

# Non-random infection across individuals and populations supports that parasites can change morphology within an adaptive radiation

P. Edelaar<sup>1,2</sup> & K. van Eerde<sup>3</sup>

<sup>1</sup> Department of Animal Ecology, University of Uppsala, Uppsala, Sweden

<sup>2</sup> Department of Conservation Biology, Biological Station of Doñana – CSIC, Sevilla, Spain

<sup>3</sup> Levimaat 13, Dwingeloo, The Netherlands

## Keywords

adaptive radiation; parasites; directional natural selection; population differentiation.

## Correspondence

Dr Pim Edelaar, Departamento de Biología de la Conservación, Estación Biológica de Doñana CSIC, Av. Americo Vespucio, s/n, 41092, Sevilla, Spain. Tel: +34 954 232 340/ +34 954466700; Fax: +34 954 621 125  
Email: edelaar@ebd.csic.es

Editor: Andrew Kitchener

Received 31 December 2009; revised 5 July 2010; accepted 2 August 2010

doi:10.1111/j.1469-7998.2010.00755.x

## Introduction

Adaptive radiation is characterized by phenotypic diversification as a result of utilizing different environments (Schluter, 2000a; Gavrillets & Losos, 2009). A large part of global biodiversity is considered to be the outcome of adaptive radiation, and thus, understanding the processes driving and limiting adaptive radiation are of considerable interest (Schluter, 2000a). However, it should be noted that not all aspects of evolutionary radiations are necessarily adaptive, and needs to be considered on a case-to-case basis (Schluter, 2000a; Losos & Miles, 2002; Gavrillets & Losos, 2009).

Evidence is accumulating that the radiation of crossbills (*Loxia curvirostra* Linnaeus – complex) is driven by adaptive specialization on distinct food resources: seeds held in (partially) closed cones of different conifer species (Benkman, 2003). Overall, the strong effect of bill size on feeding and fitness is thought to have driven the adaptive radiation of North American crossbills (Benkman, 2003).

However, Benkman *et al.* (2005) showed that a resident crossbill population did not have the predicted optimal bill depth for foraging on the local cone type due to infection by the scaly leg mite *Knemidokoptes jamaicensis*. This mite

## Abstract

Adaptive radiation is characterized by rapid phenotypic diversification as a result of utilizing different environments. Red crossbills (*Loxia curvirostra* Linnaeus – complex) have diversified in bill size and shape, and overall size, in response to differences in the conifer cones that hold the seeds they almost exclusively feed upon. However, a recent study showed how a bill of suboptimal size for foraging has evolved due to antagonistic selection by scaly leg mites *Knemidokoptes jamaicensis*. This suggests that the current variation in morphology within the adaptive radiation of crossbills may not be exclusively the result of adaptation to alternative resources. Using an independent set of populations, we found that the surprising and little-understood relationship between crossbill morphology and infection with mites is repeatable. Assuming mites depress survival, this relationship would result in directional, not stabilizing selection on morphology. We also find that the rates of infection can differ dramatically between populations, potentially depending on their ecologies. These findings suggest that morphological evolution within the adaptive radiation of crossbills may partly occur for reasons unrelated to resource use.

burrows into the legs and feet of passerines to feed on host tissue (so cannot be removed by preening), and in doing so causes progressively worsening swelling of legs and feet, deviating growth of scales and skin, and loss of scales, often up to the point that digits or entire feet become unusable or even die off (Pence *et al.*, 1999). Reduced use of legs and feet during arboreal feeding, perhaps combined with extra demands on the immune system due to responses to mites (Owen *et al.*, 2009) and secondary infection of wounds, would be expected to negatively impact upon fitness. Latta (2003) suggested for palm warbler *Dendroica palmarum* and prairie warbler *Dendroica discolor* that infection with scaly leg mites caused 100% mortality within a year (although here mortality may have been enhanced by rings causing infected, swollen legs to die off). In crossbills, infection also reduces survival, with annual survival of infected individuals estimated at 59.2% in males and 67.9% in females against 75.4% in males and 71.6% in females when uninfected (Benkman *et al.*, 2005). Interestingly, infections of mites occurred non-randomly in crossbills. For reasons that are not known, males and birds with larger bills were more likely to be infected (here used as: showing clinical signs of infection). Moreover, among those infected birds, survival

was lower in birds with larger bills. Both these effects should result in a selective force favouring birds with a smaller bill than is optimal for the local cone, and a decrease in sexual dimorphism. These patterns were observed within the time span of only a few years.

