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DIVERSIFYING COEVOLUTION BETWEEN CROSSBILLS AND BLACK SPRUCE ON NEWFOUNDLAND

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Abstract.—Coevolution is increasingly recognized as an important process structuring geographic variation in the form of selection for many populations. Here we consider the importance of a geographic mosaic of coevolution to patterns of crossbill (*Loxia*) diversity in the northern boreal forests of North America. We examine the relationships between geographic variation in cone morphology, bill morphology, and feeding performance to test the hypothesis that, in the absence of red squirrels (*Tamiasciurus hudsonicus*), black spruce (*Picea mariana*) has lost seed defenses directed at *Tamiasciurus* and that red crossbills (*L. curvirostra*) and black spruce have coevolved in an evolutionary arms race. Comparisons of cone morphology and several indirect lines of evidence suggest that black spruce has evolved defenses in response to *Tamiasciurus* on mainland North America but has lost these defenses on Newfoundland. Cone traits that deter crossbills, including thicker scales that require larger forces to separate, are elevated in black spruce on Newfoundland, and larger billed crossbills have higher feeding performances than smaller billed crossbills on black spruce cones from Newfoundland. These results imply that the large bill of the Newfoundland crossbill (*L. c. percna*) evolved as an adaptation to the elevated cone defenses on Newfoundland and that crossbills and black spruce coevolved in an evolutionary arms race on Newfoundland during the last 9000 years since glaciers retreated. On the mainland where black spruce is not as well defended against crossbills, the small-billed white-winged crossbill (*L. leucoptera leucoptera*) is more efficient and specializes on seeds in the partially closed cones. Finally, reciprocal adaptations between crossbills and conifers are replicated in black spruce and Rocky Mountain lodgepole pine (*Pinus contorta* ssp. *latifolia*), with coevolution most pronounced in isolated populations where *Tamiasciurus* are absent as a competitor. This study further supports the role of *Tamiasciurus* in determining the selection mosaic for crossbills and suggests that a geographic mosaic of coevolution has been a prominent factor underlying the diversification of North American crossbills.

Key words.—Coevolution, geographic mosaic, *Loxia curvirostra*, *Loxia leucoptera*, *Picea mariana*, species interactions, *Tamiasciurus hudsonicus*.

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A recent advance in the study of coevolution has come from the recognition that species interactions vary in outcome geographically (e.g., Thompson and Pellmyr 1992). Consequently, large-scale geographic approaches are often necessary to gain insight into the importance of coevolution as a process influencing the organization of biodiversity. Thompson's (1994, 1999a) geographic mosaic theory provides a framework for understanding how coevolution continually reshapes species interactions across and between geographic regions. The theory holds that geographic variation in the strength and form of species interactions creates selection mosaics within which some populations experience coevolution (coevolutionary hotspots) and others do not (coevolutionary coldspots). Although the extent of local adaptation can be limited by gene flow (e.g., Burdon and Thrall 1999; Lively 1999), strong divergent selection between locally adapted populations experiencing coevolution and those that are not might reduce and eventually eliminate gene flow. Such divergent selection resulting from geographic variation in species interactions can then be an important process fueling diversification within a lineage (Thompson 1999a,b; Benkman 1999; Benkman et al. 2001).

Recent work demonstrates how a geographic mosaic of coevolution results in divergent selection between red crossbill (*Loxia curvirostra* complex) populations specialized for foraging on seeds in the cones of lodgepole pine (*Pinus contorta* ssp. *latifolia*) in the Rocky Mountains and in isolated mountain ranges to the east and west (Benkman 1999; Benk-

man et al. 2001). Whether crossbills coevolve with lodgepole pine depends on the absence of red squirrels (*Tamiasciurus hudsonicus*). When *Tamiasciurus* are present they outcompete crossbills for seeds and overwhelm selection by crossbills; *Tamiasciurus* harvest and cache large numbers of cones early in the fall before crossbills have an opportunity to forage on the cones, whereas crossbills extract seeds from cones remaining on the trees. In the absence of *Tamiasciurus*, however, crossbills are resident, locally adapted, and up to 20 times more abundant. In these areas, lodgepole pine has lost defenses directed at *Tamiasciurus* and evolved defenses against crossbills and crossbills in turn have evolved deeper bills due to reciprocal selection (Benkman 1999; Benkman et al. 2001). Because conifer cones evolve different defenses in response to selection from crossbills than from *Tamiasciurus*, crossbill populations in different areas are subject to divergent selection.

Whether coevolution occurs between crossbills and other species of conifers, and thus has been an important process in the adaptive radiation of crossbills, is unknown. Benkman (1999) suggested that coevolution was especially likely for crossbills relying on conifers that produce stable annual cone crops, like Rocky Mountain lodgepole pine. Stable annual cone crops enable crossbills to be resident (e.g., Senar et al. 1993) and adapt locally. In addition, stable annual cone crops allow *Tamiasciurus* to attain high densities and outcompete crossbills.

Black spruce (*Picea mariana*) is the next most consistent cone producer relied upon by crossbills in North America. Prior research has shown that the small slender-billed white-

