprocity) [2]. Owing to the kin-structured nature of many animal societies, it has been difficult for previous research to assess the relative importance of these mechanisms [3-5]. However, one area that might allow for the relative roles of kin-selection and mutualistic benefits to be disentangled is in the resolution of conflict over territorial space [6]. While much research has focused on group-living species, the question of how cooperation can first be favoured in solitary, territorial species remains a key question. Using 22 years of data from a population of North American red squirrels, we assessed how kinship and familiarity with neighbours affected fitness in a territorial mammal. While living near kin did not enhance fitness, familiarity with neighbours 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 adversarial neighbours, offering insight into the role that mutually beneficial behaviours might play in facilitating and stabilizing social systems.

Results and Discussion

Stable social relationships among group-living species are known to play an important role in mediating intragroup conflict and enhancing fitness [3,7,8]. For territorial species, maintaining territorial boundaries can be a costly endeavor, and while kin can help to mitigate territorial aggression and enhance fitness [9], there is also potential for stable interactions between neighbours to play an important role in helping to resolve conflict over territorial space [6,10]. 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 [11,12]. Although it is not clear whether dear-enemy relationships are stabilized by reciprocity or mutualism, by minimizing negotiation of territory boundaries and alleviating costs of territoriality for both individuals, social familiarity provides a pathway to conflict resolution through mutual direct benefits.

To assess the relative importance of kinship and familiarity for resolving conflict over territorial space and thereby enhancing reproductive success and survival, we used 22 years of data from a natural population of North American red squirrels (*Tamiasciurus hudsonicus*). Red squirrels are arboreal rodents in which both sexes defend exclusive territories year-round [13]. Territories are important for overwinter survival [14] and are defended through vocalizations called 'rattles' [15], which are uniquely identifiable [16]. Juveniles typically disperse 100 m on average and females sometimes bequeath all or part of their territory to offspring [17]. As a result, neighbours are sometimes closely related and have been shown to exhibit affiliative behaviour with kin [18,19]. Red squirrels also have potential to establish long-term familiarity with neighbours since individuals rarely relocate to a vacant territory following natal dispersal

[20]. Familiarity with neighbours is demonstrated to have important benefits for red squirrels including reduced risk of territory intrusion [21] and reduced time spent on territory defence [22].

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). 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. We used average neighbourhood familiarity and relatedness as our metrics of interest, as previous research has demonstrated that these metrics have important effects on territory dynamics and behaviour in red squirrels [21,22]. Given that stable social connections have been demonstrated to reduce mortality risk and enhance longevity in other species [23], we also assessed whether maintaining familiar social relationships into later life might help to buffer squirrels against age-related declines in fitness. 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) [24,25].

Effects of familiarity

Over the 22-year period (1994-2015) in which we analyzed survival and ARS, red squirrels, on average, had 13 neighbours. Average neighbourhood familiarity ranged between 0 and 1186 days (mean = 191 ± 4 days; CV = 1.04) and average relatedness ranged between 0 and 0.5 (mean = 0.05 ± 0.001; CV = 1.09). Familiarity was not strongly correlated with either relatedness (r = -0.09) or grid-wide density (r = -0.17). We found no effect of average relatedness of the social

