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CAUSES OF VARIATION IN BIOTIC INTERACTION STRENGTH AND PHENOTYPIC SELECTION ALONG AN ALTITUDINAL GRADIENT

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Understanding the causes of variation in biotic interaction strength and phenotypic selection remains one of the outstanding goals of evolutionary ecology. Here we examine the variation in strength of interactions between two seed predators, common crossbills (*Loxia curvirostra*) and European red squirrels (*Sciurus vulgaris*), and mountain pine (*Pinus uncinata*) at and below tree limit in the Pyrenees, and how this translates into phenotypic selection. Seed predation by crossbills increased whereas seed predation by squirrels decreased with increasing elevation and as the canopy became more open. Overall, seed predation by crossbills averaged about twice that by squirrels, and the intensity of selection exerted by crossbills averaged between 2.6 and 7.5 times greater than by squirrels. The higher levels of seed predation by crossbills than squirrels were related to the relatively open nature of most of the forests, and the higher intensity of selection exerted by crossbills resulted from their higher levels of seed predation. However, most of the differences in selection intensity between crossbills and squirrels were the result of habitat features having a greater effect on the foraging behavior of squirrels than of crossbills, causing selection to be much lower for squirrels than for crossbills.

KEY WORDS: ***Loxia curvirostra*, *Pinus uncinata*, Pyrenees, *Sciurus vulgaris*, selection intensity, Spain, structural equation modeling.**

Much progress has been made in understanding patterns and consequences of spatial variation in biotic interactions over the last several decades (Thompson 1994, 2005, 2013). However, less progress has been made in understanding the underlying causes of such variation (Thompson 2005), especially in terms of the causes of phenotypic selection (MacColl 2011). Although thousands of estimates of phenotypic selection have been made (Siepielski et al. 2009), in only a relatively few cases do we know the mechanisms underlying selection and its causes of variation (Endler 1986; Wade and Kalisz 1990; MacColl 2011; Benkman 2013). Yet, understanding the causes of variation in biotic interaction strength and the resultant phenotypic selection (Benkman 2013) is critical for understanding the patterns and processes of evolution and diversification (McPeek 1996; Mittelbach et al. 2007; Schemske 2009; Schemske et al. 2009).

It is useful to consider how the upper limit for phenotypic selection varies in relation to biotic interaction strength (Benkman 2013; see also Vanhoenacker et al. 2013). In the case of antagonistic interactions such as predation, the upper limit for selection, as measured by standardized selection differentials, increases monotonically with increasing strength of the interaction (Fig. 1; see Benkman [2013] for mutualistic interactions). All else equal, the stronger the antagonistic interaction the stronger the selection. However, selection intensity is influenced also by the covariance between fitness and the traits under selection (Lande and Arnold 1983). For a given interaction strength, realized selection will increase as fitness–trait covariance increases, with truncation

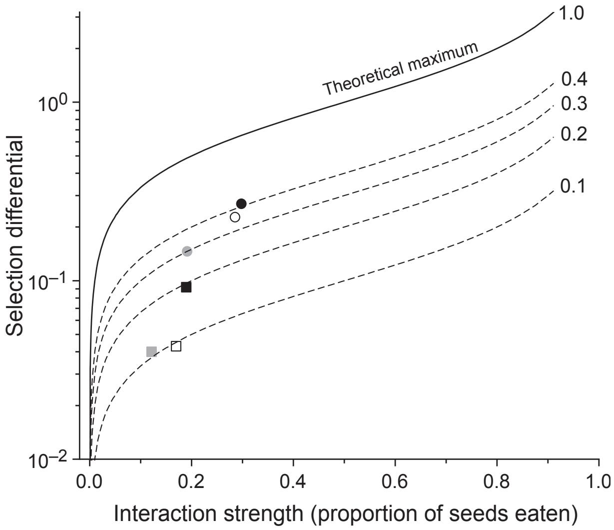

Figure 1. **The relationships between selection differentials and antagonistic interaction strengths showing the theoretical maximum (Benkman 2013), and contours representing 0.4, 0.3, 0.2, and 0.1 of the maximum. The different symbols represent values for traits at the phenotypic interface of the interaction for crossbills (scale thickness: circles; open: 2007, gray: 2008, black: 2009) and red squirrels (seed mass/cone mass: squares; open: 2007, gray: 2008, black: 2009). Note that the selection differential axis is on a $\log_{10}$ scale.**

selection required for maximum selection (Benkman 2013). Variation in the proportion of the maximum selection for a given interaction strength, or realized selection, is illustrated by the contours in Figure 1. Clearly, both interaction strength and fitness–trait covariance will be important in determining the intensity of selection. A goal, therefore, should be to tease apart their relative contributions to variation in the selection arising from biotic interactions in both time and space, and to understand the causes of this variation. For example, habitat features potentially affect the abundance of species, and hence the interaction strength, and also the fitness–trait covariance by influencing the differential use of microhabitats. Consequently, the question is not only an ecological question, but for many plant–animal interactions it is also a behavioral ecological question that will require an understanding of how habitat features affect distribution, abundance, and behavior.

Here, we address this goal for the interaction between mountain pine (*Pinus uncinata*) and its two main predispersal seed predators, common (red) crossbills (*Loxia curvirostra*) and European red squirrels (*Sciurus vulgaris*; Lescourret and Génard 1983, 1986a; Génard and Lescourret 1987; Mezquida and Benkman 2010). We quantified the strength of their interactions along an altitudinal gradient in the Pyrenees of northern Spain (Fig. 2); here the abundance of red squirrels declines with increasing elevation (Lescourret and Génard 1983, 1986a; see also Castro et al. 1999; Wauters et al. 2008), whereas the abundance of crossbills increases with increasing elevation (Génard and Lescourret 1987). The decrease in red squirrel abundance appears to be related to the decrease in tree density, because red squirrels rely extensively on seeds in conifer cones, especially from late summer to early spring (Moller 1983; Wauters and Dhondt 1987; Wauters 2000), and on the cover provided by the canopy to escape from predators (Summers and Proctor 1999; Flaherty et al. 2012; see Fig. S1); longer distances between trees increase the vulnerability of squirrels moving between trees. Crossbills rely almost exclusively on seeds in conifer cones (Newton 1972; Génard and Lescourret 1987), but do not rely on canopy cover to escape predators, and instead fly up and away from them (Benkman 1992; E. T. Mezquida, pers. obs.). Consequently, the open forests at higher elevations (Fig. S1A) may be preferred because crossbills can detect an approaching predator from farther away (Benkman 1987; Summers and Proctor 1999).

