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RESEARCH ARTICLE

Strong population structure and limited gene flow between Yellow-billed Ducks and Mallards in southern Africa

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ABSTRACT

Secondary contact and hybridization between recently diverged taxa have been increasing due to anthropogenic changes to the environment. Determining whether secondary contact leads to gene flow between species is important for understanding both the evolutionary consequences of such events (i.e. genetic swamping, speciation reversal, hybrid speciation) and for establishing proper conservation measures. Mallards (*Anas platyrhynchos*), which natively have a Holarctic distribution, have been introduced nearly worldwide due to game-farm and domestic pet releases. Their expanding range has resulted in secondary contact and increased incidences of hybridization with many closely related Mallard-like ducks that comprise the Mallard complex. Here, we assay molecular diversity for 19 nuclear introns and the mitochondrial DNA for wild Mallards (n = 50) across their Holarctic range and Yellow-billed Ducks (n = 30–75; *Anas undulata*) from southern Africa to determine population genetic structure and test for evidence of Mallard introgression into Yellow-billed Ducks. While we found limited support for contemporary gene flow across nuclear markers, we provide evidence from mitochondrial DNA that best supports ancient gene flow between Yellow-billed Ducks and Mallards. Yellow-billed Ducks best fit a single population at nuclear markers but show some location-specific mtDNA structure that suggests recent founder or bottleneck events. Although we find that introgression from Mallards into Yellow-billed Duck is limited, Yellow-billed Duck populations should be monitored to determine if expanding feral Mallard populations in southern Africa are increasing introgression.

Keywords: dabbling duck, evolution, gene flow, genetic swamping, hybridization, introgression, population genetics, population structure

Estructura poblacional fuerte y flujo génico limitado entre *Anas undulata* y *A. platyrhynchos* en el sur de África

RESUMEN

El contacto secundario y la hibridación han estado aumentado entre taxones recientemente divergidos debido a cambios antropogénicos en el ambiente. Es importante determinar si el contacto secundario promueve el flujo génico entre especies para entender las consecuencias evolutivas de estos eventos (i.e., saturación genética, reversión de especiación, especiación híbrida), y para establecer medidas de conservación adecuadas. *Anas platyrhynchos*, que presenta una distribución original holártica, ha sido introducida en casi todo el mundo debido a las liberaciones dadas por cría cinegética y uso doméstico como mascotas. Su rango en expansión ha producido un contacto secundario y aumentos en la incidencia de hibridación con muchas especies emparentadas cercanamente que forman el complejo de *A. platyrhynchos*. Aquí analizamos la diversidad molecular de 19 intrones nucleares y del ADN mitocondrial para individuos silvestres de *A. platyrhynchos* (n = 50) a través de su rango holártico y de *A. undulata* (n = 30–75) del sur de África para determinar la estructura genética poblacional y para evaluar la evidencia de introgresión de *A. platyrhynchos* en *A. undulata*. Aunque encontramos poca evidencia de flujo génico actual a lo largo de los marcadores nucleares, brindamos evidencia del ADN mitocondrial que apoya la existencia de flujo génico antiguo entre *A. undulata* y *A. platyrhynchos*. *A. undulata* se ajusta mejor a una única población considerando los marcadores nucleares, pero muestra una estructura de ADNmt específica para algunas localidades que sugiere eventos recientes de fundadores o de cuello de botella. Aunque encontramos que la introgresión de *A. platyrhynchos* en *A. undulata* es limitada, las poblaciones de *A. undulata* deberían ser monitoreadas para determinar si las poblaciones salvajes de *A. platyrhynchos* en el sur de África están aumentado la introgresión.

Palabras clave: estructura poblacional, evolución, flujo génico, genética poblacional, hibridación, introgresión, patos, saturación genética

INTRODUCTION

Human-mediated landscape changes increase the potential for secondary contact and incidences of hybridization for many species (Hanson et al. 1949, Snell 1986, Mack et al. 2000, Early et al. 2016). Understanding the historical and contemporary effects of these events on the evolutionary trajectory of species has become a major research focus of evolutionary and conservation biologists (Abbott et al. 2013, 2016; Grabenstein and Taylor 2018). For example, the intentional and unintentional geographic expansion of Mallards (*Anas platyrhynchos*) has resulted in secondary contact between Mallards and nearly all 13 taxa that comprise the Mallard complex (Johnsgard 1978, Ankney et al. 1986, Snell 1986, Lavretsky et al. 2014b, Söderquist et al. 2017). With the Mallard's high propensity to hybridize and ability to produce viable offspring (Johnsgard 1960, 1978), the genetic swamping of the New Zealand Grey Duck (*Anas superciliosa superciliosa*; Rhymer and Simberloff 1996), American Black Duck (*Anas rubripes*; Mank et al. 2004, Lavretsky et al. 2019b), Mexican Duck (*Anas platyrhynchos diazi*; Hubbard 1977), and Hawaiian Duck (*Anas wyvilliana*; Browne et al. 1993, Fowler et al. 2009) are now major conservation concerns. Here we determine whether the endemic Yellow-billed Duck (*Anas undulata*) in southern Africa harbors genetic evidence of introgressive hybridization from Mallards.

The Yellow-billed Duck is a nonmigratory, opportunistically nomadic species that is endemic to much of southern and eastern Africa (Johnsgard 1978; Figure 1). Although wild Mallards do not natively occur in southern Africa, there are increasing numbers of Mallard × Yellow-billed Duck hybrid reports on eBird (Sullivan et al. 2009).

