Articles

Journal of Avian Biology 48: 640–649, 2017
doi: 10.1111/jav.00998

Subject Editor: Darren Irwin. Editor-in-Chief: Thomas Alerstam. Accepted 2 October 2016

Divergence and gene flow in the globally distributed blue-winged ducks

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The ability to disperse over long distances can result in a high propensity for colonizing new geographic regions, including uninhabited continents, and lead to lineage diversification via allopatric speciation. However, high vagility can also result in gene flow between otherwise allopatric populations, and in some cases, parapatric or divergence-with-gene-flow models might be more applicable to widely distributed lineages. Here, we use five nuclear introns and the mitochondrial control region along with Bayesian models of isolation with migration to examine divergence, gene flow, and phylogenetic relationships within a cosmopolitan lineage comprising six species, the blue-winged ducks (genus *Anas*), which inhabit all continents except Antarctica. We found two primary sub-lineages, the globally-distributed shoveler group and the New World blue-winged/cinnamon teal group. The blue-winged/cinnamon sub-lineage is composed of sister taxa from North America and South America, and taxa with parapatric distributions are characterized by low to moderate levels of gene flow. In contrast, our data support strict allopatry for most comparisons within the shovelers. However, we found evidence of gene flow from the migratory, Holarctic northern shoveler *A. clypeata* and the more sedentary, African Cape shoveler *A. smithii* into the Australasian shoveler *A. rhynchotis*, although we could not reject strict allopatry. Given the diverse mechanisms of speciation within this complex, the shovelers and blue-winged/cinnamon teals can serve as an effective model system for examining how the genome diverges under different evolutionary processes and how genetic variation is partitioned among highly dispersive taxa.

Lineages that have broad distributions and a high degree of vagility may experience diverse speciation processes throughout their geographic range (Proches and Ramdhani 2013). For example, high dispersal capabilities leading to the colonization of new areas can result in geographically isolated populations where genetic drift and local adaptations play a major role in diversification (Jordan and Snell 2008, McCusker and Bentzen 2010). However, having a high dispersal ability also increases the chance that gene flow can persist among geographically disjunct populations, resulting in secondary contact and possibly impeding the speciation process (Lenormand 2002, Feder et al. 2012, Nosil and Feder 2012, Seehausen et al. 2014).

High dispersal capabilities have resulted in at least 59 lineages of birds having achieved cosmopolitan distributions and being deemed global (Proches and Ramdhani 2013). Among these lineages, the genus *Anas* (i.e. the dabbling ducks) seems particularly predisposed for global radiations (Johnson and Sorenson 1999, Proches and Ramdhani 2013, Lavretsky et al. 2014a), which is likely attributable to their vagile tendencies. Within the genus *Anas*, the blue-winged duck complex, named after their prominent blue wing patch, inhabits all continents except Antarctica and comprises six species, including the northern shoveler *A. clypeata*, the red shoveler *A. platalea*, the Cape shoveler *A. smithii*, the Australasian shoveler *A. rhynchotis*, the blue-winged teal *A. discors*, and the cinnamon teal *A. cyanoptera*. Owing to their global distributions and propensity for long distance movements, the blue-winged ducks provide an excellent study system to assess how highly vagile taxa diverge, as species pairs can be found under allopatry, parapatry, and sympatry. Each shoveler species occupies a separate continent (Johnson and Sorenson 1999), where environmental limitations and geographic distance may be causing these taxa to diverge under strict allopatry. Conversely, among the blue-winged/cinnamon teal, examples of sympatry, parapatry and allopatry

can be observed. For instance, *A. c. septentrionalium* and *A. discors* are sympatric in North America along the western and northwestern regions of the United States and Canada (Snyder and Lumsden 1951, Swanson et al. 1974, Hubbard 1978, Small 1994, Howell and Webb 1995); although this overlap is relatively recent as *A. discors* has only been documented west of the Rocky Mountains since 1900 (Swanson et al. 1974). *Anas discors* has also extended its breeding distribution into the northern regions of South America where it is also a common winter migrant (Rohwer et al. 2002, Wilson et al. 2011). Moreover, in South America, cinnamon teal sub-species, *A. c. cyanoptera* and *A. c. orinomus*, are parapatric at the extreme edges of their ranges in the Andean highlands of Peru and Argentina (Wilson et al. 2011, 2013). These parapatric and sympatric distributions suggest that opportunities for gene flow might be high within this group. For instance, sequence data from mtDNA and two nuclear introns support gene flow between *A. c. cyanoptera* and *A. c. orinomus* and between *A. discors* and *A. c. septentrionalium* (Wilson et al. 2011).

Ducks are well-known for their ability to hybridize and produce fertile offspring, even between deeply divergent lineages (Johnsgard 1960, Tubaro and Lijtmaer 2002). Among the blue-winged ducks, wild hybrids occur in sympatry between *A. discors* and *A. cyanoptera*, *A. discors* and *A. clypeata*, and *A. cyanoptera* and *A. clypeata*, and at least some hybrid progeny are fertile (Johnsgard 1960, Bolen 1978). Although wild hybrids have not been documented between allopatric species, vagrant *A. clypeata* occasionally occur within the ranges of the other species (Close and Jaensch 1981, Marchant and Higgins 1990, Roesler et al. 2013), resulting in opportunities for gene flow. Furthermore, Wilson et al. (2011) reported genetic evidence of gene flow between allopatric breeding populations of *A. discors* and *A. c. cyanoptera*. *Anas discors* is an uncommon migrant in Peru (some may remain year-round) and Argentina where it can occur in sympatry with *A. c. cyanoptera* (Salvador and Salvador 1990, Schulenberg et al. 2010); however, there are no breeding records of *A. discors* in southern South America. Given their capacities for interspecific hybridization and dispersing over long-distances, gene flow among species inhabiting different continents seems probable.

We used multilocus data and coalescent methods to examine the evolutionary history of this group to identify the factors influencing the evolutionary trajectories of the blue-winged ducks. Specifically, we reconstructed a species tree using sequences from mitochondrial DNA (mtDNA) and five nuclear DNA (nuDNA) introns, and compared this tree to previously published trees reconstructed from mtDNA (Johnson and Sorenson 1999) and morphology (Livezey 1991). Secondly, we determined the level of population genetic divergence and used a Bayesian model of isolation with migration to test for the presence of gene flow among species pairs diverging in allopatry and sympatry/parapatry. We predicted that taxa diverging in sympatry/parapatry (e.g. *A. c. septentrionalium* and *A. discors*) will show lower levels of divergence and higher rates of gene flow than those diverging in allopatry. However, given the high dispersal capabilities of these species, we predicted that species from different continents will also experience gene flow, especially between the migratory *A. clypeata* and the more sedentary species from the southern hemisphere. In contrast, divergence among allopatric, non-migratory species pairs will be better explained by local adaptations and stochastic processes such as genetic drift. Elucidating the interactions between population connectivity and genetic divergence within a species complex will contribute to our understanding of the dynamics of speciation in globally distributed lineages.

