

Morphological divergence among Spanish Common Crossbill populations and adaptations to different pine species

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Crossbills (Loxia spp.) provide a classical avian model of ecological specialization on food resources. Previous studies have suggested that morphometric, genetic and vocal diversification among Common Crossbill Loxia curvirostra populations is better explained by ecological distance (use of different conifers) than by geographical distance, indicating that populations have diverged adaptatively. We tested for adaptive divergence in Iberian crossbills using bill and body size measurements of 6082 crossbills from 27 sites, each consisting of a dominant or single pine (Pinus) of four possible species. Crossbills using different pines differed significantly in body size and bill size and shape. There was no correlation between geographical and morphological distance among sampling sites, consistent with the hypothesis that the morphological divergence of Iberian crossbills is shaped by their ecological differences (foraging on alternative conifers) rather than geographical distance. However, for unknown reasons, Common Crossbills foraging on Pinus sylvestris in Iberia have on average much smaller bills than Parrot Crossbills Loxia pytyopsittacus feeding on the same pine species in northern Europe. The extent to which crossbills specialize on Iberian P. sylvestris remains to be established. Specialization on conifers with overlapping geographical distributions may be facilitated by matching habitat choice of crossbills as a function of their local intake rates.

Keywords: ecological specialization, evolution, *Loxia*, morphological diversification, *Pinus*, population differentiation.

Ecological specialization is one of the main drivers promoting divergence among populations (Newton 2003). Crossbills (*Loxia* spp.) are an avian model of ecological specialization through trophic specialization on alternative conifer species (Benkman 1993, 2003). These granivorous finches have bills with crossed mandible tips that are used to separate the scales of closed conifer cones from which to obtain seeds. Crossbills feed almost exclusively on such seeds (Cramp & Perrins 1994). However, different conifers have very different cone structures (Fig. 1) and a single type of bill is unable to obtain the highest food intake on different kinds of cones (Benkman 1988, 1993).

Although the exact functional biomechanics of extracting seeds from closed cones have not been properly clarified, a few aspects appear to be important (Newton 1972, Benkman 1987, 1993, Benkman & Lindholm 1991). First, to insert the bill tips between closed cone scales, crossbills 'bite' between them. This places a lot of stress on the bill tip, and a more perpendicular entry would prevent them from breaking off the bill (Soons *et al.* 2015). For this reason, the mandibles are rounded (the scientific name '*curvirostra*' refers to this, and surprisingly not to the crossed bill tips). Hence, to prevent the tips from breaking (which strongly reduces feeding efficiency; Benkman & Lindholm 1991), the stronger the force needed to

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Figure 1. Examples of closed pine (genus *Pinus*) cones of the four pine species in Spain that are potentially suitable for Crossbills. For the Aleppo Pine *Pinus halepensis*, we show the difference between the mainland and Balearic cones. Note that, for *P. halepensis*, cones from the mainland are larger than cones from the Balearics, which would predict a larger bill. Also note that the scales of *Pinus sylvestris* and *Pinus uncinata* are very thick at the visible tip, which would predict larger, robust bills (drawings by Elena Eslava).

bite between the scales, the rounder the mandibles need to be. Rounder mandibles can be produced by a bill of a given depth that is shorter, or a bill of a given length that is deeper: either alternative results in a bill that is relatively deep compared with its length, i.e. less pointy and more robust.

Once the mandibles are between the scales, crossbills abduct their lower mandible to spread the scales apart in order to obtain the seed. Also here, the stronger the force needed, the wider the mandibles need to be to prevent them from breaking. In addition, deeper and wider mandibles provide a greater attachment area for muscles and can create a stronger downward biting and lateral abduction force (Herrel *et al.* 2005). Finally, bill

length might also be affected by cone and scale morphology, although this is less clear. It might be that stronger scales select for relatively shorter bills, again in order to prevent the bill from breaking (Soons *et al.* 2015) and to provide better muscle attachment (Herrel *et al.* 2005). Longer scales might select for relatively longer bills. Although exact details are still lacking, all of these effects can explain why there may not be a single optimal bill size or shape when there is a variety of cone types available.

The optimal bill type also affects general crossbill morphology (Benkman 1993). First, a larger bill will demand a larger head in order to provide a proper attachment to the muscle and bone (Herrel *et al.* 2005). Second, a larger bill and head weigh more and so will also demand a larger body (including legs) to support it. And, finally, a larger body also needs larger wings and tail for efficient flight (Benkman 1991). Hence, the challenge of extracting a seed from between two conifer scales may influence the requirements on the entire bird, and no single morphology will be able to fulfil all functions equally well (Benkman 1993). For this reason, some crossbills, either species or populations, have been found, or hypothesized, to be specialized in the utilization of a single, key conifer species (Benkman 1993, 2003, Edelaar 2008, Irwin 2010).

The Common Crossbill Loxia. curvirostra (henceforth 'Crossbill') ranges across forests throughout the northern hemisphere (Cramp & Perrins 1994). Only two subspecies are typically recognized to occur in Iberia: the nominate subspecies L. c. curvirostra (widespread across Eurasia), and the endemic L. c. balearica (restricted to the Balearic Islands). However, based on morphology and plumage colour pattern, some authors have included birds from southern Iberia into bal*aerica*, or have assigned it to a separate subspecies hispana (Cramp & Perrins 1994). Although current classifications reflect resource use, they are based on geographical criteria, and it is assumed that subspecies do not geographically overlap. In strong contrast to this approach, more recent research has revealed morphometric and genetic segregation according to conifer species among (partially) sympatric crossbill populations (Groth 1993, Benkman 2003, Parchman et al. 2016) that may also be applicable to Iberian Crossbills (Alonso et al. 2006, Borrás et al. 2008, Edelaar et al. 2012, Parchman et al. 2018).

