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Multi-Locus Evidence of a Late Pleistocene Divergence and Sex-Biased Dispersal in The North American Wood Duck (*Aix sponsa*)

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MULTI-LOCUS EVIDENCE OF A LATE PLEISTOCENE DIVERGENCE AND SEX-
BIASED DISPERSAL IN THE NORTH AMERICAN WOOD DUCK (*AIX SPONSA*)

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

Christopher Bigley
B.S., Wright State University, 2009

2011

Wright State University

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Christopher T. Bigley ENTITLED MULTI-LOCUS EVIDENCE OF A LATE PLEISTOCENE DIVERGENCE AND SEX-BIASED DISPERSAL IN THE NORTH AMERICAN WOOD DUCK (*AIX SPONSA*) BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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Abstract

Bigley, Christopher T. M.S., Department of Biological Sciences, Wright State University, 2011. Multi-Locus Evidence of a Late Pleistocene Divergence and Sex-Biased Dispersal in The North American Wood Duck (*Aix sponsa*).

The Pleistocene was characterized by fluctuations in climate causing repeated advances and retreats of glacial ice. The advancing ice sheets caused habitat fragmentation which initiated population divergence and speciation events between eastern and western avian populations within northern temperate forests. Based on mitochondrial DNA (mtDNA) control region sequences, North American Wood Duck (*Aix sponsa*) populations fit this model of divergence. However, mtDNA is maternally inherited, and thus may not reflect the genomic history of this species, because of male biased-dispersal, selection, or stochastic lineage sorting. To test the “Late Pleistocene divergence” hypothesis, I sequenced 11 independent nuclear introns (nuDNA) for 45 individuals sampled from eastern and western populations of Wood Ducks. Although two loci were significantly structured between East and West, overall population structure was considerably weaker for nuDNA (mean $\Phi_{ST} = 0.027$; range = 0.0 to 0.131) than for mtDNA ($\Phi_{ST} = 0.31$). Furthermore, genetic assignment tests and mark-recovery data were both consistent with male-biased dispersal between regions. Despite the influence of male-biased dispersal, estimates of time since divergence from both nuDNA and mtDNA were consistent with the last glacial advance splitting the two

populations, thus supporting the Late Pleistocene divergence hypothesis.

This study illustrates the utility of using nuDNA to test hypotheses derived from mtDNA analyses, which strengthens inferences of population history.

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Introduction

The Late Pleistocene Hypothesis

The late Pleistocene was characterized by periods of glaciations in which ice sheets advanced and retreated over much of the northern hemisphere. These cyclic events likely caused the rapid diversification of floral and faunal communities (Rand 1948, Pielou 1991, Seddon et al. 2001, Lovette 2005). The late Pleistocene origins (LPO) model asserts that advancing glaciers fragmented North America into allopatric eastern and western refugia, which promoted divergence and speciation (Mengel 1964, Knowles and Richards 2005). Mengel's (1964) model proposes that populations located in boreal forests of North America were split during four glacial advances, and each advance forced a subset of "eastern" populations to adapt to western mountain refugia during glacial maxima (see also Bermingham 1992). Furthermore, the glaciers inhibited gene flow between populations causing them to diverge in allopatry and ultimately to speciate (Mayr 1939; Mengel 1964, Rand 1948). Testing this hypothesis, Bermingham (1992) studied North American Warblers (*Parulidae*) using mitochondrial DNA (mtDNA) sequences and showed that not all western populations were descendents of eastern populations, which is inconsistent with Mengel's hypothesis. Furthermore, Zink and Slowinski (1995) tested whether the late Pleistocene was characterized by the proposed rapid diversification or if divergence was initiated in a

different time period. Zink and Slowinski (1995) assayed mtDNA for eleven avian genera, and determined that speciation events decreased from the early Pleistocene to the present. Likewise, Klicka and Zink (1997) sequenced mtDNA for 35 North American songbird species thought to have diverged in the late Pleistocene and concluded that only one species pair split in the late Pleistocene whereas ten diverged prior to the late Pleistocene and twenty-four split near the mid to late Pleistocene boundary. However, Avise et al. (1998) criticized Klicka and Zink (1997) because they did not compare sister species, but rather closely related species. Johnson and Cicero (2004) confirmed that twenty-four of the thirty-five species pairs were not sister taxa. Restricting their comparisons to sister species, Johnson and Cicero (2004) found that the majority of species diverged during the late Pleistocene, thus supporting the LPO.

Weir and Schluter (2004) later proposed the climate shift theory, which suggests that changes in climate rather than glacial ice was responsible for population fragmentation and speciation. They assayed cytochrome b and ND2 mtDNA sequences for 55 sister species of birds (see Lovette 2005). They found that divergences between sister species in areas influenced by glaciers dated to the late Pleistocene, whereas populations south of the glaciers split earlier during the early to mid Pleistocene. For instance, they found that sister species in North America diverged during the late Pleistocene whereas sister species in Mexico and

South America had a deeper divergence. These findings support the LPO hypothesis rather than the climate shift theory.