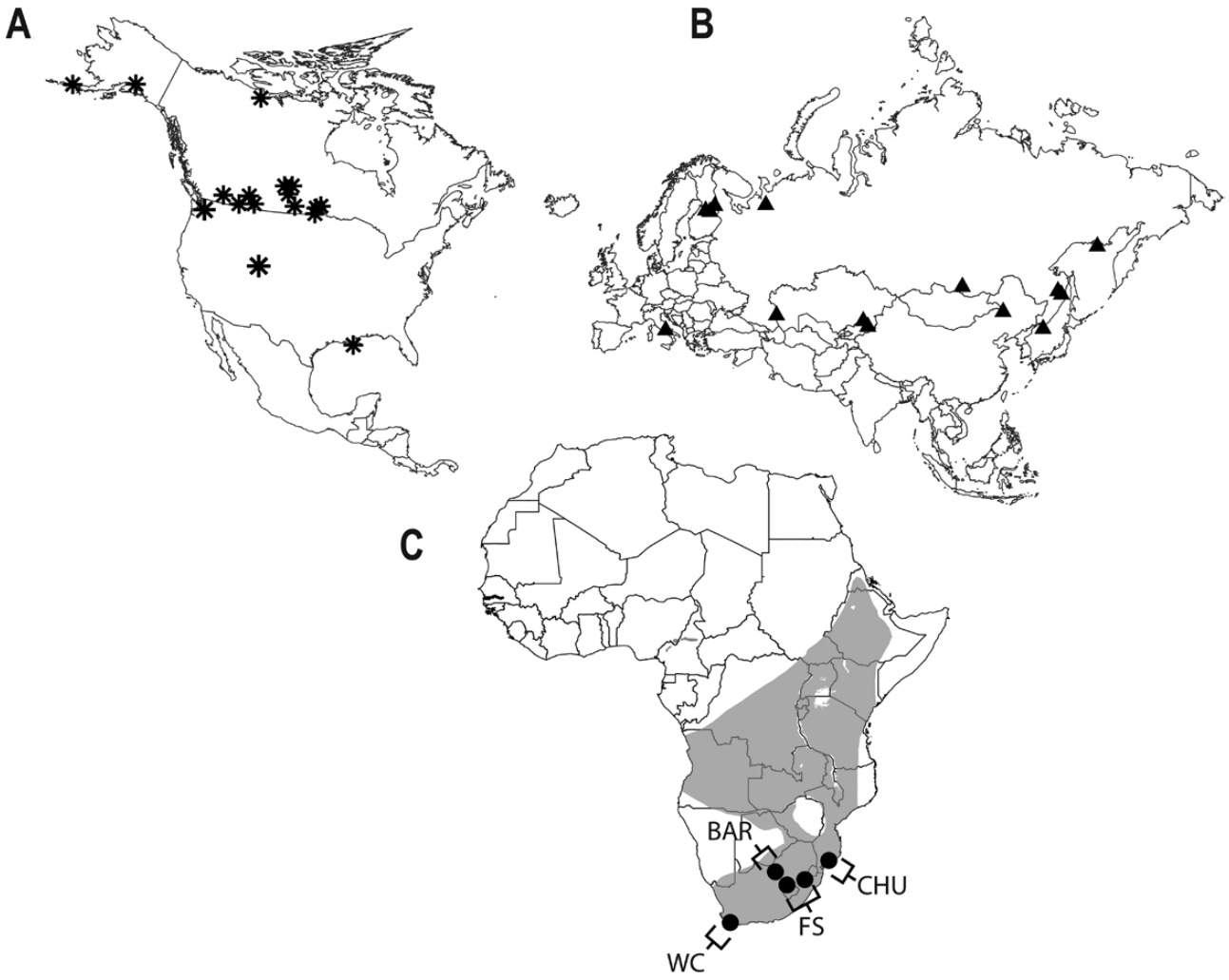

FIGURE 1. Geographic distribution of sampling for (**A**) New World (NW) Mallards from North America, (**B**) Old World (OW) Mallards from Eurasia, and (**C**) Yellow-billed Ducks from southern Africa including their geographic distribution in gray (adapted from IUCN Red List of threatened species 2016). BAR = Barberspan; CHU = Lake Chuali; FS = Free State; WC = Western Cape.

Given that recently established Mallard populations in southern Africa are best described as feral (i.e. domestic stock that has now established viable breeding populations in the wild; Dean 2000), hybridization between Yellow-billed Ducks with local feral Mallards may be of conservation concern. In particular, Mallards of domestic origins are known to significantly deviate in molecular, morphological, and ecological aspects from wild Mallards, including many traits that are expected to be highly maladaptive in the wild (Champagnon et al. 2016, Söderquist et al. 2017, Lavretsky et al. 2019b). Thus, determining whether there is gene flow between Mallards and Yellow-billed Ducks is necessary for future conservation efforts. Lavretsky et al. (2014b) reconstructed the evolutionary relationships for all taxa within the Mallard complex using nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) and reported that Mallards and Yellow-billed Ducks are significantly diverged from each other. However, a limited sample of Yellow-billed Ducks (n = 5) meant that Lavretsky et al. (2014b) were unable to test the extent of gene flow between Yellow-billed Ducks and Mallards, and whether it should be a cause for conservation concern.

