

Bigger or long-winged male common crossbills exhibit redder carotenoid-based plumage coloration

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Abstract

Carotenoid-based ornaments are often considered reliable (honest) individual condition signals because their expression implies physiological costs unaffordable for low-quality animals (handicap signals). Recently, it has been suggested that efficient cell respiration is mandatory for producing red ketocarotenoids from dietary yellow carotenoids. This implies that red colorations should be entirely unfalsifiable and independent of expression costs (index signals). In a precedent study, male common crossbills, *Loxia curvirostra*, showing a red plumage reported higher apparent survival than those showing yellowish-orange colors. The plumage redness in this species is due to ketocarotenoid accumulation in feathers. Here, we correlated the male plumage redness (a 4-level visual score: yellow, patchy, orange, and red) and the body morphology in more than 1,000 adult crossbills captured in 3 Iberian localities to infer the mechanisms responsible for color evolution. A principal component analysis summarized morphometry of 10 variables (beak, wing, tarsus length, etc.). The overall body size (PC1) and the length of flight feathers regarding body size (PC3) showed significant positive relationships with plumage redness. Plumage redness was barely correlated with bill shape measures, suggesting no constraint in acquiring carotenoids from pine cones. However, large body sizes or proportionally long flying feathers could help carotenoid acquisition via social competition or increased foraging ranges. Proportionally longer flight feathers might also be associated with a specific cell respiration profile that would simultaneously favor flying capacities and enzymatic transformations needed for ketocarotenoid synthesis. Such a phenotypic profile would agree with the hypothesis of ketocarotenoid-based colors acting as individual quality index signals.

Key words: avian carotenoids, color and biometrics, *Loxia* genus, Mediterranean crossbills, resource allocation trade-offs, shared pathway hypothesis.

In animals, the sexual selection often favors the evolution of ornaments allowing the choosy sex to estimate the quality of a potential partner (Andersson 1994). Many conspicuous colored traits may act as individual quality signals (Maynard Smith and Harper 2003; Searcy and Nowicki 2005). Colored traits evolve as signals if they must transmit reliable information that should, in some way, favor the receiver's fitness (Maynard Smith and Harper 2003). It has been hypothesized that the reliability of the information transmitted by these signals is assured by production costs, which would be disproportionately higher for low-quality individuals (i.e., “handicap signals”; reviewed in Weaver et al. 2017; Penn and Számadó 2020).

In vertebrates, carotenoid pigments give color to many yellow-to-red traits in the skin, scales, or feathers (McGraw 2006; Svensson and Wong 2011; Olsson et al. 2013). The animals need to acquire these compounds from food, as the organism cannot produce them de novo (McGraw 2006; Britton et al. 2009). Carotenoids are supposedly scarce in food resources (Endler 1980; Hill et al. 2002; McGraw 2006). Furthermore, carotenoids may contribute to maintaining homeostasis, acting as antioxidants and immune boosters (e.g., McGraw

2006; Pérez-Rodríguez 2009; Weaver et al. 2018; but see also Costantini and Møller 2008; Simons et al. 2012). Thus, it has been hypothesized that high-quality individuals would be better able to face the trade-off between acquiring and investing dietary carotenoids in coloration versus homeostasis (e.g., Møller et al. 2000; Alonso-Alvarez et al. 2008; Michael et al. 2018). The costs of solving that trade-off would guarantee the reliability of carotenoid-based traits as signals of individual quality (reviewed in Koch and Hill 2018).

Alternatively, the reliability of animal signals can be due to expression constraints. In that case, the animals could not be able to manipulate the above-cited trade-off plastically. We should consider that, for instance, some individuals could be able to perform a high investment in signal expression by delaying the physiological costs to subsequent reproductive stages. Accordingly, they would reduce its contribution to reproduction, thus letting down its mate. However, if strong physiological constraints in trait expression are present, the individuals would be forced to express the signal in only one mode, preventing any deception during the signal information transfer (Maynard and Harper 2003). These unfalsifiable traits were defined as index signals (Maynard and Harper

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2003; Biernaskie et al. 2014; Weaver et al. 2017). Certain carotenoid-based colorations could meet this criterion. Although animals cannot synthesize carotenoids from simpler molecules, they can transform yellow dietary carotenoids into red ketocarotenoids by enzymatic oxidation (McGraw 2006). This transformation seems to be made in the inner mitochondrial membrane, thus intimately linked to cell respiration enzymes (Hill et al. 2019; Cantarero and Alonso-Alvarez 2017; Cantarero et al. 2020). Therefore, only those individuals with efficient mitochondrial oxidative enzymes would be able to produce enough red pigments (see the “shared-pathway” hypothesis in Hill 2011; see also Weaver et al. 2018). In this hypothesis, the red coloration would reveal the individual quality in an unfalsifiable way (as an index signal).

