2021-08-01

Landscape Population And Evolutionary Genomics Of Several Closely Related Species Of Mallard-Like Ducks

Joshua Brown
University of Texas at El Paso

LANDSCAPE POPULATION AND EVOLUTIONARY GENOMICS OF SEVERAL CLOSELY RELATED SPECIES OF MALLARD-LIKE DUCKS

JOSHUA I. BROWN

Doctoral Program in Ecology and Evolutionary Biology

APPROVED:

Philip Lavretsky, Ph.D., Chair

Michael L. Moody, Ph.D.

Markus J. Peterson, Ph.D.

Sarah Sonsthagen, Ph.D.

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

LANDSCAPE POPULATION AND EVOLUTIONARY GENOMICS OF SEVERAL CLOSELY RELATED SPECIES OF MALLARD-LIKE DUCKS

by

JOSHUA I. BROWN, B.S.

DISSERTATION

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

August 2021

Acknowledgements

I would like to acknowledge the many individuals that have made the completion of this dissertation possible. First, my advisor, Dr. Philip Lavretsky, accepted me into his lab knowing that my interest was in waterfowl and that my understanding of evolution and genetics was limited. I am particularly grateful for his willingness to mentor me on those topics which I did not know and have enjoyed our many conversations in the duck blind, in the lab, or on the road, regarding the technical and theoretical complexities of these fields. Moreover, without his guidance and encouragement early on, I would not have pursued this dissertation in the first place. I am additionally grateful to my committee, Dr. Michael Moody, Dr. Markus Peterson, and Dr. Sarah Sonsthagen, who have provided guidance and support throughout this process.

I am deeply grateful for the friendships I have formed throughout this process, and for these friends who now feel like family. They have always accepted me for who I am and were always there for me in times of stress. I would like to offer special thanks to Christopher Wilson, who, although no longer with us, was an incredible friend and lover of science that provided many inspiring late-night conversations on evolution and biology.

Lastly, I would like to thank my family as this would have been impossible without their love, encouragement, and support. In particular, I thank my dad who instilled in me a love for everything wildlife and science related, and my mom, who always showed me what could be achieved through a meticulous and diligent work ethic.

Abstract

Divergence and speciation proceed through three major evolutionary forces (i.e., selection, genetic drift, and gene flow) that are often spatially and temporally heterogeneous across the landscape. Moreover, these forces can have differing but subtle effects within the genomes of diverging taxa, and therefore, disentangling the effects of these evolutionary mechanisms throughout the speciation process can be challenging. Here, I use a recent species radiation, the mallard complex, to investigate how strong, yet varied, evolutionary pressures influence the speciation process. The mallard complex consists of 14 mallard-like waterfowl species around the world that have some of the highest rates of hybridization among avian groups. In general, their wide distribution and lack of pre-zygotic reproductive barriers makes this group an excellent study system for investigating the genomic and adaptive effects of selection and gene flow during divergence. Additionally, anthropogenically-induced secondary contact between the mallard (*Anas platyrhynchos*) – one of the most ubiquitous species of waterfowl in the world – and the other mallard-like ducks provides unique natural experiments for testing the effects of gene flow from these non-native and captive-bred individuals into native congeners. Finally, improved sequencing and modelling technologies have increased our ability to sequence thousands of genomic markers from range-wide samples of non-model organisms.

In Chapter 1, I investigated the population genetic structure of and hybridization rate between Mexican ducks (*A. diazi*) and mallards in southwest North America using double-digest Restriction-site Associated DNA sequencing (ddRAD-seq). I sequenced 3,189 ddRAD-seq autosomal and Z-chromosome loci, as well as a portion of the mtDNA control region across 387 samples of Mexican ducks, mallards, and putative hybrids. First, I confirmed that Mexican ducks and mallards are in fact genetically distinguishable, and that Mexican ducks exhibit strong

population structure that suggests a history of sequential founder events from north to south. Moreover, Mexican ducks were characterized by three unique genetic clusters that show a southward cline in both mallard-like phenotypic traits and mallard genetic ancestry. Importantly, these results contradict past notions of pervasive introgressive hybridization from mallards into Mexican ducks, as I recovered only a few late-stage hybrids that were limited to the northern part of the Mexican duck's range. I provide additional evidence that previous estimates of hybridization were likely biased by phenotypic variation, that I conclude was due to shared ancestry and not hybridization. Finally, I discuss how both genomic and morphological comparisons are necessary for investigating the evolution of complex traits in recent species radiations and consider how such insights can help future conservation efforts.

In Chapter 2, I use range-wide samples sequenced in Chapter 1 to model evolutionary history, demographic history, and genotype-environment and genotype-phenotype associations in Mexican ducks. First, evolutionary models and genotype-environment associations (GEA) showed that Mexican ducks diverged from mallards during a glacial period in a local climate refugia around southwestern North America. Further reconstructing the demographic histories revealed that Mexican ducks diverged from mallards ~300,000 years ago during a glacial maximum and have since had cyclical population growth reflecting changing environmental conditions related to glacial and inter-glacial periods. Additionally, evolutionary and demographic models support recurring bouts of gene flow during secondary contact events, which artificially inflated Mexican duck effective population size during periods of contact. Importantly, a Mexican duck x mallard combined model of GEA showed that environmental selective pressures have played a key role in driving divergence, as there is significant genotypic turnover between species across environmental gradients. Finally, I used genotype-phenotype association testing and contend that

sexual selection has acted as a co-evolutionary process, facilitating the development of reproductive barriers that initially arose due to strong ecological partitioning. Broadly, this chapter reveals that genomic and phenotypic patterns observed during the earliest stages of divergence are complex in the way that they contribute to the evolutionary trajectory of a lineage.

In Chapter 3, I investigate the consequences of anthropogenic hybridization from introduced mallards in native New Zealand grey ducks (*A. superciliosa superciliosa*). Domestic mallards were introduced to New Zealand during the mid-1800s and have since become the dominant species of waterfowl throughout New Zealand. Alternatively, native grey ducks have been steadily declining and are at risk of complete lineage fusion due to extensive hybridization with mallards. First, I show that pockets of pure grey ducks have persisted in areas of more undisturbed habitat along the western and northwestern coasts of the South Island. In contrast, introduced mallards have experienced widespread introgression from native grey ducks, and now constitute a hybrid swarm. Additionally, I used GEA modelling to demonstrate that such extensive introgression into mallards has likely facilitated their rapid establishment and expansion throughout New Zealand. In fact, these strong selective pressures have resulted in a genetically unique New Zealand mallard that no longer resembles its original domestic stock and does not overlap the adaptive space of wild North American mallards. I showed that the New Zealand mallard hybrid swarm encompasses a more variable genetic niche space as compared to either parental species on their own, suggesting that these hybrids may be better adapted. I then measured the vulnerability of current GEAs to future climate change scenarios and found that grey ducks are at risk of losing critical adaptive habitat along the western coasts of the South Island. Finally, I discussed these findings in the larger context of conservation biology, and what should be prioritized as climate change and land use changes continue to increase cases of anthropogenic

hybridization. In general, I argue that conservation efforts should be focused on preserving core habitats of native species, as this strategy will likely have the added benefit of strengthening reproductive barriers.

While Chapter 3 emphasized the genetic and adaptive consequences of interspecific hybridization, Chapter 4 focusses on the effects of hybridization between wild and domestic conspecifics. Specifically, mallards were largely absent of eastern North America until the early 1900's when government and private organizations began supplemental stocking programs. I report that extensive hybridization continues today, and a 4-fold decrease in the prevalence of pure wild mallards as compared to estimates from a decade ago. Next, I used GEA modelling to show that wild and game-farm hybrid mallards have differing adaptive breeding ranges, with game-farm hybrids showing significant genotypic turnover across the southern border of Canada. This is likely acting as a barrier to dispersal, as game-farm hybrid genotypes were found to be most strongly influenced by winter weather conditions. Moreover, when projecting GEAs across future climate conditions, I found that while wild mallards are unlikely to be impacted in their core breeding range of the central Canadian prairies, game-farm hybrids are poised to expand as temperatures in the region increase. This suggests that unless strong efforts are made to limit wild x game-farm mallard interactions, hybridization and introgression of maladaptive domestic traits into wild populations may increase conservation risk in the future.

Overall, I provide insight into broad spatiotemporal responses to changing selective pressures across a recently diverged avian species complex. In particular, I have used novel GEA methods to demonstrate that there is a complex relationship between the environment, selection, and adaptation throughout the speciation process. More broadly, this work reveals that the evolutionary mechanisms driving speciation are not singular, and that the complex associations

between many different factors play a role in this process. Additionally, I shed light into the adaptive consequences of anthropogenic hybridization, which can have varying outcomes. Specifically, in New Zealand, extensive hybridization has acted to increase the adaptive range of non-native mallards, while in North America it is likely contributing to declining wild mallard populations. Finally, this work affirms the usefulness of reduced representation sequencing data for landscape level sample sets, especially in non-model organisms. While whole genome sequencing is the necessary next step for understanding how genomic architecture is dictated by evolutionary forces, landscape level studies can provide insight into the environmental drivers of adaptation that are critical for understanding how speciation proceeds.

Table of Contents

List of Tables

List of Figures

Chapter 1: Genomic and morphological data shed light on the complexities of shared ancestry between closely related duck species

ABSTRACT

Causes for genomic and morphological similarities among recently radiated species are often multifaceted and further convoluted among species that readily interbreed. Here, I couple genomic and morphological trait comparisons to test the extent that ancestry and gene flow explain the retention of mallard-like traits within a sister species, the Mexican duck. First, I confirm that these taxa remain genetically structured, and that Mexican ducks exhibit intra-specific structure that follows geography. Despite the assumption of wide-spread hybridization, I found only a few late-stage hybrids, all from the southwestern US. Additionally, I demonstrate that previous morphology-based estimates of hybridization were biased by the variance in phenotypic variability displayed among juvenile Mexican ducks. Rather, I conclude that the retention of molecular and morphological traits between Mexican ducks and mallards is due to recent ancestry and sequential founder events, and not hybridization. Finally, I discuss how genomic and morphological comparisons shed light into the mechanism(s) underlying the evolution of complex phenotypic traits in recent radiations, and how misunderstanding the true morphological diversity within Mexican ducks has resulted in taxonomic revisions that hinder conservation efforts.

INTRODUCTION

Divergence and speciation proceed through four major evolutionary forces (Lande, 1976; Lynch et al., 2016; Slatkin, 1987) that can be spatially and temporally variable and often result in heterogeneous genomes (Irwin et al., 2018; Turner, Hahn, & Nuzhdin, 2005; Via & West, 2008). Such genomic heterogeneity can translate into phenotypic variation within and among populations that results in biased conclusions regarding true rates of hybridization versus incomplete lineage sorting (ILS) (Hohenlohe et al., 2010; Todesco et al., 2016). In recently diverged taxa where strong reproductive barriers have yet to develop, wide-spread hybridization may act to homogenize the genome in a way that is challenging to discern from ILS (Hartman, Wetzel, Crowley, & Westneat, 2012; Lavretsky, DaCosta, Sorenson, McCracken, & Peters, 2019; Nadachowska-Brzyska et al., 2013). Thus, understanding true rates of ILS and gene flow among incipient forms, as well as resolving the phenotypic complexities that makes identifying putatively admixed individuals challenging, requires genomic and landscape-level sampling that allows us to accurately distinguish between phenotypic traits associated with hybridization versus shared ancestry (Galaverni et al., 2017). Fortunately, advances in high-throughput sequencing methods have made it possible to conduct studies of closely related groups that require thousands of genetic markers to discern subtle population structure (Lavretsky, DaCosta, et al., 2019; Leipold, Tausch, Hirtreiter, Poschlod, & Reisch, 2020; Toews et al., 2016).

Among lineages, class Aves generally shows high levels of ancestral genomic retention due to its strong dispersal ability (Greenwood, 1980), high chromosomal stasis (Ellegren 2010), and relatively slow development of breeding barriers (Grant & Grant, 1992; Price & Bouvier, 2002). These characteristics of avian evolution can result in species complexes that readily hybridize, as well as create discord in genomic and morphological divergence patterns (Hartman

et al., 2012; Lavretsky, DaCosta, et al., 2019; Nadachowska-Brzyska et al., 2013; K. Omland & Kondo, 2006). Taxa within these groups often maintain large ancestral portions of their genome, which is readily passed between hybridizing groups, while only few genomic regions under directional selection are responsible for maintaining species boundaries (Lavretsky, Dacosta, et al., 2015; Lavretsky, DaCosta, et al., 2019; Noor & Bennett, 2009; Wolf & Ellegren, 2017). For example, in the mallard complex, which consists of fourteen mallard-like ducks that have radiated around the world within the last million years (Baldassarre, 2014), taxa are morphologically diagnosable despite retaining large ancestral portions of their genomes (Lavretsky, DaCosta, et al., 2019; Lavretsky, McCracken, & Peters, 2014). Moreover, substantial proportions of these non-mallard (*Anas platyrhynchos*) taxa continue to display mallard-like phenotypic traits; however, distinguishing between introgression and ILS as a source for this phenotypic variability has been challenging (also see P. Lavretsky et al., 2020). Here, I use genetically vetted Mexican ducks (*Anas diazi*), mallards, and their hybrids to formally investigate the cause(s) of phenotypic variability in Mexican ducks.

Study System

The Mexican duck is a non-migratory desert-adapted species of waterfowl endemic to the highlands of central Mexico north into the southwestern United States. While recently being re-elevated to full species (Chesser et al., 2020), the taxonomic status of the Mexican duck has been highly contentious because very little is known about their specific biology, ecology, and evolutionary history (Hubbard, 1977; Huey, 1961; Ridgway, 1886; Scott & Reynolds, 1984). In particular, much of the debate surrounding their taxonomic status has been due to unknown rates of hybridization with mallards. Hubbard (1977) first described a clinal-like display of mallard-

phenotypes decreasing from north to south across the Mexican duck's range resulting from high rates of hybridization with mallards. This became a major conservation concern, as persistent gene flow can lead to lineage fusion and genetic swamping (Rhymer & Simberloff, 1996; Scott & Reynolds, 1984). However, more recent studies using double digest Restriction-site Associated DNA sequencing (ddRAD-seq) methods with hundreds of Mexican ducks and mallards revealed clear genetic structure between the two species and a general lack of genetic hybrids (Lavretsky, Dacosta, et al., 2015; Lavretsky, DaCosta, et al., 2019). Furthermore, Lavretsky et al. (2019) found evidence of directional selection acting in a few regions specifically throughout the genomes of mallards (e.g., Chromosomes 1,2, and the Z-sex chromosome) and Mexican ducks (e.g., Chromosome 14). Together, these findings established, for the first time, that these two species are under differing selective pressures, and that the presence of mallard-like traits in Mexican ducks might not be the result of hybridization. Given that many of the Mexican ducks with mallard traits were genetically identified as pure Mexican ducks, it seems likely that ILS is responsible for maintaining vestigial mallard characters in some Mexican duck populations (Lavretsky, Dacosta, et al., 2015).

Here, I build upon Lavretsky et al. (2015) to understand population structure within and between Mexican ducks and mallards by filling in geographical sampling gaps (i.e., Chihuahua, Sinaloa, and Southwestern USA). Although hybridization was thought to be most prevalent in the Mexican duck's northern range, these estimates were based on phenotypic characters and may be overestimating true hybridization rates (i.e., ~2%; Lavretsky et al., 2015). In fact, given the decreasing number of mallards wintering in the southwestern US, and that mallards would have to drop out of migration for hybridization to occur, hybridization is likely rarer than previously thought (Pérez-Arteaga & Gaston, 2004; Scott & Reynolds, 1984). Furthermore, I posit that

hybridization is likely due to interbreeding with feral mallards that are now prevalent throughout North America, as they have been the primary cause of interspecific hybridization for other mallard-like taxa (Fowler, Eadie, & Engilis, 2009; Lavretsky, DaCosta, et al., 2019; Lavretsky et al., 2020; Wells et al., 2019). I also aim to determine whether Mexican duck populations found in the Mexican states of Sonora and Sinaloa originated via founder events from their geographically closest interior Mexico population. Finally, I use these data to discuss whether the presence of mallard-like traits in Mexican ducks (e.g., green in the head, curled tail, black rump, etc.) is largely the result of hybridization or ancestry.

METHODS

Sampling, DNA Extraction, and ddRAD-seq Library Preparation

I analyze a total of 387 Mexican ducks, mallards, and their putative hybrids. In addition to previously published raw ddRAD sequences of Mexican ducks (N = 104; Lavretsky et al., 2015), I filled in geographical gaps by including the Mexican states of Chihuahua (*N* = 67) and Sinaloa (*N* = 18), and increasing sampling effort in southwestern USA (*N* = 59) (Fig. 1; Supplementary Materials Table S1.1). Next, a total of 138 wild (*N* = 76), domestic game-farm (*N* = 49), and feral Khaki Campbell (*N* = 13) mallards were also included in analyses. Note that game-farm mallards are domestic mallards being released on shooting preserves for hunting and/or dog training purposes and are now known to be the primary instigators of hybridization for wild populations of mallards and other mallard-like ducks (Lavretsky et al., 2020). Moreover, I used the feral Khaki Campbell mallards as reference park mallards as were caught and sampled alongside Mexican ducks and wild mallards; and thus, had the potential to interbreed with wild ducks. Having wild and domestic mallards allowed me to determine which of these posed the highest risk for

hybridization for Mexican ducks. Finally, two potential vagrant Mexican ducks collected in California were also opportunistically sampled (Figure 1.1A).

For a total of 174 new samples, genomic DNA was extracted from blood or tissue using a DNeasy Blood & Tissue kit following the manufacturer's protocols (Qiagen, Valencia, CA, USA). DNA quality was visually assessed on a 1% agarose gel to ensure high molecular weight bands, and quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA) to ensure a minimum concentration of 20 ng/μL. ddRAD-seq library preparation followed protocols outlined in DaCosta and Sorenson (2014; also see Lavretsky et al., 2015) (2014; also see Lavretsky et al. 2015a). In brief, genomic DNA was enzymatically fragmented using SbfI and EcoRI restriction enzymes. Illumina TruSeq compatible 6 base-pair barcodes were ligated to allow for future de-multiplexing. The barcode-ligated fragments were then size selected for 300-450 bp fragments using gel electrophoresis (2% low-melt agarose), followed by gel purification using a MinElute gel extraction kit (Qiagen). Size selected fragments were then PCR amplified with Phusion high-fidelity DNA polymerase (Thermo Scientific, Pittsburgh, PA, USA) and purified with AMPure XP magnetic beads (Agencourt, Beverly, MA, USA). Libraries were quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA), pooled in equimolar, and the multiplexed library sent to the University of Oregon Core Genomics Facility for 150 base-pair, single-end chemistry sequencing on an Illumina HiSeq 4000.

Raw Illumina sequence reads were processed using the custom Python scripts designed by DaCosta & Sorenson (2014; Python scripts available at http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline; also see Lavretsky et al., 2015). Sequences that pass the preliminary Illumina quality filter were parsed into individual sample files based on barcode sequences before barcodes were trimmed from each read and replaced with a "CC" sequence to construct the SbfI recognition

sequence. Low-quality reads were then filtered, and identical reads condensed while maintaining the read count and highest quality at each position. These condensed and filtered reads were then concatenated and clustered into loci using the UCLUST method in USEARCH v5 (Edgar, 2010) with an identity threshold of 0.85. Reads that had a quality score below 20 and do not cluster with other reads from the same individual at a 90% threshold were removed before further analysis. The highest quality read from each cluster was mapped to the Mallard reference genome (Accession no. SS263068950 – SS263191362; Huang et al., 2013; Kraus et al., 2011) using BLASTN v.2 (Altschul, Gish, Miller, Myers, & Lipman, 1990), and clusters with identical or nearly identical BLAST hits were combined while clusters that did not produce a BLAST hit were considered to be anonymous loci throughout the remainder of the pipeline. This step greatly improves the clustering of loci with large indel differences between samples. Then, MUSCLE v.3 (Edgar, 2010) was used to align sequences in each of the clusters (i.e. putative loci). Samples within each aligned cluster were genotyped using Python scripts written by DaCosta and Sorenson (2014). Alignments with end gaps due to indels and/ or a polymorphism in the SbfI restriction were trimmed or flagged for manual editing. Finally, using the Python script developed by DaCosta & Sorenson (2014), the aligned sequences were then genotyped. Homozygous genotypes were scored if >93% of reads were consistent with a single haplotype. Heterozygotes were scored if a second haplotype was represented by at least 29% of sequence reads. Samples with a secondary haplotype in 7-20% of reads, and putative heterozygote samples with a third haplotype in more than 10% of reads were flagged as ambiguous. Loci with multiple ambiguous genotypes are often representative of duplicated or repetitive elements in the genome. I then used the program Geneious (Biomaters Inc., San Francisco, CA, USA) to manually check flagged alignments and loci with unusually high numbers of polymorphisms or indels. To further limit the effect of

sequencing error, I required a minimum sequencing depth of 5 reads to score an allele, such that a minimum of 10 reads was required to score a locus as homozygous or heterozygous. Loci with <15% missing genotypes were retained for downstream analyses, and final output files (e.g., FASTA, NEXUS, ADMIXTURE) were generated with custom python scripts (Lavretsky et al., 2016). Moreover, ddRAD-seq loci with prefect BLAST scores to the reference genome were retained that allowed me to categorize loci as autosomal and Z-sex chromosome linked.

Mitochondrial DNA

For the same 174 new samples, primers L78 and H774 were used to PCR amplify and sequence 655 base pairs of the mtDNA control region (Sorenson, Ast, Dimcheff, Yuri, & Mindell, 1999; Sorenson & Fleischer, 1996) following Sanger Sequencing methods described in Lavretsky et al. (2014). Sanger sequencing occurred on an ABI 3730 (Applied Biosystems, Life Technologies, Carlsbad, California, USA) at the University of Texas at El Paso Border Biomedical Research Center Genomic Analysis Core Facility. Previously published mtDNA control region sequences were combined, aligned, and edited with new sequences using Sequencher v. 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Finally, haplotype relationships were visualized with a median-joining haplotype network using the program NETWORK v. 4.5.1.0 (Bandelt, Forster, & Rohl, 1999).

Nuclear Population Structure and Estimates of Molecular Diversity

Nuclear population structure was based on independent bi-allelic ddRAD-seq autosomal single nucleotide polymorphisms (SNPs) and without using a priori assignment of individuals to populations or species. Bi-allelic SNPs were extracted from a concatenated fasta file of ddRAD-

seq autosomal loci using a custom python script in plink format (i.e., ped & map files) (Lavretsky et al., 2016). Following, PLINK v1.07 (Purcell et al., 2007) was used to filter for singletons (i.e., minimum allele frequency (--maf 0.005)), any SNP missing ≥20% of data across samples (--geno 0.2), as well as any SNPs found to be in linkage disequilibrium (LD) (--indep-pairwise 2 1 0.5). One of the two SNPs was randomly excluded if an LD correlation factor (r2) > 0.5 was obtained.

First, a Principal Components Analysis (PCA) was done in the R package adegenet (Jombart, 2008), with the 'dudi.pca' function. Next, I used programs ADMIXTURE v. 1.3 (Alexander & Lange, 2011; Alexander, Novembre, & Lange, 2009) and fineRADstructure (Malinsky, Trucchi, Lawson, & Falush, 2018), with the latter shown to better differentiate between patterns of shared ancestry (i.e., ILS) and recent hybridization in Mexican ducks (Lavretsky, DaCosta, et al., 2019). Specifically, assignment probabilities based on major allele frequencies such as in ADMIXTURE can be complicated by close genetic relationship, including for taxa that show patterns of isolation-by-distance as within Mexican ducks (Lavretsky, Dacosta, et al., 2015). Rather, fineRADstructure co-ancestry matrices are calculated based on the rarest SNPs that contribute the most information, which not only allows it to account for linkage among individual ddRAD loci but has been shown better suited for studies of incipient species that often have on average very little genetic differentiation as with Mexican ducks and Mallards (Lavretsky, DaCosta, et al., 2019; Malinsky et al., 2018). For these reasons, I obtained both individual assignment and co-ancestry assignments across samples in attempt to better capture shared versus introgressed ancestry.

Following the protocol outlined by Alexander et al. (2015; also see Lavretsky et al., 2019), bi-allelic SNPs were extracted and formatted for ADMIXTURE analysis using the program Plink v. 0.67 (Purcell et al., 2007). For ADMIXTURE, I ran 100 iterations of each K for one through ten

populations. The analysis uses a ten-fold cross-validation (CV) with a quasi-Newton algorithm (Zhou, Alexander, & Lange, 2011) and a block relaxation algorithm for point estimation. Each individual run was terminated once the change in log-likelihood (i.e., delta) of the point estimates increased by <0.0001. The optimal number of populations (K) was then based on the lowest averaged CV-error across all 100 replicates per K. The package PopHelper (Francis, 2017) in R was used to convert all ADMIXTURE outputs into CLUMPP v. 1.1 (Jakobsson & Rosenberg, 2007) input files. Final assignment probabilities were based on the optimal clustering alignment across all 100 replicates per evaluated population K value using the GreedySearch algorithm for 1000 iterations as implemented in CLUMPP v. 1.1. Additionally, confidence intervals (CI) were calculated for evaluated K population value through a 1,000 bootstraps (-B1000) as implemented in ADMIXTURE (Alexander et al., 2015).

Next, I used fineRADstructure (Malinsky et al., 2018) to more closely look at shared ancestry among Mexican ducks and mallards. Briefly, fineRADstructure identifies the most recent coalescent events among sample-by-sample pairwise comparisons to infer relatedness among individual samples and is informative in cases of recent and ongoing gene flow (Brown et al., 2020; Lavretsky, DaCosta, et al., 2019). Using the same set of bi-allelic SNPs for fineRADstructure, samples were assigned to populations using 1,000,000 iterations of the tree-building algorithm to assess genetic relationships among clusters. Results were visualized as heat maps using the provided R scripts fineradstructureplot.r and finestructurelibrary.r (available at http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html).

Finally, composite pairwise estimates of relative divergence (ΦST) and nucleotide diversity (π) for mtDNA, as well as Autosomal and Z-chromosome ddRAD-seq loci were

calculated across species as well as between Mexican duck sampling groups using the package PopGenome (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014) in the program R.

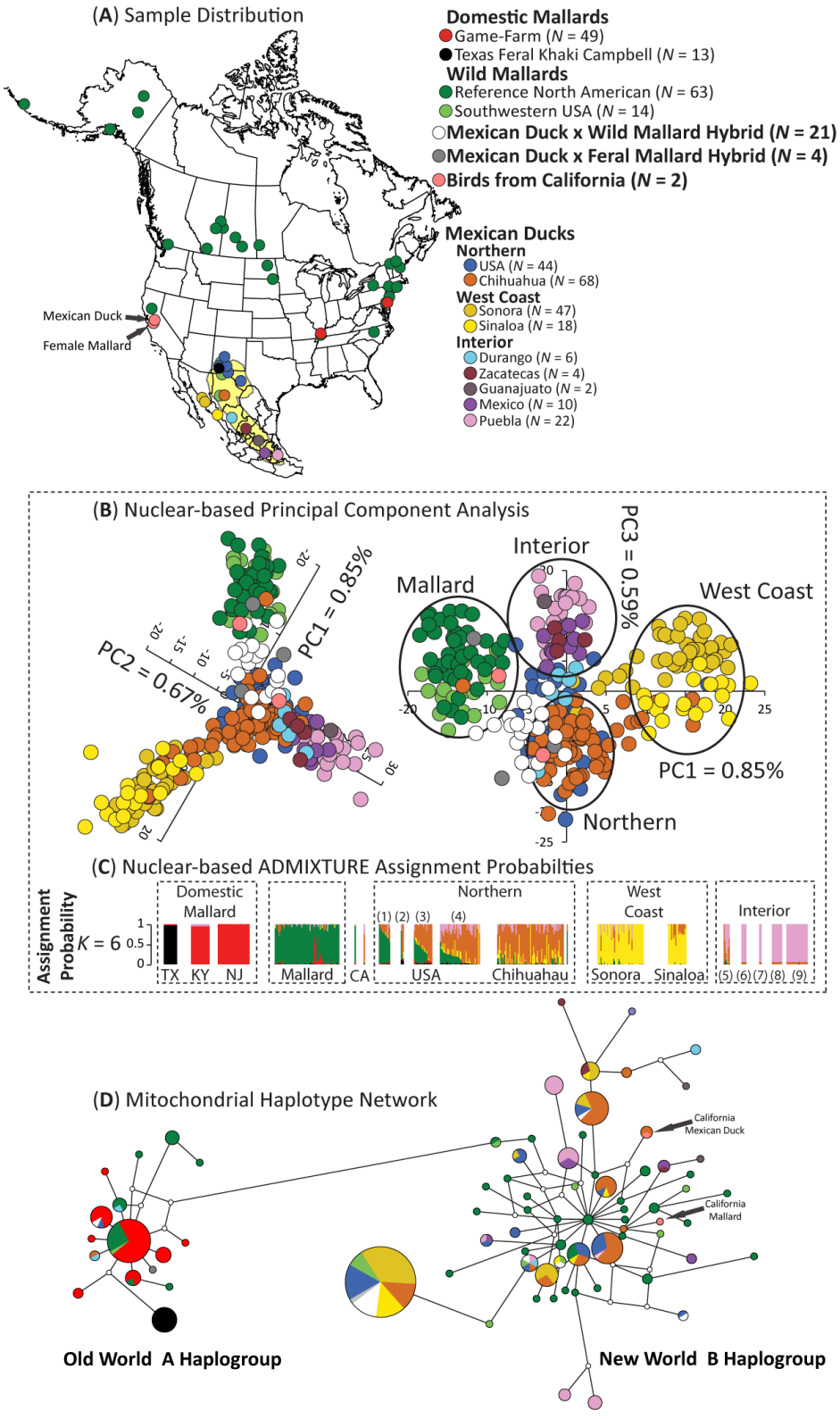

(A) Sample Distribution
Domestic Mallards
Game-Farm (N = 49)
Texas Feral Khaki Campbell (N = 13)
Wild Mallards
Reference North American (N = 63)
Southwestern USA (N = 14)
Mexican Duck x Wild Mallard Hybrid (N = 21)
Mexican Duck x Feral Mallard Hybrid (N = 4)
Birds from California (N = 2)
Mexican Ducks
Northern
USA (N = 44)
Chihuahua (N = 68)
West Coast
Sonora (N = 47)
Sinaloa (N = 18)
Interior
Durango (N = 6)
Zacatecas (N = 4)
Guanajuato (N = 2)
Mexico (N = 10)
Puebla (N = 22)
Mexican Duck
Female Mallard
(B) Nuclear-based Principal Component Analysis
PC1 = 0.85%
PC2 = 0.67%
PC3 = 0.59%
Interior
Mallard
West Coast
Northern
(C) Nuclear-based ADMIXTURE Assignment Probabilties
Assignment Probability
K = 6
Domestic Mallard
TX KY NJ
Mallard
CA
Northern
(1) (2) (3) (4)
USA
Chihuahau
West Coast
Sonora Sinaloa
Interior
(5) (6) (7) (8) (9)
(D) Mitochondrial Haplotype Network
California Mexican Duck
California Mallard
Old World A Haplogroup
New World B Haplogroup

Figure 1.1. (A) Sample size and distributions of samples. (B) PCA and (C) ADMIXTURE assignments of sampled Mexican ducks, mallards, and putative hybrids, and based on 12,696 bi-allelic ddRAD-seq nuclear SNPs. Note that these analyses were based on 370 samples that excluded domestic mallards (Supplementary Materials Figure S1.2) and all but one representative of each identified sibling group (Supplementary Materials Figure S1.3). Finally, (D) a haplotype network based on 628 sequenced base-pairs of the mitochondrial control region. Note that I identify mallards by origin (wild versus domestic) and Mexican ducks by geographical location in PCA and mitochondrial haplotype network, whereas assignment probabilities are colored by the six genetic clusters as estimated with ADMIXTURE.

RESULTS

After filtering, I recovered a total of 3,189 ddRAD-seq loci, with 3,015 (270,895 base pairs (bp) and 174 (15,609 bp) loci assigned to the autosomal and Z-sex chromosomes, respectively. The dataset consisted of an average median sequencing depth of 128 (range = 26 - 687) reads per locus per sample, and an average of 97% of alleles present per locus. Finally, 625 bp of overlapping mtDNA control region was sequenced for a total of 376 samples (of 387; Supplementary Materials Table S1.1).

Mitochondrial haplotype structure

Haplotype tree reconstruction using the mtDNA control region revealed the two known Old World (OW) A and New World (NW) B haplogroups (Figure 1.1D; Ankney, Dennis, Wishard, & Seeb, 1986; Avise, Ankney, & Nelson, 1990; Lavretsky, Hernández-Baños, & Peters, 2014). Consistent with Lavretsky et al. (2020), all domestic lineage mallards (game-farm N = 49; Khaki Campbell N = 13) carried OW A haplotypes. Notably, three Mexican ducks (1%), 22 wild mallards (31%), 1 (of 3) Mexican duck x Feral mallard hybrids, and two (of 20) Mexican duck x wild mallard hybrids contained OW A haplotypes as well. Nevertheless, the majority of genetically vetted wild mallards (69%; 50/72) and 99% of Mexican ducks were found within the NW B haplogroup (Figure. 1D). Based on a simple tally, mallards had the most unique haplotypes (N = 43), followed by Mexican ducks from Southwestern USA, Chihuahua, and then those for more southern Mexico, whereas those from the western populations of Sonora and Sinaloa both carried only five unique haplotypes.

Genetic diversity

Mexican ducks and wild mallards had similar levels of nucleotide diversity across ddRAD-seq autosomal (π_{MALL} = 0.0063, π_{MEDU} = 0.0062), Z-chromosome (π_{MALL} = 0.0027, π_{MEDU} = 0.0026; Supplementary Materials Figure S1.1A) loci, and mtDNA (π_{MALL} = 0.013, π_{MEDU} = 0.0083). Both of the domestic mallard types had lower genetic diversity across loci as compared to their wild counterparts (Supplementary Materials Figure S1.1B). Within Mexican duck sampling groups, nucleotide diversity was similar across autosomal and Z-chromosome markers (avg. π_{Aut} = 0.0060, avg. $\pi_{Z\text{-}chrom}$ = 0.0025), but varied substantially within mtDNA (range π_{mtDNA} = 0.0041-0.016). Specifically, western-coast populations (i.e., Mexican ducks sampled in the Mexican states of Sonora (π = 0.0044) and Sinaloa (π = 0.0041)) showed decreased diversity in mtDNA, which is consistent with a recent founder event (also see Lavretsky et al., 2015).

Population structure

Composite estimates of Φ_{ST} between Mexican ducks and wild mallards were 13-fold higher for ddRAD-seq Z-chromosome linked (Φ_{ST} = 0.071) than autosomal (Φ_{ST} = 0.0092; Supplementary Materials Figure S1.1A) loci, and concordant with previous estimates (Lavretsky, Dacosta, et al., 2015; Lavretsky, DaCosta, et al., 2019). Additionally, I report a ten-fold higher relative differentiation estimate between Mexican ducks and game-farm (Φ_{ST} Autosomal = 0.12, Φ_{ST} Z-Chromosome = 0.14) and feral park (Φ_{ST} Autosomal = 0.23, Φ_{ST} Z-Chromosome = 0.29) mallards as compared to wild mallards. Similarly, each of the domestic mallard types had relative genetic differentiation of >10% from wild mallards (Supplementary Materials Figure S1.1A). Finally, estimates of differentiation between Mexican duck sampling groups is relatively similar across ddRAD-seq Z-chromosome linked loci (range Φ_{ST} = 0 – 0.026), ddRAD-seq autosomal loci

(range Φ_{ST} = 0.0017– 0.048), and mtDNA (range Φ_{ST} = 0– 0.57), with increased mtDNA differentiation (Φ_{ST} = 0.099 – 0.57) among western-coast populations (Supplementary Materials Figure S1.1A).