Late Pleistocene glaciations also caused intraspecific divergences. In these cases, gene flow from relatively few migrants might have slowed down speciation (Reudink et al. 2011). Many avian intraspecific divergences show phylogeographic structure between eastern and western populations (Gill et al. 1993, Ball et al. 1998, Kimura et al. 2002, Peters et al. 2005, Spellman and Klicka 2006, Spellman et al. 2007, Spellman and Klicka 2007, Omland et al. 2000, Klicka et al. 2011). Milot et al. (2000) used mtDNA in a study of Yellow Warblers (*Dendroica petechia*) and discovered separate eastern and western genetic populations. A similar study (Gibbs et al. 2000) used nuDNA and found evidence, undetected by mtDNA, of male-biased dispersal in Yellow Warblers. The Yellow Warbler study shows that in east-west splits, mtDNA may not show a complete evolutionary history.

To date, molecular techniques used to test the LPO hypothesis have mostly been restricted to mtDNA (Klicka and Zink 1997, Avise et al. 1998, Bermingham 1992). This marker is advantageous because of its high mutation rate and rapid sorting rate (Moore 1995, Mortiz et al. 1987), which can show greater structure when compared to biparentally inherited markers (Seddon et al 2001, Hoarau 2004, Pearce et al. 2008, Hewitt 2010, Oomen 2011). However, the results of studies focusing on a single marker (e.g., mtDNA) may be unreliable owing to the stochastic effects of

genetic drift and mutation. Moreover, mtDNA is maternally inherited, and becomes particularly limiting or even misleading in species where males are the primary dispersers and females are philopatric (Doums et al. 2002). To strengthen our understanding of how ancient events (such as glacial cycles) have contributed to modern genetic diversity, it is important to use multiple biparentally inherited markers to infer population histories (i.e. gene flow, genetic drift, divergence, etc.) and to test hypotheses derived from studies of mtDNA.

Study System

The distribution of Wood Ducks (*Aix sponsa*) is divided into distinct eastern and western ranges that are separated by the prairie grasslands of the mid-west. As a result of having a large range, Wood Ducks likely occupied multiple refugia during the Pleistocene glaciations (Shafer et al. 2010). Although populations show no morphological differences, mtDNA evidence suggests two genetically distinct populations that conform to an East-West split (Figure 1; Peters et al. 2005).

Wood ducks are obligate cavity nesters, primarily found in proximity of streams and nearby riparian habitat. Due to these requirements, much of the habitat separating the eastern and western populations is unsuitable. Although Wood Ducks migrate long distances, recent molecular work with mtDNA has suggested that gene flow is

restricted between eastern and western populations and that a late Pleistocene split likely occurred (Peters et al. 2005). However, because Wood Ducks exhibit male-biased dispersal (Bellrose and Holm 1994), mtDNA might provide a biased picture of population structure for this species. In particular, nest site fidelity by females can cause the maternally inherited mtDNA to be more strongly structured than biparentally inherited nuclear DNA (Ransom 2001, Semel and Sherman 2000). In this study, I sequenced multiple biparentally inherited loci to test for population structure and to compare with results derived from mtDNA. If mtDNA reflects the genomic history of this species, I predict that the nuclear data will reveal recognizable genetic populations and little, if any, gene flow. The objectives of this study are to (1) test whether Wood Duck populations are differentiated between the East and West, (2) estimate time since divergence, and (3) estimate the level of gene flow occurring between populations.

Materials Methods

DNA isolation

I sampled 45 Wood Ducks from widely distributed locations throughout their eastern (n=25) and western (n=20) ranges (Figure 1). All samples were sequenced by Peters et al. (2005) for the mtDNA control region, except one individual from Ohio that was added later. DNA was extracted from muscle tissue following the DNeasy blood tissue kit protocol (Qiagen).

I sequenced 11 nuclear loci (nuDNA) for each individual (Table 1). Amplification was accomplished with a standard polymerase chain reaction (PCR) consisting of 25 μ L reactant per sample that included 1.5

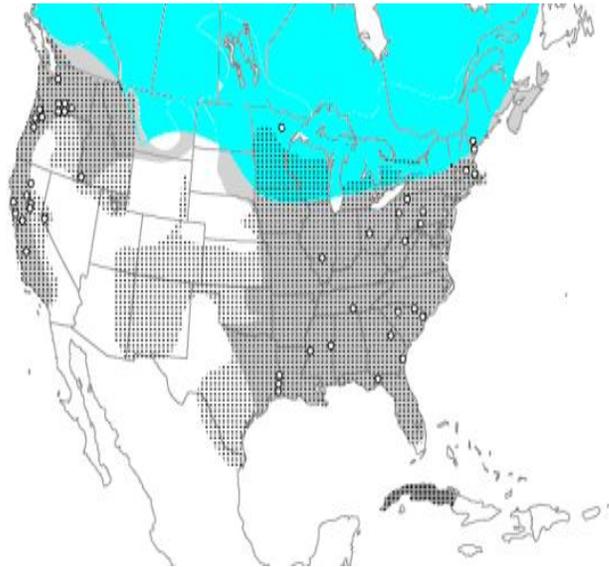


Figure 1. Wintering (stippled) and breeding distributions (shaded) of North American Wood Ducks. The extent of glacial ice during the last glacial maximum (~18,000 years ago) is shown in blue. Circles approximate sample locations. [Adapted and edited from Peters et al.

