A Coevolutionary Arms Race Causes Ecological Speciation in Crossbills

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ABSTRACT: We examined three ecological factors potentially causing premating reproductive isolation to determine whether divergent selection as a result of coevolution between South Hills crossbills (*Loxia curvirostra* complex) and Rocky Mountain lodgepole pine (*Pinus contorta latifolia*) promotes ecological speciation. One factor was habitat isolation arising because of enhanced seed defenses of lodgepole pine in the South Hills. This caused the crossbill call types (morphologically and vocally differentiated forms) adapted to alternative resources to be rare. Another occurred when crossbills of other call types moved into the South Hills late in the breeding season and feeding conditions were deteriorating so that relatively few non–South Hills crossbills bred ("immigrant infecundity"). Finally, among those crossbills that bred, pairing was strongly assortative by call type (behavioral isolation). Total reproductive isolation between South Hills crossbills and the two other crossbills most common in the South Hills (call types 2 and 5) summed to .9975 and .9998, respectively, on a scale of 0 (no reproductive isolation) to 1 (complete reproductive isolation). These extremely high levels of reproductive isolation indicate that the divergent selection resulting from the coevolutionary arms race between crossbills and lodgepole pine is causing the South Hills crossbill to speciate.

Keywords: assortative pairing, competition, divergent selection, local adaptation, *Loxia curvirostra*, sympatric speciation.

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Interest in how selection for exploiting different resources contributes to the evolution of reproductive isolation has been revived by recent studies of ecological speciation (Rice and Hostert 1993; Schluter 2000, 2001; Via 2001; Funk et al. 2002; Coyne and Orr 2004; Rundle and Nosil 2005). According to the ecological model of speciation, divergent natural selection drives the accumulation of differences, causing reproductive isolation between populations utilizing different resources or occupying different habitats (Schluter 2001). Consequently, determining how divergent selection leads to reproductive isolation is one of the challenges in the study of ecological speciation. We are gaining such an understanding from recent studies that demonstrate that phenotypic traits known to be under divergent selection are also characters used in mate choice. For example, divergent natural selection has resulted in different body sizes and shapes between sympatric ecomorphs of sticklebacks (*Gasterostreus aculatelus*; Schluter 1993, 1995), which contributes to assortative mating and reproductive isolation because females preferentially mate with males that have morphologies like their own (Nagel and Schluter 1998; Rundle et al. 2000; McKinnon et al. 2004; Boughman et al. 2005). Nevertheless, mate choice based on the same characters under divergent selection is but one mechanism by which ecological speciation can occur (Rice 1987; Schluter 2001; Ramsey et al. 2003; Coyne and Orr 2004; Nosil et al. 2005). Consequently, additional studies, especially those examining reproductive isolating barriers in addition to sexual isolation, are needed to increase our understanding of the relationship between divergent selection and reproductive isolation (Schluter 2001; Rundle and Nosil 2005).

One group where divergent selection for foraging on alternative resources has been implicated in adaptive radiation and could potentially contribute to reproductive isolation is crossbills (*Loxia*: Benkman 1993, 1999, 2003; Benkman et al. 2001, 2003; Parchman and Benkman 2002). North American red crossbills (*Loxia curvirostra* complex) are recognized as having nine separate call types as a result of differences in vocalizations, bill size (Groth 1993b; Benkman 1999), and palate structure (Benkman 1993).

Red crossbills specialize on seeds in conifer cones and use their crossed mandibles to spread overlapping cone scales apart to expose the seeds at their base (Benkman and Lindholm 1991). At least six of the call types are specialized for foraging on different species of conifers that hold seeds in partially closed cones through winter (Benkman 1993, 1999; Benkman and Miller 1996; Benkman et al. 2001; Parchman and Benkman 2002). The differences in bill and palate structure between the different call types are the result of divergent selection for foraging on different resources (Benkman 1993, 2003).

Although the factors promoting divergent selection between call types are well understood, little is known about whether divergent selection is promoting reproductive isolation between them. The different call types maintain morphological and vocal differences despite widespread sympatry, suggesting that the call types are reproductively isolated (Groth 1993*b*). Moreover, the close match between the predicted optima for foraging on the different species of conifers and the mean morphological traits of the different call types associated with these conifers (Benkman 1993; Benkman et al. 2001) indicates that gene flow does not impede adaptation. Nevertheless, direct evidence of reproductive isolation and assortative mating between the different call types has remained elusive (Groth 1993*a*; see Robb 2000; Edelaar et al. 2004 for similarly few but suggestive data for assortative pairing of call types in Europe) for at least two reasons. First, the variable nature of cone production of most conifers causes crossbills to engage in annual nomadic movements while tracking cone crop fluctuations (Newton 1972; Benkman 1987, 1992; Adkisson 1996). Second, crossbills are opportunistic breeders with the timing of breeding governed by the erratic availability of conifer seeds (Newton 1972; Benkman 1990, 1992). This combination of nomadism and erratic breeding behavior has made it difficult to observe pairing behavior on a regular basis.

The South Hills crossbill (call type 9) provides an opportunity to quantify both reproductive isolation and the nongeographic barriers contributing to reproductive isolation because the bird is resident and common in two small mountain ranges in southern Idaho (fig. 1) and periodically coexists during breeding seasons with two other wide-ranging call types (call types 2 and 5; Parchman et al. 2006). The South Hills crossbill and its coevolutionary arms race with Rocky Mountain lodgepole pine (*Pinus contorta latifolia*) have been discussed at length elsewhere (Benkman 1999; Benkman et al. 2001, 2003; Siepielski and Benkman 2004, 2005; Edelaar and Benkman 2006). In brief, South Hills crossbills are in a coevolutionary arms race with lodgepole pine because of the absence of pine squirrels (*Tamiasciurus hudsonicus*) that, throughout much of the range of lodgepole pine, preemptively outcompete

crossbills and drive the evolution of cone structure. The result is that crossbills experience divergent selection, which has arisen in the past 5,000–7,000 years, for foraging on contrasting cone structures between areas with and without pine squirrels (fig. 1; Benkman et al. 2001, 2003).