Methods

Sampling methods and compiling genetic data

We used previously published genetic datasets of South American cinnamon teal (mtDNA and 5 nuclear introns; Wilson et al. 2013) and North American cinnamon and blue-winged teal (mtDNA and 2 nuclear introns; Wilson et al. 2011). From these studies, vouchered specimens of *A. c. cyanoptera* (n = 52 individuals), *A. c. orinomus* (n = 49) *A. c. septentrionalium* (n = 70), and *A. discors* (n = 76) were collected in Argentina, Bolivia, Colombia, Peru, and the United States (Fig. 1). Homologous data for northern shovelers from widespread locations in North America (n = 48) and Eurasia (n = 36) were obtained from Peters et al. (2014). *Anas smithii* samples were collected from southern Africa (n = 24; Caron et al. 2010, Cumming et al. 2013), vouchered specimens of *A. platalea* were collected from central and southern South America (n = 24), and *A. rhynchotis* were collected from Australia (n = 19) and New Zealand (n = 4) (Fig. 1). Specimen information including locality and Genbank accession numbers from previous studies is archived at the USGS Alaska Science Center data repository (<http://dx.doi.org/10.5066/F7T72FK7>).

DNA sequencing

Genomic DNA was extracted from muscle tissue or blood using a Qiagen DNeasy tissue kit. For each sample, we amplified and sequenced five nuclear introns (ENO1, PCK1, ODC1, GRIN1, and FGB; collective total of 1501 bp) and the mitochondrial control region (658–983 bp), following protocols described by McCracken et al. (2009a, b). PCR products were cleaned using magnetic AMPure beads (Beckman Coulter, Indianapolis, IN). Direct sequencing was performed on an ABI 3730. All sequences for *A. c. cyanoptera*, *A. c. orinomus*, and *A. clypeata*, as well as mtDNA, ENO1 and ODC1 sequences for *A. c. septentrionalium* and *A. discors*, have been published previously (McCracken et al. 2009a, b, Wilson et al. 2011, 2013, Peters et al. 2014). All new sequences have been deposited in GenBank (accession numbers KX535810–KX536421).

To determine the gametic phase for each intron, we used PHASE ver. 2.1.1 (Stephens et al. 2001). The program PHASE is a Bayesian statistical method for reconstructing haplotypes form genetic data while incorporating Hardy–Weinberg equilibrium and linkage disequilibrium. We constructed input files using seqPHASE (Flot 2010), treating the blue-winged/cinnamon teal and the shoveler lineages in separate analyses. For each analysis, we ran 1000 iterations with a burn-in of 1000 generations.

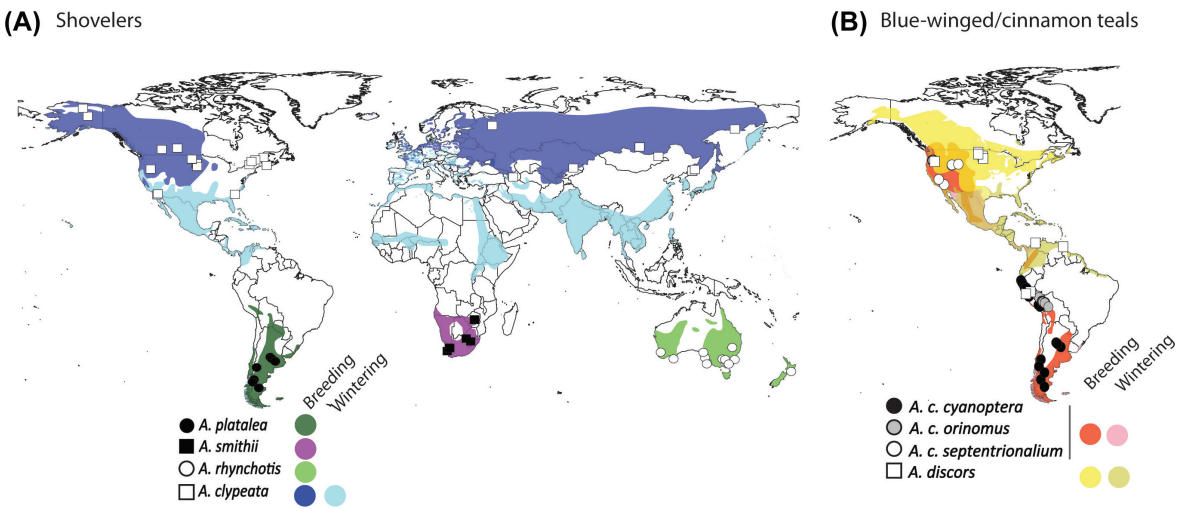

Figure 1. The distributions (shaded regions) and sampling locations (symbols) for (A) four species of shoveler and (B) two species and three sub-species from the blue-winged/cinnamon teal.

Population structure and genetic diversity

To estimate levels of genetic differentiation (F_{ST}: the proportion of total genetic diversity partitioned between populations) and nucleotide diversity (π: the average proportion of nucleotide differences for a given locus between pairs of individuals within a population) for each locus, we used DNAsp ver. 5.10 (Librado and Rozas 2009). We constructed haplotype networks for each locus using the median-joining algorithm in NETWORK ver. 5.0 (Bandelt et al. 1999). We also used the Bayesian clustering method in the program SplitsTree ver. 4.13 that computes phylogenetic networks based on genetic distances among individuals (Huson and Bryant 2006) and provides a framework for visualizing genetic variation within and among populations. Phylogenetic networks illustrate alternative connections among individuals that might arise from hybridization or recombination, and therefore, can be superior to bifurcating phylogenetic trees when examining sequences from multiple unlinked loci. We constructed splits trees using consensus sequences of the concatenated nuDNA (with heterozygous sites coded with IUPAC ambiguity codes) and a second tree using mtDNA only. We used P-distances with ambiguities treated as the average state (e.g. T vs C is a distance of 1.0, whereas Y vs C is a distance of 0.5).

Coalescent analysis

We estimated phylogenetic relationships within the blue-winged complex using the coalescent program *BEAST ver. 1.7.4 (Heled and Drummond 2010). *BEAST uses Bayesian analysis incorporating a Markov Chain Monte Carlo (MCMC) in species-tree estimation (Heled and Drummond 2010). Two separate phylogenetic analyses were performed using the constant population size prior; one analysis included the five nuclear loci and mitochondrial DNA and the other included only nuclear loci. Using MEGA ver. 6.0 (Tamura et al. 2013) we estimated the optimal substitution rate based on the maximum likelihood of different models. The HKY substitution model, which allows for different substitution rates between transitions and transversions, was selected for all loci. Each phylogeny including the puna teal *A. puna*, silver teal *A. versicolor* (McCracken et al. 2009a,b), hottentot teal *A. hottentota*, garganey *A. querquedula*, and Baikal teal *A. formosa* (unpubl.), and we treated populations of *A. clypeata* and *A. rhynchotis* from different landmasses as separate operational taxonomic units (i.e. North America vs Eurasia and Australia vs New Zealand, respectively). For each analysis, we ran 10 000 000 iterations, sampling every 1000 MCMC steps following a burn-in of 1 000 000 steps. The posterior set of trees was analyzed in DensiTree (Bouckaert 2010) to create a phylogeny where MCMC simulations are visible and not averaged together. To obtain a consensus tree with posterior distributions we used TreeAnnotator ver. 1.7 (Drummond et al. 2012) discarding the first 1000 trees as burn-in.