neighbourhood on male or female survival, number of pups sired annually, or number of pups recruited annually in either the full models or senescent models (all $|\beta| < 0.14$, all $|z| < 1.48$, all P > 0.13; Table 1). In contrast, familiarity with neighbouring individuals was associated with an increase in male and female survival ($\beta = 0.18 \pm 0.06$, $z = 2.79$, $P = 0.005$; Table 1) and an increase in the annual number of pups sired ($\beta = 0.18 \pm 0.09$, $z = 2.04$, $P = 0.04$; Table 1), although there was no overall effect of social familiarity on the annual number of pups recruited by females ($\beta = 0.06 \pm 0.05$, $z = 1.38$, $P = 0.17$; Table 1). Familiarity is, however, strongly correlated with age (and therefore survival) in early life (r = 0.60), making it difficult to disentangle whether familiarity drives survival or survival drives familiarity. However, for senescent squirrels (≥ 4 years), age and familiarity are disassociated (r = -0.04; Figure S1), such that familiarity is not driven by an individual's own survival but by the survival of its neighbours, allowing us to better disentangle these effects. When analyzing effects of familiarity in the senescent period (≥ 4 years old), we found that the benefits of social familiarity for all fitness measures were even more substantial, and were consistently at least three times greater than the effects of relatedness in the same models (Table 1). For squirrels aged 4 and older, living near familiar neighbours was associated with an increase in the probability of annual survival for both sexes ($\beta = 0.45 \pm 0.11$, $z = 3.40$, $P < 0.001$; Figure 1). For example, senescent 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. In the senescent period, living near familiar neighbours was associated with a substantial increase in the annual number of pups sired ($\beta = 0.39 \pm 0.15$, $z = 2.67$, $P = 0.008$; Figure 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. For females aged 4 and older, familiarity with neighbours was also associated with an increase in the annual number of pups recruited ($\beta = 0.23 \pm 0.09$, $z = 2.52$, $P = 0.01$; Figure 1). Senescent 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.

An important consideration is whether variation in individual quality or habitat quality might lead to a spurious correlation between familiarity and survival when the causation is actually reversed. Familiarity, however, is not a trait of an individual but a trait of an interaction *between* individuals. Therefore, an individual with 'good genes' that survives well cannot obtain high familiarity without also having neighbours that survive well. For this to be possible there would need to be spatial clustering of 'high quality' individuals or spatial variation in resource availability or predation risk that leads to local areas of consistently high survival (and therefore high familiarity). Furthermore, in order for local areas of high habitat or individual quality to confound the familiarity-fitness relationship, spatial autocorrelation in these fitness measures must persist across multiple years (because familiarity is a product of past survival). To assess this possibility, we calculated Moran's I 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. Evidence of spatial autocorrelation in any of these measures was both rare and inconsistent (Table S4). We found only one instance of spatial correlation in consecutive years (Table S4), suggesting that a spurious correlation between ARS or survival and familiarity is unlikely. When we removed the year/grid combinations with positive spatial autocorrelation from our data and reran the analyses our conclusions remained the same (Table S5). In addition, while survival and

143 familiarity might be confounded in younger squirrels where familiarity increases with age, we
144 demonstrated that familiarity still has substantial effects on fitness in older squirrels where age
145 and familiarity are independent and variation in familiarity is driven by the survival of a
146 squirrel's neighbours rather than their own survival (Figure 1; Table 1). Finally, there is no
147 mechanism by which reproductive success might affect familiarity. Therefore, there is
148 substantial evidence to suggest that familiarity is a cause rather than a consequence of high
149 fitness.

150 Although we do not have direct evidence for the mechanism by which familiarity with
151 neighbours might lead to increased reproductive success, previous work in this study system has
152 shown that social familiarity results in reduced risk of pilfering [21] and reduced time spent on
153 territory defence [22]. Conceivably then, these direct fitness benefits may result from cooperative
154 'agreements' of non-intrusion among long-term neighbours, which might increase food stores or
155 reduce time and energy allocated to territory defence. Given the particularly substantial effects of
156 familiarity on male siring success, we sought to provide some mechanistic understanding of how
157 familiarity contributes to fitness by exploring two hypotheses in male red squirrels. We
158 hypothesized first that social familiarity might directly benefit males through increased mating
159 success with familiar females in their social neighbourhood. Alternatively, we hypothesized that
160 if familiarity enhances energetic resources through reduced pilferage [21,22] that this might
161 allow male squirrels in familiar social neighbourhoods to travel farther to obtain mating
162 opportunities and thereby increase their siring success [26,27]. Our analyses revealed that there
163 was no association between familiarity and a male's siring success within his social
164 neighbourhood (≤ 130 m away; $\beta = 0.12 \pm 0.10$, $t = 1.25$, $P = 0.21$; Table 2). Instead, we found
165 evidence that males traveled farther to mate ($\beta = 19.93 \pm 8.83$, $t = 2.26$, $P = 0.03$) and sired more