Next, analyzing the complete 387 sample set, co-ancestry analyses identified 29 samples making up 12 sibling groups (see Supplementary Materials Figure S1.2 & Table S1.1). All putative sibling groups indeed were caught at the same time and location, were often flightless juvenile individuals, and all carried the same respective mtDNA haplotype. For example, three northern Mexican ducks that were all flightless juveniles sampled at the same time from the same location (Bosque del Apache, New Mexico) were identified as siblings as they had higher-than-average estimates of individual co-ancestry with one another (Supplementary Materials Fig. S1.2). Alternatively, a group of three Mexican ducks sampled in Chihuahua that had slightly higher than average co-ancestry remained in the dataset, as they were sampled on different days and wetlands, had different mtDNA haplotypes, and were therefore unlikely to be full siblings (Supplementary Materials Fig. S1.2).Given that the inclusion of full siblings strongly bias PCA and ADMIXTURE analyses, all but one representative per sibling group was subsequently excluded in final population structure analyses. In the end, PCA, ADMIXTURE, and fineRADstructure analyses were based on a total of 12,696 (of 13,835) independent bi-allelic ddRAD-seq autosomal SNPs for 370 samples.

I visualized the first three principal components in PCA, and individual assignment probabilities estimated in ADMIXTURE under a K population of six (Figure 1.1B; Supplementary Materials Figure S1.2). Despite recovering an optimum K population of three, where two groups of domestic mallards were distinguished from Mexican ducks and wild mallards, additional structure was recovered by incrementally increasing K population values to six (Supplementary

Materials Fig. S1.2C). Given that I evaluated assignment probabilities at a population K value of six, bootstrap values and associated confidence intervals per sample were based on the sum assignment probability across mallard types (i.e., wild + game-farm + feral park), and the confidence intervals as the square root sum of each standard error squared. Across all three analyses, I found wild mallards and Mexican ducks to be more closely related to one another than either were to domestic mallards, but that Mexican ducks and mallards remained genetically distinguishable from one another as well (Figures 1 & 2; Supplementary Materials Figure S1.2 & S1.3). In fact, <1% of variation was explained by any single principal component (Figure 1.1B; Supplementary Materials Figure S1.2A-B). Within Mexican ducks, I recovered the previously reported structure pattern that follows geography (Lavretsky, DaCosta, et al., 2015), as well as identify three genetically unique populations: (1) a northern population comprised of the Mexican state of Chihuahua and Mexican ducks from the southwestern USA, (2) a western-coast cluster comprised of samples from the Mexican states of Sonora and Sinaloa, and (3) an interior Mexico population comprising samples from the Mexican states of Durango, Zacatecas, Guanajuato, Mexico, and Puebla (Figure 1.1B). For fineRADstructure, I recovered a high degree of individual co-ancestry within these separate Mexican duck groups, as well as average co-ancestry to wild mallards that was on average higher than that recovered between domestic and wild mallards (Figure 1.2; Supplementary Materials Figure S1.3). Additionally, I find that western-coast Mexican ducks show a higher average co-ancestry with Mexican ducks from Chihuahua. Domestic mallards showed the highest levels of co-ancestry between individual samples, which is consistent with a history of inbreeding resulting from the domestication process (Lavretsky et al., 2020). Finally, whereas one of the two putative Mexican ducks from California was simply a female mallard, the other was indeed a genetically pure Mexican duck that had ADMIXTURE based

individual assignment probabilities similar to other Mexican ducks from the northern population, as well as clustered with other Mexican ducks from Chihuahua based on fineRADstructure co-ancestry estimates.

Hybrid identification

First, ADMIXTURE identified a total of 53 samples that had substantial assignment (>10%) to both Mexican ducks and the sum of all mallard types (i.e., domestic and wild). The majority of these were collected in southwestern USA (39/53, 74%), followed by Chihuahua (10/53, 19%), the western-coast regions of Sonora and Sinaloa (3/53, 6%), and a single sample from interior Mexico (1/53, 2%). In contrast, analyzing the full sample set with fineRADstructure identified a total of 25 samples, all of which were from the southwestern USA, with higher than average co-ancestry to both Mexican duck and mallard (wild or domestic) clusters. These samples were uniquely clustered between Mexican ducks and mallards and had a higher affinity to mallards (i.e., see dendrogram in Figure 1.2; Supplementary Materials Figure S1.3) than other Mexican duck samples, suggesting these are indeed recent hybrids. Of the 25 samples, four had higher than average co-ancestry with feral Khaki Campbell mallards (Figure 1.2). I also note that an additional five samples were inferred to be hybrids through sibling relationships, and indeed showed similar admixed co-ancestry matrices when analyzing the full dataset (Supplementary materials Fig. S1.3).

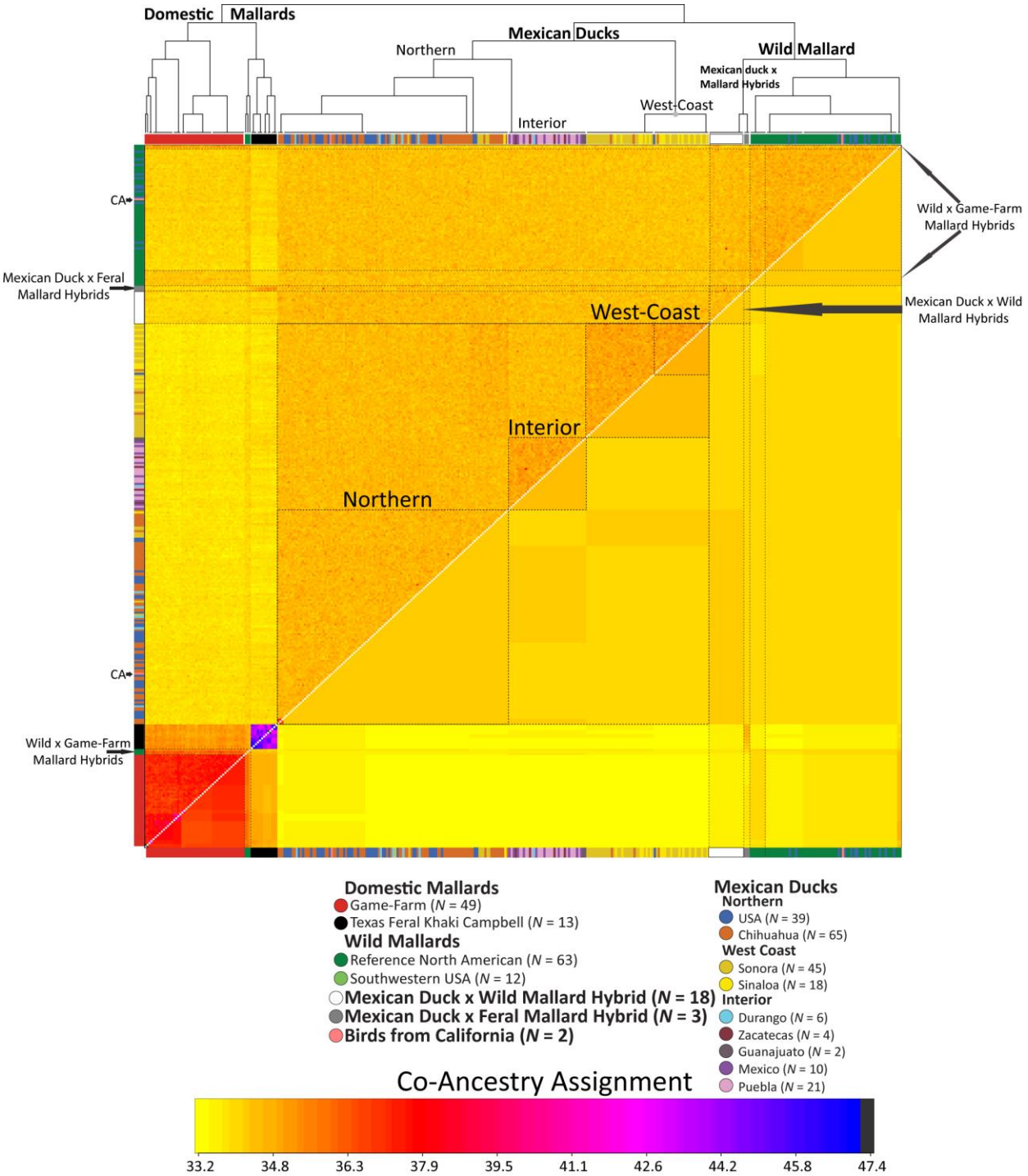


Figure 1.2. fineRADstructure individual (above diagonal) and average (below diagonal) co-ancestry coefficient matrix estimated using 12,696 bi-allelic ddRAD-seq autosomal SNPs. Note that this analysis was based on 370 samples that excluded all but one representative of each identified sibling group (Supplementary Materials Figure S1.3). The level of recent co-ancestry is color coded from low (yellow) to high (blue) is provided. I color code mallards by origin (wild versus domestic) and Mexican ducks by geographical location, as well as identify Mexican duck x (wild/feral) mallard hybrids and wild x game-farm mallard hybrids.

Earlier caution on over-interpreting ADMIXTURE results (see above) is stressed as (1) mallards and Mexican ducks have a very close genetic relationship, (2) there is fine geographical-based structure within Mexican ducks, and (3) there have been recent founder events in western-coast states of Mexico (i.e., Sonora and Sinaloa). All of these factors can result in spurious assignments and forced me to analyze ADMIXTURE at a higher than optimum K populations (Supplementary Materials Figure S1.1C). In fact, inconsistencies can be seen in the bootstrapped mallard ancestry values and associated standard errors across wild mallards and Mexican ducks (Supplementary Materials Figure S1.2D). Specifically, I see the highest standard errors among Mexican ducks comprising the Northern genetic cluster, with the majority of them overlapping zero. Similarly, I found that individuals with >50% ADMIXTURE assignment to mallard had mallard-equivalent PS values. Thus, in the case of the Mexican ducks, ADMIXTURE appears to be confounded by retained ancestry and is over-representing admixture (Figure 1.1C; Supplementary Materials Figure S1.2C). I conclude that fineRADstructure (Malinsky et al., 2018) appears to be more reliable when dealing with recently diverged taxa that have high levels of shared ancestry, as well as with fine-scale geographical structure (e.g., isolation-by-distance among Mexican ducks; also see Lavretsky et al., 2019).

DISCUSSION

Increased genomic accessibility for non-model systems has allowed me to uncover evolutionary histories and subtle population structure within and between previously indistinguishable incipient forms. Additionally, these methods permit for formal testing between potential causes of genetic and phenotypic trait retention among closely related taxa (Schweizer et al., 2019; Taylor et al., 2020). Whether ancestry, hybridization, or a combination of the two, cause

retained genetic and morphological diversity among closely related species remains inferential unless genetically vetted. These questions are particularly complicated when only a portion of a population displays ancestral morphological traits, as the most parsimonious conclusion in such scenarios is to assume these are hybrids. Alternatively, such a scenario could result from stochastic retention of genetic variation and associated phenotypic traits, which have yet to be sorted among the incipient forms. For example, the clinal expression of mallard-like traits among Mexican ducks was hypothesized to be the result of extensive hybridization, to the point that Mexican ducks in the northern part of their range were thought to be a hybrid swarm (Aldrich & Baer, 1970; Hubbard, 1977; Huey, 1961; Scott & Reynolds, 1984). However, genomic data presented here no longer support this conclusion. Rather, I provide strong evidence across the geographical range of the Mexican duck that (1) they are indeed genetically and morphologically diagnosable from mallards, (2) the few identified hybrids are confined to southwestern USA and largely in human-dominated areas, and (3) that formative male Mexican ducks, especially ones originating from the northern cluster, naturally display a high proportion of mallard traits that appear to be subsequently lost as adults. Through the comprehensive analysis regarding the geographical distribution of genetic and morphological variation of Mexican ducks, I conclude that while Hubbard (1977) correctly identified the clinal variability in the display of mallard phenotypes across the Mexican duck's range, he incorrectly concluded that it was due to high levels of hybridization with mallards. In fact, when Hernandez et al. (University of Texas at El Paso, unpublished data) applied their optimized plumage keys to historical samples from Hubbard (1977), they determined that 20% of immature males, 25% of adult males, and at least 57% of the hybrids were incorrectly identified. Once re-evaluated, these historical samples showed an almost identical distribution of plumage score (PS) values as contemporary samples collected in the same respective regions (Figure 1.3B),

and support generally low levels of hybridization. Thus, I conclude that the presence of mallard-derived genetic and phenotypic traits in Mexican ducks is the result of shared mallard ancestry and incomplete lineage sorting within Mexican ducks. This study exemplifies the intricacies of species divergence, as well as emphasizes the importance of using genomic and morphological data jointly when attempting to disentangle ancestry and hybridization as a cause for trait (genetic and/or morphological) similarities among closely related taxa.

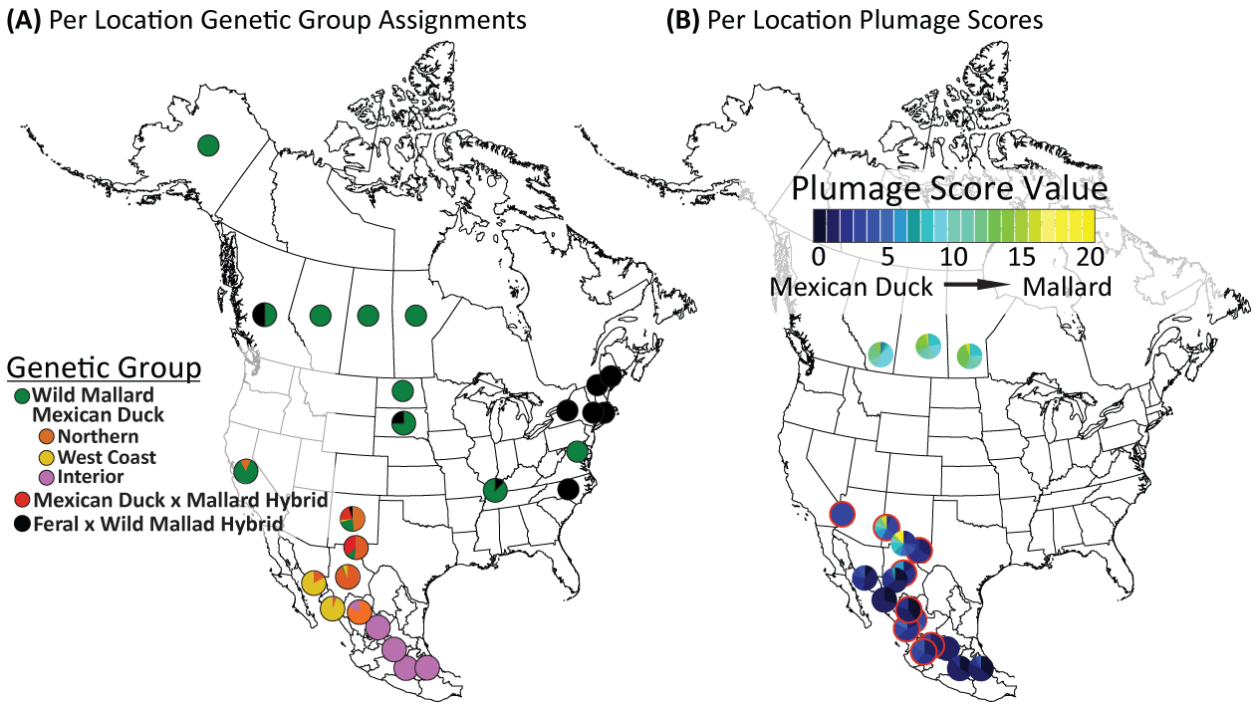


Figure 1.3. (A) Proportion of samples per geographical location with assignments to specific genetic groups as determined through population genetics analyses (Figures 1 & 2; also see Supplementary Materials Figures S1.2 & S1.3). (B) Plumage score proportions of samples per geographical location (adapted from F. Hernandez, A. Engilis Jr., P. Lavretsky, University of Texas at El Paso, unpublished data).

More generally, in concordance with evidence from Florida mottled ducks (*Anas fulvigula*; Bielefeld et al., 2016), American black ducks (*Anas rubripes*; Lavretsky, Janzen, & McCracken, 2019), and Hawaiian ducks (P. Lavretsky, Engilis, Eadie, & Peters, 2015), these results continue to support the hypothesis that the wild mallard was the ancestor to Mexican ducks and all North American monochromatic duck species. However, I note that Hawaiian ducks represent a unique hybrid origin case, as molecular work strongly suggests that the phenotypic and molecular intermediacy of the Hawaiian duck is more consistent these being a young hybrid species (Lavretsky, Engilis, et al., 2015); whereas the evolution of mainland North American monochromatic species resulted from the divergence of isolated pockets of mallards (Lavretsky, DaCosta, et al., 2019; Lavretsky et al., 2020; K. E. Omland, 1997). Sequential divergence events and unique adaptations of mallard-like species throughout North America, occurring over the last 500,000 years explains the previously perceived discord in genetic and phenotypic divergence (Lavretsky, Dacosta, et al., 2015; Lavretsky, McCracken, et al., 2014; Lavretsky et al., 2020). Additionally, retained ancestral diversity from mallards likely also explains higher than expected effective population sizes of the monochromatic species, given their substantially smaller census sizes as compared to wild mallards (Lavretsky, DaCosta, et al., 2019; Lavretsky et al., 2020). This study demonstrates the need for a holistic approach to truly understand such complex evolutionary histories as observed between Mexican ducks and wild mallards, as a single phenotypic or genetic marker type may yield spurious conclusions, which may have important implications for the conservation of many similar species complexes. For the Mexican duck, misunderstanding the cause for mallard-like phenotypes led to decades of taxonomic revisions and belief that this unique organism was largely doomed due to introgressive hybridization (Hubbard, 1977; Scott & Reynolds, 1984; USFWS 1978).

Population structure of and shared phenotypic expression in Mexican ducks explained by retained wild mallard ancestry and not hybridization

It is often assumed that the partial expression of plumage traits in a congener is evidence of hybridization; however, this assumption was not accurate in the case of the Mexican duck. Using individuals genetically vetted here, Hernandez et al. (University of Texas at El Paso, unpublished data) showed that immature male Mexican ducks naturally exhibited much greater phenotypic variation than other sex-age cohorts. Specifically, 25% (14 of 56) of genetically pure immature male Mexican ducks from the northern (~30% ; 10 of 33) and western-coast (~17% ; 4 of 23) genetic clusters innately expressed a higher proportion of mallard-like traits as compared to those from the interior Mexican ducks (i.e., 0%; F. Hernandez, A. Engilis Jr., P. Lavretsky, University of Texas at El Paso, unpublished data). In short, Hernandez et al. (University of Texas at El Paso, unpublished data) found that immature males can have combination of mallard-like traits (e.g., green in the head, elevated tail curls) that are not necessarily representative of recent hybridization.

Ultimately, the reason for the partial expression of mallard-like traits, particularly among formative male Mexican ducks remains unknown. I hypothesize that that these ancestrally derived plumage-linked genes are simply being regulated by hormones. Although the evolution of dichromatism is often believed to be a derived state, an assessment of 977 species of birds suggests that there are many cases where dichromatism has been lost in favor of monochromatism (Dale, Dey, Delhey, Kempenaers, & Valcu, 2015). Repeated losses of sexual dichromatism may have resulted from the relaxation of selection for colorful males and/or via strong environmental selection for crypsis in both sexes (e.g., dull color for crypsis; Dunn, Armenta, & Whittingham, 2015; Harrison et al., 2015; K. E. Omland, 1997). However, once sexually dimorphic traits evolve,

the raw genetic material remains, even in cases where it is lost (Dale et al., 2015; Kraaijeveld, 2014). At the point they are lost, dimorphic traits become controlled via modifiers (e.g., modifier alleles, steroids) rather than direct molecular changes (Horton et al., 2014; Kraaijeveld, 2014; Lande, 1980). In fact, the seasonal expression of dichromatic plumage in waterfowl are known to be estrogen-dependent (Kimball & Ligon, 1999), where colorful plumage develops in the absence of testosterone/estrogen or luteinizing hormones (Kimball & Ligon, 1999; Lahaye, Eens, Darras, & Pinxten, 2013; Lank, Coupe, & Wynne-Edwards, 1999; Owens & Short, 1995). Thus, monochromatic mallard-like ducks of North America that retain ancestrally derived mallard traits may control the expression of such plumage-linked genes through consistently elevated levels of testosterone or estrogen, which would induce monochromatic plumage year-round (Haase, Ito, & Wakamatsu, 1995). Regarding immature males, I hypothesize that unsuitable levels of testosterone prior to their pre-formative molt causes the partial expression of mallard-like traits in their first year. I acknowledge that the probability of expression of mallard-like traits within any single individual is likely the combination of stochastic probability regarding inheritance, and an individual's capacity to produce the necessary hormones that act to suppress the expression of those gene(s). Similarly, older male and female monochromatic mallard-like ducks and female mallards are known to regularly express male mallard-like plumage traits (Horton et al., 2014; Kraaijeveld, 2014; Lande, 1980), suggesting they are no longer able to properly regulate hormone expression. Given that hybridization rates were much lower here than previously estimated, I hypothesize that female Mexican ducks are potentially queuing and assortatively mating with males that lack mallard-like traits (i.e., maintain monochromatism). This would not only increase a female's chance to mate-pair with a knowledgeable adult male but would also act as a pre-zygotic isolating mechanism that limits incorrect mate pairings with mallards or hybrids. Future research

will benefit from mate-pairing trials or natural observations to test whether the probability of pair-bonding for immature male Mexican ducks is associated with the extent of displayed mallard-like plumage traits. Additionally, the northern populations of Mexican ducks present a natural study system to understand how phenotypic expression is controlled during molt cycles. In general, the New World mallard-like clade presents a unique set of incipient forms that can shed light into the mechanisms underlying the evolution of complex phenotypic traits as pre-zygotic reproductive barriers for recent radiations.

Low levels and confined Mexican duck x mallard hybridization

Although Mexican ducks have always been thought to be at risk of genetic swamping or extinction (Hubbard, 1977; Scott & Reynolds, 1984; USFWS 1978), I contend that this has likely never been the case. I recovered few hybrids, all of which were confined to the northern part of the Mexican duck's range (Figures 2 & 3). As predicted, I identified genetic hybrids primarily where Mexican ducks and mallards (wild and/or domestic) geographically co-occur during the breeding season. Thus, I caution that the results here may in fact be overestimating hybridization rates due to the number of samples collected in more urban areas, where mallards (wild and feral) co-exist year-round with Mexican ducks. Together, while hybridization between mallards and Mexican ducks does occur, I conclude that it is relatively rare and largely confined to their range in the southwestern USA, and more specifically in areas where resident populations of wild or domestic mallards exist year-round (Figure 1.3). Given that past taxonomic uncertainty was largely based on high rates of interbreeding with mallards, these results further support the recent taxonomic elevation of the Mexican duck to full species status (Chesser et al., 2020).

Given the general rarity and geographic distribution of identified hybrids, I posit that hybridization between mallards (wild or domestic) and Mexican ducks is often the product of forced or extra-pair copulation events during re-nesting. Male mallards, and more generally Anseriformes, are notorious for these types of forced copulation events (Cheng, Burns, & McKinney, 1982), which often act as a source of interspecific and intergeneric interactions (Gauthier, 1988; Hartman et al., 2012; Mckinney, Derrickson, & Mineau, 1983; Randler, 2005). While I conclude that hybridization with mallards is currently not a major conservation concern, continued monitoring where locally breeding mallards (wild or domestic) occur is necessary. It will be particularly important to understand what proportion of hybridization is due to interactions with domestic mallards, as I provide evidence that at least four (of 25) putative hybrids were the result of pairings between Mexican ducks and feral park ducks; additionally, I found another three Mexican ducks carrying OW A haplotypes (Figure 1.1D), which could only occur through a male Mexican duck and female domestic mallard pairing. As domestic mallards continue to be found as the primary instigator of hybridization events among mallard complex taxa (Figure 1.3A; P. Lavretsky et al., 2020; Lavretsky, DaCosta, et al., 2019; Wells et al., 2019), educating the general public regarding the conservation consequences of domestic-lineage waterfowl releases is increasingly important.

Mexican ducks evolved through sequential founder events

Population genetic analyses of landscape-level sampling not only supports the three distinct Mexican duck genetic clusters previously reported by Lavretsky et al. (2015), but also fills in geographical gaps to better understand their fine-scale structure (Figures 1 & 3A). In general, the genetic variation recovered with mtDNA and nuclear DNA support a scenario of sequential

founder events among Mexican ducks. Specifically, samples comprising the northern genetic cluster of Mexican ducks harbor all the major mtDNA haplotypes (Figure 1.1D) and are the combination of all nuclear variation found in other groups (Figures 1C & 3A). I identify Durango as the genetic transition between northern and interior populations (Figure 1.3A). Similarly, western-coast populations of Sonora and Sinaloa most closely identify with Mexican ducks of the northern genetic cluster across analyses (Figures 1C & 2). While I hypothesized that Mexican ducks from Sinaloa would be the natural extension of the closest eastern interior populations, I actually find that both Sinaloa and Sonoran Mexican ducks are genetically most similar to one another (Figure 1.1D). Additionally, of the interior clusters, I found they were closely related to Chihuahua samples based on nuclear variation and mtDNA haplotypes (Figures. 1C & 3A). Low mtDNA haplotype variation and strong partitioning in nuclear markers is consistent with reports suggesting that Mexican ducks have only recently (i.e., ~1990) colonized Mexico's western-coasts (~1990s; Lavretsky et al., 2015; Saunders & Saunders, 1981). And given that Mexican ducks from Sinaloa and Sonora are of the same genetic cluster, I conclude these populations are the result of a recent westward dispersal from Chihuahua into Sonora, followed by an expansion southward into Sinaloa along Mexico's western coast (Figure 1.3A). In fact, PCA and co-ancestry plots identify some limited structure between these western coast states, supporting the hypothesis that Sinaloa resulted from a founder event originating in Sonora (Figures 1B & 2). The westward expansion of Mexican ducks was likely in response to novel water availability due to the build-up of large dams, reservoirs and canal systems for the rapid conversion of dry, upland areas to intensive agriculture areas over the last 50 years in Mexico (Ducks Unlimited, 2019). Within interior Mexico, I posit that fine-scale structure, nuclear similarity, and mtDNA haplotype sharing likely represent ancestral founder events from north to south. Future work will benefit from

increased sampling of transitionary zones (e.g., the Mexican state of Durango) to better understand how Mexican duck genetic variation changes across the landscape. Moreover, full genome sequencing of Mexican ducks from the various genetic clusters coupled with demographic analyses (e.g., PSMC analyses; Li & Durbin, 2011) can potentially resolve whether these southward expansions may have occurred more than once through evolutionary time. With Mexican ducks being endemic to the Chihuahuan desert, I hypothesize that they would experience cyclical population expansions and contractions during glacial and inter-glacial periods, respectively, as the region shifted between wetter to drier climates.

Finally, I report for the first time a genetically pure Mexican duck collected in the San Francisco Bay Area of California, which is geographically the furthest north this species has been genetically identified (Figure 1.1). Surprisingly, while this bird was expected to be a vagrant from Mexico's western-coast due to geographic proximity, this Mexican duck shared a mtDNA haplotype with and, based on nuclear loci, clustered with Chihuahuan samples (Figures 1 & 2). Thus, this sample demonstrates that large-scale dispersal of Mexican ducks from their interior range is possible. The potential for and frequency of such migratory events to establish new Mexican duck populations outside of their current range remains unknown. In fact, it is possible that "vagrant" events may be how Mexican ducks expanded throughout time (Udvardy & Engilis, 2001). However, fine-scale population structure recovered here suggests that inter-population dispersal is infrequent (also see Lavretsky et al., 2015). In addition to whole genome data, studies of Mexican duck ecology, short- and long-distance dispersal patterns, and life-cycle phenology are increasingly needed for this recently elevated and unique North American waterfowl species. Moreover, coupling genomic data with landscape-level genotype-environment association modelling (e.g., Gradient Forest; Ellis, Smith, & Roland Pitcher, 2012) can be used to test how

genomic diversity has and will respond to shifting climatic patterns (Bay et al., 2018; Fitzpatrick & Keller, 2015).

ACKNOWLEDGEMENTS

I am grateful to US Fish and Wildlife Services and the North American Flyway Wingbees, Eduardo Carrera and Ducks Unlimited-Mexico, Ruben Del Castillo, Todd Scott and Wingshooters Lodge-Mexico, Patricio Gaudiano, Kevin G. McCracken, Alberto Lafon (with Raramuri Ranch Outfitters), and Megan Spitzer for the contributions of samples to this study. This research was funded by Ducks Unlimited Richard H. G. Bonnycastle Fellowship in Wetland and Waterfowl Biology, Ducks Unlimited Institute for Wetland and Waterfowl Research Foundation Graduate Fellowship, American Museum of Natural History Chapman Grant, the U.C. Davis Museum of Wildlife and Fish Biology, Safari Club International El Paso Chapter, and the University of Texas at El Paso. I also thank A. E. Engilis, D. Collins, and F. Hernandez for their contributions to previous drafts.

REFERENCES

Aldrich, J. W., & Baer, K. P. (1970). Status and Speciation in the Mexican Duck (*Anas diazi*). *The Wilson Bulletin*, *82*(1), 63–73.

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. doi:10.1186/1471-2105-12-246

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. doi:10.1101/gr.094052.109

Alexander, D. H., Shringarpure, S. S., Novembre, J., & Lange, K. (2015). *Admixture 1.3 Software Manual*. Los Angeles.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. doi:10.1016/S0022-2836(05)80360-2

Ankney, C. D., Dennis, D. G., Wishard, L. N., & Seeb, J. E. (1986). Low Genic Variation between Black Ducks and Mallards. *The Auk*, *103*(4), 701–709. doi:10.2307/4087181

Avise, J. C., Ankney, C. D., & Nelson, W. S. (1990). Mitochondrial Gene Trees and the Evolutionary Relationship of Mallard and Black Ducks. *Evolution*, *44*(4), 1109–1119. doi:10.1111/j.1558-5646.1990.tb03829.x

Baldassarre, G. A. (2014). *Ducks, Geese, and Swans of North America*. Baltimore, Maryland: Johns Hopkins University Press.

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Bay, R. A., Harrigan, R. J., Underwood, V. Le, Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359*(6371), 83–86. doi:10.1126/science.aan4380

Bielefeld, R. R., Engilis, A., Feddersen, J. C., Eadie, J. M., Tringali, M. D., & Benedict, R. J. (2016). Is it a mottled duck? The key is in the feathers. *Wildlife Society Bulletin*, *40*(3), 446–455. doi:10.1002/wsb.665

Brown, J. I., Lavretsky, P., Wilson, R. E., Haughey, C. L., Boyd, W. S., Esler, D., … Sonsthagen, S. A. (2020). High site fidelity does not equate to population genetic structure

for common goldeneye and Barrow's goldeneye in North America. *Journal of Avian Biology, 51*(12). doi:10.1111/jav.02600

Cheng, K. M., Burns, J. T., & McKinney, F. (1982). Forced copulation in captive mallards (*Anas platyrhynchos*): II. Temporal factors. *Animal Behaviour, 30*(3), 695–699. doi:10.1016/S0003-3472(82)80140-1

Chesser, R. T., Billerman, S. M., Burns, K. J., Cicero, C., Dunn, J. L., Kratter, A. W., … Winker, K. (2020). Sixty-first Supplement to the American Ornithological Society's Check-list of North American Birds. *The Auk, 137*(3), ukaa030. doi:10.1093/auk/ukaa030

DaCosta, J. M., & Sorenson, M. D. (2014). Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. *PLoS ONE, 9*(9), e106713. doi:10.1371/journal.pone.0106713

Dale, J., Dey, C. J., Delhey, K., Kempenaers, B., & Valcu, M. (2015). The effects of life history and sexual selection on male and female plumage colouration. *Nature, 527*(7578), 367–370. doi:10.1038/nature15509

Ducks Unlimited's International Conservation Plan for Ducks Unlimited de México. (2019).

Dunn, P. O., Armenta, J. K., & Whittingham, L. A. (2015). Natural and sexual selection act on different axes of variation in avian plumage color. *Science Advances, 1*(2), e1400155. doi:10.1126/sciadv.1400155

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. doi:10.1093/bioinformatics/btq461

Ellis, N., Smith, S. J., & Roland Pitcher, C. (2012). Gradient forests: Calculating importance gradients on physical predictors. *Ecology*, *93*(1), 156–168. doi:10.1890/11-0252.1

Fitzpatrick, M. C., & Keller, S. R. (2015). Ecological genomics meets community-level

modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters*, *18*(1), 1–16. doi:10.1111/ele.12376

Fowler, A. C., Eadie, J. M., & Engilis, A. (2009). Identification of endangered Hawaiian ducks (Anas wyvilliana), introduced North American mallards (A. platyrhynchos) and their hybrids using multilocus genotypes. *Conservation Genetics*, *10*(6), 1747–1758. doi:10.1007/s10592-008-9778-8

Francis, R. M. (2017). POPHELPER: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, *17*(1), 27–32. doi:10.1111/1755-0998.12509

Galaverni, M., Caniglia, R., Pagani, L., Fabbri, E., Boattini, A., & Randi, E. (2017). Disentangling Timing of Admixture, Patterns of Introgression, and Phenotypic Indicators in a Hybridizing Wolf Population. *Molecular Biology and Evolution*, *34*(9), 2324–2339. doi:10.1093/molbev/msx169

Gauthier, G. (1988). Territorial behaviour, forced copulations and mixed reproductive strategy in ducks. *Wildfowl*, *39*, 102–114.

Grant, P. R., & Grant, B. R. (1992). Hybridization of Bird Species. *Science*, *256*(5054), 193–197. doi:10.1126/science.256.5054.193

Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, *28*(4), 1140–1162. doi:10.1016/S0003-3472(80)80103-5

Haase, E., Ito, S., & Wakamatsu, K. (1995). Influences of Sex, Castration, and Androgens on the Eumelanin and Pheomelanin Contents of Different Feathers in Wild Mallards. *Pigment Cell Research*, *8*(3), 164–170. doi:10.1111/j.1600-0749.1995.tb00658.x

Harrison, P. W., Wright, A. E., Zimmer, F., Dean, R., Montgomery, S. H., Pointer, M. A., &

Mank, J. E. (2015). Sexual selection drives evolution and rapid turnover of male gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(14), 4393–4398. doi:10.1073/pnas.1501339112

Hartman, P. J., Wetzel, D. P., Crowley, P. H., & Westneat, D. F. (2012). The impact of extra-pair mating behavior on hybridization and genetic introgression. *Theoretical Ecology*, *5*(2), 219–229. doi:10.1007/s12080-011-0117-1

Hohenlohe, P. A., Bassham, S., Etter, P. D., Stiffler, N., Johnson, E. A., & Cresko, W. A. (2010). Population Genomics of Parallel Adaptation in Threespine Stickleback using Sequenced RAD Tags. *PLoS Genetics*, *6*(2), e1000862. doi:10.1371/journal.pgen.1000862

Horton, B. M., Hudson, W. H., Ortlund, E. A., Shirk, S., Thomas, J. W., Young, E. R., … Maney, D. L. (2014). Estrogen receptor α polymorphism in a species with alternative behavioral phenotypes. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(4), 1443–1448. doi:10.1073/pnas.1317165111

Huang, Y., Li, Y., Burt, D. W., Chen, H., Zhang, Y., Qian, W., … Li, N. (2013). The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. *Nature Genetics*, *45*(7), 776–783. doi:10.1038/ng.2657

Hubbard, J. P. (1977). The biological and taxonomic status of the Mexican Duck. *New Mexico Department of Game and Fish Bulletin, No. 16*, 1–56.