Here, we attempt to identify putative hybrids, test for signatures of gene flow from Mallards into Yellow-billed Ducks, and describe population genetic structure of Yellow-billed Ducks in southern Africa. To do this, we sequenced 19 nuDNA loci and a portion of the mtDNA control region across Yellow-billed Ducks (mtDNA n = 75; nuDNA n = 30) from southern Africa and compared them to published data for Mallards from both Eurasia (n = 25) and North America (n = 25; Figure 1; Lavretsky et al. 2014a, Peters et al. 2014b). Mallards in southern Africa are descendants of captive-bred populations (Dean 2000); however, if they are hybridizing with Yellow-billed Ducks, then any introgressed alleles in Yellow-billed Ducks will ultimately trace back to a wild Mallard ancestry. Therefore, wild Mallard genotypes serve as a good baseline for making inferences about gene flow into Yellow-billed Ducks.

METHODS

Seventy-five Yellow-billed Duck samples were obtained from the Burke Museum, University of Washington (n = 4), and by Cumming et al. (2011; n = 71) from 5 locations in southern Africa (Figure 1C; Supplementary Materials Table S1). For Mallards, 50 previously published sequences from both Eurasia (n = 25; Figure 1B) and North America (n = 25; Figure 1A) were downloaded from GenBank (Supplementary Materials Table S1; Lavretsky et al. 2014a; Peters et al. 2014a, 2014b).

Genomic DNA was isolated from Yellow-billed Duck tissue using a Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, California, USA) and following manufacturer protocols.

First, optimized primers targeting 640 base pairs (bp) of the mitochondrial (mtDNA) control region (Sorenson et al. 1999) were used to sequence 75 Yellow-billed Duck samples. Next, a subsample (n = 30) of Yellow-billed Ducks that maximized geographic coverage and better balanced sample sizes among sampling sites was sequenced using previously optimized primers for 19 nuclear introns (Lavretsky et al. 2014b; Peters et al. 2014a, 2014b; Supplementary Materials Table S1). PCR and DNA sequencing protocols are described in detail by Lavretsky et al. (2014a). Briefly, we used 1.5 µL of DNA, 2x GoTaq Green Master Mix (Promega, Madison, Wisconsin, USA), and 1.0 nM of each primer, in a total volume of 15 µL. PCR was done on an Eppendorf Mastercycler (ep*gradient*; Eppendorf, Hauppauge, New York, USA) using the following protocol: DNA denaturation at 94°C for 7 min, followed by 45 cycles of denaturation at 94°C for 20 s, primer annealing at 58°C for 20 s (52°C for mtDNA), and extension at 72°C for 1 min, and a final DNA extension at 72°C for 7 min. PCR product was cleaned with AMPure XP beads (Beckman Coulter, Brea, California, USA), and final products were sequenced at the Yale University DNA Analysis Facility on an ABI 3730 (Applied Biosystems, Life Technologies, Carlsbad, California, USA). Sequences were aligned and edited using SEQUENCHER 4.8 (Gene Codes, Ann Arbor, Michigan, USA). All Yellow-billed Duck sequences have been deposited in GenBank (Accession numbers: MN137255–MN137873).

Gametic phases of nuclear alleles were either algorithmically determined with the program PHASE 2.1.1 (Stephens and Donnelly 2003) or by applying methods described in Peters et al. (2007) for heterozygous sequences containing indels. For the latter case, we compared the ambiguous 5′-end of forward and reverse sequences to resolve the composition and placement of gaps and the linkage of polymorphisms to those gaps. Sequences resolved with the latter method were included as known alleles in PHASE (Peters et al. 2007). Additionally, sequences for Mallards were all resolved with >95% confidence from a larger dataset that included extensive allele-specific priming (Peters et al. 2014b) and were also treated as known alleles in PHASE runs. Gametic phases were determined with the program PHASE for all remaining sequences with ambiguous positions. For each locus, PHASE was run for 1,000 iterations after a burn-in of 1,000 steps and a thinning interval of 100.

Relationships and Individual Ancestry

Pairwise Φ_{ST} estimates between species as well as between Yellow-billed Duck sampling sites, within-species nucleotide diversity (π), and Tajima's D were all calculated across markers in the program ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010).

Population structure was visualized for mtDNA by constructing a haplotype network with the median-joining algorithm in the program NETWORK 5.0.0.1 (Bandelt et al. 1999). Unphased nuclear data with ambiguous positions coded in IUPAC code were concatenated and a consensus nuclear network was calculated using NeighborNet as implemented in the program SPLITSTREE4 (Huson and Bryant 2006). Additionally, population structure for nuclear markers was visualized using the program STRUCTURE 2.3.4 (Pritchard et al. 2000). STRUCTURE uses a Bayesian clustering method to assign individuals to unique genetic clusters and estimate individual ancestry. Across nuclear markers, alleles were coded as 1 to *n*, where *n* was the total number of alleles observed for a given locus. We tested *K* populations from 1 through 10 using 10 replicates of each value of *K* and 500,000 Markov chain Monte Carlo (MCMC) steps following a burn-in of 100,000 steps, and employing the admixture model with uncorrelated loci across runs. The optimum *K* was determined by calculating ΔK in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). Final STRUCTURE outputs were based on the optimal clustering alignment across all 10 replicates for each optimum *K* using a FullSearch algorithm as implemented in the program CLUMPP (Jakobsson and Rosenberg 2007).

