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A cryptic contact zone between divergent mitochondrial DNA lineages in southwestern North America supports past introgressive hybridization in the yellow-rumped warbler complex (Aves: *Dendroica coronata*)

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Using genetic data to study the process of population divergence is central to understanding speciation, yet distinguishing between recent divergence and introgressive hybridization is challenging. In a previous study on the phylogeography of the yellow-rumped warbler complex using mitochondrial (mt)DNA data, we reported limited sequence divergence and a lack of reciprocal monophyly between myrtle and Audubon's warblers (*Dendroica coronata* and *Dendroica auduboni*, respectively), suggesting very recent isolation. In the present study, we report the results obtained from a subsequent sampling of Audubon's warbler in Arizona and Utah ('*memorabilis*' race), which shows that, although this taxon is similar to *auduboni* in plumage colour, most *memorabilis* individuals sampled (93%) carry haplotypes that belong to the divergent black-fronted warbler lineage (*Dendroica nigrifrons*) of Mexico. Furthermore, the *auduboni* and *nigrifrons* lineages mix in southern Utah at a narrow, yet apparently 'cryptic' contact zone. Newly-available evidence from nuclear markers indicating marked differentiation between *auduboni* and *coronata* has focused attention on the possibility of mtDNA introgression in the absence of nuclear gene flow, and the results of the present study are consistent with the hypothesis that the mtDNA of *auduboni* was indeed historically introgressed from the *coronata* lineage. Analysis of morphological traits shows that *memorabilis* is significantly differentiated from *auduboni* and *nigrifrons* in some traits, yet is overall intermediate between the two, which is consistent with a shared common ancestor for the a*uduboni*/*memorabilis*/*nigrifrons* group. The striking, unexpected mtDNA pattern reported in the present study reveals a complex evolutionary history of the yellow-rumped warbler complex, and cautions against the exclusive use of mtDNA to infer evolutionary relationships. *Biological Journal of the Linnean Society*, 2011, **103**, 696–706.

ADDITIONAL KEYWORDS: ecomorphology – genetic diversity – haplotype cline – phylogeography.

INTRODUCTION

Analysis of neutrally-evolving molecular markers provides invaluable insight into the evolutionary history of species, and constitutes a useful complement to phenotypic data such as plumage colour and morphology that are more likely to be affected by natural selection. Genetic data are not free of interpretive problems, however, and inferring species history from mitochondrial (mt)DNA gene trees entails well-known potential risks (Irwin, 2002; Funk & Omland, 2003; Edwards *et al*., 2005). Among these risks is the particularly insidious problem of distinguishing between two very different processes: (1)

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Table 1. Sampling localities and sample sizes

Locality	Area	State	Latitude	Longitude	*N*
Hospital Flats	Pinaleno Mts	AZ	N 32.66614	W 109.87522	5
Snow Flat	Pinaleno Mts	AZ	N 32.65405	W 109.86417	4
Big Lake	White Mts	AZ	N 33.59314	W 109.36221	12
S.F. Peaks	Flagstaff	AZ	N 35.34008	W 111.60216	1
Lockett Meadows	Flagstaff	AZ	N 35.35765	W 111.62100	5
Jacob Lake	Kaibab Plateau	AZ	N 36.59873	W 112.17693	14
Dixie N.F.	Dixie N.F.	UT	N 37.52909	W 112.75322	22
Mt Timpanogos	Wasatch Mts	UT	N 40.44078	W 111.62781	4
Cache N.F.	Cache N.F.	UT	N 41.89237	W 111.63751	5
Alturas Lake	Sawtooth N.F.	ID	N 43.89835	W 114.90118	9

For sampling localities elsewhere in the yellow-rumped warbler range, see Milá *et al*. (2007).

recent population divergence, which causes incomplete lineage sorting and (2) historical introgression, by which the entire mitochondrial genome of a species can introgress into a closely-related species through hybridization. The latter has been documented or proposed in a number of animal taxa (Weckstein *et al*., 2001; Good *et al*., 2008; Irwin, Rubstov & Panov, 2009; Nunes *et al*., 2010). To rule out the introgressive hybridization hypothesis, phylogeographic and phylogenetic studies increasingly combine both mtDNA and nuclear markers, although studies using only mtDNA datasets remain common.

A case in point is our previous phylogeographic study of the yellow-rumped warbler complex (Milá, Smith & Wayne, 2007), where we used three mtDNA regions to infer the evolutionary history of the four main groups in the complex, the myrtle and Audubon's warblers of North America (*Dendroica coronata* and *Dendroica auduboni*, respectively), the black-fronted warbler of Mexico (*Dendroica nigrifrons*), and Goldman's warbler of Guatemala (*Dendroica goldmani*). These four taxa have recently been recognized as separate species (Gill & Donsker, 2010) and are treated as such in the present study. The Mexican and Guatemalan species were found to be deeply divergent from each other and from the two North American migratory species, whereas the latter formed a single undifferentiated clade. We interpreted the lack of differentiation and widespread sharing of mtDNA haplotypes between *coronata* and *auduboni* as a case of shared ancestral polymorphism and incomplete lineage sorting between these two phenotypically divergent forms. However, in a recent study on the hybrid zone between the two taxa in western Canada, Brelsford & Irwin (2009) demonstrated that *coronata* and *auduboni* are in fact well differentiated in nuclear markers, suggesting that the mtDNA pattern revealed in our previous study was likely the product of ancestral introgressive hybridization.

