

SPECIATIONAL EVOLUTION OF COLORATION IN THE GENUS *CARDUELIS*

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Sexual selection has been hypothesized to promote speciation, but evidence relating sexual selection to differences in speciation rates among taxa is equivocal. We note that evolutionary changes in ornaments are the link connecting sexual selection to speciation, and that ornament evolution is influenced by many factors so that its relationship with the strength of sexual selection may not be linear. We test if the evolution of ornamental coloration in *Carduelis* finches is related with speciation and if more ornamented lineages speciate more. We found that coloration evolves with a speciational pattern, but we found no evidence that the evolutionary changes associated with speciation are predominantly gains in ornamentation. The speciational pattern was found for both carotenoid- and melanin-based coloration, suggesting that traits putatively under stronger sexual selection by female choice (carotenoid coloration) are not the sole ones facilitating reproductive isolation. We conclude that in the genus *Carduelis* the evolutionary lability of ornaments influences speciation more than the strength of sexual selection, and we suggest that ornament lability should be considered as a possible causal factor in studies comparing cladogenesis among taxa.

KEY WORDS: *Carduelis*, coloration, ornaments, sexual selection, speciation.

The role of sexual selection in speciation is contentious. It has long been hypothesized that sexual selection promotes speciation, as many closely related species differ in sexual ornaments but are otherwise very similar (e.g., Darwin 1871; West-Eberhard 1983; Danley and Kocher 2001). However, the comparative evidence for a relationship between strength of sexual selection and cladogenesis is equivocal. In birds, some studies comparing sister taxa found that groups under stronger sexual selection speciate more (Barraclough et al. 1995; Mitra et al. 1996; Møller and Cuervo 1998; Owens et al. 1999), but larger studies that used information from entire phylogenies did not find evidence for this (Morrow et al. 2003a,b; Phillimore et al. 2006). For nonavian groups the comparative evidence is also contradictory (Arnqvist et al. 2000; Katzourakis et al. 2001; Gage et al. 2002).

One difficulty not yet considered is that the prediction that stronger sexual selection promotes speciation incorporates two

steps: (1) stronger sexual selection causes more evolutionary changes in ornaments, and (2) changes in ornaments cause reproductive isolation, that is, speciation. Step 1 is generally assumed to be true because sexually selected ornaments are highly diverse (Andersson 1994) and female preferences are predicted to change frequently (Schluter and Price 1993; Iwasa and Pomiankowski 1995; Pomiankowski and Iwasa 1998). However, there is evidence to suggest that the relationship between strength of sexual selection and amount of evolutionary change in ornaments is not linear. For example, the evolution of sexual ornaments typically alternates between gains and losses (or increases and decreases in ornamentation), with ornament losses often being more frequent than gains (e.g., Omland 1997; Kimball et al. 2001; Kraaijeveld 2003; reviewed in Wiens 2001). Also, in several bird groups the same ornament patterns appear repeatedly, showing frequent evolutionary gains and losses (Price and Pavelka 1996; Omland and

Lanyon 2000; Ödeen and Björklund 2003). Ornament losses can result from a variety of processes, such as change in female preferences, reduced sexual selection, or it being counteracted by other forms of natural selection. Although females often prefer more ornamented males (Andersson 1994), both gains and losses of ornaments could lead to reproductive isolation, especially in birds, where sexual imprinting is an important way by which young birds learn how to choose mates (Immelman 1975; Price 1998).

Under this scenario, evolutionary changes in ornaments may predict speciation better than the intensity of sexual selection. Here, we use variation in the ornamental coloration of *Carduelis* finches to test if speciation is related with changes in ornamentation, that is, if ornaments show a speciational pattern of evolution. Speciational evolution is expected for traits that are either directly involved in speciation, or change as a consequence of speciation (Moore et al. 1999). Note that speciational evolution does not imply directionality of the evolutionary changes of traits associated with speciation; regarding ornamentation, these changes can be any combination of gains and losses. The genus *Carduelis* contains a large clade that speciated very recently (the South American clade, Arnaiz-Villena et al. 1998; van den Elzen et al. 2001). This abundance of recent speciation may be useful to test for speciational evolution because the speciational signal should fade with increasing time from the speciation events, as in between pairs of species with older coalescence age there should exist a higher number of speciation events that go unnoticed due to extinction. If these unknown speciation events happen randomly through time, then speciational evolution will produce an array of phenotypes that resembles gradual evolution (i.e., change proportional to time), especially when looking at the pairs of species with older coalescence age.

