

CONSISTENCY AND VARIATION IN PHENOTYPIC SELECTION EXERTED BY A COMMUNITY OF SEED PREDATORS

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Phenotypic selection that is sustained over time underlies both anagenesis and cladogenesis, but the conditions that lead to such selection and what causes variation in selection are not well known. We measured the selection exerted by three species of predispersal seed predators of lodgepole pine (*Pinus contorta latifolia*) in the South Hills, Idaho, and found that net selection on different cone and seed traits exerted by red crossbills (*Loxia curvirostra*) and cone borer moths (*Eucosma recissoriana*) over 10 years of seed crops was similar to that measured in another mountain range. We also found that the strength of selection increased as seed predation increased, which provides a mechanism for the correlation between the escalation of seed defenses and the density of seed predators. Red crossbills consume the most seeds and selection they exert accounts for much of the selection experienced by lodgepole pine, providing additional support for a coevolutionary arms race between crossbills and lodgepole pine in the South Hills. The third seed predator, hairy woodpeckers (*Picoides villosus*), consumed less than one-sixth as many seeds as crossbills. Across the northern Rocky Mountains, woodpecker abundance and therefore selective impact appears limited by the elevated seed defenses of lodgepole pine.

KEY WORDS: Geographic mosaic of coevolution, lodgepole pine, seed predation, species interactions, trait-mediated indirect effects.

One of the outstanding challenges for evolutionary biologists is to understand what causes natural selection (Wade and Kalisz 1990; MacColl 2011), because its variation in time and space is critical to both anagenesis and cladogenesis (Futuyma 2010; Uyeda et al. 2011). Although species interactions are thought to be one of the main factors causing selection (Schluter 2000; Thompson 2005), direct evidence of biotic or even abiotic factors causing selection is surprisingly limited (MacColl 2011). In addition, understanding how species interactions influence selection can be a complex and difficult task because species often interact with an array of other species, and their interactions can vary from

direct interactions such as predation to indirect interactions that can be either density- or trait-mediated (Werner and Peacor 2003; Thompson 2005; Ohgushi et al. 2012). Furthermore, phenotypic selection can be highly temporally variable (Siepielski et al. 2009; Bell 2010) further challenging our efforts to determine whether and when the selection we measure provides much insight into longer term evolutionary trajectories.

Although we tend to focus on the frequent occurrence of temporal variation in selection with some particularly noteworthy examples such as Darwin's ground finches (*Geospiza fortis*; Grant and Grant 2008), selection may be more temporally



consistent than generally perceived (Morrissey and Hadfield 2012; see also Kingsolver and Diamond 2011). Repeated and striking patterns of phenotypic divergence indicate cases where selection has been consistent. For example, Bolnick and Lau (2008) found divergent selection presumably arising from intraspecific competition to be repeated among populations of three-spine sticklebacks (*Gasterosteus aculeatus*). In combination with evidence for character displacement in sticklebacks (Schluter 2000), this implies temporal consistency in terms of the cumulative impact. This is not to deny that the strength of competition and associated selection varies, but competition nevertheless appears to be one interaction that often leads to repeated patterns of adaptive evolution (Schluter 2000; Losos 2009; Pfennig and Pfennig 2009). The question is whether we can anticipate other situations or interactions in which selection is sustained over time.

One interaction for which we have found consistent patterns of selection and corresponding geographic variation in traits implying that selection measured recently has been repeated well into the past is that between predispersal seed predators and cone-bearing conifers (Pinaceae: Benkman 1999; Benkman et al. 2001, 2010; Siepielski and Benkman 2007a,b). For example, the presence and abundance of different seed predators and the selection they exert can account for some striking and replicated patterns of geographic variation in cone structure. This is perhaps surprising because predispersal seed predation represents a species interaction for which temporal and spatial variation has been repeatedly documented, and where our understanding of the evolutionary consequences of these interactions remains generally poor (Kolb et al. 2007). However, selection on cone-bearing conifers by predispersal seed predators may be more consistent because many conifers are engaged in coevolutionary arms races where selection on physical seed defenses has led to a relatively large allocation of resources to the woody cones that prevent most seed predators from feeding on seeds in the closed cones (Smith 1970; Hulme and Benkman 2002; Leslie 2011). Indeed, small-seeded conifers in particular allocate a relatively large fraction of their resources to seed-support structures or defense relative to that allocated by angiosperms (Greene and Johnson 1994; Lord and Westoby 2012). Presumably the escalation of seed defenses challenges the abilities of most seed predators, favoring individuals that discriminate among plants depending on their level of defense. In addition, higher levels of defense will act to limit the diversity of seed predators that can remove seeds from closed conifer cones, restricting the number of strongly interacting species and perhaps stabilizing their relative levels of impact. Finally, increasing levels of physical or chemical seed defenses tend to be negatively correlated with the extent of annual variation in seed crops (Janzen 1971, 1974). Consequently, animals that can overcome the defenses of these highly defended seeds may have more sta-

ble populations than, for example, those seed predators that rely on plants using the alternative strategy of masting to satiate seed predators.

Here we focus on the phenotypic selection exerted by predispersal seed predators on Rocky Mountain lodgepole pine (*Pinus contorta latifolia*). Because of strong selection exerted by pine squirrels (*Tamiasciurus hudsonicus*), lodgepole pine allocates a large percent of its reproductive energies to physical seed defense, as seeds comprise only about 1% of the cone mass (Smith 1970; Benkman et al. 2001, 2003, 2010). This extreme allocation to defense eliminates seed predation from all but the most specialized of predispersal seed predators (Smith 1970; Siepielski and Benkman 2004). For example, only one species of insect, the lodgepole pine cone borer moth (*Eucosma recissoriana*; Lepidoptera: Tortricidae), feeds on seeds in closed lodgepole pine cones and it consumes <0.1% of the seeds (Miller 1986). However, pine squirrels did not colonize several mountain ranges east and west of the Rocky Mountains following the glacial retreat and expansion of lodgepole pine occurring around 12,000 years ago (Benkman 1999). Here, because of relaxation of selection from pine squirrels, lodgepole pine has increased its allocation of seed mass relative to cone mass by 200–300% (Benkman 1999; Benkman et al. 2001, 2003, 2010). Consequently, seed predation by cone borer moths is over two times greater (Siepielski and Benkman 2004), and, in the absence of competition from pine squirrels, red crossbills (*Loxia curvirostra* complex), which are cardueline finches specialized for foraging on seeds in conifer cones, are up to 20 times more abundant than in ranges with pine squirrels (Benkman 1999; Siepielski and Benkman 2005). In addition, the crossbill endemic to two isolated ranges without pine squirrels, the South Hills and Albion Mountains, Idaho, appears to be undergoing ecological speciation (Smith and Benkman 2007). Its evolution of nearly complete reproductive isolation from other crossbills within only the last several thousand years might be attributable to strong and consistent divergent selection from that experienced by crossbills in ranges with pine squirrels (Benkman et al. 2003).

