GENETIC AND PHENOTYPIC DIVERGENCE WITHIN AND BETWEEN CINNAMON TEAL (*ANAS CYANOPTERA*) AND BLUE-WINGED TEAL (*A. DISCORS*)

By

Robert E. Wilson

RECOMMENDED:

Michael D. Sorenson, PhD

Kevin Winker, PhD

Mark S. Lindberg, PhD

Kevin G. McCracken, PhD
Advisory Committee Chair

Christa P. H. Mulder, PhD
Chair, Department of Biology and Wildlife

APPROVED:

Paul W. Layer, PhD
Dean, College of Natural Science and Mathematics

Lawrence K. Duffy, PhD
Dean of the Graduate School

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Date

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A

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Robert E. Wilson, B.S., M.S.

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ABSTRACT

Spatial heterogeneity in selection pressures can lead to extensive morphological variation and differences at functional genes between populations across a species' range without corresponding genetic variation at neutral loci. Divergent selection among populations may thus lead to intraspecific variation and in many cases speciation. Phenotypic and genetic structure within and between Cinnamon Teal (*Anas cyanoptera*) and the closely related Blue-winged Teal (*A. discors*) was assessed using body size measurements and neutral genetic markers in conjunction with a functional locus, hemoglobin. Cinnamon Teal are composed of five subspecies corresponding to distinct ecogeographic regions in North and South America. Subspecies and geographic regions differed significantly in overall body size, with the largest subspecies and the largest individuals found at high elevations in the central Andes (*A. c. orinomus*) and at high latitudes in southern Patagonia (*A. c. cyanoptera*). South American populations showed strong positive correlations with latitude and elevation while the migratory subspecies in North America (*A. c. septentrionalium*) showed few significant correlations with elevation and no relationship between latitude and body size. In addition, plumage differences were restricted to between North and South America as there was extensive variation observed within continents. Cinnamon Teal highland and lowland populations showed strong divergence in body size ($P_{ST} = 0.56$) and exhibited frequency differences in one non-synonymous α-globin amino acid polymorphism (Asn/Ser-α9; $F_{ST} = 0.60$), despite considerable admixture of reference loci. Selection pressures imposed by the hypoxic highland environment have likely resulted in asymmetric gene flow from the highlands

into the lowlands following a highland colonization event from the lowlands. Cinnamon Teal and Blue-winged Teal show distinct but paraphyletic mitochondrial DNA (Φ_{ST} = 0.41) and broadly shared nuclear alleles. Unlike South American Cinnamon Teal, North American Cinnamon Teal and Blue-winged Teal are characterized by high genetic diversity, large effective population size, and recent population expansion. Haplotypic and allelic sharing across continents is likely because of incomplete lineage sorting rather than ongoing gene flow. Within-continent estimates yielded higher migration rates consistent with hybridization. However, Cinnamon Teal and Blue-winged Teal are similar in body size; differences in plumage coloration may reduce hybridization events.

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INTRODUCTION

Natural selection, sexual selection, and the stochastic process of genetic drift are the key evolutionary processes leading to divergence between populations and, in many cases, speciation (Questiau 1999, Coyne and Orr 2004, Price 2008). Fully or partially isolated populations may evolve distinct morphology and/or behavioral traits in response to diversifying selection, leading to premating and postmating isolation even in the absence of genome-wide genetic differentiation (Meyer 1993, Bernatchez et al. 1996, Schluter 1998, Seehausen and van Alphen 1998, Hendry 2001, Ödeen and Bjorklund 2003). Morphological and behavioral responses to selection can cause incongruence between species limits based on phenotypic traits and gene genealogies, especially in recently diverged taxa (Funk and Omland 2003, Avise 2004, Buehler and Baker 2005, Joseph et al. 2006, Maley and Winker 2010). Of particular interest are sexual ornaments used for mate recognition, which are a major component of variation both among closely related species and within species (West-Eberhard 1983, Price 1998, Questiau 1999, Johnsen et al. 2006). Incongruence between morphological and molecular data can generate taxonomic uncertainty, but examples of incongruence also provide a valuable opportunity to gain insight into the evolutionary processes leading to speciation (Edwards et al. 2005, Johnsen et al. 2006, Joseph et al. 2006, Omland et al. 2006).

Local adaptation can occur through the substitution of alleles with large effects on phenotypes or through allelic changes with smaller effects that gradually accumulate over evolutionary time (Orr and Coyne 1992, Orr and Smith 1998, Orr 2005). In heterogeneous landscapes, selection may restrict the flow of alleles that are beneficial in

one particular environment but have reduced fitness in another (Rundle and Nosil 2005, Nosil et al. 2008, Milá et al. 2009). Furthermore, selection is likely not homogeneous across the genome and may not limit gene flow of neutral alleles, unless those alleles are closely linked to loci under selection (McKay and Latta 2002, Emelianov et al. 2004, Mallet 2005, Garant et al. 2007, Via 2009). Similarly, adaptive differentiation can still occur even in the face of countervailing gene flow, as long as the strength of selection is greater than the migration rate ($s > m$; Slatkin 1987, McKay and Latta 2002, McCracken et al. 2009a). The colonization of new habitats could thus facilitate rapid divergence in advantageous traits with little genetic differentiation at neutral markers. Therefore divergence may only be observed in a small portion of the genome (Orr and Smith 1998, Via 2009).

High-elevation regions provide excellent opportunities to investigate the molecular and morphological bases of local adaptation. Low temperatures, increased desiccation, higher atmospheric radiation, and especially hypoxic conditions (oxygen concentration ca. 40% lower at 4000 m than at sea level) can be debilitating for individuals from lowland populations (Tucker 1968, Scott et al. 2009). Highland resident species and populations have evolved a number of different strategies to survive in this extreme environment, resulting in genetically determined adaptations (Jessen et al. 1991; Storz et al. 2007, 2010; Storz 2010; Yi et al. 2010; Peng et al. 2011; Scott et al. 2011). Hemoglobin in particular has been demonstrated to evolve in response to severe hypoxia in a variety of high-altitude species (e.g., Jessen et al. 1991; Weber et al. 1993; León-Velarde et al. 1996; Weber 2002; Storz et al. 2007, 2010). Often, only one or a few

amino acid changes are observed in the Hb protein between highland and lowland conspecifics (Perutz 1983, Hiebl et al. 1987, Braunitzer and Hiebl 1988). However, when amino acid substitutions are compared across species, the same, similar, or adjacent substitutions have evolved independently in multiple highland taxa (McCracken et al. 2009b,c).

Here we investigate the population genetic structure and morphological divergence in Cinnamon Teal (*Anas cyanoptera*) and the closely related Blue-winged Teal (*A. discors*) using nucleotide sequences from the mitochondrial DNA (mtDNA) control region, five nuclear introns, and functional genes (hemoglobin), and data for a series of body-size and plumage coloration traits. Unlike Northern Hemisphere waterfowl, which are migratory and show little geographic variation, ducks in South America tend to be less migratory, more restricted in geographic range, and well differentiated into two or more subspecies (Phillips 1923, Johnsgard 1978, Williams 1991, Bulgarella et al. 2007). Cinnamon Teal are no exception: widespread throughout the Western Hemisphere, the species comprises five subspecies that inhabit distinct geographic and ecological zones (Snyder and Lumsden 1951, Wilson et al. 2010). Differences in life history traits (e.g., migratory behavior and habitat) enabled me to investigate patterns of population subdivision and gain insight into how selection has produced adaptation at both the phenotypic and molecular level.

Cinnamon Teal and Blue-winged Teal are closely related dabbling ducks that exhibit pronounced variation in body size, coloration in males, habitat choice, and behavioral traits (e.g., migratory behavior and territoriality; Gammonley 1996, Rohwer et

al. 2002). Despite considerable phenotypic differences in male breeding plumage, previous studies have found little or no genetic differentiation (Kessler and Avise 1984, Johnson and Sorenson 1999, Kerr et al. 2007), suggestive of recent divergence. Both species are widespread throughout the Western Hemisphere and are occasionally found in sympatry in western North America and in northern South America where they have been reported to occasionally hybridize (Spencer 1953).

In this study, I present analyses of both population genetic and morphological data to explore the evolutionary relationships and adaptations of Cinnamon Teal subspecies and Blue-winged Teal. The primary goals of this study were to: (1) examine morphological variation among Cinnamon Teal populations in relation to subspecific status and ecogeographic region; (2) examine genotypic and phenotypic variation between low- and high-elevation populations of Cinnamon Teal using a multilocus data from both presumably neutral and functional genes; (3) investigate the demographic history and timing of divergence between Cinnamon Teal and Blue-winged Teal; (4) characterize body size and plumage coloration differences between Cinnamon Teal and Blue-winged Teal to provide guidelines for species identification; (5) examine plumage color differences among Cinnamon Teal subspecies from the visual perspective of the birds using a model of avian color discrimination (Vorobyev and Osorio 1998); and (6) assess specimen shrinkage in waterfowl and its impact on studies involving both museum specimens and live birds.

CHAPTER 1

ECOGEOGRAPHIC VARIATION IN CINNAMON TEAL (*ANAS CYANOPTERA*) ALONG ELEVATIONAL AND LATITUDINAL GRADIENTS[1]

ABSTRACT

Cinnamon Teal (*Anas cyanoptera*) comprise five subspecies that inhabit a variety of habitats along an elevational gradient at temperate and tropical latitudes. North American and South American subspecies differ in their migratory behavior, which may have contributed to differences in body size. We measured body size of the five recognized subspecies (*A. c. cyanoptera*, *A. c. orinomus*, *A. c. borreroi*, *A. c. tropica*, and *A. c. septentrionalium*) throughout their ranges and evaluated morphometric differentiation in relation to Bergmann's rule. Subspecies and geographic regions differed significantly, with the largest subspecies and the largest individuals found at high elevations in the central Andes (*A. c. orinomus*) and at high latitudes in southern Patagonia (*A. c. cyanoptera*). Smaller-bodied individuals (*A. c. cyanoptera*) were found at the northern and southern limits of the Altiplano, where intermixing between subspecies with different body sizes might occur. However, there is no direct evidence of

[1]Wilson, R. E., T. H. Valqui, & K. G. McCracken. 2010. Ecogeographic variation in Cinnamon Teal (*Anas cyanoptera*) along elevational and latitudinal gradients. Ornithological Monographs 67: 141–161.

A. c. cyanoptera breeding at high elevations (>3,500 m). In contrast to patterns within South America, the migratory subspecies in North America (*A. c. septentrionalium*) showed few significant correlations with elevation and no relationship between latitude and body size. Morphological diversity within Cinnamon Teal appears to have arisen from spatial and temporal heterogeneity in selection pressures resulting in adaptations to their local environments.

Introduction

Geographic variation in morphology is common, and widespread patterns are often explained within an adaptive framework (Price 2008). One of the best-known ecogeographical patterns of variation in body size among vertebrates is Bergmann's rule, which states that individuals from populations in colder climates tend to be larger than those from populations in warmer climates (Bergmann 1847; Mayr 1956, 1963). Modifications to Bergmann's rule showed that larger body size would also be expected at higher latitudes and elevations, or in cooler or drier climates (Snow 1954; James 1968, 1970, 1991). Even though birds show a strong tendency to conform to modified definitions of Bergmann's rule (Ashton 2002, Meiri and Dayan 2003), the adaptive mechanisms responsible for this pattern have been debated. Various mechanisms have been proposed, such as heat conservation, fasting endurance, and competition for resources (Bergmann 1847; McNab 1971; Calder 1974, 1984; James 1991). Thus, ecotypic variation may result from complex underlying processes involving various interrelated variables (Millien et al. 2006).

South American ducks (Anseriformes: Anatidae) are particularly good candidates for a study of ecogeographic variation. Unlike their Northern Hemisphere relatives, which are migratory and show little morphological variation, ducks in South America tend to be less migratory, more restricted in geographic range, and well differentiated into two or more subspecies that differ in plumage and other morphological characters (Phillips 1923, Johnsgard 1978, Williams 1991, Bulgarella et al. 2007). For example, the ducks that inhabit the puna grasslands and wetlands of the high Andes (3,000–5,000 m)

tend to have overall larger body size and differ in conspicuous traits, such as plumage, bill color, or eye color, from those in southern Patagonia, where most breeding habitat occurs below 1,500 m (Fjeldså and Krabbe 1990). Most Andean waterfowl thus comprise one or more predominantly lowland subspecies (or species) and one or more highland subspecies (Phillips 1923).

Cinnamon Teal (*Anas cyanoptera*) are widespread throughout the Western Hemisphere, and five subspecies that inhabit distinct geographic and ecological zones are currently recognized: *A. c. cyanoptera*, *A. c. orinomus*, *A. c. borreroi*, *A. c. tropica*, and *A. c. septentrionalium* (Snyder and Lumsden 1951, Delacour 1956, American Ornithologists' Union 1957, Gammonley 1996). *Anas c. septentrionalium* breeds throughout western North America (Bellrose 1980, Madge and Burn 1988, Gammonley 1996), whereas the other four subspecies breed in South America. In South America, *A. c. borreroi* is endemic to the Colombian Andes and is replaced by *A. c. tropica* in the adjacent tropical lowlands; intermediate elevational habitat is unsuitable for either subspecies (Snyder and Lumsden 1951, Delacour 1956). *Anas c. orinomus* is endemic to the Altiplano and adjacent puna region of Argentina, Bolivia, Chile, and Peru. *Anas c. cyanoptera* occurs throughout the Andean lowlands of Peru, Bolivia, Chile, Paraguay, Brazil, Uruguay, and Argentina and is occasionally found sympatrically with *A. c. orinomus* in the high Andes (Evarts 2005). Each Cinnamon Teal subspecies thus has a distinct geographic distribution with little or no overlap, with the exception of *A. c. cyanoptera* and *A. c. orinomus* where they co-occur in the central high Andes.

We collected Cinnamon Teal throughout their range in North America and South America and compared differences in body size among geographic regions. Evidence for Bergmann's rule was evaluated to gain insight into factors shaping morphological divergence over elevational and latitudinal gradients.

Methods

Specimen collection and subspecies classification.—We collected 153 Cinnamon Teal (39 females and 114 males) from Argentina (2001, 2003), Bolivia (2001), Peru (2002), and the western United States (2002–2003) during the breeding season (Fig. 1.1 and Appendix 1.1). Voucher specimens are archived at the University of Alaska Museum (Fairbanks), Museo de Historia Natural de la Universidad de San Marcos (Lima), and Colección Boliviana de Fauna (La Paz). Measurements from Colombian vouchered specimens from the Royal Ontario Museum and Smithsonian Institution National Museum of Natural History were obtained for *A. c. borreroi* (5 females and 13 males) and *A. c. tropica* (2 females and 2 males); new specimens could not be obtained because these subspecies are endangered.

We used a combination of geography and wing chord length to classify each specimen (Snyder and Lumsden 1951, Blake 1977). Despite differences in plumage (e.g., Blake 1977), coloration is variable, and *A. c. cyanoptera*, *A. c. orinomus*, and *A. c. septentrionalium* were difficult to classify to subspecies on the basis of plumage color alone (Wilson et al. 2008). We classified all individuals from North America as *A. c. septentrionalium* because it is the only subspecies known to occur there. The Colombian specimens we used were the basis of the original subspecies descriptions (Snyder and

Lumsden 1951), and we followed them in classifying highland specimens as *A. c. borreroi* and lowland specimens as *A. c. tropica*. *Anas c. orinomus* is the most distinct of all the subspecies, which led some early researchers to consider it a separate species (Oberholser 1906). *Anas c. orinomus* was easily differentiated from *A. c. cyanoptera* by overall body size. To check the accuracy of classifications of *A. c. cyanoptera* and *A. c. orinomus* in areas of sympatry, we compared wing chord length to individuals of known classification. All initial classifications were confirmed.

Body measurements.—We took nine body-size measurements (±0.1 mm) from each bird: wing chord length (carpal joint to longest primary feather unflattened, ±1 mm), tail length (base of the uropygial gland on back to tip of the center tail feather, ±1 mm), exposed culmen length, bill length at nares (anterior edge of nares to tip of nail), tarsus bone length (tarsometatarsus), bill height (height of upper mandible at anterior edge of nares), bill width (width of upper mandible at anterior edge of nares), and body mass (g). Body mass was not available from the Colombian subspecies and therefore was only used as a secondary character in subspecies identification. Measurements for all but 45 recently collected specimens were taken the day of collection and prior to preparation as museum specimens (wet measurements), and then again several months or years after preparation (dry measurements; Appendix 1.2) by R.E.W. For 52 individuals from Argentina, Bolivia, and Colombia, only measurements from museum specimens were available (dry measurements). Specimen shrinkage during drying is a universal phenomenon and can cause analytical problems if not properly accounted for in studies that combine live or freshly killed birds and museum specimens (e.g., Winker 1996).

Fresh and dry measurements taken by the first author differed significantly (Wilson and McCracken 2008). Therefore, dry measurements of those 52 individuals could not be directly substituted for wet measurements.

We chose to analyze wet measurements, and to use individuals missing these data we used a multiple imputation (MI) procedure implemented in the program NORM (Schafer 1999) to estimate wet measurements for the 52 individuals with only dry measurements, because we had both wet and dry measurements for most of the data set. An expectation-maximization algorithm (EM) was used to obtain starting values for the multiple imputation procedure, followed by data augmentation using Markov-chain Monte Carlo to produce multiple imputations of the missing data. We used a random number seed and 10,000 iterations, with imputation every 1,000 iterations. The resulting 10 data sets were combined following Rubin's (1987) rules for scalar estimates to provide a single set of estimates for each specimen with missing data. The combined data composed of original wet measurements obtained from 123 specimens and estimated wet measurements from 52 specimens were used for all statistical analyses.

Statistical analysis of measurements.—Statistical analyses were performed with MINITAB Statistical Software (Minitab, State College, Pennsylvania). All traits were tested for normality with Kolmogorov-Smirnov tests and were normally distributed ($P_s > 0.05$). A multivariate analysis of variance (MANOVA) was performed to evaluate overall differences among subspecies and geographic regions for each sex. Geographic regions were defined as follows (with the corresponding subspecies inhabiting each area): (1) North America (*A. c. septentrionalium*); (2) Colombian highlands (*A. c. borreroi*); (3)

Colombian lowlands (*A. c. tropica*); (4) Peruvian coast (*A. c. cyanoptera*); (5) central high Andes of Argentina, Bolivia, and Peru (*A. c. orinomus* and *A. c. cyanoptera*); and (6) lowland Argentina (includes Patagonia and lowland areas of Cordoba; *A. c. cyanoptera*). Collection locations in North America (California, Oregon, and Utah) were treated as a single geographic unit, which is consistent with low levels of male breeding-site fidelity in North America (Anderson et al. 1992). Analysis of variance (ANOVA) and pairwise comparisons for each individual measurement were performed using a general linear model with Bonferroni correction for multiple comparisons. Pairwise comparisons were not made with *A. c. tropica* (lowland Colombia) because of low sample size. We used a principal component analysis to illustrate overall differences in body size among subspecies. Only those principal components with eigenvalues >1 were used for partial correlation and subspecies classification analyses.

Finally, the joint relationships between elevation and latitude and morphological variables were examined using partial correlation analysis for the following areas: all populations pooled, North America, South America, and southern South America (Altiplano and associated lowlands and Patagonia). In addition, correlations between latitude and body size were examined for *A. c. cyanoptera* (lowland and highland) separately, because it is the only subspecies with populations distributed over a large latitudinal gradient. Analyses were conducted separately for each sex, and significance levels were corrected for multiple comparisons using Bonferroni methods.

Subspecies classification.—We used two methods to evaluate subspecies identifications. We first used linear discriminant analysis to evaluate whether the

Cinnamon Teal subspecies conformed, on the basis of body-size measurements, to the 75% rule (Amadon 1949, Mayr 1969), which states that 75% of the individuals of one subspecies must be distinguishable from all other subspecies. Measurements found to be significantly different between at least two subspecies (classified based on overall body size) from the MANOVA and ANOVAs were included in this analysis. The reliability of the discriminant analysis was assessed using a cross-validation (jackknife) procedure, in which each observation was omitted one at a time and then reclassified using a classification function derived from the remaining observations (Manly 2000). Cross-validation gives a less biased error rate in classification, because it does not include observations that are used to create the classification function. We performed a discriminant analysis for each sex and locality of collection and did not include *A. c. tropica* because of low sample size.

We also tested the diagnosability of subspecies using the method of Patten and Unitt (2002), which focuses on the extent of overlap rather than detecting mean differences. Diagnosability of subspecies was determined for each measurement separately and for overall body size (PCI). An index value (D_{ij}) ≥ 0 indicates that subspecies *i* is diagnosable from subspecies *j*. Reciprocal tests were performed to determine whether subspecies *i* is diagnosable from subspecies *j* and whether subspecies *j* is diagnosable from subspecies *i*.

Results

Subspecies differed significantly in overall body size (Wilks's $\lambda = 0.05$, $F = 25.38$, df = 28 and 574, $P < 0.001$), as did the sexes (Wilks's $\lambda = 0.74$, $F = 7.16$, df = 7 and 159, $P <$

0.001; Table 1.1). There was no significant interaction between subspecies and sex (Wilks's $\lambda = 0.78$, $F = 1.46$, df = 28 and 574, $P = 0.061$). *Anas c. orinomus* was significantly larger than *A. c. tropica* (e. g., wing chord: 32.50 mm difference; tarsus: 4.01 mm difference) and *A. c. septentrionalium* (e. g., wing chord: 31.50 mm difference; tarsus: 4.83 mm difference) in most measurements, with *A. c. borreroi* and *A. c. cyanoptera* intermediate in body size (Tables 1.1 and 1.2). In addition, when individuals were grouped on the basis of collection locality instead of subspecies identification, geographic regions differed significantly in body size (Wilks's $\lambda = 0.06$, $F = 17.54$, df = 35 and 662, $P < 0.001$), as did the sexes (Wilks's $\lambda = 0.75$, $F = 7.57$, df = 7 and 157, $P < 0.001$). There was no significant interaction between geographic region and sex (Wilks's $\lambda = 0.78$, $F = 1.16$, df = 35 and 662, $P = 0.244$). The same basic overall pattern was observed, regardless of whether individuals were grouped by subspecies or by geographic region. Highland individuals were significantly larger and intermediate body sizes were found in Patagonia (*A. c. cyanoptera*) and the Colombian highlands (*A. c. borreroi*), except for some notable exceptions. Among females, the lowland Argentine population (*A. c. cyanoptera*) was not significantly different from the central high Andean population (*A. c. orinomus*) in either bill length measurements (bill length at nares: 2.04 mm difference; culmen length: 2.59 mm difference) or bill width (0.39 mm difference). In males, the lowland Argentine population was similar to the Andean populations (*A. c. cyanoptera* and *A. c. orinomus* combined) in tail length, bill length at nares, bill height, and bill width and was significantly larger than the Peruvian coastal population in most measurements (Table 1.3). Specimens of *A. c. cyanoptera* collected at high elevation in

the Andes were significantly smaller than *A. c. orinomus* for only wing chord (27.7 mm difference) and tail length (10.83 mm difference). The North American and Argentine lowland populations of *A. c. cyanoptera* had similar bill lengths, but the Argentine population had significantly greater bill height (1.17 mm difference) and bill width (0.91 mm difference). North American populations had significantly larger bill length at nares (1.78 mm difference) and culmen length (1.65 mm difference) than Peruvian coastal populations of *A. c. cyanoptera.*

Principal component analysis.—The first principal component (PC1; female eigenvalue = 3.54, male eigenvalue = 3.33) accounted for 50.5% and 47.6% of the variance for females and males, respectively, and represented an overall body size difference (Table 1.4). The second principal component (PC2; female eigenvalue = 1.57, male eigenvalue = 1.63) accounted for 23.2% and 22.5% of the variance for females and males, respectively, and represented a bill shape difference among the subspecies, as bill measurements were the most influential variables. A longer, thinner bill corresponded with a higher score. Even though plots of PC1 versus PC2 showed some overlap among subspecies, only *A. c. septentrionalium* and *A. c. cyanoptera* did not differ in PC1, and *A. c. orinomus* and *A. c. cyanoptera* did not differ in PC2 (Fig. 1.2). When subsets of *A. c. cyanoptera* were analyzed geographically (Argentina, Peruvian coast, and Andes), the Argentine population was significantly larger in overall body size (PC1), whereas the Peruvian coastal population was more similar to *A. c. septentrionalium* (North America). *Anas c. orinomus* had the largest overall body size, with *A. c. borreroi* and the lowland Argentine population and individual *A. c. cyanoptera* collected in northwest Argentina

showing intermediate body size. *Anas c. septentrionalium* had the longest bill (PC2) after controlling for variation in body size (Fig. 1.2).

Partial correlation analysis.—Several significant patterns were found after Bonferroni correction in relation to latitude and elevation (Tables 1.5 and 1.6 and Figs. 1.3–1.10). Most measurements showed a significant increase with elevation for males and females among all individuals and within South America only (elevation increase of ~4,000 m). In males, PC2 (bill shape) decreased when all individuals were pooled and increased within North America over an elevational increase of ~1,600 m.

Significant correlations with latitude were primarily restricted to males. In females, only bill height showed a positive correlation with latitude within southern populations in South America (*A. c. cyanoptera* and *A. c. orinomus*). In males, tarsus, tail length, and bill height showed a negative correlation, and bill length at nares, culmen length, and PC2 were positively correlated with increasing distance from the equator. Within southern South America, only bill height and bill width were positively correlated with increasing latitude. When only *A. c. cyanoptera* (lowland subspecies) was considered, there was a strong positive correlation between latitude and bill length at nares, culmen length, bill height, bill width, and PC1 from the Peruvian coast to southern Patagonia.

Subspecies classification.—Discriminant analysis with cross-validation correctly classified males to originally assigned subspecies with 69–100% and females with 40–100% accuracy (Table 1.7). Discriminant analysis correctly assigned 53.8–86.0% of males and 40.0–100.0% of females to their area of origin (Table 1.8). Six misclassified

male individuals from the central high Andes were assigned to the nearest lowland population adjacent to the area where they were collected, Argentina ($n = 3$) or the Peruvian coast ($n = 3$). All of these individuals were assigned correctly as *A. c. cyanoptera* in the subspecies discriminant analysis.

Male *A. c. orinomus* were diagnosable from *A. c. cyanoptera* using wing chord, tarsus, tail length, and PC1; from *A. c. septentrionalium* using wing chord, tarsus, tail length, bill height, and PC1; and from *A. c. borreroi* using wing chord, tarsus, and bill length at nares (Table 1.9). The same pattern was found in female *A. c. orinomus*, except that females could not be distinguished from *A. c. borreroi* using bill length at nares (Table 1.10). *Anas c. septentrionalium* and *A. c. borreroi* were diagnosable using bill length at nares ($D_{sb} = 9.74$, $D_{bs} = 1.50$), culmen length ($D_{sb} = 7.63$, $D_{bs} = 0.01$), bill height ($D_{sb} = 0.02$, $D_{bs} = 2.60$), and PC1 ($D_{sb} = 4.40$, $D_{bs} = 0.91$) for males. When all three subpopulations of *A. c. cyanoptera* were pooled, *A. c. cyanoptera* was not diagnosable from *A. c. septentrionalium* or *A. c. borreroi* for any single measurement or PC1. However, at the individual population level, lowland Argentina (*A. c. cyanoptera*) was diagnosable from North America (*A. c. septentrionalium*) using tarsus ($D_{sa} = 5.24$, $D_{as} = 0.90$) and PC1 ($D_{sa} = 4.01$, $D_{as} = 0.61$) and from males in the Colombian highlands (*A. c. borreroi*) using bill length at nares ($D_{ba} = 10.16$, $D_{ab} = 0.80$). The Peruvian coastal population (*A. c. cyanoptera*) was diagnosable from *A. c. septentrionalium* using bill length at nares ($D_{sp} = 4.91$, $D_{ps} = 0.06$). Female *A. c. borreroi* were diagnosable from both the Peruvian coast ($D_{pb} = 7.94$, $D_{bp} = 1.60$) and lowland Argentine ($D_{ab} = 7.12$, $D_{ba} = 0.37$) populations of *A. c. cyanoptera* using PC1.

Discussion

Cinnamon Teal are distributed along elevational and latitudinal gradients, and within these gradients climatic and habitat variables change abruptly, placing different selection pressures on different populations (e.g., subspecies). Variances in morphological characteristics appear to conform to ecogeographic regions, given that larger individuals occupied higher elevations in the Andes (*A. c. orinomus* and *A. c. borreroi*) and occur at higher latitudes in Patagonia (*A. c. cyanoptera*), whereas smaller conspecifics resided at lower elevations in temperate regions (*A. c. cyanoptera*, *A. c. septentrionalium*, and *A. c. tropica*). Environmental variables as a function of temperature and humidity have been related to body size, and modifications of Bergmann's rule have been made to take into account factors associated with high latitudes and elevations as well as arid habitats (e.g., "latitude effect," Snow 1954; "aridity effect," Hamilton 1961). However, other factors, such as hypoxia, fasting endurance, and life history traits (resource competition and migration), are also known to facilitate variation in body size (Calder 1974, 1984; Hopkins and Powell 2001; Millien et al. 2006).

The climate of the Andes changes dramatically from the warm wet temperate zone of the Colombian Andes to the colder arid climates characteristic of the Altiplano and Patagonia. Patagonia is cool, dry, and windy, with substantial seasonal and diurnal temperature fluctuations. Birds that inhabit southern Patagonia experience average low temperatures ranging from 3°C (Esquel, Chubut) to 8°C (Rio Gallegos, Santa Cruz). The Andean Altiplano is also semi-arid, with most precipitation falling during the austral summer (December to February; Garreaud et al. 2003), leaving the rest of the year cool,

dry, and windy. The average low temperatures at Cusco, Peru (3,248 m), and La Paz (4,012 m) have been reported as 5°C and 1°C, respectively (Canty and Associates 2005). Separated by an average of 115 km from the puna zone of the Andes, the lowlands of the Peruvian coast consist of scattered river valleys and associated wetlands that are also classified as semi-arid (Pearson and Plenge 1974). However, in contrast to the climates of the Altiplano and Patagonia, the Peruvian coast is, on average, 10°C warmer, with temperatures ranging from 15 to 18°C (Canty and Associates 2005). We found that individuals in the warmer, wetter climates of North America (*A. c. septentrionalium*), the Colombian lowlands (*A. c. tropica*), and the Peruvian coast (*A. c. cyanoptera*) had smaller body sizes than those in the central high Andes (*A. c. orinomus* and *A. c. cyanoptera*) and Patagonia (*A. c. cyanoptera*).

Individuals in high-altitude populations of Cinnamon Teal are significantly larger than their closest lowland relatives. The largest subspecies, *A. c. orinomus*, is found exclusively in the central high Andes, with no records of dispersal to adjacent lowland habitats. Individuals collected at mid-elevations (~2,500 m; *A. c. borreroi* and *A. c. cyanoptera* in northwest Argentina) tended to have intermediate body size (Figs. 1.7–1.10). High-altitude habitats exert selection pressures that arise from multiple factors (Monge and León-Velarde 1991). Besides having a cold, arid climate, these habitats have low air density and the partial pressure of oxygen at 4,000 m is ~60% that at sea level which may also explain, in part, why high Andean resident populations have larger body size than individuals in populations at lower elevations in the Andes with similar climatic factors (Colombia and Patagonia). Hemoglobin oxygen affinity and body size,

for example, have been found to be correlated, such that larger animals tend to have higher affinity (Schmidt-Nielsen and Larimer 1958, Hopkins and Powell 2001). By contrast, smaller-bodied animals tend to have higher metabolic requirements for oxygen, which may favor a higher venous oxygen tension (Schmidt-Nielsen and Larimer 1958, Hopkins and Powell 2001). Other waterfowl species that inhabit similar elevational gradients in the Andes also show a strong correlation between body size and elevation (Blake 1977, Bulgarella et al. 2007). Each highland population also possesses amino acid polymorphisms in the major hemoglobin genes that are likely adaptive (McCracken et al. 2009a, b). Thus, there is an overall trend among South American waterfowl. Larger individuals are found at higher elevations, whereas the adjacent lowlands are inhabited by smaller conspecifics that also differ in other important traits.

Additionally, there is a general trend for sedentary species to comply more often with Bergmann's rule than migratory species, possibly because nonmigratory species are more affected than migratory species by climatic and other factors such as food availability, in that resident populations are exposed to the same local selection pressures throughout all seasons (Meiri and Dayan 2003). Cinnamon Teal comprise both sedentary and migratory subspecies, with the migratory small-bodied *A. c. septentrionalium* showing few significant correlations with either latitude or elevation. There was a correlation with bill shape (PC2) and elevation among males, which was attributable to a decrease of <0.6 mm in bill width or bill height between Utah (1,275 m) and either Oregon or California (<700 m). However, male breeding-site philopatry is typically very low in dabbling ducks (Anderson et al. 1992). Conversely, South American subspecies,

with the exception of the southernmost populations in Argentina, may be predominantly nonmigratory and show significant correlations between morphological and geographic variables, especially *A. c. cyanoptera*, which occupies a wide range of habitats from coastal Peru to southern Patagonia.

Little information is available on the movements of individual teal between the lowlands and highlands of South America. The lowland subspecies, *A. c. cyanoptera*, occurs in the highlands in small numbers, but the extent of its distribution in the Andes is unknown. We sampled individual *A. c. cyanoptera* in the highlands at only the northern and southern edges of the Altiplano. Six individuals of this subspecies were collected at 2,141–3,369 m in northwestern Argentina (KGM 442, KGM 1110, KGM 1142) and at 3,393–4,039 m in Peru (REW 118, REW 122, REW 164). There are no records of *A. c. cyanoptera* breeding in the high Andes, and only one individual we collected was in breeding condition (KGM 1142), judging by gonad size (left testis: 30 × 10 mm), even though all individuals were in complete breeding plumage. Two individuals (REW 118, REW 122) from Jauja, Peru (3,506 m), were part of a large group that contained both highland and lowland subspecies. All other individuals were either solitary or accompanied by one or two other individuals, and no other Cinnamon Teal were found in the surrounding areas. This suggests that these individuals may have been migrants or, more likely, vagrants to these areas rather than permanent residents, as each individual was assigned to the nearest lowland population. In addition, there are no records of *A. c. orinomus* descending to coastal habitats. One *A. c. orinomus* (KGM 441) was collected at 1,468 m in Salta, Argentina, which, to our knowledge, is the lowest elevation reported

for this subspecies. Pearson and Plenge (1974) recorded occasional sightings of other Andean waterfowl species (e.g., *A. puna* and *A. flavirostris*) on the coast of Peru, which they attributed to decreased food availability at high elevations during the dry season or competition with seasonal migrants from the south. Water temperature of high Andean lakes (>4,000 m) shows little seasonal variation within the Andean tropical regions, and only the shallow ponds and lakes will freeze or dry up (R. E. Wilson pers. obs.). Cinnamon Teal populations thus face a variety of environmental factors, and phenotypic diversity appears to have arisen from spatial and temporal heterogeneity in selection pressures resulting in adaptations to the local environment.

Subspecies classification.—Morphological (plumage and body size) distinctiveness of individuals in adjacent geographic areas of North America and South America led to the naming of five Cinnamon Teal subspecies (Snyder and Lumsden 1951). However, this classification had not previously been tested. Our analyses (MANOVA and ANOVA) differentiated all subspecies for males, and female *A. c. orinomus* differed from all other subspecies. Discriminant analysis showed high accuracy of subspecies prediction of males for all subspecies and *A. c. orinomus* and *A. c. cyanoptera* females. However, diagnosability of individuals to subspecific groups using the 75% rule (Amadon 1949) showed that few characters reliably distinguished subspecies, excluding *A. c. orinomus*. Low diagnosability among subspecies for females may be attributable in part to low sample sizes. The most reliable characters that enabled diagnosis between *A. c. orinomus* and the other subspecies were wing chord, tarsus, and PC1 (overall body-size variable). Low diagnosability of *A. c. cyanoptera* with respect to

North American and Colombian subspecies could be attributable to within-subspecies variation, given that there were significant mean differences between populations of *A. c. cyanoptera* populations. When analyzed at the population level, the Argentine and Peruvian coastal populations were diagnosable from *A. c. borreroi* and *A. c. septentrionalium* using bill length measurements or PC1. Upon examination of measurements originally used to define these subspecies, the results are not surprising, because there is considerable overlap in body-size measurements, which indicates that measurements alone may not be sufficient to distinguish subspecies. Other characters, such as plumage coloration and patterns, have been proposed to differentiate subspecies (Snyder and Lumsden 1951). Although the coloration of males within and among subspecies is variable, plumage divergence in color patches that appear identical to the human eye has been reported between *A. c. septentrionalium* and South American subspecies (*A. c. orinomus* and *A. c. cyanoptera*; Wilson et al. 2008). The Colombian subspecies (*A. c. borreroi* and *A. c. tropica*) are typically darker in coloration, with spotting occurring at higher frequency (100% in *A. c. tropica*) than in the other three subspecies, but spotting also can be variable, with substantial overlap among other subspecies (Snyder and Lumsden 1951). The tone of the cinnamon color in males ranges from dark (Colombian subspecies) to pale (*A. c. orinomus*). Females are more difficult to differentiate with plumage, but in general, as with males, Colombian subspecies are darker in color. Thus, we suggest that the current subspecies classification is valid on the basis of body-size measurements (present study) and plumage coloration and as described by Snyder and Lumsden (1951) and Wilson et al. (2008).