Owing to its relief and its geographical position, the Iberian peninsula is a very diverse area from a biogeographical standpoint, and is home to several native conifer species which *a priori* seem suitable for crossbills: Aleppo Pine Pinus halepensis, Black Pine Pinus nigra, Scots Pine Pinus. sylvestris and Mountain Pine Pinus uncinata. Two more pines occur, Umbrella Pine Pinus pinea and Maritime Pine Pinus maritima, but their seeds are too large and hard for crossbills, and hence these are not suitable. For the other species, it is argued that they can host specialized crossbills because these are often seen feeding on these pines, and crossbill and pine distributions coincide, e.g. Parrot Crossbills-Scots Pine in northern Europe, resident Crossbills on the Balearics and Aleppo Pine,

resident Crossbills on Cyprus (and perhaps also Corsica) and Black Pine, and resident Crossbills in the Pyrenees and Mountain Pine. Therefore, it can be hypothesized that these pines could host viable specialized crossbill populations in Spain (but see Mezquida *et al.* 2018).

The above examples also suggest that cone structure is different enough among these pines to result in morphological divergence: the average bill width (at skin insertion) is 13.3 mm for Parrot Crossbills using Scots Pine, 11.4 mm for Cyprus Crossbills using Black Pine, 10.9 mm for Pyrenean Crossbills using Mountain Pine, and 10.5 mm for Balearic Crossbills using Aleppo Pine (measurements on museum specimens taken by P.E.; n = 14, 41, 20 and 31, respectively). However, while specialization and divergence in isolated, allopatric populations is more likely, things might be different for populations in closer geographical and genetic contact, as on the Iberian peninsula. Nonetheless, some studies have already shown that these different pines host morphologically different Crossbill populations (Alonso et al. 2006, Borrás et al. 2008, Edelaar et al. 2012). Previously, we found that variation among Crossbills from different localities was better explained by ecological distance (usage of different pines) than by geographical distance (Edelaar et al. 2012), suggesting that Iberian Crossbills are in a process of adaptive population divergence.

We tested whether morphological variation among crossbills from different sampling localities can be explained by differential resource use (i.e. it depends on the pine upon which they were feeding). However, the different pine species do not have fully overlapping geographical distributions, so we also tested an alternative hypothesis that geographical distance may explain any divergence (e.g. due to neutral divergence with limited gene flow, or due to environmental gradients; Richardson et al. 2014, Wang & Bradburd 2014). To test whether morphological variation among crossbill populations is due to ecological specialization versus geographical distance, we analysed a data set which has a much larger sample size, and covers a much wider geographical area than previous studies. For the first time, we include data from all five distinct Iberian pines (including Aleppo Pine from the Balearic Islands as a different resource) in a unified approach (Fig. 2). In addition, the data were collected by a single observer, reducing the possibility of observer effects.

We discuss to what extent morphological differences among crossbills foraging on different pines can be understood from a functional perspective (i.e. how they relate to ecological performance, and not only feeding but also temperature regulation). In addition, we discuss how divergence is possible in the first place given that the different pine species have largely overlapping geographical distributions, which should complicate, if not prevent, divergence (Lenormand 2002, Wang & Bradburd 2014).

METHODS

Data collection

Crossbill populations in Iberia were sampled at 27 localities (Fig. 2, Supporting Information Table S1), all located in patches comprising a dominant or single pine species – Scots Pine, Aleppo Pine (with those from the mainland and the Balearics, however, being considered separately), Mountain Pine or Black Pine. Data were collected from 1994 to 2016. Locality 25 (western Pyrenees) had a periodic sampling effort with about two trapping sessions per month, whereas the other sampling sites were visited once or a few times in different years (Table S1).

Birds were captured with 16-mm-mesh mistnets when coming down from the tree tops to drink water in springs or to feed on minerals on walls or sites traditionally used to provide salt for cattle (Alonso *et al.* 2017). The number of mistnets used, their position and the sampling months varied among sites, as our goal was to capture as many Crossbills as possible at each site, and the sampling efforts and periods were adjusted to local circumstances.



Figure 2. Location of the sampling localities where Crossbills were captured (numbered as in Table 1). Localities 19 and 20 correspond to the Balearic Islands. [Colour figure can be viewed at wileyonlinelibrary.com]

Once captured, each bird was ringed and its age and sex were determined (Svensson 1996). Crossbills were aged as second-year individuals (EUR-ING code 3 or 5; birds with retained, and normally shorter juvenile primary feathers) or as older than second-year individuals (EURING code 4 or 6; birds with adult primaries, which have undergone at least one complete moult in their life). We took the following measures (Svensson 1996): wing length (\pm 0.5 mm), tail length (\pm 0.5 mm), tarsus length (\pm 0.1 mm), bill length (\pm 0.1 mm), bill width (\pm 0.1 mm) and bill depth (\pm 0.1 mm) (Alonso & Arizaga 2005). All data were collected by a single observer (D.A.).

Statistical analyses

Prior to analyses, all the data were filtered to consider only birds with known age and sex (hence juveniles are excluded) and to remove possible outliers. We assumed that multivariate Mahalanobis distances were distributed according to a beta-distribution (Wilks 1963) and calculated the corresponding *P*-values for all individuals. We next examined the distribution of these *P*-values and observed an unexpected peak in the number of individuals with very small *P*-values (< 0.001), so these extreme birds were omitted from the dataset (n = 67; 1.1% of all data).

