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RESEARCH ARTICLE

Phenotypic clines across an unstudied hybrid zone in Woodhouse's Scrub-Jay (*Aphelocoma woodhouseii*)

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ABSTRACT

Woodhouse's Scrub-Jay (*Aphelocoma woodhouseii*) comprises 7 subspecies, ranging from the Rocky Mountains to southern Mexico. We quantified the phenotype of specimens throughout Mexico and found support for significant phenotypic differences between "Sumichrast's group" in southern Mexico (*A. w. sumichrasti* and *A. w. remota*) and the 2 subspecies in northern Mexico, or "Woodhouse's group" (*A. w. grisea* and *A. w. cyanotis*). Despite significant differentiation in body size and mantle color, we found no clear geographic boundary between the groups, suggesting either a geographic cline or hybridization upon secondary contact. We tested for selection against hybridization by fitting models to geographic clines for both body size and back color, and found support for a stable contact zone centered near Mexico City, with selection against intermediate back color. Based on these results, we infer that Sumichrast's and Woodhouse's groups diverged during a period of geographic and genetic isolation. The phenotypic introgression between Sumichrast's and Woodhouse's groups near Mexico City likely represents a case of recent secondary contact, with selection against hybridization maintaining a geographically stable contact zone.

Keywords: evolution, hybrid zone, morphology, systematics, taxonomy

Gradientes fenotípicos a través de una zona híbrida no estudiada en *Aphelocoma woodhouseii*

RESUMEN

Aphelocoma woodhouseii comprende siete subespecies, que van desde las Montañas Rocosas hasta el sur de México. Cuantificamos el fenotipo de especímenes a lo largo de México y encontramos apoyo para diferencias fenotípicas significativas entre el "grupo Sumichrast," en el sur de México (*A. w. sumichrasti* y *A. w. remota*) y las dos subespecies en el norte de México, o el "grupo Woodhouse" (*A. w. grisea* y *A. w. cyanotis*). A pesar de la diferenciación significativa en el tamaño corporal y el color del dorso, no encontramos un límite geográfico claro entre los grupos, sugiriendo ya sea un gradiente geográfico o hibridación luego de un contacto secundario. Evaluamos la selección contra la hibridación ajustando modelos al gradiente geográfico tanto para el tamaño corporal como para el color del dorso, y encontramos apoyo para una zona de contacto estable centrada cerca de la Ciudad de México, con selección en contra del color intermedio del dorso. Con base en estos resultados, inferimos que los grupos Sumichrast y Woodhouse de *A. woodhouseii* divergieron durante un período de aislamiento geográfico y genético. La introgresión fenotípica entre los grupos Sumichrast y Woohouse cerca de la Ciudad de México probablemente representa un caso de contacto secundario reciente, con la selección contra la hibridación manteniendo una zona de contacto geográficamente estable.

Palabras clave: evolución, morfología, sistemática, taxonomía, zona híbrida

INTRODUCTION

The processes of speciation and lineage splitting have long been sources of intrigue for evolutionary biologists (Darwin 1859, Mayr 1942, Cracraft 1983, Coyne and Orr 2004). Mayr (1942) established the still widely accepted model of avian speciation where isolating barriers lead to the accumulation of differentiation between populations due to drift and differential natural selection over millions of years. Recent studies show that this process of gradual speciation in allopatry often includes intermittent bouts of gene flow (Rheindt and Edwards 2011). A binary model of speciation assumes that these cases of secondary contact will lead either to lineage collapse and return to a single panmictic population, or to non-recognition of interspecific relatives and complete isolation via assortative mating. In reality we often see evidence for stable zones of introgression, where populations intergrade phenotypically and genetically, while maintaining species integrity on either side of the bounded area of sympatry (Swenson et al. 2006). Because we regularly observe these cases of retained interspecific fertility, it follows that pre-mating isolating barriers are especially important to maintaining species integrity in avian systems (Price and Bouvier 2002).

By studying cases of secondary contact between avian lineages, we can open a window into the evolutionary processes driving speciation and maintaining global diversity in both genotype and phenotype.

Research on avian introgression has recently seen important conceptual and methodological advances, as next-generation sequencing has facilitated the application of genome-wide approaches (Toews et al. 2015). The genic view of speciation made the case that introgression and recombination lead to genes moving independently across incipient species boundaries (Wu 2001). The "genic speciation" hypothesis can now be analytically tested via geographic cline analyses, leading to a renewed interest in studying contact zones with a focus on how individual genes transition across species boundaries (Baldassarre et al. 2014, Derryberry et al. 2014, Taylor et al. 2015). As next-generation sequencing techniques have made quantifying genome-wide patterns of introgression between recently diverged populations a reality, modern morphological and statistical methods have done the same for quantifying phenotypic introgression, with different phenotypic traits potentially moving differentially across contact zones (Baldassarre et al. 2014). The incorporation of both phenotypic and genetic data from vouchered museum specimens has created exciting new research avenues, allowing researchers to analyze introgression across temporal, spatial, genetic and phenotypic axes (Carling and Brumfield 2008, Taylor et al. 2014).

