INTERACTIONS AMONG MOTHS, CROSSBILLS, SQUIRRELS, AND LODGEPOLE PINE IN A GEOGRAPHIC SELECTION MOSAIC

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Abstract.—Repeated patterns among biological communities suggest similar evolutionary and ecological forces are acting on the communities. Conversely, the lack of such patterns suggests that similar forces are absent or additional ones are present. Coevolution between a seed predator, the red crossbill (*Loxia curvirostra* complex), and lodgepole pine (*Pinus contorta* var. *latifolia*) exemplifies the ecological and evolutionary predictions for coevolving systems. In the absence of another seed predator and preemptive competitor (pine squirrels *Tamiasciurus hudsonicus*), natural selection by crossbills results in the evolution of larger cones with thicker distal scales, while relaxation of selection by squirrels results in the evolution of cones with more seeds and a greater ratio of seed mass to cone mass. However, in one range, the Little Rocky Mountains, distal scale thickness has diverged as expected but cone size has not. In these mountains seed predation by lodgepole pine cone borer moths (*Eucosma recissoriana*) was about 10 times greater than in other ranges lacking squirrels. We quantified moth predation and cone traits and found that moths select for smaller cones with fewer seeds. Thus, selection by moths in the Little Rocky Mountains counters both selection by crossbills for large cone size and relaxation of selection by squirrels favoring more seeds per cone and accounts for the relatively small and few-seeded cones in these mountains. It is also apparent that selection by crossbills changes seed defenses in a manner that favors seed predation by moths, whereas selection by squirrels likely reduces such predation. These results demonstrate the importance of considering the evolutionary consequences of community context in locally evolved (coevolved) traits and interactions.

Key words.—Conflicting selection, *Eucosma recissoriana*, geographic mosaic theory of coevolution, *Loxia curvirostra*, *Pinus contorta* ssp. *latifolia*, predator-prey interactions, seed predation.

Repeated patterns among similarly characterized systems indicate that parallel evolutionary and ecological forces are acting on those systems. For example, the repeated patterns of the evolution in *Anolis* lizards (Losos et al. 1998), freshwater sticklebacks (Schluter and McPhail 1993), East African cichlids (Meyer 1993; Kocher et al. 1993), and of coevolution between crossbills and conifers (Benkman et al. 2001, 2003; Parchman and Benkman 2002) indicate that similar selection pressures act within each of these systems. In contrast, lack of repeated patterns in these systems would suggest that similar forces are absent or additional ones are present. Indeed, the ecological and evolutionary outcomes of species interactions often vary across large geographic areas because of variation in the abundance of interacting species, such as copollinators (Thompson and Pellmyr 1992; Thompson and Cunningham 2002), alternative hosts (Zangerl and Berenbaum 2003), or competitors (Benkman 1999; Benkman et al. 2001; Parchman and Benkman 2002). Consequently, it has become clear that approaches incorporating spatial variation in community context into the study of species interactions are needed to provide a more holistic view of the processes organizing diversity (Thompson 1999). Well-understood systems are also key for understanding the interplay between interacting species and the ecological and evolutionary patterns they produce, in part, because deviations from predicted patterns are more readily detected.

The coevolutionary arms race between red crossbills (*Loxia curvirostra* complex) and Rocky Mountain lodgepole pine (*Pinus contorta* ssp. *latifolia*) in isolated forests east and west of the Rocky Mountains (Benkman 1999; Benkman et al. 2001, 2003) exemplifies the ecological and evolutionary predictions of geographically structured coevolving systems. In this system, coevolution between crossbills and lodgepole pine occurs only in the absence of pine squirrels (*Tamiasciurus hudsonicus*), their main competitor and the primary selective agent driving cone evolution where present. Differences in selection pressures from crossbills and squirrels change cone traits in a predictable fashion that is repeated among several ranges (Benkman 1999; Benkman et al. 2001, 2003). For example, selection by crossbills favors trees with cones that are larger and have thicker distal scales. In contrast, selection from squirrels results in relatively short and wide cones that have fewer seeds.

