CONSERVATION STATUS OF BUFF-BREASTED SANDPIPERS (TRYNGITES SUBRUFICOLLIS) IN THE WESTERN HEMISPHERE: A CONSERVATION GENETIC APPROACH

by

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Abstract

Range-wide estimates of shorebird (Aves: Charadriiformes) populations suggest sharp declines in population sizes across a range of species. Efforts to accurately assess the conservation status of wild populations are becoming increasingly vital to species management. One shorebird of conservation concern, the Buff-breasted Sandpiper (*Tryngites subruficollis*), is a New World migrant which winters in southeastern South America and breeds in the arctic. To establish an updated conservation status for Buff-breasted Sandpipers, we conducted a molecular survey of wild populations on spatial and temporal scales. We analyzed patterns of global population structure, demographic trends, and phylogeography using nine polymorphic microsatellites and two mitochondrial DNA (mtDNA) markers, cytochrome *b* and the control region, among 477 individuals across their distributional range. To empirically assess the impact of population declines on genetic diversity, we also surveyed segments of the same two mtDNA markers from 220 museum specimens collected across a 135-year period. Contemporary microsatellite and mtDNA analyses revealed that Buff-breasted Sandpipers are admixed on a global scale, with effective population size estimates ranging from 2,657 to 16,400 birds and no signal of a recent genetic bottleneck. Contemporary mtDNA analyses suggested a pattern of haplotype diversity consistent with a historic radiation from a single refugium which we estimated to have occurred between 8,000−45,000 years before present. Using five measures of mtDNA diversity (haplotype and nucleotide diversity, trend analyses of haplotype richness, Watterson's estimate of theta, and phi-statistics), as well as a Bayesian Skyline reconstruction of demographic trends in effective population size (N_{ef}), we concluded that substantial mtDNA diversity and N_{ef} had not been lost as a result of the population declines in this species. While genetic diversity did not appear to

have been lost due to population losses, management efforts must focus on preventing future losses in order for wild populations to remain viable. Our results suggested that the global population of Buff-breasted Sandpipers should be treated as a single, panmictic conservation unit and that successful management must focus on preventing further declines and habitat fragmentation.

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Chapter 1 - Introduction

Life History of Buff-breasted Sandpipers

Buff-breasted Sandpipers (*Tryngites subruficollis*) are medium-sized Scolopacid shorebirds whose migratory distribution ranges from breeding sites in the arctic to wintering sites in southeastern South America (Lanctot and Laredo 1994). Buff-breasted Sandpipers use stopover sites spanning the mid-Americas for spring migration (non-breeding to breeding ground), which typically occurs between early February and late March (Myers and Myers 1979). Fall migration back to non-breeding sites occurs mainly between July and September (Oring and Davis 1966) and is categorized by adults and sub-adults primarily utilizing the same central flyway. Juveniles are more likely to be vagrants and are occasionally sighted along the Eastern flyway (Campbell and Gregory 1976). Despite the fact that many of the preferred stopover sites along these migratory routes are subject to anthropogenic alteration, most of these sites do not have protected status (Lanctot et al. 2010).

Buff-breasted Sandpipers breed in the coastal tundra ecoregion across northern Alaska, northern Canada, and eastern Russia (Lanctot et al. 2010). Both lekking males and solitary, non-lekking males breed in snow-free areas with limited vegetation (Pruett-Jones 1988). Buff-breasted Sandpipers breeding-site behavior is rare relative to other North American shorebirds in that males defend small territories where they display to females, making them the only lek-mating, North American shorebird (Lanctot and Laredo 1994). However, parentage analyses have shown that the reproductive skew typically associated with a lek-mating system is absent in Buff-breasted Sandpipers (Lanctot et al. 1997). This unexpected pattern has been attributed to the use of alternative mating strategies, such as solitary displaying males and sneaker males. While lekking is a common reproductive strategy among breeding males, multiple mating in

female Buff-breasted Sandpipers is a likely causal factor of the absence of reproductive skew (Lanctot et al 1997). Patterns of breeding site fidelity in lek-mating Buff-breasted Sandpipers are different from most socially monogamous arctic shorebirds; Buff-breasted Sandpipers do not exhibit site fidelity to lek or nest sites among breeding seasons in the arctic (Lanctot and Weatherhead 1997).

Buff-breasted Sandpipers' non-breeding habitat is characterized by intensely-grazed pastureland in Brazil, Uruguay, and Argentina (Lanctot et al. 2002). This species has also been sighted in low densities in agricultural fields used for rice production (Strum et al. 2010). The primary land cover type associated with Buff-breasted Sandpipers wintering habitat is halophytic steppe vegetation adjacent to coasts or inland bodies of water (Lanctot et al. 2004). Unlike arctic breeding grounds, Buff-breasted Sandpipers show slightly sex-biased site fidelity at wintering sites, with females being more likely to be resighted than males (Almeida et al. 2009). The presence of genetically distinct cryptic populations has been observed in other migratory bird species that show nonbreeding during winter site fidelity (Friesen et al. 2007), but this has not been studied in Buff-breasted Sandpipers.

Buff-breasted Sandpiper Conservation Status

Migratory shorebirds (Aves: Charadriiformes) are an Order characterized by an incredible diversity of natural histories, including wide variation in migration habits and mating systems (Page and Gill 1994). The increasing use of molecular techniques in studies of migratory shorebirds is adding to our understanding of demographic patterns across this diverse group, and genetic studies have allowed conservation biologists to make informed management recommendations (Haig et al. 2011). While population genetic analyses are becoming more common in shorebirds, no previous studies have used molecular techniques to elucidate spatial or

temporal population characteristics in Buff-breasted Sandpipers.

Using molecular approaches to supplement species management practices is particularly important for migratory shorebirds because this group has been subjected to severe population perturbations over the last 150 years (Morrison et al. 2006). Many species experienced sharp declines associated with intensive market hunting in the late 1800's and early 1900's (Page and Gill 1994). While the hunting of many shorebirds was halted following the enactment of the Migratory Bird Treaty Act in 1918, gradual declines continued in subsequent decades resulting from anthropogenic disturbance across these species' ranges. Buff-breasted Sandpipers, for example, are estimated to have lost substantial population numbers over the past 150 years (Jorgensen et al. 2008, Lanctot et al. 2010).

Due to continuing population losses and trends of habitat degradation, Buff-breasted Sandpipers are listed among migratory species of high conservation concern by the IUCN, U.S. Shorebird Conservation Plan, U.S. Fish and Wildlife Service, Canadian Shorebird Conservation Plan, and Audubon Watchlist (Donaldson et al. 2001; Lanctot et al. 2010). The conservation status of Buff-breasted Sandpipers ranges in severity from "near threatened" (IUCN 2011) to "highly imperiled" (U.S. Shorebird Conservation Plan 2004), and the consensus among these organizations is that management should focus on reducing ongoing population declines. While these designations are crucial in species management, Buff-breasted Sandpipers' current conservation status is entirely based on survey data. Elucidating population genetic patterns in this species allows us to make more informed conservation recommendations by supplementing current population information with measures of population viability that are not apparent from observational data. To update Buff-breasted Sandpipers' conservation status, we conducted molecular analyses of population health. Specifically, the goals of this study were to 1) assess

global migratory and genetic connectivity in the context of identifying distinct conservation units, 2) estimate the effective population size of wild Buff-breasted Sandpipers, 3) empirically assess the impact of population declines on genetic diversity, and 4) use these measures of population viability to inform future management of Buff-breasted Sandpipers.

My thesis is divided into four chapters. In this chapter, I introduce Buff-breasted Sandpipers life history and conservation status. In chapter two, I present a population genetic characterization of Buff-breasted Sandpipers using nuclear and mitochondrial markers to address hypotheses pertaining to migratory and genetic connectivity, demographic trends, and phylogeography. Specifically, I use molecular marker data to understand patterns of contemporary and historic population genetic structure within and among major biogeographical regions (breeding, stopover, and non-breeding sites). I also use DNA data to estimate global effective population size, which I present in the context of Buff-breasted Sandpipers' conservation status. In chapter three, I present results of a robust survey of mitochondrial DNA (mtDNA) diversity in 220 museum specimens of Buff-breasted Sandpipers across a 135-year period. I compare diversity indices and effective population size estimates at two mtDNA markers over time to address my hypothesis that genetic diversity has been lost as a result of anthropogenically-induced declines in population size. In the final chapter, I provide a synthesis of my results and make recommendations for future management of populations of Buff-breasted Sandpipers.

References

Almeida, J.B. 2009. Wintering ecology of Buff-breasted Sandpipers (*Tryngites subruficollis*) in Southern Brazil. Ph.D. Thesis, University of Nevada-Reno, USA.

Campbell, R.W., and P.T. Gregory. 1976. The Buff-breasted Sandpiper in British Columbia, with notes on its migration in North America. Syesis 9:123–130.

Donaldson, G.M., C. Hyslop, R.I.G. Morrison, H.L. Dickson, and I. Davidson. 2001. Canadian Shorebird Conservation Plan. Canadian Wildlife Service Special Publications. Canadian Wildlife Service, Ottawa, Canada.

Friesen, V.L., T.M. Burg, and K.D. McCoy. 2007. Mechanisms of population differentiation in seabirds. Molecular Ecology 16:1765-1785.

Haig, S.M., W.M. Bronaugh, R.S. Crowhurst, J. D'Elia, C.A. Eagles-Smith, C.W. Epps, B. Knaus, M.P. Miller, M.L. Moses, S. Oyler-McCance, W.D. Robinson, and B. Sidlauskas. 2011. Genetic Applications in Avian Conservation. Auk 128(2):205-229.

IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2 <www.iucnredlist.org>.

Jorgensen J.G., J.P. McCarty, and L.L. Wolfenbarger. 2008. Buff-breasted Sandpiper density and numbers during migratory stopover in the Rainwater Basin, Nebraska. Condor 110:63-69.

Lanctot, R.B., and C.D. Laredo. 1994. Buff-breasted sandpiper (*Tryngites subruficollis*). In The birds of North America, No. 91 (Poole, A., and F. Gill, Eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania, and The American Ornithologists' Union, Washington, D.C., USA.

Lanctot, R.B., K.T. Scribner, B. Kempenaers, and P.J. Weatherhead. 1997. Lekking without a paradox in the Buff-breasted Sandpiper. American Naturalist 149(6):1051-1070.

Lanctot, R.B., and P.J. Weatherhead. 1997. Ephemeral lekking behavior in the Buff-breasted Sandpiper, *Tryngites subruficollis*. Behavioral Ecology 8:268–278.

Lanctot, R.B., D.E. Blanco, R.A. Dias, J.P. Isacch, V.A. Gill, J.B. Almeida, K. Delhey, P.F. Petracci, G.A. Bencke, And R.A. Balbueno. 2002. Conservation status of the Buff-breasted Sandpiper: historic and contemporary distribution and abundance in South America. Wilson Bulletin 114:44–72.

Lanctot, R.B., D.E. Blanco, M. Oesterheld, R.A. Balbueno, J.P. Guerschman, and G. Piñeiro. 2004. Assessing habitat availability and use by Buff-breasted Sandpipers (*Tryngites subruficollis*) wintering in South America. Ornitologia Neotropical 15 (Suppl.): 367–376.

Lanctot R.B., J. Aldabe, J.B. Almeida, D. Blanco, J.P. Isacch, J. Jorgensen, S. Norland, P. Rocca, and K.M. Strum. 2010. Conservation Plan for the Buff-breasted Sandpiper (*Tryngites subruficollis*).Version 1.1. U. S. Fish and Wildlife Service, Anchorage, Alaska, and Manomet Center for Conservation Sciences, Manomet, Massachusetts, USA.

Morrison, R.I.G., B.J. McCaffery, R.E. Gill, S.K. Skagen, S.L. Jones, G.W. Page, C.L. Gratto-Trevor, and B.A. Andres. 2006. Population estimates of North American shorebirds. Wader Study Group Bulletin 111:67–85.

Myers, J.P., and L.P. Myers. 1979. Shorebirds of coastal Buenos Aires Province, Argentina. Ibis 121:186–200.

Oring, L.W., and W.M. Davis. 1966. Shorebird migration at Norman, Oklahoma: 1961–63. Wilson Bulletin 78:166–174.

Page, G. W. and R. E. Gill, Jr. 1994. Shorebirds in Western North America: Late 1800's to late 1900's. Studies in Avian Biology 15:147-160.

Pruett-Jones, S. 1988. Lekking versus solitary display: temporal variations in dispersion in the Buff-breasted Sandpiper. Animal Behaviour 36:1740–1752.

U.S. Shorebird Conservation Plan (USSCP). 2004. High priority shorebirds – 2004. Unpubl. Report, United States Fish and Wildlife Service, 4401 N. Fairfax Dr., MBSP 4107, Arlington, VA, 22203. USA. 5 pp.

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Chapter 2 - Range-wide conservation genetics of Buff-breasted Sandpipers (*Tryngites subruficollis*) in the Western Hemisphere

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Abstract

Range-wide estimates of shorebird (Aves: Charadriiformes) populations suggest a sharp decline in population sizes across a range of species, and efforts to accurately assess the conservation status of wild populations are becoming increasingly vital to species management. One shorebird of conservation concern, the Buff-breasted Sandpiper (*Tryngites subruficollis*), is a New World migrant which winters in southeastern South America and breeds in the arctic. To contribute to the conservation status of Buff-breasted Sandpipers, we conducted a molecular survey of samples representing each of three major migratory regions (breeding, stopover, and wintering) using nine polymorphic microsatellite loci and 1.5-kb of highly variable mitochondrial DNA (mtDNA) from two distinct mitochondrial regions (cytochrome *b* and control region). We analyzed patterns of contemporary population structure, demographic trends, and phylogeographic patterns of genetic structure. Overall, microsatellite and mtDNA analyses revealed that this population of Buff-breasted Sandpipers is panmictic at a global scale (e.g., mean F_{ST} = 0.0051, $P > 0.05$) with effective population size (N_e) estimates ranging from 2,657 to 16,400 birds with no signal of a recent genetic bottleneck. MtDNA analyses suggested a pattern of haplotype diversity consistent with a historic radiation from a single refugium (Tajima's *D*: -2.27, $P < 0.01$; Fu's F_S: -30.6, $P < 0.0001$), which we estimated to coincide with the height of the Wisconsinan glaciation. When taken together, these results suggest that Buff-breasted Sandpipers should be treated as a single conservation unit, and management efforts for this species should focus on limiting habitat fragmentation across its range.

Introduction

Range-wide estimates of population trends for multiple shorebirds (Aves: Charadriiformes) suggest a sharp decline across a range of species (for review, see: Morrison et al. 2006). Ongoing declines have been attributed to a number of disturbances across these species' ranges, including exposure to environmental contaminants, loss of suitable habitat, market hunting, and climate change (Bart et al. 2007, Strum et al. 2010). With many migratory shorebirds experiencing declines over the past several centuries, efforts to manage wild populations are becoming increasingly crucial in species conservation.

Management of migratory shorebirds based on observational data is often difficult because of the vagile and transient nature of these species. Understanding population structure and migratory connectivity in shorebirds is challenging because of low rates of resightability and lack of morphological differences among populations (Haig et al. 1997). Migratory ecology, rather than physical barriers to gene flow, appears to be important in determining population structure of migratory birds (Liebers and Helbig 2002, Davis et al. 2006, Pearce et al. 2009). Population-specific use of flyways, wintering sites, and breeding sites has been shown to shape contemporary population structure in migratory birds (Friesen et al. 2007). The presence of the aforementioned characteristics in shorebirds suggests that observational techniques alone may overlook the possibility of detecting distinct population segments with unique management needs.

Molecular techniques can elucidate cryptic population structure that typically cannot be detected by observational data alone. Non-genetic methods of assessing connectivity (e.g., stable isotope analyses) in migratory shorebirds provide relatively course resolution of patterns of migratory ecology. Also, many shorebird species are too small for satellite tracking devices to be

used successfully in the field. Genetic techniques allow us to assess patterns of historical phylogeography and contemporary population structure so conservation efforts can focus on the preservation of two important conservation units: management units (MUs) and evolutionarily significant units (ESUs; Moritz 1994). In the past two decades, migratory bird conservation has relied heavily on defining conservation units to limit the loss of cryptic populations (Haig et al. 2011). For example, several phylogeographic studies suggest that glaciation events have been largely responsible for shaping distinct ESUs in several arctic breeding migratory shorebirds, including Dunlins (*Calidris alpina*; Wenink et al. 1996) and Temminck's Stint (*Calidris temminckii*; Rönka et al. 2011) that were previously unapparent. Population genetic analyses of migratory birds have also revealed more contemporary population processes that contribute to population structure and used these data to define distinct MUs. Wennerberg et al. (2008) found distinct MUs in Southern Dunlins (*Calidris alpina schinzii*) based on contemporary allele frequencies and recommended conservation of multiple Dunlin populations. The presence of MUs has also informed conservation efforts of other shorebirds, such as Temminck's Stint (*Calidris temminckii*, Rönka et al. 2008). For migrant species, the detection of genetically distinct conservation units is crucially important in management efforts focused on maintaining genetic diversity.

Shorebird conservation is also hindered by uncertainties in estimating sizes and trends of populations. Limitations of statistical estimation techniques include potential bias from conducting surveys from roads, sparse distribution across the migratory range, the propensity to aggregate in large flocks whose detection (or non-detection) could result in uncertainty in population density estimates, and the lack of estimates on stopover duration (Lanctot et al. 2008). These limitations are common for shorebirds, often resulting in wide ranges of population size

estimates (Morrison et al. 2006). To circumvent these issues, molecular estimates of population size (i.e., effective population size, N_e) are often measured in the context of population health by comparing them to a threshold minimum value to maintain adaptive potential.

One shorebird of conservation concern, the Buff-breasted Sandpiper (*Tryngites subrifucollis*), is a New World migrant ranging from wintering sites in southeastern South America to breeding sites in the arctic (Lanctot et al. 2010). Buff-breasted Sandpipers primarily use the Central flyway of the United States, and juveniles often use the Eastern flyway for fall migration (Fig. 2.1; Skagen et al. 1999). This historically abundant species has been subject to sharp population declines in the past 150 years, attributable to market hunting in the late 1800's and habitat loss resulting from conversion of upland habitat into agricultural land (Lanctot and Laredo 1994, Page and Gill 1994, Lanctot et al. 2002). Currently, this species is listed internationally as "near threatened" by the IUCN. However, Buff-breasted Sandpipers are ranked as a Global Species that is Highly Imperiled by the U.S. Shorebird Conservation Plan (2004), a designation shared by only 4 of 54 North American species of shorebirds. Estimates of Buff-breasted Sandpipers based on a range of statistical techniques have been highly variable over the past two decades, ranging from 15,000-84,000 birds in the Western Hemisphere (Jorgensen et al. 2008, Lanctot et al. 2010). Threats to contemporary Buff-breasted Sandpipers populations include increased numbers of nest predators in the arctic, exposure to contaminants at stopover sites and non-breeding sites, and loss of nonbreeding habitat (Lanctot and Laredo 1994, Strum et al. 2008).

The goal of this study was to better understand the conservation status of Buff-breasted Sandpipers. To this end, we determined the degree of genetic connectivity among and within the three biogeographical components of migration: breeding, stopover, and wintering sites. As a

migrant with low breeding site fidelity but evidence of wintering site fidelity we expected to see patterns of low migratory connectivity and high genetic connectivity across the range of the species with possible wintering population substructure (Lanctot and Weatherhead 1997; Almeida 2009; Friesen et al. 2007). We tested this hypothesis by assessing genetic structure across the entire migratory range of this species, as well as within regional breeding and wintering ranges, to uncover potentially cryptic conservation units for management. In addition, we assessed the role of land cover and climate changes associated with events in the Late Pleistocene and Holocene on contemporary population structure and taxonomic status. We also supplemented estimates of actual population size using estimates of N_e and analyses of demographic trends in this population of Buff-breasted Sandpipers to provide a more comprehensive picture of the population and conservation status of this species.