In the present study, we explore the natural covariation of the body morphology and plumage redness of more than 1,000 male common crossbills, *Loxia curvirostra*, sampled in the wild. The role of plumage coloration in mate choice or intrasexual competition in this species still requires to be addressed by behavioral studies. However, in a closely related passerine (the house finch; *Haemorrhous mexicanus*; e.g., Payevsky 2015) with similar plumage color variability and the same carotenoid transformation pathway (yellow β -cryptoxanthin to red 3-hydroxy-echinenone; Hill and Johnson 2012; note the huge variability in carotenoid molecules among bird species; McGraw 2006), females prefer redder males as mates (Hill 1990), and redder males have better reproductive success and survival than paler animals (Hill et al. 1999). According to the latter point, we have also found higher apparent survivorship (recapture probability) of redder males in our crossbill populations (Fernández-Eslava et al. 2021, 2022).

The correlation between the level of expression of ornamental traits and body morphology is a recurrent question in evolutionary biology (e.g., reviews in Maynard Smith and Harper 2003; Cotton et al. 2004). Here, the current knowledge on crossbills' morphology and coloration may allow deducing the potential role of the above-cited mechanisms in the evolution of plumage coloration. Thus, the common crossbill bill morphology seems to have coevolved with the shape of cones from different pine species (e.g., Benkman 1987, 1993, 2010; Groth 1996; Benkman 2003). The bill shape would thus determine which tree species an individual preferably feeds on (Benkman 1993; Edelaar and Benkman 2006; Alonso et al. 2020). Moreover, we know that the carotenoid type and its concentration differ among pine species foraged by crossbills (del Val, Senar, et al. 2009). Hence, a correlation between bill morphology and plumage coloration in crossbills might arise if coloration depends on carotenoid acquisition.

On the other hand, in an early study, Völker (1957) proposed that the red carotenoid-based coloration of male common crossbills might depend not only on the diet composition but also on flying activity. Völker (1957) observed that captive male crossbills changed the initial red color to a yellow plumage after their molt, even when provided the same food they ate in freedom. He suggested that red carotenoid-based coloration could be produced from dietary yellow carotenoids if these pigments are oxidized by the animal's metabolism (see also Hill and Johnson 2012). In particular, he proposed that this oxidation should be favored by the flying metabolism, which is prevented under captivity (also Weber 1961). Interestingly, the flying effort has repeatedly been related to a

higher oxidative metabolism in avian species (Costantini et al. 2008; Jenni-Eiermann et al. 2014; Yap et al. 2017).

In general, species, populations, or individuals with proportionally longer wings are better adapted to flight and travel longer distances (Winkler and Leisler 1992; Förschler and Bairlein 2011; Nowakowski et al. 2014). Common crossbills from the circum-Mediterranean region show a trend toward being less red and more sedentary than those from northern Europe (Massa 1987; Senar et al. 1993; Newton 2006; Marquiss et al. 2012; Alonso et al. 2017; Parchman et al. 2018), also showing shorter wings (Cramp and Perrins 1994). Interestingly, longer-wing males were redder than shorter-wing males in a Spanish Pyrenees' crossbill population (Alonso and Arizaga 2013). However, that study was not conclusive since yearlings and adults were analyzed together, the first being often yellow, also presenting shorter wings than adults (Arizaga et al. 2006). Moreover, to attribute the relationship to variability in flying capacity, the feather length variability must be corrected for the size of other body measurements because the wing length is inevitably correlated to body size (Gosler et al. 1998). Furthermore, the body size might explain plumage coloration by itself. Bigger animals could gain better access to food resources due to their presumably higher competitive abilities (e.g., in passerines: Baker and Fox 1978; Richner 1989; Koivula et al. 1993; Funghi et al. 2015).

Here, we tested the potential correlation between the redness of the male common crossbill plumage and individual morphology, including several bill measures, leg size (tarsus length), and flying feathers' length. A correlation between plumage redness and bill measures or overall body size would suggest that carotenoid-based coloration could depend on differential access to dietary resources. Similarly, a positive correlation between color and flying feather length controlled for body size would indicate higher mobility favoring the acquisition of dietary carotenoids (Michael et al. 2018). However, it would also support the hypothesis that pigment production depends on the individual variability in flying-related redox metabolism (i.e., Völker 1957; Hill 2011).