In general, it is surprising that scaly leg mites had directional selective effects on morphology at all. One would normally expect birds in poorer conditions to become infected or to die due to infection, and hence selection to be stabilizing around some optimal morphology. Given these surprising, little-understood and potentially important effects of parasitic mites on adaptation at the population level, we investigated three aspects of the effect of mites on population differentiation in crossbills: (1) do mites repeatedly exert selection on morphology; (2) is selection stabilizing or directional; (3) are some populations more exposed to mites than others? We analyse a large dataset of live birds belonging to three different sympatric populations to address these questions. In addition, for a broader evaluation of the third question, we compare rates of infection with mites among all known species and subspecies of crossbill from across the world based on museum specimens.

## Materials and methods

### Data collection on morphology and vocal type of live-caught birds

Crossbills were caught under license by one of us (K. v. E.) in the Netherlands (52°49'N – 6°24'E) from July 2002 to March 2003. Individuals were attracted to decoy crossbills and small ponds for drinking and bathing and were caught in mist nets upon approach. Individuals were sexed and aged, and the following eight body and bill traits were measured: mass, wing length, tarsus length, bill depth, upper mandible length, lower mandible length, lower mandible width and length of head plus bill; see Svensson (1992) and Edelaar & Terpstra (2004) for figures and details on catching, sexing, ageing and measuring crossbills.

Crossbill flight calls were recorded upon release and assigned on sonograms to one of the six vocal types described previously in Robb (2000) by two different observers. These vocal types occur largely in sympatry (see maps in Robb, 2000), and some have been shown to differ in morphology (Edelaar, Van Eerde & Terpstra *et al.*, 2008). Vocal types also strongly mate assortatively (Edelaar, 2008a), and hence are believed to represent evolutionarily distinct populations (see 'Discussion' and Robb, 2000; Edelaar, Summers & Iovchenko, 2003; Edelaar & Terpstra, 2004; Edelaar, 2008a; Edelaar *et al.*, 2008). Here, we focus only on the three most commonly caught crossbill vocal types A, C (Robb, 2000) and X. Call type X was discovered after the publication of Robb (2000) and was previously included with call type A (Edelaar *et al.*, 2008), but is now believed to be sufficiently distinct that it warrants separate analysis. These types represented about 10, 50 and 40% of the data, respectively.

Repeatabilities of measurements (following Lessells & Boag, 1987) for 14 ringed birds recaptured and remeasured several days to months later (i.e. not within the same measuring session as often reported, which inflates repeatability) were found to be moderate to high: 0.71 (bill depth), 0.72 (mass), 0.72 (length head plus bill), 0.78 (lower mandible width), 0.83 (lower mandible length), 0.83 (wing length), 0.90 (upper mandible length) and 0.94 (tarsus length). Assignment of these 14 birds to call type was 100% identical (Edelaar *et al.*, 2008).

### Global variability in occurrence of mites in additional museum and field samples

As part of another study, one of us (P. E.) checked museum samples of virtually all species, subspecies and other distinct populations of crossbills from across the world for deviations of legs or feet. Especially the larger population samples are collected across different months, years and locations; therefore, we assume that they are representative. Based on our experience in identifying scaly leg mite infection in the wild, we feel confident that we could distinguish deviations of legs or feet caused by scaly leg mites from other deviations, such as poor preparation techniques, handling or keeping conditions, damage incurred by shooting the specimen, infection by avian pox, etc. Such an assessment of infection rates of mites in museum specimens has been done before in other species (Kirmse, 1966; see also images in Carothers & Sharber, 1974).

In addition to these museum samples, we also assessed the occurrence of mites in a few other populations sampled in the wild. Here, infected birds were easily recognized by their swollen, often pinkish grey (not black) tarsi and feet in combination with displaced (lifted) or missing tarsal scales.

### Statistical analyses

All morphology measures were  $\log_{10}$  transformed. Unsexed birds, which include potentially still growing birds in full juvenile plumage, were excluded (Groth, 1993). Outliers (apparently mostly due to measurement or entering errors) were detected using the automatic multivariate outlier detection by the EM-estimation method for correlations as implemented in SYSTAT 11.0. A total of six cases (all without mites) were removed *a priori* (3% of total). Cumulative probability plots of the log-transformed, checked data and residuals from statistical models closely resembled the straight-line expectation for normal distributions, and no automatic warnings for outliers were issued during the subsequent statistical analyses.

Before testing which biometric variables were statistically associated with infection by mites, we first explored which other, potentially confounding factors were in turn associated with the biometric variation. Therefore, we first performed a MANOVA on the biometric variables with month, sex, age, direction of bill crossing and vocal type as independent variables.

In order to enhance comparison with the results of Benkman *et al.* (2005), we present the results of univariate ANOVAs to see whether birds with mites differed in biometry from birds without mites, and whether there was an interaction between mites and sex (while correcting for the effects of sex and vocal types).

