Phylogenetics of a recent radiation in the mallards and allies (Aves: Anas): Inferences from a genomic transect and the multispecies coalescent

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A B S T R A C T

Reconstructing species trees by incorporating information from many independent gene trees reduces the confounding influence of stochastic lineage sorting. Such analyses are particularly important for taxa that share polymorphisms due to incomplete lineage sorting or introgressive hybridization. We investigated phylogenetic relationships among 14 closely related taxa from the mallard (Anas spp.) complex using the multispecies coalescent and 20 nuclear loci sampled from a genomic transect. We also examined how treating recombining loci and hybridizing species influences results by partitioning the data using various protocols. In general, topologies were similar among the various species trees, with major clades consistently composed of the same taxa. However, relationships among these clades and among taxa within clades changed among partitioned data sets. Posterior support generally decreased when filtering for recombination, whereas excluding mallards (Anas platyrhynchos) increased posterior support for taxa known to hybridize with them. Furthermore, branch lengths decreased substantially for recombination-filtered data. Finally, concordance between nuclear and morphometric topologies conflicted with those in the mitochondrial tree, particularly with regard to the placement of the Hawaiian duck (A. v. wyvilliana), Philippine duck (A. luzonica), and two spot-billed ducks (A. zonorhyncha and A. poecilorhyncha). These results demonstrate the importance of maximizing sequence length and taxon sampling when inferring taxonomic relationships that are confounded by extensive allele sharing.

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1. Introduction

Reconstructing phylogenetic relationships for recently diverged taxa can be confounded by allele sharing resulting from a recent shared ancestry (i.e. incomplete lineage sorting; Pamilo and Nei, 1988) or introgressive hybridization (Avise, 2000; Grant and Grant, 1992; Price and Bouvier, 2002). These factors result in taxa having heterogeneous genomes and discordant evolutionary histories among loci (Carstens and Knowles, 2007). Consequently, any single gene tree is unlikely to reflect the species tree (Degnan and Rosenberg, 2006). Advances in computational methods that incorporate information across numerous gene trees (Drummond and Rambaut, 2007; Kubatko et al., 2009; Liu, 2008) offer researchers the tools for reconstructing species trees derived from multispecies, genome-wide datasets (Carstens and Knowles, 2007; Jacobsen and Omland, 2011; Knowles, 2009). Although the ability of such programs to resolve relationships that are complicated by allele sharing has been tested with simulated data sets (Chung and Ané, 2011; Lanier and Knowles, 2012; Leaché and Rannala, 2011), few empirical investigations into the sensitivity of species tree reconstructions to recombination and hybridization have been conducted. The objectives of this study are to reconstruct phylogenetic relationships of 14 closely related taxa within the mallard complex (Anas platyrhynchos and allies) utilizing multispecies coalescent methods, while examining the sensitivity of results to various approaches for handling recombination and hybridizing species.

Many phylogenetic and population genetic methods require making an assumption of no intralocus recombination. Doing so, however, often requires that DNA sequences are truncated, potentially resulting in a loss of information and decreased phylogenetic resolution. Although the effects of recombination should be considered (Edwards, 2009; Rieseberg et al., 2000), simulations by Lanier and Knowles (2012) show that recombination may have little or no effect on phylogenetic inferences, and instead concluded that the negative effects introduced by ignoring recombination were offset by increasing sampling effort of loci and/or individuals. Topological comparisons between empirical datasets can be used to examine the influence of filtering for recombination, especially when comparing results to simulated data. In this study we compare trees that are reconstructed with entire gene reads (i.e. 402–411
filtering data for recombination and hybridization.

examine the sensitivity of phylogenetic inferences to methods of influences, the mallard complex is an excellent study system to species (Rhymer and Simberloff, 1996). Given these confounding 1976). Secondary contact between species pairs has resulted in rel-

berg, 1982). The mallard complex radiated around the world in the last million years (Johnson and Sorenson, 1999; Palmer, 1992) and about 20% producing viable hybrids (Scherer and Hils-

1.1. Study system

There are 11–13 extant species and three or four subspecies (depending on taxonomic authority) recognized within the mallard complex (Table 1); these species are distributed across several major continents and islands (Johnsonsgard, 1978). On the basis of these distributions, Palmer (1976) proposed an “out of Africa hypothesis” which suggests an African origin, followed by a northward and eastward radiation through Eurasia, with a step-wise progression through the South Pacific, and perhaps a single colonization of North America. An African origin is also supported by mitochondrial (mt) DNA sequences (Johnson and Sorenson, 1999).