Estimates of Gene Flow and Time Since Divergence

To test for differential rates of introgression between marker-types, gene flow between Yellow-billed Ducks and Mallards was estimated for the mtDNA and nuDNA datasets as implemented in the isolation-with-migration (IM) model (Hey and Nielsen 2004, 2007). In short, IM calculates posterior probability densities of population sizes, divergence time, and migration rates from non-recombinant sequence fragments using Bayesian Markov chain Monte Carlo (MCMC) algorithms (Nielsen and Wakeley 2001). While mtDNA has no recombination, all nuDNA sequences were filtered for recombination using the program IMgc (Woerner et al. 2007) to meet the assumption of no intra-locus recombination. In IMgc, weight was given to maximize fragment length while retaining the largest proportion of individuals for each dataset. IM analyses were run for a minimum of 10,000,000 generations following a burn-in of 1,000,000 generations or until the effective samples sizes (ESS) were ≥50 across all parameters (Hey and Nielsen 2004, 2007). Migration rates (*m*), time since divergence (*t*), and ancestral (θ_{Anc}), Mallard (θ_{MALL}), and Yellow-billed Duck (θ_{YBDU}) effective population sizes were simultaneously estimated. We used an average nuclear mutation rate of 1.2×10^{-9} substitutions per site per year (Peters et al. 2008) and an average mitochondrial mutation rate of 4.8×10^{-8} substitutions per site per year (Peters et al. 2005). Multiplying these rates by the nuclear per-locus fragment lengths (Supplementary Materials Table S2) resulted in a geometric mean of 3.07×10^{-7} substitutions per locus per year. Years since divergence (*T*) was then derived as $T = t/\mu$, where *t* is the time since divergence scaled to the geometric mean of the per-locus mutation rate (μ). Finally, converting IM parameters to effective population size (N_e) requires a calculation of generation time (*G*); we used the equation $G = \alpha + (s / (1 - s))$, where α is the age of maturity and *s* is the expected adult survival rate (Sæther et al. 2005, Peters et al. 2008). Without reliable estimates of Yellow-billed Duck adult survival, we used estimates of these parameters for Mallards only: $\alpha = 1$ and $s = 0.57$ (Baldassarre 2014). We estimated *G* to be 2.3 yr per generation. Multiplying generation time by the per-locus mutation rate gave us an estimate of 7.1×10^{-7} substitutions per locus per generation. Effective population size was then derived as $N_e = \theta/\mu$ for mtDNA and $N_e = \theta/4\mu$ for nuclear DNA, where θ is scaled to the per-locus mutation rate per generation (Peters et al. 2008).

RESULTS

Of the 1,520 nuclear sequences (80 individuals × 19 loci), the gametic phases for 1,480 (97%) sequences were resolved with >90% posterior probability. Therefore, we chose the phase reconstructions that received the highest posterior probabilities for each individual per locus for further analysis.

For Yellow-billed Duck mtDNA, mean nucleotide diversity ranged between 0 and 0.010 substitutions per site among the sampling sites (Table 1). Only Yellow-billed Ducks from Lake Chuali in Mozambique had no mtDNA variation. The overall π of 0.016 was ~1.33 times higher than that for Mallards ($\pi = 0.012$). In contrast, for nuclear markers, Mallards had nearly twice the overall nucleotide diversity of Yellow-billed Ducks ($\pi = 0.012$ and 0.007, respectively). Tajima's *D* did not significantly deviate from zero for introns across Mallards and Yellow-billed Duck populations. Conversely, in the mtDNA, a negative Tajima's *D* was recovered for Eurasian Mallards and all Yellow-billed Ducks besides Western Cape samples, suggesting either a recent population expansion or a signature of positive selection (Tajima 1989). Yellow-billed Ducks from the Western Cape showed a slightly positive Tajima's *D* in the mtDNA.

Population Structure

High Φ_{ST} estimates between Mallards and Yellow-billed Ducks for mtDNA ($\Phi_{ST} = 0.57$) and nuclear DNA ($\Phi_{ST} = 0.21$; locus-by-locus $\Phi_{ST} = 0.034–0.52$; Supplementary Materials Table S2) corresponded to previous estimates (Lavretsky et al. 2014a; Figure 2). Among Yellow-billed Duck sampling locations, mtDNA Φ_{ST} ranged between −0.05 and 0.96; the Lake Chuali population was significantly and strongly

TABLE 1. Estimates of nucleotide diversity (π) and Tajima's *D* for 19 nuclear loci and mtDNA of Mallard and Yellow-billed Duck sampling sites.

	Mitochondrial			Introns		
	Sample size (*n*)	Nucleotide diversity (π)	Tajima's *D*	Sample size (*n*)	Nucleotide diversity (π)	Tajima's *D*
Mallard		0.012	−0.72		0.012	−1.18
Eurasia	25	0.006	−1.82*	25	0.012	−0.96
North America	25	0.012	−0.043	25	0.012	−0.95
Yellow-billed Duck		0.016	0.90		0.007	−0.11
Barberspan	37	0.004	−1.58*	10	0.007	0.58
Lake Chuali	3	0.000	NA	3	0.008	0.058
Free State	4	0.003	0.00	4	0.006	−0.268
Western Cape	31	0.015	1.54	13	0.006	−0.030

*Indicates statistical significance ($P < 0.05$).