Next, because scaly leg mites have been shown to depress survival in both studies that investigated this aspect, one can view the correlations between presence of mites and biometry as estimates of natural selection on biometry (note that this excludes any synergistic effects of sexual selection, which may act against infected individuals in poor condition because sexual ornamentation is often condition dependent: see Van Doorn, Edelaar & Weissing, 2009). The invading crossbills were highly mobile and transient, and so recapture rates were too low to calculate differential survival. However, based on Latta (2003), we assume that all infected birds die within a year and have a fitness of zero, and non-infected birds have a fitness of one. This provides us with an upper bound to the strength of selection. Because fitness is then a binary variable, we estimated the effect and significance of each trait on infection with mites separately using univariate logistic regression. We estimated logistic regression parameters and their significance corrected for month, sex and vocal type (because these were found to have an influence on morphology: see below). We transformed the parameter estimates from these logistic regressions into an 'approximate selection gradient' (total selection on phenotypes, so including direct and indirect selection) following Janzen & Stern (1998). Alternatively, we use the reported survival estimates from Benkman *et al.* (2005) for infected and uninfected male and female crossbills. However, for our study, these estimates are problematic in that they include the effect of bill depth – survival is more depressed by mites in large-billed birds, and males have larger bills, which causes a sex  $\times$  mites interaction in survival (Benkman *et al.*, 2005), and we should not assume this to be true *a priori* for our data. Therefore, we only use their survival estimates for the calculation of univariate selection gradients in order to compare those alternative selection estimates with our maximum selection estimates. We calculated total selection gradients using linear regression of relative fitness on standardized biometric traits following Lande & Arnold (1983), again correcting for vocal type, sex and month in all models. The use of linear regression in this case is justifiable because fitness estimates (between 0.592 and 0.754) are not close to zero and one (Janzen & Stern, 1998).

We checked for any patterns of stabilizing or disruptive versus directional selection by fitting cubic splines and bootstrapped confidence limits to the data using GLMSWIN1.0 (Schluter, 2000b), including vocal type as a covariate.

In order to uncouple direct from indirect (correlated) selection, we analysed the difference between infected and uninfected birds also by multiple logistic regression with infection as the binary dependent variable and the biometric measures as continuous independent variables. We used an exploratory stepwise approach (backwards and forwards) in combination with model comparison to deter-

mine whether a variable significantly improved the fit of the model to the data, using the significance of the change in log-likelihood.

In order to compare infection levels among crossbill populations from across the world, we calculated for each population the proportion of infected birds simply as the total number of infected birds divided by the total number of birds checked, and then calculated the overall (global) infection level as the mean of these population infection levels. We then tested whether any populations had significantly higher-than-average infection levels by calculating for each population the probability to encounter the observed number of infected birds or more, given its sample size and the overall infection probability across all populations, using a binomial probability calculator available online at <http://stattrek.com/Tables/Binomial.aspx>. For our low overall infection level, sample sizes were too small to detect populations with significantly lower-than-average infection levels (for a two-sided test, a minimum sample size of 56 uninfected birds was required to conclude a significantly lower infection level); therefore, we only tested whether populations had a significantly higher-than-average infection level.

All analyses other than cubic spline fitting and binomial probability calculations were performed in SYSTAT 11.0.

## Results

An exploratory MANOVA ( $n = 173$ ) revealed significant effects on biometric variation due to month (Wilks'  $\lambda = 0.48$ ,  $P < 0.0001$ ), sex ( $\lambda = 0.48$ ,  $P < 0.0001$ ), bill crossing direction ( $\lambda = 0.43$ ,  $P < 0.0001$ ) and vocal type ( $\lambda = 0.79$ ,  $P = 0.003$ ); age did not have a significant effect ( $\lambda = 0.96$ ,  $P = 0.69$ ). These results suggest that we may need to take their effects into account when testing for effects of biometry on presence of mites. Moreover, these factors by themselves may be linked to presence of mites.

Exploratory analyses indeed showed a highly significant effect of age on presence of mites (logistic regression,  $n = 173$ ,  $P = 0.0079$ ). In fact, only two out of 72 (2.7%) second plumage (first-year) birds had mites, versus 75 out of 216 (25.8%) older birds (Pearson's  $\chi^2 = 18.9$ ,  $P < 0.0001$ ). To increase the power of subsequent analyses, we continued only with birds older than 1 year. Direction of bill crossing was the least related to presence of mites in older birds ( $n = 139$ ,  $P = 0.92$ ) and this variable had missing data for a fair proportion of birds in the dataset. Omitting this variable increased the available sample size for further tests to 200 birds [of which 50 (25%) had mites]. In all subsequent analyses, we use this same set of birds ( $n = 200$ ) for all statistical tests in order to keep the results and statistical estimates comparable across models.

### Effect of morphology on mite infection

Univariate ANOVAs (correcting for biometric differences between vocal types) showed that live-caught Dutch crossbills with mites had significantly longer wings and tarsi than

birds without mites (tarsus n.s. after Bonferroni correction). The interaction between mite infection and sex was never significant (Table 1).