The divergence between call types, an early stage in reproductive divergence, appears to be recent and ongoing. A study using 440 amplified fragment length polymorphic markers (AFLPs) to examine the evolutionary relationships of the North American red crossbill complex did not find evidence of call types clustering together monophyletically and revealed only minor differentiation among the types (Parchman et al. 2006). Nevertheless, the analyses revealed that the different call types are genetically differentiated from one another and form three fairly distinct clusters of call types. Three call types (fig. A1; call types 2 and 5 and the South Hills crossbill) comprise one of the clusters, and these call types are sympatric in the South Hills but differ genetically (F_{ST} values vary from .031 to .041, $P < .01$; Parchman et al. 2006). Our goal was to investigate whether the previously documented trade-offs in feeding efficiency (table 1) have led to differences in habitat use and occurrence of breeding between South Hills crossbills and other call types, thereby promoting premating reproductive isolation and reducing gene flow.

Methods

Data were collected in the South Hills between February 2001 and December 2002. One of us (J.W.S.) conducted field studies from February 1 to July 25, 2001, and from February 11 to August 1, 2002. Researcher Trevor Fetz also conducted field studies for at least 10 days of every month during the months that J.W.S. was absent (with the exception of January) in 2001 and 2002. In the South Hills, crossbills occur almost exclusively in lodgepole pine and only occasionally in the other two native and associated tree species, aspen (*Populus tremuloides*) and subalpine fir (*Abies lasiocarpa*), with rare nesting but no observed feeding in fir. The South Hills contain about 80 km² of lodgepole pine (Benkman et al. 2001) scattered in >100 forest patches within a matrix of sagebrush (*Artemisia tridentata*).

The Magnitude of Reproductive Isolation

Habitat isolation. We envision habitat isolation to occur when differences in occurrence of the different call types in the South Hills arise because, for example, bill structure and relatively poor feeding performance (table 1) cause call types 2 and 5 to remain uncommon. Call types 2 and 5 are nomadic throughout the Rocky Mountain region (table 1), apparently flying hundreds of kilometers in
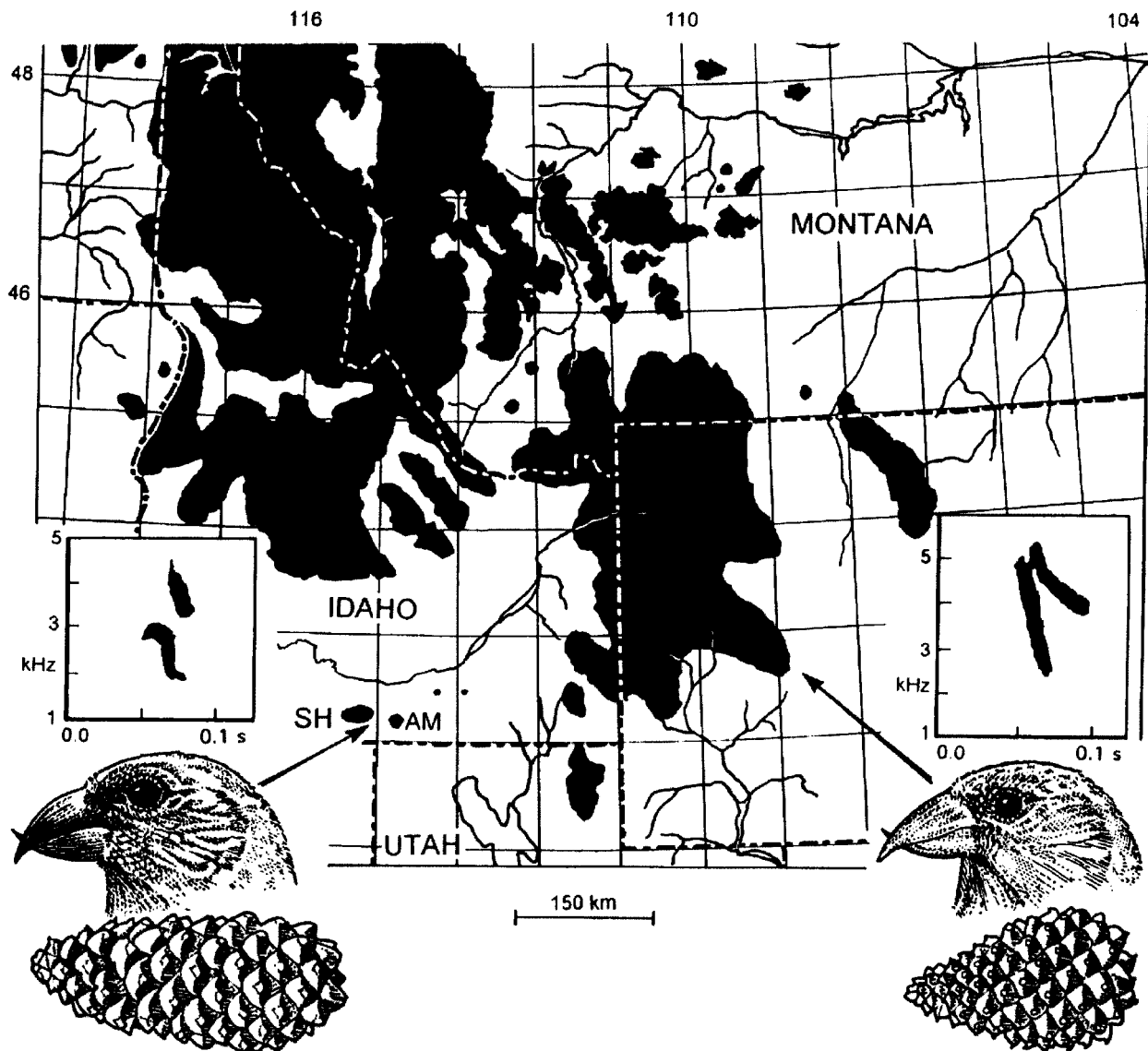
Figure 1: Distribution of Rocky Mountain lodgepole pine (*black*) and representative crossbills and cones in the Rocky Mountains (*lower right*) and in the South Hills (*SH*) and Albion Mountains (*AM; lower left*). The crossbills and cones are drawn to relative scale. Representative sonograms of flight calls are shown for the South Hills crossbill (*lower left*) and the call type 5 crossbill (*lower right*). Pine squirrels (*Tamiasciurus hudsonicus*) are found throughout the range of lodgepole pine except in some isolated mountains, including the South Hills and Albion Mountains. Rocky Mountain ponderosa pine (not shown) occurs throughout much of the geographic range of lodgepole pine in this map but at lower elevations; ponderosa pine is not found in or near the South Hills except for a few small stands that have been planted.