In the present study, we report new data that support the mtDNA introgression hypothesis of Brelsford & Irwin (2009). We sampled the '*memorabilis*' race of Audubon's warbler in Arizona, a purportedly sedentary population found in conifer forests of the sky island archipelago in Arizona and New Mexico (Fig. 1A). These populations are characterized by slightly more extensive black plumage in some individuals, although, overall, are similar to typical *auduboni* in coloration relative to *nigrifrons* (Moore, 1946). Unexpectedly, haplotypes sampled in most Arizona birds cluster with the divergent *nigrifrons* mtDNA lineage found in Mexico. Further sampling along a south–north transect into Utah and Idaho showed the existence of a narrow mtDNA contact zone in southern Utah between *nigrifrons* and *auduboni* haplotypes. Furthermore, we analyze variation in morphological and plumage colour traits to investigate whether phenotypic characters are congruent with the mtDNA pattern. Specifically, we test whether a similar contact zone exists with respect to phenotype, and the extent to which *memorabilis* is morphologically similar to *auduboni* or *nigrifrons*.

MATERIAL AND METHODS

Field sampling

Samples were obtained from ten different localities in Arizona, Utah and Idaho (Fig. 1B and Table 1) in the summers of 2006 and 2007, for a total sample size of 81 individuals. Birds were captured in the field using mist nets and song playbacks to attract individuals when necessary. Each bird was aged, sexed, weighed, and marked with a uniquely numbered aluminum band for future demographic analysis. Several voucher digital photographs were taken of each individual. For genetic analysis, we collected a blood sample by venipuncture of the subbrachial vein.