Throughout the mountain ranges east and west of the Rocky Mountains that make up the crossbill-conifer coevolutionary mosaic (Benkman 1999; Benkman et al. 2001), patterns of cone evolution are repeated in ranges lacking squirrels in a manner consistent with selection from crossbills and relaxation of selection by squirrels (Benkman et al. 2003). Moreover, as the total area of lodgepole pine among the different ranges increases so do the densities of crossbills, which presumably cause the increase in defenses directed at crossbills (e.g., thicker distal scales; Fig. 1; A. M. Siepielski and C. W. Benkman, unpubl. ms.). However, within this system one mountain range, the Little Rocky Mountains, falls outside the predicted pattern, if selection from crossbills and relaxation of selection by squirrels were the primary factors driving the evolution of seed defenses (Fig. 1). One possibility for the mismatch between predicted and observed cone structure is that an additional selective agent is operating that is absent in the other areas. A second possibility is that gene flow from other populations of lodgepole pine is causing trait mismatches. The latter is unlikely because the Little Rocky Mountains are quite isolated from other areas, and gene flow would mostly come from other ranges lacking squirrels (e.g., the Bears Paw Mountains; Benkman 1999). A third alter-
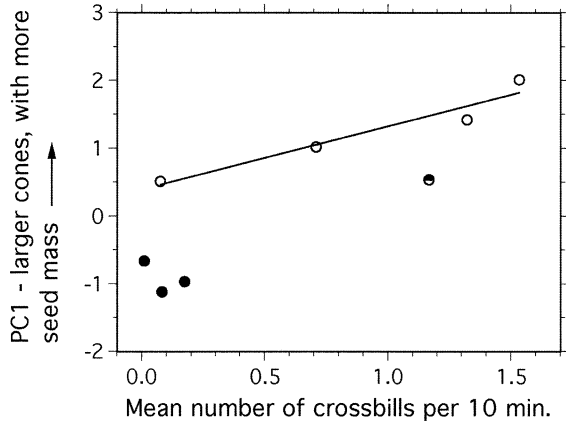
FIG. 1. The relationship between the mean number of crossbills per 10-min point count (x) and the first principal component (PC1) of seven cone measurements in lodgepole pine. The least squares linear regression only includes areas without squirrels (open circles; PC1 = 0.39 + 0.96x, $F_{1,2}$ = 41.05, r^2 = 0.95, P = 0.024) and excludes the Little Rocky Mountains (half-filled circle; from A. M. Siepielski and C. W. Benkman, unpubl. ms.), which is a significant outlier (studentized residual, t_2 = −4.181, P < 0.05). Each point represents the mean PC1 score and mean estimated density of crossbills on each range. Crossbill density is based on the mean of four survey periods during which 30 10-min point counts were conducted; because squirrels have been introduced into the Cypress Hills, its density estimate is based on the following equation for other ranges lacking squirrels (number of crossbills per point count = −0.826 + 0.492ln[km^2 of lodgepole pine], $F_{1,2}$ = 62.57, r^2 = 0.97, P = 0.016; from A. M. Siepielski and C. W. Benkman, unpubl. ms.). Ranges without squirrels are from left to right: Sweetgrass Hills, Bears Paw Mountains, Little Rocky Mountains, Cypress Hills, and South Hills. Ranges with squirrels (filled circles) are from left to right: the Little Belt, Highwood, and Judith Mountains.

native is that selection by crossbills is absent or weak, and that cones have simply evolved in response to relaxation of selection from squirrels. This alternative is unlikely because distal scales are thicker in the Little Rocky Mountains (see Fig. 2D) as expected if selection by crossbills is strong (Benkman 1999; Benkman et al. 2001, 2003). Moreover, crossbills are common (see Fig. 1), and Benkman et al. (2003) demonstrated selection by crossbills on distal scale thickness in the Little Rocky Mountains. Despite these lines of evidence indicating that selection by crossbills should dominate, cone structure in this area is significantly less like cones in other areas lacking squirrels (Fig. 1). This suggests that additional evolutionary and ecological processes are operating. During earlier studies of lodgepole pine cones in 1996, 1998, and 2000, we observed (W. C. Holimon, pers. comm., C. W. Benkman, pers. obs.) that damage by lodgepole pine cone borer moths (*Eucosma recissoriana*; Lepidoptera: Tortricidae) was elevated in the Little Rocky Mountains relative to all other areas we sampled. Here we quantify seed predation and the form of selection exerted by these moths to evaluate whether selection by moths can account for the peculiar combination of cone traits in the Little Rocky Mountains.