Because cone traits in these isolated ranges are convergent east and west of the Rocky Mountains (Benkman 1999; Parchman et al. 2011), and differ from those in the Rocky Mountains in a manner consistent with both relaxation of selection by pine squirrels and an increase in selection by especially crossbills, we have assumed selection exerted by crossbills is consistent over time and similar in form among these squirrel-less ranges (Benkman et al. 2003). However, we measured selection exerted by crossbills in only one of the mountain ranges, the Little Rocky Mountains, Montana. Our goal, therefore, was to measure selection over a nearly nonoverlapping interval (1997–2007 vs. 1990–1999, for cohorts of cones for which we measured selection exerted by crossbills) to further evaluate the temporal consistency of

selection. Because selection should be similar among similar squirrel-less areas, we measured selection exerted by seed predators in the South Hills, which is 780 km away from the Little Rocky Mountains and on the opposite (west) side of the Rocky Mountains (maps of study areas are in earlier publications [e.g., Benkman 1999; Benkman et al. 2003]). Measuring selection in the South Hills also addresses a criticism of our work on coevolution between crossbills and lodgepole pine that we used data on selection exerted by crossbills in the Little Rocky Mountains whereas we used data on selection experienced by crossbills in the South Hills (Gomulkiewicz et al. 2007). We use these data to further evaluate our claim that selection by crossbills (plus relaxation of selection by pine squirrels) is the primary driver of cone evolution in the South Hills. Finally, hairy woodpeckers (*Picoides villosus*), the only other predator of seeds in closed Rocky Mountain lodgepole pine cones, feeds much more commonly on seeds in lodgepole pine cones in the South Hills than elsewhere (C. W. Benkman, pers. obs.). Thus, we measured the form and strength of selection exerted by hairy woodpeckers to account for all predators of seeds in the closed cones. Because understanding the causes of variation in the abundance of different selective agents is critical for understanding both temporal and spatial variation in selection, we examine whether geographic variation in the cone traits that are under selection by hairy woodpeckers (and that presumably act to deter them) can account for geographic variation in woodpecker abundance.

Methods and Materials

Lodgepole pine in mountain ranges without pine squirrels presents an excellent opportunity to measure selection exerted by dispersal seed predators. First, nearly all the trees in such areas have serotinous cones (Benkman and Siepielski 2004) that are held on the branches for decades until a stand replacing fire causes the cones to open and shed their seeds. Second, in these ranges, no seed predator removes cones from the branches and seed predation by the different seed predators can be distinguished (Fig. 1). Thus, seed predation can be quantified for multiple cohorts of cones so that net selection over multiple years can be measured. Nevertheless, our measures of selection are based on correlations rather than experiments. Such correlations can lead to biased estimates of selection if, for example, local environmental conditions influence both fitness (the probability that seeds are not eaten) and the phenotype of the cones and seeds (Rausher 1992). Such biases, however, are unlikely to be substantial for crossbills based on the similarity between the selection differentials measured in the wild and those measured experimentally in aviaries for crossbills foraging on two other species of conifers (ponderosa pine [*P. ponderosa*]; Parchman and Benkman 2008; black pine [*P. nigra*];

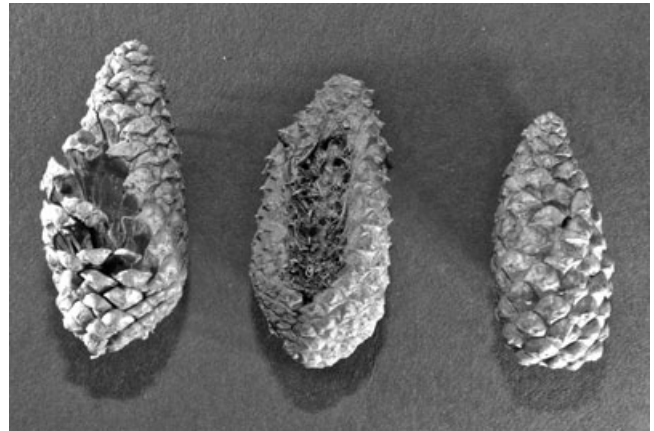


Figure 1. Photograph of cones foraged on by crossbills (left), by woodpeckers (center), and one that was not foraged on (right). Crossbills spread apart scales to retrieve the seeds from the base of the scales, whereas woodpeckers hammer with their bills to shred and remove the scales to create a cavity exposing the seeds. Moths leave small exit holes (not shown).

Benkman and Parchman 2009). In addition, we have a mechanistic understanding of the factors determining seed predation by crossbills (see Benkman et al. 2010 for a review), which strengthens inferences on a causal link between phenotype and fitness (MacColl 2011). We have discussed previously the causal basis of phenotypic selection exerted by crossbills and moths on lodgepole pine (Benkman et al. 2003; Siepielski and Benkman 2004). Finally, we note that although we measured selection over 10 years of cone production, this represents but a tenth or less of the expected reproductive life span of lodgepole pine. Consequently, we also consider whether and to what extent our measures of selection are consistent with the replicated patterns of cone evolution among multiple ranges with and without pine squirrels (Benkman 1999; Benkman et al. 2001; Siepielski and Benkman 2004) to evaluate whether our short-term measures of selection are likely representative of selection over the long term.

To quantify seed predation in relation to cone traits (i.e., phenotypic selection), we collected cones from 87 recently fallen lodgepole pine trees in various mature stands throughout the South Hills in June 2007 and 2008. Fallen trees were chosen because of the difficulty of sampling cones from standing canopy trees in mature stands, and there is no reason to suspect that sampling cones from fallen trees would bias our results. We assume that sampling cones from these trees approximates a random sample. This assumption would be violated if trees with certain cone traits were more likely to fall. We doubt that this is likely, as susceptibility to windfall appears to have a large environmental component (e.g., soil structure and moisture, exposure to wind) and is likely related to features of the trees (e.g., canopy volume, root structure) unrelated to cone structure. Five branches

that had 10 whorls of cones (≤ 10 years) were selected from the top one-third of each tree. We only included data from these cones because categorizing seed predation became increasingly difficult for cones older than 10 years. This was also the number of whorls analyzed by Benkman et al. (2003). For each branch, the number of cones produced in each whorl (\approx each year) was recorded and all cones with signs of predation were collected (Fig. 1). Additionally, at least 20 mature cones (brown cones, < 5 years old) that had no signs of predation were also collected. Three representative cones were selected from the above 20 cones from each tree for trait measurements. A sample of three cones per tree provides a reasonable estimate for among tree comparisons because variation in cone structure is significantly smaller within than among lodgepole pine trees (Smith 1968; Elliott 1974). Cone length and width were measured, and then the cones were opened in a drying oven. The following measurements were taken for each opened cone: cone mass without seeds, thicknesses of six distal and six proximal scales selected approximately equidistant around the cone, the length of six distal scales, the number of full (filled with female gametophyte) and empty seeds, and the individual masses of five full seeds (see Benkman et al. 2003 for additional information on cone measurements). All length measurements were made to the nearest 0.01 mm with digital calipers. All mass measurements were made to the nearest 0.01 mg with a digital scale after the cones had been oven-dried at 80°C for ≥ 2 days. The mean values for trees were the unit of analysis. An evolutionary response to selection on most if not all of these traits is expected because, for example, cone length (broad sense heritability, $H^2 = 0.53$), cone width ($H^2 = 0.30$), cone mass ($H^2 = 0.25$), and number of seeds per cone ($H^2 = 0.37$) are heritable in Rocky Mountain lodgepole pine (T. S. Kimbrell, C. C. Smith, J. S. Pontius, and P. F. Elliott, unpubl. ms.).