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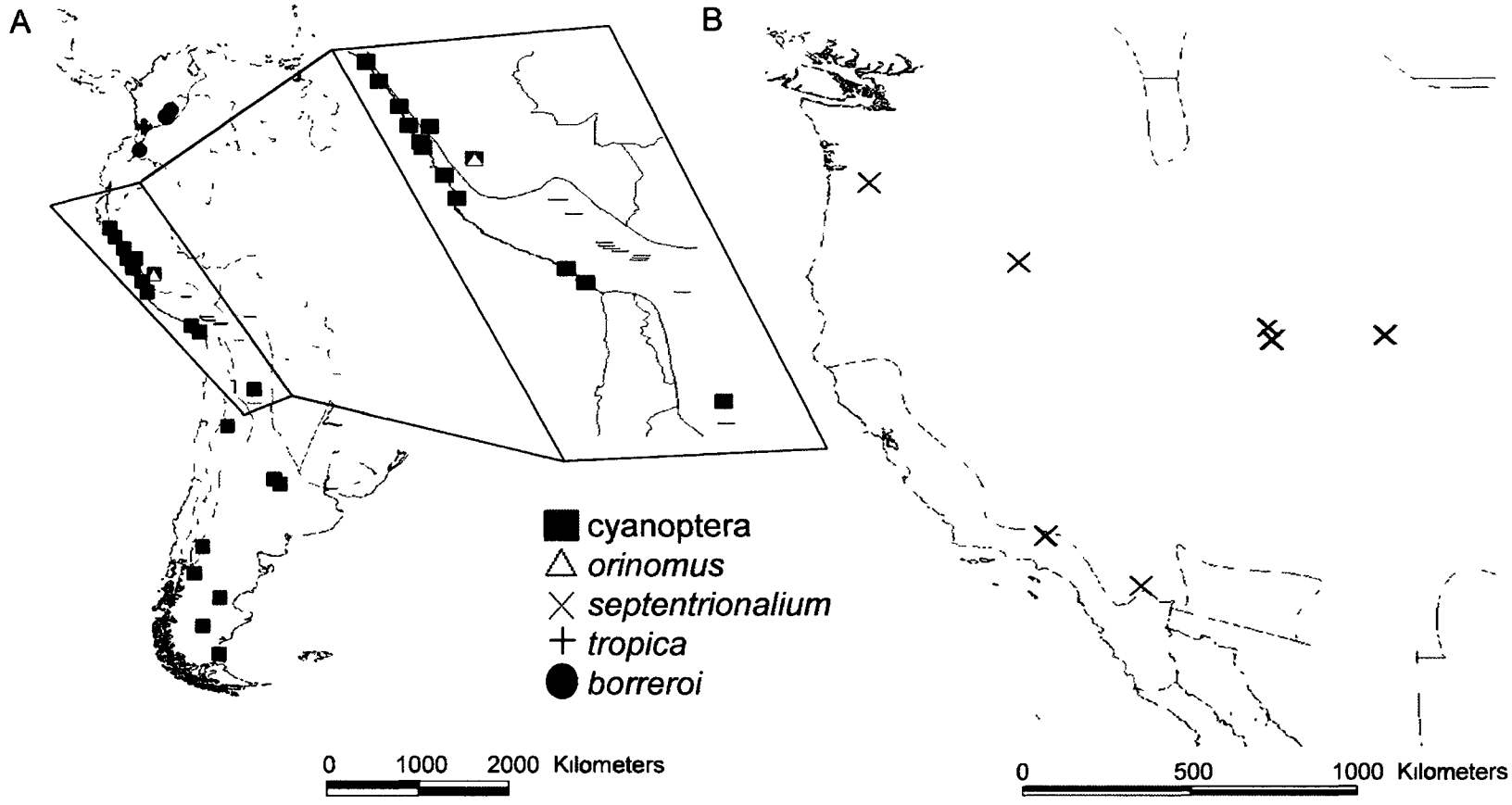

Figure 1.1. Sampling localities and geographic ranges for Cinnamon Teal (Ridgely et al. 2003).

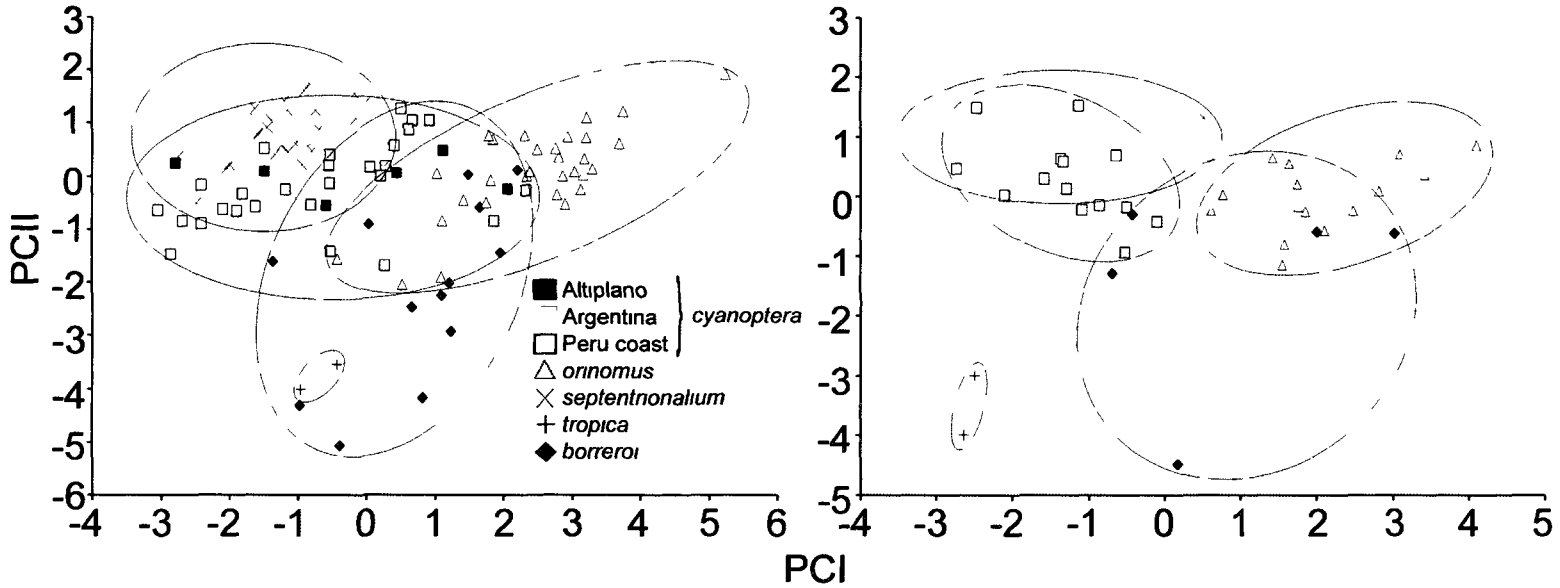

Figure 1.2. Principal component analysis (PC1 vs. PC2) of nine body-size measurements for male (left) and female (right) Cinnamon Teal.

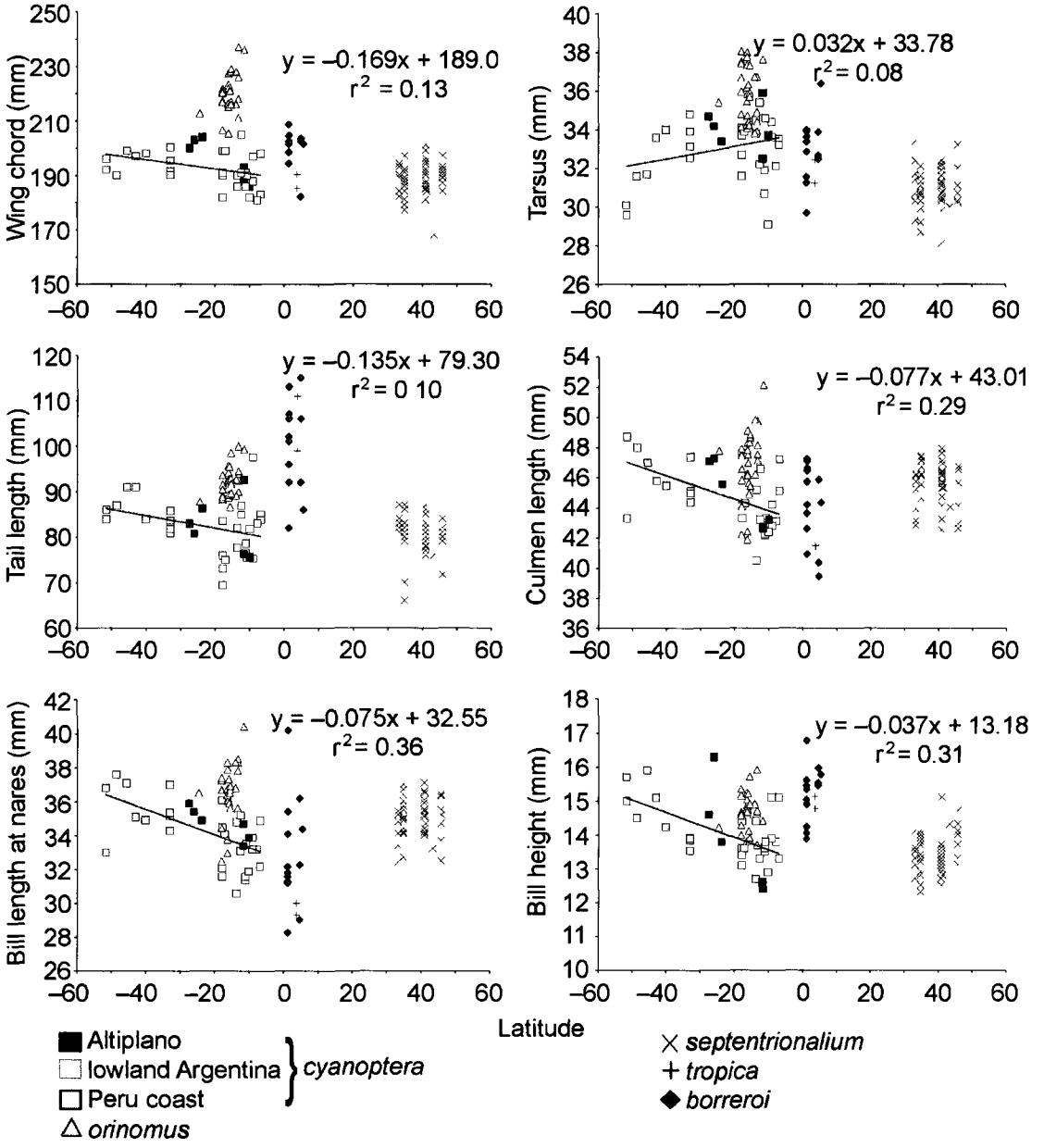

Figure 1.3. Relationships between latitude and body-size measurements for male Cinnamon Teal. Regression line is for *A. c. cyanoptera* populations only.

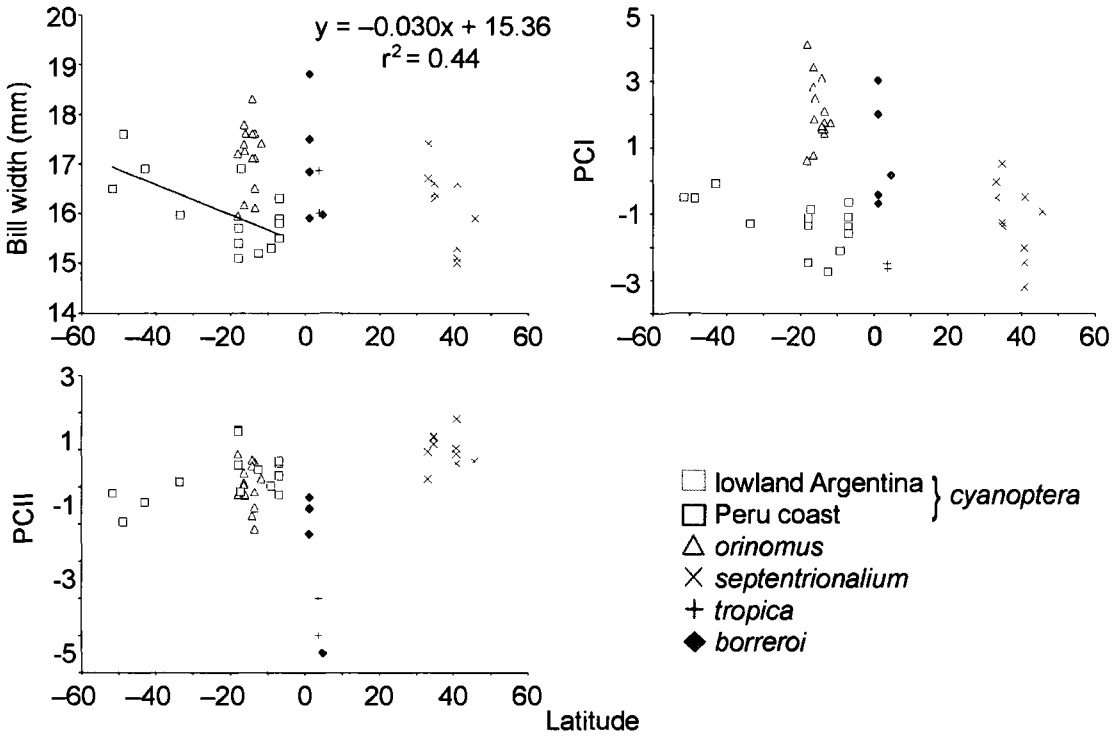

Figure 1.4. Relationships between latitude and body-size measurements for male Cinnamon Teal. Regression line is for *A. c. cyanoptera* populations only.

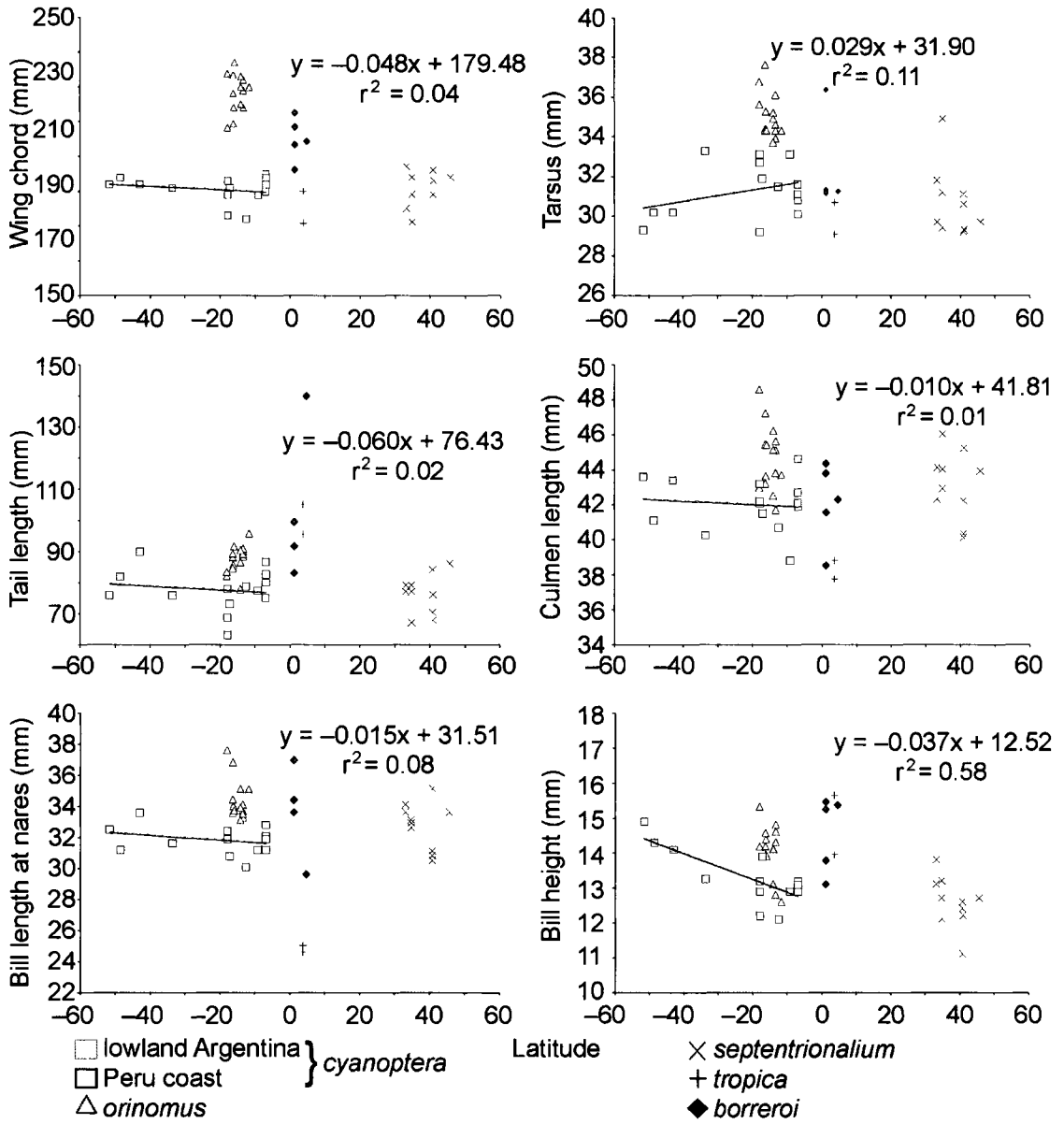

Figure 1.5. Relationships between latitude and body-size measurements for female Cinnamon Teal. Regression line is for *A. c. cyanoptera* populations only.

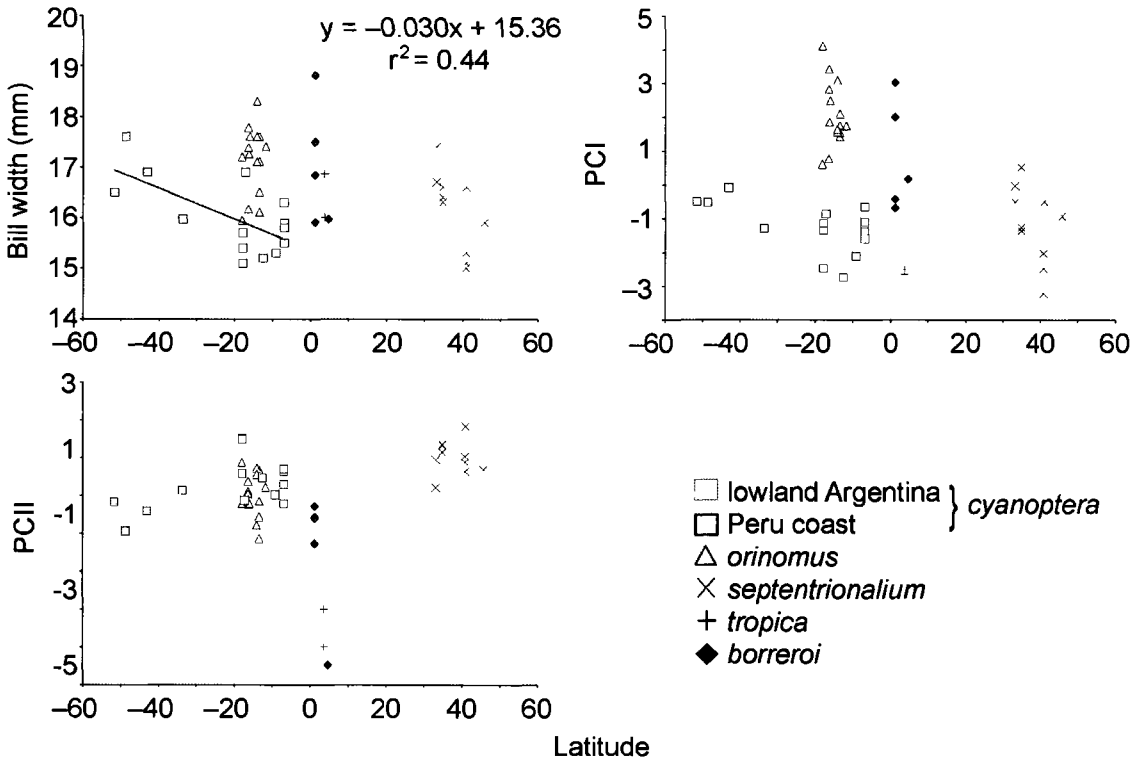

Figure 1.6. Relationships between latitude and body-size measurements for female Cinnamon Teal. Regression line is for *A. c. cyanoptera* populations only.

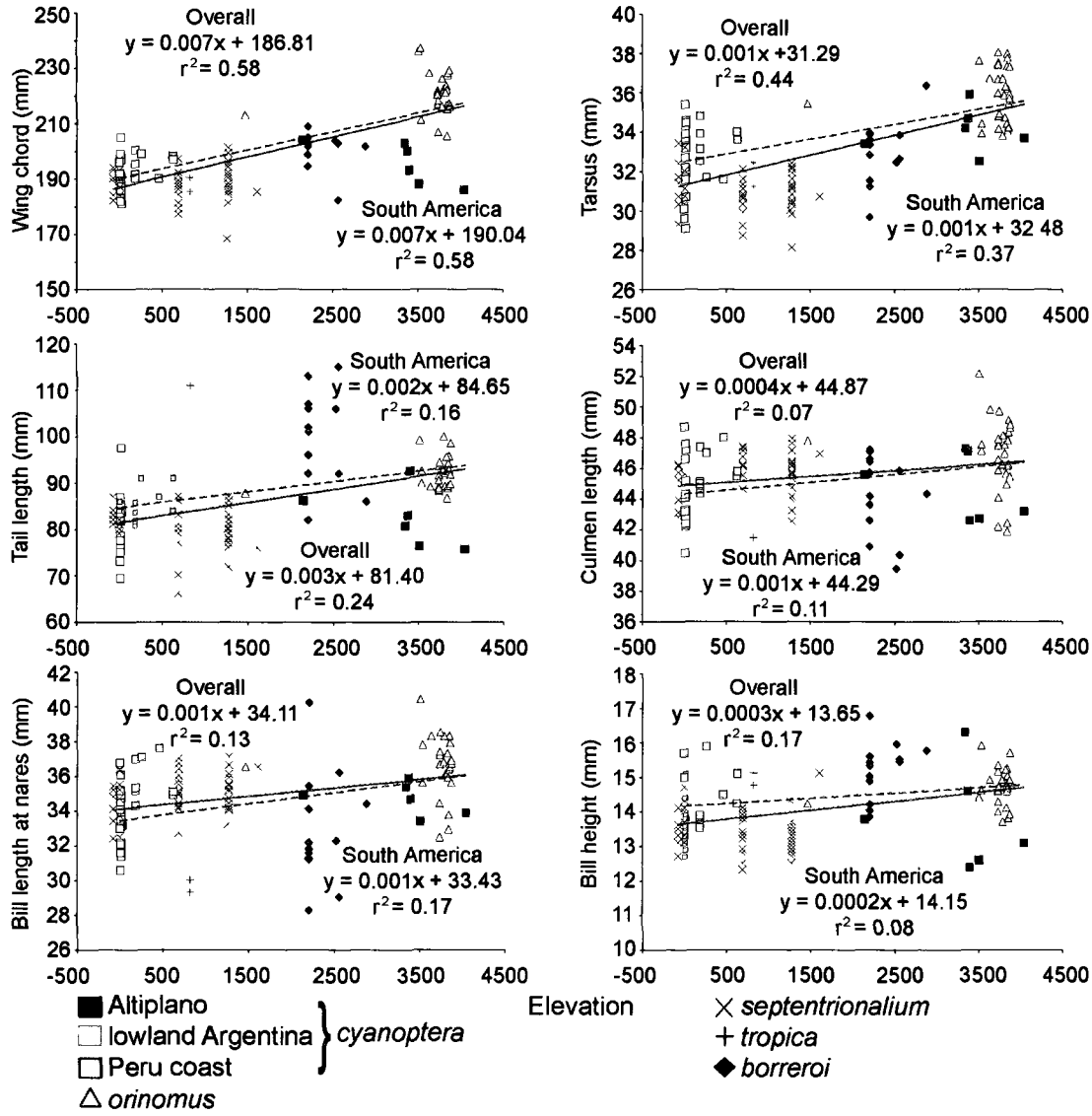

Figure 1.7. Relationships between elevation and body-size measurements for male Cinnamon Teal. Dashed regression lines are for South American individuals only.

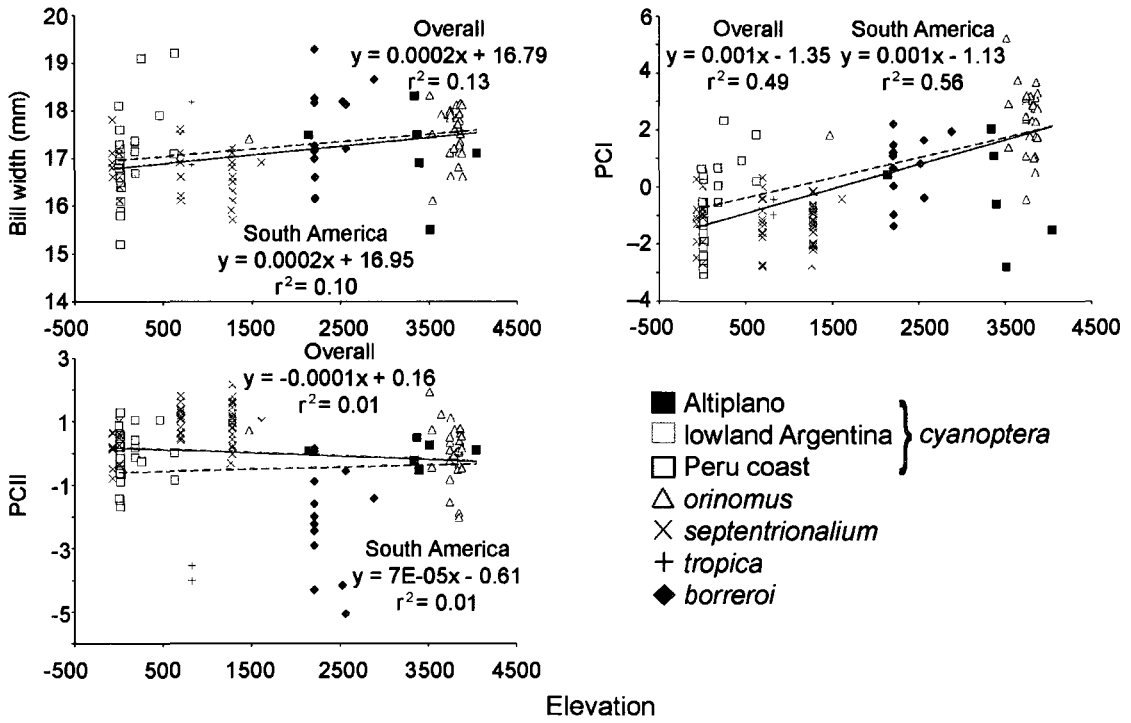

Figure 1.8. Relationships between elevation and body-size measurements for male Cinnamon Teal. Dashed regression lines are for South American individuals only.

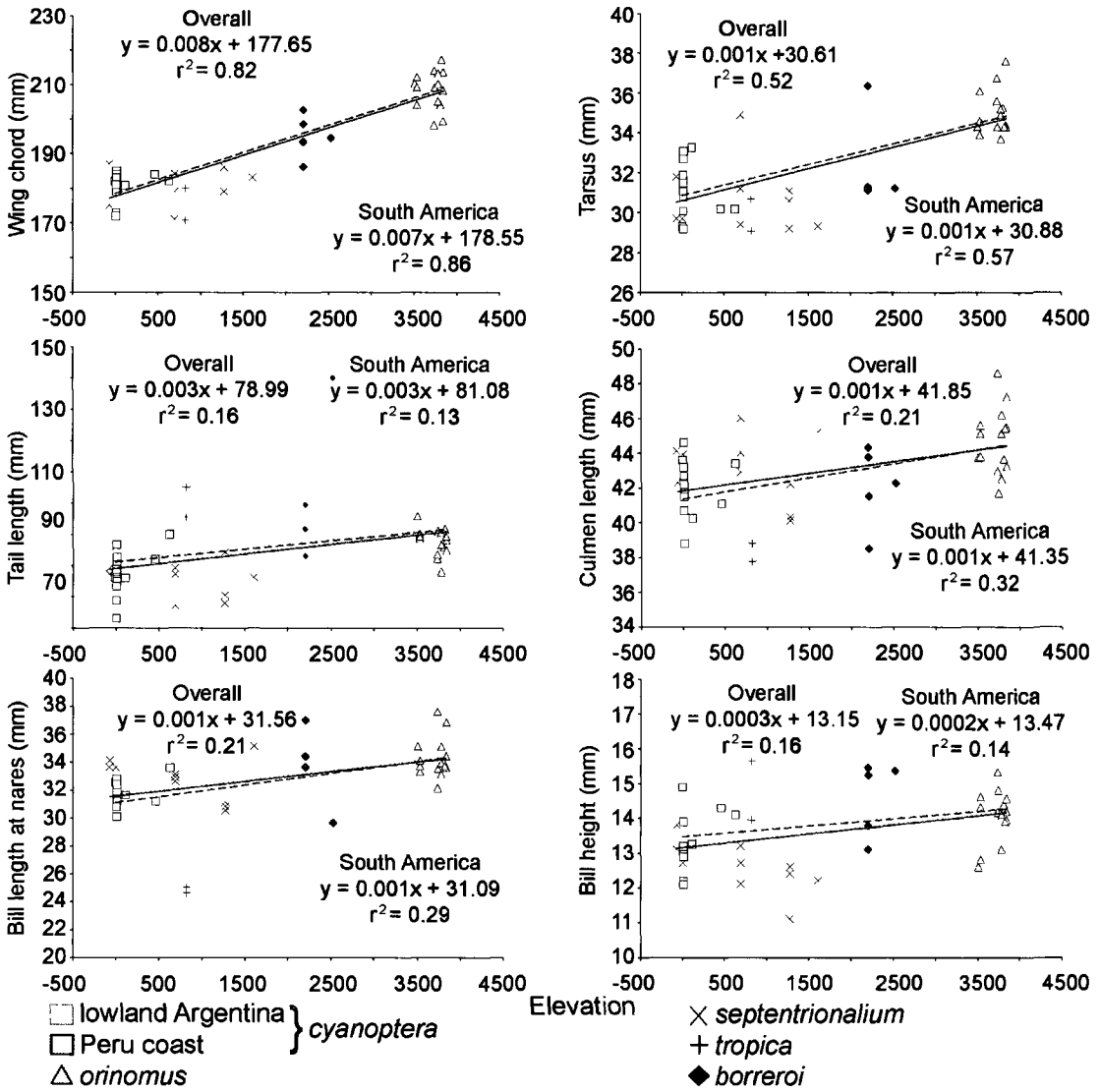

Figure 1.9. Relationships between elevation and body-size measurements for female Cinnamon Teal. Dashed regression lines are for South American individuals only.

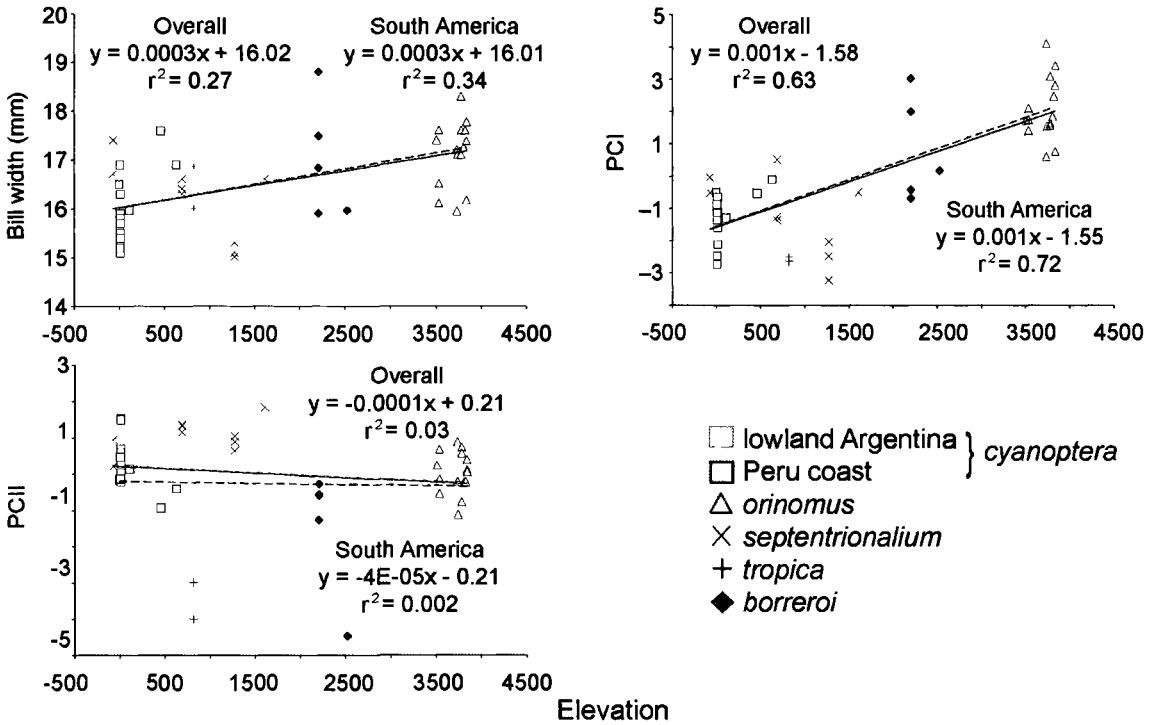

Figure 1.10. Relationships between elevation and body-size measurements for female Cinnamon Teal. Dashed regression lines are for South American individuals only.

Table 1.1. Wet measurements (mm) and body mass (g) for male *Anas cyanoptera cyanoptera*, *A. c. orinomus*, and *A. c. septentrionalium* and dry measurements for *A. c. borreroi* and *A. c. tropica*. Letters after mean value correspond to subspecies (o = *orinomus*, c = *cyanoptera*, b = *borreroi*, and s = *septentrionalium*) and indicate significant pairwise differences determined using Bonferroni corrected *P* values ($P_{adjusted} < 0.05$). Pairwise comparisons were not performed for *A. c. tropica* because of the small sample size.

		Mass	Wing chord	Tarsus	Tail	Nare	Culmen	Bill height	Bill width
orinomus	Mean	498.8	223.4 cbs	35.52 cbs	93.28 cbs	37.28 cbs	47.85 cbs	14.67 cs	17.41 cs
$n = 13$	SE	10.7	2.20	0.37	1.14	0.38	0.57	0.19	0.18
	Range	425–550	211–237	33.8–37.6	86.7–99.9	35.6–40.4	44.9–52.1	13.7–15.9	16.1–18.3
cyanoptera	Mean	414.5	191.9 ob	32.95 os	81.95 ob	33.96 o	44.55 o	14.02 obs	16.92 o
$n = 28$	SE	7.60	1.20	0.32	1.33	0.35	0.41	0.19	0.19
	Range	340–515	181–205	29.1–35.9	69.5–97.6	30.6–37.6	40.5–48.7	12.4–16.3	15.2–19.2
septentrionalium	Mean	361.8	188.8 ob	31.01 ocb	80.47 ob	35.00 ob	45.63 o	13.39 ocs	16.76 ob
$n = 50$	SE	3.30	0.90	0.15	0.58	0.17	0.20	0.08	0.07
	Range	310–420	168–201	28.1–33.4	66.0–87.0	32.4–37.1	42.5–47.9	12.3–15.1	15.7–17.8
borreroi	Mean	–	196.0 ocs	31.93 os	99.54 ocs	32.91 os	43.65 o	14.39 cs	16.57 s
$n = 13$	SE	–	1.86	0.43	2.77	0.88	0.73	0.21	0.25
	Range	–	179–205	28.7–35.2	82–115	28.3–40.2	38.9–46.7	13.1–15.9	15.2–18.2
tropica	Mean	–	184.0	30.93	105	29.68	40.99	14.13	16.54
$n = 2$	SE	–	2.50	0.59	6.00	0.36	0.03	0.17	0.62
	Range	–	182–187	30.2–31.4	99–111	29.3–30.0	40.9–41.0	13.9–14.3	15.9–17.2
P [1]		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]ANOVAs for subspecies effect based on pooled data (wet measurements and transformed dry measurements). Sample sizes: *orinomus* ($n = 30$), *cyanoptera* ($n = 34$), *septentrionalium* ($n = 50$), *borreroi* ($n = 13$), and *tropica* ($n = 2$).

Table 1.2. Wet measurements (mm) and body mass (g) for female *Anas cyanoptera cyanoptera*, *A. c. orinomus*, and *A. c. septentrionalium* and dry measurements for *A. c. borreroi* and *A. c. tropica*. Letters after mean value correspond to subspecies (o = *orinomus*, c = *cyanoptera*, b = *borreroi*, and s = *septentrionalium*) and indicate significant pairwise differences determined using Bonferroni corrected *P* values ($P_{adjusted} < 0.05$). Pairwise comparisons were not performed for *A. c. tropica* because of the small sample size.

		Mass	Wing chord	Tarsus	Tail	Nare	Culmen	Bill height	Bill width
orinomus	Mean	450.6	209.9 cbs	34.59 cbs	88.96 s	33.94 c	44.34 c	13.81 s	17.26 cs
$n = 9$	SE	11.30	1.32	0.24	1.62	0.24	0.51	0.26	0.22
	Range	390–495	204–217	33.7–36.1	77.9–95.7	33.1–35.1	41.7–46.2	12.6–14.8	16.1–18.3
cyanoptera	Mean	394.2	180.5 bo	31.14 o	77.85 b	31.85 o	42.15 o	12.29 b	16.01 o
$n = 13$	SE	10.60	1.10	0.37	1.97	0.26	0.41	0.22	0.22
	Range	340–470	172–185	29.2–33.1	63.1–90.0	30.1–33.6	38.8–44.6	12.1–14.9	15.1–17.6
septentrionalium	Mean	363.5	180.7 b	30.69 o	76.30 ob	32.74	43.10	12.59 ob	16.13 o
$n = 10$	SE	14.20	1.60	0.55	2.02	0.48	0.61	0.23	0.25
	Range	315–430	171–187	29.2–34.9	67.0–86.0	30.5–35.1	40.1–46.0	11.1–13.8	15.0–17.4
borreroi	Mean	–	190.6 ocs	31.22 o	104.8 cs	33.81	41.58	14.06 cs	15.92
$n = 5$	SE	–	2.68	0.99	10.4	1.19	1.01	0.46	0.50
	Range	–	182–198	30.1–35.2	86–145	29.6–36.9	38.1–43.8	12.6–14.9	14.9–17.6
tropica	Mean	–	171.5	28.94	104.00	24.82	37.84	14.28	15.39
$n = 2$	SE	–	4.50	0.78	5.00	0.21	0.52	0.82	0.40
	Range	–	167–176	28.2–29.7	99–109	24.6–25.0	37.3–38.4	13.5–15.1	14.9–15.8
P [1]		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003

[1]ANOVAs for subspecies effect based on pooled data (wet measurements and transformed dry measurements). Sample sizes: *orinomus* ($n = 15$), *cyanoptera* ($n = 14$), *septentrionalium* ($n = 10$), *borreroi* ($n = 5$), *tropica* ($n = 2$).

Table 1.3. Measurements (mm) and body mass (g) for populations of *Anas c. cyanoptera* in lowland Argentina, the Peruvian coast, and the central high Andes.