To estimate levels of gene flow we used the isolation with migration model IMa2 (Hey and Nielsen 2004). IMa2 is a coalescent model that uses genetic data to infer evolutionary histories and different demographic parameters (Hey and Nielsen 2004). Because the full data set was too cumbersome to analyze using IMa2, we ran four separate analyses: 1) a four-population model that included the four species of shovelers (allopatric populations), 2) a four-population model that included the blue-winged teal and the three sub-species of cinnamon teals (allopatric and parapatric populations), 3) a three-population model that included *A. clypeata* from North America, *A. discors*, and the North American cinnamon teal *A. c. septentrionalium* (parapatric and sympatric populations), and 4) a two-population model that included *A. platalea* and lowland cinnamon teal, *A. c. cyanoptera*, from South America (sympatric populations). These subsets were chosen to address specific questions: is there gene flow among species/subspecies within each sub-lineage, and is there more gene flow between lineages within sympatry? IMa2 assumes no gene flow from any other populations that are not included in the analysis, and therefore, any gene flow from another species could bias migration estimates in our models (Hey and Nielsen 2004). However, these biases are small, especially when gene flow from

Table 1. Summary of the descriptive statistics for all loci used in this study. Note that the bolded values for Tajima's D are statistically significant (p-value < 0.05).

Locus name	Length (bp)	Haplotypes	Polymorphic sites	Tajima's D
ENO1	271	40	38	–1.47819
GRIN1	274	154	52	–1.02496
FGB	216	22	21	**–1.74003**
ODC1	274	35	40	**–2.0597**
PCK1	301	19	21	–1.53843
mtDNA	652	167	106	–0.45659

an unsampled population is low (Strasburg and Rieseberg 2010), and our models generally include the taxa that are most likely to exchange genes.

Here, we estimated Θ (where $\Theta = 4N_e\mu$, and N_e is the effective population size and μ is the mutation rate per locus per generation) for each contemporary population and the ancestral populations, $2Nm$ (which is $\Theta m_{im}/2$, here m_{im} is the immigration rate relative to the mutation rate estimated in IMa2, N is the population size for the population receiving migrants, and m is the proportion of the population consisting of immigrants each generation) between each pair of species/sub-species, and t (where $t = T/\mu$, and T is the number of generations since divergence). Thus, our analyses included a total of 22 parameters for four-population models, 13 parameters for the three-population model, and 6 parameters for the two-population model). Because IMa2 assumes only recombination between loci and not within a locus, we used IMgc (Woerner et al. 2007) to detect violations of the four-gamete test and to truncate loci to be consistent with no recombination. We defined the species trees for each run based on the results of *BEAST. We ran one cold chain and 59 hot chains with geometric heating for 1 000 000 generations as a burn in and then sampled parameters every 200 generations for 30 000 000 iterations; preliminary runs indicated that this number of iterations were required for parameter convergence. The analysis was replicated with a different random number seed to check for convergence, and all effective sample size (ESS) values were > 50 in all runs (following Hey 2005).

Results

Genetic structure of the blue-winged ducks

Across all loci, the number of haplotypes varied from 19 to 167 with an overall average of 46 polymorphic sites (see Table 1 for more summary statistics). Within the blue-winged ducks and among nuclear loci, nucleotide diversity ranged between 0.0 and 0.022 substitutions/site with a mean of 0.0072 substitutions/site. For mtDNA, nucleotide diversity ranged from 0.0001 to 0.0096 substitutions/site with a mean of 0.0044 substitutions/site. Across all pairwise comparisons, F_{ST} ranged from 0.080 to 0.938 for all nuclear loci, and from 0.072 to 0.955 for mtDNA (Table 2). Overall differentiation was highest between the shovelers and the blue-wing/cinnamon teal group and among all pairwise comparisons of shoveler except *A. clypeata* and *A. rhynchotis* (pairwise $F_{ST} > 0.2$ in most cases; Table 2). Haplotype networks for each nuDNA locus revealed many shared alleles among taxa, both within and between subgroups (Supplementary material Appendix 1, Fig. A1). However, based on neighbor-net trees computed in SplitsTree for the concatenated nuDNA loci, individuals mostly clustered with individuals from the same sub-lineage; the blue-winged/cinnamon teal sub-lineage and shoveler sub-lineage (Fig. 2A). Within the shoveler sub-lineage, *A. platalea* and *A. smithii* appeared to be the most structured; all individuals from each of these species clustered with other individuals of the same species to the exclusion of other species. These patterns of clustering coincided well with pairwise F_{ST} values. However, *A. clypeata* and *A. rhynchotis* had weaker levels of genetic differentiation and clustering analysis showed a high level of admixture between these species (Fig. 2A). Additionally, the blue-winged/cinnamon teal sub-lineage had very weak clustering which also was supported by low levels of genetic differentiation (Table 2). Interestingly, four samples of *A. discors* and one sample of *A. c. septentrionalium* clustered with the shovelers, and one sample of *A. rhynchotis* clustered within the blue-winged/cinnamon teals (Fig. 2A).

In contrast to nuDNA, the mtDNA neighbor-net tree was consistent with high genetic differentiation within the

Table 2. Pairwise F_{ST} values among eight taxa of blue-winged ducks. Mitochondrial DNA above the diagonal, nuclear DNA (average among five loci) below the diagonal.

	A. rhynchotis	*A. smithii*	*A. clypeata*	*A. platalea*	*A. c. cyanoptera*	*A. c. orinomus*	*A. c. septentrionalium*	*A. discors*
A. rhynchotis	–	0.7922	0.5358	0.8741	0.8258	0.8489	0.8062	0.8328
A. smithii	0.4086	–	0.7872	0.9326	0.8993	0.9227	0.8866	0.9051
A. clypeata	0.0967	0.2434	–	0.8633	0.8113	0.8388	0.7895	0.8204
A. platalea	0.3213	0.2802	0.2946	–	0.8868	0.9131	0.8794	0.8965
A. cyanoptera								
cyanoptera	0.3086	0.4072	0.2665	0.3025	–	0.0723	0.4137	0.2527
orinomus	0.2572	0.3225	0.1815	0.2962	0.0972	–	0.4768	0.4175
septentrionalium	0.2023	0.2662	0.1343	0.2824	0.0796	0.0531	–	0.4823
A. discors	0.1864	0.2415	0.1019	0.3061	0.1435	0.0699	0.0233	–