Although species within the mallard complex were likely allopatric or parapatric historically, the mallard has responded to anthropogenic influences (e.g., releases from game farms and altered landscapes) and can now be found in sympathy with most of the other species. This secondary contact has resulted in wide-

spread hybridization with American black duck (A. rubripes; Avise et al., 1990), mottled duck (A. fulvigula; McCracken et al., 2001; Williams et al., 2005a), Chinese spot-billed duck (A. zonorhyncha; Kulikova et al., 2004), New Zealand (NZ) grey duck (A. superciliosa superciliosa; Rhymer et al., 1994), Hawaiian duck (A. wyvilliana; Griffin and Browne, 1990), and yellow-billed duck (A. undulata; Pers. Obs.). As hybridization events typically produce 100% viable offspring (Avise et al., 1990; but see Kirby et al., 2004), the taxonomy of this complex is uncertain (e.g. the Mexican Duck, Anas platyrhynchos) AOU 1983, 2010-B, Gill et al. IOC World Bird List). Because hybridization events usually involve mallards, introgressed mallard alleles shared among the other species might confound phylogenetic inferences. To examine the influence of introgression on topologies, we reconstructed phylogenies with and without mallards. If introgression does not introduce biases, we predict comparable posterior support between sets of trees (including vs. excluding mallards) as incomplete lineage sorting would have a similar influence regardless of data treatment. Alternatively, if recently introgressed mallard alleles have a strong influence on topologies or posterior support then we predict an increase in the posterior support for the relationships of the taxa that are influenced by these recently introgressed mallard derivatives.

Relationships within the mallard complex have been reconstructed with both morphometric data (Livezey, 1991) and mtDNA (Johnson and Sorenson, 1999; McCracken et al., 2001), but the topologies of these trees differed in several ways. In particular, morphometrics suggested a Pacific/southeast Asian clade that included the Pacific black duck (A. superciliosa), the Philippine duck (A. luzonica), the Indian spot-billed duck (A. poecilorhyncha), and the Chinese spot-billed duck, whereas mtDNA placed the latter three species in a clade consisting of Old World (OW) mallards to the exclusion of Pacific black ducks. Chinese spot-billed ducks and mallards have polyphyletic mtDNA haplotypes (Kulikova et al., 2004). Likewise, morphometrics suggested a sister relationship between the Hawaiian duck and the Laysan duck (A. laysanensis), but mtDNA supports a polyphyletic relationship among mallard and Hawaiian duck haplotypes that are not closely related to Laysan duck haplotypes (Fowler et al., 2009; Johnson and Sorenson, 1999). Notably, differentiation in allozymes is more consistent with morphometrics (Browne et al., 1993). Furthermore, neither of these data sets provided strong support for phylogenetic relationships among the North American monochromatic mallard-like ducks (mottled duck, American black duck, and Mexican duck), which have polyphyletic mtDNA (Avise et al., 1990; McCracken et al., 2001). Finally, mtDNA supports a prominent divergence between the Eurasian and North American mallards (Johnson and Sorenson, 1999; Kulikova et al., 2005), but at least qualitatively, there are no morphological differences between these populations. Given these conflicts between morphometric and mtDNA data, an analysis of independent characters is needed to understand the phylogenetic relationships of this recently radiated group.

Avian researchers have generally focused on mtDNA. Maternally inherited and having no recombination (Giles et al., 1980; Watanabe et al., 1985), mtDNA has a more rapid sorting rate and shorter coalescent intervals relative to biparentally-inherited, recombining nuclear DNA (nuDNA). This makes it particularly useful for recently diverged populations (Moore, 1995; Zink and Barrowclough, 2008). However, being maternally inherited and potentially under strong selection, its appropriateness for phylogenetics and phylogeography has been questioned (Bazin et al., 2006; Edwards and Bensch, 2009; Edwards et al., 2005; Hurst and Jiggins, 2005; Jacobsen and Omland, 2011b). Moreover, any single locus is sensitive to stochastic genealogical variability, which can mislead species-level phylogenies (Jacobsen and Omland, 2011b; Kubatko and Degnan, 2007; Maddison, 1997). Nevertheless, multilocus comparisons—including between and