We also test two predictions of the classic hypothesis that stronger sexual selection promotes speciation, here evaluating the strength of sexual selection by the degree of ornamentation of the species. If lineages under stronger sexual selection speciate more then (1) more ornamented species should have gone through more speciation events (Freckleton et al. 2008) and, as a result, (2) on average the last speciation event should be more recent for the more ornamented species. The second prediction focuses on the terminal branches of the phylogeny, that is, later in the radiation of the genus, when sexual selection might be more influential on speciation (Rice and Hostert 1993; Danley and Kocher 2001). Note that, contrary to the test of speciational evolution, these are explicitly directional tests, testing if speciation is associated with a higher degree of ornamentation.

Studying coloration in a single genus, where the coloration of species is expected to exhibit similar patterns of organization (Price and Pavelka 1996; Omland and Lanyon 2000; Ödeen and Björklund 2003), allows us to use detailed quantitative measures of color and of the extent of colored areas, which are homolo-

gous and therefore readily comparable among species. Furthermore, most *Carduelis* spp. have green, yellow, or red coloration, which in this genus is based on carotenoid pigments (Stradi et al. 1995, 1997; Saks et al. 2003; Shawkey et al. 2006), and also a variable amount of conspicuous black coloration, mostly on the wings, which is melanin based. This allows us to compare the evolution of carotenoid and melanin ornaments. The evolutionary patterns of these ornaments may differ because there is evidence that carotenoid coloration is a better indicator of condition than melanin coloration and, therefore, may be more strongly sexually selected (reviewed in Badyaev and Hill 2000).

Methods

SPECIMENS, COLOR MEASUREMENTS, AND COLORATION SCORES

We measured coloration on skins of the 29 species of *Carduelis* available at the ornithological collection of the Natural History Museum of London (online Supplementary Table S1). Following the molecular evidence of Arnaiz-Villena et al. (1998, 1999) and Zamora et al. (2006), we include the species *Serinus citrinella* in the genus *Carduelis*. We measured one female skin and three male skins per taxon (except for *Carduelis dominicensis*, which has a single male skin in the collection) because males often exhibit more interindividual or geographical variation in color (Price 2007).

We measured the carotenoid-based coloration in three body parts (breast, rump, and wing bar) and also the black coloration of wings. For species with no green-to-red coloration we measured the brightest area in the corresponding body part. Color measurements were made using an Ocean Optics USB2000 spectrophotometer with a deuterium-halogen light source (DT-Mini-2-GS, Ocean Optics) and a Y-shaped probe (Oceanoptics, Dunedin, FL) mounted in a holder that kept it at 3.5 mm from the feathers (38-mm² measuring area). Measurements were taken perpendicularly to the feathers' surface and were calibrated against a Micropack WS-1-SS white standard that was scanned before measuring each bird. For each body part we made three independent measurements after relocating the probe to account for possible heterogeneity of the coloration.

From the spectra of breast, rump, and wing bar we computed measures of brightness, hue, and saturation of green-to-red and of UV wavelengths ($500 < \lambda < 700$ nm and $320 < \lambda < 420$ nm, respectively, Montgomerie 2006). Brightness was computed as the mean percent reflectance for the entire avian visible spectrum ($\sum_{\lambda_{320}}^{\lambda_{700}} R_i / n$, where R_i is the percent reflectance at each wavelength class and n is the number of wavelength classes). This measure was positively skewed for our dataset and we corrected this with a square-root transformation. We computed hue as the wavelength at the reflectance midpoint between the minimum and maximum reflectance of the spectrum (λ_{R50}). As reflectance

values of individual wavelengths are sensitive to random noise in the spectra, we smoothed spectra using running averages over 10-nm intervals (e.g., Delhey et al. 2006) prior to computing hue. Carotenoid spectra are bimodal with a first reflectance plateau in the UV and a second larger one in the green-to-red range, and maximum reflectance always falls in the green-to-red plateau (for examples of typical carotenoid reflectance spectra from *Carduelis* spp. see MacDougall and Montgomerie 2003; Hill and McGraw 2004). Therefore, this measure of hue refers to variation in the green-to-red range. Note that for achromatic or nearly achromatic patches (colors with low saturation) hue is not important, and brightness is the most important parameter. Therefore, we always analyze hue in conjunction with measures of brightness and saturation. We calculated the saturation of green-to-red as the proportion of reflectance in this range relatively to the total non-UV reflectance ($\sum_{\lambda_{500}}^{\lambda_{700}} R_i / \sum_{\lambda_{420}}^{\lambda_{700}} R_i$). Similarly, UV saturation is the proportion of UV reflectance relative to total nongreen-to-red reflectance ($\sum_{\lambda_{320}}^{\lambda_{420}} R_i / \sum_{\lambda_{320}}^{\lambda_{500}} R_i$). We did not use total reflectance as the denominator in the saturation formulae because carotenoids increase the UV and green-to-red reflectance plateaus simultaneously (MacDougall and Montgomerie 2003; Bleiweiss 2005), so that the rise in the denominator would overcompensate the rise in the UV numerator (because UV has a narrower bandwidth than green to yellow) and thus create a negative correlation between the two measures. From the spectra of the wing black feathers we computed only two of these measures, brightness and UV saturation, because these feathers do not reflect appreciably on the green-to-red range but can have structurally based UV reflectance. Following Montgomerie (2006) we examined these four measures for potential outliers (which are commonly due to light contamination during scanning) using Tukey box plots and deleted them. Outliers were very few (total for the four measures is 12 out of 1431 spectra) and in all cases there remained nonoutlier spectra for the same skin and body part after these were removed. Measurements from the multiple spectra of each body part were then averaged for each skin.

