Molecular Phylogenetics and Evolution 70 (2014) 402-411

Contents lists available at ScienceDirect



Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



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



Philip Lavretsky^{a,*}, Kevin G. McCracken^b, Jeffrey L. Peters^a

^a Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Hwy, Dayton, OH 45435, USA ^b Institute of Arctic Biology, Department of Biology and Wildlife, University of Alaska Museum, 902 N. Koyukuk Dr., Fairbanks, AK 99775, USA

ARTICLE INFO

Article history: Received 6 February 2013 Revised 5 August 2013 Accepted 15 August 2013 Available online 27 August 2013

Keywords: Coalescent models Mallard complex Marker discordance Recombination Speciation Species tree

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.

© 2013 Elsevier Inc. All rights reserved.

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),

* Corresponding author. *E-mail address:* lavretsky.2@wright.edu (P. Lavretsky). 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.

^{1055-7903/\$ -} see front matter © 2013 Elsevier Inc. All rights reserved. http://dx.doi.org/10.1016/j.ympev.2013.08.008

"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; Liitmaer et al., 2003; Livezev, 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 wide-

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 (<i>A. wyvilliana</i> ; HADU)	5
Laysan duck (A. laysanensis; LADU)	5
Chinese spot-billed duck (A. zonorhyncha; SPBD _{CH})	5
Indian spot-billed duck (A. poecilorhyncha; 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

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] 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 Lavsan 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 within mitochondrial and nuclear genes—can provide insights into phenomena (e.g. historical introgression, mtDNA capture, sexbiased 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'ATGATGAGGATGAGGAA-GAGG 3')/MC1RFi (5' GTGGACCGCTACATCACCRT 3') and MC1RRi (5' TAGAGCACCAGCATGAGGA 3')/MC1RF (5' CAGTGAGGGCAACCA-GAG 3')]. These primers were designed from sequences downloaded from GenBank (accession numbers EU924091-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 μ L of template DNA (\ge 10 ng/ μ L), 2× GoTag 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, AY506947, AY506948, AY506964, AY928831, AY928841-3, AY928846, Kulikova et al. 2004, 2005).

2.1. Nuclear coalescent phylogeny and Φ_{ST} 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-

sive allele-specific priming (Peters et al. unpubl. data), and these alleles were also treated as knowns.

Filtering for recombination was based on truncating loci into putatively non-recombinant fragments containing the highest number of polymorphic positions using the program IMgc (Woerner et al., 2007). We iteratively adjusted chromosomal weighting so that a maximum of 5% of sequences were removed and so that both alleles from all taxa represented by a single individual were retained. Once thresholds were achieved sequences were manually truncated with the program Sequencher v. 4.8 (Gene Codes, Inc.) to retain sites containing >2 states that would have been automatically removed by IMgc.

We used ^{*}Beast v.1.6.1 (Drummond and Rambaut, 2007; Heled and Drummond, 2012), which employs Markov chain Monte Carlo (MCMC) to estimate the posterior distribution of the species tree given the results from each gene tree, to reconstruct a multispecies Yule tree (Species Tree: Yule Process). Analyses included (1) a nonfiltered dataset for all taxa, (2) a recombination-filtered dataset for all taxa, (3) a non-filtered dataset for all taxa excluding mallards, and (4) a recombination-filtered dataset for all taxa excluding mallards.