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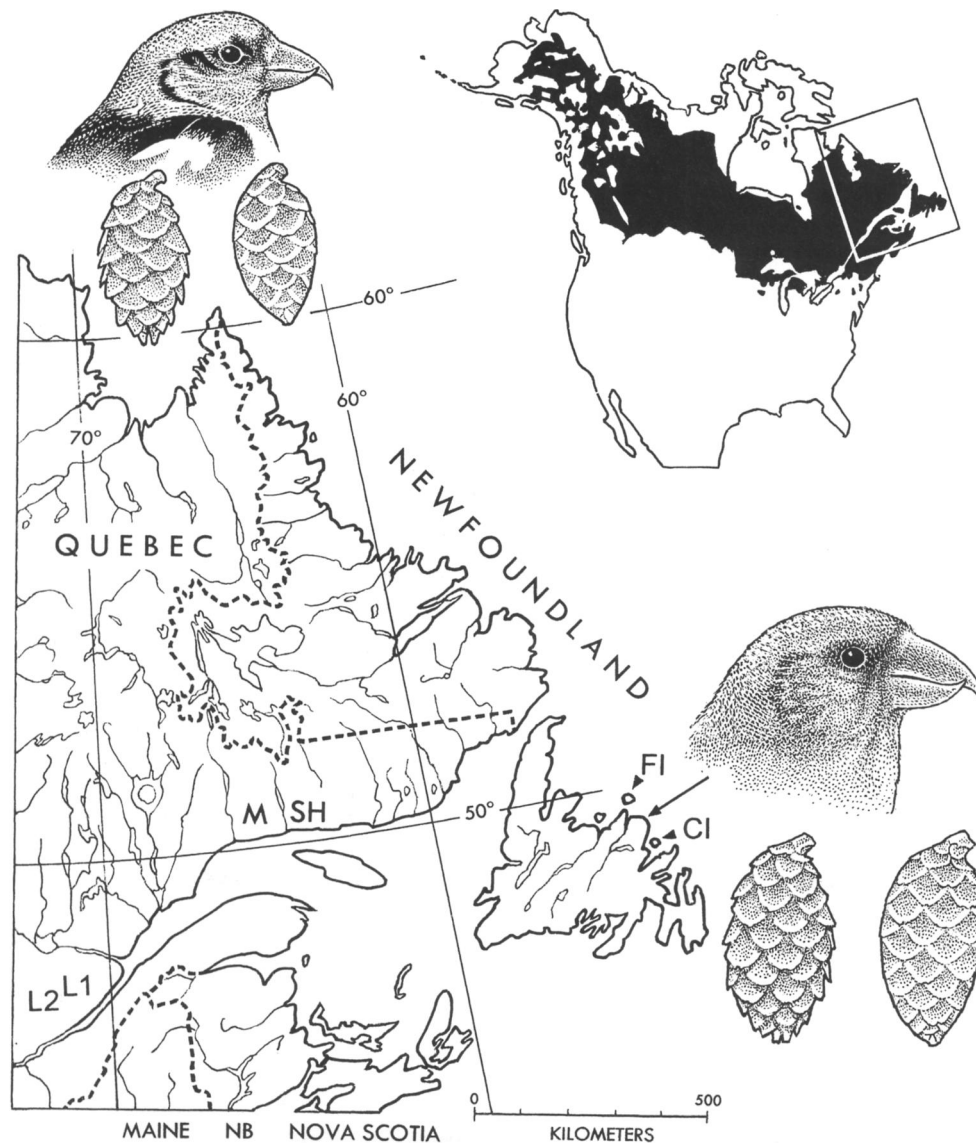


FIG. 1. The distribution of black spruce (black) in the map in the upper right, a white-winged crossbill (*Loxia leucoptera leucoptera*) (upper left) and a Newfoundland crossbill (*L. curvirostra percna*) (lower right), and representative partially closed and closed cones from the mainland (upper left) and Newfoundland (lower right). The study sites on Newfoundland were on Fogo Island (FI) and on Change and nearby Cotel islands (CI), and the study sites on the mainland were near Manitu (M) and Sheldrake (SH), and in Le Par des Grands-Jardins (L1, L2). Red squirrels (*Tamiasciurus hudsonicus*) occur throughout the spruce forests of the mainland and were introduced onto Newfoundland in 1963.

winged crossbill (*L. leucoptera leucoptera*) is specialized for foraging on the partially closed black spruce cones on the mainland (Fig. 1; Benkman 1987b, 1992). Black spruce produces "semi-serotinous" cones, with some opening their first fall after maturing, and the rest (about half) opening the following spring. However, the rigid cone scales tend to open so that only a narrow gap occurs between the overlapping scales ("partially closed") for several years (Vincent 1965). As a consequence, some seeds remain in partially closed cones for up to 19 years (Chai and Hansen 1952) and constitute a reliable resource for crossbills.

Benkman (1989a, 1993b) hypothesized a geographic selection mosaic for crossbills foraging on black spruce that was the result of the presence and absence of *Tamiasciurus*.

Black spruce and *Tamiasciurus* are widespread on the mainland (Fig. 1), but only black spruce has been present on Newfoundland for most of the past 9000 years following the retreat of glaciers (Jackson et al. 1997). Presumably black spruce colonized Newfoundland from a mainland source as it expanded north following the glacial retreat (Jackson et al. 1997). *Tamiasciurus* did not colonize Newfoundland and were absent from the island until their introduction in 1963 (Dodds 1983). Red Crossbills did colonize Newfoundland, were common, and evolved into a distinct taxa (*L. c. percna*; Fig. 1) (Benkman 1989a, 1993b). This implies that if black spruce cones on Newfoundland differ from those on the adjacent mainland, the differences reflect evolution over the past 9000 years without selection by *Tamiasciurus*. Insects

from at least five orders also feed on black spruce cones and seeds (Prévost et al. 1988). However, the same cone- and seed-feeding insects occur on Newfoundland as on the mainland (Hedlin et al. 1980), so that variation in cone structure between these two areas is unlikely to be explained by variation in selection by insects.

The goal of this paper is to evaluate whether crossbills and black spruce coevolve when *Tamiasciurus* are absent, as found for lodgepole pine (Benkman 1999; Benkman et al. 2001). First, we address the hypothesis that black spruce cone structure differs between the mainland and Newfoundland as the result of selection and relaxation of selection by *Tamiasciurus* (Benkman 1989a, 1993b). One prediction that we test is that cone traits that act to deter *Tamiasciurus*, and result in a reduction in seed production (i.e., result in an increase in cone mass relative to seed mass), should be more enhanced on the mainland than on Newfoundland. Inferring that such differences are the result of evolution is reasonable given that many black spruce cone traits are heritable (Verheggen and Farmer 1983; Khalil 1984; Stoehr and Farmer 1986). A second hypothesis is that some of the differences in cone traits between the mainland and Newfoundland are the result of increased selection by crossbills on Newfoundland. This hypothesis would be supported if cone traits that act to deter crossbills are enhanced on Newfoundland and these traits also result in an increase in cone mass, and are thus costly to the plant. If this hypothesis is rejected and the first hypothesis is not rejected, this would then be consistent with Benkman's (1989a) conjecture that differences in cone traits between the mainland and Newfoundland are simply the result of selection and relaxation of selection by *Tamiasciurus*. Finally, we test whether the large bill of the Newfoundland crossbill (Fig. 1) is an adaptation for foraging on black spruce cones on Newfoundland. We use feeding performance as a surrogate for fitness and infer that variation in feeding performance reflects the form of selection. Our recent finding that bill depth is similarly related to both feeding performance in the laboratory and survival in the wild for crossbills (C. W. Benkman, unpubl. data) strengthens this inference. Such selection, in turn, should lead to bill size evolution since bill depth is highly heritable in crossbills ($h^2 \approx 0.7$; R. Summers, pers. comm.). Unfortunately, we are limited to indirect tests of this hypothesis because the formerly abundant Newfoundland crossbill is probably extinct as a result of the introduction of *Tamiasciurus* to Newfoundland (Fig. 2; Benkman 1989a, 1993b).