μL of isolated DNA, 1.25 μL of forward and reverse primers (10nM concentration), 12.5 μL of Taq polymerase gold (Applied Biosystems) and 8.75 μL of ddH₂O. The PCR protocol started with initial denaturation at 94°C for 7 minutes followed by 45 cycles of

denaturation at 94°C for 20 seconds, annealing at 58.0°C for 20 seconds and extension at 72.0°C for 60 seconds, and a final extension step at 72.0°C for 7 minutes. Amplification was verified with 15 μL of each sample using gel electrophoresis with a 1.5% agarose gel and SYBR green for staining. PCR products were cleaned with AMPure XP beads, following Agencourt protocol (Beckman Coulter Co.)

Table 1. Measures of population differentiation (Φ_{ST}) and nucleotide diversity (π) for eastern and western Wood Ducks.

Locus	Φ_{ST}	π_{East}	π_{West}
Alpha enolase 1, intron 8	0.131	0.0455	0.0233
Lactate dehydrogenase B, intron 4	-0.010	0.0175	0.0177
Ornithine decarboxylase, intron 7	0.036	0.0105	0.0068
Alpha-B crystalline, intron 1	-0.004	0.0005	0.0002
Fibrinogen beta chain, intron 7	0.018	0.0069	0.0055
Chromo-helicase-DNA binding protein gene 1, intron 19	-0.022	0.0014	0.0008
Annexin A11, intron 2	0.059	0.0046	0.0045
Glutamate receptor, ionotropic, N-methyl D aspartate I, intron 13	0.017	0.0030	0.0029
Phosphoenolpyruvate carboxykinase, intron 9	0.007	0.0063	0.0072
Soat1-prov protein, intron 10	0.033	0.0030	0.0032
S-acyl fatty acid synthase thioesterase, intron 2	0.028	0.0115	0.0099

Sequencing was done using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) following supplier protocols: 1 μ L of Big Dye, 1.75 μ L of 5X buffer, 1 μ L of primer and 4.5 μ L of ddH₂O in a 96 well plate. Cycle sequencing started with an initial denaturation step for 1 minute at 96.0°C followed by 30 cycles of denaturation at 96.0°C for 10 seconds, annealing at 50.0°C for 5 seconds, and extension at 60.0°C for 4 minutes. Sequenced 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).

Genetic analysis

Alleles for each individual (per locus) were resolved with two methods. First, sequences that were heterozygous for indels were resolved following the methods described in Peters et al. (2007). In brief, I compared the ambiguous 3'-end with the unambiguous 5'-end of the complementary strand to resolve the length of the indel and to determine the gametic phases of each allele. Second I used the program PHASE (Stephens and Donnelly 2003), which derives the most likely phase of each allele algorithmically. Alleles resolved using the first method were treated as known alleles in the PHASE analysis.

Population Structure and Differentiation

I tested whether eastern and western individuals could be assigned to different sub-populations based on genotypes using the MCMC Bayesian method in the program STRUCTURE v.2.2.3 (Pritchard et al. 2000). STRUCTURE uses Hardy-Weinberg equilibrium and linkage disequilibrium to determine the most likely number of populations and to assign individuals to those populations (Pritchard et al. 2000). I numbered all alleles from one to n , with n being the total number of alleles for each locus. I used an admixture model with independent allele frequencies, and

a burn in period of 10,000 steps followed by 10,000 iterations of sampling. I estimated the number of genetic populations (K) (K was tested for $K = 1-5$ populations). The most likely K value was determined by choosing the value that maximized the log-likelihood. Although I found that $K = 1$ gave the highest log-likelihood, I also attempted to assign individuals to two populations based on an *a priori* hypothesis of genetically distinct eastern and western populations (Peters et al. 2005).

I calculated measures of Φ_{ST} between eastern and western populations for each locus using Arlequin (Excoffier et al. 2005). Φ_{ST} measures the proportion of the total nucleotide diversity that is partitioned among populations. Nucleotide diversity, which is the average number of pairwise differences between two random sequences within eastern and western populations, was also calculated using Arlequin (Excoffier et al. 2005). For each locus, I used the program Network v.4.6 to create haplotype networks illustrating all connections with a 95% probability of being the most parsimonious (Bandelt et al. 1999). Because I had unequal sample sizes between eastern and western populations, I used rarefaction to standardize allelic richness in the eastern population to the western sample size using the program Rarefaction Calculator (University of Alberta, Alberta, Canada). I then performed a paired t-test, with each locus treated as the paired replicate, to test for differences in allelic richness between the eastern and western populations.