pups ($\beta = 0.39 \pm 0.10$, $z = 3.79$, $P < 0.001$) outside their social neighbourhood (>130 m away) when more familiar with their neighbours (Figure 2; Table 2). This does not appear to be due to inbreeding avoidance (see Table 2). Together, these results suggest that stable social relationships with neighbours enhance male fitness by allowing them to travel farther and increase their siring success [27]. This might result from familiarity with neighbours increasing the energetic resources available to males through reduced territory defence [22] or reduced cache pilfering by neighbours [21]. While the mechanisms by which social familiarity might affect female recruitment remain unexplored, there are several pathways by which this might occur, including familiarity increasing food resources, which might lead to earlier parturition [28] or faster growth rates [29], known to be associated with enhanced juvenile survival. Alternatively the reduced time spent on defence in familiar neighbourhoods might allow females to spend more time in nest [22], and more attentive mothers have been found to have enhanced reproductive success [30]. Exploring the mechanisms by which social familiarity contributes to fitness is a viable area for future research.

Effects on senescence

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. 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 683 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 (Figure 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' [31] (Figure 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 [32], space [13], and reproductive success [33], 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 [34]. 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, although rare, 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 ($r = 0.06$) [1]. It is possible then that reduced aggression when interacting with familiar neighbours (i.e. the dear-enemy phenomenon) [12] 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

212 through increased social familiarity. In red squirrels, reduced territory defence toward familiar
213 individuals [22] may convey survival benefits to neighbours through reduced aggression or
214 lowered defense of food resources. Red squirrels have also been known to nest communally
215 during cold temperatures [18], which might enhance neighbour survival. Other mechanisms by
216 which red squirrels might reduce neighbourhood turnover such as predator alarm calling, or
217 forming defensive coalitions [e.g. 34] to prevent a territory takeover, have yet to be tested.
218 Ultimately, if cooperative behaviours enhancing the survival of familiar neighbours were to
219 become widespread enough that mean social familiarity increased with age across the population,
220 then this socially driven increase in fitness late in life could influence the force of selection and
221 consequently the evolution of senescence (Figure S2).
222
223 **Effects of relatedness**
224 Given that there is evidence of affiliative behaviour among kin in this study system [18,19], in
225 addition to localized dispersal and bequeathal of territory space to offspring [17], we expected
226 that relatedness among neighbours might play an important role in red squirrel survival and
227 reproductive success. Additionally, we were interested in the possibility that relatedness with
228 neighbours might help to mitigate the consequences of living with unfamiliar neighbours, as seen
229 in other systems [6], However, we found no evidence to suggest that kin provide fitness benefits
230 for red squirrels (Table 1), and there were no significant interactions between relatedness and
231 familiarity in any of our models. It is possible that the observed low relatedness among
232 neighbours (average neighbourhood relatedness = 0.05 ± 0.001), which likely occurs as the result
233 of low overwinter juvenile survival [35], could have constrained the potential for kin selection to
234 act at this scale. However, 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 measured these effects did not exhibit any influence of kinship. If, for instance, the primary benefits of kinship come from sharing resources such as food [21] or nests [18], relatedness of nearest neighbours (rather than all neighbours within 130 m) may be a more relevant measure to assess whether red squirrels obtain benefits from kin. Future work should therefore 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.

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 provide important fitness benefits that might feed back to limit these competitive interactions and maintain strong affiliative relationships among unrelated territorial neighbours. Similar fitness benefits of established relationships with neighbours have also been demonstrated in territorial birds such as red-winged blackbirds [36], song sparrows [37], and great tits [10]. 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 pathways, with resulting fitness benefits, independent of kinship effects [3,6]. Consequently, stable social relationships with neighbours may be an important and overlooked contributor to fitness in territorial species, and may offer insight into the relative importance of mutually beneficial 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 trap line. We are grateful to all of the field technicians who have contributed to the long-term KRSP database over the years, and J. Kapitain 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.).

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.

Declaration of Interests: The authors declare no competing interests.