Selection due to mites acted strongest against individuals with long wings, but there was also significant selection (or nearly so) against deep bills, long tarsi and large body mass (Table 2; results corrected for effects of sex, month and vocal type). In fact, all selection coefficients were negative, indicating that selection by mites in general favoured birds with smaller measures for all eight biometric traits. When assuming the milder effects of mites on survival as reported by Benkman *et al.* (2005), this resulted in considerable weaker selection gradients, and only selection on wing length remained significant. However, all selection gradients continued to be negative. Visualization of selection by cubic spline plots (not shown) confirmed that selection was directional, not stabilizing or disruptive, for each of the eight biometric traits.

In the more inclusive multiple logistic regressions, presence of mites was significantly explained by wing length

( $P = 0.014$ ) and vocal type ( $P = 0.031$ ), and nearly so by tarsus length ( $P = 0.076$ ). The remaining six biometric variables were not statistically significant (all  $P > 0.53$ ). The effects of month ( $P = 1.00$ ), sex ( $P = 0.28$ ) or month and sex together ( $P = 0.99$ ) were not significant, and hardly changed the significance of wing length, vocal type and tarsus length. Models only involving vocal type and wing length (hence ignoring the non-significant trend for tarsus length) did not show significant effects of the square of wing length (quadratic effect:  $P = 0.70$ ) or the interaction between wing length and vocal type ( $P = 0.40$ ). The interaction between wing length and sex was also not significant ( $P = 0.21$ ).

Hence, birds with longer wings were more likely to have mites (Fig. 1), and type C birds were less likely to have mites (18/97 = 18.6%) than type X birds (23/79 = 29.1%) and type A birds (9/24 = 37.5%). The fit of this final model was acceptable by all available tests (Hosmer–Lemeshow  $P = 0.59$ , Pearson's  $P = 0.45$ , deviance  $P = 0.21$ ). However, the total explanatory power of the model was limited, with

**Table 1**  $P$ -values for the effects of mites *Knemidokoptes jamaicensis*, sex and vocal type on variation in each of eight different morphological traits (ANOVA)

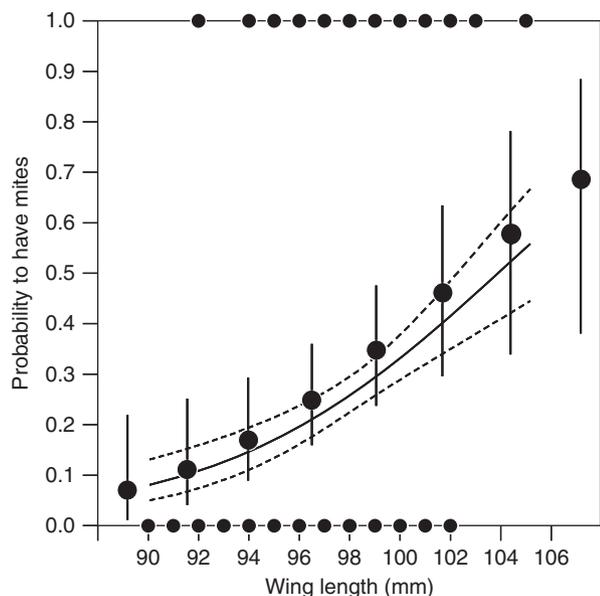
Dependent variables	Explanatory variables			
	Mites	Sex	Mites × sex	Vocal type
Mass	0.14	<0.0001	0.93	0.0004
<b>Wing</b>	<b>0.0045</b>	<0.0001	0.42	0.28
<b>Tarsus</b>	<b>0.0281</b>	0.29	0.83	0.17
Head + bill	0.19	<0.0001	0.46	0.0004
Upper length	0.40	<0.0001	0.78	0.68
Lower length	0.98	0.0001	0.40	0.57
Depth	0.15	<0.0001	0.45	<0.0001
Width	0.44	<0.0001	0.39	0.0013
Degrees of freedom	1,194	1,194	1,194	2,194

The bottom line gives the degrees of freedom used for each test ( $n=200$ ). Traits significantly related to presence of mites in bold.