Here, we use phenotypic cline analyses to investigate a previously uncharacterized contact zone within Woodhouse's Scrub-Jay (*Aphelocoma woodhouseii*) in Mexico. Woodhouse's Scrub-Jay was formerly lumped with the California Scrub-Jay (*A. californica*) under the common name Western Scrub-Jay. These 2 species were recently split based on phenotypic, ecological and genomic data, suggesting a long history of isolation and selection against hybrids in a narrow hybrid zone (Gowen et al. 2014, Chesser et al. 2016). Woodhouse's Scrub-Jay itself contains genetically and phenotypically distinct lineages that have been previously proposed as distinct species (Peterson and Navarro-Sigüenza 1999). Here, we refer to these distinct lineages as "Woodhouse's group", which comprises the 5 subspecies from eastern Oregon to Mexico City (*woodhouseii, nevadae, texana, grisea* and *cyanotis*), and "Sumichrast's group", which comprises the 2 southernmost subspecies (*sumichrasti* and *remota*) distributed from Mexico City to southeastern Oaxaca (Pitelka 1951). The 2 groups are divergent in mitochondrial DNA (McCormack et al. 2011), allozymes (Peterson 1992), microsatellites (Gowen et al. 2014) and genome-wide single-nucleotide polymorphisms (SNPs) (McCormack et al. 2016).

While there is strong evidence that Woodhouse's and Sumichrast's groups have been evolving in allopatry for 1 to 2 myr (McCormack et al. 2011), there is also evidence that these lineages hybridize in central Mexico. Pitelka (1951) described the phenotypic differences between these groups, noting, among other things, that Sumichrast's group was both significantly larger and had a brown back (or mantle), whereas the mantle of Woodhouse's group was blue (Figure 1). Pitelka (1951) also pointed to an area near Mexico City (between the ranges of subspecies *cyanotis* and *sumichrasti*) where specimens showed intermediate traits, but he described such specimens and evidence as "scant".

We use phenotypic information from specimens collected after Pitelka's (1951) work, as well as those he examined, to (i) reassess evidence for hybridization between Woodhouse's and Sumichrast's groups, and (ii) quantify geographic clines for divergent traits. Specifically, we test whether there is evidence for fixed differences between Woodhouse's and Sumichrast's groups outside of the geographic area potential zone of contact. We then assess the nature of the transition between the groups, to test the hypothesis that these traits transition rapidly, suggesting selection against hybrids and a measure of reproductive isolation between these lineages. Finally, we estimate the width and center of the transition in each trait individually, to investigate the evolutionary forces maintaining phenotypic divergence between these groups.

METHODS

Morphological and Spectral Data Collection

We examined 133 *A. woodhouseii* museum specimens spanning Mexico, from 4 subspecies: *grisea* and *cyanotis* (Woodhouse's group), and *remota* and *sumichrasti* (Sumichrast's group), as well as individuals from the putative contact zone, which appear phenotypically intermediate between *sumichrasti* and *cyanotis* (Figure 1). We collected standard morphological measurements for 6 traits: wing chord, tail length, tarsus length, bill length, bill depth and bill width (Pyle 1997). Wing chord was measured from the joint connecting the metacarpals with the ulna/radius to the end of the longest primary. Tail length was measured from the insertion point of the central rectrices to the tip of the central retrices. Tarsus length was measured from the tibiotarsal joint to the last undivided scute. Bill length, width and depth were all measured at the anterior end of the nares. Multiple researchers measured all traits on a subset of 15 specimens, 3 separate times, to confirm repeatability and accuracy scores over 0.9 for each trait in measurements by D.A.D., who would go on to collect all of the measurement data incorporated here. D.A.D. took measurements using a wing ruler to measure wing chord to the nearest 0.5 mm, and digital calipers to measure bill, tarsus and tail traits to the nearest 0.1 mm. First-year birds were excluded from the analysis after being identified on the basis of color of

FIGURE 1. Dorsal view of 5 Woodhouse's Scrub-Jay (*A. woodhouseii*) specimens showing pure (left) Woodhouse's and (right) Sumichrast's group types, as well as examples of intermediate phenotypes from the hybrid zone. From left to right, *cyanotis* (MLZ 27922), *cyanotis/sumichrasti* (USNM 144754), *cyanotis/sumichrasti* (MVZ 126143), *cyanotis/sumichrasti* (MVZ 115475) and *sumichrasti* (CAS 72079).

retained wing coverts from post-juvenal molt and the shape of rectrices and remiges (Pitelka 1945), as well as collector remarks on gonadal development.

To quantify plumage color, we used a Jaz spectrometer with an external probe and the Ocean Optics SpectraSuite (Dunedin, Florida, USA) software package to record reflectance curves from the plumage of each specimen. Each time the spectrometer was used, it was calibrated against a true white reflectance standard to ensure consistency in reflectance readings under variable ambient light. We quantified the color of the mantle (upper back) of each specimen, avoiding molting and damaged feathers and recording 10 reflectance readings for each individual. We then used the R package PAVO to smooth the 10 reflectance curves into one representative curve for each individual and formulate variables indicative of color (Maia et al. 2013). As a proxy for blue saturation, we used PAVO's variable Saturation 1B (S1B), which calculates the area under the smoothed reflectance curve from 400–510 nm, the blue portion of the color spectrum.