As in previous studies (e.g., Benkman et al. 2003), we use the percentage of seeds not eaten as a surrogate for tree fitness. Although the total number of seed produced in a lifetime is a preferred metric, we believe the percentage of seeds not eaten is suitable for long-lived trees for which we are measuring only a small fraction of the seeds they produce and where the number of seeds produced varies with tree size and age (Siepielski and Benkman 2007c). To paraphrase Siepielski and Benkman (2007c), if a tree produced cones with traits that deterred crossbills and thus few seeds were eaten, it would always be assigned a high fitness regardless of its age if we used percent of seeds not eaten, whereas its assigned fitness would largely depend on its size/age relative to other trees when the study was conducted if we used total number of seeds not eaten. Using the proportion of seeds, however, could be problematic if, for example, seed predators avoided large cones and there was a trade-off between cone size or seed number and the number of cones produced per year. Such a trade-off is not evident for lodgepole pine in the South Hills. For the 62 trees

for which we have measures of annual cone production, cone mass, and number of seeds per cone, we did not find significant correlations between the number of cones produced per branch per year and either individual cone mass ($r = 0.26$, $P = 0.06$) or the number of seeds per cone ($r = 0.25$, $P = 0.07$).

Each predated cone was scored categorically for the percentage (0%, 20%, 40%, 60%, 80%, 100%) foraged on by crossbills and woodpeckers (Fig. 1). Percent seed predation per tree by these two seed predators was estimated as the mean percent predation of all cones by that predator on the five selected branches. To convert the percentage of cones with moth exit holes into the percent of seeds consumed by moths, we first estimated the percent of seeds eaten by drying and opening cones with moth exit holes, and counting the number of full seeds. We then subtracted these values from the expected number of full seeds (i.e., the mean number of full seeds in the three undamaged cones for each tree) to estimate the percent of seeds eaten from cones with moth exit holes. This assumes that moths did not preferentially lay eggs in cones with fewer or more than the average number of seeds. Such an assumption was supported by the absence of among tree preferences for seed number (see Results). We dried and opened 43 cones with moth exit holes from 25 of the 36 trees having evidence of predation by moths; we assumed that moths did not prey upon seeds in cones without exit holes, which is supported by our previous work (Siepielski and Benkman 2004). The estimated percent of seeds eaten was negative for two of the 25 trees (i.e., more full seeds were counted in cones with moth exit holes than in cones without such holes). We set their values equal to the lowest positive estimate (24.5%) because at least some seeds must have been consumed for the moth larvae to exit the cones. For the 76 trees for which we had measures of both the proportion of cones with moth exit holes and the percent of seeds eaten in their cones, we used the product of these two values to estimate the percent seed predation by moths per tree. For the 11 trees for which we had measures of the proportion of cones with moth exit holes but not the percent of seeds eaten in their cones, we estimated the percent seed predation using the highly significant relationship between the percent seed predation by moths and the proportion of cones with moth exit holes ($\text{percent seeds eaten} = -0.016 + 0.695 [\text{percent of cones preyed upon}] + 0.019 [\text{percent of cones preyed upon} - 1.213]^2$, $df = 73$, $r^2 = 0.96$, $P < 0.0001$).

We used multiple linear regressions and resulting regression coefficients to estimate selection gradients (β) and to identify the traits under direct selection (i.e., the targets of selection; Lande and Arnold 1983). To avoid problems with multicollinearity, we examined correlation coefficients (r) between traits and variance inflation factor (VIF) scores from multiple regression models. We only included traits with absolute values for correlation coefficients < 0.50 and VIF scores < 3 ; the same set of traits was included in each multiple regression. To determine which

traits were under selection (both direct and indirect selection), we used least squares regression analyses to estimate standardized selection differentials (s) and standardized quadratic selection gradients (γ) using regression coefficients (Lande and Arnold 1983); the quadratic term was doubled (Lande and Arnold 1983; Stinchcombe et al. 2008). Relative tree fitness was estimated as 100 minus percent seed predation divided by the overall mean, and cone traits were standardized to units of SD. Relative tree fitness was estimated in relation to seed predation by each individual predator species and to the total seed predation by all three species. We used regression bootstrap to test for significance of the selection differentials and selection gradients, and to estimate standard errors because the residuals in the regressions were strongly non-normally distributed. Tests of significance were based on 1000 bootstrap replicates. We used cubic splines to further examine the form of selection on cone traits for which the quadratic term was significant ($P < 0.05$; only one quadratic regression was statistically significant). We used two composite variables: the ratio of seed mass to cone mass (a measure of the amount of energy devoted to reproduction relative to seed defense) and the ratio of cone width to cone length (a measure of cone shape). Seed mass is the product of mean individual seed mass multiplied by the mean number of full seeds per cone.

We used data from two earlier studies on seed predation and selection exerted by crossbills and moths in the Little Rocky Mountains (Benkman et al. 2003 and Siepielski and Benkman 2004, respectively) for comparative purposes and to determine the consistency of selection on lodgepole pine between two ranges lacking pine squirrels. To test for the consistency of selection by crossbills and moths, we performed separate regressions comparing the selection differentials for crossbills and moths at the two sites. A significant positive relationship would indicate consistency in selection. Because the residual distributions of these regressions were unknown, and to account for uncertainty in the estimates of the selection differentials, we used nonparametric bootstraps ($n = 1000$) to calculate confidence intervals for the regression coefficients. For each bootstrap replicate, we resampled the original data for both sites, calculated the selection differentials for each trait in the resampled datasets (as above), and then performed a linear regression on the bootstrap selection differentials for the two sites. We also calculated P -values on the hypothesis that the coefficient $b_i = 0$ using the method described in Davison and Hinkley (1997). Using the same methodology, we also performed regressions to compare the selection differentials due to crossbills, woodpeckers, and moths to the overall selection differentials resulting from total seed predation (one regression per species).