Materials and Methods

Sampling procedure

Crossbills were captured in 3 localities in northern Spain. These localities described a NE to SW axis within a maximum distance of 175 km: 1) Uztarroz (42°54'40"N, 0°59'25"W; 1,190 m a.s.l.), 2) Bigüezal (42°39'42"N, 1°09'20"W; 1,008 m a.s.l.), both in western Pyrenees (province of Navarra), and 3) El Royo (41°55'42"N, 2°39'56"W; 1,237 m a.s.l., province of Soria) situated in the Iberian System. Extensive Scot pine forests were present in the 3 sites. This pine species is naturally extended throughout Europe and the north of the Iberian Peninsula, although forestry have also expanded its distribution area (Prus-Glowacki et al. 2003).

The sampling protocol consisted of performing a trapping session every 2–4 weeks at each sampling site. Crossbills were captured using mist nets (usually 12 linear meters) that remained open 4 h from sunrise. The sampling years varied among sampling sites: 1) Bigüezal-Uztarroz, from 2001 to 2018; 2) El Royo, from 2009 to 2018. The nets were placed in places frequently used by crossbills to ingest salt (minerals).

Throughout the 18-year sampling period, 1,302 adult males presenting nonactive molt measures were captured and their

plumage color was assigned (Bigüezal, $n = 268$, Uztarroz, $n = 592$, El Royo, $n = 442$). Once captured, crossbills were ringed, and their age and sex were determined (Svensson 1998) to select only adult males. Juvenile crossbills undergo a partial molt, whereas adult crossbills do a complete molt; hence, birds with retained juvenile feathers in their wings or tails due to the partial molt were aged as first/second-year birds (1Y/2Y), whereas older birds were aged as adults (AD) (Jenni and Winkler 1994). To avoid possible biases due to the age of the birds, we only use adults (AD). The minimum age in months of adult birds was also established from their plumage features and recapture dates (method described in Fernández-Eslava et al. 2021). After aging and sexing, we also measured 9 biometric variables: wing length (± 0.5 mm, method III of Svensson 1998), tail length (± 0.5 mm), tarsus length (± 0.01 mm), head length (± 0.01 mm), beak width, depth, and length (± 0.01 mm), upper and lower mandible length (± 0.01 mm), and lower mandible width (± 0.01 mm) (see beak-associated variables in Figure 1, except beak width because of the photograph perspective). In a subsample measured twice ($n = 34$), repeatability values (R -values, following Lessells and Boag 1987) ranged from 0.80 (beak width) to 0.99 (lower mandible length) being always significant (all P -values < 0.01). We eliminated those birds molting (found them mainly between May and October) because feathers are growing (wing and tail). In addition, we also eliminated those birds found in suspended molt (that temporarily stop their molt and complete it later) (Fernández-Eslava et al. 2020) because the color at this stage can vary until the end of their molt.

Color phenotype assessment

Adult males' color was determined by using a 4-scaled classification: yellow, patchy (i.e., plumage always with yellow feathers in addition to red or orange patches), orange, and red birds (Figure 2; numbered from 1 to 4 values, respectively). The birds classified as orange may show some red feathers but not yellow patches, and the orange feathers prevail. This simple classification procedure allowed us to take a consistent color measure during an extended period (18 years) to obtain a large sample size of adult non-molting males. This color variable strongly correlates with objective color measures (del Val, Borràs, et al. 2009; see also Supplementary Material). The ordering of the 4-color categories was also coherent with the concentration of the main red

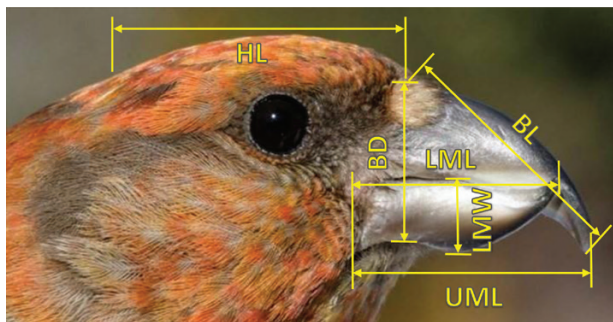


Figure 1. Some of the beak-associated measures taken on crossbills: HL, head length; BL, beak length; BD, beak depth; UML, upper mandible length; LML, lower mandible length; LMW, lower mandible width (photograph by J. Arizaga). Beak width (not shown) is taken with the bird in the frontal position.

