

The role of marker traits in the assortative mating within red crossbills, *Loxia curvirostra* complex

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Abstract

We conducted mate choice experiments to determine whether differences in calls or bill morphology might influence assortative mating between call types of red crossbills (*Loxia curvirostra* complex) that have diverged in bill structure to specialize on different species of conifers. Females preferred males that gave calls that matched their own type, but did not prefer males that more closely approximated the average or optimal bill size of the female's call type. These results were consistent with our breeding simulations, which showed that females gained an indirect fitness benefit by choosing a male of her own call type because this reduced the production of offspring with morphologies that fell between adaptive peaks. However, choice based on bill morphology within a call type provided no further benefit. Calls, which crossbills learn from their parents, likely act as a marker trait indicative of the morphological adaptations of the group, allow for easy assessment of potential mates and facilitate rapid divergence under ecological selection.

Introduction

An important issue in ecological speciation is how reproductive isolation arises between groups as a result of divergent selection. In birds, learning of selectively neutral marker traits may play an important role in speciation (Irwin & Price, 1999). Marker traits are morphological (e.g. coloration) or behavioural (e.g. vocalizations) traits that when associated with a trait under divergent selection, such as trophic morphology, provide information about group identity, allowing for divergence when assortative mating is based on the marker trait. Furthermore, if the marker trait and a preference for this trait are learned from the parents rather than being genetically inherited, then the problem of recombination between marker traits and the trait under ecological divergence (Rice & Hostert, 1993) can be eliminated. Here we suggest that divergence is facilitated by a learned marker trait in a group of incipient species that matches the divergence-

with-gene-flow model of speciation (Rice & Hostert, 1993; Orr & Smith, 1998).

Red crossbills (*Loxia curvirostra* complex) in North America are a group of incipient bird species for which we know much about the ecological conditions favouring morphological divergence and specialization on alternative resources, yet little is known about the nature of behavioural reproductive isolation (Smith & Benkman, 2007). Red crossbills are divided into nine groups in North America, referred to as call types, which are defined by their non-song vocalizations (Fig. 1; Groth, 1993a; Benkman, 1999). The divergence of several of the call types is relatively recent, most likely having occurred within the last 12 000 years (Benkman *et al.*, 2001; Parchman & Benkman, 2002; Parchman *et al.*, 2006). Furthermore, several of the red crossbill call types are highly nomadic and may have diverged in sympatry as the result of divergent selection for utilizing alternative winter resources (different conifer species whose seeds are retained in closed or partially closed cones; Benkman, 1993, 2003; Benkman & Miller, 1996; Benkman *et al.*, 2001; Parchman *et al.*, 2006; Smith & Benkman, 2007). The fitness surface for crossbills foraging on several different conifer species resembles a landscape with fitness peaks corresponding to the optimal bill structures

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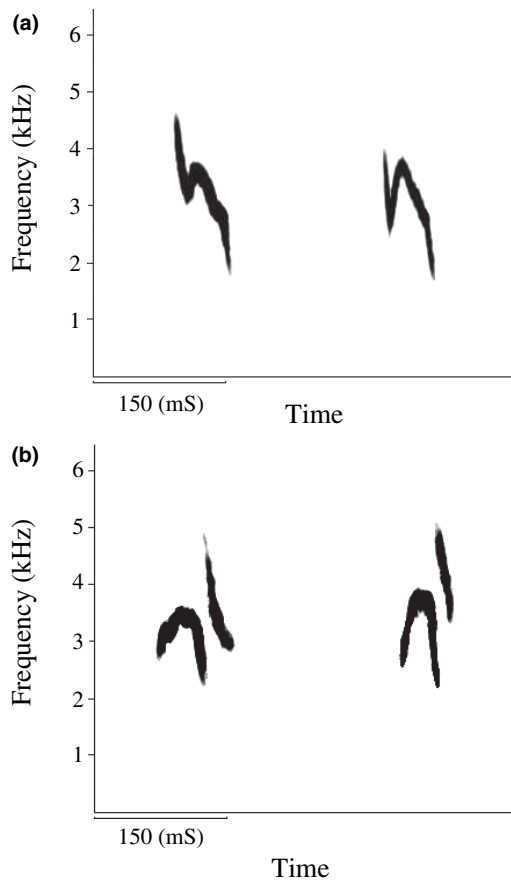