To examine the relationship between hairy woodpecker abundance and cone traits under selection by woodpeckers and to help infer a causal basis of selection exerted by woodpeckers, we use

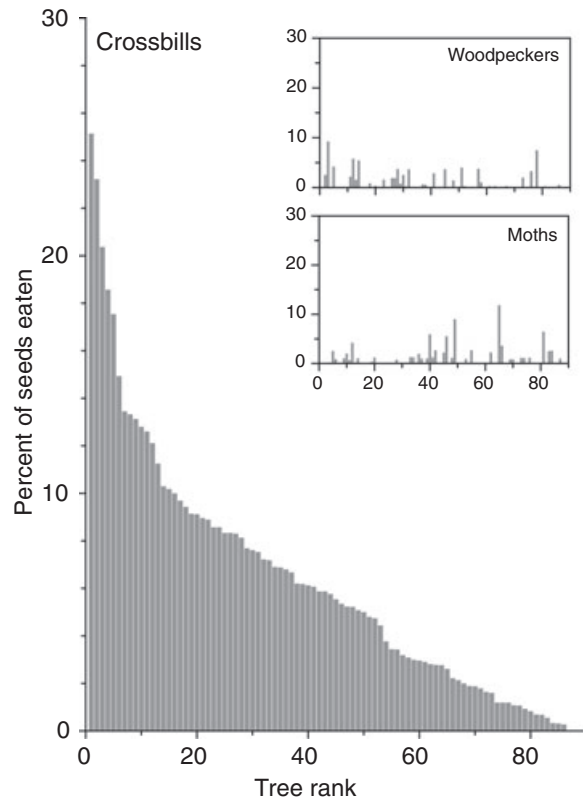


Figure 2. The percent of seeds eaten by crossbills for 87 trees ranked from the highest percent to the lowest. The insets represent data for seed predation by hairy woodpeckers and moths with the trees ranked in the same order as for crossbills.

woodpecker point-count data from Siepielski (2006) and cone data from Benkman (1999). We include data for seven ranges that represent sites where lodgepole pine dominated the forests in and near the survey points; we exclude the Judith Mountains because lodgepole pine represents relatively small patches within a much more extensive mixed forest.

Results

Crossbills consumed an average of 6.42% of the seeds per tree (Fig. 2; median = 5.76%), which was over six times greater than that consumed by either woodpeckers (mean = 0.93%, median = 0%) or moths (mean = 1.00%, median = 0%).

The percent of seeds eaten varied tremendously among trees (Fig. 2), which provides the opportunity for phenotypic selection. Crossbills tended to prefer smaller cones (Table 1; similar results were found when cone length or width was substituted for cone mass in the model, as these factors are highly correlated with cone mass: cone mass and length, $r = 0.82$, $n = 87$ trees, $P < 0.0001$; cone mass and width, $r = 0.89$, $n = 87$ trees, $P < 0.0001$), with the result that crossbills exerted selection favoring the evolution of larger, longer, and wider cones with larger

Table 1. Standardized selection gradients (β) from multiple linear regressions for selection exerted on lodgepole pine by crossbills, hairy woodpeckers, and cone moths ($n = 87$ trees). Significant P -values (< 0.05) are in bold.

Trait	Crossbills			Woodpeckers			Moths		
	$\beta \pm SE$	t	P	$\beta \pm SE$	t	P	$\beta \pm SE$	t	P
Cone width/length	0.0024 \pm 0.0061	0.34	0.75	0.0016 \pm 0.0020	0.77	0.59	-0.0022 \pm 0.0030	-0.64	0.65
Cone mass	0.0223 \pm 0.0097	2.35	0.053	0.0117 \pm 0.0030	3.90	0.0007	0.0032 \pm 0.0034	0.79	0.58
Distal-scale thickness	0.0026 \pm 0.0066	0.35	0.75	-0.0046 \pm 0.0020	-2.26	0.064	-0.0016 \pm 0.0018	-0.82	0.57
Scale length	-0.0056 \pm 0.0085	-0.71	0.62	0.0057 \pm 0.0031	1.79	0.16	-0.0078 \pm 0.0052	-1.36	0.32
Number of full seeds	-0.0047 \pm 0.0086	-0.54	0.69	-0.0085 \pm 0.0024	-3.56	0.002	-0.0044 \pm 0.0027	-1.55	0.24
Number of empty seeds	-0.0440 \pm 0.0067	-0.66	0.51	-0.0022 \pm 0.0019	-1.14	0.42	-0.0055 \pm 0.0032	-1.62	0.21
Individual seed mass	0.0077 \pm 0.0074	1.16	0.43	-0.0074 \pm 0.0024	-3.02	0.01	-0.0007 \pm 0.0022	-0.37	0.75

seeds (Table 2). Because crossbill feeding rates should increase with increases in the size of lodgepole pine seeds if other cone traits do not covary, selection exerted by crossbills favoring larger seeds presumably is mostly indirect and arises because individual seed mass is correlated with cone mass, length, and width ($r \geq 0.46$, $n = 87$ trees, $P < 0.0001$). Crossbills also tended to exert selection on cones favoring the evolution of trees with larger distal scales (Table 2), which is consistent with the results from the Little Rocky Mountains where selection favoring thicker distal scales was detected (Benkman et al. 2003). Indeed, the relationship between the selection differentials for selection exerted by crossbills in the South Hills and those in the Little Rocky Mountains was positive and significant (Fig. 3A), indicating that selection was consistent in form between these two ranges.

Woodpeckers preferred smaller cones with greater numbers of large seeds (Table 1). This resulted in selection favoring the evolution of larger cones with longer scales and especially a smaller ratio of seed mass to cone mass (Table 2). The only selection differential for woodpeckers that was larger than those for crossbills, was for the composite trait seed mass to cone mass ratio (Table 2). The only evidence of nonlinear selection was for selection exerted by woodpeckers on seed mass/cone mass (linear coefficient: -0.0072 ± 0.0021 [mean \pm SE], $t = -3.44$, $P = 0.003$; quadratic coefficient (γ): -0.0063 ± 0.0025 , $t = -2.51$, $P = 0.037$). However, selection was directional favoring the evolution of trees producing cones with less seed mass to cone mass (Fig. 4). If seed mass/cone mass, which is the trait with the strongest selection differential exerted by woodpeckers (Table 2), influences the seed availability to woodpeckers (and seed availability influences woodpecker abundance), then woodpecker abundance should increase with increases in the ratio of seed mass to cone mass. It does as shown in Figure 5.

No selection gradient (Table 1) or selection differential (Table 2) was significant for moths, although two of the selection differentials were marginally insignificant ($0.05 \leq P \leq 0.1$) including that for cone length, which was a trait for which selection was detected in the Little Rocky Mountains (Siepielski and Benkman 2004). As for crossbills, the relationship between the selection differentials for selection exerted by moths in the South Hills and Little Rocky Mountains was positive and significant (Fig. 3B), indicating that moths exerted selection that was consistent in form between these two ranges although much weaker in the South Hills.