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Table 1

<table>
<thead>
<tr>
<th>Species (Anas platyrhynchos; MALL)</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurasia (OW)</td>
<td>5</td>
</tr>
<tr>
<td>North America (NW)</td>
<td>5</td>
</tr>
<tr>
<td>American black duck (A. rubripes; ABDU)</td>
<td>5</td>
</tr>
<tr>
<td>Mottled duck (MODU)</td>
<td>5</td>
</tr>
<tr>
<td>Florida, FL (A. f. fulvigula)</td>
<td>5</td>
</tr>
<tr>
<td>Western gulf coast, WGC (A. f. maculosa)</td>
<td>5</td>
</tr>
<tr>
<td>Mexican duck (A. p. diazi; MEDU)</td>
<td>5</td>
</tr>
<tr>
<td>Hawaiian duck (A. wyvilliana; HADU)</td>
<td>5</td>
</tr>
<tr>
<td>Laysan duck (A. laysanensis; LADU)</td>
<td>5</td>
</tr>
<tr>
<td>Chinese spot-billed duck (A. zonorhyncha; SPBDm)</td>
<td>5</td>
</tr>
<tr>
<td>Indian spot-billed duck (A. poecilorhyncha; SPBDm)</td>
<td>5</td>
</tr>
<tr>
<td>Philippine duck (A. luzonica; PHDU)</td>
<td>1</td>
</tr>
<tr>
<td>African black duck (A. s. superciliosa rogersii; PBDU)</td>
<td>5</td>
</tr>
<tr>
<td>Yellow-billed duck (A. undulata; YBDU)</td>
<td>5</td>
</tr>
<tr>
<td>Miller’s duck (A. s. superciliosa; GRDU)</td>
<td>5</td>
</tr>
<tr>
<td>Pacific black duck (A. s. superciliosa; GRDU)</td>
<td>5</td>
</tr>
<tr>
<td>New Zealand grey duck (A. s. superciliosa; GRDU)</td>
<td>5</td>
</tr>
</tbody>
</table>
within mitochondrial and nuclear genes—can provide insights into phenomena (e.g. historical introgression, mtDNA capture, sex-biased dispersal) that would otherwise be lost in any single-locus analysis (Jacobsen et al., 2010; Jacobsen and Omland, 2011a; Peters et al., 2012a, 2005). Thus, we also compare phylogenetic inferences among trees derived from morphometric data, mtDNA, and nuDNA.

2. Materials and methods

We sampled one to five individuals per species, subspecies or population for a total of 64 individuals from 16 operational taxonomic units (Table 1). Mallards form the New World (NW) and Old World (OW) and mottled ducks from the western gulf coast (WGC) and Florida (FL) were partitioned into subpopulations that were previously delineated with mtDNA (Avise et al., 1990; Johnson and Sorenson, 1999; McCracken et al., 2001) or nuDNA (Williams et al., 2002, 2005a,b).

Genomic DNA was isolated from each sample using a Qiagen DNAeasy blood and tissue kit (Qiagen, Valencia, CA) following manufacturer’s protocol. We used previously optimized primers to amplify and sequence 19 nuclear introns (Table 2; Peters et al., 2012b) and 640 bp of the mtDNA control region (Sorenson et al., 1999; Sorenson and Fleischer, 1996). Additionally, we sequenced melanocortin 1 receptor (MC1R), because of its association with plumage characteristics in other birds (Mundy, 2005). Two sets of primers were designed to target 782 bp of exon sequence from the MC1R gene [primers MC1RR (5’-ATGATGGAGATTGAAGAGAAGG-3’)/MC1RFi (5’-CTGGACCCGCTACATCCG-3’)] and MC1RRi (5’-TAGAGCAGCAGCATGAGGAG-3’)/MC1RF (5’-CAGTAGGGCCACAGGAG-3’)]. These primers were designed from sequences downloaded from GenBank (accession numbers EU924209–EU924107 (Anas platyrhynchos); FJ605434–FJ605453 (Cairina moschata); Xia et al., unpubl. data).