Fig. 1 Typical flight calls recorded from (a) two type 2 and (b) two type 9 red crossbills. Type 2 calls are characterized by a zig-zag pattern starting with a decrease in frequency, followed by an increase in frequency to a frequency equal to or lower than the initial frequency, and finally decreasing in frequency again. Type 9 calls are characterized by an increase in frequency followed by a decrease, sometimes, but not always, followed by a short, down-slurred second syllable. Microstructure of calls is variable between individuals, but call types are characterized by the basic structure of their flight call.

for feeding on a particular conifer surrounded by fitness valleys in which feeding efficiency is low on all types of cones (Benkman, 2003). This fitness surface reflects both selection against intermediate individuals and divergent selection, driving call types onto different fitness peaks. Individual bill depth is the most important predictor of feeding efficiency (Benkman, 1993; Benkman & Miller, 1996) and, not surprisingly, the average bill depths of several call types reside on or near predicted fitness peaks, including the call types used in this experiment (Benkman, 1993; Benkman *et al.*, 2001; but see Benkman *et al.*, 2005).

Because individuals of most call types feed on a variety of conifers, depending on the relative availability of seed (Benkman, 1987), several call types often occur together and breed sympatrically while foraging on the same

conifer species (Groth, 1988, 1993a; Smith & Benkman, 2007), which provides the opportunity for interbreeding between call types. Red crossbills are therefore an ideal group for studying the processes involved in speciation because of our knowledge of divergent natural selection, their recent divergence, and their current, and likely historical, sympatric distributions.

Red crossbills are known to mate assortatively by call type in the wild, with levels of mixed pairing between sympatric call types less than 1% (Smith & Benkman, 2007; see also Groth, 1993b). Understanding the basis for such assortative pairing is important to our understanding of speciation. Selection against mating between call types should occur because their offspring would more likely fall in a fitness valley than if mating was strictly within call types (e.g. Grant & Grant, 1994, 1996a; Baker & Johnson, 1998); bill structure in birds is generally highly heritable (Merilä & Sheldon, 2001) and estimates of narrow sense heritability for bill depth of Scottish crossbills (*L. scotica*) are between 0.58 and 0.71 (Summers *et al.*, 2007). Reproductive isolation arising either directly or indirectly as the result of divergent selection on crossbills would constitute ecological speciation (Schluter, 2001). Because individuals of the different call types are very similar in appearance (Groth, 1993a), selection may have favoured vocalizations that rapidly identify important morphological characteristics (Smith *et al.*, 1999), such as bill depth and palate structure (Benkman, 1993, 2003). The fit between average and optimal morphology of call types suggests that calls are a good marker trait, reflecting their bearers' ecological adaptation. Such a close fit between average and optimum morphology for different call types has likely been driven by natural selection, with assortative mating allowing groups to diverge.

Red crossbills learn the structure of their flight calls (which are used to identify individual crossbills to a call type group; Groth, 1993a,b), which are short (< 60 ms), frequency-modulated chirps (Fig. 1) given when birds are in flight or perched (Groth, 1993a). Red crossbills of one call type raised with foster parents of a different call type can learn the flight calls of their foster parents (Groth, 1993a). These calls are learned early in life, as suggested by the fact that individuals cross-fostered shortly after independence do not learn the calls of the individuals they are caged with, but instead learn the calls of the individuals that raised them (Groth, 1993a). Although individuals may change the fine scale structure of their call as adults, leading to call matching between mates (Groth, 1993b), the structure of the call that distinguishes different call types (Fig. 1) remains stable over long periods in the wild (2–4 years) and call type switching is likely to be extremely unusual (P. C. Keenan, personal communication).