METHODS

Geographic Cone Variation

Recently mature and closed black spruce cones were sampled between 8 and 29 September 2000; cones mature in early September (Vincent 1965). Five sites on Newfoundland were established in black spruce dominated forests on Fogo, Change, and Cottle Islands off the northeast coast of Newfoundland, Canada (Fig. 1) where *Tamiasciurus* are absent. Sampling on these islands, therefore, avoided bias that could have occurred by sampling cones from a subset of trees avoided by squirrels. This bias was not a problem on the mainland because all the conifers in the area (*Picea mariana*, white

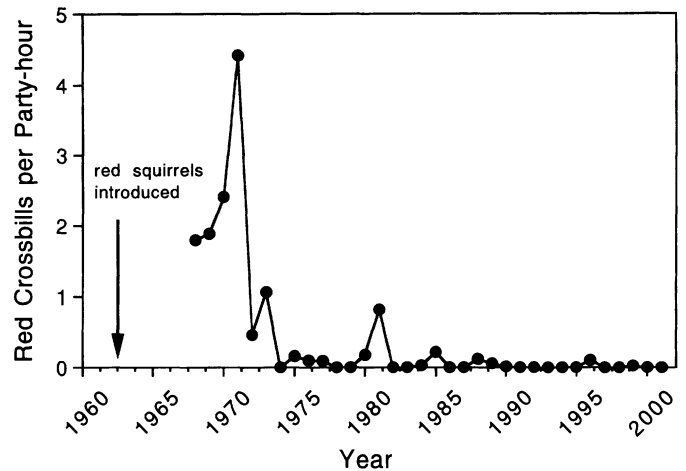


FIG. 2. The number of red crossbills per party-hour observed during annual Christmas Bird Counts in Terra Nova National Park, Newfoundland from 1968 to 2001. A "party-hour" is one hour of observation by one group of observers.

spruce *P. glauca*, and tamarack *Larix laricina*) produced large cone crops during the sampling period and during such years *Tamiasciurus* harvest few black spruce cones (Prévost et al. 1988). We sampled cones on the mainland at four sites dominated by black spruce in Québec (Fig. 1). Two sites were located near the coast in the vicinity of Manitou and Shelldrake, and two sites were located within Le Parc des Grands-Jardins in the Laurentian Mountains.

Cone traits were measured for two cones from each of 17–27 trees sampled from each site. Trees were chosen randomly within each study site at UTM coordinates obtained using a random number generator, and cones were cut with a 2-m extension pole from the crown of each tree. Only cones without apparent deformities were measured and included in the analyses. The following morphological measurements were taken on each cone: cone mass, closed cone length, closed cone width at the widest point, scale thickness at the distal edge of the seed scars of five scales from the middle third of the cone, mass of five seeds filled with kernel (female gametophyte and embryo), number of empty seeds (seed coat developed but lacking kernel), number of full seeds, and the force required to spread closed scales apart a set distance (2 mm). Length measurements were made to the nearest 0.01 mm using digital calipers. Mass measurements were made to the nearest 0.1 mg with a digital scale after cones had been oven dried for a minimum of 48 h. Prying force was measured using a prying tool with a strain gage attached. The tool was inserted between closed scales and spread apart to a set distance that approximated the gap a crossbill would need to remove a seed. The force in Newtons (N) was recorded for three separate scales in the middle third of two cones from each tree.

Two-level nested ANOVA was used to test for differences in individual cone traits among areas and among sites within the areas of Newfoundland and mainland Canada. Because trees were the experimental units, the mean values (ln-transformed except when noted otherwise) for the cone traits for each tree were used in all analyses. We tested for homogeneity of variances (Levene's test) and normality (Shapiro-

Wilkes test), and found only slight departures from normality for seed mass/cone mass ratios and force for a few of the sites. ANOVA, however, is robust to slight departures from normality. Discriminant functions analysis was used to determine which cone traits most strongly distinguish black spruce on Newfoundland from those on mainland Canada. We used total number of seeds per cone (empty and full combined) in the above analyses because it was highly correlated with the number of full seeds ($r = 0.89$) and in black spruce the percentage of full seeds is influenced by pollen availability (Caron and Powell 1989). The cross-validation procedure in SAS was used to insure that classifications were not based on the same equations used in developing the classifications.

We used principal components analysis (PCA) to characterize cone variation and to determine if patterns of cone variation between areas with and without *Tamiasciurus* were convergent between black spruce and lodgepole pine. First, we used the correlation matrix from the ln-transformed means of five lodgepole pine cone traits for each tree from four sites with and four sites without *Tamiasciurus* (see Benkman et al. 2001 for details on the sites). We used cone length, cone width, cone mass, number of seeds per cone, and individual seed mass in the analysis because each of these traits was measured in a similar manner on both lodgepole pine and black spruce cones. Second, we determined the principal component scores for each lodgepole pine tree, and then used the principal component equations derived from the lodgepole pine data to determine the principal component scores for each of the black spruce trees.

Crossbill Foraging Rates and Foraging Efficiency

Captive crossbills were kept in indoor aviaries (1.6 m × 2.7 m × 2.2 m or larger) at the New Mexico State University Animal Care Facility. The birds were fed fresh cones and Mazuri Chow (PMI Seeds Incorporated, St. Louis, MO) daily, and were provided with an abundance of grit, charcoal, and water. Each bird fed on five to seven black spruce cones each morning for two weeks prior to the experiments to ensure that the crossbills were accustomed to foraging on these cones. Beginning seven days before experiments, the crossbills' diets were controlled and the crossbills were maintained at a low body mass. To further insure consistent and motivated foraging, birds were deprived of food for about 15 hours before foraging trials. During foraging experiments the bird participating in the trial was isolated from other crossbills by a screen partition in the aviary and was allowed access to one cone at a time. The presence of crossbills on the other side of the screen eliminates variation that would otherwise occur from time spent scanning for predators (Benkman 1997). All foraging experiments were conducted between January and October 2001.

