Red squirrels cause balancing selection on the length of white spruce cones

by

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ABSTRACT

RED SQUIRRELS CAUSE BALANCING SELECTION ON THE LENGTH OF WHITE SPRUCE CONES

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Seed predators act as agents of selection on many conifer species through non-random foraging, but their effects on conifers that use mast seeding as a defense strategy are unknown. Optimal foraging models predict that when resources are scarce predators should act to maximize total energy, while when resources are abundant they should act to maximize efficiency. Here, I examined North American red squirrel (*Tamiasciurus hudsonicus*) seed predation on white spruce (*Picea glauca*) from 2007-2013, which included five low resource (non-mast) years and one high resource (mast) year in 2010. The length of white spruce cones exhibited a trade-off between total energy content and the rate at which squirrels could extract that energy; longer cones contained more total energy, but had lower rates of energy extraction than shorter cones. During mast years there was a low opportunity for selection and no selection on cone length. In contrast, in low resource years, there was natural selection for shorter cones farther from squirrel caches, but selection for longer cones closer to squirrel caches. Red squirrels harvested cones to increase efficiency closer to their caches and maximize total energy farther from their caches, which was consistent with central-place foraging theory. However, these opposing selective forces on cone length led to net selection that was typically weak and variable.

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1.0 INTRODUCTION

1.1 SEED-PREDATORS AS SELECTIVE AGENTS

Seed predators consume seeds as their main source of food, but with adverse consequences for plant fitness. If seed predators enhance their own fitness by selecting seed from plants with specific phenotypes this non-random foraging can impose natural selection on plant traits. Pre-dispersal seed predators are typically specialists (Hulme and Benkman 2002) and might, therefore, be important agents of selection on plant or seed traits. For example, high levels of pre-dispersal cone predation by squirrels and their preference for certain cone traits that maximize-energy intake make them important pre-dispersal agents of selection on many conifers (Smith 1970; Benkman et al. 2001; Benkman et al. 2003; Benkman and Siepielski 2004; Molinari et al. 2006; Siepielski and Benkman 2008). Specifically, Benkman et al. (2003) compared lodgepole pine (*Pinus contorta*) cone morphology in areas with and without North American red squirrels (*Tamiasciurus hudsonicus*) and found that the absence of squirrels resulted in cones with a greater number of seeds and larger seeds, than in areas where squirrels were present. Similar evidence of the evolution of increased seed and cone defenses in the presence of squirrels has been documented for at least six different conifer species (Molinari et al. 2006; Siepielski and Benkman 2008), but these typically involve conifers that produce relatively stable cone crops of mainly serotinous cones (Smith 1970; Elliott 1988; Benkman et al. 2001; Benkman et al. 2003; Benkman and Siepielski 2004; but see Molinari et al. 2006; Archibald et al. 2012).

1.2 WHITE SPRUCE

White spruce (*Picea glauca*) is a non-serotinous conifer that does not allocate large amounts of energy into physical seed defenses, such as thick, woody cones, but instead undergoes mast seed production (Nienstaedt and Zasada 1990; Lamontagne and Boutin 2007; Krebs et al. 2012). During mast years plants synchronously produce large quantities of seeds, followed by non-mast years when few to no seeds are produced (Kelly 1994). In many areas red squirrels are the dominant pre-dispersal seed predator of white spruce. White spruce cones open and release their seeds approximately two weeks after maturation (Archibald et al. 2012), which provides only a limited window for red squirrel pre-dispersal seed predation. Red squirrels clip and hoard cones in underground caches in late summer and early autumn (Fletcher et al. 2010). Rates of cone hoarding by red squirrels are satiated during mast years because of the limited time available to harvest cones, which increases the rate of seed escape during mast years (Fletcher et al. 2010). In non-mast years seed escape is much lower, and in extremely low cone-production years, squirrels have been found to hoard more mushrooms and fewer cones (Fletcher et al. 2010). In contrast to previous studies of red squirrels as agents of selection on conifers that produce stable cone crops (Smith 1970; Elliott 1988; Benkman et al. 2001; Benkman et al. 2003; Benkman and Siepielski 2004), the large annual changes in cone production by white spruce might cause red squirrel preferences for cone attributes to change, which could cause natural selection on cone traits to fluctuate.

The size of a prey item often leads to a trade-off between the total energy content of the prey and the rate of energy acquisition through prey consumption. Larger prey contain more overall energy, but often have greater handling times, which can lead to

reduced rates of energy extraction from larger prey. For example, Eurasian oystercatchers (*Haematopus ostralegus*) forage on mussels by cracking them open with their beaks (Meire and Ervynck 1986). Larger mussels contain more energy, but have thicker shells, which increases their handling time. In contrast, smaller mussels have higher extraction rates, but contain less energy leading to a trade-off between energy content and efficiency (Meire and Ervynck 1986). Optimal foraging theory predicts that when overall resource availability is low organisms should maximize total energy gain, but when resources are abundant they should maximize the rate or efficiency of energy acquisition (Stephens et al. 2007).

In accordance with optimal foraging theory, I hypothesized that red squirrels would disproportionately harvest cones that maximize total energy acquisition when food resources were scarce but maximize the efficiency of energy extraction when food resources were abundant. Therefore, in non-mast years white spruce trees with cones containing higher total seed mass would have lower rates of seed escape, while in mast years trees producing cones with attributes that resulted in higher rates of seed extraction by red squirrels would have lower rates of seed escape. Specific external cone traits associated with the total energy content of cones were identified by opening spruce cones of known morphology in the lab and the cone traits that affected the rate of seed extraction by red squirrels were determined by performing feeding trials with red squirrels and cones of different morphologies in the wild. Finally, the seed escape of 120 white spruce trees was measured across six years including one mast year (high resource) and five non-mast years (low resource) in order to test whether trees that produced cones with less total energy had greater seed escape in years of low cone production, and

whether trees that produced cones with lower rates of energy extraction by red squirrels

had greater seed escape in years of high cone production.

2.0 METHODS

2.1 STUDY SITE AND SPECIES

Red squirrel (*Tamiasciurus hudsonicus*) seed predation on white spruce (*Picea glauca*) was studied in the southwestern Yukon Territory, Canada (61°N, 138°W) in association with the Kluane Red Squirrel Project (KRSP). White spruce is the only conifer species in this region of boreal forest; however, trembling aspen (*Populus tremuloides Michx.*) and balsam poplar (*Populus balsamifera L.*) are also occasionally present (Krebs et al. 2012). Red squirrels harvest the majority of cones produced by white spruce trees in non-mast years (Fletcher et al. 2010). Cones are cached underground by squirrels, where seeds are unlikely to be released and dispersed by wind (Smith 1981). Cone clipping and caching are effectively equivalent to seed predation, as most clipped cones are hoarded (Archibald et al. 2012). Seeds from hoarded cones are unlikely to germinate, and seeds from cones left to open on the ground after clipping are unlikely to be wind-dispersed (Nienstaedt and Zasada 1990). Red squirrel harvesting of spruce cones is, therefore, likely to influence the evolution of mast seeding in regions where white spruce trees and red squirrels co-occur, including at this site. Unlike many other masting systems, red squirrels are not satiated by the energy in the cones, but instead by the time needed to be able to clip and cache them within a limited food hoarding season from late summer to autumn (Fletcher et al. 2010; Archibald et al. 2012). Data on white spruce cone morphology and cone production were collected in 2007, 2008, 2009, 2010, 2012 and 2013 from six red squirrel study areas at this site. 2010 was a large mast year, whereas the other years were low to moderate in cone production (Krebs et al. 2012;

Figure 1). Sampled trees did not produce any cones in 2011 (Figure 1).