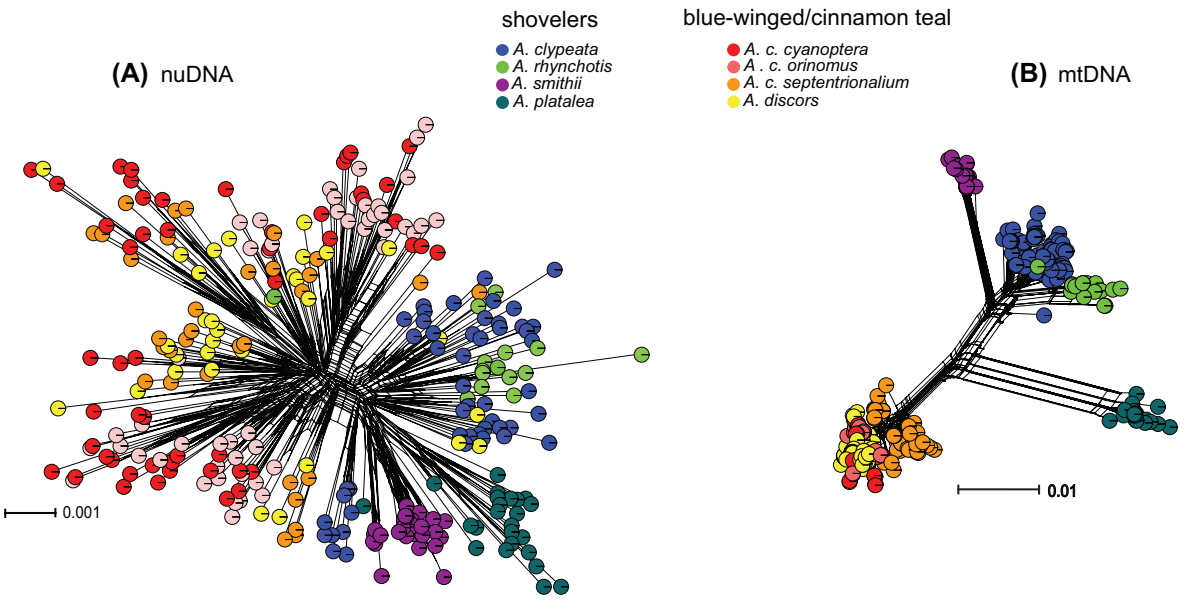

Figure 2. Neighbor-net trees constructed in the program SplitsTree for the blue-winged duck complex from (A) concatenated nuDNA at five loci (1501 aligned nucleotides) and (B) mtDNA (652 aligned nucleotides). Branch lengths are based on P-distances. Note that individuals representing the shoveler sub-lineage are displayed in 'cold' colors (i.e. blue, purple, teal, and green), and individuals representing the cinnamon teal sub-lineage are displayed in 'hot' colors (i.e. red, orange, yellow, and pink).

shoveler sub-lineage, with the vast majority of individuals grouping within species-specific lineages (mean $F_{ST} = 0.826$). The only two exceptions were that an mtDNA haplotype from one *A. clypeata* individual fell outside the *A. clypeata*/*rhynchotis* cluster, and one *A. rhynchotis* mtDNA haplotype (sampled from four individuals from New Zealand) grouped within the *A. clypeata* cluster (Fig. 2B). Much lower differentiation was observed within the blue-winged/cinnamon teal sub-lineage, although haplotypes from *A. c. septentrionalium* mostly clustered within a distinct group (also see Wilson et al. 2011).

Phylogenetic reconstruction

The analysis of nuDNA in *BEAST recovered the shoveler sub-lineage and the blue-winged/cinnamon sub-lineage to be reciprocally monophyletic (Fig. 3). This relationship was also supported by levels of genetic differentiation for both nuDNA and mtDNA ($F_{ST} = 0.254$ and 0.860 between sub-lineages, respectively). Shallow divergences were recovered between *A. c. septentrionalium* and *A. discors*, between *A. c. cyanoptera* and *A. c. orinomus* (posterior support = 1.0), between Old and New World *A. clypeata* (posterior support = 0.99) and between Australasian subspecies, *A. r. rhynchotis* and *A. r. variegata* (posterior support = 0.99). However, we found weak posterior support for *A. smithii* as sister to the *A. clypeata*/*rhynchotis* group (posterior support = 0.37), and for the monophyly of the shovelers (posterior support = 0.47). Note that an appreciable number of posterior trees recovered *A. platalea* as sister to the blue-winged/cinnamon teal group (Fig. 3), rendering the shovelers as paraphyletic. Including both mtDNA and nuDNA in the *BEAST analysis did not change the tree topology, and most relationships received higher posterior support, including the monophyly of the shoveler sub-lineage (posterior support = 0.48).

Estimates of gene flow (2Nm) from Ima2

Within the shoveler sub-lineage, we could not reject a scenario of no gene flow into *A. clypeata*, *A. smithii*, or *A. platalea* from any of the other shoveler species (Fig. 4A, B, D, respectively). In general, posterior distributions peaked at or near zero and confidence intervals were narrow. In contrast, we found evidence of low, asymmetrical gene flow into *A. rhynchotis* from both *A. clypeata* (2*Nm* = 0.5 migrants

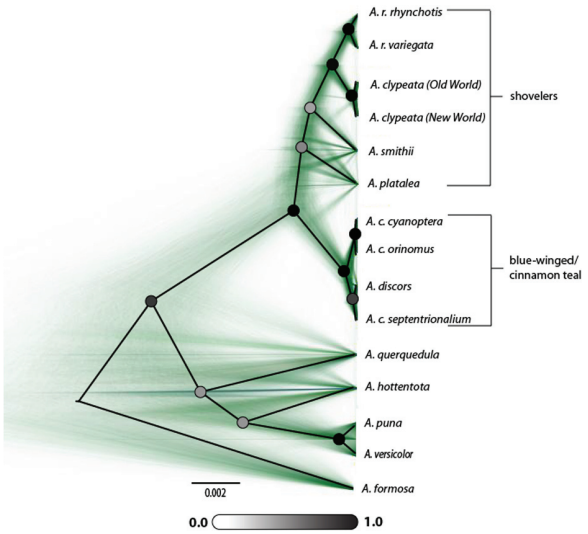

Figure 3. The evolutionary relationships of the blue-winged duck complex reconstructed using *BEAST and five nuclear introns. Shaded regions illustrate all 1000 posterior trees and the dark lines show the consensus phylogeny. The shaded circles represent the overall posterior support for each node, where lighter colors reflect lower posterior support and darker colors reflect stronger posterior support as illustrated in the lower bar. Branch lengths are given in units of mutations per site.

per generation) and *A. smithii* ($2Nm = 0.16$ migrants per generation) (Fig. 4C). In both cases, however, the 95% highest posterior distribution included our lowest bin of migration rates, suggesting that the data are also consistent with complete isolation.

In contrast, within the blue-winged/cinnamon teal sublineage, prominent traces of gene flow were detected from *A. c. orinomus* into *A. c. cyanoptera* ($2Nm =$ six migrants per generation) and in the reverse direction ($2Nm =$ two migrants per generation) (Fig. 5A, B). Strong levels of intracontinental gene flow were also found from *A. discors* into *A. c. septentrionalium* ($2Nm =$ nine migrants per generation) and in the opposite direction from *A. c. septentrionalium* into *A. discors* ($2Nm =$ five migrants per generation (Fig. 5C, D)). However, confidence intervals overlapped zero in the latter direction. Although we also found some evidence of intercontinental gene flow from *A. discors* into *A. c. cyanoptera* ($2Nm = 0.81$ migrants per generation, but note the high posterior probability associated with the lowest bin in Fig. 5B), our best estimates (the peaks in the posterior distributions) suggested more prominent gene flow between parapatric populations than between allopatric populations. Finally, we did not find evidence of gene flow between sympatric species of shovelers and *A. discors* or cinnamon teal in either North America or South America; all posterior distributions of $2Nm$ peaked at or near zero (Supplementary material Appendix 1, Fig. A2–A3).

