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Mito-nuclear discord in six congeneric lineages of Holarctic ducks (genus *Anas*)

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Abstract

Many species have Holarctic distributions that extend across Europe, Asia and North America. Most genetics research on these species has examined only mitochondrial (mt) DNA, which has revealed wide variance in divergence between Old World (OW) and New World (NW) populations, ranging from shallow, unstructured genealogies to deeply divergent lineages. In this study, we sequenced 20 nuclear introns to test for concordant patterns of OW–NW differentiation between mtDNA and nuclear (nu) DNA for six lineages of Holarctic ducks (genus *Anas*). Genetic differentiation for both marker types varied widely among these lineages (idiosyncratic population histories), but mtDNA and nuDNA divergence within lineages was not significantly correlated. Moreover, compared with the association between mtDNA and nuDNA divergence observed among different species, OW–NW nuDNA differentiation was generally lower than mtDNA divergence, at least for lineages with deeply divergent mtDNA. Furthermore, coalescent estimates indicated significantly higher rates of gene flow for nuDNA than mtDNA for four of the six lineages. Thus, Holarctic ducks show prominent mito-nuclear discord between OW and NW populations, and we reject differences in sorting rates as the sole cause of the within-species discord. Male-mediated intercontinental gene flow is likely a leading contributor to this discord, although selection could also cause increased mtDNA divergence relative to weak nuDNA differentiation. The population genetics of these ducks contribute to growing evidence that mtDNA can be an unreliable indicator of stage of speciation and that more holistic approaches are needed for species delimitation.

Keywords: Anatidae, comparative phylogeography, dabbling ducks, genetic drift, selection, sex-biased gene flow

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Introduction

Many species from taxonomically diverse groups, including more than 100 species of birds, have Holarctic distributions that extend across North America, Europe and Asia. The complex glacial history of the northern hemisphere throughout the Pleistocene, coupled with idiosyncratic dispersal capabilities among taxa, has created diverse patterns of intercontinental differentiation in mitochondrial (mt) DNA, ranging from deeply divergent lineages to weak or undetectable haplotype frequency differences among species of mammals (e.g. Hundertmark *et al.* 2002; Brunhoff *et al.* 2003; Aubry *et al.* 2009; Davison *et al.* 2011), birds (e.g. Zink *et al.* 1995; Drovetski *et al.* 2004; Buehler & Baker 2005; Humphries & Winker 2011), fishes (e.g. Brunner *et al.* 2001; Kontula & Vainola 2003; Elmer *et al.* 2008),

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invertebrates (e.g. Weider *et al.* 1999; Forister *et al.* 2004; Todisco *et al.* 2012) and plants (e.g. Eidesen *et al.* 2007). This gradient suggests wide variation in divergence times, magnitude of gene flow or both.

Most research on Holarctic birds has focused only on mtDNA, although studies of nuclear (nu) DNA sequences are emerging (Zink *et al.* 2006; Pavlova *et al.* 2008; Peters *et al.* 2008, 2012a,b; Drovetski *et al.* 2010, 2014; Sonsthagen *et al.* 2011). Under neutral coalescence, mtDNA and nuDNA are expected to provide concordant estimates of population-level parameters, because polymorphisms in both types of markers are influenced by the same species-specific evolutionary history. Although apparent discordance can arise from the faster mutation rate and coalescence of mtDNA, which has one-quarter the effective population size (N_e) of nuDNA (Moore 1995; Hudson & Turelli 2003; Zink & Barrowclough 2008), coalescent models can accommodate and adjust for these differences (e.g. Hey & Nielsen 2004). However, mito-nuclear discordance can also arise from other processes, including mtDNA introgression, male-mediated gene flow, large disparities in population sizes and selection acting on one of the genomes (reviewed in Toews & Brelsford 2012). In many cases, these processes can cause mtDNA to be structured despite high nuclear gene flow. Indeed, mito-nuclear discord might be common in Holarctic taxa (Humphries & Winker 2011; Peters *et al.* 2012a,b; Drovetski *et al.* 2014). Given the wealth of information about the evolutionary histories of Holarctic taxa obtained from mtDNA and some evidence that this single-locus approach may not accurately reflect organismal lineage history, additional studies of nuDNA differentiation are needed.

Our primary objective was to determine whether nuDNA differentiation is concordant with mtDNA divergence in six lineages of Holarctic ducks that vary in mitochondrial and phenotypic differentiation. First, we compare population genetic structure between mtDNA and 20 independent nuDNA loci under the hypothesis that genetic differentiation is correlated between marker types. Second, we compare estimates of mtDNA and nuDNA gene flow for each lineage. If mtDNA is less likely to move between continents as a result of male-biased dispersal or selection, then we expect higher estimates of gene flow from nuDNA than from mtDNA.

Study taxa

Holarctic waterfowl have wide variation in morphological and mtDNA divergence (Pearce *et al.* 2004, 2005, 2009; Kulikova *et al.* 2005; Peters *et al.* 2008, 2012a,b; Humphries & Winker 2011; Kraus *et al.* 2011; Sonsthagen *et al.* 2011). Six lineages of dabbling ducks (genus *Anas*) are codistributed across North America (NW) and Eurasia (OW; Fig. 1). These include four monotypic species (no recognized subspecies) that are morphologically undifferentiated across this range (gadwall *Anas strepera*, northern pintail *A. acuta*, northern shoveler *A. clypeata* and mallard *A. platyrhynchos*), one species that is subdivided into subspecies (common teal *A. crecca crecca* in Eurasia, green-winged teal *A. c. carolinensis* in North America) and one species pair ('northern' wigeons: Eurasian wigeon *A. penelope* and American wigeon *A. americana*).

At one extreme, the gadwall and pintail have shallow mtDNA genealogies that lack distinct phylogroups (Peters *et al.* 2005, 2008; Flint *et al.* 2009). At the other extreme, teal have OW and NW mtDNA lineages that are 5.8% divergent in mtDNA-coding regions, whereas mallard and wigeon are intermediate, ~0.6% and 2.0% divergent, respectively (Johnson & Sorenson 1999; Humphries & Winker 2011). Population differentiation for shoveler has not been examined in detail, although Johnson & Sorenson (1999) reported identical haplotypes for one individual per continent. For all species previously examined in detail (pintail, gadwall, mallard and wigeon), population structure within continents has been either undetected (Flint *et al.* 2009; Fleskes *et al.* 2010; Kulikova & Zhuravlev 2010) or limited to small, peripheral populations that differ from other regions in haplotype frequencies (Peters *et al.* 2008; Kraus *et al.* 2011; Kulikova *et al.* 2012).

