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Sexually-selected infanticide by male red squirrels in advance of a mast year

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North American red squirrels (*Tamiasciurus hudsonicus*) anticipate when white spruce (*Picea glauca)*, their primary food source, will produce large amounts of cones in infrequent and irregular mast years (Boutin et al. 2006). Cones mature in autumn and are then available as food for red squirrels, but females produce larger, and often multiple, litters the preceding spring and summer in anticipation of the upcoming mast. Because this pulse of the cone food resource follows the birth of the litter it cannot be a source of energy for the female to produce young; instead, there must be cues for increased reproductive investment by the females prior to mast

cone production, perhaps through consumption of buds on the masting trees (Boutin et al. 2006, 2013). Boutin et al. (2006) only studied females; whether male behavior anticipates mast years is still unstudied. At our study area in the Yukon, 2014 was a mast year for spruce cone production in late summer (Lamontagne et al. 2005). JAH was observing male red squirrel mating behaviour during spring 2014, giving her an unanticipated opportunity to document the previously unstudied effects of a mast year on male red squirrels.

On 25 May 2014, JAH witnessed a male squirrel committing infanticide. JAH observed a male intruding on a neighboring female's territory (both were identified using unique, color-coded ear-tags; Appendix S1: Figure S1). The male killed one of the female's pups with repeated bites to its chest and upper abdomen. On 1 June 2014, another pup from the same litter was found dead within 5 m of the nest tree with chest punctures and a partially consumed head (Figure 1, Appendix S1: Figures S2-S3). The primary predators of red squirrels do not inflict such injuries (Appendix S1: Figures S4-S5; Stuart-Smith and Boutin 1995). Rather, these match injuries inflicted on the first pup by the male. The day following infanticide the female was sitting next to her nest, apparently guarding it (Appendix S1: Figure S6). However, after she was caught in a live trap for inspection, her nipples were flaccid, suggesting that she ceased lactating which indicated the death of her entire litter of five pups (McAdam et al. 2007). Genetic analyses (Lane et al. 2008) revealed that another male, not the male that killed them, was the sole sire of this litter. Of particular note, the male who committed infanticide later became the sole sire when the female produced a second litter (see Appendix S2 for further details on these field observations). Studd et al. (2015) had speculated that infanticide by male red squirrels could occur and these observations confirmed those suspicions.

We observed other likely cases of infanticide during spring 2014. Another trapped female was lactating on April 25 and her nest was located that night using radio telemetry. On the morning of April 26, JAH saw the female chasing a male away from the vicinity of the nest; later that afternoon, we found the nest empty. That evening, the same male cached a dead, partially-eaten, pup (Appendix S1: Figures S7-S8). We confirmed with genetic analyses that this female was the pup's dam and a different male, who had been killed by a lynx (*Lynx canadensis*, Appendix S1: Figure S4), was its sire. We later confirmed with trapping that the female was no longer lactating and, thus, her pups, too, had died. She produced a second litter on 14 June 2014 sired by a third male. In 2014, other cases of possible or attempted infanticide were also

identified when dead or injured pups from several litters were found with bruising and puncture wounds (Appendix S1: Figures S9-S10), sometimes with uninjured siblings in the same nest.

Red squirrels actively defend individual, spatially dispersed and non-overlapping territories based around cached food. Thus, to commit infanticide, males would need to temporarily vacate their territory and leave their cached resources undefended. Traveling off-territory to access females during the breeding season is also energetically demanding (Lane et al. 2010). Thus, we hypothesized that infanticide might occur more often during a mast year because multiple litters are most common in mast years, giving the male committing infanticide on an earlier litter a chance to sire later litters with the mother. In non-mast years, females typically have only one litter per season (Boutin et al. 2006), reducing the likelihood that a that a male would gain paternity benefits following infanticide. Furthermore, females having larger litters with higher juvenile survival in mast years (Boutin et al. 2006, Williams et al. 2014) increases the potential fitness of males that sire more offspring with better probability of recruitment in the subsequent litter during mast years.