MATERIALS AND METHODS

Natural History of Eucosma recissoriana

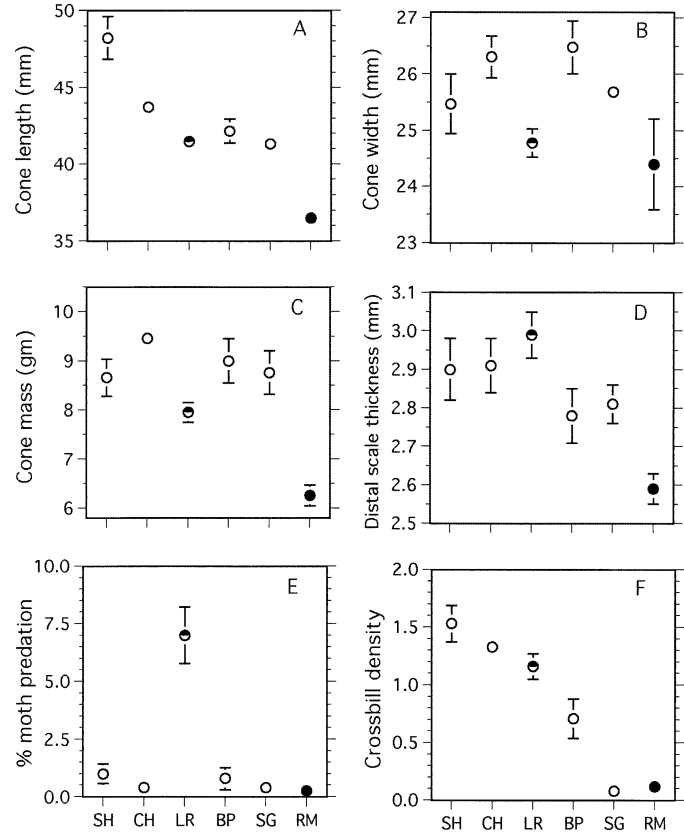The lodgepole pine cone borer moth consumes seeds in the cones of lodgepole pine, western white pine (*Pinus mon-*

FIG. 2. Four cone traits (mean ± SE) under selection by moths (A–C) or crossbills (D) in the Little Rocky Mountains, percent damaged by moths (E), and the number of crossbills observed on 10-min point counts (F). The first five acronyms along the x-axis represent ranges, ordered with regards to decreasing forest area, where lodgepole pine has evolved in the absence of squirrels (SH, South Hills; CH, Cypress Hills; LR, Little Rocky Mountains; BP, Bears Paw Mountains; and SG, Sweetgrass Hills). The sixth acronym (RM) represents the overall mean (± SE) for four ranges with squirrels within the Rocky Mountains (A–D) or the three ranges with squirrels where moth predation was measured (E, F).

ticola), whitebark pine (*P. albicaulis*), grand fir (*Abies grandis*), and subalpine fir (*A. bifolia*; Hedlin et al. 1981). These moths inhabit coniferous forests throughout the Rocky Mountains, Sierra Nevada, and Cascade Mountains in western North America (Hedlin et al. 1981). Adult moths emerge in late spring and most commonly lay eggs on second-year cones (Keen 1958; Hedlin 1974; Lavender 1978). However, little is known of their egg-laying biology on lodgepole pine. On western white pine, females lay on average 16 eggs in a triangular mass at the juncture of two overlapping scales (Ollieu and Schenk 1966). Larvae hatch and bore between successive cone scales to reach and feed on underlying seeds and scale tissue, leaving small entrance holes (< 2 mm in diameter) before exiting after completing the fifth instar. Mature larvae vacate cones and drop to the ground to pupate in late summer.

Geographic Patterns of Seed Predation by Moths

We quantified seed predation in three mountain ranges with squirrels and five ranges without them. The ranges with squir-

rels were the Highwood, Judith, and Little Belt Mountains, Montana. The ranges without squirrels were the Sweetgrass Hills, Bears Paw, and Little Rocky Mountains, Montana; the Cypress Hills, Alberta; and the South Hills, Idaho. Squirrels were introduced to the Cypress Hills in 1950 and are now quite common (Benkman 1999). We include the Cypress Hills with the ranges without squirrels because lodgepole pine in the Cypress Hills probably evolved in the absence of squirrels for up to 12,000 years and the cone traits there are representative of ranges without squirrels (Benkman 1999; Benkman et al. 2001).