2.2 CONE SAMPLING AND MORPHOLOGY

White spruce cones were sampled from the same 120 marked trees over six years

between the end of July and beginning of August, once cones had matured, but before

squirrel harvesting began. In 2013, 160 additional trees, for a total of 280 trees, were

sampled to produce a larger sample size for a non-mast year. Cones were knocked off

each tree using an extendable pole (Wonder Pole® 621ProHigh), placed in labeled plastic

bags and stored in a cooler. 10 cones were collected from trees with 20 or more cones, six

cones were collected from trees with less than 20 cones, and all cones were collected

from trees with six or less cones. Previous research has shown that a sample of between

three and five cones per tree can accurately assess variation among trees in most cone and

seed traits (Garcia et al. 2009). Cone traits were measured in the field within seven days

of removal from the tree. Wet mass, chirality, insect damage of cone scales and cone

deformities were recorded for all cones. These were the only measurements taken on

deformed cones because their bent shape would have likely biased the size

measurements. For all normal cones, calipers were used to measure the length and width

at the widest point of each cone. A vertical line was drawn down the center axis of the

cone, and the number of cone scales intersecting this line was recorded. This was then

divided by cone length (mm) to get a measure of scale density in the cone (number of

scales intersected per mm). Three cones were chosen at random from all sampled cones

from each tree and brought back to the lab for further measurements. Cones were kept in

the lab in a -20 freezer prior to measurements.

2.3 INSECT PREDATION

Seed damage by insect larvae was quantified on two of the cones sampled from each tree from 2008-2013. The proportion of visible seeds that were destroyed by insect larvae was determined by cross-sectioning whole cones longitudinally with a razor blade. Destroyed seeds were evident by their reddish-brown colour instead of yellow. The number of insect larvae in these bisected cones was enumerated by dissecting cones and counting the larvae. Larvae of spruce cone flies (*Strobilomyia neanthracina*) and gall midges (*Cecidomyiidae* spp.) were found. These insects might also act as agents of selection on white spruce cones, but previous studies have found insects to have weak selective pressure on cones (Benkman et al. 2012) and in this system seed predation by insect larvae resulted in a much lower opportunity for selection than seed predation by red squirrels (Table 1), so their potential effects were not considered further.

2.4 SEED MEASUREMENTS

In order to determine the relationship between external cone traits and the total seed mass of spruce cones, 440 cones were collected between 2007 and 2012 and all external traits previously described were measured. In addition, these cones were allowed to open and release their seeds in the lab in individual paper bags. Individual seeds were removed from seed coats by pinching off the wing and gently rubbing seed between two fingers to remove the seed coat. The number of seeds in each cone was recorded. Seeds were weighed using a XP26 Microbalance (Mettler Toledo; ± 0.001 mg). Total seed mass was divided by the number of seeds in each cone to determine the mean seed mass. The number of seeds that were destroyed by insect larvae, evident by reddish-brown colouration and holes in seeds, was also recorded. Two seeds were found at the base of

every scale during lab measurements, which is supported by previous studies of white spruce cone morphology (Brink and Dean 1966). Previous studies have investigated the energy content of white spruce seeds and found that North American white spruce seeds have an average energy content of 6615 cal/g ($\pm$ 92 SE) (Brink and Dean 1966). This low variability suggests that using total seed mass is a reasonable proxy for the total energy content of cones. Therefore, using total seed mass as a measure of energy content and rate of seed extraction (g seed eaten per second) as a measure of energy acquisition seems reasonable.

2.5 CONE FEEDING TRIALS

In order to determine how cone morphology affected the rate of seed extraction by red squirrels, the time taken for squirrels to remove seeds from measured cones was recorded. White spruce cones used in the feeding trials were collected from multiple squirrel middens not located on current study areas. Cones selected for trials were hard, unopened and had no visible signs of mould or decay, but had been cached for at least one winter. External cone traits of these cones were measured in the field, following the protocol outlined above. After all measurements were recorded, each cone was placed in an individually labeled plastic bag. Cones were stored in an underground cooler to prevent them from opening, and carried to feeding trials on ice using an insulated travel mug to prevent them from opening and releasing their seeds.

Individually marked red squirrels were live-trapped as part of the Kluane Red Squirrel Project following methods outlined in McAdam et al. (2007). Eighty feeding trials were performed on 80 individually marked red squirrels; each squirrel only participated in one trial. Squirrels were not held in traps for more than two hours. Live

traps containing the captured squirrel were placed on a 35 x 50 cm plastic tray to collect

seeds, wings and scales that were not eaten by the squirrel and an additional 30 x 40 cm

white plastic tray was placed behind the trap to provide a neutral backdrop for video

recording, and to encourage the squirrel to face toward the video camera. A video camera

mounted on a tripod was placed approximately 5m away from the trap to record each

trial. Each squirrel was presented with one cone of known morphology (see above)

through the bars of the live trap to begin the trial. The observer watched each trial from

behind the video camera and limited movement and noise to avoid distracting the

squirrel. If the squirrel failed to completely husk the cone after 10 minutes the trial was

abandoned and the squirrel was released. Trials were repeated after a minimum of three

days for squirrels that did not eat the cone in their initial trial. After the cone was

consumed, the squirrel was released and all cone debris on the tray was collected in a

labeled plastic bag. Red squirrels husk spruce cones starting at the base of the cone and

remove scales with their teeth to uncover the underlying seeds as they rotate the cone.

Wings are typically not consumed by squirrels and are tossed aside with the uneaten

scales (Brink and Dean 1966; Figure 2). Consistent with cached cones consumed by red

squirrels, the cones provided in the feeding trials were moist from being stored

underground through the winter, which prevented scales, seeds and wings from blowing

away from the collection tray underneath the live-trap, so all debris not consumed by the

squirrel was collected. Since wings and seed coats often broke into multiple pieces and all

cones measured in the lab had two seeds at the base of each scale, the number of scales

collected beneath the live-trap was used as an indicator of the number of seeds that were

in the cone.

Videos were analyzed using JWatcher (Blumstein et al. 2012) and the time at which each scale was removed was recorded. Three distinct scale removal behaviours were noted (Figure 3). First, when squirrels started eating a cone they typically removed several scales without eating any seeds. This was noted in the videos by the absence of a chewing motion by the squirrel and noticeably faster rates of scale removal. Second, squirrels occasionally paused eating or even dropped the cone, resulting in very slow instantaneous rates of seed extraction. This was noted in the videos by long pauses and was classified as inactive feeding. Finally, the most common feeding behaviour was referred to as active feeding, which involved consistent and rapid removal of scales using repetitive and coordinated mouth and paw movements whereby the underlying seeds were consumed as indicated by noticeable chewing. The time taken to remove 10 successive scales (i.e. eat 20 seeds) during a randomly selected, uninterrupted bout of active feeding (see Figure 3) was used as the measure of seed extraction time. This was converted to a rate of seed extraction by dividing 20 seeds by the time in seconds taken to extract 20 seeds during the bout.