Methods

Study site and sample collection

We sampled breeding Buff-breasted Sandpipers (primarily males; n = 206) at leks and solitary sites (i.e., males displaying away from leks) at three main sites ~315-km apart in Northern Alaska: Barrow (71.30^{o}N, 156.77^{o}W), Canning River (70.07^{o}N, 146.71^{o}W), and Prudhoe Bay (70.33^{o}N, 148.71^{o}W) between 1993 and 2009. We weighed, measured, and ringed each bird with unique colored tarsal bands and a U.S. Fish and Wildlife Service metal band. We collected blood from captured adults with micro-hematocrit capillary tubes following venipuncture of the brachial vein with a 28 gauge needle for DNA extraction and analysis.

Between 2006 and 2007, we captured adult Buff-breasted Sandpipers along the Central flyway with mist nets, drop nets, and night lighting. Blood samples collected at stopover sites

represented individuals from Anahuac National Wildlife Refuge, Chambers County, TX (29.34°N, 94.32°W), Quivira National Wildlife Refuge, Stafford County, KS (38.08°N, 98.29°W), Konza Prairie Biological Station, Riley County, KS (39.04°N, 96.33°W) and Kissinger Wildlife Management Area, Clay County, NE (40.26°N, 98.06°W). In Latin America, we sampled birds from Uruguay (34.41°S, 54.16°W) and Argentina (30.43°S, 60.11°W; Strum et al. 2010). We also sampled birds at nocturnal roosts at wintering sites in Brazil from 2001-2005 using spotlights and dip nets (Almeida 2009). These samples represented individuals from two sites in Brazil separated by ~350-km: Lagoa do Peixe (30.25^{o}S, 50.96^{o}W) and Taim (32.59^{o}S, 52.59^{o}W).

Genomic DNA from stopover and wintering samples was available from previous molecular work with these samples (Almeida 2009; Strum et al. 2010). We extracted genomic DNA from breeding site blood samples using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, California, USA).

Microsatellite amplification

We surveyed a total of forty candidate microsatellite loci (11 developed for Pectoral Sandpipers from Carter and Kempenaers [2007] and 29 developed for Buff-breasted Sandpipers in our laboratory) to establish genotype profiles for all individuals. Of the forty candidate loci screened, two of the loci developed in our laboratory (6A3F: 5'-TGAGTTTAAAGCCTCAGAGC-3'; 6A3R: 5'-CACACAAGACCCTGGTAACT-3'; 6A12F: 5'-GTGCTGCCAGAAGAAATCAC-3'; 6A12R: 5'-CAGACGAAATGGCTCGATAT-3') and seven from Carter and Kempenaers (2007) consistently amplified using polymerase chain reaction (PCR) and were polymorphic. We deposited sequences for our two novel microsatellites into Genbank (accession numbers

JX123420 and JX123421). These nine loci were used in our analyses (Table 2.1).

PCR mixtures contained 20-100 ng genomic DNA, 2 µL 1X reaction buffer (PROMEGA), 2mM $MgCl_2$, 0.2mM of each dNTP, 0.5 µM of reverse primer (Table 2.1), 0.3 µM of forward primer, and 0.3 µM of M-13 universal primers (Schuelke 2000) labeled with a fluorescent dye (HEX, PET, NED, or FAM; Operon Biotechnologies, Huntsville, AL) attached to the 5' end, 0.1 µg bovine serum albumen (BSA), and 0.1 units of GoTaq polymerase (PROMEGA). We denatured these mixtures in an Eppendorf Mastercycler Pro thermal cycler (Brinkman Inc. Westbury, NY), for one 4 min cycle at 94^{o}C followed by 30 thermal cycles combining a 30 s denaturation step at 94^{o}C, a 30 s annealing step at Tm^{o}C (Table 2.1), and a 30 s extension at 72^{o}C followed by another 10 thermal cycles combining a 30 s denaturation step at 94^{o}C, a 30 s annealing step at 54^{o}C, and a 30 s extension at 72^{o}C and a final extension step for 10 min at 72^{o}C. We multiplexed PCR products for fragment analysis (ABI 3730; Applied Biosystems) and sized fragments against 500-LIZ size standard (Applied Biosystems). We used Program Genemarker (v1.95; Soft Genetics LLC, State College, PA) to visualize amplified fragments. We reran samples that were scored as homozygotes, as well as a random subset of heterozygotes (10% of the total heterozygotes at that locus), to assess allelic dropout rates.

Mitochondrial DNA amplification

We amplified a total of 1,543 bp of mitochondrial DNA (mtDNA) for two variable regions using primers described in Appendix A. The region was comprised of the cytochrome *b* gene (967 bp) and 576 bp at the 5'-end of the mitochondrial control region. We amplified these regions using PCR mixtures at a volume of 10µL contained 20-100 ng genomic DNA, 2 µL 1X reaction buffer (PROMEGA), 2mM $MgCl_2$, 0.2mM of each dNTP, 0.5 µM of each primer (Appendix A), 0.1 µg bovine serum albumen (BSA), and 0.1 units of GoTaq polymerase

(PROMEGA). We denatured these reactions in an Eppendorf Mastercycler Pro thermal cycler (Brinkman Inc. Westbury, NY), for one 2 min cycle at 95^{o}C followed by 30 thermal cycles combining a 30 s denaturation step at 94^{o}C, a 30 s annealing step (^{o}C, Appendix A), and a 30 s extension at 72^{o}C, and a final extension step for 10 min at 72^{o}C. We bidirectionally sequenced PCR products at University of Kentucky AGTC Sequencing Center via BigDye reactions using an ABI 3730 DNA Sequencer with the same forward and reverse primers used in amplification.

After checking chromatographs for errors, we compiled consensus sequences using the resulting forward and reverse sequences in Program Bioedit (v7.0.5.3; Hall 1999). We then aligned consensus sequences using a ClustalW approach and by eye in Program MEGA4 (Tamura et al. 2007) for each of the five amplified regions. We assembled the three segments of cytochrome *b* and two segments of the control region, respectively, for phylogenetic analyses. We deposited sequence data for all novel haplotypes in Genbank (accession numbers JX121967−JX122073).

Microsatellite analyses of genetic diversity and structure

We analyzed microsatellite characteristics across all individuals with complete genotype profiles (n = 477) using several software packages. For table-wide analyses of significance, we used a sequential Bonferroni correction for multiple pairwise comparisons (Rice 1989). We calculated the number of alleles (N_A), size ranges, and assessed private alleles using Microsatellite Toolkit (v3.1.1; Park 2001). We performed exact locus-by-locus tests for deviation from Hardy Weinberg equilibrium (HWE) using a 1,000,000-step Markov Chain Monte Carlo simulation in Program Arlequin (H_o and H_e values given in Table 2.1; v3.5 Excoffier et al. 2005). We also tested for pairwise linkage disequilibrium (LD) between all microsatellite pairs using Fisher's exact test with 5,000 permutations in Program Arlequin. Loci that deviated

significantly from HWE are noted in Table 2.1, and we conducted all microsatellite tests independently with and without these loci. We also assessed the possible presence of null alleles using a homozygosity excess test in Program MICROCHECKER (v2.2.3; van Oosterhout 2004).

After categorizing all novel microsatellite loci, we used a number of independent approaches to assess population substructure. We used a Bayesian clustering approach based on K = 1 through K = 5 on all loci in Program Structure (v2.3; Pritchard et al. 2000). We performed this analysis on the entire population (n = 477) with and without defining putative populations. We defined putative populations as each biogeographically distinct region (breeding, stopover, and wintering) to assess migratory connectivity. At $K \geq 2$, individuals were admixed among the clusters, with no individuals being strongly assigned to any one group, and thus no population substructure was evident using this method. However, due to some violations of the model assumptions (i.e., no HWE) of this Bayesian clustering approach, we opted to supplement this method with a descriptive approach to assessing genetic differentiation among and within sampling sites. We conducted a two-dimensional principal coordinate analysis (PCA) based on genetic distance, implemented in Genalex (v6.3; Peakall and Smouse 2006). We also calculated Wright's F-statistics and performed analyses of molecular variance (AMOVA) for these biogeographical regions in Program Arlequin.

To test our hypothesis regarding regional genetic structure, we performed the same population structure analyses on smaller geographic subsets of the overall species range: the four sites within the wintering region ($n_{Argentina} = 5$; $n_{Uruguay} = 14$; $n_{Taim} = 39$; $n_{L.Peixe} = 195$) and three sites within the breeding region ($n_{Prudhoe} = 153$; $n_{Barrow} = 15$; $n_{Canning} = 10$). For these regional analyses, we treated each disjunct site as a putative population and tested for pairwise genetic differences among sites. A recent study from Almeida (2009) suggested a sex bias in strength of

site fidelity at Brazilan wintering sites. Since females were more likely to show wintering site fidelity than males, we also excluded males and partitioned females between our two Brazilian wintering sites to elucidate possible genetic structure among females. To assess temporal changes in microsatellite diversity, we partitioned our global sample by decades. Breeding samples from the 1990's (n = 65) were treated as a separate population from samples collected the 2000's (n = 412), and we assessed private alleles to determine if alleles had been lost over time.

Effective population size and population trend analysis

We used microsatellite data to estimate effective population size (N_e) for our total contemporary population of Buff-breasted Sandpipers (n = 477). Using a linkage disequilibrium (LD) method implemented in Programs LDNe (v1.31; Waples and Do 2008) and NeEstimator (v1.3; Peel et al. 2004), we estimated 95% confidence intervals for N_e. For LDNe, we excluded alleles with a frequency ≤ 0.001 (i.e., singleton private alleles). We chose the LD method over the temporal method because in order for the latter to be applicable, samples should be between five and 10 generations apart for the influence of drift to be greater than the noise associated with sampling that population (Waples and Yokota 2007). Moreover, since microsatellite data suggested that this is one admixed population, the bias associated with sampling this population multiple times over small intervals is unpredictable (Waples and Yokota 2007).

Since Buff-breasted Sandpipers were subject to declines in population numbers due to market hunting 150 years ago and more recently with habitat loss and possible chemical contamination, we used two independent methods for detecting signatures of a population bottleneck from genotypic data. We used the *M*-ratio method, which relates the total number of alleles to the overall range in allele sizes. Using the conservative parameterization suggested by

Garza and Williamson (2001) for the two-phase mutation (TPM) model, we assumed a proportion of multi-step mutations (p_g) = 0.10, an average size of multi-step mutations (Δ_g) = 3.5, and a mutation rate (μ) of 5.0E-4 mutations/locus/generation. Since pre-bottleneck N_e is not known for this population, and this value is required to estimate critical threshold values of *M* (M_c), we used a range of estimated pre-bottleneck N_e to calculate a range of θ (where $\theta = 4N_e\mu$) and tested for *M* across this range. We found the *M*-ratio for each locus and averaged this value over all loci using Program M_P_Val (Garza and Williamson 2001). When compared with M_c thresholds (generated using Program CRITICAL_M) the *M*-ratio can be used to differentiate between a recent population bottleneck and a population that has remained small over time (Garza and Williamson 2001). Our second method for assessing population bottleneck was to use a mode-shift indicator and a test for heterozygosity excess under the TPM model in program BOTTLENECK (Cornuet and Luikart 1997). Using the same conservative parameterization for the TPM model we used for the *M*-ratio test, we tested the entire sample of Buff-breasted Sandpipers for potential bottlenecks.

Phylogeographic analysis using mtDNA data

We successfully amplified and analyzed 967 bp of the cytochrome *b* gene in 438 Buff-breasted Sandpipers sampled across the species' distributional range. We confirmed sequence identity by alignment with a published Buff-breasted Sandpiper cytochrome *b* sequence (Genbank accession number EF373162.1). We translated sequences from nucleotide to amino acid sequences in MEGA4 using the vertebrate mitochondrial genetic code to confirm that sequences showed no evidence of pseudogene amplification (i.e., fully coding with no frameshifts or premature stop codons; Rodríguez et al. 2007). We calculated standard molecular diversity indices (number of haplotypes, haplotype diversity, and nucleotide diversity) as well as

Fu's F_S (Fu and Li 1993) and Tajima's *D* (Tajima 1989) tests for selective neutrality in Program DnaSP (v5; Librado and Rosaz 2009). To elucidate phylogeographic patterns of gene flow, we also calculated Φ-statistics using haplotypic sequence data in Program Arlequin (Excoffier 2005). To visualize phylogeographic patterns graphically, we constructed a minimum-spanning haplotype network in Program Network (v4.610; Bandelt et al. 1999). For the 576 bp region of the mtDNA control region we amplified in 446 Buff-breasted Sandpipers, we calculated the same indices.

For estimates of divergence time, we used several approaches for a range of mutation rates using the coding cytochrome *b* gene. Since the molecular clock for cytochrome *b* in Buff-breasted Sandpipers has not been estimated, we used the reported range for Charadriiformes proposed by Weir and Schluter (2008) of 1.59 - 4.31 % per MY. We used this range of mutation rates for 967 bp of the cytochrome *b* gene to estimate a range of mutation rates (1.53E-5−4.15E-5 substitutions/site/year). We then used the mutation rates to estimate divergence times via the average number of mutations separating ancestral and descendent haplotypes (ρ-statistic) in Program Network (Forster et al. 1996). To test the validity of these estimates, we also employed a Bayesian coalescent-based approach to estimate divergence time in program BEAST (v1.6.2; Drummond and Rambaut, 2007). We used the AIC method to select the best-fit nucleotide substitution model for this gene Program jModelTest (v3.7; Posada 2008). Then, using a MCMC approach and imposing a Bayesian skyline plot as our demographic model, we estimated divergence time to approximate the time period that the population began to diverge from the most pervasive haplogroup.

Results

Migratory and genetic connectivity

Microsatellite characterization revealed high polymorphism in all loci across all geographic sites (mean alleles across all loci ± SD = 8.89 ± 4.96, Table 2.1). Across our entire sample, four of the nine loci analyzed showed significant deviation from Hardy-Weinberg equilibrium due to heterozygosity deficit (sequential Bonferroni corrected $P = 0.01$; Table 2.1). These same four loci showed evidence for null alleles according to Program MICROCHECKER ($P < 0.01$). No loci showed significant deviation from LD ($P = 0.001$).

Measures of population differentiation did not indicate significant population substructure among breeding, stopover, and wintering sites (Table 2.2). With the exception of the inbreeding coefficient (F_{IS}), all measures of differentiation were similarly close to 0 in relaxed tests using all nine loci and more conservative tests omitting four loci with evidence of null alleles. While statistically significant fixation indices suggested a degree of genetic distinction among these regions, the index for differentiation between regions was small, indicating high genetic connectivity across the distributional range of Buff-breasted Sandpipers ($F_{ST} = 0.004$, $P = 0.004$). AMOVAs using microsatellite marker data suggested that 0.42% of variation can be attributed to genetic distance among regions, whereas 85.6% can be attributed to variation within individuals. High genetic connectivity was supported when using a descriptive approach. A PCA based on genetic distance explained 39.14% of variation among genotypes and did not indicate the presence of genetic structure (Fig. 2.2). Taken together, these results indicated that Buff-breasted Sandpipers are currently a single, panmictic population at a global scale. Thus, we assessed the coefficient of inbreeding using our entire sample of Buff-breasted Sandpipers. Using the entire suite of microsatellite loci, the population showed evidence of inbreeding (F_{IS} =

0.14, $P < 0.0001$). After eliminating loci with null alleles, however, the inbreeding coefficient was not significantly different from 0 ($F_{IS} = 0.020$, $P > 0.05$). Since null alleles can inflate values of genetic differentiation due to falsely perceived homozygotes, we chose to use our conservative dataset to assess inbreeding (Carlsson 2008). Moreover, summary statistics for temporal analyses of all samples did not indicate genetic differences in samples grouped by decade (1990's vs. 2000's). Overall measures of genetic diversity (H_o) were not substantially different between decades. Any private alleles present in either temporal bin were present at low frequencies, implying that no alleles were substantially lost over the past two decades.

Locally, results of within-region analyses suggested high levels of genetic connectivity among geographically disjunct sites within breeding and wintering regions. Microsatellite AMOVAs provided no evidence for population structure among breeding sites (Table 2.3). For three Alaskan breeding sites separated by ~315-km (Barrow, Prudhoe Bay, and Canning), pairwise F_{ST} indicated high levels of gene flow among all sites ($F_{ST} = 0.01$, $P > 0.10$). Pairwise F_{ST} among the Argentinian, Uruguayan, and two Brazilian wintering sites revealed the same patterns of gene flow ($F_{ST} = 0.045$, $P > 0.019$). PCA based on genetic distance among wintering ground individuals explains 41.75% of variation and supports absence of structure (Fig. 2.3). When males were excluded from the Brazilian wintering site analyses, females did not show significant population structure between sites ($F_{ST} = 0.002$, $P > 0.41$).

Estimates of population differentiation among the three major migratory regions using mtDNA sequence data also supported the inferences drawn from microsatellite data and suggested high levels of gene flow ($\Phi_{ST} \leq 0.0004$, $P > 0.30$). An unrooted median-joining haplotype network analysis indicated mtDNA admixture between biogeographical regions (Fig. 2.4).

Effective population size and trend analyses

Since Buff-breasted Sandpipers appear to be one admixed population, we included all individuals in the analysis of N_e. We calculated two estimates; one using a conservative subset of loci omitting the four that showed evidence of null alleles and one using the full suite of microsatellite loci. Point estimates of N_e were variable and depended largely on the subset of microsatellite loci used (Table 2.4). For LDNe, point estimates of N_e ranged from 4,634 to 16,400 effective breeders when using the conservative and liberal datasets, respectively. For NeEstimator, point estimates of N_e ranged from 2,657 to 4,869 effective breeders. Jackknife and parametric 95% CI, however, were similar between the two methods for both subsets of loci used (Table 2.4). All estimation techniques resulted in upper bound estimates of N_e = infinite. Our results should be treated with caution, since N_e estimates with infinite confidence limits indicate that model assumptions were not met. However, despite noncompliance with model assumptions, our estimate of N_e is the first for Buff-breasted Sandpipers and can still serve an informative starting point. When we compared the range of N_e point estimates to the estimated minimum to maintain adaptive potential (1,000-5,000 effective breeders; Lynch and Lande 1998), all estimates fell within or above this range, implying that contemporary Buff-breasted Sandpipers are currently a genetically secure population.

For the range of estimated pre-bottleneck effective population sizes, the *M*-ratio results for bottleneck tests were the same (Table 2.5). The observed *M*-ratio was significantly higher than M_c in all tests, supporting the absence of any recent genetic bottlenecks. Our results were supported by heterozygosity excess tests (Wilcoxon test: $P > 0.9$) and mode-shift indicator (normal L-shape for all tests). These results were consistent with and without the presence of potential null alleles (Table 2.5), which agrees with several recently-published studies of

population bottlenecks (e.g., Carlsson 2008, Sastre et al. 2011). Overall, our independent tests on microsatellite data did not indicate any signal of recent population bottlenecks.