Methods

We sampled 50 individuals (25 per continent) from each of the six duck lineages from widely distributed locations across the Holarctic (Fig. 1) for a total of 300 individuals (Table S1, Supporting information). Samples were collected at various times of the year and included breeding, migrating and wintering individuals. We categorized samples as OW or NW based on their sampling locality for all species except wigeon, which have diagnostic plumage differences in both sexes. Two individuals sampled from North America were OW wigeon by plumage (Peters *et al.* 2005).

We sequenced 20 noncoding nuclear loci, covering more than 6 kbp of sequence and mapping to 20 different chromosomes in the chicken (*Gallus gallus*) genome (Peters *et al.* 2012c). Primers and protocols followed the study by Peters *et al.* (2012c; see Table S2, Supporting information, for additional details). Some of these data were published previously, including one locus in a subset of wigeon, 8 loci in teal, 20 loci in gadwall and 20 loci in a subset of our mallard samples (Peters *et al.* 2005, 2012b,c, 2014; Lavretsky *et al.* 2014; Table S3,

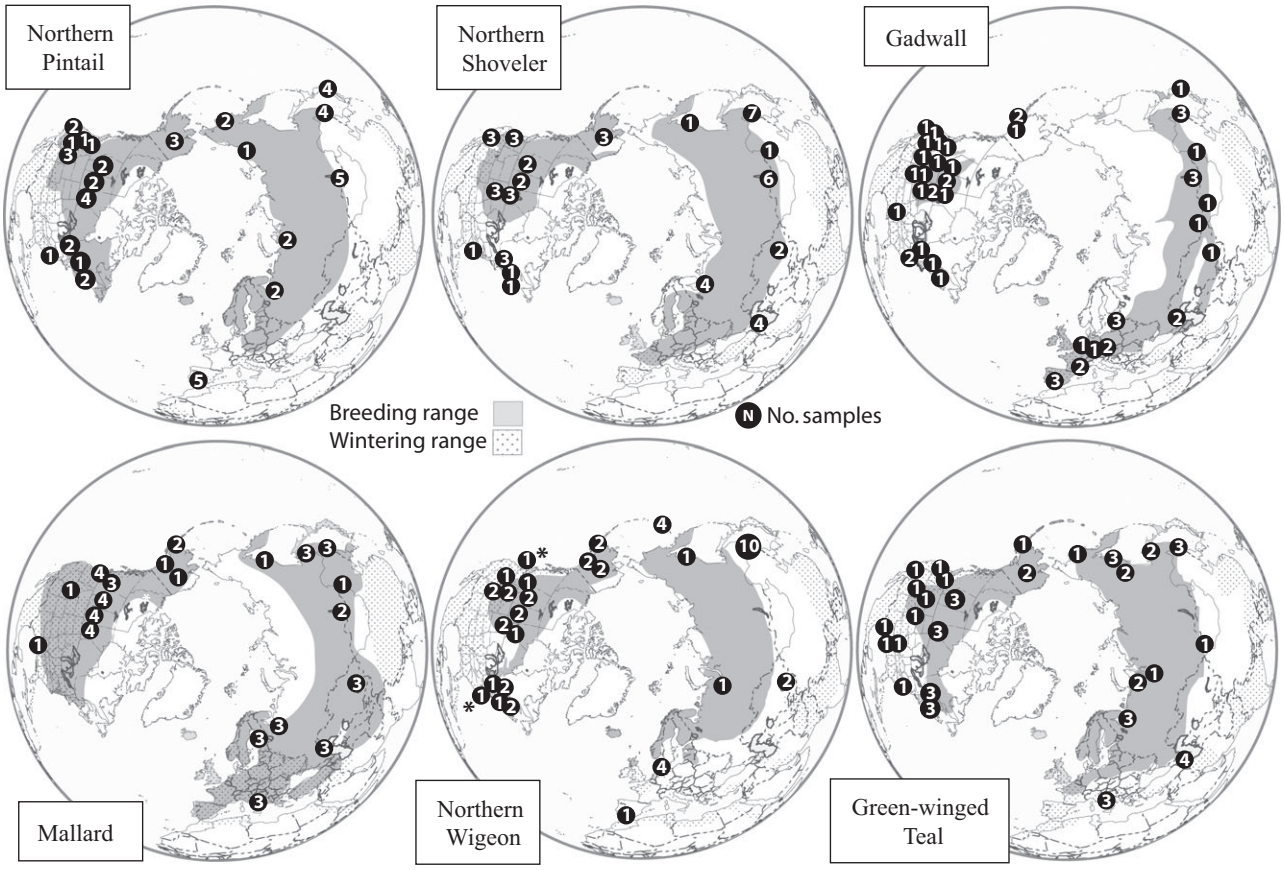

Fig. 1 Distributions of six lineages of *Anas* ducks and sample origins for 300 individuals (25 individuals/continent/lineage) sequenced for the mtDNA control region and 20 nuclear introns. The asterisks (*) in the northern wigeon panel indicate two Eurasian wigeon sampled from North America. Numbers within circles indicate the number of individuals sampled for nuDNA from each site [see Table S1 (Supporting information) for additional details and the distribution of mtDNA samples].

Supporting information). We obtained 658–667 bp of mtDNA control region sequences for each species from published data sets (Kulikova *et al.* 2005, 2012; Peters *et al.* 2005, 2008, 2012b, 2014; Lavretsky *et al.* 2014; Table S3, Supporting information), supplementing these data with new sequences. We used mtDNA sequences for 33–86 individuals/continent/lineage, totalling 590 individuals (Table S1, Supporting information).

We used three strategies to resolve the gametic phases of nuDNA haplotypes when sequences contained multiple polymorphisms. First, sequences that were heterozygous for indels were resolved by comparing the ambiguous 3′-end and unambiguous 5′-end of the forward and reverse chromatograms. Because indels shift peaks downstream of the indel, we could determine linkage among polymorphic sites and the indel, thus resolving gametic phase (Peters *et al.* 2007). Second, we used Bayesian methods in PHASE 2.1 to reconstruct alleles from diploid consensus sequences and calculate the allele pair probabilities (Stephens *et al.* 2001; Stephens & Donnelly 2003); input files were created using SEQPHASE (Flot 2010). Third, when the probabilities of reconstructed alleles were <0.90, we used allele-specific priming to determine gametic phases (Bottema *et al.* 1993). PHASE was rerun to confirm that all reconstructions received probabilities ≥0.90.