The polymerase chain reaction (PCR) was used to amplify each locus using 1.5 μL of template DNA (≥ 10 ng/μL), 2× GoTaq Green Master Mix (Promega), and 1.0 nM of each primer, in a total volume of 15 μL. PCR was conducted using an Eppendorf Mastercycler (epgradient) under the following conditions: 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 (at 52 °C for mtDNA) for 20 s, and extension at 72 °C for 1 min, and a final DNA extension at 72 °C for 7 min. Amplification was verified using gel electrophoresis with a 1.5% agarose gel, and PCR products were cleaned with AMPure XP beads following the Agencourt protocol (Beckman Coulter Co.). Sequencing was done using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) following manufacturer protocols using a 1/8 reaction. Final products were sent to the DNA Analysis Facility at Yale University for automated sequencing on an ABI 3730. Sequences were aligned and edited using Sequencher v. 4.8 (Gene Codes, Inc.). All sequences have been submitted to GenBank (accession numbers: KF607919-KF609252; AY506871, AYS06947, AY506948, AY506964, AY928831, AY928841-3, AY928846, Kulikova et al., 2004, 2005).

2.1. Nuclear coalescent phylogeny and ΨST estimates

The gametic phases of nuclear alleles were determined by resolving sequences with the program PHASE v. 2.1.1 (Stephens and Donnelly, 2003), which derives the most likely state of each allele algorithmically. Additionally, indels were resolved using methods described in Peters et al. (2007) that determined gametic phases based on base-pair peak shifts within the chromatograms. Sequences resolved with this method were included as known alleles in the PHASE analyses. Mallard sequences were all resolved with >95% confidence from a larger data set that included exten-
Table 2
Characteristics of 20 nuclear loci sequenced in this study: locus name, chromosomal location, and the total length, number of polymorphic sites, and number of parsimony-informative sites of non-filtered and filtered (in parentheses) datasets.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Locationa</th>
<th>Non-filtered (filtered)</th>
<th>Number of polymorphic sites</th>
<th>Number of parsimony-informative sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromo-helicase-DNA binding protein gene 1, intron 19</td>
<td>Z</td>
<td>326 (326)</td>
<td>10 (10)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Lactate dehydrogenase 1, intron 4</td>
<td>1</td>
<td>520 (520)</td>
<td>7 (7)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>S-acyl fatty acid synthase thioesterase, intron 2</td>
<td>2</td>
<td>303 (303)</td>
<td>10 (10)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Ornithine decarboxylase, intron 7</td>
<td>3</td>
<td>302 (151)</td>
<td>37 (20)</td>
<td>24 (13)</td>
</tr>
<tr>
<td>Fibrinogen beta chain, intron 7</td>
<td>4</td>
<td>437 (246)</td>
<td>27 (15)</td>
<td>17 (8)</td>
</tr>
<tr>
<td>Serum amyloid A, intron 2</td>
<td>5</td>
<td>322 (133)</td>
<td>37 (12)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>Annexin A11, intron 2</td>
<td>6</td>
<td>440 (382)</td>
<td>39 (34)</td>
<td>26 (23)</td>
</tr>
<tr>
<td>Myostatin, intron 2</td>
<td>7</td>
<td>281 (168)</td>
<td>26 (16)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Soat 1-grov protein, intron 10</td>
<td>8</td>
<td>332 (332)</td>
<td>13 (13)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Nucleolin, intron 12</td>
<td>9</td>
<td>359 (98)</td>
<td>49 (16)</td>
<td>40 (14)</td>
</tr>
<tr>
<td>Melanocortin 1 receptor</td>
<td>11</td>
<td>782 (782)</td>
<td>28 (25)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Preproghrelin, intron 3</td>
<td>12</td>
<td>305 (290)</td>
<td>18 (17)</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Glutamate receptor, ionotropic, N-methyl D aspartate 1, intron 13</td>
<td>17</td>
<td>300 (85)</td>
<td>22 (1)</td>
<td>14 (0)</td>
</tr>
<tr>
<td>Sex determining region Y-box, intron 2</td>
<td>18</td>
<td>402 (120)</td>
<td>60 (12)</td>
<td>46 (11)</td>
</tr>
<tr>
<td>Carboxypeptidase D, intron 9</td>
<td>19</td>
<td>332 (127)</td>
<td>43 (18)</td>
<td>34 (13)</td>
</tr>
<tr>
<td>Phosphoehospholate dehydrogenase, intron 9</td>
<td>20</td>
<td>333 (333)</td>
<td>12 (12)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Alpha enolase 1, intron 8</td>
<td>21</td>
<td>294 (179)</td>
<td>19 (11)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>Alpha-B crystallin, intron 1</td>
<td>22</td>
<td>323 (323)</td>
<td>8 (8)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Growth hormone 1, intron 3</td>
<td>23</td>
<td>380 (379)</td>
<td>22 (21)</td>
<td>16 (12)</td>
</tr>
<tr>
<td>Lechithin-cholesterol acyltransferase, intron 3</td>
<td>Unk</td>
<td>323 (154)</td>
<td>36 (15)</td>
<td>22 (10)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7396 (5431)</td>
<td>523 (293)</td>
<td>332 (170)</td>
</tr>
</tbody>
</table>