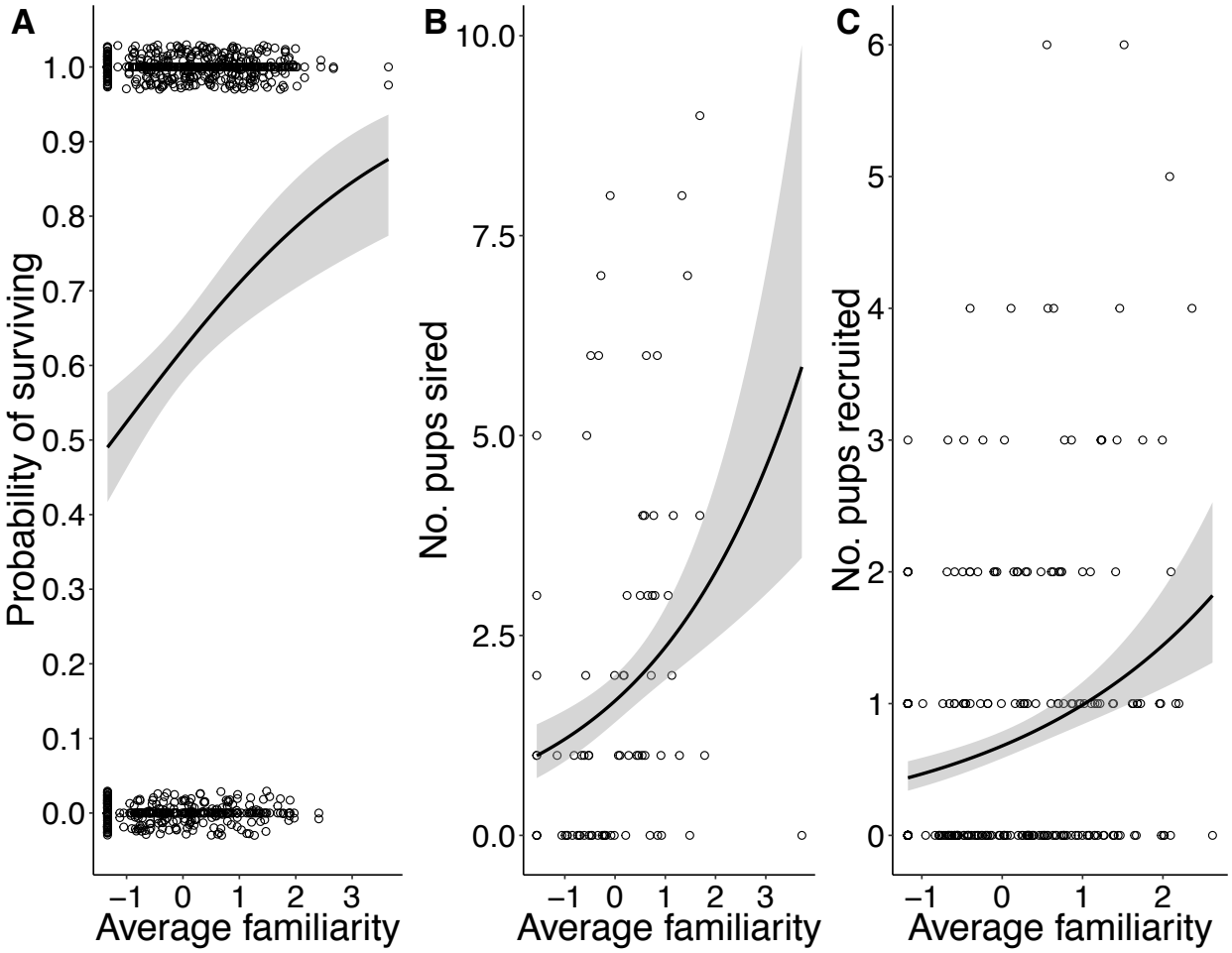


Figure 1. Effects of average neighbourhood familiarity on A) annual survival (N = 512), B) male annual reproductive success (ARS; N = 78), and C) female ARS (N = 268) during the senescent period (≥ 4 years old). Shaded grey bars indicate 95% confidence intervals. Values on x-axis are standardized measures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