All loci were independently analyzed for substitution and clock models prior to species tree reconstruction. Substitution models were tested in MEGA v. 5.1 (Tamura et al., 2011) and ranked based on Bayesian Information Criterion (BIC) scores that identified the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al., 1985) as the most appropriate model for all datasets. Although additional parameters were not required for the truncated fragments, 12 loci within the full datasets required a gamma distribution across sites, with five of these having some proportion of invariable sites. Gene trees for each locus were analyzed with a strict clock (null model) and a Bayesian uncorrelated log-normal relaxed clock (alternative model) in ^{*}Beast v.1.6.1 and compared using Bayes Factors (BF) in Tracer v.1.5 (Rambaut and Drummond, 2009). A log BF < 3 (or $\log BF > -3$)(Li and Drummond, 2012) provided support for the null hypothesis of a strict clock for 13 loci in datasets ignoring recombination and 17 loci in recombination-filtered datasets. Species trees were then reconstructed with the appropriate substitution model and molecular clock defined for each locus (Table S1). A piecewise linear and constant root population size model with UPGMA starting trees (Sneath and Sokal, 1973) were used for each analysis. Sampling occurred every 2000 iterations with runs continuing until effective sample sizes (ESS) across parameters were ≥ 100 . Burnin was set to 10% of the total number of sampled trees, and final species trees were constructed using TreeAnnotator and viewed in FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree).

To examine overall levels of shared genetic variation, average pairwise Φ_{ST} was calculated for the 20 nuclear loci in Arlequin 3.5 (Excoffier and Lischer, 2010).

2.2. Mitochondrial phylogeny

Two separate analyses were conducted using mtDNA, including a Bayesian derived individual tree reconstructed using MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and a species tree reconstructed in 'Beast v.1.6.1 (Drummond and Rambaut, 2007; Heled and Drummond, 2012). An HKY substitution model with a gamma distribution across sites and a invariable sites model was determined as the best model based on BIC scores obtained in MEGA v. 5.1 (Tamura et al., 2011). Molecular clocks were tested with similar methods as above by reconstructing species trees in 'Beast v.1.6.1 (Drummond and Rambaut, 2007; Heled and Drummond, 2012) and using Bayes Factors to compare them. A strict molecular clock was accepted, suggesting that rate variation across taxa is negligible and sequences are evolving in a clock-like fashion, which corroborates previous mtDNA studies

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 parsimonyinformative sites of non-filtered and filtered (in parentheses) datasets.

Locus	Location ^a	Non-filtered (filtered) length ^b	Number of polymorphic sites	Number of parsimony-informative sites			
Chromo-helicase-DNA binding protein gene 1, intron 19	Z	326 (326)	10 (10)	3 (3)			
Lactate dehydrogenase 1, intron 4	1	520 (520)	7 (7)	3 (3)			
S-acyl fatty acid synthase thioesterase, intron 2	2	303 (303)	303) 10 (10) 4 (4)				
Ornithine decarboxylase, intron 7	3	302 (151)	37 (20)	24 (13)			
Fibrinogen beta chain, intron 7	4	437 (246)	27 (15)	17 (8)			
Serum amyloid A, intron 2	5	322 (133)	37 (12)	20 (5)			
Annexin A11, intron 2	6	440 (382)	39 (34)	26 (23)			
Myostatin, intron 2	7	281 (168)	26 (16)	11 (7)			
Soat1-prov protein, intron 10	8	332 (332)	13 (13)	7 (7)			
Nucleolin, intron 12	9	359 (98)	49 (16)	40 (14)			
Melanocortin 1 receptor	11	782 (782)	28 (25)	10 (9)			
Preproghrelin, intron 3	12	305 (290)	18 (17)	9 (8)			
Glutamate receptor, ionotropic, N-methyl D aspartate I,	17	300 (85)	22 (1)	14 (0)			
intron 13							
Sex determining region Y-box 9, intron 2	18	402 (120)	60 (12)	46 (11)			
Carboxypeptidase D, intron 9	19	332 (127)	43 (18)	34 (13)			
Phosphenolpyruvate carboxykinase, intron 9	20	333 (333)	12 (12)	10 (10)			
Alpha enolase 1, intron 8	21	294 (179)	19 (11)	14 (8)			
Alpha-B crystallin, intron 1	24	323 (323)	8 (8)	2 (2)			
Growth hormone 1, intron 3	27	380 (379)	22 (21)	16 (12)			
Lecithin-cholesterol acyltransferase, intron 3	Unk	323 (154)	36 (15)	22 (10)			
Total		7396 (5431)	523 (293)	332 (170)			
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.