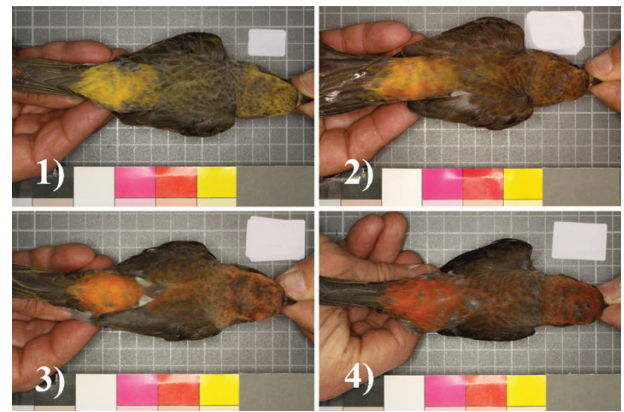


Figure 2. Plumage color scores of adult male crossbills: yellow (1), patchy (2), orange (3), and red (4).

ketocarotenoid (3-hydroxy-echinenone) in common crossbill feathers (Supplementary Material; see also Cantarero et al. 2020). Moreover, precedent work classifying a subsample of individuals in these 4 categories proved to be highly repeatable when measured twice by the same person (DA) blind to bird identity, the 2 measures separated by a week (Spearman's $r = 0.96$, $n = 47$; supplementary information in Cantarero et al. 2020). The same observer (DA) recorded all measurements in the present sample, including color classification. Adult individuals can change color during life (Cantarero et al. 2020). Because some birds were captured more than once (approximately 10%), we only analyzed the capture event when the bird presented the highest color score (i.e., repeated measures were not tested).

Statistical analyses

First, a nonparametric chi-square test was performed to test whether the frequency of each color category varied throughout the year. Then, a principal component analysis (PCA) was performed to reduce the number of morphological variables.

Precedent studies on crossbills' morphology tested beak variables separated from other body parameters (e.g., Borràs et al. 2008; Alonso et al. 2020). However, the diet composition (presumably related to beak size/shape) and flying capacity (related to flying feather length) could affect color expression simultaneously because the less nomadic birds might concurrently be adapted to feed on specific conifer species (with certain cone shapes). Therefore, determining the relevance of one mechanism (dietary or flying-related) over the other requires testing the individual's global phenotypic profile. Accordingly, our PCA included the 6 beak variables, that is, beak width, depth and length, lower and upper mandible length, and lower mandible width (see Figure 1) and the 4 body measures: head length (also Figure 1), wing length, tail length, and tarsus length. Unfortunately, not all the morphometric variables were obtained in all the birds, 1,008 individuals being finally analyzed in the PCA. Additional analyses separating beak and body variables are provided in Supplementary Materials, which allows comparing the results with previous studies (i.e., Alonso et al. 2020). We did not include body mass in the PCA as this trait exhibits a large within-individual variability throughout the lifetime (e.g., Fernández-Eslava et al. 2021). However, the alternative PCs

that included body mass provided the same findings when testing its relationship with plumage color (Supplementary Materials).

PCs with an eigenvalue > 1 (PC1–PC3) were extracted from all the crossbill morphometric variables. They were tested as covariates in generalized linear mixed models (GLMMs) that analyzed plumage coloration variability (below). The sum of the 3 main PCs explained 59% of the variance (Table 1). The PC1 showed positive factor loadings for all the variables. Therefore, it would represent an overall measure of body size. The PC2 showed negative factor loadings for the beak length and upper and lower mandible length and positive factor loadings for the rest of the measures, especially for head, wing, and tail length. Hence, birds with higher PC2 scores showed proportionally shorter bills in larger-sized heads and longer flight feathers than those with lower PC2 scores (Table 1). Finally, the PC3 showed positive loadings for wing and tail length and negative ones for some beak measures, the head and tarsus length. Higher PC3 scores thus represented longer flight feathers in birds with narrower bills, smaller heads, and shorter legs. PC2 and PC3 would describe similar flight feather lengths but different beak shapes (PC2: beak length; PC3: beak thickness) and body sizes (head and tarsus length). In PC3, the opposite sign of flight feathers (positive) and head and tarsus length (negative) suggests that this PC should better describe the flying efficiency (longer flight feathers relative to body size) compared with PC2.