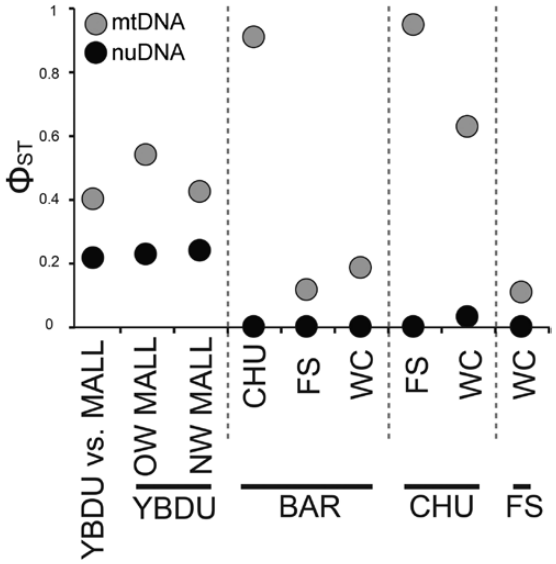

FIGURE 2. Dot plot of Φ_{ST} estimates per pairwise comparisons for 19 nuclear loci and mtDNA.

differentiated from all others. However, populations were either unstructured or weakly so across nuclear markers (mean Φ_{ST} range: −0.0077 to 0.023; Figure 2).

Visualizing structure within mtDNA, we recovered 4 major haplogroups within the haplotype network (Figure 3A). First, all Mallard samples were clustered within previously identified Eurasian, Old World (OW) A (n = 31; 63%) or North American, New World (NW) B (n = 18; 37%) haplogroups (Avise et al. 1990, Johnson and Sorenson 1999, Kulikova et al. 2005). Yellow-billed Duck haplotypes formed 3 distinct haplogroups. Most individuals (n = 63 of 71 individuals; 89%) grouped within a Yellow-billed Duck specific haplogroup, whereas a Lake Chuali specific haplogroup (n = 3 individuals) was more closely related to the OW A Mallard haplogroup than to the other Yellow-billed Duck haplogroup. The remaining 5 (7%) Yellow-billed Ducks, all from the Western Cape, were nested within the OW A haplogroup. For nuDNA, SplitsTree analysis clearly differentiated between Yellow-billed Ducks and Mallards, with no additional substructuring within either species (Figure 3B).

We identified an optimum K of 2 populations when analyzing nuDNA from Mallards and Yellow-billed Ducks in STRUCTURE (Supplementary Materials Figure S1). At K = 2, all samples were assigned to their respective species with >90% assignment, with none of the samples clearly identifiable as a hybrid (Figure 4). Additional resolution was not achieved when visualizing higher values of K. Finally, analyzing Mallards and Yellow-billed Ducks independently in STRUCTURE recovered no additional substructuring (Supplementary Materials Figure S2).

Estimating Gene Flow, Divergence Time, and Effective Population Size

Despite IM estimating a 6-fold higher gene flow rate from Mallards into Yellow-billed Ducks ($m_{MALL \rightarrow YBDU}$ = 0.48; 95% CI = 0–2.075) than from Yellow-billed Ducks into Mallards ($m_{YBDU \rightarrow MALL}$ = 0.075; 95% CI = 0–0.475) for nuclear markers, all estimates were <1 migrant per generation and with the posterior distribution overlapping zero (Figure 5A). IM analysis of mtDNA also resulted in low rates of gene flow between Mallards and Yellow-billed Ducks. Although we were still unable to reject non-zero levels of gene flow from Yellow-billed Ducks into Mallards ($m_{YBDU \rightarrow MALL}$ = 0.01; 95% CI = 0–0.170), we found evidence for non-zero levels of gene flow from Mallards into Yellow-billed Ducks ($m_{MALL \rightarrow YBDU}$ = 0.15; 95% CI = 0.03–0.73).

Although IM did not converge on an estimate of Mallard N_e for mtDNA, ancestral N_e ($N_{e\ Anc}$ = 633,808; 95% CI = 343,631–1,222,493) was estimated to be nearly double that of Yellow-billed Ducks ($N_{e\ YBDU}$ = 309,962; 95% CI = 171,815–573,759; Figure 5B). Based on nuclear markers, the effective population size (N_e) estimated for the Mallard and Yellow-billed Duck ancestor was 536,915 individuals (95% CI = 407,706–706,203 individuals) and Mallards ($N_{e\ MALL}$ = 2,016,706 individuals; 95%