Discussion

Phylogenetic relationships

In this study we used a multi-locus approach to examine the processes shaping patterns of genetic divergence within the blue-winged duck complex. Our multi-locus dataset supported reciprocal monophyly between the shovelers and blue-winged/cinnamon teals, which agrees with a morphological tree reconstructed from 157 phenotypic characters (Livezey 1991). In contrast, a mtDNA gene tree recovered the shovelers as being paraphyletic with respect to the blue-winged/cinnamon teals (Johnson and Sorenson 1999). Specifically, *A. platalea* was sister to a clade comprising the remaining shovelers, blue-winged teal, and cinnamon teals. However, this sister relationship received low (< 50%) bootstrap support, and the many reticulations in

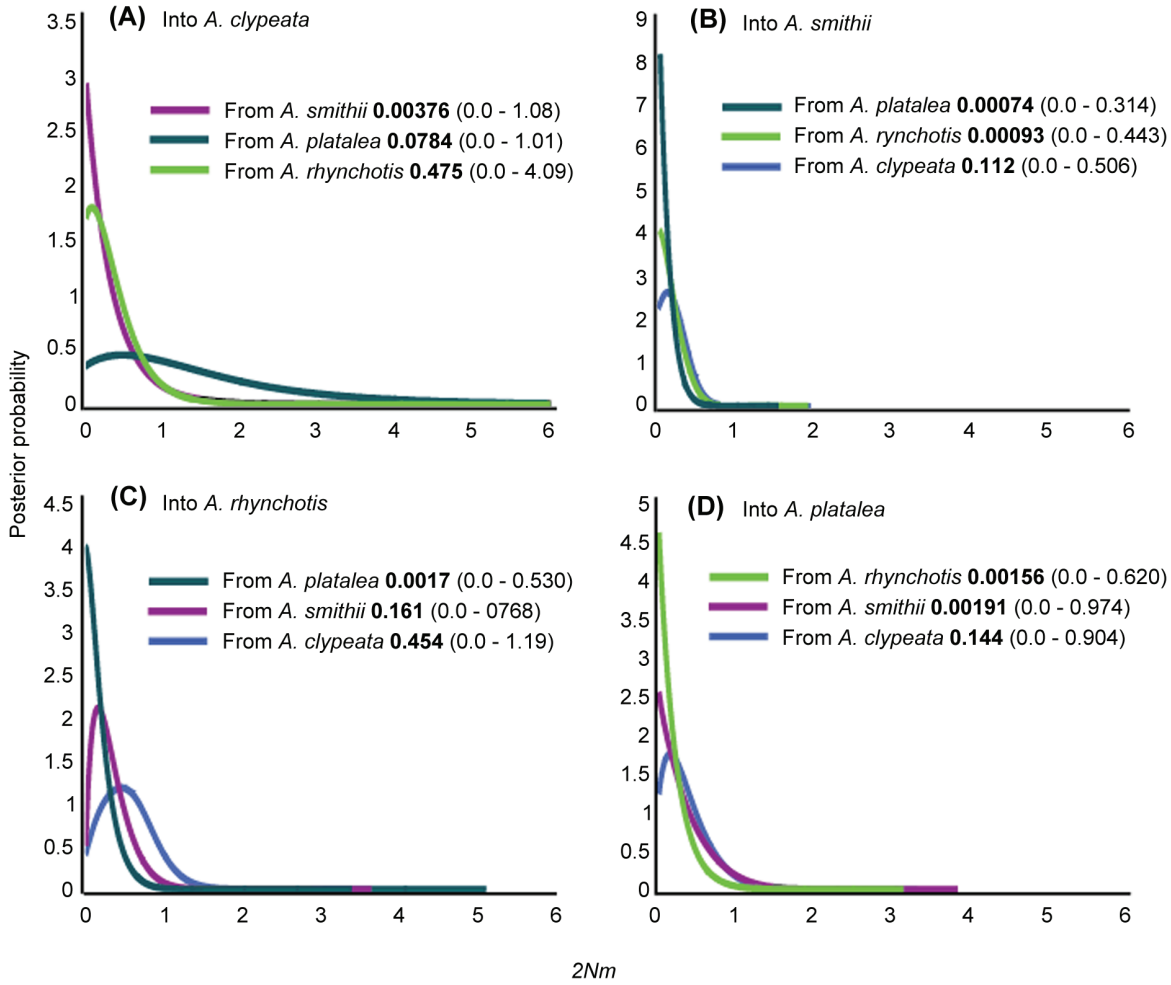

Figure 4. Estimates of the number of immigrants per generation for each of the shoveler sub-lineages: *A. clypeata* (A), *A. smithii* (B), *A. rhynchotis* (C), and *A. platalea* (D). Values in bold represent the highest posterior probability and the values in parentheses are the 95% highest posterior densities.

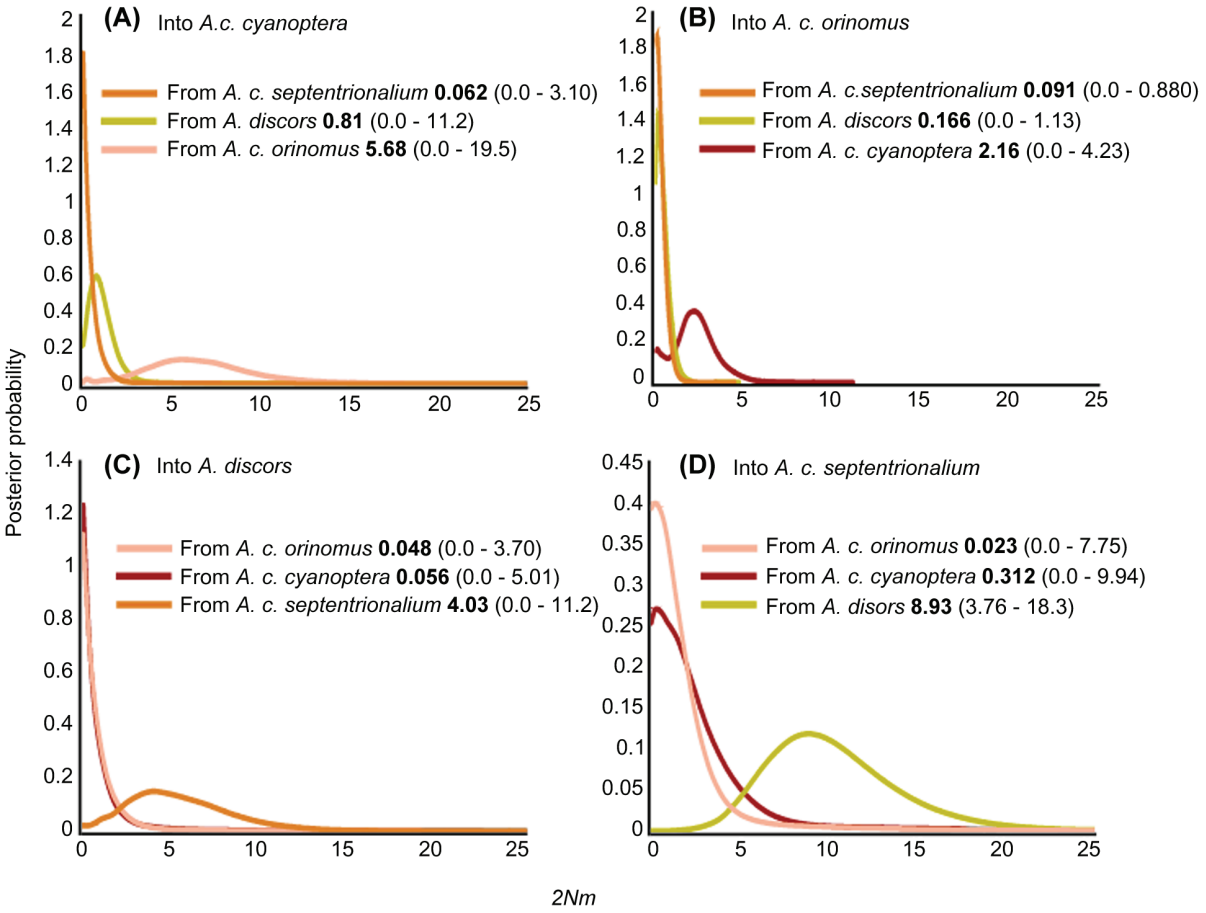

Figure 5. Estimates of the number of immigrants per generation for each taxon of the blue-winged/cinnamon teal complex: *A. c. cyanoptera* (A), *A. c. orinomus* (B), *A.discors* (C), and *A. c. septentrionalium* (D). Values in bold represent the highest posterior probability and the values in parentheses are the 95% highest posterior densities.

our neighbor-net tree further illustrates the uncertainty in the mtDNA gene tree (Fig. 2B). In our multi-locus species tree reconstructions, we did find a small set of posterior trees supporting the paraphyly of the shovelers, but in this case, *A. platalea* was sister to the blue-winged/cinnamon teals (Fig. 3). Although a monophyletic shoveler lineage was the best-supported species tree overall, this lineage also received low posterior support (< 50%), and therefore, additional data may be needed to fully resolve phylogenetic relationships within this group of ducks.