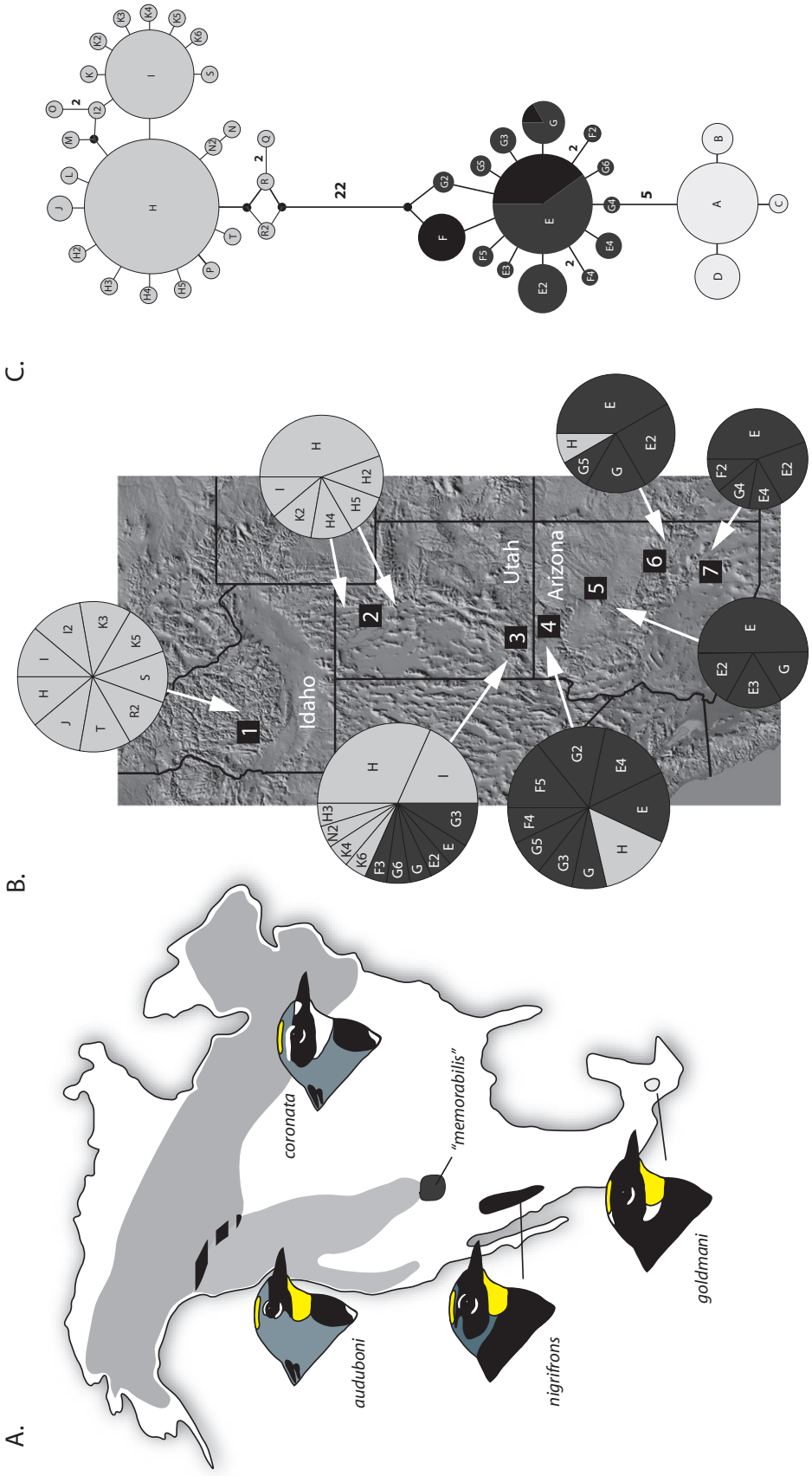

Figure 1. A, breeding range of the yellow-rumped warbler complex in North and Central America. Black stripes indicate the approximate location of a hybrid zone between *auduboni* and *coronata*. B, haplotype frequencies in each sampling locality. Locality codes are: 1 = Sawtooth N.F., Idaho; 2 = Northern Utah (Cache N.F and Wasatch Mts); 3 = Dixie N.F., Utah; 4 = Kaibab Plateau, AZ; 5 = Flagstaff, AZ; 6 = White Mts, AZ; 7 = Pinaleno Mts, AZ. For further locality details, see Table 1. C, median-joining network of mitochondrial DNA haplotypes, where each branch corresponds to one substitution, and numbers next to branches represent the number of substitutions if greater than one. Haplotypes are coloured according to taxon: *auduboni* and *coronata* (medium grey), *nigrifrons* (black), *memorabilis* (dark grey), and *goldmani* (light grey).

Blood was stored in lysis buffer (Seutin, 1991) and kept at ambient temperature until storage at –80 °C in the laboratory.

Molecular data and analysis

We extracted genomic DNA with a Qiagen extraction kit and used the polymerase chain reaction to amplify a 648-bp fragment that spanned 358 bp of the *ATP-synthase 6* gene and the entire *ATP-synthase 8* gene (hereafter referred to jointly as ATPase). Primer sequences, amplification conditions, and sequencing details are provided in See Milá *et al*. (2007). Sequences were aligned using SEQUENCHER, version 4.1 (GeneCodes) and variable sites were checked visually for accuracy. To verify the mitochondrial origin of sequences, we translated them into their amino acid sequence and looked for evidence of stop codons and anomalous amino acids. All new haplotypes sampled in this study have been deposited in GenBank under accession numbers JF830586–JF830610.

Estimates of haplotype diversity (h) and nucleotide diversity (π) were obtained using ARLEQUIN, version 3.5. (Excoffier & Lischer, 2010). We constructed a network of haplotypes using the reduced median-joining algorithm in the software NETWORK, version 4.5.1.0 (Forster, Forster & Watson, 2007). In these networks, haplotypes are represented as circles at the nodes of a tree instead of at the tips, with the size of the circle being proportional to the frequency of the haplotype in the population. For comparative purposes, we constructed a network using both the new haplotypes sampled in the present study and those in Milá *et al*. (2007), which cover the range of the entire *D. coronata* complex.

To test for past population expansions, we used Fu's test of neutrality (Fu, 1997), which specifically detects departures from neutrality in cases characterized by an excess of rare alleles and young mutations in nonrecombining sequences, and used ARLEQUIN, version 3.5. to generate values of F_S. Significant, large negative values of F_S indicate an excess of recent mutations and reject population stasis (Fu, 1997).

To estimate the location of the centre of the mtDNA cline, as well as width (defined as the inverse of the maximum slope), we used the CFIT (Gay *et al*., 2008) to determine the best fitting sigmoid for the *ATPase* haplotype frequencies, morphological traits, and plumage characters. The transition of allele frequencies and phenotypic traits across many hybrid zones can be modeled effectively by a simple sigmoidal curve (Barton & Hewitt, 1985; Szymura & Barton, 1986). Given the proposed north–south orientation of the transition in mtDNA, we used the average latitude for each population to represent its location across the contact zone. Because of the high variance in morphological measurements, we were unable to confidently fit clines for these traits.

Plumage colour analysis

We quantified plumage colour differences across groups by scoring the amount of black in three body areas (dorsum, nape, and face) from voucher digital photographs. In dorsum and nape, the amount of black plumage was coded in accordance with the index: 0 = < 5%, 1 = 5–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–95%, 5 = > 95%. For the face, the scores used were: 0 = lores and auriculars 100% grey; 1 = black feathers in lores and around the eye; 2 = black extends beyond eye into the auriculars, but does not reach the posterior edge of the auricular region; 3 = black feathers present throughout the lores and auriculars, but grey covers > 5% of the area; 4 = lores and auriculars > 95% black. Only adult males were used, and sample sizes were as follows: *auduboni* ($N = 14$); *memorabilis* ($N = 34$); *nigrifrons* ($N = 24$); and *goldmani* ($N = 19$).

Morphological measurements and analysis

A wing ruler was used to measure the unflattened wing chord (i.e. the distance from the carpal joint to the tip of the longest primary) to the nearest 0.5 mm. Dial calipers of 0.1-mm precision were used to measure the length of each of the nine primary feathers, defined as the distance from the tip of the feather to the point where it enters the skin (numbered p1 to p9 from proximal to distal), tail length (from the uropygial gland to the tip of the longest rectrix), tarsus length (from the intertarsal joint to the most distal undivided scute on the tarsometatarsus), bill length (from the base of the bill at the cranium to the tip of the upper mandible), and bill width and depth (both measured at the anterior end of the nares). All measurements were taken by B.M.