In addition, we measured the vertical extension of the breast bright area, categorized the extent of carotenoid and melanic coloration in the head and in the wing as the number of parts or feather types comprised, categorized the pattern of pigmentation of the breast, back, rump and undertail from cryptically mottled to smooth, and categorized tail featheredges, beak color and leg color in increasing order of conspicuousness. We give the values of all 26 measures for each species and sex, and their detailed definitions in the online Supplementary Table S1.

We obtained a one-dimensional coloration score by summing the significant Principal Components (PCs) of a Principal Component Analysis (PCA) on the 26 measures from each species and sex. This PCA returned five PCs with eigenvalues that are significant by the broken-stick criterion (Jackson 1993), and together

they explain 66% of the variation. All these five PCs are characterized by strong positive trait loadings (online Supplementary Table S2), whereas negative trait loadings are much fewer and generally lower in absolute value (note that, unlike the other traits, for the trait “brightness of wing dark feathers” lower values mean more conspicuousness, i.e., deeper black). Therefore, we interpret these PCs as axes of coloration conspicuousness. We computed a total coloration score as the sum of those five PC scores scaled by their eigenvalues.

We also ran a PCA on the set of 16 color measures obtained from the areas in which *Carduelis* species most often have carotenoid coloration, the breast, wing, tail featheredges, and rump. This returned three significant PCs by the broken-stick criterion, which together explain 61% of variation. We calculated the carotenoid coloration score as the sum of these PC scores scaled by their eigenvalues. Similarly, we ran a PCA on the four measures derived from black coloration. The eigenvalue of the first PC from these PCA is lower than the critical broken-stick value, but the eigenvalues of the first two PCs are both greater than unity and together explain 65% of the variation. Therefore, we computed the melanin coloration score as the sum of these two PC scores scaled by their eigenvalues. As in the PCA with all coloration traits, these PCs are characterized by several strong positive trait loadings and fewer negative loadings (online Supplementary Table S2), and therefore quantify conspicuousness (as before, note that lower values of “brightness of wing dark feathers” mean more conspicuousness).

We measured tarsus length, beak length, and depth on the same bird skins. We also computed an index of beak shape as beak depth/beak length. For each species we averaged these measurements for all skins, male and female alike, because *Carduelis* spp. are not sexually size dimorphic. We ran a PCA on the three metric measurements and use the score of the first PC as a measure of body size. This is the only PC with an eigenvalue that is significant by the broken-stick criterion (1.901, 63% of variation); all trait loadings on this PC are greater than 0.732.

PHYLOGENY RECONSTRUCTION

We used the mitochondrial cytochrome b sequences of Arnaiz-Villena et al. (1998, 25 species and 4 outgroup species), which comprise all the *Carduelis* species with cytochrome b sequences currently available. Based on those sequences we reconstructed the phylogeny of the genus by Bayesian inference using the software MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). We used a General Time Reversible model with a proportion of invariable sites and gamma-distributed rate variation across sites, to account for multiple nucleotide substitutions in the same sites. We then made a chronogram by smoothing branch lengths on this tree using penalized maximum likelihood with the software r8s 1.71 (Sanderson 2002). The phylogenetic