search of large cone crops during most years; they are not prevented by geographic barriers or historical accidents from occurring and increasing in abundance in the South Hills (Benkman 1987; Siepielski and Benkman 2005; Parchman et al. 2006). Call types 2 and 5 would be uncommon if poorly adapted individuals died (immigrant inviability; Nosil et al. 2005) or, alternatively, if they did not remain because they determined that the habitat is

unsuitable (i.e., feeding intake rates are low). We suspect the latter is more common, because crossbills closely track shifts in changes in seed profitability and are strong fliers (Benkman 1987). Consequently, without habitat isolation, the frequency of call type 2 crossbills should approximate the frequency of South Hills crossbills. This assumption is supported by the large populations of call type 2 crossbills that exist in isolated ranges east of the Rocky Moun-

Table 1: Characteristics of the three red crossbill call types in this study

Call type	Mean bill depth (n)	Morphologically specialized to feed on these seeds[a]	Foraging efficiency in South Hills	Occurrence in South Hills[b]	Geographic distribution in the Rocky Mountain region[c]
South Hills	9.85 (476)	Rocky Mountain lodgepole pine in South Hills and Albion Mountains	1.00	Common, highly sedentary	Resident in South Hills and Albion Mountains
2	9.56 (103)	Rocky Mountain ponderosa pine	.57	Much less common, nomadic	Nomadic throughout Rocky Mountains, especially in ponderosa pine and in lodgepole pine in isolated ranges east and west of Rocky Mountains
5	9.29 (33)	Rocky Mountain lodgepole pine	.35	Least common, nomadic	Nomadic throughout Rocky Mountains, especially at higher elevations in lodgepole pine and Engelmann spruce (*Picea engelmannii*)

Note: Bill depth (mm) shown as average of means for male and female, with n = total number of adults measured. Foraging efficiency is shown relative to the South Hills crossbills and is inversely proportional to the time required for a crossbill to meet its daily energy requirement; this is based on foraging data from 28 crossbills and on equations in Benkman (2003; see also Benkman et al. 2001), estimated for an individual with an average bill structure for each of the call types.

[a] Benkman 1993, 1999, 2003; Benkman and Miller 1996; Benkman et al. 2001.
[b] Observations by C. W. Benkman, T. Fetz, P. Keenan, L. Santisteban, and J. W. Smith from 1997 to 2006.
[c] Benkman 1993, 1999; Groth 1993*b*; Adkisson 1996; Siepielski and Benkman 2005; Edelaar and Benkman 2006; C. W. Benkman, personal observation.

tains, where lodgepole pine is the main conifer and its cones are convergent with those from the South Hills (the two northeasternmost ranges in fig. 1; Siepielski and Benkman 2005). Because there is no evidence or reason to suspect that different call types prefer different microhabitats within the South Hills (see Benkman 1987), our measure of habitat isolation pertains to only the occurrence of the call types in the South Hills during the breeding season. The individual contribution of habitat isolation (reproductive isolation [RI]$_1$) between South Hills and call type 2 crossbills was therefore estimated as

$$\mathrm{RI}_1 = \frac{\sum_{i=1}^{6} 1 - \{\mathrm{Ty2}_i / [(\mathrm{Ty2}_i + \mathrm{SH}_i)/2]\}}{6},$$

where $\mathrm{Ty2}_i$ and SH_i represent the number of call type 2 and South Hills crossbills counted, respectively, for each month (i) for both years combined (e.g., March 2001 and March 2002 combined). Monthly estimates of habitat isolation could range from -1 to 1, where negative values indicate increased opportunities for gene flow relative to random expectations, 0 equals no reproductive isolation, and 1 equals complete reproductive isolation. Estimates were made for each of the 6 months from when pairing began in February (see "Results") until the end of the nesting cycle in July, and RI$_1$ is the monthly mean. Habitat isolation between South Hills and call type 5 crossbills was estimated in the same manner by substituting values of call type 5 for call type 2. Call type 5 crossbills are much

less efficient at foraging on cones from the South Hills than are call type 2 (table 1) crossbills; therefore, one prediction of the ecological speciation hypothesis (Schluter 2001) is that habitat isolation would be stronger for call type 5 crossbills than for call type 2 crossbills. See the appendix, available in the online edition of the *American Naturalist*, for additional details on measuring habitat isolation.

Immigrant infecundity. Call types 2 and 5 commonly breed from late winter through summer (Adkisson 1996), which is when South Hills crossbills breed; however, the low feeding efficiencies of call types 2 and 5 on cones from the South Hills (table 1) may prevent them from regularly breeding in the South Hills. We term this "immigrant infecundity." Immigrant infecundity is analogous to immigrant inviability, but instead of the immigrants dying, the immigrants survive but do not breed or breed less frequently. We estimated the individual contribution of immigrant infecundity (RI$_2$) between South Hills and call type 2 crossbills as

$$\mathrm{RI}_2 = \left[\sum_{i=1}^{6} 1 - (\mathrm{BrTy2}_i / \{(\mathrm{BrTy2}_i + \mathrm{BrSH}_i) \right.$$
$$\left. \times\ [\mathrm{Ty2}_i / (\mathrm{Ty2}_i + \mathrm{SH}_i)]\})\right]/6,$$

where $\mathrm{BrTy2}_i$ and BrSH_i represent the number of breeding call type 2 and South Hills crossbills, respectively, for each of 6 months (i, years combined) used to estimate habitat

isolation. Immigrant infecundity between South Hills and call type 5 crossbills was estimated in the same manner by substituting values of call type 5 for call type 2. Values range from $-\infty$ to 1 and are interpreted similarly to values for habitat isolation. Because immigrant infecundity is measured with respect to the observed numbers of each of the call types in the South Hills, this measure is independent of habitat isolation. See the appendix for additional details on field research and analyses for determining immigrant infecundity.

Behavioral isolation. Evidence for behavioral isolation is inferred if heterotypic mating is less frequent than the random mating rate, a situation that could arise if, for example, call types flock assortatively and choose mates from within flocks (Smith et al. 1999) or if females preferentially mate with individuals of their own call type. We used the estimator I_{PSI} (pair sexual isolation) to estimate the individual contribution of behavioral isolation (RI_3; Rolán-Alvarez and Caballero 2000; Pérez-Figueroa et al. 2005). The I_{PSI} ranges from -1 (complete negatively assortative mating) to 1 (complete positive assortative mating), with 0 representing random mating by call type. Detailed analyses show that in many cases, including where data has characteristics similar to our data, I_{PSI} has better estimation properties than other estimators of sexual isolation (Pérez-Figueroa et al. 2005). It requires data on the four possible pairwise pairing combinations, which were determined using the pairing data gathered to estimate temporal isolation. The I_{PSI} compares the observed pairs with the pairs expected when assuming random mating (see Rolán-Alvarez and Caballero 2000 for the formula). We estimated behavioral isolation for each month of each year when more than one call type bred. We used the mean of the monthly estimates as our measure of the individual contribution of behavioral isolation. Because our measure of behavioral isolation includes only crossbills that were breeding during the same months and I_{PSI} factors out the effects of breeding frequency (Rolán-Alvarez and Caballero 2000), it is independent of our measures of habitat isolation and immigrant infecundity. We assumed that behavioral monogamy corresponds to genetic monogamy (i.e., extrapair paternity is rare; see appendix for justification).