For comparison, in our analyses, we included morphological measurements from samples of *auduboni*, *coronata*, *nigrifrons*, and *goldmani* populations used in a previous study (Milá, Wayne & Smith, 2008). Only adult males (> 2 years) were used, which reduced the dataset to the following sample sizes per population: *coronata* ($N = 16$), *auduboni* ($N = 37$); *memorabilis* ($N = 24$); *nigrifrons* ($N = 16$); and *goldmani* ($N = 21$). Birds in Arizona and Utah were coded as *auduboni* if they carried *auduboni* haplotypes, and as *memorabilis* if they carried *nigrifrons* haplotypes. To determine the degree of differentiation between all five groups (*coronata*, *auduboni*, *memorabilis*, *nigrifrons*, and *goldmani*), we ran a discriminant function analysis on all morphological traits, and used one-way analysis of variance (ANOVA) (with population

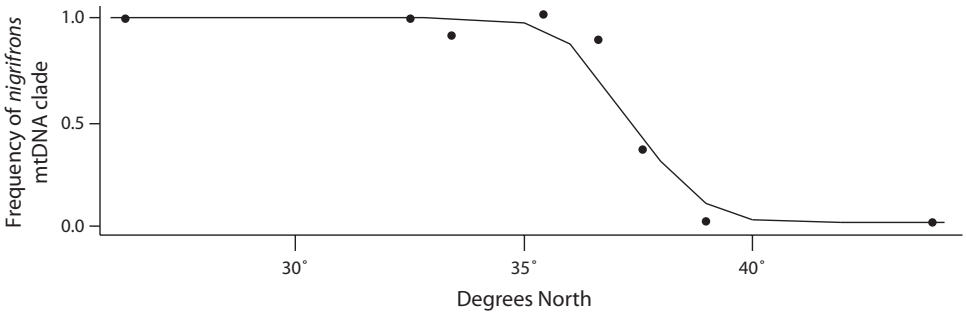

Figure 2. Mitochondrial DNA haplotype cline in yellow-rumped warblers at a north–south transect in Arizona and Utah. Points are frequencies of *ATPase* haplotypes and locations are averaged distances (degrees latitude). The line is a simple sigmoid curve with center and width parameters as estimated by CFIT (Gay *et al*., 2008).

as fixed factor) to compare the means for specific traits among populations. All analyses were carried out with SPSS, version 11.5. (SPSS Inc.).

RESULTS

Mitochondrial DNA data

We sequenced 81 individuals for *ATPase* from Arizona, Utah, and Idaho, and found 30 haplotypes, with 25 of them not being found in our previous study (Table 2). Most individuals from Utah and Idaho carry haplotypes that cluster with typical *auduboni*. However, the majority of haplotypes sampled in Arizona and southern Utah cluster with *nigrifrons* haplotypes from Mexico (Fig. 1C, Table 2). Haplotype 'E', the most common haplotype in Mexico, was found in all Arizona localities, whereas only three out of 41 individuals (7%) carried *auduboni* haplotype 'H' there. The Dixie N.F. locality ($N = 22$) was the only one to contain haplotypes from both lineages at high frequencies (32% *nigrifrons*, 68% *auduboni*) (Fig. 1B, Table 2).

Genetic diversity was similar in *auduboni* and *memorabilis* populations (*auduboni*: $h = 0.714 \pm 0.045$, $\pi = 0.0016 \pm 0.0012$; *memorabilis*: $h = 0.853 \pm 0.039$, $\pi = 0.0021 \pm 0.0015$), yet considerably lower in *nigrifrons* ($h = 0.569 \pm 0.071$, $\pi = 0.0010 \pm 0.0009$). Fu's F_S test of population expansion revealed clear patterns of rapid population growth in *auduboni* ($F_S = -18.04$, $P < 0.0001$) and *memorabilis* ($F_S = -8.04$, $P = < 0.0001$), but not *nigrifrons* ($F_S = 0.10$, $P = 0.422$).

The best sigmoid fit to the mtDNA haplotype frequencies (log-likelihood = –26.93) had a centre at 37.16566°N latitude and a width of 297 km (2.67°N latitude; Fig. 2). By contrast, none of the morphological and plumage traits fit a sigmoidal curve, indicating an incongruent pattern between mtDNA and phenotype across the contact zone (see below).

Plumage colour

Quantification of the amount of black plumage in male individuals from *auduboni*, *memorabilis*, *nigrifrons*, and *goldmani* groups revealed a lack of differentiation between *auduboni* and *memorabilis* ($P = 1$ for all three traits, one-way ANOVA), with both being significantly differentiated from *nigrifrons* and *goldmani* ($P < 0.0001$ for all traits, one-way ANOVA). The latter taxon was completely black for all traits (Fig. 3). A more detailed analysis of plumage colour is necessary in additional parts of the body to fully assess colour differences between *auduboni* and *memorabilis*. However, these results illustrate the general pattern observed in the field, showing that *memorabilis* cannot be readily distinguished from typical *auduboni*. *Memorabilis* has been described as having on average a larger amount of black in the breast area (Hubbard, 1970; Hunt & Flaspohler, 1998) yet, as a result of considerable individual variation in male plumage coloration, this characteristic was not obvious in the field and was difficult to quantify on digital photographs. These results are consistent with Moore (1946), who found *memorabilis* to be similar to nominal *auduboni* and diagnosably distinct from Mexican *nigrifrons*.