**Table 2** Estimates of total univariate (direct plus indirect) directional selection on eight biometric variables due to infection by scaly leg mites *Knemidokoptes jamaicensis* (corrected for the effects of sex, vocal type and month)

Variables	Based on logistic regression		Based on linear regression	
	$\beta_{\text{approx}}$	$P$ -value	$\beta$	$P$ -value
Mass	-0.078	0.056	-0.010	0.12
Wing	<b>-0.173</b>	<b>0.005</b>	<b>-0.020</b>	<b>0.006</b>
Tarsus	<b>-0.073</b>	<b>0.049</b>	-0.008	0.13
Head + bill	-0.050	0.24	-0.009	0.15
Upper length	-0.026	0.56	-0.004	0.56
Lower length	-0.004	0.92	-0.002	0.73
Depth	<b>-0.143</b>	<b>0.014</b>	-0.011	0.11
Width	-0.051	0.19	-0.009	0.12

Left columns indicate approximate selection gradients based on logistic regression, assuming all infected birds die and have a fitness of zero, versus one for uninfected birds. Right columns indicate selection gradients based on linear regression, assuming fitness as equivalent to average survival of infected and uninfected crossbills as reported in Benkman *et al.* (2005). For each variable, we give its selection gradients and associated  $P$ -values (significant values in bold, nearly significant values in italics).



**Figure 1** Crossbills *Loxia curvirostra* with longer wings are more likely to show signs of infection with scaly leg mites *Knemidokoptes jamaicensis*. Given are the fitted cubic spline  $\pm 1$  SE following Schluter (2000b) (solid and dotted curves), and the estimated mean occurrence with 95% confidence interval (large dots with vertical bars) based on our best logistic regression model comprising the effects of wing length and vocal type. Small dots are actual observations of birds with ( $n=50$ ) or without mites ( $n=150$ ).

McFadden's  $\rho^2 = 0.058$  (0.20 would be a good model) and a total proportion of successfully predicted cases of 64.9%.

### Global population differences in occurrence of mites

As shown above for the sample of crossbills from the Netherlands, the presence of mites differed dramatically between age classes in the other populations from across the world. Combining the three populations showing the highest proportion of mite infection, the percentage of individuals infected in juveniles was 0.00% (0/18), in second plumage birds it was 6.25% (1/16) and in birds after their second plumage it was 29.8% (14/47). Because population samples differed in age composition, next we only focused on individuals after their second plumage to improve estimates of differences in the proportion of birds infected among populations. The overall level across populations of mite infection among these older birds was 5.28% (Table 3). With this average infection level, three populations stood out as having significantly higher levels of infection: Corsica, southern Italy and Cyprus (Table 3).

Combining these three populations with the highest proportion of infected birds (in order to get enough infected birds in the sample), we did not find statistical support for morphology explaining the presence of mites (i.e. no current selection on morphology due to mites – results not shown). This sample did confirm our earlier result of the absence of a

difference between the sexes in infection rates ( $P = 0.79$ ): the proportion of infected individuals was 30.8% (4/13) in females and 29.4% (10/34) in males.

### Discussion

Perhaps most importantly, our results confirm the surprising and little-understood relationship between the morphology of crossbills and the likelihood to be infected with scaly leg mites. When analysed in isolation (and after correcting for effects of month, sex and vocal type, Table 2), we confirm the significant relationship between bill depth and infection as found by Benkman *et al.* (2005). Because these mites were more common in birds with deeper bills and assuming that mites cause a 100% mortality as found by Latta (2003), this implies that mites can have antagonistic selective effects on bill traits that optimize food intake rate.

The multivariate analysis indicates that in our dataset, the selection against deep bills is probably largely explained as an indirect effect through its correlation with wing length. Nonetheless, the estimated univariate approximate selection gradients represent the overall strength of selection that acts on each trait and in principle an evolutionary response would be expected, be it by direct or indirect selection (under the usual assumption of positive heritabilities, and similarity of phenotypic and genetic covariance matrices – see Summers, Dawson & Phillips (2007) and Stepan, Phillips & Houle (2002) for support). The selection gradients of all eight measured biometric variables were in the same direction, suggesting that selection by mites in general favoured birds with smaller values for any trait. Our results therefore lend partial support to the hypothesis of Benkman *et al.* (2005), which indicates that not the size of any particular trait but the overall size is (somehow) mechanically involved in explaining the presence of mites, perhaps through its correlation with (unmeasured) hormonal or immunological parameters (Benkman *et al.*, 2005). Note that assuming a milder mortality due to mites yielded the same estimates of direction of selection and remained significant for wing length.

Benkman *et al.* (2005) found that male crossbills had a higher probability of infection than females, and that the relation between infection by mites and morphology differed between the sexes. We did not find the same results for the sample from the Netherlands (Table 1), nor for the three combined Mediterranean samples with the highest infection level. It therefore seems that a reduction in sexual dimorphism due to increased mortality of larger males as reported by Benkman *et al.* (2005) may not be a general feature of this host–parasite interaction.