Total reproductive isolation. We used the methods outlined in Coyne and Orr (1989) and extended to more than two reproductive isolating barriers by Ramsey et al. (2003) to calculate the absolute contribution of each sequentially and independently acting component (AC_n). Because habitat isolation acts first, $AC_1 = RI_1$. The absolute contribution of the second-acting barrier, immigrant infecundity (AC_2), equals $RI_2(1 - AC_1)$. The absolute contribution of behavioral isolation (AC_3) equals $RI_3[1 - (AC_1 + AC_2)]$. Total reproductive isolation is the sum of the absolute contributions of habitat isolation, immigrant infecundity, and behavioral isolation ($AC_1 + AC_2 + AC_3$).

Feeding Rates in the South Hills

We measured feeding rates of South Hill crossbills throughout the study in 2001 and 2002 as well as in July, September, and October 2000 to provide a mechanistic understanding of the reproductive isolating barriers; bill structure influences feeding rates (table 1; Benkman 1993, 2003), and feeding rates influence crossbill movements, habitat use (Benkman 1987, 1992), and the timing of breeding (Benkman 1990). Feeding rates were measured by recording the number of seeds consumed during timed intervals, as in Benkman (1997). Foraging crossbills were observed with $\times 20$–$\times 60$ Kowa and $\times 40$ Questar telescopes. See appendix for details concerning the tabulation and analysis of these data.

Results

Habitat Isolation

South Hills crossbills occurred at much higher frequencies than other call types, and call type 2 was usually about 10 times more common than call type 5 (fig. 2). The frequency of call types 2 and 5 increased through spring to more than 20% of the individuals recorded in June or July (fig. 2). Beginning in August, call type 2 and, especially, call type 5 became very rare, accounting for less than 2% of the crossbills encountered. The trends in frequencies were similar in both years, although both call types were more common in 2002 than in 2001 (fig. 2). Limiting the analyses to the period from active courtship through nesting (February to July; see next section), habitat isolation (RC_1) was larger between South Hills and call type 5 crossbills than between South Hills and call type 2 crossbills (table 2), consistent with the low abundance of call type 5 crossbills. The comparatively low foraging efficiency of call type 5 crossbills (table 1) may explain why call type 5 is rare in the area.

Timing of Breeding and Immigrant Infecundity

The number of breeding pairs increased by approximately three to four times from March to April and remained high through June (fig. 3). Nest building was first observed on March 30, 2001, and on April 3, 2002, and continued until June 18, 2001, and July 19, 2002 (fig. A2). Many pairs initiated nesting in April (fig. A2), and many of the later nests presumably represented second nesting attempts (e.g., three different pairs of South Hills crossbills with dependent juveniles were observed building nests).
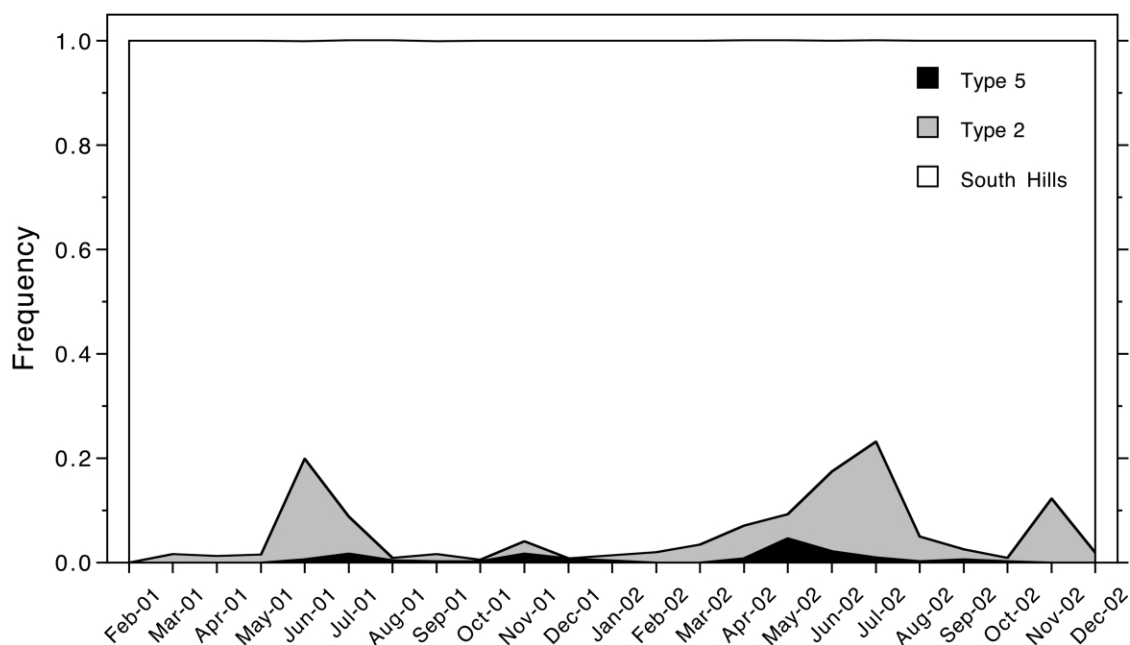
Figure 2: Relative frequencies of South Hills, call type 2, and call type 5 crossbills in the South Hills during each month from February 2001 to November 2002 (data were not gathered in January 2002, so values were extrapolated). The frequency of each call type is equal to the height of its respective fill type, with the total summing to 1 during each month. Each month, an average of 488 South Hills crossbills (range 128–1,536), 30 call type 2 crossbills (range 0–206), and four call type 5 crossbills (range 0–22) were recorded.

