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Hybrid origin of Audubon's warbler

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Abstract

Several animal species have recently been shown to have hybrid origins, but no avian examples have been documented with molecular evidence. We investigate whether the Audubon's warbler (*Dendroica auduboni*), one of four visually distinct species in the yellow-rumped warbler complex, has originated through hybridization between two other species in this group, the myrtle warbler (*D. coronata*) and black-fronted warbler (*D. nigrifrons*). Analysis of nuclear amplified fragment length polymorphism (AFLP) and sequence markers shows that Audubon's warblers are genetically intermediate and carry a mixture of alleles otherwise found only in one or the other of their putative parental species. Audubon's warblers also carry two deeply divergent mitochondrial DNA lineages, each shared with only one putative parental form. Broad clines between Audubon's and black-fronted warblers in AFLP markers call into question the validity of these two forms as full species; nevertheless, our results suggest that the Audubon's warbler probably originated through hybridization between two long-diverged species. It is likely that more cases of avian species of hybrid origin will be revealed by surveys of variation in nuclear DNA and other traits.

Keywords: *Dendroica auduboni*, *Dendroica coronata*, *Dendroica goldmani*, *Dendroica nigrifrons*, hybrid speciation, hybrid zone, mitochondrial capture

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Introduction

Botanists and zoologists have traditionally held different views on the evolutionary importance of hybridization (Harrison 1993; Arnold 1997). Zoologists have generally emphasized the role of hybridization in preventing or reversing divergence between incipient species (e.g., Mayr 1963), while botanists have pointed to the possibility of cross-species spread of beneficial mutations and the formation of novel lineages (e.g., Stebbins 1959). Hybrid speciation has long been known to occur in plants, often involving polyploidy (Otto & Whitton 2000) but in some cases without it (Rieseberg 1997). In recent years, several cases of homoploid hybrid speciation have been documented in animals (reviewed in Mavárez & Linares 2008), including fish (e.g., Nolte *et al.* 2005; Meyer *et al.* 2006), insects (e.g., Gompert *et al.* 2006; Mavárez *et al.* 2006) and water fleas (Taylor *et al.* 2005), suggesting that this phenomenon may be more important in animals than was previously thought.

Hybrid origins have been proposed for a few bird taxa, but evidence for these cases is sparse or equivocal (Price 2008). The Adelaide rosella *Platycercus elegans adelaidae* was proposed as a possible hybrid species (Serventy 1953; Price 2008) on the basis of intermediate colour pattern between two related forms with nearby distributions, the crimson rosella *P. e. elegans* and yellow rosella *P. e. flaveolus*. Joseph *et al.* (2008) examined mtDNA and microsatellite variation across this complex with ambiguous results; the intermediate form may have originated through hybridization, but may have been ancestral to the two extreme types, forming an

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'incomplete ring species'. The Italian sparrow (*Passer domesticus italiae*) presents another possible case of hybrid species (Johnston 1969; Price 2008) on the basis of morphological and geographic intermediacy between the house sparrow (*P. d. domesticus*) and Spanish sparrow (*P. hispaniolensis*). Experimental hybrids between house and Spanish sparrows resemble wild Italian sparrows (Johnston 1969), and molecular evidence suggests a hybrid origin (Hermansen JS, Sæther SA, Elgvin TO, Borge T, Hjelle E, Sætre GP unpublished).

The yellow-rumped warbler (*Dendroica coronata*) complex is composed of four well-marked forms, the relationships and taxonomic status of which have been extensively debated (Fig. 1; Hubbard 1969; Barrowclough 1980; Zink & McKitrick 1995; Rohwer & Wood 1998; Johnson *et al.* 1999; Milá *et al.* 2007; Brelsford & Irwin 2009; Gill & Donsker 2010). The four forms were initially described as separate species *Dendroica coronata* (Linnaeus 1766), *D. auduboni* (Townsend 1837), *D. nigrifrons* (Brewster 1889) and *D. goldmani* (Nelson 1897). Later, *nigrifrons* and *goldmani* were considered subspecies of *auduboni* on the basis of similar plumage (Oberholser 1921), while *coronata* was maintained as a full species, presumably because of its distinct facial plumage pattern (Fig. 1). The discovery of a hybrid zone between *coronata* and *auduboni* (Hubbard 1969) led to all four forms being considered conspecific. A study of mitochondrial DNA (mtDNA) by Milá *et al.* (2007) found a surprising similarity between *auduboni* (sampled in Washington, Oregon, and California) and *coronata* (sampled in Alaska and Maine), and a deep divergence (approximately 1.7 million years) between these migratory forms and the two sedentary southern forms. The suggested interpretation of this pattern was that *coronata* diverged very recently from *auduboni* and rapidly evolved its distinctive male breeding-season plumage, presumably under strong sexual selection (Milá *et al.* 2007). By analysing nuclear DNA variation in the hybrid zone, Brelsford & Irwin (2009) showed that the *coronata/auduboni* hybrid zone is stable and maintained largely by postmating reproductive barriers. Intrinsic postzygotic barriers evolve slowly in birds (Price & Bouvier 2002), leading Brelsford & Irwin (2009) to hypothesize that the mtDNA similarity between *coronata* and *auduboni* could be a result of cytoplasmic capture rather than very recent divergence from a common ancestor. On the basis of recent molecular work on the complex (Milá *et al.* 2007; Brelsford & Irwin 2009), the International Ornithological Congress restored all four taxa to full species status (Gill & Donsker 2010). For simplicity, we hereafter refer to the four taxa as species while recognizing that their taxonomic treatment remains controversial.