Population structure

Exons were trimmed from all sequences so that only introns were included in data analyses. We also removed alleles containing large gaps (>20 bp) and nucleotide sites containing transposable elements, inversions or large insertions (>20 bp; Table S2, Supporting information). We calculated nucleotide diversity and pairwise Φ_{ST} (the proportion of the total genetic variance partitioned between populations) values within lineages, partitioning samples into OW and NW populations, using ARLEQUIN v.3.5 (Excoffier & Lischer 2010). Significance was tested with 10 000 bootstrap replicates. To correct *P*-values for multiple comparisons, we applied a false discovery rate (FDR) to each pairwise comparison

(Benjamini & Hochberg 1995). For mtDNA, we calculated Φ_{ST} using ARLEQUIN v.3.5 and net sequence divergence (d_A; total divergence between groups minus mean divergence within groups) using MEGA 6.0 (Tamura *et al.* 2013) for each OW–NW comparison.

We tested for associations between nuDNA and mtDNA divergence using PGLS analyses and the Caper package in R (Orme 2013). This method implements general least-squares models to account for dependence resulting from shared phylogenetic history. For each of the six lineages, we used mtDNA cytochrome *b* and ND2 sequences from Johnson & Sorenson (1999) to reconstruct a maximum-likelihood tree in MEGA v. 6 (Tamura *et al.* 2013) using a general time-reversible substitution model with a gamma distribution and invariant sites.

We also calculated Φ_{ST} and d_A for all between-species pairwise comparisons ($n = 15$ comparisons) to examine the relationship between mtDNA and nuDNA differentiation at deeper divergences. When data were available, we further compared each of the Holarctic lineages to their sister species (or another closely related species) to examine the relationship between mtDNA and nuDNA for more recent divergence times. These comparisons included mtDNA control region and five nuclear loci for the following: mallard vs. mottled duck (*A. fulvigula*) (Peters *et al.* 2014), northern shoveler vs. South American cinnamon teal (*A. cyanoptera cyanoptera*) (Wilson *et al.* 2013), North American green-winged teal vs. speckled teal (*A. flavirostris flavirostris*) (McCracken *et al.* 2009a) and northern pintail vs. yellow-billed pintail (*A. georgica*) (McCracken *et al.* 2009b).

For mtDNA, we constructed haplotype networks using the median-joining algorithm in the program NETWORK v.4.6.1.1 (Bandelt *et al.* 1999) and a neighbour-net tree using uncorrected *P*-distances in SPLITSTREE 4.12.6 (Huson & Bryant 2006). For the 20 nuclear loci, we concatenated the consensus sequences (using IUPAC ambiguity codes for heterozygous positions) for each individual, for a total of 6379 aligned nucleotide positions, and constructed neighbour-net trees using uncorrected *P*-distances and average states for heterozygous positions.

To estimate the number of genetic populations and to assign individuals to those populations, we used the 20-locus nuDNA data set and the program STRUCTURE 2.2.3 (Pritchard *et al.* 2000). For each locus, we coded alleles from 1 to n, where n is the number of alleles observed. For some loci, few alleles were shared between individuals (Table S2, Supporting information), and we therefore excluded autapomorphies to group closely related alleles. To determine the number of populations (K), we estimated ln $\Pr(X|K)$ for $K = 1$ to 5 populations for each lineage separately without a priori information regarding sampling locations. STRUCTURE was run using an admixture model and independent allele frequencies for 100 000 burn-in and 500 000 sampling generations. We replicated each analysis 10 times and calculated ΔK to determine the most likely number of populations (Evanno *et al.* 2005) using STRUCTURE HARVESTER (Earl & vonHoldt 2012).

Gene flow

We fit the data from each lineage to isolation-with-migration models in the program IM (Hey & Nielsen 2004) by treating OW and NW as separate populations. IM uses MCMC Bayesian methods to estimate six demographic parameters scaled to the substitution rate per locus (u), including θ (where $\theta = 4N_e u$; N_e is the effective population size) for the ancestral population (θ_A) and each of the two daughter populations (θ_{OW} & θ_{NW}), immigration rates (M, where $M = m/u$; m is the rate at which alleles enter the population through immigration) for each daughter population (M_{OW} & M_{NW}) and time since divergence (t, where $t = Tu$; T is the number of years since population divergence). To test for mito-nuclear discordance, we analysed mtDNA and nuDNA in separate analyses. For gadwall and teal, we used a larger fragment of mtDNA control region that included domain III (totalling 956–987 bp), because these sequences were previously available and provided higher resolution (Peters 2006; Peters *et al.* 2012b).

Because IM assumes no intralocus recombination, we used IMgc (Woerner *et al.* 2007) to select the fragment containing the highest number of polymorphic sites consistent with no recombination. We preferentially removed nucleotides over sequence copies by iteratively adjusting the chromosomal weighting to remove a maximum of 5% of copies. By doing so, we presumably removed rare recombinant alleles and PCR/editing errors without dramatically altering allele frequencies. IMgc was run for each lineage separately, and the recombination-filtered data were used for IM analyses.

We defined priors containing the entire posterior distributions for each parameter determined from preliminary runs. However, some posterior distributions were flat over a broad range of values. In these cases, we used a priori information to set priors. For mtDNA, we used the 95% highest posterior distribution (HPD) of *TMRCA* (time to most recent common ancestor) to set an upper prior for t for pintail, wigeon and teal; by doing so, we assumed that t could not be older than the deepest coalescent (see Peters *et al.* 2007). For deeply diverged species (wigeon & teal), which lacked information regarding ancestral population sizes in mtDNA, we set upper priors on θ_A based on the ratio θ_A:($\theta_{OW} + \theta_{NW}$) estimated from nuDNA (see Peters *et al.* 2012b). Finally, for θ_{OW} in pintail and θ_{NW} in mallard,

we used information from census sizes, ~2–10 million individuals per continent (Delany & Scott 2006), to set an upper bound of θ at 1500, which corresponds to population sizes of ~4 000 000 individuals, when assuming a mtDNA substitution rate of 4.8×10^{-8} substitutions/site/year and a generation time of 3 years (Peters *et al.* 2008). Given uncertainties in mutation rate calibrations and generation times, our upper priors on θ reflect approximations only. Furthermore, N is generally ten times larger than N_e (Frankham 1995), and our priors were much wider than the posterior distributions for other populations (Fig. S1, Supporting information). Therefore, our priors were probably sufficiently wide to include actual values of θ.