We also examine how these interactions translate into phenotypic selection exerted on the seed cones of mountain pine. Numerous studies have shown that crossbills exert selection on conifer cone structure that is repeated in form within and among conifers because crossbills forage in a stereotypic manner (Benkman et al. 2010 for review; see also Benkman et al. 2013). In particular, selection often favors an increase in cone scale thickness because it deters crossbills from spreading apart the scales to reach the underlying seeds at the base of the scales. In addition, the form of selection is consistent across habitats and in both aviaries and the wild, indicating that other environmental variables have relatively little impact on the form of selection exerted by crossbills (Benkman and Parchman 2013). Tree squirrels (*Sciurus* and *Tamiasciurus*) also forage in a stereotypic manner, and they exert selection that favors an increase in cone mass relative to seed mass because it approximates the amount of cone mass that squirrels need to bite through to access seeds (Benkman et al. 2010). Tree squirrels bite off successive scales starting at the base of the cone to get access to underlying seeds that are located mostly in the distal half to two-thirds of the cone. However, studies on tree squirrels indicate that their tree preferences, and thus the covariance between fitness and cone traits, might be influenced by forest structure (Mezquida and Benkman 2010; Flaherty et al. 2012).

Earlier, we (Mezquida and Benkman 2010) examined seed predation and phenotypic selection exerted by both crossbills and red squirrels on mountain pine, but our focus was on geographic comparisons between multiple sites across the Pyrenees to sites in two small, isolated range in the central part of the Iberian Peninsula. We found that geographic differences in the strength of the interactions and associated differences in the form and intensity of selection account for the phenotypic differences in cone traits between regions; cone traits in conifers are known to

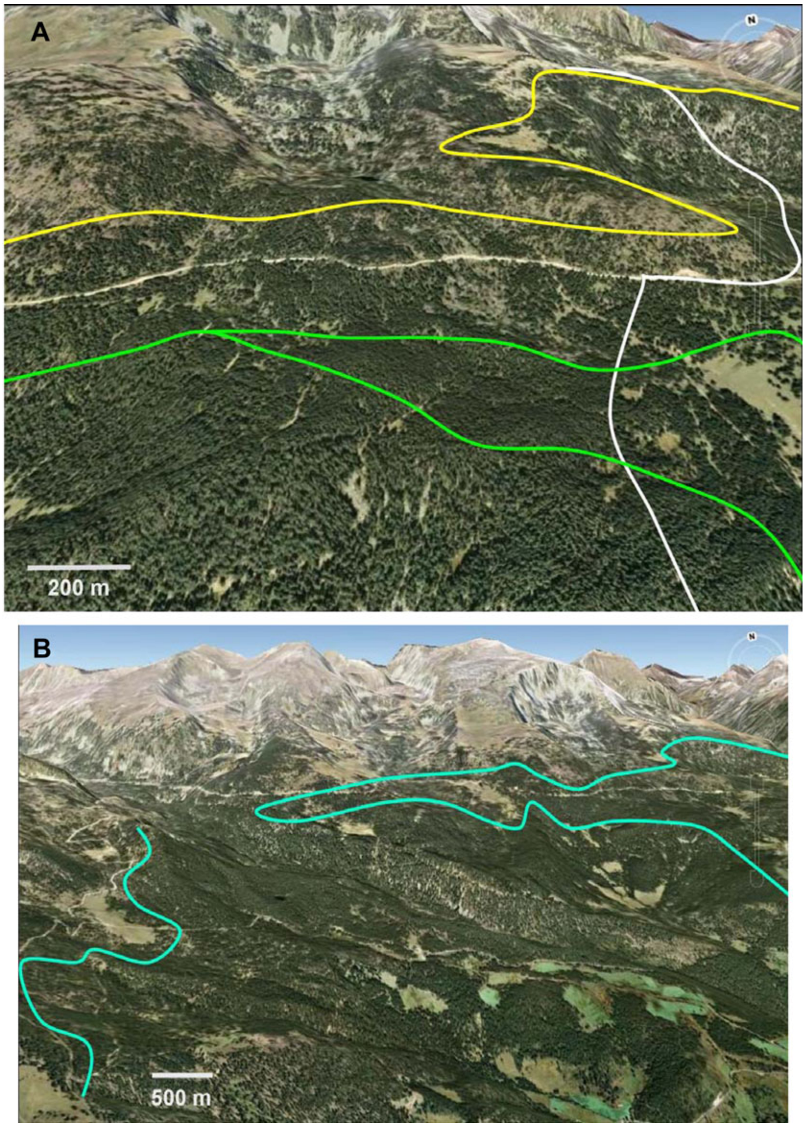

Figure 2. The approximate location of the three transects. (A) The white line represents the first 2008 transect, and the yellow and green lines represent the high- and low-elevation transects, respectively, in 2008. (B) The blue line depicts the 2009 transect. For orientation, the large sloping meadow above tree limit at the right of center of B is the same meadow as found in the upper left of A.

have high heritabilities and therefore should evolve in response to selection (Singh and Chaudhary 1993; Matziris 1998). These results implied an escalated coevolutionary arms race between crossbills and pine in the Pyrenees. Our focus here is on how tree and forest features (number of cones produced, and tree density and canopy closure) influence the strength of the species interactions (i.e., seed predation) and the intensity of selection on different phenotypic traits, and especially on traits known to be the target of selection (scale thickness for crossbills, ratio of seed mass to cone mass for red squirrels). Both crossbills and red squirrels are exclusively seed predators (i.e., do not disperse seeds) in their interactions with mountain pine. We focus on crossbills and red squirrels because they, especially crossbills, are dominant predispersal seed predators in this system (Lescourret and Génard 1986a,b). We did not detect any seed predation by great spotted woodpeckers (*Dendrocopos major*), and very little seed predation by insects comparable or less than what has been recorded previously (up to ~3.5 % of seeds; Lescourret and Génard 1983).