Here, we use mate-choice experiments to investigate the roles of two possible cues that might lead to assortative mating and adaptation of red crossbills: flight calls and bill depth. We also perform breeding simula-

tions to determine the fitness consequences of choosing mates based on marker traits (calls) or on morphology directly. We consider bill depth as the morphological character for female assessment in our mate-choice design, because bill depth is highly correlated with other size-related traits (Groth, 1993a) and it is an important trait under natural selection for foraging on key conifers (Benkman, 1993, 2003; Benkman & Miller, 1996).

Methods

Mate choice

Two call types of red crossbills were used in mate-choice experiments. Call type 2 is nomadic and specializes on Rocky Mountain ponderosa pine (*Pinus ponderosa scopulorum*). The estimate of its optimal bill depth is 9.56 mm (Benkman, 1993; Benkman *et al.*, 2001). Call type 9 is resident in the South Hills and Albion Mountains, Idaho, where it specializes on Rocky Mountain lodgepole pine (*P. contorta latifolia*). The estimate of its optimal bill depth is 9.99 mm (Benkman *et al.*, 2001). We chose these two call types because type 2 birds are the most common breeding call type, other than type 9, in the South Hills (Smith & Benkman, 2007) and genetic analyses indicate that type 2 and type 9 cluster together genetically along with type 5 (Parchman *et al.*, 2006). In addition, the range of natural variation in the bill depth of type 2 birds allowed us to create pairs of type 2/type 9 males with similar bill depths to test for preference for vocalizations, as well as pairs of type 2 males with a range of size differences to test for preferences for bill morphology.

We captured type 2 crossbills in the Bears Paw Mountains, Montana (48°09.5'N, 109°40.2'W), near Nederland, Colorado (39°98.1'N, 105°52.0'W), and in the Sandia Mountains, New Mexico (35°13.1'N, 106°24.9'W) in August–October 2003, and type 9 crossbills in the South Hills, Idaho (42°10.7'N, 114°15.3'W) in July–August 2003. Vocalizations of all crossbills were recorded using a Marantz PMD222 recorder (Aurora, IL, USA) and Sennheiser directional microphone (Old Lyme, CT, USA), and analysed by creating spectrograms using Raven 1.2.1 (Cornell Lab of Ornithology, Ithaca, NY, USA). We visually compared these spectrograms (Fig. 1) to the red crossbill call types defined by Groth (1993a) and Benkman (1999). A total of 21 female (bill depth: range 9.00–9.78 mm, mean 9.33 mm) and 12 male (bill depth: range 8.98–10.24 mm, mean 9.73 mm) type 2 birds, and 20 female (bill depth: range 9.30–10.24 mm, mean 9.65 mm) and 5 male (bill depth: range 9.72–10.12 mm, mean 9.91 mm) type 9 birds were used in these experiments.

Birds were housed in indoor flight rooms (2.7 m × 1.6 m × 2.2 m or larger) at the New Mexico State University Animal Care Facility in single sex flocks that were visually isolated from individuals of the opposite sex. They were kept on a natural light schedule for the

South Hills, fed pelleted food (Mazuri Chow) *ad libitum* and supplied water with vitamins. In addition, birds received fresh pine cones daily and pine branches weekly. We conducted 120-min mate choice trials in an outdoor mate choice arena at the Animal Care Facility. The arena consisted of two parallel lanes (1.8 m × 1.2 m × 2.2 m each) for holding males and a third lane (2.4 m × 0.9 m × 2.2 m) that held the female being tested (Fig. 2). The female could move freely between perches located in front of each of the male lanes, allowing her to assess the males, or utilize a perch behind a solid barrier along the top of her cage where she was visually isolated from both males ('no choice' area). Food (Mazuri Chow) and water were available to all birds during trials. Food and water were located centrally in the female's no choice area and in the rear of the males' lanes. Males were visually isolated from each other by a solid partition (Fig. 2). We coloured males with red permanent marker (Sharpie® brand, Sanford Corp., Oak Brook, IL, USA) to standardize their colour to avoid any bias for carotenoid coloration, which is a common sexual signal in passerines (Hill & Benkman, 1995; Hill, 2002).