Isolation with Migration

I used the coalescent program IM (Hey and Nielsen 2004) to fit the data to an isolation with migration model. I first obtained the largest non-recombinant fragment using IMgc (Woerner et al. 2007) to meet the assumption of no recombination. I interactively changed the chromosomal weighting so that a maximum of five percent of sequences were removed from the analysis. A two population model was used to estimate migration rates (m_1 and m_2), divergence times (t), effective population sizes (Θ_1 and Θ_2), and the ancestral effective population size (Θ_0). Effective population size was determined as $\Theta = 4N_e\mu$, where N_e is the effective population size and μ is the mean substitution rate per locus. Time since divergence (t) scaled to μ was estimated as $t = T\mu$, with T being the number of years since divergence. Migration rates were estimated as m_1 and m_2 for the eastern and western populations, respectively, where $m = M/\mu$, with M being the proportion of the population consisting of migrants each generation. The splitting parameter s was used to test for founder events that might be predicted for western populations (Hey 2005). To convert parameters estimated in IM to biologically informative values, I used the average μ (1.2×10^{-9} substitutions per locus per site per year) for five nuclear loci estimated in Peters et al. (2008), which is similar to rates calculated in other studies of birds (e.g., Morris-Pocock et al. 2011). In this study the geometric mean of the per locus mutation rate was 3.1×10^{-7} substitutions per locus per year. Generation time for ducks of 3.2 years per

generation was also obtained from Peters et al. (2008), and was used to convert estimates of Θ to N_e (the substitution rate per locus per generation is necessary for this conversion).

Banding Data

I examined 141,841 mark-recovery records for Wood Ducks banded and recovered in North America from 1926-2007 (Bird banding Laboratory Patuxent, MD). The records were sorted into eastern and western populations depending on banding location, with the Rocky Mountains as the divider between them. Fifty-seven records were removed, because they could not be assigned to an Eastern or Western population with confidence; these included intermediate states and provinces (Utah, Montana, Colorado, Alberta, Arizona, New Mexico, Wyoming, and Northwest territories), Europe, Colombia, Dominican Republic/Haiti, Bahamas, and Honduras. I also only included recoveries from the hunting season, because I assumed that these recoveries were more random than recaptures (e.g., nest-box monitoring; but see Christensen 2001). Additionally all records missing sex and location information were omitted. I then calculated the proportion of individuals recovered within the same or a different region (East or West) from the banding location. The data were partitioned into males and females to test for evidence of sex-biased dispersal between regions. A chi-squared test

was performed to determine whether the proportion of individuals moving between regions differed between males and females. Although movements between regions do not necessarily equate to gene flow (almost all recoveries were from the nonbreeding period), I use these movement patterns as indices of opportunities for gene flow.

Results

Genetic diversity

Values of nucleotide diversity (π) were generally larger in the east than the west (Table 1) but did not differ significantly (paired t-test; $t = 1.31$; $df = 10$; $P = 0.22$). The haplotype network showed a total of 116 different haplotypes (Figure 2). The eastern population of Wood Ducks contained 41 alleles not shared by the west, and the western population had 17 alleles not shared by the east. A total of 58 alleles were shared between populations. Western Wood Ducks had an average of 6.6 alleles per locus (± 3.6 StDev). Using rarefaction to standardize sample sizes to that of western samples, eastern Wood Ducks had an average of 8.7 alleles per locus (± 3.6 StDev). Overall, the eastern population had significantly higher allelic richness than the western population (paired t-test; $t = 2.79$; $df = 10$; $P = 0.019$).

Population Structure

Among the 11 loci, Φ_{ST} ranged between 0.0 and 0.13 (mean $\Phi_{ST} = 0.027 \pm 0.042$ StDev; table 1). I found significant differences ($P < 0.05$) between eastern and western populations at two loci only: alpha enolase 1 (ENO1) and annexin A11 (ANXA11). The best supported value of K from structure analysis indicated a one population model, but to examine the two-population hypothesis I forced K to equal two. Wood Ducks sampled in the East were assigned to population 1 with a mean assignment probability of 0.527 (± 0.053 StDev), whereas Wood Ducks from the West were assigned to population 1 with a probability of 0.473 (± 0.066 StDev). Although these assignments were low, there was a trend for eastern Wood Ducks having higher assignment probabilities to population 1 than did western Wood Ducks (t-test, $t = 3.32$, $df = 1$, $P = 0.068$). In addition, eleven males and three females were incorrectly assigned to their respective populations, which is consistent with male-biased dispersal, although the differences were not significant ($\chi^2 = 3.00$, $P = 0.083$; Fig. 3).