Assuming that the mtDNA gene tree accurately reflects the history of this marker and that our species tree accurately reflects the history of population divergence and speciation, our results suggest conflict between mitochondrial and nuclear markers. Mito-nuclear discord can result from a number of processes, including stochastic lineage sorting, hybridization, sex-biased gene flow, and selection (Toews and Brelsford 2012). For example, because the effective population size (N_e) of mtDNA is a quarter the N_e of nuDNA, divergence and lineage sorting can happen at a much faster rate for mtDNA, causing evolutionary discord between the two genomes. The short internodes at the base of the mtDNA and nuDNA phylogenies (Johnson and Sorenson 1999; Fig. 2, 3) suggest rapid diversification, and therefore, we can expect high levels of conflict among different loci being driven by stochastic lineage sorting. As found in other studies (Machado et al. 2002, Faure et al. 2009, Bulgarella et al. 2012, Paupério et al. 2012, Lavretsky et al. 2014b), using a multi-locus approach allows us to account for the stochastic lineage sorting and conflict among loci that might otherwise lead to biases when inferring evolutionary relationships based on a single marker type. However, this conflict likely contributes to low support for phylogenetic relationships in the absence of large datasets.

Despite the conflict in relationships between shovelers and blue-winged/cinnamon teals recovered from mitochondrial and nuclear markers, our species tree does agree with the mitochondrial genealogy to some extent (Johnson and Sorenson 1999). For example, both mtDNA and our species tree support a sister relationship between *A. smithii* and the *A. clypeata*/*rhynchotis* group, whereas morphological characteristics (including skeletal and plumage characteristics) suggest that *A. clypeata*/*rhynchotis* is sister to *A. platalea* (Livezey 1991). Patterns of dichromatism are prevalent in *A. platalea*, *A. clypeata*, and *A. rhynchotis* but not in *A. smithii*, which might have contributed to this morphological grouping. In addition, our results agree with the mtDNA gene tree in suggesting that cinnamon teals are paraphyletic with respect to *A. discors*, although in that case *A. discors* was sister to *A. c. cyanoptera* rather than *A. c. septentrionalium* (Johnson and Sorenson 1999, Wilson et al. 2011). Wilson et al. (2011) suggested a South American origin for the

blue-winged/cinnamon teal complex; specifically, a South American cinnamon teal ancestor might have given rise to *A. discors* and *A. c. septentrionalium*, although the number of colonization and the order of divergence events were ambiguous (Wilson et al. 2011). The paraphyly of *A. cyanoptera* with respect to *A. discors* in our multi-locus species tree supports a single colonization of North America, followed by divergence between *A. discors* and *A. c. septentrionalium*. However, estimating species trees in *BEAST requires an assumption of no gene flow between operational taxonomic units (Heled and Drummond 2010), but we found strong evidence of gene flow between *A. discors* and *A. c. septentrionalium*. Gene flow, in this case, could have resulted from secondary contact following two independent colonizations of North America, which could mislead phylogenetic reconstructions. Analyzing larger datasets with methods that jointly estimate migration and phylogenies may be necessary to conclusively resolve the placement of *A. discors* within the blue-winged/cinnamon teal complex.

Estimated number of migrants per generation (2Nm)

The high dispersal abilities and high capacity for hybridization of the blue-winged duck complex have led to bouts of gene flow within both sub-lineages. Specifically, within the shoveler sub-lineage, evidence of unidirectional gene flow was found among allopatric species from different continents (from *A. clypeata* into *A. rhynchotis* and from *A. smithii* into *A. rhynchotis*), although confidence intervals included zero for both comparisons (Fig. 5A). Although gene flow is unexpected from *A. smithii*, the wintering range of the migratory *A. clypeata* has expanded into the Philippines and Malaysia (Van Weerd and Van der Ploeg 2004), and vagrant individuals from Australia and New Zealand were reviewed in Close and Jaensch (1981) and Marchant and Higgins (1990), as well as reported in public databases such as eBird (<www.ebird.org>; Sullivan et al. 2009) and the Atlas of Australian Birds (<www.birddats.com.au>). These vagrants provide opportunities for hybridization and gene flow between these otherwise allopatric species. For hybridization to occur it is likely that a small fraction of individuals must take up at least semi-residency in either Australia or New Zealand during the breeding season. As sightings in the Philippines have been reported between May and August (Van Weerd and Van der Ploeg 2004), it appears that at least some vagrant *A. clypeata* males have at least partially suspended their migration back to the typical northern breeding grounds. Although females of these two species are difficult to differentiate and therefore it is unknown if females are present year-round as well, the year-round presence of males raises the possibility they could be reproductively active thus providing an avenue for potential hybridization. Interestingly, all four of our samples of *A. rhynchotis* from New Zealand had mtDNA haplotypes that clustered more closely with haplotypes from *A. clypeata* than with *A. rhynchotis* from Australia suggesting mtDNA introgression and past female-mediated gene flow. To our knowledge there are no confirmed wild hybrids, suggesting that hybridization is probably rare.

Congruent with previous research (Wilson et al. 2011), we found evidence of gene flow between parapatric taxa in South America (*A. c. cyanoptera* and *A. c. orinomus*) and North America (*A. c. septentrionalium* and *A. discors*) within the blue-winged/cinnamon teal sub-lineage. Within central South America, cinnamon teal subspecies occur in sympatry at the extreme edges of the distribution of *A. c. orinomus* within the central High Andes (Wilson et al. 2011, 2013). Although sightings of lowland taxa in the highlands, and vice versa, have been reported for many species, the hypoxic environment of the High Andes likely restricts gene flow into the highlands as seen in other waterfowl (McCracken et al. 2009a, b, Bulgarella et al. 2012). Intermixing between subspecies also could occur at mid-elevations as observed in the crested duck *Lophonetta specularioides*, which is characterized by a well-defined hybrid zone at an elevation of ~ 2000 m (Bulgarella et al. 2012); however, cinnamon teal appear to be rare at mid-elevation as the lowest record for *A. c. orinomus* is approximately 1500 m based on a single bird (Wilson et al. 2013).