Trials took place between dawn and 16:30 h between 13 February and 17 March 2004. During these months, crossbills often form pair bonds in the wild (Smith & Benkman, 2007). To increase female receptiveness to

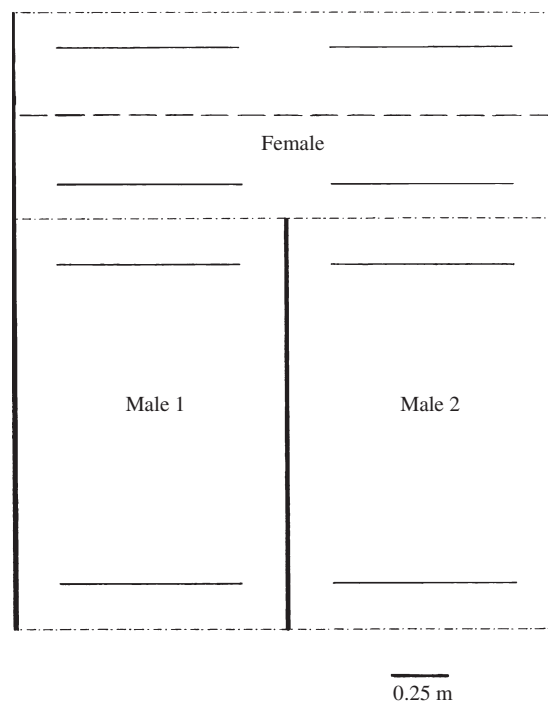


Fig. 2 Schematic diagram of the mate-choice arena. Dark solid lines represent solid partitions, dash-dot lines represent mesh partitions, heavy dash lines represent female no-choice barrier and light solid lines represent perches.

males, we implanted each female with a 6 mm length of silastic tubing, of which 4 mm was filled with 17- β -oestradiol, at least 3 weeks before their participation in trials. Each crossbill was placed in small single-sex groups in the mate choice arena for at least 8 h to become familiar with its layout before trials began.

To test for a preference for similar vocalizations (vocal choice), we presented female red crossbills with one type 2 and one type 9 male that had similar bill sizes (difference in bill depth < 0.1 mm, average difference 0.06 mm). Each pair of males was presented in random order to both a type 2 female and a type 9 female. We thus expected each male in a pair to be preferred by one female.

To test for a preference for the optimal or average bill size (morph choice), we presented both type 2 and type 9 females with pairs of type 2 males. We used only type 2 males because of the wide variation present in bill size in type 2 (Siepielski & Benkman, 2005). One male in each pair was close to the optimal (average) bill depth for the call type of female being tested; the other had a bill depth at least 0.2 mm larger or smaller than the optimum (range of differences: 0.23–0.97 mm, average 0.47 mm). Although 0.2 mm may seem like a small difference in size, differences of this magnitude have large effects on feeding efficiency and survival in the wild (about a 20% reduction in survival; Benkman, 2003). Moreover, if preferences based on bill depth are important for reproductive isolation between call types, then the differences in bill sizes we used should be adequate to detect such preferences because the mean bill depth interval between the four most common call types in western North America is 0.46 mm (data in Groth, 1993a). Two observers (C. W. Benkman and J. S. Colquitt) measured the bill depths, which were generally highly repeatable both between individuals and between years (Benkman *et al.*, 2005). Because type 2 and type 9 birds have different optimal bill depths, some males that were used as the optimal male for females of one type were used as the non-optimal male for females of the other type. Bill depth is correlated with other morphological measures of size (Benkman, 1993). The regressions between body mass of male crossbills in the wild (with little if any observable fat) and bill depth (C. W. Benkman, unpublished data) for both type 2 ($r^2 = 0.21$, d.f. = 68, $P < 0.0001$) and type 9 ($r^2 = 0.14$, d.f. = 46, $P = 0.008$) were significant and did not differ in slope (ANCOVA, $F_{1,114} = 0.056$, $P = 0.81$) or intercept (ANCOVA, $F_{1,115} = 1.509$, $P = 0.22$). This suggests that males with similar bill depth should be similar in other size measures, irrespective of call type identity. We focus here on bill depth because of its ecological importance.