Across all years of data collection, observations of injured pups were only recorded anecdotally during systematic recording of female reproduction. Thus, we augmented these field observations by assessing the frequency of litter mortality over 12 years (2003-2014) on two study areas using trapping to assess female reproductive status and genetic analyses to assign pup paternity (see General Field Procedures in Appendix S2, McAdam et al. 2007, Lane et al. 2008). Three years (2005, 2010, 2014) were determined to be mast years of cone production. First litters were less likely to survive in the spring preceding a mast as opposed to other years (proportion of first litters surviving during mast years: mean = 0.56, 95% confidence interval = 0.45-0.68, n = 178 litters and 96 females; proportion of first litters surviving during non-mast years: mean = 0.91, 95% confidence interval = 0.85-0.95, n = 495 litters from 262 females). Undoubtedly, pup mortality occurs for myriad reasons (e.g., insufficient maternal resources; see also Studd et al. 2015), and these could be especially pronounced in mast years which follow several years of low seed production. Our results reveal another, previously under-appreciated, cause of litter loss: infanticide by males.

Infanticide has been documented in a wide range of animals including other rodent species (Hrdy 1979, Ebensperger 1998). Several adaptive explanations for infanticide have been suggested (Hrdy 1979), but our observations point to a sexually selected competitive advantage

in competition between males. There are two predictions from this hypothesis (Hrdy 1979). First, the offspring killed are unlikely to be the progeny of the perpetrator but instead will lower the reproductive success of its competitors. Second, committing infanticide will lead to more opportunities for the perpetrator to breed (Hrdy 1979), typically measured as a shorter inter-birth period of the female suffering infanticide (Hrdy 1979, Ebensperger 1998). Sexually-selected infanticide occurs more frequently in species where males can defend or monopolize access to females (Lukas and Huchard 2014), for example in social groups (e.g., Packer and Pusey 1983), and typically occurs due to the appearance of a new sire (Ebensperger 1998).

Both predictions of the sexual selection hypothesis were supported by our observations. In 2014, the two male red squirrels observed or suspected of committing infanticide were not the sires of the pups they killed. During three mast years (re-nesting is uncommon during non-mast years, see below), there was a shorter interval between first and second litter parturition dates following a failed first litter than following a successful one (mean days between birth dates of first and second litters following failed first litter: 51.0 days, mean days following successful first litter: 67.9 days, Welch's t-test: $t = 7.9$, $df = 40$, $p<0.001$, $n = 47$ failed and 23 successful litters for which birth date was known for first and second litters). In addition, litter success and the occurrence of a mast year influenced whether females produced a second litter (Williams et al. 2014) and thus whether males could achieve paternity benefits from committing infanticide. During mast years, only 26% of females re-nested following a successful litter but 76% of females re-nested following litter loss (determined when their pups were <25 days old, i.e., when we typically enter the nest to tag the pups, Williams et al. 2014). During non-mast years, re-nesting following litter loss was less common (39% of females) and was very rare following a successful litter (<1% of females, Williams et al. 2014). Infanticide has been thought to be less common in annual, seasonally-breeding species that may produce only one litter (Lukas and Huchard 2014), such as red squirrels usually do, because paternity benefits can not immediately be realized (Hrdy 1979). Infanticide that triggers subsequent breeding attempts, especially in a mast year, may be an under-appreciated way that seasonally-breeding males could gain paternity benefits.

The strength or direction of sexual selection may shift with environmental or other factors (Cornwallis and Uller 2010), and our observations suggest that male red squirrels have environmentally-induced, alternative reproductive strategies resulting in higher incidences of

sexually-selected infanticide in mast years. This suggests that male red squirrels can manipulate siring opportunities to gain fitness benefits during a year when females are likely to re-nest following a failed reproductive attempt. How females anticipate a mast is poorly understood (Boutin et al. 2006), and whether males are sensitive to the same cues or whether they respond to increased female reproductive effort needs further exploration. Regardless of the cue, our observations of sexually-selected infanticide in a solitary species with a scramble competition mating system (Lane et al. 2009) stands in contrast to previous evidence coming predominantly from species where males can monopolize access to females (Lukas and Huchard 2014) and suggest a mechanism promoting infanticide in some years.

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Figure Legends

Figure 1. A dead pup whose littermate was killed by an adult male red squirrel. This pup was found dead within 5 m of the nest where the incidence of infanticide was observed. Blood on its chest indicates chest wounds, and its head was partially missing, presumably consumed. The pup who was killed by infanticide had similar wounds inflicted to its chest and upper abdomen.

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