One of us (A. M. Siepielski) examined 10 three- to seven-year-old old serotinous cones (cones mature when two years old) from each of 50 randomly selected trees in each range, and recorded the total number of cones with larval exit holes. We used the percent of cones per tree with exit holes as a measure of seed predation; therefore, our estimate of predation is an average over five years of cone production. The correlation between predation quantified in this manner and the percent of seeds eaten in the Little Rocky Mountains (r_s = 0.81, n = 34 trees, $P < 0.0001$; see below) indicates that this is a reasonable measure of seed predation. A Kruskal-Wallis test was used to test for differences in predation by moths among ranges because the assumptions for parametric tests were not met. We used Dunn's procedure for pairwise comparisons to test whether seed predation was significantly greater in the Little Rocky Mountains than in other ranges. A one-tailed t-test was used to determine if moth predation (mean percent predation in each range) was greater in ranges without squirrels (excluding the Little Rocky Mountains) than in ranges with them.

The Form and Targets of Natural Selection by Moths

One of us (C. W. Benkman) collected 20 mature three- to four-year-old serotinous cones from 34 trees in a lodgepole pine dominated forest in the Little Rocky Mountains on 14–15 August 2001 to examine how cone traits were related to seed predation by moths. Trees were sampled atop a ridge (elevation 1440 m) 3.8 km north-northeast of Zortman, Montana, under the constraint that 20 cones could be reached unaided from the ground. A. M. Siepielski measured three cones from each of the 34 trees. The three cones measured were undamaged by moths and represented the average cone width and length (selected by visual inspection) of the 20 cones from each tree. We assumed that the cones preyed upon by moths or avoided had the same average traits as those measured for the tree. This assumption is reasonable given that within-tree variance in lodgepole pine cone traits is significantly smaller than the between-tree variance (Smith 1968; Elliott 1974). If moths were selective of cones within a tree, then our measurements should bias us against detecting trends among trees preyed upon or avoided by moths. The following cone traits were measured (see fig. 2 in Benkman et al. 2003) as described by Benkman et al. (2001): closed cone length and width, cone mass with seeds removed, the thickness of the distal end of six scales in the middle of the distal third of the cone and in the middle of the proximal third of the cone (scales were selected approximately equidistant around the cone), number of seeds and number of full

seeds (i.e., filled with female gametophyte), and the individual masses of five full seeds without their wings. 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 60–70°C for at least two days. The number of seeds eaten by moths was estimated by drying and opening all cones with exit holes and counting the number of full seeds not damaged by moths and subtracting 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). Percent predation per tree was the mean percent of seeds eaten in the 20 cones. We assume that cones without exit holes were not preyed upon by moths. This is a reasonable assumption given that no evidence of moth damage was found within the 102 cones examined without exit holes. Cone morphometrics from other areas are from Benkman (1999).

To determine which traits were the targets of selection (direct selection), we used a multiple regression model between estimated tree fitness in relation to moth predation and five cone traits standardized to units of standard deviations. Tree fitness was estimated as 100 minus percent seed predation divided by the overall mean. The standardized partial regression coefficients (β) estimate the direct selection gradients (Lande and Arnold 1983). To avoid problems with multicollinearity, we removed highly correlated ($r > 0.5$, $P < 0.05$) variables or grouped variables together where appropriate. Two composite variables were used: 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.

To determine which traits were under selection by moths (both direct and indirect selection), we used least squares regression analyses to estimate standardized selection gradients (β') via regression coefficients (Lande and Arnold 1983). Tree fitness was estimated as above, and cone traits were standardized to units of standard deviations. We used the paired regression bootstrap technique of Efron and Tibshirani (1993) to test for significance of the selection gradients because the residuals in most of the regressions were strongly nonnormally distributed. Tests of significance were based on 1000 bootstrap replicates. Second-order models were also examined to test for nonlinear selection. We used cubic splines (program provided by D. Schluter) to further examine the form of selection on cone traits for which the quadratic term was significant.

To determine if selection by moths was related to the deviation in the first principal component (PC1) scores in the Little Rocky Mountains in comparison to other ranges without squirrels (Fig. 1), we used a cubic spline to determine the relationship between percent predation and the first principal component score of seven cone traits for each of the 34 trees. We used the principal components equation derived in Benkman (1999) to estimate PC1 scores.