Here, we survey mitochondrial and nuclear genetic variation across the full range of the species complex to test whether *auduboni* may have originated through hybridization between *coronata* and *nigrifrons*. In a taxon of recent hybrid origin, we expect genetic intermediacy between the two parental forms as well as a lack of

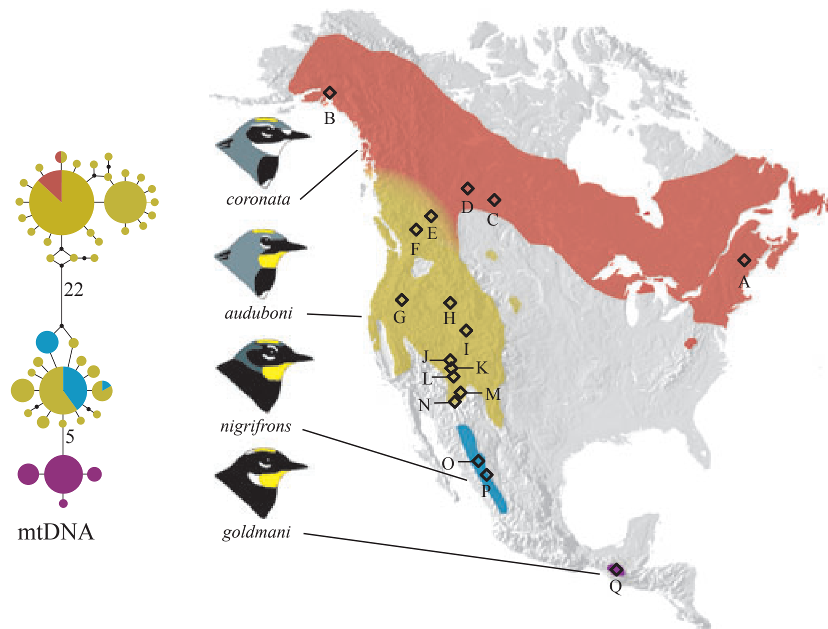

Fig. 1 Range map, sampling locations, facial plumage pattern and mitochondrial DNA (ATPase 6 and 8) haplotype network for four yellow-rumped warbler species. In the haplotype network, all *auduboni* in sites E–I group with *coronata*, while 93% of *auduboni* in sites K–N group with *nigrifrons*.

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alleles unique to that taxon. If *auduboni* is a lineage of hybrid origin, it raises the possibility that hybrid speciation may be occurring in this and other groups of birds.

Methods

Field sampling

Warblers ($n = 187$) were captured on their breeding grounds in 2001–2007 (see Fig. 1 and Table 1 for locations) using mist nets and song playbacks. We chose sampling sites to broadly cover the range of the species complex and to enable analysis of clinal genetic variation along a transect spanning the *coronata*, *auduboni* and *nigrifrons* forms. Blood samples were obtained by brachial venipuncture and stored in Queens lysis buffer (Seutin *et al.* 1991), and birds were released after capture. For one site (site G), muscle tissue samples were obtained from the Burke Museum (UWBM No. 64371, 64373, 64374, 64440). We excluded sites within the narrow *auduboni/coronata* hybrid zone from the present analysis, as that hybrid zone was analysed in detail by Brelsford & Irwin (2009), and our goal here was to identify the origins of *auduboni* sampled outside of that hybrid zone.