Methods and Materials

We conducted three sets of transects near Lles de Cerdanya (42° 25′N, 1° 40′E) during two years to address how seed predation and phenotypic selection exerted by crossbills and squirrels on mountain pine vary in relation to forest structure along an altitudinal gradient. Lles de Cerdanya is located on the southern slopes of the La Cerdanya Valley, eastern Pyrenees, Spain, where mountain pine forests occupy extensive areas below tree limit (Mezquida and Benkman 2010). The first transect was completed in 2008, in which seed predation was quantified along an altitudinal transect (Fig. 2A; white line). A second transect was completed in 2008, in which phenotypic selection was quantified at high and low elevations (Fig. 2A; yellow and green lines, respectively). A third transect was completed in 2009 (Fig. 2B), in which selection again was quantified, but instead of dividing the transects into high and low elevations, the elevation of each tree was recorded so that elevation could be included as a continuous variable, as in the first transect. The data we collected over time changed because of refinement of our predictions, and thus our analyses vary with each dataset.

VARIATION IN SEED PREDATION BY CROSSBILLS AND SQUIRRELS IN RELATION TO ELEVATION

During late October 2008, we started at a point haphazardly chosen in the upper tree limit at an elevation of 2330 m and walked downslope to an elevation of 1930 m (Fig. 2A). We selected a tree haphazardly every 10 to 20 m and recorded, for a total of 150 trees (approximately one tree every 2.7-m change in elevation), elevation, distance to nearest cone-bearing tree, and the number of cones depredated by crossbills and squirrels at the tree's base. Seed predation by crossbills and squirrels can be easily quantified and distinguished, because the cone remains differ, and both species remove the cones from the branches and drop them below the tree (Mezquida and Benkman 2010). To test whether seed predation by crossbills and squirrels varied with elevation and tree density (using the distance to the nearest tree as a proxy for tree density), we used negative binomial regressions because of the skewed distribution of counts and variances were much greater than the means for both count variables.

PHENOTYPIC SELECTION EXERTED BY CROSSBILLS AND SQUIRRELS IN RELATION TO FOREST STRUCTURE AND ELEVATION

We estimated the targets and form of phenotypic selection exerted by crossbills and squirrels on cone structure by quantifying seed predation in relation to cone traits of trees along a second set of transects also surveyed during late October 2008 (Fig. 2A). The survey was divided into high- and low-elevation transects, with the high-elevation transect located above an elevation of 2100 m (Fig. 2A). We chose trees haphazardly and recorded the number of cones depredated by crossbills and squirrels underneath each tree. We also counted the number of cones remaining on the tree with the aid of binoculars.

We used a branch cutter attached to an extendable pole to collect four cones that survived predation from each tree. We measured the following traits of each cone (Benkman et al. 2003): maximum length and width of the closed cone, cone mass without seeds, number of full seeds (i.e., filled with female gametophyte), number of empty seeds, mass of five filled seeds without their wings, and the thickness of five scales and length of three scales in the middle part of the cone. We measured scales at similar positions owing to the asymmetry of the cones (Mezquida and Benkman 2010). Length measurements were made to the nearest 0.01 mm with digital calipers and mass measurements were made on oven-dried (65°C for >36 h) cones and seeds to the nearest 0.01 mg with a digital scale. We calculated the ratio of seed mass to cone mass (a measure of the amount of energy devoted to reproduction relative to seed defense; Benkman 1999), as the total seed mass in grams (number of full seeds times individual seed mass) divided by cone mass in grams. Cone measurements were averaged for each tree because trees were the experimental units in the statistical analyses. Sample sizes for these analyses were 50 trees for the upper transect and 47 trees for the lower transect; storms prevented us from sampling more trees. Because we sampled cones that remained after crossbills and squirrels foraged, our measures underestimate (sensu Hadfield 2008) the intensity of selection if crossbills and squirrels were selective of cones within a tree in addition to being selective among trees. However, this effect should be small because variation within trees averages about one half the variation among trees (Garcia et al. 2009 and references therein). If there is a resulting bias in our comparisons, the underestimation of selection intensity should increase with increasing predation, as fewer cones will remain for us to sample as seed predation increases. This could cause us, for example, to underestimate the difference in the intensity of selection between crossbills and squirrels.

We used multiple regression models between relative tree fitness and cone traits to determine the targets of selection (direct selection) by crossbills (Lande and Arnold 1983). Tree fitness in relation to crossbill predation was estimated as one minus the proportion of cones foraged on (i.e., the number of cones foraged on by crossbills divided by the total number of cones). Absolute tree fitness was converted into relative tree fitness by dividing individual tree fitness by mean population fitness, and cone traits were standardized to zero mean and unit variance. We used the proportion of seeds not eaten as a measure of fitness because we sought a measure of fitness during one episode of selection that would be representative of relative fitness in response to selection exerted by seed predators over the lifetime of a long-lived iteroparous tree whose annual seed output increases with size and age. By using the proportion of seeds not eaten as a surrogate for fitness, trees that produce cones with traits that deter seed predators will be assigned a high relative fitness regardless of whether they are young and produce relatively few cones or they are older, larger, and produce many more cones (Siepielski and Benkman 2007a). In contrast, if we used the total number of seeds not eaten, then our measure of relative fitness will depend more on the age and size of the tree during the year of the study and therefore be less representative of the tree's expected lifetime fitness relative to other individuals.

To avoid multicollinearity, we examined correlation coefficients between traits and checked variance inflation factor scores from regression models. We removed two cone size variables (cone length and width) because of their high variance inflation factor scores. These scores were <2 for the remaining six cone and seed traits included in the model. To test for the effect of stand structure on seed predation by crossbills and to control for its potential influence on selection coefficients, we included elevation of transect (low or high elevation; Fig. 2A) as an independent dummy variable in the multiple regression model (Gómez 2003). We estimated linear selection gradients and checked for nonlinear selection by examining a multiple regression model with quadratic and cross-product terms (Lande and Arnold 1983). All quadratic terms were doubled (Stinchcombe et al. 2008).