The 16 breeding individuals of call type 2 seen between March and May (fig. 3) matched the number expected (i.e., 464 breeding South Hills and call type 2 crossbills times the relative frequency of call type 2 [.0345] during March–May; fig. 2). However, the 11 breeding individuals of call type 2 seen during June and July (fig. 3) were significantly fewer ($\chi_1^2 = 55.54$, $P < .0001$) than the 66.6 expected breeding individuals (382 breeding South Hills and call type 2 crossbills times the relative frequency of call type 2 crossbills [.1743] during June and July; fig. 2). These patterns were consistent with our monthly estimates of immigrant infecundity (table A1), which increased progressively from March to July, and indicate that a disproportionate number of call type 2 crossbills in the South

Hills during June and July did not breed and thus could not hybridize with South Hills crossbills. Our monthly estimates for immigrant infecundity between South Hills and call type 5 crossbills were more variable (table A1), although the overall average (RC_2) was similar to that for South Hills and call type 2 crossbills (table 2).

Behavioral Isolation

A total of 170 pairs was recorded in 2001 (fig. 3*A*). One hundred and sixty-four were South Hills pairs, four were call type 2 pairs, and two were heterotypic pairs (South Hills/call type 2). The observed frequency of heterotypic pairs was significantly lower than that expected under ran-

Table 2: Three components of premating reproductive isolation and their individual (RI_i) and absolute contributions (AC_i) to reproductive isolation between South Hills crossbills and call types 2 and 5 crossbills in the South Hills, Idaho (0 = unimpeded gene flow, 1 = no gene flow)

Isolating barrier	Individual components of reproductive isolation		Absolute contribution to total reproductive isolation	
	South Hills vs. type 2	South Hills vs. type 5	South Hills vs. type 2	South Hills vs. type 5
Habitat isolation	.8469	.9785	.8469	.9785
Immigrant infecundity	.1988	.2756	.0304	.0059
Behavioral isolation	.9800	.9888	.1202	.0154
Total isolation			.9975	.9998

dom mating (Fisher's exact test, $P < .0001$) indicating that pairing was highly assortative. The first heterotypic pair was observed in early April, and the female of this pair was the only call type 2 crossbill recorded during the first 2 weeks of April. A second heterotypic pair was found in late April, when only three call type 2 crossbills were recorded, including the female of the heterotypic pair and a breeding pair of call type 2. Individuals of call type 5 were even less common than call type 2 (fig. 2), and no call type 5 crossbills were found paired in 2001 (fig. 3A).

In 2002, when both call types 2 and 5 were more frequent than in 2001 (fig. 2), a total of 258 pairs were recorded, of which four were heterotypic (fig. 3*B*). As in 2001, the observed frequency of heterotypic pairs was significantly lower than that expected under random mating (Fisher's exact tests, $P < .0001$), indicating that pairing was highly assortative. Overall, only six (three males and three females) out of a total of 820 paired South Hills crossbills (.7%) recorded in 2001 and 2002 were involved in heterotypic pairs. Such strong assortative pairing resulted in high values of behavioral isolation (table 2; behavioral isolation could be estimated only for 7 months for South Hills and call type 2 crossbills [monthly estimates ranged from .925 to 1.0] and for three months combined from May to July in 2002 for South Hills and call type 5 crossbills). However, the absolute contributions of behavioral isolation to total reproductive isolation were small (table 2) because the other isolating barriers were also strong and acted before behavioral isolation. We found complete assortative pairing between call types 2 and 5 (11 and four assortative pairs, respectively; Fisher's exact test, $P = .0007$), suggesting that they were completely reproductively isolated (though sample size is small).

Feeding Rates in the South Hills

Feeding rates for South Hills crossbills varied seasonally each year (fig. 4). Feeding rates were 60% higher during April and May, when nesting was most frequent (fig. A2), than in November (fig. 4). In addition to foraging on lodgepole pine cones in trees, crossbills in the South Hills also foraged on cones on fallen branches. These cones became available to crossbills in April as snow melted. During June 2001 and May–July 2002, South Hills crossbills spent substantial amounts of time foraging on fallen cones (fig. A3). Higher temperatures near the ground caused the cones to open and seeds to be readily accessible so that even the less specialized pine siskin (*Carduelis pinus*) commonly foraged on seeds in these cones. South Hills crossbill feeding rates on these fallen cones were significantly higher than their feeding rates on cones in trees during the same months ($t = -13.58$, df $= 831$, $P < .0001$); feeding bouts on the ground were excluded from
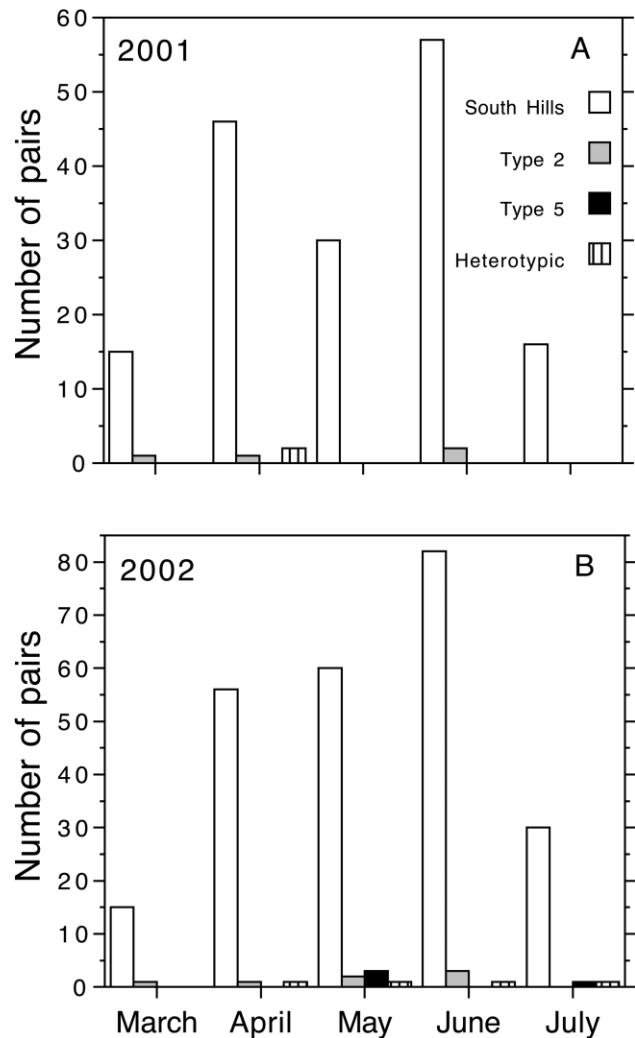