 Φ_{ST} estimates (Table 3) followed phylogenetic relationships. On average, $\geq 40\%$ of the variability was explained by differences among taxa within the African group and between the African, South Pacific, and Australasian groups, whereas 17% of the genetic variability was explained when comparing African taxa to NW and OW taxa. Whereas pairwise $\Phi_{\rm ST}$ estimates were on average 14% among the Hawaiian duck, Philippine duck, and the NZ grey/Pacific black duck, an average of 57% of differences were explained between these taxa and the Laysan duck. Finally, the lowest levels of differences were observed among NW taxa, OW mallards, and both spot-billed duck species ($\Phi_{\rm ST} \leq 2$ %). Notably, the two mallard populations were indistinguishable from each other and the American black duck ($\Phi_{\rm ST} < 0$).

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 grouped 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 spotbilled 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.

Table 3

Average pairwise Φ_{ST} estimates for 20 nuclear loci across taxa of the mallard complex (abbreviations are defined in Table 1). $\Phi_{ST} \leq 0.05$ indicates a high proportion of shared polymorphisms and are shown in bold text.

	AFBD	YBDU	MELL	HADU	LADU	PHDU	GRDU	PBDU	MALLow	SPBDch	SPBDin	MALLnw	MEDU	ABDU	MODUwgc
AFBD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YBDU	0.342	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MELL	0.545	0.320	-	-	-	-	-	-	-	-	-	-	-	-	-
HADU	0.210	0.276	0.336	-	-	-	-	-	-	-	-	-	-	-	-
LADU	0.889	0.633	0.919	0.387	-	-	-	-	-	-	-	-	-	-	-
PHDU	0.467	0.327	0.474	0.247	0.884	-	-	-	-	-	-	-	-	-	-
GRDU	0.215	0.273	0.251	0.145	0.495	0.088	-	-	-	-	-	-	-	-	-
PBDU	0.270	0.306	0.293	0.175	0.542	0.187	0.015	-	-	-	-	-	-	-	-
MALLow	0.167	0.220	0.169	0.096	0.460	0.090	0.065	0.089	-	-	-	-	-	-	-
SPBDch	0.176	0.206	0.151	0.112	0.484	0.151	0.083	0.089	0.000	-	-	-	-	-	-
SPBDin	0.414	0.285	0.467	0.172	0.878	0.382	0.151	0.187	0.038	0.069	-	-	-	-	-
MALLnw	0.161	0.179	0.148	0.094	0.463	0.109	0.085	0.121	-0.001	0.025	0.025	-	-	-	-
MEDU	0.168	0.199	0.173	0.095	0.480	0.114	0.078	0.106	0.004	0.017	0.070	0.023	-	-	-
ABDU	0.165	0.194	0.144	0.101	0.466	0.091	0.083	0.106	-0.010	0.015	0.052	-0.011	0.012	-	-
MODUwgc	0.136	0.168	0.146	0.104	0.474	0.120	0.094	0.116	0.018	0.026	0.056	0.004	0.023	0.013	-
MODUfl	0.126	0.196	0.188	0.107	0.489	0.153	0.123	0.147	0.046	0.063	0.056	0.026	0.055	0.024	0.027

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. -49% 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 locus 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),



Fig. 2. Mean and 95% highest posterior density for the root height of nuDNA species trees obtained from the four methods of data treatment (see Fig. 1).



Fig. 3. Mitochondrial gene tree reconstructed in MrBayes using 690 base pairs of the control region for 64 individuals.

phylogenetic (Fig. 1) and Φ_{ST} estimates (Table 3) suggest an almost simultaneous split between the Australasian clade, the Hawaiian clade, and OW mallards/NW taxa. Consequently, results from nuD-NA 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 Φ_{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. 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 $\Phi_{\rm ST}$ 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 super clade 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 super clade 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 spotbilled 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 introgress 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 Mariana mallard (*A. oustaleti*), 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

and the Pacific black duck. Additional data are are required to test quantitatively these alternative hypotheses, which will need larger sample sizes (see Jacobsen and Omland, 2011a) and additional analyses (e.g. program STEM-hy; Kubatko, 2009). Finally, the mito-nuclear discordance for the Philippine duck and spot-billed ducks should be interpreted cautiously because we had only a single captive Philippine duck, which could complicate inferred relationships as ducks are well known for hybridizing in captivity (Johnsgard, 1960), and the phylogenetic placement of the spotbilled ducks received low posterior support.