Huey, W. S. (1961). Comparison of Female Mallard with Female New Mexican Duck. *The Auk*, *78*(3), 428–431. doi:10.2307/4082284

Irwin, D. E., Milá, B., Toews, D. P. L., Brelsford, A., Kenyon, H. L., Porter, A. N., … Irwin, J. H. (2018). A comparison of genomic islands of differentiation across three young avian species pairs. *Molecular Ecology*, *27*(23), 4839–4855. doi:10.1111/mec.14858

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*(14), 1801–1806. doi:10.1093/bioinformatics/btm233

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. doi:10.1093/bioinformatics/btn129

Kimball, R. T., & Ligon, J. D. (1999). Evolution of avian plumage dichromatism from a proximate perspective. *American Naturalist*, *154*(2), 182–193. doi:10.1086/303228

Kraaijeveld, K. (2014). Reversible Trait Loss: The Genetic Architecture of Female Ornaments. *Annual Review of Ecology, Evolution, and Systematics*, *45*(1), 159–177. doi:10.1146/annurev-ecolsys-120213-091550

Kraus, R. H., Kerstens, H. H., Van Hooft, P., Crooijmans, R. P., Van Der Poel, J. J., Elmberg, J., … Groenen, M. A. (2011). Genome wide SNP discovery, analysis and evaluation in mallard (Anas platyrhynchos). *BMC Genomics*, *12*(1), 150. doi:10.1186/1471-2164-12-150

Lahaye, S. E. P., Eens, M., Darras, V. M., & Pinxten, R. (2013). Hot or Not: The Effects of Exogenous Testosterone on Female Attractiveness to Male Conspecifics in the Budgerigar. *PLoS ONE*, *8*(8), e74005. doi:10.1371/journal.pone.0074005

Lande, R. (1976). Natural Selection and Random Genetic Drift in Phenotypic Evolution. *Evolution*, *30*(2), 314–334. doi:10.1111/j.1558-5646.1976.tb00911.x

Lande, R. (1980). Genetic Variation and Phenotypic Evolution During Allopatric Speciation. *The American Naturalist*, *116*(4), 463–479. doi:10.1086/283642

Lank, D. B., Coupe, M., & Wynne-Edwards, K. E. (1999). Testosterone-induced male traits in female ruffs (*Philomachus pugnax*): autosomal inheritance and gender differentiation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1435),

2323–2330. doi:10.1098/rspb.1999.0926

Lavretsky, P., Dacosta, J. M., Hernández-Baños, B. E., Engilis, A., Sorenson, M. D., & Peters, J. L. (2015). Speciation genomics and a role for the Z chromosome in the early stages of divergence between Mexican ducks and mallards. *Molecular Ecology*, *24*(21), 5364–5378. doi:10.1111/mec.13402

Lavretsky, P., DaCosta, J. M., Sorenson, M. D., McCracken, K. G., & Peters, J. L. (2019). ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. *Molecular Ecology*, *28*(10), 2594–2609. doi:10.1111/mec.15091

Lavretsky, P., Engilis, A., Eadie, J. M., & Peters, J. L. (2015). Genetic admixture supports an ancient hybrid origin of the endangered Hawaiian duck. *Journal of Evolutionary Biology*, *28*(5), 1005–1015. doi:10.1111/jeb.12637

Lavretsky, P., Hernández-Baños, B. E., & Peters, J. L. (2014). Rapid radiation and hybridization contribute to weak differentiation and hinder phylogenetic inferences in the New World Mallard complex (*Anas spp.*). *The Auk*, *131*, 524–538. doi:10.1642/AUK-13-164.1

Lavretsky, P., Janzen, T., & McCracken, K. G. (2019). Identifying hybrids & the genomics of hybridization: Mallards & American black ducks of Eastern North America. *Ecology and Evolution*, *9*(6), 3470–3490. doi:10.1002/ece3.4981

Lavretsky, P., McCracken, K. G., & Peters, J. L. (2014). Phylogenetics of a recent radiation in the mallards and allies (Aves: Anas): Inferences from a genomic transect and the multispecies coalescent. *Molecular Phylogenetics and Evolution*, *70*(1), 402–411. doi:10.1016/j.ympev.2013.08.008

Lavretsky, P., McInerney, N. R., Mohl, J. E., Brown, J. I., James, H. F., McCracken, K. G., &

Fleischer, R. C. (2020). Assessing changes in genomic divergence following a century of human-mediated secondary contact among wild and captive-bred ducks. *Molecular Ecology*, *29*(3), 578–595. doi:10.1111/mec.15343

Lavretsky, P., Peters, J. L., Winker, K., Bahn, V., Kulikova, I., Zhuravlev, Y. N., … McCracken, K. G. (2016). Becoming pure: Identifying generational classes of admixed individuals within lesser and greater scaup populations. *Molecular Ecology*, *25*(3), 661–674. doi:10.1111/mec.13487

Leipold, M., Tausch, S., Hirtreiter, M., Poschlod, P., & Reisch, C. (2020). Sampling for conservation genetics: how many loci and individuals are needed to determine the genetic diversity of plant populations using AFLP? *Conservation Genetics Resources*, *12*(1), 99–108. doi:10.1007/s12686-018-1069-1

Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. *Nature*, *475*(7357), 493–496. doi:10.1038/nature10231

Lynch, M., Ackerman, M. S., Gout, J.-F., Long, H., Sung, W., Thomas, W. K., & Foster, P. L. (2016). Genetic drift, selection and the evolution of the mutation rate. *Nature Reviews Genetics*, *17*(11), 704–714. doi:10.1038/nrg.2016.104

Malinsky, M., Trucchi, E., Lawson, D. J., & Falush, D. (2018). RADpainter and fineRADstructure: Population Inference from RADseq Data. *Molecular Biology and Evolution*, *35*(5), 1284–1290. doi:10.1093/molbev/msy023

Mckinney, F., Derrickson, S. R., & Mineau, P. (1983). Forced Copulation in Waterfowl. *Behaviour*, *86*, 250–293. doi:10.1163/156853983X00390

Nadachowska-Brzyska, K., Burri, R., Olason, P. I., Kawakami, T., Smeds, L., & Ellegren, H. (2013). Demographic Divergence History of Pied Flycatcher and Collared Flycatcher

Inferred from Whole-Genome Re-sequencing Data. *PLoS Genetics*, *9*(11), e1003942. doi:10.1371/journal.pgen.1003942

Noor, M. A. F., & Bennett, S. M. (2009, December 18). Islands of speciation or mirages in the desert Examining the role of restricted recombination in maintaining species. *Heredity*. Nature Publishing Group. doi:10.1038/hdy.2009.151

Omland, K. E. (1997). Correlated rates of molecular and morphological evolution. *Evolution*, *51*(5), 1381–1393. doi:10.1111/j.1558-5646.1997.tb01461.x

Omland, K., & Kondo, B. (2006). S18-2 Phylogenetic studies of plumage evolution and speciation in New World orioles (Icterus). *Current Zoology*, *52*, 320–326.

Owens, I. P. F., & Short, R. V. (1995). Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology & Evolution*, *10*(1), 44–47. doi:10.1016/S0169-5347(00)88967-3

Pérez-Arteaga, A., & Gaston, K. J. (2004). Wildfowl population trends in Mexico, 1961-2000: A basis for conservation planning. *Biological Conservation*, *115*(3), 343–355. doi:10.1016/S0006-3207(03)00088-0

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. doi:10.1093/molbev/msu136

Price, T. D., & Bouvier, M. M. (2002). The Evolution of F1 Postzygotic Incompatabilities in Birds. *Evolution*, *56*(10), 2083–2089. doi:10.1111/j.0014-3820.2002.tb00133.x

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P. C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics*, *81*(3), 559–575.

doi:10.1086/519795

Randler, C. (2005). Do Forced Extrapair Copulations and Interspecific Brood Amalgamation Facilitate Natural Hybridisation in Wildfowl. *Behaviour*, *142*(4), 477–488.

Rhymer, J. M., & Simberloff, D. (1996). Extinction by Hybridization and Introgression. *Annual Review of Ecology and Systematics*, *27*(1), 83–109. doi:10.1146/annurev.ecolsys.27.1.83

Ridgway, R. (1886). Preliminary Descriptions of Some New Species of Birds from Southern Mexico, in the Collection of the Mexican Geographical and Exploring Commission. *The Auk*, *3*(3), 331–331. doi:10.2307/4625406

Saunders, G. B., & Saunders, D. C. (1981). *Waterfowl and Their Wintering Grounds in Mexico, 1937-64*. Washington D.C.: US Department of the Interior, Fish and Wildlife Service.

Schweizer, M., Warmuth, V., Alaei Kakhki, N., Aliabadian, M., Förschler, M., Shirihai, H., … Burri, R. (2019). Parallel plumage colour evolution and introgressive hybridization in wheatears. *Journal of Evolutionary Biology*, *32*(1), 100–110. doi:10.1111/jeb.13401

Scott, N. J., & Reynolds, R. P. (1984). Phenotypic Variation of the Mexican Duck (*Anas platyrhynchos diazi*) in Mexico. *The Condor*, *86*, 266–274.

Slatkin, M. (1987). Gene Flow and the Geographic Structure of Natural Populations. *Science*, *236*(4803), 787–792. doi:10.1126/science.3576198

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-Based Approach to Mitochondrial Genome Sequencing in Birds and Other Vertebrates. *Molecular Phylogenetics and Evolution*, *12*(2), 105–114. doi:10.1006/MPEV.1998.0602

Sorenson, M. D., & Fleischer, R. C. (1996). Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proceedings of the National*

Academy of Sciences of the United States of America, *93*(26), 15239–15243. doi:10.1073/PNAS.93.26.15239

Species Profile for Mexican duck (Anas diazi). (n.d.). Retrieved from https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=B007

Taylor, R. S., Manseau, M., Horn, R. L., Keobouasone, S., Golding, G. B., & Wilson, P. J. (2020). The role of introgression and ecotypic parallelism in delineating intraspecific conservation units. *Molecular Ecology*, *29*(15), 2793–2809. doi:10.1111/mec.15522

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S., … Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary Applications*, *9*(7), 892–908. doi:10.1111/eva.12367

Toews, D. P. L., Campagna, L., Taylor, S. A., Balakrishnan, C. N., Baldassarre, D. T., Deane-Coe, P. E., … Winger, B. M. (2016). Genomic approaches to understanding population divergence and speciation in birds. *The Auk*, *133*(1), 13–30. doi:10.1642/AUK-15-51.1

Turner, T. L., Hahn, M. W., & Nuzhdin, S. V. (2005). Genomic Islands of Speciation in Anopheles gambiae. *PLoS Biology*, *3*(9), e285. doi:10.1371/journal.pbio.0030285

Udvardy, M. D., & Engilis, J. . A. (2001). Migration of northern pintail across the Pacific with reference to the Hawaiian Islands. *Studies in Avian Biology*, *22*, 124–132.

Via, S., & West, J. (2008). The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Molecular Ecology*, *17*(19), 4334–4345. doi:10.1111/j.1365-294X.2008.03921.x

Wells, C. P., Lavretsky, P., Sorenson, M. D., Peters, J. L., DaCosta, J. M., Turnbull, S., … Engilis, A. (2019). Persistence of an endangered native duck, feral mallards, and multiple hybrid swarms across the main Hawaiian Islands. *Molecular Ecology*, *28*(24), 5203–5216. doi:10.1111/mec.15286

Wolf, J. B. W., & Ellegren, H. (2017). Making sense of genomic islands of differentiation in light of speciation. *Nature Reviews Genetics*, *18*(2), 87–100. doi:10.1038/nrg.2016.133

Zhou, H., Alexander, D., & Lange, K. (2011). A quasi-Newton acceleration for high-dimensional optimization algorithms. *Statistics and Computing*, *21*(2), 261–273. doi:10.1007/s11222-009-9166-3

Chapter 2: Evolutionary and ecological drivers of local adaptation and speciation in a North American avian species complex

ABSTRACT

Throughout the speciation process, the genomes of diverging populations can be differentially impacted by selective pressures, as well as rates of gene flow and genetic drift. Disentangling the effects of these evolutionary mechanisms throughout the divergence process remains challenging, especially for non-model organisms. Accounting for complex evolutionary histories and contemporary population structure often requires sufficient sample sizes for which the expense of full genomes remains prohibitive. Here, I demonstrate the utility of coupling evolutionary models with partial-genome sequence data for range-wide samples to shed light into the divergence process of two closely related ducks, the Mexican duck (*Anas diazi*) and mallard (*A. platyrhynchos*). I determine the role of selective and neutral processes in the speciation process of these ducks by integrating evolutionary and demographic modelling with genotype-environment (GEA) and genotype-phenotype association testing. First, evolutionary models and GEA analyses support the hypothesis that Mexican ducks originally diverged from mallards during a glacial period in a local climate refugia in southwestern North America, and that subsequent ecological partitioning via differing environmental selective pressures played a key role in divergence. In fact, demographic analyses revealed that the two species retained an identical ancestral effective population size until they diverged ~300,000 years ago. While mallards continuously increased in effective population size, Mexican ducks showed cyclical patterns that likely reflected repeated range expansions and contractions, along with bouts of gene flow with mallards during glacial cycles. Finally, I provide evidence that sexual selection acted on several phenotypic traits as a co-evolutionary process, facilitating the development of reproductive barriers that initially arose due to strong ecological selection. More broadly, this work reveals that

the genomic and phenotypic patterns observed across species complexes are the result of myriad factors that contribute in dynamic ways to the evolutionary trajectories of a lineage.

INTRODUCTION

During the earliest stages of speciation, the various mechanisms of evolution (i.e., selection, and gene flow) can differentially impact genomes among populations in response to changing environmental conditions (Byers, Xu, & Schlüter, 2017; Harvey, Singhal, & Rabosky, 2019; Tobias, Ottenburghs, & Pigot, 2020). While such heterogeneous pressures can often cause local adaption (Lenormand, 2012; Meier, Marques, Wagner, Excoffier, & Seehausen, 2018; Rundle & Nosil, 2005), whether these adaptive differences result in speciation largely depends on the balance between selection and other factors such as effective population size and gene flow (Martin & Pfennig, 2009; Payne, Polechová, & Payne, 2020; Savolainen, Lascoux, & Merilä, 2013). Given that pre-zygotic reproductive barriers are slow to develop during the earliest stages of divergence (Kautt et al., 2020; T. D. Price & Bouvier, 2002), pervasive gene flow can act to homogenize the genome and swamp out locally adaptive alleles (Kautt et al., 2020; Tigano & Friesen, 2016). In addition, demographic processes (e.g., population bottlenecks) can stochastically decrease the frequency of adaptive traits due to the differential impacts of drift (Allendorf, 1986; Chen et al., 2019). Recent species radiations – particularly those adapted to more extreme habitats (e.g., deserts) – provide unique opportunities to assess how strong, yet varied evolutionary pressures influence the speciation process (Nevo, 2011; Tobler, Kelley, Plath, & Riesch, 2018). Landscape-level population sampling in conjunction with genomic data is often required to uncouple the influence of these varied evolutionary forces on the species divergence process (Ellegren, 2014).

Here, I integrate evolutionary and demographic modelling with genotype-environment and genotype-phenotype association testing to determine the role of selective (e.g., environmental and sexual) versus neutral (e.g., demographic) processes in driving divergence between mallards (*Anas*

platyrhynchos) and their closely related sister species, the Mexican duck (*Anas diazi*). Mexican ducks are hypothesized to have diverged from mallards within the last 500,000 years as they became isolated in a glacial refugia and adapted to the arid habitats of southwest North America (Kulikova et al., 2005; Lavretsky et al., 2015; Lavretsky, Hernández-Baños, & Peters, 2014). However, incomplete lineage sorting and the potential for hybridization has made it challenging to reconstruct their true evolutionary history (Lavretsky, Hernández-Baños, et al., 2014; Lavretsky, McCracken, & Peters, 2014). In fact, a high prevalence of mallard-like morphological traits found in some Mexican duck populations once resulted in the inference of pervasive hybridization with mallards (Hubbard, 1977); however, recent genomic work found this to be incorrect, and rather determined that the retention of such traits in Mexican ducks was due to ancestry (Brown et al., *in review*; Lavretsky et al., 2015). Additionally, Lavretsky et al. (Lavretsky, DaCosta, Sorenson, McCracken, & Peters, 2019) found that while a significant proportion of the genome was shared between these taxa, several locations were under divergent selection in either the mallard (i.e., Z-sex chromosome and autosomal chromosomes 1-4) or Mexican duck (Chromosome 14), which suggests that these regions may be associated with traits responsible for maintaining reproductive barriers. Overall, the rapid divergence of these two species caused by extreme environmental conditions provides an excellent study system for disentangling the effects that different evolutionary mechanisms have on the process of speciation.

Though speciation research has often focused on how genomic barriers develop (Cruickshank & Hahn, 2014; Feder, Egan, & Nosil, 2012; Turner, Hahn, & Nuzhdin, 2005; Wolf & Ellegren, 2017), relating these barriers to non-genic (e.g., environment and phenotype) variables is an essential next step in truly understanding the speciation process (Huang, Huang, Huang, & Liao, 2017; Seehausen et al., 2014; J. Wang, Street, Park, Liu, & Ingvarsson, 2020). Whereas

ecological niche models have traditionally been used to approximate a species' relationship with environmental variables (Sillero, 2011), these methods fail to consider the underlying intra-specific genomic variation and local adaptation driving these connections (Layton et al., 2021; Razgour et al., 2019). Instead, using genotype-environment associations (GEA) to model genetic niche space has been shown to improve forecasting (Capblancq, Fitzpatrick, Bay, Exposito-Alonso, & Keller, 2020; Rhoné et al., 2020), as these methods allow for the exploration of how genomic diversity is influenced by the environment (Razgour et al., 2019; Smith, Godsoe, Rodríguez-Sánchez, Wang, & Warren, 2019; T. Wang, O'Neill, & Aitken, 2010). Additionally, by estimating 'genomic offset' or 'genomic vulnerability' – the degree of mismatch between genomic variation modelled under current versus past or future climate conditions – GEA approaches are being applied to approximate species' historical and future genetic niche spaces (Bay et al., 2018; Fitzpatrick & Keller, 2015; Ruegg et al., 2018). In particular, understanding how contemporary genetic variation is related to historical climate conditions can shed light into the origins of intra-specific adaptation and fine-scale population structure (Bemmels, Title, Ortego, & Knowles, 2016; Theodoridis et al., 2020; Yannic et al., 2014).

In this study, I demonstrate how environmental selection, gene flow, and demographic patterns acted both individually and jointly through time to initiate divergence between these two species, and that secondarily, sexual selection has proceeded to reinforce reproductive barriers. First, I test among evolutionary scenarios, and estimate divergence time, rates of gene flow, and effective population size between Mexican ducks and mallards. I also estimate time-series demographic models for both species (Brown et al., *in review*) to understand the influence of North American glacial cycles on fluctuations in demographic history. Next, I implement a multivariate machine-learning program, gradient forest (GF), to model the relationship between allele

frequency changes and environmental variables. In addition to reconstructing the contemporary adaptive landscape for each of the species, I identify unique genetic niche space using a model that combines genomic information from each species. I then hindcasted the model of genotype turnover for Mexican ducks across historic climate conditions, to identify the circumstances under which Mexican ducks may have speciated. Next, given that sexual selection often acts secondarily on phenotypic differences arising from environmental selection, I use redundancy analysis (RDA) to measure the effects of genetic diversity on phenotypic variation found across Mexican ducks and mallards. I use this as a proxy for measuring whether sexual selection has been an important aspect of assortative mating and has played a significant role in maintaining species boundaries since divergence. Finally, I estimated the adaptive potential of Mexican ducks by projecting their GF model across future climate conditions, identifying areas where they are vulnerable to future climate change.

METHODS

Sampling

A total of 208 and 64 samples representing the North American ranges of Mexican ducks and mallards, respectively, were included in analyses (Supplementary Materials Table S2.1). I used published ddRAD-seq data for Mexican ducks (BioProject PRJNA516035, Lavretsky et al., 2015; Brown et al., *in review*) collected from the southwest US (N = 40); Chihuahua, Mexico (N = 65); the western coast of Mexico (i.e., Sinaloa and Sonora; N = 63), and interior Mexico (i.e., Puebla, Durango, Zacatecas, Mexico, Guanajuato; N = 40). In order to limit the effects of recent introgression with mallards (Brown et al., *in review*) on GEA and demographic analyses, only Mexican ducks of pure ancestry were included (Bay et al., 2018; Fitzpatrick & Keller, 2015). In

addition, only ddRAD-seq data published for wild mallards collected from North American breeding grounds were used (BioProject PRJNA516035, Lavretsky et al., 2015; Brown et al., *in review*).

Gene flow at an evolutionary scale

First, I used the diffusion approximation program, ∂a∂i (Gutenkunst, Hernandez, Williamson, & Bustamante, 2010), to test empirical data against specified evolutionary models of divergence between Mexican ducks and mallards. Briefly, ∂a∂i uses a diffusion approximation approach to create a model SFS based on a specified evolutionary scenario to test against an empirical SFS. I calculated the best fit model for comparisons between mallards and all Mexican ducks. Using custom python scripts I created a folded two-dimensional SFS for each pairwise comparison that was then projected down to account for missing data and a lack of shared variants – Mexican ducks (N = 325 alleles) and mallards (N = 100 alleles. I tested empirical data against five different evolutionary models including Isolation-with-Migration, Isolation-without-Migration, Split-Migration (i.e., secondary contact), Split-without-Migration, and Neutral-No-Divergence. I determine the best-fit model based on the best log-likelihood of the optimal parameters across five replicates of each model. I then performed 50 independent parameter optimization runs and used the geometric mean of these results as the final optimal parameters, as well as to calculate uncertainty metrics (i.e., standard deviation; Coffman, Hsieh, Gravel, & Gutenkunst, 2016; Gutenkunst, Hernandez, Williamson, & Bustamante, 2009). Evolutionary models in ∂a∂i simultaneously estimate time since divergence ($t = T$ x $2N_{ANC}$; t = time since divergence in generations), contemporary ($N_i = \nu_i$ x N_{ANC}) and ancestral ($\theta = 4N_{ANC}$ x μ; N_{ANC} = Ancestral effective population size) effective population sizes, and migration rates ($m_{i \leftarrow j} = M_{i \leftarrow j}$/

($2N_{ANC}$); $m_{i \leftarrow j}$ = proportion of migrants/generation in population *i* from population *j*) using a scaling factor θ (Gutenkunst et al., 2009).

To convert parameter estimates from ∂a∂i demographic models into biologically informative values, I estimated generation time (G) and overall mutation rates (μ). First, generation time (G) is calculated as $G = \alpha + (s/(1-s))$, where α is the age of maturity and *s* is the expected adult survival rate (Sæther et al., 2005). For both Mexican ducks and mallards, I used an age of maturity (α) of 1 (Baldassarre, 2014). Additionally, given that data on Mexican duck survival is lacking, I used the adult survival (*s*) as estimated in mallards ($s = 0.574$; Reynolds, Blohm, Nichols, & Hines, 1995). Finally, to obtain a scaled mutation rate for autosomal markers, I started with a mutation rate of 1.2×10^{-9} substitutions/site/year, which is considered to be the mean nuclear mutation rate for various mallard complex species (Peters, Roberts, Winker, & McCracken, 2012; Peters et al., 2014). This mutation rate was then scaled to the generation time for each species ($G = 2.35$) before being multiplied by the total number of base pairs ($N = 270{,}895$) to get a final mutation rate scaled to substitutions/site/generation (s/s/g).

Modelling demographic history through time

Long-term demographic histories for Mexican ducks and mallards were estimated following the approach of Hernández, Brown, Kaminski, & Lavretsky, *in review*), which uses ∂a∂i to model changes in effective population size through time. Briefly, I used custom python scripts (scripts available at https://github.com/jibrown17/Dove_dadi.demographics; Hernández et al., *in review*) to calculate a one-dimensional site-frequency spectrum (SFS) from Nexus formatted concatenated sequencing data. Each species' SFS was folded and masked (Gutenkunst et al., 2010; Gutenkunst et al., 2009; Hernández et al., *in review*) before being projected down to account for

missing data between groups (N_{MEDU} = 400 alleles, N_{MALL} = 140 alleles). Next, based on a custom demographic model (Hernandez et al. *in review*; https://github.com/jibrown17/Dove_dadi.demographics) that uses 100 iterations of the single population integration function ('*Integration.one_pop*' in ∂a∂i), ∂a∂i creates a model SFS which is used to estimate the optimum parameters of effective population ($N_n = \nu_n$ x N_{Anc}, N_n = effective population size at the n^{th} time interval) and time intervals ($t_n = T_n$ x 2 x N_{Anc} x G, t_n = total years before present at the n^{th} time interval & G = generation time) for each integration step. These optimum parameters are then scaled to the empirical data using θ ($\theta = 4N_{ANC}$ x μ; N_{ANC} = Ancestral effective population size), which is then used to calculate the actual effective population size through time (for detailed methods on custom demographic models see Hernández et al., *in review*). I then used the geometric mean calculated across 50 replicates of parameter optimization for each model and estimate the goodness of fit for each model by calculating log-likelihood of the model given the empirical data. Finally, I estimated confidence intervals (CI) using parameter uncertainty metrics included in ∂a∂i (Coffman et al., 2016; Gutenkunst et al., 2009). Uncertainty metrics were calculated across a range of step sizes ($\varepsilon = 10^{-2} - 10^{-7}$) in order to maximize the number of parameters for which ∂a∂i is able to return a true estimate of uncertainty (Blischak, Barker, & Gutenkunst, 2020; Coffman et al., 2016; for detailed methods on uncertainty metrics see Hernández et al., *in review*). I converted each ∂a∂i parameter into biologically informative values as described above.

Genotype-environment association modelling with gradient forest

For GEA testing, I obtained contemporary environmental data that is available at a high resolution from several public databases. I chose a total of 27 environmental variables that are

thought to have impacts on bird physiology and ecology, and that might be strong drivers of adaptation (Supplementary Materials Table S2.2; Bay et al., 2018).

I downloaded and used as predictors a suite of 19 climate variables from the WorldClim v. 1.4 database (https://www.worldclim.org/version1, 30 arc-second (~1 km) resolution; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005); Landsat Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), and Net Primary Productivity (NPP) data from the USGS AppEEARS database (https://lpdaacsvc.cr.usgs.gov/appeears); and elevation data from the Global Land Cover Facility (http://www.landcover.org). In order to differentiate the effects of annual vs seasonal vegetation processes, I calculated an average annual, summer (June), and winter (December) value for NDVI and EVI based on data collected from 2000 – 2019.

Following the approach of Bay et al. (Bay et al., 2018; Fitzpatrick & Keller, 2015), I used a GF analysis as implemented by the R package gradientForest (Ellis, Smith, & Roland Pitcher, 2012) to test which environmental predictor variables most strongly explain patterns of allele frequency turnover in Mexican ducks and mallards. GF analysis was originally created to detect the effects of environmental predictor variables on species turnover across a landscape (Ellis et al., 2012), but has since been adapted for measuring allele frequency turnover (Bay et al., 2018; Fitzpatrick & Keller, 2015). Briefly, GF uses a machine learning regression tree-based algorithm (i.e. random forests) to detect shifts in allele frequency across an environmental gradient, where a function is built for each individual SNP (the response) before an aggregate function is created for all SNPs across each independent predictor variable (Fitzpatrick & Keller, 2015).

For use in GF, I converted SNP data into minor allele frequencies using the package PopGenome (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014) in the program R and subsequently filtered any SNP that was polymorphic in fewer than five total sampling sites

(Fitzpatrick & Keller, 2015). Using a large number of trees (N = 5,000), GF produced an R^2 ranked list of weighted importance for all environmental variables. To assess the performance of actual GF models for both species, and to rule out the chance that results were influenced by spurious correlations, the environmental predictor data was randomized in relation to sampling sites. I then compared the performance of 100 models created with randomly generated data to the observed model. To visualize the GF model for each species across North America, I extracted values for the top five environmental variables from random points generated across their home range. I then used a PCA to summarize the transformed values from the top five predictor variables (based on R^2 weighted importance) for each point. Finally, I transformed the top three principal components to create a RGB color scale that was used to visualize different patterns of adaptive genetic diversity across the landscape. In the end, colors reflect associations between allele frequencies and the environmental predictor variables that allow me to draw conclusions about how the environment has affected genetic diversity and putatively driven adaptation.

Finally, in a novel use of GF analysis, I combined independent species' models of allele frequency turnover to test for partitioning of genetic niche space between species. Briefly, the *combinedGradientForest* function in R acts to standardize independent models to one another by calculating a combined function of cumulative importance, which represents the overall relationship in both species between allele frequency turnover and the environmental predictor variables. Additionally, during standardization, cumulative importance functions for each variable are weighted based on the total R^2 value of the combined gradient forest. As previously described, I visualized the combined model by predicting the GF object across geographic space before converting the PCA into a standardized RGB color scale.

Modelling Past and Future Patterns of Diversity

In order to estimate the potential threat of climate change to Mexican ducks, I used GF to model GEA across future climate conditions and subsequently measure the genetic offset (i.e., Euclidean distance between contemporary and future GF models) from current GEA space (Bay et al., 2018; Fitzpatrick & Keller, 2015). Additionally, I extended this method to measure the offset between contemporary and historical patterns of genetic diversity in order to better understand evolution and adaptation of Mexican ducks through time. Future and past bioclimatic variables from Global Climate Models (GCM) were downloaded at the highest available resolution: Last inter-glacial (~130 ky BP; 30 arc-second resolution; Otto-Bliesner, Marshall, Overpeck, Miller, & Hu, 2006); Last Glacial Maximum (~22 ky BP; CCSM4 at 2.5 arc-minute resolution; Hijmans et al., 2005); Mid-Holocene (~6 ky BP; CCSM4 at 30 arc-second resolution; Hijmans et al., 2005); two future (2070) scenarios of climate change (rcp2.6 and rcp8.5; CCSM4 at 30 arc-second resolution; Hijmans et al., 2005).

RDA phenotype-genotype association testing

Using a subset of individuals (N_{MEDU} = 165; N_{MALL} = 6; Supplementary Materials Table S2.1) for which phenotypic data was available, I used Redundancy Analysis (RDA) to test for associations between genotypes and phenotypic traits that could be important for assortative mating. The traits used here were identified by Brown et al. (Brown et al., *in review*) as significant for differentiating pure Mexican ducks from mallards and their hybrids (for a detailed description of each trait see Brown et al., *in review*). I compared all samples and each sex separately using a concatenated Autosomal+Z-sex chromosome dataset. Additionally, I also tested for significant

associations within the Z-sex chromosome only as this region was predicted to be linked to phenotypic traits between these two species (Lavretsky et al., 2015).

Following the procedure of Talbot et al. (Talbot et al., 2017), I used a Principal Components Analysis (PCA) to summarize genotypic variability from bi-allelic SNP loci and reduce the number of predictor variables to be included in RDA. Briefly, bi-allelic SNPs were filtered for linkage-disequilibrium in PLINK v1.07 (Purcell et al., 2007) and reformatted as a STRUCTURE file. I then performed a PCA in the R package *Adegenet* (Jombart, 2008) with the '*dudi.pca*' function; I kept the top 50 PCs (hereafter referred to as genotypic PCs) for association testing with RDA. For each of the datasets described, I tested the effect of genotypic PCs on phenotypic variability using the '*rda*' function in the R package *vegan* (Dixon, 2003; Legendre & Gallagher, 2001). To account for bias created by population structure, I assigned each individual sample a value of 1-5 that corresponded to previously identified genetic clusters (Brown et al., *in review*; Lavretsky et al., 2015), and used this variable as a confounding factor. Here, RDA was used to account for multiple response variables and provided an estimate of the effect of genotypic variation on the phenotypic traits as a whole (Talbot et al., 2017). Additionally, I identified individual traits within the tails of the distribution for RDA loadings ($\alpha = 0.1$), as well as the genotypic PCs most strongly associated with them. I then returned to the initial genetic PCA to count the number of SNPs found in the tails of the distribution of significant genotypic PCs ($\alpha = 0.05$). Finally, I used the '*envfit*' function from the R package *vegan* (Dixon, 2003) to test if these individual genotypic PCs identified by RDA significantly explain the response variables.

RESULTS

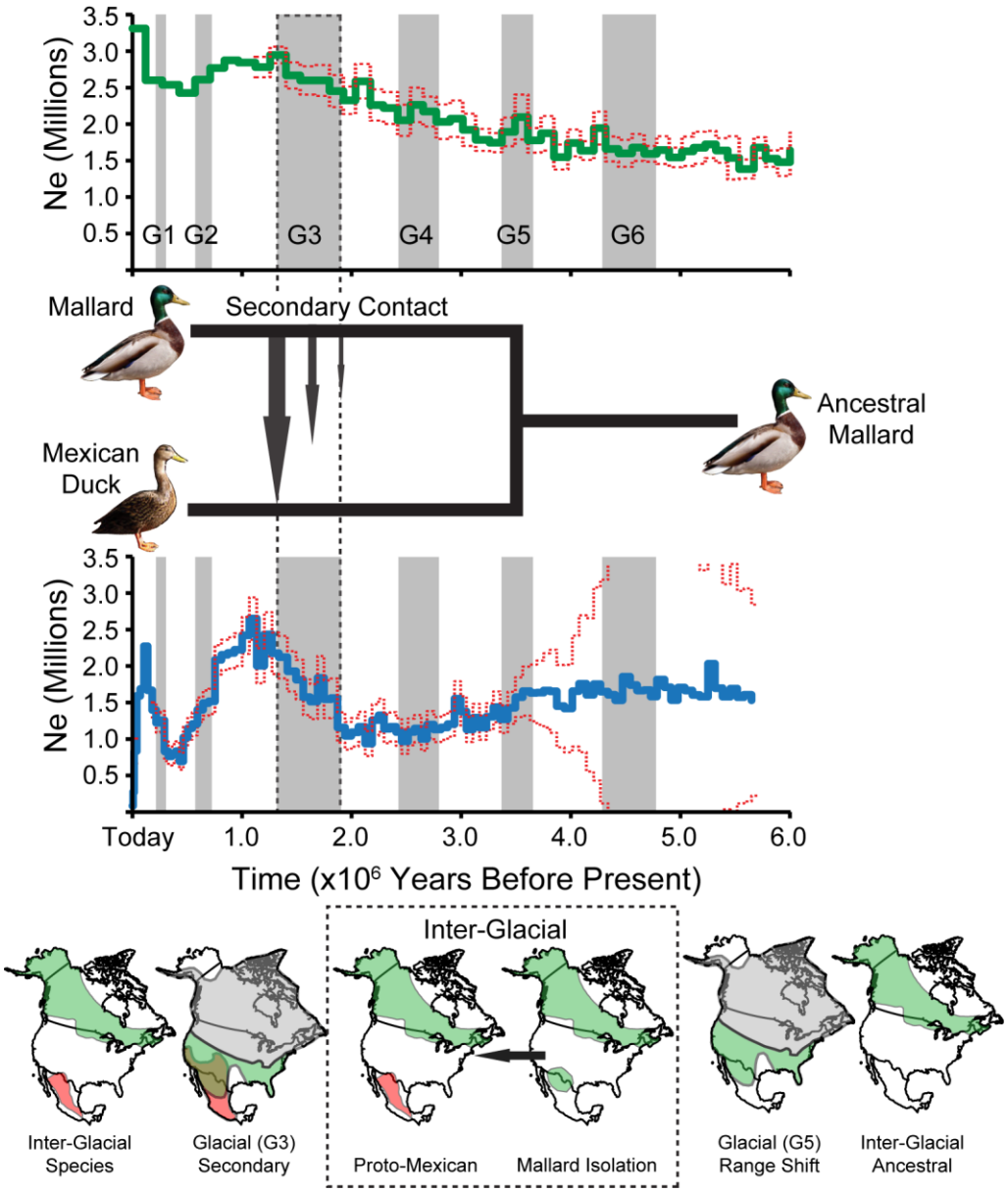


Figure 2.1. Time-series ∂a∂i demographic models for Mexican ducks and mallards. Dotted red lines indicate 95% confidence intervals. Glacial and inter-glacial periods are denoted in grey and white bars, respectively, with glacial advancements also identified (Batchelor et al., 2019). An evolutionary model of how Mexican ducks and mallards diverged, including how glacial cycles impacted this process is overlaid.