Subsequently, we removed the effects of age, sex, year and month for each measure (the last two variables were included to control for variation due to any unwanted methodological changes in measuring and the effect of feather and bill wear, respectively). For this, we performed generalized linear mixed models (GLMMs) for each logtransformed response variable with an identity link function and a normal distribution of errors. We included age, sex, pine species, year and month as fixed categorical factors, with sampling site included as a random factor (we added pine species and sampling site to remove any confounding effects of the other variables). We then calculated corrected individual estimates according to the derived model parameters based on specific pine and site effects, but keeping all other variables constant. These corrected values thus retained the differences among pines and localities, while controlling for age, sex, year and month.

Principal components analyses (PCAs) on the corrected estimate derived from the above procedure were used to obtain a number of independent, uncorrelated summary variables that captured most of the size and shape variation. We ran two PCAs: one with only the variables related to body size and the flight apparatus (wing, tail and tarsus length; PCAbody) and the other with only the bill variables (length, depth and width; PCA_{bill}). This separation simplifies biological interpretation and probably reflects better the morphological and genetic correlations within these two sets of traits. The main components (PC) of each PCA were then used as dependent variables in GLMMs with pine species as a fixed factor to test for ecologically driven morphological divergence and sampling site as a random factor (unnested design). We used the R packages *lmerTest* (Kuznetsova et al. 2017) and stats (R Core Team 2014) for the analyses.

Finally, we conducted Mantel tests to check whether morphological differences were associated with geographical distance between sampling sites, as greater distances might reduce gene flow and thereby promote (neutral) divergence. Mantel tests, however, are not recommended when autocorrelation between compared variables is suspected (Guillot & Rousset 2013), something that is expected here because our sampling sites (particularly those with Black and Mountain Pine) are not homogeneously geographically distributed (Fig. 2). To overcome this potential bias, we ran two sets of Mantel tests separately: one for the Scots Pine localities only and one for the Aleppo Pine localities only, because for these pines we had large sample sizes and a relatively broad geographical range in Iberia (Fig. 2). Although this does not test the global effect of geographical distance on population divergence for the entire dataset, it gives us a good idea of the pattern within the two pine species with the largest geographical coverage, and therefore for the dataset as a whole (involving 21 of 27 localities). For each pine, we investigated the scores on the first and second principal components, separate for the PCAbody and the PCA_{bill} (i.e. four dependent variables). We therefore constructed four distance matrices, with each representing the Euclidean distance between populations in PC scores. In this analysis, the population from the Balearic Islands was omitted, as its connectivity is probably greatly affected by the sea compared with inland localities. The geographical distance matrix was built using the ecodist R package (Goslee & Urban 2007). In the absence of a significant positive correlation in this analysis, we inferred that any morphological variation between localities was independent of the geographical distance between them. All statistical analyses were carried out in R (R Core Team 2014).

RESULTS

GLMMs showed a significant (P < 0.05) effect of month, age, sex and year for all biometric variables (Supporting Information Table S2). The effects of these variables were subsequently statistically removed for further analyses (see above). In addition, there was a significant effect of species of pine on wing and tail length as well as bill depth, and approaching significant effects (P < 0.10) for the remaining three morphological variables – tarsus length, bill length and bill width. A detailed description of the data can be seen in Supporting Information Table S3.

The PCA on the body variables (corrected for age, sex, year and month) provided a PC1_{body} with positive factor loadings of fairly similar size for all traits (Table 1), so this axis of variation can be seen to describe body size, birds with higher PC1_{body} scores being larger. The PC2_{body} showed negative factor loadings for the wing and tail length, but a positive loading for the tarsus length, so Crossbills with positive, higher PC2_{body} scores had a proportionally longer tarsus for a given wing and tail length. The PCA on the bill variables (corrected for age, sex, year and month) also provided a PC1_{bill} with positive factor loadings of similar size for all traits, so also here it is best interpreted as reflecting overall bill size, and those birds with higher PC1_{bill} scores had larger bills. The PC2_{bill} showed a positive factor loading for bill length, but negative, almost equal-sized factor loadings for bill depth and width, so Crossbills with higher PC2_{bill} scores had thinner, pointier bills whereas birds with lower (negative) scores had broader, stockier bills. PC1_{bill}, PC2_{bill} and PC1_{body}, but not PC2_{body}, differed significantly among Crossbill populations using different pine species (Table 2, Fig. 3).

The Mantel tests did not reveal significant positive correlations between the geographical and the morphological distance matrices (Table 3). The test was significant for $PC2_{body}$ for the *sylvestris* localities, but the correlation was negative, indicating that localities closer to each other were more different than localities further away, a pattern

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that is opposite to the hypothesized isolation-bydistance effect. When corrected for multiple testing of the same hypothesis, no correlations were of statistical significance.

DISCUSSION

In support of the hypothesis that crossbills are ecologically adapted through trophic specialization, we found morphological differences among Iberian Crossbill populations utilizing different conifer species (Fig. 3, Table 3). Although much additional lower-level variation among individuals and among localities exists (Tables 2 and Table S3, see low R^2 values), these differences among pines are not due to Crossbill age or sex differences, because this was statistically taken into account (Table S1). Because there were no significant positive Mantel correlations between geographical and morphological distances between sampling localities for the two most widespread pines (Table 3), we conclude that these differences among Crossbills using different conifers are apparently also not a consequence of a reduction in gene flow with increasing geographical distance (which could create a spatial population structure via neutral processes or be driven by unknown ecological gradients that could confound correlations with pine use). Evidence for low or absent effects of geographical distance on crossbill species divergence has also been found for crossbill vocal types in America, for morphology and vocalizations (Groth 1993) and genetics (Parchman et al. 2016), and for a smaller dataset than the current one for the Iberian peninsula (Edelaar et al. 2012). We conclude, therefore, that the morphological divergence of Iberian Crossbills is shaped by their ecological distance (the difference in pine species used) and (assuming the results for the two pines tested can be generalized to all) is independent of the geographical distance between sampling localities.