Quantification of Differences between Sumichrast's and Woodhouse's groups

For each trait, we used a pairwise Welch's *t*-test to test for sexual dimorphism, and for significant differences between Sumichrast's and Woodhouse's groups (Table 1). We used Welch's *t*-test because it is robust to unequal sample sizes and unequal variance between groups. We used a Shapiro-Wilk test to confirm the normality of the residuals for each variable, to quantitatively verify the assumptions of Welch's *t*-test. Because we were repeatedly testing for differences in 7 separate traits, we used the "false discovery rate" adjustment on our significance threshold as we interpreted the *p*-values from 21 pairwise tests (Benjamini and Hochberg 1995). We detected significant sexual dimorphism in 5 of 6 morphological traits measured, with males displaying 2–5% larger body size on average (Table 1). To account for this sexual dimorphism in body size, we tested for differences between Woodhouse's and Sumichrast's groups separately for male and female populations. No plumage dimorphism was detected between sexes (Table 1). We ran a principal components analysis (PCA) on a correlation matrix of variables to quantify and visualize major axes of differentiation in appearance between each subspecies, and to identify phenotypic structure within the species. We homed in on the transition between Woodhouse's and Sumichrast's groups in central Mexico by visualizing PC1 scores and the plumage variable S1B on violin/dot plots.

Cline Analysis

We conducted cline analyses by fitting georeferenced locations across the putative hybrid area to a linear sampling transect. In total, 108 georeferenced specimens were collapsed into 10 sampling locations (A–J in Figure 2) along a transect from the northern end of the range of *cyanotis* to the southern end of the range of *sumichrasti*. We used the R package hzar to fit cline models to phenotypic characters along the sampling transect (Derryberry et al. 2014). For each character, the best model was chosen based on lowest AICc score. Hzar also provides an estimate of the center and width of the transition for each phenotypic character. Plots of the clines for PC1 (general body size) for males and females, and S1B (back color) are reported in the text.

RESULTS

Differences between Sumichrast's and Woodhouse's groups

PC1 describes 48.4% of the total variation, and can be considered a proxy for general body size, as all 6 morphological variables loaded heavily on this axis in the same direction (Table 2, Figure 3). Sumichrast's and Woodhouse's groups show separation on PC1, indicating divergent body size. The 24 specimens measured from the putative contact zone between *cyanotis* and *sumichrasti* display intermediate trait sizes (Figures 3 and 4). We found significant differences between Sumichrast's and Woodhouse's groups in 6 out of 7 traits measured for males, and 5 out of 7 traits in females (Table 1, Appendix Figure 1).

TABLE 1. Sample size and means for each variable reported, by group, and sex. Wing, tail, tarsus, bill length, bill width and bill depth are reported in mm, S1B is a reflectance measure of the area under the spectral curve between 400 and 510 nm. Group analyses do not include *cyanotis/sumichrasti* individuals. Asterisk (*) denotes significant (false discovery rate adjusted) differences in a given trait based on pairwise Welch's 2-sample *t*-tests. Standard error reported in parentheses next to each trait value.

Categorization method	*n*	Wing	Tail	Tarsus	Bill length	Bill width	Bill depth	S1B
Group (male)								
Woodhouse's	36	*135.2 (0.8)	*148.0 (0.9)	*39.6 (0.3)	19.2 (0.2)	*7.4 (0.1)	*8.7 (0.1)	*0.302 (0.003)
Sumichrast's	32	*143.6 (1.1)	*152.9 (1.1)	*40.8 (0.3)	19.4 (0.1)	*7.7 (0.1)	*9.2 (0.1)	*0.251 (0.002)
Group (female)								
Woodhouse's	21	*131.2 (0.9)	*142.6 (1.2)	*38.8 (0.3)	18.2 (0.2)	7.2 (0.1)	*8.2 (0.1)	*0.298 (0.004)
Sumichrast's	20	*140.2 (1.2)	*146.2 (1.1)	*40.5 (0.3)	18.4 (0.2)	7.4 (0.1)	*8.9 (0.1)	*0.249 (0.003)
Sex								
Male	81	*139.3 (0.7)	*150.4 (0.7)	*40.3 (0.2)	*19.2 (0.1)	*7.6 (<0.1)	*9.0 (<0.1)	0.275 (0.003)
Female	52	*136.0 (0.8)	*144.4 (0.7)	*39.7 (0.2)	*18.2 (0.1)	*7.3 (0.1)	*8.6 (0.1)	0.271 (0.004)