Phylogeography

For the 438 individuals (90% of the total sample) successfully sequenced at the cytochrome *b* gene, mtDNA analysis revealed 31 variable sites producing 33 unique haplotypes. We estimated an average haplotype diversity (H_d) of 0.436 ± 0.030 and nucleotide diversity (π) of 7.2E-4 ± 6.0E-5 (Table 2.6). A total of 328 individuals out of the 438 sampled (75%) fell into the most predominant haplogroup. High haplotype diversity and low nucleotide diversity are considered indicative of populations that have undergone rapid demographic expansion. To test this hypothesis in our study system, we conducted two tests for neutrality. Both tests were significant: Tajima's D = -2.27 ($P < 0.01$) and Fu's F_S = -30.6 ($P < 0.0001$), indicating that this population is likely undergoing genetic radiation from a single historic refugium.

Using an estimated value of the average number of mutations separating ancestral and descendent haplotypes (ρ-statistic = 0.349 ± 0.098) estimated from Program Network, we approximated a divergence time from the ancestral haplotype. Estimated divergence times ranged from 8,416.1 (± 2,366) to 22,816 (± 6,416) years before present (BP) based on our range of mutation rates (4.15E-5 and 1.53E-5 substitutions/site/year, respectively). To assess the validity of our estimates, we also combined two independent runs of 10^8 iterations (discarding the first 10^7 as burn-in) of a Bayesian coalescent-based approach for estimating divergence time in Program BEAST. Using the Tamura-Nei model (TrN; Tamura and Nei 1993) of nucleotide substitution with invariable sites and gamma distribution (TrN+I+G model, base frequencies of A = 0.284, C = 0.313, G = 0.132, T = 0.270) and a relaxed lognormal clock, we estimated approximate population divergence time. Estimates ranged from 17,000 to 45,000-BP depending

on the mutation rate used in the analysis (4.15E-5 and 1.53E-5 substitutions/site/year, respectively). Historic demographic patterns reconstructed using our Bayesian skyline plot analysis implied steady growth from this radiation (Fig. 2.5). The inference of population expansion is confirmed by the starburst topology of the haplotype network (Avise 2009; Fig. 2.4).

The patterns of historical demography inferred from the coding cytochrome *b* gene were supported by analyses of the non-coding mitochondrial control region. For 449 individuals (92% of total sample) successfully sequenced at the mitochondrial control region, analyses revealed 51 variable sites producing 74 unique haplotypes with an average haplotype diversity (H_d) of 0.822 ± 0.017 and nucleotide diversity (π) of 3.24E-3 ± 1.6E-4 (Table 2.6), the same pattern consistent with recent expansion that we observed in cytochrome *b*. Neutrality tests were also significant: Tajima's *D*: -2.11 ($P < 0.01$); Fu's F_S: - 27.14 ($P < 0.0001$), which supported inferences drawn from cytochrome *b* sequence data.

Discussion

In this study, we constructed genotype profiles for nine polymorphic microsatellite loci and haplotypes for ~1.5-kb of variable mtDNA in individuals representing the major migratory regions across the entire distributional range of Buff-breasted Sandpipers. We tested hypotheses of within and among-site connectivity by assessing levels of contemporary gene flow among breeding and wintering sites, as well as among breeding, stopover, and wintering regions. We were successful in inferring phylogeographic patterns similar to what has been observed in other migratory shorebirds using mtDNA data. These data were used to test population status hypotheses using several independent approaches.

Migratory and genetic connectivity

We found no signal of genetic structure at breeding grounds or wintering grounds, which is consistent with predictions for highly vagile migratory birds. Migratory species with low breeding site fidelity seldom show strong population structure, and panmixia was consistent in Buff-breasted Sandpipers at all biogeographical levels. Independent tests for population substructure using microsatellite data (Bayesian clustering, AMOVA, PCA, and analyses of Wright's *F*-statistics) and mtDNA sequence data (Φ-statistics) support our hypothesis that this species is a single, admixed population on a global scale. Moreover, while we only sampled a small geographic subset of the species' overall breeding and wintering ranges, our stopover site individuals should adequately represent the global population since individuals from all sites funnel through the Great Plains flyway during migration (Fig. 2.1).

To examine possible genetic structure associated with the high site fidelity reported at Brazilian wintering grounds, we examined microsatellite and mtDNA differentiation at these sites (Almeida 2009). Despite being ~300-km apart, we saw no evidence for genetic substructure between our two Brazilian wintering locations in either sex. When we expanded analyses to include samples from Uruguay and Argentina, we did not detect a signal of population structure based on geographic locality. Lack of genetic structure was an unexpected result, since other species of migratory birds with high site fidelity at multiple non-breeding sites have shown stronger genetic structure than those that lack such structure (Friesen et al. 2007). However, it is possible that the small sample size at three of or four wintering sites lead to incomplete detection of overall genetic diversity in this region, which could explain the perceived absence of genetic structure.

Effective population size and trend analyses

Genetic assessments of demographic trends can serve as powerful tools for inferring the impacts of historical demographic processes on contemporary populations. To elucidate a potential genetic response to population declines, we estimated a range of N_e and the likelihood of a recent population bottleneck. Estimates of N_e were sufficiently high to maintain adaptive potential. Populations with a high N_e tend to lose genetic diversity more slowly than those with a low N_e, implying that Buff-breasted Sandpipers should be resilient to the genetic effects of declining population size (Nunney 1995). It is also important to note that Buff-breasted Sandpipers are a lek-mating species, with lek sites located across the arctic (Lanctot et al. 1997). Reproductive skew can bias estimates of N_e, and we expect a lek-mating system to have a reduced N_e compared to a non-lekking system. However, due to the use of alternate mating strategies (e.g., solitary displays, sneaker males, and multiple mating females), the male reproductive skew usually associated with a lek-mating system is absent in Buff-breasted Sandpipers (Lanctot et al. 1997). Thus, we can be confident that the mating system does not bias our estimates of N_e.

Our independent tests for a population bottleneck did not show any evidence for the heterozygosity excess associated with a recent genetic bottleneck in Buff-breasted Sandpipers. Lack of a bottleneck was somewhat unexpected considering the numerous population pressures leading to population declines over the last two centuries. However, high levels of gene flow in a single, globally admixed population has been shown to alleviate the genetic effects of population declines in Black-tailed Godwits (*Limosa limosa*, Trimbos et al. 2011), and it is possible that this is also the case for Buff-breasted Sandpipers.

Phylogeography

Understanding the historical demography of migrants is vital to species conservation. Linking patterns of phylogeographic structure to known historical events can help conservation biologists understand the forces that shape contemporary population structure, and this information can be used to prevent the loss of taxonomic diversity within species (ESU's). Furthermore, because of the slower mutation rate of mtDNA relative to microsatellites, it is possible to detect signals of genetic structure on the mitochondrial level that are absent when assessing only rapidly-evolving microsatellite data. Thus, phylogeographic analyses played an important role in our conservation recommendation for Buff-breasted Sandpipers.

For both mtDNA regions, Buff-breasted Sandpipers showed high haplotype diversity and low nucleotide diversity. This pattern, coupled with significantly negative values for our independent tests for neutrality, was indicative of a recent radiation event (Fu 1997). This historical demographic pattern can be visualized in the starburst topology shown in our haplotype network for cytochrome *b* (Avise 2009; Fig. 2.4). Together, these results suggested that Buff-breasted Sandpipers are undergoing a radiation from a single refugium.

Our estimates of divergence time for our study population suggested that Buff-breasted Sandpipers began to radiate from a single refugium in the arctic ~8,500 to 45,000-BP. This estimate coincides with the height of the Wisconsinan glaciation in North America (Munyikwa et al. 2011). During this recent glaciation event, ranging from ~110,000 to 10,000-BP, much of the eastern extent of the Buff-breasted Sandpipers arctic breeding range was covered with ice. It is possible that, with much of the species' breeding range rendered unusable by ice sheets, Buff-breasted Sandpipers were confined to breed in a smaller region in eastern Russia, western Canada, and Alaska. Once the ice sheets retreated and breeding habitat expanded, the population

size grew. Similar phylogeographic patterns have been reported in other species of arctic breeding shorebirds including Dunlins (*Calidris alpina*; Wenink et al. 1996) and Temminck's Stint (*Calidris temminckii*; Rönka et al. 2008).

Conservation status

Moritz (1994) suggests that carefully monitoring both contemporary and historical conservation units (MUs and ESUs, respectively) is vital in species conservation. Several recent studies suggest carefully monitoring both contemporary and historical conservation units for management, specifically in the context of migratory birds (Haig et al. 2011). We did not observe a signal of population structure either phylogeographically or contemporarily at any biogeographic level. Thus, we recommend that Buff-breasted Sandpipers should be managed as one conservation unit. It also appears from our demographic trend analyses that the high level of admixture observed in this species could be helping to maintain global diversity and is an important aspect of maintaining population viability. Thus, management efforts must focus on maintaining this global genetic connectivity. Anthropogenic habitat fragmentation is common across the range of this species, and successful management efforts should concentrate on international efforts to mitigate any negative impacts this fragmentation may have on genetic connectivity.

Conservation biology is often a crisis-driven discipline, and it is important to focus on management of declining species before populations suffer severe losses. For species that are difficult to monitor, it is particularly important to use a number of different approaches to establishing conservation units for management. Here, our goal was to update the conservation status of a species that is currently undergoing population declines by supplementing its conservation and taxonomic status with novel population genetic and phylogeographic data.

While estimates of N_e and bottleneck tests are excellent starting points for understanding the impact of population declines on Buff-breasted Sandpipers, it would be ideal to sample populations before and after the major events that caused their decline (Busch et al. 2007). Thus, we conducted a molecular survey of museum samples representing Buff-breasted Sandpipers from the past 150 years to determine how genetic diversity was impacted by varying magnitudes of population declines (Chapter 3). We hope to supplement the findings in this current study with patterns of historic phylogeography in order to fully understand the conservation status of Buff-breasted Sandpipers.

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References

Almeida, J.B. 2009. Wintering ecology of Buff–Breasted Sandpipers (*Tryngites subruficollis*) in Southern Brazil. PhD Dissertation, University of Nevada, Reno, Nevada, USA.

Avise, J.C. 2009. Phylogeography: retrospect and prospect. Journal of Biogeography 36:3–15.

Bandelt, H.J., P. Forster, and A. Röhl. 1999. Median–joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Bart, J., S. Brown, B. Harrington and R.I.G. Morrison. 2007. Survey trends of North American shorebirds: population declines or shifting distributions? Journal of Avian Biology 38:73–82.

Busch, J.D., P.M. Waser, and J.A. DeWoody. 2007. Recent demographic bottlenecks are not accompanied by a genetic signature in banner–tailed kangaroo rats (*Dipodomys spectabilis*). Molecular Ecology 16:2450–2462.

Carter, K.L. and B. Kempenaers. 2007. Eleven polymorphic microsatellite markers for paternity analysis in the pectoral sandpiper, *Calidris melanotos*. Molecular Ecology Notes 7(4):658–660.

Carlsson, J. 2008. Effects of microsatellite null alleles on assignment testing. Journal of Heredity. 99:616–623.

Cornuet, J.M. and G. Luikart. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Davis, L.A., E.H. Roalson, K. L. Cornell, K.D. McClanahan, and M.S. Webster. 2006. Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. Molecular Ecology 15:2141–2152.

Drummond, A.J. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Forster, P., R. Harding, A. Torroni, and H.–J. Bandelt. 1996. Origin and evolution of Native American mtDNA variation: a reappraisal. American Journal of Human Genetics 59:935–945.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66:95–107.

Frankham R., J.D. Ballou, and D.A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK.

Friesen, V. L., T. M. Burg, and K. D. McCoy. 2007. Mechanisms of population differentiation in seabirds. Molecular Ecology 16:1765–1785.

Fu Y–X and Li W–H. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Fu Y–X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Garza J.C. and E.G., Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10:305–318.

Haig, S.M., C.L Gratto–Trevor, T.D. Mullins, and M.A. Colwell. 1997. Population identification of Western Hemisphere shorebirds throughout the annual cycle. Molecular Ecology 6:413–427.

Haig, S.M., W.M. Bronaugh, R.S. Crowhurst, J. D'Elia, C.A. Eagles–Smith, C.W. Epps, B. Knaus, M.P. Miller, M.L. Moses, S. Oyler–McCance, W.D. Robinson, and B. Sidlauskas. 2011. Genetic applications in avian conservation. Auk 128(2):205–229.

Hall, T.A. 1999. BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Jorgensen, J.G., J.P. McCarty, and L.L. Wolfenbarger. 2008. Buff–breasted Sandpiper density and numbers during migratory stopover in the Rainwater Basin, Nebraska. Condor 110:63–69.

Lanctot, R.B. and C. D. Laredo. 1994. Buff–breasted Sandpiper (*Tryngites subruficollis*). Birds of North America, No. 91.

Lanctot, R.B., K.T. Scribner, B. Kempenaers, and P.J. Weatherhead. 1997. Lekking without a paradox in the Buff–Breasted Sandpiper. The American Naturalist 149(6):1051–1070.

Lanctot, R.B., D.E. Blanco, R.A. Dias, J.P. Isacch, V.A. Gill, J.B. Almeida, K. Delhey, P.F. Petracci, G.A. Bencke, And R.A. Balbueno. 2002. Conservation status of the Buff–breasted Sandpiper: historic and contemporary distribution and abundance in South America. Wilson Bulletin 114:44–72.

Lanctot, R.B., A. Hartman, L.W. Oring, and R.I.G. Morrison. 2008. Response to: limitations of statistically derived population estimates, and suggestions for deriving national population estimates for shorebirds. Auk 125(4):983–985.

Lanctot, R.B., J. Aldabe, J.B. Almeida, D. Blanco, J.P. Isacch, J. Jorgensen, S. Norland, P. Rocca, and K.M. Strum. 2010. Conservation Plan for the Buff–breasted Sandpiper (*Tryngites subruficollis*).Version 1.1. U. S. Fish and Wildlife Service, Anchorage, Alaska, and Manomet Center for Conservation Sciences, Manomet, Massachusetts, USA.

Librado, P. and J. Rosaz. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Liebers D. and A.J. Helbig. 2002. Phylogeography and colonization history of Lesser Black–backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15:1021–1033.

Lynch and Lande 1998. The critical effective size for a genetically secure population. Animal Conservation 1(1):70–72.

Moritz, C. 1994. Defining Evolutionarily Significant Units for conservation. Trends in Ecology and Evolution 9(10):373–375.

Morrison, R.I.G., B.J. McCaffery, R.E. Gill, S.K. Skagen, S.L. Jones, G.W. Page, C.L. Gratto–Trevor, and B.A. Andres. 2006. Population estimates of North American shorebirds. Wader Study Group Bulletin 111:67–85.

Munyikwa, K., Feathers J.K., Rittenour T.M., Shrimpton H.K. 2011. Constraining the Late Wisconsinan retreat of the Laurentide ice sheet from western Canada using luminescence ages from postglacial aeolian dunes. Quaternary Geochronology 6:407–422.

Nunney, L .1995. Measuring the ratio of effective population size to adult numbers using genetic and ecological data. Evolution 49:389–392.

Palstra, F.P. and D.E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology 17(15):3428–3447.

Page, G.W. and R.E. Gill Jr. 1994. Shorebirds in western North America: late 1800s to late 1900s. Studies in Avian Biology 15:147–160.

Park, S.D.E. 2001. Trypanotolerance in West African Cattle and the population genetic effects of selection, Ph.D. thesis, University of Dublin, Ireland.

Pearce J.M., K.G. McCracken, T.K. Christensen, Y.N. Zhuravlev. 2009. Migratory patterns and population structure among breeding and wintering Red–breasted (*Mergus serrator*) and Common Mergansers (*M. merganser*). Auk 126: 784–798.

Peakall, R. and P.E. Smouse. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288–295.

Peel, D., J.R. Ovenden, and S.L. Peel. 2004. NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25(7):1253–1256.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43(1):223–225.

Rodríguez, F., J. Albornoz, and A. Domínguez. 2007. Cytochrome *b* pseudogene originated from a highly divergent mitochondrial lineage in genus *Rupicapra*. Journal of Heredity 98(3):243–249.

Rönka, A., L. Kvist, J. Karvonen, K. Koivula, V. Pakanen, D. Schamel, and D.M. Tracy. 2008. Population genetic structure in the Temminck's Stint *Calidris temminckii*, with an emphasis on Fennoscandian populations. Conservation Genetics 9:29–37.

Rönka, N., L. Kvist, V. Pakanen, A. Rönka, V. Degtyaryev, P. Tomkovich, D.M. Tracy, and K. Koivula. 2011. Phylogeography of the Temminck's Stint (*Calidris temminckii*): historical vicariance but little present genetic structure in a regionally endangered Palearctic wader. Diversity and Distributions 18(5):1–13.

Sastre, N., C. Vila, M. Salinas, V.V. Bologov V. Urios, A. Sanchez O. Francino, and O. Ramırez. 2011. Signatures of demographic bottlenecks in European wolf populations. Conservation Genetics 12(3):701–712.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18(2):233–234.

Strum, K.M. 2008. Exposure of migratory shorebirds to organophosphorus and carbamate pesticides at migratory stopover and non–breeding sites in the western hemisphere. M.Sc. thesis, Kansas State University, Manhattan, Kansas, USA.

Strum, K.M., M.J. Hooper, K.A. Johnson, R.B. Lanctot, M.E. Zaccagnini, and B.K. Sandercock. 2010. Exposure of nonbreeding migratory shorebirds to cholinesterase–inhibiting contaminants in the Western Hemisphere. Condor 112(1):15–28.

Skagen, S. K., P.B. Sharpe, R.G. Waltermire, and M.B. Dillon. 1999. Biogeographical profiles of shorebird migration in midcontinental North America. Biological Science Report USGS/BRD/BSR—2000–0003. U.S. Government Printing Office, Denver, CO.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3):512–526.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596–1599.
Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Trimbos K.B., C.J.M. Musters, Y.I. Verkuil, R. Kentie, T. Piersma, and G.R. de Snoo. 2011. No evident spatial genetic structuring in the rapidly declining Black–tailed Godwit *Limosa limosa limosa* in The Netherlands. Conservation Genetics 12:629–636.

U.S. Shorebird Conservation Plan (USSCP). 2004. High priority shorebirds – 2004. Unpubl. Report, United States Fish and Wildlife Service, 4401 N. Fairfax Dr., MBSP 4107, Arlington, VA, 22203. USA. 5 pp.

van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Waples, R.S., and M. Yokota. 2007. Temporal estimates of effective population size in species with overlapping generations. Genetics 175:219–233.

Waples, R.S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.

Waples, R.S. 2010. Spatial–temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. Molecular Ecology Resources 10(5):785–796.

Weir, J.T. and D. Schluter. 2008. Calibrating the avian molecular clock. Molecular Ecology 17:2321–2328.

Wenink, P.W., A.J. Baker, H.–U. Rosner, and M.G. J. Tilanus. 1996. Global mitochondrial DNA phylogeography of Holarctic breeding Dunlins (*Calidris alpina*). Evolution 50(1):318–330.

Wennerberg, L., G. Marthinsen, and J.T. Lifjeld. 2008. Conservation genetics and phylogeography of southern dunlins *Calidris alpina schinzii*. Journal of Avian Biology 39:423–437.

Figures and Tables

Figure 2.1: Range map for Buff-breasted Sandpipers modified from Lanctot et al. 2010. Green shaded areas represent the species' range. Solid black lines represent primary migratory routes and the dashed line indicates the route used by juveniles during fall migration. Yellow circles indicate sampling sites.