To select the structure of the models for testing the relationship between color and the cited morphometry variables, we first established the probable causality among different traits. We should consider that the skeleton structure finishes its growth in about 2 months in passerines (e.g., Badyaev 2010). Therefore, the expression level (length) of morphometries (tarsus, wing, beak lengths, etc.) should be fully established earlier than the plumage color of adult males. Note that the birds in our sample were all adults. They have made at least one complete molt, so they are 13 months old minimum. Thus, it is unlikely that plumage color could affect morphometries. Instead, morphometries can reasonably influence the plumage color because the beak structure or body size can determine the feeding behavior, affecting the carotenoid content in the diet. Similarly, the wing feather length would influence the flying

effort, perhaps involving the mitochondrial metabolism (see Introduction).

Accordingly, the color score was tested as the dependent ordinal variable in GLMMs, including the morphometries as the independent (explanatory) terms (covariates). The plumage color variable was analyzed using a multinomial distribution with a cumulative logit function. The procedure GLIMMIX in SAS9.4 software was used. The dependent variable was considered an ordinal variable following a low-to-high redness scale (i.e., yellow < patchy < orange < red; 1–4 values, respectively; see also Supplementary Materials). This procedure adjusts the probability from the lower end of the color scale (yellow). Hence, the odds ratio for any morphometry covariate higher than one indicate a decrease of redness with the size of that covariate (Rodríguez 2007; Kiernan 2018; Fernández-Eslava et al. 2021). This implies that the model estimates for morphometry covariates must be interpreted in the opposite direction. Thus, the negative slopes indicate an increase in plumage redness with the size of the body measures (Kiernan 2018; see also Fernández-Eslava et al. 2021).

The capture site was tested as a fixed factor (3 levels). Alternative models, including this factor as a random term, provided the same significant correlations between color and PC1 and PC3 covariates (Supplementary Material). Only the capture year was tested as a random factor, reporting a significant effect (Table 2). We alternatively tested the year as a continuous covariate, but it did not significantly influence plumage color ($P = 0.30$). Hence, the color measurement showed differences among years but not a time-related trend (increase or decrease). Additionally, the capture month and estimated minimum age were also tested as potential covariates in the same model but again did not report any significant influence (both $P > 0.63$), being removed. Satterthwaite degrees of freedom were used.

Results

Most captures of common crossbills occurred from late winter to spring, with a peak in March in Bigüezal and April in the other 2 sampling localities (dots in Figure 3). An oscillation in the proportion of each color score among adult males captured across the year was detected (bars in Figure 3). The red phenotype was proportionally less abundant during the summer months (color score \times calendar month: $\chi^2 = 57.56$, $df = 30$, $P < 0.001$).

Table 1. Factor loadings of the 3 components with eigenvalues > 1 obtained from a PCA conducted on crossbill morphometries

| | PC1 | PC2 | PC3 |
|------------------------|------|-------|-------|
| Beak length | 0.42 | -0.39 | 0.18 |
| Upper mandible length | 0.40 | -0.34 | 0.08 |
| Lower mandible length | 0.35 | -0.38 | 0.11 |
| Lower mandible width | 0.23 | 0.13 | -0.39 |
| Beak width | 0.36 | 0.13 | -0.33 |
| Beak depth | 0.37 | 0.16 | -0.35 |
| Head length | 0.08 | 0.39 | -0.27 |
| Tarsus length | 0.30 | 0.17 | -0.13 |
| Wing length | 0.28 | 0.41 | 0.48 |
| Tail length | 0.24 | 0.43 | 0.50 |
| Standard deviation | 1.73 | 1.24 | 1.15 |
| Explained variance (%) | 30 | 15 | 13 |

Table 2. Generalized linear mixed model testing the effect of principal components derived from morphometries on plumage color variability (color score, multinomial distribution; see Statistical Analyses section; $n = 1,008$)

| Covariates | Estimate | SE | df | F | P |
|-------------|----------|-------|---------|-------|--------|
| PC1 | -0.081 | 0.036 | 1,1000 | 5.19 | 0.023 |
| PC2 | -0.057 | 0.051 | 1,1000 | 1.28 | 0.258 |
| PC3 | -0.128 | 0.054 | 1,1000 | 5.56 | 0.019 |
| Locality | | | 2,559.8 | 43.79 | <0.001 |
| Random term | Estimate | SE | Z | P | |
| Year | 0.106 | 0.063 | 1.64 | 0.047 | |