∂a∂i evolutionary models

∂a∂i analyses of autosomal SNPs supported an optimum evolutionary model of split-with-migration (Supplementary Materials Table S2.3). Parameters were converted into biologically informative values using a mutation rate of 7.63 x 10^{-4} s/s/g. First, ancestral N_e for Mexican ducks and mallards ($N_{e\ anc}$ = 451,092; 95% CI = ± 11,032) was lower than contemporary estimates (Supplementary Materials Table S2.3). Contemporary effective population size of mallards (N_e = 1,372,661; 95% CI = ± 97,899) is ~2 times larger than Mexican ducks (N_e = 608,035; 95% CI =± 21,590). Divergence time between Mexican ducks and mallards was 995,227 years before present (YBP) (95% CI = ± 25,796), which was nearly three times those estimated from previous studies (Lavretsky et al., 2015, 2019). Finally, with bi-directional gene flow assumed in the split-with-migration model and in terms of chromosomes/generation, I scaled these values to the N_e of each population to get the number of migrants/generation. Migration was significant in both directions, but with estimates of migration from mallards into Mexican ducks ($m_{MALL\text{-}MEDU}$ = 20; 95% CI = ± 0.60) being ~2 times higher than from Mexican ducks into mallards ($m_{MEDU\text{-}MALL}$ = 9; 95% CI = ± 0.27).

∂a∂i demographic modelling

Models of demographic history estimated N_e up to at least 500,000 YBP (Supplementary Materials Table S2.1), and demarcated distinct demographic histories for mallards and Mexican ducks. First, I find that mallards retained an N_e of ~1.6 million individuals (95% CI = ~1,500,000 - ~1,900,000) until ~500K YBP and have since experienced an exponential increase to a contemporary N_e of ~3.3 million individuals (Figure 2.1). For Mexican ducks, I recovered cyclical trends in their N_e. Specifically, Mexican ducks retained a consistent N_e of ~1.6 million individuals

(95% CI = 0 - ~2,000,000) that broadly overlapped mallards until ~350,000 YBP. At this time, Mexican ducks diverged in N_e by declining slightly until ~200,000 YBP, followed by a gradual rise to ~2.5 million individuals; this was nearly identical to the N_e of mallards at that time point (Figure 2.1, see discussion). Finally, this increase was followed by two distinct bottleneck events that occurred within the last 100,000 years, with the most recent one providing a contemporary estimate of ~130,000 Mexican ducks (Figure 2.1).

Genotype-environment association modelling

The GF model for Mexican ducks found associations between genotype and environment in 1,005 out of 9,158 SNPs (11.0% of SNPs had a positive R^2 value), while the mallard model recovered 410 out of 4,386 SNPs (9.3% of SNPs had a positive R^2 value). The number of SNPs with positive R^2 values (N = 1,005) and the mean R^2 value (N = 0.134) for the Mexican duck dataset was consistently greater than the upper 95% quartile of values from randomized datasets (Supplementary Materials Figure S2.2), indicating that associations between genotypes and environmental variables reported in the model are not spurious. While GF analysis for mallards was not significant compared to randomized models (SNPs with positive R^2 value: N = 410; mean R^2 value: N = 0.114), there were a number of loci (N = 172) with an R^2 value greater than that of the average for the randomized data (Supplementary Materials S2.2). Finally, the combined Mexican duck x mallard GF model did have more SNPs with positive R^2 values (N = 1,450) and a mean R^2 value (N = 0.125) higher than that of the combined randomized datasets, indicative of a model that represented real genotype/environment associations across both species' ranges.

Of the top five environmental predictors identified by GF for Mexican ducks, three were related to seasonal changes (Bio15, NDVI-winter, Bio11), while the second most predictive

variable was elevation (Figure 2.2). Variables related to precipitation, geography, vegetation, and temperature were all found to be important, suggesting that a variety of environmental factors play a role in driving Mexican duck allele frequencies across their range (Supplementary Materials Figure S2.3). While GF models for mallards did not perform better than the randomized models, three of the top five variables were similarly related to seasonal changes (Bio10, Bio5, EVI-winter); although temperature and vegetation growth played a more important role in driving genotype frequencies than precipitation (Supplementary Materials Figure S2.3).

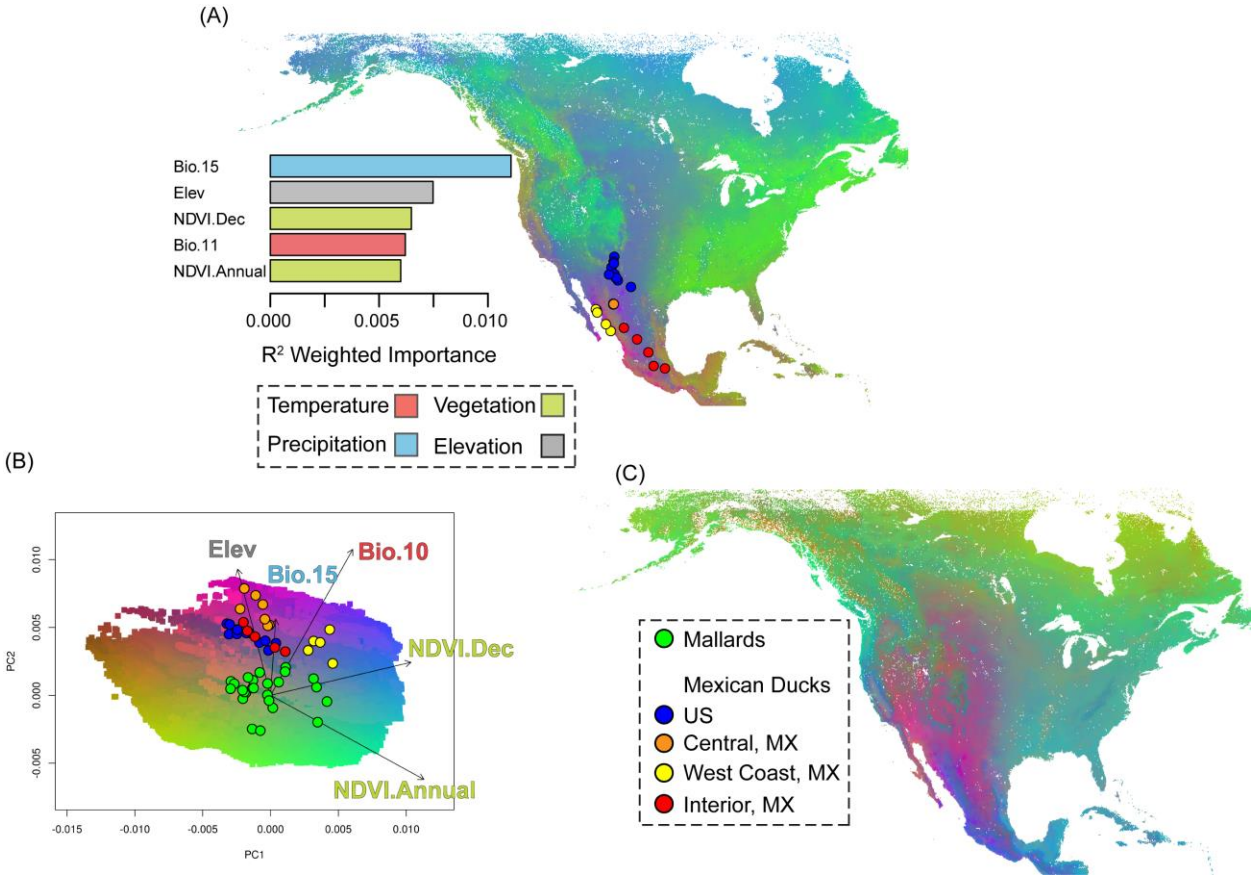


Figure 2.2. Genotype environment association models from gradient forest (GF) mapped across North America for (A) Mexican ducks. Inset represents the top five most predictive environmental variables based on cumulative R^2 weighted importance. The combined Mexican duck and mallard GF model (B) PCA and (C) map.

Graphing principal components of GF outputs for Mexican ducks showed a signal of local adaptation, with different environmental predictors having the strongest effects in different populations (e.g., allele frequencies in west coast samples are more strongly affected by the temperature variable Bio11; Supplementary Materials Figure S2.4). I then generated a map of environmentally associated allelic turnover across North America based on the PCAs for Mexican duck, mallard, and combined GF models (Figure 2.2). In Mexican ducks, GF showed the most significant genomic turnover outside of its native range (i.e., the rocky-mountain region, the central Canadian prairies, and the eastern US). More subtle differences that correspond with population structure occurred within its range in the central highlands of Mexico (i.e., Southwestern USA and Chihuahua, Mexico) and along the western coast of Mexico (i.e., Sinaloa and Sonora, Mexico). Alternatively, mallards show minimal genotype turnover across their primary breeding grounds in central Canada, which is reflected by the tighter clustering of samples in GF PCA results (Supplementary Materials Figure S2.4). Finally, the combined GF model showed significant genotype turnover concordant with the native range of Mexican ducks in southwestern North America, and with the combined PCA clearly partitioning GEA space between the two species. Together, this suggests that environmental selective pressures are differentially driving divergence in allele frequencies between Mexican ducks and mallards.

Genotype-phenotype association testing

RDA analysis showed a significant effect of genotypic PCs on phenotypic traits for the full dataset as well as in the male-only datasets; there were no significant effect found in females (Table 2.1). All associations were significant when accounting for population structure (Table 2.1). All of the concatenated Autosomal/Z-sex chromosome datasets as well as the Z-sex chromosome male

only dataset had a similar percentage of genetic markers identified as being significantly associated with phenotypic variation (12.6% – 13.6%), which was ~3 times the percentage identified within female Z-sex chromosome markers (4.7%). Phenotypic traits identified as significant varied across datasets, with male variation generally being associated with wing characteristics and females being characterized by a mix of traits on the wing, head, and belly (Supplementary Materials Table S2.4; Figure S2.7). Finally, strong partitioning between Mexican duck and mallard samples plotted along RDA axes showed that genetic variation within these species can explain at least a portion of their phenotypic variation (Supplementary Materials Figure S2.8).

Genomic offset from past and future climate conditions

Using only the top five temperature and precipitation variables (Bio4, Bio8, Bio11, Bio15, Bio18) I subtracted GF modelled allele frequencies under contemporary climate from differing historic and future climate conditions, to get a measure of genomic offset (the difference between genomic variation as related to environment through time) in Mexican ducks (Figure 2.3; Supplementary Materials Figure S2.5). Models of genotype turnover for Mid-Holocene (~6,000 YBP) climate conditions resembled contemporary models, with the only noticeable offset being predicted along the western coast of Mexico (Supplementary Materials Figure S2.6). Next, there is significant offset from the Last Glacial Maximum (LGM; ~22,000 ybp) and the Last Inter-glacial (LIG; ~130,000 ybp) periods. During the LGM, genetic offset identified a significant increase in the Mexican duck's southwestern US range where their genetic diversity is associated with favorable environmental conditions. In contrast, LIG climate conditions likely caused a restriction in adaptive niche space for Mexican ducks in the northern and eastern parts of its contemporary range, leaving their core adaptive range restricted to deep interior Mexico. Finally, genetic offset

from future climate (2070 rcp2.6 & rcp8.5) identified severe habitat loss under the most severe climate change conditions only (rcp 8.5), with habitats along the western coast of Mexico and in the central Mexican highlands of Chihuahua to be most impacted (Figure 2.3; Supplementary Materials Figure S2.5 & S2.6).

Table 2.1. Significance and R2 of RDA testing for phenotype-genotype associations. Bold values indicate a model where genetic markers explain a significant proportion of the phenotypic variation (P-value ≤ 0.05).

	Aut & Z Chromosomes	Aut & Z Chromosomes Males	Aut & Z Chromosomes Female	Z Chromosome Males	Z Chromosome Females
P-value	**0.005**	**0.015**	0.843	**0.039**	0.185
*Conditional R^2	0.1282	0.1463	0.20445	0.1463	0.205
**Constrained R^2	0.3492	0.4585	0.769	0.433	0.791
# of Predictive SNPs	1178 (12.6%)	1247 (13.2%)	1082 (13.0%)	30 (13.6%)	11 (4.7%)

*Proportion of the variation explained by conditional variables (i.e., population structure)

**Proportion of the variation explained by constrained axes

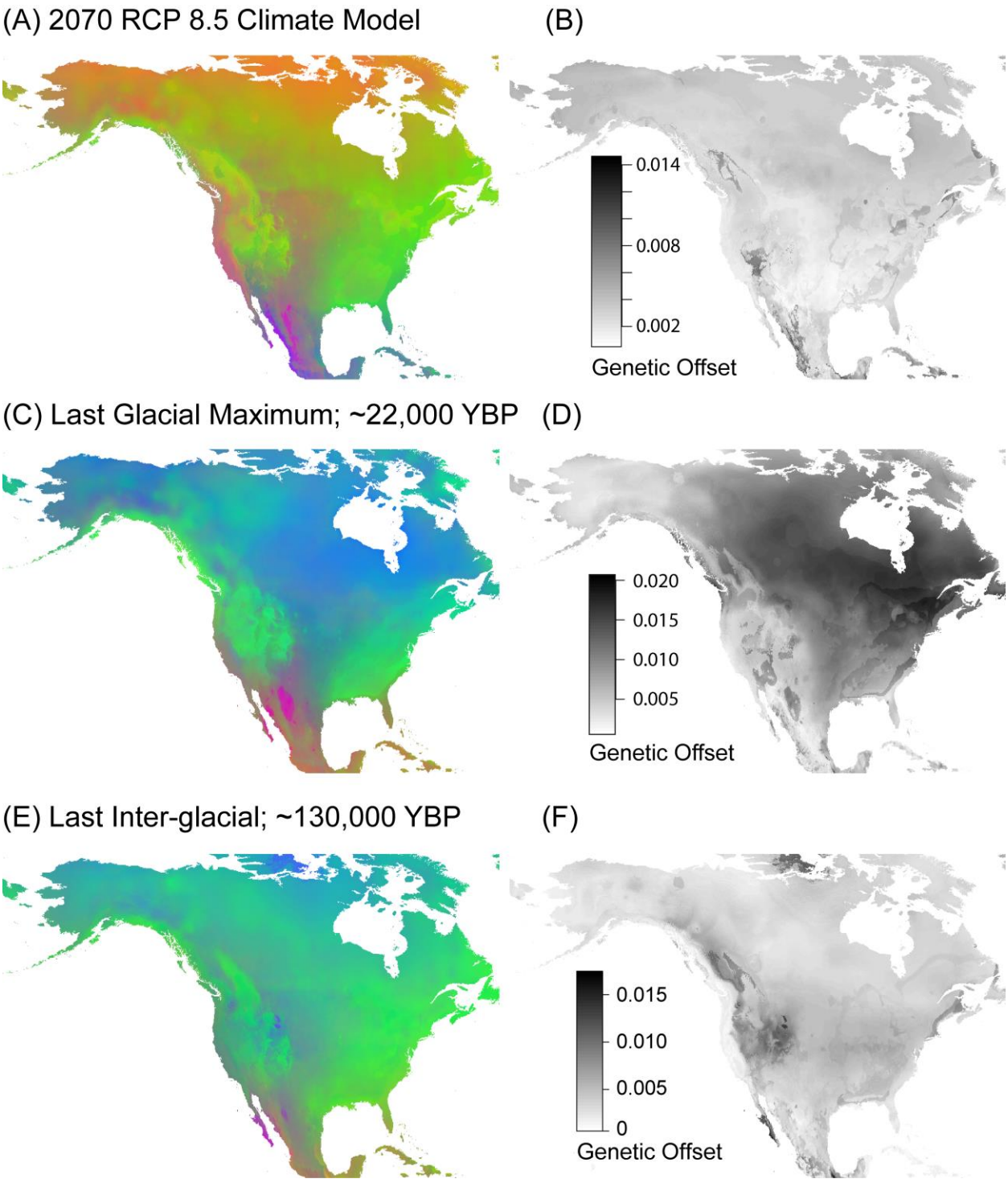


Figure 2.3. Mexican duck genotype-environment association models from GradientForest (GF) based on only the top five most predictive temperature and precipitation variables. Associations are modelled across historic and future environmental data for (A) 2070 under the most extreme (RCP 8.5) projections of climate change, (C) the Last Glacial Maximum (~22,000 YBP), and (E) the Last Inter-glacial (~130,000 YBP). (B, D, F) Genomic offset calculated from the Euclidean distance between models based on contemporary and historic climate conditions mapped across North America.

DISCUSSION

Glacial cycles induce divergence, secondary contact, and range shifts between two closely related species of ducks

While vicariance resulting from glacial advancement has been directly responsible for the diversification of many North American avian taxa (Johnson & Cicero, 2004; Licciardi, Clark, Brook, Elmore, & Sharma, 2004; Weir & Schluter, 2004), arid habitats of the Southwest were indirectly impacted by heterogeneous climate conditions across the landscape (Hewitt, 2000; Hewitt, 1996). This kind of environmental heterogeneity would result in habitat fragmentation, subsequently giving rise to isolated 'climate refugia' (Gavin et al., 2014; Jaeger, Riddle, & Bradford, 2005). Taxa with wide distributions and high adaptive potential (e.g., mallards) are more likely to use these fragmented climate refugia during glacial periods (Douglas, Douglas, Schuett, & Porras, 2006; Stewart, Lister, Barnes, & Dalén, 2010), which can often result in rapid divergence in isolation (Hewitt, 2000; Stewart et al., 2010). First, this study further supports the importance of these climate refugia in southwestern North American species divergence. In doing so, I provide concordant evidence for the hypothesis that Mexican ducks originated when strong selective pressures allowed a subset of isolated mallards to persist within this southwestern climate refugia after glacial retreat (Figure 2.1). In particular, mapping combined genetic niche space across North America not only recapitulated current breeding ranges of both species, but also demarcated significant genotypic turnover in transition zones of their distributions (Figure 2.2). Such partitioning in genetic niche space indicates that ecologically-driven divergent selection was an important force that differentially impacted the genetic diversity of Mexican ducks and mallards.

In reconstructing the evolutionary history of the Mexican duck, all analyses support a scenario in which Mexican ducks and mallards diverged in the last 500,000 years but have come

into secondary contact one or more times since. Such an evolutionary scenario is supported by both species' comparative (Supplementary Materials Table S2.3) and species-specific demographic (Figure 2.1) models. Specifically, effective population sizes remained identical until ~350,000 YBP (Figure 2.1), a time of divergence that is identical to earlier estimates (Lavretsky, Hernández-Baños, et al., 2014), suggesting that this time-period was when a proto-Mexican duck population first began to evolve independently from an ancestral mallard population. At this time, the advancing Laurentide ice sheet likely initiated species divergence by creating isolated pocket(s) of mallards in known southwestern North American glacial refugium (Figure 2.1; Hernández et al., *in review*; Sarabia, VonHoldt, Larrasoaña, Uríos, & Leonard, 2020). Although I am unable to model genomic turnover for this particular glacial period when the Mexican duck's demographic history diverged from mallards, climate patterns likely resembled those during the LGM (~22,000 ybp; Berger et al., 2016). And in fact, GF models of the LGM show high genomic offset for Mexican ducks throughout much of northern and eastern North America, and identify southwestern North America as a likely climate refugia (Figure 2.3; Batchelor et al., 2019).

While the expansion and contraction of the Laurentide ice sheet during the G5 glaciation created an ideal ecological niche space that allowed Mexican ducks to initially diverge from mallards, subsequent glacial periods continued to cause fluctuations in adaptive range that would bring about cycles of isolation and secondary contact. Following the initial divergence in demographic history ~350,000 YBP, these two species continued on independent trajectories until Mexican ducks began increasing ~200,000 YBP, reaching an effective population size nearly identical to that of mallards (N_e = ~2,000,000; Figure 2.1). Although it is possible that Mexican duck numbers did significantly increase over this 50,000 year period, estimates of effective population size are unlikely to reflect this type of sudden increase, as they often lag behind

increasing census sizes (Gasca-Pineda, Cassaigne, Alonso, & Eguiarte, 2013; Lonsinger, Adams, & Waits, 2018; Miller & Waits, 2003). Instead, these patterns are more consistent with the effects of gene flow, which can artificially increase estimates of diversity (Sato et al., 2020); and thus, the convergence of effective population suggests that a major gene flow event occurred during the G3 glacial period ~150,000 to ~200,000 years ago (Figure 2.1). Given that mallard populations continued growing during the G4-G3 inter-glacial period, I contend that the advancing ice sheets of the G3 glacial period led to secondary contact with the recently diverged Mexican ducks. Also note that the G3 glacial period is the longest of those occurring since Mexican ducks diverged from mallards; and thus, the time in sympatry during other episodes of glacial advancements was likely insufficient to result in significant gene flow (Figure 2.1). Pervasive gene flow occurring during this major period of secondary contact is therefore likely responsible for the unbalanced number of migrants moving from mallards into Mexican ducks as detected by my evolutionary models (Supplementary Materials Table S2.3). Moreover, models of GEA and genomic offset confirm that glacial periods were more conducive to a northern expansion, as suitable genetic niche space for Mexican ducks during the LGM was more expansive throughout the Southwest and intermountain regions of North America (Figure 2.3). Alternatively, inter-glacial periods showed a more fragmented and limited range of stable niche space restricted deep in western interior Mexico; significant turnover across the northern parts of this range also indicates strong barriers to expansion during inter-glacial periods. Together, I hypothesize that a simultaneous northern expansion of Mexican ducks and southern push of the mallard's range during glacial periods results in increased range overlap, while warm inter-glacial periods result in phases of isolation and limited gene flow (Figure 2.1; Moodley et al., 2020; Yamasaki et al., 2020).

Since this major secondary contact event, I find that mallards and Mexican ducks have maintained divergent effective population sizes. Specifically, mallard populations continued growing while Mexican ducks fluctuated in response to glacial advancement and retreat (Figures 2.1 & 2.3). Given that Mexican ducks currently maintain low levels of hybridization despite the capacity to interbreed with mallards (i.e., ~0% – 5%; Brown et al., *in review*), as well as show significant divergence from mallards in genetic diversity associated with ecologically (Figure 2.2) and sexually selected traits (Table 2.1; Supplementary Materials Table S2.4), I posit that the major gene flow event occurring early in the divergence process likely acted to facilitate the development of strong reproductive barriers (Butlin & Smadja, 2018; Feder et al., 2012). In general, here I provide support for the role of glacial cycles in facilitating local adaptation and subsequent species divergence, as well as demonstrate how glaciers may significantly influence genomic diversity and adaptation throughout the entire speciation process. Moreover, this study further demonstrates that when working with landscape-level species sampling, partial genome sequencing data is effective in modeling complex evolutionary histories, estimating fine-scale demographic changes, and identifying associations between genotypes and phenotypes or the environment.

Environmental drivers of adaptive divergence

GF models indicate that not only did glacial events provide the strong ecological selection necessary to induce divergence of proto-Mexican duck populations from mallards, but that their partitioned niche space continues to have differing selective impacts on contemporary genomic diversity. First, unlike mallards, for which GF found that allele frequencies are minimally impacted by the environment, genetic diversity among sampled Mexican ducks showed a significant association with various environmental factors (Figure 2.2 Supplementary Materials Figure S2.3).

Specifically, precipitation seasonality (Bio15) was identified as the most important environmental variable affecting Mexican ducks (Figure 2.2), which was expected given that water availability is limited across its range (Lecomte, Gauthier, & Giroux, 2009; Perez-Arteaga, Gaston, & Kershaw, 2002; Scott & Reynolds, 1984). Additionally, I found that variables related to geography (i.e., elevation), seasonal vegetation, and seasonal temperature changes are also important, suggesting that allele frequencies are responding to changes in seasonal weather patterns (excluding elevation) as opposed to annual precipitation and temperature. This kind of response to seasonal weather shifts is consistent with other desert adapted waterfowl (e.g., Grey Teal (*A. gracilis*) and Pacific Black ducks (*A. superciliosa rogersi*) in Australia, McEvoy, Ribot, Wingfield, & Bennett, 2017; Roshier, Klomp, & Asmus, 2006), which generally breed year-round when habitat and climate conditions become ideal (Cumming & Ndlovu, 2015).

Next, whereas temperate species often experience increased habitat suitability and population growth during inter-glacial periods (Hewitt, 1999; Provan & Bennett, 2008), the GF models provide further evidence supporting the claim that species in more arid habitats respond in a contradictory manner (Stewart et al., 2010). In concordance with patterns seen in other avian taxa from more arid habitats (e.g., white-throated butcherbirds, *Cracticus subgenus Bulestes*; Kearns, Joseph, Toon, & Cook, 2014) I show that increased Mexican duck habitat suitability occurs during glacial periods when temperate vegetation is more abundant across the southwest, while contracting during inter-glacial periods when this range becomes arid (Metcalfe, Say, Black, McCulloch, & O'Hara, 2002). Specifically, in a pattern that was likely repeated throughout the Pleistocene (Lockwood, 2001), southwestern habitats of the LGM were dominated by continuous woodlands of pinyon pine and juniper, which became fragmented by expanding desert vegetation under the extreme drought conditions that began during the current inter-glacial period (~13,000

years ago; Betancourt, 2004; Thompson & Anderson, 2000). Therefore, I contend that reduced habitat connectivity during inter-glacial periods likely limits dispersal across environmental gradients, facilitating strong intra-specific genetic structure and local adaption. And in fact, this fragmentation is most evident in the contemporary inter-glacial model for Mexican ducks, where the central highlands of Chihuahua are acting as montane habitat 'islands' surrounded by mountainous regions (i.e., the Sierra Madre Occidental to the west and the Sierra Madre Oriental in the east) and which are represented by areas of high genomic turnover (Figure 2.2). Consequently, the fine-scale geographically-associated population structure that occurs across the Mexican duck's current range (Brown et al., *in review*; Lavretsky et al., 2015) is likely the product of these repeated range expansions and contractions occurring in concert with glacial cycles. Overall, these results demonstrate not only the inverse responses of southern and northern latitude taxa to glacial cycles, but the potential impact these cycles have on future intra-specific genetic diversity and local adaption of southern latitude groups.

Sexual selection as a post-divergence co-evolutionary mechanism

Sexual selection often acts as a co-evolutionary process that promotes divergence on top of other evolutionary mechanisms (Rundle & Rowe, 2018). This secondary process occurs as selection arising from ecological differences accentuates variance in plumage characteristics that simultaneously act as species recognition cues (Price, 1998). For Mexican ducks, it is now evident that the loss of dichromatic mallard-like traits occurred as proto-Mexican duck populations diverged and eventually became monochromatic. This phenomenon is common in southerly species facing harsh habitat conditions (i.e., deserts; Hill, 1994), as sexual selection is often relaxed where natural selection is strongest (Lavretsky et al., 2020; Omland, 1997; Stuart–Fox & Ord,

2004). However, the partitioning between Mexican ducks and mallards in genetic diversity that is significantly associated with phenotypic characters related to female mate-choice suggests that sexual selection has secondarily acted to promote assortative mating (Supplementary Materials Figure S2.8; Marchetti, 1993; Seddon et al., 2013). Among traits, those associated with wings were the most significant across analyses (Supplementary Materials Table S2.4, Figures S2.7 & S2.8), with the speculum being particularly important. Given that wing markings and speculum color are known cues for waterfowl mate pairing (Eliason & Shawkey, 2012; Omland, 1996), this suggests that the drab plumage coloration originally arising due to strong environmental selection on proto-Mexican duck populations has since become an important species recognition cue that acts as a pre-zygotic reproductive barrier (Seddon et al., 2013). Moreover, sexual selection working in concert with environmental selection can reduce the propensity for lineage fusion during secondary contact events (Cooney, Tobias, Weir, Botero, & Seddon, 2017). Therefore, sexual selection acting secondarily on plumage traits in Mexican ducks has likely helped to maintain species boundaries during the early stages of divergence, specifically, preventing the swamping of locally adaptive alleles during glacial periods when Mexican ducks and mallards likely came in contact. Future work to understand the role of sexual selection in the speciation process would benefit from mate-pair studies to determine how female mate choice occurs in Mexican ducks and mallards. Additionally, ddRAD-sequencing loci used here are non-coding and are therefore unlikely to be under the direct influence of selection (DaCosta & Sorenson, 2014; Lavretsky et al., 2015); thus, whole genome sequencing will be necessary for a more fine-scale investigation of potential linkage between sexually and environmentally selected traits.

Vulnerability to future climate conditions

Understanding the genetic basis for adaptation has become a major component of evaluating the vulnerability of natural populations under future climate change scenarios (Razgour et al., 2019). Incorporating adaptive potential allows us to better predict areas where contemporary diversity may harbor alleles that remain adaptive under future climate conditions, as well as identify migratory pathways to suitable habitat that becomes newly available (Gougherty, Keller, & Fitzpatrick, 2021; Meester, Stoks, & Brans, 2018). While GEA modelling under the mildest estimate of climate change (rcp2.6) shows very little offset from contemporary conditions (Supplementary Materials Figure S2.6), the more extreme model (rcp8.5) suggests that Mexican ducks may be vulnerable to future maladaptation throughout the core of their range in central Chihuahua as well as along the western coast of Mexico (Figure 2.3). In fact, the extreme offset in central Chihuahua is consistent with past inter-glacial periods where intense drought conditions throughout the region have caused large bodies of water to be reduced or lost altogether (Castiglia & Fawcett, 2006); suggesting that critical Mexican duck breeding habitat will continue to be lost as the current inter-glacial period progresses. Moreover, wetland loss will likely be exacerbated by land use changes, which cannot be accounted for in GF modelling, as wetlands throughout the central highlands of Mexico are being drained at a rapid pace for agricultural purposes (Perez-Arteaga et al., 2002). This has already led to a serious decline in local waterbird populations throughout the region as critical breeding habitat is lost (Eric Mellink, Luévano, & Riojas-López, 2018; Perez-Arteaga et al., 2002). Along the western coast of Mexico, increasing surface temperatures in the Pacific Ocean are affecting sea-levels and regular climate oscillations (Lim et al., 2019). Specifically, sea-level rise is associated with saline intrusion along coastal wetlands, which can subsequently lead to habitat loss through native plant mortality and invasive plant

encroachment (Saintilan, Rogers, Kelleway, Ens, & Sloane, 2019). Additionally, El Nino events have become weaker as the region warms, which has been shown to negatively affect breeding and molting phenology in local populations (Mellink, 2000; Wingfield, Ramos-Fernandez, Nuñez-De La Mora, & Drummond, 1999). While incorporating genomic diversity allows us to more effectively model how adaptive potential can mitigate the effects of habitat loss in the future, I cannot predict how these negative trends may act to exacerbate the effects of the bottlenecks Mexican ducks have experienced over the last 13,000 years, as the loss of genetic diversity often limits a species' adaptive potential (Willi, Van Buskirk, & Hoffmann, 2006). However, I note that without more explicit data on movement and migratory patterns of Mexican ducks, I cannot rule out the possibility of newly suitable habitat being colonized as they abandon deteriorating habitat conditions.

Advancements and conclusions

Identifying the relationship between genomic divergence and myriad evolutionary mechanisms that underly these patterns is the critical next step in understanding the speciation process. However, recognizing such relationships can be especially challenging when the effects of selection, drift, and gene flow are working together in a way that creates only subtle signals of divergence in the observed, contemporary genome. Here, I overcome this by using an extensive range-wide sample set of Mexican ducks to model GEAs and genotype-phenotype associations occurring throughout the genome; and find that GF models identified a significant subset of SNPs in Mexican ducks that are strongly associated with environmental variables. While standard outlier methods based on relative differentiation (i.e., F_{ST}) have alternatively been used to identify islands of differentiation (Irwin et al., 2018; Turner et al., 2005), these methods depend on the strength of

selection and background divergence. In particular, loci putatively under selection can be missed in cases of local adaptation not linked to reproductive barriers, as relative divergence often remains low when selection is acting on many alleles of small effect (Le Corre & Kremer, 2012; Yeaman, 2015). For example, while Lavretsky et al. (2015, 2019) was able to use F_{ST} outlier methods (e.g., Bayescan) to identify a few genomic regions under strong divergent selection in either Mexican ducks or mallards, the GEA analysis finds evidence of selection acting throughout the genome. Overall, I contend that when full genome sequencing data is unavailable, a landscape level GEA analysis of reduced-representation sequencing data can be more effective at detecting evidence of selection acting on alleles of small effect than traditional F_{ST} outlier methods.

Finally, this study provides insight into broad spatiotemporal responses to changing selective pressures in a uniquely desert adapted species of waterfowl, demonstrating the role of climate refugia and glacial cycles in driving intra- and inter-specific divergence. Looking at models of evolutionary and demographic histories, GEAs, and phenotype-genotype associations demonstrates that a complex relationship between the environment, selection, and adaptation exists throughout the speciation process. Additionally, I report that GEA methods could be effective at demonstrating ecologically-based divergent selection between closely related species, as well as at visualizing how past climatic conditions act to structure contemporary genetic diversity on the landscape. Specifically, I find that Mexican ducks likely diverged within a climate refugia arising during a glacial period, and that cyclical population expansions and contractions in response to these glacial cycles subsequently facilitated intra-specific population structure (Figure 2.1). More broadly, this work reveals that the evolutionary mechanisms driving speciation are not singular, and that the complex associations between many different factors play a role in this process. Additionally, I demonstrate the importance of incorporating adaptive potential when predicting

vulnerability to future climate conditions, as these types of landscape-level datasets can help to identify areas where conservation efforts will be most critical. Finally, I demonstrate that reduced-representation molecular data for landscape-level sample sets remain useful and powerful in providing insight into the evolutionary history of non-model systems. Nevertheless, future work would benefit from whole genome sequencing, which would allow for a more nuanced look at the effects of neutral versus selective processes on genomic architecture.

ACKNOWLEDGEMENTS

I am grateful to Ducks Unlimited for the Richard H. G. Bonnycastle Fellowship in Wetland and Waterfowl Biology, to Ducks Unlimited Canada Institute for Wetland and Waterfowl Research for the Waterfowl Research Foundation Graduate Fellowship, and to the University of Texas at El Paso for providing financial support throughout this study. I also want to thank R. Harrigan for his contributions in analytical methods and revisions of previous drafts.

REFERENCES

Allendorf, F. W. (1986). Genetic drift and the loss of alleles vs heterozygosity. *Zoo Biology*, *5*, 181–190.

Baldassarre, G. A. (2014). *Ducks, Geese, and Swans of North America*. Baltimore, Maryland: Johns Hopkins University Press.

Batchelor, C. L., Margold, M., Krapp, M., Murton, D. K., Dalton, A. S., Gibbard, P. L., … Manica, A. (2019). The configuration of Northern Hemisphere ice sheets through the Quaternary. *Nature Communications*, *10* (1), 1–10. doi:10.1038/s41467-019-11601-2

Bay, R. A., Harrigan, R. J., Underwood, V. Le, Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018).

Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359* (6371), 83–86. doi:10.1126/science.aan4380

Bemmels, J. B., Title, P. O., Ortego, J., & Knowles, L. L. (2016). Tests of species-specific models reveal the importance of drought in postglacial range shifts of a Mediterranean-climate tree: insights from integrative distributional, demographic and coalescent modelling and ABC model selection. *Molecular Ecology*, *25* (19), 4889–4906. doi:10.1111/mec.13804

Berger, B., Crucifix, M., Hodell, D. A., Mangili, C., McManus, J. F., Otto-Bliesner, B., … Vazquez Riveiros, N. (2016). Interglacials of the last 800,000 years. *Reviews of Geophysics*, *54* (1), 162–219. doi:10.1002/2015RG000482

Betancourt, J. L. (2004). Arid Lands Paleobiogeography: The Rodent Midden Record in the Americas. In M. V. Lomolino & L. R. Heaney (Eds.), *Frontiers of Biogeography: New Directions in the Geography of Nature*. Sunderland, Massachusetts: Sinauer Associates Inc.