Morphology and plumage colouration

We extracted data on wing length and throat colour from previously published studies (Hubbard 1969, 1970). Additionally, one of us (AB) scored the extent of black in the cheek patch from photographs taken in the field. Scoring criteria were as follows: 0) lores and auriculars completely 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 and 4) lores and auriculars >95% black.

Molecular techniques

Mitochondrial DNA. We used mitochondrial ATPase 6 and 8 sequences obtained by Milá *et al.* (2007, GenBank accession numbers DQ855192–DQ855209), supplemented by sequencing 81 additional *auduboni* individuals sampled along a transect from Idaho to Arizona (localities H through N, Fig. 1 and Table 1). PCR and sequencing protocols followed Milá *et al.* (2007).

Amplified fragment length polymorphism (AFLP). Amplified fragment length polymorphism markers are widely used to test hypotheses of hybrid speciation (e.g., Gompert *et al.* 2006; Koblmüller *et al.* 2007; Kronforst *et al.* 2007) because of their reproducibility and ease of generating large numbers of markers. AFLP reactions were carried out according to Vos *et al.* (1995), with minor modifications described by Toews & Irwin (2008). Fluorescently labelled AFLP products were separated by 6.5% polyacrylamide gel electrophoresis, visualized on a 4300 DNA analyzer (LI-COR, Lincoln, Nebraska) and scored manually using SAGA 2.0 (LI-COR, Lincoln, Nebraska). AFLP analysis was carried out on 119 individuals, 38 of which were run twice starting from a new DNA extraction. We used 10 primer combinations (Table S1 in supporting information), which yielded 311 polymorphic markers with average repeatability of 0.986.

AFLP sequences. We isolated and sequenced two AFLP markers that showed high differentiation among *coronata*, *auduboni* and *nigrifrons*, following the protocol of Brugmans *et al.* (2003) with slight modifications. Markers to be sequenced were selected from those which had frequency differences >0.6 between any two of the three non-*goldmani* species. We followed the minisequencing protocol of Brugmans *et al.* (2003), using a generalized set of 12 degenerate primers to determine three additional selective bases for each M primer. We then carried out a PCR reaction using the band-specific M primer and original E primer, separated the multiple PCR products on a 4% agarose gel and excised a plug from the target band. The plug was incubated in 50 μL of ultra-pure water for 1 h at 55 °C, and this solution was used as template for an additional PCR reaction using the band-specific M primer and original E primer. The resulting amplicons were sequenced bidirectionally by Macrogen, Inc., using the amplification primers. We then sequenced outward in both directions from each AFLP fragment using a DNA Walking SpeedUp kit (SeeGene, Inc., Seoul, Korea) and designed primers to amplify a fragment of approximately 800 bp spanning the entire AFLP marker. These sequences aligned unambiguously to the zebra finch genome; one maps to chromosome three near the predicted gene *KIAA1383*, while the other maps to chromosome six within an intron of the gene *ARID5B*.

Nuclear sequences. We sequenced 12 nuclear loci in at least five individuals of each species. Loci sequenced included nine introns, two AFLP-derived loci (described above) and one nuclear sequence of mitochondrial origin (numt). Because of limited quantities of template DNA available from some sites, we used nested PCR to amplify loci for sequencing. Primers, primer sources and reaction conditions are shown in Table S2 (Supporting information). Amplicons were sequenced bidirectionally by Macrogen Inc. or by the Genome Quebec McGill Innovation Centre, using ABI 3730XL sequencers.

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Analysis

Sequences were aligned and proofread using BioEdit (Hall 1999). For nuclear loci, the phase of heterozygous sites was determined using Phase (Stephens & Donnelly 2003). We used TCS (Clement *et al.* 2000) to reconstruct haplotype networks, and DNAsp (Rozas *et al.* 2003) to calculate pairwise F_{ST} values between species. Multidimensional scaling based on Jaccard distances (Kingston & Rosel 2004) was used to graphically display AFLP-derived genetic distances among individuals in two dimensions; this analysis was implemented in R (R Development Core Team) using the package Vegan (Oksanen *et al.* 2008). We used Bayesian clustering implemented in Structure 2.3 (Hubisz *et al.* 2009) to assess population structure and used the methods of Evanno *et al.* (2005) to determine the most likely number of populations in the sample (assuming for this analysis that this group consists of discrete populations with some admixture). To enable comparisons of clinal genetic and phenotypic variation, we measured the minimum straight-line distance from each sampled population to the centre of the *coronata/auduboni* hybrid zone (as described in Brelsford & Irwin 2009).