Male		Mass	Wing chord	Tarsus	Tail	Nare	Culmen	Bill height	Bill width
Argentina	Mean	429.2	194.96	32.50	85.53	35.64	46.02	14.56	17.68
$n = 10$	SE	9.67	1.20	0.55	1.08	0.46	0.54	0.26	0.28
	Range	450–540	190–200	29.6–34.8	80.9–91.0	33.0–37.6	43.3–48.7	13.5–15.9	16.7–19.2
Peruvian coast	Mean	394.4	190.5	33.06	80.39	33.21	43.98	13.74	16.51
$n = 18$	SE	4.88	1.61	0.37	1.52	0.36	0.43	0.16	0.14
	Range	340–430	181–205	29.1–35.4	69.5–97.6	30.6–36.1	40.5–47.6	12.7–15.1	15.2–17.6
Andes	Mean	429.2	195.68	34.07	82.45	34.71	44.45	13.79	17.13
n = 6	SE	14.4	3.18	0.48	2.61	0.38	0.89	0.60	0.38
	Range	380–470	186–204	32.5–35.9	75.7–92.6	33.4–35.9	42.6–47.3	12.4–16.3	15.5–18.3
Female									
Argentina	Mean	418.8	182.23	30.75	81	32.24	42.09	14.14	16.73
$n = 4$	SE	21.4	0.65	0.87	3.32	0.53	0.84	0.34	0.34
	Range	365–470	181–184	29.3–32.3	76.0–90.0	31.2–33.6	40.3–43.6	13.3–14.9	15.9–16.9
Peruvian coast	Mean	387.0	179.8	31.5	76.4	31.68	41.98	12.95	15.71
$n = 10$	SE	10.4	1.37	0.40	2.15	0.26	0.48	0.16	0.18
	Range	340–430	172–185	29.2–33.1	63.1–86.7	30.1–32.8	38.8–44.6	12.1–13.9	15.1–16.9

Table 1.4. Principal components (PC1 and PC2), eigenvectors, eigenvalues, and percent of variance calculated from male and female Cinnamon Teal (*Anas cyanoptera*).

	Males		Females	
	PC1	PC2	PC1	PC2
Wing chord	0.48	0.01	0.48	–0.06
Tarsus bone	0.41	–0.09	0.42	0.05
Tail	0.32	–0.48	0.14	–0.66
Bill length–Nare	0.33	0.56	0.39	0.41
Culmen length	0.33	0.54	0.40	0.36
Bill height	0.36	–0.39	0.30	–0.51
Bill width	0.39	–0.09	0.42	–0.12
Eigenvalue	3.33	1.63	3.54	1.57
Variance (%)	47.6	23.2	50.5	22.5
Cumulative %	47.6	70.9	50.5	73.0

Table 1.5. Partial correlation coefficients between latitude[1] and body measurements and principal components for Cinnamon Teal (*A. cyanoptera*). Significant values determined using Bonferroni corrected *P* values ($P_{adjusted} < 0.05$) are in bold.

	Male				Female			
	Pooled data	*A. c. cyanoptera*	Southern South America	North America	Pooled data	*A. c. cyanoptera*	Southern South America	North America
Wing chord	–0.096	0.381	0.097	0.145	–0.092	0.044	–0.061	0.329
Tarsus bone	**–0.343**	–0.280	–0.213	0.029	–0.178	–0.219	–0.290	–0.371
Tail	**–0.425**	0.319	0.184	0.039	–0.362	–0.321	0.041	0.449
Bill length–Nare	**0.476**	**0.619**	0.382	0.088	0.223	0.081	0.113	–0.101
Culmen	**0.358**	**0.544**	0.294	–0.155	0.291	0.081	0.052	–0.173
Bill height	**–0.278**	**0.557**	**0.434**	0.197	–0.236	0.681	**0.569**	–0.334
Bill width	–0.005	**0.662**	**0.560**	–0.33	0.022	0.407	0.438	–0.589
PC1	–0.065	**0.629**	0.383	–0.013	0.007	0.320	0.271	–0.340
PC2	**0.582**	0.259	0.133	–0.111	0.398	–0.240	–0.366	–0.261

[1]Latitude is calculated in degrees as the absolute value of distance from the equator.

Table 1.6. Partial correlation coefficients between elevation and body measurements and principal components for Cinnamon Teal (*Anas cyanoptera*). Significant values determined using Bonferroni corrected *P* values ($P_{adjusted} < 0.05$) are in bold.

	Male			Female		
Elevation	Pooled data	South America	North America	Pooled data	South America	North America
Wing chord	**0.724**	**0.782**	–0.038	**0.905**	**0.928**	–0.122
Tarsus bone	**0.603**	**0.619**	–0.018	**0.705**	**0.754**	0.018
Tail	**0.417**	**0.354**	–0.265	0.388	0.333	–0.529
Bill length–Nare	**0.381**	**0.547**	0.270	**0.454**	**0.575**	–0.356
Culmen	**0.262**	**0.426**	0.312	**0.458**	**0.611**	–0.167
Bill height	**0.292**	0.275	–0.243	0.357	0.373	–0.590
Bill width	**0.265**	**0.356**	–0.188	**0.497**	**0.605**	–0.461
PC1	**0.649**	**0.710**	–0.040	**0.784**	**0.861**	–0.419
PC2	**–0.018**	0.214	**0.494**	–0.118	0.007	0.584

Table 1.7. Classification of predicted Cinnamon Teal (*Anas cyanoptera*) subspecies based on body-size measurements and discriminant analysis with (inside parentheses) and without (outside parentheses) cross-validation. The percent of individuals that were assigned to their initial subspecific classification are in bold text.

	Predicted			
Initially classified as:	*cyanoptera*	*orinomus*	*septentrionalium*	*borreroi*
		Male (n = 127)		
cyanoptera	**76.5 (76.5)**	0.0 (0.0)	14.7 (14.7)	8.8 (8.8)
orinomus	0.0 (0.0)	**100 (100)**	0.0 (0.0)	0.0 (0.0)
septentrionalium	8.0 (10.0)	0.0 (0.0)	**92.0 (90.0)**	0.0 (0.0)
borreroi	7.7 (30.8)	0.0 (0.0)	0.0 (0.0)	**92.3 (69.2)**
Total correct:	89.8 (86.6)			
		Female (n = 44)		
cyanoptera	**85.7 (71.4)**	0.0 (0.0)	0.0 (28.6)	0.0 (0.0)
orinomus	0.0 (0.0)	**100 (100)**	0.0 (0.0)	0.0 (0.0)
septentrionalium	30.0 (50.0)	0.0 (0.0)	**70.0 (50.0)**	0.0 (0.0)
borreroi	0.0 (20.0)	0.0 (40.0)	0.0 (0.0)	**100 (40.0)**
Total correct:	88.6 (72.7)			

Table 1.8. Classification of predicted area of origin of individual Cinnamon Teal (*Anas cyanoptera*) based on body-size measurements and discriminant analysis with (inside parentheses) and without (outside parentheses) cross-validation. The percent of individuals that were assigned to their collection locality are in bold text.

Initially classified as:	Predicted				
	Central high Andes	Argentina	Peruvian Coast	North America	Colombia highlands
			Male (n = 127)		
Central high Andes	**83.3 (83.3)**	8.3 (8.3)	8.3 (8.3)	0.0 (0.0)	0.0 (0.0)
Argentina	0.0 (0.0)	**80.0 (40.0)**	20.0 (30.0)	0.0 (20.0)	0.0 (10.0)
Peruvian Coast	5.6 (5.6)	0.0 (0.0)	**77.8 (77.8)**	11.1 (11.1)	5.6 (5.6)
North America	0.0 (0.0)	6.0 (8.0)	4.0 (6.0)	**90.0 (86.0)**	0.0 (0.0)
Colombian highlands	7.7 (7.7)	23.1 (23.1)	0.0 (15.4)	0.0 (0.0)	**69.2 (53.8)**
Total correct:	83.5 (77.2)				
			Female (n = 44)		
Central high Andes	**100 (100)**	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Argentina	0.0 (0.0)	**75.0 (75.0)**	25.0 (25.0)	0.0 (0.0)	0.0 (0.0)
Peruvian Coast	0.0 (0.0)	20.0 (20.0)	**60.0 (60.0)**	20.0 (20.0)	0.0 (0.0)
North America	0.0 (0.0)	10.0 (10.0)	20.0 (40.0)	**70.0 (50.0)**	0.0 (0.0)
Colombian highlands	0.0 (20.0)	0.0 (20.0)	0.0 (0.0)	0.0 (0.0)	**100 (40.0)**
Total correct:	81.8 (70.5)				

Table 1.9. Pairwise diagnosability index values (D_{ij}/D_{ji}) for males of Cinnamon Teal (*Anas cyanoptera*) subspecies. D_{ij} values greater than zero indicate that population *i* is diagnosable from population *j* and are in bold.

	Wing chord	Tarsus	Tail	Nare	Culmen	Bill height	Bill width	PC1
orinomus								
and *cyanoptera*	**37.91/**	**5.57/**	**21.27/**	**5.41/**	**5.35/**	**2.50/**	**2.27/**	**0.37/**
	14.22	**0.46**	**5.30**	–0.45	–2.04	–0.02	–0.15	**4.79**
and *borreroi*	**34.13/**	**6.23/**	–16.81/	**11.02/**	**8.30/**	–1.27/	–2.25/	–0.50/
	7.73	**0.58**	**9.06**	**1.82**	–1.03	**1.31**	**0.65**	**3.68**
and	**40.98/**	**6.34/**	**18.65/**	**3.25/**	**3.02/**	**2.25/**	**1.48/**	**2.60/**
septentrionalium	**17.92**	**2.15**	**5.85**	–1.64	–3.37	**0.33**	–0.24	**5.89**
cyanoptera								
and *borreroi*	–7.06/	**3.33/**	–5.47/	**8.55/**	**6.30/**	–0.33/	–1.47/	**3.31/**
	17.32	–2.52	**24.28**	–0.66	–2.33	**2.85**	**1.99**	–1.32
and	**13.92/**	**3.43/**	**7.30/**	–0.77/	–1.01/	**1.31/**	**0.69/**	–0.20/
septentrionalium	–7.18	–0.96	–9.40	**4.13**	**4.67**	–1.21	–1.57	**3.53**
septentrionalium								
and *borreroi*	–3.40/	–1.64/	–4.94/	**9.74/**	**7.63/**	**0.02/**	–1.55/	**4.40/**
	20.39	**3.30**	**21.66**	**1.50**	**0.01**	**2.60**	**1.19**	**0.91**

Table 1.10. Pairwise diagnosability index values (D_{ij}/D_{ji}) for females of Cinnamon Teal (*Anas cyanoptera*) subspecies. D_{ij} values greater than zero indicate that population *i* is diagnosable from population *j* and are in bold.

	Wing chord	Tarsus	Tail	Nare	Culmen	Bill height	Bill width	PCI
orinomus								
and *cyanoptera*	**34.35/**	**6.67/**	**24.91/**	**3.84/**	**5.37/**	**2.31/**	**2.63/**	**0.64/**
	16.45	**1.80**	**3.07**	–0.64	–1.14	–0.62	–0.13	**0.27**
and *borreroi*	**32.65/**	**10.63/**	–67.43/	**9.47/**	**9.91/**	–3.02/	**4.18/**	–4.18/
	3.85	**1.58**	**9.29**	–1.25	–0.54	**1.69**	–0.74	**2.62**
and	**38.24/**	**8.46/**	**26.19/**	**4.82/**	**5.74/**	**3.01/**	**2.75/**	**0.69/**
septentrionalium	**17.13**	**2.65**	**4.21**	–1.10	–1.91	**0.05**	–0.22	**4.96**
cyanoptera								
and *borreroi*	–5.80/	–6.70/	–55.88/	–7.38/	–7.48/	–2.22/	–2.99/	**1.27/**
	20.13	**2.96**	**24.89**	**2.38**	**2.35**	**2.54**	**2.07**	–4.51
and	–11.40/	**4.53/**	**14.64/**	–2.73/	–3.32/	**2.22/**	–1.58/	–1.14/
septentrionalium	**6.83**	–1.89	–12.21	**2.22**	**3.71**	–0.84	**1.55**	**2.77**
septentrionalium								
and *borreroi*	–5.11/	–5.84/	–54.72/	–7.84/	**8.24/**	–1.54/	–3.09/	**7.28/**
	24.01	**4.75**	**26.17**	**3.36**	–2.73	**3.24**	**2.19**	–0.01

Appendix 1.1. Specimens of *Anas cyanoptera* examined, with collection locality. KGM, JT, and REW specimens are catalogued at University of Alaska Museum, Fairbanks.

A. c. borreroi
COLOMBIA: Dept. Putumayo, Sibundoy
ROM 79230, ROM 79231, ROM 79232, ROM 79233,ROM 79234, ROM 91946, ROM 91947, ROM 91948, ROM 91949, ROM 91950, ROM 91954, SM437473, SM437474
COLOMBIA: Dept. Cundinamarca, La Hererra
ROM 91943, ROM 91953
COLOMBIA: Dept. Cundinamarca, Laguna Fuquene
SM 437475
COLOMBIA: Dept. Cundinamarca, Sabana de Bogota
ROM 91944, SM437472
A. c. tropica
COLOMBIA: Dpto. Valle del Cauca, Vijes
ROM 91957, ROM 91958, ROM 91959, ROM 91960
A. c. septentrionalium
USA: Utah, Weber Co., 41°14'59.7"N, 112°07'55.8"W, 1,275 m
REW 075
USA: Utah, Salt Lake Co., 40° 50'50.7"N, 112°01'50.9"W, 1,275 m
REW 077, REW 078, REW 079
USA: Oregon, Columbia Co., 45°45'18.1"N, 122°50'51.4"W, 1 m
REW 797, REW 398, REW 399, REW 400, REW 401, REW 402, REW 403, REW 404, REW 406
USA: California, Imperial Co., 33°11'24.0"N, 115°35'18.5"W, –68 m
REW 411, REW 412, REW 414, REW 416, REW 418, REW 419, REW 421
USA: California, Imperial Co., 33°11'39.0"N, 115°34'46.2"W, –73 m
REW 415, REW 420
USA: California, Kerns Co., 34°47'43.5"N, 118°07'11.3"W, 693 m
REW 422, REW 423, REW 424, REW 425, REW 426, REW 427, REW 428, REW 429, REW 430, REW 431, REW 432, REW 433, REW 434, REW 435, REW 436, REW 437
USA: Utah, Salt Lake Co., 40°50'45.1"N, 112° 01'41.7"W, 1,275 m
REW 438, REW 439, REW 440, REW 441, REW 442, REW 443, REW 444, REW 445, REW 446, REW 447, REW 448, REW 449, REW 450, REW 451, REW 452, REW 453, REW 454, REW 455, REW 456
USA: Colorado, Moffat Co., 40°59'10.7"N, 108°59'10.5"W, 1,609 m
REW 457, REW 458
USA: Oregon, Harney Co., 48°43'53.7"N, 118°50'25.3"W, 1,260 m
REW 464
A. c. cyanoptera
ARGENTINA: Neuquen, Rio Collon Cura, R.N. 40, 40°12'45"S, 70°38'58"W, 625 m[1]
KGM 268
ARGENTINA: Cordoba, Laguna La Felipa, 33°04'17"S, 63°31'33"W, 184 m [1]
KGM 310, KGM 313, KGM 311, KGM 312

Appendix 1.1 continued.

ARGENTINA: Cordoba, S. Canals, 33°36'23"S, 62°53'16"W, 112 m [a]
KGM 322
ARGENTINA: Jujuy, S. Purmamarca, 23°49'13"S, 65°28'34"W, 2,141 m
KGM 442
PERU: Dpto. Lima, S Huacho, 11°10'12.9"S, 77°35'31.4"W, 15 m
REW 081, REW 082
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11°44'14.5"S, 75°29'32.7"W, 3,506 m
REW 118, REW 122
PERU: Dpto. Ancash, Laguna Conococha, 10°07'10.8"S, 77°17'00.7"W, 4,039 m
REW 164
PERU: Dpto. Lambayeque, ca. Puerto Eten, 06°54'51.9'S, 79°52'22.4"W, 13 m
REW 193, REW 194, REW 195, REW 196
PERU: Dpto. Lambayeque, Playa Monsefu, 06°54'03.7"S, 79°53'42.4"W, 12 m
REW 198, REW 199
PERU: Dpto. La Libertad, Magdalena de Cao, 07°51'54.3"S, 79°20'51.2"W, 23 m
REW 200
PERU: Dpto. Ancash, Chimbote, 09°07'26.0"S, 78°33'11.3"W, 15 m
REW 203, REW 204, REW 205
PERU: Dpto. Ancash, Puerto Huarmey, 10°05'52.0"S, 78°09'10.3"W, 14 m
REW 206
PERU: Dpto. Lima, Albufera de Medio Mundo, 10°55'25.9"S, 77°40'10.8"W, 14 m
REW 207
PERU: Dpto. Ica, Pisco, 13°41'46.8"S, 76°13'07.3"W, 7 m
REW 235
PERU: Dpto. Ica, Pisco, 13°40'47.2"S, 76°12'56.6"W, 9 m
REW 236
PERU: Dpto. Tacna, Ite, 17°52'47.2"S, 71°01'05.9"W, 10 m
REW 298, REW 299, REW 300, REW 301, REW 302, REW 303, REW 304
PERU: Dpto. Arequipa, Punta de Bombon-Islay, 17°11'31.9"S, 71°46'19.4"W, 8 m
REW 305, REW 306
PERU: Dpto. Lima, 2 km N. La Laguna, 12°33'13.0"S, 76°42'42.1"W, 9 m
REW 315, REW 316, REW 317
ARGENTINA: Chubut, Laguna Terraplen, 42°59'50.7"S, 71°30'55.1"W, 630 m
KGM 712, KGM 713
ARGENTINA: Santa Cruz, Estancia Angostura, 48°38'33.9"S, 70°38'37.3"W, 460 m
KGM 766, KGM 767
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51°37'35.7"S, 69°00'59.4"W, –3 m
KGM 797, KGM 798
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51°36'54.9"S, 68°59'26.6"W, 0 m
KGM 799
ARGENTINA: Chubut, S. Lago Colhue Huapi, 45°38'49.6"S, 68°56'45.1"W, 256 m
KGM 808

Appendix 1.1 continued.

ARGENTINA: Catamarca, Antofogasta de la Sierra, Laguna La Alumbrera, 26°06'46.4"S 67°25'26.7"W, 3,338 m
KGM 1110
ARGENTINA: Catamarca, Embalse Cortaderas, 27°33'21.2"S, 68°08'41.9", 3,369 m
KGM 1142
A. c. orinomus
ARGENTINA: Salta, NE La Caldera, 24° 33'01"S, 65° 22'15"W, 1,468 m
KGM 441
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16°11'45"S, 68°37'28"W, 3,808 m
KGM 485, KGM 486, KGM 487
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16°20'13"S, 68°41'20"W, 3,854 m
KGM 499
BOLIVIA: Dpto. Oruro, Lago Uru Uru, 18°02'03"S, 67°08'46"W, 3,735 m
KGM 527, KGM 528, KGM 529, KGM 530, KGM 531, KGM 532, KGM 533, KGM 534, KGM 535
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16°25'28"S, 68°51'43"W, 3,850 m
KGM 557
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21'03"S, 68° 37'40"W, 3,839 m
KGM 559, KGM 560
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21'02"S, 68°37'48"W, 3,840 m
KGM 561, KGM 562
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16°21'07"S, 68°38'06"W, 3,845 m
KGM 563, KGM 564, KGM 565, KGM 566
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11°44'14.5"S, 75°29'32.7"W, 3,506 m
REW 125, REW 126
PERU: Dpto. Cusco, Laguna Chacan, 13°26'02.6"S, 72°07'49.6"W, 3,533 m
REW 238, REW 239, REW 240, REW 241, REW 242
PERU: Dpto. Cusco, ca. Chinchero, 13°25'49.3"S, 72°03'41.7"W, 3,789 m
REW 248
PERU: Dpto. Cusco, Urubamba Valley, 13°25'22.9"S, 72°02'38.2"W, 3,743 m
REW 253, REW 254
PERU: Dpto. Cusco, ca. Laguna Pomacanchi, 14°06'51.9"S, 71°27'56.6"W, 3,781 m
REW 255, REW 256, REW 257, REW 258, REW 259
PERU: Dpto. Puno, Lago Titicaca, Jaru Jaru, 15° 59'05.6"S, 69° 36'24.3"W, 3,824 m
REW 268, REW 269
PERU: Dpto. Puno, Lago Titicaca, ca. Puno, 15°52'01.2"S, 69°56'21.3"W, 3,830 m
REW 271
PERU: Dpto. Puno, Lago Umayo, Sillvstani, 15°42'45.8"S, 70°09'00.0"W, 3,853 m
REW 272
PERU: Dpto. Puno, Deustva, 15°33'50.0"S, 70°14'33.1"W, 3,871 m
REW 284, REW 285, REW 286

Appendix 1.1 continued.

[1]These elevation values are interpolated from the U.S. Geological Survey's GTOPO30 digital elevation model (available at eros.usgs.gov/); all other elevations were measured with a GPS receiver.

Appendix 1.2. Dry body-size measurements (mm) for three subspecies of Cinnamon Teal.

	A. c. orinomus [1]			*A. c. cyanoptera* [1]			*A. c. septentrionalium* [1]		
Male	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Wing chord	215.4	1.05	200–229	186.8	1.39	176–201	185.5	1.0	163–199
Tarsus bone	34.75	0.30	32.2–37.3	32.31	0.21	30.1–34.1	30.32	0.14	27.7–32.1
Tail	96.07	1.08	82.0–108.0	86.52	1.22	75–102	78.75	0.53	66.0–85.0
Bill length–Nare	36.61	0.32	32.4–39.6	34.32	0.37	30.9–37.6	35.05	0.16	32.0–36.8
Culmen	47.19	0.44	42.0–52.4	44.36	0.49	40.1–49.3	44.70	0.20	41.7–47.1
Bill height	14.36	0.23	12.5–17.6	13.34	0.14	11.5–15.2	12.58	0.10	11.0–14.4
Bill width	16.49	0.13	14.9–17.4	16.05	0.18	14.1–17.7	15.58	0.15	12.6–17.4
Female									
Wing chord	202.4	1.7	193–217	177.9	2.0	167–191	178.5	1.8	169–186
Tarsus bone	33.59	0.54	30.6–37.2	31.29	0.45	29.3–33.5	30.29	0.43	28.8–32.8
Tail	91.87	1.48	84.0–102.0	83.94	2.85	71.3–102.0	77.10	1.86	69.0–88.0
Bill length–Nare	34.16	0.44	32.2–37.9	31.83	0.34	29.9–33.1	32.83	0.49	30.5–35.4
Culmen	44.57	0.55	41.7–49.8	41.41	0.62	38.6–43.8	42.37	0.54	39.7–44.9
Bill height	13.82	0.24	12.5–15.5	12.80	0.29	11.1–14.1	11.83	0.28	10.2–13.1
Bill width	15.70	0.28	13.6–17.2	15.04	0.16	14.2–16.2	15.07	0.39	13.1–17.3

[1]Sample sizes: *A. c. orinomus* (29 male, 14 female), *A. c. cyanoptera* (27 male, 10 female), *A. c. septentrionalium* (47 male, 10 female).

CHAPTER 2

GENETIC AND PHENOTYPIC DIVERGENCE BETWEEN HIGH- AND LOW-ELEVATION POPULATIONS OF TWO RECENTLY DIVERGED CINNAMON TEAL SUBSPECIES[1]

ABSTRACT

Geographic variation in selection often leads to divergent selection between populations occupying different parts of a species' range, ultimately leading to population divergence. The colonization of new areas can thus facilitate divergence in beneficial traits with little genetic differentiation at neutral markers. We investigated genetic and phenotypic patterns of divergence between high- and low-elevation populations of Cinnamon Teal (*Anas cyanoptera*) in the Andes and adjacent lowland regions of South America (normoxia vs. hypoxia environments). Cinnamon Teal showed strong divergence in body size (PC1; $P_{ST} = 0.56$) and exhibited significant frequency differences in a single non-synonymous α-globin amino acid polymorphism (Asn/Ser-α9; $F_{ST} = 0.60$) between environmental extremes, despite considerable admixture of reference loci ($F_{ST} = 0.004$–0.168). Inferences of strong population segregation were further supported

[1]Wilson, R. E. and K. G. McCracken. Genetic and phenotypic divergence between high- and low-elevation populations of two recently diverged Cinnamon Teal subspecies. Prepared for AUK.

by the observation of few mismatched individuals in either environmental extreme. Coalescent analyses indicated that the highlands were most likely colonized from lowland regions more recently than other waterfowl species and since divergence gene flow has been asymmetric from the highlands into the lowlands. Multiple selection pressures associated with high elevation habitats, including cold and hypoxia, have likely shaped divergence within South American Cinnamon Teal.

Introduction

Species are often comprised of populations distributed across ecologically different environments. As populations colonize new environments, particular traits can become modified via divergent selection enabling individuals to exploit differences in habitat or to gain advantages in competition for contested resources (Mayr 1963; West-Eberhard 1983; Endler 1986; Schluter 1998, 2001). In heterogeneous landscapes, gene flow may be restricted by selection because alleles and traits that are beneficial in one particular environment may result in reduced fitness in another environment (Rundle and Nosil 2005, Nosil et al. 2008, Milá et al. 2009). The strength of selection is not likely to be homogeneous across the genome, as selection may not limit the dispersal of neutral alleles, unless those alleles are linked to loci under selection (McKay and Latta 2002, Emelianov et al. 2004, Mallet 2005, Garant et al. 2007, Via 2009). Similarly, adaptive differentiation can still occur even in the face of countervailing gene flow, so long as the strength of selection is greater than the force of migration ($s > m$; Slatkin 1987, McKay and Latta 2002, McCracken et al. 2009a). The colonization of new environments could thus facilitate divergence in advantageous traits with little genetic differentiation at neutral markers and may only result in divergence in a small portion of the genome (Orr and Smith 1998, Via 2009).

Isolation is also a strong barrier to gene flow and promotes the evolution of subsequent isolating barriers. For example, individual preferences for different habitats may reduce the likelihood of encounters between individuals of diverging populations, facilitating pre-zygotic isolation when mating occurs in or near preferred habitat (Funk et

al. 2002, Rundle and Nosil 2005, Hendry et al. 2007). Furthermore, the limitation of gene flow between populations adapted to spatially segregated environments may promote population divergence. When isolation develops between populations, divergent selection through adaptation may act selectively on the genome, effecting rapid changes at key loci while leaving the rest of the genome virtually unchanged (Wu 2001, Via 2009).

A comparative approach contrasting functional genes and phenotypic traits with an independent set of putatively neutral markers can help determine a population's response to differing environmental selection pressures in the context of heterogeneous landscapes. Spatial differences in allelic variation among genes that are associated with potential agents of selection versus allelic variation at neutral molecular markers can provide insight into the evolutionary history of adaptive divergence. This approach is especially effective in the early stages of population divergence, when neutral markers typically still reflect a combination of unresolved ancestral polymorphisms, recent gene flow, and the stochastic effects of the coalescent process (Maddison 1997, Via 2009). The premise of this approach is that the effects of selection are likely to be locus-specific, whereas demographic processes (nonadaptive processes) are expected to have uniform effects across the genome (Cavalli-Sforza 1966, Lewontin and Krakauer 1973, Storz and Dubach 2004, Beaumont 2005).

High-elevation regions provide an excellent opportunity to investigate the molecular and morphological bases of local adaptation imposed by strong selection. The selection pressures imposed by high-elevation habitats are relatively well understood, and

numerous genes under selection have been identified. The low temperatures, increased desiccation, higher atmospheric radiation, and especially hypoxic conditions (oxygen concentration approx. 40% lower at 4,000 m than at sea level) can be debilitating for lowland individuals (Tucker 1968, Scott et al. 2009). Populations that exist in highland areas have evolved a number of different strategies, resulting in genetically based adaptations (Jessen et al. 1991; Storz et al. 2007, 2010; Storz 2010; Yi et al. 2010; Peng et al. 2011; Scott et al. 2011). Hemoglobin in particular has repeatedly been demonstrated to exhibit an important evolutionary response to severe hypoxia in high-elevation species (e.g., Jessen et al. 1991; Weber et al. 1993; León-Velarde et al. 1996; Weber 2002; Storz et al. 2007, 2010). It is often the case that only one or a few amino acid changes are found in the hemoglobin protein (Perutz 1983, Hiebl et al. 1987, Braunitzer and Hiebl 1988), but when compared across species it has been shown that the same, similar, or adjacent substitutions appear in multiple highland taxa (McCracken et al. 2009b,c). In addition, body size is often correlated with hemoglobin oxygen affinity, with larger animals tending to have higher oxygen affinity (Schmidt-Nielson and Larimer 1958, Hopkins and Powell 2001). This is often interpreted as a thermoregulatory adaptation to the colder arid climates associated with high elevation. Functional changes in hemoglobin structure and an increase in body size provide two important mechanisms in coping with high elevations.

Waterfowl (Anatidae) are well known for their ability to thrive in extreme environments, and they are well suited to cope with hypoxic stress in high elevation habitats (Faraci 1991, Weber et al. 1993, Hopkins and Powell 2001). Amino-acid

polymorphisms that are likely targets of selection in the major hemoglobin genes have been identified in all lineages of high-elevation waterfowl surveyed to date (McCracken et al. 2009b,c). Cinnamon Teal (*Anas cyanoptera*), in particular, are excellent candidates for studying the molecular and morphological basis of adaptation to high-elevation. Two subspecies of Cinnamon Teal (*Anas cyanoptera*) inhabit southern South America. A small-bodied subspecies *A. c. cyanoptera* (340–515 g, 181–205 mm wing length) is widespread in lowland habitats (< 1,000 m) from the Pacific coast of Peru to southern Argentina, but it is replaced by the larger-bodied subspecies *A. c. orinomus* (425–550 g, 211–237 mm wing length) at elevations of 3,500–4,600 m in the central high Andes (Wilson et al. 2010). Cold temperatures at high elevations is one environmental factor that has likely contributed to the larger body size of this subspecies (Wilson et al. 2010), but other factors such as hypoxia also can contribute to body size differences (Hopkins and Powell 2001) and have likely shaped the evolution of hypoxia resistance in this species.

Using a series of vouchered specimens collected from the high Andes and adjacent lowlands of South America, we sought evidence of divergent selection in hemoglobin and phenotypic characteristics in contrast to divergence in neutral markers, consisting of five nuclear introns and the mitochondrial DNA (mtDNA) control region. Specifically, we aim to assess which evolutionary mechanism, genetic drift following isolation or recent divergence with divergent selection, more likely explains the observed patterns in genetic and morphologic variation between highland and lowland populations. If functional traits have diverged greater than neutral loci, this would provide evidence

that those traits have diversified more than expected by genetic drift alone indicating that selection has played a role in the genetic or morphological structure observed. In addition, using classic population genetic approaches coupled with coalescent theory, we assessed how adaptations to high elevation influence population genetic structure and gene flow between environments.

Methods

Specimen collection and DNA extraction.—We collected 52 *A. c. cyanoptera* and 50 *A. c. orinomus* from low- and high-elevation regions of Argentina, Bolivia, and Peru between 2001 and 2005 (Fig. 2.1, Appendix 2.1). We used published subspecific morphological characters to classify each specimen to subspecies (Snyder and Lumsden 1951, Blake 1977, Wilson et al. 2010). Six small-bodied individuals were collected in highland localities (> 2,100 meters) at the northern and southern limits of the *orinomus* distribution. Based on morphological characters, these individuals were assigned to *cyanoptera* and treated as part of the lowland population (Wilson et al. 2010). Preliminary molecular analysis showed no significant structure among lowland localities in Argentina and the west slope of the Andes in Peru (mtDNA: $F_{ST} = 0.03$, P-value 0.07; nuclear introns: $F_{ST} = 0.00$); therefore all lowland populations were treated as a single population. Vouchered specimens and frozen tissues are archived at the University of Alaska Museum (Fairbanks, Alaska), Museo de Historia Natural de la Universidad de San Marcos (Lima, Peru), and Colección Boliviana de Fauna (La Paz, Bolivia). Genomic DNA was extracted from muscle tissue using standard protocols and a QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA).

DNA Sequencing.—We sequenced the two adult hemoglobin genes (αA; 677 bp and βA; 1582 bp) that comprise the major hemoglobin isoform (HbA). Five autosomal introns that map to different locations in the chicken genome also were sequenced to be compared to hemoglobin genes (Table 2.1): ornithine carboxylase intron six (ODC1; 351 bp), α-enolase intron eight (ENO1; 312bp), beta fibrinogen intron 7 (FGB; 245 bp), N-methyl D aspartate receptor type I intron 11 (GRIN1; 330bp), and phosphoenolpyruvate carboxykinase intron 9 (PCK1; 345bp). Polymerase chain reaction (PCR), sequencing protocols and primers are described by McCracken et al. (2009b). We also sequenced 1,272 bp of the mtDNA control region and adjacent phenylalanine tRNA and 12S rRNA gene using the overlapping primer pairs L78–H774 and L736–H1530 (Sorenson and Fleischer 1996, Sorenson et al. 1999), and two additional primers (L627: 5'–TAAGCCTGGACACACCTGCGTTATCG–3'; H693: 5'–CAGTGTCAAGGTGATTCCC–3') designed specifically for Cinnamon Teal.

Sequences from opposite strands were reconciled using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences that contained double-peaks, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms. Indels were resolved by comparing the unambiguous 5'-ends of sequences to the 3'-ambiguous ends between forward and reverse strands (Peters et al. 2007). Gaps resulting in shifted peaks in the chromatograms, thus, enabled us to resolve length polymorphisms within the sequences. All sequences were aligned by eye using the sequence alignment editor Se-Al 2.0a11 (Rambaut 2007). Sequences and voucher information including georeferenced localities are deposited in GenBank (accession

numbers GQ269364–GQ269772, GQ271146–GQ271246, GQ271884–GQ271985, JF914653–JF919754).

Gametic phase of nuclear allele sequences.–The allelic phase of each nuclear sequence that was heterozygous at two or more nucleotide positions was determined using allele-specific priming and the software PHASE 2.1 (Stephens et al. 2001). PHASE uses a Bayesian method to infer haplotypes from diploid genotypic data with recombination and the decay of linkage disequilibrium (LD) with distance. Each data set was analyzed using the default values (100 main iterations, 1 thinning interval, 100 burn-in) followed by 1,000 main iterations and 1,000 burn-in (-X10 option) for the final iteration. The PHASE algorithm was run five times automatically (-x5 option) from different starting points, selecting the result with the best overall goodness of fit. We next selected individuals with allele pair probabilities <80% and designed allele-specific primers to amplify one allele but not the other (Bottema et al. 1993, Peters et al. 2005). The resulting haploid allele sequence was then subtracted from the diploid consensus sequence to obtain the gametic phase of the second haplotype. Each data set was then analyzed five more times using PHASE and the additional known allele sequences (-k option). The gametic phases of 97.1% (n = 692) of the 713 individual autosomal sequences that we analyzed were identified experimentally or with >95% posterior probability, and 98.0% (n = 699) were identified with >90% posterior probability.

Estimation of Genetic Diversity.—Nucleotide diversity (π), expected and observed heterozygosities, and linkage disequilibrium (LD) between nuclear introns were calculated in ARLEQUIN 3.11 (Excoffier et al. 2005). Allelic networks were

constructed in NETWORK 4.5.1 (Fluxus Technology Ltd. 2004) using the reduced median algorithm (Bandelt et al. 1995), to illustrate possible reticulations in the gene trees due to homoplasy or recombination. Gaps were treated as a fifth base, and indels were treated as a single insertion/deletion event.

To test for departures from neutrality in scenarios characterized by an excess of rare alleles, we calculated Tajima's *D* (Tajima 1989). Significantly negative values for this test statistic may indicate a population evolving under non-random processes such as directional or balancing selection, or demographic expansion or contraction.

Estimation of Population Subdivision.—To assess levels of population subdivision between highland (*orinomus*) and lowland (*cyanoptera*) populations, we calculated pairwise Φ_{ST} and F_{ST} for sequence data in ARLEQUIN using the best-fit nucleotide substitution model, as identified in MODELTEST 3.06 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC; Akaike 1974). *P*-values were adjusted for multiple comparisons using permutations (3,000) or Bonferroni corrections ($\alpha = 0.05$).

We used STRUCTURE 2.2 (Pritchard et al. 2000) to examine population differentiation. STRUCTURE uses a Bayesian method to assign individuals to populations by maximizing Hardy-Weinberg equilibrium and minimizing linkage disequilibrium. Data were analyzed using an admixture model without priori knowledge of specimen localities, assuming correlated frequencies with a burn-in period of 100,000 iterations, 1,000,000 Markov chain Monte Carlo iterations. Four analyses were performed, one including only the five autosomal introns and three additional analyses

with five introns and mtDNA plus either the αA subunit or βA subunit. No prior population information was used, and analyses were performed for one and two population models (K = 1 or 2) to compute the probability of assignment to the lowland or highland population and identify individuals with admixed lowland and highland genotypes.

Estimation of Gene Flow and Timing of Divergence.—We estimated gene flow between highland and lowland populations using two methodologies: IM (Hey and Nielsen 2004, Hey 2005) and BayesAss 1.3 (Wilson and Rannala 2003). IM uses the isolation-with-migration coalescent model, which treats divergence t (μT) and population splitting (s) as independently estimated parameters in addition to the effective population size parameter ($\theta = 4N_e\mu$) and gene flow ($M = m/\mu$; Hey and Nielsen 2004, Hey 2005). BayesAss uses an assignment methodology, which does not incorporate genealogy (Wilson and Rannala 2003). Estimates of the gene flow rate can thus be interpreted differently at different temporal scales with IM estimating gene flow since population divergence, whereas BayesAss reflects gene flow that occurred only in the past several generations.

For the IM analyses, we simultaneously estimated the following parameters scaled to the mutation rate per locus: effective population sizes (θ), immigration rates (M), and time since population divergence (t). We also estimated the splitting parameter (s) to test for the genetic signature of the direction of colonization.

IM assumes that the loci are free from intralocus recombination. We tested for recombination within each nuclear intron using a four-gamete test in DNAsp v. 4.10

(Rozas et al. 2003) and included the largest independently segregating block of sequence consistent with no recombination. Only ENO1 and GRIN1 showed evidence of recombination and were truncated to the 5' end positions 15–312 and 60–206, respectively. For the hemoglobin genes, the longest fragment with no recombination that included all non-synonymous amino acid replacements were 1–338 for the αA subunit and 118–587 for the βA subunit. The remaining loci had no detectable recombination; therefore the full sequences were used in the analysis. Additionally, we verified the four-gamete tests with an independent estimate of the overall recombination rate (r) for each locus using LAMARC 2.1.6 (Kuhner 2006) with the upper and lower limits for r set to 0 and 10, respectively.