Blischak, P. D., Barker, M. S., & Gutenkunst, R. N. (2020). Inferring the Demographi History of Inbred Species from Genome-Wide SNP Frequency Data. *Molecular Biology and Evolution*, *37* (7), 2124–2136. doi:https://doi.org/10.1093/molbev/msaa042

Brown, J. I., Hernández, F., Engilis, Jr., A., Hernandez, B., Collins, D., & Lavretsky, P. (*in review*). Genomic and morphological data shed light on the complexities of shared ancestry between closely related duck species. *In Review*.

Butlin, R. K., & Smadja, C. M. (2018). Coupling, Reinforcement, and Speciation. *The American Naturalist*, *191* (2), 155–172. doi:10.1086/695136

Byers, K. J. R. P., Xu, S., & Schlüter, P. M. (2017). Molecular mechanisms of adaptation and speciation: why do we need an integrative approach? *Molecular Ecology*, *26* (1), 277–290. doi:10.1111/mec.13678

Capblancq, T., Fitzpatrick, M. C., Bay, R. A., Exposito-Alonso, M., & Keller, S. R. (2020). Genomic Prediction of (Mal)Adaptation across Current and Future Climatic Landscapes. *Annual Review of Ecology, Evolution, and Systematics*, *51* (1), 245–269. doi:10.1146/annurev-ecolsys-020720-042553

Castiglia, P. J., & Fawcett, P. J. (2006). Large Holocene lakes and climate change in the Chihuahuan Desert. *Geology*, *34* (2), 113–116. doi:10.1130/G22036.1

Chen, N., Juric, I., Cosgrove, E. J., Bowman, R., Fitzpatrick, J. W., Schoech, S. J., … Coop, G. (2019). Allele frequency dynamics in a pedigreed natural population. *Proceedings of the National Academy of Sciences of the United States of America*, *116* (6), 2158–2164. doi:10.1073/pnas.1813852116

Coffman, A. J., Hsieh, P. H., Gravel, S., & Gutenkunst, R. N. (2016). Computationally Efficient Composite Likelihood Statistics for Demographic Inference. *Molecular Biology and Evolution*, *33* (2), 591–593. doi:https://doi.org/10.1093/molbev/msv255

Cooney, C. R., Tobias, J. A., Weir, J. T., Botero, C. A., & Seddon, N. (2017). Sexual selection, speciation and constraints on geographical range overlap in birds. *Ecology Letters*, *20* (7), 863–871. doi:10.1111/ele.12780

Cruickshank, T. E., & Hahn, M. W. (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular Ecology*, *23* (13), 3133–3157. doi:10.1111/mec.12796

Cumming, G. S., & Ndlovu, M. (2015). Satellite telemetry of Afrotropical ducks: methodological details and assessment of success rates. *African Zoology*, *46* (2), 425–434.

DaCosta, J. M., & Sorenson, M. D. (2014). Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. *PLoS ONE*, *9* (9), e106713.

doi:10.1371/journal.pone.0106713

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14* (6), 927–930. doi:10.1111/j.1654-1103.2003.tb02228.x

Douglas, M. E., Douglas, M. R., Schuett, G. W., & Porras, L. W. (2006). Evolution of rattlesnakes (Viperidae; *Crotalus*) in the warm deserts of western North America shaped by Neogene vicariance and Quaternary climate change. *Molecular Ecology*, *15* (11), 3353–3374. doi:10.1111/j.1365-294X.2006.03007.x

Eliason, C. M., & Shawkey, M. D. (2012). A photonic heterostructure produces diverse iridescent colours in duck wing patches. *Journal of The Royal Society Interface*, *9* (74), 2279–2289. doi:10.1098/rsif.2012.0118

Ellegren, H. (2014). Genome sequencing and population genomics in non-model organisms. *Trends in Ecology and Evolution*, *29* (1), 51–63. doi:10.1016/j.tree.2013.09.008

Ellis, N., Smith, S. J., & Roland Pitcher, C. (2012). Gradient forests: Calculating importance gradients on physical predictors. *Ecology*, *93* (1), 156–168. doi:10.1890/11-0252.1

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation-with-gene-flow. *Trends in Genetics*, *28* (7), 342–350. doi:10.1016/j.tig.2012.03.009

Fitzpatrick, M. C., & Keller, S. R. (2015). Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters*, *18* (1), 1–16. doi:10.1111/ele.12376

Gasca-Pineda, J., Cassaigne, I., Alonso, R. A., & Eguiarte, L. E. (2013). Effective Population Size, Genetic Variation, and Their Relevance for Conservation: The Bighorn Sheep in Tiburon Island and Comparisons with Managed Artiodactyls. *PLoS ONE*, *8* (10), e78120. doi:10.1371/journal.pone.0078120

Gavin, D. G., Fitzpatrick, M. C., Gugger, P. F., Heath, K. D., Rodríguez-Sánchez, F., Dobrowski, S. Z., … Williams, J. W. (2014, October 1). Climate refugia: Joint inference from fossil records, species distribution models and phylogeography. *New Phytologist*. Blackwell Publishing Ltd. doi:10.1111/nph.12929

Gougherty, A. V., Keller, S. R., & Fitzpatrick, M. C. (2021). Maladaptation, migration and extirpation fuel climate change risk in a forest tree species. *Nature Climate Change, 11* (2), 166–171. doi:10.1038/s41558-020-00968-6

Gutenkunst, R., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2010). Diffusion Approximations for Demographic Inference: *∂a∂i*. *Nature Precedings*, *5*, 1–1. doi:10.1038/npre.2010.4594.1

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the Joint Demographic History of Multiple Populations from Multidimensional SNP Frequency Data. *PLoS Genetics*, *5* (10), e1000695. doi:10.1371/journal.pgen.1000695

Harvey, M. G., Singhal, S., & Rabosky, D. L. (2019). Beyond Reproductive Isolation: Demographic Controls on the Speciation Process. *Annual Review of Ecology, Evolution, and Systematics*, *50* (1), 75–95. doi:10.1146/annurev-ecolsys-110218-024701

Hernández, F., Brown, J. I., Kaminski, M., & Lavretsky, P. (*in review*). Species genomics reveals rare hybridization, robust species trees, and large demographic changes in the evolutionary histories of four dove species. *In Review*.

Hewitt, G. M. (2000). The genetic legacy of the quaternary ice ages. *Nature*, *405* (6789), 907–913. doi:10.1038/35016000

Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, *58* (3), 247–276. doi:10.1111/j.1095-

8312.1996.tb01434.x

Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, *68* (1–2), 87–112. doi:10.1111/j.1095-8312.1999.tb01160.x

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25* (15), 1965–1978. doi:10.1002/joc.1276

Hill, G. E. (1994). Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. *Behavioral Ecology*, *5* (1), 64–73. doi:10.1093/beheco/5.1.64

Huang, B. H., Huang, C. W., Huang, C. L., & Liao, P. C. (2017). Continuation of the genetic divergence of ecological speciation by spatial environmental heterogeneity in island endemic plants. *Scientific Reports*, *7* (1), 1–13. doi:10.1038/s41598-017-05900-1

Hubbard, J. P. (1977). The biological and taxonomic status of the Mexican Duck. *New Mexico Department of Game and Fish Bulletin, No. 16*, 1–56.

Irwin, D. E., Milá, B., Toews, D. P. L., Brelsford, A., Kenyon, H. L., Porter, A. N., … Irwin, J. H. (2018). A comparison of genomic islands of differentiation across three young avian species pairs. *Molecular Ecology*, *27* (23), 4839–4855. doi:10.1111/mec.14858

Jaeger, J. R., Riddle, B. R., & Bradford, D. F. (2005). Cryptic Neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas. *Molecular Ecology*, *14* (10), 3033–3048. doi:10.1111/j.1365-294X.2005.02645.x

Johnson, N. K., & Cicero, C. (2004). New mitochondrial DNA data affirm the importance of pleistocene speciation in North American birds. *Evolution*, *58* (5), 1122–1130.

doi:10.1111/j.0014-3820.2004.tb00445.x

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24* (11), 1403–1405. doi:10.1093/bioinformatics/btn129

Kautt, A. F., Kratochwil, C. F., Nater, A., Machado-Schiaffino, G., Olave, M., Henning, F., … Meyer, A. (2020). Contrasting signatures of genomic divergence during sympatric speciation. *Nature*, *588* (7836), 106–111. doi:10.1038/s41586-020-2845-0

Kearns, A. M., Joseph, L., Toon, A., & Cook, L. G. (2014). Australia's arid-adapted butcherbirds experienced range expansions during Pleistocene glacial maxima. *Nature Communications*, *5* (1), 1–11. doi:10.1038/ncomms4994

Kulikova, I. V, Drovetski, S. V, Gibson, D. D., Harrigan, R. J., Rohwer, S., Sorenson, M. D., … Kevin, G. (2005). Phylogeography of the Mallard (*Anas platyrhynchos*): Hybridization , Dispersal , and Lineage Sorting Contribute to Complex Geographic Structure. *The Auk*, *122* (3), 949–965.

Lavretsky, P., Dacosta, J. M., Hernández-Baños, B. E., Engilis, A., Sorenson, M. D., & Peters, J. L. (2015). Speciation genomics and a role for the Z chromosome in the early stages of divergence between Mexican ducks and mallards. *Molecular Ecology*, *24* (21), 5364–5378. doi:10.1111/mec.13402

Lavretsky, P., DaCosta, J. M., Sorenson, M. D., McCracken, K. G., & Peters, J. L. (2019). ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. *Molecular Ecology*, *28* (10), 2594–2609. doi:10.1111/mec.15091

Lavretsky, P., Hernández-Baños, B. E., & Peters, J. L. (2014). Rapid radiation and hybridization contribute to weak differentiation and hinder phylogenetic inferences in the New World

Mallard complex (*Anas spp.*). *The Auk, 131*, 524–538. doi:10.1642/AUK-13-164.1

Lavretsky, P., McCracken, K. G., & Peters, J. L. (2014). Phylogenetics of a recent radiation in the mallards and allies (Aves: Anas): Inferences from a genomic transect and the multispecies coalescent. *Molecular Phylogenetics and Evolution*, *70* (1), 402–411. doi:10.1016/j.ympev.2013.08.008

Lavretsky, P., McInerney, N. R., Mohl, J. E., Brown, J. I., James, H. F., McCracken, K. G., & Fleischer, R. C. (2020). Assessing changes in genomic divergence following a century of human-mediated secondary contact among wild and captive-bred ducks. *Molecular Ecology*, *29* (3), 578–595. doi:10.1111/mec.15343

Layton, K. K. S. S., Snelgrove, P. V. R. R., Dempson, J. B., Kess, T., Lehnert, S. J., Bentzen, P., … Bradbury, I. R. (2021). Genomic evidence of past and future climate-linked loss in a migratory Arctic fish. *Nature Climate Change*, *11* (2), 158–165. doi:10.1038/s41558-020-00959-7

Le Corre, V., & Kremer, A. (2012). The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology, 21* (7), 1548–1566. doi:10.1111/j.1365-294X.2012.05479.x

Lecomte, N., Gauthier, G., & Giroux, J.-F. (2009). A link between water availability and nesting success mediated by predator–prey interactions in the Arctic. *Ecology, 90* (2), 465–475. doi:10.1890/08-0215.1

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia, 129* (2), 271–280. doi:10.1007/s004420100716

Lenormand, T. (2012). From local adaptation to speciation: Specialization and reinforcement. *International Journal of Ecology*. doi:10.1155/2012/508458

Licciardi, J. M., Clark, P. U., Brook, E. J., Elmore, D., & Sharma, P. (2004). Variable responses

of western US glaciers during the last deglaciation. *Geology*, *32* (1), 81–84. doi:10.1130/G19868.1

Lim, E. P., Hendon, H. H., Hope, P., Chung, C., Delage, F., & McPhaden, M. J. (2019). Continuation of tropical Pacific Ocean temperature trend may weaken extreme El Niño and its linkage to the Southern Annular Mode. *Scientific Reports*, *9* (1), 1–15. doi:10.1038/s41598-019-53371-3

Lockwood, J. G. (2001). Abrupt and sudden climatic transitions and fluctuations: a review. *International Journal of Climatology*, *21* (9), 1153–1179. doi:10.1002/joc.630

Lonsinger, R. C., Adams, J. R., & Waits, L. P. (2018). Evaluating effective population size and genetic diversity of a declining kit fox population using contemporary and historical specimens. *Ecology and Evolution*, *8* (23), 12011–12021. doi:10.1002/ece3.4660

Marchetti, K. (1993). Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature*, *362* (6416), 149–152. doi:10.1038/362149a0

Martin, R. A., & Pfennig, D. W. (2009). Disruptive Selection in Natural Populations: The Roles of Ecological Specialization and Resource Competition. *The American Naturalist*, *174* (2), 268–281. doi:10.1086/600090

McEvoy, J. F., Ribot, R. F. H., Wingfield, J. C., & Bennett, A. T. D. (2017). Heavy rainfall triggers increased nocturnal flight in desert populations of the Pacific black duck (*Anas superciliosa*). *Scientific Reports*, *7* (1), 1–9. doi:10.1038/s41598-017-17859-0

Meester, L. De, Stoks, R., & Brans, K. I. (2018). Genetic adaptation as a biological buffer against climate change: Potential and limitations. *Integrative Zoology*, *13* (4), 372–391. doi:10.1111/1749-4877.12298

Meier, J. I., Marques, D. A., Wagner, C. E., Excoffier, L., & Seehausen, O. (2018). Genomics of

Parallel Ecological Speciation in Lake Victoria Cichlids. *Molecular Biology and Evolution*, *35* (6), 1489–1506. doi:10.1093/molbev/msy051

Mellink, E. (2000). Breeding of brown boobies in the Gulf of California: Seasonality and apparent effects of El Nino. *Waterbirds*, *23* (3), 494–499. doi:10.2307/1522189

Mellink, Eric, Luévano, J., & Riojas-López, M. E. (2018). Half a century of changes in waterbird populations in a semiarid wetland system. *Wetlands Ecology and Management*, *26* (6), 1047–1060. doi:10.1007/s11273-018-9630-y

Metcalfe, S., Say, A., Black, S., McCulloch, R., & O'Hara, S. (2002). Wet conditions during the last glaciation in the Chihuahuan Desert, Alta Babicora Basin, Mexico. *Quaternary Research*, *57* (1), 91–101. doi:10.1006/qres.2001.2292

Miller, C. R., & Waits, L. P. (2003). The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): Implications for conservation. *Proceedings of the National Academy of Sciences of the United States of America*, *100* (7), 4334–4339. doi:10.1073/pnas.0735531100

Moodley, Y., Westbury, M. V, Russo, I.-R. M., Gopalakrishnan, S., Rakotoarivelo, A., Olsen, R.-A., … Bruford, M. W. (2020). Interspecific Gene Flow and the Evolution of Specialization in Black and White Rhinoceros. *Molecular Biology and Evolution*, *37* (11), 3105–3117. doi:10.1093/molbev/msaa148

Nevo, E. (2011). Evolution Under Environmental Stress at Macro- and Microscales. *Genome Biology and Evolution*, *3* (1), 1039–1052. doi:10.1093/gbe/evr052

Omland, K. E. (1996). Female mallard mating preferences for multiple male ornaments: I. Natural variation. *Behavioral Ecology and Sociobiology*, *39* (6), 353–360. doi:10.1007/s002650050300

Omland, K. E. (1997). Examining two standard assumptions of ancestral reconstructions: Repeated loss of dichromatism in dabbling ducks (*Anatini*). *Evolution, 51* (5), 1636–1646. doi:10.1111/j.1558-5646.1997.tb01486.x

Otto-Bliesner, B. L., Marshall, S. J., Overpeck, J. T., Miller, G. H., & Hu, A. (2006). Simulating arctic climate warmth and icefield retreat in the last interglaciation. *Science, 311* (5768), 1751–1753. doi:10.1126/science.1120808

Payne, P., Polechová, J., & Payne, P. (2020). Sympatric ecological divergence with coevolution of niche preference: Sympatric divergence with niche choice. *Philosophical Transactions of the Royal Society B, 375* (1806). doi:10.1098/rstb.2019.0749

Perez-Arteaga, A., Gaston, K. J., & Kershaw, M. (2002). Population trends and priority conservation sites for Mexican Duck Anas diazi. *Bird Conservation International, 12* (1), 35–52. doi:10.1017/S0959270902002034

Peters, J. L., Roberts, T. E., Winker, K., & McCracken, K. G. (2012). Heterogeneity in Genetic Diversity among Non-Coding Loci Fails to Fit Neutral Coalescent Models of Population History. *PLoS ONE, 7* (2), e31972. doi:10.1371/journal.pone.0031972

Peters, J. L., Winker, K., Millam, K. C., Lavretsky, P., Kulikova, I., Wilson, R. E., … McCracken, K. G. (2014). Mito-nuclear discord in six congeneric lineages of Holarctic ducks (genus Anas). *Molecular Ecology, 23* (12), 2961–2974. doi:10.1111/mec.12799

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology and Evolution, 31* (7), 1929–1936. doi:10.1093/molbev/msu136

Price, T. (1998). Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society B, 353* (1366), 251–260. doi:10.1098/rstb.1998.0207

Price, T. D., & Bouvier, M. M. (2002). The Evolution of F1 Postzygotic Incompatabilities in Birds. *Evolution*, *56* (10), 2083–2089. doi:10.1111/j.0014-3820.2002.tb00133.x

Provan, J., & Bennett, K. D. (2008, October 1). Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution*. Elsevier Current Trends. doi:10.1016/j.tree.2008.06.010

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P. C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics*, *81* (3), 559–575. doi:10.1086/519795

Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J., Ibáñez, C., … Manel, S. (2019). Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences of the United States of America*, *116* (21), 10418–10423. doi:10.1073/pnas.1820663116

Reynolds, R. E., Blohm, R. J., Nichols, J. D., & Hines, J. E. (1995). Spring-Summer Survival Rates of Yearling versus Adult Mallard Females. *The Journal of Wildlife Management*, *59* (4), 696. doi:10.2307/3801945

Rhoné, B., Defrance, D., Berthouly-Salazar, C., Mariac, C., Cubry, P., Couderc, M., … Vigouroux, Y. (2020). Pearl millet genomic vulnerability to climate change in West Africa highlights the need for regional collaboration. *Nature Communications*, *11* (1), 1–9. doi:10.1038/s41467-020-19066-4

Roshier, D. A., Klomp, N. I., & Asmus, M. (2006). Movements of a nomadic waterfowl, Grey Teal Anas gracilis, across inland Australia – results from satellite telemetry spanning fifteen months. *Ardea*, *94* (3), 461–475.

Ruegg, K., Bay, R. A., Anderson, E. C., Saracco, J. F., Harrigan, R. J., Whitfield, M., … Smith, T. B. (2018). Ecological genomics predicts climate vulnerability in an endangered southwestern songbird. *Ecology Letters, 21* (7), 1085–1096. doi:10.1111/ele.12977

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters, 8* (3), 336–352. doi:10.1111/j.1461-0248.2004.00715.x

Rundle, H. D., & Rowe, L. (2018). The contribution of sexual selection to ecological and mutation-order speciation. *Evolution, 72* (11), 2571–2575. doi:10.1111/evo.13599

Sæther, B.-E., Lande, R., Engen, S., Weimerskirch, H., Lillegård, M., Altwegg, R., … Visser, M. E. (2005). Generation time and temporal scaling of bird population dynamics. *Nature, 436* (7047), 99–102. doi:10.1038/nature03666

Saintilan, N., Rogers, K., Kelleway, J. J., Ens, E., & Sloane, D. R. (2019). Climate Change Impacts on the Coastal Wetlands of Australia. *Wetlands, 39* (6), 1145–1154. doi:10.1007/s13157-018-1016-7

Sarabia, C., VonHoldt, B., Larrasoaña, J. C., Uríos, V., & Leonard, J. A. (2020). Pleistocene climate fluctuations drove demographic history of African golden wolves (*Canis lupaster*). *Molecular Ecology*, *Online*. doi:10.1111/mec.15784

Sato, Y., Ogden, R., Kishida, T., Nakajima, N., Maeda, T., & Inoue-Murayama, M. (2020). Population history of the golden eagle inferred from whole-genome sequencing of three of its subspecies. *Biological Journal of the Linnean Society*, *130* (4), 826–838. doi:10.1093/biolinnean/blaa068

Savolainen, O., Lascoux, M., & Merilä, J. (2013, November 18). Ecological genomics of local adaptation. *Nature Reviews Genetics*. Nature Publishing Group. doi:10.1038/nrg3522

Scott, N. J., & Reynolds, R. P. (1984). Phenotypic Variation of the Mexican Duck (*Anas*

platyrhynchos diazi) in Mexico. *The Condor, 86*, 266–274.

Seddon, N., Botero, C. A., Tobias, J. A., Dunn, P. O., MacGregor, H. E. A., Rubenstein, D. R., … Safran, R. J. (2013). Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society B, 280* (1766), 20131065. doi:10.1098/rspb.2013.1065

Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., … Widmer, A. (2014). Genomics and the origin of species. *Nature Reviews Genetics, 15* (3), 176–192. doi:10.1038/nrg3644

Sillero, N. (2011). What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecological Modelling, 222* (8), 1343–1346. doi:10.1016/j.ecolmodel.2011.01.018

Smith, A. B., Godsoe, W., Rodríguez-Sánchez, F., Wang, H. H., & Warren, D. (2019, March 1). Niche Estimation Above and Below the Species Level. *Trends in Ecology and Evolution*. Elsevier Ltd. doi:10.1016/j.tree.2018.10.012

Stewart, J. R., Lister, A. M., Barnes, I., & Dalén, L. (2010). Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B, 277* (1682), 661–671. doi:10.1098/rspb.2009.1272

Stuart–Fox, D. M., & Ord, T. J. (2004). Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proceedings of the Royal Society B, 271* (1554), 2249–2255. doi:10.1098/rspb.2004.2802

Talbot, B., Chen, T.-W., Zimmerman, S., Joost, S., Eckert, A. J., Crow, T. M., … Manel, S. (2017). Combining Genotype, Phenotype, and Environment to Infer Potential Candidate Genes. *Journal of Heredity, 108* (2), 207–216. doi:10.1093/jhered/esw077

Theodoridis, S., Fordham, D. A., Brown, S. C., Li, S., Rahbek, C., & Nogues-Bravo, D. (2020).

Evolutionary history and past climate change shape the distribution of genetic diversity in terrestrial mammals. *Nature Communications*, *11* (1), 1–11. doi:10.1038/s41467-020-16449-5

Thompson, R. S., & Anderson, K. H. (2000). Biomes of western North America at 18,000, 6000 and 0 14C yr bp reconstructed from pollen and packrat midden data. *Journal of Biogeography*, *27* (3), 555–584. doi:10.1046/j.1365-2699.2000.00427.x

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*, 2144–2164. doi:10.1111/mec.13606

Tobias, J. A., Ottenburghs, J., & Pigot, A. L. (2020). Avian Diversity: Speciation, Macroevolution, and Ecological Function. *Annual Review of Ecology, Evolution, and Systematics*, *51* (1), 533–560. doi:10.1146/annurev-ecolsys-110218-025023

Tobler, M., Kelley, J. L., Plath, M., & Riesch, R. (2018, February 1). Extreme environments and the origins of biodiversity: Adaptation and speciation in sulphide spring fishes. *Molecular Ecology*. Blackwell Publishing Ltd. doi:10.1111/mec.14497

Turner, T. L., Hahn, M. W., & Nuzhdin, S. V. (2005). Genomic Islands of Speciation in Anopheles gambiae. *PLoS Biology*, *3* (9), e285. doi:10.1371/journal.pbio.0030285

Wang, J., Street, N. R., Park, E., Liu, J., & Ingvarsson, P. K. (2020). Evidence for widespread selection in shaping the genomic landscape during speciation of *Populus*. *Molecular Ecology*, *29* (6), 1120–1136. doi:10.1111/mec.15388

Wang, T., O'Neill, G. A., & Aitken, S. N. (2010). Integrating environmental and genetic effects to predict responses of tree populations to climate. *Ecological Applications*, *20* (1), 153–163. doi:10.1890/08-2257.1

Weir, J. T., & Schluter, D. (2004). Ice sheets promote speciation in boreal birds. *Proceedings of*

the Royal Society B, *271* (1551), 1881–1887. doi:10.1098/rspb.2004.2803

Willi, Y., Van Buskirk, J., & Hoffmann, A. A. (2006). Limits to the Adaptive Potential of Small Populations. *Annual Review of Ecology, Evolution, and Systematics*, *37* (1), 433–458. doi:10.1146/annurev.ecolsys.37.091305.110145

Wingfield, J. C., Ramos-Fernandez, G., Nuñez-De La Mora, A., & Drummond, H. (1999). The effects of an "El Nino" southern oscillation event on reproduction in male and female blue-footed boobies, *Sula nebouxii*. *General and Comparative Endocrinology*, *114* (2), 163–172. doi:10.1006/gcen.1998.7243

Wolf, J. B. W., & Ellegren, H. (2017). Making sense of genomic islands of differentiation in light of speciation. *Nature Reviews Genetics*, *18* (2), 87–100. doi:10.1038/nrg.2016.133

Yamasaki, Y. Y., Kakioka, R., Takahashi, H., Toyoda, A., Nagano, A. J., Machida, Y., … Kitano, J. (2020). Genome-wide patterns of divergence and introgression after secondary contact between Pungitius sticklebacks. *Philosophical Transactions of the Royal Society B*, *375* (1806). doi:10.1098/rstb.2019.0548

Yannic, G., Pellissier, L., Ortego, J., Lecomte, N., Couturier, S., Cuyler, C., … Côté, S. D. (2014). Genetic diversity in caribou linked to past and future climate change. *Nature Climate Change*, *4* (2), 132–137. doi:10.1038/nclimate2074

Yeaman, S. (2015). Local Adaptation by Alleles of Small Effect. *The American Naturalist*, *186* (S1), S74–S89. doi:10.1086/682405

Chapter 3: Anthropogenically-induced hybridization promotes the re-wilding of introduced domestic and extirpation of endemic populations in New Zealand

ABSTRACT

Anthropogenic hybridization can lead to several outcomes, with the most severe being hybrid swarming and the complete genetic extirpation of local populations. With increasing frequency of such events, understanding the adaptive consequences for both the endemic and invasive populations today and into the future is critical; particularly, as climbing extinction rates force the debate over naturalization of invasive species that may be considered as biological replacements. However, such secondary contact events are often too recent and/or complicated from ongoing supplemental stocking that testing for such adaptive responses is difficult or impossible. Here, I investigate the consequences of domestic mallard introductions in New Zealand on the genetic integrity and adaptiveness of endemic New Zealand grey ducks. First, though once considered to be genetically extinct, I identify that pockets of pure grey ducks persist, with the northwestern portion of the South Island as the core of their remaining range. In contrast, I conclude that introduced mallards have experienced widespread adaptive introgression from grey ducks that facilitated their rapid establishment and expansion throughout New Zealand. In fact, I demonstrate that adaptive introgression and strong environmental selective pressures has resulted in a genetically unique New Zealand mallard that no longer resembles the original wild stock. Instead, these mallards constitute a hybrid swarm that encompasses a more variable genetic niche space as compared to New Zealand grey ducks. Importantly, modeling genotypic turnover across future climate scenarios suggests that New Zealand mallards appear to be better adapted to future environmental conditions. I discuss whether this self-sustaining mallard population is a candidate for naturalization, and their potential to act as an ecological replacement in New Zealand. However, I maintain that conserving New Zealand's grey duck should remain the priority, with efforts focused on preserving core habitat and limiting further contact with mallard populations.

Finally, I discuss the potentials and pitfalls of anthropogenic hybridization on conservation strategies into the future.

INTRODUCTION

There is growing conservation concern over anthropogenic hybridization that occurs when previously isolated taxa come into contact due to human disturbance(s) (Grabenstein & Taylor, 2018; Lande, 1998; McFarlane & Pemberton, 2019; Wayne & Shaffer, 2016). Specifically, species that have been historically allopatric are increasingly experiencing human-mediated secondary contact due to range expansion through habitat degradation and/or direct introductions (Crispo, Moore, Lee-Yaw, Gray, & Haller, 2011; Hasselman et al., 2014). In cases of human introduction, domestic-origin conspecifics have often been used to supplement existing wild populations, or to establish self-sustaining populations for recreational purposes (Michaelides, Goodman, Crombie, & Kolbe, 2018; Sheridan, 1995). Given that wild and domestic conspecifics are unlikely to have strong barriers to gene flow (Lavretsky et al., 2020), pervasive introgression from the introduced taxon can lead to the formation of a hybrid swarm, or in extreme cases, complete lineage fusion (Hasselman et al., 2014; Rhymer & Simberloff, 1996). Such cases are especially problematic for native species, as locally adapted genotypes and gene combinations can be quickly broken-up (Pfaff et al., 2001) or simply swamped by the introduced taxon (McFarlane, Senn, Smith, & Pemberton, 2021; Wells et al., 2019). Alternatively, hybridization without backcrossing represents lost reproductive potential if hybrids are less viable or unable to interbreed with parental taxa (Lavretsky, Janzen, & McCracken, 2019; Lavretsky et al., 2020), which is required for gene flow or introgression to occur (Rhymer & Simberloff, 1996). Ultimately, the outcome of hybridization largely depends on the balance between the strength of selection against hybrids and the rate of gene flow between species; both of which are spatially and temporally heterogeneous (Hoskin, Higgie, Mcdonald, & Moritz, 2005; Liou & Price, 2006). Therefore, landscape- and genomic-level

sampling data is increasingly necessary to study the impacts of anthropogenic hybridization on the genomic and adaptive outcomes for the endemic and invasive groups.

Hybrid swarming generally indicates that reproductive barriers are lacking and that negative selection against hybrids is weak or absent altogether (Gow, Peichel, & Taylor, 2006). Without a significant fitness disadvantage, introgression into the parent populations can proceed uninhibited (Gilman & Behm, 2011; Rhymer & Simberloff, 1996), and as novel genetic variants are continuously passed between species, admixed individuals may be able to take advantage of an expanded niche space that was previously maladaptive for either species on its own (Glotzbecker, Walters, & Blum, 2016). Although newly introduced taxa may be initially maladaptive, these populations can be sustained by the hybrid swarm, which maintains a low prevalence of locally adaptive alleles until they begin increasing in frequency among the invasive taxa (Drake, 2006). Furthermore, while populations introduced from domestic stock may have a high genetic load due to the pressures of artificial selection (Marsden et al., 2016; Schubert et al., 2014), novel genetic variation introgressed from the native taxa can act to quickly purge such deleterious alleles (Ingvarsson & Whitlock, 2000). While this kind of adaptive introgression can provide novel genetic variation that increases evolutionary potential, if the direction of gene flow is primarily into the newly introduced taxon, then locally adaptive alleles from native populations may facilitate rapid range expansion at the cost of their own extirpation (Pfennig, Kelly, & Pierce, 2016). Here, I investigate a putative hybrid swarm consisting of native New Zealand grey ducks (*Anas superciliosa superciliosa;* "grey duck") and recently introduced mallards (*Anas platyrhynchos*) to better understand the costs of anthropogenic hybridization, as well as to determine the process and role that introgressive hybridization played in facilitating the expansion of the grey duck's non-native congener.

Grey ducks are a subspecies of the Pacific black duck (*Anas superciliosa*) and one of fourteen mallard-like taxa that comprise the mallard complex (Lavretsky, McCracken, & Peters, 2014). Grey ducks likely colonized New Zealand from Australia within the last 100,000 years (Brown et al., 2021; Rhymer, Williams, & Braun, 1994; Rhymer, Williams, & Kingsford, 2004), where they remained the only endemic *Anas* species until mallards were introduced in the mid 1800's (Dyer & Williams, 2010; Gillespie, 1985). Acclimatisation society records from New Zealand show that >30,000 mallards were released from domestic European and North American stocks starting in the 1860's (Dyer & Williams, 2010; M. Williams, 1981). Releases continued until ~1970, when self-sustained feral mallard populations were established and began expanding throughout New Zealand (Caithness, Williams, & Nichols, 1991; Dyer & Williams, 2010; M. Williams, 2017). Contemporary estimates of mallard populations are around three million, which comprises ~90% of all waterfowl in New Zealand, while grey ducks have been in severe decline (Gillespie, 1985; M. Williams, 2017). Additionally, given that mallard x grey duck hybrids face no apparent reduction in fertility (Haddon, 1984), a century of human induced secondary contact has led to extensive anthropogenic introgressive hybridization (Gillespie, 1985; Rhymer et al., 1994, 2004). Despite the looming threat of lineage fusion, it seems that grey duck and mallard population trends have stabilized in the last three decades, with true grey ducks making up 5% – 10% of New Zealand's waterfowl populations (Rhymer et al., 2004; M. Williams, 2017). However, both phenotypic characters and the limited amount of genetic information used thus far have been unreliable in identifying hybrid backcrosses past the F1 generation (Braithwaite & Miller, 1975; Rhymer et al., 2004; M. Williams & Roderick, 1973). Therefore, I apply thousands of nuclear loci across landscape-level sampling efforts to determine the true extent of hybridization, and whether grey ducks persist in New Zealand today or are genetically extinct.

First, I aim to describe the population structure and geographic distribution of grey ducks, mallards, and hybrids throughout New Zealand. Given that South Island hybridization rates appear to have been stable over the last three decades (Gillespie, 1985; Rhymer et al., 1994, 2004; M. Williams, 2017), I hypothesize that hybridization may be limited to areas of range overlap, and that geographic barriers (i.e., Cook's strait and the Southern Alps) and differences in habitat preference have preserved grey duck populations in areas of undisturbed native habitat. Next, using a multivariate machine-learning program, Gradient Forest (GF), I created individual and combined models of genotype-environment associations (GEA) for mallards, grey ducks, and their hybrids to visualize differences in genotypic turnover between these groups. I hypothesize that models of genotypic turnover will show non-overlapping associations between grey ducks and mallards/mallard-like hybrids. Alternatively, the introgression of adaptive alleles from grey ducks into mallards could result in increased adaptive potential for hybrids that would have facilitated the mallard's known range expansion throughout New Zealand. If this is the case, I expect that the genetic niche space occupied by hybrids may encompass and possibly expand on the adaptive space occupied by both species together. Finally, I model GEAs across future projected climate conditions to determine the extent that changing environmental conditions may have on grey ducks, mallards, and hybrids. I predict that introduced mallards and not endemic grey ducks will likely be better adapted to predicted land-use changes as the former has a high affinity for urban and agricultural habitats (Figley & VanDruff, 1982).

METHODS

Sampling, DNA extraction, ddRAD-seq library preparation & de-multiplexing

From 2016-2018, a total of 584 grey ducks, mallards, and putative hybrids were sampled throughout the North and South Islands of New Zealand (Supplementary Materials Table S3.1). Given the history of multiple mallard stocks used in New Zealand, comparable sequence data previously published for wild North American mallards (N = 74) and known game-farm mallards (N = 31) were included as reference populations for comparison (BioProject PRJNA591912, Lavretsky et al., 2020)

For the 584 new samples, genomic DNA was extracted from blood or tissue using a DNeasy Blood & Tissue kit following the manufacturer's protocols (Qiagen, Valencia, CA, USA). DNA quality was visually assessed on a 1% agarose gel to ensure high molecular weight bands, and quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA) to ensure a minimum concentration of 20 ng/μL. ddRAD-seq library preparation followed protocols outlined in DaCosta and Sorenson (2014; Lavretsky et al., 2015). In brief, genomic DNA was enzymatically fragmented using SbfI and EcoRI restriction enzymes. Illumina TruSeq compatible 6 base-pair barcodes were ligated to allow for future de-multiplexing. The barcode-ligated fragments were then size selected for 300 – 450 bp fragments using gel electrophoresis (2% low-melt agarose), followed by gel purification using a MinElute gel extraction kit (Qiagen); and following manufacturer protocols. Size selected fragments were then PCR amplified with Phusion high-fidelity DNA polymerase (Thermo Scientific, Pittsburgh, PA, USA) and purified with AMPure XP magnetic beads (Agencourt, Beverly, MA, USA). Libraries were quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA), pooled in equimolar, and the multiplexed library

sent to the University of Oregon Core Genomics Facility for 150 base-pair, single-end chemistry sequencing on an Illumina HiSeq 4000.