Within North America, bouts of gene flow may be dictated by seasonal breeding migrations between *A. c. septentrionalium* and *A. discors*. Distributions of *A. c. septentrionalium* and *A. discors* overlap more extensively in the western region of the United States and Canada where hybridization does occur (Lokemoen et al. 1990, Gammonley and Fredrickson 1995, Howell and Webb 1995, Wilson et al. 2011), although the extent of hybridization is unknown. We observed non-zero gene flow (CIs do not overlap zero) indicating that these two taxa maintained some level of genetic connectivity after divergence. This level of genetic exchange and directionality could potentially reflect the recent expansion of *A. discors* breeding ranges, although more data are needed to test this hypothesis. Overall, our results suggested more prominent gene flow between parapatric populations than between allopatric populations, although the broad posterior distributions overlapped zero for most of our comparisons.

Interestingly, Wilson et al. (2011) detected non-zero values for migration rates between *A. c. cyanoptera* and *A. c. septentrionalium*, suggesting inter-continental gene flow within the sub-lineage. However, this was not the case in this study where only intra-continental gene flow was detected as all confidence intervals between *A. c. cyanoptera* and *A. septentrionalium* broadly overlapped zero. One reason for this could reside in resolution power within genetic markers. For example, Wilson et al. (2011) used a total of three loci (two nuclear loci and one mitochondrial locus), which may result in a lack of resolution for coalescent simulations (Cruickshank and Hahn 2014). By using six total loci, we have increased the power and resolution. An alternative contributing factor could be Wilson et al.'s (2011) use of pairwise comparisons in the two-population IM models. Specifically, one of the assumptions when using IM is that there is no gene flow among populations that are not included in the analysis (Hey and Nielsen 2004). Thus, when running only a two-population model, any shared genetic variation that cannot be explained by ancestral variation between the two taxa may give a false positive of non-zero migration rates. In contrast, running analyses that include all taxa incorporate a full-migration matrix, accounting for sources of variation from all other taxa, and potentially reducing the occurrence of false positives. Overall, our estimates of gene flow within the blue-winged/cinnamon teal suggest that geographic distance and other environmental variables between North

and South America likely create strong barriers to gene flow resulting in allopatric divergence and speciation.

Conclusions

Studying the evolutionary histories among closely related taxa, at different stages of the speciation continuum, can reveal the propensity of different evolutionary forces to act on the process of divergence and ultimately speciation (Johnson and Sorenson 1999, Lenormand 2002). Here we show that the evolutionary relationships within the blue-winged duck complex suggest two reciprocally monophyletic sub-lineages. We suggest that gene flow is most common among parapatric sister taxa (especially in the case of *A. c. septentrionalium* and *A. discors*) and that more distantly related taxa experience nearly complete isolation and may be diverging via allopatric speciation. In the latter case, larger geographical structures (e.g. mountain ranges, bodies of water, and geographical distance) may be playing a role in reducing long distance dispersal events and subsequently decreasing the probability of hybridization. However, the high dispersal capability of the migratory *A. clypeata* may facilitate gene flow among otherwise allopatric populations. Because the blue-winged ducks show diverse patterns of speciation, this complex can serve as a model system for identifying how the genome diverges under different evolutionary processes and how genetic variation is partitioned among highly dispersive taxa.

Acknowledgements – This work was supported by a Start-up Grant and a Research Initiation Grant from the College of Science and Mathematics at Wright State Univ. to JLP. We thank the Univ. of Washington Burke Museum and the Louisiana State Univ. Museum of Natural Science for tissue loans. Additional sample collection was supported by grants from the Inst. of Arctic Biology, Alaska EPSCoR (NSF EPS-0092040 and EPS-0346770) and NSF (DEB-0444748) to KGM, by USAID via subcontract to the Wildlife Conservation Society's GAINS programme to GSC, and grants from the Delta Waterfowl Foundation and Frank M. Chapman Fund at the American Museum of Natural History to REW. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U. S. Government.

References

Bandelt, H., Forster, P. and Roehl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. – Mol. Biol. Evol. 16: 37–48.

Bolen, E. G. 1978. Notes on blue-winged teal × cinnamon teal hybrids. – Southwest. Nat. 23: 692–696.

Bouckaert, R. R. 2010. DensiTree: making sense of sets of phylogenetic trees. – Bioinformatics 26: 1372–1373.

Bulgarella, M., Peters, J. L., Kopuchian, C., Valqui, T., Wilson, R. E. and McCracken, K. G. 2012. Multilocus coalescent analysis of haemoglobin differentiation between low- and high-altitude populations of crested ducks (*Lophonetta specularioides*). – Mol. Ecol. 21: 350–368.

Caron, A., Abolnik, C., Mundava, J., Gaidet, N., Burger, C. E., Mochotlhoane, B., Bruinzeel, L., Chiweshe, N., de Garine-Wichatitsky, M. and Cumming, G. S. 2010. Persistence of low pathogen avian influenza virus in waterfowl in a southern African ecosytem. – Ecohealth 8: 109–115.

Close, D. H. and Jaensch, R. P. 1981. Northern shoveler at Coongie Lake. – S. Aust. Ornithol. 28: 178–179.

Cruickshank, T. E. and Hahn, M. W. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. – Mol. Ecol. 23: 3133–3157.

Cumming, G. S., Shepard, E., Okanga, S., Caron, A., Ndlovu, M. and Peters, J. L. 2013. Host associations, biogeography, and phylogenetics of avian malaria in southern African waterfowl. – Parasitology 140: 193–201.

Drummond, A. J., Suchard, M. A., Xie, D. and Rambaut, A. 2012. Bayesian phylogenetics with BEAUti: and BEAST 1.7. – Mol. Biol. Evol. 29: 1969–1973.

Faure, B., Jollivet, D., Tanguy, A., Bonhomme, F. and Bierne, N. 2009. Speciation in the deep sea: multi-locus analysis of divergence and gene flow between two hybridizing species of hydrothermal vent mussels. – PLoS One 4: e6485.

Feder, J. L., Egan, S. P. and Nosil, P. 2012. The genomics of speciation-with-gene-flow. – Trends Genet. 28: 342–350.

Flot, J. 2010. SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. – Mol. Ecol. Resour. 10: 162–166.

Gammonley, J. H. and Fredrickson, L. H. 1995. Life history and management of the blue-winged teal. – In: Cross, D. H. and Vohs, P. (eds), Waterfowl management handbook. United States Dept of the Interior, pp. 13.1.8.

Heled, J. and Drummond, A. J. 2010. Bayesian inference of species trees from multilocus data. – Mol. Biol. Evol. 27: 570–580.

Hey, J. 2005. On the number of New World founders: a population genetic portrait of the peopling of the Americas. – PLoS Biol. 3: e193.

Hey, J. and Nielsen, R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis.* – Genetics 167: 747–760.

Howell, S. N. and Webb, S. 1995. A guide to the birds of Mexico and northern Central America. – Oxford Univ. Press.

Hubbard, J. P. 1978. Revised check-list of the birds of New Mexico. – New Mexico Ornithological Society.

Huson, D. H. and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. – Mol. Biol. Evol. 23: 254–267.

Johnsgard, P. A. 1960. Hybridization in the Anatidae and its taxonomic implications. – Condor 62: 25–33.

Johnson, K. P. and Sorenson, M. D. 1999. Phylogeny and biogeography of dabbling ducks (genus: *Anas*): a comparison of molecular and morphological evidence. – Auk 116: 792–805.

Jordan, M. A. and Snell, H. L. 2008. Historical fragmentation of islands and genetic drift in populations of Galapagos lava lizards (*Microlophus albemarlensis* complex). – Mol. Ecol. 17: 1224–1237.