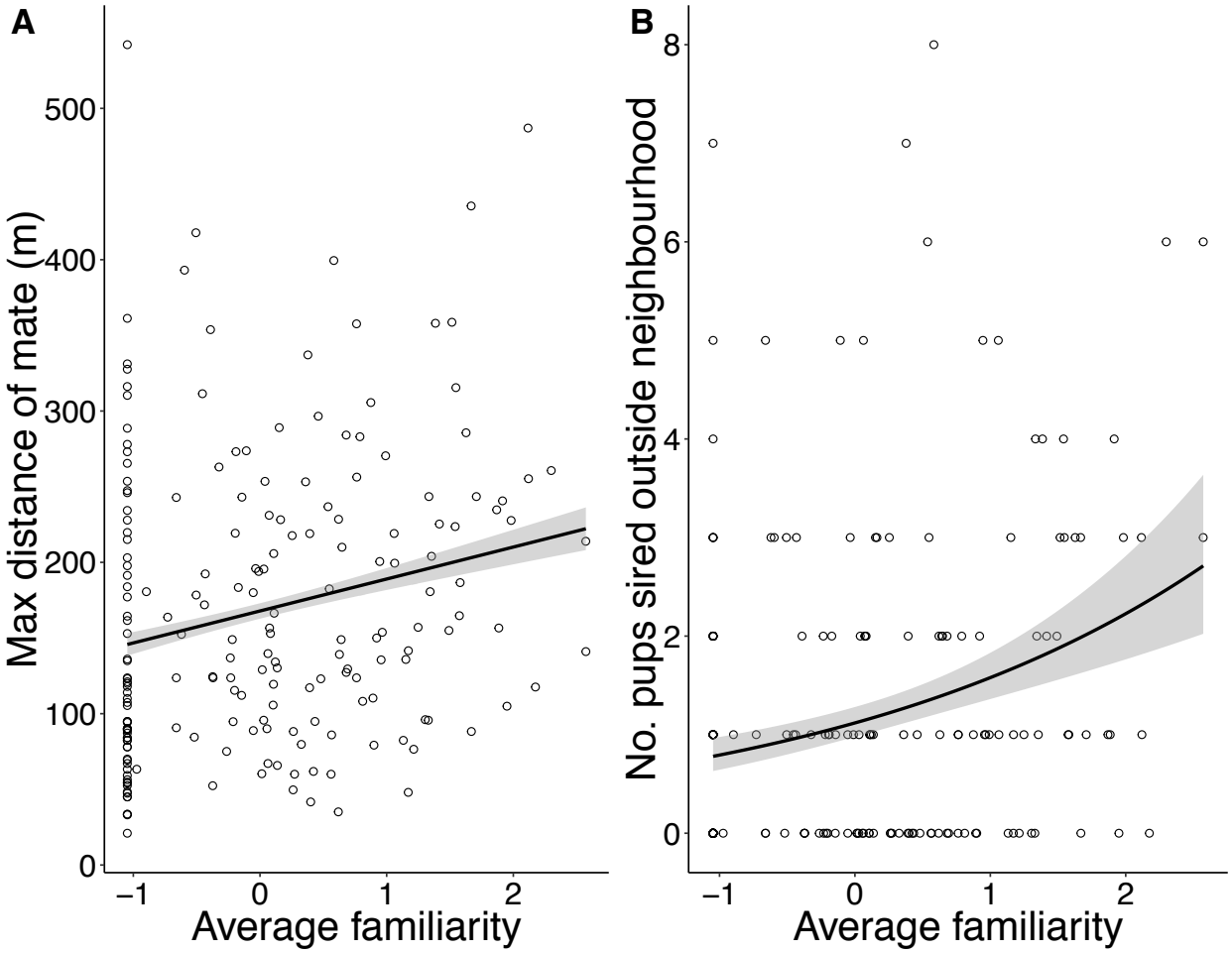


Figure 2. Effects of average neighbourhood familiarity on A) the distance that males traveled to mate and B) number of pups sired outside the social neighbourhood (i.e. 130 m radius; N = 199). Shaded grey bars indicate 95% confidence intervals. Values on x-axis are standardized measures of average familiarity. Points indicate raw data. See also Table 2 and S2.