Annual Variation in Predation by Moths

Because the form of natural selection often varies from year to year, it is important to assess the reliability of mea-

TABLE 1. Estimates of multivariate selection gradients using regression analysis between relative tree fitness, because of seed predation (100 minus mean percent of seeds eaten, divided by the overall mean), and several standardized cone and seed traits in the Little Rocky Mountains, Montana ($n = 34$ trees). Full model: $F_{5, 28} = 8.09$, $R^2 = 0.59$, $P < 0.001$.

Trait	$\beta \pm SE$	t	P
Cone width/cone length	0.020 ± 0.007	3.01	0.005
Cone mass	-0.032 ± 0.007	-4.33	0.0002
Distal scale thickness	-0.004 ± 0.008	-0.50	0.62
Number of full seeds	-0.014 ± 0.007	-2.23	0.034
Number of empty seeds	0.008 ± 0.007	1.23	0.229

sures of selection made over one or two years. This is essentially a question of repeatability; if we measure a relatively high level of moth predation in one year for a given tree, do we find a relatively high level the next year. We expected that the form of selection would vary little between years because cone (seed) production in the Little Rocky Mountains is more stable from year to year ($CV = 5\%$) than any known plant species (Benkman et al. 2003). Nevertheless, we quantified the occurrence of moth predation as done described above (*Geographic Patterns of Seed Predation by Moths*) for 424 cones that had matured over the previous three years from 15 trees in the Little Rocky Mountains in July 2003 to measure the repeatability of moth predation. We estimated repeatability, which is the proportion of variance in moth predation that occurs among rather than within trees, as described by Lessells and Boag (1987).

RESULTS

Geographical Variation in Seed Predation by Moths

Predation levels were significantly different among the eight mountain ranges (Kruskal-Wallis $\chi^2 = 112.01$, $P < 0.0001$) and were significantly higher in the Little Rocky Mountains than in any of the other ranges (Fig. 2E; Dunn's

procedure, all pairwise comparisons $P \leq 0.0001$). When the Little Rocky Mountains were excluded from the analysis, predation levels did not differ significantly among the remaining mountain ranges (Kruskal-Wallis $\chi^2 = 7.17$, $P = 0.31$). Excluding the Little Rocky Mountains, predation by moths tended to be higher in areas where squirrels were absent (mean = 0.65%) than present (mean = 0.27%; Fig. 2E; one-tailed t-test on the means of each range, $t_5 = 1.66$, $P = 0.079$), although power was low (power = 0.272).

Natural Selection by Moths in the Little Rocky Mountains

The multiple regression indicates that moths preferred heavier, relatively long, narrow cones with many seeds (Table 1). That is, the targets of selection (direct selection) were cone size and shape and the number of seeds. These preferences caused selection that favored decreases in cone mass and length and resulted in indirect selection on correlated traits ($r > 0.5$, $P < 0.005$) including distal and proximal scale thickness (Table 2), which were not under direct selection (Table 1). Such indirect selection apparently overwhelmed direct selection favoring narrower cones, resulting in negative selection on cone width (Table 2). Direct selection favored trees that produced cones with fewer full seeds (Table 1); however, the selection gradient (both direct and indirect selection) for the number of full seeds was not significant (Table 2). Indirect selection was unlikely to counter direct selection on the number of full seeds per cones because it was not correlated with other cone traits under direct selection (e.g., $r = 0.003$, $P = 0.98$ between cone length and $r = 0.26$, $P = 0.14$ between cone mass; see also Benkman et al. 2003). Direct selection on other traits such as cone mass would act to increase the variance in the estimate of the selection gradient, so that statistical significance would be less likely. In addition, the relationship between estimated fitness and the number of full seeds appeared nonlinear although monotonic (e.g., Spearman rank correlation was significant: $r_s = -0.42$, $P = 0.014$), which suggests that selection was directional.