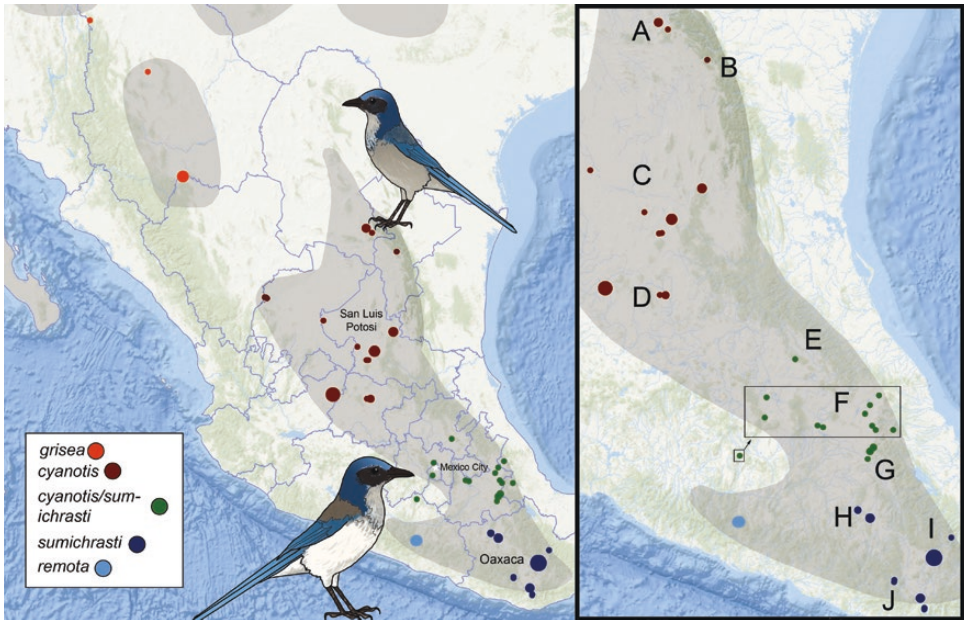

FIGURE 2. Map showing localities of specimens used in this study (*n* = 133), with contact zone individuals labeled *sumichrasti/cyanotis*. Inset displays the hybrid zone sampling transect with localities arbitrarily divided into populations A–J moving southeast (*n* = 108). A bounding box is drawn around individuals at Site F. Shaded background is a modified version of the NatureServe range map manually adjusted to reflect updated distribution information based on recent sampling.

PC2 describes 15.5% of the total variation, and is driven mainly by S1B, a quantification of the saturation of blue on the mantle. Bill length and width are also driving some variation in PC2, with Sumichrast's group characterized by a larger overall bill. Woodhouse's group displays significantly more blue coloration on the mantle than Sumichrast's group with limited overlap between the groups (Table 1, Figure 4). Individual subspecies within each group are not phenotypically diagnosable (Appendix Table 1).

Specimens measured from the contact zone between *cyanotis* and *sumichrasti* display a wide range in blue saturation of the mantle, largely overlapping both Woodhouse's and Sumichrast's groups (Figure 4). The distribution of contact zone individuals is bimodal, with 6 specimens falling within the range of blue saturation of Woodhouse's group and 18 falling within Sumichrast's group (Figure 4). A linear model relating mantle color and body size for contact zone individuals indicates a non-significant but detectable relationship between these traits, indicating that despite differing cline widths, trait divergence is generally coupled across the contact zone (Appendix Figure 2).

TABLE 2. PC loadings on phenotypic variables.

Character	PC1	PC2	PC3
Wing	−0.463	−0.199	0.273
Tail	−0.415	0.103	0.400
Tarsus	−0.392	−0.065	0.331
Bill length	−0.316	0.614	0.033
Bill width	−0.333	0.175	−0.706
Bill depth	−0.425	0.029	−0.341
S1B	0.259	0.733	0.202

Cline Analysis

Given the intermediate appearance of specimens from central Mexico, we investigated phenotypic intergradation using geographic cline analysis. For morphology, PC1 shows a gradual increase in body size from *cyanotis* in San Luis Potosí to *sumichrasti* in Oaxaca (Figure 5). This is reflected in the gradual clines for both males and females, with estimated widths of 370 and 686 km, respectively (Table 3). The clines for both sexes concordantly estimate the transition center between Woodhouse's and