We did find that rate of infection increased dramatically with age. This may suggest that birds do not become infected by their parents as nestlings, but become infected at a later age. Alternatively, it may take over 1 year for an infection to become visually detectable. We cannot exclude the possibility that the rate of infection keeps increasing with age and that, despite our focus on older birds only, some of the results are confounded if there are age differences among

**Table 3** Variation among crossbill *Loxia* populations in the proportion of individuals<sup>a</sup> infected with scaly leg mites *Knemidokoptes jamaicensis*

Population <sup>b</sup>	Resource	Sample size	# Infected	Proportion	<i>P</i> -value <sup>c</sup>
<i>Mediterranean</i>					
<i>L. c. balearica</i>	<i>Pinus halepensis</i>	11	0	0.00	1.00
<i>L. c. corsicana</i>	<i>Pinus nigra</i>	7	4	0.57	<b>0.00024</b>
<i>L. c. guillemardi</i>	<i>P. nigra</i>	22	4	0.18	<b>0.027</b>
<i>L. c. poliogyna</i>	<i>Pinus halepensis</i>	21	0	0.00	1.00
<i>L. c.</i> Alicante, Spain	<i>P. halepensis</i>	12	0	0.00	1.00
<i>L. c.</i> Pyrenees, Spain	<i>Pinus uncinata</i>	13	1	0.08	0.51
<i>L. c.</i> Calabria, Italy	<i>P. nigra</i>	18	6	0.33	<b>0.00023</b>
<i>Northern Europe</i>					
<i>L. (p.) pytyopsittacus</i>	<i>Pinus sylvestris</i>	8	0	0.00	1.00
<i>L. (p.) scotica</i>	<i>P. sylvestris?</i>	21	2	0.10	0.31
<i>Asia</i>					
<i>L. c.</i> 'himalayensis'	<i>Tsuga/Larix?</i>	45	1	0.02	0.91
<i>L. c. japonica</i>	<i>Picea jezoensis?</i>	43	0	0.00	1.00
<i>L. c. luzoniensis</i>	<i>Pinus kesiya?</i>	12	0	0.00	1.00
<i>L. c. meridionalis</i>	<i>P. kesiya</i>	28	0	0.00	1.00
<i>L. c.</i> East Asia	unknown	7	0	0.00	1.00
<i>L. (l.) bifasciata</i>	<i>Larix russica</i>	17	0	0.00	1.00
<i>America</i>					
<i>L. c. mesamericana</i>	<i>Pinus caribaea</i>	13	0	0.00	1.00
<i>L. c. perna</i>	<i>Picea mariana</i>	23	0	0.00	1.00
<i>L. c.</i> type 2	<i>Pinus ponderosa</i>	26	1	0.04	0.76
<i>L. c.</i> type 3	<i>Tsuga heterophylla</i>	30	0	0.00	1.00
<i>L. c.</i> type 4	<i>Pseudotsuga mensiezii</i>	21	0	0.00	1.00
<i>L. c.</i> type 5	<i>Pinus contorta</i>	24	0	0.00	1.00
<i>L. c.</i> type 6	<i>Pinus oocarpa?</i>	36	0	0.00	1.00
<i>L. (l.) leucoptera</i>	<i>Picea</i> spp.	40	0	0.00	1.00
<i>L. (l.) megaplaga</i>	<i>Pinus occidentalis</i>	13	0	0.00	1.00
Total		506	19	0.0528	–

For each population sample, we give its taxonomic assignment, the (putative) food resource it depends upon, the number of individuals checked (samples <5 excluded), the number of individuals visibly infected with mites, the proportion of individuals infected and the cumulative *P*-value (significant *P*-values in bold) to find that same number or more infected individuals, given the observed overall proportion of infected individuals presented at the bottom.

<sup>a</sup>Only older birds = those that have completed a full post-breeding moult.

<sup>b</sup>*L. c.* = *Loxia curvirostra*, *L. c.* type 2–6 = five taxonomically unassigned populations (possibly biological species), *L. (l.)* = the superspecies *Loxia leucoptera*, *L. (p.)* = the superspecies *Loxia pytyopsittacus*. *L. c.* 'himalayensis' includes both *L. c. himalayensis* and *L. c. bangsi* (Edelaar, 2008b).

<sup>c</sup>For all samples without infected birds, the *P*-value is 1.00 because we only tested whether the proportion of infected birds in a population was higher than the overall proportion of 0.0528.

samples (sexes, vocal types, different populations across the world). This is a potential problem that also affects previous studies on scaly leg mites (e.g. Latta, 2003; Benkman *et al.*, 2005) or on other parasites. Age-specific effects of parasites and age-related malformation due to parasite infestation are known from other studies. For example, Galligan & Kleindorfer (2009) showed permanent effects on bill size and shape in a Darwin's finch due to infection with the parasitic fly *Philornis downsi* that is exclusive to the nestling stage.