Figure 3: Number of breeding pairs of crossbills recorded in the South Hills from March to July during 2001 (*A*) and 2002 (*B*).

the earlier analyses (i.e., fig. 4). These fallen and open cones may have been critical to the presence and successful breeding of small-billed call type 5 crossbills because they were observed foraging only on fallen cones. By August, crossbills rarely foraged on fallen cones, presumably because seeds in these cones were depleted. The greater availability of seeds in fallen cones in 2002 than in 2001 (fig. A3), which was apparently due to more abundant fallen branches (T. Fetz, personal communication), may explain why call type 2 and especially call type 5 crossbills were more common in 2002 than in 2001 (fig. 2). This between-year variation further suggests that the abundance of other call types was determined by the availability of seeds, which in turn influenced the opportunity for breeding and potential hybridization, especially for call type 5.
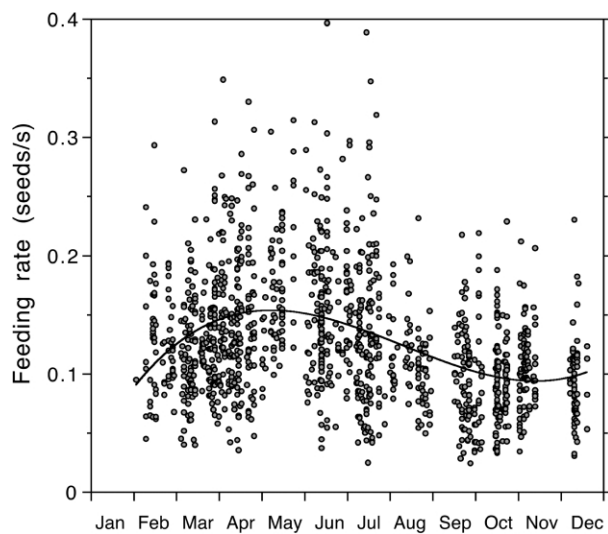
Figure 4: Feeding intake rates (seeds/s) for South Hills crossbills foraging in trees varied seasonally. Circles represent an individual feeding bout or the mean of several bouts from a given individual and the solid line represents a third-order polynomial fit to the data ($r^2 = .15$, $F = 75.96$, df $= 3, 1,262$, $P < .0001$; fit is significantly better than for a second-order polynomial, $F = 49.35$, df $= 1, 1,262$, $P < .0001$). Data were gathered over 3 years.

Discussion

The results of this study indicate that the trade-offs in feeding efficiency causing divergent selection between South Hills and other Rocky Mountain crossbills and favoring local adaptation by South Hills crossbills (Benkman et al. 2001, 2003; Benkman 2003) have caused differences between them in habitat use and the occurrence of breeding. Such differences in addition to behavioral isolation lead to high levels of reproductive isolation (table 2), sufficient to cause a reduction in gene flow between South Hills and other Rocky Mountain crossbills (Parchman et al. 2006). Here we discuss how and why feeding rates vary throughout the year, because feeding rates determine so many aspects of crossbill ecology from conifer (habitat) use and movements between regions (Benkman 1987, 1992) to the timing of breeding (Benkman 1990). We then discuss how such variation contributes to patterns of breeding and the occurrence of hybridization. Finally, we discuss the context in which reproductive isolation has probably evolved in the South Hills crossbill.

Seasonal Variation in Feeding Rates, Occurrence of Breeding, and the Potential For Hybridization

Outside of late autumn, crossbills initiate breeding when intake rates are sufficient for females to produce eggs and

will be sufficient to feed nestlings (Benkman 1990). The consistent changes in intake rates in the South Hills presumably account for the observed regular breeding season of the South Hills crossbill (figs. 3, 4). Nest building was initiated mostly in April (fig. A2), when feeding rates neared their maximum (fig. 4), and breeding behavior declined as feeding rates and the availability of seeds in open cones on the ground decreased (figs. 4, A3). Such variation in seed availability presumably also influenced both the occurrence of call types 2 and 5 and their potential for breeding and hybridizing with South Hills crossbills.

Call types 2 and 5 tended to increase in frequency in the South Hills in May, June or July (fig. 2) coinciding with the period when nomadic crossbills in North America search for developing cone crops on which to breed (Benkman 1987, 1990, 1992; Hahn 1998). These call types, however, did not remain for long in the South Hills, departing mostly in July or August (fig. 2). Because both call types 2 and 5 commonly begin breeding in July and August in the Rocky Mountains (C. W. Benkman, personal observation), we suspect that the declining feeding rates over this period (fig. 4) deterred these crossbills from breeding and remaining in the South Hills (fig. 2). Because this decline in seed availability may have been caused by the increase in energy demand by breeding South Hills crossbills, they are competitively excluding other call types. In 2002, call type 2 also increased in November (fig. 2; call type 5 increased slightly in November 2001), which is another period when crossbills often move in search of seeds (Benkman 1987, 1992). However, most of these crossbills departed by December (fig. 2), presumably because they are relatively inefficient at feeding on cones in the South Hills (table 1). This emigration from the South Hills by call types 2 and 5 resulted in their being rare in the South Hills over winter and into early spring (fig. 2), when South Hills crossbills initiated courtship and began nesting, and was responsible for the high levels of habitat isolation early in the breeding season (table A1).

The relatively low frequency of occurrence of call type 5 compared with the call type 2 frequency further indicates that the smaller-billed call type 5 crossbill is more adversely affected by the increase in seed defenses in the South Hills (table 1) that have evolved as a result of the arms race between crossbills and lodgepole pine. This could also reflect the fact that call type 5 crossbills tend to be less nomadic than call type 2 crossbills and are less common outside of the main ranges in the Rocky Mountain region (Benkman 1993; Siepielski and Benkman 2005; Edelaar and Benkman 2006). Nonetheless, the coevolutionary process that has caused strong divergent selection that favors the evolution of the locally adapted and resident South Hills crossbills (Benkman 1999; Benkman et al. 2001, 2003; Siepielski and Benkman 2005) makes it difficult for less

well-adapted crossbills to reside in the South Hills and breed successfully. The result is that call types 2 and 5 are competitively excluded from the South Hills and persist in only small numbers or are at times absent from the South Hills even though they commonly immigrate into the South Hills every year and, if seed availability were sufficient, could potentially increase to high densities (e.g., Benkman 1987). Indeed, call type 2 occurs in comparable abundances in comparably isolated areas of lodgepole pine where South Hills crossbills are absent (Siepielski and Benkman 2005).