TABLE 2. Estimates of standardized bivariate selection gradients using least squares regression analyses between relative tree fitness, because of seed predation (100 minus mean percent of seeds eaten, divided by the overall mean), and various standardized cone traits in the Little Rocky Mountains, Montana ($n = 34$ trees). Bootstrapping was used to establish statistical significance of β'. The second-order models are also presented when the quadratic term was significant ($P < 0.05$).

| Trait	Least squares regressions		
	$\beta' \pm SE$	t	P
First-order models			
Cone length	-0.030 ± 0.009	-3.26	<0.005
Cone width	-0.022 ± 0.007	-2.90	<0.01
Cone width/cone length	0.008 ± 0.009	0.96	<0.4
Cone mass	-0.030 ± 0.009	-3.52	<0.005
Distal scale thickness	-0.017 ± 0.007	-2.50	<0.01
Proximal scale thickness	-0.012 ± 0.006	-1.93	<0.05
Individual seed mass	-0.012 ± 0.008	-1.39	<0.2
Number of full seeds	-0.016 ± 0.010	-1.56	<0.2
Number of empty seeds	-0.0001 ± 0.014	-0.004	>0.9
Seed mass/cone mass	-0.004 ± 0.008	-0.57	>0.5
Second-order models			
Cone length	0.269 ± 0.083	3.24	<0.005
(Cone length)^2	-0.299 ± 0.086	-3.48	<0.005
Cone mass	0.107 ± 0.032	3.56	<0.005
(Cone mass)^2	-0.138 ± 0.033	-4.43	<0.001
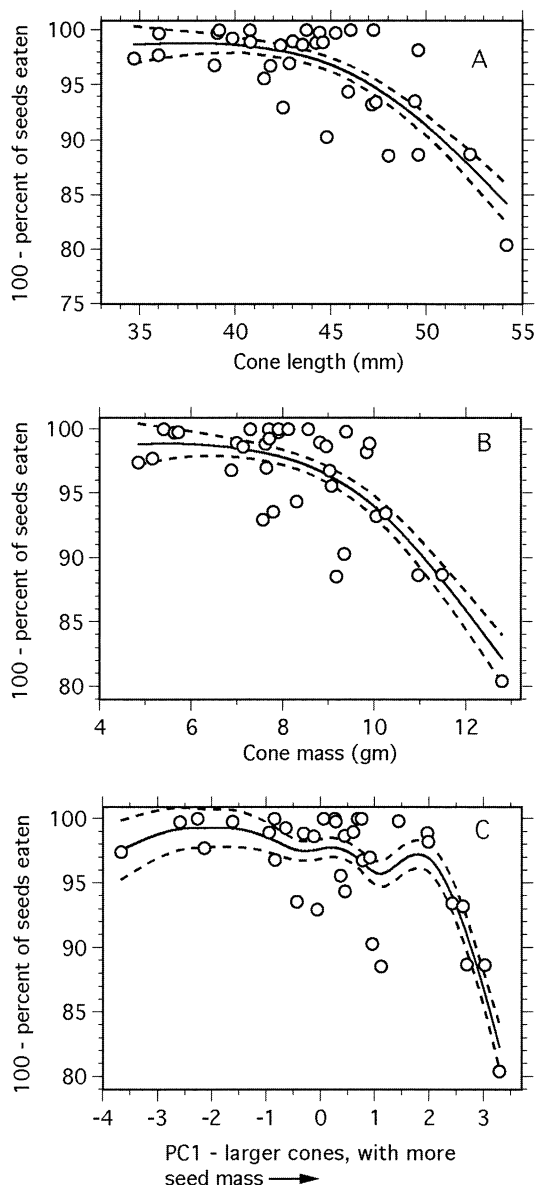
FIG. 3. The relationships between 100 minus the mean percent of seed eaten per tree (3.7% ± 0.8 [mean ± SE], n = 34 trees) by lodgepole pine cone borer moths and (A) cone length; (B) cone mass; and (C) the first principal component scores of seven cone traits in the Little Rocky Mountains. The solid curves represent cubic splines and the dashed curves represent ± one SE based on 50 bootstrap replicates.

The selection gradient was nonlinear for both cone length and cone mass (Table 2) with tree fitness (100—percent seed predation) decelerating as cone length and cone mass increased (Fig. 3A, B). Although the significant quadratic terms (Table 2) suggest that fitness should decline at smaller values of cone length and cone mass, the cubic splines show that selection was not stabilizing and that fitness leveled out as cone size decreased (Fig. 3A, B). Similarly, tree fitness decreased with increases in PC1 (Fig. 3C), where larger values of PC1 reflect longer, wider, and heavier cones (these three cone traits have the highest loadings on PC1; Benkman 1999).

Annual Variation in Predation by Moths

Our estimate of repeatability of moth predation for individual trees over a three-year period is 0.82. This relatively high value indicates that measures of selection made over one year (ours was two years) would likely represent well the form of selection over a longer time.