Twenty-one female type 2 and 20 female type 9 crossbills were tested for their preferences. We randomized the type of choice in which a female first participated and allowed at least 3 weeks between trials of individual females. No female was presented with the same male more than once. We videotaped all trials for later analysis

to avoid disturbance to the birds that might be caused by a human observer. From the 120 min videotape of each trial, we recorded the female's position every 30 s. We considered all time in which the female was on the perch or wire in front of a male's cage to be time spent in association with that male and used this as a proxy for mating preference. Physical association or orientation towards an individual is commonly used as a proxy for mate preference in laboratory studies in birds (Clayton, 1990; Hill, 1990, 1993; Enstrom, 1993; Johnson *et al.*, 1993; Nolan & Hill, 2004) as well as other taxa such as fish (Forsgren, 1992; Hankison & Morris, 2003; Cummings & Mollaghan, 2006; Wong & Rosenthal, 2006). Moreover, time in association during such mate-choice experiments is a good predictor of mating behaviour in captive birds (Clayton, 1990). Nevertheless, even if we are simply measuring affinity for association, this could lead to assortative mating between groups if females prefer to associate with males of their own call type and assortative mating is a byproduct of such association.

We also considered behaviour in individual trials to determine whether females were showing behaviours consistent with mate preference. We discarded trials in which the female did not respond to either male with behaviours such as sideways movement along the perch and movement towards the male (i.e. trials in which the female primarily stayed in one place), trials in which one or both males did not respond to the female with behaviours similar to those as listed for the female, or trials in which the female did not associate with the males for more than 30 min. We arcsine transformed our frequency data before performing statistical tests; the distributions of the transformed data did not differ from normal distributions. We performed one-sample *t*-tests, with the null hypothesis that the mean preference for the predicted male would be 0.5236 (i.e. 0.5 arcsine transformed).

Computer simulation

We used simulations to estimate the relative advantages, in terms of expected offspring survivorship, of different methods of mate choice. We estimated the average expected survivorship of offspring for type 2 females that mated randomly with respect to both vocalizations and morphology (simulation 1), mated assortatively by call type but randomly in relation to bill depth (simulation 2), and mated assortatively by call type with a male closest to the optimal bill depth (9.56 mm for type 2) of her own call type out of a set of *N* males (simulation 3). These simulations allowed us to assess the indirect fitness benefits of assortative mating by call type in comparison with random mating, and of assortative mating by call type with a preference for mates having a bill depth closest to the optimum in comparison with when mating is assortative only by call type.

We used type 2 and type 5 individuals in these simulations rather than type 2 and type 9 (as used in the mate-choice experiments) because we have estimates of optimal bill depth and groove width for both type 2 and type 5 and these call types match their predicted optima well. We used measurements of wild crossbills to estimate the distribution of bill depths and groove widths for males and females of type 2 and type 5. We assumed mean bill depths of 9.45 mm for female type 2, 9.60 mm for male type 2, and 9.37 mm for male type 5 with a variance of 0.08 mm² for each group. We assumed mean groove widths of 1.92 mm for type 2 and 1.68 mm for type 5, with a variance of 0.02 mm² for each type. We assumed normal distributions for both bill depth and husking groove width. We drew bill depths and groove widths independently for each individual because these morphological characters are not correlated for either call type (type 2: $r = 0.15$, d.f. = 27, $P = 0.44$; type 5: $r = 0.11$, d.f. = 11, $P = 0.74$; Benkman, 1993; C. W. Benkman, unpublished data). In simulations 1 and 2 above, we paired females randomly with males from both call types or from within the population of their own call type respectively. In simulation 3, we paired the female with the male with the bill depth closest to 9.56 mm out of a set of 2, 4, 6 or 10 males of her call type. In general, we would expect that a larger sampling effort would lead to the choice of a male that was better adapted and therefore would increase offspring fitness.