For the analyses, we considered that Crossbills caught in a forest with a particular pine are utilizing and putatively specialized to that particular pine. There would be at least two reasons why this is a simplification. First, Crossbills are mobile and may fly many kilometres to reach favourite or available sites for drinking or feeding on minerals (D.A. pers. obs.). This could mean that birds feeding on other pines elsewhere might have been included in our samples. Second, Crossbills could also feed on other species



Figure 3. Mean (\pm 95% confidence interval) values of principal components on body size and bill, representing the morphological variation in average morphology between Crossbill populations inhabiting different types of conifer forests (bale = *Pinus halepensis* in the Balearics; hale = *P. halepensis*; nigr = *Pinus nigra*; sylv = *Pinus sylvestris*; unci = *Pinus uncinata*). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Factor loadings obtained from principal component analyses on the body variables (PCA_{body}) and on the bill variables (PCA_{bill}).

Variable	PC1	PC2
PCA _{body}		
Wing length	+0.68	-0.15
Tail length	+0.64	-0.36
Tarsus length	+0.36	+0.92
Eigenvalue	1.63	0.92
Explained variance (%)	54.4	30.6
PCA _{bill}		
Bill length	+0.52	+0.84
Bill width	+0.59	-0.48
Bill depth	+0.62	-0.24
Eigenvalue	1.81	0.71
Explained variance (%)	60.5	23.6

apart from their key pine species, especially when the cones are opening and seeds are much easier to obtain (Benkman 1993). Also, dispersing Crossbills on the move may temporarily feed on conifers to which they are not specialized. As a result, it must be acknowledged that our samples may be composed of unknown fractions of birds which do not typically feed on the focal pines, adding noise and perhaps some bias to our data.

Other criteria could be and are being developed to assign Crossbills to specific, given pines, e.g. vocalizations (Groth 1993), genetics (Edelaar et al. 2012, Parchman et al. 2016), isotopic signatures (Marquiss et al. 2008, 2012, Arizaga et al. 2014, 2015) and spatial tracking studies (Alonso et al. 2017). Until this work is completed, this potential bias in the data cannot be fully assessed. Similarly, we also assumed that cone structure is constant among sampling sites of given pine species, whereas genetic and environmental differences may also exist among localities. Geographical variation in cone structure that is relevant for foraging Crossbills has been described, including variation due to the presence of food competitors (squirrels, insects, woodpeckers, etc.) driving overall cone evolution (Benkman et al. 2001, Mezquida & Benkman 2005). If Crossbills specialize on these local variations in cone structure, this will add more variation to the data, but in the absence of detailed data on such variability and how this may

PCA components	F	Р	hale	nigr	sylv	unci
$\frac{PC1_{body}}{(larger body size)}$ $\frac{R_{GLMM(m)}^{2} = 0.040}{R_{GLMM(m)}^{2} = 0.102}$	8.45	<0.001	+1.32 (0.36)	+1.07 (0.39)	+1.87 (0.36)	+1.62 (0.49)
$\begin{aligned} & \text{PC2}_{\text{body}} \\ & \text{(relatively longer legs)} \\ & R_{\text{GLMM(m)}}^2 = 0.003 \\ & R_{\text{GLMM(c)}}^2 = 0.030 \end{aligned}$	1.54	0.245	+0.36 (0.22)	+0.29 (0.24)	+0.22 (0.23)	+0.01 (0.29)
PC1 _{bill} (larger bill size) $R_{GLMM(m)}^2 = 0.019$ $R_{GLMM(c)}^2 = 0.140$	3.36	0.027	+1.00 (0.47)	+0.12 (0.54)	+0.95 (0.48)	+1.66 (0.68)
$PC2_{bill}$ (pointier bill) $R_{GLMM(m)}^{2} = 0.036$ $R_{GLMM(c)}^{2} = 0.158$	3.18	0.036	+0.28 (0.29)	+0.31 (0.34)	-0.16 (0.30)	-0.44 (0.43)

Table 2. Results of general linear mixed models used to test for the effect of pine species on PCA components, with sampling site included as a random factor.

The estimates (\pm se) for the effect of pine species are also indicated, with *Pinus halepensis* in the Balearic Islands as the reference (effect = 0); *hale = P. halepensis* on the continent; *nigr = Pinus nigra*; *sylv = Pinus sylvestris*; *unci = Pinus uncinata*. For each principal component we indicate (in parentheses) what a larger positive score implies. We also provide the marginal $R_{GLMM(m)}^2$ (Naka-gawa & Johnson 2017), a measure of the variance explained by the fixed effects (pine species), and the conditional $R_{GLMM(m)}^2$, a measure of the variance explained by the fixed and random effects combined (pine species and sampling site) as calculated with the R package MuMIn (Barton 2014).

affect Crossbills, this interesting aspect cannot be incorporated (or statistically controlled for) in our analyses.

Perhaps due to the above issues, statistical support for divergence among Crossbills feeding on different pines is limited (Table 2). Leaving these complications and statistical significance aside for now, based on their average PC scores (Fig. 3, Table 3), the Crossbills using each pine might be described as follows. Crossbills from the Balearic Islands feeding on *P. halepensis* are smaller birds with small, but relatively robust, bills. Birds feeding on continental P. halepensis are medium-sized birds, with a larger, proportionately longer bill. Those feeding on *P. nigra* are similar to the latter, but have much smaller bills (but these are poorly defined; see below on large variation among localities). Crossbills feeding on P. sylvestris are large birds and have a larger, relatively more robust bill. Finally, those feeding on *P. uncinata* are also large, have the largest bills, and their bills are especially robust. The Balearic birds and those feeding on P. uncinata are the most clearly differentiated, with the latter having the larger bill and body. Less differentiated, but still significantly different are the Crossbills feeding on mainland *P. halepensis* and *P. sylvestris* for body size (P = 0.028) and body and bill shape (P = 0.021, P < 0.001, respectively; no difference in bill size: P = 0.62; same GLMMs on PC scores as before, but only for this subset of data). Overall, therefore, we detected evidence supporting a noticeable morphological difference in Crossbills feeding on different pines.