Morphometrics

Memorabilis birds from Arizona and southern Utah were found to be morphologically intermediate between *auduboni* and *nigrifrons*, and all three taxa formed a group distinctly differentiated from *coronata* and *goldmani* (Fig. 4). The first canonical function in a discriminant-function analysis separated three main groups: *coronata*, *auduboni*/*memorabilis*/*nigrifrons*, and *goldmani*. Highest eigenvalues were observed for wing length and outer primary lengths. In turn, *memorabilis* and *nigrifrons* separated from *auduboni* along the second function, with inner primary lengths showing the highest correlations (Table 3).

Analysis of trait means revealed that *memorabilis* individuals were intermediate between *auduboni* and *nigrifrons* in wing, tail, tarsus, and bill lengths, yet

Table 2. Haplotype frequencies in each sampling area

Hap	ME	AK	WA	OR	CA	ID	NUT (UT)	DIX (UT)	KAI (AZ)	FLAG (AZ)	WMT (AZ)	PINA (AZ)	MEX	GUAT	Totals
A														20	20
B														3	3
C														1	1
D														5	5
E								1	2	3	5	4	9		24
E2								1		1	3	2			7
E3										1					1
E4									2			1			3
F													7		7
F2												1			1
F3								1							1
F4									1						1
F5									2						2
G								1	1	1	2		1		6
G2									2						2
G3								2	1						3
G4												1			1
G5									1		1				2
G6								1							1
H	4	3	4	8	5	1	4	7	2		1				39
H2							1								1
H3								1							1
H4							1								1
H5							1								1
I			4	5	3	1	1	4							18
I2						1									1
J	1					1									2
K				1											1
K2							1								1
K3						1									1
K4								1							1
K5						1									1
K6								1							1
L	1														1
M	1														1
N		1													1
N2								1							1
O		1													1
P		1													1
Q		1													1
R		1													1
R2						1									1
S						1									1
T						1									1
Totals	7	8	8	14	8	9	9	22	14	6	12	9	17	29	172

Haplotypes from localities Maine (ME), Alaska (AK), Washington (WA), Oregon (OR), California (CA), Mexico (MEX), and Guatemala (GUAT) are obtained from Milá *et al*. (2007).

ID, Idaho; NUT, Northern Utah (Wasatch Mts and Cache NF); DIX, Dixie NF (Utah); KAI, Kaibab Plateau (Arizona); FLAG, Flagstaff (Arizona); WMT, White Mts (Arizona); PINA, Pinaleno Mts. (Arizona).

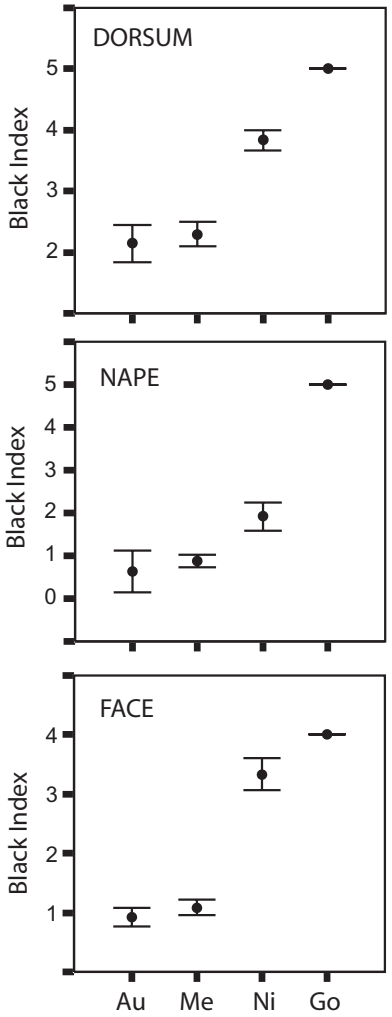

Figure 3. Plumage differences between taxa as estimated by the amount of black plumage on dorsum, nape and face. Au, *auduboni*; Me, *memorabilis*; Ni, *nigrifrons* (Mexico); Go, *goldmani* (Guatemala).

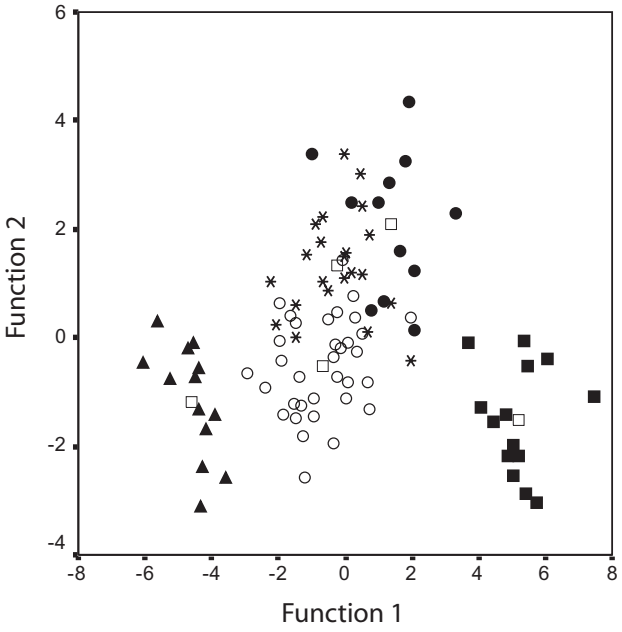

Figure 4. Discriminant function analysis of morphological traits in five yellow-rumped warbler taxa: *coronata* (filled triangles), *auduboni* (empty circles), *memorabilis* (asterisks), *nigrifrons* (filled circles), and *goldmani* (filled squares). Group centroids are represented by empty squares.