For nuDNA, the posterior distribution of t for gadwall contained a clear peak but a broad tail that did not approach zero. We set the upper prior to 0.1, because this prior contained the entire posterior distribution of t from a model that included exponential growth (Peters *et al.* 2012c). Likewise, t rose sharply and peaked at the upper prior for pintail and shoveler, regardless of the priors used, and we arbitrarily set the upper prior to 0.1 to reflect the shallow mtDNA divergence. Finally, posteriors for migration rates were flat for pintail, shoveler and mallard, and we set upper priors to $m = 100$ for these species. The posterior distributions for all other parameters were contained within our priors, which we assumed to be uninformative.

For mtDNA, we defined an inheritance scalar of 0.25 so that parameter estimates were reported on the same scale as autosomal loci. Thus, estimates of the effective number of migrants ($2Nm = \theta M/2$) were directly comparable between mtDNA and nuDNA. We used Metropolis coupling with a geometric heating scheme, one cold chain and 39 heated chains. We ran a burn-in of 1 000 000 generations and sampled parameters every 20 generations for >10 000 000 generations. We repeated each analysis with a different random number seed to confirm that replicates converged on the same stationary distributions.

Results

Population structure

Nucleotide diversity for the mtDNA control region ranged between 0.001 and 0.013 among populations for the six duck lineages (Fig. 2; Table S2, Supporting

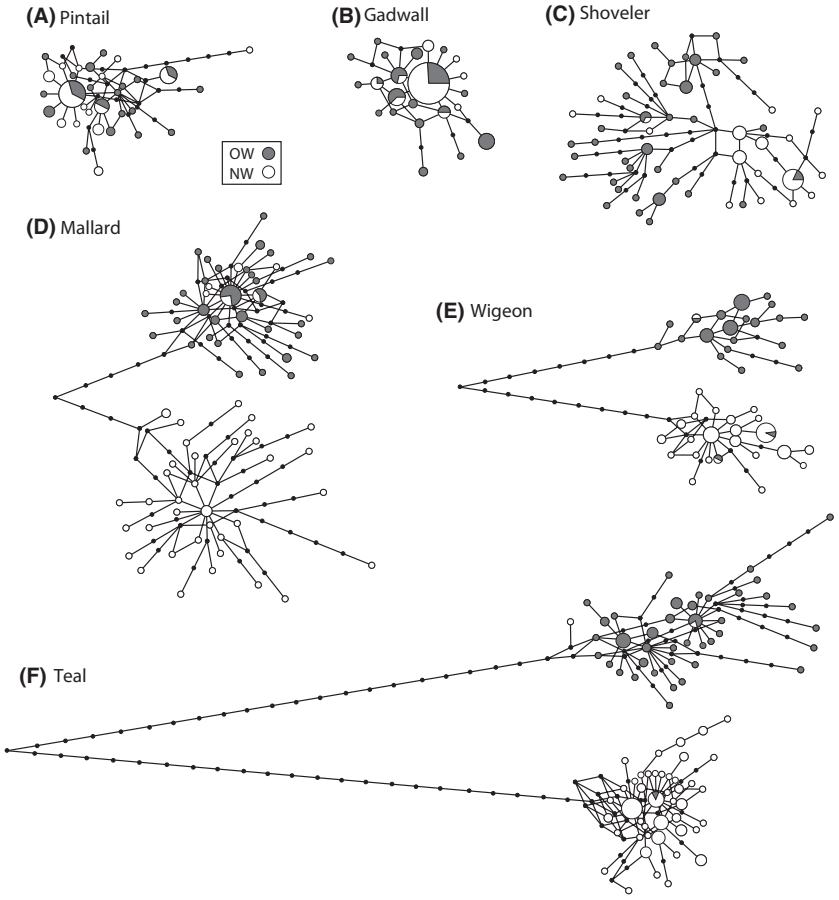

Fig. 2 Mitochondrial DNA haplotype networks illustrating the range of divergence between OW (shaded) and NW (open) individuals for (A) northern pintail, (B) gadwall, (C) northern shoveler, (D) mallard, (E) 'northern' wigeon and (F) green-winged teal. Each circle corresponds to a different haplotype, circle area is proportional to the number of individuals with that haplotype, and mutations are indicated as lines separating sampled haplotypes or intermediate haplotypes that were not sampled (small, black circles).

information). Pintail had a shallow genealogy with nonsignificant differentiation between OW and NW (Fig. 2A, Table 1), whereas both gadwall and shoveler had shallow mtDNA genealogies with significant haplotype frequency differences (Fig. 2B,C, Table 1). Consistent with previous studies, mallard, wigeon and teal had deeply divergent mtDNA haplogroups between OW and NW (Fig. 2D–F, Table 1), although all three species had some haplotypes that grouped with haplotypes from the other continent (12.0% in mallard, 2.9% in wigeon and 2.1% in teal; Fig. 2D–F).

Mean nucleotide diversity for nuDNA ranged between 0.010 and 0.016 among the six lineages (Table S2, Supporting information). Pairwise Φ_{ST} values indicated that neither pintail nor shoveler was significantly differentiated between OW and NW at any nuclear locus (mean $\Phi_{ST} < 0.0$; Table 1). OW and NW mallards were weakly differentiated ($\Phi_{ST} = 0.016$), with only two loci exhibiting significant frequency differences (CHD1Z & LDHB; Table S2, Supporting information). Gadwall ($\Phi_{ST} = 0.060$), wigeon ($\Phi_{ST} = 0.046$) and teal ($\Phi_{ST} = 0.050$) were significantly differentiated between OW and NW at 11, 10 and 6 loci, respectively (Table S2, Supporting information).

Table 1 Genetic differentiation in mtDNA and nuDNA (mean and range for 20 introns) sequenced from six lineages of Holarctic ducks [see Table S2 (Supporting information) for additional details]

	mtDNA		nuDNA
	Uncorrected d_A	Φ_{ST}	Φ_{ST}
Northern pintail	0.00011	0.019	<0.0 (<0.0–0.012)
Gadwall	0.00035	0.100*	0.060 (<0.0–0.19)*
Northern shoveler	0.0019	0.190*	<0.0 (<0.0–0.015)
Mallard	0.0084	0.498*	0.016 (<0.0–0.23)*
Northern wigeon	0.026	0.834*	0.046 (<0.0–0.41)*
Green-winged teal	0.062	0.879*	0.050 (<0.0–0.38)*

*$P < 0.05$ for mtDNA or ≥ 2 nuDNA loci.