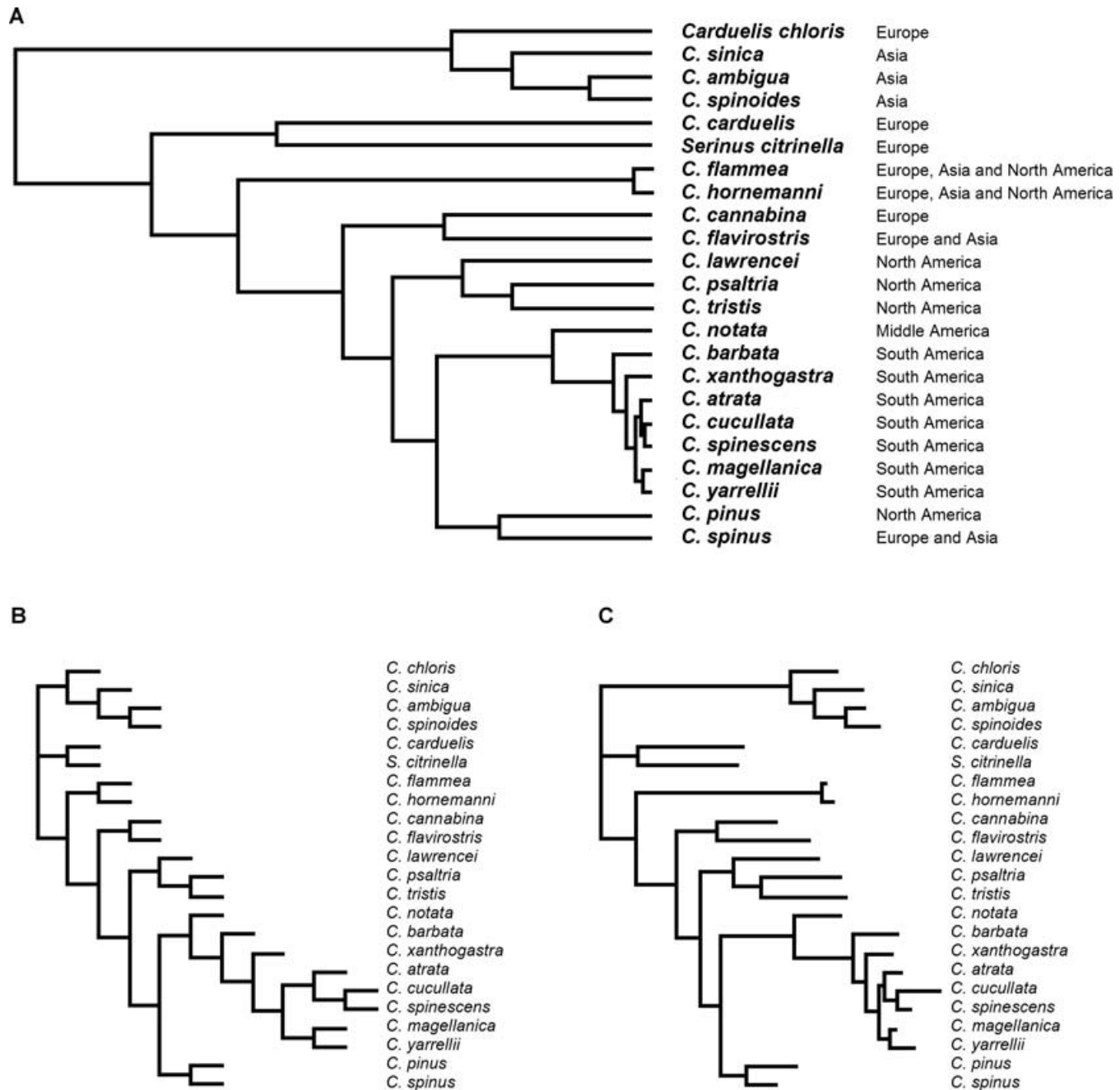


Figure 1. (A) Rooted chronogram of the 23 *Carduelis* spp. with genetic and coloration data. Continents comprising the main geographical distribution of each species are given (from Clement et al. 1993). (B) Speciation evolution tree. This is the same as (A) with all branches of equal length. (C) Genetic distances tree or phylogram. This is the same as (A) before rate smoothing.

tree comprises 23 of the species in our dataset of color measurements (Fig. 1A), and we base our subsequent phylogenetic comparative analyses on this subset of species.

TESTING EVOLUTIONARY MODELS

We compared the fit of coloration in each sex to different models of evolutionary change using the likelihood differences method of Mooers et al. (1999). Calculations were done with a version of

the CONTML program of PHYLIP (Felsenstein 1993) modified by Mooers et al. (1999).

We are most interested in comparing the fit of the coloration data to the gradual versus speciation evolution models. Gradual evolution refers to the case in which phenotypic change is proportional to time, so that phenotypic distances between species are similar to the phylogenetic chronogram (Fig. 1A). Speciation evolution refers to the case in which phenotypic evolution

is associated with speciation events, so that phenotypic distances reflect a speciation tree (Fig. 1B). We also consider a genetic distances model in which the processes causing molecular substitutions influence phenotypic evolution so that phenotypic distances reflect the phylogram of the group (Fig. 1C). We report the likelihoods of these models relative to a nonhistorical model, which is depicted as a star phylogeny and represents the situation in which there is no phylogenetic signal in the data. This method has greater statistical power when analyzing a set of uncorrelated phenotypes instead of a single, one-dimensional phenotype (Mooers et al. 1999). Therefore, we analyze the scores of the set of PCs used in the computation of each coloration score (total coloration, carotenoid coloration, or melanin coloration).