Habitat choice, which is favored when there are ecologically divergent selection and local adaptation, is probably an important reproductive isolating barrier for many species (Rice 1987), including, especially, phytophagous insects (Berlocher and Feder 2002; Drès and Mallet 2002; Funk et al. 2002), which, like crossbills, are resource specialists. In addition, the apparent learned habitat preferences by crossbills, whereby they prefer to forage on conifers providing the highest intake rates (Benkman 1987, 1992), should facilitate speciation (Beltman and Metz 2005). In this situation, morphological divergence will lead to habitat divergence without requiring linkage disequilibrium with habitat preference alleles (i.e., habitat preference based on a one-allele mechanism that causes individuals to prefer foraging in habitats where they feed most efficiently; Felsenstein 1981).

Immigrant infecundity appears to arise between South Hills crossbills and especially call type 2 crossbills because the ability of call type 2 to breed in the South Hills decreases more rapidly than that of South Hills crossbills (table A1) as feeding rates decrease (fig. 4). Much as immigrant inviability has been argued to be an important reproductive isolating mechanism (Nosil et al. 2005), we suspect that immigrant infecundity is probably important, especially during the early stages of divergence. This is when adaptations to a given resource or resources are still evolving and do not prevent or only slightly compromise the use of the alternative resources. Most call types should also experience such reproductive isolation even when foraging on the same conifers because the timing of reproduction is determined by feeding intake rates (Benkman 1990), which are also determined by bill structure (Benkman 1993). Because bill size determines performance, conifer use, and timing of reproduction (Benkman 1987, 1990, 1993), reproductive isolation will commonly arise between groups of crossbills that differ in bill size, facilitating the evolution of local adaptation (Hendry et al. 2001) as well as promoting speciation.

Habitat isolation and immigrant infecundity would serve to limit hybridization even if crossbills breed randomly; however, crossbills breed highly assortatively by call type, further contributing to reproductive isolation (table 2). Assortative pairing arises as a byproduct of assortative flocking by call type (Smith et al. 1999). Assortative flocking is favored because of resource assessment benefits of flocking with like morphologies (Smith et al. 1999), and mate choice occurs mostly between flock members (Newton 1972). Ongoing studies are designed to determine what cues females use in mate choice and how this contributes to behavioral isolation. Mate choice is likely to be an especially important component to reproductive isolation where multiple call types come together on the same species of conifer for reproduction (e.g., *Picea engelmannii* in the Rocky Mountains). In addition, divergent selection should lead to selection against hybrids (Benkman 1993, 2003). This would create a postmating reproductive barrier between call types and further contribute to reproductive isolation, resulting in even higher levels of total reproductive isolation than reported here (table 2). However, even with selection against hybridization, a low level of hybridization is likely to continue between call types because the strength of selection against hybridization decreases with decreasing frequencies of hybridization (Spencer et al. 1986; Coyne and Orr 2004, p. 371).

Unfortunately, no other study on birds has quantified reproductive isolation in the manner of Coyne and Orr (1989, 2004) and Ramsey et al. (2003), and most studies that have estimated the frequency of hybridization between recognized bird species, although reporting higher frequencies of hybridization than we found for South Hills crossbills, are from hybrid zones (e.g., Johnson and Johnson 1985; Confer and Larkin 1998; Veen et al. 2001) and therefore are not directly comparable to our estimates. Nevertheless, the extremely high values of reproductive isolation that we found ($>.99$) are similar to those estimated for pairs of "good" plant species (Ramsey et al. 2003; Kay 2006). If we use this comparison as a guide, or if one employs the biological species concept allowing a low level of gene flow (Coyne and Orr 2004), South Hills crossbills could be recognized as a species or at least an incipient species near the species boundary.

Ecological Speciation in the South Hills Crossbill

This example is of special interest because these reproductive isolating barriers have presumably increased gradually over only the past 5,000–7,000 years as lodgepole pine in the South Hills has increased its seed defenses directed at crossbills in a coevolutionary arms race (Benkman 1999; Benkman et al. 2001, 2003). In addition, South Hills crossbills have diverged in spite of considerable yearly immigration into the South Hills by crossbills from surrounding areas. Indeed, because divergent selection increased gradually as the cones diverged, the level of habitat

isolation must have increased gradually as well. As cones diverged, selection would have favored increasingly larger-billed crossbills to become resident, a type of phenotypic sorting within a call type that we find in smaller forested ranges without pine squirrels east of the Rocky Mountains (Siepielski and Benkman 2005). The resident crossbills would have become engaged in a coevolutionary arms race with lodgepole pine, leading to increasingly stronger divergent selection, with adaptation by the resident crossbills to the stable seed supply preventing nomadic and less well-adapted crossbills from persisting in large numbers. This would have led to habitat isolation and selection favoring assortative flocking by bill size as bill size differences between resident and nomadic individuals increased (Smith et al. 1999). Divergence in calls would have been favored to facilitate assortative flocking (Smith et al. 1999), and divergence in vocalizations would have led to assortative pairing (L. Snowberg and C. W. Benkman, unpublished data).

This evolution of a largely reproductively isolated resident population within the much larger area over which call types 2 and 5 wander nomadically each year, and where there have been no apparent physical barriers to their movements during the period during which they could have diverged, fits the divergence-with-gene-flow model of speciation (Rice and Hostert 1993; Orr and Smith 1998) and appears to be an example of incipient sympatric speciation. This is consistent with other examples of sympatric speciation, which show conspicuous divergence in resource use and assortative mating as byproducts of adaptation to alternative resources (Coyne and Orr 2004). It also supports the conclusions of Coyne and Orr (2004, p. 175), that sympatric speciation is as likely among the most highly mobile of species, such as crossbills, as among less mobile species.

Acknowledgments

Literature Cited

Adkisson, C. S. 1996. Red crossbill (*Loxia curvirostra*). Pages 1–24 *in* A. Poole and F. Gill, eds. The birds of North America. No. 256. Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, DC.

Beltman, J. B., and J. A. J. Metz. 2005. Speciation: more likely through a genetic or through a learned habitat preference. Proceedings of the Royal Society B: Biological Sciences 272:1455–1463.

Benkman, C. W. 1987. Food profitability and the foraging ecology of crossbills. Ecological Monographs 57:251–267.