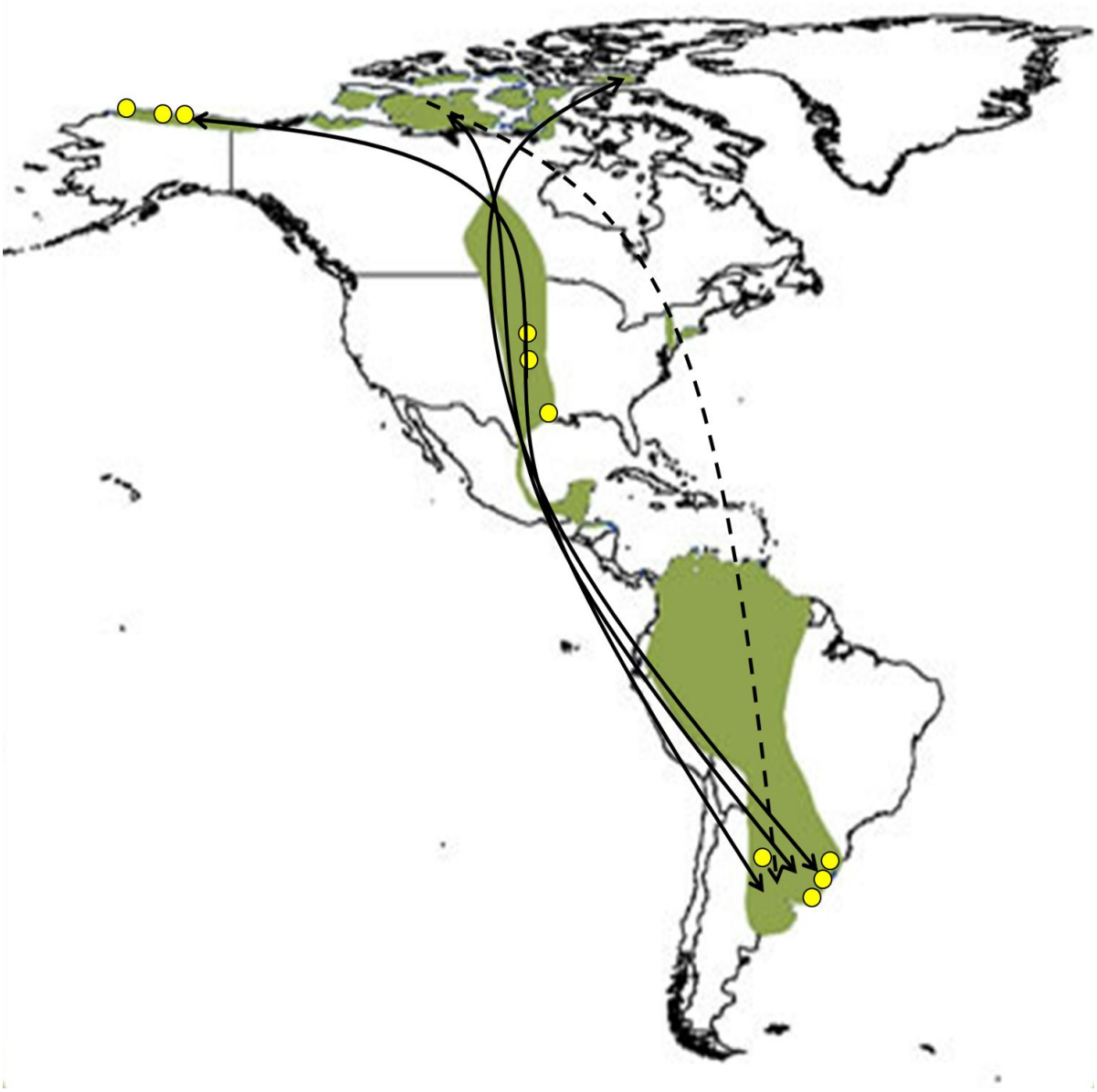

Figure 2.2: Principal Coordinate Analysis (PCA) based on genetic distance among all individuals. 20.42% of variation is explained by Coordinate 1 and 18.73% by Coordinate 2. Filled black circles = breeding sites, unfilled red triangles = stopover sites, filled green circles = wintering sites.

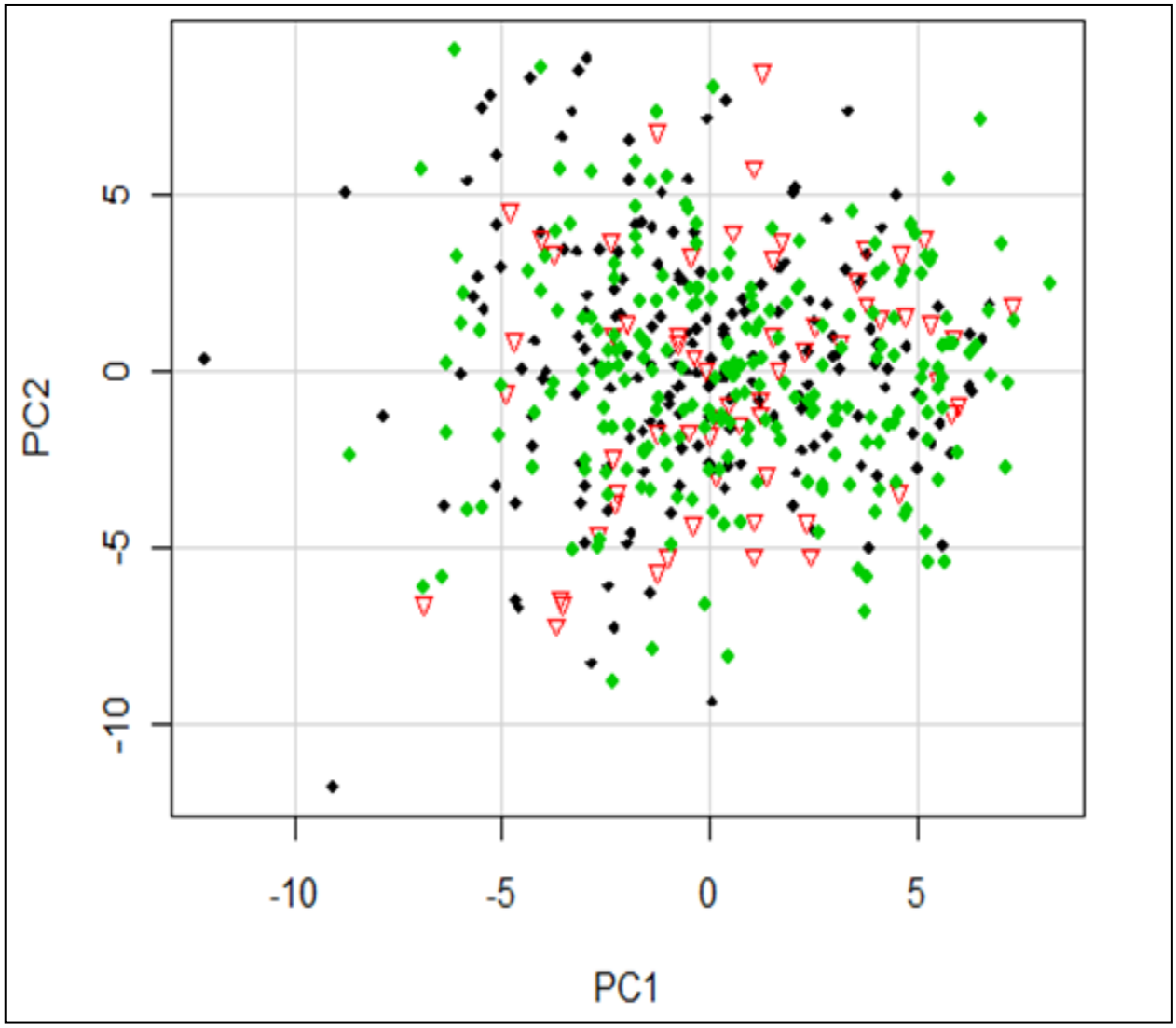

Figure 2.3: Principal Coordinate Analysis (PCA) based on genetic distance among wintering site individuals. 23.17% of variation is explained by Coordinate 1 and 18.59% by Coordinate 2. Filled black boxes = Argentina; filled blue triangles = Uruguay; filled green circles = Taim, Brazil; and unfilled red circles = Lagoa do Peixe, Brazil.

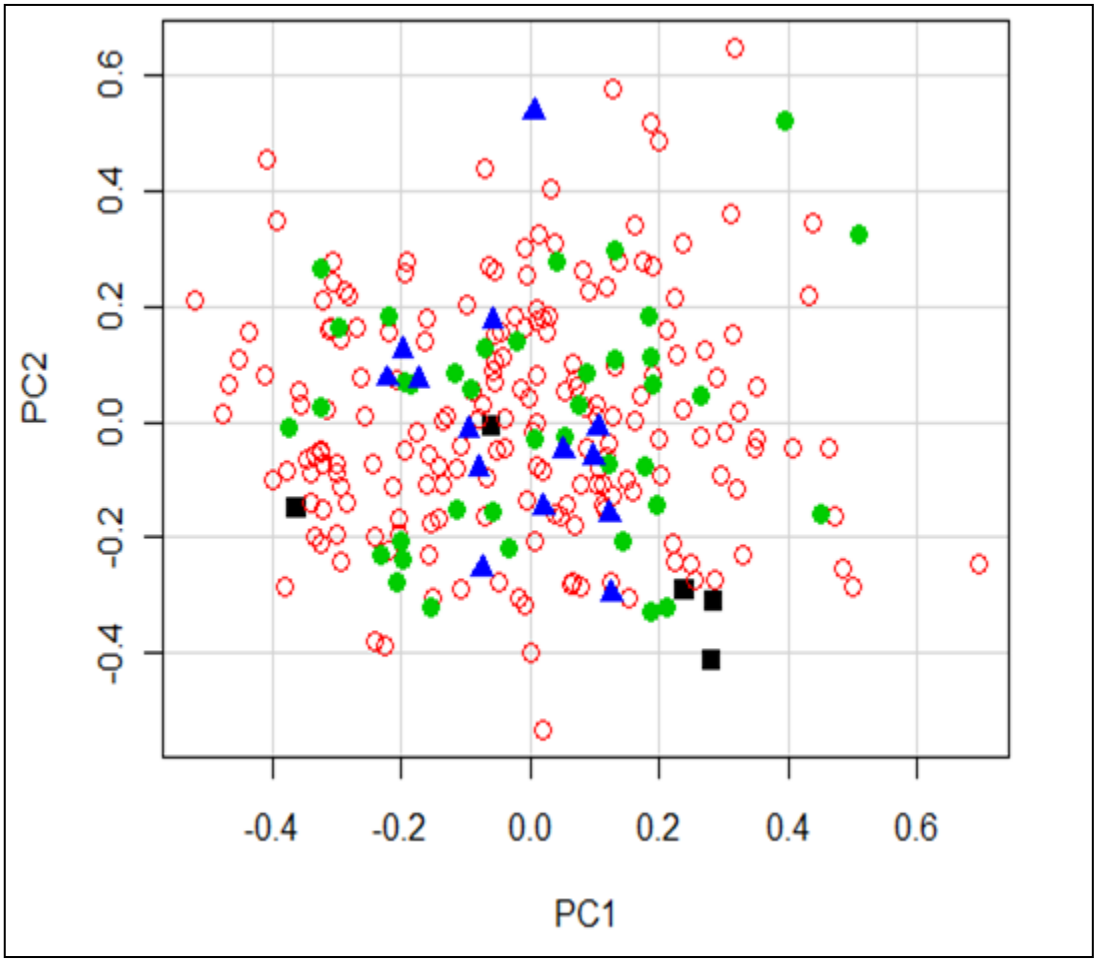

Figure 2.4: Minimum-spanning haplotype network for Buff-breasted Sandpipers based on a 967 bp region of the cytochrome *b* gene (n = 438 birds). Node size indicates relative size of each haplogroup and branch length indicates number of mutations (standard branch length is one mutation). Circles are color-coded to correspond with biogeographical region (white = breeding, black = stopover, and gray = wintering). Assumed mutations that were not found in our sample are represented by red circles along branches.

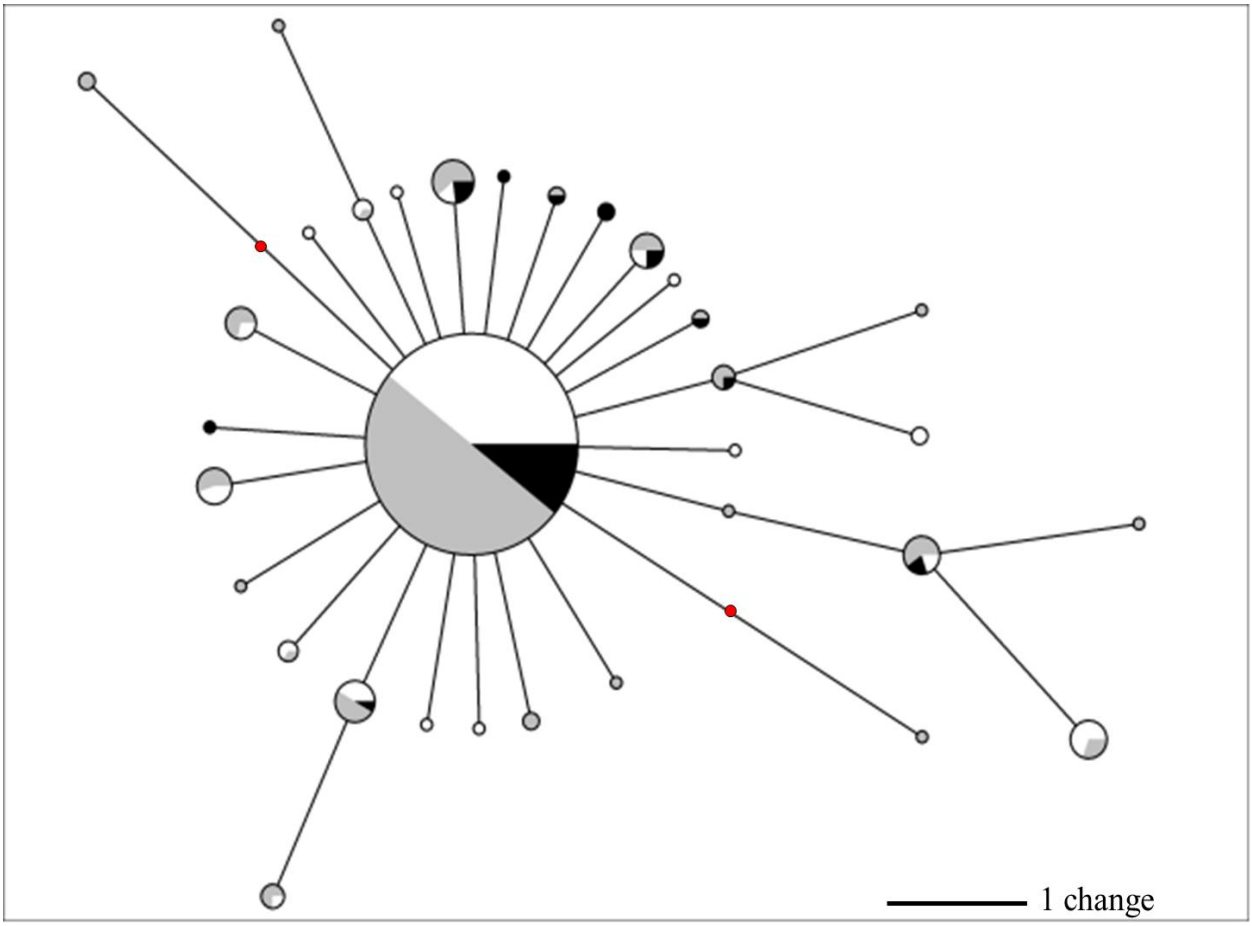

Figure 2.5: Reconstruction of demographic population fluctuations based on a Bayesian skyline plot derived from cytochrome *b* sequence data. The solid black line represents median population size estimate ($N_e\theta$) and the solid gray lines represent a 95% CI.

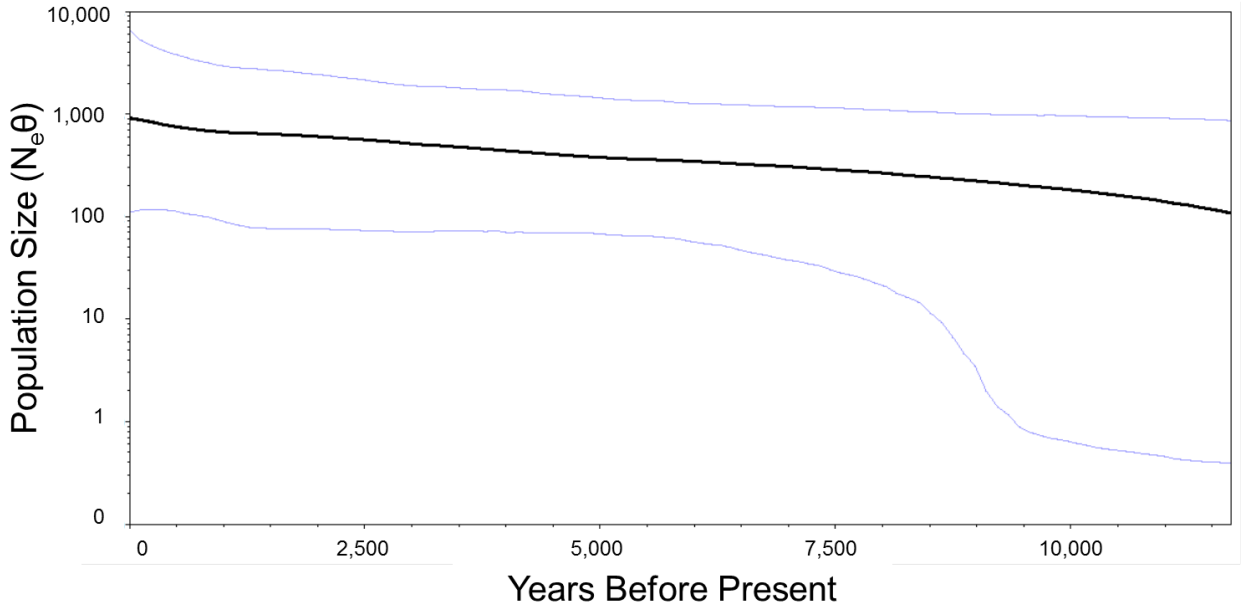

Table 2.1: Characterization of microsatellite loci used in the study. "CME" loci were adapted from Pectoral Sandpipers (Carter and Kempenaers 2007) and "6A" primers were developed in our laboratory for Buff-breasted Sandpipers. All forward primers were M-13 tagged for fluorescent visualization. H_o values with a * indicate loci that show significant deviation from HWE (Bonferroni corrected final $P < 0.01$).

Locus ID	Repeat motif	N_A	Tm (^oC)	Size Range	H_o	H_E
6A12	$(CT)_{11}$	4	58	247-259	0.356	0.431*
6A3	$(TCTT)_5$	8	56	212-226	0.711	0.745*
CME1	$(CA)_{19}$	9	58	102-120	0.755	0.787
CME2	$(GT)_{15}AT(GA)_3$	7	58	155-171	0.415	0.423
CME6	$(CA)_8$	18	61	201-235	0.554	0.888*
CME8	$(CA)_6$	12	61	209-235	0.480	0.764*
CME9	$(GT)_{13}$	3	61	161-165	0.317	0.320
CME10	$(CA)_{14}$	5	56	202-210	0.327	0.348
CME12	$(CT)_3(GT)_{13}$	14	56	192-218	0.755	0.740
Mean ± SD		8.89 ± 4.96			0.519 ± 0.182	0.605 ± 0.220

Number of alleles (N_A), annealing step temperature (Tm), allelic size range (number of bp), observed heterozygosity (H_o), and expected heterozygosity (H_E).