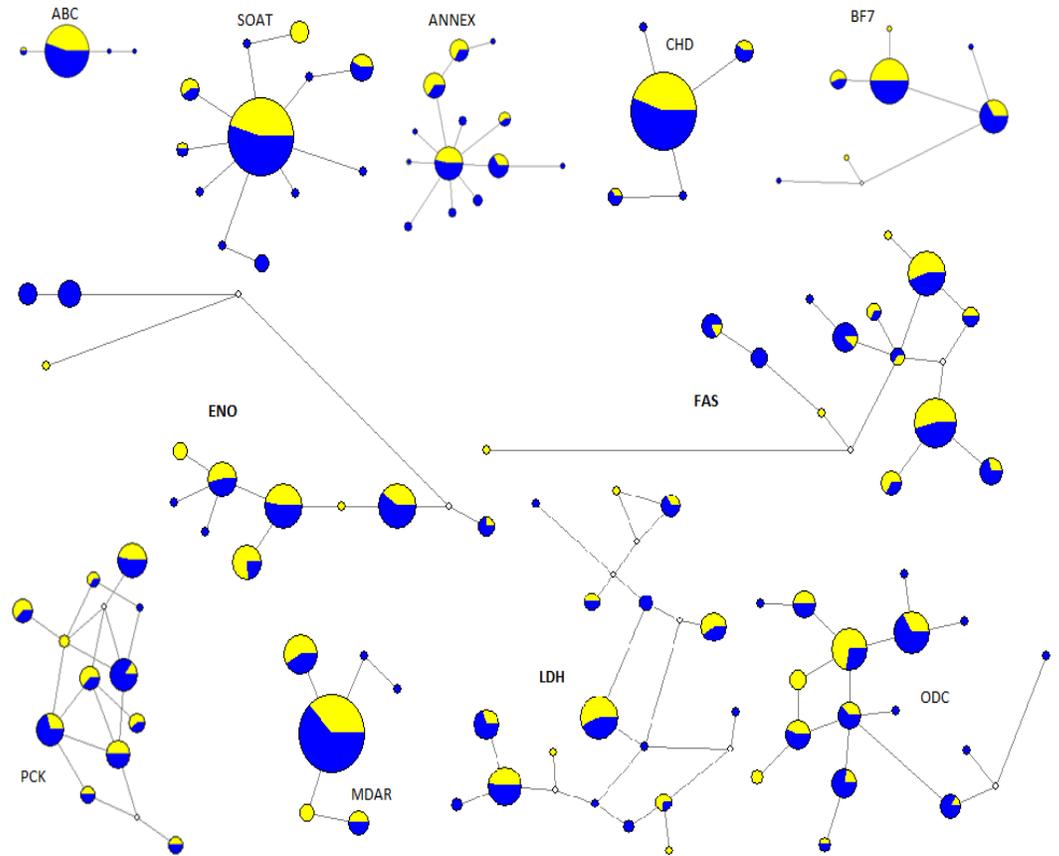


Figure 2. Parsimony networks illustrating haplotypes western (yellow), eastern (blue), and intermediate unsampled haplotypes (open circles). Circle size is proportionate to the number of alleles sampled. Connections have a 95% probability of being the most parsimonious.

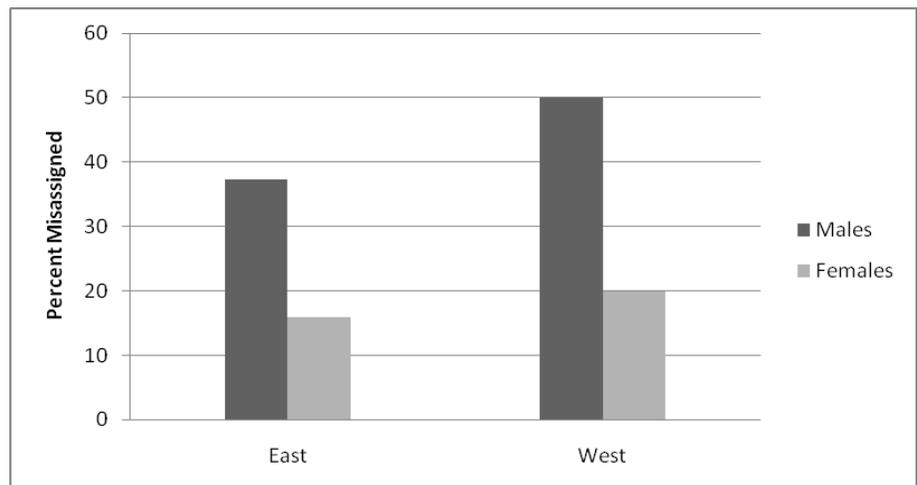


Figure 3. The percent of Wood Ducks that were incorrectly assigned to their population of origin by Structure analyses.