We also tested the fit to these models of two nonornamental traits: body size, and beak shape. For body size or beak shape we only have one-dimensional measures, and therefore these tests have lower statistical power. To allow a fair comparison between the results of coloration and nonornamental traits, we repeated the above tests for coloration, this time using the one-dimensional scores of coloration, obtained by summing scaled PC scores (see above).

SPECIATIONAL EVOLUTION AND AGE OF SPECIES DIVERGENCE

We predicted that the fit to the speciation model decreases with the age of species divergence. This is because, due to extinctions, more speciation events are likely to go unnoticed in between pairs of species with older coalescence age and, therefore, our expectation of how much these pairs should have diverged by speciation change becomes inaccurate. To test for this possible effect we compared the regressions of the fit of the pairs of species to speciation or gradual evolution on the age of divergence of those pairs of species. First, we computed the fit of the pairs of species to the speciation or gradual model as the absolute values of residuals from the regression through the origin of phenotypic distances on phylogenetic distances (i.e., on distances in the speciation tree, Fig. 1B, or in the chronogram, Fig. 1A, respectively). We then regressed the fit to speciation or gradual evolution on the age of divergence of the pairs of species, and compared these two regression slopes with a *T*-test (Zar 1996, pp. 353–355, intercepts were subtracted to make each regression pass through the origin without changing its slope). Datapoints from either the phylogenetic or phenotypic distance matrixes are not independent because the same species is involved in each line or column. Therefore, statistical significance of the above correlations should be evaluated with Mantel tests using random but symmetrical permutations of the columns and lines in the dependent distance matrix (Legendre et al 1994), which corresponds to shuffling the position of the species in the phylogenetic tree. We did 999 permutations and

from each computed the test statistics, obtaining null distributions against which we evaluated the significance of the tests.

DIRECTIONAL RELATIONSHIP BETWEEN COLORATION AND SPECIATION

If lineages under stronger sexual selection speciate more, we expect that coloration scores are correlated with the number of speciation events that occurred in each lineage, and that species with higher coloration scores tend to have diverged more recently than species with lower coloration scores. Following Freckleton et al. (2008), we tested the first prediction using generalized least squares (GLS) tests (Martins and Hansen 1997; Pagel 1997) relating the number of speciation events counted from the root to each extant species with the coloration scores of each species. We tested the second prediction with similar GLS tests relating terminal branch lengths with the coloration scores. GLS tests were made with the software COMPARE 4.6b (Martins 2004). These tests are affected both by speciation and extinction rates: if more ornamented lineages tend to speciate more but also tend to go extinct more often, no differences in cladogenesis will be observed. The second test focuses on the most recent part of the phylogeny, and therefore may be more robust to the influence of extinctions.

Note that these analyses are distinct from relating number of speciation events with the amount of evolutionary changes, which creates an artifact known as the node density effect (Venditti et al. 2006; Hugall and Lee 2007). This artifact arises because in more speciose lineages (lineages with more “nodes”) we have more sample points and therefore greater ability to detect multiple evolutionary changes on the same trait or multiple nucleotide substitutions on the same site; then, through circular reasoning, this can erroneously lead to the conclusion that evolutionary change is related to the number of speciation events. In one analysis of this section we do use the number of speciation events but we do not relate it with the amount of inferred evolutionary change; we relate it with the extant phenotypes of the species (Freckleton et al. 2008), which are measured rather than inferred. Thus, the node density effect is not an issue here. In the previous sections too, we never used inferred evolutionary changes, which is the source of inaccuracy behind the node density effect.

Results

The fit of coloration and morphometric data to gradual evolution was in most cases similar to or worse than the fit to the nonhistorical model, indicating that those traits are very evolutionarily labile. Nevertheless, for coloration the speciation model often fit significantly better than the nonhistorical one (Fig. 2). Speciation change explained the evolution of coloration significantly better than either gradual change or the model of genetic distances (i.e., log-likelihood differences were greater than 2, Edwards 1992;