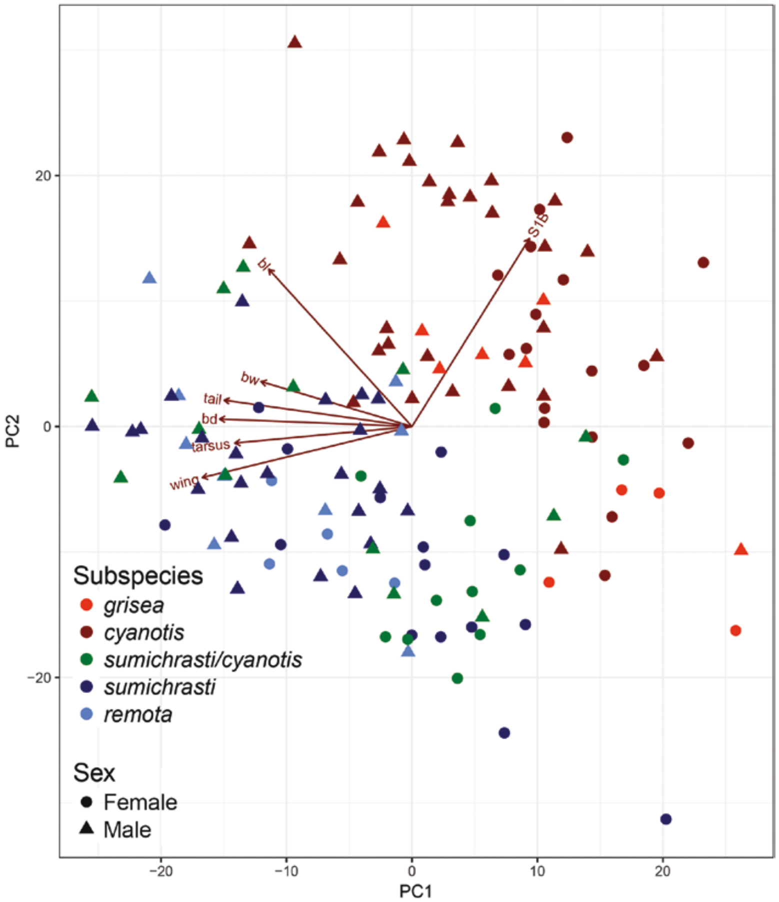

FIGURE 3. PCA of 133 individuals based on 6 morphological characteristics and one variable associated with the blue saturation of the back. Individuals from the contact zone (sites E–G) are labeled as *sumichrasti/cyanotis*.

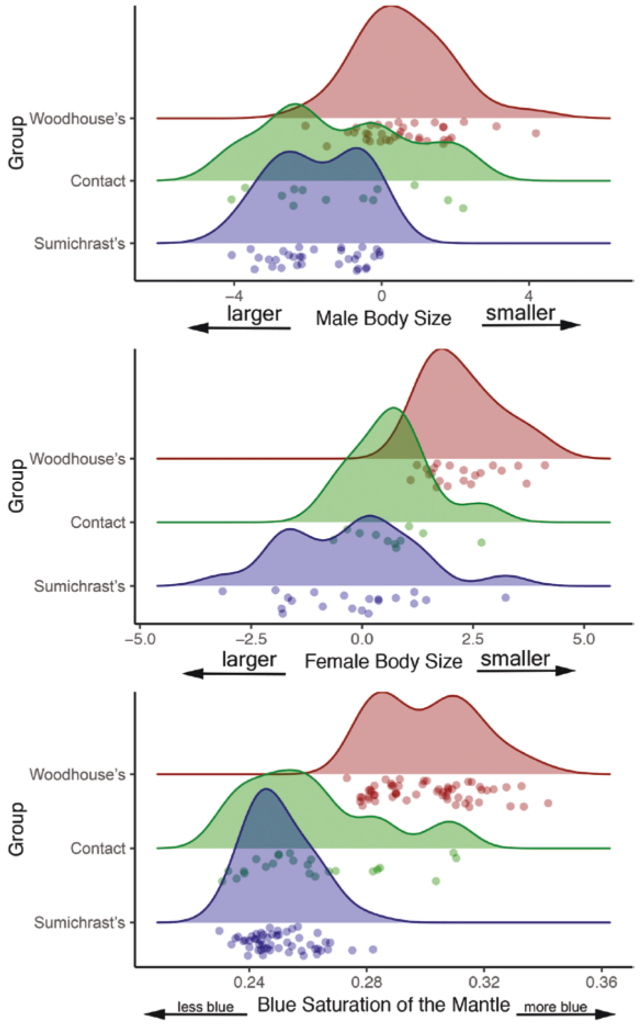

FIGURE 4. Density and scatterplots based on PC1 score (general body size) for males and females, and S1B (blue saturation of back plumage) for all individuals from Woodhouse's and Sumichrast's groups. Individuals from sites E, F and G (Figure 2) are considered the contact zone.

Sumichrast's groups at between 765–770 km into the sampling transect.

In contrast, the cline for plumage (S1B) shows a steep transition, and a correspondingly narrow cline width of 257 km (Figure 5). The center of the transition in plumage color between Woodhouse's and Sumichrast's groups is estimated to occur 656 km into the sampling transect. For both PC1 and S1B, "fixN" was chosen as the best-fit model based on lowest AICc score, indicative of a clinal transition between 2 populations with differential, fixed traits on either end.