2.6 SEED ESCAPE

The number of cones escaping red squirrel seed predation at the end of the hoarding season was measured to determine how cone attributes of each tree affected the proportion of their cones that escaped squirrel predation. The number of cones produced by each of 580 trees, including the 120 cone sampling trees, on six study areas was measured in each year near the end of July to beginning of August before squirrel harvesting. New cones within the top 3m were counted from one side of each tree using binoculars (Krebs et al. 2012). The number of cones on each tree was ln(x+1)

transformed, and the average of the transformed values represented the index of cone availability in each year (Boutin et al. 2006; Lamontagne and Boutin 2007). Old, open cones that remained on the tree from previous years, which contained no accessible seeds, were also counted in 2009, 2010, 2012 and 2013. When there were more than 100 cones a digital photograph of the tree was taken (Krebs et al. 2012), and cones were counted in the lab using ImageJ software (Schneider et al. 2012). All trees that produced new cones were recounted at the end of September after the remaining cones on the trees had released their seeds. Seed escape was determined using the number of open cones, which successfully escaped squirrel predation and remained on the tree during the second count, subtracted by the number of old cones from previous years that were present when initial cone counts were completed. The number of old cones from previous years was not recorded in 2007 and 2008, so trees with more open cones counted post-squirrel harvesting than new cones produced pre-squirrel harvesting in these years were not included in the analyses (5% of trees in these years). 2009 was comparable in cone production to 2007 and 2008. Measuring seed escape by subtracting the number of old cones (as we did for 2009 to 2013) or by ignoring old cones and excluding those trees with more open cones after squirrel harvesting than new cones produced were highly correlated in the 2009 data ($r = 0.78$, $t = 20.75$, $df = 281$, $P < 0.001$) and there was no difference in mean seed escape between these two techniques (mean seed escape correcting for open cones from previous years = 0.21, mean seed escape without correcting for open cones and excluding trees with more open cones = 0.19; $t = 0.80$, $df = 613.65$, $P = 0.42$).

2.7 STATISTICAL ANALYSIS

In order for selective harvesting by red squirrels to cause natural selection on cone attributes, squirrels must harvest enough cones to result in a high opportunity for selection (Arnold and Wade 1984). Red squirrels have previously been shown to harvest a high proportion of white spruce cones (Fletcher et al. 2010), but I explicitly quantified the opportunity for selection by red squirrels in each of the six years by measuring the variance in relative seed escape (Table 1). Relative seed escape was calculated by dividing the proportion of cones escaping seed predation for each tree by the average proportion of cones escaping seed predation that year and the variance of this measure of relative fitness was used to calculate the opportunity for selection (Arnold and Wade 1984). The opportunity for selection on white spruce trees by insect larvae was also calculated by measuring the variance in relative seed escape (Table 1). While squirrels depredate entire cones, insect larvae depredate individual seeds, so the absolute fitness of each tree was calculated as the average proportion of seeds not destroyed by insect larvae for each cone sampled for that tree. Relative fitness was calculated as the average proportion of seeds escaping insect predation divided by the average proportion of seeds not destroyed by insect larvae in that year.

Cone traits preferred by squirrels must be repeatable (i.e. significantly differ among trees), otherwise selective harvesting by squirrels would result in within-tree rather than among-tree covariation between cone attributes and seed escape. Variation in cone traits and seed escape among trees is, therefore, necessary for natural selection on cone attributes (Price 1970; Frank 1995). White spruce cone attributes have been previously found to be repeatable (Khalil 1974) and high heritabilities of cone traits have

been measured for other conifer species (Matziris 1998), but I tested whether cone

attributes were repeatable in this population of white spruce by calculating variances in

cone and seed traits among trees and across years. Repeatabilities were calculated using

mixed effect models for cone traits and seed escape over the six years of the study (Table

2). In this study across-year repeatability was calculated using the among-tree variance

from the random effect of tree ID in mixed effect models. Within-year repeatability was

calculated using the random effect of year nested within tree ID. Repeatability models

also included year as a fixed effect, so all repeatabilities are relative to the annual mean

value for that trait. Likelihood ratio tests were used to test whether traits were

significantly repeatable by assessing the significance of the tree ID random effect.

The effects of measured external cone traits on total seed mass and rate of seed

extraction by red squirrels were calculated using multiple regression models including all

external cone traits measured as independent variables. Variance inflation factors were

checked in all regression models to assess potential multicollinearity (Lande and Arnold

1983). Cone mass was removed from all models due to its high collinearity with length

and width. Since there may be more fluctuations in mass due to water content at the time

of measurement, length and width were kept in models. The remaining traits in the

models had VIF scores <5 after mass was removed.

Seed escape was measured using data from 120 trees sampled over six years of

the study. First, data from all six years were combined in one analysis, including cone

predictors of total seed mass and rate of seed extraction, as well as annual cone

abundance and local squirrel density. Then, data were split into non-mast years (2007,

2008, 2009, 2012 and 2013) and mast years (2010) because different cone attributes were

predicted to experience selection depending on annual cone availability, and because the opportunity for selection was found to differ between mast and non-mast years. Generalized linear models with binomial error distribution (logit link function) that accounted for overdispersion were used to predict seed escape. Seed escape was calculated as the weighted proportion of cones escaping squirrel predation and releasing their seeds relative to cones not escaping squirrel predation and being removed from trees before seed dispersal. Annual cone abundance was calculated as the cone index described above. Local squirrel density was included in statistical models because if squirrels act as agents of selection on white spruce their density ought to affect selection on cone traits (i.e. selection should be stronger when more squirrels are present). Local squirrel density was measured as the number of squirrels that owned a midden within 1ha of each sampled tree (mean = 2.90; range = 0 to 9 squirrels/ha). Separate statistical models including distance to nearest squirrel midden (mean = 21.07; range = 0 to 72.12 m) were calculated because foraging distance from central cache might also impact selection on cone traits. Both local squirrel density and distance to nearest squirrel midden were determined using squirrel census and location data collected by the Kluane Red Squirrel Project.

Linear regression models were used to estimate linear selection gradients separately for each year of the study to obtain measures of direct selection on each trait (Lande and Arnold 1983). Relative fitness was calculated as seed escape for an individual tree divided by the average seed escape of all trees within that year. Length, width, scale density, chirality and the proportion of deformed cones were standardized to zero mean and unit variance and included in the selection gradient models. Selection differentials

were also calculated for cone length each year as a measure of total phenotypic selection on each trait. Mass was once again excluded from model because of high collinearity with length and width. In each year all remaining traits in the models had VIF scores <5. Standard errors were calculated using a delete-one jackknife procedure (Mitchell-Olds and Shaw 1987). The standard errors were used to generate 95% confidence intervals on each selection gradient following an assumed t-distribution. All calculations and analyses were determined using R version 2.15.1 (R Core Team 2012).

3.0 RESULTS

During non-mast years red squirrels harvested over 75% of white spruce cones produced. This corresponded with a high opportunity for selection through squirrel pre-dispersal seed predation that ranged from 1.95 to 4.50. In contrast, during the one mast year (2010) squirrels predated just over 40% of the cones and the opportunity for selection by squirrels was low (I=0.33). Insect larvae infested less than 19% of seeds, which resulted in a low opportunity for selection through insect seed predation in all years (range among years: 0.01 to 0.17; Table 1).

All cone and seed traits were significantly repeatable among trees within a year (range: 0.23 to 0.61; Table 2), but were less repeatable across years (range: 0.07-0.36). The repeatability of seed escape was not significantly different from zero (Table 2), but all other cone traits were significantly repeatable both within and across years (Table 2). All cone traits also exhibited significant variation among years (Table 2). In most cases, this resulted from differences between cones produced during the mast year and those produced during the non-mast years. On average trees produced cones with 12% more seeds and 56% larger seeds in the mast year than in the non-mast years, but length, width and scale density were similar between mast and non-mast years (43.86 mm vs. 44.85 mm, 13.60 mm vs. 14.58 mm, 0.38 scales/mm vs. 0.34 scales/mm respectively).