DISCUSSION

Thompson's (1994, 1999) geographic mosaic theory of coevolution predicts that variation in geographic structuring of species interactions influences their ecological and evolutionary outcome. Our results support this prediction by showing that variation in the community composition of interacting species influences the evolutionary outcome of the interactions. In combination with previous work (Benkman 1999; Benkman et al. 2001, 2003), our data suggests that the evolution of seed defenses in lodgepole pine in the Little Rocky Mountains have evolved in response to a mosaic of species interactions.

Seed Predation and the Targets of Selection by Moths

Oviposition site preferences are a critical feature of female moth ecology and evolution because they determine habitat use, and hence resource availability, for their offspring (Jaenike 1978; but see Wilkund 1977). Importantly, oviposition site preferences by moths are thought to have a genetic basis (Jaenike and Holt 1994) and should be under strong selection (Craig et al. 1989; Fidgen et al. 1998; but see Larsson and Ekbom 1995). Our results suggest that moths prefer large cones with many seeds (Table 1) that causes selection against trees with such cones (Table 2). An evolutionary response to such selection is expected because 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.).

Other studies have also found cone- and seed-eating insects to preferentially oviposit on larger, and in particular, longer cones. For example, McClure et al. (1998) found that larch cone maggots (*Strobilomyia laricis*) preferred longer cones for oviposition. Similarly, Fidgen et al. (1998) found that the proportion of black spruce (*Picea mariana*) and white spruce (*P. glauca*) seeds eaten by cone maggots (*Strobilomyia* spp.) increased with increases in cone length. In previous studies, cone length has been correlated with total number of seeds per cone (e.g., Fidgen et al. 1998), which reflects resource abundance to seed-eating larvae. However, we did not find a correlation between cone length and total number of seeds ($r = 0.003$, $n = 34$ trees, $P = 0.98$). Because moths preferentially oviposited in cones with more seeds, this suggests that ovipositing moths somehow assessed seed abundance, but cone length was unlikely the cue they used.

As with cone maggots, a preference for cones with more seeds may be adaptive if it results in larger larvae with higher fitness (e.g., McClure et al. 1996, 1998; Fidgen et al. 1998). Such a preference could also be important if several larvae compete for seeds in a cone. The presence of more than one

exit hole on average in our study (mean = 1.45, range = 1–4) indicates that often several larvae were within a lodgepole pine cone. Studies of late instar *E. recissoriana* foraging on western white pine seeds showed that larvae vacate heavily foraged on cones and move to nearby cones before dropping to pupate (Ollieu and Schenk 1966). Dispersal from cones is presumably due to resource depletion owed to overcrowding in the cone (Groot 1998). We have no evidence of intraspecific competition. The fact that many intact seeds remained in most cones suggests that if resource competition is important, it occurs locally within a cone.

Why moth predation is significantly greater in the Little Rocky Mountains in comparison to other ranges is enigmatic. For example, cones in the South Hills are heavier (Fig. 2C) and have more full seeds (mean = 50.0) than in the Little Rocky Mountains (mean = 29.0), yet predation by moths was only one-seventh of that in the Little Rocky Mountains (Fig. 2E). The low frequency of seed predation in other areas without squirrels suggests that susceptibility to moth damage could be related to environmental factors that might vary between ranges. Herbivorous insects on conifers are consistently more abundant in environments that are stressful to plants, including low availability of nutrients and chronic drought (Waring and Cobb 1992). In the Little Rocky Mountains, soils are composed mostly of limestone, they are rockier, appear more poorly developed, and lodgepole pine appear to have lower growth rates in comparison to the other ranges (C. W. Benkman and A. M. Siepielski, pers. obs.), suggesting poor growing conditions that could make trees more susceptible to moths.