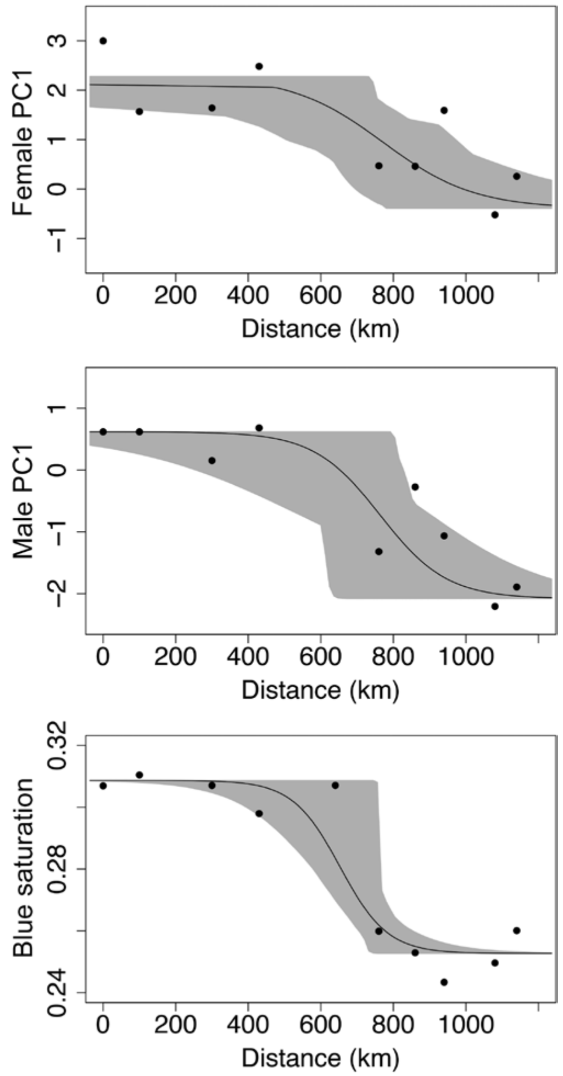

FIGURE 5. Geographic clines for PC1 score (general body size) for males and females, and S1B variable (blue saturation of the mantle) for individuals along a transect from Coahuila to Oaxaca, Mexico. Each point represents the average value of all individuals at a given sampling locality. The line represents the predicted model with the lowest AICc score, and the shaded area represents the 2 log likelihood confidence interval for each cline.

DISCUSSION

We demonstrate diagnosable differences between divergent lineages within Woodhouse's Scrub-Jay in Mexico, as well as the existence of a zone of intergradation. While physical differences between these lineages have long been described (Pitelka 1951), our results lend quantitative statistical evidence to the overall divergence between them. Additionally, our results lend support to Pitelka's notion, based on just a few specimens available to him at the time, that these lineages come into limited contact. Our cline analyses characterize the complexity of this contact area by showing that plumage color transitions much more quickly than body size. Combined with prior evidence of significant genetic divergence, current evidence suggests that the Woodhouse's and Sumichrast's lineages diverged in

TABLE 3. Cline center (km) and width (km) for each cline modeled in hzar for 7 characters plus PC1 scores, reported with 95% confidence intervals (n = 108). Model chosen based on lowest AICc score, with “fixN” chosen as the best-fit model for all 8 clines.

Character	Center	Width
Bill depth	811 (636–962)	465 (207–1008)
Bill width	786 (553–989)	368 (120–1109)
Bill length	907 (426–1100)	143 (0–1109)
Wing chord	642 (511–770)	1097 (743–1110)
Tarsus	381 (70–699)	1107 (386–1107)
Tail length	708 (531–924)	418 (1–1109)
PC1 (female)	769 (620–877)	685 (276–865)
PC1 (male)	767 (582–871)	370 (116–1109)
S1B	656 (577–758)	257 (2–443)

allopatry, where they evolved genetic and phenotypic differences. Later, likely sometime in the Pleistocene (Cicero and Johnson 2002, Cicero 2004, Lovette 2005), these lineages came back into secondary contact, forming the contact zone we see today.

While the adaptive value of plumage color and body size are as yet unknown in these jays, a narrow cline suggests that mantle color is under strong divergent selection, in contrast with the wide cline for body size. This result builds on prior research identifying variable cline widths among traits, depending on the strength of selection against intermediates (Baldassarre et al. 2014, Ottenburghs et al. 2017). Unique plumage traits are often evolved in avian systems via divergent sexual selection, which can drive reproductive isolation (Barraclough et al. 1995, Safran et al. 2013, 2016, Toews et al. 2016). However, sexual selection is not thought to drive diversification in *Aphelocoma*, evidenced by the lack of male ornamentation in any known lineage. Instead, it is likely that the traits studied here diverged during an extended period of genetic and geographic isolation between these groups. Future behavioral and genomic studies will be necessary to reveal the roles of body size and plumage traits in mediating current interactions between these lineages.

The differences in body size shown here do not conform to Bergmann’s ecogeographic rule, which predicts a positive relationship between the absolute value of latitude and body size (Bergmann 1847). In this case, the more northern Woodhouse’s group is smaller than Sumichrast’s group to the south. However, Bergmann’s rule has usually been studied in systems without the deep intraspecies divergences of *Aphelocoma*, and a recent meta-analysis suggests that Bergmann’s rule only holds in roughly 25% of birds and mammals (Riemer et al. 2018). A similar lack of adherence to Bergmann’s rule is also seen in the closely related Mexican Jay (*A. wollweberi*) and California Scrub-Jay (*A. californica*) (Pitelka 1951, McCormack et al. 2008). These cases suggest that population structure and unusually deep intraspecies divergences within *Aphelocoma* repeatedly confound expected ecological patterns such as Bergmann’s rule. In sum, these results highlight the complex evolutionary and geographic forces mediating diversification within *Aphelocoma*. Future research should seek to quantify evolutionary forces that have contributed to phenotypic diversification in *Aphelocoma*, where all stages of the speciation continuum (sensu de Queiroz 1998) are represented.