Percent change between filtered and non-filtered datasets

-27.0%  -44.0%  -49.0%

a Location: chromosomal location based on chicken genome (Hillier et al., 2004).
b Length: base-pairs.

(Weir and Schluter, 2008). The Beast species tree obtained during the molecular clock analysis was used for direct comparison with the nuclear derived tree. A Bayesian tree illustrating relationships among all haplotypes was reconstructed in MrBayes using the same substitution and molecular clock models. The tree search comprised two concurrent runs, 3 million MCMC generations with sampling occurring every 2000 generations, and persisted until the average standard-deviation between runs was <0.01. The first 25% of trees were discarded as burn-in and the final tree was summarized and viewed in FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).

3. Results

3.1. Nuclear species tree

In general, topologies across the four nuDNA coalescent trees (Fig. 1) were similar and included a basal African lineage consisting of the African black duck (A. sparsa), the yellow-billed duck, and the Meller’s duck (A. melleri), an Australasian clade composed of the Philippine duck, NZ grey duck, and Pacific black duck, a Hawaiian clade with the Hawaiian duck and Laysan duck, and a New World clade with the NW mallard, Mexican duck, American black duck, FL mottled duck, and WGC mottled duck. In addition, analyses ignoring recombination yielded a South Pacific super clade with the Hawaiian and Australasian clades being sister, that also included the Chinese and Indian spot-billed ducks as sister lineages. Relationships within the NW clade and the OW mallard were poorly supported; however, the highest posterior support within the NW clade was obtained with the exclusion of mallards and ignoring recombination.

3.2. Ignoring vs. filtering for recombination

After filtering for recombination, the total number of nucleotides, polymorphic sites, and informative sites decreased by 27%, 44%, and 49%, respectively (Table 2). All major groups were present with the filtered dataset; however, posterior support across nodes substantially decreased with the exception of those within the Hawaiian and Australasian clades. Although poorly supported across analyses, both spot-billed ducks (Indian and Chinese) were grouped within the Hawaiian and/or Australasian clades when ignoring recombination, but placed within the NW clade and elsewhere when analyzed with the recombination-filtered dataset. Neither dataset conclusively resolved relationships of NW taxa. Interestingly, on average, branch lengths substantially decreased when filtering for recombination (Fig. 1), and strongly corresponded to treatment (i.e. ignoring vs. filtering for recombination) and not the inclusion/exclusion of mallards (Fig. 2).

3.3. Including vs. excluding mallards

Excluding mallards had no effect on overall relationships and little effect on posterior support of basal lineages. However, posterior support among the remaining NW taxa increased slightly when mallards were excluded (Fig. 1).
3.4. Mitochondrial Derived Topologies