Vocal type C birds had a significantly lower likelihood to be infected with mites. This observation strengthens the interpretation that these populations are independent biological entities despite their sympatric occurrence. On the other hand, it provides the possibility that (a part of) the morphological difference reported between type C and type A/X could be attributed to a stronger selection against large

birds in the more infected type A/X birds, and not to adaptation to differential resource use (Edelaar *et al.*, 2008).

We found that the presence of mites can differ dramatically among allopatric populations (Table 3). Benkman *et al.* (2005) attribute an observed decline in male bill depth of 0.12 mm in just a few years to mite infection. This decline in bill depth represents a sizeable proportion of the general difference in bill depth among ecologically specialized North American populations (around 0.3 mm: Benkman, 1993; Groth, 1993). Relatively large effects of selection due to mite infection, which differs across populations, could confirm our interpretation of patterns of morphological adaptation using a resource-based framework.

It is also apparent from our data that the highest levels of mite infestation were observed in all three populations utilizing Black pine *Pinus nigra*, whereas no mites were

observed in nearby populations utilizing Aleppo pine *Pinus halepensis* (the sample size for Corsica was small, but high infection rates here are supported by finding 1/5 live birds infected in 2007). This suggests that shifts in morphology due to selection by mites might be systematically related to particular ecologies/resources. If so, then this represents another argument why overall patterns within the crossbill radiation might be influenced by mites.

However, longer term effects on crossbill morphology due to infection with mites depend on consistency of the kinds of effects reported here and by Benkman *et al.* (2005). As parasite virulence may reduce after a new host is colonized, this is not guaranteed. In fact, the lack of a relationship between morphology and infection in the museum samples suggests that selective effects may not be constant across populations or across time. On the other hand, our data do not suggest that mites necessarily go through populations as short-term epidemics. For the population from Calabria, we found infected specimens across the entire collection range (24 years from 1939 to 1963), and the same for Corsica (42 years from 1884 to 1926; even extended to 123 years if we include the field observation of mites in 2007), with no clear changes in rates of infection over time.

Overall, both this study and that of Benkman *et al.* (2005) caution that historic (but unknown) levels of infection by scaly leg mites might have significantly altered the patterns of differentiation in bill traits in a system that otherwise is a classic example of morphological adaptation to resource use (Lack, 1944; Newton, 1972; Krebs, 1991; Benkman, 1993, 2003; Groth, 1993). We have shown here that infection by mites is related to morphological traits, that this relationship predicts not stabilizing but directional selection on morphology and that the rates of infection differ between populations, perhaps because of their ecology. The relationship between infection by scaly leg mites and ecologically important morphological traits in crossbills is not well understood and may seem unlikely, but we showed it to be a repeatable pattern for some populations. Parasites have been implicated in population differentiation and even speciation due to their effects on a range of traits such as resistance (Buckling & Rainey, 2002; Eizaguirre *et al.*, 2009), age at maturation (Maccoll, 2009), sexual ornamentation (Hamilton & Zuk, 1982) and habitat use (Piersma, 1997); therefore, the capacity of parasites to drive or constrain the evolution of ecological traits during adaptive population differentiation is perhaps more overlooked than unusual and warrants further investigation in additional systems.

## Acknowledgements

We would like to thank Magnus Robb for confirming identification of recordings to vocal type and many useful discussions, Mats Björklund for comments on the paper and hosting, Danny Pence and Herman Cremers for information on identification and effects of scaly leg mites, Craig Benkman and four anonymous reviewers for useful comments

and questions, and the American Museum of Natural History, the British Museum of Natural History, the Field Museum of Natural History, the Louisiana State University Museum of Natural Science, the Milan Museum of Natural History, the Museum of Comparative Zoology, the Museum of Vertebrate Zoology, the Smithsonian National Museum of Natural History, the Naturalis National Museum of Natural History, the Peapody Museum of Natural History and the Texas Cooperative Wildlife Collections for loans or access to specimens. The study was financially supported by a European Commission Marie-Curie OIF grant to P.E. (this paper reflects the author's opinion only, and the EU denies any responsibility for use of this information).