We ran each simulation with 10 000 females. Each pairing produced one offspring, randomly assigned as either male or female. The offspring was drawn from a distribution with mean $\mu_{\text{sex}} + h^2 \{[(z_f + z_m)/2] - [(\mu_f + \mu_m)/2]\}$ and variance $V_p [1 - (1/2)h^2]$, where μ_{sex} is the mean phenotype of the offspring, which depends on the sex of the offspring, μ_m and μ_f are the mean phenotypes of males and females, and z_m and z_f are the phenotypes of the male and female parents. We used a heritability (h^2) estimate of 0.6 (similar to that measured for heritability of bill depth in Scottish crossbills by Summers *et al.*, 2007) and also varied this estimate between 0.4 and 1.0 to explore how changing heritability affected the predictions. We used the equations in Benkman (2003) to calculate survival of each of the offspring when foraging on ponderosa pine. We then summed the fitnesses of all offspring and divided by the total number of offspring to generate a mean fitness for offspring from each simulation run.

Results

Mate choice

Both type 2 and type 9 females showed a significant preference for males of their own call type (Fig. 3a; type 2: $t_{16} = 2.914$, $P = 0.005$; type 9: $t_{17} = 4.237$, $P = 0.0003$). Fourteen of seventeen type 2 females (82%) preferred the type 2 male to the type 9 male (Fig. 3a).

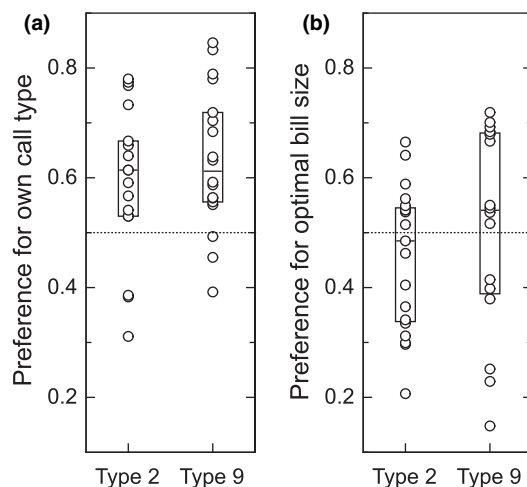


Fig. 3 Percentage of time individual females spent in association with the male (a) of her call type and (b) with the optimal morphology for her call type. Graphs are box plots with bars representing lower quartile, median and upper quartile, with each female plotted as a circle.

Fifteen of eighteen type 9 females (83%) preferred the type 9 male to the type 2 male (Fig. 3a). Trials for four type 2 and two type 9 females were discarded. Type 9 females tended to prefer males of their own call type more strongly than did type 2 females (Fig. 3a), although this trend was not statistically significant (two-sample t -test, $t_{33} = -0.867$, $P = 0.39$). Although it was not possible to determine the frequency of calling by individuals used in trials, crossbills produced flight calls frequently and we have no evidence that crossbills of either call type called disproportionately.

Neither type 2 nor type 9 females consistently preferred the bill depth considered optimal or average for their call type (Fig. 3b). Nine of nineteen type 2 females (47%; $t_{18} = -1.339$, $P = 0.20$) and 10 of 16 type 9 females (63%; $t_{15} = 0.380$, $P = 0.71$) preferred the male closer to the optimal bill depth (Fig. 3b). Trials of two type 2 females and four type 9 females were discarded. Alternatively, females may only prefer the male closest to the optimum when the size difference between the males is large. However, their preference for the male closest to the optimum did not increase as the size difference between the males increased (type 2 females: $\chi^2_1 = 0.418$, $P = 0.52$; type 9 females: $\chi^2_1 = 2.302$, $P = 0.13$).