The mtDNA gene tree derived with Bayesian methods provided estimates of relationships among individuals, whereas the coalescent methods inferred species relationships. Although the Bayesian methods revealed a large polytomy and failed to resolve relationships among clades (Fig. 3), memberships within groups were generally well supported and consistent with previous studies. However, NW species, OW mallards, and both spot-billed ducks were polyphyletically intermixed, with some NW mallards grouping within the OW clade and some Chinese spot-billed ducks grouping within the NW clade (Kulikova et al., 2004, 2005). Chinese spot-billed duck haplotypes within the NW clade were consistent with a monophyletic subclade (Kulikova et al., 2004). Within the NW clade, the placement of Hawaiian ducks was consistent with a monophyletic group (see also Fowler et al., 2009) that was sister to three of the five FL mottled ducks. In addition, a well-supported subclade consisted exclusively of Mexican ducks and WGC mottled ducks (Fig. 3). FL and WGC mottled duck haplotypes were fairly divergent and consistent with previous studies (McCracken et al., 2001). The Philippine duck group within the OW clade that consisted of OW mallards, Chinese spot-billed ducks, and Indian spot-billed ducks (Fig. 3). Otherwise, mtDNA haplotypes for the remaining species clustered into monophyletic groups clades. Two divergent groups were recovered for Pacific/New Zealand grey duck, one of which was exclusive to New Zealand (Rhymer et al., 2004). Relationships within the coalescent derived species tree provided similar relationships with the exception being that the Philippine duck was recovered as sister to the yellow-billed duck (Fig. 4).

3.5. Morphometrics vs. MtDNA vs. NuDNA

Relationships provided by the three trees based on different character sets varied extensively, especially with regards to the placement of the Philippine duck, both Chinese and Indian spot-billed ducks, and the Hawaiian duck. The discrepancy, however, mostly lies with mtDNA, whereas topologies were nearly identical between morphometric data and nuDNA. Specifically, the sister relationship of the Philippine duck to the Pacific black duck/NZ grey duck lineage and the sister relationship between the Hawaiian duck and Laysan duck (Fig. 1) were consistently well supported by

Fig. 1. Nuclear multispecies coalescent trees reconstructed from 20 nuclear loci and 16 species/subspecies/populations of ducks. Analyses were conducted for the full dataset (ignoring recombination), recombination-filtered datasets, and including or excluding mallards, which hybridizes extensively with the other species.
nuDNA and morphology, but not mtDNA (Fig. 4). Additionally, while the relationships of the two spot-billed ducks were poorly supported with nuDNA, they were found to be closer to the Hawaiian and Australasian clades with datasets where recombination was ignored, which once again corresponded to relationships derived from morphometric data. However, the topology of the nuDNA trees obtained from truncating sequences showed some evidence of the spot-billed ducks grouping with the NW/OW mallards, which was consistent with the mtDNA topology.

4. Discussion

Applying coalescent methods to a 20-locus dataset provided a fairly well resolved phylogeny for taxa within the mallard complex. Topologies across all protocols for handling recombination and hybridizing species were similar with major groups strongly supported. However, nodal support declined when filtering the data for recombination. Support for the more divergent lineages especially decreased, corroborating findings from simulated datasets showing that ignoring recombination decreased nodal support for deeper relationships within recently radiated taxa that have unsorted loci (Lanier and Knowles, 2012). The success of resolving relationships between taxa that are only statistically distinguishable based on allele frequencies is dependent on the presence of sufficient data (Knowles et al., 2012; Maddison and Knowles, 2006). Specifically, as loci are truncated and informative variation is removed (e.g. ~45% of parsimony-informative positions in this study; Table 2), the power for resolving relationships decreases. In contrast, ignoring recombination maximizes the number of nucleotides and individuals per taxon, which presumably enhances the phylogenetic signal obtained from statistically diagnostic markers. In general, while biases may be present when ignoring recombination, phylogenetic reconstructions of recently radiated taxa appear to be robust to violating the assumption of no recombination (Lanier and Knowles, 2012). Given the overall similarity in topologies from the recombination-filtered and the full datasets, our results are consistent with this generalization.

Unlike the tree topology, branch lengths were strongly affected when filtering the dataset for recombination (Figs. 1 and 2). Although taxonomic relationships are generally corroborated, discrepancies in branch lengths between the two datasets suggest that estimating divergence times and the rate of diversification will be sensitive to how the data are treated (Fig. 2). However, it is not clear which of these methods give more realistic branch lengths. On the one hand, ignoring recombination might inflate branch lengths, because more mutations will be inferred when recombination creates new alleles. On the other hand, filtering for recombination can result in the exclusion of the most variable portions of the loci and the most variable sequences in the data set. This bias is supported by simulated data showing that filtering for recombination underestimates effective population sizes (Woerner et al., 2007). Thus, analyzing recombination-filtered datasets likely biases branch lengths downwards, which would lead to underestimating divergence times. The true branch length is likely intermediate between these two extremes.