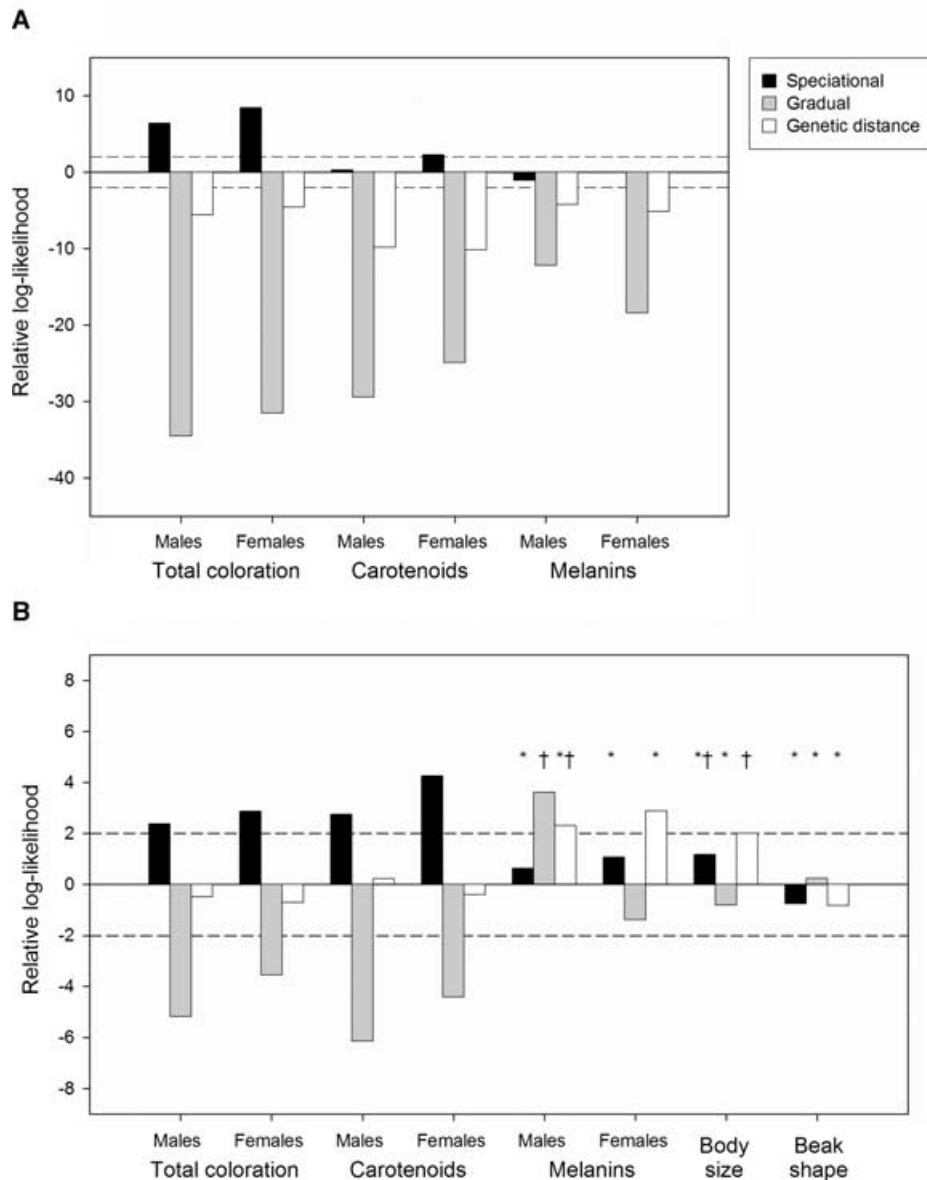


Figure 2. Log-likelihoods of models of evolutionary change relative to the nonhistorical model. Horizontal broken lines indicate the threshold for significant differences (± 2 log-likelihood differences) from the nonhistorical model. (A) Likelihoods calculated using Principal Components of coloration measures. (B) Likelihoods calculated using one-dimensional coloration and morphometric scores. Within each type of data, symbols group models whose likelihoods are not significantly different.

Mooers et al. 1999) for all coloration scores in either sex (total coloration, carotenoids, and melanins, Fig. 2A). Except for melanin coloration, the better fit of speciation relative to gradual change remained significant when analyzing the one-dimensional scores instead of the coloration PCs (Fig. 2B). On the contrary, for body size and beak shape the differences of fit to the speciation and gradual models were not significant (Fig. 2B).

The fit of coloration scores to speciation evolution increased in value (larger absolute values of residuals meaning worse fit) with the age of divergence of the pairs of species (Table 1). This was significant for the scores of carotenoid- and melanin-based

coloration in either sex (all regression slopes $\beta > 0.21$, all $P < 0.04$) but not for total coloration (males: $\beta = 0.16$, $P = 0.08$; females: $\beta = 0.10$, $P = 0.19$). The worse fit of pairs of species with older coalescence age to speciation evolution could reflect general uncertainties of phylogenetic reconstruction deeper in the phylogeny, and not be related to the speciation model per se. To control for this possibility we compared the previous regression slopes with similar ones that use fit to the gradual model. Comparing how the fit to speciation evolution and the fit to gradual evolution vary with age of divergence, we can also cancel out noise caused by the high evolutionary lability of coloration, as

Table 1. Standardized regression coefficients (β) of the fit to the speciation model on the age of divergence of pairs of species, and T -tests comparing the slopes of regressions of fit to speciation or gradual evolution on the age of divergence of the pairs of species.