Partial correlation between the time required to remove and eat seeds from black spruce cones by captive red crossbills and the various cone traits was used to determine which cone traits deter crossbills. Time per seed is a good measure of seed defense because crossbills maximize feeding rates (mg seed/s) and thus avoid trees where time per seed is high (Benkman 1987a, 1989b). Five red crossbills (one ponderosa

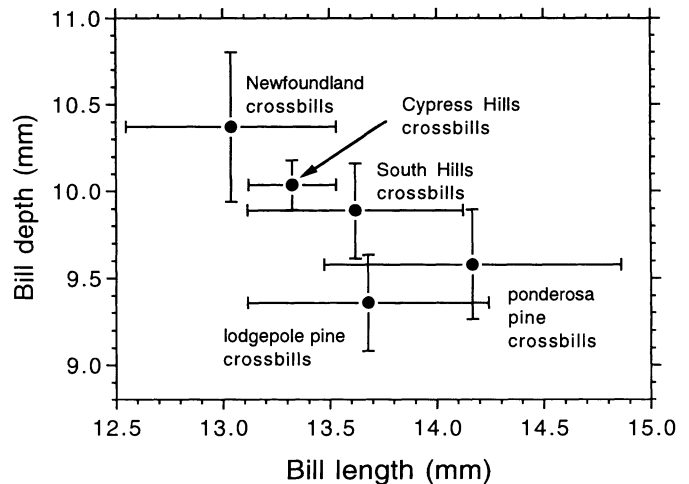


FIG. 3. Bill depth in relation to bill length (mean \pm SD) for five taxa of red crossbills. Sample sizes were 19 Newfoundland crossbills, 10 Cypress Hills crossbills, 58 South Hills crossbills, 32 lodgepole pine crossbills, and 61 ponderosa pine crossbills. All crossbills were alive when measured except the Newfoundland and Cypress Hills crossbills, which are both presumably extinct.

pine or type two crossbill of Groth [1993] and four South Hills crossbills of Benkman [1999]) were each timed foraging on 10 seeds from one closed black spruce cone from each of 91 trees spanning the full range of variation between Newfoundland and the mainland. These crossbills were chosen because their bill sizes (mean bill depth at the anterior end of the nares = 10.08 mm) were large and relatively similar to the bill size of the presumably extinct Newfoundland crossbill (Fig. 3). The total time to consume 10 seeds was recorded to the nearest 0.1 sec beginning after the bird ate the first seed and ended when the eleventh seed was eaten (see Benkman 1993a for more details on aviary protocols). Experimental cones were stored in a freezer and thawed two days prior to foraging trials. All of the cones used in trials were taken from trees for which morphological measurements were obtained as described previously. We assumed that the cones used in the foraging trials had the same average cone traits as those measured for the tree. This assumption is reasonable as long as within-tree variance in cone traits is substantially smaller than the between-tree variance. We have not tested this assumption for black spruce, although this seems reasonable based on visual impression and on data from lodgepole pine (Smith 1968; Elliott 1974). Cones were given to each crossbill in a random order with respect to geographic location and cone traits, and each bird was timed foraging on one cone from each tree. We have no reason to suspect that our results would have differed qualitatively if we had used partially closed rather than closed cones.

In earlier studies (e.g., Benkman 1993a; Benkman et al. 2001), analyses were based on the time taken to remove seeds from cones ("prying time"), which is estimated as the difference between mean total time per seed and husking time. Because we did not gather husking times from each crossbill foraging on cones from each tree used in the experiments, we present feeding rates in relation to total time per seed. We also present the results of analyses based on prying time

for the experiments for which we measured husking time. In no case did analyses based on time per seed differ substantially from comparable analyses based on prying time. Because individual trees were the experimental units, the mean foraging rate of the five crossbills on cones from each tree was used in the partial correlation analysis. Cone length, cone width, cone mass, and seed mass were excluded because these traits were highly correlated with other measured cone traits ($r > 0.63$). Once these traits were excluded, the highest correlation was between scale thickness and force ($r = 0.52$).

To test further whether defenses were elevated on Newfoundland, we timed 11 red crossbills (eight South Hills and three lodgepole pine or type five crossbills of Groth [1993]) each foraging on eight to 10 partially closed cones from and representative of Newfoundland, and on six to 11 partially closed cones from and representative of the mainland. We created partially closed cones by allowing closed cones to dry and open at room temperature. Partially closed cones were those that opened but had only narrow gaps between the scales (Fig. 1). Cones qualified as "representative" when length, width, and mass measurements were all within one standard deviation of the mean for black spruce from the respective area. Foraging trials were run as described previously. Because we tested for higher feeding times on cones from Newfoundland than from the mainland, we used one-tailed paired *t*-tests to compare the mean time per seed of each crossbill on each cone type.

To test the hypothesis that the Newfoundland crossbill was adapted for foraging on black spruce on Newfoundland, the relationship between bill depth and foraging efficiency was quantified. We used data from the above foraging experiment involving 11 crossbills (bill depths ranging from 9.22 mm to 10.04 mm) foraging on partially closed cones from and representative of Newfoundland. Ideally, we would have used Newfoundland crossbills in this experiment so that we could have tested whether their average bill depth (10.37 mm; Fig. 3) matched the most efficient bill depth predicted for foraging on black spruce cones (e.g., Benkman 1993a). Because the Newfoundland crossbill was substantially larger than other North American crossbills north of Mexico, the range of bill sizes tested did not encompass bill depths as large as that of the Newfoundland crossbill. Nevertheless, if relatively large-billed crossbills have higher feeding performances on Newfoundland black spruce, this would suggest that selection would have favored the evolution of a large bill for crossbills isolated on the island.

Feeding efficiency is inversely related to the foraging time necessary to meet daily energy demands. We estimated feeding efficiency as the inverse of the product of time per seed and estimated daily energy demands of a bird in the wild (Benkman 1993a; Benkman and Miller 1996). Daily energy demands were estimated as $(\text{body mass})^{0.6052}$ (Walsberg 1983). Body mass depends upon a variety of factors including the amount of fat stored and, especially in captivity, the loss of flight muscle mass. The crossbills used in the experiments varied in body fat at time of capture. Consequently, body mass was estimated from the allometric relationship between body mass (Y , in g) and bill depth (X , in mm): $Y = -3.78 + 3.82X$ ($r^2 = 0.68$, $df = 77$, $P < 0.0001$), where body mass

was measured at time of capture of crossbills lacking much fat (Benkman et al. 2001).