The results were not confounded by lane preferences ($\chi^2_1 = 0.23$, $P = 0.63$). Every male was preferred by at least one female, suggesting all males were acceptable as potential mates.

Simulation

By choosing mates assortatively by call type, females had an estimated 12.4% increase in indirect fitness relative to

when choosing mates randomly. Surprisingly, if females also chose within their call type the male closest to the optimum bill depth they did not accrue additional indirect fitness benefits. Our simulations showed a decrease of 0.1–0.2% in indirect fitness relative to choosing a mate of the same call type without reference to morphology. Changing the estimate of heritability led to slight differences in the estimates of indirect fitness benefits, especially when comparing random mate choice with mate choice that was assortative by call type. The estimates of indirect fitness benefits for heritabilities of 0.4, 0.6, 0.8 and 1.0 were 10.5%, 12.4%, 13.9% and 15.0%, respectively.

Discussion

These experiments suggest that female red crossbills use differences in vocalizations, which likely act as a marker trait of trophic specialization, to choose a mate with a matching call type, but show no consistent preference for an optimal or average bill depth (Fig. 3). Although we cannot rule out potential differences in behaviour or other unmeasured factors having led to female preferences, taken along with previous research, this study supports the importance of calls in the assortative mating and divergence of red crossbills. Because call structure, which distinguishes call types (Fig. 1), is learned in crossbills (Mundinger, 1979; Groth, 1993a), the use of calls in mate choice suggests that a sexual imprinting process might play an important role in reproductive isolation in crossbills. Sexual imprinting is a process by which an adult chooses a mate based on a template developed at a young age, and is often based on some parental characteristic.

Perhaps the best study implicating a role for learning and sexual imprinting in speciation is that of the brood-parasitic indigobirds (*Vidua chalybeata*; Sorenson *et al.*, 2003). Males of this species learn their song from their foster father (Payne *et al.*, 1998), whereas females sexually imprint on the song of their foster father and later choose a mate that sings a similar song (Payne *et al.*, 2000). In the case of indigobirds, groups ('cultural species') diverge to specialize on different host species following host shifts. In the case of crossbills, the mechanism of vocal learning and imprinting is likely similar, but call types have diverged to specialize on different food resources (Benkman, 1993, 2003). Like in indigobirds, sexual imprinting has probably played a major role in the diversification of crossbills. Sexual imprinting is widespread in birds (ten Cate & Vos, 1999; Irwin & Price, 1999) and may be important to speciation in many different groups (Laland, 1994; Irwin & Price, 1999), as it eliminates the need for a genetic linkage to develop between the trait under divergent natural selection and the trait used in mate choice (Verzijden *et al.*, 2005).

The role of calls as a reproductive isolating mechanism in red crossbills is not surprising. Most research into the role of vocalizations in speciation among birds has focused on song (Ratcliffe & Grant, 1985; Grant & Grant, 1996b; Grant & Grant, 1997, 1998; Martens, 1996; Payne *et al.*, 2000; Slabbekoorn & Smith, 2000), although the role of song learning in speciation remains controversial (Baker & Cunningham, 1985; Baptista & Trail, 1992; Lachlan & Servedio, 2004). Calls are generally believed to be innate, but crossbills (Groth, 1993a) and perhaps other cardueline finches (Mundinger, 1979), learn some aspects of the structure of their calls (i.e. that which is responsible for variation within and between call types). This allows calls to function evolutionarily in a manner similar to that of song in most songbirds. Differences in calls have the potential to be more important than differences in song because both sexes produce calls (Groth, 1993b), making mutual mate choice for similar calls possible. Furthermore, assortative mating based on similarity of marker traits possessed by both sexes should theoretically facilitate speciation (Kondrashov & Kondrashov, 1999; Doebeli, 2005).