4.1. Relationships within the mallard complex

Topologies corresponded to those predicted by the “Out of Africa” hypothesis (Palmer, 1976), including basal African lineages (see also Johnson and Sorenson, 1999). However, whereas the “Out of Africa” hypothesis is based on a step-wise progression through the South Pacific after colonization of the OW (Palmer, 1976),
phylogenetic (Fig. 1) and \( \Phi_{ST} \) estimates (Table 3) suggest an almost simultaneous split between the Australasian clade, the Hawaiian clade, and OW mallards/NW taxa. Consequently, results from nuDNA are inconclusive regarding the step-wise progression proposed by Palmer (1976).

Few differences exist within and between NW taxa and OW mallards (\( \Phi_{ST} \leq 2\% \)), demonstrating the extent to which the genome is shared among them (e.g. Kraus et al., 2012; Kulikova et al., 2004). Moreover, of the two spot-billed ducks, pairwise \( \Phi_{ST} \) estimates were lowest when comparing the NW and OW mallards to the Chinese spot-billed duck (Table 3). In fact, the Chinese spot-billed duck appears to be indistinguishable from OW mallards (\( \Phi_{ST} \leq 0\% \)), which corroborates previous research from mtDNA suggesting high levels of hybridization between these two taxa (Kulikova et al., 2004). In general, the inability to resolve relationships within these groups can be attributed to a recent ancestry (i.e. Upper Pleistocene; Heusmann, 1974) and ongoing introgressive hybridization between each species and the mallard. A larger sample size of individuals will be needed to increase the signal from allele-frequency differences, which can strengthen phylogenetic inferences for recently diverged taxa (Knowles, 2009; Knowles and Maddison, 2002; Lanier and Knowles, 2012). However, methods that incorporate introgression might be necessary to fully resolve these phylogenetic relationships.

4.2. The introgressive effect

We predicted that relationships among NW and OW taxa would be most influenced by the presence/absence of mallards because of the high incidence of hybridization between mallards and other taxa (Avise et al., 1990; Kulikova et al., 2004). Specifically, if relationships are significantly confounded by introgressed alleles then posterior support should increase when the introgressing species (i.e. mallard) is removed. Conversely, if relationships are predominantly affected by incomplete lineage sorting (ILS) then relationships and posterior support should remain fairly similar between

Fig. 3. Mitochondrial gene tree reconstructed in MrBayes using 690 base pairs of the control region for 64 individuals.
Fig. 4. Phylogenetic relationships of 16 species/subspecies/populations from the mallard complex derived from (A) morphometric data (Reconstructed with data from Livezey, 1991), (B) mitochondrial DNA (mtDNA) control region, and (C) 20 nuclear loci (nuDNA; ignoring recombination). Both species mtDNA and nuDNA species trees were reconstructed in Beast v.1.6.1 (Drummond and Rambaut, 2007; Heled and Drummond, 2012) (see Section 2).

tree pairs. In general, relationships remained similar and poorly supported within the NW/OW group across runs; however, the posterior support for NW taxa doubled when mallards were excluded and recombination was ignored. Similar patterns were not observed between trees derived from recombination-filtered datasets, where the overall decreased resolution across topologies is likely attributable to the number of parsimony-informative sites that were lost (Table 2). Thus, results between datasets ignoring recombination suggest that the genomes of the various taxa are not swamped by mallard alleles, and while they do not have taxon-specific markers, they are likely distinguishable through frequency differences. However, when the mallard was included, shared alleles due to mallard introgression reduces the signal of these diagnostic markers. In general, these results demonstrate that the inclusion of the inrogressing taxa does in fact influence the support of those taxa it is interacting with and that high rates of introgression may be having an important influence on inferences of phylogenetic relationships among the NW taxa.