The selection differentials (composite traits excluded; see Table 2) for all three seed predators combined were strongly correlated with those for crossbills (Fig. 6A); much more so than for those of the other two seed predators (Fig. 6B, C). The linear regression between the selection differentials from overall selection and those from crossbills ($selection\ differentials_{overall} = -0.0031 + 1.112 [selection\ differentials_{crossbills}]$, $P < 0.001$) had an intercept that did not differ from zero ($P = 0.25$) and the confidence interval for the slope overlapped one (95% CI: 0.064, 1.552). This indicates that we could not detect an effect of seed predation by woodpeckers and moths on the overall selection differentials relative to that exerted by crossbills alone. Thus, selection exerted by crossbills accounts for most of the selection on individual traits exerted by predispersal seed predators.

The strength of selection tended to increase as seed consumption by a seed predator increased. For example, crossbills consumed nearly seven times as many seeds as woodpeckers and over six times as many seeds as moths (Fig. 2), and the selection differentials (absolute values; excluding seed mass/cone mass ratio) for crossbills were 4.3 and 3.0 times larger than those for woodpeckers and moths, respectively (Table 2; Fig. 6). Moths consumed about one-seventh as many seeds in the South Hills as

Table 2. Selection differentials (*s*) for selection exerted by crossbills, hairy woodpeckers, cone moths, and the overall selection resulting from the total predation of lodgepole pine seeds by these three species (*n* = 87 trees). Significant *P*-values (<0.05) are in bold.

Trait	Crossbills			Woodpeckers			Moths			Overall		
	<i>s</i> ± SE	<i>t</i>	<i>P</i>	<i>s</i> ± SE	<i>t</i>	<i>P</i>	<i>s</i> ± SE	<i>t</i>	<i>P</i>	<i>s</i> ± SE	<i>t</i>	<i>P</i>
Cone length	0.0159 ± 0.0060	2.61	0.03	0.0048 ± 0.0019	2.57	0.03	-0.0051 ± 0.0024	-2.04	0.10	0.0158 ± 0.0071	2.29	0.06
Cone width	0.0215 ± 0.0058	3.71	0.001	0.0033 ± 0.0016	2.04	0.10	-0.0044 ± 0.0022	-1.95	0.12	0.0207 ± 0.0057	3.60	0.002
Cone width/length	0.0028 ± 0.0052	0.54	0.69	-0.0021 ± 0.0018	-1.17	0.40	0.0012 ± 0.0018	0.64	0.65	0.0020 ± 0.0068	0.20	0.78
Cone mass	0.0210 ± 0.0056	3.80	< 0.001	0.0052 ± 0.0018	2.94	0.01	-0.0045 ± 0.0026	-1.81	0.16	0.0221 ± 0.0063	3.58	0.002
Distal-scale thickness	0.0105 ± 0.0054	1.90	0.13	0.0002 ± 0.0013	0.08	0.79	-0.0044 ± 0.0029	-1.50	0.26	0.0061 ± 0.0066	0.96	0.50
Proximal-scale thickness	0.0082 ± 0.0063	1.35	0.32	0.0020 ± 0.0012	1.55	0.24	-0.0032 ± 0.0021	-1.50	0.26	0.0070 ± 0.0065	1.09	0.44
Scale length	0.0094 ± 0.0063	1.45	0.28	0.0054 ± 0.0022	2.43	0.04	-0.0059 ± 0.0033	-1.70	0.19	0.0089 ± 0.0086	1.10	0.43
Number of full seeds	0.0061 ± 0.0055	1.14	0.41	-0.0023 ± 0.0015	-1.55	0.24	-0.0015 ± 0.0017	-0.87	0.54	0.0019 ± 0.0067	0.24	0.77
Number of empty seeds	0.0022 ± 0.0051	0.44	0.72	0.0009 ± 0.0019	0.48	0.71	-0.0037 ± 0.0030	-1.30	0.34	-0.0008 ± 0.0058	-0.18	0.78
Individual seed mass	0.0155 ± 0.0066	2.43	0.04	-0.0003 ± 0.0019	-0.13	0.79	-0.0038 ± 0.0018	-2.12	0.09	0.0114 ± 0.0079	1.50	0.26
Seed mass/cone mass	-0.0032 ± 0.0059	-0.51	0.70	-0.0074 ± 0.0020	-3.38	0.004	-0.0010 ± 0.0018	-0.62	0.65	-0.0012 ± 0.0075	-1.63	0.21

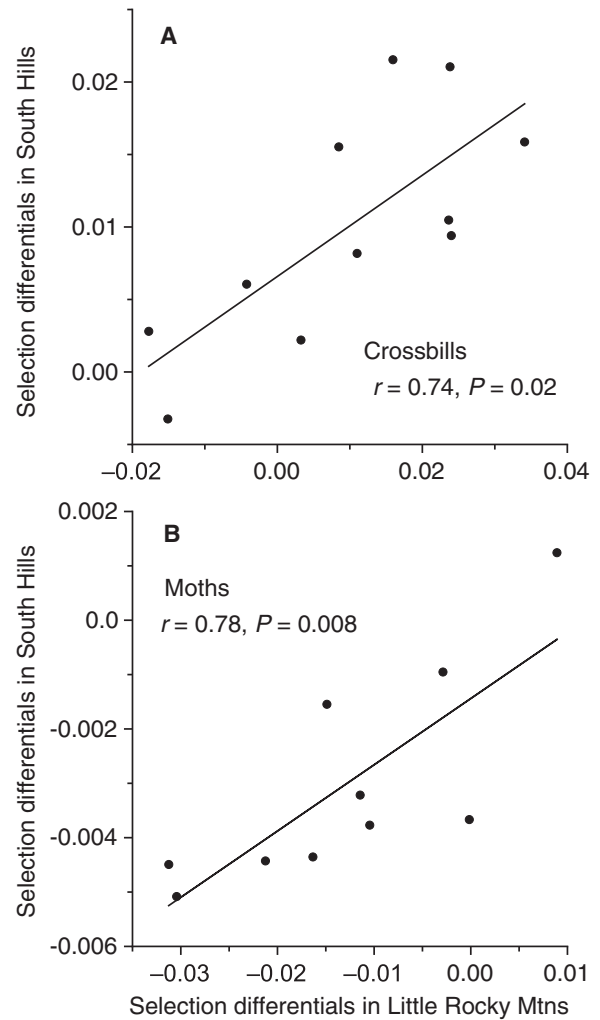


Figure 3. The selection differentials are positively related between those in the South Hills and Little Rocky Mountains for both (A) crossbills and (B) moths. The traits included were all those in Table 1 with the exception of scale length for selection exerted by moths because this trait was not measured in the study on moths in the Little Rocky Mountains (Siepielski and Benkman 2004).

in the Little Rocky Mountains, which may explain why the moth selection differentials in the South Hills were only about one-fifth of those in the Little Rocky Mountains (mean absolute values: 0.0033 vs. 0.015, respectively; paired *t*-test, *t* = 4.07, *P* = 0.003). We cannot make a similar comparison for crossbills because most of the trees sampled were not chosen randomly in the Little Rocky Mountains.