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 relationships, and more importantly branch lengths were reduced when filtering for recombination. Regardless, clade membership of taxa was generally supported by consistency 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.

Acknowledgments

We are grateful to the following people and institutions for their contributions of samples to this study: Andy Engilis, Department of Wildlife, Fish and Conservation Biology, University of California, Davis; Leo Joseph, the Australian National Wildlife Collection; Graeme Cumming, the Percy FitzPatick Institute, University of Cape Town; Yuri N. Zhuravlev and Irina Kulikova, Institute of Biology and Soil Sciences, Far Eastern Branch of the Russian Academy of Sciences; John Dyer and David Klee, New Zealand Fish and Game; Sylvan Heights Waterfowl Park in Scotland Neck, North Carolina, and the Burke Museum, University of Washington. This research was funded by Ducks Unlimited Richard H. G. Bonnycastle Fellowship in Wetland and Waterfowl Biology, the National Science Foundation (DEB-0926162), and the College of Sciences and Mathematics at Wright State University. The collection of yellow-billed ducks was supported by USAID via subcontract to the Wildlife Conservation Society's GAINS programme to G. Cumming. We also thank L. Joseph and G. Cumming, as well as the anonymous reviewers for insightful comments on earlier drafts of this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.08. 008.

References

- Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.
- Avise, J.C., Ankney, D.C., Nelson, W.S., 1990. Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. Evolution 44, 1109–1119.
- Bazin, E., Glémin, S., Galtier, N., 2006. Population size does not influence mitochondrial genetic diversity in animals. Science 312, 570–572.

