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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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ABSTRACT

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. 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.

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 multilocus, genome-wide datasets (Carstens and Knowles, 2007; Jacobsen and Omland, 2011b; 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 multilocus 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.

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"ignoring recombination") or with datasets where loci have been truncated to be consistent with no recombination (i.e., "recombination-filtered"). Based on simulated datasets (Lanier and Knowles, 2012) we expect few changes in the relationships among taxa but a decrease in the posterior support, particularly for the deepest nodes, when data is filtered for recombination.

Discordance among loci resulting from hybridization has been an important issue in avian phylogenetics (Degnan and Rosenberg, 2009; Jacobsen and Omland, 2011b; Weckstein et al., 2001). The high proportion of shared polymorphisms among species has been attributed to dispersal ability (Greenwood, 1980), chromosomal stasis (Ellegren, 2010), and relatively low levels of reinforcement (Grant and Grant, 1997) in birds. Among avian orders, waterfowl (Anseriformes) experience among the highest rates of hybridization (Johnsgard, 1960; Lijtmaer et al., 2003; Livezey, 1986), with 30–40% of species being capable of interbreeding (Grant and Grant, 1992) and about 20% producing viable hybrids (Scherer and Hilsberg, 1982). The mallard complex radiated around the world in the last million years (Johnson and Sorenson, 1999; Palmer, 1976). Secondary contact between species pairs has resulted in relatively high rates of introgressive hybridization, especially between the geographically widespread mallard and the other species (Rhymer and Simberloff, 1996). Given these confounding influences, the mallard complex is an excellent study system to examine the sensitivity of phylogenetic inferences to methods of filtering data for recombination and hybridization.

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 (Johnsgard, 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 stepwise 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 sympatry with most of the other species. This secondary contact has resulted in widespread 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*] *diazi*; 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 tree 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 supported a Pacific/southeast Asian clade that included the Pacific black duck (*A. superciliosa*), the Philippine duck (*A. luzonica*), the Indian spot-billed duck (*A. poeciliorhyncha*), 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 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

Table 1
Species, sub-species, and populations of the mallard complex included in analyses with their respective sample sizes.

Species	Sample size
Mallard (*Anas platyrhynchos*; MALL)	
Eurasia (OW)	5
North America (NW)	5
American black duck (*A. rubripes*; ABDU)	5
Mottled duck (MODU)	
Florida, FL (*A. f. fulvigula*)	5
Western gulf coast, WGC (*A. f. maculosa*)	5
Mexican duck (*A. p. diazi*; MEDU)	5
Hawaiian duck (*A. wyvilliana*; HADU)	5
Laysan duck (*A. laysanensis*; LADU)	5
Chinese spot-billed duck (*A. zonorhyncha*; $\mathrm{SPBD_{CH}}$)	5
Indian spot-billed duck (*A. poecilorhyncha*; $\mathrm{SPBD_{IN}}$)	1
Philippine duck (*A. luzonica*; PHDU)	1
African black duck (*A. sparsa*; AFBD)	1
Yellow-billed duck (*A. undulata*; YBDU)	5
Meller's duck (*A. melleri*; MELL)	1
Pacific black duck (*A. superciliosa rogersii*; PBDU)	5
New Zealand grey duck (*A. s. superciliosa*; GRDU)	5