Evolution of Seed Defenses in Response to Selection from Moths

The repeated pattern of evolution of seed defenses in isolated mountain ranges during the past 10,000 years where squirrels are absent can largely be accounted for by selection from crossbills and relaxation of selection by squirrels (Benkman 1999; Benkman et al. 2001, 2003). However, the observed preference for several heritable cone traits by lodgepole pine cone borer moths, and the fact that selection of oviposition sites by lepidopterans are often heritable (Jaenike and Holt 1991), show that moths have the potential to be a selective force in the evolution of seed defenses as well. Indeed, the discrepancy between PC1 scores in the Little Rocky Mountains and the other ranges without squirrels (Fig. 1) is explained well by selection from moths (Fig. 3, Table 3). Four of the six traits under selection by moths in the Little Rocky Mountains differed from expected in a manner consistent with selection by moths (Table 3). For example, selection by moths favors trees with smaller (shorter, narrower, and less heavy) cones (Table 2, Fig. 3), whereas selection by crossbills favors larger cones (Table 3). Thus, cones in the Little Rocky Mountains should tend to be smaller than in other ranges without squirrels because of conflicting selection pressures by moths. This is exactly what was found (Figs. 1, 2A–C). The number of seeds in cones where squirrels are absent increases apparently because of relaxation of selection by squirrels (Benkman et al. 2003). However, the Little Rocky Mountains have relatively few seeds per cone (Table

TABLE 3. The direction of selection exerted by moths (Table 2; see also text), crossbills, and squirrels (Benkman et al. 2003) on various lodgepole pine cone traits and the observed changes in these cone traits in the Little Rocky Mountains relative to that expected based on cone traits in other similar-sized ranges without squirrels. Plus and minus symbols refer to selection favoring increases and decreases in a cone trait (parentheses enclose those that are marginally nonsignificant, $0.08 > P > 0.05$), respectively. Zeroes refer to no statistical support for selection on the trait.

	Selection by			Observed
	Moths	Crossbills	Squirrels	Δ
Cone length	−	+	(−)	−
Cone width	−	(+)	0	−
Cone width/length	0	−	+	0
Cone mass	−	+	0	−
Distal scale thickness	−	+	0	0
Proximal scale thickness	−	0	0	0
Individual seed mass	0	0	0	0
Number of full seeds	−	0	−	−
Seed mass/cone mass	0	−	−	0

3), presumably because moths select against trees that have more seeds per cone (Table 2; although the linear selection gradient was not significant, directional selection was suggested by a significant Spearman rank correlation). Selection by moths also favored trees that produced cones with thinner distal and proximal scales (Table 2). Scale thickness in the Little Rocky Mountains, however, is as expected if only selection by crossbills favored thicker distal scales (Table 3, Fig. 2D). Presumably, strong selection on distal scale thickness by crossbills (Benkman et al. 2003) overwhelms counter selection by moths. Overall, selection by crossbills favors the evolution of larger cones with thicker distal scales, whereas selection by moths favors the evolution of smaller cones with fewer seeds. The balance between these conflicting selection pressures determines the evolutionary equilibrium and can account for the cones in the Little Rocky Mountains that are relatively small and have relatively few seeds but thick distal scales.

A Selection Mosaic for Moths

The observed tendency for seed predation by moths to consistently be over two times greater in the absence of squirrels (Fig. 2E) suggests that lodgepole pine cone borer moths may also experience a selection mosaic. Although the magnitude of seed predation by moths was not repeated among ranges without squirrels, relaxation of selection by squirrels and perhaps competition from them appear critical for allowing moths to become an important selective agent on seed defenses. Likewise, the presence and absence of squirrels determines the selection mosaic for crossbills (Benkman 1999; Benkman et al. 2001, 2003; Parchman and Benkman 2002). A comparison of the traits preferred by squirrels (Benkman et al. 2001, 2003) and moths (Table 1) shows that both species prefer some of the same traits. For example, cones that are relatively narrow and have more seeds are preferred by both moths (Table 1) and squirrels (Benkman et al. 2003). Thus, the evolution of a trait that deters predation by squirrels would also deter predation by moths. Indeed, percent seed predation by moths is much smaller as PC1

scores in the Little Rocky Mountains (Fig. 3C) approach those of mean PC1 scores for ranges where squirrels drive the evolution of seed defenses (Fig. 1). Because defenses aimed at deterring squirrel predation are prominent throughout most of the distribution of lodgepole pine, this may explain why moth predation is relatively infrequent throughout the Rocky Mountains ($< 0.1\%$; Miller 1986) and throughout most of the other ranges sampled where squirrels were present (Fig. 2E). This reaffirms the central importance of squirrels in structuring the geographic selection mosaic in lodgepole pine. It also substantiates the importance of considering the evolution and ecology of interactions between multiple species, especially for those whose distributions and abundances are not completely coincident.

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