Table 3. Intra-group correlations between morphological variables and the first two standardized canonical discriminant functions (97% of the variance)

	Function 1	Function 2
Wing length	0.698	0.428
P1	0.263	0.657
P2	0.414	0.699
P3	0.413	0.600
P4	0.463	0.361
P5	0.576	0.607
P6	0.603	0.544
P7	0.693	0.377
P8	0.607	0.413
P9	0.527	0.435
Tail length	0.388	0.433
Tarsus length	0.180	0.042
Bill length	0.091	0.127
Bill width	0.192	–0.327
Bill depth	0.357	–0.118

showed significantly deeper and wider bills than *auduboni* (Fig. 5). When measurements were adjusted for overall size, *memorabilis* were similar to *auduboni* and *nigrifrons* in most traits, yet had longer wings and wider bills than both *auduboni* and *nigrifrons*, and deeper bills than *auduboni* (Fig. 6). With regard to wing shape, *memorabilis* size-adjusted primary lengths were intermediate between *auduboni* and *nigrifrons*, except for slightly shorter inner primaries (p1–p3; Fig. 7).

DISCUSSION

Sampling of yellow-rumped warbler mtDNA haplotypes in Arizona and southern Utah populations revealed the presence of *nigrifrons*-like haplotypes in the southern end of the *auduboni* distribution, whereas everywhere else *auduboni* individuals carry mtDNA haplotypes that are shared with, or closely related to, those found in *coronata* (Milá *et al*., 2007). By contrast with mtDNA data, recent evidence by

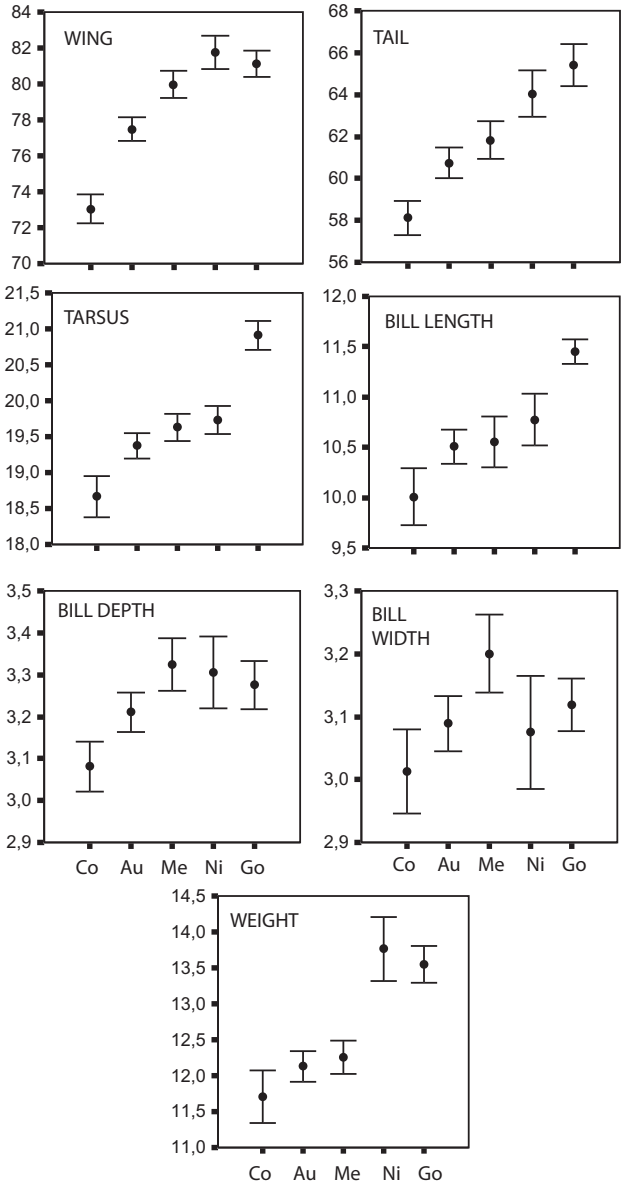

Figure 5. Means (95% CI) of six morphological variables and weight in five yellow-rumped warbler taxa: Co, *coronata*; Au, *auduboni*; Me, *memorabilis*; Ni = *nigrifrons* (Mexico); Go, *goldmani* (Guatemala).