We defined inheritance scalars in IM for mtDNA as 0.25 (maternally inherited) and for autosomal introns as 1.0 (biparentally inherited) to reflect differences in effective population sizes. We used the HKY model of mutation for mtDNA and infinite sites model for the nuclear introns. We initially ran IM using large, flat priors for each parameter. Based on the results of these runs, we defined narrower upper bounds for each parameter that encompassed the full posterior distributions from each initial run. Using those priors, we then used a burn-in of 500,000 steps and recorded results every 50 steps for more than 2 million steps. Effective sample sizes for each parameter exceeded 100. We repeated the analyses three times using a different random number seed to verify that independent runs converged on the same values.

To convert IM estimates to biologically informative values, we estimated the mutation rate (μ per locus) using a mutation rate of 4.8×10^{-8} substitutions/site/year

(s/s/y) for the mtDNA control region (range: $3.1 \times 10^{-8} - 6.9 \times 10^{-8}$ s/s/y; Peters et al. 2005) and calibrated mutation rates for introns on the goose-duck split following methods outlined by Peters et al. (2007, 2008). Using the geometric mean of substitution rates averaged for mtDNA and introns (7.57×10^{-7} s/s/y), we converted *t* and TMRCA to years before present (T) using $t = T\mu$ and by dividing the IM estimate of TMRCA by geometric mean of μ.

For the BayesAss analysis genotypic allelic data was grouped as follows: (1) nuclear introns, (2) five nuclear introns and βA subunit, and (3) five nuclear introns, βA subunit, and αA subunit. BayesAss was initially run with the default delta values for allelic frequency (P), migration rate (m), and inbreeding (F). Subsequent runs incorporated different delta values to ensure that proposed changes between chains at the end of the run were between 40–60% of the total chain length to maximize log likelihood values and ensure the most accurate estimates (Wilson and Rannala 2003). Final delta values used were $\Delta P = 0.06$, $\Delta m = 0.03$, and $\Delta F = 0.09$. We performed five independent runs (50 million iterations, 5 million burn-in, and sampling frequency of 2000) with different random seeds to ensure convergence across runs.

Simulated Neutral Genetic Diversity.— Using the parameters inferred from the isolation-with-migration coalescent model, we simulated genetic data under a model of selective neutrality in the program ms (Hudson 2002). We also included locus-specific recombination rates estimated in LAMARC as well as mutation rates based on empirical data for the five nuclear introns and mtDNA control region described above. We simulated a total of 1,000 data sets from which the distribution of pairwise Φ_{ST} expected

under selective neutrality was calculated and compared to estimates of αA and βA subunits as well as morphometric divergence (P_{ST}) to determine if a neutral trait could generate the divergence estimates observed.

Estimation of Morphometric Divergence.—Phenotypic differentiation was assessed using a phenotypic-Q_{ST} (P_{ST}; Sæther et al. 2007, Whitlock 2008) for seven previously published body size measurements taken from a recent analysis of Cinnamon Teal morphology (Wilson et al. 2010): wing chord length (carpal joint to longest primary feather unflattened, ±1 mm), tail length (base of the uropygial gland on back to tip of the center tail feather, ±1 mm), exposed culmen length, bill length at nares (anterior edge of nares to tip of nail), tarsus bone length (tarsometatarsus), bill height (height of upper mandible at anterior edge of nares), and bill width (width of upper mandible at anterior edge of nares). Due to low female sample size, we used a Bartlett's equal of variance test with Bonferroni-correction for multiple comparisons to determine whether male and female data sets could be pooled. No significant differences ($P > 0.05$) were found; therefore, female values were adjusted to male equivalents by adding the mean difference between sexes to females. A principal components analysis was performed on the seven body measurements excluding body mass to extract an overall body size index. In addition, a discriminant analysis was performed to estimate the probability of assignment to highland or lowland population based on all seven measurements.

To assess the degree of differentiation in phenotypic traits, we partitioned the morphological variation between subspecies by calculating a phenotypic-Q_{ST} (P_{ST}; Sæther et al. 2007, Whitlock 2008). Phenotypic-Q_{ST} can be interpreted as an F_{ST} analogue for

quantitative traits provided that within- and between-population variance in trait values is exclusively attributable to additive genetic effects (Wright 1943, 1951; Rogers and Harpending 1983). Otherwise, P_{ST} estimates may be biased if within- and between-population components of environmental variance are not proportional (Merilä and Crnokrak 2001). Comparisons of P_{ST} and F_{ST} are typically interpreted as follows: $P_{ST} > F_{ST}$, the trait(s) that P_{ST} was calculated from has diversified more than expected based on genetic drift alone; $P_{ST} < F_{ST}$, the trait(s) are under stabilizing selection that maintained the same value across the heterogeneous landscape in spite of genetic drift; and $Q_{ST} = F_{ST}$, there is insufficient evidence to suggest that selection is acting differentially or uniformly across the landscape and genetic drift cannot be ruled out as a driving force in diversification (Rogers 1986, Lande 1992, Whitlock 1999, Merilä and Crnokrak 2001, McKay and Latta 2002).

P_{ST} variance measures were calculated as described in Storz (2002) and Sæther et al. (2007). Phenotypic variation was partitioned into within- and between-group components using a variance component model (Model II ANOVA) on PC1 and PC2 scores and individual measurements from Wilson et al. (2010). As the data are solely phenotypic, assumptions about the heritability of the traits were made as outlined in Storz (2002) and Merilä (1997) where morphological traits were assumed to have a narrow-sense habitability of 0.5 based on heritabilities of quantitative traits in avian and mammalian taxa (e.g., Boag and van Noordwijk 1987, Larsson 1993, Merilä and Gustafsson 1993, Falconer and Mackay 1996). In addition, morphological trait differences (e.g., wing chord) have been shown to be genetically controlled along an

elevational gradient in passerines (*Junco hyemalis*; Rasner et al. 2004, Bears et al. 2008) and other Andean waterfowl (e.g., *Anas puna* and *Lophonetta specularioides*) bred in captivity in lowlands show morphological differences observed in the wild. Furthermore, it has been shown that only an extremely large environmental component of between group variance would affect the estimation of P_{ST} and lead to accepting the null hypothesis of neutral phenotypic divergence (Merilä 1997, Storz 2002, Sæther et al. 2007).

It is important to note that comparisons of P_{ST} and F_{ST} provide an initial starting point to identify potential characters that may be under selection as detailed experiments such as reciprocal transplant experiments, physiological studies, and correlation of morphology with environmental conditions are needed to confirm findings of these types of comparisons (Whitlock 2008). However, the selection pressures imposed by high elevation are well defined, and two main environmental factors (lower temperatures and hypoxia) are known to facilitate morphological variation (Bergmann 1894, James 1968, 1970, 1991, Hopkins and Powell 2001). Therefore, trait(s) showing an elevated level of phenotypic divergence (P_{ST}) would be predicted to be a strong candidate to be under directional selection associated with high elevation.

Results

Genetic Diversity.—Five to 22 alleles were identified in the five autosomal introns, with three to 18 polymorphic sites per locus. Observed heterozygosity was moderate to high (0.48 to 0.92) for all reference loci and similar between subspecies (Table 2.2). Genetic diversity, in terms of number of alleles and polymorphic sites, was higher than the introns

for both the αA and βA subunits (Table 2.2), which had 17 and 21 alleles with 15 and 63 polymorphic sites, respectively. Twenty-seven mtDNA haplotypes characterized by 18 variable sites were identified within Cinnamon Teal (Fig. 2.2).

Observed heterozygosity at the αA and βA subunits was similar to levels observed for autosomal introns for lowland *cyanoptera* (Ho = 0.58 and 0.77, respectively; Table 2.2). In contrast, observed heterozygosity for the αA subunit was considerably lower than levels observed for the nuclear introns for highland *orinomus*; most individuals in the highlands were homozygous for a single αA subunit allele (Ho = 0.06; Table 2.2, Fig. 2.2). All reference loci were in Hardy-Weinberg equilibrium except for FGB; *cyanoptera* exhibited heterozygote deficiency (Table 2.2). In contrast to the five introns, both αA and βA subunits were out of Hardy-Weinberg equilibrium. Heterozygote deficiency was observed the αA subunit when highland and lowland populations were pooled (42% vs. 68%; $P < 0.001$), however, *orinomus* and *cyanoptera* were in Hardy-Weinberg equilibrium when analyzed separately ($Ps > 0.5$). In addition, the lowland population was found to be heterozygote deficient (58% vs. 78%) for the βA subunit, whereas the highland population was in Hardy-Weinberg equilibrium (Table 2.2). The five autosomal introns were in linkage equilibrium ($Ps > 0.05$).

Tajima's *D* was not significant for any comparisons involving the five nuclear introns, βA subunit, or mtDNA ($Ps > 0.50$; Table 2.2). However, *orinomus* exhibited a significant Tajima's D, indicating a significant excess of rare alleles for the αA subunit in the highland population (Table 2.2; Fig. 2.2).

Population Subdivision.—Significant variance in the spatial distribution of allelic and haplotypic frequencies was observed across loci within Cinnamon Teal. Low to moderate levels of genetic structure were observed across the five introns (Φ_{ST} = 0.007–0.087; Table 2.2, Fig. 2.2). In contrast, very high differentiation was observed for the αA subunit between lowland *cyanoptera* and highland *orinomus* populations falling outside the 95% confidence limit of simulated data (Φ_{ST} = 0.551, Table 2.2, Fig. 2.2 and 2.3). Despite high levels of subdivision observed for the αA subunit, the βA subunit showed levels of structure similar to variance estimates calculated for the autosomal introns (Φ_{ST} = 0.061; Table 2.2, Fig. 2.2).

Little evidence of population structure was detected within Cinnamon Teal using Structure and a two-population model (K = 2) with either the autosomal introns or the mtDNA data set (posterior assignment probabilities averaged 50.8% and 50.7%, respectively; Fig. 2.4). Results from the combined analysis of five introns and mtDNA with the βA subunit were similar (posterior assignment probabilities = 51.4 ± 3.2% SD; Fig. 2.4). However, with the inclusion of the αA subunit, lowland *cyanoptera* individuals and highland *orinomus* individuals were assigned to two clusters with high posterior probabilities (94.5 ± 9.4% SD; Fig. 2.4).

Hemoglobin amino acid substitutions.—Two nonsynonymous substitutions were observed on the αA subunit. One substitution resulted in an amino acid replacement (Asn → Ser-α9), which showed highly significant allele frequency differences between *cyanoptera* and *orinomus* (F_{ST} = 0.94) and has not been recorded in any other waterfowl species (McCracken et al. 2009b). Ninety-four percent of individual *cyanoptera* were

homozygous for Asn, whereas 94% of individual *orinomus* were homozygous for Ser. Three (6%) heterozygous individuals were found in *cyanoptera* and three in *orinomus*. All six *cyanoptera* individuals collected above 2,000 m and at the northern and southern borders of Altiplano were homozygous for the allele that occurred at high frequency in the lowland population, Asn-α09. The second substitution we observed was Ala → Thr-α28. One *orinomus* (KGM499) collected on Lake Titicaca and four *cyanoptera* in Patagonia were heterozygous Ala/Thr-α28 with all remaining individuals homozygous Ala/Ala-α28. Ala is a synapomorphy for Cinnamon Teal and other members of the blue-winged duck group, whereas Thr-α28 is the ancestral state found in all other dabbling ducks (McCracken 2009b). The βA subunit possessed only silent (i.e., synonymous) polymorphisms.

Time since divergence.—Based on joint estimate for mtDNA and five nuclear introns, time since divergence peaked at 0.13 (95% CI = 0.05–0.72; Fig. 2.5), suggesting that lowland and highland subspecies began diverging about 171,730 years before present (ybp; range = 62,500–901,750). Assuming an exponential growth model, the posterior distribution of the splitting parameter, *s*, peaked at 94.95 % (8.25–98.15) as the percent of the ancestral population that contributed to *cyanoptera*, indicating a highland colonization from the lowlands.

Gene flow.—IM estimated the joint gene flow rate for mtDNA and nuclear introns into the lowlands M_c to be approximately 28.56 times greater than the mutation rate (95% CI = 6.82–83.81), which exceeded the migration rate into the highlands (M_o= 1.94; 95% CI = ~0.00–65.39; Fig. 2.5). There was considerable overlap in estimates; however, we

could not reject a hypothesis of no gene flow into the highlands. Multiplying through by θ, the joint estimate indicated that there were on average 27.2 migrants/generation into the lowlands and effectively no migrants into the highlands. The posterior distribution of the time at which each gene flow event was recorded peaked at $t = 0$ for all loci, suggesting that the majority of the gene flow occurred after divergence, which peaked at 0.13 (Fig. 2.5). Single-locus IM analysis for the αA subunit could not estimate population parameters, presumably because one allele predominated the highland population causing the effective population size to be zero.

Consistent with the pattern observed for the isolation-with-migration model (IM), an asymmetrical downslope migration rate for the five nuclear introns was also observed under the BayesAss assignment model. There was restricted upslope migration, with approximately 0.8% (0.0–3.1%) of the highland population comprised of migrant origin with 0.272 (0.161–0.325) of the lowland population having a highland origin. Similar results were obtained with nuclear introns and βA subunit combined. However, there was restricted gene flow estimated in both directions when the αA subunit was included, with 0.7% (0.0–2.6%) of the highland population and 1.5% (0.1–4.4%) of the lowland population showing a migrant origin. The inference of restricted gene flow in the αA subunit was further suggested by the higher proportion of nonmigrant individuals estimated in the *cyanoptera* population than in the *orinomus* population (0.985, 95% CI 0.956–0.999, and 0.993, 95% CI 0.974–1.000, respectively).

Population Size.—The population size parameter,Θ, estimated in IM for mtDNA and nuclear introns combined was higher for *cyanoptera* than *orinomus* (Fig. 2.5). The

effective population sizes (N_e) for *cyanoptera* and *orinomus* were estimated to be 98,091 (47,497–256,071) and 49,560 (20,650–120,807), respectively. Also, posterior distributions were smaller than the ancestral size (397,510; 187,923–971,630), suggesting population contractions following divergence. The effective population size and census size were in close agreement, as current population estimates for *cyanoptera* range from 25,000–100,000 (Rose and Scott 1997) and 10,000–100,000 for *orinomus* (Wetlands International 2002).

Morphometric divergence.—The first principal component (PC1) accounted for 59.9% of the variance in morphology (eigenvalue = 4.20 and represented an overall body size vector, as factor loadings for all measurements were uniformly high and positive. The second principal component (PC2) accounted for 11.2% of the variance and represented a bill shape difference, as bill measurements were the most influential variables. Highland (*orinomus*) individuals were significantly larger than lowland (*cyanoptera*) individuals (ANOVA: $F_{1,93} = 135.57$, $P < 0.001$). Discriminant analysis showed a high overall probability of subspecific assignment (Fig. 2.3). For males, only one *orinomus* (KGM 441; 92%) and one *cyanoptera* (REW 316; 80%) had an assignment probability of less than 95% to subspecies. All females had 100% probability of assignment.

Body size (PC1) divergence between subspecies was approximately five times larger ($P_{ST} = 0.586$) than the highest F_{ST} value (0.103, FGB) from the five nuclear introns. Among individual measurements, P_{ST} estimates for wing chord (0.822), tarsus (0.470), and tail length (0.640) showed similar pattern as PC1 (body size), with P_{ST} falling far

outside the 95% confidence limit of simulated neutral genetic data (Fig. 2.3). Divergences in bill shape were roughly similar to F_{ST} estimates falling within the simulated data: bill length at nares (P_{ST} = 0.000), culmen length (0.217), bill width (0.127), and bill height (0.135). In addition, there were significant added variance components ($Ps < 0.05$) except for bill length at nares, indicating that individuals from different environments differ more from each other than do individuals from the same environment. All P_{ST} values were similar when males and females were analyzed separately (data not shown).

Discussion

Hemoglobin and phenotypic divergence.—Cinnamon Teal showed strong divergence in hemoglobin and body size despite a pattern of considerable admixture of reference loci between environmental extremes. The lack of significant allelic frequency differences across all nuclear introns (F_{ST} = 0.004, $P > 0.05$) and STRUCTURE analysis indicated little evidence for genetic structuring among sampled sites. However, a single nonsynonymous substitution on the αA hemoglobin subunit (Asn → Ser-α9; F_{ST} = 0.94) and large body size (PC1; P_{ST} = 0.560) were two characteristics of highland individuals, whereas lowland individuals generally lacked this allele and possessed a smaller body size. The observed amino acid substitution (α9) is located on an exterior, solvent-accessible position on the A helix (McCracken et al. 2009c), which is known to undergo an important conformational change during the transition from the deoxy to the oxy state suggesting this amino acid may confer a functional response to hypoxia (Perutz 1990).

If genetic drift has played a major role in shaping adaptive traits, then differences between highlands and lowlands should be roughly equivalent to divergence found in neutrally evolving genetic markers. Both multivariate (first principal component) and univarate (wing chord and tarsus) indicators of overall body size (Rising and Somers 1986, Senar and Pascual 1997) as well as αA hemoglobin subunit showed elevated divergence in comparison to reference loci. Divergence estimates (P_{ST} and Φ_{ST}) for body size and αA hemoglobin subunit were outside the distribution of simulated values of neutral divergence indicating it would be unlikely that a neutral trait could generate the observed levels of divergence. This is suggestive that local adaptation to high elevation has influenced body size and hemoglobin structure. The increase in body size observed with elevation is consistent with Bergmann's rule, which predicts that individuals in colder arid environments tend to be larger in size (Wilson et al. 2010). In addition, high elevation habitats exert multiple environmental pressures (Monge and León-Velarde 1991), which may act synergistically with the resulting interacting effects, posing a greater challenge than each factor alone. Therefore it is not surprising that hemoglobin-oxygen affinity and body size are often correlated, such that larger animals tend to have higher hemoglobin-O_2 affinity (Schmidt-Nielsen and Larimer 1958, Hopkins and Powell 2001).

Pre-zygotic isolation can occur when populations are separated in space. When habitat segregation occurs due to genetically-based adaptations, isolation can arise that reduces the likelihood of heterospecific encounters (Rice and Salt 1990, Johnson et al. 1996, Rundle and Nosil 2005). The spatial distributions of hemoglobin alleles and body

types suggest that there is a strong tendency for individuals to remain in their native elevation, as there were only a few individuals with mismatched genotypes and/or phenotypes to their environment. Each population contained three individuals (3% overall) that were heterozygous (Asn/Ser) at their typical elevation, while there were six mismatched, small-bodied individuals found in the highlands that possessed the lowland αA hemoglobin allele. Of these six individuals, only two were found in sympatry with *orinomus* at Laguna de Paca (Junin, Peru) where *orinomus* is not known to breed. The remaining four individuals were collected at the periphery of the range of *orinomus* and were either observed as solitary pairs or a small group of no more than four individuals. In addition, no other lowland Cinnamon Teal were encountered in the surrounding areas or within the main breeding area of *orinomus*; thus the likelihood of intermixing of highland and lowland populations appears to be low.

Gene flow.—Although gene flow estimates were restricted between highland and lowland populations when the full data set was analyzed (reference loci and hemoglobin), dispersal is likely much higher than the rate of gene flow (Garant et al. 2007). Transplant experiments have demonstrated that lowland birds have difficulty successfully breeding at high elevation (Monge and León-Velarde 1991) and that selection imposed by hypoxia are the main cause of low hatchability of eggs (Visschedijk et al. 1980). At elevations of 4,000 m or greater, there is a shift in physiological mechanism regulating gas exchange from conservation of water and CO_2 at middle elevation to mechanisms improving O_2 availability (Carey 1994). Adult hemoglobin has been shown to appear by day six during embryonic development (León-Velarde and Monge-C 2004), and if the observed amino

acid substitution confers a higher oxygen affinity, it would likely ensure higher O_2 content required for embryonic growth and development at high elevations. Individuals possessing mismatched genotypes were found in Cinnamon Teal in this study and in a recent study of Yellow-billed Pintail (*A. georgica*, McCracken et al. 2009a), indicating that individuals can disperse into the highlands, perhaps because they can initially acclimate to hypoxia via multiple physiological pathways. However, the susceptibility of the avian embryo to hypoxia most likely limits these individuals from successfully breeding in the highlands.

Furthermore, reference loci (mtDNA and introns) showed asymmetrical gene flow, with a greater immigration into the lowlands from the highlands, whereas immigration into the highlands was indistinguishable from zero. Highland species have been successfully bred in captivity in lowland environments from wild stock with no difficulty (Delacour 1956), and these captive stock Andean waterfowl continue to possess the molecular (hemoglobin) and morphological (body size) adaptations of wild populations (*Chloephaga melanoptera* Hiebl et al. 1987, *Anser indicus* Scott et al. 2011, *A. puna* pers. observ.). Thus, it is unlikely that high-elevation adaptations restrict highland birds from breeding in the lowlands. Andean coots (*Fulica ardesiaca*) breeding at sea level on the west slope of Peru, for example, have been shown to have elevated hemoglobin-O_2 affinity that is sharply left-shifted and indistinguishable from individuals from the highlands (Monge and León Velarde 1991). It is likely that such Andean west slope populations may actually be of highland origin, as highland birds more often descend to the west slope (Pearson and Plenge 1974, Fjeldså 1985). However, we found

no highland individuals in the lowlands except for one individual at approximately 1,500 m, suggesting that movement into the lowlands is uncommon even though the populations are separated by only < 100 km in some parts of their range. Therefore, factors other than high-elevation adaptations are likely restricting gene flow to the lowlands (e.g., migratory behavior, availably of forging habitat, etc.).

Timing and direction of colonization and adaptive trait change.—Residents of high elevations often develop long-term, genetic adaptations, in particular to the hemoglobin molecule, to reduce the physiological stress impose by hypoxic environment (León-Velarde 1996; Weber 2007; McCracken 2009b,c). Adaptations can accumulate over a long period of time or can occur over a very short time period, as evident in high-elevation populations of chickens in the Andes that have likely acquired higher Hb-O_2 affinity within the last 500 years (León-Velarde et al. 1991). Coalescent analyses suggest that lowland *cyanoptera* and highland *orinomus* have been diverging for at least 62,500 years. Compared to other waterfowl species, Cinnamon Teal have a shallower divergence (e.g., *Lophonetta specularioides* Bulgarella unpublished data, *Anas flavirostris* McCracken et al. 2009b, and *A. puna/A. versicolor* Johnson and Sorenson 1999), which maybe indicative of a shorter period of isolation in the highlands. Therefore, Cinnamon Teal may be a more recent Andean resident. This conclusion is consistent with the observation that only a single, derived amino acid polymorphism (Asn/Ser-α9) exhibited significant frequency differences between the lowlands and the highlands.

The splitting parameter provided further evidence that the highlands were most likely colonized from the lowlands, suggesting that less than 10% of the ancestral population contributed to the highland *orinomus*. However, the splitting parameter had a large confidence interval with some values being inconsistent with a colonization event (95% CI of s = 8–98%), indicating that the possibility that the ancestral population contributed equally to *cyanoptera* and *orinomus* could not be completely rejected. However, the alternative hypothesis of lowland colonization from the highlands is unlikely, as values indicative of this direction had extremely low probability. The splitting parameter suggested that the majority (s = 95%) of the ancestral population contributed to the lowland *cyanoptera,* which would correspond to other Andean avifauna that appear to have colonized the highlands from the southern lowlands (Fjeldså 1985, Vuilleumier 1986, McCracken et al. 2009b). Therefore it is likely that Cinnamon Teal also had its origins in the lowlands.

Assuming that the highlands were colonized from the lowlands, we can infer that small body size and Asn-α9 are the ancestral traits. Many closely related species differ in elevational distributions, which led to the suggestion that habitat change might have been the initial step in divergence, and that body size differences and other traits arose later (Diamond 1986, Richmond and Price 1992). As seen in Cinnamon Teal, other Andean waterfowl show similar patterns of body size (Blake 1977, Bulgarella et al. 2007) and hemoglobin structure changes (McCracken et al. 2009b,c) between lowland and highland counterparts. Given that Cinnamon Teal began diverging well after the uplift of the Altiplano/Puna region and associated climate change (Gregory-Wodzicki 2000), it is

unlikely that the divergence originated within a single lowland habitat and that differentiated populations later gave rise to different forms when additional habitats (e.g. Andean Altiplano) become available.

Our conclusions on the adaptive function of morphological and hemoglobin divergence rely on three assumptions about the evolutionary mechanisms underlying divergence observed: (i) divergent selection in different habitats has caused the differences in traits, (ii) hemoglobin amino acid polymorphism likely alters hemoglobin function, and (iii) morphological traits are heritable and not subject to substantial phenotypic plasticity. The parallel patterns of hemoglobin amino acid substitution and consistent body size changes across all Andean waterfowl (Blake 1977, Bulgarella et al. 2007, McCracken et al. 2009a,b) are consistent with functional predictions to deal with a hypoxic, cold habitat and suggest that these morphological and physiological differences are a product of directional selection (Bulgarella et al. 2007; McCracken et al. 2009b,c; Wilson et al. 2010). However, the structural and functional effect of the observed Asn → Ser-α9 is still unknown, and further research is required to determine its role in the oxygen transport system. Although the structural and functional effects of the observed amino acid change are yet to be determined, the genetic and morphological divergence observed has likely resulted due to strong selection imposed by high-elevation environment.

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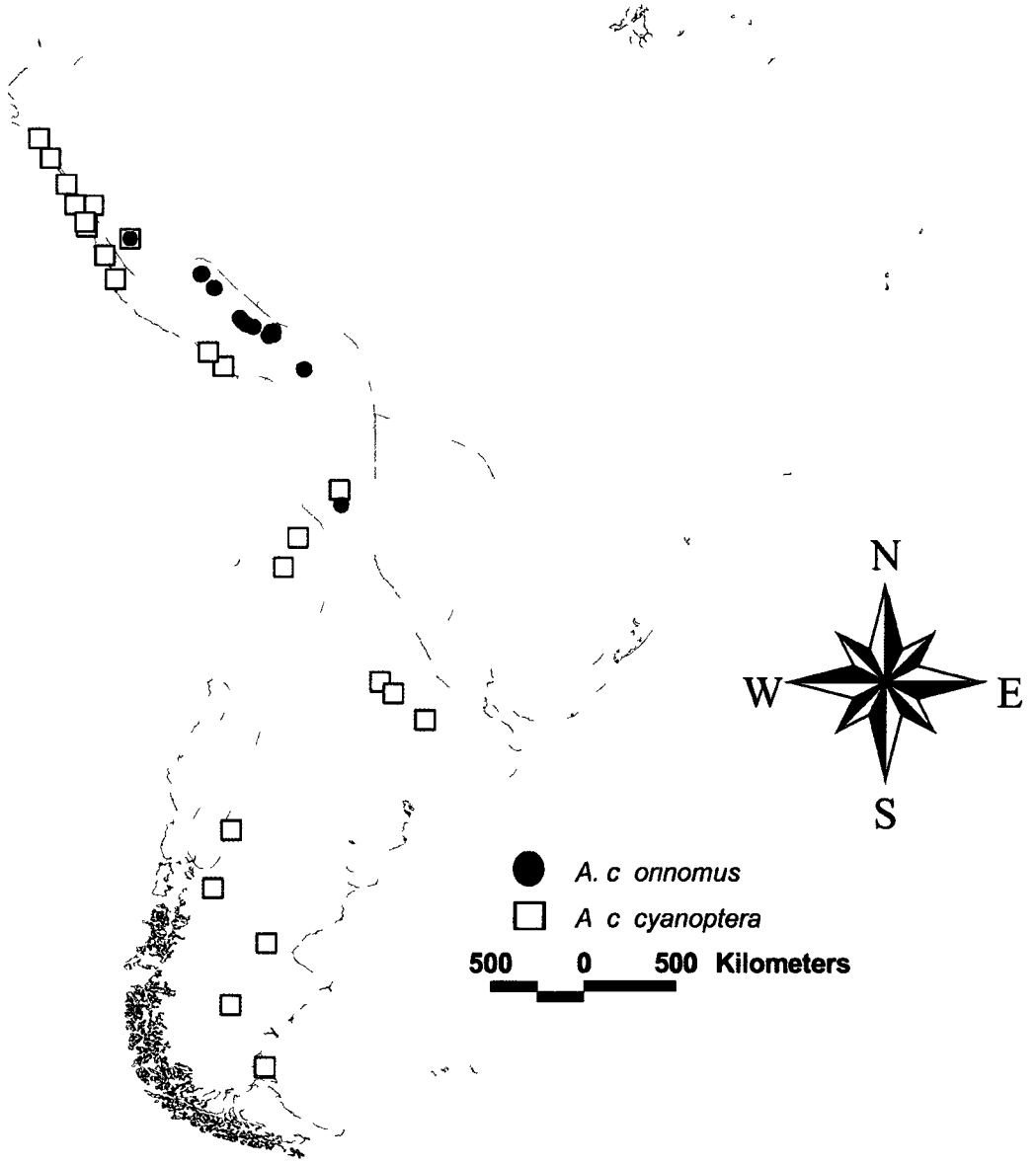

Figure 2.1. Sampling localities and geographic ranges for Cinnamon Teal (*Anas cyanoptera*) in this study (Ridgely et al. 2003).

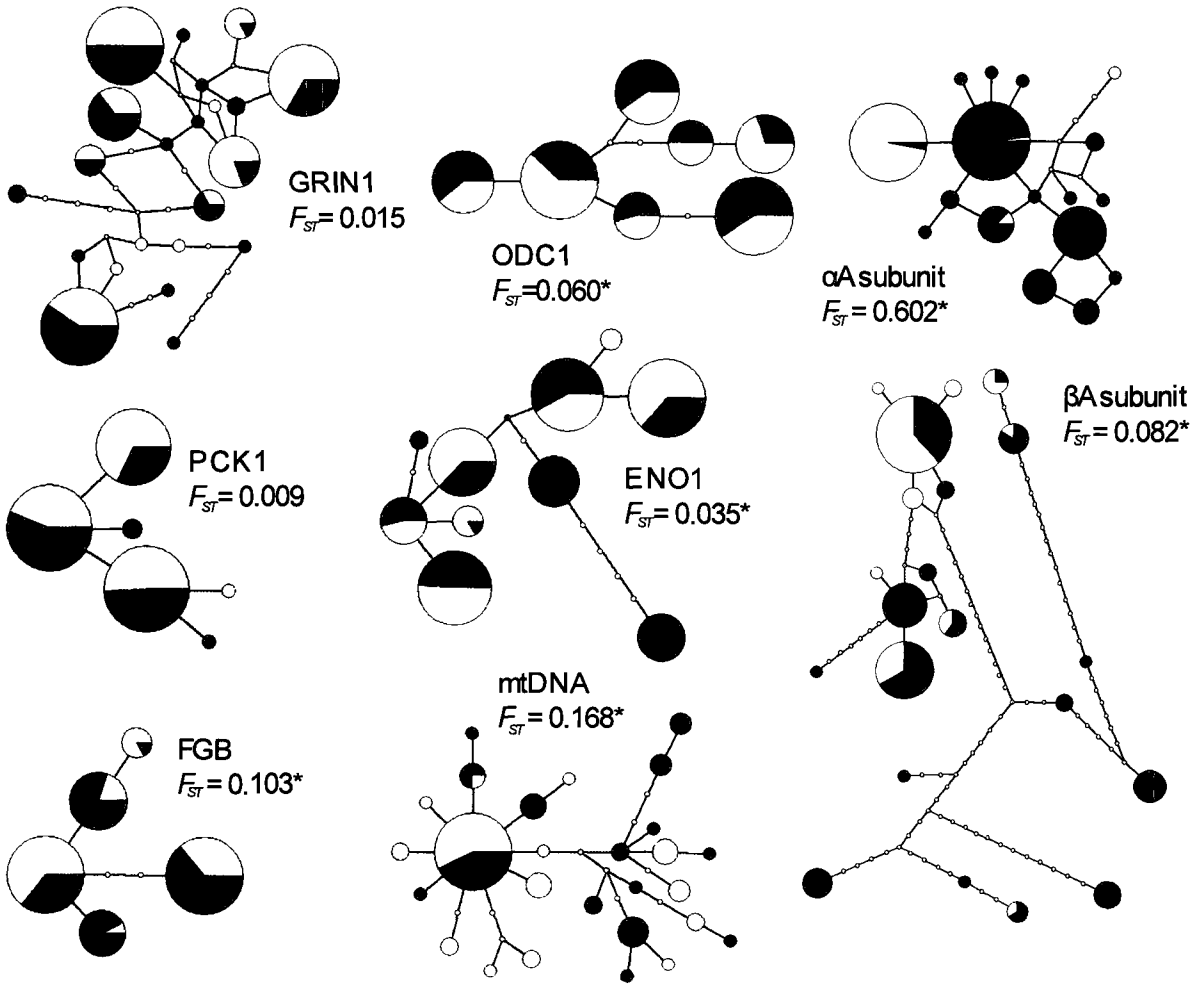

Figure 2.2. Allelic networks for eight loci. Alleles for *A. c. cyanoptera* are shown in black, and alleles for *A. c. orinomus* are shown in white. Significant F_{ST} ($P < 0.05$) is indicated by an asterisk. Circle area is proportional to the number of each allele found and small gray circles indicate intermediate alleles not sampled.

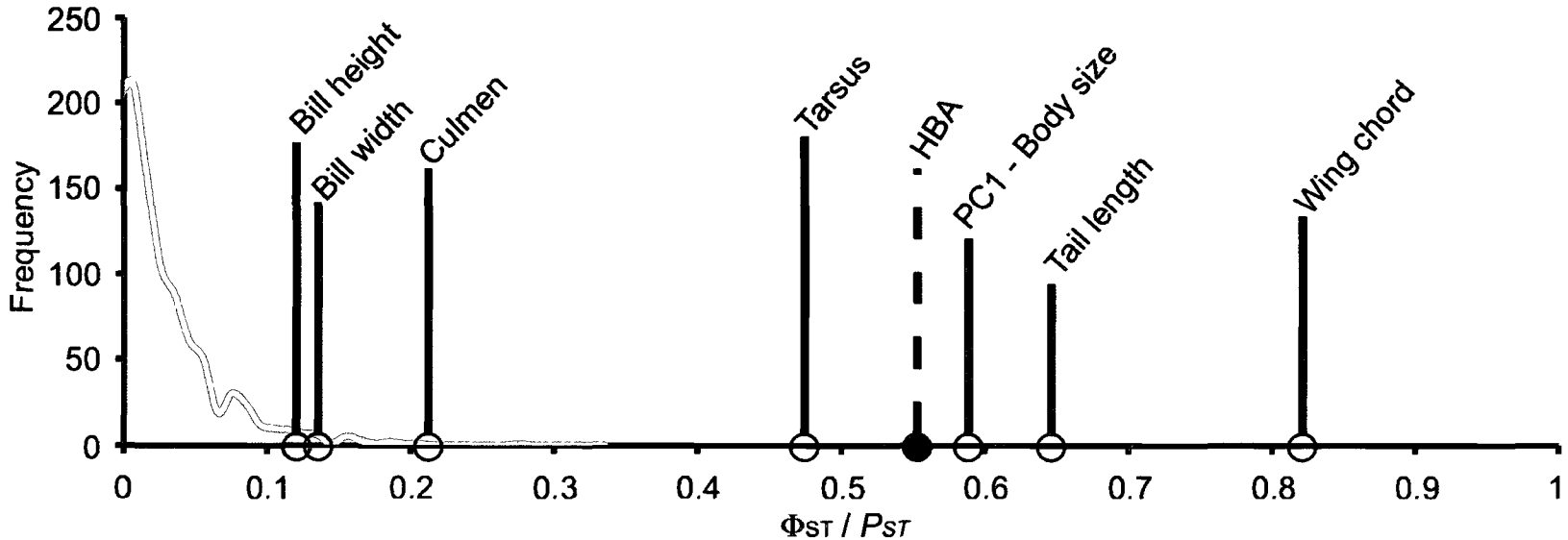

Figure 2.3. Simulated values of Φ_{ST} for 1,000 simulated data sets for neutral loci (grey line) and empirical values for the αA hemoglobin subunit (HBA; closed circle and dashed line) and phenotypic-Q_{ST} (P_{ST}, open circles and solid black line) for morphological traits.