RESULTS

Geographic Cone Variation

Each cone trait differed significantly between Newfoundland and mainland Canada ($P < 0.0001$; Table 1). Black spruce cones from the Newfoundland sites were larger, had more and heavier seeds, and had thicker scales requiring larger forces to separate. Cones from Newfoundland also had a higher seed mass to cone mass ratio ($P < 0.0001$) (Table 1). Cone length, the total number of seeds per cone, scale thickness, the ratio of seed mass to cone mass, and the scale prying force were also significantly different among sites within Newfoundland and mainland Canada (Table 1).

The canonical discriminant functions correctly classified 92% of the trees from Newfoundland ($n = 91$ trees) and 91% of the trees ($n = 79$ trees) from the Canadian mainland. Based on the canonical discriminant functions, black spruce on Newfoundland is most importantly characterized by heavier cones with more seeds and thicker scales that require greater forces to spread apart.

*Has Black Spruce on Newfoundland Reduced Defenses that Might Have Deterred *Tamiasciurus*?*

Two types of evidence in combination indicate that black spruce on Newfoundland have reduced their cone defenses directed at *Tamiasciurus* as a result of relaxation of selection. First, cones on Newfoundland have more seeds per cone and a higher ratio of seed mass to cone mass than on the mainland (Table 1). We expected this result if *Tamiasciurus* prefer the same cone traits as found in lodgepole pine (Smith 1970; Elliott 1974, 1988; Benkman 1999; Benkman et al. 2001). *Tamiasciurus* prefer cones with more seeds and a greater ratio of seed mass to cone mass, because *Tamiasciurus* harvest and cache cones whole and more seeds per cone results in more energy cached. In addition, a greater ratio of seed mass to cone mass should result in higher feeding rates because *Tamiasciurus* would need to bite through less cone mass as they bite through successive scales to reach underlying seeds. Second, these differences are not related to the foraging rates of crossbills, nor can they be interpreted as responses to selection by crossbills (Table 2). In contrast to lodgepole pine, we did not expect cone width or relative cone width (cone width/cone length) to necessarily decrease as a result of relaxation of selection on Newfoundland. Black spruce cones are not sessile to the branch like lodgepole pine cones, thus greater relative cone width would not make black spruce cones more difficult to remove from the tree.

Has Black Spruce on Newfoundland Evolved in Response to Crossbills?

Two cone traits, scale thickness and the force (N) required to separate cone scales, were positively and significantly correlated with time per seed (Table 2). Increases in both scale thickness and the force required to separate cone scales increase their resistance to crossbills attempting to spread apart cone scales to reach underlying seeds. These two traits are

TABLE 1. Mean cone measurements for sites on Newfoundland and the Canadian mainland. The *P*-values give the significance of the difference between areas and of the differences between separate sites within the areas of Newfoundland and mainland Canada based on two-level nested ANOVAs (data ln-transformed except for seed mass/cone mass which was arcsine transformed). The ratio of seed mass/cone mass is individual seed mass in mg times the number of full seeds per cone divided by cone mass in mg. Analyses were based on the mean for each tree, with two cones measured from each tree.

Measurement	Newfoundland					Mainland Canada					Between Newfoundland and mainland Canada			Among sites in Newfoundland and mainland Canada		
	Fogo Island I		Fogo Island II		Change Island	Sheldrake	Manitou	Laurentides Reserve East	Laurentides Reserve West	<i>F</i> (df = 1,8)		<i>P</i>	% of total	<i>F</i> (df = 8,201)	<i>P</i>	% of total
Cone length, mm	31.91	35.04	34.61	32.65	33.05	28.87	27.63	29.32	29.71	133.8	<0.0001	53.8	3.8	<0.0007	4.5	
Cone width, mm	16.06	15.92	16.29	15.55	15.8	14.46	14.26	14.86	15.07	57.2	<0.0001	36.7	7.7	=0.11	0.32	
Cone mass, gm	2.33	2.55	2.65	2.39	2.33	1.63	1.61	1.67	1.81	151.4	<0.0001	62.3	1.6	=0.15	0.67	
Number of seeds/cone	71.27	61.5	83	80.88	75.84	43.8	37.99	50.44	53.88	313.6	<0.0001	65.3	11.8	<0.0001	10.8	
Scale thickness, mm	1.14	1.16	1.23	1.19	1.17	0.98	1.09	1.01	1.01	124	<0.0001	59.7	3.2	=0.003	4.3	
Individual seed mass, mg	1.31	1.4	1.38	1.33	1.42	1.07	1.04	1.09	1.07	95.8	<0.0001	51.9	0.7	=0.68	0	
Seed mass/cone mass	0.025	0.024	0.029	0.031	0.029	0.013	0.015	0.023	0.023	106.4	<0.0001	53.2	6.3	<0.0001	6.5	
Force to open scales, N	0.778	0.741	0.786	0.816	0.786	0.708	0.651	0.705	0.729	146.1	<0.0001	14.2	7.9	<0.0001	48.2	
Number of trees per site	20	24	19	17	19	20	18	27	26							

TABLE 2. Partial correlation coefficients between various cone traits and prying time and total time per seed (*n* = 91 trees, all ln-transformed) for five red crossbills foraging on black spruce cones.

	Prying time	Total time per seed
Number of full seeds	0.022	-0.011
Number of empty seeds	0.142	0.154
Scale thickness	0.315**	0.320**
Force to separate scales	0.268*	0.259*

* *P* < 0.02, ** *P* < 0.005.

also elevated on Newfoundland compared with the mainland (Table 1). Two other traits, the number of full and the number of empty seeds, were not correlated with time per seed (Table 2).

Time per seed for crossbills foraging on both closed and partially closed cones from Newfoundland was significantly greater than on comparable cones from the mainland (paired *t*-tests, *t*₄ = 10.06, *P* = 0.0003 [*P* = 0.0002 for prying time] and Fig. 4A, *t*₁₀ = 8.97, *P* < 0.0001, respectively). These results indicate that black spruce on Newfoundland has evolved cone defenses directed at crossbills, in the form of increased cone scale thickness and resistance, in addition to losing defenses directed at *Tamiasciurus*. Alternatively, the thicker scales on Newfoundland could be a correlated response to relaxation of selection by *Tamiasciurus*, if, for example, scale thickness increased with increases in the number of seeds per cone. Scale thickness, however, is not correlated with the number of seeds per cone within either the mainland or Newfoundland (Fig. 5; *P* > 0.4).