We estimated selection differentials using least squares regression analyses between relative tree fitness and each of the nine cone and seed traits to determine which traits were under selection (both direct and indirect selection) due to predation by crossbills (Lande and Arnold 1983). We examined quadratic regression models to check for nonlinear selection on all traits, and used cubic splines to further visualize the form of selection (Schluter 1988). We followed similar procedures and analyses to estimate the targets and form of selection exerted by squirrels on mountain pine.

To characterize stand structure of the high- and low-elevation transects, we estimated tree density using the point-centered quarter method. For every third sampled tree, we located a point at a direction and distance (1–30 m) randomly chosen. At each point, we recorded the elevation and the distance to the nearest cone-bearing tree in each of four quadrants, for a total of 17 locations in the high-elevation transect and 13 in the low-elevation transect. We used a one-tailed *t*-test to test the prediction that tree density was greater at low than high elevations.

To further explore the targets and form of selection exerted by crossbills and squirrels on mountain pine taking into account canopy structure around each tree, we quantified canopy closure and seed predation, and sampled cones from 100 trees along a third elevation transect during late October and early November 2009 (Fig. 2B). We used the same general methods to choose trees, estimate seed predation, and collect and measure cones as described above. To estimate canopy closure around each tree, we took eight hemispherical photographs, two at each of the four cardinal directions two and four meter apart from the canopy edge. Photographs were taken using a Nikon Coolpix 4500 with a Nikon FC-E8 fisheye lens, fixing the optical axis to the zenith direction. We used Hemiview 2.1 Canopy Analysis Software (Delta-T Devices, Burwell, Cambridge, UK) to calculate the proportion of visible sky in each photograph. The sky map of each hemispherical photograph was divided into 16 angular sectors (corresponding to azimuth angles of 22.5°) and nine angular rings (corresponding to zenith angles of 10°), and the proportion of the sky that was visible was calculated for each sector. The overall proportion for each photograph was the sum of the 16 sectors, not including sectors with erroneous calculations due to excessive brightness or reflections. Canopy closure around each tree was estimated as one minus the mean proportion of the sky that was visible in the eight photographs. We recorded the elevation of each sampled tree. We did not record the elevation of each sampled tree in the "second" 2008 transect.

We used multiple linear and nonlinear (quadratic) regressions to determine the targets of selection for crossbills and squirrels. To estimate selection gradients we followed similar procedures as described for the "second" 2008 transect, and included canopy closure around each tree as an independent continuous variable in the regression models. To determine the form of selection exerted by crossbills and squirrels (selection differentials), we used pairwise regressions between relative fitness and cone traits as described above.

TEASING APART THE VARIOUS FACTORS AFFECTING SEED PREDATION BY CROSSBILLS AND SQUIRRELS

We used data from the 2009 transect and structural equation modeling (SEM) to estimate selection on cone traits by crossbills and squirrels while considering the influence of other plant traits and abiotic factors on both seed predators, and ultimately tree fitness. Structural equation modeling allows for the analysis of a set of hypothesized relationships among fitness and variables (e.g., traits, environmental variables), mediated by other variables (e.g., seed predators; Mitchell 1992; Scheiner et al. 2000). We first built an a priori full model using previous information on this and similar systems (Benkman et al. 2010; Mezquida and Benkman 2010). We hypothesized that cone traits affect seed predation by crossbills and squirrels, so we connected the same six cone traits used in the above multiple regressions (cone mass, number of full and empty seeds, individual seed mass, and scale thickness and length) to the proportion of cones depredated by crossbills and squirrels. To test for the effect of canopy closure on the incidence of predation, we included paths from this environmental variable to both predators. Moreover, to control for the covariation between canopy closure and elevation, we also included elevation in the full model and connected elevation to both seed predators. The number of cones produced by each tree may further influence foraging preferences, which may also covary with canopy closure and elevation due to competition among trees and abiotic conditions. Therefore, we included the number of cones produced per tree in the initial saturated model, and added paths between this variable and the incidence of both seed predators. Finally, the incidence of each seed predator was connected to tree fitness, which was estimated as the proportion of cones surviving predation by crossbills and squirrels.

The occurrence of seed predation by crossbills and squirrels was negatively correlated, so we included the correlation between them in the initial full model. Correlations were also allowed between different cone traits, and between the number of cones, canopy closure, and elevation. Some variables in the model were transformed to improve normality and linearity, and path coefficients were estimated using generalized least squares as the type of discrepancy function. The resulting standardized total path coefficients calculated by SEM can be interpreted as the total direct selection acting on each phenotypic trait (Scheiner et al. 2000).

The initial full structural equation model (SE model) was compared to a set of alternative nested models that were built constraining some of the paths to zero. To select the best fitting model, we calculated the second-order Akaike Information Criterion (AICc), which is recommended when sample size is small relative to the number of parameters in the model to avoid overfitting (Burnham and Anderson 2002). AICc includes a penalization to the fit of models with more parameters, so the model with the minimum AICc value is preferred from a set of candidate models (Burnham and Anderson 2002). We present the best model (i.e., lowest AICc value). We show results from Chi-squared goodness-of-fit tests to assess the fit of the best model to the data.

In our hypothesized SE model of causal relationships between fitness, plant traits, and environmental variables, we used the proportion of cones surviving predation (which approximates the proportion of seeds surviving) as a surrogate for tree fitness. We also built a similar SE model using the number of full seeds surviving predation by crossbills and squirrels as an additional surrogate for tree fitness. In this model, seed predation by crossbills and squirrels was estimated as the number (instead of the proportion) of seeds (cones) eaten by each predator. In addition, we added a path from the number of cones produced to tree fitness because, all things being equal, trees that produce more cones will disperse more seeds. We followed similar procedures to solve this model as described for the previous SE model.

Results

FOREST STRUCTURE ALONG ALTITUDINAL TRANSECTS

In the first transect in 2008, tree density decreased with increasing elevation ($r = -0.24$, $P = 0.003$). Similarly, tree density in the second set of transects in 2008 was 2.1 times higher in the lower transect (702.0 ± 129.0 trees/ha) than in the upper transect (334.3 ± 60.9 trees/ha; $t_{28} = 2.8$, $P = 0.005$). In the 2009 transects, canopy closure was negatively correlated with elevation ($r = -0.34$, $P < 0.001$) as expected if tree density decreases with elevation.