Table 2.2: Pairwise-F_{ST} values estimated from microsatellites for the three migratory regions (left) and Φ_{ST} values from mitochondrial control region (right). Although pairwise F_{ST} values were small, they were all significantly different from 0. Φ_{ST} values were not significantly different from 0.

	Breeding	Stopover	Wintering
Breeding	-	0.000	0.000
Stopover	0.007	-	-0.003
Wintering	0.004	0.004	-

Table 2.3: Microsatellite AMOVA and fixation indices for three arctic breeding sites in Barrow Bay, Prudhoe Bay, and Canning, Alaska.

Source of Variation	d.f.	Sum of squares	Variance component	Percentage of Variation	Fixation Index
Among Populations	2	3.09	0.0025	0.19	$F_{ST} = 0.002$
Among Individuals Within Populations	174	251	0.101	7.50	$F_{IS} = 0.075$
Within Individuals	177	219	1.24	92.31	
Total	353	473	1.34		

Table 2.4: Independent point estimates of effective population size (N_e) ± 95% CI using the full suite of nine microsatellite loci as well as a conservative subset of five loci that showed no significant deviation from HWE. Estimates from Programs LDNe and NeEstimator are given.

	LDNe	95% CI	NeEstimator	95% CI
N_e - 9 loci	16,400	1,093 - infinity	4,869	1,621 - infinity
N_e - 5 loci	4,634	603- infinity	2,657	802- infinity

Table 2.5: Tests for genetic bottleneck using all loci and the conservative subset of five loci that showed no significant deviation from HWE. For all TPM model runs, $\sigma^2 = 12$, $p_g = 0.10$. Sign tests are given in ratios of heterozygosity excess vs. deficiency.

	Parameters	Result
Test - 9 loci		
Heterozygosity excess	Sign test	3:6; $P = 0.120$
	Wilcoxon	$P = 0.980$
M-Ratio	$N_e = 50$, $\theta = 0.1$, $M_C = 0.841$	M = 0.948
	$N_e = 1{,}000$, $\theta = 2$, $M_C = 0.755$	M = 0.948
	$N_e = 2{,}500$, $\theta = 5$, $M_C = 0.747$	M = 0.948
	$N_e = 7{,}500$, $\theta = 15$, $M_C = 0.761$	M =0.948
Test - 5 loci		
Heterozygosity excess	Sign test	1:4; $P = 0.100$
	Wilcoxon	$P = 0.970$
M-Ratio	$N_e = 50$, $\theta = 0.1$, $M_C = 0.800$	M = 0.936
	$N_e = 1{,}000$, $\theta = 2$, $M_C = 0.718$	M = 0.936
	$N_e = 2{,}500$, $\theta = 5$, $M_C = 0.712$	M =0.936
	$N_e = 7{,}500$, $\theta = 15$, $M_C = 0.734$	M = 0.936

Table 2.6: Molecular diversity indices for cytochrome *b* and control region across the range of Buff-breasted Sandpipers.

	Cytochrome *b*				Control Region			
	N	*h*	H_d	π x 10^3	*N*	*h*	H_d	π x 10^3
Breeding	166	18	0.39 ± 0.05	0.69 ± 0.11	172	50	0.80 ± 0.03	3.17 ± 0.27
Stopover	40	10	0.44 ± 0.10	0.57 ± 0.15	43	17	0.84 ± 0.05	3.16 ± 0.49
Wintering	232	24	0.47 ± 0.01	0.75 ± 0.09	227	51	0.83 ± 0.02	3.27 ± 0.22
All	438	33	0.44 ± 0.03	0.72 ± 0.01	449	74	0.82 ± 0.02	3.24 ± 0.16

Sampling site, number of individuals (*N*), number of haplotypes (*h*), haplotype diversity (H_d) ± SD, and nucleotide diversity (π) ± SD.

The following chapter is formatted for publication in Conservation Genetics.

Chapter 3 - Stable effective size and genetic diversity in a declining population: 135 years of mtDNA diversity in Buff-breasted Sandpipers (*Tryngites subruficollis*)

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Abstract

The maintenance of genetic diversity has been a cornerstone for management efforts across a wide range of threatened taxa, largely because populations with greater genetic diversity are resistant to the stochastic effects of genetic drift and inbreeding associated with low diversity. Modern and historic processes that limit contemporary population size can contribute to the loss of genetic diversity. Like many species of migratory shorebirds, Buff-breasted Sandpipers (*Tryngites subruficollis*) have suffered gradual population losses over the past century, with a quick, large-scale decline associated with intensive market hunting in the late 1800's and early 1900's. To empirically assess the impact of ongoing population declines on genetic diversity, we surveyed two mitochondrial DNA (mtDNA) markers, cytochrome *b* and the control region, from 220 museum specimens of this species across a period of 135 years. Measures of mtDNA diversity did not change substantially over time (e.g., trend analysis on haplotype richness in control region: $\chi^2 = 0.171$, $P = 0.679$). Using estimates of θ_w as a measure of effective female population size (N_{ef}) and a Bayesian Skyline reconstruction of demographic trends, we concluded that N_{ef} size was constant during our study period. While genetic diversity and N_{ef} did not appear to have been lost as a result of market hunting or steady declines in Buff-breasted Sandpipers, management efforts must focus on preventing future losses for wild populations to remain viable.

Introduction

The maintenance of genetic diversity has been a cornerstone for management efforts across a wide range of threatened taxa (Spielman et al. 2004). This management objective is based on populations with greater genetic diversity being more resilient to environmental stochasticity and the deleterious effects of inbreeding than populations with low diversity (Soulé 1991, Brook et al. 2002). Modern and historic processes that limit contemporary population size can contribute to the loss of genetic diversity, and the impacts of these processes are often inferred from genetic patterns in contemporary populations. The increasing use of DNA extracted from museum specimens has allowed conservation genetic studies to elucidate the genetic impacts of past events empirically rather than by inference from contemporary data (Ramakrishnan and Hadly 2009). For example, distinct mitochondrial lineages that have been lost as a result of population declines would be absent in contemporary samples but detectable in pre-decline populations, and DNA derived from museum specimens can provide a crucial historical perspective on events like large-scale population declines that may not be apparent from contemporary sampling alone (Wandeler et al. 2007). Moreover, museum collections may allow for robust sampling over multiple centuries, which allows us to more accurately detect and assess the genetic impacts of subtle, long-term declines in population size.

Migratory shorebirds (Aves: Charadriiformes) have been subjected to severe population declines for the past 150 years (Morrison et al. 2006). These losses have been attributed to a number of anthropogenic disturbances across these species' ranges, with initial large-scale declines associated with intensive market hunting of migratory birds in the late 1800's and early 1900's. Impacted species included American Golden Plovers (*Pluvialis dominica*, Clay et al. 2010); Long-billed Curlews (*Numenius americanus,* Allen 1980); Oystercatchers (*Haematopus*

palliatus, George 2002); and American Woodcocks (*Scolopax minor*, Weik 2001). Harvest was a contributing factor in the extinction of at least two migratory bird species, including Eskimo Curlews (*Numenius borealis,* Roberts et al. 2009) and Passenger Pigeons (*Ectopistes migratorius,* Fultona et al. 2012). With the enactment of the Migratory Bird Treaty Act in 1918, commercial harvest of these birds was slowed and eventually halted for most species, and some wild populations recovered. While population numbers of some species were estimated to have become more stable during the period following the ban on hunting (Burleigh 1958, Tudor 2000), the genetic effects of early declines on migratory shorebirds are not well understood. Several studies have attributed signals of recent genetic bottlenecks in shorebird species to commercial harvest (e.g., Baker and Stauch 1988), but to date no studies have investigated the impacts of market hunting on genetic diversity in this group. Conservation biologists are presented with a unique opportunity to use museum specimens to empirically assess the genetic impacts of this bottleneck on shorebird populations over long time periods.

Population pressures on migratory shorebirds have likely changed over the past century. While some shorebird species recovered following the Migratory Species Act, habitat degradation and other anthropogenic factors have continued the gradual decline of shorebirds throughout the twentieth century (Butler et al. 2004, Morrison et al. 2006). Since substantial losses of genetic diversity and fitness have been attributed to long-term population declines, elucidating the genetic impacts of gradual declines directly is also crucial to shorebird management (Westemeier et al. 1998).

The Buff-breasted Sandpiper (*Tryngites subrifucollis*) is a New World migratory shorebird ranging from wintering sites in southeastern South America to breeding sites in the arctic (Lanctot and Laredo 1994). Like other shorebird species, this historically abundant species

underwent substantial population declines in the past century. The short-term drastic decline of Buff-breasted Sandpipers has been largely attributed to market hunting at the turn of the twentieth century, with pre-hunting estimates of Buff-breasted Sandpipers based on anecdotal sightings ranging into the hundreds of thousands (Lanctot et al. 2010). In the past century, population pressure from anthropogenic disturbances along migratory routes, including habitat loss resulting from conversion of upland habitat to agricultural land and exposure to toxic environmental contaminants, is continuing and has likely resulted in gradual population losses (Page and Gill 1994, Lanctot et al. 2010, Strum et al. 2010). Contemporary estimates of Buff-breasted Sandpipers based on a range of statistical techniques have been highly variable over the past two decades, ranging from 15,000-84,000 birds (Jorgensen et al. 2008, Lanctot et al. 2010).

With populations declining dramatically over the past century, this species provides a rare opportunity to empirically assess temporal changes in genetic characteristics in a shorebird population with a known population bottleneck followed by a century of other population pressures. The goal of this study was to empirically estimate the impact of both intense, short-term and gradual, long-term population declines on mitochondrial DNA (mtDNA) diversity in Buff-breasted Sandpipers. We sampled birds from ten U.S. museums (Appendix B.1) to elucidate population genetic patterns in mtDNA diversity that coincided with the decline in the global population size. Specifically, we sought to evaluate changes in mtDNA diversity and effective population size, as well as the possible extirpation of evolutionarily distinct lineages. We hypothesized that mtDNA diversity would be greater before the large-scale market hunting of Buff-breasted Sandpipers than in the decades following the introduction of the Migratory Bird Treaty Act in 1918. We also predicted a steady decay in genetic diversity throughout the 20th

century as Buff-breasted Sandpipers populations continued to decline due to anthropogenic disturbance.

Methods

Sampling and DNA extraction

We obtained a total of 220 Buff-breasted Sandpiper tissue samples from ten domestic museums as either shafts of intact contour flank feathers or toe pad shavings. Museum specimens represented individuals collected from 1841 to 1981 across the species' distributional range (Appendix B.1).

We extracted DNA from all 220 tissue samples at Kansas State University between October 2011 and December 2011 using the phenol-chloroform extraction procedures modified from Wisely et al. (2004). Depending on the tissue we received for each museum skin, we extracted one to three feather shafts or one toe pad per individual. All samples were chopped finely with a clean razorblade and incubated overnight in lysis buffer before starting the phenol-chloroform extraction. We eluted extracted DNA to 150 µL in sterile water and stored it in the laboratory at -20^{o}C.

To minimize possible contamination of historic DNA, we performed extractions in a genetics laboratory dedicated to the handling of historic samples. We bleached surfaces and equipment using a 10% bleach solution before and after every extraction to reduce the risk of cross-contamination. Every set of twelve extractions contained one or more negative controls which followed the entire tissue extraction procedure but used a water blank instead of a tissue sample. We tested each negative control for contamination via polymerase chain reactions (PCR) amplification. To minimize all possible sources of PCR contamination, all personnel entering the

laboratory showered prior to entry and wore protective clothing that was kept exclusively in the laboratory.

DNA amplification and sequencing

We amplified DNA samples by PCR using primers developed in the Conservation Genetic and Molecular Ecology Laboratory at Kansas State University (Appendix B.2). Since historic DNA is typically degraded and low-quality, we designed novel primers to amplify 4 short, overlapping regions within the cytochrome *b* gene and 2 regions within the mitochondrial control region. Prior to processing museum specimens, we optimized primer conditions using contemporary Buff-breasted Sandpipers DNA samples. Once primers were optimized, we ordered primer oligonucleotides to be used exclusively in the ancient DNA lab to avoid possible contamination from the contemporary molecular laboratory.

We amplified DNA from museum specimens in PCR mixtures at a volume of 25 µL containing 2 µL template DNA in final elution, 5 µL 1X reaction buffer ($MgCl_2$ included; Thermo Scientific), 0.2mM of each dNTP, 0.5 µM of each primer (Appendix B.2), 2.5 µg bovine serum albumen (BSA), and 0.1 units of Phire Hot Start II DNA polymerase (Thermo Scientific). We performed these reactions in an Eppendorf Mastercycler Pro thermal cycler (Brinkman Inc., Westbury, NY) for one 30 s thermal cycle at 98^{o}C followed by 35 cycles combining a 5 s denaturation step at 98^{o}C, a 5 s annealing step at 50^{o}C, and a 10 s extension at 72^{o}C, and a final extension step for 1 min at 72^{o}C. We bidirectionally sequenced PCR products at University of Kentucky AGTC Sequencing Center via BigDye reactions with the same forward and reverse primers used in amplification.

To confirm sequence identity, we thoroughly assessed chromatographs for errors in the resulting forward and reverse sequences in Program Bioedit (v7.0.5.3, Hall 1999). We then

compiled forward and reverse sequences into consensus sequences in Bioedit. We aligned consensus sequences by eye in Program MEGA4 (Tamura et al. 2007) for each of the six amplified regions. We then independently assembled the four regions of cytochrome *b* and two regions of the control region, respectively, for analyses. Since museum DNA is typically low quality, we were not successful in amplifying our full target cytochrome *b* segment in all individuals. Thus, we conducted cytochrome *b* analyses on all individuals that amplified for the full 780 bp segment (n = 97) as well as the individuals that only amplified at three of the four segments, CB2-CB4 (552 bp, n = 152). We deposited sequences representing each of the haplotypes visualized from museum specimens with voucher information into Genbank (accession numbers JX123379−JX123419).

mtDNA diversity

For comparison with contemporary populations of Buff-breasted Sandpipers (1993-2009), we supplemented our historical sequence database with 438 contemporary cytochrome *b* sequences and 460 control region sequences from a previous study (Chapter 2). Before conducting phylogenetic analyses, we trimmed contemporary sequences to cover the same regions amplified in the museum samples. We also translated cytochrome *b* sequences from nucleotide to amino acid sequences in MEGA4 using the vertebrate mitochondrial genetic code to confirm that sequences showed no evidence of nuclear pseudogene amplification (i.e., fully coding with no frameshifts or premature stop codons; Rodríguez et al. 2007).

To assess temporal changes in genetic diversity, we binned individuals into four groups spanning the time between 1874 and 2009 (see: Table 3.1). Since analyses of genetic connectivity in contemporary Buff-breasted Sandpipers suggested a single, panmictic population, we pooled all museum samples regardless of geographic locality (Chapter 2). For each temporal

bin, we calculated standard molecular diversity indices (number of haplotypes, *h;* haplotype diversity, H_d; and nucleotide diversity, π) for both mtDNA regions in Program DnaSP (v5; Librado and Rozas 2009). We also estimated Φ-statistics with analyses of molecular variance (AMOVA) on temporal groups to determine a signal of population differentiation between time points using 1,000 permutations in Program Arlequin (v3.5, Excoffier et al. 2005). To test for a trend in haplotype richness, we estimated the ratio of haplotypes to individuals (*h/N*) for all temporal groups and performed a χ^2 trend analysis on these proportions in Program R (v2.12.1, R Development Core Team 2010, http://www.r-project.org/). To account for sample size differences among temporal bins, we also conducted a rarefaction correction for the number of haplotypes expected from a larger sample in program EstimateS (v8.2.0, Colwell 2009). To circumvent any bias in diversity estimates associated with the larger sample sizes in our contemporary population, we performed 50 iterations of random resampling using an adjusted sample size equal to the mean size of our museum samples. All contemporary (1993-2009) diversity indices reported are mean values across all 50 random subsamples.

To directly assess the genetic impacts of market hunting on Buff-breasted Sandpipers, we divided our total sample into groups of individuals collected prior to a ban on commercial harvest of migrating birds (pre-1920) and post-ban (1920-2009) and performed the same analyses. Since other species of migratory birds have shown sex bias in diversity indices, we also performed the analyses independently on males and females within each temporal group and across all temporal groupings (Ruokonen et al. 2010).

Demographic reconstruction

To empirically test for trends in demography across our study period, we employed several methods of temporal demographic reconstruction. First, to assess changes in effective

population size (N_e), we calculated female effective population size (N_{ef}) for each temporal bin using Watterson's θ (θ_w) estimated from Program DnaSP (Librado and Rozas 2009). Since mtDNA is maternally inherited, effective population size estimates derived from mtDNA haplotype data are expressed in numbers of breeding females following the equation $\theta = 2N_{ef}\mu$, where N_{ef} is the female effective population size and μ is the mutation rate per site per generation. When calculating N_{ef}, we assumed a range of cytochrome *b* mutation rates equal to the reported range for Charadriiformes of 1.59 - 4.31 % per MY proposed by Weir and Schluter (2008). We used this range of mutation rates for 967 bp of the cytochrome *b* gene to estimate a range of mutation rates (1.53E-5−4.15E-5 substitutions/site/generation). Since the control region is extremely variable among species and its mutation rate has not been estimated for Buff-breasted Sandpipers, we based estimates of N_{ef} solely on cytochrome *b* sequence data. To visualize patterns of demography over time graphically, we constructed a minimum-spanning haplotype network in Program Network (v4.610, Bandelt et al. 1999) for each of our four temporal bins.

To assess robustness of these N_{ef} estimates, we used tip-dated mtDNA sequence data to visualize temporal demographic trends graphically. To choose the best-fit nucleotide mutation model for cytochrome *b*, we used the AIC method in Program jModelTest (v3.7, Posada 2008). We then estimated changes in population size over time using a MCMC approach and imposing a Bayesian skyline plot as our demographic model in Program BEAST (v1.6.2, Drummond and Rambaut, 2007).

Results

MtDNA diversity

Due to the low quality of DNA derived from museum specimens, we were unable to visualize full haplotypes for our 335 bp segment of the mitochondrial control region in 68 of our 220 (31%) historic samples. For the 152 museum specimens we successfully sequenced at the control region, we observed 24 unique haplotypes, eight of which were not present among our contemporary Buff-breasted Sandpipers (Appendix B.1). In all time periods, the most common haplotype (CRM1) comprised ~50% of the total population (54% in the pre-1920 sample, 49% in post-1993 sample, Fig. 3.1). Haplotype CRM2 was present in ~20% of each historical population and 15% of the contemporary population. All other haplotypes occurred in $\leq$ 10% of the individuals in each temporal group. Moreover, all unique haplotypes present only in historic (i.e., pre-1990) populations were represented by a single individual. We did not observe any major shifts in haplotype frequencies over our timescale for the mitochondrial control region (Fig. 3.1).

Haplotype and nucleotide diversity estimates for the control region remained stable over time (Fig. 3.2). AMOVAs did not indicate significant differentiation between any temporal groups. This pattern was consistent for our 4 temporal bins (Φ_{ST} were not significantly different than 0; P for each pairwise comparison > 0.65) as well as our combined pre- and post-1920 samples (Φ_{ST} = -0.011, P = 0.960). One measure of haplotype richness, the proportion of distinct haplotypes to individuals, was also stable over time (trend analysis: χ^2 = 0.171, P = 0.679). When we corrected our richness estimates for variation in sample size with a rarefaction analysis, the expected number of haplotypes appeared to increase over time. However, large standard errors in the rarefaction corrected number of haplotypes suggested that no substantial increase occurred (Table 3.1). We also found no evidence for a consistent sex-bias across diversity estimates.