Are Changes in Black Spruce and Lodgepole Pine Cone Structure in the Presence and Absence of Tamiasciurus Convergent?

Black spruce and lodgepole pine cones differ in similar ways between areas with and without *Tamiasciurus* (Fig. 6). This indicates that in the absence of *Tamiasciurus*, conifers that produce relatively stable annual cone crops evolve in parallel. Equally striking were the very similar ratios of seed mass to cone mass for black spruce (Table 1) and lodgepole pine (table 1 in Benkman et al. 2001) in areas with *Tamiasciurus* (0.019 and 0.012) and in areas without *Tamiasciurus* (0.028 and 0.029), respectively. These results indicate further that in the absence of *Tamiasciurus* black spruce has reduced defenses directed at *Tamiasciurus* and increased defenses directed at crossbills.

Was the Newfoundland Crossbill Adapted to Black Spruce on Newfoundland?

Time per seed decreased significantly (Fig. 4A; *r*² = 0.50, *df* = 9, *P* = 0.015) and feeding efficiency increased with increases in bill depth (Fig. 4B; *r*² = 0.36, *df* = 9, *P* = 0.051) on partially closed black spruce cones from Newfoundland. This suggests that a larger, deeper bill such as that of the Newfoundland crossbill would have been adaptive for feeding on black spruce on Newfoundland.

Foraging data on partially closed cones from the mainland indicate that a large-billed crossbill like the Newfoundland

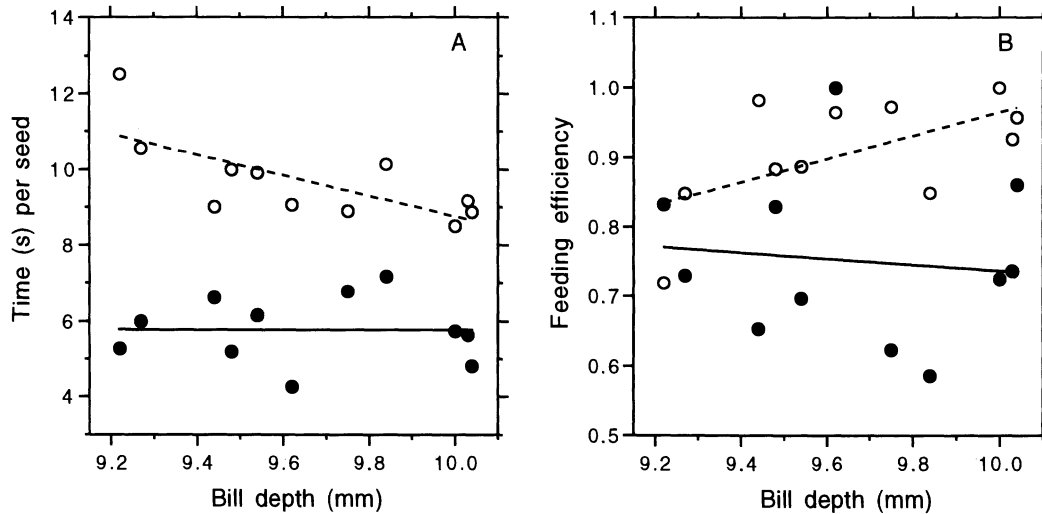


FIG. 4. Time per seed (A) and estimated feeding efficiency (B) in relation to bill depth of crossbills foraging on partially closed black spruce cones from Newfoundland (open circles and dashed lines) and from the Canadian mainland (filled circles and solid lines). Each symbol represents the mean of a single bird.

crossbill would not be favored (Fig. 4). Time per seed was unrelated to bill depth (Fig. 4A; $r^2 = 0.00$, $df = 9$, $P = 0.98$) and foraging efficiency tended to decrease slightly as bill depth increased because of increasing energy requirements with increasing bill size (Fig. 4B; $r^2 = 0.01$, $df = 9$, $P = 0.76$). These results indicate that larger billed crossbills are favored for foraging on partially closed black spruce cones on Newfoundland, but there is no advantage of a larger bill on the mainland.

DISCUSSION

Our study indicates that the Newfoundland crossbill co-evolved with black spruce in a predator-prey arms race on

Newfoundland during the last 9000 years. Black spruce on mainland Canada evolved in response to selection from *Tamiasciurus*, whereas black spruce on Newfoundland evolved in response to the relaxation of selection from *Tamiasciurus* and in response to selection from crossbills. The Newfoundland crossbill in turn evolved a deep bill, which increased foraging efficiency on the partially closed cones of black spruce on Newfoundland. On the mainland, where black spruce is not as well defended against crossbills, the small-billed white-winged crossbill specializes on seeds in partially closed black spruce cones (Benkman 1987b, 1992). The results of this and other recent studies (Benkman 1999; Benkman et al. 2001) show that variation in the presence and absence of *Tamiasciurus* determines whether coevolution occurs between crossbills and conifers, and creates a geographic mosaic of selection for both crossbills and conifers. Below we discuss the evidence that each of these species have evolved reciprocally in response to selection imposed by the other. The repeated and convergent nature of coevolution between crossbills and conifers in isolated populations where *Tamiasciurus* are absent (Fig. 6) will also be discussed as it pertains to patterns of crossbill diversity.