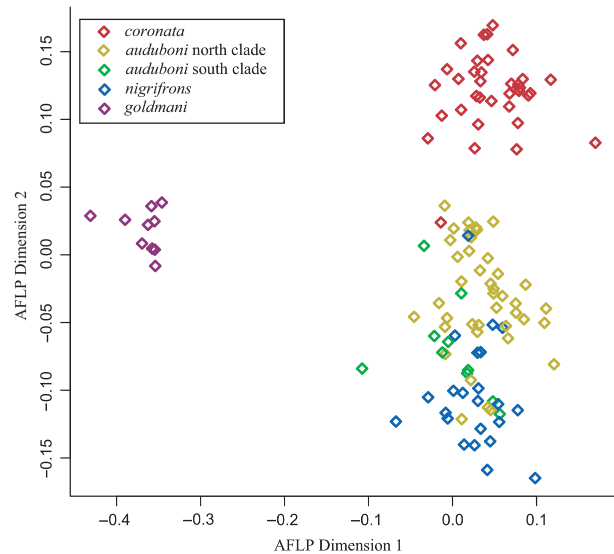

Fig. 2 Multidimensional scaling plot of amplified fragment length polymorphism variation. Dimension 1 separates the disjunct *goldmani* species from the other three more widespread forms, which are arrayed along dimension 2. Northern populations of *auduboni* are intermediate between *nigrifrons* and *coronata*, while southern *auduboni* (sampled in Arizona) are closer to *nigrifrons*.

Results

Mitochondrial and AFLP analysis identified three distinct clusters in the yellow-rumped warbler complex (Figs 1, 2 and 3), with additional geographic structure present within some clusters. Both types of markers separated *coronata*, *nigrifrons* and *goldmani* from each other, but the placement of *auduboni* differed. Multidimensional scaling of AFLP data showed *goldmani* to be the most genetically distinct species in the complex (discussed further below). Furthermore, *coronata* and *nigrifrons* formed well-separated clusters, and the *auduboni* cluster was located between *coronata* and *nigrifrons*, partly overlapping with *nigrifrons* (Fig. 2). This suggests that most of the genetic differentiation between *auduboni* and *nigrifrons* is a result of the presence of alleles characteristic of *coronata*, which are more common in northern *auduboni* populations. Analysis of AFLP data using Structure produced broadly consistent results, grouping *auduboni* mostly with *nigrifrons* but with considerable introgression from *coronata* (Fig. 3). In contrast, mitochondrial DNA of most *auduboni* grouped with *coronata*, consistent with the results of Milá *et al.* (2007). Southern populations of *auduboni*, newly sampled for this study, were an exception to this pattern: 38 of 41 sampled in Arizona (sites K-N, Table 1) belonged to the same mitochondrial clade as *nigrifrons*.

We found private AFLP alleles in three of the four species, with *auduboni* being the exception. Private alleles were defined as the presence or absence of an AFLP band at frequency >0.2 in the focal species and <0.05 in all other species. The number of private alleles in each species was 4 (*coronata*), 0 (*auduboni*), 3 (*nigrifrons*) and 7 (*goldmani*).

The *goldmani* species was the most genetically distinct form on the basis of AFLPs, largely because of its much lower within-population variation (Fig. 2, Table S3, Supporting information). The proportion of bands that were polymorphic was much lower in *goldmani* (0.29) than in *coronata* (0.90), *auduboni* (0.97) or *nigrifrons* (0.84); correcting for differences in sample size, at $n = 10$ for all species, the values are 0.29, 0.68, 0.70 and 0.67, respectively. Over half of the scored markers (161 of 311) were fixed in *goldmani* and polymorphic in each of the other three species. This suggests that the genetic distinctiveness of *goldmani* is due partly to its loss of genetic variation present in the other species, presumably because of its historically small population size.

Nuclear sequences (Table S4 and Fig. S1, Supporting information) were consistent with the close relationship between *auduboni* and *nigrifrons* seen in AFLPs. Of 12 nuclear sequence loci screened, most showed very little differentiation among the four taxa (Table S4, Supporting information). Two of these loci, *CHD1Z* and *Numt-Dco1*, were highly differentiated between *coronata* and