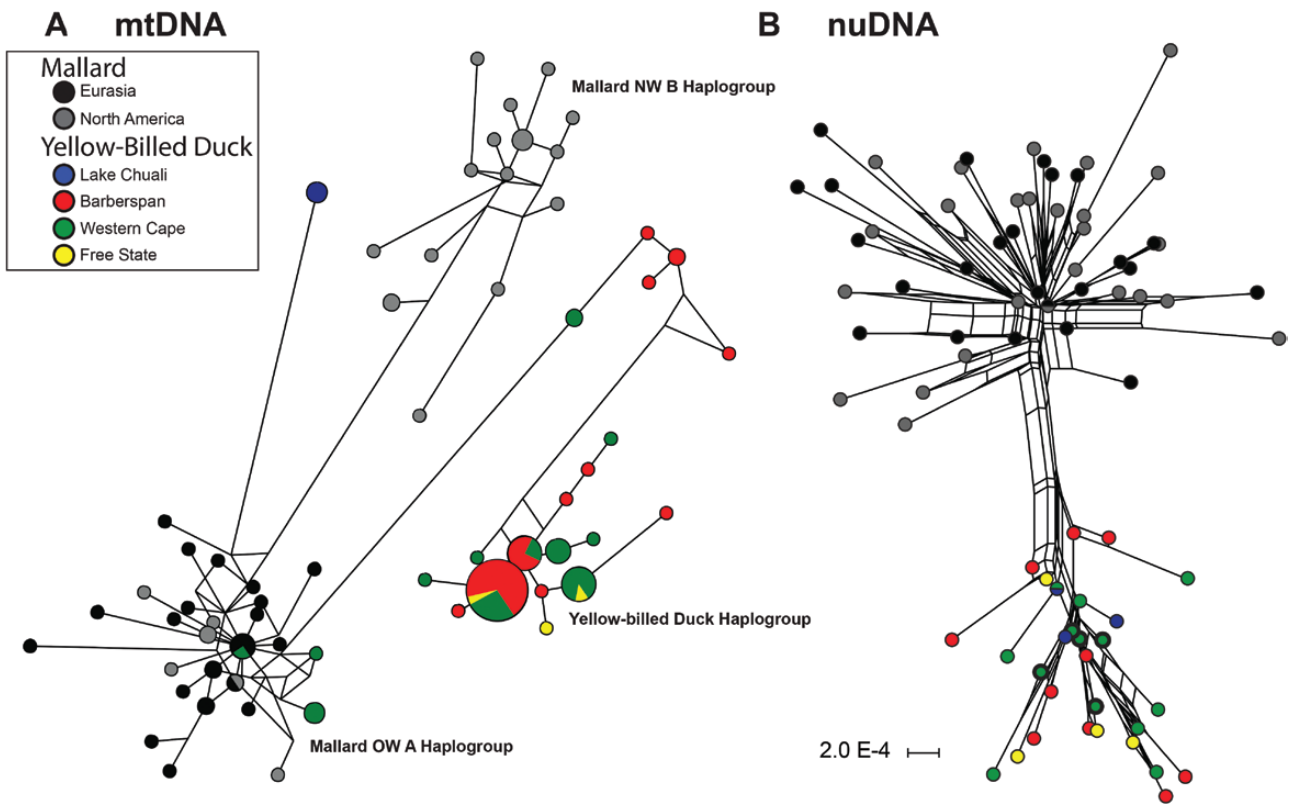

FIGURE 3. (**A**) Mitochondrial DNA median-joining network. Size of circles corresponds to total number of individuals (range: 1–10) with that haplotype; branch lengths indicate the number mutations separating haplotypes. (**B**) SplitsTree nuclear network from 19 nuclear loci. Bolded circles indicate Western Cape Yellow-billed Ducks that share OW Mallard A haplotypes.

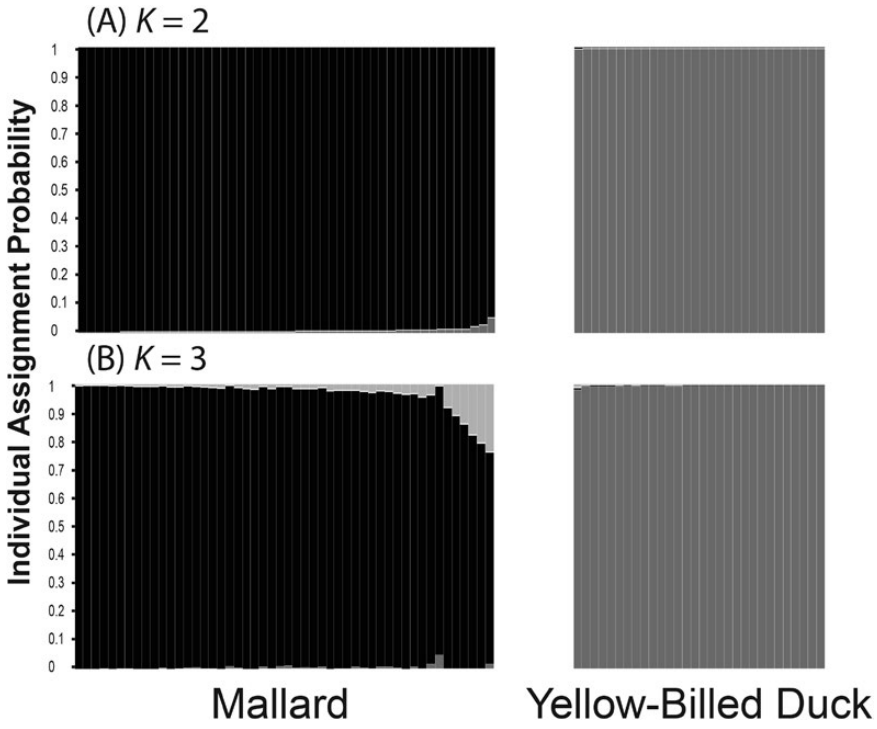

FIGURE 4. STRUCTURE assignment probabilities based on 19 nuclear loci for Mallards (black) and Yellow-billed Ducks (gray) for $K = 2$–3.