Cone length and scale density were both important predictors of total seed mass (Table 3), but cone length was the only significant predictor of the rate of seed extraction (Table 4). Cone length, therefore, represents a single cone trait that exhibited my predicted trade-off between total seed mass and rate of seed extraction in mast and non-mast years; longer cones contained more total seed mass (Table 3, Figure 4), but had

lower rates of seed extraction by red squirrels (Table 4, Figure 4).

Across all years, seed escape was affected by the interactive effects of cone length, squirrel density and annual cone abundance (i.e. three-way interaction; Table 5). Due to the importance of annual cone abundance to this interaction, I also analyzed the data for mast (2010) and non-mast years (2007, 2008, 2009, 2012 and 2013) separately (Table 6, Figure 5). In the mast year, there was a negative effect of squirrel density on seed escape, but contrary to my prediction there was no effect of cone length on seed escape (Table 6). In non-mast years, there was a significant interaction between squirrel density and cone length (Table 6). Trees that produced longer cones had lower rates of seed escape when squirrel density was low, but higher rates of seed escape when squirrel density was high (Figure 5).

There was a negative relationship between squirrel density and the distance to the nearest squirrel midden ($\beta = -1.85 \pm 0.34$, $t_{505} = -5.45$, $P < 0.0001$), so similar patterns were present in separate mast and non-mast year models that included distance to nearest squirrel midden instead of squirrel density (Table 7, Figure 6). In the mast year, there was a positive effect of the distance to the nearest squirrel midden, but no effect of cone length on seed escape (Table 7). In non-mast years, there was a significant interaction between cone length and distance to nearest squirrel midden (Table 7). Trees farther from squirrel middens had greater seed escape when they produced shorter cones, whereas trees closer to squirrel middens had higher seed escape if they produced longer cones (Figure 6).

Squirrel density (and distance to the nearest squirrel midden) varied both within and among years, which resulted in overall selection gradients that fluctuated from one year to the next (Table 8). In 2008 and 2013, when squirrel density was high (mean = 3.5-4

squirrels per hectare), cone length selection gradients and differentials were positive indicating selection for longer cones, whereas in 2007, 2009 and 2010 when squirrel density was lower (mean = 1.9-2.7 squirrels per hectare) the selection differentials for length were negative showing selection for shorter cones. There was also large spatial variation in squirrel density (range = 0 to 9 squirrels per ha) and distance to the nearest squirrel midden (range = 0 to 72.12 m), which would reduce net selection and increase uncertainty in selection gradients (Table 8) given the opposing relationships for cone length that I have documented (Figure 5, Figure 6).

4.0 DISCUSSION

My original hypothesis was based on the concept of a trade-off for red squirrels between maximizing either energy or efficiency through selection for different cone attributes. Cone length was found to be the most significant indicator of total energy content and rate of energy extraction, since longer cones contained more total seed mass, but had slower rates of seed extraction. This is consistent with previous literature indicating that larger prey contain more energy, but smaller prey often have faster handling times (Meire and Ervynck 1986). There was, therefore, potential for squirrels to act to maximize energy or efficiency by selecting longer or shorter cones to harvest. While scale density was an important predictor for total seed mass, it explained less of the variation than cone length and was not significant in predicting seed extraction rates. Cone traits were also repeatable within individuals and variable among individual trees, which means that selective harvesting of cones by squirrels could result in natural selection on cone traits. In particular, cone length had a repeatability of 0.54 among trees within a year and 0.34 across years, and has been found to have a broad sense heritability of 0.74 in Aleppo pine (*Pinus halepensis*; Matziris 1998).

4.1 SQUIRREL FORAGING BEHAVIOUR

In the mast year there was no support for my prediction of squirrels harvesting shorter cones to increase efficiency. At high squirrel densities and when trees were closer to squirrel middens there was decreased rates of seed escape, but there was no effect of cone length on seed escape. The opportunity for selection by pre-dispersal seed predation was much lower than in non-mast years (84% reduction from non-mast years; Table 1),

indicating limited potential for red squirrels to act as agents of selection on cone traits during mast years. Increased resource availability can lead to high mean fitness when all trees have high rates of seed escape, which will result in low opportunity for selection due to low variance in seed escape (Rundle and Vamosi 1996; Benkman 2013). There is, however, selection for increased synchrony of cone maturation in mast years; white spruce tress whose cones mature more synchronously with neighbouring trees are more likely to escape red squirrel predation in mast and non-mast years (Archibald et al. 2012). Synchronization in cone maturation and masting cycles of white spruce have been found to be highest in areas with higher densities of red squirrels (Lamontagne and Boutin 2007), giving more evidence for squirrels selecting for synchrony among trees.

In contrast, I found partial support for my initial prediction that squirrels would preferentially harvest longer cones when food abundance was low (i.e. in non-mast years) to maximize total energy, however, there was a spatial dimension that was not originally anticipated. As predicted tress with shorter cones had higher rates of seed escape, but this was only the case for trees at low squirrel densities (i.e. farther away from squirrel middens). At average squirrel densities this trend disappeared and at high squirrel densities (i.e. trees were closer to squirrel middens) squirrels preferentially harvested shorter cones, from which they were more efficient at extracting seeds. In these cases, squirrels appear to be maximizing efficiency rather than total energy content. Although not part of my original predictions, these effects of distance to the nearest squirrel midden on the relationship between cone length and seed escape are consistent with central-place foraging theory (Orians and Pearson 1979; Elliott 1988; Wetterer 1989).

Red squirrel territories do not overlap (LaMontagne 2007; Fletcher et al. 2010), so squirrel density would not affect the number of squirrels foraging on an individual tree.

The local density of squirrels around each tree, however, will affect the shortest distance between the tree and the nearest squirrel midden, which we assume is the squirrel that will forage on that tree. Red squirrels have a central cache or midden where they store and defend their food (Smith 1968; Fletcher et. al 2010). Central-place foraging (CPF) theory is used to describe how such animals maximize the rate at which resources are transferred to their central cache (Elliott 1988; Wetterer 1989). The Orians and Pearson (1979) model within CPF theory predicts that prey with higher rates of energy efficiency will be selected in patches that are close to the cache, while prey with higher total energy content will be selected in patches farther from the cache, regardless of handling time (Elliott 1988; Wetterer 1989). This model is consistent with my findings for non-mast years in which trees with longer cones had lower rates of seed escape when farther from squirrel middens, but higher rates of seed escape when the tree was closer to squirrel middens. Previous research on red squirrels foraging on lodgepole pine have suggested that as they forage farther from their cache and the cost of bringing the cone back to a central cache increases, squirrels preferentially harvest cones with higher total energy content (Elliott 1988; Wetterer 1989). Red squirrels in my study, therefore, appear to be non-randomly harvesting spruce cones in a manner consistent with CPF theory.

In addition to affecting the distance between the tree and the nearest red squirrel midden, the local density of squirrels might also directly affect red squirrel foraging behaviour. At high densities, red squirrels spend more time being vigilant and less time feeding (Dantzer et al. 2012). This would result in a need to increase feeding efficiency at higher squirrel densities, which is supported by my finding that shorter cones are preferentially harvested at high squirrel densities. Harvesting shorter cones might relieve short-term demands for efficient feeding, but could have potentially long-term

consequences for squirrels because shorter cones have less total energy. Squirrels at low densities, which are relieved of the time constraint caused by increased vigilance, harvested longer cones with more overall energy.