Isolation-with-migration

Based on IM analyses, θ_1 (eastern population) peaked at 2.79 (95% confidence interval (CI) = 1.52 – 5.00), whereas θ_2 (western population) peaked at 0.20 (95% CI = 0.091-0.54). Thus there was no overlap between the estimates of the two population sizes, with the eastern population being larger than the western population. Converting these values using generation time and mutation rates, I estimated a population size of about 700,000 individuals in the east and 49,000 individuals in the west. The estimate for θ_0 was 2.27 (95% CI = 1.72 - 2.97), or approximately 575,000 ancestral individuals. This analysis suggests that the eastern population size is similar to the ancestral size, but the western population is about an order of magnitude smaller. The estimate of m_1 (migrants from the western population into the eastern) peaked at 73.0 (CI = 36.9 – 100.0), which corresponds to about 102 migrants per generation. The estimate of m_2 from east to west peaked at 34.4 (CI = 0.75 – 90.5) or 3.4 migrants per generation. However the posterior distributions for each m had a long, flat tail which suggests that higher migration rates might be consistent with the data. My estimated value for t was 0.014 (95% CI = 0.001-0.071), suggesting that eastern and western wood ducks began diverging about 45,000 years before present. Although the s parameter peaked near 1.0, suggesting that less than 1% of the ancestral population

contributed to western Wood Ducks, the posterior distribution was flat over much of the parameter space (Figure 4g).

Banding data

To examine whether sex-biased dispersal might have contributed to differential gene flow between eastern and western populations of Wood Ducks, I examined 141,920 mark-recovery records from across North America that span from 1926-2007 (Bird banding Laboratory Patuxent, MD.). Of 85,232 males banded in the east, 28 (0.033%) were recovered in the west, whereas only 9 of 52,991 (0.017%) females banded in the east were recovered in the west ($\chi^2 = 3.07$; $P = 0.080$; Fig. 5). Of birds banded in the west, 6 of 1435 (0.42%) males were recovered in the east whereas 4 of 2262 (0.17%) females were recovered in the east ($\chi^2 = 1.06$; $\Phi_{ST} = 0.30$; Fig. 5). Although neither test was not significant, effects from both samples were in a direction consistent with male-biased gene flow connecting the two populations.