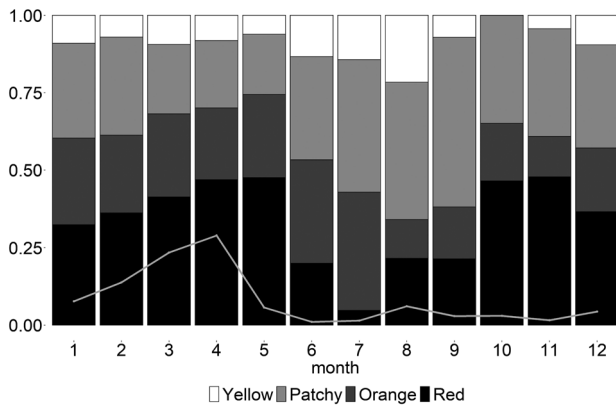


Figure 3. Percentage of captures by months (points) and by type of color in each month (bars) of male adult crossbills in the 3 sampling locations in Scot pine forests in northern Spain.

In the GLMM that tested if color variability was related to morphometries, significant effects for PC1 and PC3, but not PC2, were detected (Table 2). These effects showed a negative slope. Thus, redder birds showed larger body sizes (i.e., PC1) or proportionally longer flight feathers regarding other body measures (i.e., PC3). To illustrate this, we also reported raw data uncorrected for random effects (i.e., Figure 4A and B). Here, note that the relationship with PC1 (Figure 4A) was mostly driven by the difference between red and orange birds. Also, note that each PC is entirely independent of the other, meaning that the sample of birds driving one of these 2 relationships was not the same that the sample that favored the second correlation. PC1 and PC3 remained significant (both $P < 0.021$) when removing PC2 from the model.

The factor site was highly significant (Table 2). The proportion of red individuals decreased from NE to SW (64%, 46%, and 23% for Uztarroz, Bigüezal, and El Royo, respectively). In contrast, paler birds showed the opposite pattern: orange (12%, 24%, and 35%), patchy (22%, 21%, and 28%), and yellow (3%, 9%, and 14%, respectively). The locality factor did not significantly interact with any PC covariate (all P -values > 0.10).

To understand the locality effect, we also run GLMMs testing each PC as a normally distributed dependent variable, the locality as a fixed factor, and the year as the random term. Only the PC1 showed a trend to significance ($F_{2,835.2} = 2.73$, $P = 0.066$). The birds from El Royo (least square mean \pm SE: -0.33 ± 0.19) were significantly smaller birds than those from Bigüezal and Uztarroz (0.03 ± 0.17 and 0.03 ± 0.16 , respectively; LSD post hoc $P < 0.04$; Bigüezal vs. Uztarroz $P = 0.99$). The other 2 PCs did not show a locality effect (PC2: $F_{2,849.6} = 0.65$, $P = 0.520$; PC3: $F_{2,871.7} = 1.72$, $P = 0.179$).

Finally, we ran an alternative (simpler) model testing the standardized residuals obtained from an ordinary least squares (OLS) linear regression between the wing and tarsus lengths ($r = 0.22$, $P < 0.001$). These residuals were tested as a covariate in an alternative GLMM on the color score to clarify whether the PC3 effect indeed had a relationship between flight capacity and plumage color. In this case, 1,237 birds were tested. Agreeing with the PC3 interpretation, the cited residuals were highly correlated with color redness ($F_{1,1231} = 13.99$, $P = 0.0002$; estimate \pm SE: -0.206 ± 0.055 ; locality fixed factor: $F_{2,597.1} = 51.9$, $P < 0.001$; year random factor: $Z = 1.78$, $P = 0.038$). Redder crossbills showed proportionally longer wings (Figure 5).

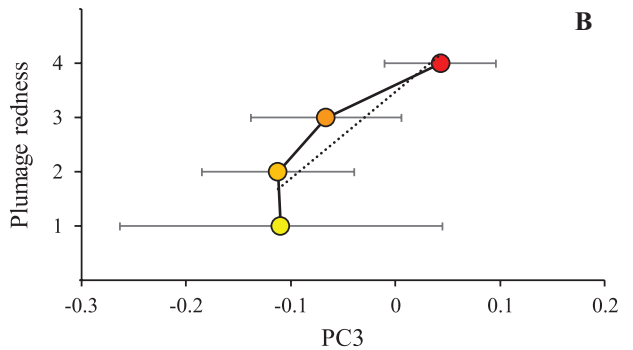
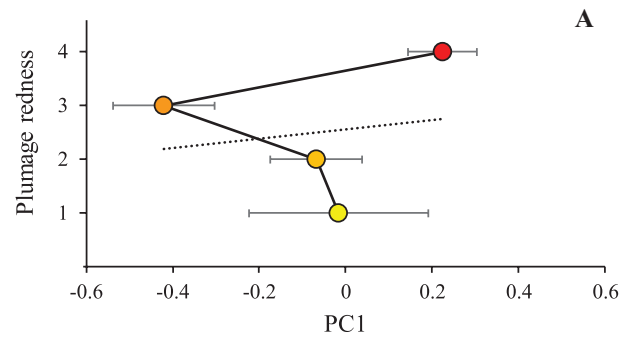