4.2 CONSEQUENCES OF SQUIRRELS FOR SELECTION

Understanding the foraging behaviour of red squirrels gives important insights into their ecology and potential evolution of white spruce cone traits caused by selection. There was high opportunity for selection imposed by red squirrels during non-mast years, which can lead to selection against preferred cone traits. There appears to be an underlying mechanistic relationship where squirrels are harvesting different cone lengths at different densities, but rather than this resulting in clear patterns of fluctuating selection across years, these opposing patterns are occurring mostly within years and this is resulting in weak net selection within any one year. Selection gradient analyses can be limited by their assumptions of homogeneity, while agents of selection within an environment may have heterogeneous distributions (Heisler and Damuth 1987). Squirrel density was heterogeneous which affected the relationships between cone length and seed escape within the population. Therefore, there were not significant selection gradients and differentials of cone length in any one year. In addition to the heterogeneous relationships between cone length and seed escape within each year, the lack of significance of the selection gradients for cone length could also be due to limited sample sizes (Hersch and Phillips 2004; Kingsolver and Pfennig 2007). Although 120 trees were sampled in each year, we could not measure selection on cone length for trees that did not produce any cones in a given year. As a result sample sizes for each year were reduced (range = 55 to 118 trees). Uncertainty around selection gradients can also be increased as a result of

correlations among traits (i.e. collinearity; Mitchell-Olds and Shaw 1987). There were

weak correlations between the cone size measurements, cone length, width, scale density

and mass. Mass was removed due to high collinearity values, but width and scale density

remained in the models since VIF scores were <5. The remaining collinearity was

unlikely to still be affecting standard errors because it was very weak, and standard errors

for selection differentials, which include no correlations between other size

measurements, remained fairly consistent with those of selection gradients. In this

analysis I measured only one component of tree fitness, pre-dispersal seed escape,

however, post-dispersal seed predation and recruitment success also play a role in total

tree fitness. There could still be other effects after seeds have been dispersed or trade-offs

between pre-dispersal seed escape and other fitness components, such as longevity.

However, previous studies on conifers and seed predators have also successfully used

seed escape and pre-dispersal predation as their measure of fitness (Benkman et al. 2003;

Benkman et al. 2012). Therefore, the variability in direction of selection on cone length

within years leading to weak net selection was a novel observation within such a system.

When compared to pine cones, white spruce cones are relatively efficient to open,

however, their seeds are extremely small and acquiring enough total energy is likely a

challenge for their main predators, especially in non-mast years. Their morphology

differences may also lead to selection on different cone traits. Previous studies on

lodgepole pine involve multiple agents of selection, including red squirrels and crossbills

(*Loxia curvirostra*), as well multiple sites with and without red squirrels present

(Benkman et al. 2001; Benkman et al. 2003). Increased seed defenses and reduced seed

mass and number of seeds within cones have been documented for pine trees where red

squirrels are present (Benkman et al. 2001; Benkman et al. 2003). Cone selection by

Eurasian red squirrels (*Sciurus vulgaris*) on Norway spruce (*Picea abies*), another

masting conifer, has been investigated (Molinari et al. 2006). Squirrels targeted trees with

more seeds per cone, which was also found to be positively correlated with cone length (r

= 0.55; Molinari et al. 2006). While the correlation between number of seeds and cone

length was lower in white spruce (r = 0.40), red squirrels showed similar selective

foraging behaviours for longer cones when farther from their cache. However, these

previous studies did not reveal evidence of red squirrels altering foraging behaviour and

direction of selection on cone length within populations. Future studies comparing white

spruce cone attributes in areas with and without red squirrels could help reveal if there are

differences in cone traits or if selection by red squirrels is too weak over years to result in

any major evolutionary differences between populations.

5.0 CONCLUSIONS

Red squirrels experience a trade-off between energy content and efficiency of energy extraction of white spruce cones, which was supported by lab and field studies, as well as seed escape rates at different distances from their central cache. In contrast to previous studies that have found red squirrels to be an important agent of selection on various species of pine (Smith 1970; Benkman et al. 2001; Benkman et al. 2003; Benkman and Siepielski 2004; Molinari et al. 2006; Siepielski and Benkman 2008), I found a clear, but heterogeneous relationship between cone length and seed escape from red squirrel predation. In low resource years, distance to nearest squirrel midden and squirrel density were found to impact foraging behaviour and squirrel selection on cone length, as predicted by central-place foraging theory. Red squirrels in this system have the potential to impact seedling recruitment and forest structure by harvesting from trees with preferred cone phenotypes and reducing white spruce fitness. However, rates of seed escape are highest in mast years when selection pressures by squirrels are low. This may lead to very slow evolution for trees producing cones that are less susceptible to squirrel predation. Unlike pine, white spruce systems are highly variable and while squirrels appear to preferentially harvest white spruce cones in predictable ways, the overall patterns at the population scale of trees cancel out. This leads to balancing selection and the potential for the persistence of increased variation among cone traits, which is exacerbated by low selection pressures in mast years with highest rates of seed escape.

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7.0 TABLES

Table 1. Opportunity for selection (I) imposed by pre-dispersal seed predation by North American red squirrels (*Tamiasciurus hudsonicus*) and insect larvae (*Strobilomyia neanthracina* and *Cecidomyiidae* spp.).

		N	$\overline{w}$	I
Red squirrels				
	2007	332	0.19	1.95
	2008	123	0.13	4.50
	2009	335	0.22	2.56
	2010*	675	0.64	0.33
	2012	373	0.14	3.91
	2013	454	0.25	2.20
	All years	358	0.51	0.43
	All non-mast years	301	0.14	2.08
Insect seed predation				
	2010	119	0.95	0.01
	2012	73	0.62	0.17
	2013	151	0.85	0.04

Notes:
The opportunity for selection was calculated as the variance in relative fitness, which was the proportion of seed escape of each tree divided by average seed escape ($\overline{w}$).
Proportion of seed escape with respect to squirrel predation was measured as the number of cones that remained on the tree after squirrel harvesting divided by the total number of cones produced by the tree that year. Proportion of seed escape with respect to insect seed predation was measured as the average number of seeds not destroyed by insect larvae in a cross-section of two sampled cones per tree divided by the total number of seeds in cross-section of those cones.
N represents the number of trees used in each analysis.
* 2010 was the only mast year.
No cones were produced in 2011.

Table 2. Within-year and across-year repeatabilities calculated from repeated measures of white spruce (*Picea glauca*) traits.