- Brelsford, A., MilÁ, B., Irwin, D.E., 2011. Hybrid origin of Audubon's warbler. Molecular Ecology 20, 2380–2389.
- Browne, R.A., Griffin, C.R., Chang, P.R., Hubley, M., Martin, A.E., 1993. Genetic divergence among populations of the Hawaiian duck, Laysan duck, and mallard. Auk 110, 49–56.
- Carstens, B.C., Knowles, L.L., 2007. Estimating species phylogeny from gene-tree probabilities despite incomplete lineage sorting: an example from Melanoplus grasshoppers. Systematic Biology 56, 400–411.
- Chung, Y., Ané, C., 2011. Comparing two Bayesian methods for gene tree/species tree reconstruction: simulations with incomplete lineage sorting and horizontal gene transfer. Systematic Biology 60, 261–275.
- Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. PLoS Genetics 2, e68.
- Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology & Evolution 24, 332–340.
- Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7, 214.
- Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? Evolution 63, 1–19.
- Edwards, S., Bensch, S., 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Molecular Ecology 18, 2930–2933.
- Edwards, S.V., Kingan, S.B., Calkins, J.D., Balakrishnan, C.N., Jennings, W.B., Swanson, W.J., Sorenson, M.D., 2005. Speciation in birds: genes, geography, and sexual selection. Proceedings of the National academy of Sciences of the United States of America 102, 6550–6557.
- Ellegren, H., 2010. Evolutionary stasis: the stable chromosomes of birds. Trends in Ecology & Evolution 25, 283–291.
- Excoffier, L, Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10, 564–567.
- Fowler, A., Eadie, J., Engilis, A., 2009. Identification of endangered Hawaiian ducks (*Anas wyvilliana*), introduced North American mallards (*A. platyrhynchos*) and their hybrids using multilocus genotypes. Conservation Genetics 10, 1747– 1758.
- Giles, R.E., Blanc, H., Cann, H.M., Wallace, D.C., 1980. Maternal inheritance of human mitochondrial DNA. Proceedings of the National Academy of Sciences 77, 6715– 6719.
- Grant, P.R., Grant, B.R., 1992. Hybridization of bird species. Science 256, 193–197.
- Grant, P.R., Grant, B.R., 1997. Hybridization, sexual imprinting, and mate choice. The American Naturalist 149, 1–28.
- Greenwood, P.J., 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28, 1140–1162.
- Griffin, C.R., Browne, R., 1990. Genetic Variation and Hybridization in Hawaiian Duck and Mallards in Hawaii. U.S. Fish and Wildlife Service, Hawaiian and Pacific Islands National Wildlife Refuge Complex.
- Hasegawa, M., Kishino, H., Yano, T.-a., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22, 160– 174.
- Heled, J., Drummond, A.J., 2012. Calibrated tree priors for relaxed phylogenetics and divergence time estimation. Systematic Biology 61, 138–149.
- Heusmann, H.W., 1974. Mallard-black duck relationships in the northeast. Wildlife Society Bulletin 2, 171–177.
- Hillier, L.W., Miller, W., Birney, E., Warren, W., Hardison, R.C., Ponting, C.P., Bork, P., Burt, D.W., Groenen, M.A.M., Delany, M.E., Dodgson, J.B., 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432, 695–716.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
- Hurst, G.D.D., Jiggins, F.M., 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society B: Biological Sciences 272, 1525–1534.
- Jacobsen, F., Omland, K.E., 2011a. Increasing evidence of the role of gene flow in animal evolution: hybrid speciation in the yellow-rumped warbler complex. Molecular Ecology 20, 2236–2239.
- Jacobsen, F., Omland, K.E., 2011b. Species tree inference in a recent radiation of orioles (Genus Icterus): multiple markers and methods reveal cytonuclear discordance in the northern oriole group. Molecular Phylogenetics and Evolution 61, 460–469.
- Jacobsen, F., Friedman, N.R., Omland, K.E., 2010. Congruence between nuclear and mitochondrial DNA: combination of multiple nuclear introns resolves a wellsupported phylogeny of New World orioles (Icterus). Molecular Phylogenetics and Evolution 56, 419–427.
- Johnsgard, P.A., 1960. Hybridization in the Anatidae and its taxonomic implications. Condor 62, 25–33.
- Johnsgard, P.A., 1978. Ducks, Geese, and Swans. Univ. Nebraska Press, Lincoln.
- Johnson, K.P., Sorenson, M.D., 1999. Phylogeny and biogeography of dabbling ducks (genus: Anas): a comparison of molecular and morphological evidence. Auk 116, 792–805.
- Kirby, R.E., Sargeant, G.A., Shutler, D., 2004. Haldane's rule and American black duck \times mallard hybridization. Canadian Journal of Zoology 82, 1827–1831.
- Knowles, L.L., 2009. Estimating species trees: methods of phylogenetic analysis when there is incongruence across genes. Systematic Biology 58, 463–467.
- Knowles, L.L., Maddison, W.P., 2002. Statistical phylogeography. Molecular Ecology 11, 2623–2635.