	Regression of fit to the speciation model on the age of divergence of pairs of species		T -test for the difference of regression slopes	
	β	P^*	T	P^*
Total coloration				
Males	0.16	0.076	2.46	0.021
Females	0.10	0.190	4.27	0.003
Carotenoid coloration				
Males	0.24	0.010	3.65	0.002
Females	0.23	0.005	4.24	0.001
Melanin coloration				
Males	0.22	0.033	1.64	0.249
Females	0.27	0.016	4.22	0.001

* P values from Mantel tests with null distributions obtained by randomly shuffling the position of species in the phylogenetic tree.

evolutionary liability should worsen the fit to both models. With the exception of melanin coloration in males ($T = 1.64$, $P = 0.25$, Table 1), the regression slope of the fit to speciation evolution on the age of divergence of the pairs of species was significantly higher than the corresponding slope for gradual evolution (all $T > 2.45$, all $P < 0.03$, Table 1). This means that the coalescence age of species pairs affects the fit to speciation evolution more than to gradual evolution.

Because most of the pairs of species that diverged recently are in the South American clade (Fig. 1A), we tested if the pattern of speciation evolution was driven entirely by this group. We simply repeated the log-likelihood tests of coloration using the same PC scores as before, after removing the South American clade. In general the log-likelihood differences between evolutionary models decreased (not shown), but the difference between speciation and gradual evolution remained significant in all cases (for any classification of coloration and sex, all log-likelihood differences > 4.49).

We found no evidence that more ornamented lineages (i.e., lineages with higher coloration scores) speciate more. Correlations of any coloration score with number of speciation events were low (for any coloration score and sex, $r < 0.21$, $P > 0.33$, $N = 23$), and coloration scores were also not negatively related with terminal branch lengths in either sex (for any coloration score and sex, $r > -0.21$, $P > 0.33$, $N = 23$). With our sample size these tests have low statistical power. Thus, although these results indi-

cate absence of a strong directional association between the degree of ornamental coloration and cladogenesis in *Carduelis*, it is possible that larger datasets find correlations of this magnitude to be significant (Freckleton et al. 2008). These null results can also be due to extinction rates counteracting a possible difference in speciation rate, although the test that uses only the information on terminal branch lengths is expected to be robust to the influence of extinctions.

Discussion

We found that the evolution of coloration was very labile in the genus *Carduelis*, and that it fits speciation evolution better than gradual evolution. This is true both for carotenoid- and melanin-based coloration, in either sex, but not for nonornamental morphology. We found no evidence that more ornamented lineages have higher diversification rates, or that more ornamented species tend to have diverged more recently. This suggests that lineages putatively under stronger sexual selection do not speciate appreciably more. Taken together these results indicate that, in *Carduelis* finches, the evolution of ornamental coloration is related with speciation, but the changes in coloration occurring during speciation do not seem to be necessarily increases in ornamentation.

All speciation events in which one of the daughter species went extinct are, of course, not present in the phylogeny that we used. Also, we only have coloration and molecular data for 23 of the 32 extant species of *Carduelis* finches (Clement et al. 1993), and it was recently shown that *Loxia* crossbills are a monophyletic clade within *Carduelis* (Zamora et al. 2006). These missing speciation events worsen the prediction of how much phenotypic evolution should have occurred by speciation evolution and make the speciation model less accurate. Because of this, we predicted that testing for speciation evolution would be conservative, especially deeper in the phylogeny where more speciation can go unnoticed due to extinctions, and we found that the speciation signal in the data is indeed weaker for pairs of species with longer coalescence periods. This suggests that a complete knowledge of speciation events would yield an overall stronger speciation pattern, and also warns that tests of speciation evolution may often be conservative.