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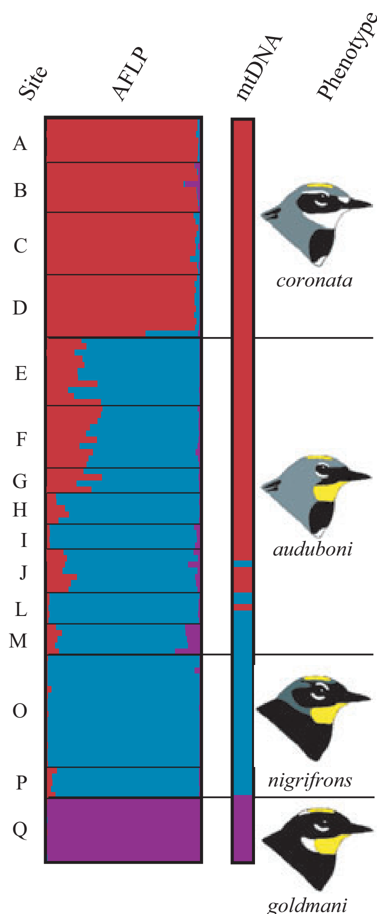

Fig. 3 Amplified fragment length polymorphism (AFLP)-based population assignment from Structure, mtDNA clade and phenotypic group for four yellow-rumped warbler species. Both mtDNA and AFLPs show three clusters in the species complex, corresponding to *coronata*, *nigrifrons* and *goldmani*, while *auduboni* shows evidence of genetic contribution from both *coronata* and *nigrifrons* in both marker types. Mitochondrial DNA was not directly sampled in locations B–G; however, all of the 92 individuals sampled by Milá *et al.* (2007) in nearby locations carried *coronata*-clade mtDNA.

auduboni (Brelsford & Irwin 2009) and in both cases showed little differentiation between *auduboni* and *nigrifrons*.

Haplotype networks of these two loci and two sequenced AFLP markers (Fig. S1, Supporting information) supported a close relationship between *auduboni* and *nigrifrons*, with considerable introgression from *coronata* into *auduboni*. In three of the four informative loci (Fig. S1B, C, D, Supporting information), the distribution of haplotypes in *auduboni* and *nigrifrons* was nearly identical, with *coronata* having substantially different haplotypes. In the fourth locus (Fig. S1A, Supporting information), differentiation between *coronata* and *nigrifrons* was high, but *auduboni* contained haplotypes characteristic of both forms. Overall, these sequencing results were highly consistent with the AFLP data, which integrate patterns from a much larger number of markers; both showed that *auduboni* is genetically intermediate to *coronata* and *nigrifrons*, although more similar to *nigrifrons*.

Clinal variation in different markers and traits also showed congruence among some traits and incongruence among others. The frequency of southern-clade mtDNA had a sharp break near the Arizona/Utah border (Fig. 4d), while the two nuclear markers *CHD1Z* and *Numt-Dco1* showed sharp changes in frequency at the hybrid zone between *coronata* and *auduboni* (Fig. 4h, i). The population mean of AFLP dimension 2 (which incorporates the bulk of differentiation among the three northern species) showed a small but abrupt change across the *coronata/auduboni* hybrid zone, and a gradual change across the range of *auduboni* (Fig. 4g). Wing length (measured by Hubbard 1970) showed a similar pattern to AFLPs (Fig. 4c), while throat colour (scored by Hubbard 1969, 1970) was similar to *CHD1Z* and *Numt-Dco1* (Fig. 4a), and cheek patch distinguished *auduboni* from both *coronata* and *nigrifrons*.

Discussion

Taken together, our evidence indicates that the history of the species in the yellow-rumped warbler complex cannot be represented as a simple bifurcating tree. Rather, the Audubon's warbler (*D. auduboni*) as defined by previous taxonomy (Townsend 1837; Oberholser 1921; Hubbard 1970) clearly represents an admixture between two long-divergent lineages, *coronata* and *nigrifrons*, which differ substantially in plumage, morphology, migratory behaviour and both mitochondrial and nuclear DNA. The *auduboni* species most closely resembles its southern neighbour *nigrifrons* in its male breeding-season plumage traits (including yellow throat, white wing patch and lack of eye stripe). In contrast, over most of its range, *auduboni* shares with *coronata* its mtDNA clade and migratory behaviour. In nuclear allele frequencies (Figs 2 and 4) and wing length (Fig. 4c), northern *auduboni* are intermediate between *coronata* and *nigrifrons*, while southern *auduboni* closely resemble *nigrifrons*. Finally, of the four species, only *auduboni* lacks private AFLP alleles.