We note two important aspects of this pattern: function (how morphology affects ecological performance) and mechanism (how these differences arise and are maintained). With respect to the first point, some aspects of the observed differences fit with the expectations based on the abiotic conditions of each population. We see an increase in body size as we go from low to high altitudes, seemingly in line with Bergmann's ecogeographical rule (but see Groth 1993). Elevation could also explain bill shape to some extent, according to Allen's rule, as more robust bills will reduce heat loss in colder environments. However, our sample size (four populations; five if we consider 'halepensis' from the Balearics as a source differentiated from the mainland 'halepensis') is very small, and a comparison across more

	<i>P. sylves</i> localities	tris	<i>P. halepensis</i> localities	
PC component	Mantel r	P-value	Mantel r	P-value
PC1 _{body} (overall body size) PC2 _{body} (relative leg length) PC1 _{bill} (overall bill size) PC2 _{bill} (bill shape)	-0.27	0.16	+0.08	0.69
	-0.33	0.034 ^a	-0.22	0.08
	-0.21	0.28	-0.01	0.52
	-0.27	0.19	+0.15	0.82

Table 3. Results for Mantel correlation tests for the *sylvestris* and *halepensis* localities (considered separately), testing for potential correlations between geographical and morphological distances between localities.

^aNot significant after correcting for multiple testing of the same hypothesis.

populations (preferably at a global scale) would be useful to test whether such a pattern is general for Crossbills.

Crossbills from the Balearic Islands do not. however, completely fit the above pattern, as their bills are quite robust. This might be due to these crossbills using a local kind of Aleppo Pine which has different cone traits. Observing feeding Common, Scottish and Parrot Crossbills, Summers et al. (2010) proposed that massive bills were needed to get into the closed cones of Scots Pine (confirmed by feeding trials with Common Crossbills; McNab et al. 2019). Mezquida and Benkman (2005) provided evidence supporting an effect of squirrel presence on the evolution of Aleppo Pine cones. Squirrels (e.g. of the genera Sciurus or Tamiasciurus) eat many pine seeds, and in concordance with previous studies involving different pine species (Benkman et al. 2001), predation by squirrels on Aleppo Pine is selective and depends on certain cone traits. In response, over many generations, pines have evolved cone traits that defend their seeds against squirrels. Interestingly, squirrels are absent from the Balearic Islands. Following expectations for relaxed selection of defences against squirrels, cones on the Balearic Islands are smaller and have shorter and thinner scales than cones on the mainland. How exactly this affects selection on bill traits is not yet established, but it is probable that Balearic cones allow easier access to seeds, and hence smaller and perhaps shorter bills would suffice, as observed.

Following on from the above, Crossbills foraging on mainland Aleppo Pine, which has evolved larger and more defended cones in response to squirrel predation, should have larger bills. While Mezquida and Benkman (2005) noted that these cones appeared to have evolved levels of defence that have excluded Crossbills foraging on this pine, Crossbills are indeed not common, but are nonetheless rather widespread in Aleppo Pine forests. Genetic data indicate they are apparently resident, at least in southern Iberia, and presumably only forage on Aleppo Pine cones just as on the Balearics and in northern Africa (Parchman et al. 2018). We have confirmed here that they have a distinct biometry, suggesting they are well adapted to this mainland Aleppo Pine variety. This includes a proportionately longer bill, and we hypothesize that this may have to do with the characteristics of Aleppo Pine cone scales (see Fig. 1): this pine has the longest scales of all Spanish pines (requiring a long bill), but the scales are relatively thin and flexible (perhaps not requiring such a thick and robust bill).

Similar reasoning appears valid for birds utilizing P. uncinata. This pine has the thickest scales of all Iberian pines (Fig. 1; Mezquida & Benkman 2010). In line with observations on other crossbill populations (e.g. Benkman et al. 2001, Parchman & Benkman 2002, Mezquida & Benkman 2005, Parchman et al. 2007), thicker scales require deeper (more robust) bills, because deeper bills can exert a greater force to separate the overlapping scales. As expected, the Crossbills utilizing this pine had the largest and most robust bills. This observation is in line with a recent capture-recapture analysis of Crossbills in P. uncinata forests (Gómez-Blanco et al. 2019), which showed that apparent (i.e. local) survival is highest for birds having bills that are larger than the mean bill sizes of populations using other pines, supporting local adaptation in Crossbills using P. uncinata.

The relationship between bill and cone structure is less clear for those Crossbills utilizing *P. nigra* and *P. sylvestris*. The cones of *P. nigra* are fairly large and some resident Crossbills exploiting this pine elsewhere in Europe have very large bills, e.g. the endemic subspecies *L. c. guillemardi* on Cyprus (Cramp & Perrins 1994). We have sampled only three localities situated in forests of *P. nigra*, and found a large variation among these localities, with one locality hosting birds with very large bills, so some additional sampling seems necessary for this specific pine (we also cannot confirm yet whether it is used year-round). Finally, *P. sylvestris* has the smallest cones and scales of all lberian pines, and does not seem to be particularly well-defended. In this respect, it may appear strange that Crossbills exploiting this pine have such robust, large bills. However, the massively billed Parrot Crossbill from northern Scandinavia and Russia also specializes on this pine species, and Common Crossbills from Scotland (subsp. *curvirostra*) with much smaller bills than Parrot Crossbills could not open closed cones (McNab *et al.* 2019), so it would appear that feeding on *P. sylvestris* somehow requires robust, large bills.