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. See also Table S1 for random effects.

Data set	N	Response	Fixed effect	Parameter ± SE	*z*	*P*
Full data						
	2346	Probability of survival	**Familiarity**	**0.18 ± 0.06**	**2.79**	**0.005**
			Age	**-0.27 ± 0.08**	**-3.47**	**0.001**
			Age^2	**-0.11 ± 0.04**	**-2.63**	**0.01**
			Relatedness	0.01 ± 0.05	0.16	0.88
			Density	0.10 ± 0.10	0.92	0.36
			Grid-SU	0.17 ± 0.10	1.67	0.09
	412	No. pups sired *(Male ARS)*	**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	-0.12 ± 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 *(Female ARS)*	Familiarity	0.06 ± 0.05	1.38	0.17
			Age	0.08 ± 0.05	1.42	0.16
			Age^2	**-0.10 ± 0.04**	**-2.72**	**0.007**
			Relatedness	0.03 ± 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**	**4.00**	**<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 *(Male ARS)*	**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 *(Female ARS)*	**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

Table 2. Fixed effects from models assessing effects of familiarity on (i) the farthest distance that males traveled to mate, (ii) the number of pups sired inside the neighbourhood, and (iii) the number of pups sired outside the neighbourhood (N = 199 observations over 129 males). Regression coefficients are standardized and significant effects are indicated in bold. See also Table S2 for random effects.

Response	Fixed Effect	Parameter ± SE	Test-statistic	*P*
Distance of farthest mate	**Familiarity**	**19.93 ± 8.83**	**2.26**	**0.03**
	Relatedness	6.91 ± 6.82	1.01	0.31
	Age	-4.67 ± 9.56	-0.49	0.63
	Density	**-24.86 ± 7.49**	**-3.32**	**0.001**
No. pups sired inside	Familiarity	0.12 ± 0.10	1.24	0.21
	Relatedness	0.01 ± 0.07	0.18	0.85
	Age	0.03 ± 0.11	0.24	0.81
	Density	**0.27 ± 0.12**	**2.32**	**0.02**
No. pups sired outside	**Familiarity**	**0.39 ± 0.10**	**3.78**	**<0.001**
	Relatedness	-0.003 ± 0.09	-0.04	0.97
	Age	-0.09 ± 0.12	-0.77	0.44
	Density	**-0.25 ± 0.09**	**-2.66**	**0.008**

STAR Methods

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Erin Siracusa (erinsiracusa@gmail.com).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

Data are available from the Figshare Repository (https://doi.org/10.6084/m9.figshare.8813564).

Experimental Model and Subject Details

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 [17,25]. 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 [38], with an average of three pups per litter [39]. 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.

334 ***Ethical guidelines***

335 This study required trapping individuals using Tomahawk live traps to determine territory
336 ownership and assess reproductive status. Traps were checked every 60-90 min and squirrels
337 were never left in a trap for longer than 120 min. We also entered the natal nest when pups were
338 1-2 days old and 25 days old to collect DNA, measure pup growth, and tag individuals. We
339 returned the pups to the natal nest immediately after processing to minimize time spent away
340 from the dam. These procedures had no detectable negative effects on the survival or welfare of
341 the study animals. This research was approved by the University of Guelph Animal Care
342 Committee (AUP number 1807).

343

344 **Method Details**

345 ***Measuring familiarity***

346 We completed a full census of the population twice annually in May and August and determined
347 territory ownership using the aforementioned methods. Both male and female red squirrels
348 defend exclusive territories year round, which are centered around a larder hoard of food
349 resources called a 'midden' [13]. We defined each squirrel's social neighbourhood to be all
350 conspecifics whose middens (i.e. the center of the territory) were within a 130 m radius of the
351 focal squirrel's midden. One hundred and thirty meters is the farthest distance that red squirrel
352 territorial vocalizations ('rattles') are known to be detectable to the human ear [40], and is
353 similar to the distance at which red squirrels have been demonstrated to be most responsive to
354 changes in local density (150 m) [41]. This suggests that 130 m is a reasonable measure of the
355 distance at which squirrels can receive and respond to information about their social environment.
356 We measured the pairwise familiarity between the territory owner and each neighbour as the