Lavretsky, P., Hernández-Baños, B. E. and Peters, J. L. 2014a. Rapid radiation and hybridization contribute to weak differentiation and hinder phylogenetic inferences in the New World mallard complex (*Anas* spp.). – Auk 131: 524–538.

Lavretsky, P., McCracken, K. G. and Peters, J. L. 2014b. Phylogenetics of a recent radiation in the mallards and allies (Aves: *Anas*): inferences from a genomic transect and the multispecies coalescent. – Mol. Phylogenet. Evol. 70: 402–411.

Lenormand, T. 2002. Gene flow and the limits to natural selection. – Trends Ecol. Evol. 17: 183–189.

Librado, P. and Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. – Bioinformatics 25: 1451–1452.

Livezey, B. C. 1991. A phylogenetic analysis and classification of recent dabbling ducks (Tribe Anatini) based on comparative morphology. – Auk 108: 471–507.

Lokemoen, J. T., Duebbert, H. F. and Sharp, D. E. 1990. Homing and reproductive habits of mallards, gadwalls, and blue-winged teal. – Wildl. Monogr. 106: 3–28.
Machado, C. A., Kliman, R. M., Markert, J. A. and Hey, J. 2002. Inferring the history of speciation from multilocus DNA sequence data: the case of *Drosophila pseudoobscura* and close relatives. – Mol. Biol. Evol. 19: 472–488.
Marchant, S. and Higgins, P. J. 1990. Handbook of Australian, New Zealand and Antarctic birds. Volume 1. Part B. Australian pelican to ducks. – Oxford Univ. Press.
McCracken, K. G., Barger, C. P., Bulgarella, M., Johnson, K. P., Kuhner, M. K., Moore, A. V., Peters, J. L., Trucco, J., Valqui, T. H. and Winker, K. 2009a. Signatures of high-altitude adaptation in the major hemoglobin of five species of andean dabbling ducks. – Am. Nat. 174: 631–650.
McCracken, K. G., Bulgarella, M., Johnson, K. P., Kuhner, M. K., Trucco, J., Valqui, T. H., Wilson, R. E. and Peters, J. L. 2009b. Gene flow in the face of countervailing selection: adaptation to high-altitude hypoxia in the betaA hemoglobin subunit of yellow-billed pintails in the Andes. – Mol. Biol. Evol. 26: 815–827.
McCusker, M. R. and Bentzen, P. 2010. Historical influences dominate the population genetic structure of a sedentary marine fish, Atlantic wolffish (*Anarhichas lupus*), across the North Atlantic Ocean. – Mol. Ecol. 19: 4228–4241.
Nosil, P. and Feder, J. L. 2012. Genomic divergence during speciation: causes and consequences. – Phil. Trans. R. Soc. B 367: 332–342.
Paupério, J., Herman, J., Melo-Ferreira, J., Jaarola, M., Alves, P. and Searle, J. 2012. Cryptic speciation in the field vole: a multilocus approach confirms three highly divergent lineages in Eurasia. – Mol. Ecol. 21: 6015–6032.
Peters, J. L., Winker, K., Millam, K. C., Lavretsky, P., Kulikova, I., Wilson, R. E., Zhuravlev, Y. N. and McCracken, K. G. 2014. Mito-nuclear discord in six congeneric lineages of Holarctic ducks (genus *Anas*). – Mol. Ecol. 23: 2961–2974.
Proches, S. and Ramdhani, S. 2013. Eighty-three lineages that took over the world: a first review of terrestrial cosmopolitan tetrapods. – J. Biogeogr. 40: 1819–1831.
Roesler, I., Hofland, R., De Lange, G. G., Hendriks, K. P., Duivenvoorde, F. V., Overman, W. G., Schrijvershof, P. G. and Roos, M. T. 2013. El pato cuchara boreal (*Anas clypeata*) en Argentina: ¿divagante austral o escapado? – Nuestras Aves 58: 23–34.
Rohwer, F. C., Johnson, W. P. and Loos, E. R. 2002. Blue-winged teal (*Anas discors*). – In: Poole, A. (ed.), The birds of North America online. Cornell Lab of Ornithology, Ithaca, <http://bna.birds.cornell.edu/bna/species/625>.
Salvador, S. A. and Salvador, L. A. 1990. Nuevos hallazgos en Argentina de *Anas discors*, *Lophornis chalybea* y *Tyrannus tyrannus*. – Hornero 13: 178–179.
Schulenberg, T. S., Stotz, D. F., Lane, D. F., O'Neill, J. P. and Parker III, T. A. 2010. Birds of Peru. – Princeton Univ. Press.
Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., Peichel, C. L., Saetre, G., Bank, C., Braennstroem, A., Brelsford, A., Clarkson, C. S., Eroukhmanoff, F., Feder, J. L., Fischer, M. C., Foote, A. D., Franchini, P., Jiggins, C. D., Jones, F. C., Lindholm, A. K., Lucek, K., Maan, M. E., Marques, D. A., Martin, S. H., Matthews, B., Meier, J. I., Moest, M., Nachman, M. W., Nonaka, E., Rennison, D. J., Schwarzer, J., Watson, E. T., Westram, A. M. and Widmer, A. 2014. Genomics and the origin of species. – Nat. Rev. Genet. 15: 176–192.
Small, A. 1994. California birds. Their status and distribution. – IBIS Press.
Snyder, L. L. and Lumsden, H. G. 1951. Variation in *Anas cyanoptera*. – Occ. Pap. R. Ontario Mus. Zool. 10: 1–18.
Stephens, M., Smith, N. J. and Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. – Am. J. Hum. Genet. 68: 978–989.
Strasburg, J. L. and Rieseberg, L. H. 2010. How robust are "isolation with migration" analyses to violations of the IM model? A simulation study. – Mol. Biol. Evol. 27: 297–310.
Sullivan, B. L., Wood, C. L., Iliff, M. J., Bonney, R. E., Fink, D. and Kelling, S. 2009. eBird: a citizen-based bird observation network in the biological sciences. – Biol. Conserv. 142: 2282–2292.
Swanson, G. A., Meyer, M. I. and Serie, J. R. 1974. Feeding ecology of breeding blue-winged teals. – J. Wildl. Manage. 38: 396–407.
Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. – Mol. Biol. Evol. 30: 2725–2729.
Toews, D. P. and Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. – Mol. Ecol. 21: 3907–3930.
Tubaro, P. L. and Lijtmaer, D. A. 2002. Hybridization patterns and the evolution of reproductive isolation in ducks. – Biol. J. Linn. Soc. 77: 193–200.
Van Weerd, M. and Van der Ploeg, J. 2004. Surveys of wetlands and waterbirds in Cagayan valley, Luzon, Philippines. – Forktail 20: 33–39.
Wilson, R. E., Eaton, M. D., Sonsthagen, S. A., Peters, J. L., Johnson, K. P., Simarra, B. and McCracken, K. G. 2011. Speciation, subspecies divergence, and paraphyly in the cinnamon teal and blue-winged teal. – Condor 113: 747–761.
Wilson, R. E., Peters, J. L. and McCracken, K. G. 2013. Genetic and phenotypic divergence between low-and-high-altitude populations of two recently diverged cinnamon teal subspecies. – Evolution 67: 170–184.
Woerner, A. E., Cox, M. P. and Hammer, M. F. 2007. Recombination-filtered genomic datasets by information maximization. – Bioinformatics 23: 1851–1853.

Supplementary material (Appendix JAV-00998 at <www.avianbiology.org/appendix/jav-00998>). Appendix 1.