Discussion

Our results, especially when compared to the results of previous studies on seed predation and selection in another isolated range without pine squirrels, have important implications for studies on crossbill-lodgepole pine coevolution, the repeatability of selection

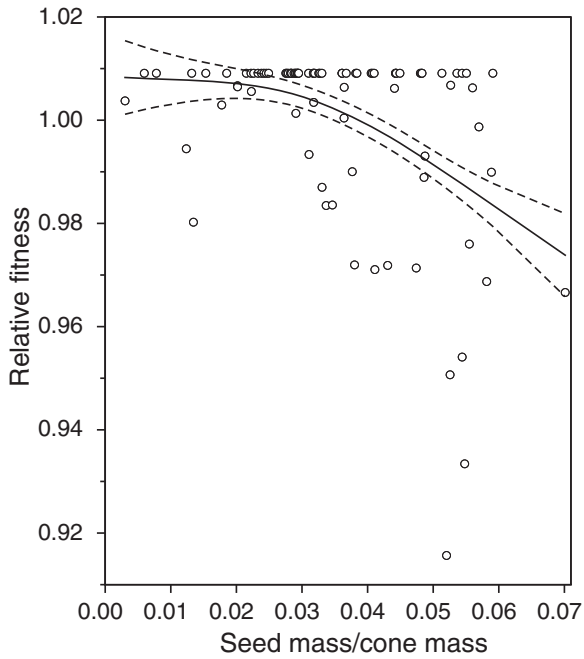


Figure 4. Relative tree fitness with respect to seed predation by woodpeckers decreases nonlinearly with increases in the ratio of seed mass to cone mass. The solid curve is a cubic spline, and the dashed lines represent 1 SE around the curve.

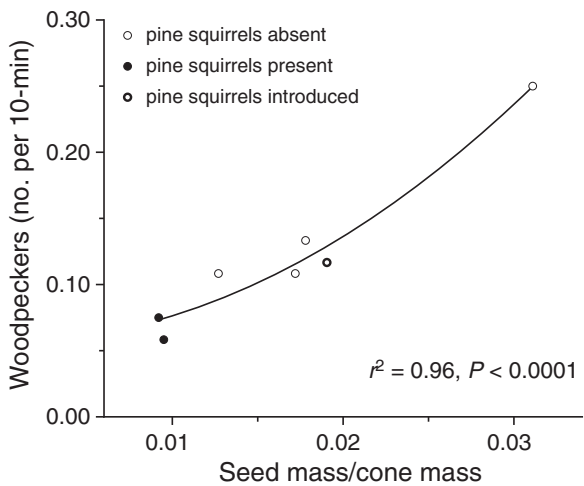


Figure 5. The densities of woodpeckers increase with increases in the ratio of seed mass to cone mass among seven mountain ranges with and without pine squirrels ($\text{woodpecker density} = 0.06 + 199.81 [\text{seed mass}/\text{cone mass}]^2$, $r^2 = 0.96$, $df = 5$, $P < 0.0001$; linear regression provides nearly as good of a fit: $r^2 = 0.93$, $df = 5$, $P = 0.0004$). The South Hills is the point in the upper right of the figure, and when excluded the relationship remains significant but is linear ($r^2 = 0.77$, $df = 4$, $P = 0.02$). Pine squirrels were introduced in the Cypress Hills in 1950 and are now more abundant than in the areas originally having them (Benkman 1999).

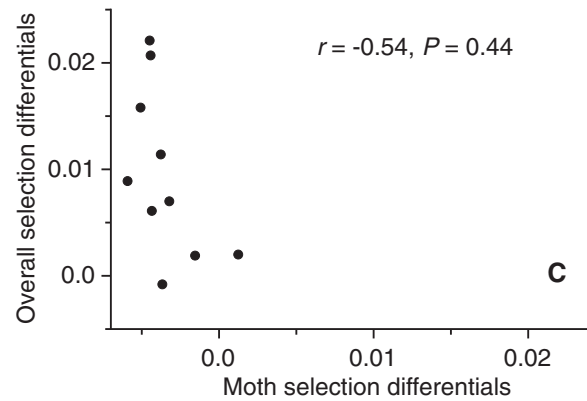
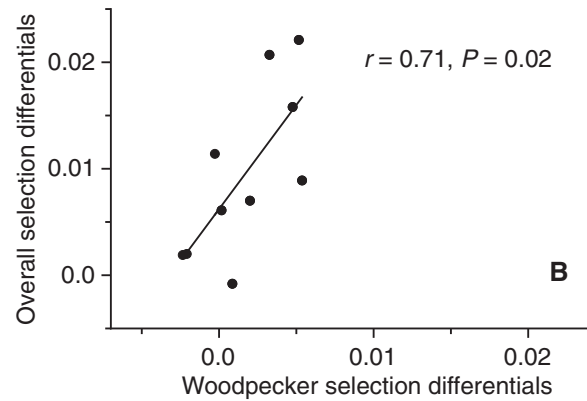
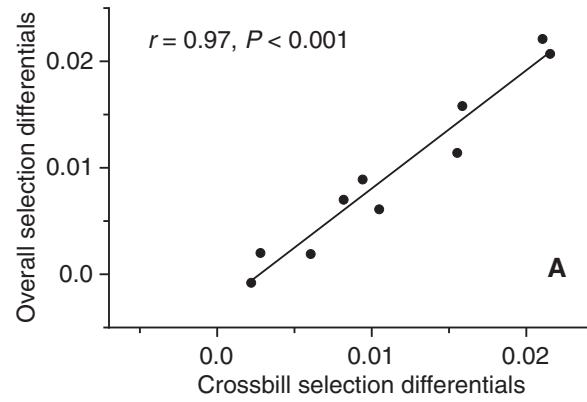


Figure 6. The relationships between the selection differentials arising from the total seed predation by all three predispersal seed predators and each of the three seed predators alone. The solid lines are based on least square linear regressions. The selection differentials exclude seed mass to cone mass ratio (see Table 1).

by different selective agents, and the relationship between the amount of predation and the strength of selection. Below we discuss these implications.

CROSSBILL-LOGEPOLE PINE COEVOLUTION

The substantially stronger selection, as measured by selection differentials, exerted by crossbills than by woodpeckers and moths

Table 3. Direction of selection (+ = favoring increase, - = favoring decrease, 0 = no detectable selection; $*0.1 \geq P \geq 0.05$) exerted by crossbills, hairy woodpeckers, cone moths (Table 1), and pine squirrels (Benkman et al. 2003) on cone traits of lodgepole pine, and the observed changes in these traits in the South Hills (and other ranges where pine squirrels are absent) relative to ranges with pine squirrels (Benkman et al. 2001). The second symbol for crossbills and moths is from studies in the Little Rocky Mountains (Benkman et al. 2003; Siepielski and Benkman 2004). Note that if crossbills, woodpeckers, and moths are important selective agents then the predicted changes in cone traits should be in the same direction of selection, whereas if selection by pine squirrels is important then the changes in their absence (relaxation of selection) should be in the opposite direction. Scale length is not shown because we have not measured it in ranges with pine squirrels.