357 number of days that both individuals had occupied their current territories within 130 m of each
358 other. We then averaged all pairwise measures of familiarity to obtain a measure of each
359 individual's average familiarity with its neighbours. For all analyses we used average familiarity
360 values calculated in May, as this aligned most closed with the reproductive season. The timing of
361 our semi-annual censuses meant that we could only update each squirrel's familiarity with its
362 neighbours twice per year. Therefore, pairwise familiarities could increase from one year to the
363 next in increments of either 273 days (new neighbour in August survived until May), or 365 days
364 (neighbour survived from previous May until current May). Average familiarity, however, could
365 take on many possible values because pairwise familiarities were averaged across all neighbours.

366 ***Measuring relatedness***

367 We temporarily removed juveniles from their natal nest immediately following parturition to
368 weigh, sex, and take ear tissue samples. Observing pups in the natal nest also allowed us to
369 assign maternity with certainty. Starting in 2003, we determined paternity by genotyping all
370 individuals at 16 microsatellite loci [42] and assigned paternity with 99% confidence using
371 CERVUS 3.0 [43]. Genotyping error based on known mother-offspring pairs was 2%. Additional
372 details on the genetic methods can be found in Lane et al. (2008). Using the established
373 multigenerational pedigree, we calculated the coefficient of relatedness between the territory
374 owner and each neighbour. We averaged all pairwise measures of relatedness to provide a
375 measure of the average relatedness between each squirrel and all of its neighbours.

376 ***Fitness measures***

377 For individuals alive between 1994 and 2015, we measured annual survival as the probability of
378 surviving to the following year (N = 2346 records over 1009 individuals). Given that long-term
379 monitoring of individuals in this population first began in 1987, we excluded data prior to 1994

380 to ensure accurate measurement of familiarity between neighbours. Our ability to successfully
381 redetect adults in the population each year is estimated to be 1.0 [44].
382 We measured female annual reproductive success (ARS) as the number of pups recruited
383 each year (i.e. surviving overwinter). We included data from 450 breeding females between 1994
384 and 2015 (N = 981 records). All females with a known parturition date were considered to have
385 bred; all other non-breeding females were excluded from analysis. Most non-breeding females
386 were yearlings that were too young to breed. Since red squirrel males do not contribute to the
387 raising of pups and the male's social environment is, therefore, unlikely to affect pup recruitment,
388 we defined male ARS as the number of pups sired each year. We used data from 207 males
389 between 2003 and 2014 (N = 412 records). We excluded male reproductive success outside of
390 this timeframe because paternity data was not available prior to 2003 and after 2014. For all
391 analyses we only used data for adults (≥ 1 year old) whose age could be assigned with certainty
392 (i.e. individuals tagged in nest as juveniles).
393 It is possible that our ability to accurately assess recruitment is somewhat confounded by
394 dispersal beyond the borders of our study grids [45]. On average, juveniles typically disperse less
395 than 100 m [17,46], and estimates of juvenile survival do not differ between the center and edge
396 of our study grids, which might be expected if dispersing juveniles were mistakenly considered
397 to have died [25]. However, 37% of our population is comprised of immigrants from outside our
398 study grids [45], which suggests that some juvenile recruitment outside the study area is likely to
399 have been missed by our methods.
400
401 **Quantification and Statistical Analysis**
402 ***Annual survival and ARS***