Raw reads were de-multiplexed based on perfect barcode matches using the script *ddRADparser.py* (DaCosta & Sorenson, 2014). Using the program trimmomatic (Bolger, Lohse, & Usadel, 2014), I trimmed and discarded poor quality sequences. The remaining quality reads were mapped to the mallard reference genome (Accession no. SS263068950 – SS263191362; Huang et al., 2013; Kraus et al., 2011) using the Burrows Wheeler Aligner v. 07.15 (bwa; Li & Durbin, 2011). Next, Samtools v. 1.6 was used to sort, index, and genotype samples. Only high-quality sequences were retained by setting a base pair PHRED and strand quality scores at ≥30. With a minimum base-pair sequencing depth of 5X (i.e., 10X per genotype), I used PGDspider v2.1.1.2 (Excoffier & Lischer, 2010) to convert VCF files into FASTA file format. Sequences where then further filtered for positions with <80% of alleles present.

Finally, sex was assigned to each sample based on differences in sequencing depth across autosomal and sex chromosome-linked loci (Lavretsky, Janzen, et al., 2019). Specifically, for the homogametic sex (i.e., males = ZZ), I expect to find near-zero levels of sequencing depth across W-sex chromosome linked loci but near equal depth for Z-sex chromosome linked loci when compared to autosomal loci. For the heterogametic sex (i.e., females = ZW), I expect to recover about half the sequencing depth at both W- and Z-sex chromosome linked loci as compared to autosomal loci.

Mitochondrial DNA

Primers L78 and H774 were used to sequence 655 base pairs of the mtDNA control region (Sorenson, Ast, Dimcheff, Yuri, & Mindell, 1999; Sorenson & Fleischer, 1996) following

protocols outlined in Lavretsky et al. (2014). Final products were sequenced on an ABI 3730 (Applied Biosystems, Life Technologies, Carlsbad, California, USA) machine at the University of Texas at El Paso BBRC Genomic Analysis Core Facility. Sequences were then aligned and edited using Sequencher v. 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Structure across mtDNA haplotypes was visualized with a median-joining haplotype network calculated in the program Network (Bandelt, Forster, & Rohl, 1999).

Nuclear population structure and estimates of genetic diversity

Nuclear population structure was based on independent bi-allelic ddRAD-seq autosomal single nucleotide polymorphisms (SNPs) and without using a priori assignment of individuals to populations or species. First, a custom python script (Lavretsky et al., 2020) was used to extract bi-allelic SNPs from a concatenated FASTA file of all autosomal loci. Next, PLINK v1.07 (Purcell et al., 2007) was used to filter for singletons (i.e., minimum allele frequency (--maf 0.002)), any SNP missing ≥20% of data across samples (--geno 0.2), as well as any SNPs found to be in linkage disequilibrium (LD) (--indep-pairwise 2 1 0.5). One of the two SNPs was randomly excluded if an LD correlation factor (r^2) > 0.5 was obtained.

Population structure was first visualized with a Principal Components Analysis (PCA) done in the R package Adegenet (Jombart, 2008) using the '*dudi.pca*' function. Next, assignment probabilities were estimated with the program ADMIXTURE v. 1.3 (Alexander & Lange, 2011; Alexander, Novembre, & Lange, 2009). For ADMIXTURE, I ran 100 iterations of each K for one through ten populations. The analysis uses a ten-fold cross-validation (CV) with a quasi-Newton algorithm (Zhou, Alexander, & Lange, 2011) and a block relaxation algorithm for point estimation. Each individual run was terminated once the change in log-likelihood (i.e., delta) of the point

estimates increased by <0.0001. The optimal number of populations (K) was then based on the lowest averaged CV-error across all 100 replicates per K. The package PopHelper (Francis, 2017) in R was used to convert all ADMIXTURE outputs into CLUMPP v. 1.1 (Jakobsson & Rosenberg, 2007) input files. Final assignment probabilities were based on the optimal clustering alignment across all 100 replicates per evaluated population K value using the GreedySearch algorithm for 1,000 iterations as implemented in CLUMPP v. 1.1.

Finally, composite estimates of relative divergence (ΦST) for concatenated Autosomal and Z-chromosome ddRAD-seq loci as well as mtDNA sequences were calculated across species as well as between North and South Island groups using the package PopGenome (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014) in the program R.

Establishing hybrid indices

To assign admixed individual to hybrid or backcross generations, I followed methods outlined in Lavretsky et al. (2016). Briefly I simulated expected assignment probabilities for first-generation hybrids (F1) and nine generations of backcrosses (F2-F10) into either parental population (i.e., grey ducks or mallards). I first generated ten simulated F1 hybrids by randomly sampling alleles from the grey duck and mallard 'gene pool' across bi-allelic SNPs, each position was randomly sampled based on a probability proportional to the allelic frequency in each respective gene pool. I then simulated five of these F1 hybrids each backcrossing into either the grey duck or mallard parental populations for up to nine generations. Hybrid indices were constructed using only pure individuals (i.e., assignment probability ≥95%) to limit potential biases (Lavretsky, DaCosta, Sorenson, McCracken, & Peters, 2019). Ten independent simulations were run, with outputs subsequently put into ADMIXTURE to estimate assignment probabilities

for a K of 2 and 3. At each K, 25 iterations were run per simulation for a total of 250 ADMIXTURE outputs generated per K, which were then combined and converted in PopHelper (Francis, 2017) into CLUMPP input files. I employed the Large Greedy algorithm and 1,000 random permutations with final admixture proportions for each K and per sample assignment probabilities based on CLUMPP analyses of all 250 replicates per K. Per generation expected assignment probabilities were based on the average of either all ten (F1) or each of the five (F2–F10) backcrosses, along with each lower and upper limit. Empirical ADMIXUTRE data was then fit to the simulated hybrid indices in order to calculate the proportion of samples that fall within each hybrid generation. Samples that fell outside the lower/upper limit of each generation were considered hybrid swarm backcrosses (Lavretsky, DaCosta, et al., 2019; Lavretsky, Janzen, et al., 2019).

Genotype-environment association modelling with gradient forest

Contemporary environmental data across New Zealand were obtained at a high resolution from several public databases. I used a total of 27 environmental variables thought to have impacts on bird physiology and ecology (Supplementary Materials Table S3.3; Bay et al., 2018). Environmental datasets were downloaded at a 30 arc-second resolution, and included (1) 19 climate variables from the WorldClim v. 1.4 database (https://www.worldclim.org/version1; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005); Landsat Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), and Net Primary Productivity (NPP) data from the USGS AppEEARS database (https://lpdaacsvc.cr.usgs.gov/appeears), and (2) elevation data from the Global Land Cover Facility (http://www.landcover.org). In order to differentiate the effects of annual versus seasonal vegetation processes, I calculated an average annual, summer (June), and winter (December) value for NDVI and EVI based on data collected from 2000 – 2019.

Samples were categorized as pure grey duck, grey duck backcross, pure mallard, mallard-backcross, and early-generation hybrids (i.e., F1-F3) based on the hybrid indices classification established from ADMIXTURE models (see above). Following the approach of Bay et al. (2018), I used a GF analysis as implemented by the R package gradientForest (Ellis, Smith, & Roland Pitcher, 2012) to test which environmental predictor variables most strongly explain patterns of allele frequency turnover in each of these groups. The GF analysis was originally created to detect the effects of environmental predictor variables on species turnover across a landscape (Ellis et al., 2012) but has since been adapted for measuring allelic frequency turnover (Bay et al., 2018; Fitzpatrick & Keller, 2015). Briefly, GF uses a machine learning regression tree-based algorithm to detect shifts in allele frequency across an environmental gradient, where a function is built for each individual SNP before an aggregate function is created for all SNPs across each independent predictor variable (Fitzpatrick & Keller, 2015).

For use in GF, I converted all SNP data into minor allele frequencies using the R package PopGenome (Pfeifer et al., 2014), and subsequently filtered any SNP that was polymorphic in fewer than five total sampling sites (Fitzpatrick & Keller, 2015). Using a large number of trees (N = 5,000), GF produced an R^2 ranked list of weighted importance for all environmental variables. To assess the performance of GF models for both species, environmental predictor data was randomized in relation to sampling sites. I then compared the performance of 100 models created with randomly generated data to those generated for empirical data. To visualize the GF model for each species across New Zealand, I extracted values for each environmental variable from points generated across this area. I then used a PCA to summarize the transformed values from the top five predictor variables (based on R^2 weighted importance) for each point. I then transformed the top three principal components to create a RGB color scale that was used to visualize different

patterns of adaptive genetic diversity across the landscape. In the end, colors reflect associations between allele frequencies and the environmental predictor variables that allow me to draw conclusions about how the environment has affected genetic diversity and putatively driven adaptation. Finally, I combined independent species' models as well as early-generation hybrid models of allele frequency turnover to identify differences in genetic niche space between species. Briefly, the *combinedGradientForest* function in R acts to standardize independent models to one another by calculating a combined function of cumulative importance, which represents the overall relationship in both species between allele frequency turnover and the environmental predictor variables. Additionally, during standardization, cumulative importance functions for each variable are weighted based on the total R^2 value of the combined gradient forest. As previously described, I visualized the combined model by predicting the GF object across geographic space before converting the PCA into a standardized RGB color scale.

In a novel use of GF modelling, I projected North American mallards across New Zealand as a proxy for testing whether these two populations occupy different adaptive spaces. First, I visualized New Zealand mallard genotypes in PCA space as previously described by creating an RGB color scale from a PCA of the top five predictor variables. Next, I independently transformed a GF model for pure North American mallards (Lavretsky et al., 2020). Using the *predict.prcomp* function from the R base package, I then projected the individual PCs of this model in the New Zealand mallard GEA space. In general, colors reflect associations between New Zealand mallard allele frequencies and environmental predictor variables, and points of North American mallards projected across this space represent how allele frequencies from this group are related to environmental gradients relative to New Zealand mallards.

Finally, in order to estimate the potential threat of climate change to grey ducks, as well as the adaptive potential of introduced mallards and their hybrids, I used GF to model GEA across future climate conditions and subsequently measure the genetic offset (i.e., Euclidean distance between contemporary and future GF models) from current GEA space. Future bioclimatic variables from Global Climate Models (GCM) were downloaded at the highest available resolution for two future (2070) scenarios of climate change (rcp2.6 and rcp8.5; CCSM4 at 30 arc-second resolution; Hijmans et al., 2005).

RESULTS

After filtering, a total of 3,121 ddRAD-seq loci with 2,943 (369,313 bp) an178 (22,527 bp) loci assigned the autosomal and Z-sex chromosomes, respectively. An additional 13 loci were assigned to the W-sex Chromosome (1,680 bp). My dataset consisted of an average median sequencing depth of 141 (range = 25 - 246) reads per locus per sample, and an average of 98% of alleles present per locus. Plotting sequencing depth ratios between sex and autosomal linked loci reliably determined sex across samples (Supplementary materials Figure S3.1). Finally, 600 base pairs of overlapping mtDNA COI was sequenced for a total of 681 samples (of 689; Supplementary Materials Table S3.1).

Population structure and hybrid indices

Population structure was visualized by plotting the first two principal components of the PCA, and ADMIXTURE results were based on an optimum K population of five. First, genetic partitioning was recovered for wild North American mallards, game-farm mallards, grey ducks, and New Zealand's mallards; with the latter being further broken down into North and South Island

clusters (Figure 3.1). Putative hybrids were represented by those samples of admixed assignment probabilities and intermediate scattering in PCA space between each primary genetic group (Figure 3.1). I note that the majority of putatively grey duck x mallard hybrids clustered closer to New Zealand's mallards. Additionally, although New Zealand's mallards were either assigned to or the combination of the two unique mallard genetic clusters, many had small interspecific assignment (i.e., ≤ 5%) to the game-farm genetic cluster, including a single sample from the South Island more evidently being in intermediate PCA space and with substantial (~27%) assignment to game-farm mallards. Unlike New Zealand's mallards, putative grey ducks were assigned to a single genetic cluster without any assignment to reference wild or game-farm mallards (Figure 3.1).

Given the variance in interspecific assignment among New Zealand samples (Figure 3.1), formal assignment across samples was based on indices derived from simulations of expected assignment probability (Figure 3.2A). For simulations, individuals with ≥95% assignment to either grey duck or mallard genetic clusters, with the latter being the summation of a sample's assignment to the North and South Island mallard genetic clusters as recovered in initial ADMIXTURE results (see above) were used; doing so takes any potential retained ancestry and handles small frequencies of introgression into consideration (Lavretsky, DaCosta, et al., 2019). At a K population of two, assignment probabilities to grey duck or mallard plateaued at 100% and ~99%, respectively after six generations; results were statistically similar when analyzing a *K* population of three (two-tailed t-test $p = 0.92$; Figure 3.2A; Supplementary Materials Table S3.2). Regardless of population model, I find predictable declines in interspecific assignment with each generation of backcrossing (Lavretsky et al., 2020, 2016), with individual lineages becoming genetically indistinguishable from their backcrossed population by the third generation of backcrossing (i.e., F4 generation;

Supplementary Materials Table S3.2); which is concordant to previous work in other ducks (Lavretsky, Janzen, et al., 2019; Lavretsky et al., 2016). Given the evident inter-Island population structure of New Zealand's mallards, empirical samples were assigned to backcrossed indices established from a K population of model of three.

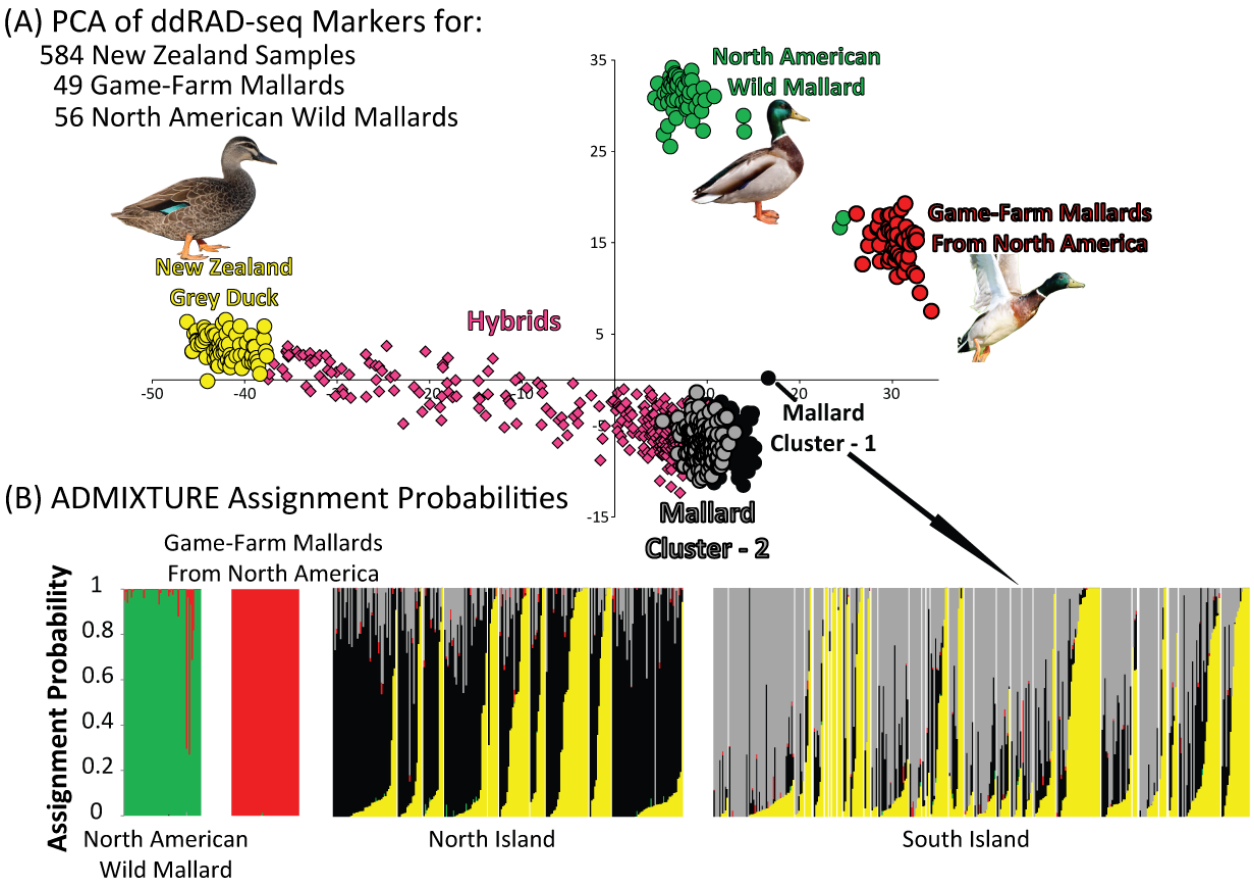


Figure 3.1. (A) PCA and (B) ADMIXTURE assignments of sampled grey ducks, New Zealand mallards, and reference North American wild and game-farm mallards, based on independent bi-allelic ddRAD-seq nuclear SNPs.

Simulation-based reassignments of New Zealand samples recovered a total of 47 and 88 samples across islands with expected assignment probabilities of a genetically pure grey duck or New Zealand mallard, respectively (Figure 3.2B - F). Assignment probabilities of 11 samples were within expectations of a F1 hybrid, and with a total of 37 and 94 samples classified within the remaining backcrossed generations (i.e., F2 – F4) towards grey ducks or mallards, respectively (Figure 3.2F). Finally, the remaining 307 samples fell in between simulated ranges across hybrid indices, suggested these are hybrid x hybrid pairings of unknown generations, and were therefore considered as part of a hybrid swarm (N = 51 grey duck backcrosses; N = 256 mallard backcrosses).

Individual samples were assigned to groups for pairwise Φ_{ST} estimates based on ADMIXTURE derived genetic assignments. Patterns of differentiation were similar across all three marker types (i.e., Autosomal, Z-chromosome, mtDNA; Supplementary Materials Figure S3.2). Pure grey ducks showed very little structure between the North and South Islands (Autosomal Φ_{ST} = 0.0026; Z-chromosome Φ_{ST} = 0.00098). Divergence between islands for New Zealand mallards was ~1.2 (Φ_{ST} = 0.0032) and ~4.6 (Φ_{ST} = 0.0045) times that of grey ducks for Autosomal and Z-chromosome loci, respectively. Next, divergence in autosomal markers between grey ducks and New Zealand mallards (Φ_{ST} = 0.11) was only ~0.5 and ~0.8 of that with game-farm (Φ_{ST} = 0.22) and North American mallards (Φ_{ST} = 0.14), respectively.

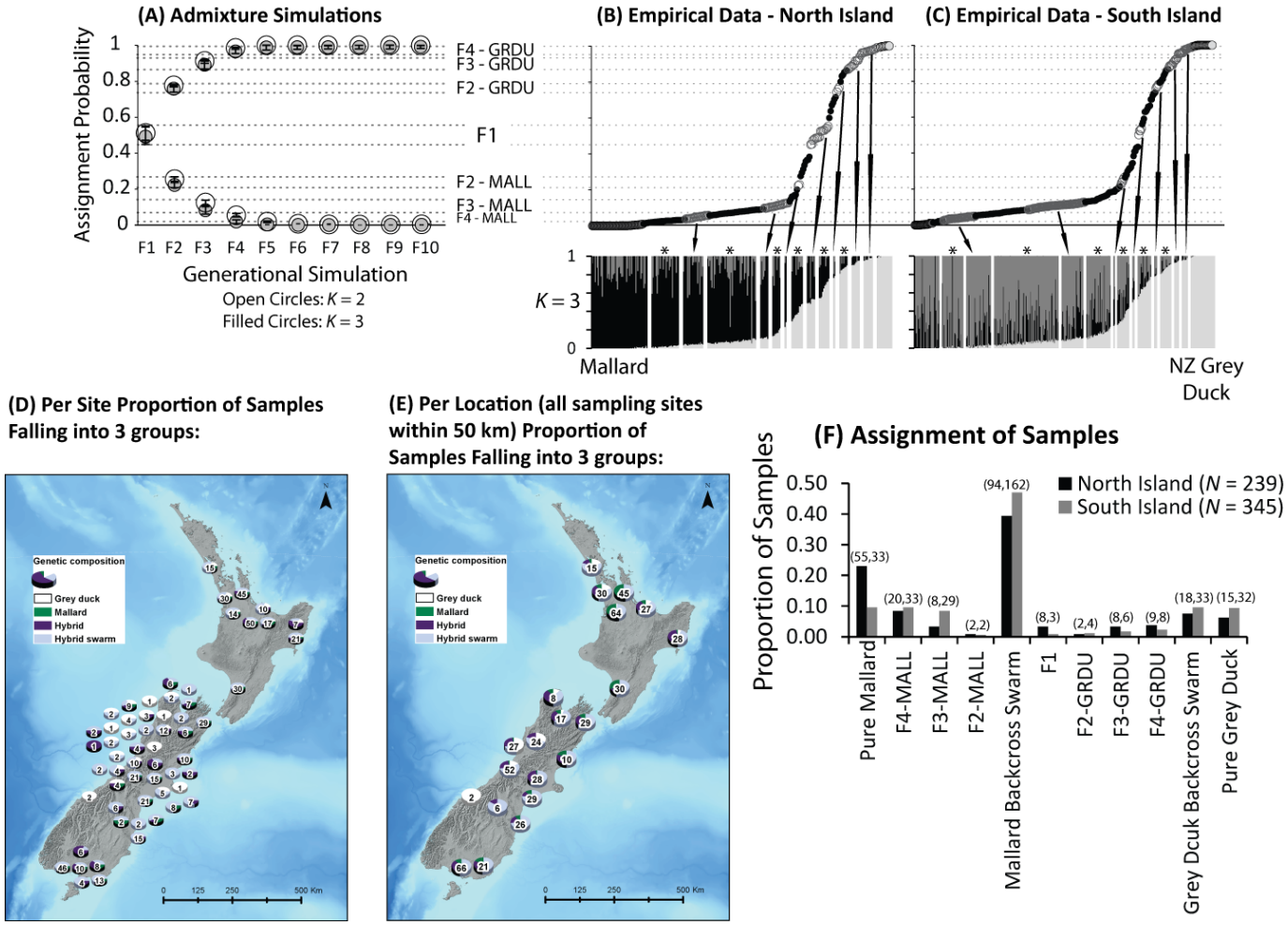


Figure 3.2. (A) Simulation based hybrid indices (F1 – F10) and (B) empirical assignment probabilities using a K of 3 for grey ducks and mallards sampled throughout New Zealand. Proportion of samples assigned to each hybrid backcross generation for (D) each sample site and (E) sample sites within 50 km grouped. (F) Proportion of all samples assigned to pure parental species, each hybrid backcross generation, and hybrid swarm backcrosses.

Mitochondrial DNA haplotype structure

Haplotype tree reconstruction using the mtDNA control region revealed two known group I and group II haplogroups previously identified in Pacific black ducks (Figure 3.3; Brown et al., 2021; Rhymer et al., 1994, 2004), as well as the known Old World (OW) A and New World (NW) B haplogroups (Figure 3.3; Avise, Ankney, & Nelson, 1990; Kulikova et al., 2005). The majority of genetically pure grey ducks (87%; 41 of 47) and grey duck-like samples (75%; 100 of 134) contained a Pacific black duck group I or group II haplotype (Figure 3.3), while mallards and mallard-like samples collected throughout New Zealand rarely shared a Pacific black duck haplotype (6%; 27 of 462). Of the 18 and 23 unique haplotypes found in each of the groups I and II haplogroups, four and nine were shared between grey duck and mallard-like samples, respectively. First generation hybrids (F1) were split between the group I (N = 1), group II (N = 3), and OW A (N = 7) haplogroups. Interestingly, 11 (2%) New Zealand mallards shared a single NW B haplotype, while no grey ducks did. The OW A haplogroup contained the majority of New Zealand samples (71%; 441 of 572); and despite the presence of 52 unique haplotypes, 69% of these individuals (304 of 441) shared a single haplotype (Figure 3.3).

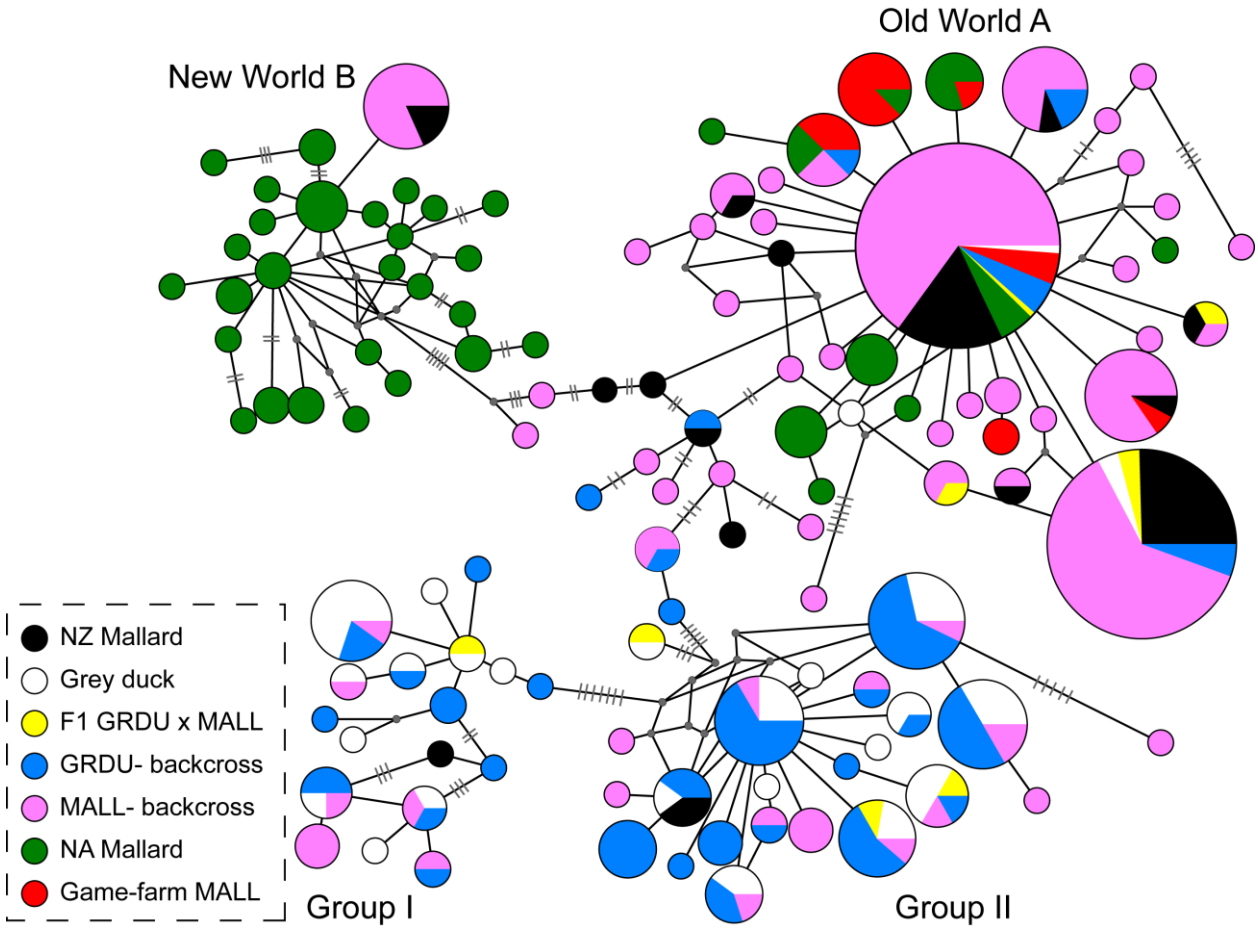


Figure 3.3. A haplotype network based on the mitochondrial control region sequenced for grey ducks, New Zealand mallards, and reference wild North American and game-farm mallards. Note that I identify pure parental groups and hybrid backcrosses based ADMIXUTRE assignment probabilities.

Genotype-environment association modelling

The mean R^2 value for GF models of pure grey ducks (R^2 = 0.114) and New Zealand mallards (R^2 = 0.127) did not perform better than the randomized datasets, likely due to the limited number of sample sites that contained genetically pure individuals (N_{GRDU} = 26, N_{MALL} = 25; Supplementary Materials Figure S3.3). Alternatively, GF recovered a mean R^2 value for grey duck (R^2 = 0.094) and mallard (R^2 = 0.093) backcrosses that was greater than the upper 95% quartile of values from the randomized datasets, indicating that the models performed better than random (Supplementary Materials Figure S3.3). A total of 810/8,026 (10.1%) and 1,023/12,234 (8.3%) SNPs with a positive R^2 were identified in grey duck and mallard backcrosses, respectively (Supplementary Material Figure S3.3). Results presented hereafter represent models of grey duck and mallard backcrosses. Next, early-generation hybrids had a mean R^2 value of 0.102, which was greater than the 95% quartile for randomized datasets (Supplementary Materials Figure S3.3). Finally, the combined grey duck x mallard backcross (R^2 = 0.091, N = 1,833) and grey duck x mallard x hybrid (R^2 = 0.095; N = 2,662) models both had a mean R^2 value greater than that of combined randomized datasets.

Four of the top five most predictive environmental variables identified in grey ducks were related to seasonal and annual vegetation growth (i.e., NDVI and EVI; Supplementary Materials Figure S3.4). Alternatively, GEAs in mallards were primarily driven by seasonal temperature variables (Bio4, Bio6, Bio8, Bio9), however, annual temperature (Bio1) was also found to be important (Supplementary Materials Figure S3.4). Hybrids were more strongly affected by temperature variables and NPP, which suggests that allele frequency turnover in admixed individuals is more strongly dictated by mallard GEAs. Combined GF models of grey ducks x mallards and grey ducks x hybrids x mallards are being dictated by variables that are important in

both species (i.e., Elev, Bio9, NPP); additionally, the combined grey duck x hybrid x mallard model showed that NPP was the only top variable influenced by hybrids (Supplementary Materials Figure S3.4).

Graphing principal components of GF outputs showed that the primary adaptive range of grey ducks in New Zealand had minimal variability and that they are widely adapted across major environmental gradients (Figure 3.4). This can be seen in the map of GEAs projected across New Zealand, as turnover was relatively homogenous, and the only areas of significant genotype turnover were limited to mountainous regions in the South Island (Figure 3.4). Alternatively, both mallards and early-generation hybrids were more variable in PCA and had noticeable genotype turnover that was spread across both islands (Figure 3.4). First, mallards projected in PCA space putatively showed evidence of allele frequency differences across sites, as sample sites were highly variable within GEA space. Genotype turnover mapped across New Zealand for this model corresponds with ADMIXTURE frequencies, in that the western parts of the North and South Islands are both at the edge of the adaptive range for mallards (Figure 3.4). I note that patterns of genetic diversity could also be dictated by land-use variability, however the high-resolution data required for GF analyses are not currently available. Interestingly, the model for early-generation hybrids shows an intermediate pattern of genotype turnover, with the most significant turnover occurring along the Southern Alps and the southern half of the South Island. A more homogenous landscape for hybrids indicates the importance of grey duck alleles in adaptation throughout New Zealand. Next, significant overlap in the grey duck x mallard combined model suggests that ecological portioning overall may be weak as introgression continues to homogenize genomes (Supplementary Materials Figure S3.5). The combined grey duck x hybrid x mallard model however, was more similar to patterns seen in the distribution of ADMIXTURE assignments.

Specifically, turnover mapped across New Zealand recapitulated the putative remaining range of grey ducks in the northwest portions of the North and South Islands (Figure 3.4). Additionally, mallards and hybrids similarly encompass the entire range of adaptive space in the GF PCA. Given that the majority of samples collected were mallard-like backcrosses, this combined model suggests that signals of genotype turnover described here are primarily being influenced by the GEAs of hybrid swarm individuals and grey ducks.

Next, I projected a model of North American mallard GEAs across the PC space of the New Zealand mallard GF model. Broadly, New Zealand mallards fall closer to grey ducks than their North American counterparts, suggesting that they share more adaptive alleles with grey ducks (Figure 3.5). This shows that North American mallards are not adapted to the same environmental space, and that the genetic response to environmental pressures differs between these two mallard groups.

Finally, genetic offset from future climate conditions (2070 rcp2.6 & rcp8.5) differed significantly between grey ducks and mallards. First, grey ducks are, by far, the most vulnerable group, as both the mild (rcp2.6) and extreme (rcp8.5) estimates of climate change show significant genomic offset across nearly all of New Zealand (Figure 3.6). More specifically, adaptiveness to native west coast habitat, which acts as the primary refuge for grey duck populations, is at risk of being completely lost. Even more severe, climate change across the North Island could threaten the few grey ducks that remain in the region. Alternatively, mallard models under mild estimates of climate change show limited offset from contemporary conditions, with isolated areas of vulnerability constrained to the more arid mountain plateaus of both islands (Figure 3.6). Under more extreme climate conditions, mallards showed widespread vulnerability similar to grey ducks.

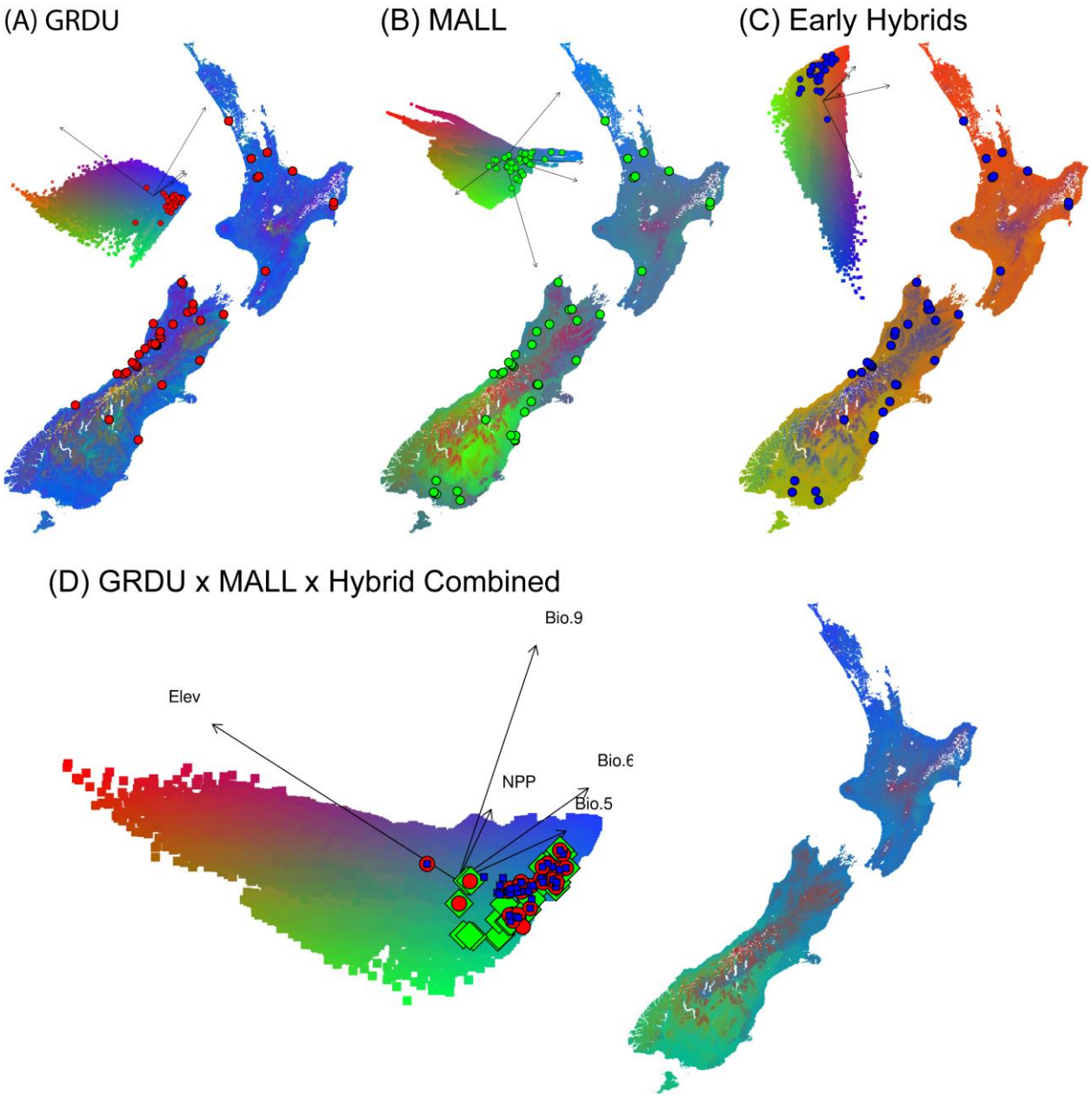


Figure 3.4. Genotype-environment association models from gradientForest (GF) mapped across New Zealand for (a) grey duck backcrosses, (b) New Zealand mallard backcrosses, (c) early generation hybrids, and (d) the combined model. Insets represent PCA results from GF modelling.