Cone trait	Selection exerted by					Observed Δ in absence of squirrels
	Crossbills	Woodpeckers	Moths	Combined	Pine squirrels	
Cone length	+, +	+	-, -	+*	-*	+
Cone width	+, +*	+*	0, -	+	0	+
Cone width/length	0, -	0	0, 0	0	+	-
Cone mass	+, +	+	0, -	+	0	+
Distal-scale thickness	0, +	0	0, -	0	0	+
Proximal-scale thickness	0, 0	0	0, -	0	0	-
Number of full seeds	0, 0	0	0, -	0	-	+
Individual seed mass	+, 0	0	-, 0	0	0	+
Seed mass/cone mass	0, -	-	0, 0	0	-	+

(Table 2) and the strong correlation between the selection differentials arising from crossbill predation and overall seed predation (Fig. 6A) support previous studies that have emphasized selection exerted by crossbills on lodgepole pine in the South Hills (Benkman 1999; Benkman et al. 2001, 2003). Moreover, the combination of selection exerted by crossbills in the South Hills and relaxation of selection by pine squirrels appears to be sufficient to account for seven of the nine trait changes that have occurred in lodgepole pine in the South Hills in comparison to lodgepole pine in ranges with pine squirrels (Table 3). Distal scales are thicker in the South Hills than in ranges with pine squirrels yet pine squirrels were not found to exert selection on this trait (Benkman et al. 2003) nor did we detect selection by crossbills on distal-scale thickness in the South Hills (Tables 2 and 3). However, we detected selection by crossbills in the Little Rocky Mountains favoring thicker distal scales (Table 3), and in the South Hills there was a tendency for crossbills to exert selection favoring thicker distal scales (Table 2: $s = 0.0105$, $P = 0.12$). These results indicate that with increased sample sizes in the South Hills, especially directed sampling that increases the number of trees at the extremes (high and low seed predation by crossbills), as done in the Little Rocky Mountains (Benkman et al. 2003), would likely reveal selection exerted by crossbills on distal-scale thickness. Distal-scale thickness is the one trait that we have found to be under selection by crossbills in other conifers and has evolved in response to such selection (Parchman et al. 2007; Parchman and Benkman 2008; Benkman and Parchman 2009; Benkman et al. 2010; Mezquida and Benkman 2010). This makes functional sense because thicker scales impede the spreading apart of scales by crossbills and

most of the seeds are in the distal part of the cone (although note in Fig. 1 that crossbills do not forage exclusively at the distal end). Concerning the decrease in proximal-scale thickness in the absence of pine squirrels, observations by Smith (1970) and Elliott (1974, 1988) of pine squirrels foraging on lodgepole pine indicate that proximal-scale thickness experiences selection and should decrease with the relaxation of selection by pine squirrels (assuming thicker scales, and more generally any increase in cone mass, require the allocation of additional resources and are thus costly to produce). Thus, our study combined with earlier studies indicate that the observed changes in all nine cone traits between areas with and without pine squirrels (e.g., South Hills) can be explained by selection or relaxation of selection by the various predispersal seed predators.

Finally, our study rectifies a prior criticism that coevolution between crossbills and lodgepole pine in the South Hills was inferred using measurements of selection exerted by crossbills in the Little Rocky Mountains rather than in the South Hills (Gomulkiewicz et al. 2007). In combination with previous studies (Benkman et al. 2003), we have now documented reciprocal selection and evolution between crossbills and lodgepole pine in the South Hills further strengthening these studies as an example of the geographic mosaic of coevolution (Benkman 1999; Benkman et al. 2001, 2003; Thompson 2005; Gomulkiewicz et al. 2007).

CONSISTENCY AND VARIATION IN SELECTION

The extent of variation in selection in time and space has important evolutionary implications, including the extent of phenotypic stasis and evolutionary divergence between populations. If

selection varies in direction over time then phenotypic stasis is a potential outcome, whereas if net selection is directional then phenotypic evolution is likely with phenotypic divergence between populations experiencing divergent selection. Some studies that have examined selection over multiple years find that selection varies, often considerably from year to year (Siepielski et al. 2009), but recent analyses indicate that directional selection is usually consistent over time (Morrissey and Hadfield 2012).

Our measures of selection integrate seed predation over 10 annual cohorts of cones so we are integrating selection over an extended period of time. For moths, it represents 10 years of seed predation because each year they attack only cones maturing that year, whereas for crossbills and woodpeckers our measures probably represent at most five years of seed predation because few cones less than five years of age are preyed upon. Although these estimates of selection do not allow us to examine annual variation in selection directly, our measures allow us to detect if there are net trends in selection and if so this suggests that fluctuations in selection do not eliminate overall or cumulative directional selection (Kingsolver and Diamond 2011).

The relative strengths of selection exerted by crossbills on the different cone traits in the South Hills were similar to those exerted in the Little Rocky Mountains (Fig. 3A). A comparable pattern was also found for selection exerted by moths in these two mountain ranges (Fig. 3B), although the selection they exerted was much weaker than that exerted by crossbills. This indicates that patterns of selection exerted on lodgepole pine by these two predispersal seed predators are repeated from location to location. Such consistent patterns of net selection strongly support previous interpretations (Benkman 1999; Benkman et al. 2001; 2003). Namely, the replicated patterns of cone evolution among different mountain ranges in the absence of pine squirrels east and west of the Rocky Mountains are the result of selection exerted by crossbills and relaxation of selection exerted by pine squirrels. Although we have not measured selection exerted by pine squirrels in multiple locations, our previous study (Benkman et al. 2001, 2003) combined with the observations and data in Smith (1970) and Elliott (1974, 1988) indicate that selection exerted by pine squirrels is also likely replicated among locations. In combination with genetic studies of the crossbills (Parchman et al. 2006) and lodgepole pine (Parchman et al. 2011), we can now conclude with even greater confidence that coevolution has occurred independently and repeatedly in isolated ranges east and west of the Rocky Mountains.