Consistent with overall weak nuDNA differentiation, OW and NW individuals were broadly intermixed in the nuDNA neighbour-net tree for each lineage (Fig. 3A), which contrasted markedly with strong clustering by continent observed in the mtDNA haplotype networks (Fig. 2D–F) and neighbour-net tree for mallard, wigeon and teal (Fig. 3B). Moreover, after correcting for phylogeny, the regressions between mean Φ_{ST} for nuDNA and both Φ_{ST} and d_A for mtDNA were not significant (Fig. 4A; $R^2 = 0.29$, $F_{1,5} = 1.61$, $P = 0.27$; $R^2 = 0.31$, $F_{1,5} = 1.83$, $P = 0.25$; respectively). In contrast, the regression between Φ_{ST} and d_A for mtDNA was significant ($R^2 = 0.74$, $F_{1,5} = 11.55$, $P = 0.027$). The gadwall appears to be an outlier in the comparisons between mtDNA and nuDNA, and given the small sample size, the regression could be particularly sensitive to this outlier. Indeed, removing the gadwall from analyses resulted in a significant regression between Φ_{ST} for nuDNA and both Φ_{ST} and d_A for mtDNA ($R^2 = 0.96$, $F_{1,5} = 68.17$, $P = 0.0037$; $R^2 = 0.79$, $F_{1,5} = 11.08$, $P = 0.045$; respectively).

Among the 15 between-species comparisons, Φ_{ST} for nuDNA was significantly correlated with uncorrected d_A for mtDNA (Fig. 4B, also see Table S4, Supporting information; Mantel test, $r = 0.82$, $P = 0.018$), and a similar trend was observed for the four sister-species comparisons (Fig. 4B; $R^2 = 0.85$, $P = 0.078$). Relative to the depth of mtDNA divergence, nuDNA differentiation between OW and NW populations was substantially lower than nuDNA differentiation among the different

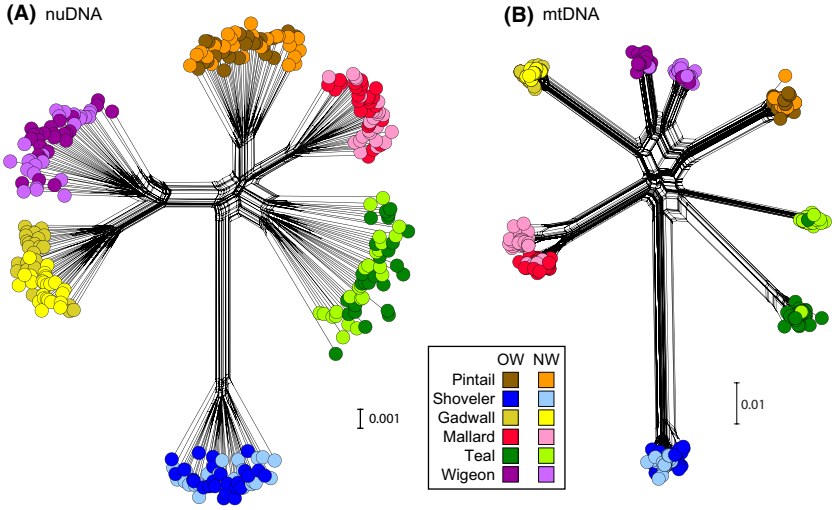

Fig. 3 Neighbour-net trees for (A) nuDNA (6379 aligned nucleotides from 20 independent loci) and (B) mtDNA control region (679 aligned nucleotides) illustrating genetic distances for six Holarctic duck lineages.

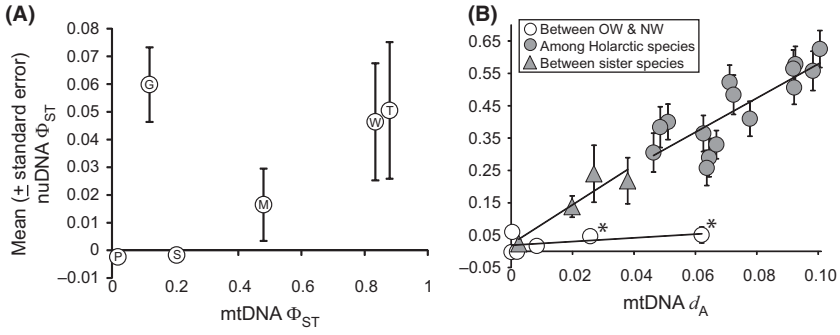

Fig. 4 Relationship between mtDNA and nuDNA differentiation between OW and NW for six Holarctic duck lineages. (A) Φ_{ST} between OW and NW populations within lineages: pintail (P), gadwall (G), shoveler (S), mallard (M), wigeon (W) and teal (T). (B) nuDNA Φ_{ST} compared with mtDNA divergence (d_A) within lineages (open circles), among Holarctic species (shaded circles) and between sister species (shaded triangles). The two strong outliers with high mtDNA d_A but low nuDNA Φ_{ST} (marked with an asterisk) correspond to wigeon and teal, respectively.

species, especially at deep mtDNA divergences (Fig. 4B).

On the basis of ΔK, the nuDNA genotypes from each lineage best fit a two-population model. However, Ln (Pr | K) peaked at one for pintail, shoveler and mallard, suggesting $K = 1$ as a better model (because calculations of ΔK depend on how Ln (Pr | K) changes with increasing numbers of populations, ΔK cannot support a one-population model; Evanno *et al.* 2005). Indeed, assigning individuals of these three lineages to a two-population model did not result in any signal of population structure – the probability of being assigned to population 1 (Q_1) was near 0.5 for all individuals (Fig. 5). In contrast, Ln (Pr | K) peaked at $K > 1$ for gadwall, wigeon and teal. Overall, 100% of gadwall, 94% of wigeon and 98% of teal were assigned with other individuals from the same population, and most assignment scores were ≥0.90 (Fig. 5).

