Appendix from J. W. Smith and C. W. Benkman, "A Coevolutionary Arms Race Causes Ecological Speciation in Crossbills"
(Am. Nat., vol. 169, no. 4, p. 455)

Further Information on Methods

Relative abundance of call types. We actively searched for crossbills each day to determine the number of each call type present each month. When crossbills were encountered, recordings were made of their flight calls using an ME 66 Sennheiser directional microphone and a Marantz PDM-222 tape recorder. We made sonograms of the recorded flight calls using Canary 1.2.4 (Cornell Laboratory of Ornithology, Ithaca, NY) and assigned each individual to a call type (fig. A1; Groth 1993*b*; Benkman 1999); the fine structure of the calls (i.e., call type) can be learned from their parents (Groth 1993*b*) and is apparently often modified to match the call of their mates (Groth 1993*a*; the calls of the male and female were virtually identical in two-thirds of the recorded breeding pairs in our study [see Mundinger 1972]). If only one call type was recorded from a flock, then we assigned all flock members to a single call type. When more than one call type was recorded from a flock (<5% of the flocks), we used the number of different calls recorded to estimate the proportion of individuals of each call type.

Although call matching could lead us to underestimate the number of individuals of a given call type in a flock, this should not result in a consistent bias in our estimates because call matching by mates appears general to crossbills (e.g., Groth 1993*a*). We also have no reason to suspect that individuals of uncommon call types are more likely to remain silent. Nevertheless, many crossbills were undoubtedly recorded on more than one occasion. Although this would inflate the degrees of freedom, no statistical tests were conducted using these data, and this should not bias our estimate of the relative frequencies of the call types.

Immigrant mating success. When a pair was encountered, they were observed for 1 h or more, and the flight calls of both the male and female were recorded and analyzed as described above. Crossbills exhibit several characteristic behaviors when paired (Newton 1972; Benkman 1992; Adkisson 1996). Pairs were included in the analyses if the male and female stayed close together for at least 20 min and included at least one characteristic behavior (e.g., male courtship, feeding the female).

Flight calls differ among individuals within a call type (fig. A1; Groth 1993*a*, 1993*b*) but are very stable within an individual over a summer (Smith 2005), making it possible to distinguish between different pairs on the basis of their flight calls, and this minimizes the possibility that we were unable to recognize a given pair recorded on more than one occasion. Using variation between individuals, we attempted to identify pairs that had been recorded multiple times by visually inspecting the sonograms of males and females of pairs from each of 12 subregions within the South Hills that were each isolated by at least several hundred meters of unsuitable habitat (e.g., sagebrush). Because South Hills crossbills move relatively short distances, especially during the breeding season, breeding South Hills crossbills were unlikely to move between these subregions. For example, three banded pairs were resighted on several different occasions (separated by between 20 and 57 days) within approximately 250 m of the initial sighting location; banded pairs were never resighted at locations greater than 250 m from the initial sighting location. In blind tests, we included a second set of sonograms of some of the pairs and 96% of the time correctly identified them. This indicated that we were able to eliminate most pairs that had been recorded on more than one occasion. Twenty-four pairs were considered to be duplicates, and they were eliminated from further analysis. We did not attempt to determine whether the same individuals were recorded in both years; therefore, we limited inferential statistics to each year separately.

Extra-pair copulation. Because male crossbills closely guard their mate until all her eggs are laid and provide most of the food to the young (Newton 1972; Benkman 1990), with such care probably being critical to the breeding success of the female (Benkman 1989), the frequency of extrapair paternity is likely low (Møller 2000; Arnold and Owens 2002). Furthermore, Møller and Briskie (1995) showed that extrapair paternity is generally uncommon in species when males have relatively small testes compared to their body mass. Based on the mean

testis length for 30 male call type 2 crossbills (5.5 mm, SE $=$ .15 mm; T. P. Hahn, unpublished data), we estimated testis mass using both the relationship between testis length and width for call type 2 crossbills ($r^2 =$ .97, df $=$ 11, $P < .0001$; T. P. Hahn, unpublished data) and the equation in Møller and Briskie (1995). Call type 2 and presumably other call types have relatively small testes compared to their body mass (mean male call type 2 body mass $=$ 33.8 gm, $n =$ 30; residual value for testis mass of call type 2 $= -.42$ on a ln-ln plot of data from Møller and Briskie 1995; only seven of 54 species had more negative values), which further suggests that extrapair paternity is rare in red crossbills.