These patterns strongly suggest that *auduboni* arose through hybridization of *coronata* and *nigrifrons*. Two alternative scenarios could produce in *auduboni* similar patterns of genetic intermediacy between the two putative parental species, but we view these alternatives as less likely. First, it may be that the intermediate characteristics of *auduboni* are the ancestral condition, rather than a result of admixture, and that *coronata* and

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Table 1 Locations and AFLP, mtDNA and cheek patch data for 17 localities sampled in this study. AFLP Dim. 2 refers to dimension 2 of the multidimensional scaling analysis, which is the main axis along which *coronata*, *auduboni* and *nigrifrons* differ. Cheek patch score is the extent of black coloration on the cheek patch, ranging from 0 (completely grey) to 4 (completely black)

Site	Latitude	Longitude	Species (morphology)	Mean AFLP Dim. 2	ARID5B AFLP band freq.	KIAA1383 AFLP band freq.	*n* (AFLP)	South clade mtDNA freq.	*n* (mtDNA)	Cheek patch	*n* (cheek patch)
A	44.98	−70.66	*coronata*	0.125	0.14	0.29	7	0.00	21		
B	61.15	−149.74	*coronata*	0.132	0.00	0.38	8			3.20	22
C	54.72	−110.09	*coronata*	0.115	0.10	0.30	10			3.40	10
D	55.48	−114.85	*coronata*	0.112	0.20	0.20	10				
E	52.49	−121.71	*auduboni*	−0.016	0.45	1.00	11				
F	49.21	−121.36	*auduboni*	−0.021	0.30	0.90	10			1.10	10
G	42.78	−122.08	*auduboni*	−0.011	0.33	1.00	4				
H	43.92	−114.86	*auduboni*	−0.052	0.60	1.00	5	0.00	9		
I	40.43	−111.64	*auduboni*	−0.101	0.25	1.00	4	0.00	4	0.75	4
J	37.57	−112.84	*auduboni*	−0.029	0.29	1.00	7	0.32	22	1.00	10
K	36.71	−112.22	*auduboni*					0.86	14	1.00	12
L	35.36	−111.62	*auduboni*	−0.046	0.80	1.00	5	1.00	6	1.00	5
M	33.87	−109.40	*auduboni*	−0.094	0.40	1.00	5	0.92	12		
N	32.66	−109.87	*auduboni*					1.00	9	1.25	8
O	26.47	−106.39	*nigrifrons*	−0.105	0.72	1.00	18	1.00	15	3.33	24
P	24.95	−105.92	*nigrifrons*	−0.100	0.80	1.00	5	1.00	17		
Q	15.53	−91.52	*goldmani*	0.018	0.00	1.00	10			3.94	16

AFLP, amplified fragment length polymorphism.

nigrifrons have independently diverged from *auduboni*. In this case, we would expect to find private alleles equally common in all three widespread forms. Instead, we find private AFLP alleles only in *coronata* (four loci) and *nigrifrons* (three loci), while at all loci, *auduboni* shares alleles with *coronata*, *nigrifrons* or both. Second, the divergence of *auduboni* from *nigrifrons* may have pre-dated the hybridization with *coronata* that gave rise to its admixed genome. Even if this scenario applies, the absence of private alleles in *auduboni* and its intermediate position in allele-frequency space (Fig. 2) indicate that any genetic divergence between *auduboni* and *nigrifrons* prior to hybridization with *coronata* was insubstantial. Our evidence suggests that only *auduboni* lacks a long history of independent evolution.

When did the hybridization that gave rise to *auduboni* occur? One possibility is during a previous interglacial period. Range shifts and fragmentation associated with glacial cycles could have caused geographic isolation between *auduboni* and one or both of its parental forms, with subsequent expansion from a refugium producing a widespread species of hybrid origin. Alternatively, *auduboni* may have formed at the beginning of the current interglacial period as a result of secondary contact and hybridization between *coronata* and *nigrifrons*. If this contact occurred in the southwestern US, the *coronata* alleles (including mtDNA) found in *auduboni* would represent a neutral wake left behind by a hybrid zone that has moved northward (e.g., Krosby & Rohwer 2009). If instead the initial contact between *coronata* and *nigrifrons* occurred in the Canadian Rockies, the *coronata* alleles found in *auduboni* would have introgressed southward, most likely under positive selection although possibly by drift. We find secondary contact in the Rockies more plausible for two reasons: First, the Canadian Rockies form a suture zone for many west—east pairs of boreal forest taxa (Swenson & Howard 2005; Toews & Irwin 2008; Irwin *et al.* 2009), making it a likely location for initial contact. Second, the transition between the two mitochondrial clades in Arizona and Utah has a width of the same order as that of the *coronata/auduboni* hybrid zone in Canada (132 km), which is maintained by selection (Brelsford & Irwin 2009). If the northern-clade mtDNA is neutral and was left behind after hybrid zone movement, more mixing should have occurred at the interface of the two clades in Arizona and Utah (Endler 1977; Brelsford & Irwin 2009). We find it more plausible that some form of selection caused *coronata* mtDNA to flow into *auduboni* and spread south to the current boundary between the clades. Environmental selection on mitochondrial function has been inferred in other systems (e.g., Cheviron & Brumfield 2009), and further research could test the possibility that selection maintains this relatively narrow mitochondrial contact zone in the absence of strong morphological or nuclear genetic differentiation.