SEED PREDATION BY CROSSBILLS AND SQUIRRELS

Seed predation by crossbills increased with increasing elevation (a 10% increase in seed predation for every 13-m increase in elevation, as estimated by the regression model; $Z = 2.7$, $P = 0.006$), and also tended to increase with decreases in tree density although not significantly (a 10% increase in predation with an increase in 0.4 m in the distance to the nearest tree; $Z = 1.6$, $P = 0.11$). Seed predation by squirrels was not linearly correlated with elevation or tree density ($Z < 0.27$, $P > 0.78$, for both variables). Overall, seed predation by crossbills (mean number of depredated cones/tree: 10.8 ± 1.9 SE, $n = 150$ trees) was 2.3 times higher than that by squirrels (4.8 ± 0.9, $n = 150$ trees). This latter comparison was based on the first 2008 transect as it provided estimates of seed predation at regular intervals along the whole elevational gradient.

PHENOTYPIC SELECTION AND FOREST STRUCTURE

The multiple linear regressions indicated that the targets of selection for crossbills in both years were cone mass and scale thickness (Table S1). Crossbills also preferentially foraged on trees in more open habitat (Table S1). The multiple nonlinear regressions showed that the nonlinear selection gradient for cone mass was significant in 2008 (Table S3); correlational selection was detected for only one pair of traits in one year (Table S3). Nonlinear selection differentials were also significant for the three cone size traits and scale thickness in both years and for the number of empty seeds in 2009 (Table S5). However, cubic splines showed that selection on the three cone-size traits and scale thickness was directional (the intensity of selection increased monotonically but at a decelerating rate) rather than stabilizing (see Fig. 3A), as in an earlier study in a different location (Mezquida and Benkman 2010). Overall, selection (direct and indirect; simple linear regressions) exerted by crossbills favored the evolution of trees that produced larger cones with thicker and longer scales, and heavier seeds in both years (Table S2). We used a cubic spline (mgcv package [version 1.7–27] in R 3.0 [R Development Team]) to examine the relationship between relative tree fitness, scale thickness (the cone trait consistently under selection by crossbills in this and previous studies [Benkman et al. 2010]), and canopy closure in relation to seed predation by crossbills (Fig. 3A). Positive selection on scale thickness was strong and decelerating, with crossbills tending to avoid more closed canopies (see also Tables S1 and S3). Comparing the slopes of the surface along the two axes, tree use was influenced much more by cone traits than habitat features (Fig. 3A).

Both the linear (Table S1) and nonlinear (Table S4) multiple regressions for selection exerted by squirrels in 2008 were

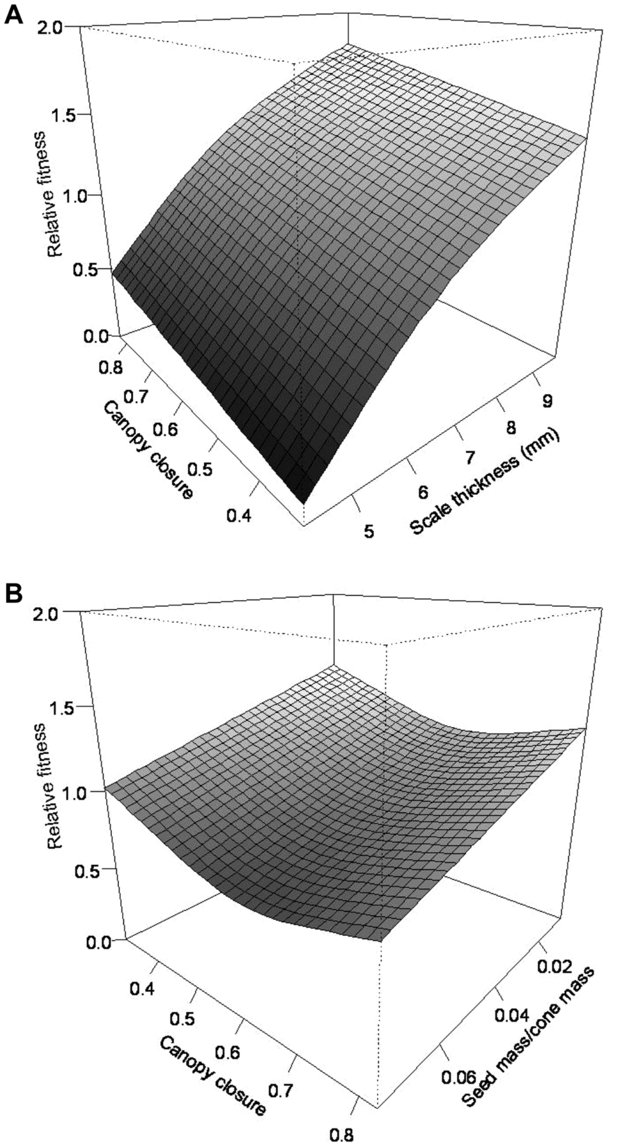

Figure 3. Variation in selection exerted on mountain pine by (A) crossbills and (B) red squirrels in relation to canopy closure and the two cone traits at the interface of their interactions with mountain pine, scale thickness and seed mass/cone mass, respectively.

not significant, whereas in 2009 both of these regressions were significant (Tables S1 and S3). The targets of selection in 2009 were cone mass, the number of full seeds, seed mass, and scale thickness (Table S1). Nonlinear selection gradients were not significant for any of the cone traits in 2009 but the quadratic coefficient was significant for canopy closure (Table S4), indicating that squirrels tended to forage on trees at intermediate values of canopy cover avoiding trees in denser and more open habitat (see Fig. 3B). No consistent patterns of correlational selection were detected other than the number of empty seeds was always involved (Table S4). The only trait that experienced selection (direct and indirect) by squirrels in both years was seed mass/cone mass ratio (Table S2), favoring the evolution of more cone mass relative to seed mass. Nonlinear selection differentials were significant for the three cone-size traits in 2009 (Table S6), suggesting disruptive rather than directional selection (cubic splines; not shown). The quadratic equation for cone length indicates that the preferred cone length (in which tree fitness is at a minimum) was 53 mm. The relationship between relative tree fitness, seed mass/cone mass (the one trait consistently under selection by tree squirrels in this and in previous studies [Benkman et al. 2010]), and canopy closure in relation to seed predation by squirrels is illustrated in Figure 3B. Selection on seed mass/cone mass was directional and relatively weak compared to that exerted by crossbills on scale thickness (Fig. 3). Squirrels preferred intermediate levels of canopy closure with tree use influenced as much by habitat features as by cone traits (Fig. 3B).