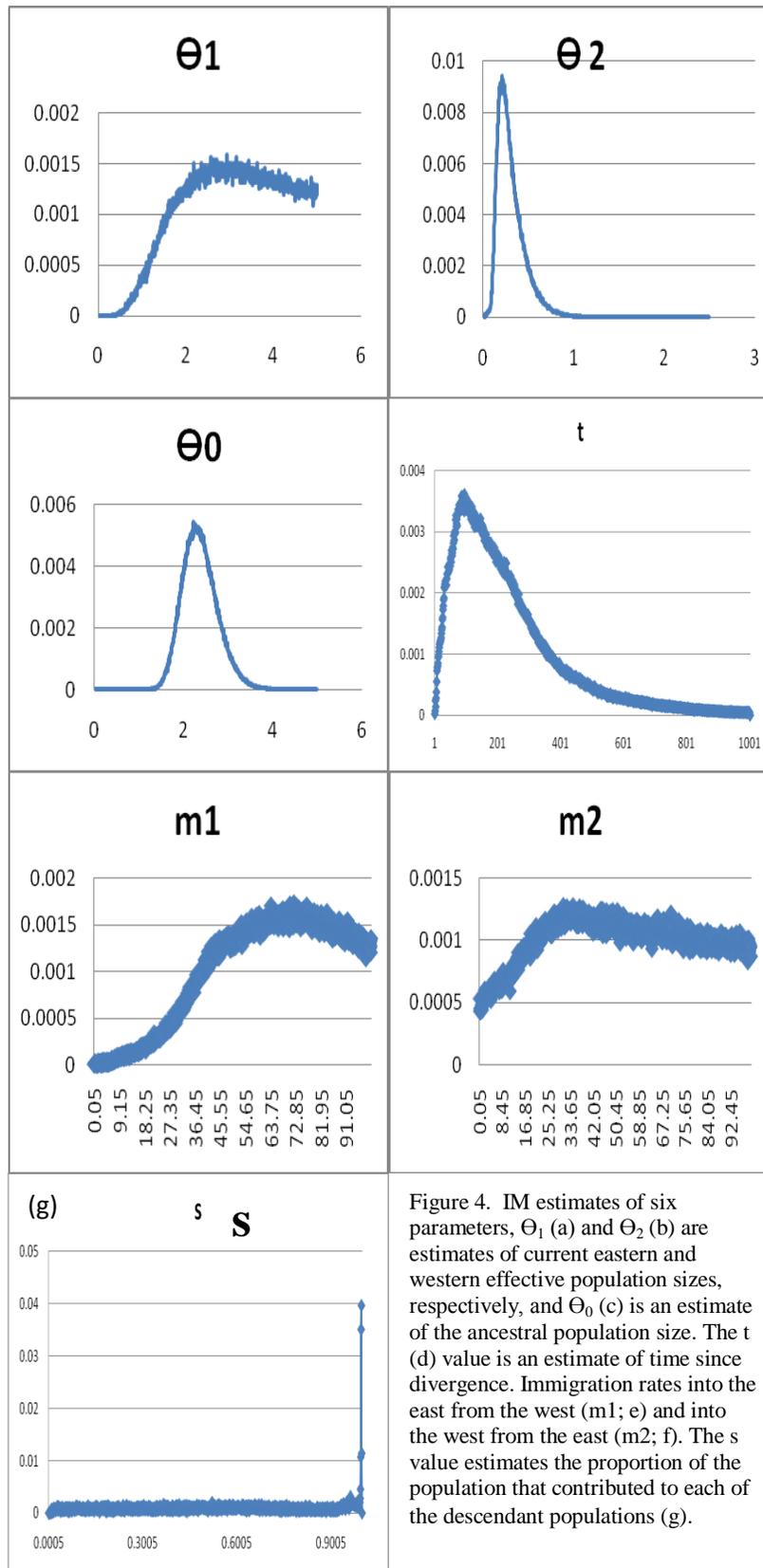


Figure 4. IM estimates of six parameters, Θ_1 (a) and Θ_2 (b) are estimates of current eastern and western effective population sizes, respectively, and Θ_0 (c) is an estimate of the ancestral population size. The t (d) value is an estimate of time since divergence. Immigration rates into the east from the west ($m1$; e) and into the west from the east ($m2$; f). The s value estimates the proportion of the population that contributed to each of the descendant populations (g).

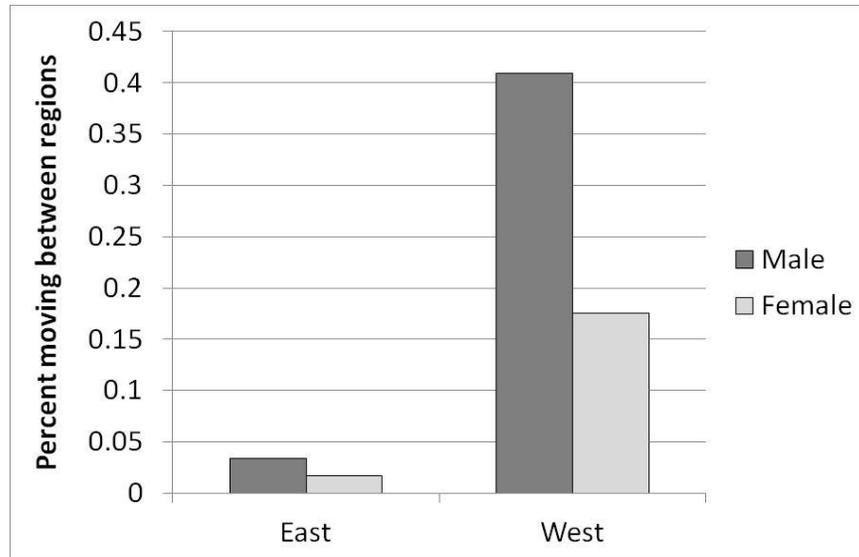


Figure 5. Left: Percent of male and female Wood Ducks that were banded in the east and recovered in the west. **Right:** Percent of male and female Wood Ducks that were banded in the west and recovered in the east. (Bird Banding Laboratory, unpubl. data, Patuxent,MD.)

Discussion

Comparisons of eastern and western Wood Ducks based on nuDNA revealed three main findings. First, there is not a large amount of differentiation in nuDNA between populations. Second, there is evidence of male biased dispersal influencing genomic differentiation. Third, the time since divergence estimated from nuDNA is a close match to that estimated from mtDNA and supports the late Pleistocene divergence hypothesis.

Population structure and gene flow

Overall, I found weak differentiation between eastern and western populations of Wood Ducks at nuDNA. Only two loci showed significant differentiation, and structure analyses suggested that the data best fit a one-population model. Furthermore, when forcing a two-population model, population assignment probabilities were low for all individuals, despite some evidence that eastern and western individuals were generally assigned to different populations. In contrast, Peters et al. (2005) found that eastern and western Wood Ducks were significantly structured at mtDNA. Comparing these results supports the contention that mtDNA, owing to its maternal inheritance, can be inadequate for inferring population-level processes (Moore 1995, Hoelzer 1997, Gibbs et al. 2000, Hudson and Turelli 2003, Hoarau et al. 2004), and that eastern and western populations of Wood Ducks are more strongly connected than suggested by Peters et al. (2005). Fewer than one migrant per generation needs to contribute to gene flow for populations to be considered distinct (Spieth 1974), and my estimates of gene flow from nuDNA are well above this level. Thus, these seemingly allopatric populations are unlikely to be evolutionarily independent.