It has been hypothesized that very large bills are necessary to open wet cones that are frozen in winter (Knox 1990). Nonetheless, even if this is so, it is not clear why Crossbills exploiting this pine in Spain, or even *P. uncinata* in the even colder highaltitude subalpine zone, do not have bills as large as Parrot Crossbills (Cramp & Perrins 1994). One possibility is that Crossbills specialized on Scots Pine in Spain do not exist, and that Crossbills specialized on Mountain and/or Aleppo Pine only use Scots Pine when the seeds are available in spring (this may also be true for Crossbills using *P. nigra*). This hypothesis is argued by Mezquida et al. (2018), who observed that the period between seed fall of one generation of cones and the production of seeds by the new generation of cones is much greater in southern than in northern Europe, which could lead to the starvation of a specialist population. Indeed, Alonso and Arizaga (2017) reported much higher densities of Crossbills in late winter and spring in a Pyrenean Scots Pine area, when cones open and the seeds are available. However, they also reported that Crossbills were present throughout the whole annual cycle, and that a proportion of these birds even bred in the summer, presumably in *P. sylvestris*. There is also evidence that these Crossbills are faithful to this resource: Alonso and Arizaga (2013) recaptured many Crossbills in the same site for several years, and stable isotope analysis revealed that birds using Scots Pine and nearby Aleppo Pine forests did not mix freely (Arizaga et al. 2014). Preliminary genomic data, however, indicated that birds feeding on Scots and Aleppo Pine in northern Iberia were not clearly differentiated (D.A. unpubl. data). Together, this suggests that specialist birds are present but that the population is augmented with Crossbills from elsewhere when cones open.

Whatever the exact links between cone and Crossbill morphologies, it is surprising to find morphological divergence among Crossbills using pines with largely overlapping distributions (Fig. 2). Movements of Crossbills in search of food are well documented in the Iberian peninsula (Senar et al. 1993) and elsewhere (e.g. Newton 2006), and if subsequent settlement and breeding were random. it would quickly lead to the homogenization of populations (Newton 2003). Divergence could hence only be possible when natural selection acts against locally maladapted morphologies, or if individual birds select their habitat according to its suitability (Edelaar & Bolnick 2012, 2019. Richardson et al. 2014, Wang & Bradburd 2014, Nicolaus & Edelaar 2018). Indeed, at a smaller scale these pines are normally spatially segregated, especially by elevation. This would facilitate the selection of the appropriate pine species (if based either on genetic preference alleles, on imprinting, or on performance-based comparisons: for details see Akcali & Porter 2017), especially for such a mobile bird as the crossbill (Ravigne et al. 2009, Edelaar et al. 2017). With respect to the latter mechanism, Benkman (2017) provided evidence supporting performance-based habitat choice (also called matching habitat choice; Edelaar et al. 2008) as responsible for the disappearance (or emigration) of locally maladapted individuals that previously immigrated into a population of an ecologically specialized endemic crossbill species in the USA. They concluded that the selective emigration of locally maladapted individuals contributes to the maintenance of endemic species' morphological, vocal and genetic distinctiveness (Edelaar 2018). Similarly, Gómez-Blanco et al. (2019) presented the results of a capture-recapture analysis of Crossbills using P. uncinata in the Spanish Pyrenees, which also provided indications for the emigration of locally maladapted individuals. It is likely that both natural selection and habitat choice operate in Crossbills and contribute to local adaptation and population divergence. However, measuring the relative contribution of each mechanism requires data on the dispersal and survival of individuals across different habitats, which is hard to obtain (but see Edelaar et al. 2019).

The radiation of Crossbills at a relatively small geographical scale suggests that Spain is an important region for the conservation of Crossbill diversity in Europe. Genomic research is underway to determine to what extent this ecological specialization and morphological divergence is linked to genetics. Preliminary results already have shown that genetic differences between presumably conspecific Crossbill populations within Spain are far greater than between heterospecific Common and Parrot Crossbills (Parchman et al. 2018). In view of the potential for movement and habitat selection by Crossbills, it is important to improve our understanding of dispersal of these birds (e.g. Alonso et al. 2017) and to what extent given populations are able to respond to habitat loss or degradation. Ringing recoveries and telemetry may contribute to this, as well as other approaches. For example, Arizaga et al. (2015) showed that stable isotope signatures of Crossbills foraging on Scots Pine were different from those using Aleppo Pine, and that the presence of Crossbills from northern Europe was rare. The conservation of native pine forests may be critical (Summers et al. 2002) because the ability of Iberian Crossbills to cope with loss of habitat (e.g. due to fires or global change) vs. colonization and exploitation of reforested areas (with younger and denser tree stands) remains unknown.

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DATA AVAILABILITY STATEMENT

The data used in this work are available under request to the authors.

REFERENCES

- Akcali, C.K. & Porter, C. 2017. Comment on Van Belleghem et al 2016: Habitat choice mechanisms in speciation and other forms of diversification. *Evolution* **71**: 2754–2761.
- Alonso, D. & Arizaga, J. 2005. Efecto de la edad, el sexo y el tiempo en la biometría del piquituerto común (*Loxia curvirostra curvirostra*) en Navarra. *Munibe* 56: 133–144.
- Alonso, D. & Arizaga, J. 2013. The impact of vagrants on apparent survival estimation in a population of Common Crossbills (*Loxia curvirostra*). J. Ornithol. 154: 209–217.
- Alonso, D. & Arizaga, J. 2017. Seasonal abundance patterns of common crossbills *Loxia curvirostra* L., 1756 in two localities of the Navarran Pyrenees and implications for its survey through ringing. *Munibe* 65: 95–105.