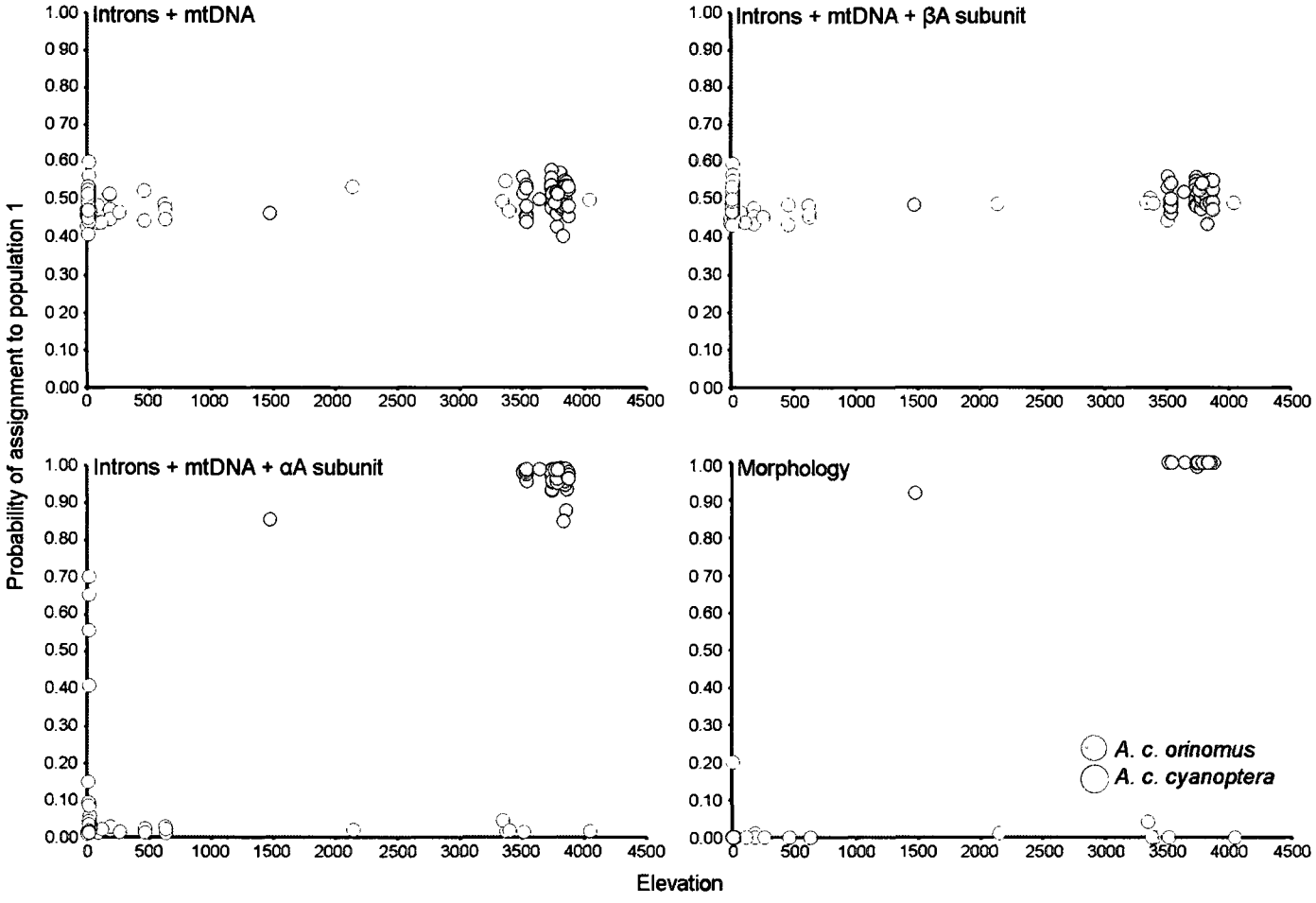

Figure 2.4. Posterior probability of assignment to the highland population versus elevations for genetic data using STRUCTURE 2.2 and morphological data using a discriminant analysis.

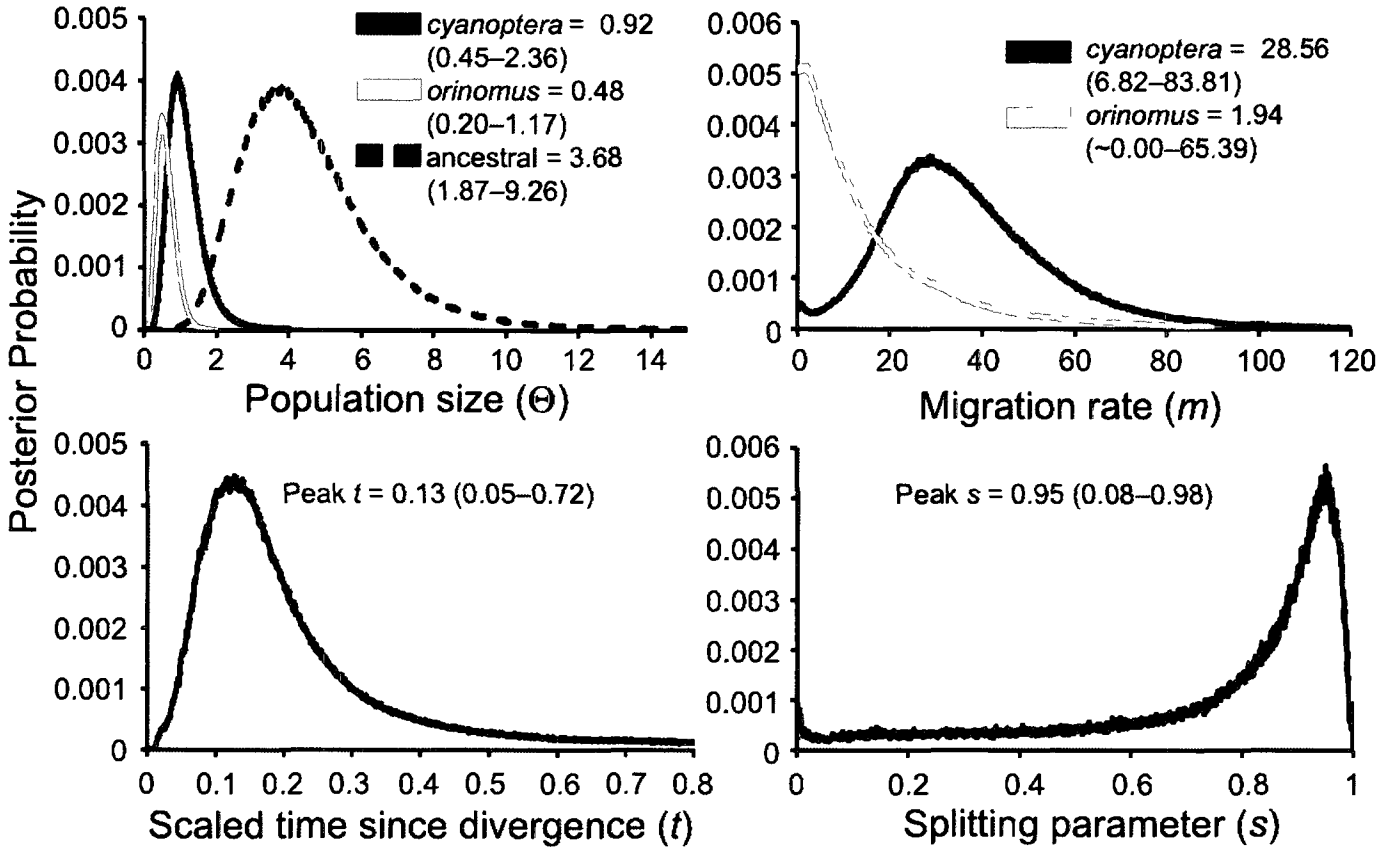

Figure 2.5. Posterior distributions of estimated migration rates (m), effective population size (Θ), time since divergence (t), and splitting parameter (s) calculated using IM (scaled to the neutral mutation rate, u). Peak estimates for each parameter are given and the 95% highest posterior distribution is shown in parentheses.

Table 2.1. Genes sequenced and their chromosomal positions in the chicken genome.

Locus	Base pairs sequenced	Chicken chromosome[1]
mtDNA control region (mtDNA)	1,270–1,272	mtDNA
Ornithine decarboxylase intron 5 (ODC1)	351	3
α enolase intron 8 (ENO1)	312	21
β fibrinogen intron 7 (FGB)	245	4
N-methyl D aspartate 1 glutamate receptor intron 11 (GRIN1)	330	17
Phosphoenolpyruvate carboxykinase intron 9 (PCK1)	345	20
α A hemoglobin subunit (HBA1)	678	14
βA hemoglobin subunit (HBB)	1,578–1,582	1

[1]Location in the chicken genome as defined by Hillier et al. (2004).

Table 2.2. Number of alleles, nucleotide diversity, heterozygosity (observed Ho and expected He), F_{ST}, and Φ_{ST} for five unlinked autosomal introns, mtDNA control region, and the αA and βA hemoglobin subunits from *A. c. cyanoptera* (lowland) and *A. c. orimonus* (highland) subspecies of Cinnamon Teal.

	Population	No. polymorphic sites	No. Alleles	Nucleotide Diversity (π/site)	Tajima's D	Ho/He[1]	F_{ST}[2]	Φ_{ST}[2]
Ornithine decarboxylase	*cyanoptera*	9	7	0.0078	1.86	0.81/0.79	**0.060**	0.011
	orinomus	9	7	0.0079	1.71	0.74/0.81		
α enolase	*cyanoptera*	14	9	0.0129	-0.11	0.77/0.86	**0.035**	**0.087**
	orinomus	7	7	0.0077	-0.05	0.74/0.78		
β fibrinogen	*cyanoptera*	5	4	0.0078	2.05	**0.65/0.70**	**0.103**	**0.043**
	orinomus	6	5	0.0056	0.38	0.50/0.50		
N-methyl D aspartate receptor type I intron 13	*cyanoptera*	18	18	0.0161	1.97	0.75/0.80	0.015	0.019
	orinomus	12	12	0.0146	2.77	0.92/0.83		
Phosphoenolpyruvate	*cyanoptera*	4	5	0.0019	-0.33	0.48/0.60	0.009	0.007
carboxykinase	*orinomus*	3	4	0.0022	0.59	0.60/0.63		
Averaged introns	*cyanoptera*	—	—	0.0092	—	—	0.004	**0.036**
	orinomus	—	—	0.0075	—	—		

Table 2.2 continued.

	Population	No. polymorphic sites	No. Alleles	Nucleotide Diversity (π/site)	Tajima's D	Ho/He[1]	F_{ST}[2]	Φ_{ST}[2]
mtDNA control region	*cyanoptera*	29	16	0.0034	-1.45	—	**0.168**	**0.072**
	orinomus	21	13	0.0019	-1.47	—		
αA hemoglobin	*cyanoptera*	12	16	0.0025	-0.75	0.77/0.71	**0.602**	**0.551**
	orinomus	7	4	0.0002	**-1.94**	0.06/0.06		
βA hemoglobin	*cyanoptera*	59	15	0.0076	0.40	**0.58/0.78**	**0.082**	**0.061**
	orinomus	54	11	0.0042	-1.07	0.48/0.46		

[1]Populations out of Hardy-Weinberg equilibrium are shown in bold.
[2]Significant values ($P < 0.05$) are shown in bold.

Appendix 2.1. Localities of *Anas cyanoptera* specimens. KGM, JT, and REW specimens are cataloged at University of Alaska Museum.

A. c. cyanoptera
ARGENTINA: Neuquen, Rio Collon Cura, R.N. 40, 40° 12' 45" S, 70° 38' 58" W, 625 m[1]
KGM 268
ARGENTINA: Cordoba, Laguna La Felipa, 33° 04' 17" S, 63° 31' 33" W, 184 m[1]
KGM 310, KGM 313, KGM 311, KGM 312
ARGENTINA: Cordoba, S. Canals, 33° 36' 23" S, 62° 53' 16" W, 112 m[1]
KGM 322
ARGENTINA: Jujuy, S. Purmamarca, 23° 49' 13" S, 65° 28' 34" W, 2,141 m
KGM 442
PERU: Dpto. Lima, S Huacho, 11° 10' 12.9" S, 77° 35' 31.4" W, 15 m
REW 081, REW 082
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11° 44' 14.5" S, 75° 29' 32.7" W, 3,506 m
REW 118, REW 122
PERU: Dpto. Ancash, Laguna Conococha, 10° 07' 10.8" S, 77° 17' 00.7" W, 4,039 m
REW 164
PERU: Dpto. Lambayeque, ca. Puerto Eten, 06° 54' 51.9" S, 79° 52' 22.4" W, 13 m
REW 193, REW 194, REW 195, REW 196
PERU: Dpto. Lambayeque, Playa Monsefu, 06° 54' 03.7" S, 79° 53' 42.4" W, 12 m
REW 198, REW 199
PERU: Dpto. La Libertad, Magdalena de Cao, 07° 51' 54.3" S, 79° 20' 51.2" W, 23 m
REW 200
PERU: Dpto. Ancash, Chimbote, 09° 07' 26.0" S, 78° 33' 11.3" W, 15 m
REW 203, REW 204, REW 205
PERU: Dpto. Ancash, Puerto Huarmey, 10° 05' 52.0" S, 78° 09' 10.3" W, 14 m
REW 206
PERU: Dpto. Lima, Albufera de Medio Mundo, 10° 55' 25.9" S, 77° 40' 10.8" W, 14 m
REW 207
PERU: Dpto. Ica, Pisco, 13° 41' 46.8" S, 76° 13' 07.3" W, 7 m
REW 235
PERU: Dpto. Ica, Pisco, 13° 40' 47.2" S, 76° 12' 56.6" W, 9 m
REW 236
PERU: Dpto. Tacna, Ite, 17° 52' 47.2" S, 71° 01' 05.9" W, 10 m
REW 298, REW 299, REW 300, REW 301, REW 302, REW 303, REW 304
PERU: Dpto. Arequipa, Punta de Bombon-Islay, 17° 11' 31.9" S, 71° 46' 19.4" W, 8 m
REW 305, REW 306
PERU: Dpto. Lima, 2 km N. La Laguna, 12° 33' 13.0" S, 76° 42' 42.1" W, 9 m
REW 315, REW 316, REW 317
ARGENTINA: Chubut, Laguna Terraplen, 42° 59' 50.7" S, 71° 30' 55.1" W, 630 m
KGM 712, KGM 713

Appendix 2.1 continued.

ARGENTINA: Santa Cruz, Estancia La Angostura, 48° 38' 33.9" S, 70° 38' 37.3" W, 460 m
KGM 766, KGM 767
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51° 37' 35.7" S, 69° 00' 59.4" W, -3 m
KGM 797, KGM 798
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51° 36' 54.9" S, 68° 59' 26.6" W, 0 m
KGM 799
ARGENTINA: Chubut, S. Lago Colhue Huapi, 45° 38' 49.6" S, 68° 56' 45.1" W, 256 m
KGM 808
ARGENTINA: Catamarca, Antofogasta de la Sierra, Laguna La Alumbrera, 26° 06' 46.4" S 67° 25' 26.7" W, 3,338 m
KGM 1110
ARGENTINA: Catamarca, Embalse Las Cortaderas, 27° 33' 21.2" S, 68° 08' 41.9", 3,369 m
KGM 1142
ARGENTINA: Buenos Aires, 34° 52' 27" S, 61° 23' 19.2", 86 m
JT 011
ARGENTINA: Buenos Aires, 34° 53' 15" S, 61° 21' 51", 86 m
JT 046, JT 047
A. c. orinomus
ARGENTINA: Salta, NE La Caldera, 24° 33' 01" S, 65° 22' 15" W, 1,468 m
KGM 441
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 11' 45" S, 68° 37' 28" W, 3,808 m
KGM 485, KGM 486, KGM 487
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 20' 13" S, 68° 41' 20" W, 3,854 m
KGM 499
BOLIVIA: Dpto. Oruro, Lago Uru Uru, 18° 02' 03" S, 67° 08' 46" W, 3,735 m
KGM 527, KGM 528, KGM 529, KGM 530, KGM 531, KGM 532, KGM 533, KGM 534, KGM 535
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 25' 28" S, 68° 51' 43" W, 3,850 m
KGM 557
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 03" S, 68° 37' 40" W, 3,839 m
KGM 559, KGM 560
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 02" S, 68° 37' 48" W, 3,840 m
KGM 561, KGM 562
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 07" S, 68° 38' 06" W, 3,845 m
KGM 563, KGM 564, KGM 565, KGM 566
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11° 44' 14.5" S, 75° 29' 32.7" W, 3,506 m
REW 125, REW 126
PERU: Dpto. Cusco, Laguna Chacan, 13° 26' 02.6" S, 72° 07' 49.6" W, 3,533 m
REW 238, REW 239, REW 240, REW 241, REW 242

Appendix 2.1 continued.

PERU: Dpto. Cusco, ca. Chinchero, 13° 25' 49.3" S, 72° 03' 41.7" W, 3,789 m REW 248
PERU: Dpto. Cusco, Urubamba Valley, 13° 25' 22.9" S, 72° 02' 38.2" W, 3,743 m REW 253, REW 254
PERU: Dpto. Cusco, ca. Laguna Pomacanchi, 14° 06' 51.9" S, 71° 27' 56.6" W, 3,781 m REW 255, REW 256, REW 257, REW 258, REW 259
PERU: Dpto. Puno, Lago Titicaca, Jaru Jaru, 15° 59' 05.6" S, 69° 36' 24.3" W, 3,824 m REW 268, REW 269
PERU: Dpto. Puno, Lago Titicaca, ca. Puno, 15° 52' 01.2" S, 69° 56' 21.3" W, 3,830 m REW 271
PERU: Dpto. Puno, Lago Umayo, Sillvstani, 15° 42' 45.8" S, 70° 09' 00.0" W, 3,853 m REW 272
PERU: Dpto. Puno, Deustva, 15° 33' 50.0" S, 70° 14' 33.1" W, 3,871 m REW 284, REW 285, REW 286

[1]These elevation values are interpolated from the U.S. Geological Survey's GTOPO30 (http://eros.usgs.gov); all other elevations were measured with a GPS receiver.

CHAPTER 3

SPECIATION, SUBSPECIES DIVERGENCE, AND PARAPHYLY IN CINNAMON TEAL AND BLUE-WINGED TEAL[1]

ABSTRACT

Divergent selection can lead to extensive morphological and behavioral differences despite low neutral genetic differentiation. We examined the evolutionary history of two closely related waterfowl species, Cinnamon Teal (*Anas cyanoptera*) and Blue-winged Teal (*A. discors*) that are morphologically distinct but have paraphyletic mitochondrial DNA (mtDNA) and shared allozyme alleles. Our results based on mtDNA and nuclear intron sequence data revealed that North American Cinnamon Teal ($n = 70$) and Blue-winged Teal ($n = 76$) are characterized by high genetic diversity, a large effective population size, and a recent population expansion. In contrast, South American Cinnamon Teal ($n = 102$) have less genetic diversity, a smaller effective population size, and have had more stable effective population sizes. We found 91 unique mtDNA haplotypes with only a few haplotypes shared among Cinnamon Teal

[1]Wilson, R. E., M. D. Eaton, S. A. Sonsthagen, J. L. Peters, K. P. Johnson, B. Simarra, and K. G. McCracken. Speciation, subspecies divergence, and paraphyly in Cinnamon Teal and Blue-winged Teal. Condor: in press.

subspecies or between species, but haplotypes were intermixed in a polyphyletic relationship and diagnostic phylogroups were not observed. Moreover, populations were strongly differentiated for mtDNA ($\Phi_{ST} = 0.41$) compared to nuclear introns ($\Phi_{ST} = 0.04$–0.06). Isolation with migration (IM) analyses indicated that haplotypic and allelic sharing across continents is most likely attributable to incomplete lineage sorting rather than gene flow, whereas within-continent estimates yielded higher migration rates. The oldest divergence was between North American Cinnamon Teal and the other taxa, whereas Blue-winged Teal likely split from South American Cinnamon Teal more recently. However, there was considerable overlap in divergence confidence intervals suggesting that these taxa diversified rapidly.

INTRODUCTION

Natural selection, sexual selection, and stochastic processes such as genetic drift and founder events are important evolutionary forces leading to divergence between populations and ultimately, in many cases, to speciation (Questiau 1999, Coyne and Orr 2004, Price 2008). As populations colonize new environments, particular traits become modified to exploit new resources or to gain advantages in social competition for contested resources such as food and mates (West-Eberhard 1983). Incipient species thus can develop distinct morphology or behavior in response to varying selection that result in premating and postmating isolation, with little or no neutral genetic differentiation (Meyer 1993, Bernatchez et al. 1996, Schluter 1998, Seehausen and van Alphen 1998, Hendry 2001, Ödeen and Bjorklund 2003). These morphological and behavioral responses can cause incongruence between species limits based on phenotypic traits and gene genealogies, especially in recently diverged taxa (Funk and Omland 2003, Avise 2004, Buehler and Baker 2005, Joseph et al. 2006, Maley and Winker 2010). Furthermore, a major component of variation among closely related species or subspecies often results from differences in sexual ornaments used for mate recognition (West-Eberhard 1983, Price 1998, Questiau 1999, Johnsen et al. 2006). Such discrepancies between morphological and molecular data have resulted in questions regarding species status; however, they provide a valuable opportunity to gain insight into species' biology and the evolutionary processes leading to speciation (Edwards et al. 2005, Johnsen et al. 2006, Joseph et al. 2006, Omland et al. 2006).

Cinnamon Teal (*Anas cyanoptera*) and Blue-winged Teal (*A. discors*) are two species of closely related dabbling ducks that are particularly well suited for studying divergence and gene flow between paraphyletic species and subspecies with shallow genetic differentiation. These species exhibit pronounced differences in body size, coloration, habitat choice, and aspects of their behavior (e.g., migratory behavior and territoriality; Gammonley 1996, Rohwer et al. 2002), but mitochondrial DNA (mtDNA) suggests a recent divergence (Kessler and Avise 1984, Johnson and Sorenson 1999, Kerr et al. 2007). Both species are widespread throughout the Western Hemisphere and are occasionally found in sympatry in western North America and in northern South America (Fig. 3.1). Cinnamon Teal are composed of five morphologically distinct subspecies that are distinguished by unique geographic and ecological zones: *A. c. cyanoptera* (lowland South America and occasionally the high Andes), *A. c. orinomus* (endemic to the high Andes), *A. c. borreroi* (Colombian Andes), *A. c. tropica* (Colombian Andean lowlands), and *A. c. septentrionalium* (North America; Snyder and Lumsden 1951, American Ornithologists' Union 1957, Gammonley 1996, Wilson et al. 2010). Blue-winged Teal are found throughout most of North America but occur at low densities in western North America where they are sympatric with *A. c. septentrionalium*. Although Blue-winged Teal are common wintering migrants to Central America and northern South America, only a small number of individuals occur year round in Colombia and Peru (Fjeldså and Krabbe 1990); a small breeding population was recently established in Colombia (G. Stiles pers. comm.).

Breeding plumages of male Cinnamon Teal and Blue-winged Teal are distinctive. Cinnamon Teal males are reddish brown throughout, and Blue-winged Teal males have a characteristic steel-blue neck and head with a white facial crescent. Despite these striking plumage and coloration differences in males, females and juveniles are difficult to distinguish, as is often the case between closely related avian species (West-Eberhard 1983). Additionally, post-zygotic isolation in ducks is weak, and hybridization is common among waterfowl (Tubaro and Lijtmaer 2002); most reproductive isolation occurs by other mechanisms that usually involve premating behaviors. Hybridization between Cinnamon Teal and Blue-winged Teal has been reported infrequently in the wild (only hybrid males can be recognized based on plumage), perhaps because of limited overlap in their breeding distributions. However, interbreeding occurs freely in captivity (Delacour and Mayr 1945), suggesting that Cinnamon Teal and Blue-winged Teal diverged too recently for the evolution of strong pre- or post-mating isolation mechanisms.

Here we investigate the evolutionary histories of Cinnamon Teal and Blue-winged Teal sampled from widespread locations throughout their breeding distributions by comparing sequences from the hyper-variable mtDNA control region and two independent nuclear loci. We evaluate whether there are distinct lineages or haplotypic frequency differences between Cinnamon Teal subspecies and Blue-winged Teal. We also use coalescent methods to examine the demographic history of this species complex (Nielsen and Wakeley 2001, Hey and Nielsen 2004). We compare times of divergence and gene flow within and between continents and between species to evaluate the roles

these two factors play in the shallow genetic divergence and mitochondrial paraphyly observed between the taxa.

METHODS

SPECIMEN COLLECTION

Vouchered specimens of 52 *A. c. cyanoptera*, 50 *A. c. orinomus*, 70 *A. c. septentrionalium*, and 76 *A. discors*, were collected in Argentina (2001, 2003, 2005), Bolivia (2001, 2005), Colombia (2004), Peru (2002), and the United States (2002, 2003; Fig. 3.1, Appendix 3.1). For Cinnamon Teal, we used published subspecific morphological characters to classify each specimen to subspecies (Snyder and Lumsden 1951; Wilson et al. 2010). *A. c. borreroi* and *A. c. tropica* were not included because they are critically endangered (Black 1998) and sufficient specimens of these subspecies do not exist.

DNA EXTRACTION, PCR, AND DNA SEQUENCING

Genomic DNAs were extracted from muscle tissue using a QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, California). We amplified 1,272 bp of the mtDNA control region, phenylalanine tRNA, and part of the 12S rRNA gene using the overlapping primer pairs L78–H774 and L736–H1530 (Sorenson and Fleischer 1996, Sorenson et al. 1999) and two additional primers (L627: 5'–TAAGCCTGGACACACCTGCGTTATCG–3'; H693: 5'–CAGTGTCAAGGTGATTCCC–3'). PCR amplifications were carried out in a 50 μL volume; 2–100 ng genomic DNA, 0.5 μM each primer, 1.0 μM dNTPs, 10X PCR buffer,

2.5 μM $MgCl_2$, and 0.2 units Taq Polymerase. PCR reactions began with 94°C for 7 min followed by 45 cycles of 94°C for 20 sec, 52°C for 20 sec, and 72°C for 1 min with a 7 min final extension at 72°C. PCR products were gel-purified and both strands were sequenced using BigDye Terminator Cycle Sequencing Kits on an ABI 3100 or 377 DNA sequencer (Applied Biosystems, Foster City, California). Sequences from opposite strands were reconciled using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan).

We also sequenced two independent nuclear introns: ornithine decarboxylase (ODC1) intron five (353 bp) and α-enolase (ENO1) intron eight (312 bp) (Peters et al. 2008, McCracken et al. 2009a). These two introns were sequenced using techniques described above with an annealing temperature of 60°C and AmpliTaq Gold PCR Master Mix (Applied Biosystems, Foster City, California). Sequences that contained double-peaks, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms. Insert/deletions (indels) were resolved by comparing the unambiguous 5'-ends of sequences to the 3'-ambiguous ends between forward and reverse strands (Peters et al. 2007). Gaps resulting in shifted peaks in the chromatograms enabled us to resolve length polymorphisms within the sequences. All sequences were aligned by eye using the sequence alignment editor Se-Al 2.0a11 (Rambaut 2007). Sequences were deposited in GenBank (accession numbers GQ269364–GQ269567 and JF914362–JF914900).

GAMETIC PHASE OF ALLELE SEQUENCES

We used a two-step procedure to determine the gametic phase of each intron sequence that was heterozygous at two or more nucleotide positions. We first analyzed the diploid consensus sequences of each individual using PHASE 2.1 (Stephens et al. 2001). PHASE uses a Bayesian method to infer haplotypes from diploid genotypic data with recombination and the decay of linkage disequilibrium (LD) with distance. Each dataset was analyzed using the default values (100 main iterations, 1 thinning interval, 100 burn-in) followed by 1000 main iterations and 1,000 burn-in (-X10 option) for the final iteration. The PHASE algorithm was run five times automatically (-x5 option) from different starting points, selecting the result with the best overall goodness-of–fit. We next selected individuals with low allele pair probabilities (<80%) and designed allele-specific primers to selectively amplify one allele (Bottema et al. 1993, Peters et al. 2005). The resulting haploid allele sequence was then subtracted from the diploid consensus sequence to obtain the gametic phase of the second haplotype. Each dataset was then analyzed five more times using PHASE and the additional known allele sequences (-k option). The gametic phases of 92% (n = 456) of 495 individual autosomal sequences were identified experimentally or with >95% posterior probability, and 95% (n = 469) were identified with >90% posterior probability.

GENETIC DIVERSITY AND POPULATION SUBDIVISION

Nucleotide diversity (π), expected and observed heterozygosities, and LD between ODC1 and ENO1 were calculated in ARLEQUIN 3.11 (Excoffier et al. 2005). Allelic richness

was standardized to the smallest sample size ($n = 50$). Allelic networks were constructed in NETWORK 4.5.1 (Fluxus Technology Ltd.) using the reduced median algorithm (Bandelt et al. 1995) to illustrate possible reticulations in the gene trees due to homoplasy or recombination. Gaps were treated as a fifth state, and indels were treated as a single insertion/deletion event regardless of length.

Preliminary analyses showed no significant genetic differentiation (Φ_{ST}) among populations within *A. cyanoptera* subspecies or between North America and Colombia *A. discors*. Thus all analyses were done at the subspecific and species level. To assess levels of population structure between species (*A. discors* and *A. cyanoptera*) and among subspecies (*A. cyanoptera*), we calculated pairwise Φ_{ST} for sequence data in ARLEQUIN using the best-fit nucleotide substitution model, as identified in MODELTEST 3.06 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC; Akaike 1974). Additionally, a hierarchical analysis of molecular variance (AMOVA) was performed in ARLEQUIN to analyze spatial variance in haplotypic and allelic frequencies between species and among populations. *P*-values were adjusted for multiple comparisons using permutations (3000) or Bonferroni corrections ($\alpha = 0.05$).

We used STRUCTURE 2.2.3 (Pritchard et al. 2000) to evaluate the number of genetic clusters (K) present in our dataset. STRUCTURE assigns individuals to populations by maximizing Hardy-Weinberg equilibrium and minimizing linkage disequilibrium. For this analysis, we coded each mtDNA or nuclear DNA haplotype as a separate allele. MtDNA and nuclear sequence data were analyzed using an admixture model without *a priori* information about specimen identification or collection locality.

The analysis was run for K = 1–15 populations with 100 000 burn-in iterations and 1 000 000 Markov chain Monte Carlo iterations; the analysis was repeated ten times to ensure consistency across runs. We used the ΔK method of Evanno et al. (2005) to determine the most likely number of groups at the uppermost level of population structure.

To test for past changes in effective population size, we calculated Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) based on the site-frequency spectrum of segregating sites for mtDNA. Negative values of Tajima's *D* or Fu's *F*s result when there is an excess of low frequency polymorphisms, which can result from rapid population expansion or a selective sweep acting on linked polymorphisms. Conversely, a positive value for either test statistic can be indicative of a population decline. Additionally, mismatch distributions of mtDNA haplotype data were calculated in ARLEQUIN. Mismatch distributions that are multimodal in shape indicate a population that is at demographic equilibrium, whereas a unimodal distribution is consistent with a population that has undergone a recent expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992). We used parametric bootstrapping based on the sum of square deviation (SSD) between observed and expected distributions to test the fit of the stepwise expansion model. In addition, we used a coalescent model in LAMARC 2.1.3 (Kuhner 2006) to calculate the population growth rate parameter (g) for mtDNA from each Cinnamon Teal subspecies and from *A. discors* (each population was treated independently). We used Bayesian analyses with 1 million recorded genealogies sampled every 50 steps, with a burn-in of 100 000 (10%) genealogies. Priors were flat with the upper limit for growth set to 15 000.

COALESCENT ANALYSES–GENE FLOW AND TIME OF DIVERGENCE

We used a coalescent model in IM (Hey and Nielsen 2004, Hey 2005) to determine whether patterns of differentiation between species and subspecies were the result of incomplete lineage sorting, gene flow, or a combination of both. We simultaneously estimated the following parameters scaled to the mutation rate: time since divergence between populations (t), immigration rates (m), and effective population sizes of ancestral (θ_A) and contemporary populations (θ_1 and θ_2). In addition, we ran each set of comparisons assuming constant population size and incorporating exponential population growth with the splitting parameter (s). We ran paired two-population analyses for a combined analysis of mtDNA and two nuclear loci, ODC1 and ENO1: (1) within North America (*A. c. septentrionalium* vs. *A. discors*), (2) within South America (*A. c. cyanoptera* vs. *A. c. orinomus*), and (3) between continents (*A. c. cyanoptera* vs. *A. discors* and *A. c. cyanoptera* vs. *A. c. septentrionalium*). Within-continent comparisons were used to test hypotheses about gene flow patterns between partially sympatric taxa. Comparisons between continents were used to determine if shared haplotypes and mtDNA paraphyly resulted from incomplete lineage sorting or ongoing gene flow and to test for the genetic signature of the direction of colonization (the splitting parameter, s; Hey 2005). The isolation-with-migration model assumes that the two populations being compared are each panmictic and are not exchanging genes with other populations or species (Hey and Nielsen 2004, Won et al. 2005), which would likely be violated. However, simulations suggest that IM is fairly robust to violations of those assumptions

(Strasburg and Rieseberg 2010). In addition, the species tree for this group is unknown precluding a four-population analysis using IMa2. Thus, paired two-population analyses are the most suitable for analyzing patterns of divergence and gene flow within this species complex.

IM further assumes that loci are selectively neutral with no intralocus recombination. We tested for recombination within each nuclear intron using a four-gamete test in DNAsp v. 4.10 (Rozas et al. 2003) and included the largest independently segregating block of sequence consistent with no recombination. ODC1 and ENO1 were truncated to the 5' end positions 82–327 and 152–312, respectively. We defined inheritance scalars for mtDNA as 0.25 (maternally inherited) and for autosomal introns as 1.0 (biparentally inherited) to reflect differences in effective population sizes. We used the HKY model of mutation for mtDNA and infinite sites model for the nuclear introns. We initially ran IM using large, flat priors for each parameter. Based on the results of these runs, we defined narrower upper bounds for each parameter that encompassed the full posterior distributions from each initial run. However, estimates of current population sizes sometimes contained distinct peaks but the tails did not approach zero. In those cases, we used priors that contained the peak and the point near where the distribution began flattening. Using those priors, we used a burn-in of 500 000 steps and recorded results every 50 steps for more than 1×10^6 steps. Effective sample sizes for each parameter exceeded 100. We repeated the analyses three times using a different random number seed to verify that independent runs converged on the same values. We converted t to real time (t) using $t = t\mu$. We used mutation rates of 4.8×10^{-8}

substitutions/site/year (s/s/y) for mtDNA control region (Peters et al. 2005), 1.0 x 10^{-9} s/s/y for ENO1, and 1.2 x 10^{-9} s/s/y for ODC1 (Peters et al. 2008). The geometric mean of substitution rates among the three loci was used for conversions.

RESULTS

POPULATION STRUCTURE AND GENETIC DIVERSITY

The 248 individuals of Blue-winged Teal and Cinnamon Teal surveyed contained 91 unique mtDNA control region haplotypes comprising 76 variable sites. No fixed differences were observed among subspecies or between species, but rather haplotypes were intermixed in a polyphyletic relationship (Fig. 3.2). Ten haplotypes were shared among species or subspecies; five haplotypes were shared between *A. c. septentrionalium* and *A. discors* in North America, three haplotypes were shared between *A. discors* and the South American Cinnamon Teal subspecies, and two haplotypes were shared between the South American subspecies *A. c. cyanoptera* and *A. c. orinomus* (Fig. 3.2, Appendix 3.2). *A. c. septentrionalium* did not share any haplotypes with either of the South American subspecies. Overall, North American taxa had higher genetic diversity than South American taxa (Table 3.1).

The global Φ_{ST} for mtDNA control region was high, with 41% of the genetic diversity explained by differences among taxa ($\Phi_{ST} = 0.41$, $P < 0.001$). Inter-subspecies Φ_{ST} values ranged from 0.07–0.51 (Table 3.2). The highest Φ_{ST} was between *A. c. septentrionalium* and *A. discors* in North America, and the lowest Φ_{ST} was between *A. c. cyanoptera* and *A. c. orinomus* in South America. Variance in mtDNA haplotype

frequencies among groups was maximized when samples were grouped based on (sub)species ($\Phi_{CT} = 0.43$, $P < 0.001$) rather than geographic proximity ($\Phi_{CT} = 0.12$, $P = 0.14$).

Twenty-three ODC1 alleles comprising 20 variable sites and 38 ENO1 alleles comprising 29 variable sites were found in the autosomal intron sequences. Most alleles were broadly shared among all four taxa (Fig. 3.2). Nucleotide diversity was consistently higher for the introns relative to mtDNA, and heterozygosity ranged from 65.2–96.1% (Table 3.1). All taxa were in Hardy-Weinberg equilibrium, and no LD was detected between ODC1 and ENO1, confirming that these loci are independent. Both introns were significantly structured between most taxa ($\Phi_{ST} = 0.00$–0.12 for ENO1 and 0.00–0.08 for ODC1; Table 3.2). In contrast to mtDNA, the among group variance for both nuclear introns combined was maximized when taxa were grouped on the basis of geographic proximity (i.e., North America versus South America; $\Phi_{CT} = 0.05$, $P = 0.01$) rather than by (sub)species ($\Phi_{CT} = 0.03$, $P = 0.03$), but the differences between the two models were small.

The Bayesian clustering analysis in STRUCTURE using mtDNA and both nuclear introns supported a two-population model (Fig. 3.3). Most *A. c. cyanoptera* (87%) and *A. c. orinomus* (94%) were assigned to one genetic cluster, whereas all *A. c. septentrionalium* and *A. discors* where assigned to a second cluster with high probability (98%). Seven *A. c. cyanoptera* individuals assigned to the North American cluster with probability >66% were collected at lowland sites in Argentina (JT 011, JT 046, KGM 322, KGM 798, KGM 808) or the Peruvian coast (REW 081, REW 303). Three *A. c.*

orinomus individuals (KGM 441, REW 698, REW 708) were also assigned to the North American cluster with probability >50%. In summary, majority of individuals were assigned to clusters corresponding to their geographic location in South America (*A. c. cyanoptera* and *A. c. orinomus*) or North America (*A. c. septentrionlium* and *A. discors*).

IM COALESCENT ANALYSES

In general, the population size parameter (Θ) was larger for North American *A. c. septentrionlium* and *A. discors* (Fig. 3.4). Within the North American comparison, Θ was similar for *A. discors* and *A. c. septentrionalium* (7.79; 3.59–12.94 and 6.04; 2.75–15.25, respectively). Both population sizes were larger than the ancestral population size (3.31; 2.07–10.83), suggesting population expansions, but posterior distributions broadly overlapped. In the South American comparison, the population size parameter was larger for *A. c. cyanoptera* (1.22; 0.49–4.67) than for *A. c. orinomus* (0.60; 0.24–2.02). Posterior distributions were smaller than the ancestral size (9.08; 3.23–44.23), suggesting population contractions following divergence.

The most probable estimate for the migration rate (*m*) between continents was low (0.00–1.35), and confidence intervals broadly overlapped zero in all directions, except into *A. c. septentrionalium* from *A. c. cyanoptera* (95% CI: 0.39–5.70; Fig. 3.4). Comparing the North American taxa yielded higher migration rates. Although confidence intervals were broadly overlapping, the most probable estimates suggested that migration rates were higher into *A. c. septentrionalium* (1.49; 0.39–5.76) than into *A. discors* (0.49; ~0.00–3.34). In the South American comparison, IM suggested low

migration into the highlands (0.33; 0.00–42.87), and we could not reject a hypothesis of no gene flow into *A. c. orinomus* from *A. c. cyanoptera*. However, no gene flow was rejected in the opposite direction as the posterior distribution of *m* into *A. c. cyanoptera* did not overlap with zero (18.05; 7.45–79.05).