DISCUSSION

The population structure of grey ducks and mallards of New Zealand

Genotyping hundreds of putative grey ducks, mallards, and hybrids across New Zealand revealed that (1) isolated pockets of pure grey ducks still exist, (2) that New Zealand mallards are genetically unique and show inter-Island population structure, and (3) that the majority of samples collected were hybrid swarm backcrosses (Figures 3.1 – 3.3). Despite over a century of human-induced secondary contact with introduced mallards, pure grey ducks continue to persist in New Zealand (Figures 3.1 & 3.3), with 6% and 10% of all samples being of pure grey duck ancestry on the North and South Islands, respectively. These are concordant with previous estimates of hybridization over the last four decades (Gillespie, 1985; Rhymer et al., 1994, 2004), which suggests that gene flow has stabilized and that limited reproductive isolation has prevented complete lineage fusion. Additionally, while the presence of early-stage hybrids (i.e., F1 – F3, Figure 3.3) supports that hybridization continues, the limited number of first-generation hybrids (F1) suggests that inter-specific pairings between pure parentals are rare (Culumber, Ochoa, & Rosenthal, 2014).

Whereas grey ducks were largely scattered across North Island sampling locations, the majority of pure grey ducks were recovered in the northwestern area of the South Island. In fact, except for a single individual, grey ducks were completely absent from the eastern coast of the South Island. This suggests that geographically, grey ducks persist in areas of more undisturbed habitat such as the mountainous streams, lakes, and tidal estuaries that make up the western coasts (Figure 3.2; G. R. Williams, 1964). Additionally, grey ducks do not show inter-island population structure similar to mallards (Figures 3.1 & 3.2), which indicates that grey ducks likely exhibit longer distance dispersal and that the geographic scattering of these birds across the North Island

is due to sporadic movement from their core habitat on the South Island. Given that mallards appear to be reluctant to disperse long distances (<25 mi.; Caithness et al., 1991), I contend that major geographic features such as Cook strait and the Southern Alps are acting as pre-zygotic reproductive barriers that reduce gene flow. I also note that breeding phenology could also reduce the opportunity for inter-specific pairings as grey ducks have a shorter breeding season that peaks in spring and early summer (October-December), while mallard breeding pairs peak in late summer and early fall (March-September; Balham, 1952; Braithwaite & Miller, 1975); although, I note that mallards in New Zealand have been documented breeding year-round (Balham, 1952). In general, future work will require fine-scale telemetry data to better understand how grey duck and mallard movement patterns differ, and whether seasonal dispersal is affecting population structure and their propensity to come in contact during their breeding seasons.

Next, the presence of OW and NW mallard haplotypes in New Zealand confirms that both European domestic and North American wild stocks were used. Moreover, while the New Zealand mallard lineage contains only a single NW B haplotype, 29 OW A haplotypes can be found across mallards and hybrids; these results are consistent with the number of Eurasian (OW) and North American (NW) mallards used across stocking events (Dyer & Williams, 2010). Interestingly, nuclear DNA shows that New Zealand mallards have since become strongly differentiated from both of their original stocks (Figures 3.1 & 3.3; Supplementary Materials Figure S3.2). Furthermore, the inter-Island structure found among mallards and hybrids is in line with telemetry data, which suggests that these populations rarely move between islands (Caithness et al., 1991). Unlike grey ducks that are confined to a relatively small undisturbed area of the South Island, mallards and hybrids can be found throughout the two Islands, and particularly in more human-dominated agricultural and urban habitats (Figure 3.2).

In general, I contend that human-induced habitat changes and the introgression of locally adaptive alleles from grey ducks (see discussion below) has allowed New Zealand's mallards to expand as a self-sustaining population. Specifically, I find that mallards are genetically intermediate to other groups in PCA (Figure 3.1), supporting a scenario in which the grey duck and New Zealand mallard genomes have become widely admixed to the point that they represent an intermediate form as compared to their parental groups – i.e., New Zealand mallards, and not grey ducks, are the hybrid swarm. Moreover, combined GEA analysis shows that New Zealand mallards more closely resemble grey ducks in terms of adaptive environmental space, suggesting that New Zealand's mallards have significantly diverged from their original domestic stock due to local selective pressures, gene flow, and genetic drift (Figures 3.4 & 3.5). While mallard fitness cannot be directly tested in New Zealand, survival and breeding metrics of these birds are now comparable to wild North American populations (Sheppard, 2017), emphasizing the role of selection in promoting local adaptation in what was once maladaptive domestic stock. Overall, these results beg the question of whether a once domestically-derived lineage may now be a unique evolutionary unit? While these results suggest that New Zealand mallards could be in the incipient stages of hybrid speciation, whole genome sequencing would be necessary to differentiate genomic contributions from each parent species and identify the selective pressures that underlie their adaptations.

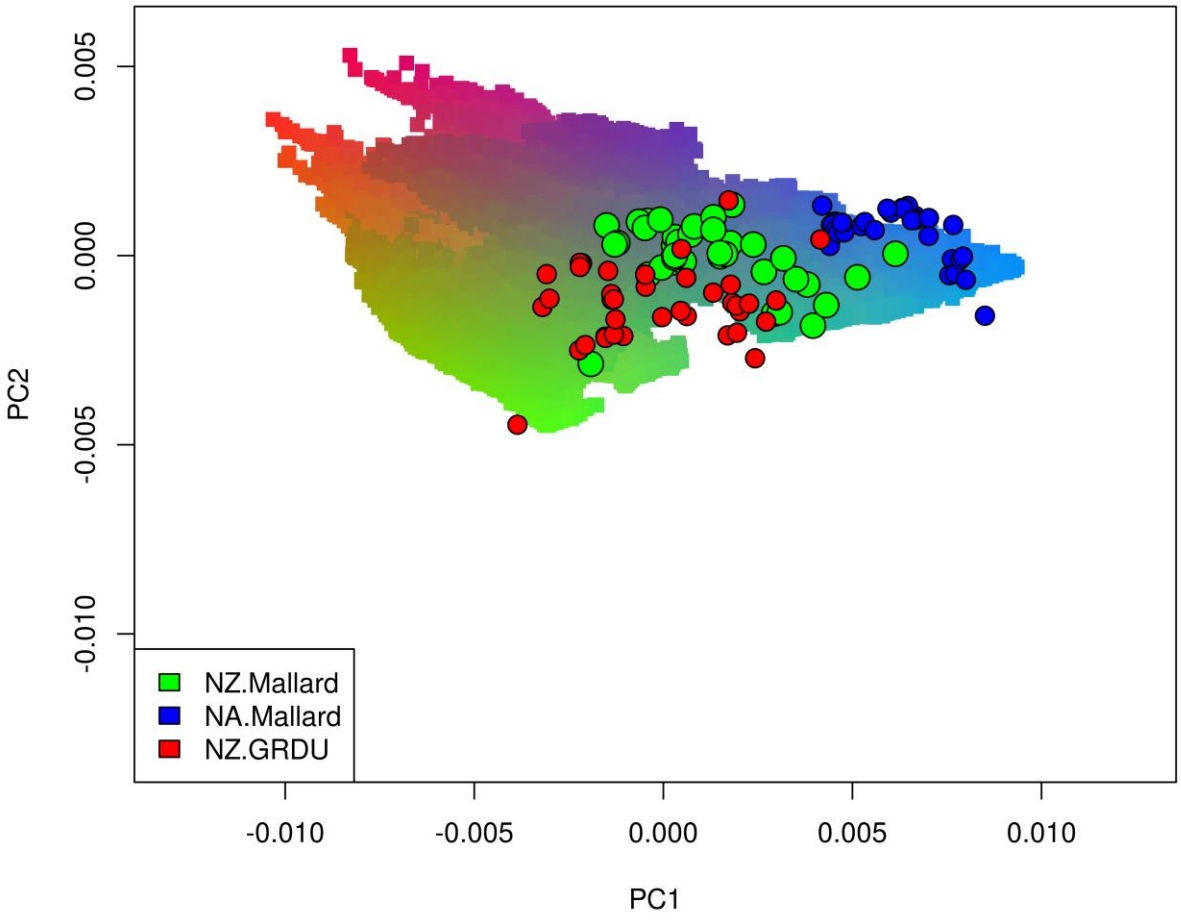


Figure 3.5. Grey duck, New Zealand mallard, and North American mallard gradientForest (GF) values projected across a PCA of genotype-environment associations for New Zealand mallards.

Human-induced breakdown of reproductive barriers and adaptive introgression facilitating non-native species expansion

Anthropogenic hybridization is often variable across the landscape, as heterogeneous selective pressures are artificially influenced by changes in land use and habitat degradation (Foley et al., 2005). This type of transformation can lead to a breakdown of reproductive isolation in some areas, as anthropogenic changes interrupt selective pressures that drive natural behaviors (Malukiewicz et al., 2015). Variable rates of hybridization among grey ducks and mallards across New Zealand suggest that reproductive isolation has broken down in areas where the two species come in contact. Starting with European colonization ~200 years ago, mallard populations have been able to expand throughout this region, as native wetlands and forest lands were quickly replaced with farmland (Holdaway, Wiser, & Williams, 2012; Taylor & Dizon, 1999). Such human disturbance likely acted to facilitate hybridization, as areas with the most severe habitat degradation generally overlap areas where hybrid swarming is now concentrated (e.g., eastern coast of the South Island; Figure 3.2). In fact, the genotypic turnover in the combined grey duck x mallard GF model not only recapitulates the putative range of the remaining grey duck population but identifies it as distinct niche space from areas dominated by mallards and hybrids (i.e., the eastern parts of the South and North Islands; Figure 3.4; Supplementary Materials Figure S3.5). Together, I conclude that the timing in mallard introductions coinciding with human-induced habitat changes facilitated the quick establishment and spread of feral mallard populations throughout New Zealand.

While the theoretical complexities of biological invasions have been widely discussed, empirical evidence on the roles that various evolutionary mechanisms play in this process is lacking (Colautti & MacIsaac, 2004; Davis & Thompson, 2000; Richardson et al., 2000; Valéry,

Fritz, Lefeuvre, & Simberloff, 2008). Here, I provide evidence that the spread of a largely feral mallard population throughout New Zealand was facilitated by extensive introgressive hybridization with locally adapted grey ducks. During species invasion, establishment and expansion are two separate processes which are differentially affected by novel selective pressures, standing genetic variation, and gene flow with closely related native taxa (Drake, 2006). In a kind of 'catapult' effect, I contend that heterosis (i.e., hybrid vigor) – which occurs when hybrid offspring are superior in fitness to either parental species with respect to growth or reproduction (Lippman & Zamir, 2007) – has enabled the establishment of mallard populations in New Zealand, while adaptive introgression from grey ducks has subsequently facilitated their rapid expansion (Verhoeven, Macel, Wolfe, & Biere, 2011). In fact, combined models of GEA suggest that while genotypic turnover within early-generation hybrids encompass the genetic variation of both parental species, admixed individuals and mallard backcrosses have an increased adaptive range (Figure 3.4). These results demonstrate how introgression can act to increase establishment probability for non-native taxa through heterosis and re-affirm the important role of hybridization in biological invasions (Keller & Taylor, 2010; Verhoeven et al., 2011).

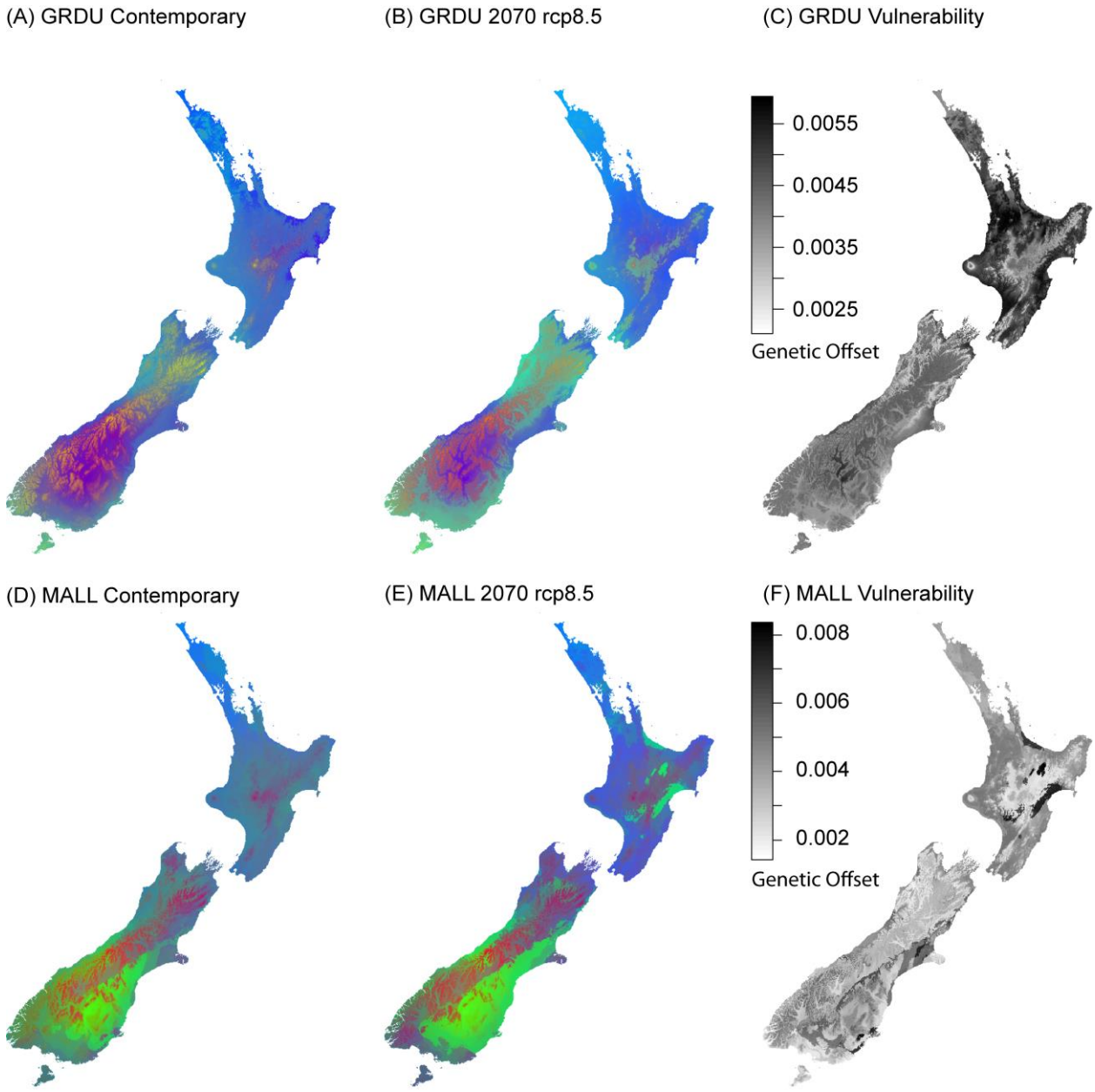


Figure 3.6. (A, D) Grey duck and mallard genotype-environment association models from gradientForest (GF) based on only the top five most predictive temperature and precipitation variables. (B, E) Associations are modelled across future environmental data for 2070 under the most extreme (rcp8.5) projections of climate change. (C, F) Genomic offset calculated from the Euclidean distance between models based on contemporary and future climate conditions mapped across North America.

Future threat of climate change and landscape use changes

Understanding the relationship between genotype and the environment has become a major tool for evaluating the vulnerability of natural populations under future climate change scenarios (Owens & Samuk, 2020). Additionally, given that many invasive taxa stand to thrive due to climate change (Macinnis-Ng et al., 2021), I can use these same GEA methods to predict adaptive potential of invasive taxa under future environments and identify areas where expansion might further threaten local populations. Here, GEA modelling for the most extreme estimates (rcp8.5) of climate change show differing areas of vulnerability for mallards and grey ducks (Figure 3.6). Specifically, mallard genotypes show a strong potential for becoming maladaptive across nearly the entirety of the North Island, which may act to further isolate North and South Island populations as allele frequencies respond differently to the environment. However, the projected Northern expansion of South Island genotypes suggests that introgression of novel alleles into northern populations may help to mitigate these effects (Figure 3.6). Given that New Zealand mallards have restricted movement patterns (i.e., <25km; (Caithness et al., 1991), and that are reflected in inter-island population structure (Figure 3.1), whether they can persist under future climate conditions without supplemental stocking may depend on increased dispersal between islands. Moreover, while incorporating standing genetic variation allows me to better estimate adaptive potential, GF models cannot account for land use change in the future. Since human colonization began ~700 years ago, it is estimated that more than 70% of the native wetlands throughout New Zealand have been drained for agricultural purposes or urban development (Ausseil, Chadderton, Gerbeaux, Stephens, & Leathwick, 2011; Myers, Clarkson, Reeves, & Clarkson, 2013); if this trend continues, New Zealand mallards would be the most likely to benefit,

as they are known to take advantage of these agricultural and high-disturbance areas (English, Robertson, Peck, & Mallory, 2017; Figley & VanDruff, 1982).

Unlike mallards, grey ducks are extremely vulnerable to climate change across their entire range, with the most severe offset across the North Island. In the South Island, significant genetic offset from contemporary conditions were identified in the eastern and southeastern regions (Figure 3.6); however, I note these areas may already be unsuitable given the general lack of grey duck samples from this area (Figure 3.6; McGlone, 2009). More importantly, GEA models predict that remaining core grey duck habitat along the western coasts will be threatened due to climate change (Figure 3.6). If grey ducks are unable to rapidly adapt and are pushed out of this range, ecological partitioning and reproductive barriers will continue to break down, increasing the chance for complete lineage fusion. Additionally, while I cannot rule out the possibility of grey ducks rapidly adapting to changing environmental conditions, breeding habitat along these coastal wetlands will undeniably be lost to sea level rise and saline intrusion (Cieraad, Walker, Price, & Barringer, 2015). Saltwater intrusion can have a particularly detrimental effect on plant x herbivore interactions (Traill, Whitehead, & Brook, 2009; White & Kaplan, 2017), as freshwater plants and invertebrates that act as essential food sources for grey ducks would likely be replaced. In general, I conclude that conservation of native grey duck habitat should be prioritized, as changing environmental conditions will certainly lead to increased contact and gene flow with mallards. Specifically, native wetland restoration across the South Island would likely increase suitable grey duck habitat, which could simultaneously reinforce the development of reproductive barriers through ecological partitioning.

Considerations of adaptive potential and conservation in the Anthropocene

The general role for conservation biology has long been debated (Soulé, 1985; Wilson, 2000), as specific conservation goals are not always congruent across political or organizational boundaries (Dallimer & Strange, 2015). However, in general, the main principle of conservation is to preserve as much unique biodiversity as possible, including, but not limited to the most specialized habitats and the various distinctive taxa that have evolved to thrive within them (Hunter Jr. & Gibbs, 2007; Wilson, 2000). More recently, calls to reconsider how anthropogenic hybridization is viewed in an era of rapid environmental change have gone against this guiding principle (Hirashiki, Kareiva, & Marvier, 2021). Specifically, Hirashiki et al. (2021) argued that introgression from invasive taxa may actually provide the genetic variation necessary for rapid adaptation to occur under uncertain future environmental conditions. Taking this idea a step further, they suggested that in cases where genetic swamping occurs and the resulting hybrid swarm is at least equally as fit as the parent species, then biodiversity is not lost as a new entity (i.e., the hybrid swarm) continues to fill the ecological niche. For example, an ancestral hybridization event between mallards and Laysan ducks (*A. laysanensis*) resulted in hybrids that were unexpectedly better adapted to changing landscapes driven by Polynesian arrival, permitting the Hawaiian duck (*A. wyvilliana*) to survive while its original congener (the Laysan duck) nearly went extinct (Lavretsky, Engilis, Eadie, & Peters, 2015). In this case, the Hawaiian duck is one of few clear examples of a natural hybridization event providing the necessary diversity rapid adaptation to ongoing ecological change. While the Hawaiian duck is one such example of how hybridization can increase biodiversity in a rapidly changing niche space, I provide the first evidence of anthropogenic hybridization resulting in a sustained feral x wild population that appears to be better adapted to current and future environmental conditions then their endemic

wild parental species. However, the complex ecological effects originating from extensive introgression are unpredictable and can easily break the equilibrium between selection and gene flow that was previously acting to maintain diversity from both parental taxa. Therefore, I implore that attempts to conserve New Zealand's endemic grey duck are absolutely necessary in order to preserve the unique natural diversity contained within this species.

Naturalization of introduced species, especially those of domestic origins remains contentiously debated within the conservation sciences (Sol, 2008). The ecological roles played by these introduced populations are important to consider when deciding their fate, as adaptive introgression from native taxa can provide those variants necessary to thrive. For instance, though their ancestors were largely of domestic origins, New Zealand's mallards today have not only been under the strong selective pressures from a novel environment for over a century, but their genomes show significant introgression from their wild congener, the grey duck. In general, these mallards are uniquely adapted to the local selective pressures of New Zealand (Figure 3.5), and no longer genetically resemble their parental populations (Figure 3.5). Given stable population trends (McDougall & Amundson, 2017) with survival and fecundity estimates being comparable to other wild populations (Sheppard, 2017), New Zealand's mallards potentially constitute a case of naturalization. I argue that the adaptive advantages of the introduced and/or hybrid individuals require careful evaluation on a case-by-case basis with special consideration still being given to conserving the endemic species. Though I demonstrate the usefulness of reduced-representation sequence data with landscape-level sample sets for investigating the evolutionary history of non-model systems, future work will require continued genetic monitoring as the negative effects of introgression may yet to be realized.

ACKNOWLEDGEMENTS

I am thankful to all participating New Zealand Game & Fish individuals, J. Sheppard, I. Engilis, and M. Mayfield for providing samples. I am grateful to Ducks Unlimited for the Richard H. G. Bonnycastle Fellowship in Wetland and Waterfowl Biology, to Ducks Unlimited Canada Institute for Wetland and Waterfowl Research for the Waterfowl Research Foundation Graduate Fellowship, A. Engilis Jr. and the UC-Davis Museum of Wildlife and Fish Biology, as well as the University of Texas at El Paso for providing financial support throughout this study.

REFERENCES

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. doi:10.1186/1471-2105-12-246

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research, 19*(9), 1655–1664. doi:10.1101/gr.094052.109

Ausseil, A.-G. E., Chadderton, W. L., Gerbeaux, P., Stephens, R. T. T., & Leathwick, J. R. (2011). Applying systematic conservation planning principles to palustrine and inland saline wetlands of New Zealand. *Freshwater Biology*, *56*(1), 142–161. doi:10.1111/j.1365-2427.2010.02412.x

Avise, J. C., Ankney, C. D., & Nelson, W. S. (1990). Mitochondrial gene trees and the evolutionary relationships of Mallard and Black ducks. *Evolution*, *44*(4), 1109–1119. doi:10.1111/j.1558-5646.1990.tb03829.x

Balham, R. W. (1952). Grey and Mallard Ducks in the Manawatu District, New Zealand. *Emu*, *52*(3), 163–191. doi:10.1071/MU952163

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Bay, R. A., Harrigan, R. J., Underwood, V. Le, Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359*(6371), 83–86. doi:10.1126/science.aan4380

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. doi:10.1093/bioinformatics/btu170

Braithwaite, L. W., & Miller, B. (1975). The Mallard, *Anas platyrhynchos*, and Mallard-Black Duck, *Anas superciliosa rogersi*, Hybridization. *Wildlife Research*, *2*(1), 47–61. doi:10.1071/WR9750047

Brown, J. I., Lavretsky, P., Joseph, L., Roshier, D., Guay, P. J., & Peters, J. L. (2021). A multi-locus approach to discern conservation units and adaptive potential of Pacific black ducks across Australia and surrounding islands. *Emu - Austral Ornithology*, 1–12. doi:10.1080/01584197.2021.1911601

Caithness, T., Williams, M., & Nichols, J. D. (1991). Survival and Band Recovery Rates of Sympatric Grey Ducks and Mallards in New Zealand. *The Journal of Wildlife Management*, *55*(1), 111–118. doi:10.2307/3809247

Cieraad, E., Walker, S., Price, W., & Barringer, J. (2015). An updated assessment of indigenous cover remaining and legal protection in New Zealand's land environments. *New Zealand Journal of Ecology*, *39*(2), 309–315.

Colautti, R. I., & MacIsaac, H. J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions*, *10*(2), 135–141. doi:10.1111/j.1366-9516.2004.00061.x

Crispo, E., Moore, J.-S., Lee-Yaw, J. A., Gray, S. M., & Haller, B. C. (2011). Broken barriers: Human-induced changes to gene flow and introgression in animals. *BioEssays*, *33*(7), 508–518. doi:10.1002/bies.201000154

Culumber, Z. W., Ochoa, O. M., & Rosenthal, G. G. (2014). Assortative mating and the maintenance of population structure in a natural hybrid zone. *The American Naturalist*, *184*(2), 225–232. doi:10.1086/677033

DaCosta, J. M., & Sorenson, M. D. (2014). Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. *PLoS ONE*, *9*(9), e106713. doi:10.1371/journal.pone.0106713

Dallimer, M., & Strange, N. (2015). Why socio-political borders and boundaries matter in conservation. *Trends in Ecology and Evolution*, *30*(3), 132–139. doi:10.1016/j.tree.2014.12.004

Davis, M. A., & Thompson, K. (2000). Eight Ways to Be a Colonizer; Two Ways to Be an Invader: A Proposed Nomenclature Scheme for Invasion Ecology. *Bulletin of the Ecological Society of America*, *81*(3), 226–230.

Drake, J. M. (2006). Heterosis, the catapult effect and establishment success of a colonizing bird. *Biology Letters*, *2*(2), 304–307. doi:10.1098/rsbl.2006.0459

Dyer, J., & Williams, M. (2010). An introduction most determined: Mallard (Anas platyrhynchos) to New Zealand. *Notornis*, *57*(4), 178–195.

Ellis, N., Smith, S. J., & Roland Pitcher, C. (2012). Gradient forests: Calculating importance gradients on physical predictors. *Ecology*, *93*(1), 156–168. doi:10.1890/11-0252.1

English, M. D., Robertson, G. J., Peck, L. E., & Mallory, M. L. (2017). Agricultural food resources and the foraging ecologies of American black ducks (*Anas rubripes*) and mallards (*Anas*

platyrhynchos) at the northern limits of their winter ranges. *Urban Ecosystems*, *20*(6), 1311–1318. doi:10.1007/s11252-017-0683-0

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. doi:10.1111/j.1755-0998.2010.02847.x

Figley, W. K., & VanDruff, L. W. (1982). The Ecology of Urban Mallards. *Wildlife Monographs*, *81*, 3–39.

Fitzpatrick, M. C., & Keller, S. R. (2015). Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters*, *18*(1), 1–16. doi:10.1111/ele.12376

Foley, J. A., Defries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., … Snyder, P. K. (2005). Global consequences of land use. *Science*, *309*(5734), 570–574. doi:10.1126/science.1111772

Francis, R. M. (2017). POPHELPER: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, *17*(1), 27–32. doi:10.1111/1755-0998.12509

Gillespie, G. D. (1985). Hybridization, Introgression, and Morphometric Differentiation between Mallard (Anas platyrhynchos) and Grey Duck (Anas superciliosa) in Otago, New Zealand. *The Auk*, *102*(3), 459–469. doi:10.2307/4086641

Gilman, R. T., & Behm, J. E. (2011). Hybridization, species collapse, and species reemergence after disturbance to premating mechanisms of reproductive isolation. *Evolution*, *65*(9), 2592–2605. doi:10.1111/j.1558-5646.2011.01320.x

Glotzbecker, G. J., Walters, D. M., & Blum, M. J. (2016). Rapid movement and instability of an

invasive hybrid swarm. *Evolutionary Applications*, *9*(6), 741–755. doi:10.1111/eva.12371

Gow, J. L., Peichel, C. L., & Taylor, E. B. (2006). Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Molecular Ecology*, *15*(3), 739–752. doi:10.1111/j.1365-294X.2006.02825.x

Grabenstein, K. C., & Taylor, S. A. (2018). Breaking Barriers: Causes, Consequences, and Experimental Utility of Human-Mediated Hybridization. *Trends in Ecology and Evolution*, *33*(3), 198–212. doi:10.1016/j.tree.2017.12.008

Haddon, M. (1984). A Re-Analysis of Hybridization between Mallards and Grey Ducks in New Zealand. *The Auk*, *101*(1), 190–191.

Hasselman, D. J., Argo, E. E., McBride, M. C., Bentzen, P., Schultz, T. F., Perez-Umphrey, A. A., & Palkovacs, E. P. (2014). Human disturbance causes the formation of a hybrid swarm between two naturally sympatric fish species. *Molecular Ecology*, *23*(5), 1137–1152. doi:10.1111/mec.12674

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. doi:10.1002/joc.1276

Hirashiki, C., Kareiva, P., & Marvier, M. (2021). Concern over hybridization risks should not preclude conservation interventions. *Conservation Science and Practice*, *3*(4), e424. doi:10.1111/csp2.424

Holdaway, R. J., Wiser, S. K., & Williams, P. A. (2012). Status Assessment of New Zealand's Naturally Uncommon Ecosystems. *Conservation Biology*, *26*(4), 619–629. doi:10.1111/j.1523-1739.2012.01868.x

Hoskin, C. J., Higgie, M., Mcdonald, K. R., & Moritz, C. (2005). Reinforcement drives rapid allopatric speciation. *Nature*, *43*(7063), 1353. doi:10.1038/nature04004

Huang, Y., Li, Y., Burt, D. W., Chen, H., Zhang, Y., Qian, W., … Li, N. (2013). The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. *Nature Genetics*, *45*(7), 776–783. doi:10.1038/ng.2657

Hunter Jr., M. L., & Gibbs, J. P. (2007). *Fundamentals of Conservation Biology* (Third). Oxford, UK: Blackwell Publishing.

Ingvarsson, P. K., & Whitlock, M. C. (2000). Heterosis increases the effective migration rate. *Proceedings of the Royal Society B: Biological Sciences*, *267*(1450), 1321–1326. doi:10.1098/rspb.2000.1145

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*(14), 1801–1806. doi:10.1093/bioinformatics/btm233

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. doi:10.1093/bioinformatics/btn129

Keller, S. R., & Taylor, D. R. (2010). Genomic admixture increases fitness during a biological invasion. *Journal of Evolutionary Biology*, *23*(8), 1720–1731. doi:10.1111/j.1420-9101.2010.02037.x

Kraus, R. H., Kerstens, H. H., Van Hooft, P., Crooijmans, R. P., Van Der Poel, J. J., Elmberg, J., … Groenen, M. A. (2011). Genome wide SNP discovery, analysis and evaluation in mallard (Anas platyrhynchos). *BMC Genomics*, *12*(1), 150. doi:10.1186/1471-2164-12-150

Kulikova, I. V, Drovetski, S. V, Gibson, D. D., Harrigan, R. J., Rohwer, S., Sorenson, M. D., … Kevin, G. (2005). Phylogeography of the Mallard (*Anas platyrhynchos*): Hybridization ,

Dispersal , and Lineage Sorting Contribute to Complex Geographic Structure. *The Auk*, *122*(3), 949–965.

Lande, R. (1998). Anthropogenic, Ecological and Genetic Factors in Extinction and Conservation. *Researches on Population Ecology*, *40*(3), 259–269. doi:10.1007/BF02763457

Lavretsky, P., Dacosta, J. M., Hernández-Baños, B. E., Engilis, A., Sorenson, M. D., & Peters, J. L. (2015). Speciation genomics and a role for the Z chromosome in the early stages of divergence between Mexican ducks and mallards. *Molecular Ecology*, *24*(21), 5364–5378. doi:10.1111/mec.13402

Lavretsky, P., DaCosta, J. M., Sorenson, M. D., McCracken, K. G., & Peters, J. L. (2019). ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. *Molecular Ecology*, *28*(10), 2594–2609. doi:10.1111/mec.15091

Lavretsky, P., Engilis, A., Eadie, J. M., & Peters, J. L. (2015). Genetic admixture supports an ancient hybrid origin of the endangered Hawaiian duck. *Journal of Evolutionary Biology*, *28*(5), 1005–1015. doi:10.1111/jeb.12637

Lavretsky, P., Janzen, T., & McCracken, K. G. (2019). Identifying hybrids & the genomics of hybridization: Mallards & American black ducks of Eastern North America. *Ecology and Evolution*, *9*(6), 3470–3490. doi:10.1002/ece3.4981

Lavretsky, P., McCracken, K. G., & Peters, J. L. (2014). Phylogenetics of a recent radiation in the mallards and allies (Aves: Anas): Inferences from a genomic transect and the multispecies coalescent. *Molecular Phylogenetics and Evolution*, *70*(1), 402–411. doi:10.1016/j.ympev.2013.08.008

Lavretsky, P., McInerney, N. R., Mohl, J. E., Brown, J. I., James, H. F., McCracken, K. G., &

Fleischer, R. C. (2020). Assessing changes in genomic divergence following a century of human-mediated secondary contact among wild and captive-bred ducks. *Molecular Ecology*, *29*(3), 578–595. doi:10.1111/mec.15343

Lavretsky, P., Peters, J. L., Winker, K., Bahn, V., Kulikova, I., Zhuravlev, Y. N., … McCracken, K. G. (2016). Becoming pure: Identifying generational classes of admixed individuals within lesser and greater scaup populations. *Molecular Ecology*, *25*(3), 661–674. doi:10.1111/mec.13487

Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. *Nature*, *475*(7357), 493–496. doi:10.1038/nature10231

Liou, L. W., & Price, T. D. (2006). Speciation by Reinforcement of Premating Isolation. *Evolution*, *48*(5), 1451. doi:10.2307/2410239

Lippman, Z. B., & Zamir, D. (2007). Heterosis: revisiting the magic. *Trends in Genetics*, *23*(2), 60–66. doi:10.1016/j.tig.2006.12.006

Macinnis-Ng, C., Mcintosh, A. R., Monks, J. M., Waipara, N., White, R. S., Boudjelas, S., … Peltzer, D. A. (2021). Climate-change impacts exacerbate conservation threats in island systems: New Zealand as a case study. *Frontiers in Ecology and the Environment*, *19*(4), 216–224. doi:10.1002/fee.2285

Malukiewicz, J., Boere, V., Fuzessy, L. F., Grativol, A. D., de Oliveira e Silva, I., Pereira, L. C. M., … Stone, A. C. (2015). Natural and Anthropogenic Hybridization in Two Species of Eastern Brazilian Marmosets (*Callithrix jacchus* and *C. penicillata*). *PLOS ONE*, *10*(6), e0127268. doi:10.1371/journal.pone.0127268

Marsden, C. D., Vecchyo, D. O. Del, O'Brien, D. P., Taylor, J. F., Ramirez, O., Vilà, C., … Lohmueller, K. E. (2016). Bottlenecks and selective sweeps during domestication have

increased deleterious genetic variation in dogs. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(1), 152–157. doi:10.1073/pnas.1512501113

McDougall, M. B., & Amundson, C. L. (2017). Harvest dynamics and annual survival of mallards and grey ducks. *The Journal of Wildlife Management*, *81*(3), 449–460. doi:10.1002/jwmg.21213

McFarlane, S. E., & Pemberton, J. M. (2019). Detecting the True Extent of Introgression during Anthropogenic Hybridization. *Trends in Ecology and Evolution*, *34*(4), 315–326. doi:10.1016/j.tree.2018.12.013

McFarlane, S. E., Senn, H. V., Smith, S. L., & Pemberton, J. M. (2021). Locus-specific introgression in young hybrid swarms: Drift may dominate selection. *Molecular Ecology*, *30*(9), 2104–2115. doi:10.1111/mec.15862

McGlone, M. S. (2009). Postglacial history of New Zealand wetlands and implications for their conservation. *New Zealand Journal of Ecology*, *33*(1), 1–23. doi:10.1016/j.quascirev.2007.09.011

Michaelides, S. N., Goodman, R. M., Crombie, R. I., & Kolbe, J. J. (2018). Independent introductions and sequential founder events shape genetic differentiation and diversity of the invasive green anole (*Anolis carolinensis*) on Pacific Islands. *Diversity and Distributions*, *24*(5), 666–679. doi:10.1111/ddi.12704

Myers, S. C., Clarkson, B. D. R., Reeves, P. N., & Clarkson, B. D. R. (2013). Wetland management in New Zealand: Are current approaches and policies sustaining wetland ecosystems in agricultural landscapes? *Ecological Engineering*, *56*, 107–120. doi:10.1016/j.ecoleng.2012.12.097

Owens, G. L., & Samuk, K. (2020). Adaptive introgression during environmental change can

weaken reproductive isolation. *Nature Climate Change*, *10*(1), 58–62. doi:10.1038/s41558-019-0628-0

Pfaff, C. L., Parra, E. J., Bonilla, C., Hiester, K., McKeigue, P. M., Kamboh, M. I., … Shriver, M. D. (2001). Population structure in admixed populations: Effect of admixture dynamics on the pattern of linkage disequilibrium. *American Journal of Human Genetics*, *68*(1), 198–207. doi:10.1086/316935

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. doi:10.1093/molbev/msu136

Pfennig, K. S., Kelly, A. L., & Pierce, A. A. (2016). Hybridization as a facilitator of species range expansion. *Proceedings of the Royal Society B*, *283*(1839). doi:10.1098/rspb.2016.1329

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P. C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics*, *81*(3), 559–575. doi:10.1086/519795

Rhymer, J. M., & Simberloff, D. (1996). Extinction by Hybridization and Introgression. *Annual Review of Ecology and Systematics*, *27*(1), 83–109. doi:10.1146/annurev.ecolsys.27.1.83

Rhymer, J. M., Williams, M. J., & Braun, M. J. (1994). Mitochondrial Analysis of Gene Flow between New Zealand Mallards (Anas platyrhynchos) and Grey Ducks (A . superciliosa). *The Auk*, *111*(4), 970–978.