403 To assess the effects of familiarity on ARS and survival, we used generalized linear mixed
404 effects models (GLMMs) with a BOBYQA optimizer. We used a binomial distribution (logit-
405 link) to assess probability of annual survival and a Poisson distribution (log-link) for male and
406 female ARS. For all models we fitted average familiarity, average relatedness, a linear and
407 quadratic term for age, and grid-wide density as continuous predictors and included study grid as
408 a categorical fixed effect (Table 1). For the survival models we checked for an effect of sex, but
409 sex was not significant and so was not considered further. We included a random effect of
410 squirrel ID and year to account for repeated measures of individuals and temporal variation in
411 resource availability, respectively (Table S1). To account for inherent spatial structure in our
412 data, we grouped squirrels into 150 m squares (within each year) based on their known spatial
413 locations and included this 'spatial ID' as a random effect in all of our models. Our results
414 remained unchanged even when this spatial grouping term was defined to be a 75 m or 300 m
415 square (see Table S3). Fitting separate spatial autocorrelation terms for each year would have
416 been ideal, however, it was not possible to obtain convergence from our data using this method.
417 To account for the fact that familiarity and age are strongly correlated in early, but not later, life
418 (Figure S1), we also fit models for just the senescent period (≥ 4 years old). Models for the
419 senescent period included the same covariates as the full models described above, but only
420 included a linear effect of age. Finally, we conducted an additional analysis to test for spatial
421 autocorrelation by calculating Moran's I (ape package version 5.1) [47] for each study grid and
422 year (Table S4). We removed the year/grid combinations with positive spatial autocorrelation
423 from our data and reran the analyses above (Table S5).

424 ***Mechanisms underlying male reproductive success***

425 We conducted a post-hoc analysis to reveal potential mechanisms by which familiarity with

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

436 ***Force of selection***

437 If the fitness benefits of stable social relationships were sufficiently widespread in the red
438 squirrel system then an increase in the mean number of reproductive opportunities later in life
439 associated with social familiarity would lead to a less rapid decline in the force of natural
440 selection with age. This would delay the arrival of the 'wall of death', a spike in mortality caused
441 by the accumulation of deleterious alleles, leading to the evolution of slower rates of aging [31].
442 We conducted a post-hoc analysis to explore this possibility in red squirrels by creating a
443 'simulated' population where social interactions were excluded from the calculation of age-
444 specific survival and fecundity (Figure S2). To do this, we fitted the same models as above,
445 except that we included both breeding and non-breeding females and the response variable for
446 females was now number of pups born rather than number recruited. For the first set of models
447 we included age, a quadratic term for age, density and grid as fixed effects, as well as squirrel ID
448 and year as random effects. We extracted the parameter estimates for age in each of these models

449 and used those parameters to estimate age-specific fecundity and survival for females and males
450 separately. This represented our 'observed' population, and provided us with the estimates of
451 age-specific fecundity and survival as observed and as potentially confounded by social
452 familiarity among squirrels. We then measured what the effects of age would have been had
453 there not be been variation in familiarity by including familiarity as a continuous predictor in the
454 same models described above, and thus statistically controlling for its effects. Our parameter
455 estimates for age now represented the effects of age in the absence of social effects. We again
456 used these parameters to estimate age-specific fecundity and survival for both sexes. We
457 calculated force of selection separately for males and females in our 'observed' population and
458 'simulated' population (where the benefits of social relationships were statistically excluded) by
459 using the following formula [48]:

460

$$S(x) = \sum_{y=x+1} e^{-ry}\, l(y)m(y)$$

461

462 Here $S(x)$ represents the force of selection against a deleterious allele in the population, where r
463 is the intrinsic rate of increase, $l(y)$ and $m(y)$ are the survivorship and fecundity functions and y
464 is used to sum up the net expected reproduction over all ages after age x.

465 ***Data analysis***

466 We conducted statistical analyses using R version 3.4.1 [49] and fitted models using the lme4
467 package (version 1.1-13) [50]. We obtained *P* values for the LMMs using the package lmerTest
468 (version 3.0-1) [51]. For all models, we checked for significant non-linearities between the
469 predictor and response variables by fitting generalized additive models. For GLMMs we checked
470 for overdispersion by assessing whether the sum of squared Pearson residuals approximated a

Chi-squared distribution with *N-P* degrees of freedom [52]. 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). (Results remained the same even when these 25 neighbourhoods were removed from the analysis). To facilitate direct comparison of effect sizes [53] 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 ± SE, unless otherwise stated, and consider all differences statistically significant at $\alpha < 0.05$.

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