Gene flow

Estimates of gene flow varied considerably among lineages and between marker types (Fig. 6). In general, gene flow was high for pintail, moderately high for shoveler and mallard and comparatively low for gadwall, wigeon and teal. Whereas estimates of gene flow were similar between mtDNA and nuDNA for pintail and gadwall (Fig. 6A,B), gene flow was substantially higher in one

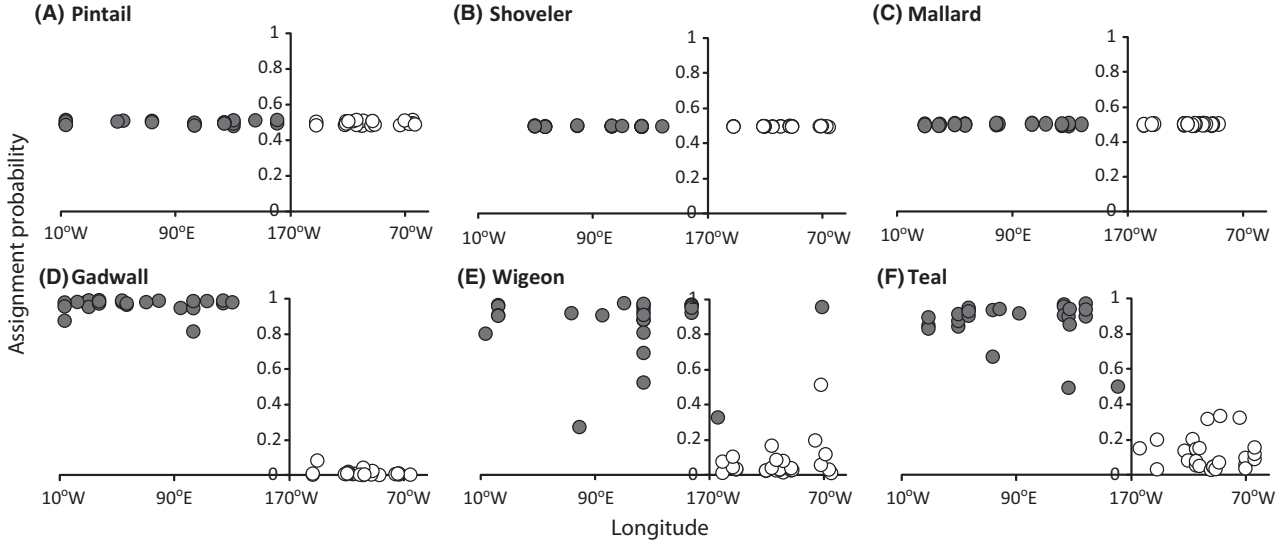

Fig. 5 Population assignment probabilities of individuals sampled from OW (shaded circles) and NW (open circles) populations of six Holarctic duck lineages using two-population models. Pintail (A), shoveler (B) and mallard (C) best fit one-population models and did not contain diagnosable differences between OW and NW. Gadwall (D), wigeon (E) and teal (F) best fit two-population models, and most (≥94%) individuals were correctly assigned to their respective populations.

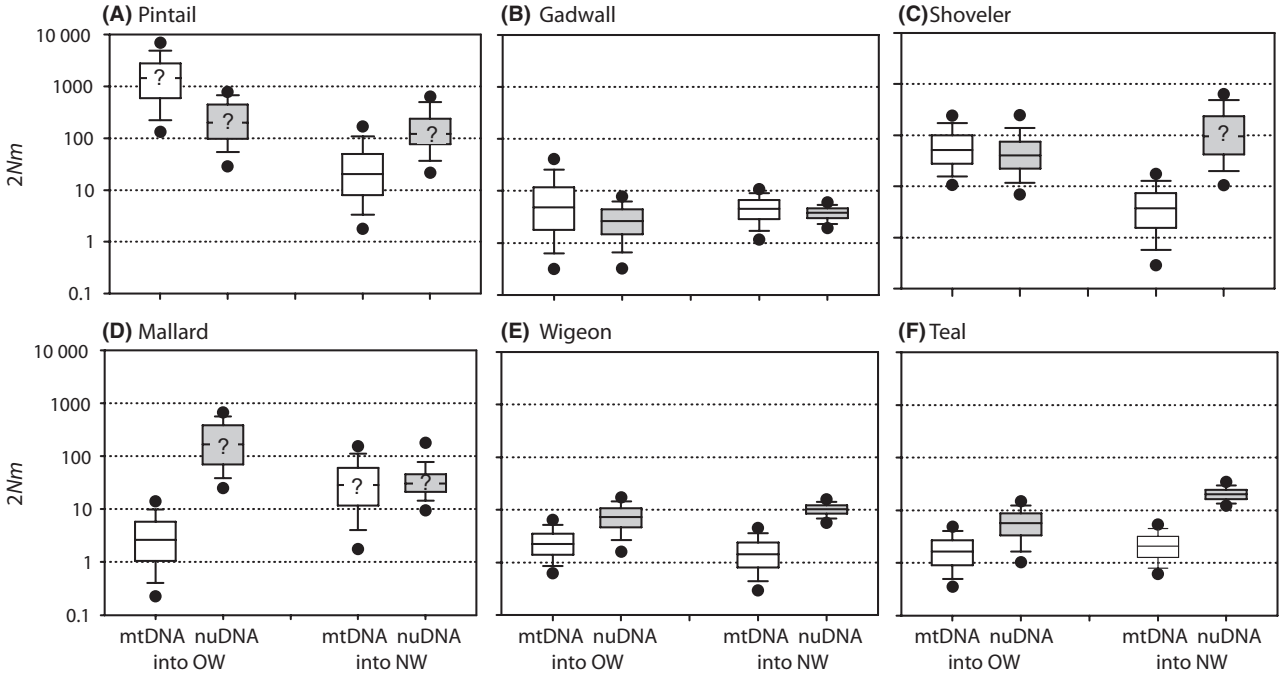

Fig. 6 Number of effective migrants ($2Nm$) estimated from mtDNA and nuDNA for A) northern pintail, B) gadwall, C) northern shoveler, D) mallard, E) 'northern' wigeon and F) green-winged teal. The box plots show the 25th and 75th percentile (boxes), the 10th and 90th percentile (error bars) and the 5th and 95th percentile (points) of the posterior distributions of $2Nm$. Question marks indicate that the posterior distribution for either θ or migration (m/u) was flat, and therefore, the estimate of $2Nm$ might have been sensitive to the priors used (Fig. S1 & Table S5, Supporting information). Note that each lineage, except gadwall, contained evidence of higher nuDNA relative to mtDNA gene flow in at least one direction.

direction for nuDNA compared with mtDNA for four lineages (nonoverlapping 90% confidence intervals): OW→NW for shoveler, wigeon and teal, and NW→OW for mallard (Fig. 6C–F). Gene flow estimates were also considerably higher for nuDNA than mtDNA in three additional comparisons (nonoverlapping 75% confidence intervals): OW→NW for pintail and NW→OW for wigeon and teal. Thus, the general pattern supports higher rates of intercontinental movements for nuDNA than mtDNA, and we found no cases where mtDNA gene flow was compellingly higher than nuDNA gene flow.