The cline widths for measured morphological and plumage traits differ, but their centers are concordant, placing the middle of the hybrid zone just north of Mexico City, almost exactly where Pitelka (1951) surmised it was. Just north of this region, in Hidalgo and Guanajuato, there is a gap in the sampling of museum specimens. This does not indicate an impermeable range gap, as there are a number of eBird records from this area, but the density of observations is much lower, suggesting unsuitable habitat in this region might explain the origin of allopatric divergence between lineages. Prior research suggests that species in parapatry often intergrade within a bounded area of contact (Gee 2004, Carling and Brumfield 2008). Based on the differentiated plumage patterns of these 2 groups and the rarity of intermediate phenotypes, we infer that Mexico City represents a bounded contact area between the evolutionarily distinct Woodhouse’s and Sumichrast’s groups, which is maintained by selection on back color (or other linked plumage traits) and the reduced gene flow imposed by an area of low population density between the groups.

Accurate taxonomy based on evolutionary history is an essential basis for connecting ecological and evolutionary theory to the realities of global biodiversity (Frost and Hillis 1990, Mace 2004). Based on fossil-calibrated molecular phylogenies, Woodhouse’s and Sumichrast’s groups likely diverged 1–2 MYA (McCormack et al. 2011, Gowen et al. 2014) and across multiple multilocus phylogenies; Sumichrast’s group has consistently been recovered as a monophyletic group, sister to the rest of *A. woodhouseii* (Delaney et al. 2008, McCormack et al. 2011, Gowen et al. 2014). We found Woodhouse’s and Sumichrast’s groups significantly differentiated in phenotype, adding further support to the growing evidence for the isolation of these lineages. Although there is a zone of intergradation between the groups, it is limited, and there appears to be selection against hybrids in plumage traits. While future study of the extent of genome-wide introgression will hold the key to revealing the histories of these groups, the existing evidence makes a compelling case that the 2 groups are distinct evolutionary lineages that could qualify as species under both phylogenetic and biological species concepts (de Queiroz 1998).

Future research should assess how individual genetic loci move across the hybrid zone between these 2 lineages (e.g., Walsh et al. 2016, Gompert et al. 2017), to help identify the potential adaptive loci under selection in this potential case of speciation-in-action. The genomic basis of plumage divergence between the groups is another fruitful avenue for research, as previous research in other avian systems has uncovered unique polymorphisms in genes related to feather color, sometimes as the only clear genetic differences among species (Uy et al. 2009, Toews et al. 2016). Additional work investigating the ecology of these distinct groups will allow us to identify possible pre-mating barriers to reproduction such as divergence in niche, vocalizations, or behavior.

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Author contributions: J.E.M. and J.M.M conceived the idea, design and methodology. D.A.D conducted all specimen measurements. All authors participated in data analysis. D.A.D. wrote the original draft of the manuscript, and all authors contributed substantially to edits and revisions of the final manuscript.

Data deposits: The full dataset including morphological and plumage variables for all 133 included specimens, and original R scripts to perform the analyses shown in this manuscript, can be accessed from the following GitHub repository: https://github.com/DevonDeRaad/Woodhouses-morphology.