Black Spruce Evolution in Response to Seed Predation

Although we did not directly address the form of selection exerted by *Tamiasciurus* on black spruce, several lines of evidence suggest that black spruce on Newfoundland has lost some of its defenses directed at *Tamiasciurus*. First, both the number of seeds per cone and the ratio of seed mass to cone mass have increased on Newfoundland. Similar increases have occurred in lodgepole pine (Benkman 1999; Benkman et al. 2001) and limber pine (*Pinus flexilis*; Benkman 1995) in areas where *Tamiasciurus* are absent and can be related to *Tamiasciurus* cone preferences and foraging behavior. In contrast, no evidence indicates that selection by crossbills alone in the absence of *Tamiasciurus* would favor these changes. For example, the time for crossbills to remove and eat seeds

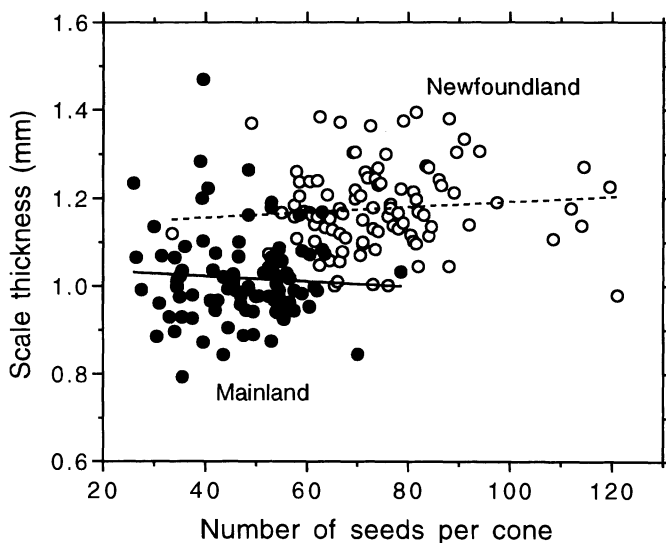


FIG. 5. Scale thickness in relation to the number of seeds per cone. The linear relationships between these two variables were not significant for Newfoundland (open circles and dashed line) or the mainland (filled circles and solid lines) ($P > 0.4$). Each symbol represents the means of one tree.

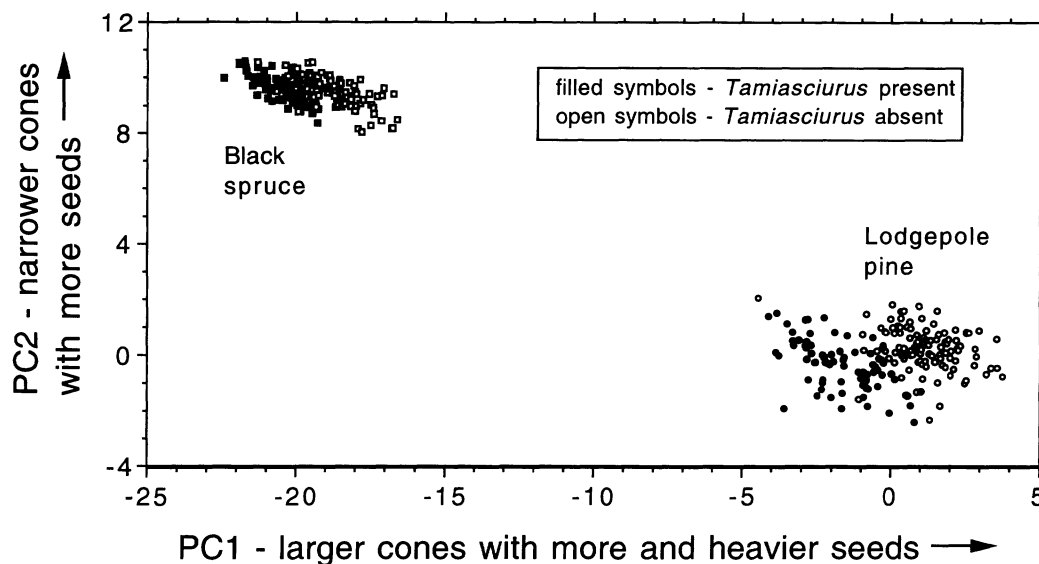


FIG. 6. Variation in cone structure between areas with and without *Tamiasciurus* in relation to the first two principal components of five cone and seed traits of black spruce (squares) and lodgepole pine (circles).

is unrelated to the number of seeds per cone (Table 2) because crossbills forage on seeds individually and avoid areas of the cone with sterile scales. Second, introduced *Tamiasciurus* on Newfoundland remove a much greater percentage of the black spruce cone crop by early autumn (64–95%; West 1989) than do *Tamiasciurus* on the mainland (19% at most; Prévost et al. 1988). Third, densities of *Tamiasciurus* are greater in black spruce dominated habitat on Newfoundland than in comparable habitats on the mainland (Benkman 1989a, 1993b). These patterns are consistent with other studies (see Benkman 1993b) indicating that black spruce cones are avoided by *Tamiasciurus* on the mainland but readily used on Newfoundland, and suggest that black spruce on Newfoundland has lost defenses directed at *Tamiasciurus* in response to relaxation of selection by *Tamiasciurus*.

Geographic variation in black spruce cone traits is also consistent with the hypothesis that in the absence of *Tamiasciurus* crossbills are more common and exert stronger selection on cone traits than when *Tamiasciurus* are present. The traits that evolve to deter crossbill predation increase resistance to the forces crossbills exert while separating overlapping cone scales to extract seeds (Benkman 1999; Benkman et al. 2001), and are expected to be elevated on Newfoundland where crossbills were common (Benkman 1989a, 1993b) and presumably were the primary seed predator. Partial correlation between time per seed and various cone traits shows that scale thickness and the force required to pry overlapping cone scales apart are the traits that slow crossbill foraging rates (Table 2). These are the traits that should evolve in response to crossbill predation, and they are traits that are elevated in black spruce on Newfoundland. The greater amounts of time spent extracting seeds from cones from Newfoundland than from the mainland (Fig. 4A) further suggest that black spruce on Newfoundland evolved cone defenses in response to selection by crossbills.

Finally, these results are consistent with other studies on crossbills. For example, time per seed for red crossbills in-

creases with increases in the scale thickness of lodgepole pine cones (Benkman et al. 2001). Moreover, thicker scales are interpreted as traits that have evolved in response to crossbill predation in areas of the Rocky Mountains where *Tamiasciurus* are absent and crossbills are the main selective agent (Benkman 1999; Benkman et al. 2001). Similarly, Eurasian common crossbills (*L. c. curvirostra*) prefer to forage on shorter cones than on longer cones of Scots pine (*Pinus sylvestris*), presumably because shorter cones have thinner scales (Summers and Proctor 1999). Such preferences would lead to selection for thicker scales and larger cones.