Variable	Number of Cones	Number of Trees	Fixed Effect of Year	Within-year repeatability	Across-year repeatability
Cone Traits					
Length (mm)	5150	596	$F_{5,\,399} = 40.2^*$	**0.54**	**0.34**
Width (mm)	5150	596	$F_{5,\,399} = 41.0^*$	**0.61**	**0.36**
Scales intersecting	5147	596	$F_{5,\,399} = 119.7^*$	**0.39**	**0.23**
Scale density (scales/mm)	5147	596	$F_{5,\,399} = 160.1^*$	**0.23**	**0.13**
Mass (g)	5152	596	$F_{5,\,399} = 51.3^*$	**0.55**	**0.34**
Seed Traits					
Number of seeds	421	149	$F_{4,\,46} = 11.4^*$	**0.33**	**0.07**
Total seed mass (g)	421	149	$F_{4,\,46} = 73.2^*$	**0.35**	**0.13**
Average seed mass (mg)	421	149	$F_{4,\,46} = 124.8^*$	**0.50**	**0.33**
Number of seeds destroyed	171	60	$F_{2,\,14} = 10.2^*$	**0.25**	**0.24**
Tree Fitness					
Cone production	2283	698	$F_{5,\,1580} = 218.0^*$		**0.14**
Cone production (non-mast only)	1611	609	$F_{4,\,998} = 15.5^*$		**0.17**
Seed escape	2283	698	$F_{5,\,1580} = 178.4^*$		**0.06**
Seed escape (non-mast only)	1611	609	$F_{4,\,998} = 8.4^*$		0.20

Notes:
Mixed effect models were used to calculate variances in cone, seed and tree fitness traits across six years of study, except seed traits, which were not measured in 2013. Within-year repeatability was calculated as the proportion of variance due to the among-tree variance. The across-year repeatability was calculated as the proportion of variance due to differences between years in trees. Repeatability models also included year as a fixed effect. Repeatabilities indicated in bold were significant based on a likelihood ratio test. Non-mast only does not include the 2010 mast year.
* $P < 0.002$

Table 3. Cone length and scale density were both positively associated with the total seed mass of white spruce (*Picea glauca*) cones.

Cone Trait	Estimate (β) ± SE	t	P-value
Length (mm)	0.002 ± 0.0003	5.22	< 0.0001
Scale density (scales/mm)	0.09 ± 0.03	3.39	0.0008
Width (mm)	0.001 ± 0.001	0.91	0.36
External insect damage	0.001 ± 0.003	0.44	0.66
Chirality$_{dextral}$	0.001 ± 0.003	0.38	0.71

Notes:

Results are presented for a general linear model of total seed mass predicted by five external cone traits measured on 440 cones collected between 2007 and 2012 from 100 trees ($F_{5, 414}$= 11.95, adjusted R^2= 0.12, P < 0.0001). Total seed mass was measured as total mass of seeds without wings or seed coats in milligrams. Cone mass was not included in the model due to its high collinearity with other traits.

Table 4. Cone length had a significant negative effect on the rate of seed extraction from white spruce (*Picea glauca*) cones.

Cone Trait	Estimate (β) ± SE	t	P-value
Length (mm)	-0.07 ± 0.03	-2.01	0.04
Scale density (scales/mm)	-4.37 ± 4.74	0.92	0.36
Width (mm)	-0.04 ± 0.15	-0.25	0.80
Chirality$_{dextral}$	-0.07 ± 0.41	-0.16	0.87

Notes:
Results are presented for a general linear model of rate of seed extraction collected from 80 red squirrel (*Tamiasciurus hudsonicus*) feeding trials ($F_{4, 70}$= 3.07, adjusted R^2= 0.10, P < 0.02). The rate of seed extraction (seeds consumed per second) was calculated based on the time taken for red squirrels to extract and consume 20 seeds. Cone mass was not included in the model due to its high collinearity with other traits.

Table 5. Cone length (mm), annual cone abundance and local red squirrel (*Tamiasciurus hudsonicus*) density had complex interactive effects on white spruce (*Picea glauca*) seed escape.

Variable	Estimate (β) ± SE	t	P-value
Cone Length (mm)	-0.19 ± 0.087	-2.21	0.03
Cone Index	-1.18 ± 0.87	-1.36	0.17
Squirrel Density	-2.49 ± 1.10	-2.27	0.02
Length : Cone Index	0.05 ± 0.02	2.35	0.02
Length : Squirrel Density	0.06 ± 0.02	2.56	0.01
Cone Index : Squirrel Density	0.65 ± 0.27	2.39	0.02
Length : Cone Index : Squirrel Density	-0.02 ± 0.01	-2.85	0.005

Notes:
Seed escape was measured for 120 white spruce trees sampled over six years and variation in seed escape was modeled using a generalized linear model with binomial error distribution. Seed escape is the weighted proportion of cones escaping red squirrel predation and releasing their seeds over cones not escaping squirrel predation and being removed from trees before seed dispersal. Estimates are reported on a log-odds scale. Separate analyses for mast and non-mast years are reported in Table 6. Overdispersion was accounted for using quasibinomial model. The number of cones counted on one side of each tree was ln(x+1) transformed, and the average of the transformed values represented the cone index in each year (Boutin et al. 2006; Lamontagne and Boutin 2007). Local squirrel density was the number of squirrels owning territories within 1ha of each tree.

Table 6. Cone length (mm) and local red squirrel (*Tamiasciurus hudsonicus*) density were significant predictors of white spruce (*Picea glauca*) seed escape in non-mast years, while in the mast year only squirrel density was a significant predictor.

Analysis	Variable	Estimate (β) ± SE	t	P-value
Non-mast years	Length	-0.10 ± 0.03	1.73	0.006
	Squirrel Density	-1.26 ± 0.44	-2.74	0.004
	Length : Squirrel Density	0.03 ± 0.01	3.04	0.003
Mast year (2010)	Length	-0.004 ± 0.02	-0.21	0.84
	Squirrel Density	-0.26 ± 0.09	-3.03	0.003

Notes:

Seed escape was measured for 120 white spruce trees sampled over five non-mast years and one mast year and variation in seed escape was modeled using a generalized linear model with binomial error distribution. Seed escape is the weighted proportion of cones escaping red squirrel predation and releasing their seeds over cones not escaping squirrel predation and being removed from trees before seed dispersal. Estimates are reported on a log-odds scale.

Overdispersion was accounted for using quasibinomial model. Local squirrel density was the number of squirrels owning territories within 1ha of each tree.

Table 7. Cone length (mm) and distance to nearest red squirrel (*Tamiasciurus hudsonicus*) midden (m) were significant predictors of white spruce (*Picea glauca*) seed escape in non-mast years, while in the mast year only distance to nearest squirrel midden was a significant predictor.

Analysis	Variable	Estimate (β) ± SE	t	P-value
Non-mast years	Length	0.08 ± 0.03	2.48	0.01
	Squirrel Distance	0.14 ± 0.05	2.86	0.004
	Length : Squirrel Distance	-0.003 ± 0.001	-2.58	0.01
Mast year (2010)	Length	-0.01 ± 0.02	-0.50	0.62
	Squirrel Distance	0.03 ± 0.01	2.24	0.03

Notes:

Seed escape was measured for 120 white spruce trees sampled over five non-mast years and one mast year and variation in seed escape was modeled using a generalized linear model with binomial error distribution. Seed escape is the weighted proportion of cones escaping red squirrel predation and releasing their seeds over cones not escaping squirrel predation and being removed from trees before seed dispersal. Estimates are reported on a log-odds scale.

Overdispersion was accounted for using quasibinomial model.

Table 8. Standardized selection gradients ($\beta \pm$ one standard error) for white spruce (*Picea glauca*) cone traits and standardized selection differentials (S $\pm$ one standard error) for cone length (mm) over 6 years of study. Cone length (mm), width (mm), scale density (scales/mm), chirality and proportion of deformed cones were included in each model.