DISENTANGLING THE VARIOUS FACTORS INFLUENCING SEED PREDATION BY CROSSBILLS AND SQUIRRELS

The SE model with the lowest AICc value from the set of candidate models contained 47 parameters (Fig. 4). This simpler model presented a good fit to the data ($\chi^2_{31} = 38.0$, $P = 0.18$). The model indicated that both crossbills and squirrels preferentially foraged on trees having more cones, whereas elevation had contrasting affects on crossbills and squirrels: seed predation by crossbills increased whereas seed predation by squirrels decreased with increasing elevation (Figs. 4 and 5). These opposing effects of elevation plus the contrasting tree preferences of crossbills and squirrels for trees having cones with thinner and thicker scales, respectively, led to a negative correlation between seed predation by crossbills and squirrels (Fig. 4). However, this negative correlation between crossbills and squirrels also suggests that they compete for cones, which is consistent with many trees having most of their cones removed by both crossbills and squirrels.

The SE model with the lowest AICc value from the set of models using the number of full seeds not eaten presented a poorer fit to the data ($\chi^2_{29} = 49.9$, $P = 0.01$). Nonetheless, the ratio χ^2/df for this model (1.7) was much lower than the suggested threshold value of 5, indicating that the model was satisfactory (e.g., Rey et al. 2006). The results of this model were similar and consistent with those from the above SE model (Table 1). In this model, the environmental variable influencing seed predators, and ultimately fitness, was canopy closure instead of elevation. Crossbills preferentially foraged on trees in more open habitat whereas squirrels preferred trees in more closed habitat (Table 1).

Discussion

The two seed predators, crossbills and squirrels, differed in their use of habitat and in their cone preferences resulting in spatial

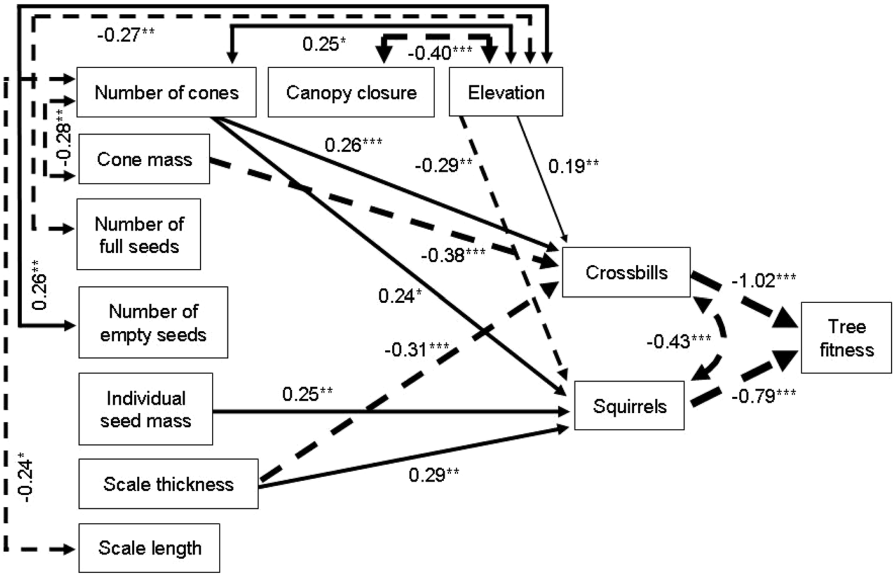

Figure 4. Best structural equation model for the relationships among cone and seed traits, number of cones per tree, habitat structure, elevation, and seed predation by crossbills and red squirrels, and tree fitness. Negative effects are indicated with dashed arrows, positive effects with solid arrows. Two-headed arrows show the correlations that were significant in the final model between environmental variables, between these variables and cone traits, and the correlation between the incidence of seed predation by both crossbills and squirrels. Correlations between cone traits are not shown for simplicity. The width of the arrows is proportional to the path or correlation coefficients, which are shown by the arrows. Asterisks indicate significance levels (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

variation in strength of their interactions with and selection experienced by mountain pine (Fig. 3). Although crossbills and squirrels differed in habitat use and cone trait preferences, there was also evidence of resource competition. Below we discuss patterns and causes of habitat use, and phenotypic selection exerted by crossbills and squirrels and its causes and variation.

HABITAT USE BY CROSSBILLS AND SQUIRRELS

As expected based on earlier population surveys (Lescourret and Génard 1983, 1986a; Génard and Lescourret 1987), crossbills foraged more commonly at higher elevations where the canopy was more open, whereas squirrels foraged more commonly at lower elevations where the canopy was more closed (Figs. 2 and S1; see Camarero et al. 2000; Ninot et al. 2008 for mountain pine forest structure in the Pyrenees). In addition, cone mass and scale length were negatively correlated with the number of cones produced by a tree (Fig. 4). Thus, the preference by crossbills for more open habitat and trees producing more cones might in part be the result of a preference for trees with smaller cones. Summers and Proctor (1999) found that cones were smaller in larger Scots pine growing in more open woodland, and they suggested the preference by crossbills for larger trees in more open areas was at least partly the result of a preference by crossbills for smaller cones with smaller (thinner) scales. This is consistent with our analyses that showed that cone trait preferences were much more important than canopy closure for tree use by crossbills (Fig. 3A; see also Benkman and Parchman 2013). However, crossbills preferred to forage in more open habitat when controlling for cone traits (Fig. 3A, Tables 1, S1, and S3).