Figure 4. Variability of PC1 values with plumage redness of adult male crossbills. Means \pm SE from raw PC1 (A) and PC2 (B) values. A simple linear adjustment is added (dotted line). The slopes from the multinomial mixed model are reported in Table 2.

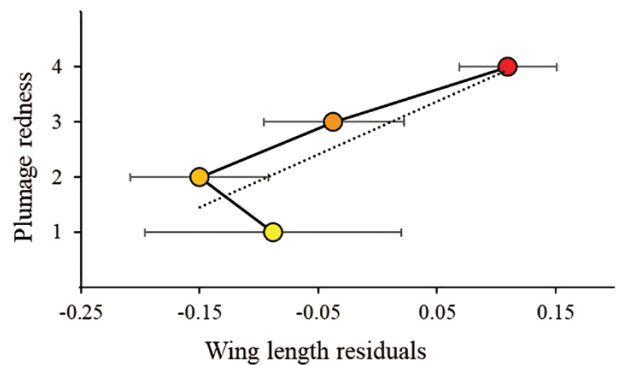


Figure 5. Relationship between plumage redness and wing length variability controlled for body size (tarsus length). The standardized residuals obtained from a regression of wing length on tarsus length were used (see Results section). Means \pm SE from raw data is reported. A simple linear adjustment (dotted line) is added. The slope from the multinomial mixed model is reported in the Results section.

Discussion

The plumage redness of adult male common crossbills was significantly correlated with body size, and also flight feather length controlled for body size variability. The results, as a whole, suggest that color expression depends on individual morphology in male common crossbills. We should remember that each PC is fully independent (uncorrelated) of the other. Hence, adult males are redder when they show one of the 2 different morphological configurations (phenotypic profiles). We should also consider that the large sample size favored detecting small effects (see the low slopes in Table 2). Nonetheless, the results were coherent with the idea of red carotenoid-based colorations signaling individual quality, the

reddest birds being bigger or showing longer flight feathers proportionally to body size.

At first glance, the relationship between color and PC1 (Table 2) indicates that larger individuals (high PC1 values) could do better in attaining the redder plumage than smaller birds. Bigger individuals could obtain more potentially limited resources such as carotenoids (e.g., Hill et al. 2002). Body size would favor competitive abilities, chasing competitors (Baker and Fox 1978; Richner 1989; Funghi et al. 2015), which could augment the intake of macronutrients. This would improve general body condition, increasing carotenoid absorption and energy needed for carotenoid metabolism (Hill 1996; McGraw 2006). Dominant access to carotenoids in food or improved assimilation would make enzymatic transformations unconstrained by resource availability. An experiment with captive red-legged partridges, *Alectoris rufa*, in which the availability of yellow carotenoids in diet was increased and oxidative stress manipulated, revealed that carotenoid enzymatic transformations could depend not only on having a well-adjusted oxidative status (Hill 2011) but also on obtaining enough high amounts of yellow carotenoid with food (García-de Blas et al. 2016). However, whether body size indeed favors food acquisition is still unknown in wild male crossbills.

Additionally, higher PC1 birds might be redder due to their origin. Some individuals might belong to other populations that evolve under different environmental conditions, perhaps with better carotenoid availability in food. The carotenoid content varies according to the pine species (del Val, Senar, et al. 2009) and the climate, with lower levels when the temperature is higher or in arid environments (Yudina et al. 2017; Sirgedaitė-šėžiene et al. 2019). Moreover, a large body size seems to be favored in colder climates to improve thermoregulation (Bergmann's rule; e.g., Salewski and Watt 2017). Interestingly, we found higher frequencies of redder birds and bigger animals in the 2 northern locations. We should also consider the low proportion of captures and the disappearance of redder birds during the summer (Figure 3; also Alonso et al. 2017). We may here interpret that big redder crossbills arriving from Central Europe could avoid the extreme conditions of the Iberian summer. The year random factor was also significant, again suggesting that color variability was influenced by the occasional arrival of birds from other populations (e.g., Cramp and Perrins 1994; Newton 2006).