Whereas $\Phi_{ST}$ estimates suggest that the two spot-billed ducks are more similar to NW taxa and OW mallards than to others, only trees reconstructed with no mallards and recombination-filtered datasets place them within the OW/NW group, and the tree reconstructed with mallards has the Chinese spot-billed duck as sister to the OW mallard (Fig. 1). Otherwise, trees reconstructed with datasets ignoring recombination placed them within the Australasian clade, which closely resembled the tree derived from morphometric data (Fig. 4A; Livezey, 1991). Furthermore, unlike posterior support of NW taxa that increased when excluding mallards, those of the two spot-billed ducks remained low and unchanged across analyses. Consequently, relationships of the two spot-billed ducks seem to be more influenced by how the data are processed rather than the inclusion/exclusion of mallards, despite extensive hybridization between the Chinese spot-billed duck and the mallard (Kulikova et al., 2004). In general, the Pacific relationship is likely due to retention of ancestral states that are similar to those within the South Pacific superclade but missing in NW taxa, while extensive sharing of polymorphisms with NW taxa maintains low posterior support for these relationships (Fig. 3). Moreover, the loss of the spot-billed duck from the South Pacific superclade in trees derived from recombination-filtered datasets is likely due to the loss of the ancestral states during the filtering process, which then draws the spot-billed ducks closer with OW/NW taxa.

4.3. Marker comparison and speciation within the mallard complex

We found strong discrepancies between morphometric, mitochondrial, and nuclear based phylogenies (Fig. 4). Generally, however, where the nuDNA and mtDNA topologies conflicted, the nuDNA was corroborated by morphometrics (Livezey, 1991). For example, mtDNA places the Hawaiian duck within the NW clade, whereas both morphology and nuDNA place it as the sister-taxon of the Laysan duck. The close affinity between the Hawaiian duck and Laysan duck is also supported by allozyme studies (Browne et al., 1993). Similarly, whereas both morphology and nuDNA place the Philippine duck sister to the Pacific black duck and NZ grey duck, mtDNA suggests that it is part of the OW clade. These sister relationships received high posterior support in all four nuDNA trees. However, the placement of the Chinese and Indian spot-billed ducks is more ambiguous in the nuDNA trees and varied with the manner of treating data. When ignoring recombination, these species grouped with the South Pacific superclade; the inclusion of spot-bills and Australasian ducks within the same clade to the exclusion of Northern Hemisphere mallards is consistent with morphometrics but conflicts with mtDNA. In contrast, when filtering for recombination, spot-bills had a tendency to group with the Northern Hemisphere ducks, which is more consistent with mtDNA relationships.

Such mito-nuclear conflict can result from a number of processes including stochastic lineage sorting and hybridization. It seems unlikely that this discord results from stochastic lineage sorting given the deep mtDNA branch lengths among the major clades, the shallow mtDNA divergence among species within clades, and the strong nodal support for the nuDNA topology. However, mitochondrial capture (Brelsford et al., 2011) or hybrid speciation (Jacobsen and Omland, 2011a; Mallet, 2007) could explain this discord. First, considering the relationships presented with mtDNA and nuDNA, generations of introgressive events between female mallards and male heterospecifics can cause mtDNA to inrogress and become fixed within the invaded species, resulting in mitochondrial capture. The strong support for the 20-locus nuDNA topology suggests close genomic affinities between Hawaiian and Laysan ducks and between Philippine and Pacific black ducks, which is consistent with introgression of mallard mtDNA into a genomically divergent species.

Alternatively, hybrid speciation theory dictates that a novel species evolves from historical hybridization events between two parental taxa (Seehausen, 2004). Such a mechanism for speciation within the mallard group has been suggested for the extinct Marian mallard (A. australis), which is believed to be descended from hybridization between the mallard and Pacific black duck (Reichel and Lemke, 1994). Thus, it is possible that the Hawaiian duck arose from hybridization between a NW mallard-like duck and Laysan duck and that the Philippine duck, and perhaps the spot-billed ducks, arose from hybridization between an OW mallard-like duck.
4.4. Conclusions

The data presented in this study represent the most comprehensive phylogeny, both in terms of sample sizes and genomic coverage, for the mallard clade. This study illustrates the effectiveness of multilocus data and coalescent methods in resolving phylogenetic relationships among taxa with extensive sharing of polymorphisms. Generally, posterior support across analyses and relatively strong posterior support for some nodes. Finally, the discordance in the placement of the Hawaiian duck, Philippine duck, as well as Indian and Chinese spot-billed ducks demonstrates how comparing trees based on different character sets can reveal phenomena that would otherwise be lost with a single tree. Testing the causes of this discordance can be important in reconstructing and understanding evolutionary history and speciation.

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Appendix A. Supplementary material

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References