———. 1989. Breeding opportunities, foraging rates, and parental care in white-winged crossbills. Auk 106:483–485.

———. 1990. Intake rates and the timing of crossbill reproduction. Auk 107:376–386.

———. 1992. White-winged crossbills. Pages 1–20 *in* A. Poole, P. Stettenheim, and F. Gill, eds. The birds of North America. No. 27. Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, DC.

———. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. Ecological Monographs 63:305–325.

———. 1997. Feeding behavior, flock size dynamics and variation in sexual selection in crossbills. Auk 114:163–178.

———. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. American Naturalist 153(suppl.):S75–S91.

———. 2003. Divergent selection causes the adaptive radiation of crossbills. Evolution 57:1176–1181.

Benkman, C. W., and A. K. Lindholm. 1991. The advantages and evolution of a morphological novelty. Nature 349:519–520.

Benkman, C. W., and R. E. Miller. 1996. Morphological evolution in response to fluctuating selection. Evolution 50:2499–2504.

Benkman, C. W., W. C. Holimon, and J. W. Smith. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. Evolution 55:282–294.

Benkman, C. W., T. L. Parchman, A. Favis, and A. M. Siepielski. 2003. Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. American Naturalist 162:182–194.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual Review of Entomology 47:773–815.

Boughman, J. W., H. D. Rundle, and D. Schluter. 2005. Parallel evolution of sexual isolation in sticklebacks. Evolution 59:361–373.

Confer, J. L., and J. L. Larkin. 1998. Behavioral interactions between golden-winged and blue-winged warblers. Auk 115:209–214.

Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution 43:362–381.

———. 2004. Speciation. Sinauer, Sunderland, MA.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philosophical Transactions of the Royal Society B: Biological Sciences 357:471–492.

Edelaar, P., and C. W. Benkman. 2006. Replicated population divergence caused by localized coevolution? a test of three hypotheses

in the red crossbill-lodgepole pine system. Journal of Evolutionary Biology 19:1651–1659.

Edelaar, P., M. Robb, K. van Eerde, K. Terpstra, R. Bijlsma, and E. Maassen. 2004. Zijn er meerdere soorten "Gewone" Kruisbek in Nederland? Limosa 77:31–38.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124–138.

Funk, D. J., K. E. Filchak, and J. L. Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica 116:251–267.

Groth, J. G. 1993a. Call matching and positive assortative mating in red crossbills. Auk 110:398–401.

———. 1993b. Evolutionary differentiation in morphology, vocalizations, and allozymes among nomadic sibling species in the North American red crossbill (*Loxia curvirostra*) complex. University of California Publications in Zoology 127:1–143.

Hahn, T. P. 1998. Reproductive seasonality in an opportunistic breeder, the red crossbill, *Loxia curvirostra*. Ecology 79:2365–2375.

Hendry, A. P., T. Day, and E. B. Taylor. 2001. Population mixing and adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. Evolution 55:459–466.

Johnson, N. K., and C. B. Johnson. 1985. Speciation in sapsuckers (*Sphyrapicus*). II. Sympatry, hybridization, and mate preference in *S. rubber daggetti* and *S. nuchalis*. Auk 102:1–15.

Kay, K. M. 2006. Reproductive isolation between two closely related hummingbird-pollinated Neotropical gingers. Evolution 60:538–552.

McKinnon, J. S., S. Mori, B. K. Blackman, L. David, D. M. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.

Mundinger, P. C. 1972. Vocal imitation and individual recognition of finch calls. Science 168:480–482.

Nagel, L., and D. Schluter. 1998. Body size, natural selection, and speciation in sticklebacks. Evolution 52:209–218.

Newton, I. 1972. Finches. Collins, London.

Nosil, P., T. H. Vines, and D. J. Funk. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59:705–719.

Orr, M. R., and T. B. Smith. 1998. Ecology and speciation. Trends in Ecology & Evolution 13:502–506.

Parchman, T. L., and C. W. Benkman. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. Evolution 56:1663–1672.

Parchman, T. L., C. W. Benkman, and S. Britch. 2006. Patterns of genetic variation in the adaptive radiation of New World crossbills (Aves: *Loxia*). Molecular Ecology 15:1873–1887.

Pérez-Figueroa, A., A. Caballero, and E. Rolán-Alvarez. 2005. Comparing the estimation properties of different statistics for measuring sexual isolation from mating frequencies. Biological Journal of the Linnean Society 85:307–318.

Ramsey, J., H. D. Bradshaw Jr., and D. W. Schemske. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). Evolution 57:1520–1534.

Rice, W. R. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evolutionary Ecology 1:301–314.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47:1637–1653.

Robb, M. 2000. Introduction to vocalizations of crossbills in northwestern Europe. Dutch Birding 22:61–107.

Rolán-Alvarez, E., and A. Caballero. 2000. Estimating sexual selection and sexual isolation effects from mating frequencies. Evolution 54:30–36.

Rundle, H. D., and P. Nosil. 2005. Ecological speciation. Ecology Letters 8:336–352.

Rundle, H. D., L. Nagel, and D. Schluter. 2000. Natural selection and parallel speciation in sticklebacks. Science 287:306–308.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. Ecology 74:699–709.

———. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–9.

———. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

———. 2001. Ecology and the origin of species. Trends in Ecology & Evolution 16:372–380.

Siepielski, A. M., and C. W. Benkman. 2004. Interactions among moths, crossbills, squirrels, and lodgepole pine in a geographic selection mosaic. Evolution 58:95–101.

———. 2005. A role for habitat area in the geographic mosaic of coevolution between red crossbills and lodgepole pine. Journal of Evolutionary Biology 18:1042–1049.

Smith, J. W. 2005. The role of coevolution in promoting ecological speciation in red crossbills (*Loxia curvirostra* complex). PhD diss. New Mexico State University, Las Cruces.

Smith, J. W., C. W. Benkman, and K. Coffey. 1999. The use and misuse of public information by foraging red crossbills. Behavioral Ecology 10:54–62.

Spencer, H. G., B. H. McArdle, and D. M. Lambert. 1986. A theoretical investigation of speciation by reinforcement. American Naturalist 130:958–962.

Veen, T., T. Borge, S. C. Griffith, G. Saetre, S. Bures, L. Gustafsson, and B. C. Sheldon. 2001. Hybridization and adaptive mate choice in flycatchers. Nature 411:45–5.

Via. S. 2001. Sympatric speciation on animals: the ugly duckling grows up. Trends in Ecology & Evolution 16:381–39.