Feeding rates in the South Hills. The analyses of foraging rates were based on a total of 1,454 timed foraging bouts for adult South Hills crossbills foraging on cones in trees from which 9,671 seeds were consumed in a total of 90,514 s. When more than one foraging bout per individual crossbill was recorded on the same day (some crossbills were color banded, and sometimes successive foraging bouts were recorded from an individual), we used the overall mean in the analyses (sample unit was an individual bird on a given day; for analyses $n =$ 1,266). Forty-seven banded crossbills were recorded foraging, and 10 of them were recorded foraging on 2 or 3 days (average of 2.3 days), with an average of 58 days between measurements (range 8–147 days). Because we were interested in seasonal variation in foraging rates, the time between measurements of individual crossbills was generally long, and data over a 2-year period were combined, we do not consider including such repeated measurements of the same individuals over time a problem. We limited the analyses to South Hills crossbills, because few foraging data were gathered from the other call types. Because two observers collected foraging data, it was necessary to determine whether their measurements were comparable. During March 2001 and June–July 2001 and 2002, both observers were in the South Hills and independently collected foraging data. We compared their feeding rate measurements (ln transformed to meet the normality assumption) during these months using a two-way ANOVA with month and observer as effects. The observer effect was significant ($F =$ 1.08, df $= 1, 383$, $P =$ .002) but not the month effect ($F =$ .02, df $= 4, 383$, $P =$ .88). We adjusted the foraging data by subtracting 1.08 s for each seed eaten from all bouts recorded by T. Fetz (based on the least squares means of the untransformed data) instead of entering observer as a covariate, because seasonal variation in feeding rates was nonlinear and the two observers gathered data over mostly nonoverlapping time periods.

Table A1

Monthly estimates of habitat isolation and immigrant infecundity (data from 2001 and 2002 combined) between South Hills crossbills and call types 2 and 5 crossbills in the South Hills, Idaho

	Habitat isolation		Immigrant infecundity	
Month	South Hills vs. type 2	South Hills vs. type 5	South Hills vs. type 2	South Hills vs. type 5
February	.9841	1.0	NE	NE
March	.9414	1.0	−1.1344	NE
April	.9211	.9912	.1706	1.0
May	.9311	.9435	.2194	−.1361
June	.6567	.9634	.7984	.8050
July	.6470	.9731	.9397	−.5663
Monthly average (RI_i)	.8469	.9785	.1988	.2756