Year	N	β_{length}	β_{width}	$\beta_{scale\ density}$	$\beta_{chirality}$	$\beta_{deformed}$	S_{length}
2007	87	-0.15 ± 0.15	-0.22 ± 0.12	0.22 ± 0.15	-0.08 ± 0.14	0.17 ± 0.19	**-0.34 ± 0.15**
2008	56	0.29 ± 0.40	-0.03 ± 0.38	0.33 ± 0.40	-0.41 ± 0.42	0.31 ± 0.52	0.20 ± 0.26
2009	64	0.12 ± 0.37	**-0.53 ± 0.21**	0.05 ± 0.29	0.23 ± 0.28	0.25 ± 0.27	-0.13 ± 0.30
2010*	119	0.02 ± 0.10	-0.06 ± 0.07	0.03 ± 0.09	-0.04 ± 0.06	-0.01 ± 0.06	-0.04 ± 0.05
2012	66	-0.26 ± 0.27	0.27 ± 0.30	0.03 ± 0.26	0.01 ± 0.22	-0.09 ± 0.17	-0.16 ± 0.18
2013	115	0.03 ± 0.14	-0.18 ± 0.12	-0.16 ± 0.10	-0.16 ± 0.12	0.13 ± 0.11	0.03 ± 0.12

Notes:
Positive cone chirality values indicate selection for right spiraling or dextral cones, while negative values indicate selection for left spiraling or sinistral cones. Standard errors were calculated using a delete-one jackknife procedure (Mitchell-Olds and Shaw 1987). The standard errors were used to generate 95% confidence intervals on each selection gradient following an assumed t-distribution, which were used to estimate significance.
P<0.05 are indicated in bold.
* 2010 was the only mast year.
No cones were produced in 2011.
Only trees sampled that produced new cones were used in these analyses.
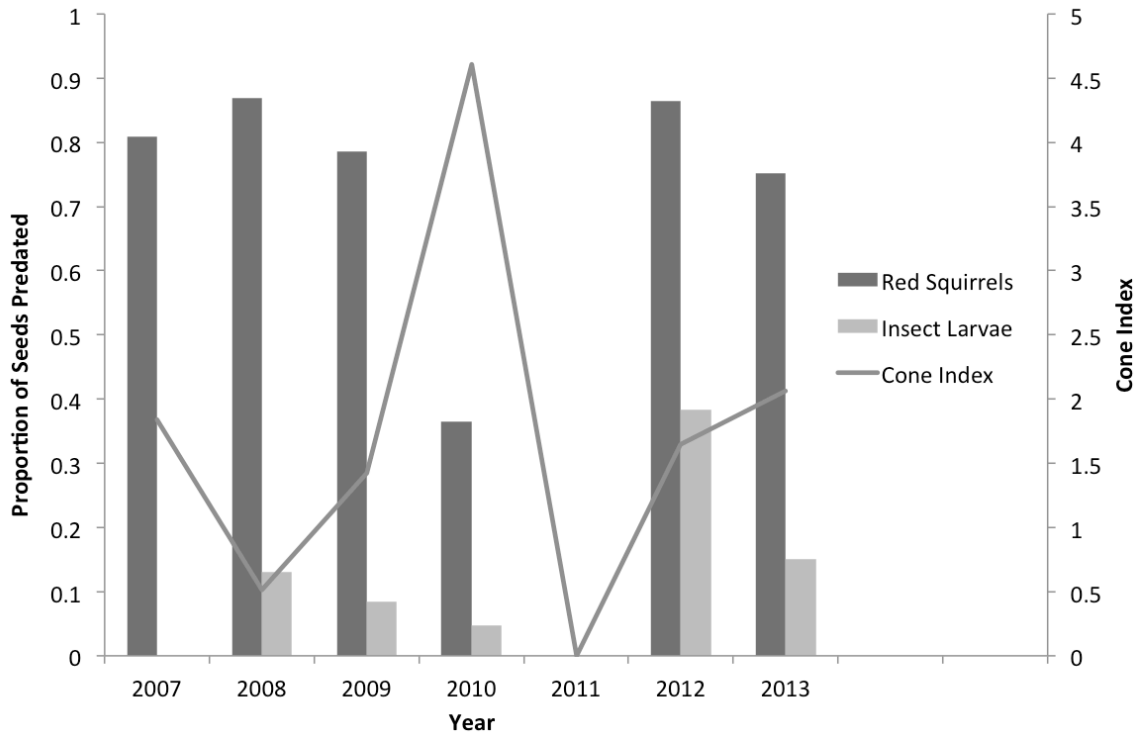
8.0 FIGURES

Figure 1. Red squirrels (*Tamiasciurus hudsonicus*) harvested a high proportion of white spruce (*Picea glauca*) cones in all years except for 2010, which was a mast year. Pre-dispersal seed predation by insect larvae (*Strobilomyia neanthracina* and *Cecidomyiidae* spp.) was generally low.

Notes:
Insect larvae predation was not measured in 2007, and in 2011 sampled trees produced no cones so there was no squirrel or insect larvae predation. The number of cones counted on one side of each tree was ln(x+1) transformed and averaged within each year as an index of cone production (Boutin et al. 2006; Lamontagne and Boutin 2007).