Our data do not allow us to distinguish whether *auduboni* originated in the current or a previous intergla-

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cial period. The mitochondrial introgression from *coronata* into *auduboni* can be dated very approximately to 16 000 years ago (Milá *et al.* 2007), which might suggest that *auduboni* originated in the current interglacial period. However, there is a large degree of uncertainty around that timing estimate, and it is also possible that *auduboni* existed as a hybrid species before the most recent episode of mitochondrial introgression. The genetic and phenotypic similarity over most of *auduboni*'s range (excluding Arizona and Utah) might imply that the species expanded from a small refugium where it persisted through at least one glacial cycle. We suggest that hybridization in a previous interglacial period followed by persistence in a glacial refugium and subsequent expansion across western North America may be the most straightforward explanation for the existence of *auduboni* as a distinct form with a recognizable phenotype and genotype over a broad region, rather than a wide hybrid zone between *coronata* and *nigrifrons*, but further research will be necessary to test this hypothesis.

If *auduboni* truly represents an evolutionarily distinct form, it is one of the best-documented cases thus far of the origin of a bird species through hybridization. However, the broad cline in AFLPs and discordance between narrow plumage and mtDNA clines between *nigrifrons* and *auduboni* call into question the designation of these two forms as full species. It is possible that there is no reproductive isolation between *nigrifrons* and *auduboni* and that the two forms will eventually fuse. However, despite apparent interbreeding between these taxa, phenotypic intermediates have only been found in a small area of southernmost Arizona (Moore 1946; Hubbard 1970), suggesting the possibility of a narrow and stable hybrid zone. Further study of the contact zone between these forms is clearly warranted to determine the extent of reproductive isolation between them, as information is especially lacking from the area south of the U.S./Mexico border.

Arnold (2006) defines hybrid speciation as 'the process in which natural hybridization results in the production of an evolutionary lineage that is at least partially reproductively isolated from both parental lineages and which demonstrates a distinct evolutionary and ecological trajectory'. In the case of *D. auduboni*,