Discussion

Comparative analysis of mtDNA control region and 20 nuclear introns for six lineages of codistributed Holarctic ducks revealed wide variation in population differentiation both among species (idiosyncratic population histories) and between marker types within lineages. Differentiation between OW and NW populations ranged from a lack of detectable structure in both marker types (e.g. northern pintail) to deeply divergent mtDNA haplogroups with overall weak nuDNA differentiation (e.g. 'northern' wigeon and green-winged teal). Intermediate between these extremes, we found moderate frequency differences in both marker types (e.g. gadwall), high frequency differences in mtDNA in the absence of detectable nuDNA structure (e.g. northern shoveler) and divergent mtDNA haplogroups with very weak nuDNA differentiation (e.g. mallard). Differentiation between mtDNA and nuDNA was not significantly correlated among these lineages, and the regression line fit to these data was substantially shallower than that found for comparisons among the different species (Fig. 4), revealing mito-nuclear discord in these taxa.

Mito-nuclear discord

Apparent mito-nuclear discord can result from the faster sorting rate of mtDNA, which has one-quarter the effective population size of nuDNA and accumulates interpopulation differences faster than nuDNA (Moore 1995; Hudson & Turelli 2003; Zink & Barrowclough 2008). We can reject this explanation for Holarctic ducks from two lines of evidence. First, differences in sorting rates should also affect the among-species comparisons. We found a significant correlation between mtDNA and nuDNA differentiation among different species, yet for wigeon and teal (and perhaps mallard and shoveler), nuDNA differentiation was much lower than expected for the observed mtDNA divergence (Fig. 4B). Second, coalescent estimates of population history, which account

for differences in effective population size, and thus sorting rates, supported significantly higher rates of gene flow for nuDNA than mtDNA in shoveler, mallard, wigeon and teal (Fig. 6). Thus, other factors besides sorting rates have contributed to the mito-nuclear discord in these ducks.

Two of the most commonly cited causes of mito-nuclear discord are selection and sex-biased dispersal (reviewed in Toews & Brelsford 2012). Sex-biased dispersal seems the most likely cause of the weak nuDNA differentiation relative to mtDNA divergence observed for Holarctic ducks. As a general rule, female waterfowl display natal philopatry, whereas males disperse greater distances (Rohwer & Anderson 1988), which can restrict movements of the maternally inherited mtDNA despite males causing effective gene flow of nuclear alleles among populations. These behavioural differences between the sexes have often been invoked to explain apparent mito-nuclear discord in waterfowl (Tiedemann *et al.* 1999; Scribner *et al.* 2001; Kulikova *et al.* 2004; Pearce *et al.* 2009; Sonsthagen *et al.* 2011; Peters *et al.* 2012b), although few studies have quantitatively demonstrated a role for sex-biased dispersal and/or rejected differences in molecular sorting rates (Pearce *et al.* 2005; Lecomte *et al.* 2009; Peters *et al.* 2012a). Our coalescent analyses, suggesting restricted gene flow for mtDNA relative to nuDNA, provide strong support for male-mediated gene flow between North America and Eurasia. Under this hypothesis, we further predict that gene flow will be highest among Z-linked loci (males carry two copies, whereas females carry a single copy). Our single Z-linked locus (CHD1Z) was among the most structured loci for mallard and wigeon (Table S2, Supporting information), which seems inconsistent with high gene flow; however, given the low number of polymorphisms in CHD1Z, additional data are needed to quantitatively test this prediction.

An alternative interpretation of the discord is that selection has influenced polymorphisms in one or both marker types. Signatures of selection are likely to arise more rapidly in the mitochondrial genome, because it is a single linkage group and is therefore especially sensitive to the effects of genetic hitchhiking (Ballard & Whitlock 2004; Bazin *et al.* 2006; Meiklejohn *et al.* 2007). Most evidence for a role of selection in generating mito-nuclear discord supports adaptive introgression of mtDNA, which results in low mtDNA divergence relative to nuDNA divergence (reviewed in Toews & Brelsford 2012). However, this is not the case for Holarctic ducks and other birds with female-biased rather than male-biased dispersal (Humphries & Winker 2011), which have increased mtDNA divergence.

Selection could increase mtDNA divergence if adaptations to local environments reduce effective gene flow (Cheviron & Brumfield 2009; Ribeiro *et al.* 2011; Pavlova *et al.* 2013) or if female hybrids incur negative fitness in accord with Haldane's rule (Tegelström & Gelter 1990; Carling & Brumfield 2008). Holarctic ducks are distributed at similar latitudes and use a wide variety of habitats on both continents; therefore, local adaptations seem unlikely to explain mtDNA divergence in these species. However, Haldane's rule posits that hybrids of the heterogametic sex (females in birds) will suffer negative fitness consequences before the homogametic sex (Haldane 1922), and female hybrid ducks are generally rarer than male hybrids, suggesting lower viability (Tubaro & Lijtmaer 2002; Kirby *et al.* 2004). Haldane's rule could potentially apply to species with deep mtDNA divergence and subspecies/species-level plumage differences between OW and NW (teal and wigeon), restricting intercontinental movements of mtDNA. Reduced fitness for the heterogametic sex should also inhibit gene flow for Z-linked loci (Naisbit *et al.* 2002; Carling & Brumfield 2008; Backström *et al.* 2010), and we found the Z-linked CHD1Z to be significantly differentiated in mallard, wigeon and teal (Table S2, Supporting information). More extensive sampling of the genome is needed to determine whether the Z chromosome is generally more divergent than autosomal DNA (Ellegren *et al.* 2012) and whether Haldane's rule has had a prominent role in generating the mito-nuclear discordance observed in these ducks. Importantly, Z-linked loci offer an opportunity to distinguish between Haldane's rule (high mtDNA, low autosomal and high Z differentiation) and male-mediated gene flow (high mtDNA, low autosomal and low Z differentiation).

In contrast to the patterns observed between OW and NW populations, there was general mito-nuclear concordance among species. Specifically, we found a significant correlation between mtDNA d_A and nuDNA Φ_{ST} among the six Holarctic species and a similar trend between these species and their sister species (Fig. 4B). These comparisons support a tight coupling between mitochondrial and nuclear divergence, as would be expected following the cessation of gene flow. Although hybridization among species of *Anas* ducks is well documented (Johnsgard 1960; Tubaro & Lijtmaer 2002; Kraus *et al.* 2012), interspecific gene flow might be sufficiently rare for both marker types to prevent the emergence of mito-nuclear discord. Regardless, the mito-nuclear *con*cordance observed among species emphasizes the prominent *dis*cordance observed within lineages.