- Knowles, L.L., Lanier, H.C., Klimov, P.B., He, Q., 2012. Full modeling versus summarizing gene-tree uncertainty: method choice and species-tree accuracy. Molecular Phylogenetics and Evolution 65, 501–509.
- Kraus, R.H., Kerstens, H.H., van Hooft, P., Megens, H.-J., Elmberg, J., Tsvey, A., Sartakov, D., Soloviev, S.A., Crooijmans, R.P., Groenen, M.A., 2012. Widespread horizontal genomic exchange does not erode species barriers among sympatric ducks. BMC Evolutionary Biology 12, 45.
- Kubatko, L.S., 2009. Identifying hybridization events in the presence of coalescence via model selection. Systematic Biology 58, 478–488.
- Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology 56, 17–24.
- Kubatko, L.S., Carstens, B.C., Knowles, L.L., 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. Bioinformatics 25, 971– 973.
- Kulikova, I.V., Zhuravlev, Y.N., McCracken, K.G., 2004. Asymmetric hybridization and sex-biased gene flow between eastern spot-billed ducks (*Anas zonorhyncha*) and mallards (*A. platyrhynchos*) in the Russian far east. Auk 121, 930– 949.
- Kulikova, I.V., Drovetski, S.V., Gibson, D.D., Harrigan, R.J., Rohwer, S., Sorenson, M.D., Winker, K., Zhuravlev, Y.N., McCracken, K.G., 2005. Phylogeography of the mallard (*Anas platyrhynchos*): hybridization, dispersal, and lineage sorting contribute to complex geographic structure. Auk 122, 949–965.
- Lanier, H.C., Knowles, L.L., 2012. Is recombination a problem for species-tree analyses? Systematic Biology.
- Leaché, A.D., Rannala, B., 2011. The accuracy of species tree estimation under simulation: a comparison of methods. Systematic Biology 60, 126–137.
- Li, W.L.S., Drummond, A.J., 2012. Model averaging and Bayes factor calculation of relaxed molecular clocks in Bayesian phylogenetics. Molecular Biology and Evolution 29, 751–761.
- Lijtmaer, D.A., Mahler, B., Tubaro, P.L., Dunn, P., 2003. Hybridization and postzygotic isolation patterns in pigeons and doves. Evolution 57, 1411–1418.
- Liu, L., 2008. BEST: Bayesian estimation of species trees under the coalescent model. Bioinformatics 24, 2542–2543.
- Livezey, B.C., 1986. A phylogenetic analysis of recent Anseriform Genera using morphological characters. Auk 103, 737–754.
- Livezey, B.C., 1991. A phylogenetic analysis and classification of recent dabbling ducks (Tribe Anatini) based on comparative morphology. Auk 108, 471–507.
- Maddison, W.P., 1997. Gene trees in species trees. Systematic Biology 46, 523–536. Maddison, W.P., Knowles, L.L., 2006. Inferring phylogeny despite incomplete lineage sorting, Systematic Biology 55, 21–30.
- Mallet, J., 2007. Hybrid speciation. Nature 446, 279-283.
- McCracken, K.G., Johnson, W.P., Sheldon, F.H., 2001. Molecular population genetics, phylogeography, and conservation biology of the mottled duck (*Anas fulvigula*). Conservation Genetics 2, 87–102.
- Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrialgene trees versus nuclear-gene trees. Evolution 49, 718–726.
- Mundy, N.I., 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proceedings of the Royal Society B: Biological Sciences 272, 1633–1640.
- Palmer, R.S., 1976. Handbook of North American Birds. New Haven, Connecticut. Pamilo, P., Nei, M., 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5, 568–583.
- Peters, J.L., McCracken, K.G., Zhuravlev, Y.N., Lu, Y., Wilson, R.E., Johnson, K.P., Omland, K.E., 2005. Phylogenetics of wigeons and allies (Anatidae: Anas): the importance of sampling multiple loci and multiple individuals. Molecular Phylogenetics and Evolution 35, 209–224.
- Peters, J.L., Zhuravlev, Y., Fefelov, I., Logie, A., Omland, K.E., 2007. Nuclear loci and coalescent methods support ancient hybridization as cause of mitochondrial paraphyly between gadwall and falcated duck (Anas spp.). Evolution 61, 1992–2006.
- Peters, J.L., Bolender, K.A., Pearce, J.M., 2012a. Behavioural vs. molecular sources of conflict between nuclear and mitochondrial DNA: the role of male-biased dispersal in a Holarctic sea duck. Molecular Ecology 21, 3562–3575.