Figure 2. Typical contents of a white spruce (*Picea glauca*) cone eaten by a red squirrel (*Tamiasciurus hudsonicus*). 60 cone scales (a), 112 seed wings (b) and 72 seed coats (c) were all dried after being collected beneath a trap used for a cone handling trial. 120 seeds (d) were removed from a cone with 60 scales that was air dried in the lab. Lab measurements and cones opened in the field indicated that each scale had two seeds located at its base.
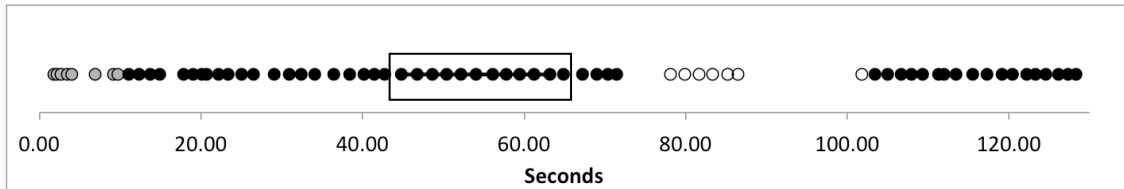
Figure 3. Example graphic of a red squirrel (*Tamiasciurus hudsonicus*) feeding on a white spruce (*Picea glauca*) cone during a feeding trial. Each point represents that time at which a scale was removed. Grey points indicate scale removal without consuming seeds, white points indicate inactive feeding, and black points indicate active feeding. The time taken to remove 10 successive scales during a randomly selected, uninterrupted bout of active feeding was used as the measure of seed extraction time and is surrounded by a box.
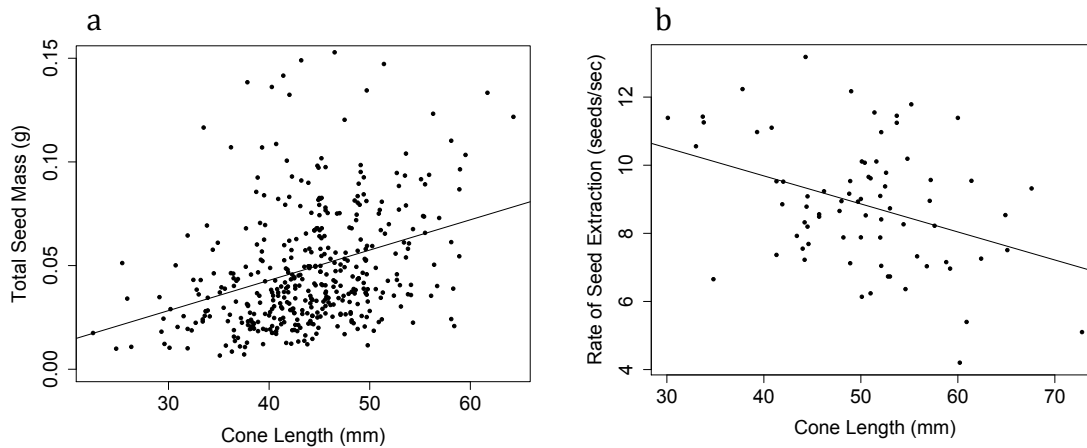
Figure 4. White spruce (*Picea glauca*) cone length was positively associated with total seed mass (a), but negatively associated with rate of red squirrel (*Tamiasciurus hudsonicus*) seed extraction (b). Total seed mass, not including seed coats or wings, and cone length were measured for 400 cones in the lab. Rate of seed extraction was calculated based on the time taken for red squirrels to extract 20 seeds from cones of known length in each of 80 feeding trials.
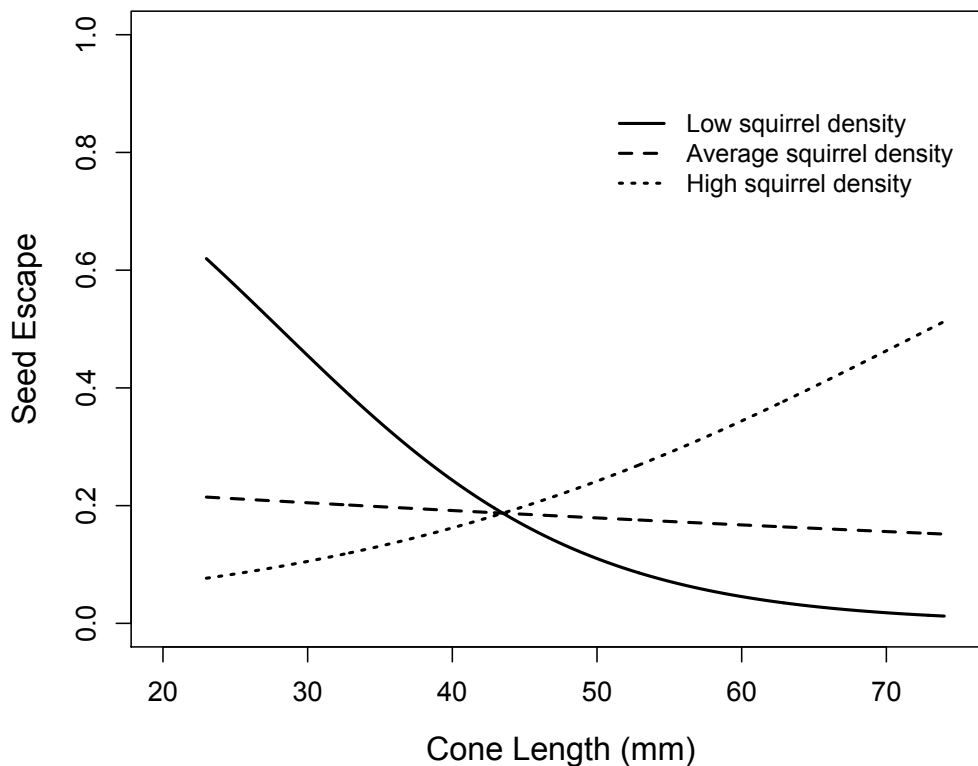
Figure 5. The effect of white spruce (*Picea glauca*) cone length on pre-dispersal seed escape depended on the local density of red squirrels (*Tamiasciurus hudsonicus*). Seed escape was higher for shorter cones when squirrel density was low (density = 0 squirrels/ha shown), but was higher for longer cones when squirrel density was high (density = 5 squirrels/ha shown). There was no effect of cone length on seed escape at a typical squirrel density (3 squirrels per ha shown).

Notes:
Lines represent the predicted seed escape based on a generalized linear model (binomial error distribution, logit link) of seed escape 120 trees sampled over five non-mast years. Seed escape is the weighted proportion of cones escaping red squirrel predation and releasing their seeds over cones not escaping squirrel predation and being removed from trees before seed dispersal.
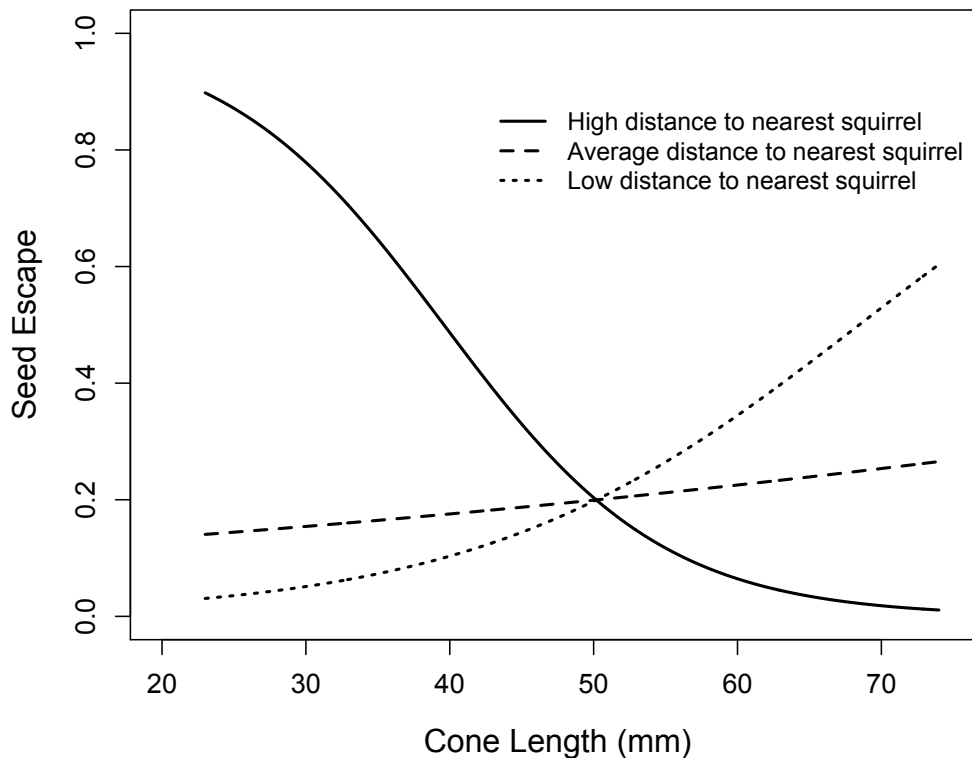
Figure 6. The effect of white spruce (*Picea glauca*) cone length on pre-dispersal seed escape depended on the distance to nearest red squirrel (*Tamiasciurus hudsonicus*) midden (m). Seed escape was higher for shorter cones when distance was high (distance = 72 m shown), but was higher for longer cones when distance was low (distance = 0 m shown). There was a minor effect of cone length on seed escape at a typical squirrel density (distance = 21 m shown).

Notes:
Lines represent the predicted seed escape based on a generalized linear model (binomial error distribution, logit link) of seed escape 120 trees sampled over five non-mast years. Seed escape is the weighted proportion of cones escaping red squirrel predation and releasing their seeds over cones not escaping squirrel predation and being removed from trees before seed dispersal.