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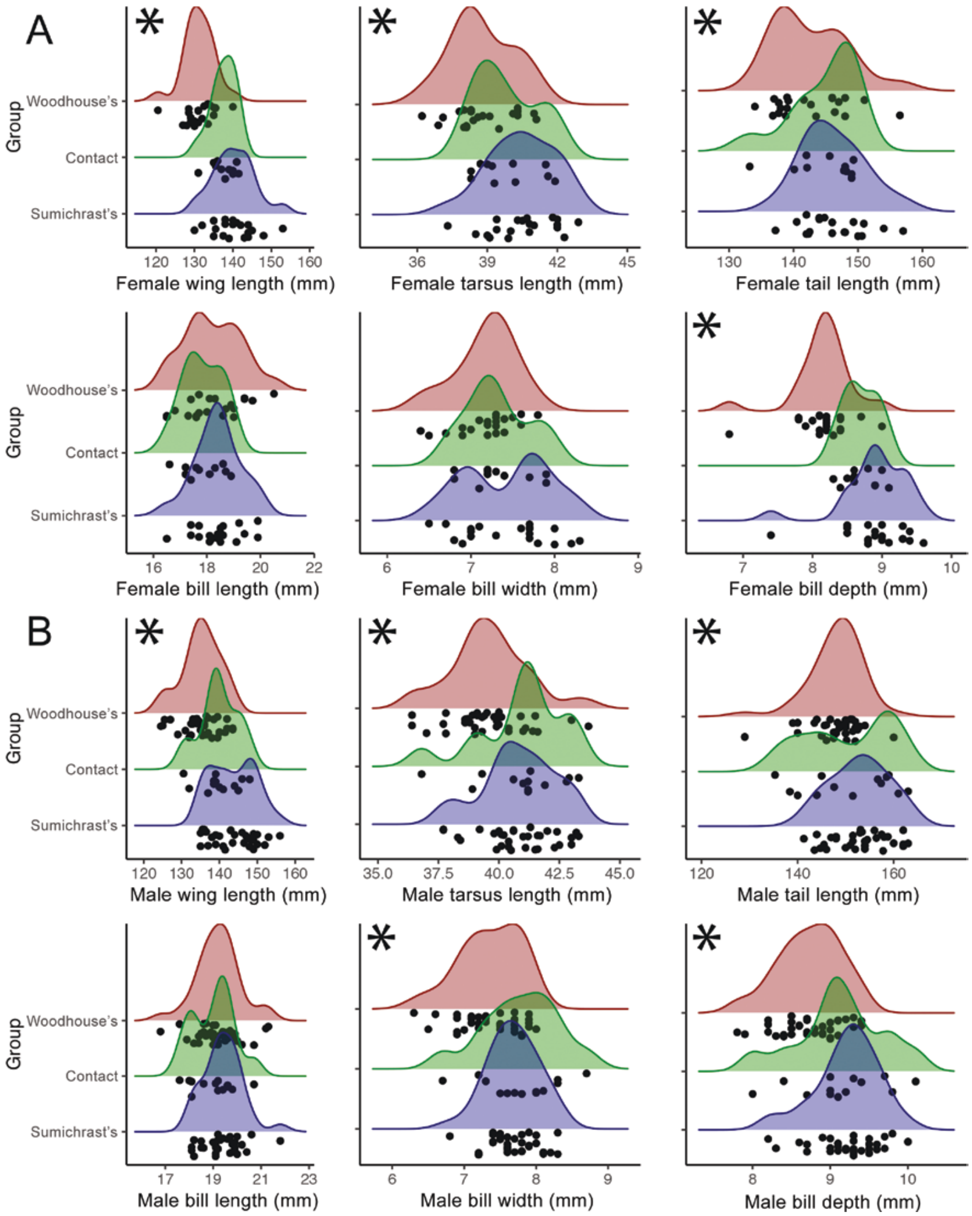
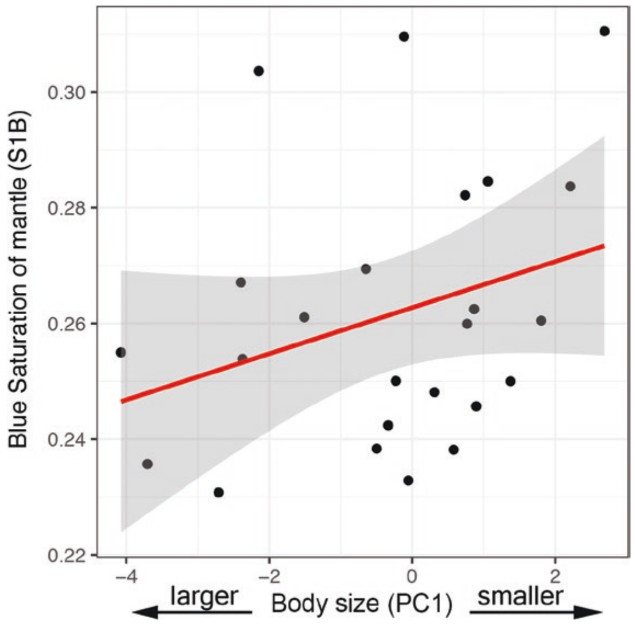
APPENDIX



APPENDIX FIGURE 1. Visualizations by group assignment for all 6 morphological variables for (**A**) only female specimens and (**B**) only male specimens. Asterisk (*) denotes a significant difference in a given trait, based on pairwise Welch's *t*-tests adjusted to account for false discovery rate.



APPENDIX FIGURE 2. Scatterplot showing the relationship between body size and blue saturation of the mantle for the 24 individuals from the contact zone. Red line shows the fit of a linear regression model to the data, which has an *R*-squared value of 0.095 ($F = 2.3$, $p = 0.14$) reflecting a positive relationship between decreasing size and increasing blue saturation of the mantle.

APPENDIX TABLE 1. Measurements and sample sizes for each of the subspecies individually. Wing, tail, tarsus, bill length, bill width and bill depth are reported in mm, S1B is a reflectance measure of the area under the spectral curve between 400 and 510 nm.

Subspecies	*n*	Wing	Tail	Tarsus	Bill length	Bill width	Bill depth	S1B
grisea	11	132.0 (1.3)	145.0 (1.5)	38.4 (0.4)	18.2 (0.4)	7.2 (0.1)	8.5 (0.1)	0.287 (0.002)
cyanotis	46	134.2 (0.8)	146.3 (0.9)	39.5 (0.2)	19.0 (0.1)	7.3 (0.1)	8.5 (0.1)	0.304 (0.003)
cyanotis/sumichrasti	24	138.9 (0.9)	148.0 (1.7)	40.4 (0.3)	18.5 (0.2)	7.6 (0.1)	8.9 (0.1)	0.261 (0.005)
sumichrasti	38	141.5 (1.0)	151.3 (1.1)	40.5 (0.2)	18.9 (0.1)	7.5 (0.1)	9.1 (0.1)	0.250 (0.002)
remota	14	144.5 (1.0)	147.6 (1.1)	41.3 (0.5)	19.3 (0.3)	7.7 (0.1)	9.2 (0.1)	0.251 (0.003)