Overall, our measures of mtDNA control region diversity appeared to be stable over time, with the highest estimated values present in our resampled contemporary population.

We observed a haplotype distribution pattern similar to that of the control region in both cytochrome *b* datasets (short and long); with the most pervasive haplotype (CBM1) accounting for over 75% of the haplotypes across all temporal groups (Fig. 3.1). For the 151 individuals successfully sequenced at the shorter cytochrome *b* segment, we observed 14 distinct haplotypes, five of which were not present in contemporary samples (Appendix B.1). For the full 780 bp segment, we observed only nine distinct cytochrome *b* haplotypes among all museum specimens, two of which were not present in our contemporary Buff-breasted Sandpiper population.

Diversity indices in the short segment of cytochrome *b* were larger in the pre-1920 sample (H_d = 0.416 ± 0.080, π = 0.011 ± 2.80E-4) than in the 1960-1987 sample (H_d = 0.236 ± 0.074, π = 0.005 ± 1.00E-4; Fig. 3.2). Measures of haplotype richness (with and without sample-size correction) were also larger in the pre-1920 group than the 1960-1987 group ($A_{pre\text{-}1920}$ = 33.0 ± 19.1, $A_{1960\text{-}1987}$ = 7.00 ± 3.00; Table 3.2). However, when contemporary (1993-2009) samples were considered, the pattern of declining genetic diversity did not persist (Fig. 3.2). Trend analysis on haplotype richness was non-significant over time (χ^2 = 1.42, P = 0.234), implying that the observed decline in haplotype richness was not statistically significant at a Type I error rate (α) of 0.05. Φ_{ST} values estimated from standard AMOVAs did not differ significantly from 0 (all P > 0.15), which supported the absence of population differentiation among temporal groups. When partitioned by sex, we found no consistent pattern of sex-biased mtDNA differentiation over time (Table 3.3). For the full segment of cytochrome *b*, diversity indices followed a similar pattern. Haplotype and nucleotide diversity estimates appeared higher in the pre-1920 group than in the 1960-1987 group (Table 3.2). Haplotype richness (*h/N*) was also higher in the pre-1920

group than in the 1960-1987 sample, but a rarefaction correction for the latter could not be estimated due to the absence of doubletons (Chao 1984). Like the shorter segment, haplotype richness for the full cytochrome *b* sequence did not show a significant trend over time (χ^2 = 0.008, P = 0.927). While cytochrome *b* showed weak temporal signals of a decline, it appeared that cytochrome *b* diversity, like control region diversity, had been relatively stable over time despite evidence of population declines.

Demographic reconstruction

Empirically estimating changes in effective population size across a temporal gradient provides insights into demographic trends that cannot be inferred from contemporary populations alone. For cytochrome *b*, we observed the highest estimates of N_{ef} in the pre-1920 sample (N_{ef} = 30,000−83,000 females) and lowest estimates in the 1960-1987 sample (N_{ef} = 10,482−29,000 females; Table 3.4). While we did not estimate N_{ef} directly for the control region due to uncertainty in mutation rates, we were able to use θ_w estimates to infer relationships in N_{ef} for this mtDNA region. Estimates of θ_w for the control region were similar over time, implying that there was no substantial change in N_{ef}.

Using the Tamura-Nei model (TrN; Tamura and Nei 1993) of nucleotide substitution with invariable sites (TrN+I model, base frequencies of A=0.284, C=0.313, G=0.132, T=0.27) and a relaxed lognormal clock, we conducted a Bayesian Skyline reconstruction of population size trends over our sampling period. This reconstruction implied a constant effective population size following the ban on market hunting, a result consistent with our estimates of N_{ef} (Fig. 3.3).

Discussion

In Chapter 2, I used mtDNA sequence data to elucidate historic phylogeography and admixture in contemporary populations of Buff-breasted Sandpipers. The goal of this chapter

was to empirically estimate the genetic impact of varying magnitudes of population declines on wild Buff-breasted Sandpipers. We hypothesized a decrease in mtDNA diversity associated with the anthropogenically-induced decline in population size, a pattern that has been suggested in other bird species of conservation concern (Martinez-Cruz et al. 2007, Solovyeva and Pearce 2011). To elucidate patterns of genetic characteristics over time, we compared several measures of diversity at two rapidly-evolving mtDNA regions across a 135-year period that coincides with a decline in population size.

For the mitochondrial control region, we observed stable diversity indices over time (Fig. 3.2). While we expected Buff-breasted Sandpipers to show a decrease in mtDNA diversity, other studies have observed similar patterns of stable control region diversity in declining bird populations (e.g., Hawaii Akepas *Loxops coccineus coccineus*, Reding et al. 2010). This pattern has also been observed in declining migratory birds that have undergone recent population bottlenecks including Canadian Peregrine Falcons (*Falco peregrinus*, Brown et al. 2007) and a vulnerable albatross (*Phoebastria albatrus*, Kuro-o et al. 2010). In these declining populations, high levels of mtDNA diversity are thought to have been maintained primarily due to high pre-decline genetic diversity or effective population sizes, as well as conservation-focused management efforts. Given the large pre-decline estimates of population size in Buff-breasted Sandpipers, it is possible that the same factors are responsible for the patterns we observed in this species.

The pattern of stable diversity indices and Watterson's θ over time suggest that control region diversity was resistant to population declines. Since large populations are typically more resistant to the genetic effects of population reduction than small ones (Frankham et al. 2004), it is possible that the initial large population size and concomitant high genetic diversity of Buff-

breasted Sandpipers, coupled with high genetic connectivity in the global population prior to market hunting, helped sustain genetic diversity in the control region throughout the 20th century. The pattern of stable genetic diversity was supported by haplotype network topology and haplotype distributions. While the identity of singleton haplotypes varied across our study period, the overall patterns of diversity (starburst topology) and haplotype frequencies remained unchanged. Declining bird populations have also shown signals of increasing control region diversity over time (Ruokonen et al. 2010). A temporal increase in diversity is thought to have been maintained by gene flow between distinct populations. Since Buff-breasted Sandpipers appear to be a single, panmictic population, it is unlikely that diversity has been maintained via migration between distinct subpopulations. While we did not find evidence for unique subpopulations of Buff-breasted Sandpipers, gene flow may be responsible for maintenance of genetic diversity in this species. It is possible that high levels of global genetic connectivity, coupled with large pre-decline population size and genetic diversity, are responsible for the maintenance of mtDNA diversity in Buff-breasted Sandpipers.

For the cytochrome *b* gene, museum samples of Buff-breasted Sandpipers showed signals of a subtle decline in haplotype diversity, haplotype richness, diversity indices, and the population parameter θ_w over time. These patterns did not persist if we included individuals from our contemporary samples, implying that overall genetic diversity and N_{ef} have not been substantially lost over time. Haplotype distribution patterns were similar to the control region, with the identity and number of singleton haplotypes changing over time but no change in the overall topology or haplotype frequency (Fig. 3.1). Bayesian Skyline reconstruction supported a stable effective size over our sampling period. When taken together, the results of these rigorous mtDNA analyses suggest that diversity and effective population size have not substantially

changed over time.

Here, we illustrated the utility of museum specimens to assess trends of genetic diversity in a declining population of migratory shorebirds. Assuming that diversity in the mitochondrial genome is an accurate reflection of overall genetic diversity, this information can provide a crucial historic perspective on the impact of anthropogenic disturbance on the future adaptability in a population. However, our analyses of historic mtDNA diversity in Buff-breasted Sandpipers emphasize the importance of cautious interpretation of museum sequence data. For example, even exhaustive sampling of museum specimens can result in small sample sizes (e.g., Martinez-Cruz et al. 2007) which could reduce the power of analyses. Resampling efforts for cytochrome *b* revealed that diversity index estimates from even the large museum sample size presented in this study (mean *N* across temporal groups = 51) can result in a wide range of values when sampled randomly from a larger population (min H_d = 0.187, max H_d = 0.495). Thus, the subtle decline in diversity observed in cytochrome *b* across museum samples could be the result of sampling rather than the result of demographic processes or conversely, a true signal of decline could have been minimized by the noise in the data. Since cytochrome *b* mutates at an estimated 1.59 - 4.31% per MY (Weir and Schluter 2008), it is more likely that the higher diversity indices observed in our contemporary sample are the result of sampling rather than novel mutations. We also observed a large range of haplotype diversity estimates in the control region when we resampled contemporary Buff-breasted Sandpipers (min H_d = 0.462, max H_d = 0.824), emphasizing the sensitivity of haplotype diversity estimates to sampling.

Haplotype and nucleotide diversity are often the sole indices from which inferences of temporal changes in mtDNA diversity are drawn in studies that use DNA sequence data derived from museum specimens (Brown et al. 2007, Anderson et al. 2008, Reding et al. 2010).

However, as our resampling of contemporary Buff-breasted Sandpipers showed, these indices are sensitive to which individuals are sampled from the total population. Inferences drawn from these estimates must be taken in the context of several other measures of diversity in order to fully understand the impact of population declines on genetic diversity in wild populations. For example, it has been shown that a population of Whooping Cranes (*Grus americana*) recovering from a critically small population of 14 individuals can retain higher levels of control region nucleotide diversity than we observed in Buff-breasted Sandpipers (Glenn et al. 1999). By using a wide range of techniques on a large historic sample instead of simply comparing standard diversity indices, we were able to confidently address our hypothesis that Buff-breasted Sandpipers had lost genetic diversity due to population losses.

In the present study, we were successful in elucidating patterns of mtDNA diversity and demographic trends across a 135-year period in a declining population. Our results suggest that overall genetic diversity and effective population size has remained stable, and no evolutionarily distinct lineages were lost in Buff-breasted Sandpipers over the past century as a result of anthropogenically-induced declines in population size. However, while it appears as though substantial mtDNA diversity has not been lost, we must still carefully manage this species. Genetic diversity is only one of several crucial factors in determining population viability, and it must always be interpreted in the context of demographic processes (Gregory et al. 2011). Despite showing signs of stable genetic diversity, if population declines continue unabated, Buff-breasted Sandpipers will become critically sensitive to the genetic impacts of stochastic demographic processes. It is fortunate that this species has maintained genetic viability in the face of population losses because conservation efforts focusing on its recovery will be more manageable than if genetic diversity was critically low. Successful management of Buff-breasted

Sandpipers will require that population sizes be kept as stable as possible to avoid reducing populations to critically low levels.

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References

Anderson SJ, Conrad KF, Gillman MP, Woiwod IP, and Freeland JR (2008) Phenotypic changes and reduced genetic diversity have accompanied the rapid decline of the garden tiger moth (*Arctia caja*) in the U.K. Ecological Entomology 33:638–645.

Allen JN (1980) The Ecology and Behavior of the Long–Billed Curlew in Southern Washington. Wildlife Monographs 73:3–67.

Baker AJ and Strauch JG (1988) Genetic variation and differentiation in shorebirds. Proceedings of the XIX International Ornithological Conference:1639–1645.

Bandelt HJ, Forster P, and Röhl A (1999) Median–joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Brook BW, Tonkyn DW, O'Grady JJ, and Frankham R (2002) Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology 6:16.

Brown JW, Van Coeverden de Groot PJ, Birt TP, Seutin G, Boag PT, and Friesen VL (2007) Appraisal of the consequences of the DDT–induced bottleneck on the level and geographic distribution of neutral genetic variation in Canadian Peregrine Falcons, *Falco peregrinus*. Molecular Ecology 16:327–343.

Burleigh TD (1958) Georgia birds. University of Oklahoma, Norman, OK.

Butler RW, Ydenberg RC, Donaldson GD, and Brown S (2004) Hypotheses to explain census declines in North American shorebirds. Shorebird Research Group of the Americas Report 1 (http://www.shorebirdresearch.org/workinggroups.htm)

Chao A (1984) Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11:265–270.

Clay RP, Lesterhuis AJ, and Johnson O (2010) Conservation Plan for the American Golden–Plover (*Pluvialis dominica*).Version 1.1. Manomet Center for Conservation Sciences, Manomet, Massachusetts.

Colwell RK (2009) EstimateS: Statistical estimation of species richness and shared species from samples. Version 8.2. User's Guide and application published at: http://purl.oclc.org/estimates.

Drummond AJ and Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

Excoffier L, Laval G, and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Fulton TL, Wagner SM, Fisher C, and Shapiro B (2012) Nuclear DNA from the extinct Passenger Pigeon (*Ectopistes migratorius*) confirms a single origin of New World pigeons. Annals of Anatomy 194:52–57.

Frankham R, Ballou JD, and Briscoe DA (2004) A Primer of Conservation Genetics. Cambridge University Press, Cambridge, UK.

George RC (2002) Reproductive Ecology of the American Oystercatcher (*Haematopus palliatus*) in Georgia. M.Sc thesis, The University of Georgia, Athens, Georgia, USA.

Glenn TC, Stephan W, and Braun MJ (1999) Effects of a population bottleneck on Whooping Crane mitochondrial DNA variation. Conservation Biology 13(5):1097–1107.

Gregory AJ, Kaler RSA, Prebyl TJ, Sandercock BK, and Wisely SM (2011) Influence of translocation strategy and mating system on the genetic structure of a newly established population of Island Ptarmigan. Conservation Genetics 13:465–474.

Hall TA (1999) BioEdit: a user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium 41:95–98.

Jorgensen JG, McCarty JP, and Wolfenbarger LL (2008) Buff–breasted Sandpiper density and numbers during migratory stopover in the Rainwater Basin, Nebraska. Condor 110:63–69.

Kuro–o M, Yonekawa H, Saito S, Eda M, Higuchi H, Koike H, and Hawegaway H (2010) Unexpectedly high genetic diversity of mtDNA control region through severe bottleneck in vulnerable albatross *Phoebastria albatrus.* Conservation Genetics 11:127–137.

Lanctot RB and Laredo CD (1994) Buff–breasted Sandpiper (*Tryngites subruficollis*). Birds of North America, No. 91. Philadelphia, PA, USA.

Lanctot RB, Aldabe J, Almeida JB, Blanco D, Isacch JP, Jorgensen J, Norland S, Rocca P, and Strum KM. (2010) Conservation Plan for the Buff–breasted Sandpiper (*Tryngites subruficollis*).Version 1.1. U. S. Fish and Wildlife Service, Anchorage, Alaska, and Manomet Center for Conservation Sciences, Manomet, Massachusetts, USA.

Librado P and Rosaz J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Martinez–Cruz B, Godoy JA, and Negro JJ (2007) Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. Molecular Ecology 16:477–486.

Morrison RIG, McCaffery BJ, Gill RE, Skagen SK, Jones SL, Page GW, Gratto–Trevor CL, and Andres BA (2006) Population estimates of North American shorebirds. Wader Study Group Bulletin 111:67–85.

Page GW and Gill RE Jr. (1994) Shorebirds in western North America: late 1800s to late 1900s. Studies in Avian Biology 15:147–160.

Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25(7):1253–1256.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. ISBN 3–900051–07–0, URL http://www.R–project.org/.

Ramakrishnan U and Hadly EA (2009) Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies. Molecular Ecology 18:1310–1330.

Reding DM, Freed LA, Cann RL, and Fleischer RC (2010) Spatial and temporal patterns of genetic diversity in an endangered Hawaiian honeycreeper, the Hawaii Akepa (*Loxops coccineus coccineus*). Conservation Genetics 11:225–240.

Roberts D, Elphick CS, and Reed, MJ (2009) Identifying anomalous reports of putatively extinct species and why it matters. Conservation Biology 24(1):189–196.

Rodríguez F, Albornoz J, and Domínguez A (2007) Cytochrome *b* pseudogene originated from a highly divergent mitochondrial lineage in genus *Rupicapra*. Journal of Heredity 98(3):243–249.

Ruokonen M, Aarvak T, Chesser RK, Lundqvista A–C, and Merilä J (2010) Temporal increase in mtDNA diversity in a declining population. Molecular Ecology 19:2408–2417.

Soulé ME (1991) Conservation: tactics for a constant crisis. Science 253(5021):744 –750.

Solovyeva DV and Pearce JM (2011) Comparative mitochondrial genetics of North American and Eurasian mergansers with an emphasis on the endangered Scaly–sided Merganser (*Mergus squamatus*). Conservation Genetics 12:839–844.

Spielman D, Brook BW, and Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Science 101(42):15261–15264.

Strum KM, Hooper MJ, Johnson KA, Lanctot RB, Zaccagnini ME, and Sandercock BK (2010) Exposure of nonbreeding migratory shorebirds to cholinesterase–inhibiting contaminants in the Western Hemisphere. Condor 112(1):15–28.

Tamura K and Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3):512–526.

Tamura K, Dudley J, Nei M, and Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596–1599.

Tudor L (2000) Migratory shorebird assessment. Maine Department of Inland Fisheries and Wildlife, Resource Assessment Section, Bird Group, Bangor, Maine, USA.

Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. Trends in Ecology and Evolution 22:634–642.

Weik AP (2001) Woodcock Assessment. Maine Department of Inland Fisheries and Wildlife, Bangor, Maine, USA.

Weir JT and Schluter D (2008) Calibrating the avian molecular clock. Molecular Ecology 17:2321–2328.

Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, and Paige KN (1998) Tracking the long–term decline and recovery of an isolated population. Science 282(5394):1695−1698.

Wisely SM, Maldonado JE, and Fleischer RC (2004) A technique for sampling ancient DNA that minimizes damage to museum specimens. Conservation Genetics 5:105–107.

Tables and Figures

Table 3.1: **Molecular diversity indices for the mtDNA control region. *N*, number of individuals; H_d, haplotype diversity ± SD; π, nucleotide diversity ± SD; *h*, total number of haplotypes; *A*, rarefaction estimate of haplotypes ± SD; *h*/*N*, the proportion of haplotypes to the total number of individuals in the population. 1993-2009 indices represent means over 50 iterations of resampling.**

Samples	*N*	H_d	π x 10^3	*h*	*A*	*h*/*N*
1871-1919	41	0.669 ± 0.071	3.40 ± 0.660	10	28.0 ± 15.1	0.244
1920-1959	49	0.713 ± 0.059	3.25 ± 0.500	13	44.0 ± 23.3	0.245
1960-1987	53	0.708 ± 0.058	4.16 ± 0.760	13	64.0 ± 32.6	0.264
1993-2009	48	0.733 ± 0.060	4.01 ± 0.694	14	45.4 ± 20.1	0.292

Table 3.2: Molecular diversity indices for both segments of cytochrome *b* across temporal groups. *N*, number of individuals; H_d, haplotype diversity ± SD; π, nucleotide diversity ± SD; *h*, total number of haplotypes; A, rarefaction estimate of haplotypes ± SD; *h*/*N*, the proportion of haplotypes to the total number of individuals in the population. 1993-2009 indices represent means over 50 iterations of resampling.

	Cytochrome *b* - Full					
Samples	*N*	H_d	π x 10^3	*h*	*A*	*h*/*N*
1871-1919	28	0.390 ± 0.115	0.710 ± 0.240	6	14.0 ± 8.31	0.214
1920-1959	33	0.280 ± 0.099	0.380 ± 0.140	4	4.25 ± 0.613	0.121
1960-1987	36	0.257 ± 0.093	0.400 ± 0.160	4	N/A	0.139
1993-2009	32	0.397 ± 0.104	0.791 ± 0.267	6.52	16.4 ± 5.75	0.204
	Cytochrome *b* - Short					
Samples	*N*	H_d	π x 10^3	*h*	*A*	*h*/*N*
1871-1919	46	0.416 ± 0.092	1.06 ± 0.280	10	33.0 ± 19.1	0.227
1920-1959	51	0.322 ± 0.085	0.630 ± 0.180	7	8.00 ± 1.90	0.120
1960-1987	55	0.236 ± 0.074	0.500 ± 0.170	5	7.00 ± 3.00	0.091
1993-2009	51	0.343 ± 0.083	0.921 ± 0.239	7.22	14.29 ± 6.05	0.142

Table 3.3: Sex-specific measures of genetic diversity over time. *N*, number of individuals; *h*, number of haplotypes; *h/N*, ratio of haplotypes to individuals.