Intriguingly, however, the relationship between plumage redness and body size (PC1) was not organized in the order of the color scale (i.e., yellow < patchy < orange < red; Figure 4A). It was driven by the difference between orange and red birds. We should remember that the color scale was based on objective characteristics. Patchy birds included individuals with yellow feathers, whereas orange crossbills did not show noticeable yellow patches and reported higher concentrations of red ketocarotenoids in feathers than patchy individuals (Supplementary Materials). Surprisingly, the orange birds seem to be the smallest individuals (Figure 4A). To explain this, we may turn to the hypothesis of 2 populations (i.e., itinerant big red birds and local small orange individuals) probably sharing the same habitat (see also Alonso et al. 2020). In that case, the intermediate body size of yellow and patchy birds (Figure 4A) needs to be interpreted from a life-history point of view. The plumage color of male common crossbills can change throughout the lifetime, birds becoming less red at older ages probably due to aging-related oxidative

stress constraining carotenoid conversion (Fernandez-Eslava et al. 2021). Accordingly, big red or small orange birds could become patchy or yellow animals when they age. However, the estimated age was tested and did not report a significant influence on color, and the locality factor was fixed in the models. The correlations between color and different PCs were thus theoretically independent of the locality and age effects (Table 2). Hence, the reason for the small size of orange birds remains to be explained.

Regarding the PC3, common crossbills with proportionally longer flight feathers would also have been able to attain a redder plumage. The individuals with longer flight feathers may have a better flying capacity than the short-winged birds (e.g., Fiedler 2005; von Rönn et al. 2016; de la Hera et al. 2020). This capacity could mean that redder individuals could also have a larger home range, favoring better access to carotenoid resources. The argument would be similar to that used for the case of body size (PC1; above). However, because the flying effort has repeatedly been linked to a different antioxidant status in birds (Costantini et al. 2008; Jenni-Eiermann et al. 2014; Yap et al. 2017), the result may also support the hypothesis that a better flight-related metabolism favors the oxidative enzymatic reactions needed to produce red carotenoids (i.e., Völker 1957). In that case, the carotenoid transformation rate, and the resulting red coloration, could be linked to individual quality via constraints associated with cell (mitochondrial) metabolism (Hill and Johnson 2012; Cantarero and Alonso-Alvarez 2017; Cantarero et al. 2020; Weaver et al. 2018; Hill et al. 2019). We have previously reported that redder crossbills have higher recapture probabilities (i.e., apparent survival; Fernández-Eslava et al. 2021, 2022). That result supports the hypothesis that plumage color expression is associated with individual quality in male common crossbills. However, higher survivorship of red birds could still be due to their better capacity to acquire those limiting resources favoring survival.

Regarding the latter point, the PC3 also shows that birds with longer flight feathers have smaller heads and narrow beaks, suggesting that they are adapted to feed on different trophic resources. Thus, their supposedly higher-flying capacities might have favored the adaptation to exploit a larger range of food items, narrow beaks being considered more versatile (see Badyaev 2010). Nevertheless, the PC3 loads of the beak variables are closer to zero than those obtained from flight feathers (r -values around 0.50; Table 1), thus being less relevant to explaining color variability. Furthermore, the PCs obtained only from head measurements (Supplementary Table S2) did not explain color variability (Supplementary Table S5). Moreover, the residuals of wing length on tarsus length confirmed the interpretation of flying capacity explaining plumage color variability.

To conclude, the finding that a redder plumage was correlated with longer flight feathers suggests that the expression of red carotenoid-based colorations would depend not only on obtaining carotenoid-rich resources. It could also be linked to a certain flight-related oxidative metabolism favoring enzymatic carotenoid transformation, as initially proposed by Völker (1957). However, more studies are needed to disentangle the relative importance of these 2 mechanisms in the evolution of red carotenoid-based colorations in animal species. In common crossbills, we still need to know the diet composition and migratory capacities of wild birds with different colorations and morphologies. Experimental work

is also necessary to demonstrate that the metabolism associated with the flying effort in red and yellow crossbills differs according to animal signaling theory. Experiments under captivity could address these points (e.g., Cantarero et al. 2020), but studies in free-ranging animals are preferable to demonstrate that the involved mechanisms indeed act under natural selection pressures.

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Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary Material

Supplementary data are available at *Current Zoology* online.

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