Black spruce cones are larger on Newfoundland than on the mainland (Fig. 1, Table 1). Likewise, lodgepole pine also has substantially larger cones in areas where the evolution of cone defense has been in response to crossbill predation, in comparison to areas where evolution has been in response to predation by *Tamiasciurus* (Fig. 6; Benkman 1999; Benkman et al. 2001). One explanation for these general size differences is that selection on specific cone traits leads to correlated responses in other traits affecting cone size. For example, the combination of relaxation of selection by *Tamiasciurus*, which favors trees having cones with more seeds, and selection by crossbills, which favors trees having cones with thicker scales, may result in the evolution of larger cone size as a correlated response (Benkman et al. 2001).

Our evidence indicates that crossbills have had a much stronger impact on black spruce cone evolution on Newfoundland than on the mainland. Crossbills have a negligible impact on lodgepole pine cone evolution in the presence of *Tamiasciurus* because they remove most of the cones before crossbills have the opportunity to forage on them (Benkman 1999; Benkman et al. 2001). Any selection crossbills exert on cones is therefore restricted to the relatively small subset of cones left by *Tamiasciurus* (Benkman 1999). On the mainland, however, *Tamiasciurus* harvest a small fraction of the black spruce cone crop. Consequently, preemptive competition by *Tamiasciurus* alone cannot explain why white-

winged crossbills have not had much of a selective impact on black spruce. We believe that white-winged crossbills have had relatively little selective impact because they prefer to feed on white spruce and tamarack, and tend to avoid black spruce except in late winter and spring when most of the seeds in the other conifers have been shed or eaten (Benkman 1987a, 1992). We suspect that Newfoundland crossbills would have also preferred foraging on white spruce and tamarack cones when available. The cone crops of these conifers, however, fluctuate tremendously from year to year (e.g., Benkman 1987a) and during much of most years black spruce was probably the main seed available to Newfoundland crossbills. White-winged crossbills are able to avoid regional cone failures of white spruce and tamarack during most years by wandering across the extensive boreal forests of North America (Benkman 1987a, 1992). Newfoundland crossbills likely relied more extensively on black spruce seed than do white-winged crossbills, and consequently the Newfoundland crossbill had a greater impact on black spruce cone evolution.

In sum, differences in black spruce cone structure between Newfoundland and mainland Canada reflect different selective regimes resulting from the foraging behavior of *Tamiasciurus* and crossbills. Black spruce on the mainland have experienced *Tamiasciurus* as a primary selective agent, and have evolved cone traits that serve as defenses against *Tamiasciurus*. In contrast, black spruce on Newfoundland have evolved in the absence of selection from *Tamiasciurus*, but with crossbills as an important selective agent.

Crossbill Evolution in Response to Changes in Black Spruce Cone Structure on Newfoundland

Crossbills with deeper bills have higher feeding rates and presumably higher fitness feeding on the cones of black spruce from Newfoundland than would a shallower billed crossbill (Fig. 4). This should result in selection favoring a bill depth greater than 10 mm. However, we are unable to determine how feeding efficiency varies as bill depth increases above 10 mm and thus determine if 10.37 mm, the mean depth of the Newfoundland crossbill (Fig. 3), approximates the optimal bill depth (e.g., Benkman 1993a). Nonetheless, all evidence indicates that the key resource for the Newfoundland crossbill was the seeds in partially closed black spruce cones, which on the mainland is the key resource for the white-winged crossbill (Benkman 1987b, 1993a).

Selection for foraging on partially closed black spruce cones on the mainland likely favors smaller bills and is divergent from selection on Newfoundland. Although our experiments are equivocal concerning selection on bill size on the mainland, earlier experiments (Benkman 1987b) are not equivocal. They show that white-winged crossbills, which have smaller bills (mean bill depth of 7.94 mm; Benkman 1992) than any of the crossbills used in our experiments, would be favored over larger billed crossbills. White-winged crossbills required substantially less time to remove seeds from partially closed cones from the mainland (mean \pm SE = 1.98 \pm 0.08 sec) than did red crossbills with bill depths averaging 9.6 mm (mean \pm SE = 3.59 \pm 0.16 sec; *t*-test, *t*₅ = 8.09, *P* < 0.0005) (Benkman 1987b).

Replicate Coevolution

Benkman (1999; Benkman et al. 2001) demonstrated that replicate coevolution between crossbills and lodgepole pine has occurred in several mountain ranges east and west of the Rocky Mountains where *Tamiasciurus* are absent. Our study shows that the patterns replicated in lodgepole pine are replicated in another genus of conifer (Fig. 6). All measured cone traits common to both study systems show changes in the same direction and roughly the same magnitude between areas with and without *Tamiasciurus*. Reciprocal adaptation also led to the evolution of a large stout bill for the Newfoundland crossbill, a change parallel to that seen in crossbill populations coevolving with Rocky Mountain lodgepole pine in the Cypress Hills, Alberta, Saskatchewan, Canada, and in the South Hills, Idaho, U.S. (Fig. 3). The replicate nature of the outcome of coevolution between crossbills and conifers indicates similar nonrandom processes (i.e., natural selection) have been important and is analogous to that seen in the replicate radiations of *Anolis* lizards in the Greater Antilles (Losos 1992; Losos et al. 1998), radiations of fish in post-glacial lakes (Schluter and McPhail 1993), and cichlid radiations in the East African great lakes (e.g., Meyer 1993). Losos (1992) suggests that strong interspecific interactions enhance the predictability of faunal evolution. This suggestion is consistent with and describes well the interactions between crossbills and some conifers.

Conclusions

Thompson's (1994, 1999a) geographic mosaic theory of coevolution is based on the idea that variation in the strength and occurrence of species interactions can lead to a selection mosaic in which some populations experience coevolution and others do not. The repeated and convergent outcomes of coevolution between crossbills and conifers in different geographic locations and among different genera of conifers suggests a geographic mosaic of coevolution has been an important source of variation in selection regimes experienced by crossbill populations. This study further suggests that geographic variation in species interactions may have fueled crossbill diversification across a selection mosaic. A critical feature of the selection mosaic that crossbills experience is that coevolution is a prominent process only where *Tamiasciurus* are absent as a preemptive competitor (Benkman 1999; Benkman et al. 2001). The effectiveness of *Tamiasciurus* as a competitor, especially when conifers have lost "squirrel" defenses, is demonstrated by the likely extinction of both the Newfoundland crossbill and a unique population of crossbills in the Cypress Hills following the introduction of *Tamiasciurus* (Benkman 1999). These studies clearly demonstrate the need for interpreting community-level processes from both evolutionary and ecological perspectives (e.g., McPeck 1996).

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