TIME SINCE DIVERGENCE

The coalescent analyses suggested that the oldest divergence (*t*, scaled divergence times) was between *A. c. septentrionalium* and the other taxa, although there was broad overlap among comparisons (Fig. 3.5). The divergence between *A. c. septentrionalium* compared to *A. c. cyanoptera* peaked at 0.19 (0.11–0.77) suggesting a divergence of approximately 95 000 ybp (years before present; range = 33 000–700 000 ybp). Posterior distributions of *t* for *A. discors* compared to *A. c. septentrionalium* (0.14; 0.09–0.42) and to *A. c. cyanoptera* (0.13; 0.07–0.26) were similar, which when converted to years, suggested that *A. discors* diverged around 70 000 ybp (range = 27 000–385 000 ybp; within continent comparison) or 65 000 ybp (range = 21 000–238 000 ybp; between continent comparison). For the South American comparison, peak values spanned *t* of the other comparisons (0.13–0.20) and the tail of the distribution did not approach zero in all replicates; therefore an accurate estimate of divergence times could not be obtained.

Assuming an exponential growth model, the posterior distribution of the splitting parameter, *s*, for the South American comparison peaked at 99.5 % (3.1–100%) as the percent of the South American ancestral population that contributed to *A. c. cyanoptera*. For all other comparisons, the splitting parameter distribution did not contain a single

peak, but rather a plateau, and the highest likelihood appeared to be associated with a range of values indicating an ambiguous colonization.

HISTORICAL DEMOGRAPHY

In agreement with coalescent analyses, North American populations of *A. c. septentrionalium* and *A. discors* showed evidence of recent population expansion as indicated by a single high frequency haplotype accompanied by numerous rare mtDNA haplotypes (Fig. 3.2) and significantly negative Fu's *Fs* and Tajima's *D* values (Fig. 3.6). Furthermore, the mismatch distributions for *A. c. septentrionalium* and *A. discors* were unimodal and fit the expansion model curve ($Ps > 0.68$; Fig. 3.6). Recent population expansion also was supported by the population growth rate parameter (g) estimates obtained from LAMARC for mtDNA as 95% confidence limits did not contain zero.

In contrast, the South American subspecies (*A. c. cyanoptera* and *A. c. orinomus*) exhibited mtDNA patterns consistent with long-term population stasis and a lack of clear demographic expansion. Neither Tajima's *D* nor Fu's *Fs* were significant for mtDNA control region. The mismatch distributions were multimodal in shape but the Harpending's raggedness index was not significant ($Ps > 0.66$). However LAMARC's estimate of the 95% confidence interval for population growth overlapped zero; the data were consistent with a stable population size (Fig. 3.6).

DISCUSSION

GENETIC STRUCTURE AMONG BLUE-WINGED TEAL AND CINNAMON TEAL

Blue-winged Teal and Cinnamon Teal exhibit marked male plumage differentiation, whereas Cinnamon Teal subspecies exhibit pronounced body size variation and subtle plumage differentiation (Snyder and Lumsden 1951, Wilson et al. 2008, 2010). Despite this phenotypic discord, previous studies based on more conservative regions of mtDNA found little or no genetic differentiation between these two species (Kessler and Avise 1984, Johnson and Sorenson 1999, Kerr et al. 2007), suggesting a recent divergence or high gene flow. Concordant with these studies, we observed low genetic distances (0.3–0.6%) with no fixed differences between species or among subspecies. However, there were strong haplotypic frequency differences in mtDNA ($\Phi_{ST} = 0.41$), which is similar to differentiation observed among other waterfowl subspecies and populations (McCracken et al. 2001, Peters et al. 2005, Sonsthagen et al. 2011). Likewise, we found significant differentiation in nuclear introns (albeit lower levels, $\Phi_{ST} = 0.04–0.06$) that was similar to levels found between allopatric populations of other *Anas* ducks (Peters et al. 2008). Coalescent analyses of mtDNA and nuclear introns suggested that Blue-winged Teal have been diverging from South American Cinnamon Teal for at least 21 000 years and from North American Cinnamon Teal for at least 27 000 years (Fig. 3.5); therefore it is unlikely that selectively neutral nuclear markers would have had enough time to sort due to the longer coalescence time associated with a larger effective size compared to mtDNA (Avise 2004, Zink and Barrowclough 2008).

Despite the lack of distinct mtDNA phylogroups indicative of long-term isolation, few haplotypes were shared between taxa. Furthermore, shared haplotypes were mostly confined to central or centrally connected positions within the network, suggesting Blue-winged Teal and Cinnamon Teal are at an intermediate stage of divergence. These teal species appear to be approaching local fixation of haplotypes leading to the eventual loss of ancestral haplotypes as taxa move towards reciprocal monophyly (Omland et al. 2006). A similar pattern was observed within the nuclear network, as alleles shared between continents tended to be at central positions, whereas more derived alleles tended to be taxon specific or shared within continents. Limited sharing of alleles and haplotypes between continents suggests long-term genetic isolation between Northern Hemisphere and Southern Hemisphere taxa following divergence. Genetic isolation is also supported by the transcontinental migration rates peaking at or broadly overlapping zero, which is consistent with no gene flow between continents, except into North American *A. c. septentrionalium* from South American *A. c. cyanoptera.* The transcontinental gene flow might represent an ancient migration into North America, as present day gene flow between *A. c. cyanoptera* and *A. c. septentrionalium* is unlikely due to completely segregated distributions (Evarts 2005, Camacho and Wilson 2011). However, large numbers of wintering Blue-winged Teal occur in sympatry with South American Cinnamon Teal as far south as Peru (Botero and Rusch 1988). Restricted gene flow between the continents could most likely be due to differences in timing of breeding cycle during periods of sympatry (Rohwer et al. 2002) indicated by the fact the three Blue-winged Teal males collected in Peru were not in breeding condition (average left

testis of non-breeding individuals: 11 X 4 mm and breeding individuals: 25 X 11 mm). Thus, the occurrence of mixed (sub)species pairs is probably rare and localized. Therefore, sharing of mtDNA haplotypes and nuclear alleles observed among species and subspecies from different hemispheres is more attributable to incomplete lineage sorting rather than gene flow.

ORIGIN OF BLUE-WINGED TEAL AND CINNAMON TEAL

The geographic origin of many dabbling duck groups is difficult to determine because of their high dispersal ability. However, Johnson and Sorenson (1999) reported a general trend of a Southern Hemisphere origin with multiple colonization events of the Northern Hemisphere. Cinnamon Teal appear to conform to this trend, suggested by the asymmetrical gene flow that was found from South American Cinnamon Teal into North America. North American taxa are characterized by large effective population sizes that underwent a recent population expansion, whereas South American subspecies have smaller effective population sizes that did not undergo a recent population expansion. This contrast in demographic history could be explained by a founder event(s) (South America to North America) followed by a population expansion in North America. However, recent population expansions are common among Northern Hemisphere birds (Zink 1997, Avise 2000) and are often interpreted as a postglacial expansion from Late Wisconsin refugia (see Lessa et al. 2003). Although the splitting parameter showed an ambiguous divergence between continents, population expansions in North America combined with asymmetrical gene flow into North America from South America

supports a South American origin for the Cinnamon Teal and Blue-winged Teal species complex.

Geographic isolation between North America and South America appears to have been a strong barrier to gene flow for Blue-winged Teal and Cinnamon Teal after the initial colonization event. In addition, east-west genetic differentiation is found among many widespread temperate migratory birds in North America (Milot et al. 2000, Kimura et al. 2002, Ruegg and Smith 2002, Newton 2003, Lovette et al. 2004, Peters et al. 2005) and suggests two major glacial refugia on either side of the Rocky Mountains or Great Plains during the Pleistocene (Colbeck et al. 2008). Thus, North American Cinnamon Teal and Blue-winged Teal might be descendant from a common ancestor that colonized North America from South America, and then diverged in allopatry on either side of the Rocky Mountains. Consistent with that hypothesis, the nuclear introns supported a greater similarity between pairs within continents than between continents, and the strong mtDNA divergence is suggestive of at least two refugia.

Alternatively, there might have been multiple dispersal events from a South American ancestor independently giving rise to Blue-winged Teal and North American Cinnamon Teal. Multiple colonization scenarios into North America have been proposed for several groups of species (Temple 1972, Weir et al. 2009), and this hypothesis might explain the closer mtDNA relationship of Blue-winged Teal to South America Cinnamon Teal (see also Johnson and Sorenson 1999). Furthermore, IM results suggested that *A. c. septentrionalium* was the most divergent lineage. Additional evidence of a closer affinity between Blue-winged Teal and South American Cinnamon Teal can be found in plumage

patterns. Although male breeding plumage in Blue-winged Teal is distinctive, the spotted body feathers bear a striking resemblance to an "archaeo-adult" spotted breeding plumage (first nuptial plumage) seen frequently in young male South American Cinnamon Teal (Snyder and Lumsden 1951). This plumage series is absent in North American Cinnamon Teal. A closer relationship between Blue-winged Teal and South American Cinnamon Teal would suggest two independent colonization events into North America: one ~95 000 ybp giving rise to *A. c. septentrionalium* in western North America and a more recent event ~65 000 ybp giving rise to Blue-winged Teal in central and eastern North America, followed by subsequent gene flow between two independently diverged populations. Regardless, additional loci are needed to test the single-colonization versus the dual-colonization hypotheses as confidence intervals for divergence times overlapped considerably.

WITHIN-CONTINENT DIVERGENCE

Within North America, at least two areas acted as temperate glacial refugia during the Pleistocene and were often separated by the Great Plains or Rocky Mountains (Gorman 2000, Milot et al. 2000, Ruegg and Smith 2002, Newton 2003, Shafer et al. 2010). Concordance in phylogenetic breaks observed between closely related species identified suture zones that congregated around mountain ranges and have been proposed to act as a barrier to gene flow during glacial and interglacial periods (Swenson and Howard 2004, 2005). Cinnamon Teal and Blue-winged Teal exhibited strong mtDNA divergence corresponding to an east-west divide. Concordant with the recent westward expansion of

Blue-winged Teal since the 1930s (Wheeler 1965, Connelly 1978), we observed a higher migration rate into Cinnamon Teal (~ nine migrants per generation) than into Blue-winged Teal (~ four migrants per generation), although confidence intervals broadly overlapped and symmetrical gene flow could not be rejected. Blue-winged Teal often co-occur on ponds with Cinnamon Teal (Connelly and Ball 1984), with mixed populations disproportionally represented by Cinnamon Teal (Bellrose 1980). Despite being similar ecologically and behaviorally, hybridization is infrequent (Spencer 1953). Males and females both possess plumage color and vocalization differences that are used in courtship as well as other social behaviors. These differences may serve as mate recognition cues on shared wintering grounds where pairing occurs, thus decreasing hybridization events.

In South America, the Andes impose not only a physical barrier but also extreme environmental selection associated with high elevation, and this likely restricts gene flow between resident low- and high-elevation populations (Milá et al. 2009, McCracken et al. 2009a). Colonization within South America appears to have been from the lowlands to the highlands, conforming to the general trend observed in other Andean avifauna (Fjeldså 1985, Vuilleumier 1986, McCracken et al. 2009a). The cold, hypoxic conditions prevalent at high elevation likely requires a physiological mechanism to deal with such environmental stressors, which have led to both phenotypic and genetic differences in high-elevation populations of Cinnamon Teal as well as other Andean waterfowl (Bulgarella et al. 2007, McCracken et al. 2009a, 2009b, Wilson et al. 2010). Traits evolved in response to local adaptation could restrict dispersal from lowland populations

to the highlands and vice versa (McCracken et al. 2009a, 2009b). However, traits that are beneficial at high elevation (e.g. hemoglobin with high oxygen affinity) are well tolerated in the lowlands (Monge and León-Velarde 1991, León-Velarde et al. 1993, León-Velarde et al. 1996), and our estimates of gene flow support asymmetrical gene flow into the lowlands from the highlands. We encountered and collected *A. c. cyanoptera* specimens at the northern and southern geographical limits of the Altiplano, which is outside the typical breeding distribution of *A. c. orinomus*, but not elsewhere in the Andes. Such sympatry could result in the intermixing of *A. c. cyanoptera* and *A. c. orinomus*. However there is no direct evidence of *A. c. cyanoptera* breeding at high elevations (>3500 m) and no records of *A. c. orinomus* in the lowlands.

IM ASSUMPTIONS

Isolation-with-migration makes a number of assumptions (Hey and Nielsen 2004, Hey 2005), and many of these are violated to some extent in most systems (see Hey 2005, Peters et al. 2008), including within the Cinnamon Teal and Blue-winged Teal complex. Violating these assumptions could potentially affect inferences of population history parameters (Becquet and Przeworkski 2009). For example, IM assumes that the two populations being compared are panmictic and not exchanging genes with other populations. In particular, gene flow from a third species can cause divergence times to be overestimated and can result in spurious inferences of asymmetrical gene flow (Strasburg and Rieseberg 2010). These biases might be particularly important when conducting pairwise comparisons between North and South American taxa, because the

comparisons do not account for gene flow within continents. An alternative approach would be to use a four-population model in IMa2; however, that approach requires *a priori* information about the order of divergence events, which is not known for these taxa. In addition, more independent loci will be required to estimate parameters under this more complex model. Despite these limitations, IM is generally fairly robust to small to moderate violations of those assumptions, and therefore, broad aspects of our pairwise comparisons are likely informative.

CONCLUSIONS

Cinnamon Teal and Blue-winged Teal are closely related species that exhibit pronounced plumage differences, and Cinnamon Teal are comprised of morphologically distinct subspecies. We found strong haplotypic frequency differentiation and little haplotype sharing between species and among Cinnamon Teal subspecies. North American and South American Cinnamon Teal studied here have limited contact while Blue-winged Teal winter in sympatry with South American Cinnamon Teal during for part of the year. This limited overlap in breeding and/or overwintering distributions along with timing of breeding has likely restricted gene flow between continents following divergence. Although divergence times were broadly overlapping, this result would be expected in species complexes that have diverged rapidly which is likely the case here. Where Cinnamon Teal and Blue-winged Teal are parapatric or partially sympatric within continents, environmental selection associated with high altitude (South America) and

sexual selection (North America) might have played a major role in the diversification of this group.

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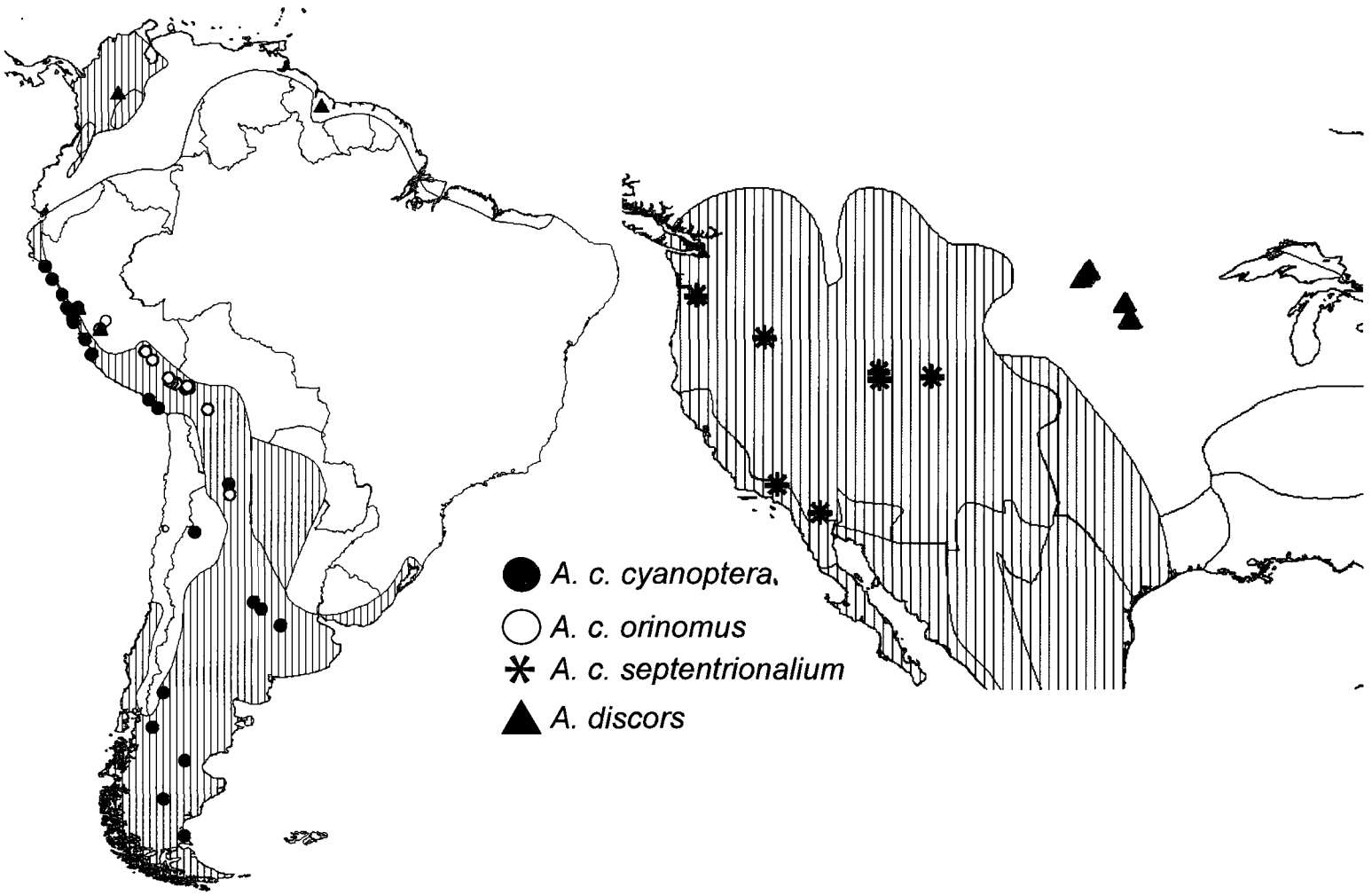

FIGURE 3.1. Sampling localities and geographic ranges (breeding and wintering) for Cinnamon Teal (stripes: *Anas cyanoptera*) and Blue-winged Teal (grey: *Anas discors*) (Ridgely et al. 2003).

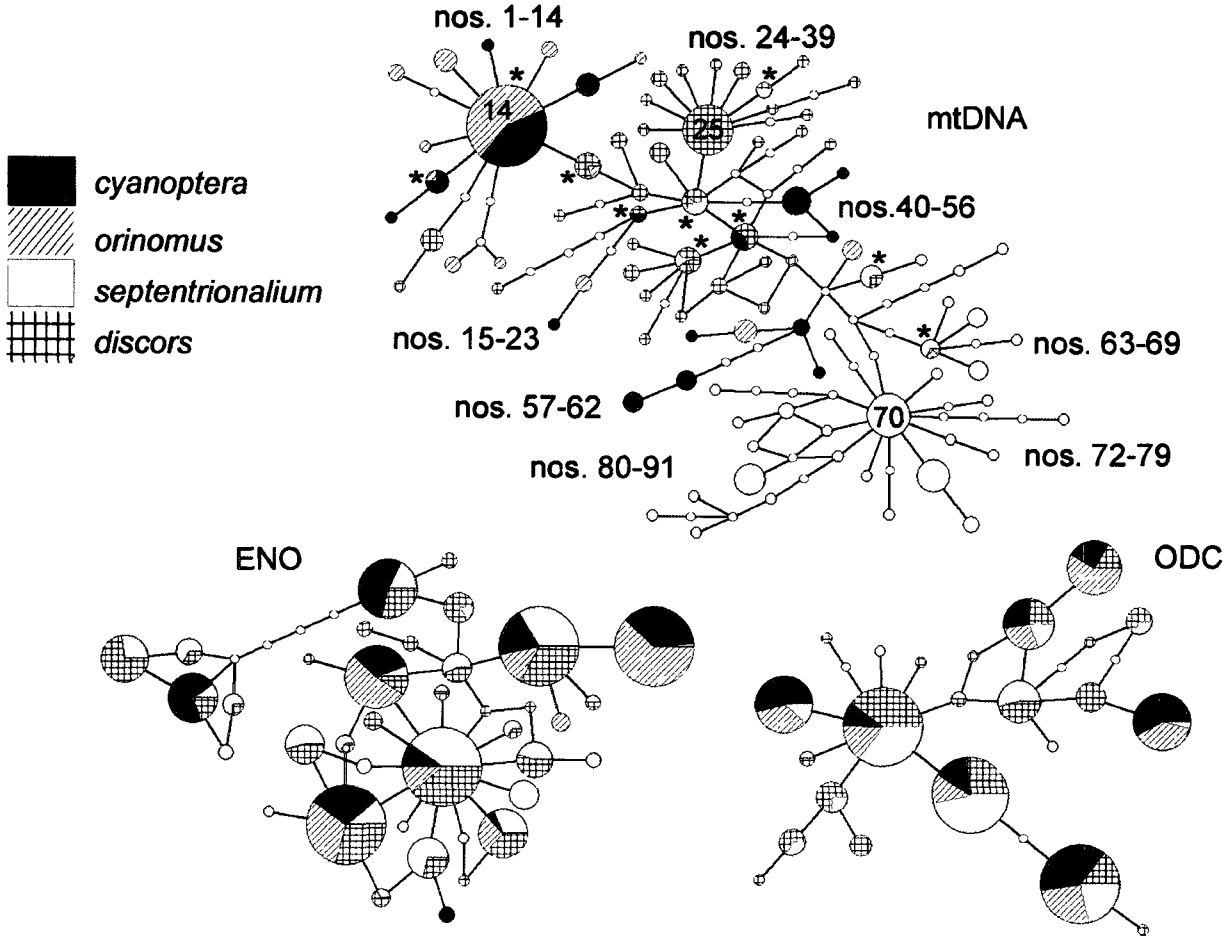

FIGURE 3.2. Unrooted allelic networks for mtDNA control region and two nuclear introns, ODC1 and ENO1. Circles are proportional to the frequency of each allele observed. Small white circles indicate putative ancestral alleles not sampled.

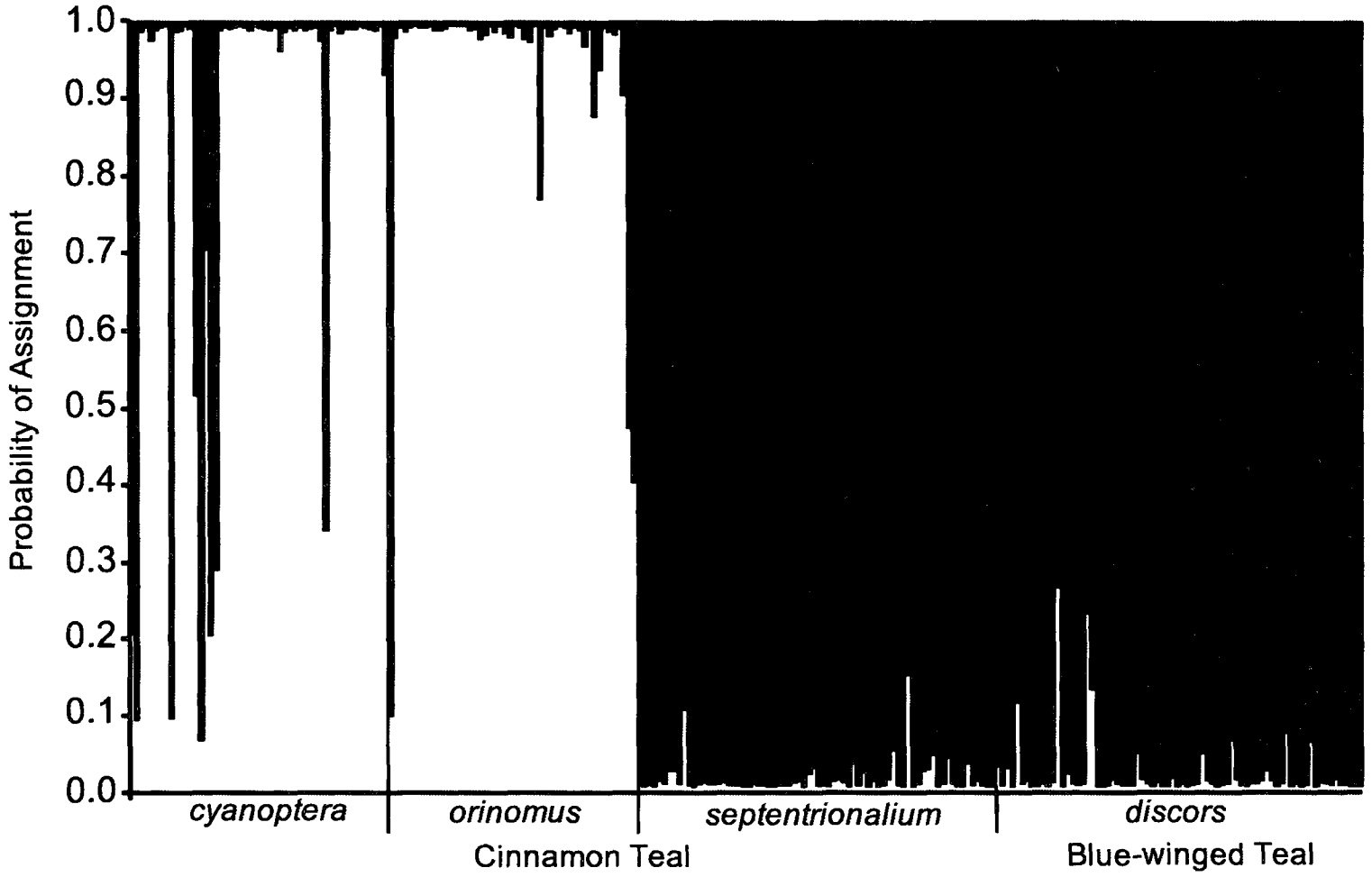

FIGURE 3.3. STRUCTURE 2.2 analysis showing posterior probability of assignment of individuals to each ($K = 2$) genetic cluster. White bar represents the estimated probability of assignment to cluster one, and black bar is the estimated probability of assignment to cluster two.

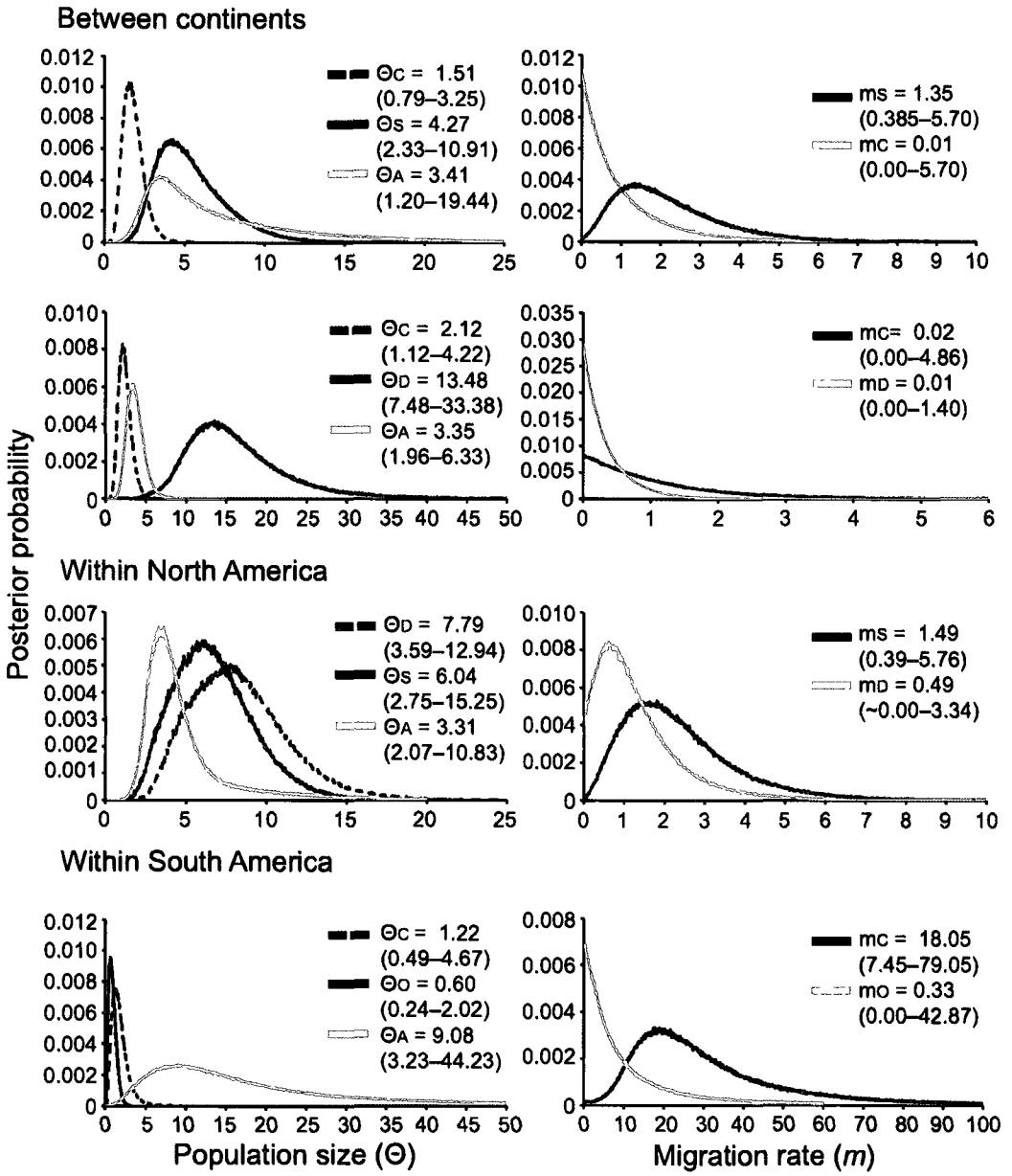

FIGURE 3.4. Posterior distributions of effective population size, Θ, and immigration rates, *m*, calculated using IM (scaled to the neutral mutation rate, μ). Peak estimates for each parameter are given and the 95% highest posterior distribution is shown in parentheses. Letters correspond to Cinnamon Teal subspecies (C = *A. c. cyanoptera*, S = *A. c. septentrionalium*, O = *A. c. orinomus*), Blue-winged Teal (D), or ancestral population for each paired comparison (A).

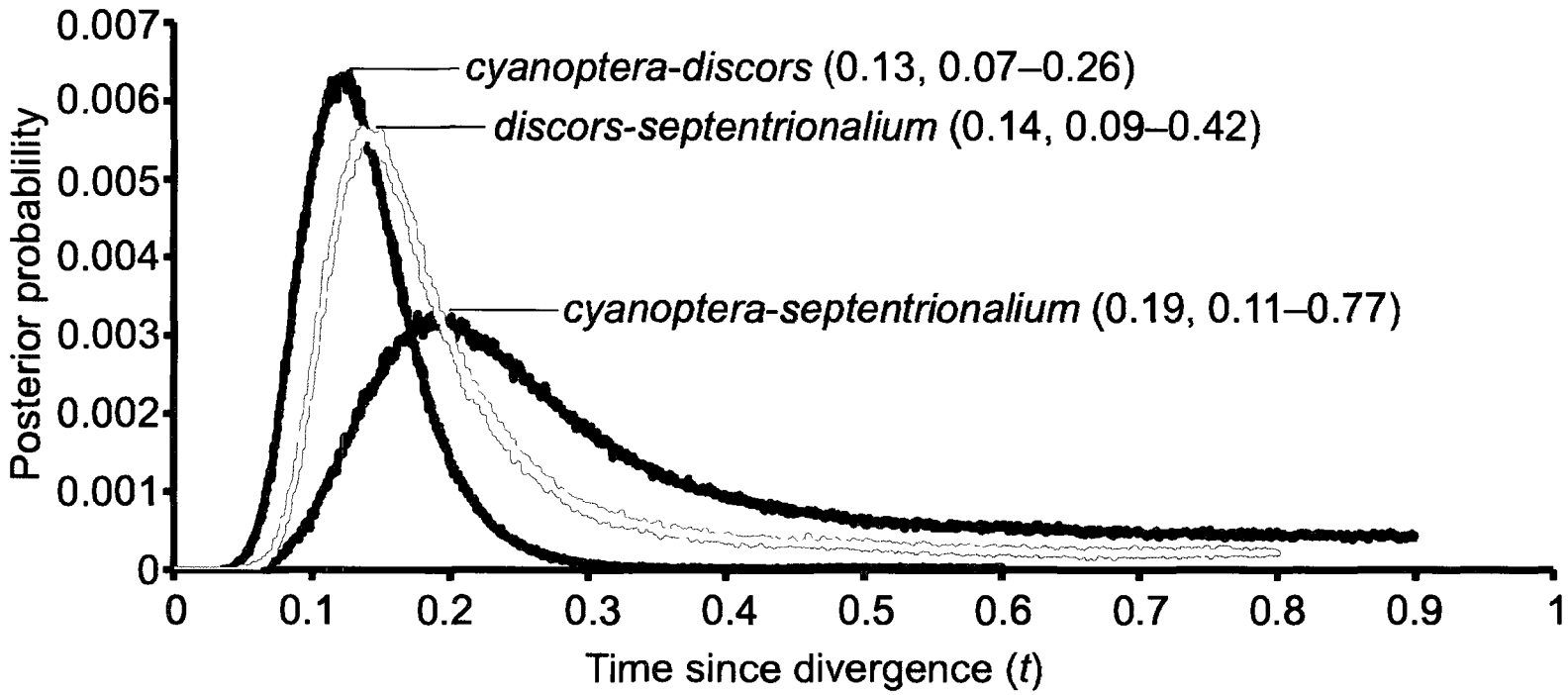

FIGURE 3.5. Posterior distribution of time since divergence (t) calculated in IM.

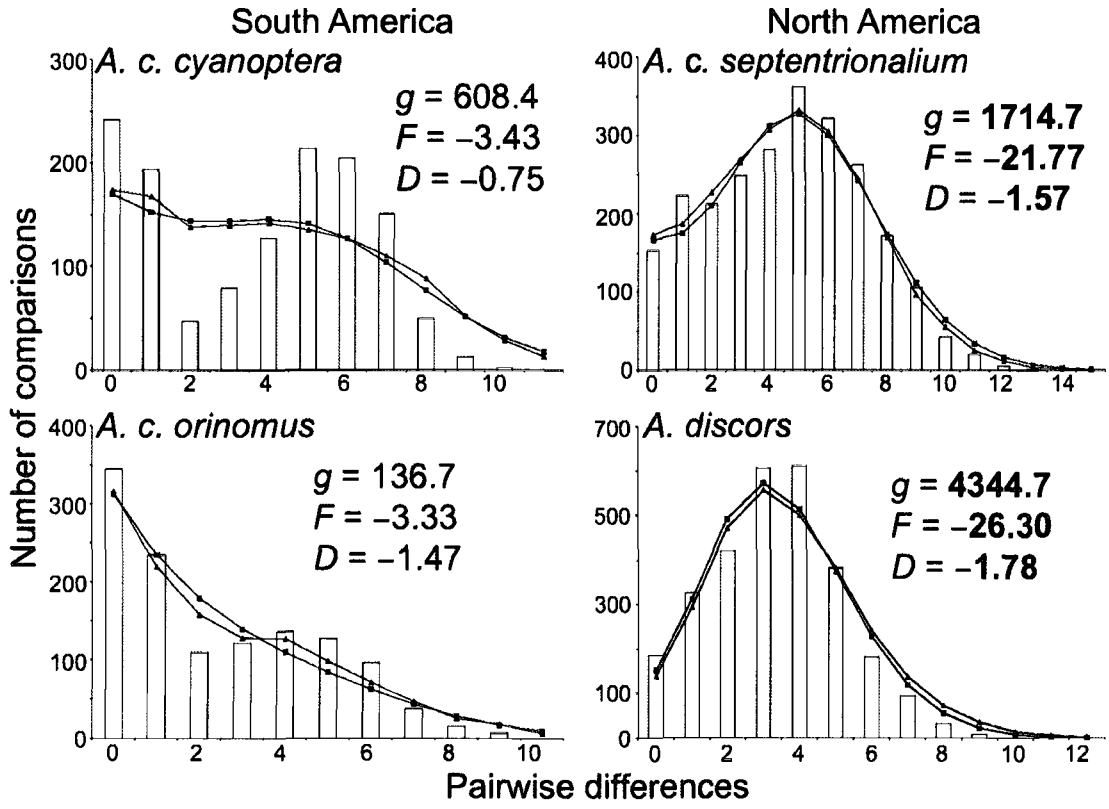

FIGURE 3.6. Results of mismatch distribution and population demographic parameters for North American and South American subspecies of Cinnamon Teal (*Anas cyanoptera*) and Blue-winged Teal (*A. discors*). Bars represent the frequency of pairwise differences. The line with triangle depicts the theoretical distribution under sudden expansion model, whereas the line with square depicts the distribution under a spatial expansion model. Significant *P*–values for Fu's *Fs* (F, $P < 0.02$), Tajima's *D* (D, $P < 0.05$), and population growth parameter (g) that did not overlap with zero are in bold text.

Table 3.1. Number of haplotypes/alleles per population, observed (H_o) and expected (H_e) heterozygosity, allelic richness (r), and nucleotide diversity (π) for the mtDNA control region, ODC1, and ENO1.

		mtDNA			ODC1				ENO1			
Population	*n*	No. haplotypes	r^1	π	No. alleles	Ho/He (%)	*r*	π	No. alleles	Ho/He (%)	*r*	π
A. discors	76	38	35.0	0.003	19	73.7/72.9	16.2	0.006	28	96.1/91.0	24.2	0.013
A. c. septentrionalium	70	34	32.1	0.004	14	65.2/70.6	12.4	0.005	28	88.6/90.4	25.1	0.013
A. c. cyanoptera	52	16	16.0	0.003	7	80.8/79.4	7.0	0.008	9	76.9/85.6	9.0	0.013
A. c. orinomus	50	13	13.0	0.002	7	74.0/81.2	7.0	0.008	7	74.0/78.4	7.0	0.008

[1]Allelic richness based on smallest sample among subspecies and within subspecies.

Table 3.2. Pairwise Φ_{ST} for mtDNA control region, ODC1, and ENO1 among three Cinnamon Teal subspecies and Blue-winged Teal. Significant comparisons are marked with an asterisk.

	mtDNA	ODC1	ENO1
discors			
– *septentrionalium*	0.51*	0.02*	0.00
– *cyanoptera*	0.25*	0.08*	0.04*
– *orinomus*	0.40*	0.03*	0.12*
septentrionalium			
– *cyanoptera*	0.43*	0.06*	0.06*
– *orinomus*	0.47*	0.04*	0.12*
cyanoptera			
– *orinomus*	0.07*	0.01	0.09*

[1]Best-fit nucleotide substitution models for mtDNA (HKY+I+G), ODC1 (K80+I+G), and ENO1 (TVM+I).

Appendix 3.1. Localities of *Anas cyanoptera* and *A. discors* specimens. KGM, JT, and REW specimens are cataloged at University of Alaska Museum.