As elevation increased, seed predation by squirrels declined more rapidly than seed predation by crossbills increased (Table 1, Fig. 5). This smaller change in seed predation by crossbills than by squirrels with changing elevation indicates that general habitat features had less of an influence on crossbills than on squirrels. This inference is further supported by the greater magnitude of the effects of canopy closure on squirrels than on crossbills (Fig. 3, Table 1). As mentioned earlier, squirrels require trees for escape from predators and thus are tied to relatively high densities of trees. Squirrels also tended to avoid foraging in the most densely forested habitat (Fig. 3B), although why requires study. Crossbills on the other hand fly long distances, between—for example—water sources and feeding trees, and thus their use of particular trees appears tied more to their cone traits (Benkman and Parchman 2013) than to other habitat features (e.g., water, forest structure, etc.).

FORM OF SELECTION EXERTED BY CROSSBILLS AND SQUIRRELS

Crossbills exerted selection that favored the evolution of trees having larger cones with thicker scales (Tables 1 and S1; Figs. 3 and 4); such selection by crossbills has been found in previous studies (Benkman et al. 2010) including a study of crossbills

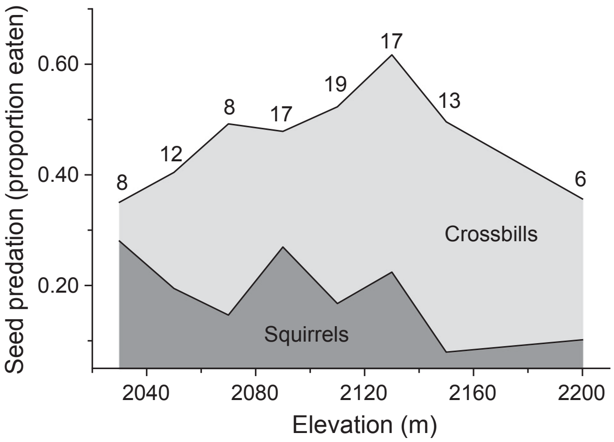

Figure 5. The proportion of seeds eaten increased with increasing elevation until the highest elevations near tree limit (see Figs. 2 and S1; 2009 transects), because seed predation by crossbills increased with elevation more rapidly than seed predation by red squirrels decreased. Shown along the top is the number of trees sampled within each 20-m elevation interval (except for the highest, which was an 80-m interval because of the lack of trees to sample).

foraging on mountain pine 215 km west of our study site (Mezquida and Benkman 2010). Thicker scales in particular deter crossbills from spreading the scales apart to reach the underlying seeds.

Squirrels preferentially foraged on trees having smaller cones with more seeds (Table S1) causing selection that favored trees with a lower ratio of seed mass to cone mass (Fig. 3, Table S2). Such a preference by tree squirrels for a higher ratio of seed mass to cone mass has been found repeatedly since Smith's (1970) classic study on pine squirrels (*Tamiasciurus*), and can be explained by squirrels attempting to minimize the amount of cone mass that they need to bite through to reach a given seed. Squirrels were also found to preferentially forage on trees having cones with larger seeds and thicker scales favoring the evolution of trees having cones with smaller seeds with thinner scales (Tables S1 and S2). A preference for larger seeds makes sense because this too would increase seed kernel intake rates. The preference for trees having cones with thicker scales is surprising. However, we measured scale thickness at the enlarged apophyses on distal ends of the scales (see Fig. 1 in Mezquida and Benkman 2010), whereas squirrels bite through the scales at the base well away from the apophyses (see Coffey et al. 1999). Thus, our measure of scale thickness is less relevant to squirrels than to crossbills. Finally, we found evidence for disruptive selection on cone size in one year (2009), which is consistent with previous studies. This result presumably arises because squirrels prefer an intermediate cone size, however squirrels preferred slightly smaller cones (trees with 53-mm-long cones) than in previous studies on other conifers (60 to 80 mm; Mezquida and Benkman 2005; Parchman and Benkman 2008; Benkman et al. 2010). One explanation for the preference for smaller cones is that red squirrels in the Pyrenees (formerly described as subspecies *S. v. alpinus*) are relatively small (Purroy 2002).

INTENSITY OF SELECTION EXERTED BY CROSSBILLS AND SQUIRRELS

Crossbills exerted much stronger selection than did squirrels. The absolute values of the selection differentials (direct and indirect selection) were 7.5 and 2.6 times greater (median differences) for crossbills than squirrels in 2008 and 2009, respectively (Table S2). In the earlier study west of the current site (Mezquida and Benkman 2010), the selection differentials for crossbills were 5.8 times greater (median difference) than those for squirrels. The greater selection intensities by crossbills than squirrels can be accounted for by the generally sparse nature of much of the mountain pine forests (Fig. 2; Ruiz de la Torre 2006); note that at the lowest elevations, where tree densities are high (see Fig. S1B), seed predation by squirrels exceeded that by crossbills (Fig. 5). In 2008 and 2009, respectively, crossbills consumed 2.3 and 1.6 times more seeds than did squirrels. In 2007, crossbills consumed 3.3 times more seeds than squirrels did (Mezquida and Benkman 2010; 1.7 times more at the site where selection was measured); Lescourret and Génard (1986a,b) found comparable and even proportionately greater predation by crossbills than squirrels. Because stronger antagonistic interactions (i.e., the more seeds consumed) result in a greater opportunity for selection (Benkman 2013), crossbills should and did exert stronger selection than squirrels (Fig. 1). In addition, habitat features (canopy closure) in relatively open mountain pine forests are much more important in influencing the foraging behavior and tree selection of squirrels than of crossbills (Fig. 3, Table 1).

When features other than the phenotype of the prey influence the foraging decisions of a predator, then the realized selection relative to the potential (theoretical maximum) selection will be reduced (Fig. 1). That is, the proportion of the maximum possible selection is reduced for a given amount of predation. This presumably explains why, when controlling for the level of seed predation, the selection exerted by squirrels is less than half of that exerted by crossbills (Fig. 1). Crossbills exerted selection that was between 0.3 and 0.4 of the maximum, whereas squirrels exerted selection that was only between 0.1 and 0.2 of the maximum (Fig. 1). Consequently, even though the magnitudes of direct selection on cone traits might be similar for crossbills and squirrels when controlling for habitat variables (Fig. 4), the overall intensities of selection (selection differentials) on cone traits differ greatly in magnitude (Fig. 1, Table S2). Thus, habitat structure strongly influences the intensity of selection exerted by squirrels by affecting their density, as measured by the proportion of seeds eaten (see McKinney and Fiedler [2010] for support

Table 1. Total direct selection on mountain pine phenotypic traits in relation to elevation and canopy closure according to the structural equation models.