- Alonso, D., Arizaga, J., Miranda, R. & Hernandez, M.A. 2006. Morphological diversification of Common Crossbill *Loxia curvirostra* populations within Iberia and the Balearics. *Ardea* 94: 99–107.
- Alonso, D., Arizaga, J., Meier, C.M. & Liechti, F. 2017. Light-level geolocators confirm resident status of a Southern European Common Crossbill population. J. Ornithol. 158: 75–81.
- Arizaga, J., Alonso, D. & Hobson, K. 2014. Disentangling the origin of crossbills using morphology and isotopic (δ^2 H) characters. Are southern European crossbills restricted to population-specific key resources? *J. Ornithol.* **155**: 1027–1035.
- Arizaga, J., Alonso, D. & Edelaar, P. 2015. Stable isotopes in a southern European crossbill population indicate restricted movement between regions with different pine species. J. Zool. 295: 49–55.
- Barton, K. 2014. *MuMIn: Multi-model inference. R package version 1.10.5.* Vienna: R Foundation for Statistical Computing.
- Benkman, C.W. 1987. Crossbill foraging behaviour, bill structure, and patterns of food profitability. *Wilson Bull.* 99: 351–368.
- Benkman, C.W. 1988. Seed handling efficiency, bill structure, and the cost of bill specialization for crossbills. *Auk* **105**: 715–719.
- Benkman, C.W. 1991. Predation, seed size partitioning and the evolution of body size in seed-eating finches. *Evol. Ecol.* 5: 118–127.
- Benkman, C.W. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. *Ecol. Monogr.* 63: 305–325.
- Benkman, C.W. 2003. Divergent selections drives the adaptive radiation of crossbills. *Evolution* 57: 1176–1181.
- Benkman, C.W. 2017. Matching habitat choice in nomadic crossbills appears most pronounced when food is most limiting. *Evolution* 71: 778–785.
- Benkman, C.W. & Lindholm, A.K. 1991. The advantages and evolution of a morphological novelty. *Nature* 349: 519– 520.
- Benkman, C.W., Holimon, W.C. & Smith, J.W. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. *Evolution* **55**: 282–294.
- Borrás, A., Cabrera, J. & Senar, J.C. 2008. Local divergence between Mediterranean crossbills occurring in two different species of pine. *Ardeola* 55: 169–177.
- Cramp, S. & Perrins, C.M. 1994. Handbook of the Birds of Europe, the Middle East and North Africa, 8th edn. Oxford: Oxford University Press.
- Edelaar, P. 2008. Rediscovery of a second kind of crossbill for The Himalayan region, and the hypothesis that ecological opportunity drives crossbill diversification. *Ibis* **150**: 405–408.
- Edelaar, P. 2018. Ecological speciation: when and how variation among environments can drive population divergence. In Tietze, D.T. (ed.) *Bird Species How They Arise, Modify and Vanish: 195–215.* Cham: Springer.
- Edelaar, P. & Bolnick, D.I. 2012. Non-random gene flow: an underappreciated force in ecology and evolution. *Trends Ecol. Evol.* 27: 659–665.

- Edelaar, P. & Bolnick, D.I. 2019. Appreciating the multiple processes increasing individual or population fitness. *Trends Ecol. Evol.* 34: 435–446.
- Edelaar, P., Siepielski, A.M. & Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* 62: 2462– 2472.
- Edelaar, P., Alonso, D., Lagerveld, S., Senar, J.C. & Björklund, M. 2012. Population differentiation and restricted gene flow in Spanish crossbills: not isolation-by-distance but isolation-by-ecology. J. Evol. Biol. 25: 417–430.
- Edelaar, P., Jovani, R. & Gomez-Mestre, I. 2017. Should I change or should I go? Phenotypic plasticity and matching habitat choice in the adaptation to environmental heterogeneity. *Am. Nat.* **190**: 506–520.
- Edelaar, P., Banos-Villalba, A., Quevedo, D.P., Escudero, G., Bolnick, D.I. & Jordan-Andrade, A. 2019. Biased movement drives local cryptic coloration on distinct urban pavements. *Proc. Biol. Sci.* 286: 20191343.
- Gómez-Blanco, D., Santoro, S., Borrás, A., Cabrera, J., Senar, J.C. & Edelaar, P. 2019. Beak morphology predicts apparent survival of crossbills: due to selective survival or selective dispersal? J. Avian. Biol. 50. (in press).
- Goslee, S.C. & Urban, D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *J. Stat. Softw.* 22: 1–19.
- Groth, J.G. 1993. Evolutionary differentiation in morphology, vocalizations, and allozymes among nomadic sibling species in the North American Red Crossbill (Loxia curvirostra) complex. Univ. California Publication in Zoology 127.
- Guillot, G. & Rousset, F. 2013. Dismantling the Mantel tests. *Methods Ecol. Evol.* 4: 336–344.
- Herrel, A., Podos, J., Huber, S.K. & Hendry, A.P. 2005. Evolution of bite force in Darwin's finches: a key role for head width. J. Evol. Biol. 18: 669–75.
- Irwin, K. 2010. A new and cryptic call type of the Red Crossbill. Western Birds 41: 10–25.
- Knox, A.G. 1990. The sympatric breeding of Common and Scottish Crossbills *Loxia curvirostra* and *L. scotica* and the evolution of crossbills. *Ibis* **132**: 454–466.
- Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. 2017. ImerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82: 1–26.
- Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17: 183–189.
- Marquiss, M., Hobson, K.A. & Newton, I. 2008. Stable isotope evidence for different regional source areas of common crossbill *Loxia curvirostra* irruptions into Britain. *J. Avian Biol.* **39**: 30–34.
- Marquiss, M., Newton, I.A.N., Hobson, K.A. & Kolbeinsson, Y. 2012. Origins of irruptive migrations by Common Crossbills *Loxia curvirostra* into northwestern Europe revealed by stable isotope analysis. *Ibis* 154: 400–409.
- McNab, E., Summers, R., Harrison, G. & Park, K.J. 2019. How important are different non-native conifers in Britain to Common Crossbills *Loxia curvirostra curvirostra*? *Bird Study* 66: 64–72.
- Mezquida, E.T. & Benkman, C.W. 2005. The geographic selection mosaic for squirrels, crossbills and Aleppo pine. J. Evol. Biol. 18: 348–357.
- Mezquida, E.T. & Benkman, C.W. 2010. Habitat area and structure affect the impact of seed predators and the

potential for coevolutionary arms races. *Ecology* **91**: 802-814.