A. c. septentrionalium
USA: Utah, Weber Co., 41° 14' 59.7" N, 112° 07' 55.8" W, 1,275 m
REW 075
USA: Utah, Salt Lake Co., 40° 50' 50.7" N, 112° 01' 50.9" W, 1,275 m
REW 077, REW 078, REW 079
USA: Oregon, Columbia Co., 45° 45' 18.1" N, 122° 50' 51.4" W, 1 m
REW 797, REW 398, REW 399, REW 400, REW 401, REW 402, REW 403, REW 404, REW 406
USA: California, Imperial Co., 33° 11' 24.0" N, 115° 35' 18.5" W, -68 m
REW 411, REW 412, REW 414, REW 416, REW 418, REW 419, REW 421
USA: California, Imperial Co., 33° 11' 39.0" N, 115° 34' 46.2" W, -73 m
REW 415, REW 420
USA: California, Kerns Co., 34° 47' 43.5" N, 118° 07' 11.3" W, 693 m
REW 422, REW 423, REW 424, REW 425, REW 426, REW 427, REW 428, REW 429, REW 430, REW 431, REW 432, REW 433, REW 434, REW 435, REW 436, REW 437
USA: Utah, Salt Lake Co., 40° 50' 45.1" N, 112° 01' 41.7" W, 1,275 m
REW 438, REW 439, REW 440, REW 441, REW 442, REW 443, REW 444, REW 445, REW 446, REW 447, REW 448, REW 449, REW 450, REW 451, REW 452, REW 453, REW 454, REW 455, REW 456
USA: Colorado, Moffat Co., 40° 59' 10.7" N, 108° 59' 10.5" W, 1,609 m
REW 457, REW 458
USA: Oregon, Harney Co., 48° 43' 53.7" N, 118° 50' 25.3" W, 1,260 m
REW 459, REW 460, REW 461, REW 462, REW 463, REW 464, REW 465, REW 466, REW 467
A. c. cyanoptera
ARGENTINA: Neuquen, Rio Collon Cura, 40° 12' 45" S, 70° 38' 58" W, 625 m[1]
KGM 268
ARGENTINA: Cordoba, Laguna La Felipa, 33° 04' 17" S, 63° 31' 33" W, 184 m[1]
KGM 310, KGM 313, KGM 311, KGM 312
ARGENTINA: Cordoba, S. Canals, 33° 36' 23" S, 62° 53' 16" W, 112 m[1]
KGM 322
ARGENTINA: Jujuy, S. Purmamarca, 23° 49' 13" S, 65° 28' 34" W, 2,141 m
KGM 442
PERU: Dpto. Lima, S Huacho, 11° 10' 12.9" S, 77° 35' 31.4" W, 15 m
REW 081, REW 082
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11° 44' 14.5" S, 75° 29' 32.7" W, 3,506 m
REW 118, REW 122
PERU: Dpto. Ancash, Laguna Conococha, 10° 07' 10.8" S, 77° 17' 00.7" W, 4,039 m
REW 164
PERU: Dpto. Lambayeque, ca. Puerto Eten, 06° 54' 51.9" S, 79° 52' 22.4" W, 13 m
REW 193, REW 194, REW 195, REW 196

Appendix 3.1 continued.

PERU: Dpto. Lambayeque, Playa Monsefu, 06° 54' 03.7" S, 79° 53' 42.4" W, 12 m
REW 198, REW 199
PERU: Dpto. La Libertad, Magdalena de Cao, 07° 51' 54.3" S, 79° 20' 51.2" W, 23 m
REW 200
PERU: Dpto. Ancash, Chimbote, 09° 07' 26.0" S, 78° 33' 11.3" W, 15 m
REW 203, REW 204, REW 205
PERU: Dpto. Ancash, Puerto Huarmey, 10° 05' 52.0" S, 78° 09' 10.3" W, 14 m
REW 206
PERU: Dpto. Lima, Albufera de Medio Mundo, 10° 55' 25.9" S, 77° 40' 10.8" W, 14 m
REW 207
PERU: Dpto. Ica, Pisco, 13° 41' 46.8" S, 76° 13' 07.3" W, 7 m
REW 235
PERU: Dpto. Ica, Pisco, 13° 40' 47.2" S, 76° 12' 56.6" W, 9 m
REW 236
PERU: Dpto. Tacna, Ite, 17° 52' 47.2" S, 71° 01' 05.9" W, 10 m
REW 298, REW 299, REW 300, REW 301, REW 302, REW 303, REW 304
PERU: Dpto. Arequipa, Punta de Bombon-Islay, 17° 11' 31.9" S, 71° 46' 19.4" W, 8 m
REW 305, REW 306
PERU: Dpto. Lima, 2 km N. La Laguna, 12° 33' 13.0" S, 76° 42' 42.1" W, 9 m
REW 315, REW 316, REW 317
ARGENTINA: Chubut, Laguna Terraplen, 42° 59' 50.7" S, 71° 30' 55.1" W, 630 m
KGM 712, KGM 713
ARGENTINA: Santa Cruz, Estancia La Angostura, 48° 38' 33.9" S, 70° 38' 37.3" W, 460 m
KGM 766, KGM 767
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51° 37' 35.7" S, 69° 00' 59.4" W, -3 m
KGM 797, KGM 798
ARGENTINA: Santa Cruz, ca. Punta Loyola, 51° 36' 54.9" S, 68° 59' 26.6" W, 0 m
KGM 799
ARGENTINA: Chubut, S. Lago Colhue Huapi, 45° 38' 49.6" S, 68° 56' 45.1" W, 256 m
KGM 808
ARGENTINA: Catamarca, Antofogasta de la Sierra, Laguna La Alumbrera, 26° 06' 46.4" S 67° 25' 26.7" W, 3,338 m
KGM 1110
ARGENTINA: Catamarca, Embalse Las Cortaderas, 27° 33' 21.2" S, 68° 08' 41.9", 3,369 m
KGM 1142
ARGENTINA: Buenos Aires, 34° 52' 27" S, 61° 23' 19.2", 86 m
JT 011
ARGENTINA: Buenos Aires, 34° 53' 15" S, 61° 21' 51", 86 m
JT 046, JT 047

Appendix 3.1 continued.

A. c. orinomus
ARGENTINA: Salta, NE La Caldera, 24° 33' 01" S, 65° 22' 15" W, 1,468 m
KGM 441
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 11' 45" S, 68° 37' 28" W, 3,808 m
KGM 485, KGM 486, KGM 487
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 20' 13" S, 68° 41' 20" W, 3,854 m
KGM 499
BOLIVIA: Dpto. Oruro, Lago Uru Uru, 18° 02' 03" S, 67° 08' 46" W, 3,735 m
KGM 527, KGM 528, KGM 529, KGM 530, KGM 531, KGM 532, KGM 533, KGM 534, KGM 535
BOLIVIA: Dpto. La Paz, Lago Titicaca, 16° 25' 28" S, 68° 51' 43" W, 3,850 m
KGM 557
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 03" S, 68° 37' 40" W, 3,839 m
KGM 559, KGM 560
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 02" S, 68° 37' 48" W, 3,840 m
KGM 561, KGM 562
BOLIVIA: Dpto. La Paz, Lago Titicaca, Cohani, 16° 21' 07" S, 68° 38' 06" W, 3,845 m
KGM 563, KGM 564, KGM 565, KGM 566
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11° 44' 14.5" S, 75° 29' 32.7" W, 3,506 m
REW 125, REW 126
PERU: Dpto. Cusco, Laguna Chacan, 13° 26' 02.6" S, 72° 07' 49.6" W, 3,533 m
REW 238, REW 239, REW 240, REW 241, REW 242
PERU: Dpto. Cusco, ca. Chinchero, 13° 25' 49.3" S, 72° 03' 41.7" W, 3,789 m
REW 248
PERU: Dpto. Cusco, Urubamba Valley, 13° 25' 22.9" S, 72° 02' 38.2" W, 3,743 m
REW 253, REW 254
PERU: Dpto. Cusco, ca. Laguna Pomacanchi, 14° 06' 51.9" S, 71° 27' 56.6" W, 3,781 m
REW 255, REW 256, REW 257, REW 258, REW 259
PERU: Dpto. Puno, Lago Titicaca, Jaru Jaru, 15° 59' 05.6" S, 69° 36' 24.3" W, 3,824 m
REW 268, REW 269
PERU: Dpto. Puno, Lago Titicaca, ca. Puno, 15° 52' 01.2" S, 69° 56' 21.3" W, 3,830 m
REW 271
PERU: Dpto. Puno, Lago Umayo, Sillvstani, 15° 42' 45.8" S, 70° 09' 00.0" W, 3,853 m
REW 272
PERU: Dpto. Puno, Deustva, 15° 33' 50.0" S, 70° 14' 33.1" W, 3,871 m
REW 284, REW 285, REW 286

Appendix 3.1 continued.

A. discors
USA: South Dakota, Day Co.
REW 001, REW 002, REW 003, REW 004, REW 005, REW 006, REW 007, REW 008, REW 009, REW 010, REW 011, REW 013, REW 014, REW 015
USA: South Dakota, Kingsbury Co.
REW 021, REW 022, REW 023, REW 028, REW 029, REW 032, REW 033, REW 034, REW 035, REW 036, REW 037
USA: North Dakota, Kidder Co.
REW 038, REW 039, REW 040, REW 041, REW 042, REW 043, REW 044, REW 045, REW 046, REW 047, REW 048, REW 049, REW 050, REW 052, REW 053, REW 054, REW 056, REW 061, REW 062, REW 063, REW 065, REW 066, REW 067, REW 068
USA: Oregon, Columbia Co., 45° 45' 18.1" N, 122° 50' 51.4" W, 1 m
REW 405
PERU: Dpto. Junin, Jauja, Laguna de Paca, 11° 44' 14.5" S, 75° 29' 32.7" W, 3,506 m
REW 121, REW 124
PERU: Dpto. Ancash, Laguna Conococha, 10° 07' 10.8" S, 77° 17' 00.7" W, 4,039 m
REW 163
GUYANA
ANSP 8442
COLOMBIA
F(23), M(16)1–M(16)4, M(19)1–M(19)3, M(24)1–M(24)8, M(9)1–M(9)6

[1]These elevation values are interpolated from the U.S. Geological Survey's GTOPO30 (http://eros.usgs.gov); all other elevations were measured with a GPS receiver.

Appendix 3.2. Geographic areas, sampling sites, number of each mtDNA control region haplotype observed, and total sample size per area included in the present study.

Geographic area	Sampling site	Haplotypes observed (count)	*n*
		A. c. cyanoptera	
Peru	coastal regions	12 (4), 14 (15), 40 (3), 41 (1), 43 (1), 61 (3), 62 (2)	29
Peru	Andes (highland)	14 (2), 40 (1)	3
Argentina	Catamarca (highland)	8 (2)	2
Argentina	Jujuy (highland)	42 (1)	1
Argentina	Patagonia	3(1), 8 (1), 9 (1), 14 (1), 23 (1), 57 (2), 59 (1), 62 (1)	9
Argentina	Cordoba and Buenos Aires	14 (3), 20 (1), 40 (2), 43 (1), 60 (1)	8
		A. c. orinomus	
Peru	Altiplano & puna region	2 (1), 4 (2), 7 (1), 8 (1), 14 (12), 15 (1), 22 (1), 54 (2), 58 (2)	23
Bolivia	Altiplano	1 (2), 2 (3), 5 (2), 6 (1), 13 (1), 14 (13), 22 (1), 54 (1), 58 (2)	26
Argentina	Salta	14 (1)	1
		A. c. septentrionalium	
Utah	Salt Lake Co.	19 (1), 56 (1), 63 (1), 65 (1), 67 (1), 69 (2), 70(7), 71 (1), 75 (1), 76 (1), 78 (1), 79 (1), 83 (1), 86 (1), 87 (4)	25
Colorado	Moffat Co.	77 (1), 94 (1)	2
Oregon	Columbia Co. and Harney Co.	32 (1), 55 (2), 65 (1), 66 (1), 69 (1), 70 (1), 72 (1), 73 (1), 77 (4), 78 (1), 81 (1), 89 (1), 90 (1), 91 (1)	18
California	Imperial Co. and Kerns Co.	19 (1), 44 (1), 55 (1), 64 (1), 67 (2), 68 (1), 70 (6), 74 (1), 77 (3), 80 (1), 82 (1), 83 (1), 85 (1), 87 (3), 88 (1)	25

Appendix 3.2 continued.

		A. discors	
North Dakota	Kidder Co.	10 (2), 15 (1), 18 (1), 19 (2), 24 (1), 25 (8), 28 (1), 29 (1), 34 (1), 43 (1), 44 (1), 46 (1), 47 (1), 51 (1), 55 (1)	24
South Dakota	Day Co. and Kingsbury Co.	10 (2), 11 (1), 17 (2), 24 (1), 25 (5), 30 (1), 31 (1), 32 (1), 37 (1), 38 (1), 39 (1), 43 (1), 44 (1), 45 (1), 49 (1), 50 (2), 52 (1), 53 (1)	25
Oregon	Columbia Co.	25 (1)	1
Colombia	Barranquilla	15 (3), 16 (2), 19 (1), 20 (1), 21 (1), 24 (1), 25 (3), 26 (1), 27 (1), 28 (1), 31 (1), 33 (1), 36 (1), 43 (1), 44 (1), 48 (1), 65 (1)	22
Guyana		35 (1)	1
Peru	Andes (highland)	25 (1), 44 (1), 46 (1)	3

CHAPTER 4

PLUMAGE AND BODY SIZE DIFFERENTIATION IN BLUE-WINGED TEAL AND CINNAMON TEAL[1]

ABSTRACT

Blue-winged Teal (*Anas discors*) and Cinnamon Teal (*A. cyanoptera septentrionalium*) are two closely related dabbling duck species that are ecologically equivalent, inhabiting different breeding areas in North America. Cinnamon Teal are primarily restricted to regions west of the Great Plains, whereas Blue-winged Teal occur primarily in the central and eastern part of the continent, having only recently expanded their range westward within the last 75 years. Males of the two species exhibit striking plumage differences that make them easy to distinguish, but females are difficult to differentiate by either plumage or body size. Here we reassess previously recognized body size differences and quantify new differences in plumage color using avian color discrimination modeling. Like previous studies, significant differences were found for bill morphology; mean bill length was 7-10% longer in Cinnamon Teal. Based on visual modeling and plumage reflectance data, plumage color differences between species were found for most female feather patches, with breast coloration ($\Delta S = 3.41$) representing a

[1]Wilson, R. E., M. D. Eaton, and K. G. McCracken. Plumage and body size differentiation in Blue-winged Teal and Cinnamon Teal. Submitted to Wilson Journal of Ornithology.

potentially important and previously unrecognized inter-specific signal. Male breeding plumages are extremely different between the species as illustrated by cheek coloration ($\Delta S = 11.4$), but novel color differences were also detected between species in the wing speculum ($\Delta S = 3.24$) that are indistinguishable to human vision. Although color reflectance data yielded higher accuracy than morphometrics for identifying females, body size measurements (in addition to plumage) also proved to be reliable in correctly classifying males of each species. The use of color reflectance data presents a potentially useful identification and management tool for avian species that are otherwise difficult to distinguish.

INTRODUCTION

Blue-winged Teal (*Anas discors*) and Cinnamon Teal (*A. cyanoptera septentrionalium*) are common North American waterfowl species that are closely related, behaviorally, ecologically, and genetically (McKinney 1970, Connelly and Ball 1984, Johnson and Sorenson 1999). Blue-winged Teal breed primarily in the north-central United States and prairie provinces of Canada, whereas the Cinnamon Teal's breeding range occurs west of the Great Plains and into central Mexico (Gammonley 1996, Rohwer et al. 2002, Evarts 2005). Within the last 75 years, Blue-winged Teal have expanded their breeding range west of the Great Plains, where they were not known to occur prior to 1860 (Wheeler 1965, Connelley Jr. 1978). Both species can now be observed on the same ponds at many locations throughout western North America, and hybridization is known to occur in areas of sympatry (Harris and Wheeler 1965, Bolen 1978, Lokemoen and Sharp 1981). Despite this until recent allopatric breeding distribution, Cinnamon Teal and Blue-winged Teal are known to co-occur on wintering areas. Like most migratory waterfowl these two species form pair bonds on the wintering grounds; therefore female choice is based solely on morphology and/or behavior of males (McKinney 1992).

Blue-winged and Cinnamon teal exhibit pronounced variation in male breeding coloration, but reportedly show little variation in body size, or in plumage among non-breeding males, females, and juveniles. Cinnamon Teal males are reddish brown throughout, and Blue-winged Teal males have a characteristic steel blue neck and head with a white facial crescent. Some differences regarding the overall tone of female

coloration have been suggested with Cinnamon Teal females described as more reddish brown (Wallace and Ogilvie 1977), however such a subtle distinction would likely require observing the two species side by side. Palmer (1976) furthermore noted that female plumages are quite variable among individuals within each species, thus suggesting overall color tone may not be a reliable indicator of species identification. As in plumage characters, females of these two species can be difficult to tell apart based solely on body size measurements. Previous reports have suggested that culmen length and other bill length measures are potential discriminating characters, as it has been noted that Cinnamon Teal bills are slightly longer and of a more spatula shape than Blue-winged Teal with no overlap in measurements (Spencer 1953, Stark 1979). However, Johnsgard (1975) reported that there is overlap in bill measurements, and, thus, bill characteristics may not be adequate to correctly identify species.

Here, we reassess body size variation between Blue-winged Teal and Cinnamon Teal and report novel plumage coloration based on spectral reflectance data. Objective measurements of color using reflectance spectrophotometry are necessary, given that birds see plumage colors differently than humans (Cuthill et al. 2000, Vorobyev 2003, Bennett and Thery 2007, Hart and Hunt 2007), including sensitivity to ultraviolet reflectance, which is a prevalent aspect of avian plumage (Eaton and Lanyon 2003). Furthermore, human visual assessment and interpretations of feather coloration might be inadequate, given that models of avian color discrimination suggest human vision often does not see plumage color differences potentially visually discernable to birds (Vorobyev et al. 1998, Eaton 2005, Hastad et al. 2005, Benites et al. 2007). Hence, we

tested for plumage color differences between Blue-winged Teal and North American Cinnamon Teal from the visual perspective of the birds, for six color patches on males and eight color patches on females. Our main goal with respect to body size data was to rigorously reassess morphometric variation among these two species, using large numbers of freshly collected and measured specimens to avoid biases due to specimen shrinkage (Winker 1993, 1996; Wilson and McCracken 2008). Overall, our results represent a more through quantification of plumage and morphological differences between Blue-winged Teal and Cinnamon Teal, and offer diagnosable characters for species identification.

METHODS

Morphometrics.—Body measurements were taken from adult Cinnamon Teal (10 females, 50 males) from California, Colorado, Oregon, and Utah, and adult Blue-winged Teal (13 females, 34 males) from North Dakota, Oregon, and South Dakota, U.S.A. (2002–2003). We took nine body-size measurements (±0.1 mm) from each bird: wing chord length (carpal joint to longest primary feather unflattened, ±1 mm), tail length (base of the uropygial gland on back to tip of the center tail feather, ±1 mm), exposed culmen length, bill length at nares (anterior edge of nares to tip of nail), tarsus bone length (tarsometatarsus), bill height (height of upper mandible at anterior edge of nares), bill width (width of upper mandible at anterior edge of nares), and body mass (g). Measurements were taken the same day individuals were collected prior to preparation as museum specimens. Body mass was not used for comparisons as individuals were in

different reproductive states, which influenced body mass differences (e.g., female Blue-winged Teal were in the laying stage but female Cinnamon Teal were not).

Statistical analyses were performed with Statistica 9.1 Software (StatSoft 2010). All traits were tested for normality with Kolmogorov-Smirnov tests and were normally distributed ($Ps > 0.05$). A multivariate analysis of variance (MANOVA) was performed to evaluate overall differences between species. Analysis of variance (ANOVA) and pairwise comparisons for each individual measurement were performed using a general linear model with Bonferroni correction for multiple comparisons. We tested the diagnosability of species using the method of Patten and Unitt (2002), which focuses on the extent of overlap rather than detecting mean differences. Diagnosability of species was tested for each measurement separately at the 75% level to determine if at least 75% of the distribution of one species lies outside the distribution of the other species. An index value (D_{ij}) ≥ 0 indicates that species *i* is diagnosable from species *j*. Reciprocal tests were performed to determine whether species *i* is diagnosable from subspecies *j* and subspecies *j* is diagnosable from species *i*.

We also performed a forward step-wise general discriminant analysis to evaluate whether the Cinnamon Teal and Blue-winged Teal could be accurately identified using a subset of morphometric variables. The reliability of the discriminant analysis was assessed using a cross-validation procedure. Cross-validation samples give a less biased error rate in classification, because it does not include observations that are used to create the classification function. The cross-validation sample consisted of ten individuals from each species for males, and two females due to the lower sample size.

Colorimetric Plumage Measurements.—To evaluate potential plumage color differences between Blue-winged Teal (8 females, 10 males) and Cinnamon Teal (12 females, 12 males), we collected reflectance data using an Ocean Optics S2000 fiber optic spectrophotometer following methods described in Wilson et al. (2008). Plumage color data were not collected concurrent with collection of morphometric data from freshly collected specimens. At the time plumage data were collected, round study skins were not available for most of these particular individuals, and thus, plumage measurements were taken from round study skins housed at the University of Alaska Museum (12 Cinnamon Teal males), Smithsonian Museum of Natural History (10 Blue-winged Teal males), and the Field Museum of Natural History (all female specimens). In addition, we were able to measure, and include data from, 43 Blue-winged Teal and 17 Cinnamon Teal males for all wing-patch measurements (representing some of the same individuals used for morphometric data collection).

Plumage spectral properties of museum skins may change over time due to fading (Endler and Théry 1996, Hausmann et al. 2003) thus potentially not representing accurately the color of living birds. However, it has been shown that specimens collected within the last 50 years change little in color (Armenta et al. 2008), and even studies including very old specimens do not report effects of specimen age on plumage color (Benites et al. 2010, Seddon et al. 2010). While the collection dates for most of the male specimens were from 2001 or 2002, specimen age did range from 1896 to 2002. Hence, we performed all analyses using all individuals and using only the most recent individuals (< 50 years) and we obtained the same qualitative results for both datasets,

indicating that year of collection did not bias our results. In addition, linear regression of the reflectance variables against the year of specimen collection showed no significant relationship (all $P > 0.05$); therefore the results shown are from all individuals.

Measurements were taken from male specimens at six different feather patches: cheek, crown, blue wing patch, white greater wing coverts, speculum, and tertials; and from female specimens at eight feather patches: cheek, crown, blue wing patch, white greater wing coverts, speculum, tertials, breast, and flanks. All homologous male and all homologous female feather patches measured appear identical, or very similar in coloration (e.g., breast coloration of females), between Cinnamon Teal and Blue-winged Teal to human vision, except for male cheek (cinnamon color in Cinnamon Teal vs. bluish grey in Blue-winged Teal). This latter plumage patch was included to serve as a representative value of color difference within avian perceptual color space, corresponding to a clear difference in human visual assessment of color, by which to compare other avian color space values (see below).

We subsequently calculated color difference (ΔS) between Blue-winged Teal and Cinnamon Teal for each plumage patch within each sex using the Vorobyev-Osorio (1998) color discrimination model, with detailed methods described by Eaton (2005) and Wilson et al. (2008). Briefly, the model calculates a linear distance (ΔS) between two colors (e.g., reflectance measurements from the same patch of a female Cinnamon Teal and a female Blue-winged Teal) in avian perceptual color space, defined by the spectral sensitivity functions of the four different single-cone cell photoreceptors (see Vorobyev et al. 1998). The units of ΔS are jnd (just noticeable differences), where 1.0 jnd is, by

definition, the threshold value for discrimination of colors (Vorobyev et al. 1998). Thus, ΔS values < 1.0 jnd indicate two colors are visually indistinguishable, whereas values > 1.0 jnd indicate the magnitude of discrimination above the threshold (Vorobyev et al. 1998, Vorobyev 2003, Siddiqi et al. 2004). Generally, at jnd = 1.0 for threshold, two colors are barely distinguishable under ideal conditions, and as jnd becomes larger two colors are more easily discernable under worsening viewing conditions (Siddiqi et al. 2004).

Statistical analysis of spectral data.—Average coloration for each feather patch within species and sexes was used in the color discrimination model, and thus differences interpreted by the model might not be biologically functional if the variance in coloration of two homologous feather patches overlaps to the point that it is not a reliable visual indicator of taxonomy. To test the reliability of color indicators, MANOVAs and ANOVAs with Bonferroni correction for multiple comparisons were performed for each feather patch within each sex to evaluate overall differences in color between species and differences in the visual signal of each avian cone-cell type for color from each plumage patch. To assess the reliability of plumage coloration in species identification between species and to determine which feather patches best discriminate species, we used a forward stepwise general discriminant analysis with cross-validation sample of two males and two females to assess reliability, as in the morphometric analysis.

RESULTS

Morphometrics.—Overall morphology differed between species (Wilks' $\lambda = 0.38$, $F_{(8, 96)} = 19.83$, $P < 0.0001$) and between sexes (Wilks' $\lambda = 0.52$, $F_{(8, 96)} = 11.18$, $P <$

0.0001). There was no significant interaction between species and sex (Wilks' $\lambda = 0.91$, $F_{(8, 96)} = 1.15$ $P = 0.338$) Mean differences between species were restricted to three bill measurements (culmen length, length at nares, and bill width) for both males and females, with Cinnamon Teal approximately 7–10 % larger on average (Table 4.1). Using the diagosability index (D_{ij}), Blue-winged Teal and Cinnamon Teal were not diagnosable from each other as all index values were less than zero for both males and females, indicated by considerable overlap in ranges (Table 4.1, Fig. 4.1).

The final discriminant function included three variables (culmen, tail length, wing chord) for males and only one variable (bill width) for females (Table 4.2). Overall, male Cinnamon Teal and Blue-winged Teal were correctly assigned in 94.0% and 100.0% of cases, respectively. Female classification was lower, with Cinnamon Teal and Blue-winged Teal classified correctly in 70.0% and 92.3% of cases, respectively. All cross-validation samples were correctly assigned for both males and females.

Color divergence.—As expected, color difference in avian perceptual color space between Cinnamon Teal and Blue-winged Teal was greatest for cheek color of males (ΔS =11.15), which corresponds to an easily distinguishable difference in coloration to human vision. Unexpectedly, there were also color differences most likely large enough to be visually discernable to birds ($\Delta S = \sim 3$) for several plumage patches that are visually identical to the human eye: male speculum, female breast, and female cheek (Tables 4.3–4.4). All other patches were very near, or less than, the threshold for discrimination as different colors in avian perceptual color space, with the exception of female flank which had $\Delta S = 1.99$. We observed statistical differences between Cinnamon Teal and Blue-

winged Teal for at least one photoreceptor signal (i.e., quantum catches, Q_1–Q_4; Tables 4.3–4.4) for coloration taken from each of the following plumage patches: all male patches, except tertial; and for females, crown, breast, speculum, and tertial.

Final discriminant analysis showed that speculum (Q1 and Q2) best discriminated species for male wings, and crown (Q4), speculum (Q3 and Q4), and cheek (Q1 and Q2) best discriminated species for females (Table 4.2). When only female wing reflectance measurements were used, speculum (Q3 and Q4) showed the best predictability. Males were correctly classified in 76.5% and 81.4% of cases for Cinnamon Teal and Blue-winged Teal, respectively. All Blue-winged Teal males used in the cross-validation sample ($n = 5$) were correctly classified, while only 66.7% (2 out of 3) of Cinnamon Teal were. For females, all individuals for both models (full body and wing only), including the cross-validation sample ($n = 2$), were correctly classified based on color variables across plumage patches.

DISCUSSION

Blue-winged Teal and Cinnamon Teal are very similar in both morphology and plumage characters making species identification difficult, especially among female individuals. As with other studies (Spencer 1953, Stark 1979), we confirm inter-specific mean differences in bill morphology. However, there was overlap in each measurement, which is not surprising as on average differences only correspond to a 3 mm (length) and 1 mm (width) difference and habitat use and feeding strategies are virtually identical (Connelly and Ball 1984). Although being morphologically similar in size, Blue-winged Teal and Cinnamon Teal males show strikingly divergent plumage, not only in overall body

coloration, but also in head and neck coloration. This was clearly reflected in our color discrimination analyses, with male cheek reflectance measurements yielding a large distance in avian perceptual color space between the two species ($\Delta S = 11.4$). This, of course, corresponds with a difference in coloration that humans easily perceive as distinct (see plate 20 in Kear 2005). In addition to the large male plumage divergences, from the visual perspective of a duck we found that several female plumage patches showed color differences between species, although to a lesser degree than male color differences. This is often the case between closely related avian species where a major component of variation often results from differences in sexual ornaments used for mate recognition with little variation among juvenile and female plumages (West-Eberhard 1983, Price 2008).

Biological plumage differences between species.— Plumage is an integral part of signaling behavior of waterfowl, and color patches have evolved to increase the effectiveness of displays in social situations such as pair formation (McKinney 1992, Price 2008). As with many closely related dabbling duck species, Cinnamon Teal and Blue-winged Teal males perform the same display repertoire and the accompanying vocal and plumage signals are often used for mate recognition (Johnsgard 1963, McKinney 1970). In both species, the speculum is used in a common courtship display (lateral dabbling), along with other distinguishing plumage traits such as cheek and flank feathers. Although wing coloration of the two species is indistinguishable to human vision, the inter-specific color difference ($\Delta S = 3.24$) of the speculum likely represents a novel species-specific plumage signal in males. Furthermore, the color divergence

between species in the green speculum was absent in females ($\Delta S < 1$), suggesting that sexual selection might play a role in evolution of speculum color divergence in male Blue-winged Teal and Cinnamon Teal.

Previous descriptions of female Blue-winged Teal and Cinnamon Teal reported overall body and head coloration differences, although these lacked rigorous quantification (Spencer 1953, Wallace and Ogilive 1977, Bellrose 1980). From an avian visual perspective, we quantified inter-specific differences in cheek, breast, and flank plumage coloration that should be visually distinguishable to the birds, given their respective linear distances in avian perceptual color space between homologous feather patches (i.e., ΔS, Table 4.4). These objective plumage color differences support previous subjective descriptions of female coloration. Statistically, only color variables representing the breast plumage were different between females of the two species, with intra-specific variation being too large for cheek and flank color (Table 4.4). These ambiguous results between statistical analyses of color and color discrimination model analyses might reflect the confusing historical descriptions of plumage variability among and within these teal species (Palmer 1976). Potentially, these female differences in coloration (e.g., breast plumage) could serve as recognition cues for potential mates (e.g., species recognition), and thus, might reinforce divergence through decreasing hybridization events, assuming hybrids have lower fitness due to such factors such as susceptibility to parasitism (Mason and Clark 1990) or disadvantages in securing a mate (Morton 1998, Sorenson et al. 2010). Ideally, behavioral choice experiments are needed to confirm female plumage signals as biologically functional species identifiers.

Identification of species.—Aside from male breeding plumage, Blue-winged Teal and Cinnamon Teal have historically been difficult to differentiate, and this confusion in part has likely played a role in lack of accurate population and harvest estimates especially for Cinnamon Teal as counts of these two species are combined during aerial surveys, banding records, and waterfowl parts surveys (Gammonley 1996, Rohwer et al. 2002, Raftovich et al. 2010). In addition, identification of females based on an accompanying male can be misleading (Phillips 1975) as these two species do hybridize albeit infrequently (Spencer 1953).

Differences between species have been reported as potential discriminating variables, particularly in bill measurements. However, there is no consensus across reports indicating that a single measurement or plumage feature can accurately differentiate these two species. In agreement with Johnsgard (1975), but in contrast with Stark (1979), we found considerable overlap in bill measurements as well as other measurements, indicated by the lack of diagnosability, e.g., less than 75% of individuals from Blue-winged Teal lie outside the range of Cinnamon Teal and vice versa. Using a multivariate discriminate function (wing chord, tail, and culmen length), males could be correctly identified with high accuracy (96.4%). Even though this model is based on adult males, this model can be applicable to immature males of eight weeks or older when full growth is essentially obtained (Stark 1979). Females were particularly problematic to identify. The low power for female assignment could be attributable to low sample size. However, variation in Blue-winged Teal measurements typically overlapped the means of Cinnamon Teal measurements. Therefore, it is unlikely that a

larger sample size would substantially increase accuracy of the assignments. Also differences between species in bill measurements were extremely small (< 3mm) therefore any error in measuring, even 1 mm, could cause a misidentification.

Plumage features such as overall color tone, facial pattern, and presence of eye stripe have also been proposed as possible discriminating characteristics. However most of the descriptions are subjective such as more "reddish brown" or "streakier" which would require comparing both species side by side. Using plumage reflectance data to quantify color differences allows for the potential to accurately identify individuals without reference specimens and takes away observer subjectivity in such factors as what constitutes more "reddish brown". Plumage coloration did show higher accuracy in identification between females than did morphometrics. The typical reddish brown was evident in all Cinnamon Teal females, but female coloration in Blue-winged Teal was variable, as indicated by others (e.g., Palmer 1976). However, the discriminate function based on reflectance data from cheek, crown, and speculum correctly assigned all females to species (Table 4.2). In addition, males could be assigned with high accuracy based on wing coloration (although not 100% as in females). Whereas identification requiring precise bill measurements where any error in measurement could result in misidentification, plumage reflectance data have the potential to provide additional confirmation on species identification or accurately identify problematic individuals. Thus, the use of plumage reflectance information may serve as a useful new tool for wildlife managers, in combination with morphometrics, to more accurately identify species of unknown individuals.

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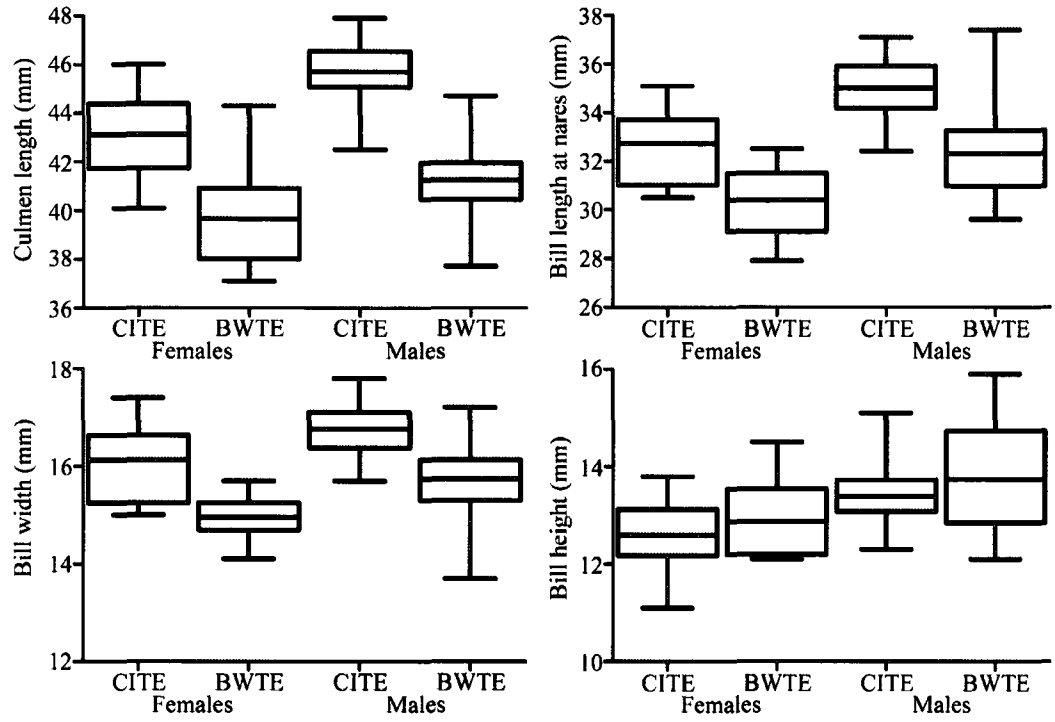

Figure 4.1. Variation in bill measurements between Blue-winged Teal (BWTE; 13 females and 34 males) and Cinnamon Teal (CITE; 10 females and 50 males) males and females. Results are means (line) within 95% confidence limits (box). Vertical lines represent minimum and maximum values to illustrate the amount of overlap in ranges.

Table 4.1. Body size (mm) and body mass (g) measurements for Cinnamon Teal and Blue-winged Teal.

	A. c. septentrionalium[a]			*A. discors*[a]				
Sex	Mean	SE	Range	Mean	SE	Range	% Dimorphism[b]	*P*-value[c]
Males								
Mass	361.8	3.33	310-420	386.1	4.69	330-460		
Wing chord	188.8	0.87	168-201	187.6	0.70	180-199	0.53	1.00
Tarsus	31.01	0.15	28.1-33.4	30.50	0.16	29.1-32.5	1.67	1.00
Tail	80.47	0.58	66.0-87.0	73.51	1.03	62.9-84.6	9.47	< 0.001
Nare	35.00	0.17	32.4-37.1	32.30	0.30	29.6-37.4	8.36	< 0.001
Culmen	45.63	0.20	42.5-47.9	41.31	0.22	37.7-44.7	10.46	< 0.001
Bill height	13.39	0.08	12.3-15.1	13.74	0.18	12.1-15.9	-2.55	0.40
Bill width	16.76	0.07	15.7-17.8	15.74	0.13	13.7-17.2	6.48	< 0.001
Females								
Mass	363.5	14.20	315-430	410.4	9.63	335-465		
Wing chord	180.7	1.60	171-187	177.2	1.38	171-186	1.96	0.76
Tarsus	30.69	0.55	29.2-34.9	30.05	0.17	29.1-31.2	2.07	1.00
Tail	76.30	2.02	67.0-86.0	72.90	1.32	66.9-81.4	4.46	0.69
Nare	32.74	0.48	30.5-35.1	30.37	0.38	27.9-32.5	7.24	< 0.001
Culmen	43.10	0.61	40.1-46.0	39.65	0.62	37.1-44.3	8.01	< 0.001
Bill height	12.59	0.23	11.1-13.8	12.88	0.25	12.1-14.5	-2.28	1.00
Bill width	16.13	0.25	15.0-17.4	14.95	0.13	14.1-15.7	7.34	< 0.001

[a]Sample sizes: *A. c. septentrionalium* (50 male, 10 female), *A. discors* (34 male, 13 female).
[b]Dimorphism calculated based on mean for *A. c. septentrionalium* divided by *A. discors*.
[c]Bonferroni corrected *P* values ($P_{adjusted} < 0.05$).