Environmental and phenotypic variables	Effect Mediated Via		Proportion of seeds surviving	Effect Mediated Via		Number of full seeds surviving
	Crossbills	Red squirrels		Crossbills	Red squirrels	
Elevation	0.189	−0.291	0.038			
Canopy closure				−0.196	0.304	0.049
No. of cones	0.259	0.238	−0.452	0.523	0.464	0.374
Cone mass	−0.380	0.000	0.388	−0.259	0.000	0.309
No. of full seeds				−0.176	0.000	0.210
Seed mass	0.000	0.247	−0.196	0.000	0.251	−0.153
Scale thickness	−0.306	0.294	0.079	−0.265	0.290	0.139

The first model estimated tree fitness as the proportion of seeds surviving predispersal predation by crossbills and red squirrels, and the second model as the number of full seeds surviving predispersal predation. The magnitude of the direct effects between each environmental variable or phenotypic trait and each seed predator is also shown. The number of empty seeds and scale length were included in the initial full models, but they are not included in the table because these traits were not in the two selected models.

of this assumption for tree squirrels), and by affecting the relative importance of cone traits in foraging decisions. These results are also consistent with other studies on red squirrels (Molinari et al. 2006), which did not detect selection by red squirrels near tree limit in arolla pine (*Pinus cembra*) but did detect selection at lower elevations in Scots pine (*P. sylvestris*), where tree densities were 50% higher (Wauters et al. 2008). Similarly, Fedriani (2005) found that the consumption of *Helleborus foetidus* fruits by mice (*Apodemus sylvaticus*) was influenced more by variation in habitat features, which apparently altered predator risk for the mice, than by variation in plant traits.

Risk of predation in particular has the potential to influence which individual plants its antagonist or mutualist visits, because predation risk generally has a strong impact on foraging decisions (Lima and Dill 1990; Lima 1998). Foraging animals need to balance resource gain with the risk of predation. For example, bees avoid plants where danger or perceived danger is relatively high, and hence predators on bees can act to alter the plants they visit and thus plant fitness (Dukas 2001, 2005; Dukas and Morse 2003; Romero et al. 2011; Llandres et al. 2012). In particular, less profitable patches or plants (e.g., for squirrels, trees having, for example, low ratios of seed mass to cone mass) might be preferentially visited if more profitable ones are more risky to exploit (Gilliam and Fraser 1987; Jones 2010; Llandres et al. 2012). We suspect that when the relationship between predation risk and the plant traits related to profitability (and are the targets of selection) is random, or especially positively related (e.g., Heiling and Herberstein 2004), then realized selection will be depressed relative to the maximum possible selection (see Jones 2010). This may often be the case for tree squirrels in more open forests. In earlier studies (Siepielski and Benkman 2007a,b), we argued that low densities of limber pine (*P. flexilis*) and whitebark pine (*P. albicaulis*) result in lower densities of pine squirrels (*Tamiasciurus* spp.), which in turn reduces their antagonistic selective impact and thereby allows the evolution of effective seed dispersal by Clark's Nutcrackers (*Nucifraga columbiana*). The focus was on how low tree density negatively affected squirrel density. However, this should be reexamined, as low tree density is likely to also depress realized selection. Similarly, the framework provided in Figure 1 might be useful for examining how variation in interaction strengths between plants, pollinators, and predators of pollinators influence the intensity of selection pollinators exert on plants. Such a framework could also be used to address the extent to which the variation in abiotic conditions (e.g., Herrera 1995) depresses selection by altering interaction strength versus realized selection.

Conclusions

Variation in biotic interaction strength and the resulting natural selection is one of the most, if not the most, important factors influencing patterns of evolution and diversification (Darwin 1859; Thompson 1994, 2005, 2013). Yet, understanding how variation in biotic interaction strength and selection are related, as well as the causes of variation in selection, remain outstanding questions in evolutionary ecology (McPeek 1996; MacColl 2011; Benkman 2013). We found that crossbills consumed more seeds and exerted much stronger selection on cone traits than did squirrels in the relatively open high-elevation mountain pine forests. The higher seed predation rates by crossbills arose because crossbills preferred more open forests whereas squirrels avoided them. Higher seed predation rates lead to greater opportunities for selection and greater maximum potential selection (Fig. 1; Benkman 2013). However, the more intense selection exerted by crossbills than by squirrels was also related to crossbills foraging mostly in relation to cone traits that deter them rather than to habitat features, whereas habitat variation, in particular canopy closure, had a large impact on the feeding preferences of squirrels resulting in weaker realized selection on cone traits (Fig. 1). Future studies on biotic interactions would benefit from teasing apart the relative importance of biotic interaction strength and how other factors affect the intensity of selection relative to the maximum potential selection.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Photographs of mountain pine forests at (A) high elevations near tree limit where density of trees is low and trees are widely spaced, and (B) at low elevations where density of trees is high and the canopy more closed.
Table S1. Multivariate linear selection gradients (β) for phenotypic selection exerted by crossbills and red squirrels on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).
Table S2. Univariate linear selection differentials (β') for phenotypic selection exerted by crossbills and red squirrels on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).
Table S3. Nonlinear (γ) and correlational (γij) selection gradients for phenotypic selection exerted by crossbills on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).
Table S4. Nonlinear (γ) and correlational (γij) selection gradients for phenotypic selection exerted by red squirrels on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).
Table S5. Nonlinear selection differentials (γ') for phenotypic selection exerted by crossbills on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).
Table S6. Nonlinear selection differentials (γ') for phenotypic selection exerted by red squirrels on mountain pine along altitudinal transects in La Cerdanya, eastern Pyrenees, Spain in 2008 ($n = 97$ trees) and in 2009 ($n = 100$ trees).