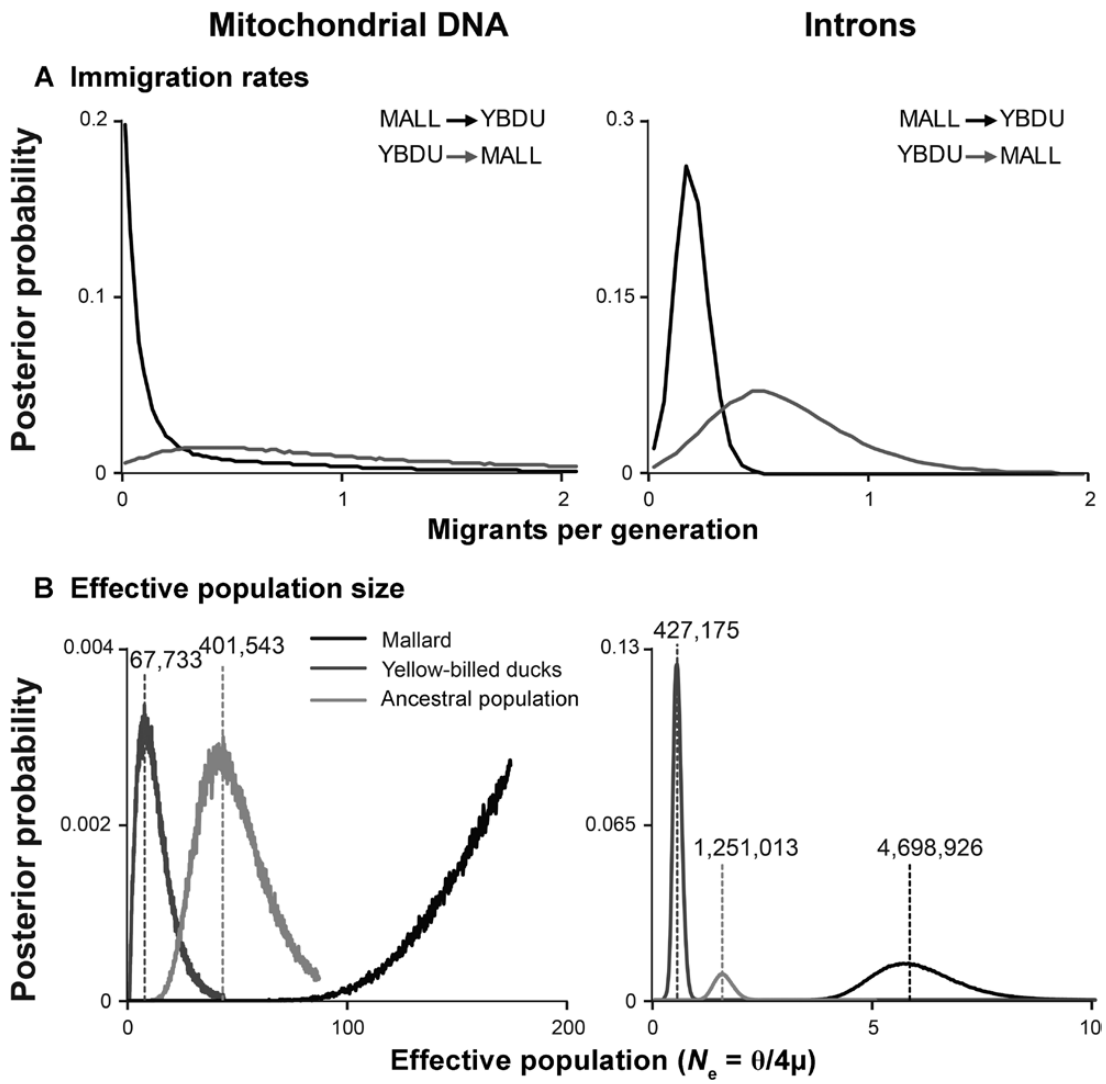

FIGURE 5. (**A**) Immigration rates estimated in IM (Hey and Nielsen 2004, 2007) for Mallard and Yellow-billed Ducks at 19 nuclear loci and mtDNA. (**B**) Effective population size (N_e) estimated in IM for Mallards, Yellow-billed Ducks, and their ancestor for 19 nuclear loci and mtDNA. The best-supported N_e is depicted by a dashed line estimated as N_e = (θ/4μ) individuals. MALL = Mallards; YBDU = Yellow-billed Ducks.

CI = 1,524,316–2,854,819 individuals) had ~11 times larger N_e than Yellow-billed Ducks ($N_{e\,YBDU}$ = 183,337 individuals; 95% CI = 130,955–256,672 individuals).

We were unable to reliably estimate time since divergence for mtDNA, but time since divergence based on nuclear markers was 514,562 yr before present (95% CI = 381,772–766,474 yr before present) between Mallards and Yellow-billed Ducks (Supplementary Materials Figure S3).

DISCUSSION

Unlike similar scenarios of secondary contact and subsequent introgressive hybridization between Mallards and other Mallard-like ducks (e.g., New Zealand Grey Ducks [Rhymer and Simberloff 1996], Hawaiian Ducks [Browne et al. 1993], American Black Ducks [Mank et al. 2004, Lavretsky et al. 2019b], Mottled Ducks [Peters et al. 2014a, Ford et al. 2017], or between feral Mallards and wild Mallards of North America [Lavretsky et al. 2019a] and Eurasia [Söderquist et al. 2017]), we conclude that Mallard gene flow into Yellow-billed Ducks does not appear to be a current threat. First, Yellow-billed Ducks were strongly differentiated across markers (Supplementary Materials Table S2), and we found no evidence of Mallard × Yellow-billed Duck hybrids in SPLITSTREE (Figure 3) or STRUCTURE analyses (Figure 4). There was also no evidence of recent Yellow-billed Duck backcrosses, as all samples showed >98% assignment to their respective species, indicating a lack of recent hybridization or introgression. Furthermore, we estimated migration rates of <1 migrant

per generation and could not reject estimates of no gene flow for nuDNA markers due to their posterior distribution overlapping zero (Figure 5A). Thus, based on sampled individuals, we conclude that there is little evidence to support contemporary introgressive hybridization of Mallards into Yellow-billed Duck populations.