Species-specific histories

Given the mito-nuclear discord in our data set and similar results among other avian lineages in this region

(Humphries & Winker 2011), inferences about Holarctic phylogeography need to consider the two marker types separately. Based on the observation that five lineages are significantly differentiated between OW and NW, the Bering Strait and the Bering and Chukchi seas seem to be formidable, albeit porous, barriers to gene flow. Indeed, estimates of intercontinental gene flow from mtDNA are low to moderate for these lineages. In contrast, nuDNA supports moderate to high gene flow, suggesting that intercontinental dispersal is common. In the latter case, gene flow is probably sufficient to offset the effects of drift and selection driving population divergence, stalling speciation short of completion.

For pintails, genetic evidence suggests a lack of structure between OW and NW for mtDNA, nuclear introns and microsatellites (this study, Flint *et al.* 2009). Studies of marked individuals have documented recurrent intercontinental movements for both males and females (Miller *et al.* 2005; Flint *et al.* 2009; Hupp *et al.* 2011), especially during drought years (Henny 1973). In this dispersive species, gene flow is probably high for both sexes, and OW and NW populations have not effectively diverged.

In contrast to the pintail, the gadwall is moderately differentiated between OW and NW at both marker types, and coalescent estimates of gene flow are consistent with equal dispersal rates between the sexes (Fig. 6B). Genetic data suggest that gadwall recently colonized North America from Eurasia (perhaps within the past 100 000 years), and a founder effect resulted in reduced genetic diversity and shifted allele frequencies in the NW population, increasing genomic differentiation (Peters *et al.* 2008, 2012c). This founder event might explain why OW and NW gadwall were more diagnosable using nuDNA (Fig. 5D) than wigeon or teal, despite the shallower mtDNA genealogy. Furthermore, in contrast to the other lineages, the gadwall is distributed at lower latitudes, and its range contains a wider OW–NW disjunction (Fig. 1). This disjunction probably inhibits gene flow for both sexes, limiting the manifestation of mito-nuclear discord.

This study presents the first population genetics data for the northern shoveler. Despite a shallow genealogy, mtDNA haplotype frequencies were strongly differentiated between OW and NW (Table 1). Qualitatively, NW haplotypes seemed to be nested within OW haplotypes (Fig. 2C), suggesting that like gadwall, shoveler might have colonized North America from Eurasia. However, unlike the gadwall, shoveler nuDNA does not appear to contain signatures of a founder effect: none of the 20 introns were significantly structured, and genetic diversity was similar between OW and NW (Table S2, Supporting information). It is possible that male-mediated gene flow has been sufficiently high to erase this signature in biparentally inherited DNA, whereas female-mediated gene flow has been sufficiently rare to retain the signature of an ancestral colonization event. The shoveler warrants further study to test the influence of population history and sex-biased dispersal on the observed mito-nuclear discord.

Mallards have had a complex phylogeographic history confounded by hybridization and a recent radiation (Lavretsky *et al.* 2014). Indeed, the two mtDNA clades that are paraphyletic/polyphyletic with respect to other species of mallard-like ducks have been the focus of extensive debate regarding the role of incomplete lineage sorting vs. introgression (Avise *et al.* 1990; Omland 1997; Johnson & Sorenson 1999; McCracken *et al.* 2001; Kulikova *et al.* 2004; Peters *et al.* 2014). The near absence of nuDNA differentiation is surprising given the fairly deep mtDNA divergence (also see Kraus *et al.* 2013), and this supports the possibility of recent adaptive introgression of mtDNA from North American mallard-like species, such as the American black duck (*A. rubripes*) and mottled duck (*A. fulvigula*), that share the NW haplogroup (McCracken *et al.* 2001; Lavretsky *et al.* 2014). Regardless, the absence of similar NW haplotypes in Eurasia (Kulikova *et al.* 2005; Kraus *et al.* 2011) suggests that female-mediated gene flow is rare despite male-mediated gene flow homogenizing nuDNA.

Among these six lineages, the wigeon and teal have the most similar population histories. Both lineages have relatively deep mtDNA divergences and diagnosable plumage differences between OW and NW. Although nuDNA differentiation is weak, allelic frequency differences are sufficient for assigning individuals to their respective populations in most cases (Fig. 5E,F). Peters *et al.* (2012b) argued that teal have probably experienced a long history of parapatric divergence and that male-mediated gene flow has been sufficient to prevent the completion of speciation despite the deep mtDNA divergence. This scenario seems to fit the wigeon as well. Although Eurasian and American wigeons are recognized as separate species on the basis of morphology, male-mediated gene flow probably inhibits broadscale genomic differentiation and the evolution of strong isolating mechanisms. The Eurasian wigeon has become more common on the Pacific coast of North America during winter (Edgell 1984), and hybridization with American wigeon might be increasing. This species pair provides an excellent opportunity for studying mechanisms of speciation and factors contributing to mito-nuclear discord.

Conclusions

There is increasing evidence of mito-nuclear discord in Holarctic birds, and our data suggest that in Holarctic

Anas ducks, it is associated with differences in the rates at which loci from the different genomes move between populations. Although male-biased dispersal seems to be an important contributing factor in these lineages, we cannot rule out the possibility that selection (e.g. Haldane's rule) inhibits mtDNA gene flow. Regardless, nuDNA gene flow has probably stalled speciation short of completion in some species despite deep mtDNA divergences (e.g. teal and wigeon), emphasizing that the evolution of reproductive barriers can be a slow process (Kronforst 2008; Gill 2014) and that more holistic approaches are needed in studies of species divergences (Nadachowska-Brzyska *et al.* 2013). With more than 100 species of birds with varying dispersal mechanisms distributed across the Holarctic, this region provides an excellent natural laboratory for studying factors contributing to mito-nuclear discord and the speciation process in general.

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Data accessibility

DNA sequences: GenBank Accession Nos. KJ821022–KJ825676 (also see Table S3, Supporting information).

Other data files (e.g. FASTA files containing resolved alleles; NEXUS files; IM, STRUCTURE and ARLEQUIN input files): Dryad accession doi: 10.5061/dryad.67170.

Supporting information

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

Fig. S1 Posterior distributions of demographic parameters estimated from (A) mtDNA and (B) nuDNA for six lineages of Holarctic ducks.

Table S1 Specimen data for 590 individual Holarctic ducks.

Table S2 Genetic diversity in six lineages of Holarctic ducks.

Table S3 GenBank accession numbers for sequences used in this study.

Table S4 Genetic differentiation among different species of ducks.

Table S5 Demographic parameters estimated from (A) mtDNA and (B) nuDNA for six lineages of Holarctic ducks.