Although the patterns of selection among traits are consistent both for crossbills and moths, the strength of selection on individual traits varied greatly depending on the abundance of the seed predator. Higher abundances and greater seed predation appear to lead to larger selection differentials as found in our

comparisons among the three predators in this study. The percent of seeds consumed by crossbills in the South Hills was 1.2 times greater than that found in the Little Rocky Mountains (5.2% eaten; estimated using the subset of trees from Benkman et al. 2003 that were selected randomly [$n = 29$]), which presumably reflects the 1.3 times greater densities of crossbills in the South Hills than in the Little Rocky Mountains (1.5 vs. 1.2 crossbills/10-min point-count, respectively; Siepielski and Benkman 2005). Similarly, Siepielski and Benkman (2005) found that in four ranges without pine squirrels, the first principal component (PC1) of seven lodgepole pine cone traits increased linearly with increases in the density of crossbills. Because crossbills in the South Hills exert selection that would cause an increase in PC1 (based on a comparable analysis using principal components: 0.0102 ± 0.0027 [$s \pm SE$], $t = 3.84$, $P < 0.001$), our results further strengthen the interpretation that increasing densities of crossbills lead to increased seed predation and to concordant increases in selection causing an escalation in lodgepole pine seed defenses. Because crossbill densities increase with the forest area of lodgepole pine (Siepielski and Benkman 2005), increases in forest area lead to increases in lodgepole pine seed defenses directed at crossbills. Such a result has also been found for black pine (Benkman and Parchman 2009) and mountain pine (*P. uncinata*; Mezquida and Benkman 2010) in southern Europe. In the latter study, seed predation by crossbills was also found to be greater where forests were extensive (Pyrenees) than in smaller isolated forests. The presence and absence of tree squirrels also potentially affect the abundance of crossbills. Crossbills occurred in densities that averaged about 20 times higher in the South Hills than in the Rocky Mountains where pine squirrels are present (Benkman 1999; Siepielski and Benkman 2005). The much lower densities of crossbills in ranges with pine squirrels should result in minimal selection exerted by crossbills on lodgepole pine. In sum, forest area and the occurrence of tree squirrels influences the densities of crossbills, which in turn influences the level of seed predation and selection, with consequences for the extent of escalation in seed defenses and coevolution between crossbills and conifers, and the extent of divergent selection between areas with and without tree squirrels (Benkman et al. 2010).

INDIRECT EFFECTS ON POPULATION DENSITIES

Seed defenses may also act to limit the abundances of seed predators, and thus influence the relative abundance of seed predators and the selection they exert. The ratio of seed mass to cone mass is the main trait under selection by pine squirrels, and likely explains why this ratio's value is so low in ranges with pine squirrels (Smith 1970; Benkman 1999; Benkman et al. 2003, 2010); only about 1% of the cone's mass consists of seeds where pine squirrels are common (Fig. 5; Smith 1970; Benkman et al. 2010). Note that selection by woodpeckers and perhaps crossbills should favor

a decrease in the ratio of seed mass to cone mass (Table 2) yet this ratio increases in the absence of pine squirrels presumably because of relaxation of even stronger selection by pine squirrels (Table 3). Presumably woodpeckers prefer such cones (Tables 1 and 2; Fig. 4) because they have less cone mass that needs to be shredded and removed to reach a given amount of underlying seed mass (see Fig. 1). Clark's Nutcrackers (*Nucifraga columbiana*) also use their bills to hammer and shred cone scales (but do not forage on lodgepole pine) like woodpeckers, and nutcrackers also preferentially forage on cones with high ratios of seed to cone mass (Siepielski and Benkman 2007a).

Given the avoidance by woodpeckers of cones with low ratios of seed mass to cone mass (Fig. 4), we predicted that if lodgepole pine seeds form an important component of their diet then woodpecker densities should increase with increases in the ratio of seed mass to cone mass. The relationship should be reversed if selection exerted by woodpeckers was driving the variation in the ratio of seed mass to cone mass. Woodpeckers increased with increases in the ratio of seed mass to cone mass (Fig. 5). These results (Figs. 4, 5) imply that woodpeckers are less common in lodgepole pine where seeds are less accessible because selection by pine squirrels has caused the evolution of cones with less seed mass relative to cone mass. This represents a trait-mediated indirect effect of pine squirrels (Benkman et al. 2012). In contrast, the competitive (i.e., a density-mediated) effect from pine squirrels is minimal. Three of the seven ranges currently have pine squirrels (Fig. 5), and when the presence and absence of pine squirrels is included as a covariate in the regression, woodpeckers only tended to be more abundant in the absence of pine squirrels ($P = 0.12$) while the effect of seed mass/cone mass remained significant ($P = 0.0004$).

CONCLUSIONS

We found two seed predators exerting selection on lodgepole pine that was consistent between two mountain ranges, with the cumulative impact of such selection by predispersal seed predators over thousands of years presumably accounting for the replicated patterns of trait divergence between these and other mountain ranges. Such consistency in selection is perhaps the result of the elevated levels of physical seed defenses in cone-bearing conifers. First, elevated defenses likely act to restrict access by all but the most specialized seed predators and then limit their abundances depending on their abilities to access seeds. Where pine squirrels are present, they are very strong preemptive competitors that exert strong selection that leads to extreme defenses that limit the abundance of both cone borer moths (Siepielski and Benkman 2004) and hairy woodpeckers (Fig. 5). Crossbills are also uncommon but this is more likely the result of exploitative competition (Benkman 1999; Siepielski and Benkman 2005). The result is

that where pine squirrels are present, the selection they exert overwhelms and drives the evolution of lodgepole pine cones. Second, elevated defenses should favor feeding selectivity that causes consistent selection. For example, if seed defenses were few then the importance of variation in these defenses to the choices of seed predators may be minimal and overwhelmed by other environmental factors influencing resource use. If on the other hand the defenses are so great that a given seed predator can access only a small fraction of the seeds, then other environmental variables may have little influence on the food choices of the seed predators. Third, the level of physical and chemical defenses of seeds tends to be inversely related to temporal fluctuations in seed availability (Janzen 1971, 1974). Thus, highly defended seeds like those in lodgepole pine usually present a relatively stable resource for seed predators (Smith 1970).

In isolated ranges where pine squirrels are absent, the abundances of other seed predators increase accordingly. The consistent and relatively strong selection exerted by crossbills in the South Hills drives cone divergence from areas where pine squirrels are present, which leads to increasingly strong divergent selection on crossbills (Benkman et al. 2003). This increasingly strong (and consistent) divergent selection may account for why South Hills crossbills have evolved nearly complete reproductive isolation in perhaps only the last 3–5000 years (Smith and Benkman 2007). Consistent rather than fluctuating selection is critical to ecological speciation whereby divergent ecological selection causes reproductive isolation (Price 2008). When the environment fluctuates over time, as for seed size and hardness in the Galápagos, selection can vary from strong selection against hybrids to selection favoring hybrids leading to the potential collapse of diverging populations of Darwin's ground finches (*Geospiza* spp.; Grant and Grant 2008). Indeed, the nearly ubiquitous coevolution between crossbills and conifers (Benkman et al. 2010) and the resulting consistent directional and divergent selection must have contributed to the rapid radiation (mostly post-Pleistocene) and speciation within the crossbill complex (Parchman et al. 2006; Smith and Benkman 2007). Because speciation appears critical for sustained evolution (Futuyma 2010), and speciation is most likely when selection is consistent and strong (Price 2008), determining the conditions and situations that cause such selection remains a central problem in evolutionary biology (Nosil et al. 2009; Futuyma 2010).

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