- Mezquida, E.T., Svenning, J.-C., Summers, R.W. & Benkman, C.W. 2018. Higher spring temperatures increase food scarcity and limit the current and future distributions of crossbills. *Divers. Distrib.* 24: 473–484.
- **Nakagawa, S., Johnson, P.C.D. & Schielzeth, H.** 2017. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *J. R. Soc. Interface* **14**: 20170213.
- Newton, I. 1972. Finches. London: Collins.
- **Newton, I.** 2003. *Speciation and Biogeography of Birds.* London: Academic Press.
- Newton, I. 2006. Movement patterns of Common Crossbills Loxia curvirostra in Europe. Ibis 148: 782–788.
- Nicolaus, M. & Edelaar, P. 2018. Comparing the consequences of natural selection, adaptive phenotypic plasticity, and matching habitat choice for phenotype–environment matching, population genetic structure, and reproductive isolation in metapopulations. *Ecol. Evol.* 8: 3815–3827.
- Parchman, T.L. & Benkman, C.W. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. *Evolution* 56: 1663–1672.
- Parchman, T.L., Benkman, C.W. & Mezquida, E.T. 2007. Coevolution between hispaniolan crossbills and pine: Does more time allow for greater phenotypic escalation at lower latitude ? *Evolution* 61: 2142–2153.
- Parchman, T.L., Buerkle, C.A., Soria-Carrasco, V. & Benkman, C.W. 2016. Genome divergence and diversification within a geographic mosaic of coevolution. *Mol. Ecol.* 25: 5705–5718.
- Parchman, T.L., Edelaar, P., Uckele, K., Mezquida, E.T., Alonso, D., Jahner, J.P., Summers, R.W. & Benkman, C.W. 2018. Resource stability and geographic isolation are associated with genome divergence in western Palearctic crossbills. J. Evol. Biol. 31: 1715–1731.
- **R Core Team.** 2014. *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing.
- Ravigne, V., Dieckmann, U. & Olivieri, I. 2009. Live where you thrive: joint evolution of habitat choice and local adaptation facilitates specialization and promotes diversity. *Am Nat* 174: E141–169.
- Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. 2014. Microgeographic adaptation and the spatial scale of evolution. *Trends Ecol. Evol.* 29: 165–176.
- Senar, J.C., Borras, A., Cabrera, T. & Cabrera, J. 1993. Testing for the relationship between coniferous crop stability and Common Crossbill residence. J. Field Ornithol. 64: 464– 469.
- Soons, J., Genbrugge, A., Podos, J., Adriaens, D., Aerts, P., Dirckx, J. & Herrel, A. 2015. Is beak morphology in Darwin's Finches tuned to loading demands? *PLoS ONE* 10: e0129479.
- Summers, R.W., Jardine, D.C., Marquiss, M. & Rae, R. 2002. The distribution and habitats of crossbills *Loxia* spp. in Britain, with special reference to the Scottish Crossbill *Loxia scotica. Ibis* **144**: 393–410.
- Summers, R.W., Dawson, R.J.G. & Proctor, R. 2010. Temporal variation in breeding and cone size selection by three species of crossbills *Loxia* spp. in a native Scots pinewood. *J. Avian Biol.* **41**: 219–228.

- Svensson, L. 1996. *Guía para la Identificación de los Paseriformes Europeos.* Madrid: Sociedad Española de Ornitología.
- Wang, I.J. & Bradburd, G.S. 2014. Isolation by environment. *Mol. Ecol.* 23: 5649–5662.
- Wilks, S.S. 1963. Multivariate statistical outliers. *Sankhya A* 25: 407–426.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sampling localities where Crossbills were captured. Codes are the same as in Figure 2. Pine (genus *Pinus*) species: bale = *P. halepensis* from the Balearic Islands; hale = continental *P. halepensis* (Aleppo Pine); unci = *P. uncinata* (Mountain Pine); sylv = *P. sylvestris* (Scots Pine); nigr = *P. nigra* (Black Pine). Localities with a pine species in parentheses mean that crossbills were captured in a given pine patch surrounded by larger patches of other pine species (in parentheses); thus, hale (nigr) means

that birds were caught in a zone of *P. halepensis* surrounded by forests/patches of *P. nigra*.

Table S2. Single-variable effects provided by general linear mixed models (GLMMs) with age, sex, year, month and species of pine as fixed categorical factors (locality included as random effect). Within each cell, we show the *F*-values with the numerator and denominator associated degrees of freedom (df) and the associated *P*-value (in italics) as well.

Table S3. Mean $(\pm$ se) morphological values of Crossbills captured at 27 localities (site codes as in Table 1) in Spain. Month, sex, age and year effects have been removed. Abbreviations: WL = wing length, TL = tail length, LL = tarsus (leg) length, BL = bill length, BD = bill depth, BW = bill width (all variables are in mm). For further details on principal components (PC) see Table 1. Within each column, the sex and age-associated beta-parameter estimates have been included. Reference values for sex and age are females and young birds (EURING 3/5), so to obtain the value of the males and old birds (EURING 4/6), the corresponding beta-parameters must be added.