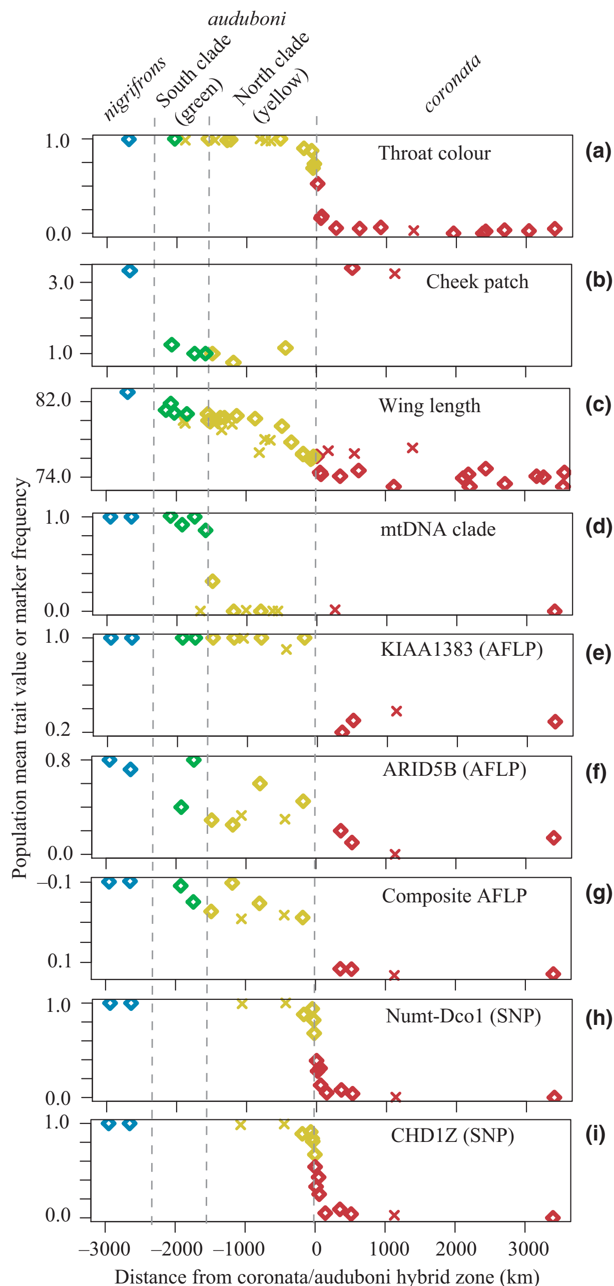

Fig. 4 Geographic variation in traits and genetic markers that differ between *nigrifrons* and *coronata* demonstrate incongruent patterns in the putative hybrid *auduboni*. The *auduboni* form resembles *nigrifrons* in throat colour (a) and three nuclear markers (e, h, i). In contrast, in wing length (c), one nuclear marker (f) and multilocus amplified fragment length polymorphism (AFLP) score (g), *auduboni* is intermediate between *nigrifrons* and *coronata*. The sharp phylogeographic break between northern and southern mtDNA lineages (d) occurs within the range of *auduboni* and is not accompanied by an abrupt cline in phenotype or any other known genetic marker. Throat colour and wing length (a, c) were taken from Hubbard (1969, 1970). Mitochondrial clade frequencies (d) were taken from this study (sites H–N) and Milá *et al.* (2007). SNP frequencies (h, i) were taken from this study (sites A, B, G, O, P) and Brelsford & Irwin (2009). Because the latitudinal gradient in wing length differs between coastal and interior populations, we use ''X'' symbols to denote sample locations near the Pacific coast (California, Oregon, Washington, western British Columbia, Yukon and Alaska); interior and eastern sites are represented by diamonds.

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reproductive isolation from *nigrifrons* has not yet been demonstrated but is likely to some degree, as discussed earlier. Furthermore, sympatric overlap between wintering *auduboni* and breeding *nigrifrons* takes place in western Mexico during the spring (B. Milá, personal observation), apparently without interbreeding. Regardless of whether *auduboni* is considered a species, as Nolte & Tautz (2009) observe, a diagnosable lineage of hybrid origin can provide substantial insight into the process of hybrid speciation.

The geographically isolated fourth species in the complex, *D. goldmani*, showed a much lower level of nuclear genetic diversity than in the other three forms, confirming this aspect of Milá *et al*.'s (2007) mtDNA results. The *goldmani* form has a small effective population size, restricted range and long history of independent evolution, which suggests the need for increased conservation attention. This result also highlights the sensitivity of allele-frequency-based measures of genetic differentiation (i.e., most analyses of microsatellites and AFLPs) to differences in population size (Ehrich *et al*. 2009), unlike measures based on DNA sequence divergence (Zink & Barrowclough 2008).

We have shown that the *coronata* and *nigrifrons* species diverged from each other long ago and that the *auduboni* form represents an admixed population in which alleles from the two divergent parental types occur in novel combinations. The two parental types remain highly distinct despite this admixture, providing additional evidence that hybridization need not reverse speciation (Arnold 1997). Previous research has demonstrated partial reproductive isolation between *auduboni* and *coronata* (Brelsford & Irwin 2009), and it is possible that some reproductive isolation exists between *auduboni* and *nigrifrons*, making this a potential case of hybrid speciation. Such a widespread taxon of hybrid origin has not yet been shown in birds, but others may exist (e.g., the Italian sparrow, Hermansen *et al*. unpublished). Such cases can best be recognized through surveys of many independent genetic markers. Ongoing advances in genomic methods and greater attention to the possibility of hybrid speciation may reveal many more examples in birds and other animals.

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Data accessibility

DNA sequences: Genbank accessions JF309758–JF310275.

AFLP data table: DRYAD entry doi:10.5061/dryad.8573.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Haplotype networks for 4 nuclear loci that differ between *coronata* and *nigrifrons*.

Table S1 AFLP primer combinations used, and number of polymorphic loci obtained from each.

Table S2 Primers used for amplification and sequencing of nuclear loci. External primer sources are (1) Borge *et al.* 2005; (2) Backström *et al.* 2008, (3) Fridolfsson & Ellegren 1999, and (4) this study; all internal primer sequences were developed for this study.

Table S3 AFLP-based differentiation among yellow-rumped warbler forms *coronata*, *auduboni* north clade, *auduboni* south clade, *nigrifrons*, and *goldmani.*

Table S4 Divergence among the three widespread forms of yellow-rumped warbler at 12 nuclear loci, measured by F_{ST} and net sequence divergence (D_a).

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