Note: Negative values indicate increased opportunities for gene flow relative to random expectations. 0 = random mating, 1 = no opportunities for gene flow. NE = no estimate, when immigrant infecundity could not be estimated before pairs separated from flocks and pairs could be distinguished (i.e., February) or when one of the call types was absent (i.e., March for call type 5).
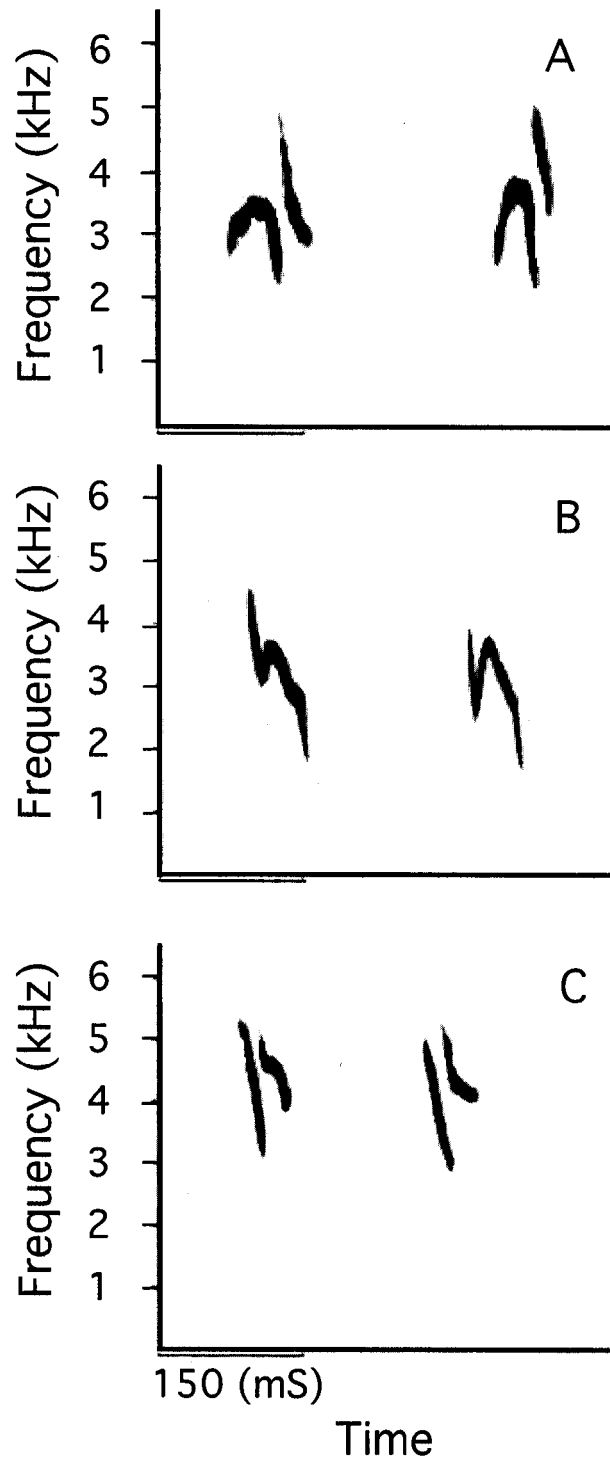
Figure A1: Representative flight calls from two individuals of South Hills (*A*), call type 2 (*B*), and call type 5 (*C*) crossbills. Flight calls differ between call types in the presence of a second element (e.g., call type 2 usually lacks it), frequency (e.g., call type 5 has highest average frequency), and slope of the frequency modulations (e.g., frequency increases in the beginning of the South Hills crossbill call but not in the others; Groth 1993*b*; Smith 2005).
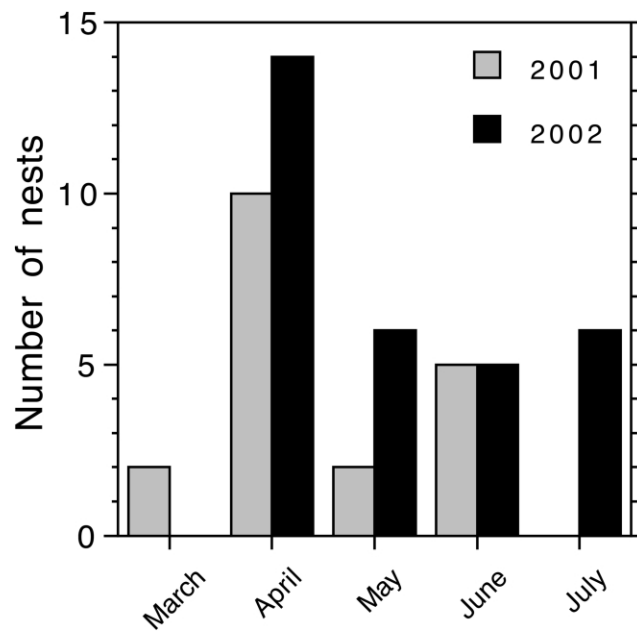
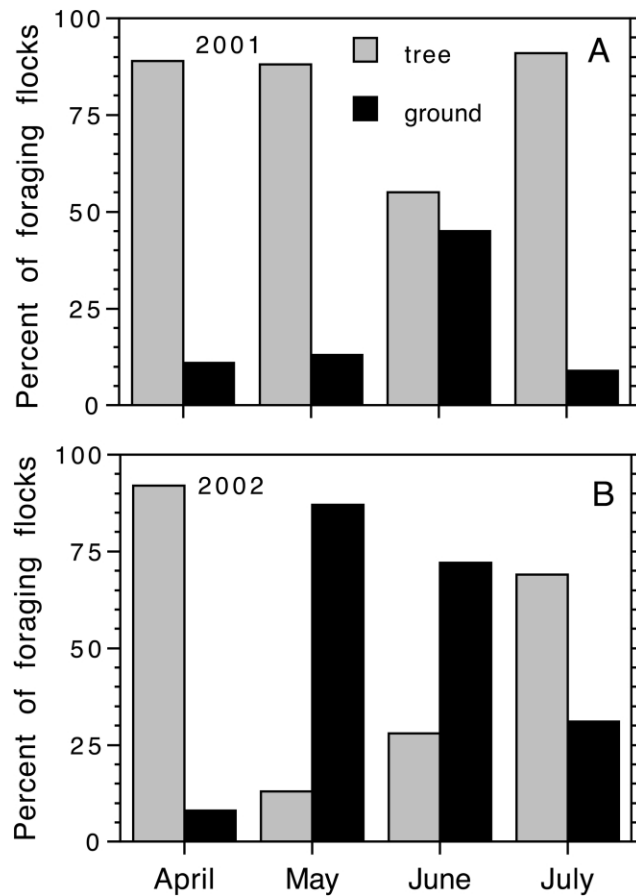**Figure A2:** Number of nests found during each month in 2001 and 2002.

Figure A3: Percent of South Hills crossbill flocks foraging on cones in trees and on the ground from April to July in 2001 (*A*) and 2002 (*B*).

Literature Cited Only in Appendix

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Møller, A. P. 2000. Male parental care, female reproductive success, and extrapair paternity. Behavioral Ecology 11:161–168.

Møller, A. P., and J. V. Briskie. 1995. Extra-pair paternity, sperm competition and the evolution of testis size in birds. Behavioral Ecology and Sociobiology 36:357–365.