Sex biased dispersal has been established by the observation of female site fidelity (Bellrose and Holm 1994). Structure analysis and banding data do not provide significant evidence of male-biased dispersal occurring in Wood Ducks, although the differences between males and females were in the predicted direction (i.e., more males moved between

regions and were assigned to the “wrong” population). However, comparing the results from mtDNA and nuDNA suggests that male-biased dispersal likely maintains genomic similarity between eastern and western populations. This can be observed in the haplotype networks and in IM results. The haplotype network for mtDNA (Peters et al. 2005) showed no shared haplotypes between the east and the west, whereas the nuDNA showed 58 shared haplotypes among the 11 loci. Estimates for mtDNA gene flow were 0 to 74 migrants per generation (Peters et al. 2005), whereas gene flow for nuDNA was around 105 (95% CI 51 – 148) migrants per generation, but the posterior distribution contained a long tail suggesting that migration rates might be higher than I estimated. This phenomenon is not typical in birds as they usually have female biased dispersal (Pusey and Wolf 1996). However, as a general rule, males are the dispersing sex in waterfowl (Rohwer and Anderson 1988).

Late Pleistocene divergence hypothesis

My estimation of time since divergence (t) was 45,000 years (95% HPD = 32,000–229,000 years) before present. This value matches well with Peters et al.’s (2005) estimate from mtDNA of 34,000 years (95% HPD = 10,000–124,000 years) before present. Importantly, whereas the mtDNA estimate suggests that the divergence might have occurred after the last glacial maximum, my estimate from nuDNA suggests that the divergence pre-dated the last glacial advance. Collectively, the similar estimates of time since divergence between mtDNA and nuDNA provide

compelling evidence that a late Pleistocene population split occurred in North American Wood Ducks, but populations may not have remained separate after glaciers retreated. Thus after the populations were split in the Late Pleistocene, it appears that they were reconnected through male biased dispersal as the glaciers retreated.

Many intraspecific avian divergences have been dated to the Late Pleistocene using mtDNA (Gill et al. 1993, Ball et al. 1998, Kimura et al. 2002, Peters et al. 2005, Spellman and Klicka 2006, Spellman et al. 2007, Spellman and Klicka 2007, Omland et al. 2000, Klicka et al. 2011). The use of a single locus mtDNA can potentially be affected by stochasticity in mutation rates and genetic drift and may lead to results that overestimate or underestimate divergence (Peters et al. 2005, Hudson and Turelli 2003). My research used multiple nuclear loci to account for the stochasticity of genetic processes and likely provided a more robust estimate of divergence times and a better test of the LPO hypothesis. Furthermore, the similar estimates of divergence times between marker types suggest that differences in dispersal between the sexes did not mislead these inferences.

Effective population sizes

My estimates of effective population size suggest that the eastern population is about 14 times larger than the western. The difference in population size is also illustrated in the nuDNA haplotype networks, as the

eastern population has more alleles than the western. Estimates from mtDNA suggest that the eastern population size is 36 times larger than the western size (Peters et al. 2005), and census data suggest the eastern size is approximately 50 times larger (Bellrose and Holm 1994). Fluctuations in population sizes, either over the long-term or in recent time, could cause ratios of effective population sizes to differ between census data and genetic estimates. For example, in the early part of the 20th century, deforestation and hunting brought the species to the brink of extinction and resulted in a ban on Wood Duck hunting in both the United States and Canada (Lowery 1974). Also, based on mtDNA, the eastern Wood Duck seems to have undergone a population expansion, perhaps from a glacial refugium, whereas the western population seems to have been more stable or to have contracted (Peters et al. 2005). Regardless, census data, mtDNA, and nuDNA all support a larger population size in the East, strengthening that inference.

Conclusion

This study illustrates the use of nuDNA to test hypotheses generated from mtDNA. Although inferences from mtDNA were well-supported in terms of confidence intervals, nuDNA illustrates that mtDNA provides an incomplete picture of population history. Specifically, eastern and western populations of Wood Ducks are likely connected by higher

levels of gene flow than suggested by mtDNA. On the other hand, nuDNA confirmed the conclusion of a Late Pleistocene divergence and differences in long-term effective population sizes that were inferred from mtDNA. Thus, whereas the Late Pleistocene glaciations do appear to have split the Wood Duck populations, secondary contact mediated through male-biased dispersal has likely reconnected those populations; this inference was not apparent in mtDNA analyses (Peters et al. 2005). Given the wealth of information about population histories that has been uncovered from mtDNA, it is important to test these hypotheses using independent data. Doing so will provide a better and more robust understanding of the processes that have influenced population structure observed in modern populations, and thereby, strengthen our understanding of the processes generating and maintaining genetic diversity. More specifically, a multi-locus approach can provide greater confidence in the role of the Late Pleistocene glaciations in driving diversification and population divergence.

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