## References

- 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 selection drives the adaptive radiation of crossbills. *Evolution* **57**, 1176–1181.
- Benkman, C.W., Colquitt, J.S., Gould, W.R., Fetz, T., Keenan, P.C. & Santisteban, L. (2005). Can selection by an ectoparasite drive a population of red crossbills from its adaptive peak? *Evolution* **59**, 2025–2032.
- Buckling, A. & Rainey, P.B. (2002). The role of parasites in sympatric and allopatric host diversification. *Nature* **420**, 496–499.
- Carothers, S.W. & Sharber, N.J. (1974). Scaly-leg (Knemidokoptiasis) in a population of Evening Grosbeaks. *Wilson Bull.* **86**, 121–124.
- Edelaar, P. (2008a). Assortative mating also indicates that common crossbill *Loxia curvirostra* vocal types are species. *J. Avian Biol.* **39**, 9–12.
- Edelaar, P. (2008b). 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., Summers, R. & Iovchenko, N. (2003). The ecology and evolution of crossbills *Loxia* spp.: the need for a fresh look and an international research program. *Avian Sci.* **3**, 85–93.
- Edelaar, P. & Terpstra, K. (2004). Is the nominate subspecies of the Common Crossbill *Loxia c. curvirostra* polytypic? I. Morphological differences among years at a single site. *Ardea* **92**, 93–102.
- Edelaar, P., Van Eerde, K. & Terpstra, K. (2008). Is the nominate subspecies of the common crossbill *Loxia c. curvirostra* polytypic? II. Differentiation among vocal types in functional traits. *J. Avian Biol.* **39**, 108–115.
- Eizaguirre, C., Lenz, T.L., Traulsen, A. & Milinski, M. (2009). Speciation accelerated and stabilized by pleiotropic major histocompatibility complex immunogenes. *Ecol. Lett.* **12**, 5–12.

- Galligan, T.H. & Kleindorfer, S. (2009). Naris and beak malformation caused by the parasitic fly, *Philornis downsi* (Diptera: Muscidae), in Darwin's small ground finch, *Geospiza fuliginosa* (Passeriformes: Emberizidae). *Biol. J. Linn. Soc.* **98**, 577–585.
- Gavrilets, S. & Losos, J.B. (2009). Adaptive radiation: contrasting theory with data. *Science* **323**, 732–737.
- Groth, J.G. (1993). *Evolutionary differentiation in morphology, vocalizations, and allozymes among nomadic sibling species in the North American red crossbill (Loxia curvirostra) complex*. Berkeley: University of California.
- Hamilton, W.D. & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384–387.
- Janzen, F.J. & Stern, H.S. (1998). Logistic regression for empirical studies of multivariate selection. *Evolution* **52**, 1564–1571.
- Kirmse, P. (1966). Cnemidocoptic mite infestations in wild birds. *Bull. Wildl. Dis. Assoc.* **2**, 86–99.
- Krebs, J.R. (1991). The case of the curious bill. *Nature* **349**, 465.
- Lack, D. (1944). Correlation between beak and food in the crossbill, *Loxia curvirostra* Linnaeus. *Ibis* **86**, 552–553.
- Lande, R. & Arnold, S.J. (1983). The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226.
- Latta, S.C. (2003). Effects of scaly-leg mite infestations on body condition and site fidelity of migratory warblers in the Dominican Republic. *Auk* **120**, 730–743.
- Lessells, C.M. & Boag, P.T. (1987). Unrepeatable repeatabilities – a common mistake. *Auk* **104**, 116–121.
- Losos, J.B. & Miles, D.B. (2002). Testing the hypothesis that a clade has adaptively radiated: iguanid lizard clades as a case study. *Am. Nat.* **160**, 147–157.
- Maccoll, A.D.C. (2009). Parasites may contribute to 'magic trait' evolution in the adaptive radiation of three-spined sticklebacks, *Gasterosteus aculeatus* (Gasterosteiformes: Gasterosteidae). *Biol. J. Linn. Soc.* **96**, 425–433.
- Newton, I. (1972). *Finches*. London: Collins.
- Owen, J.P., Delany, M.E., Cardona, C.J., Bickford, A.A. & Mullens, B.A. (2009). Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*). *Int. J. Parasitol.* **39**, 789–799.
- Pence, D.B., Cole, R.A., Brugger, K.E. & Fischer, J.R. (1999). Epizootic podoknemidokoptiasis in american robins. *J. Wildl. Dis.* **35**, 1–7.
- Piersma, T. (1997). Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? *Oikos* **80**, 623–631.
- Robb, M. (2000). Introduction to vocalizations of crossbills in north-western Europe. *Dutch Birding* **22**, 61–107.
- Schluter, D. (2000a). *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Schluter, D. (2000b). Estimating fitness functions using the cubic spline: glms 4.0 and glmsWIN 1.0. Available at <http://www.zoology.ubc.ca/~schluter/software.html#spline> (accessed 12 September 2009)
- Steppan, S.J., Phillips, P.C. & Houle, D. (2002). Comparative quantitative genetics: evolution of the G matrix. *Trends Ecol. Evol.* **17**, 320–327.
- Summers, R.W., Dawson, R.J.G. & Phillips, R.E. (2007). Assortative mating and patterns of inheritance indicate that the three crossbill taxa in Scotland are species. *J. Avian Biol.* **38**, 153–162.
- Svensson, L. (1992). *Identification guide to European passerines*. Stockholm: Svensson.
- Van Doorn, G.S., Edelaar, P. & Weissing, F.J. (2009). On the origin of species by natural and sexual selection. *Science* **326**, 1704–1707.