Table 4.2. Step-wise discriminant function coefficients for identification of male and female Cinnamon Teal and Blue-winged Teal using morphometric and plumage reflectance data.

Male			Female		
		Morphometrics			
	A. c. septentrionalium	*A. discors*		*A. c. septentrionalium*	*A. discors*
Intercept	-1102.00	-991.86	Intercept	-275.87	-236.43
Culmen	22.62	20.06	Bill width	34.10	31.57
Tail	3.04	2.50			
Wing chord	4.89	5.12			
% Correctly Classified	94.0	100.0	% Correctly Classified	70.0	92.3
		Plumage			
	Wing only			Full body/wing only	
Intercept	-11.65	-13.95	Intercept	-37.15/-17.34	-21.74/-6.05
Q2 speculum	0.05	0.03	Q4 crown	-0.02	0.04
Q3 speculum	0.01	0.03	Q3 speculum	-1.25/-0.48	0.66/-0.06
			Q4 speculum	0.94/0.39	-0.44/0.08
			Q1 cheek	-0.21	0.04
			Q2 cheek	0.24	-0.04
% Correctly Classified	76.5	81.5	% Correctly Classified	100.0	100.0

Table 4.3. Average receptor quantum catches (Qi) of each of the four single cone cell types, and color discriminability (ΔS) using the Vorobyev-Osorio color discrimination model for each feather patch on male Cinnamon Teal and Blue-winged Teal. $\Delta S > 1.0$ just noticeable difference indicates distinguishable differences in color to the avian visual system under ideal viewing conditions.

Feather	*A. discors* Mean (SE)	*A. c. septentrionalium* Mean (SE)	P^a	ΔS
Blue wing patch				
Q1[b]	2495.55 (64.5)	2134.0 (101.0)	0.004	0.49
Q2	1607.11 (39.0)	1393.2 (63.7)	0.005	
Q3	1199.37 (27.2)	1055.7 (47.1)	0.008	
Q4	1372.99 (29.3)	1230.2 (56.1)	0.017	
Speculum border				
Q1	3837.96 (86.7)	4232.0 (226.0)	0.050	0.61
Q2	2825.89 (68.4)	3123.0 (141.0)	0.038	
Q3	2340.03 (60.0)	2603.0 (105.0)	0.027	
Q4	2973.70 (78.0)	3320.0 (126.0)	0.022	
Speculum				
Q1	534.14 (16.7)	491.0 (23.6)	0.16	3.24
Q2	340.22 (10.5)	330.7 (18.4)	0.69	
Q3	631.23 (19.8)	515.4 (27.9)	0.002	
Q4	529.31 (22.0)	470.4 (23.7)	0.13	
Crown				
Q1	278.41 (26.9)	408.5 (28.5)	0.007	0.09
Q2	213.92 (19.9)	314.8 (24.7)	0.009	
Q3	208.45 (18.9)	307.1 (26.9)	0.014	
Q4	328.82 (30.1)	481.6 (47.7)	0.025	
Cheek				
Q1	674.26 (40.1)	381.5 (30.9)	< 0.001	11.4
Q2	517.07 (27.1)	321.6 (26.1)	< 0.001	
Q3	467.07 (22.8)	388.9 (29.0)	0.04	
Q4	671.93 (31.8)	854.2 (50.7)	0.005	

Table 4.3 continued.

Feather	*A. discors* Mean (SE)	*A. c. septentrionalium* Mean (SE)	P^a	ΔS
Blue tertial				
Q1	2163.49 (68.3)	2144.0 (107.0)	0.88	0.49
Q2	1433.62 (43.2)	1375.9 (70.3)	0.48	
Q3	1022.91 (31.8)	971.0 (45.1)	0.35	
Q4	1094.26 (27.4)	1049.4 (48.1)	0.42	

[a]Bonferroni adjusted *P*-value.
[b]Q1 is receptor quantum catch of the violet sensitive cone (VS), Q2 the short-wave sensitive cone (SWS), Q3 the middle-wave sensitive cone (MWS), and Q4 the long-wave sensitive cone (LWS).

Table 4.4. Average receptor quantum catches (Qi) of each of the four single cone cell types, and color discriminability (ΔS) using the Vorobyev-Osorio color discrimination model for each feather patch on female Cinnamon Teal and Blue-winged Teal. $\Delta S > 1.0$ just noticeable difference indicates distinguishable differences in color to the avian visual system under ideal viewing conditions.

Feather	*A. discors* Mean (SE)	*A. c. septentrionalium* Mean (SE)	P^a	ΔS
Crown				
Q1[b]	447.9 (45.4)	324.4 (22.8)	0.016	1.05
Q2	356.6 (38.6)	259.0 (17.8)	0.020	
Q3	348.7 (37.9)	259.7 (17.7)	0.029	
Q4	543.8 (58.1)	422.8 (28.7)	0.054	
Cheek				
Q1	1188.0 (123.0)	908.7 (78.9)	0.059	2.92
Q2	1053.0 (105.0)	892.8 (69.1)	0.20	
Q3	1023.7 (97.5)	941.1 (67.5)	0.48	
Q4	1511.0 (136.0)	1465.0 (101.0)	0.79	
Breast				
Q1	1292.0 (119.0)	853.8 (45.8)	< 0.001	3.41
Q2	1143.2 (90.8)	834.9 (43.2)	0.003	
Q3	1135.6 (78.9)	911.9 (45.5)	0.017	
Q4	1715.0 (105.0)	1488.5 (70.6)	0.078	
Flank				
Q1	470.0 (41.0)	439.4 (32.4)	0.56	1.99
Q2	412.3 (38.0)	410.7 (29.1)	0.97	
Q3	439.6 (38.1)	462.6 (31.2)	0.65	
Q4	734.6 (57.7)	806.3 (50.4)	0.37	
Blue wing patch				
Q1	1735.0 (135.0)	1481.8 (55.0)	0.064	1.26
Q2	1169.7 (84.0)	1033.9 (35.4)	0.11	
Q3	920.0 (57.0)	835.8 (25.0)	0.15	
Q4	1102.8 (58.3)	1042.0 (28.0)	0.31	
Speculum border				
Q1	2169.0 (310.0)	2276.0 (282.0)	0.81	1.04
Q2	1614.0 (219.0)	1787.0 (222.0)	0.60	
Q3	1383.0 (178.0)	1563.0 (188.0)	0.52	
Q4	1822.0 (223.0)	2083.0 (239.0)	0.46	

Table 4.4 continued.

Feather	*A. discors* Mean (SE)	*A. c. septentrionalium* Mean (SE)	P^a	ΔS
Speculum				
Q1	278.6 (25.2)	402.9 (36.9)	0.023	0.92
Q2	225.5 (19.3)	325.1 (28.9)	0.020	
Q3	219.5 (17.3)	315.4 (25.9)	0.013	
Q4	313.7 (26.0)	477.2 (36.6)	0.004	
Blue tertial				
Q1	194.4 (16.9)	282.4 (27.1)	0.026	0.54
Q2	162.3 (13.0)	233.3 (21.0)	0.021	
Q3	168.0 (12.5)	240.4 (19.6)	0.013	
Q4	261.9 (19.3)	388.3 (29.8)	0.005	

[a]Bonferroni adjusted *P*-value.
[b]Q1 is receptor quantum catch of the violet sensitive cone (VS), Q2 the short-wave sensitive cone (SWS), Q3 the middle-wave sensitive cone (MWS), and Q4 the long-wave sensitive cone (LWS).

CHAPTER 5

COLOR DIVERGENCE AMONG CINNAMON TEAL (*ANAS CYANOPTERA*) SUBSPECIES FROM NORTH AMERICA AND SOUTH AMERICA[1]

[1]Wilson, R. E., M. Eaton, and K. G. McCracken. Color divergence among Cinnamon Teal (*Anas cyanoptera*) subspecies from North America and South America. Ornitologia Neotropical 19:307–314.

INTRODUCTION

Plumage is an integral part of signaling behavior of waterfowl, especially during courtship and pair formation. There is a vast array of display repertoires among dabbling ducks (genus *Anas*) with many species performing the same displays. While many closely related species perform displays in similar form, the accompanying vocal and plumage signals differentiate species (McKinney 1970). Modifications in display frequencies have been proposed to evolve in association with slight plumage or morphological differences (Johnsgard 1960, McKinney 1961, McKinney 1965). In addition, color patches have evolved to increase the effectiveness of the displays in social situations such as pair-formation, hostile or territorial encounters, maintaining contact with mate, and flock activities (McKinney 1970, Price 2008).

Cinnamon Teal (*Anas cyanoptera*) is composed of five subspecies (*A. c. borreroi*, *A. c. cyanoptera*, *A. c. orinomus*, *A. c. septentrionalium*, and *A. c. tropica*; Snyder & Lumsden 1951), and each performs a variety of movements during social courtship that are accompanied by postures using different plumage areas. The color of the "cinnamon" feathers in males is known to be variable among and within subspecies (Snyder & Lumsden 1951), however color of other plumage patches among subspecies appear identical to human visual assessment (Delacour 1956, Blake 1977, Johnsgard 1978, Evarts 2005). During Cinnamon Teal displays, such as turn-back-of-head and lateral dabbling, differences in color of feather patches could potentially provide information about subspecies identification or male quality, because plumage is known to be important in avian signaling and mate choice (e.g., Cooke & McNally 1975, Klint 1980,

Holmberg *et al.* 1989, Weidmann 1990, Sorenson & Derrickson 1994, Omland 1996a, 1996b; Bridge & Eaton 2005).

However, all birds studied to date see plumage colors differently than humans (Cuthill *et al.* 2000, Bennett & Thery 2007, Hart & Hunt 2007), and recent analyses of plumage colors quantified through spectrophotometry suggest birds might detect plumage color differences not detectable through human vision (Eaton 2005, Benites *et al.* 2007). Thus, human visual assessment of feather coloration is inadequate for proper study and interpretation of many biological questions. To overcome this problem, herein, we test for color differences from the visual perspective of the birds, using a model of avian color discrimination (Vorobyev & Osorio 1998). For several plumage patches that appear identical in coloration to humans, including those used during courtship displays, we quantify both male and female plumage color differences (i.e., divergence) among the three most widespread and abundant Cinnamon Teal subspecies (*A. c. cyanoptera*, *A. c. orinomus*, and *A. c. septentrionalium*). The other subspecies are not common, and museum collections lack very recently collected specimens needed for comparisons in this study.

METHODS

Study species. In general, the male breeding plumage consists of a reddish brown to bright reddish chestnut color. The abdomen color ranges from brownish to black, and the crown is typically black. The wings have blue upper-wing coverts (wing patch) and a metallic green speculum that is duller on females and these areas are separated by white greater wing coverts. Although the coloration of males within and among subspecies is

variable, there are some general trends that have been used in conjunction with morphological measurements to distinguish subspecies (Snyder & Lumsden 1951). Male *A. c. septentrionalium* tends to have more cinnamon red color than the other subspecies and lacks the spots on the breast, flanks, and belly that can be found on *A. c. cyanoptera* (Blake 1977). *A. c. cyanoptera* is usually a rich chestnut color. *A. c. tropica* and *A. c. borreroi* generally have a darker overall chestnut color with a higher frequency of spotting. The chestnut color of *A. c. orinomus* is typically lighter than *A. c. cyanoptera.* Female coloration range from mottled tan brown to red brown and tone is also quite variable ranging from pale to moderately dark (Gammonley 1996). *A. c. tropica* and *A. c. borreroi* are generally darker than the other subspecies. *A. c. orinomus* females tend to have darker streaking and are more reddish than *A. c. cyanoptera. A. c. septentrionalium* females are extremely variable in both color and tone (Blake 1977, Gammonley 1996).

Spectral analysis of plumage colors. In 2004, we measured 17 adult *A. c. orinomus* (7 females, 10 males), 29 *A. c. cyanoptera* (8 females, 21 males), and 15 *A. c. septentrionalium* (3 female, 12 males) collected from Argentina (2001, 2003), Peru (2002), and western United States (2002-2003). To avoid any potential bias introduced from color degradation from older specimens, we only used very recently collected specimens. Voucher specimens are archived at the University of Alaska Museum (Fairbanks, Alaska). All individuals were determined to be in complete breeding plumage and there was no evidence of color fading. Feather patch locations measured were chosen based on their overall visibility during social displays (McKinney 1970) and conspicuousness when compared to surrounding feathers. Streaked or barred regions of

the plumage were not used because those patches are smaller than the ~4 mm^2 measuring area, and thus reliable measurements could not be made. Measurements were taken of seven different feather locations for males: crown, cheek, breast, blue wing patch, white greater wing coverts, green speculum, and blue tertial feathers. Due to the streakiness of female plumage, only two readings (blue wing patch and green speculum) were taken, both from the wing.

Spectral reflectance data were collected with an Ocean Optics S-2000 spectrometer (Dunedin, FL, USA) equipped with an R200-7-UV/VIS reflectance probe (fiber diameter = 200 microns) and a PX-2 pulsed xenon light source. Data collected were calibrated against a Spectralon white reflectance standard with the following settings: msec = 100, average = 10. These settings determined the pulse rate of the light source, and the number of scans averaged per spectrum saved, respectively. The reflectance probe was housed in a black rubber tube, which blocked ambient light, maintained the distance from the probe to the feather surface constant (approximately 2 mm), and achieved a 90-degree measurement angle relative to the feather surface. The spectrometer was recalibrated after all measurements were taken for each individual specimen. Raw reflectance data were averaged to yield percent light reflected every 10 nm between 300 and 700 nm, using the SAS statistical software package (SAS Institute, Cary, NC, USA).

Avian visual system modeling. We evaluated color divergence among three of the *A. cyanoptera* subspecies for each feather patch using the Vorobyev-Osorio (1998) color discrimination model. The model calculates a distance in avian color space (ΔS), defined

by the quantum catches of each receptor type in the avian retina. Thus, Q_1 represented the receptor quantum catch of the violet sensitive cone (VS), Q_2 the short-wave sensitive cone (SWS), Q_3 the middle-wave sensitive cone (MWS), and Q_4 the long-wave sensitive cone (LWS). The model assumes only that discrimination of color within this perceptual space is limited by noise originating in the receptors, and no visual signal results when a stimulus and background differ only in intensity (Vorobyev *et al.* 1998). The model is supported from behavioral data for several bird species, bees, and humans (Vorobyev *et al.* 1998, Vorobyev *et al.* 2001, Goldsmith & Butler 2003).

To calculate ΔS (color divergence) for each subspecies comparison, we used methods described in detail by Eaton (2005), substituting spectral sensitivity and relative photoreceptor abundance data from the peacock for those of the blue tit. Spectral sensitivity data do not exist for *A. cyanoptera* or other ducks, so we used the peacock data as an approximation. These data provide a good estimate for *A. cyanoptera*, as the visual pigment characteristics of other Anseriformes are similar to those of the peacock, and thus photoreceptor sensitivities are highly conserved between these taxa for much of the visual range (Hart 2001). The units of ΔS are jnd (just noticeable differences), where 1.0 jnd is the threshold value for discrimination of colors. Thus, ΔS values < 1.0 jnd indicate two colors are visually indistinguishable, whereas values > 1.0 jnd indicate the magnitude of discrimination above threshold (Vorobyev et al. 1998, Vorobyev 2003, Siddiqi *et al.* 2004). Thus, ΔS values represent the divergence of color between Cinnamon Teal subspecies in relation to anseriform visual capabilities. Generally, at jnd = 1.0 for threshold, two colors are barely distinguishable under ideal conditions, and as jnd

becomes larger two colors are more easily discernable under worsening viewing conditions (Siddiqi *et al.* 2004).

Statistical analysis of spectral data. Average receptor quantum catches for each feather patch were used in the color discrimination model, and thus color differences among subspecies generated by the model might be misleading if the variance in coloration between subspecies is too large. Thus, a multivariate analysis of variance (MANOVA) was performed to evaluate the overall differences in receptor quantum catch of each cone (Q_1–Q_4) among subspecies for each sex. Analysis of variance (ANOVA) and pairwise comparisons for the average receptor quantum catch of each cone for each feather patch were performed using a general linear model with Bonferroni-correction for multiple comparisons.

RESULTS

Color divergence was greatest for most plumage areas between *A. c. septentrionalium* and either *A. c. cyanoptera* or *A. c. orinomus* (Table 5.1). For example, considering the crown and speculum of males, ΔS comparing North American to South American subspecies was ~2–5 times larger than ΔS comparing between South American subspecies. The same pattern was observed for both female plumage patches, as well. Color divergence between South American subspecies was relatively low for all plumage patches for males and females, with the exception of male cheek color. ΔS values for this plumage area were relatively large among all three subspecies (Table 5.1).

We observed statistical differences in overall color in males (MANOVA: Wilks' $\lambda = 0.0381, F_{(56, 26)} = 1.92, P = 0.036$) but not in females (MANOVA: Wilks' $\lambda = 0.2225$,

$F_{(16, 14)} = 0.98$, $P = 0.52$). Significant differences for male plumage in average receptor quantum catches in each cone (Q_1–Q_4) were found only between *A. c. septentrionalium* and the two South American subspecies (Table 5.2). No significant differences were observed for quantum catches in any cone among any of the subspecies for female plumage (Table 5.3).

DISCUSSION

There was striking plumage color divergence among Cinnamon Teal subspecies, when color differences were analyzed from an avian visual perspective. Some areas of the plumage (e.g., crown and speculum) differed to a degree that should be easily distinguishable to the ducks, thus representing novel plumage signals (e.g., $\Delta S > 2$). Additionally, some plumage areas have diverged to a lesser degree, but still above the threshold for visual discrimination (e. g., ΔS values between 1 and 2). These differences represent potentially biologically significant differences for birds (Siddiqi *et al.* 2004, Eaton 2005), and thus could function as visual signals to the ducks, although the large variances in coloration for many of these plumage patches raise questions about their utility as reliable subspecies visual indicators (Tables 5.2 and 5.3). Nonetheless, the variation in color shown herein provides the raw material for selection to operate on plumage colors in Cinnamon Teal populations, assuming that coloration is heritable for a plumage area.

Signaling systems and color patterns are subject to a variety of selection pressures influenced by many aspects of life (e.g., mating success and foraging; Burtt, Jr. 1981, Endler 1992, Saetre 2000), as well as stochastic processes (e.g., genetic drift). It is

unclear if the observed color divergence in Cinnamon Teal is a result of (1) genetic drift, (2) local natural selection acting upon plumage patterns to maximize signal strength in the particular environment of each subspecies, or (3) sexual selection acting to promote assortative mating. However, our results reveal plumage color differences that, to date, were unknown for Cinnamon Teal, thus providing the contextual basis for testing evolutionary hypotheses as future behavioral and genetic data are collected. Furthermore, use of avian visual modeling for analyses of plumage color morphology offers a powerful tool for quantifying geographic variation, and even individual variation, of color patterns among birds.

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Table 5.1. Color discriminability of (ΔS) among Cinnamon Teal subspecies (*Anas cyanoptera orinomus*, 7 females, 10 males; *A. c. cyanoptera*, 8 females, 21 males; and *A. c. septentrionalium*, 3 female, 12 males) using the Vorobyev-Osorio color discrimination model. Values > 1.0 just noticeable differences indicate distinguishable differences using the avian visual system under ideal viewing conditions.

	Pairwise comparisons		
	cyanoptera vs *orinomus*	*cyanoptera* vs *septentrionalium*	*orinomus* vs *septentrionalium*
Male			
Crown	1.07	3.30	2.25
Cheek	1.76	1.32	2.42
Breast	1.30	1.19	0.82
Blue wing patch	0.29	1.03	0.76
White wing covert	0.25	0.37	0.59
Speculum	0.61	3.16	3.57
Blue tertial	0.12	0.34	0.39
Female			
Blue wing patch	0.59	1.65	2.23
Speculum	1.03	1.56	2.58

Table 5.2. Average receptor quantum catches of each of the four cones for each feather patch on male Cinnamon Teal (*Anas cyanoptera*) subspecies.

Feather	*A. c. orinomus* (n = 10)	*A. c. cyanoptera* (n = 21)	*A. c. septentrionalium* (n = 12)	P^1
	Mean (SE)	Mean (SE)	Mean (SE)	
Crown				
Q_1[2]	371.3 (25.8)	341.5 (13.6)*	408.5 (28.5)*	0.071
Q_2	269.0 (22.1)	237.1 (9.7)*	314.8 (24.7)*	0.007
Q_3	247.8 (20.6)	215.5 (8.7)*	307.1 (26.9)*	0.001
Q_4	365.7 (32.8)^	307.1 (13.7)*	481.6 (47.7) ^*	0.001
Breast				
Q_1	546.0 (47.6)	523.9 (27.6)	563.6 (30.7)	0.684
Q_2	398.6 (32.9)	386.7 (21.8)	427.9 (24.6)	0.506
Q_3	398.6 (28.4)	404.3 (22.9)	441.6 (26.5)	0.503
Q_4	769.2 (40.9)	808.9 (41.4)	826.8 (45.2)	0.721
Cheek				
Q_1	362.2 (38.2)	306.5 (12.2)*	381.5 (30.9)*	0.057
Q_2	282.1 (27.3)	238.9 (10.8)*	321.6 (26.1)*	0.010
Q_3	311.5 (24.3)^	278.7 (13.2)*	388.9 (29.0)^*	0.001
Q_4	675.8 (37.3)^	642.3 (27.9)*	854.2 (50.7)^*	0.001
Blue wing patch				
Q_1	2010.0 (128.0)	2372.0 (136.0)	2071.0 (126.0)	0.138
Q_2	1282.4 (70.9)	1494.1 (80.1)	1352.0 (78.6)	0.183
Q_3	956.6 (46.4)	1104.1 (54.9)	1023.1 (56.9)	0.202
Q_4	1080.4 (50.3)	1246.2 (59.6)	1184.5 (66.3)	0.209
White wing covert				
Q_1	4377.0 (224.0)	4610.0 (195.0)	4278.0 (318.0)	0.579
Q_2	3141.0 (187.0)	3360.0 (137.0)	3145.0 (200.0)	0.544
Q_3	2583.0 (161.0)	2775.0 (114.0)	2625.0 (147.0)	0.558
Q_4	3261.0 (212.0)	3505.0 (147.0)	3347.0 (176.0)	0.592
Speculum				
Q_1	400.0 (24.4)	437.0 (26.3)	498.3 (32.0)	0.111
Q_2	301.0 (15.7)	335.2 (22.0)	348.9 (22.6)	0.414
Q_3	491.9 (36.4)	526.1 (37.8)	514.7 (36.2)	0.839
Q_4	350.2 (31.3)	387.5 (30.5)*	467.5 (32.3)*	0.081

Table 5.2 continued.

Feather	*A. c. orinomus* (n = 10)	*A. c. cyanoptera* (n = 21)	*A. c. septentrionalium* (n = 12)	P^1
	Mean (SE)	Mean (SE)	Mean (SE)	
Blue tertial				
Q_1	1920.3 (83.0)	2024.0 (88.6)	2144.0 (107.0)	0.372
Q_2	1223.8 (48.8)	1302.3 (54.4)	1375.9 (70.3)	0.312
Q_3	859.2 (35.8)	913.3 (33.7)	971.0 (45.1)	0.215
Q_4	912.8 (38.4)	971.0 (31.3)	1049.4 (48.1)	0.098

[1]ANOVAs for subspecies effect. Means with same symbol within a row are different as determined using Bonferroni corrected *P*-values ($P_{adjusted} < 0.1$).
[2]Q_1 is receptor quantum catch of the violet sensitive cone (VS), Q_2 the short-wave sensitive cone (SWS), Q_3 the middle-wave sensitive cone (MWS), and Q_4 the long-wave sensitive cone (LWS).

Table 5.3. Average receptor quantum catches of each of the four cones for each feather patch on female Cinnamon Teal (*Anas cyanoptera*) subspecies.

Feather	*A. c. orinomus* (n = 7)	*A. c. cyanoptera* (n = 8)	*A. c. septentrionalium* (n = 3)	P^1
	Mean (SE)	Mean (SE)	Mean (SE)	
Blue wing patch				
Q_1[2]	1722.0 (149.0)	1711.3 (97.3)	1601.3 (61.7)	0.844
Q_2	1137.0 (80.7)	1146.5 (55.4)	1123.8 (35.8)	0.981
Q_3	899.8 (49.6)	913.8 (37.3)	932.0 (40.3)	0.912
Q_4	1058.1 (48.9)	1102.1 (36.6)	1177.6 (81.9)	0.368
Speculum				
Q_1	370.5 (64.4)	350.3 (47.4)	510.0 (165.0)	0.420
Q_2	286.5 (45.3)	275.5 (32.9)	412.0 (141.0)	0.331
Q_3	268.0 (40.0)	260.5 (28.6)	402.0 (141.0)	0.260
Q_4	354.0 (55.2)	361.3 (41.9)	592.0 (225.0)	0.190

[1]ANOVAs for subspecies effect.
[2]Q_1 is receptor quantum catch of the violet sensitive cone (VS), Q_2 the short-wave sensitive cone (SWS), Q_3 the middle-wave sensitive cone (MWS), and Q_4 the long-wave sensitive cone (LWS).

CHAPTER 6

SPECIMEN SHRINKAGE IN CINNAMON TEAL[1]

ABSTRACT

Body size measurements from freshly collected birds and dried museum specimens were used to evaluate specimen shrinkage in Cinnamon Teal (*Anas cyanoptera*). Six of seven body measurements of female Cinnamon Teal differed significantly after specimen drying, whereas five of seven male body measurements differed. The largest amount of shrinkage was in bill height, bill width, and tarsus length. Bill length at nares showed no significant shrinkage, suggesting it is a more conservative measurement than exposed culmen and, therefore, a more reliable method for accurately measuring bill length. Correction values for body size measurements are reported for future waterfowl studies combining measurements of both live birds and museum specimens.

[1]Wilson, R. E. and K. G. McCracken. 2008. Specimen shrinkage in Cinnamon Teal. Wilson Journal of Ornithology 120:390–392.

INTRODUCTION

Specimen shrinkage during the process of drying is common. Shrinkage can cause analytical problems if not properly corrected in studies involving live or freshly killed birds and museum specimens (e.g., Winker 1996). Correction for shrinkage is needed before applying to live birds when developing classification criteria for sex, subspecies, or species based on morphological features from museum specimens (Greenwood 1979, Jenni and Winkler 1989, Winker 1993). For example, Mueller (1990) reported that a shrinkage value of 1.72% would comprise 34% of wing length differences between male and female Saw-whet Owls (*Aegolius acadicus*). In addition, the amount of shrinkage varies among body parts and species (Winker 1993).

Shrinkage values from one taxon may have limited use outside that particular taxon or similar morphological species because of the morphological diversity of birds and variety of preparation techniques (Jenni and Winkler 1989, Winker 1993). Specimen shrinkage in waterfowl has yet to be investigated. This paper reports shrinkage values for Cinnamon Teal (*Anas cyanoptera*), which may be used to develop correction values for similar sized (~350–550 g) waterfowl.

METHODS

Cinnamon Teal are widespread throughout the Western Hemisphere and five subspecies currently are recognized (Snyder and Lumsden 1951, Delacour 1956, American Ornithologists' Union 1957, Johnsgard 1978, Gammonley 1996). The three most widespread subspecies of Cinnamon Teal (*A. c. cyanoptera*, *A. c. orinomus*, and *A. c. septentrionalium*; 26 females, 80 males) were collected in Argentina (2003), Peru

(2002), and western United States (2002–2003) as part of a larger population genetic and morphological study. Even though subspecies are distinct in overall body size, there is overlap in measurements among subspecies (R. E. Wilson, unpubl. data). Therefore, different subspecies were pooled for each sex to ascertain the extent of shrinkage for each measurement.

Seven body measurements were recorded for each bird (± 0.1 mm unless otherwise indicated; Baldwin et al. 1931): wing chord length (carpal joint to longest primary feather unflattened; ± 1 mm), tail length (± 1 mm), exposed culmen length (edge of forehead feathers to anterior edge of nail), bill length at nares (anterior edge of nares to anterior edge of nail), total tarsus length (top of bent knee to bottom of foot), bill height (height of upper mandible at nares), and bill width (width of upper mandible at nares). Measurements were taken the same day specimens were collected prior to preparation as museum specimens (fresh measurements), and subsequently 9 months to 2 years after preparation (dry measurements from standard museum round skins) by the same individual (R. E. Wilson) with the same set of calipers. The right wing and tarsus were used for fresh and dry measurements of each specimen. Voucher specimens are archived at the University of Alaska Museum (Fairbanks, Alaska). A paired *t*-test was used to compare differences between fresh and dry measurements. Pearson correlation values were used to examine the relationship between body mass and percent shrinkage.

RESULTS

Five of the seven measurements for males had significant differences after drying (Table 6.1). There was no significant change in tail length or bill length at nares, but both

measurements showed an increase after drying. All other measurements had > 1% percent decrease with bill height and bill width having the largest shrinkage. Percent shrinkage of total tarsus length (Pearson correlation = 0.255, $P = 0.023$) and culmen length (Pearson correlation = –0.356, $P = 0.001$) had a significant relationship with body mass.

Six of the seven body measurements for females had significant differences after drying (Table 6.1). Bill length at nares had no significant difference. All measurements except tail length decreased after drying with bill width and total tarsus having the greatest amount of shrinkage. There were no significant relationships between any of the shrinkage measurements and body mass.

DISCUSSION

Cinnamon Teal had significant changes after specimen preparation for most measurements. Specimen preparation may have contributed to differences between measurements besides the drying process. The bills of specimens were tied to keep them closed during the drying process in the field. Tying of bills may have squeezed the bill together, slightly decreasing bill width. Tail length increased after drying for House Sparrows (*Passer domesticus*) and was attributed to the retraction of the intercalamal skin (Bjordal 1983).

Bill length is an important descriptor for studying feeding ecology (Borras et al. 2000) and subspecies classification (e.g., Ridgway 1902, Hall 1996). Therefore, it is critical to have a bill measurement that is repeatable and accurate. There are several ways to measure bill length, with the three main alternatives being total culmen length,

exposed culmen length, and length from the nares (Baldwin et al. 1931). Fjeldså (1980) suggested the amount of shrinkage of the exposed culmen will vary according to bill anatomy and, thus, one universal correction factor would not be applicable to all bird species. This has led to the suggestion that bill length from the anterior edge of the nares is the most reliable bill measurement as both end points are easily defined (Winker 1998, Borras et al. 2000). This study confirms the recommendation, in particular for waterfowl, that bill length should be measured from the nares, especially if no correction factors are available.

The range of shrinkage values of –3.7 to 6.4% for Cinnamon Teal is comparable to other studies, which report values ranging from –1.5 to 4.0% depending on the body measurement. Correction values to convert dry measurements ranged from 1.000 to 1.068 for measurements that decreased and 0.887 (males, not significant) and 0.965 (females) for tail lengths, which increased (Table 6.1). Winker (1993) suggested correction values that ranged from 0.960–0.996 (fresh to dry) which converts to 1.004–1.040 (dry to fresh). No previous data describing specimen shrinkage have been reported for waterfowl to our knowledge; the values reported here provide general correction factors for future studies of morphology in similar sized waterfowl.

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Table 6.1. Effects of shrinkage on body measurements of Cinnamon Teal with correction values from dried specimens to live birds.

		Mean length (mm)								Correction
Sex	*n*	Fresh	SE	Dry	SE	t^1	*P*	Shrinkage %	SE	factor
Males										
Wing chord	80	194.51	1.57	191.00	1.49	8.44	<0.001	2.12	0.25	1.018
Total tarsus	80	41.92	0.26	40.46	0.23	11.21	<0.001	3.43	0.30	1.036
Tail	80	83.04	0.74	93.66	0.95	-0.91	0.366	-0.83	0.83	0.887
Bill nare	80	35.07	0.20	35.08	0.19	-0.04	0.968	-0.03	0.18	1.000
Bill culmen	80	45.63	0.23	45.10	0.26	4.29	<0.001	1.16	0.27	1.012
Bill height	80	13.72	0.09	12.97	0.10	8.01	<0.001	5.36	0.66	1.058
Bill width	80	16.89	0.08	15.95	0.11	11.98	<0.001	6.44	0.54	1.059
Females										
Wing chord	26	189.69	2.88	185.46	2.59	5.25	<0.001	2.17	0.39	1.023
Total tarsus	26	41.56	0.42	39.89	0.42	4.64	<0.001	3.94	0.84	1.042
Tail	26	81.54	1.58	84.49	1.89	-2.35	0.027	-3.73	1.53	0.965
Bill nare	26	32.79	0.28	32.7	0.30	0.71	0.483	0.28	0.38	1.003
Bill culmen	26	43.04	0.38	42.55	0.38	2.54	0.018	1.11	0.44	1.012
Bill height	26	13.06	0.17	12.6	0.20	2.48	0.020	3.45	1.42	1.037
Bill width	26	16.34	0.19	15.3	0.20	5.29	<0.001	6.21	1.15	1.068

[1] *t*-value from paired sample *t*-test.

CONCLUSIONS

Cinnamon Teal (*Anas cyanoptera*) are distributed along elevational and latitudinal gradients, and within these gradients climatic and habitat variables change abruptly, resulting in spatial heterogeneity in selection pressures across the species' range. High elevation appears to have played a major role in influencing the spatial variation in morphological and molecular divergence within South American Cinnamon Teal; patterns of variation in morphological characteristics correspond to highland and lowland subspecies pairs in the Colombian and central high Andes. Larger individuals occupied higher elevations in the Andes (*A. c. orinomus* and *A. c. borreroi*) and occurred at higher latitudes in Patagonia (*A. c. cyanoptera*), whereas smaller conspecifics resided at lower elevations in temperate regions (*A. c. cyanoptera*, *A. c. septentrionalium*, and *A. c. tropica*), a pattern consistent with Bergmann's Rule.

Spatial variance in morphometrics is coupled with striking plumage color divergence among three Cinnamon Teal subspecies when color differences were analyzed from an avian visual perspective. Some areas of the plumage (e.g., crown and speculum) differed to a degree that should be easily distinguishable to the ducks, thus representing novel plumage signals (e.g., $\Delta S > 2$). These signals may promote assortative mating, thus increasing the mating success of the most common genotype in a particular environment (Lenormand 2002) and further reinforcing phenotypic and genetic divergence observed within Cinnamon Teal. Additionally, some plumage areas have diverged to a lesser degree, but still above the threshold for visual discrimination (e.g., ΔS values between 1 and 2). These differences represent potentially biologically

significant differences for birds (Siddiqi et al. 2004, Eaton 2005), and thus could function as visual signals to the ducks, although the large variances in coloration for many of these plumage patches raise questions about their utility as reliable subspecies visual indicators. Nonetheless, the variation in color shown herein provides the raw material for selection to operate on plumage colors in Cinnamon Teal populations, assuming that coloration is heritable for a plumage area.

Individuals occurring at high elevations in the central high Andes are confronted with multiple selection pressures, such as a colder and hypoxic environment, which present a physiological challenge to living at elevations above 3,500 m. Little evidence of genetic subdivision was detected between highland (*A. c. orinomus*) and lowland (*A. c. cyanoptera*) for mitochondrial DNA and five nuclear introns. However, highland Cinnamon Teal were shown to have a single amino acid polymorphism at the α-globin (Asn/Ser-α9) and to have much larger body size, whereas lowland individuals generally lacked this allele and have a smaller body size. This amino acid substitution is located on the exterior of the A helix and is known to undergo an important conformational during the transition from the deoxy to the oxy state, and alterations to this site may result in a higher oxygen affinity (Perutz 1990, McCracken et al. 2009b). Higher oxygen affinity in hemoglobin has been shown repeatedly to be an important evolutionary response to hypoxia (McCracken et al. 2009a,b).

Coalescent analyses revealed strong restricted gene flow for the αA subunit (< 1 migrant per generation) compared to neutral nuclear markers over evolutionary time, and both highland and lowland populations showed a high immigrant ancestry assignment

based on all nuclear markers (hemoglobin and introns) combined to their native elevation. Transplant experiments have demonstrated that lowland bird populations have difficulty successfully breeding at high elevation (Monge and Leon-Velarde 1991), suggesting that selection pressures imposed by hypoxia are the main cause of low hatchability of eggs (Visschedijk et al. 1980). At elevations of 4,000 m or greater there is a shift in physiological mechanism regulating gas exchange from conservation of water and CO_2 at low altitudes to mechanisms improving O_2 availability (Carey 1994). Adult hemoglobin appears by day six during embryonic development (León-Velarde and Monge-C 2004), and if the observed amino acid substitution confers a higher oxygen affinity it would ensure a high O_2 content in the blood necessary for embryonic development and growth at high elevations. Individuals possessing mismatched genotypes were found in Cinnamon Teal and Yellow-billed Pintail (*A. georgica*; McCracken et al. 2009c), indicating that individuals can disperse into the highlands as they can initially acclimate to hypoxia via multiple physiological pathways. However, the susceptibility of the avian embryo to hypoxia most likely limits these individuals from breeding in the highlands.

Finally, Cinnamon Teal and Blue-winged Teal (*A. discors*) are two closely related species with shallow genetic divergence. Although being morphologically similar in size, Blue-winged Teal and Cinnamon Teal males show strikingly divergent plumage, not only in overall body coloration, but also in head and neck coloration. This was clearly reflected in color discrimination analyses, with male cheek reflectance measurements yielding a large distance in avian perceptual color space between the two species ($\Delta S =$

11.4). Females also showed color differences between species in plumage patches, although this was restricted to breast plumage and was of a lesser degree than the differences found between males. Shallower plumage divergence observed for females is often the case between closely related avian species, wherein a major component of variation often results from differences in sexual ornaments used for courtship displays with little variation among juvenile and female plumages (West-Eberhard 1983, Price 1998). In addition, strong haplotype frequency differentiation and little haplotype sharing was observed between species and among Cinnamon Teal subspecies. North American and South American Cinnamon Teal studied here have limited contact, while wintering Blue-winged Teal individuals occur in sympatry with South American Cinnamon Teal during for part of the year. This limited overlap in breeding and/or overwintering distributions along with timing of breeding has likely restricted gene flow between continents following divergence. Although divergence times were broadly overlapping, this result would be expected in species complexes that have diverged rapidly, which is likely the case here. Where the taxa are parapatric or partially sympatric within continents, environmental selection pressures associated with high elevation (South America) and sexual selection (North America) might have played a major role in the diversification of this group.

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