The hypothesis that sexual selection promotes speciation is usually tested in a directional way, that is, stronger sexual selection causing more speciation (e.g., Morrow and Pitcher 2003; Morrow et al. 2003a). We found no evidence that more ornamentation promotes speciation. Although the statistical power of our directional tests is low, this suggests that in *Carduelis* there is not a strong relationship between strength of sexual selection and speciation. An explanation for this is that the direct link with speciation is not sexual selection per se but evolutionary changes in ornaments, and that changes in ornaments are not driven solely

by sexual selection. Strong sexual selection is indeed a possible cause for changes in ornaments, for example via changing female preferences (Schluter and Price 1993) or via arms race in sexual conflict (van Doorn and Weissing 2006), but it is not the only one. For example, ornaments also change due to habitat properties (e.g., Marchetti 1993; Endler and Théry 1996), reinforcement under secondary contact (e.g., Sætre et al. 1997), or ecologically driven interspecific mimicry (e.g., Jiggins et al. 2001; Balakrishnan and Sorenson 2006; Puebla et al. 2007). More importantly, many evolutionary changes in ornaments, perhaps the majority, are losses or reductions (Wiens 2001), and these losses can contribute to reproductive isolation (e.g., Hill and McGraw 2004). Ornament losses may happen by a variety of reasons, including reduced sexual selection relative to other forms of natural selection (Wiens 2001). Thus, the evolutionary lability of ornaments and the opportunity for reproductive isolation may not be greater in taxa experiencing strong sexual selection, as unrelated factors, including reduced sexual selection, may facilitate the alternation between gains and losses of ornaments.

The speciation evolution of coloration, but not of morphology, likely implies that speciation in *Carduelis* was mediated by changes in ornaments. However, this result does not allow us to distinguish among the different possible ways in which evolutionary changes in ornaments can contribute to reproductive isolation (Price 1998; Omland and Kondo 2006). Ornaments may actively promote speciation if they change and create reproductive isolation before populations are ecologically differentiated. Alternatively, ornaments may prevent gene flow between already differentiated or partly differentiated populations if they change as a consequence of ecological differences between populations or by reinforcement. For example, the rapid speciation of *Carduelis* in South America is likely due to the variable topography and high temperatures of the Andean habitats that these birds colonized, which is known to affect avian species richness (van den Elzen et al. 2001; Davies et al. 2007). Our result suggest that, had those ancestral populations not changed in coloration, either less species would have formed or more would have merged upon secondary contact. In either case, the ability of populations to change their ornaments (by sexual selection, withdrawal of sexual selection, reinforcement, or any other process) would contribute to cladogenesis in the long run.

There is evidence that carotenoid coloration is a better indicator of condition than melanin coloration (reviewed in Badyaev and Hill 2000; but see also Owens and Hartley 1998; Griffith et al. 2006) and, therefore, it may be a preferential target of sexually selected female preferences. For example, in different *Carduelis* spp. carotenoid coloration (but not melanin ornaments; McGraw and Hill 2000; Rosen and Tarvin 2006) correlates with measures of male condition (Merilä et al. 1999; McGraw and Hill 2000; Lindström and Lundström 2001; Rosen and Tarvin 2006) and is

object of female preferences (Hill and McGraw 2004; Senar et al. 2005). Therefore, if reproductive isolation in this genus were due to female preferences for condition-dependent signals, we would expect carotenoid coloration to show a stronger speciation pattern than melanin ornamentation. We found speciation evolution both for carotenoid- and melanin-based ornaments, not lending support to the idea that sexually selected female preferences were paramount for achieving reproductive isolation.

It was argued before that genetically determined sexual preferences may not be efficient isolating mechanisms because, being often directional and open-ended, they can lead to females from less-ornamented populations preferring more-ornamented males even if from different populations or species (Price 1998; e.g., Hill 1994; Collins and Ludden 2002). On the contrary, sexual imprinting offers a simple means for achieving reproductive isolation, because in each generation individuals learn to recognize the sexual traits of their own population and, in the case of birds, imprinting is a widespread phenomenon (Immelman 1975; Price 1998). Whether genetically determined sexual preferences or imprinting mediate reproductive isolation, we would expect changes in male coloration to be more important for speciation because female birds are generally the choosier sex (Andersson 1994). The finding that both male and female coloration evolved with a speciation pattern, though, is not inconsistent with this because males and females share many coloration traits (Clement et al. 1993; online Supplementary Table S1).

We conclude that evolutionary changes in ornaments are associated with speciation in *Carduelis*. We did not find evidence for a directional relation of speciation with the degree of ornamental coloration, suggesting that stronger sexual selection was not a major promoter of reproductive isolation in this group. Sexual selection may contribute to speciation insofar as it causes ornament evolution, but there are other causes for evolutionary changes in ornaments that may be equally important. Based on this insight, we suggest that the evolutionary lability of ornaments, which is not necessarily correlated with the overall intensity of sexual selection, can be considered as a possible explanation for differences in cladogenesis among taxa.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Coloration scores of *Carduelis* spp. and traits measured.

Table S2. Eigenvalues and trait loadings on Principal Components.

This material is available as part of the online article from:

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