Although Yellow-billed Ducks and Mallards were strongly differentiated at nuclear markers, 5 Western Cape samples and all Lake Chuali samples were nested within or closely related to the Mallard OW A haplogroup (Figure 3A). Given that domestic Mallards generally carry OW A haplotypes that are slightly diverged from wild Mallard OW A haplotypes (Söderquist et al. 2017, Lavretsky et al. 2019b), we are unable to rule out recent introgression with unsampled local feral Mallards as the source of identical OW A haplotypes shared by 2 Western Cape Yellow-billed Ducks. However, this scenario seems unlikely given that nuclear markers showed no evidence of introgression in both STRUCTURE and IM. Furthermore, the remaining closely related Yellow-billed Duck haplotypes were separated by several mutations, including 2–4 and ~13 mutations for the Western Cape and Lake Chuali samples, respectively, and the accumulation of these mutations requires significant time. Additionally, mtDNA is expected to have a smaller effective population size and shorter time for complete lineage sorting as compared to nuclear markers (Zink and Barrowclough 2008). Finally, although <1 migrant per generation, IM did recover non-zero rates of gene flow from Mallards into Yellow-billed Ducks for mtDNA (Figure 5A). Together, the data suggests that divergence between OW Mallard A haplotypes and the Yellow-billed Duck haplotypes closely related to them is likely due to older incidences of gene flow, rather than from feral Mallard populations that only became established over the last ~70 yr in southern Africa (i.e. ~1950; Matthews and Brand 2004). Alternatively, it is possible that these more closely related mtDNA haplotypes are simply due to incomplete lineage sorting; however, our IM results of non-zero gene flow from Mallards into Yellow-billed Ducks do not support this. Despite the increased probability of interspecific interactions between Yellow-billed Ducks and local feral Mallards in southern Africa, we conclude that introgressive hybridization does not seem to be a major threat to Yellow-billed Ducks. Future work will require the genetic assessment of local breeding Mallards from southern Africa to not only determine the source of these populations (i.e. domestic versus wild), but more comprehensively assess whether gene flow with these local Mallards is a conservation threat to Yellow-billed Ducks

Finally, species undergoing a rapid radiation (e.g., those in the Mallard complex) often share a large amount of ancestral variation throughout the genome due to insufficient time for complete lineage sorting to occur (Takahashi et al. 2001, Lavretsky et al. 2014a, Peters et al. 2014a). Thus, a general lack of fixed differences between Mallards and Yellow-billed Ducks across nuclear (Φ_{ST} = 0.21; locus-by-locus Φ_{ST} = 0.037–0.52) markers could be the result of an insufficient time since divergence (514,562 yr before present; 95% CI = 381,772–766,474 yr before present) that is necessary for lineage sorting. However, it is also possible that bouts of ancestral gene flow have prevented the fixation of molecular differences across the genomes of Mallards and Yellow-billed Ducks.

Yellow-Billed Duck Population Structure and Molecular Diversity

The most recent population census for Yellow-billed Ducks across all of Africa suggests the species has been stable around 130,000–210,000 individuals (Scott and Rose 1996), which is in accordance with the overall effective population size range estimated from nuclear markers ($N_{e\ YBDU}$ = 183,337 individuals; 95% CI = 130,955–256,672 individuals), but slightly lower than those estimated from mtDNA ($N_{e\ YBDU}$ = 309,962 individuals; 95% CI = 171,815–573,759 individuals: Figure 5B). Among the Yellow-billed Duck sampling areas, we found similar levels of nucleotide diversity for nuclear markers (π = 0.0061–0.0084) but recovered some differences in mtDNA markers. For example, the low mtDNA nucleotide diversity (π = 0; Table 1) and positive Tajima's *D* in Lake Chuali samples possibly reflect a recent founder event. However, Lake Chuali was fixed for a deeply divergent haplotype that was more closely related to the OW mtDNA Mallard A haplotypes than to Yellow-billed Duck haplotypes. Although ancestral gene flow with Mallards is likely the cause of this deep divergence, fixation of this haplotype could result from differences in movement ecology between sub-populations (Cumming et al. 2012); Yellow-billed Ducks from Lake Chuali and other coastal areas of East Africa might be partially isolated from other southern African populations that we sampled. More extensive sampling from this location and nearby locations is needed to better understand the evolutionary relevance of this finding.

Despite some mitochondrial structure, Yellow-billed Ducks are unstructured across nuclear markers, and we conclude that Yellow-billed Ducks should be considered as a single population. The mito-nuclear discordance observed here likely reflects Mallard mitochondrial capture, along with localized demographic processes that are often observed in waterfowl (Moore 1995, Kulikova et al. 2005, Zink and Barrowclough 2008), rather than the rise of mito-nuclear incompatibilities as is often the case for diverged taxa (Peters et al. 2014b). Specifically, waterfowl display strong female nest-site fidelity, leading to a high degree of mtDNA structure, and male-biased dispersal, which leads to an absence of structure in nuclear markers (Rohwer and Anderson 1988, Anderson et al. 1992, Peters et al. 2014b). In general, these results further demonstrate

why multi-locus comparisons are essential for population structure analysis of taxa in which demographic processes can disproportionally affect different markers.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *The Condor: Ornithological Applications* online.

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Author contributions: JLP, GSC, and PL conceived the idea and performed lab work; JIB, PL, and JLP wrote the paper and developed the methods; JIB analyzed the data.

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