- Peters, J.L., Roberts, T.E., Winker, K., McCracken, K.G., 2012b. Heterogeneity in genetic diversity among non-coding loci fails to fit neutral coalescent models of population history. PLoS One 7, e31972.
- Price, T.D., Bouvier, M.M., 2002. The evolution of F1 postzygotic incompatibilities in birds. Evolution 56, 2083–2089.
- Rambaut, A., Drummond, A.J., 2009. Tracer v1.5.
- Reichel, J.D., Lemke, T.O., 1994. Ecology and extinction of the Mariana mallard. The Journal of Wildlife Management 58, 199–205.
- Rhymer, J.M., Simberloff, D., 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27, 83–109.
- Rhymer, J.M., Williams, M.J., Braun, M.J., 1994. Mitochondrial analysis of gene flow between New Zealand mallards (*Anas platyrhynchos*) and grey ducks (*A. superciliosa*). Auk 111, 970–978.
- Rhymer, J.M., Williams, M.J., Kingsford, R.T., 2004. Implications of phylogeography and population genetics for subspecies taxonomy of grey (Pacific black) duck *Anas superciliosa* and its conservation in New Zealand. Pacific Conservation Biology 10, 57–66.
- Rieseberg, L.H., Baird, S.J.E., Gardner, K.A., 2000. Hybridization, introgression, and linkage evolution. Plant Molecular Biology 42, 205–224.
- Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
- Scherer, V.S., Hilsberg, T., 1982. Hybridisierung und verwandtschaftsgrade innerhalb der Anatidae – eine systematische un evolutionstheoretische betrachtung. Journal für Ornitologie 123, 357–380.
- Seehausen, O., 2004. Hybridization and adaptive radiation. Trends in Ecology & Evolution 19, 198–207.
- Sneath, P.H.A., Sokal, R.R., 1973. Numerical Taxonomy. San Francisco.
- Sorenson, M.D., Fleischer, R.C., 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proceedings of the National Academy of Sciences 93, 15239–15243.
- Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T., Mindell, D.P., 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Molecular Phylogenetics and Evolution 12, 105–114.
- Stephens, M., Donnelly, P., 2003. A comparison of bayesian methods for haplotype reconstruction from population genotype data. The American Journal of Human Genetics 73, 1162–1169.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using Maximum Likelihood, evolutionary distance, and Maximum Parsimony methods. Molecular Biology and Evolution.
- Watanabe, T., Mizutani, M., Wakana, S., Tomita, T., 1985. Demonstration of the maternal inheritance of avian mitochondrial DNA in chicken-quail hybrids. Journal of Experimental Zoology 236, 245–247.
- Weckstein, J.D., Zink, R.M., Blackwell-Rago, R.C., Nelson, D.A., 2001. Anomalous Variation in mitochondrial genomes of white-crowned (*Zonotrichia leucophrys*) and golden-crowned (*Z. atricapilla*) sparrows: pseudogenes, hybridization, or incomplete lineage sorting? Auk 118, 231–236.
- Weir, J.T., Schluter, D., 2008. Calibrating the avian molecular clock. Molecular Ecology 17, 2321–2328.
- Williams, C.L., Brust, R.C., Rhodes Jr., O.E., 2002. Microsatellite polymorphism and genetic structure of Florida mottled duck populations. The Condor 104, 424– 431.
- Williams, C.L., Brust, R.C., Fendley, T.T., Tiller, G.R., Rhodes Jr., O.E., 2005a. A comparison of hybridization between mottled ducks (*Anas fulvigula*) and mallards (*A. platyrhynchos*) in Florida and South Carolina using microsatellite DNA analysis. Conservation Genetics 6, 445–453.
- Williams, C.L., Fedynich, A.M., Pence, D.B., Rhodes Jr., O.E., 2005b. Evaluation of allozyme and microsatellite variation in Texas and Florida mottled ducks. The Condor 107, 155–161.
- Woerner, A.E., Cox, M.P., Hammer, M.F., 2007. Recombination-filtered genomic datasets by information maximization. Bioinformatics 23, 1851–1853.
- Zink, R.M., Barrowclough, G.F., 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17, 2107–2121.