Sample	Sex	Cytochrome *b* - short			Control region		
		N	*h*	*h/N*	*N*	*h*	*h/N*
1871-1919	Males	21	8	0.38	14	10	0.71
	Females	13	1	0.08	14	5	0.36
1920-1959	Males	23	3	0.13	23	6	0.26
	Females	20	4	0.20	21	8	0.38
1960-1987	Males	24	2	0.08	20	13	0.65
	Females	14	4	0.29	10	3	0.30

Table 3.4: Population parameters and estimates of effective population sizes from a 552 bp segment of cytochrome *b* and the mtDNA control region across temporal periods. *N*, sample size; θ_w, Watterson's estimate of θ ± SD; N_{ef}, range of female effective population sizes calculated from point estimates of θ_w assuming a range of mutation rates from 1.53E-5 to 4.15E-5 substitutions/site/generation. N_{ef} for the control region was not estimated because of uncertainty in mutation rates.

	Cytochrome *b*		
Samples	*N*	θ_w	N_{ef}
1871-1919	46	2.49 ± 1.00	30,000 - 83,000
1920-1959	51	1.33 ± 0.64	16,024 - 44,333
1960-1987	55	0.87 ± 0.93	10,482 - 29,000
1993-2009	51	1.63 ± 0.30	19,639 - 54,333
	Control region		
1871-1919	42	2.32 ± 0.96	N/A
1920-1959	50	2.23 ± 0.91	N/A
1960-1987	52	3.54 ± 1.28	N/A
1993-2009	48	2.70 ± 0.56	N/A

Figure 3.1: Minimum-spanning haplotype networks for temporal bins for control region and a 552 bp fragment of cytochrome *b*. Each node represents a unique haplotype (a = CRM1, b = CRM2, and c = CBM1) and node size is proportional to the number of individuals representing that haplotype. Branch length represents the number of nucleotide substitutions separating each haplotype. Assumed mutations are represented by small solid dots along branches. A, control region 1874-1919; B, control region 1920-1959; C, control region 1960-1987; D, cytochrome *b* 1874-1919; E, cytochrome *b* 1920-1959; F, cytochrome *b* 1960-1987.

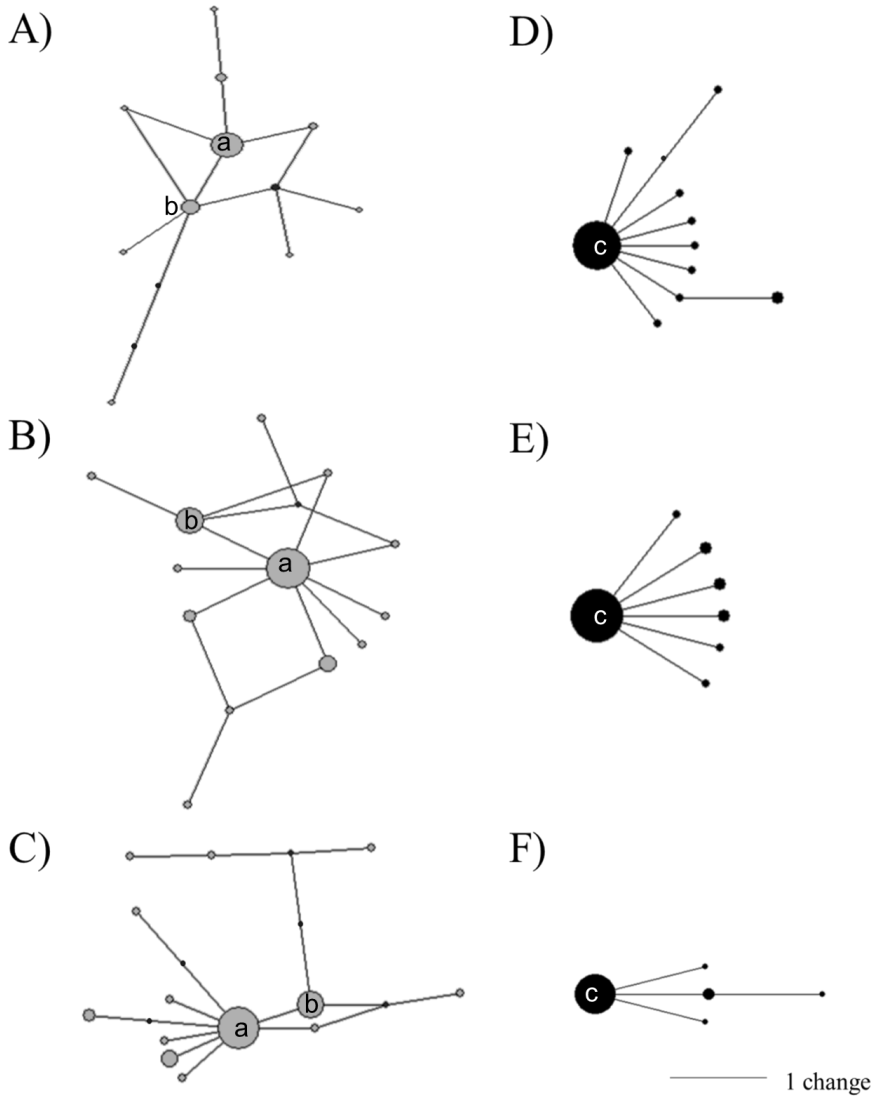


Figure 3.2: Haplotype diversity (H_d ± SD) for the mtDNA control region (black circles) and cytochrome *b* (white circles) for each time period. For contemporary samples (1993-2009), H_d represents the mean (± SD) of 50 resamplings.

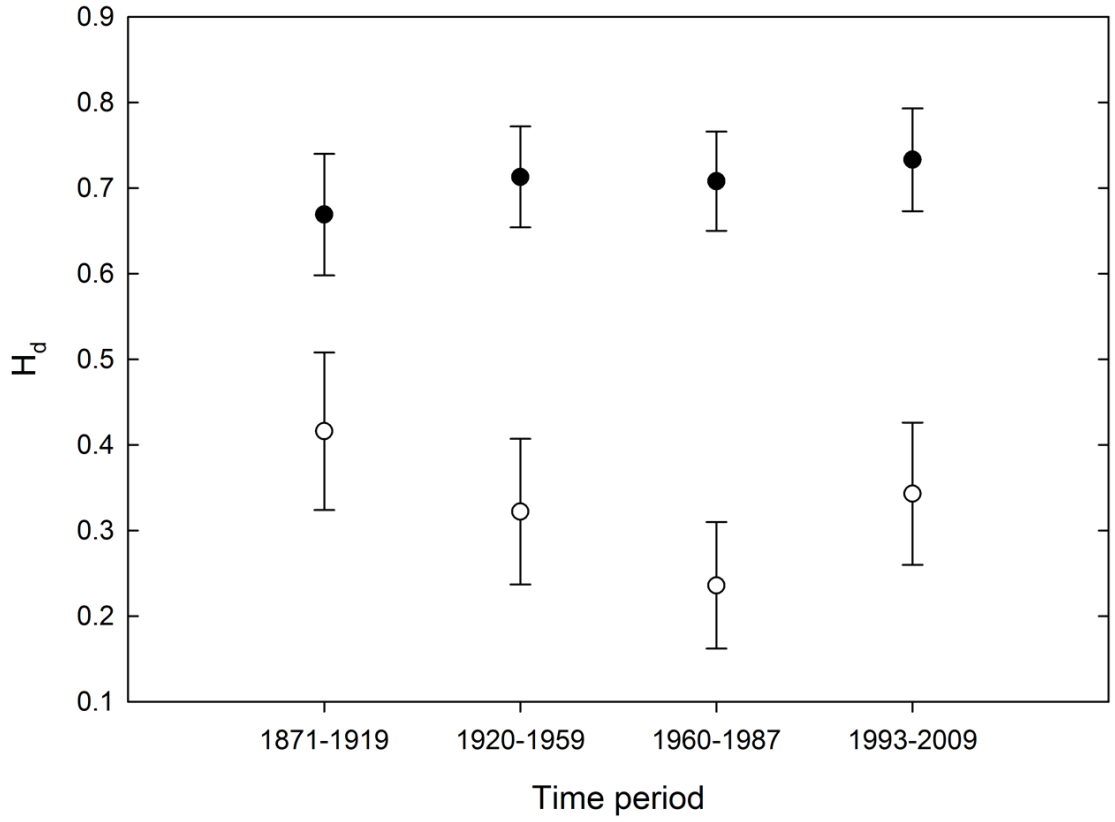
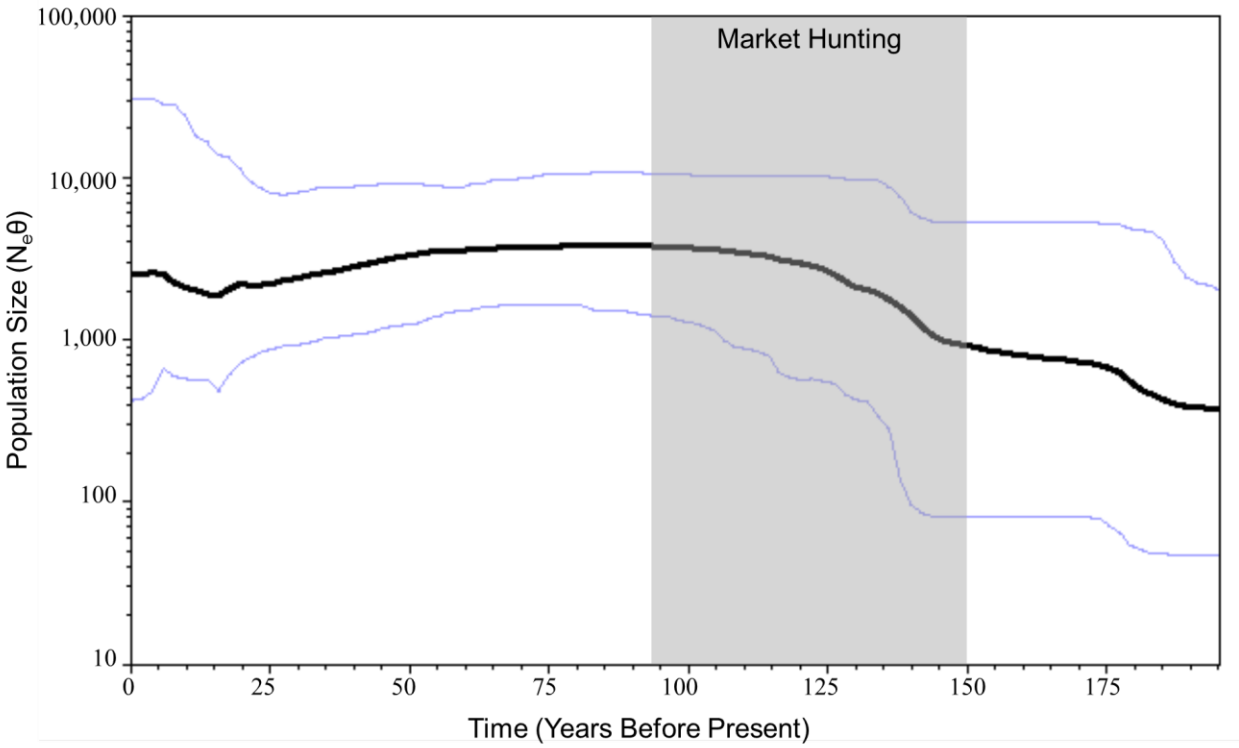


Figure 3.3: Reconstruction of population fluctuations based on a Bayesian skyline plot derived from 552 bp of cytochrome *b* sequence data. The solid black line represents the median population size estimate and the solid colored lines represent a 95% CI. For reference, we highlighted the period during which migrating Buff-breasted Sandpipers were hunted.



Chapter 4 - Conclusions

Despite conservation efforts, many species of migratory shorebirds are continuing to decline, mostly as the result of anthropogenic disturbances across their distributional ranges (Butler et al. 2004, Morrison et al. 2006, Bart et al. 2007). These disturbances take many forms, including illegal hunting (Clay et al. 2010), environmental contamination from pesticides or other chemicals (Strum et al. 2010), and habitat degradation (Lanctot et al. 2010). As hemispheric migrants, many shorebirds travel across thousands of kilometers, and disturbances at sites anywhere in their global distributions can have detrimental impacts on population viability. To address the genetic impacts of anthropogenically-induced population declines on our study species, the Buff-breasted Sandpiper, I presented the results of rigorous population genetic analyses on spatial and temporal scales.

As a migrant with low breeding site fidelity but some evidence of wintering site fidelity (Almeida 2009), we expected low levels of migratory connectivity and high levels of genetic connectivity with possible population substructure at wintering grounds. Population genetic analyses of contemporary samples suggested a single, panmictic population of Buff-breasted Sandpipers (Chapter 2). Measures of population differentiation and historic phylogeography among and within the major biogeographical regions (breeding, stopover, and wintering grounds) were consistent with the hypothesis of high levels of genetic connectivity. Contrary to our predictions, however, we did not detect any signal of genetic differentiation at wintering sites despite evidence for site fidelity (Almeida 2009). It is possible that we did not detect population differentiation because the small sample size three of our four wintering sites limited the power of our analyses, so we strongly urge future studies to more closely examine wintering site genetic structure in Buff-breasted Sandpipers. Since we did not find evidence for modern or historic

population structure, we recommend that Buff-breasted Sandpipers be treated as one global conservation unit (Moritz 1994).

The presence of population substructure can limit effective population size (N_e). As migration between subpopulations decreases, local N_e in subpopulations decreases (Waples 2010). If populations of Buff-breasted Sandpipers become isolated by anthropogenic habitat fragmentation, it is likely that they will lose genetic variability and global N_e will be greatly decreased. Estimates of N_e in Buff-breasted Sandpipers were sufficiently high to maintain adaptive potential. While N_e estimates suggest population viability, maintaining the global connectivity we observed in this study will still be critical in successful management of this species. We recommend that Buff-breasted Sandpipers habitat be closely managed to decrease the likelihood of population fragmentation.

Another goal of this study was to empirically estimate the impact of both intense, short-term and gradual, long-term population decline on mtDNA variability in Buff-breasted Sandpipers. Analyses of genetic diversity across our study period revealed stable mtDNA diversity despite varying magnitudes of population losses over the past 150 years (Chapter 3). This result was unexpected because populations undergoing steady declines are expected to lose genetic diversity over time (Westemeier et al. 1998). It is possible that high levels of mtDNA variability have been maintained due to high pre-decline genetic variability, large effective population sizes, and a highly admixed population. With pre-decline population estimates ranging into the hundreds of thousands, a large, highly admixed global population was likely well-buffered from demographic stochasticity. It is also likely that the location of the market hunting of this species contributed to the patterns we observed in this study. Since Buff-breasted Sandpipers were hunted primarily along their migratory routes, it is unlikely that any one distinct

population, if present, was preferentially hunted.

When taken in the context of demographic trends, the results of our phylogeographic and population genetic analyses provided invaluable insight into Buff-breasted Sandpipers' conservation status. It appeared that, on the molecular level, Buff-breasted Sandpipers were buffered against the genetic impacts of population losses. Substantial genetic diversity and distinct evolutionary lineages do not appear to have been lost over time, and there was no compelling evidence for a recent population bottleneck at nuclear or mtDNA markers. Therefore, Buff-breasted Sandpipers have retained sufficient genetic diversity necessary for successful future population recovery. There are several critical steps to take in order to ensure that this common species remains common.

Buff-breasted Sandpipers are a hemispheric migrant whose distribution spans many countries, so preliminary steps to limit degradation and fragmentation of this species' habitat should focus on preserving major breeding, stopover, and wintering sites. Since Buff-breasted Sandpipers have such a wide latitudinal breeding range, arctic habitat conservation will require joint efforts from the United States, Canada, and Russia, all of which have recently expressed concern for Buff-breasted Sandpipers conservation in their respective Shorebird Conservation Plans. Specifically, anthropogenic habitat degradation from infrastructure development (e.g., gas and oil drilling; Lanctot et al. 2010) needs to be monitored closely for its impact on Buff-breasted Sandpipers. Major stopover sites along the central U.S. flyway (e.g., the Rainwater Basin of Nebraska; Jorgensen et al. 2008) need to be managed to prevent further loss of suitable habitat and chemical contamination (Strum et al. 2010). Moreover, connectivity between wintering grounds in Brazil, Argentina, and Uruguay must also be maintained in order to limit wintering population isolation. Successful conservation of Buff-breasted Sandpipers will require

international efforts to limit habitat degradation and population fragmentation.

The most critical implication of this study is that management of Buff-breasted Sandpipers must focus on preventing further population and habitat losses. This species has experienced drastic declines over the past two centuries and, despite these losses, has remained genetically viable. However, it is imperative that we avoid misinterpreting the results of this study and subsequently making Type II errors in conservation recommendations. That is, conservation recommendations cannot focus solely on the contemporary genetic health of Buff-breasted Sandpipers, but rather must integrate these data with current demographic trends. If we presume that Buff-breasted Sandpipers are not a species of conservation concern from genetic viability alone, we risk putting them in jeopardy of declining to critical levels in the near future. As their numbers continue to dwindle, this species is becoming increasingly prone to the genetic impacts of limiting population size. Buff-breasted Sandpipers' genetic health does not seem to have suffered from population losses, but this does not mean that they are not a species of conservation concern. While molecular tools are crucial in species management, maintaining genetic diversity becomes meaningless if there are no individuals remaining to manage. Our management recommendation is to limit the impacts of demographic stochasticity by preserving Buff-breasted Sandpipers' habitat connectivity and keeping population numbers high by limiting disturbance at all three major biogeographical regions.

References

Almeida, J.B. 2009. Wintering ecology of Buff–Breasted Sandpipers (*Tryngites subruficollis*) in Southern Brazil. PhD dissertation, University of Nevada, Reno, Nevada, USA.

Bart, J., S. Brown, B. Harrington and R.I.G. Morrison. 2007. Survey trends of North American shorebirds: population declines or shifting distributions? Journal of Avian Biology 38:73–82.

Butler RW, Ydenberg RC, Donaldson GD, and Brown S (2004) Hypotheses to explain census declines in North American shorebirds. Shorebird Research Group of the Americas Report 1 (http://www.shorebirdresearch.org/workinggroups.htm)

Clay, R.P., A.J. Lesterhuis, and O. Johnson. 2010. Conservation Plan for the American Golden–Plover (*Pluvialis dominica*).Version 1.1. Manomet Center for Conservation Sciences, Manomet, Massachusetts, USA.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66:95–107.

Jorgensen J.G., J.P. McCarty, and L.L. Wolfenbarger. 2008. Buff–breasted Sandpiper density and numbers during migratory stopover in the Rainwater Basin, Nebraska. Condor 110:63–69.

Lanctot, R.B., J. Aldabe, J.B. Almeida, D. Blanco, J.P. Isacch, J. Jorgensen, S. Norland, P. Rocca, and K.M. Strum. 2010. Conservation Plan for the Buff–breasted Sandpiper (*Tryngites subruficollis*).Version 1.1. U. S. Fish and Wildlife Service, Anchorage, Alaska, and Manomet Center for Conservation Sciences, Manomet, Massachusetts, USA.

Moritz, C. 1994. Defining Evolutionarily Significant Units for conservation. Trends in Ecology and Evolution 9(10):373–375.