The role of call dialects in population divergence has been studied in Amazon parrots (*Amazona auropalliata*), which also learn their calls, but this system differs greatly from that of crossbills. Importantly, parrot dialects are geographically defined and individual parrots may produce calls of more than one dialect (Wright, 1996). These dialects show very little genetic differentiation, suggesting that call dialects do not constrain the movement of individuals between groups of different dialects (Wright & Wilkinson, 2001; Wright *et al.*, 2005) and that individuals may learn calls throughout their life, depending on the social situation (Wright, 1996). Given that crossbills maintain the same call type that they learned from their parents (P. C. Keenan, unpublished data), this would have greater potential to reduce gene flow between groups than if call type could be changed easily (Ellers & Slabbekoorn, 2003).

What factors originally promoted the divergence of calls among red crossbills is unknown. The differences in calls are unlikely to be caused by differences in morphology. In one family of birds (Emberizidae), differences in vocalizations are related to differences in morphology (Podos, 1997). For instance, the frequency bandwidth and rates of syllable repetition of Darwin's finches (*Geospiza* spp.) are affected by beak structure and body size (Podos, 2001). Morphological divergence, however, does not always lead to vocal divergence. For example, the black-bellied seed-cracker (*Pyrenestes ostrinus*), which has two distinct bill morphs specialized for feeding on the seeds of different sedges (Smith, 1993), does not show vocal differences between the morphs (Slabbekoorn & Smith, 2000). We believe that changes in bill structure are unlikely to have led to call divergence between red crossbill call types, because each call type produces a discrete call note despite considerable overlap in bill morphology between call

types. Instead, cultural drift among different groups could have produced call variation, which was later co-opted for assortative flocking and mating. The study of call divergence in cardueline finches is promising because several different species show call variation that corresponds to morphological differences (e.g. pine grosbeaks, *Pinicola enucleator*, Adkisson, 1981; evening grosbeaks, *Coccothraustes vespertinus*, Sewall *et al.*, 2004).

Our simulations examining the relative advantages, in terms of expected offspring survivorship, of different methods of mate choice suggest that assortative mating by vocalizations increases the fitness of offspring compared with random mating. Whether such indirect fitness benefits could drive the evolution of mate-choice preferences depends largely on the magnitude of the genetic component for fitness. The importance of indirect fitness benefits in the evolution of mate choice has been questioned (Arnqvist & Kirkpatrick, 2005; Charmantier & Sheldon, 2006; Qvarnström *et al.*, 2006). However, the genetic component for fitness may be large in divergent groups, such as crossbills, suggesting that indirect as well as direct fitness benefits could be driving the evolution of mating preferences in this group.

Our simulation revealed no additional indirect fitness benefit to mate choice based on bill morphology within a call type. This may explain the observed lack of female preference for bill size, both within their own call type and within another call type. By simply choosing a male within their own call type, females are on average choosing a well-adapted mate. With no additional indirect benefit of choosing based on bill depth, it is not surprising that females have not evolved the ability to directly assess bill morphology. Moreover, palate groove width, which influences seed handling rates (Benkman, 1993), cannot be visually detected making any visual assessment of bill structure incomplete. Nevertheless, females would likely benefit directly by choosing a morphologically well-adapted mate within their call type. For example, if females choose males that are faster foragers, this would provide direct fitness benefits to the female and may also reflect both the bill depth and palate groove width of the male.

Finally, our results support the idea that the evolution of different vocalizations among red crossbill call types has allowed for the assessment of ecological adaptation (Smith *et al.*, 1999). Crossbills assess food patch quality more rapidly by foraging with individuals with similar feeding abilities (Smith *et al.*, 1999), which should favour assortative flocking by trophic phenotype and may account for their strong tendency to flock assortatively by call type (Smith, 2005). Our results show that crossbills also preferentially choose mates of the same call type. The use of distinct calls as a marker trait enabling crossbills to assess trophic adaptation of potential mates further reduces gene flow between groups specialized on alternative resources, thereby promoting ecological speciation.

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