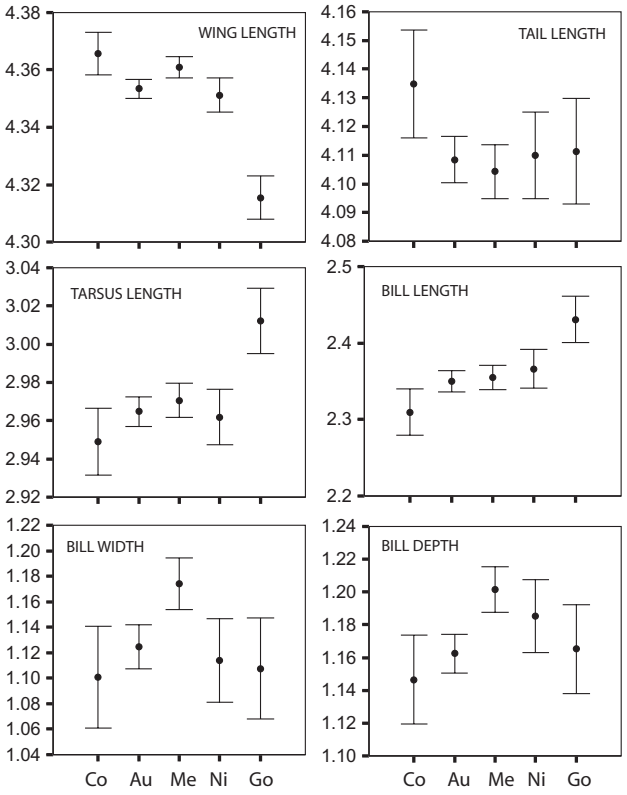

Figure 6. Size-adjusted trait means (95% CI) of six morphological variables in five yellow-rumped warbler taxa: Co, *coronata*; Au, *auduboni*; Me, *memorabilis*; Ni, *nigrifrons* (Mexico); Go, *goldmani* (Guatemala).

Brelsford & Irwin (2009) revealed that there is marked divergence in the nuclear genomes of *coronata* and *auduboni*. Moreover, a recent analysis of genome-wide amplified fragment length polymorphism data including the *memorabilis* samples from Arizona in the present study, showed that *auduboni*, *memorabilis*, and *nigrifrons* form a single genetic unit, clearly differentiated from *coronata* to the north and *goldmani* to the south (Brelsford, Milá & Irwin, 2011). This pattern is consistent with our phenotypic results. *Memorabilis* individuals were found to be phenotypically similar to nominal *auduboni* in plumage coloration, and their morphology is overall intermediate between *auduboni* and *nigrifrons*. Consequently, this pattern of variation is consistent with a latitudinal gradient in morphometric traits that could be driven by selection on body size and wing shape related to migratory distance, as demonstrated previously (Milá *et al*., 2008).

Combined with recent molecular evidence from nuclear markers, our mtDNA and phenotypic results strongly suggest that the lack of mtDNA divergence between *coronata* populations and *auduboni* populations north of Arizona is likely the result of ancestral introgressive hybridization, and not incomplete lineage sorting, as we previously proposed (Milá *et al*., 2007). The approximate time since introgression likely corresponds to the divergence time between *auduboni* and *coronata*, which we estimated to be approximately 16 000 years ago using coalescence analysis in our previous study. Our data also suggest that the replacement of *auduboni* mtDNA genomes by those from *coronata* is not complete, and individuals in Arizona and southern Utah likely represent an