Morrison, R.I.G., B.J. McCaffery, R.E. Gill, S.K. Skagen, S.L. Jones, G.W. Page, C.L. Gratto–Trevor, and B.A. Andres. 2006. Population estimates of North American shorebirds. Wader Study Group Bulletin 111: 67–85.

Palstra, F.P. and D.E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology 17(15):3428–3447.

Strum, K.M., M.J. Hooper, K.A. Johnson, R.B. Lanctot, M.E. Zaccagnini, and B.K. Sandercock. 2010. Exposure of nonbreeding migratory shorebirds to cholinesterase–inhibiting contaminants in the Western Hemisphere. Condor 112(1):15–28.

Waples, R.S. 2010. Spatial–temporal stratifications in natural populations and how they affect understanding and estimation of effective population size. Molecular Ecology Resources 10(5):785 –796.

Westemeier R.L., J.D. Brawn, S.A. Simpson, T.L. Esker, R.W. Jansen, J.W. Walk, E.L. Kershner, J.L. Bouzat, and K.N. Paige. 1998. Tracking the long–term decline and recovery of an isolated population. Science 282(5394):1695−1698.

Supplementary Material for Chapter 2

Table 0.1: Primer pairs for each of five regions amplified and optimal annealing temperatures (^{o}C).

Region	Forward Primer	Reverse Primer	^{o}C
Cytochrome *b* 1	5'-TAG GAT CAT TCG CCC TAT CCA T-3'	5'-CGA AAG CGG TTG CTA TTA G-3'	56
Cytochrome *b* 2	5'-TGG AAT ACA GGA GTC ATC C-3'	5'-GAA GTT TTC TGG GTC TCC -3'	56
Cytochrome *b* 3	5'-CTC TTC CTA CTA ACC CTT G-3'	5'-TAA AGT AGG TGA GGG ATG CTA GT-3'	56
Control Region 1	5'-GCA TGT AAT TTG GGC ATT TTT TG-3'	5'-ATT TCA CGT GAG GAG CT-3'	58
Control Region 2	5'-CGA AAT ACA TAC AAG CCG -3'	5'-CCT GAG GGC CAA AAT AAG -3'	50

Supplementary Material for Chapter 3

Table 0.1: Individual Buff-breasted Sandpipers sampled from ten U.S. museums and their respective haplotypes. Museum codes: ANSP = Academy of Natural Sciences, Philadelphia; DMNH = Delaware Museum of Natural History, Wilmington; MCZ = Harvard University Museum of Comparative Zoology, Cambridge; MVZ = University of California Museum of Vertebrate Zoology, Berkeley; OMNH = University of Oklahoma Sam Noble Museum of Natural History, Norman; UMMZ = University of Michigan Museum of Zoology, Ann Arbor; USNM = Smithsonian Museum of Natural History, Washington D.C.; KU = University of Kansas Natural History Museum, Lawrence; UNSM = University of Nebraska State Museum, Lincoln. LCW and DAE are original collection names of samples to be sent to KU.

Museum ID	Sex	State/Province	Country	Year collected	CR Haplotype	CB Haplotype
ANSP37549	male	Alaska	USA	unknown	CRM2	
ANSP37550	female	Alaska	USA	unknown	CRM1	
ANSP177677	female	Loreto	Peru	unknown		
ANSP183814	female	Napo	Ecuador	unknown	CRM2	
ANSP183815	male	Napo	Ecuador	unknown	CRM1	
ANSP11256	undetermined	unknown	Brazil	unknown		
ANSP11254	female	San Juan	Nicaragua	unknown		
ANSP11255	male	San Juan	Nicaragua	unknown	CRM10	
ANSP82997	male	Loreto	Peru	unknown	CRM1	CBM1
DMNH28887	male	Oklahoma	USA	1962	CRM22	
DMNH52821	male	Nunavat	Canada	1962	CRM1	CBM1
DMNH52822	male	Nunavat	Canada	1962	CRM1	CBM1
DMNH52823	male	Nunavat	Canada	1966		
DMNH52824	male	Nunavat	Canada	1962	CRM1	CBM1
DMNH52825	female	Nunavat	Canada	1966	CRM2	CBM1
DMNH52826	male	Nunavat	Canada	1966	CRM2	CBM1
DMNH52827	female	Nunavat	Canada	1966	CRM2	CBM1
DMNH52828	male	Nunavat	Canada	1962	CRM14	CBM1
DMNH52829	male	Nunavat	Canada	1962		
DMNH52830	female	Nunavat	Canada	1962	CRM1	CBM1

DMNH52831	female	Nunavat	Canada	1962	CRM1	CBM1
DMNH52832	male	Nunavat	Canada	1962	CRM5	
DMNH52833	male	Nunavat	Canada	1962		
DMNH5862	male	Manitoba	Canada	1939	CRM1	CBM1
DMNH5863	female	Manitoba	Canada	1939	CRM3	CBM1
MCZ100429	female	Massachusetts	USA	1878	CRM24	CBM1
MCZ100431	undetermined	Texas	USA	1887		
MCZ116348	male	unknown	Costa Rica	1898		CBM1
MCZ137768	male	unknown	Ecuador	1926	CRM1	CBM9
MCZ137769	female	unknown	Ecuador	1926		
MCZ150090	male	Minnesota	USA	1912		CBM1
MCZ182586	male	Texas	USA	1922	CRM2	
MCZ182587	male	Texas	USA	1922	CRM6	
MCZ182588	female	Texas	USA	1922		
MCZ182589	female	Texas	USA	1922	CRM1	
MCZ182590	undetermined	Massachusetts	USA	1904	CRM4	CBM1
MCZ182591	male	Massachusetts	USA	1904	CRM5	
MCZ182592	female	Massachusetts	USA	1906		CBM1
MCZ182593	male	Massachusetts	USA	1890		CBM6
MCZ242664	male	Minnesota	USA	1889		CBM1
MCZ255493	female	Massachusetts	USA	1886	CRM1	CBM1
MCZ255494	female	Massachusetts	USA	1908	CRM1	CBM1
MCZ255495	undetermined	New Hampshire	USA	1909	CRM1	CBM1
MCZ255496	male	Kansas	USA	1908		CBM4
MCZ255497	female	Kansas	USA	1909	CRM2	CBM1
MCZ255498	male	Alberta	Canada	1923	CRM2	CBM1
MCZ255499	female	Alberta	Canada	1923	CRM21	
MCZ255500	male	Alberta	Canada	1924	CRM1	CBM1
MCZ255517	male	Texas	USA	1914		
MCZ255518	male	Texas	USA	1914	CRM3	CBM1
MCZ271740	male	Texas	USA	1880		CBM1
MCZ271741	female	Texas	USA	1880		
MCZ301922	female	Illinois	USA	1874		CBM1
MCZ301923	female	Illinois	USA	1874		CBM1
MCZ314458	male	Texas	USA	1909	CRM6	CBM1
MCZ314459	male	Texas	USA	1909		CBM1
MCZ315720	male	Kansas	USA	1909		CBM1
MCZ319322	female	Texas	USA	1912	CRM1	CBM1
MCZ321645	female	Alaska	USA	1914	CRM2	
MCZ321646	male	Alaska	USA	1914		
MCZ327770	male	Alberta	Canada	1924	CRM1	CBM11
MCZ327771	female	Alberta	Canada	1923	CRM12	
MCZ53712	male	Kansas	USA	1909		

MCZ53713	male	Kansas	USA	1909		
MCZ53714	male	Kansas	USA	1909		CBM1
MCZ53715	female	Texas	USA	1910		
MCZ53716	male	Texas	USA	1910	CRM17	
MCZ54791	female	N. Dakota	USA	1901		
MCZ54792	male	N. Dakota	USA	1901	CRM7	
MCZ54793	male	N. Dakota	USA	1901	CRM1	
MCZ66932	male	Siberia	Russia	1913	CRM1	
MCZ68675	female	Alaska	USA	1914	CRM1	
MCZ68676	male	Alaska	USA	1913	CRM2	CBM8
MCZ68677	male	Alaska	USA	1913	CRM1	CBM1
MCZ68678	female	Alaska	USA	1913		
MCZ68679	female	Alaska	USA	1914	CRM2	
MVZ101107	male	Ontario	Canada	1883		CBM1
MVZ106887	female	Texas	USA	1910	CRM2	CBM1
MVZ126731	male	Alaska	USA	1951		CBM1
MVZ126732	male	Alaska	USA	1951	CRM13	CBM1
MVZ126734	male	Alaska	USA	1951		
MVZ133566	male	Alaska	USA	1955		CBM1
MVZ137340	male	Alaska	USA	1958	CRM2	CBM1
MVZ137341	female	Alaska	USA	1958		CBM1
MVZ137342	female	Alaska	USA	1958	CRM18	CBM5
MVZ137343	undetermined	Alaska	USA	1958	CRM2	CBM1
MVZ137344	undetermined	Alaska	USA	1958	CRM1	CBM1
MVZ137345	male	Alaska	USA	1958		
MVZ137346	female	Alaska	USA	1958		CBM1
MVZ142038	male	Alaska	USA	1960		CBM1
MVZ158255	male	Alaska	USA	1960	CRM9	CBM1
MVZ163389	male	Alaska	USA	1972		CBM1
MVZ164929	female	Prov. Buenos Aires	Argentina	1974	CRM1	CBM1
MVZ164930	male	Prov. Buenos Aires	Argentina	1974		CBM1
MVZ164932	male	Prov. Buenos Aires	Argentina	1974		
MVZ164933	male	Prov. Buenos Aires	Argentina	1974		CBM1
MVZ164934	female	Prov. Buenos Aires	Argentina	1974		CBM12
MVZ164936	male	Prov. Buenos Aires	Argentina	1974	CRM3	CBM1
MVZ166490	male	Prov. Buenos Aires	Argentina	1974	CRM2	CBM1
MVZ166491	male	Prov. Buenos Aires	Argentina	1974		
MVZ166492	female	Prov. Buenos Aires	Argentina	1974		CBM1
MVZ166493	female	Prov. Buenos Aires	Argentina	1974	CRM1	CBM2
MVZ166494	male	Prov. Buenos Aires	Argentina	1974		CBM1
MVZ166495	male	Prov. Buenos Aires	Argentina	1974	CRM7	CBM1
MVZ166496	male	Prov. Buenos Aires	Argentina	1974	CRM4	CBM1
MVZ166497	male	Prov. Buenos Aires	Argentina	1974		

MVZ166498	undetermined	Prov. Buenos Aires	Argentina	1974	CRM6	
MVZ166501	female	Prov. Buenos Aires	Argentina	1974	CRM3	CBM1
MVZ166502	male	Prov. Buenos Aires	Argentina	1974	CRM1	CBM2
MVZ169783	female	Prov. Buenos Aires	Argentina	1974		CBM1
MVZ31862	male	Texas	USA	1890		CBM1
OMNH2719	female	Oklahoma	USA	1956		CBM1
OMNH2732	female	Oklahoma	USA	1956	CRM1	CBM1
OMNH3141	male	Oklahoma	USA	1957	CRM1	CBM1
OMNH3142	male	Oklahoma	USA	1957	CRM1	CBM1
OMNH3143	male	Oklahoma	USA	1957	CRM1	CBM1
OMNH3144	female	Oklahoma	USA	1957	CRM1	CBM1
OMNH3969	male	Oklahoma	USA	1960	CRM1	CBM1
OMNH3970	male	Oklahoma	USA	1960	CRM1	CBM2
OMNH4333	female	Oklahoma	USA	1960	CRM1	CBM1
OMNH4360	female	Oklahoma	USA	1960	CRM2	CBM2
OMNH4891	male	Oklahoma	USA	1961	CRM2	CBM1
OMNH4954	male	Oklahoma	USA	1961	CRM3	CBM1
OMNH5129	male	Oklahoma	USA	1962	CRM1	CBM1
OMNH5130	male	Oklahoma	USA	1962	CRM1	CBM1
OMNH5131	female	Oklahoma	USA	1962	CRM2	CBM6
OMNH5280	male	Oklahoma	USA	1963	CRM1	CBM1
OMNH8533	male	Oklahoma	USA	1957		
OMNH8534	female	Oklahoma	USA	1957	CRM1	CBM1
UMMZ118397	female	Alaska	USA	1934	CRM4	CBM1
UMMZ124333	male	Florida	USA	1936	CRM1	CBM1
UMMZ124334	female	Louisiana	USA	1940	CRM2	CBM1
UMMZ124335	male	Alberta	USA	1933	CRM1	CBM1
UMMZ124336	male	Alberta	USA	1933	CRM2	CBM1
UMMZ124337	female	Alberta	USA	1933	CRM2	CBM2
UMMZ124338	female	Alaska	USA	1944	CRM1	CBM1
UMMZ124339	female	Alaska	USA	1944	CRM1	CBM1
UMMZ124340	female	Alaska	USA	1945	CRM2	CBM1
UMMZ124341	male	Alaska	USA	1945	CRM4	CBM1
UMMZ124342	male	Alaska	USA	1946		CBM1
UMMZ124343	female	Alaska	USA	1946	CRM1	CBM1
UMMZ124344	undetermined	Alaska	USA	1946	CRM3	
UMMZ124345	male	Alaska	USA	1946	CRM1	CBM1
UMMZ165396	female	Texas	USA	1931	CRM1	CBM1
UMMZ165397	male	Texas	USA	1935	CRM2	CBM1
UMMZ210851	female	Michigan	USA	1966		
UMMZ230950	male	Alberta	Canada	1923	CRM1	CBM1
UMMZ30228	undetermined	Michigan	USA	1875	CRM1	CBM14
UMMZ52408	male	Texas	USA	1913	CRM1	CBM3

UMMZ54729	female	North Dakota	USA	1924	CRM1	CBM1
UMMZ54730	female	North Dakota	USA	1923	CRM21	CBM1
UMMZ59521	female	Alberta	Canada	1923	CRM3	CBM3
UMMZ59522	male	Alberta	Canada	1923	CRM1	CBM1
UMMZ72066	undetermined	Michigan	USA	1931	CRM1	CBM3
UMMZ83851	male	Manitoba	Canada	1936	CRM1	CBM1
UMMZ83852	male	Manitoba	Canada	1936		
UMMZ83853	female	Manitoba	Canada	1936	CRM2	CBM2
UMMZ83854	female	Manitoba	Canada	1936	CRM1	CBM1
UMMZ99973	female	Nebraska	USA	1882		
UMMZ99974	female	Nebraska	USA	1882		
UMMZ99975	male	Nebraska	USA	1882	CRM2	CBM1
USNM119888	male	San Jose	Costa Rica	1890		CBM7
USNM119889	female	San Jose	Costa Rica	1890	CRM1	
USNM121417	female	New York	USA	1888	CRM1	CBM1
USNM13075	undetermined	Nebraska	USA	1889		
USNM164766	male	Texas	USA	1899		CBM1
USNM165956	male	Texas	USA	1900	CRM1	CBM1
USNM167039	male	Manitoba	Canada	1900	CRM1	CBM4
USNM167040	female	Manitoba	Canada	1900	CRM4	CBM1
USNM176083	undetermined	San Jose	Costa Rica	1890		
USNM173474	undetermined	Rhode Island	USA	1900	CRM1	CBM2
USNM176082	undetermined	San Jose	Costa Rica	1890	CRM3	CBM1
USNM176083	female	San Jose	Costa Rica	1890	CRM3	CBM1
USNM19954	male	Northwest Territories	Canada	1860		
USNM220472	female	Massachusetts	USA	1892	CRM1	
USNMA4458	undetermined	Washington	USA	1855		
USNM45495	undetermined	Texas	USA	unknown	CRM1	
USNM552	unknown	New York	USA	1841		
USNM565464	male	Indiana	USA	1892		
USNM84654	male	Massachusetts	USA	1871		
USNM84656	female	Minnesota	USA	1877	CRM2	CBM1
USNM93225	male	Alaska	USA	1883	CRM1	CBM1
USNM93232	male	Alaska	USA	1883		
USNM93238	female	Alaska	USA	1883		CBM1
DAE1005	male	Missouri	USA	1965	CRM1	CBM1
DAE1185	female	Missouri	USA	1966	CRM2	
KU101354	undetermined	Kansas	USA	1955	CRM3	CBM10
KU103199	undetermined	Kansas	USA	1968	CRM2	CBM1
KU107793	undetermined	Kansas	USA	1983	CRM1	CBM1
KU12096	undetermined	Kansas	USA	1921		
KU31962	undetermined	Kansas	USA	1954	CRM11	CBM5

KU31963	undetermined	Kansas	USA	1954	CRM15	CBM1
KU65115	undetermined	Kansas	USA	1971	CRM7	CBM1
KU71775	undetermined	Kansas	USA	1879		
KU71776	undetermined	Kansas	USA	1879		
LCW433	female	Missouri	USA	1967	CRM1	CBM1
LCW434	female	Missouri	USA	1967	CRM1	CBM1
LCW435	female	Missouri	USA	1967	CRM1	CBM1
LCW439	female	Missouri	USA	1967	CRM1	CBM1
LCW440	female	Missouri	USA	1967	CRM1	CBM1
LCW441	undetermined	Missouri	USA	1967	CRM3	CBM1
LCW445	female	Missouri	USA	1967	CRM1	CBM1
LCW446	female	Missouri	USA	1967	CRM1	
LCW447	female	Missouri	USA	1967	CRM23	
LCW448	female	Missouri	USA	1967	CRM20	CBM1
LCW451	female	Missouri	USA	1967	CRM1	CBM1
LCW452	female	Missouri	USA	1967	CRM1	CBM4
LCW455	undetermined	Missouri	USA	1967	CRM1	CBM1
LCW456	female	Missouri	USA	1967	CRM2	CBM1
LCW458	female	Missouri	USA	1967	CRM2	CBM1
LCW459	female	Missouri	USA	1967	CRM16	CBM1
UNSM12687	male	Nebraska	USA	1909	CRM2	CBM1
UNSM12688	male	Nebraska	USA	1912	CRM1	CBM1
UNSM12689	male	Nebraska	USA	1912	CRM1	
UNSM12690	female	Nebraska	USA	1912	CRM1	CBM1
UNSM6198	female	Nebraska	USA	1919	CRM1	CBM1
UNSM6199	male	Nebraska	USA	1904	CRM19	CBM13
UNSM6201	undetermined	Nebraska	USA	1916	CRM1	
UNSM6203	undetermined	Nebraska	USA	1916	CRM1	

Table 0.2: Primers for each of the six regions amplified across historical samples. CytB = cytochrome *b* and CR = mtDNA control region. All primers are optimized with an annealing temperature of 50^{o}C.

Region ID	Primer Name	Sequence (5' - 3')
CytB1	CytB_Mus2.F	GCC TCG GAA CAC AAA TC
	CytB_Mus3.R	CGA AAG CGG TTG CTA TTA G
CytB2	CytB_Mus4.F	TGG AAT ACA GGA GTC ATC C
	CytB_Mus5.R	GGC CTG CGA TTA TGA ATG
CytB3	CytB_Mus6.F	CAC TAA CCC GAT TCT TCG
	CytB_Mus7.R	GAA GTT TTC TGG GTC TCC
CytB4	CytB_Mus8.F	CTC TTC CTA CTA ACC CTT G
	CytB_Mus9.R	GGA TTT GTG GAG AAG TGG
CR1	CR_Mus2.F	CGA AGC AAT GAA CCT AG
	CR_Mus3.R	ATT TCA CGT GAG GAG CT
CR2	CR_Mus4.F	ATA CAA GCC GTA CCA G
	CR_Mus5.R	GCC AAC CAG ATG TAT TCG