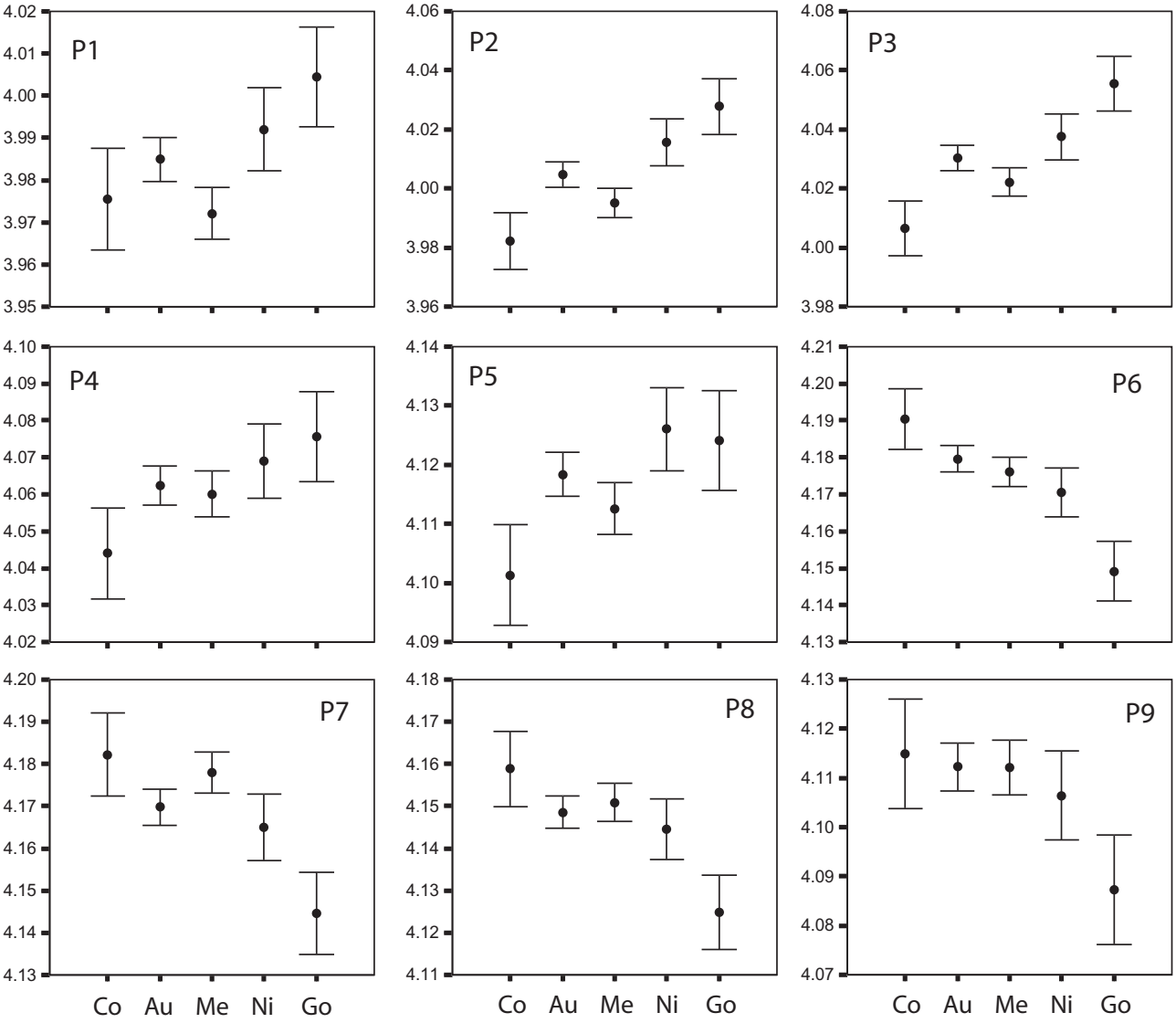

Figure 7. Size-adjusted means (95% CI) of primary feather lengths in five yellow-rumped warbler taxa: Co, *coronata*; Au, *auduboni*; Me, *memorabilis*; Ni, *nigrifrons* (Mexico); Go, *goldmani* (Guatemala).

unintrogressed population that has maintained its original mitochondrial genome.

We suggest two alternative hypotheses regarding the origin of *auduboni* that could explain observed patterns of variation in genetic and phenotypic data. One is that *auduboni* differentiated from *nigrifrons* before introgressive hybridization with *coronata*. This hypothesis is supported by the observation that the grey *memorabilis* plumage is similar to other *auduboni* populations and divergent from its darker *nigrifrons* ancestor. According to this scenario, *coronata* mtDNA introgressed into an ancestral population of *auduboni* through hybridization in a small, isolated population. Following hybridization, the introgressed *auduboni* population subsequently spread throughout western North America, which is consistent with patterns of genetic diversity and results from our tests of population expansion (significant in *auduboni* and *memorabilis*, but not *nigrifrons*). During this expansion, *auduboni* eventually came into contact with *coronata* in western Canada where it formed a narrow hybrid zone (Brelsford & Irwin, 2009), and with *memorabilis* in southern Utah and northern Arizona, giving rise to the contact zone documented in the present study. An alternative hypothesis is that *auduboni* is of hybrid origin, and resulted from the hybridization between *coronata* and *nigrifrons* (Brelsford *et al.*, 2011). If the

contact between *coronata* and *nigrifrons* occurred in the south-western USA, the *coronata* haplotypes found in *auduboni* could correspond to those left behind as the hybrid zone moved northward following the postglacial expansion of suitable habitat (Krosby & Rohwer, (2009). Alternatively, contact between the two taxa could have taken place first in the Canadian Rockies, near the present location of the *coronata*/*auduboni* hybrid zone. Thereafter, the *coronata* haplotypes would have introgressed southward through positive selection.

Testing these alternative hypotheses will require an in-depth analysis of contact zones between groups, replicated transects across the regions of interest, as well as the use of genome-wide markers. Further research will also be necessary to understand the evolutionary mechanisms maintaining the contact zone between *auduboni* and *nigrifrons* haplotypes in southern Utah, and to determine the relative roles of neutral and selective factors in maintaining this 'cryptic' contact zone. The fact that it is over twice as wide as the *coronata*/*auduboni* hybrid zone in Canada (132 km), which is maintained by selection (Brelsford & Irwin, 2009), suggests a weaker role for selective factors. This conclusion is consistent with the observed lack of marked phenotypic differentiation across the mtDNA contact zone, although the possibility of a selective sweep on mtDNA cannot be ruled out at present.

The present study provides a clear example of the importance of extensive geographic sampling when attempting to infer the evolutionary history of widespread species (Funk & Omland, 2003). Sampling of the relatively small population of *auduboni* in Arizona has revealed the close relationship between this taxon and *nigrifrons* (and not *coronata*), showing that the *coronata*-like mtDNA found in *auduboni* populations elsewhere is likely a result of past introgressive hybridization, and not recent divergence from *coronata* as previously proposed (Milá *et al*., 2007). Evidence for cases of partial introgressive hybridization are increasingly being reported (Rohwer, Bermingham & Wood, 2001; Weckstein *et al*., 2001; Good *et al*., 2008; Plötner *et al*., 2008; Nunes *et al*., 2010), and cases in which complete mtDNA capture has taken place are more difficult to detect and thus probably under-represented (Irwin *et al*., 2009), yet perhaps no less common. Therefore, despite the undeniable utility of mtDNA markers in many studies on avian phylogeography (Zink & Barrowclough, 2008), the results reported in the present study exemplify the potential risks involved in drawing inference from mtDNA datasets in the absence of complementary nuclear loci (Edwards & Bensch, 2009) and complete geographic sampling.

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