Rhymer, J. M., Williams, M. J., & Kingsford, R. T. (2004). Implications of phylogeography and population genetics for subspecies taxonomy of Grey (Pacific Black) Duck Anas superciliosa and its conservation in New Zealand. *Pacific Conservation Biology*, *10*, 57–66.

Richardson, D. M., Pysek, P., Rejmanek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, *6*(2), 93–107. doi:10.1046/j.1472-4642.2000.00083.x

Schubert, M., Jónsson, H., Chang, D., Der Sarkissian, C., Ermini, L., Ginolhac, A., … Orlando, L. (2014). Prehistoric genomes reveal the genetic foundation and cost of horse domestication. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(52), E5661–E5669. doi:10.1073/pnas.1416991111

Sheppard, J. L. (2017). *Breeding Ecology and Productivity of Mallards and Mallard-grey Duck Hybrids in New Zealand*. University of Auckland, Auckland, NZ.

Sheridan, A. K. (1995). The Genetic Impacts of Human Activities on Wild Fish Populations. *Reviews in Fisheries Science*, *3*(2), 91–108. doi:10.1080/10641269509388568

Sol, D. (2008). Artificial selection, naturalization, and fitness: Darwin's pigeons revisited. *Biological Journal of the Linnean Society*, *93*(4), 657–665. doi:10.1111/j.1095-8312.2008.00957.x

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-Based Approach to Mitochondrial Genome Sequencing in Birds and Other Vertebrates. *Molecular Phylogenetics and Evolution*, *12*(2), 105–114. doi:10.1006/MPEV.1998.0602

Sorenson, M. D., & Fleischer, R. C. (1996). Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(26), 15239–15243. doi:10.1073/PNAS.93.26.15239

Soulé, M. E. (1985). What Is Conservation Biology? *BioScience*, *35*(11), 727–734.

Taylor, B. L., & Dizon, A. E. (1999). First policy then science: Why a management unit based

solely on genetic criteria cannot work. *Molecular Ecology*, *8*(s1), S11–S16. doi:10.1046/j.1365-294x.1999.00797.x

Traill, L. W., Whitehead, P. J., & Brook, B. W. (2009). How will climate change affect plantherbivore interactions? A tropical waterbird case study. *Emu - Austral Ornithology*, *109*(2), 126–134. doi:10.1071/MU09003

Valéry, L., Fritz, H., Lefeuvre, J. C., & Simberloff, D. (2008, December 1). In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions*. Springer. doi:10.1007/s10530-007-9209-7

Verhoeven, K. J. F., Macel, M., Wolfe, L. M., & Biere, A. (2011). Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society B*, *278*(1702), 2–8. doi:10.1098/rspb.2010.1272

Wayne, R. K., & Shaffer, H. B. (2016). Hybridization and endangered species protection in the molecular era. *Molecular Ecology*, *25*(11), 2680–2689. doi:10.1111/mec.13642

Wells, C. P., Lavretsky, P., Sorenson, M. D., Peters, J. L., DaCosta, J. M., Turnbull, S., … Engilis, A. (2019). Persistence of an endangered native duck, feral mallards, and multiple hybrid swarms across the main Hawaiian Islands. *Molecular Ecology*, *28*(24), 5203–5216. doi:10.1111/mec.15286

White, E., & Kaplan, D. (2017). Restore or retreat? saltwater intrusion and water management in coastal wetlands. *Ecosystem Health and Sustainability*, *3*(1), e01258. doi:10.1002/ehs2.1258

Williams, G. R. (1964). Extinction and the Anatidae of New Zealand. *The Wildfowl Trust Fifteenth Annual Report*, *15*, 140–146.

Williams, M. (1981). *The Duckshooter's Bag: An Understanding of New Zealand's Wetland Gamebirds*.

Williams, M. (2017). The changing relative abundance of grey duck (Anas superciliosa) and mallard (A . platyrhynchos) in New Zealand. *Notornis*, *64*, 211–228.

Williams, M., & Roderick, C. (1973). The breeding performance of Grey duck, Anas superciliosa, mallard, Anas platyrhynchos, and their hybrids in captivity. *International Zoo Yearbook*, *13*(1), 62–69. doi:10.1111/j.1748-1090.1973.tb02105.x

Wilson, E. O. (2000). On the future of conservation biology. *Conservation Biology*, *14*(1), 1–3.

Zhou, H., Alexander, D., & Lange, K. (2011). A quasi-Newton acceleration for high-dimensional optimization algorithms. *Statistics and Computing*, *21*(2), 261–273. doi:10.1007/s11222-009-9166-3

Chapter 4: Lost adaptive potential due to hybridization with domestic conspecifics: are we losing wild mallards in North America?

ABSTRACT

Anthropogenic hybridization resulting from growing feral populations has become a leading conservation concern for their respective wild conspecifics. Such interactions can result in the introgression of maladaptive alleles that lower fitness in wild populations, as domestic populations often face artificial selective pressures during the domestication process. For example, the release of domestically derived game-farm mallards has resulted in extensive interbreeding with wild mallards in North America. Here, I assay thousands of nuclear loci and the mitochondrial DNA control region across a range-wide sampling of wild North American mallards to determine current rates of hybridization and the adaptive consequences of these interactions. First, I find a 4-fold decrease in the prevalence of pure wild mallards as compared to a decade ago, with mallards in eastern North America (i.e., Atlantic flyway) now comprising a hybrid swarm as ~98% of samples possessed significant game-farm ancestry. Next, I use genotype-environment association modelling to show that wild and game-farm mallards have differing relationships to environmental selective pressures. Moreover, contemporary climate conditions are likely limiting the expansion of game-farm mallards, as significant genotypic turnover across the southern Canadian border is likely acting as a barrier to dispersal. Using future climate conditions, I show that game-farm mallards are likely to find an expanded adaptive breeding space as temperatures warm, especially since this group is most strongly affected by winter weather conditions. Broadly, these results further demonstrate how the relationship between selection, gene flow, and the environment has a complex effect on fitness. Finally, I discuss the utility of including landscape genomics in the conservation decision making process, as increasing anthropogenically-induced ecological changes are felt around the world.

INTRODUCTION

Domestic releases have resulted in increased anthropogenic hybridization across landscapes (Chapin III et al., 2000) and are now a major conservation concerns for their respective wild conspecific populations (e.g., *Neovison vison*, Kidd, Bowman, Lesbarreres, & Schulte-Hostedde, 2009; *Canis lupus*, Vilá & Wayne, 1999; and *Sus scrofa*, Scandura, Iacolina, & Apollonio, 2011; *Anas platyrhynchos*, Lavretsky et al., 2020). Given that domesticated individuals do not face the same selective pressures as their wild counterparts, their introduction can lead to the introgression of maladaptive alleles that lower overall fitness in wild populations (Ford, 2002). For instance, Araki, Cooper, & Blouin (2007) found that the fitness of captive reared Steelhead trout (*Onchorynchus mykiss*) declined by ~40% per captive-bred generation, and that the cumulative decrease in fitness across subsequent generations among wild populations was driven by their augmented genomes and not environmental effects. In general, traits favored during the domestication process can lead to decreased predator avoidance (McPhee, 2004), lowered feeding efficiency (T. Ellis, Hughes, & Howell, 2002), and irregular breeding behaviors (Ruzzante, 1994). Even with these known threats posed by the introduction of domestic individuals, supplemental stocking programs continue to be a common management tool for popular game species (Griffith, Scott, Carpenter, & Reed, 1989; Laikre, Palmé, Josefsson, Utter, & Ryman, 2006); however, supplemental stocking efforts often continue indefinitely as many of these programs are unable to establish or re-establish self-sustaining populations (e.g., salmon (*Salmo Salar*), Blanchet, Páez, Bernatchez, & Dodson, 2008; walleye (*Stizostdion vitreum*), Parsons & Pereira, 2001; mallards, Champagnon et al., 2012; northern bobwhite quail (*Colinus virginianus*), Evans et al., 2017; red deer (*Cervus elaphus*), Iacolina, Corlatti, Buzan, Safner, & Šprem, 2019). Improving sequencing technologies have increased our ability to genetically monitor these populations and has shed light

into the true conservation consequences of such stocking programs (Angeloni, Wagemaker, Vergeer, & Ouborg, 2012; Garner et al., 2016). For example, domestically-derived game-farm mallards have been released in North America for over a century with the belief that these releases would artificially increase mallards as a game resource with no maladaptive impact on wild populations (Heusmann, 2017; Heusmann & Sauer, 1997). However, recent molecular assessments of wild populations identified wide-spread introgressive hybridization, with ~92% of eastern populations containing significant game-farm ancestry (Lavretsky, Janzen, & McCracken, 2019; Lavretsky et al., 2020), which is also hypothesized to partially explain recent mallard population declines in eastern North America (Champagnon et al., 2016; Heusmann, 2017). While extensive hybridization between domestic and wild mallards has already been identified, this study aims to investigate the genetic and adaptive consequences of such interactions.

Variability in selective pressures across a species' range can act to create genotype-environment associations that lead to the development of locally adaptive traits within a population (i.e., local adaptation; Kawecki & Ebert, 2004; Williams, 1966). Given that the relative fitness advantage of locally adapted traits is most strongly associated with the proximate environment, introgression and genetic swamping from captive-bred individuals may counteract potential fitness advantages (i.e., outbreeding depression; Edmands, 1999). Although in this case I might expect purifying selection to purge deleterious alleles being introduced by domestic conspecifics, the outcome largely depends on the balance between the number of new domestic individuals being released and the selective pressures that dictate introgression (i.e., gene flow, negative selection; Ford, 2002; Savolainen, Pyhäjärvi, & Knürr, 2007; Slatkin, 1987). Deleterious alleles can be readily maintained in local populations where environmental selection is relaxed, and releases are more common (Robert, 2009; Waples, Zabel, Scheurell, & Sanderson, 2008). Additionally,

introgression from domestic stock can differentially affect wild populations across their range, as the selective pressures that dictate this process are spatially and temporally heterogeneous across the landscape (Forester, Jones, Joost, Landguth, & Lasky, 2016; Vangestel, Eckert, Wegrzyn, St. Clair, & Neale, 2018). Overall, hybridization between wild and domestic conspecifics provides 'wild experiments' that can be used to improve our understanding of how selection and gene flow interact to facilitate or prevent the genetic swamping of locally adaptive alleles (Petren, Grant, Grant, & Keller, 2005; Rieseberg & Burke, 2001). Therefore, I employ genotype-environment association (GEA) testing on North American mallard populations, which are now comprised of a wild x feral hybrid swarm, to investigate how various environmental selective pressures create genomic gradients that affect the distribution of adaptive and maladaptive traits among these mallard types (Holderegger & Wagner, 2008; Manel et al., 2010; Manel, Schwartz, Luikart, & Taberlet, 2003).

Study system

Over the past two centuries, mallard populations in eastern North America have undergone dramatic fluctuations due to shifts in range and local management practices (Heusmann, 1991; Johnsgard, 1967). Prior to the early 1800s, mallards were largely restricted to areas west of the Mississippi River and were considered vagrants in Atlantic flyway states (i.e., the east coast; Heusmann, 1991). However, mallards became one of the most common waterfowl species in eastern North America by the 1950s. The exponential increase of mallards east of the Mississippi river is thought to be a result of changes in boreal forest habitat and augmented management practices (Osborne, Swift, & Baldassarre, 2010; USFWS, 2013). First, the systematic conversion of eastern boreal forest habitats of Northeastern North America into agricultural lands allowed for

the eastward expansion of wild mallards (Hanson, Rogers, & Rogers, 1949; Snell, 1986). Additionally, both government and private organizations augmented local eastern populations by supplementing them with game-farm (i.e., captive-bred) mallards (Heusmann, 1974, 1991; USFWS, 2013). It is estimated that nearly 500,000 game-farm mallards were released per year along the Atlantic flyway from the 1920s to the late 1970s, with ~250,000 still being released today (Hepp, Novak, Scribner, & Stangel, 1988; Heusmann, 1974; Soutiere, 1986; USFWS, 2013). As a result, eastern mallards are now comprised of descendants from both wild and captive-bred sources (Lavretsky, Janzen, et al., 2019; Lavretsky et al., 2020).

Even with continued game-farm releases, mallard numbers peaked in the Atlantic flyway by the mid-1990s, and overall population numbers as well as local breeding pairs have since steadily declined throughout the region (Alisauskas, Arnold, Leafloor, Otis, & Sedinger, 2014; Heusmann, 2017). To date, there is little known about the proximate cause(s) for the recent population declines of eastern mallards (Heusmann, 2017; Johnsgard, 1967; Merendino & Ankney, 1994). However, the physiological and behavioral differences, as well as overall lower survival of captive bred as compared to wild mallards may be playing a significant role in this decline (Ankney, Dennis, Wishard, & Seeb, 1986; Söderquist, Gunnarsson, Elmberg, & Dessborn, 2021). While captive bred mallards are known to have an overall lower survival (Hepp et al., 1988; Söderquist et al., 2021), they are able to maintain large populations by exploiting urban habitats and agricultural lands (Ankney et al., 1986; Cheng, Shoffner, Phillips, & Lee, 1979). Originally, differences in habitat preference (i.e., urban versus non-urban) was thought to act as a pre-zygotic reproductive barrier between game-farm and wild mallards, however, ecological partitioning has proven to be insufficient due to continuing land use changes and high release numbers (Hepp et al., 1988). In fact, Lavretsky et al. (2019) sampled range-wide mallards across North America and

found that ~92% of Atlantic flyway mallards sampled in 2009 – 2010 were assigned to a genetically unique non-western mallard population that was later confirmed to have originated from known game-farm mallards (Lavretsky et al., 2020). Furthermore, these findings not only suggest extensive introgression of captive-bred genetic material into wild mallards, but that game-farm mallards have likely established viable breeding populations (i.e., feral; Lavretsky et al., 2019).

Modeling introgressive hybridization between domestic and wild conspecifics has demonstrated that detrimental genomic effects take time to become quantifiable (Tufto, 2017). For example, captive-bred mallards have been released throughout Europe on a large-scale since the 1970s (Champagnon et al., 2013), and while introgression is somewhat limited by decreased survival of released mallards, continued large-scale release programs have led to a loss of genetic integrity in some wild mallard populations in Europe (Champagnon et al., 2013; also see Söderquist et al., 2017). Consequently, it is possible that a century of introgression from captive-bred mallards into wild populations has introduced maladaptive genes that are partially responsible for recent declines seen in eastern North American mallard populations.

Here, I use genomic sequencing data from a landscape-level sampling of wild mallard populations to first describe population structure and hybridization rates between wild and game-farm mallards across North America. Given the extent of hybridization detected in previous samples (i.e., ~92% of sampled mallards in 2009-2010; Lavretsky et al., 2019), I hypothesize that eastern populations (i.e., the Atlantic and Mississippi flyways) are now almost entirely comprised of feral and feral x wild hybrid individuals (i.e., hybrid swarm). Additionally, under the hypothesized westward expansion of feral and feral x wild mallards, I expect that the number of hybrids will have increased over the last decade in central North American breeding grounds (i.e.,

the prairie pothole region) as compared to previous estimates (Lavretsky, Janzen, et al., 2019). Next, I implement a multivariate machine-learning program, gradient forest (GF), to model individual and combined relationships between allele frequency changes and environmental variables for wild, feral, and feral x wild hybrid mallards. In general, if feral and hybrid individuals are less adapted in the wild, then I expect higher rates of genotypic turnover reflecting reduced adaptive range as compared to their wild counterparts. Finally, I test for the adaptive capacity of wild and hybrid individuals to adapt to climate change by modeling GEAs across future projected climate conditions. I posit that rising temperatures will act to restrict the northern breeding range of wild mallards, while hybrid individuals will take advantage of more urbanized and agricultural habitats. The genomic evaluation of such domestic x wild interactions will not only shed light into current population dynamics, but also into unintended consequences of management strategies that can result in decreased adaptive evolutionary potential.

METHODS

Sampling, DNA extraction, ddRAD-seq library preparation & de-multiplexing

From 2017 – 2020, a total of 1,393 wild mallards and putative game-farm hybrids were sampled throughout North America (Supplementary Materials Figure S4.1). Additionally, previously published sequences for known game-farm mallards (N = 49) were included as reference populations for comparison (BioProject PRJNA591912, Lavretsky et al., 2020).

For the 1,393 new samples, genomic DNA was extracted from blood or tissue using a DNeasy Blood & Tissue kit following the manufacturer's protocols (Qiagen, Valencia, CA, USA). DNA quality was visually assessed on a 1% agarose gel to ensure high molecular weight bands, and quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA) to ensure a minimum

concentration of 20 ng/μL. ddRAD-seq library preparation followed protocols outlined in DaCosta and Sorenson (DaCosta & Sorenson, 2014; also see Lavretsky et al., 2015). In brief, genomic DNA was enzymatically fragmented using SbfI and EcoRI restriction enzymes. Illumina TruSeq compatible 6 base-pair barcodes were ligated to allow for future de-multiplexing. The barcode-ligated fragments were then size selected for 300 – 450 bp fragments using gel electrophoresis (2% low-melt agarose), followed by gel purification using a MinElute gel extraction kit (Qiagen); and following manufacturer protocols. Size selected fragments were then PCR amplified with Phusion high-fidelity DNA polymerase (Thermo Scientific, Pittsburgh, PA, USA) and purified with AMPure XP magnetic beads (Agencourt, Beverly, MA, USA). Libraries were quantified using a Qubit 3 Flourometer (Invitrogen, Carlsbad, CA, USA), pooled in equimolar, and the multiplexed library sent to the University of Oregon Core Genomics Facility or Novogenetics for 150 base-pair, single-end chemistry sequencing on an Illumina HiSeq 4000 or X, respectively.

Raw reads were de-multiplexed based on perfect barcode matches using the script *ddRADparser.py* (DaCosta & Sorenson, 2014). Next, the program trimmomatic (Bolger, Lohse, & Usadel, 2014) was used to trim and discard poor quality sequences. The remaining quality reads were mapped to the mallard reference genome (Accession no. S263068950 – S263191362; Huang et al., 2013; Kraus et al., 2011) using the Burrows Wheeler Aligner v. 07.15 (bwa; Li & Durbin, 2011). Subsequently, SAMtools v. 1.6 (Li et al., 2009) was used to sort, index, and genotype samples. Only high-quality sequences were retained by setting a base pair PHRED and strand quality scores at ≥30. With a minimum base-pair sequencing depth of 5X (i.e., 10X per genotype), I used PGDspider v2.1.1.2 (Lischer & Excoffier, 2012) to convert VCF files into FASTA file format. Sequences were then further filtered for positions with <80% of alleles present using custom in-house python scripts (Python scripts available at https ://github.com/jonmohl/PopGen).

Finally, sex was assigned to each sample based on differences in sequencing depth across autosomal and sex chromosome-linked loci (Lavretsky, DaCosta, Sorenson, McCracken, & Peters, 2019). Specifically, for the homogametic sex (i.e., males = ZZ), I expect to find near-zero levels of sequencing depth across W-sex chromosome linked loci but near equal depth for Z-sex chromosome linked loci when compared to autosomal loci. For the heterogametic sex (i.e., females = ZW), I expect to recover about half the sequencing depth at both W- and Z-sex chromosome linked loci as compared to autosomal loci.

Mitochondrial DNA

Mallards are characterized by the old world (OW) A and new world (NW) B mitochondrial (mtDNA) haplogroups, which distinguish individuals of Eurasian or North American descent, respectively (Ankney et al., 1986; Avise, Ankney, & Nelson, 1990; Lavretsky, McCracken, & Peters, 2014). Importantly, being of Eurasian descent, all domestically-derived mallards carry OW A haplotypes, thus, making OW A haplotypes a distinguishing marker when assessing game-farm mallard introgression within a wild mallard lineage in North America (Lavretsky, 2020; Lavretsky et al., 2020). In short, primers L78 and H774 were used to sequence 655 base pairs of the mtDNA control region (Sorenson, Ast, Dimcheff, Yuri, & Mindell, 1999; Sorenson & Fleischer, 1996) following protocols outlined in Lavretsky et al. (2014). Final products were sequenced on an ABI 3730 (Applied Biosystems, Life Technologies, Carlsbad, California, USA) machine at the University of Texas at El Paso BBRC Genomic Analysis Core Facility. Sequences were then aligned and edited using Sequencher v. 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Samples were sorted among OW A and NW B mtDNA haplogroups using a median-joining haplotype network calculated in the program Network (Bandelt, Forster, & Rohl, 1999).

Nuclear population structure

Nuclear population structure was based on independent bi-allelic ddRAD-seq autosomal single nucleotide polymorphisms (SNPs), and without using a priori assignment of individuals to populations or species. First, a custom python script (Lavretsky et al., 2020) was used to extract bi-allelic SNPs from a concatenated FASTA file of all autosomal loci. Next, PLINK v1.07 (Purcell et al., 2007) was used to filter for singletons (i.e., minimum allele frequency (--maf 0.002)), any SNP missing ≥20% of data across samples (--geno 0.2), as well as any SNPs found to be in linkage disequilibrium (LD) (--indep-pairwise 2 1 0.5). One of the two SNPs was randomly excluded if an LD correlation factor (r^2) > 0.5 was obtained.

Population structure was first visualized with a Principal Components Analysis (PCA) in the R package Adegenet (Jombart, 2008) using the '*dudi.pca*' function. Next, assignment probabilities were estimated with the program ADMIXTURE v. 1.3 (Alexander & Lange, 2011; Alexander, Novembre, & Lange, 2009). For ADMIXTURE, I ran 100 iterations of each K for one through ten populations. The analysis uses a ten-fold cross-validation (CV) with a quasi-Newton algorithm (Zhou, Alexander, & Lange, 2011) and a block relaxation algorithm for point estimation. Each individual run was terminated once the change in log-likelihood (i.e., delta) of the point estimates increased by <0.0001. The optimal number of populations (K) was then based on the lowest averaged CV-error across all 100 replicates per K. The package PopHelper (Francis, 2017) in R was used to convert all ADMIXTURE outputs into CLUMPP v. 1.1 (Jakobsson & Rosenberg, 2007) input files. Final assignment probabilities were based on the optimal clustering alignment across all 100 replicates per evaluated population K value using the GreedySearch algorithm for 1,000 iterations as implemented in CLUMPP v. 1.1.

Establishing hybrid indices

To assign admixed individual to hybrid or backcross generations, I followed methods outlined in Lavretsky et al. (2016). Briefly I simulated expected assignment probabilities for first-generation hybrids (F1) and nine generations of backcrosses (F2 – F10) into either parental population (i.e., wild or game-farm mallards). First, ten simulated F1 hybrids were generated by randomly sampling alleles from the game-farm and mallard 'gene pool' across bi-allelic SNPs; each position was randomly sampled based on a probability proportional to the allelic frequency in each respective gene pool. Next, backcrossing was simulated by taking five of these F1 hybrids and backcrossing into either the wild or game-farm mallard parental gene pool for nine generations. Note, to limit potential biases from including highly admixed individuals, simulations were based on initial ADMIXTURE assigned probabilities across individuals (see above), and only those samples with ≥95% assignment to either game-farm or wild mallards were included (also see Lavretsky, Janzen, et al., 2019). Ten independent simulations were run, with outputs subsequently put into ADMIXTURE to estimate assignment probabilities for a population K of 2. I ran 25 iterations per simulation for a total of 250 ADMIXTURE outputs generated, which were then combined and converted in PopHelper (Francis, 2017) into CLUMPP input files. I employed the Large Greedy algorithm and 1,000 random permutations with final admixture proportions for each sample assignment probabilities based on CLUMPP analyses of all 250 replicates. Per generation expected assignment probabilities were based on the average of either all ten (F1) or each of the five (F2 – F10) backcrosses, along with each lower and upper limit. Empirical ADMIXUTRE data was then fit to the simulated hybrid indices in order to calculate the proportion of samples that fall within each hybrid generation. Samples outside the lower/upper limit of each

generation were considered as hybrid swarm backcrosses (i.e., hybrid x hybrid combinations of unknown generation; Lavretsky, Janzen, et al., 2019).

Genotype-environment association modelling with gradient forest

Contemporary environmental data across North America were obtained at a high resolution from several public databases. I used a total of 27 environmental variables thought to have impacts on bird physiology and ecology (Supplementary Materials Table S4.1; Bay et al., 2018). Environmental datasets were downloaded at a 30 arc-second resolution, and included (1) 19 climate variables from the WorldClim v. 1.4 database (https://www.worldclim.org/version1; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005); Landsat Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), and Net Primary Productivity (NPP) data from the USGS AppEEARS database (https://lpdaacsvc.cr.usgs.gov/appeears), and (2) elevation data from the Global Land Cover Facility (http://www.landcover.org). In order to differentiate the effects of annual versus seasonal vegetation processes, I calculated an average annual, summer (June), and winter (December) value for NDVI and EVI based on data collected from 2000 – 2019.

To test for differences in adaptive landscapes for breeding mallards across North America, only summer collected birds were included in GEA analysis. Using sample assignments based on simulated hybrid indices (see above), models were independently created for pure wild mallards, early generation game-farm hybrids (i.e., F1 – F3), and those identified as part of the hybrid swarm between the F1 – F3 hybrid generations. All reference game-farm mallards were excluded from GEA analyses as these were taken from release preserves and thus cannot be represented on the wild landscape. Following the approach of Bay et al. (2018), I used a GF analysis as implemented by the R package gradientForest (N. Ellis, Smith, & Roland Pitcher, 2012) to test which

environmental predictor variables most strongly explain patterns of allele frequency turnover in each of these groups. The GF analysis was originally created to detect the effects of environmental predictor variables on species turnover across a landscape (N. Ellis et al., 2012) but has since been adapted for measuring allelic frequency turnover (Bay et al., 2018; Fitzpatrick & Keller, 2015). Briefly, GF uses a machine learning regression tree-based algorithm to detect shifts in allele frequency across an environmental gradient, where a function is built for each individual SNP before an aggregate function is created for all SNPs across each independent predictor variable (Fitzpatrick & Keller, 2015).

For use in GF, I converted all SNPs into minor allele frequencies using the R package PopGenome (Pfeifer, Wittelsbürger, Ramos-Onsins, & Lercher, 2014), and subsequently filtered any SNP that was polymorphic in fewer than five total sampling sites (Fitzpatrick & Keller, 2015). Using a large number of trees (N = 5,000), GF produced an R^2 ranked list of weighted importance for all environmental variables. To assess the performance of GF models for both species, environmental predictor data was randomized in relation to sampling sites. I then compared the performance of 100 models created with randomly generated data to those generated for my empirical data. To visualize the GF model for each species across North America, I extracted values for each environmental variable from points generated across this area. I then used a PCA to summarize the transformed values from the top five predictor variables (based on R^2 weighted importance) for each point. I then transformed the top three principal components to create a RGB color scale that was used to visualize different patterns of adaptive genetic diversity across the landscape. In the end, colors reflect associations between allele frequencies and the environmental predictor variables that allow us to draw conclusions about how the environment has affected genetic diversity and putatively driven adaptation. Next, I combined independent models from

wild and game-farm hybrid mallards to identify differences in genetic niche space between these groups. Briefly, the *combinedGradientForest* function in R acts to standardize independent models to one another by calculating a combined function of cumulative importance, which represents the overall relationship in both species between allele frequency turnover and the environmental predictor variables. Additionally, during standardization, cumulative importance functions for each variable are weighted based on the total R^2 value of the combined gradient forest. As previously described, I visualized the combined model by predicting the GF object across geographic space before converting the PCA into a standardized RGB color scale.

Finally, in order to estimate the potential effects of climate change on wild and game-farm mallard distributions, I used GF to model GEA across future climate conditions and subsequently measure the genetic offset (i.e., Euclidean distance between contemporary and future GF models) from current GEA space. Future bioclimatic variables from Global Climate Models (GCM) were downloaded at the highest available resolution for two future (2070) scenarios of climate change (rcp2.6 and rcp8.5; CCSM4 at 30 arc-second resolution; Hijmans et al., 2005).

RESULTS

After filtering, a total of 2,709 ddRAD-seq loci were retained, with 2,575 (235,348 bp) and 134 (18,515 bp) loci assigned the autosomal and Z-sex chromosomes, respectively. My dataset consisted of an average median sequencing depth of 147 (range = 34 – 242) reads per locus per sample, and an average of 98% of alleles present per locus. Plotting sequencing depth ratios between sex and autosomal linked loci reliably determined sex across samples (Supplementary materials Fig. S4.2).

Population structure and hybrid indices

A total of 18,895 independent autosomal bi-allelic SNPs that met filtering criteria were used across analyses of population genetics and in simulating hybrid indices. Population structure was visualized by plotting the first two principal components of the PCA, and assignment probabilities visualized from ADMIXTURE results based on a K population model of two (Figure 4.1). First, wild and game-farm mallards were clearly distinguished in PCA space and assigned to different genetic clusters in the ADMIXTURE analysis (Figure 4.1). Putative hybrids were represented in intermediate PCA space and with interspecific assignment to each parental group. There was a decreasing probability of hybrid ancestry moving westward (Figure 4.2). In fact, ~98% of Atlantic flyway samples recovered had interspecific assignment and fell intermediate within PCA space. Overall, PCA and ADMIXTURE results showed that the majority of hybrid backcrosses were genetically closer to the wild mallard clusters.

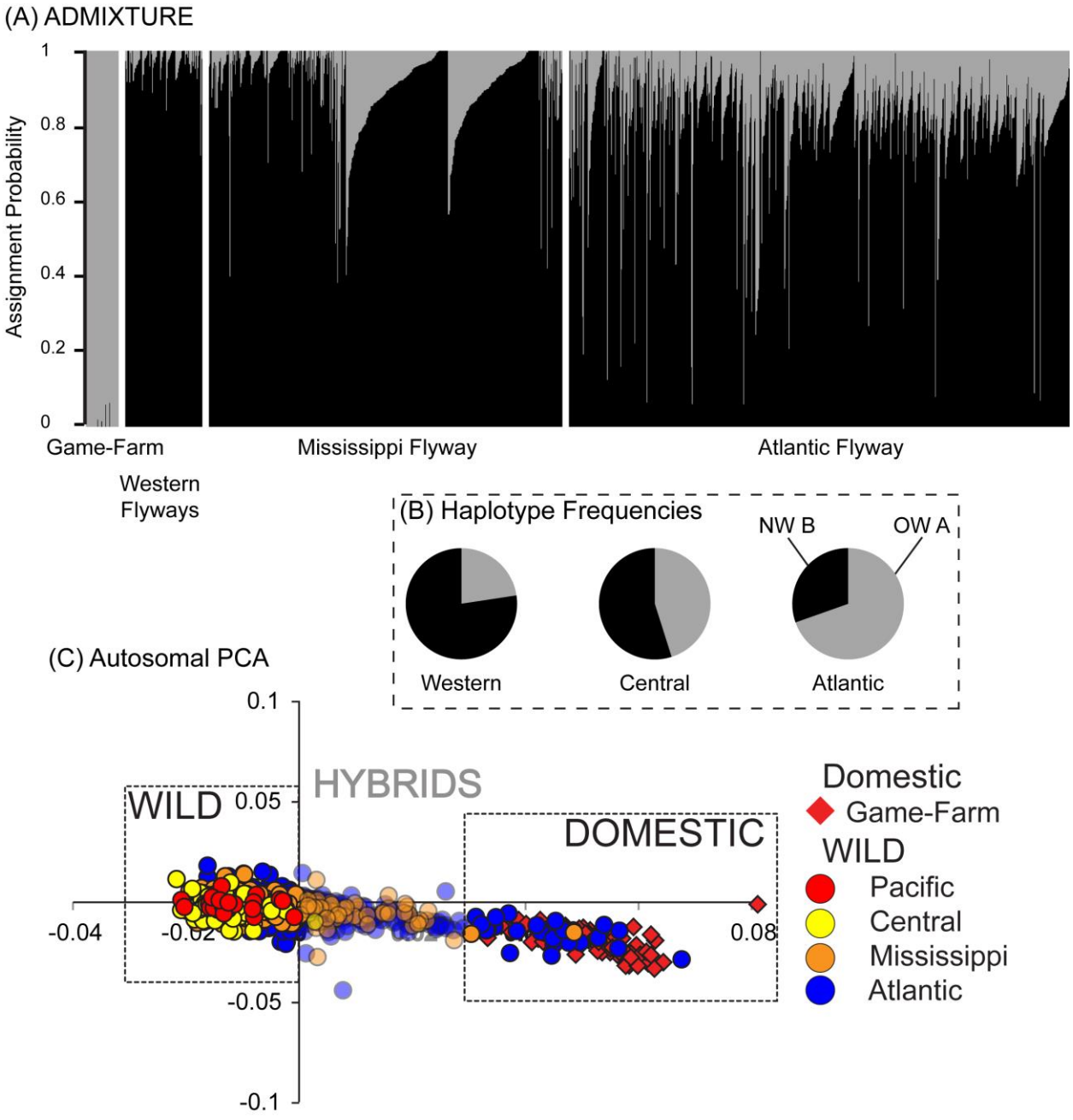


Figure 4.1. (A) ADMIXTURE and (C) PCA assignments of sampled wild North American mallards, game-farm hybrids, and reference known game-farm mallards based on 18,895 independent bi-allelic ddRAD-seq nuclear SNPs. (B) Frequency of OW A and NW B haplotypes from sampled mallards in each flyway.

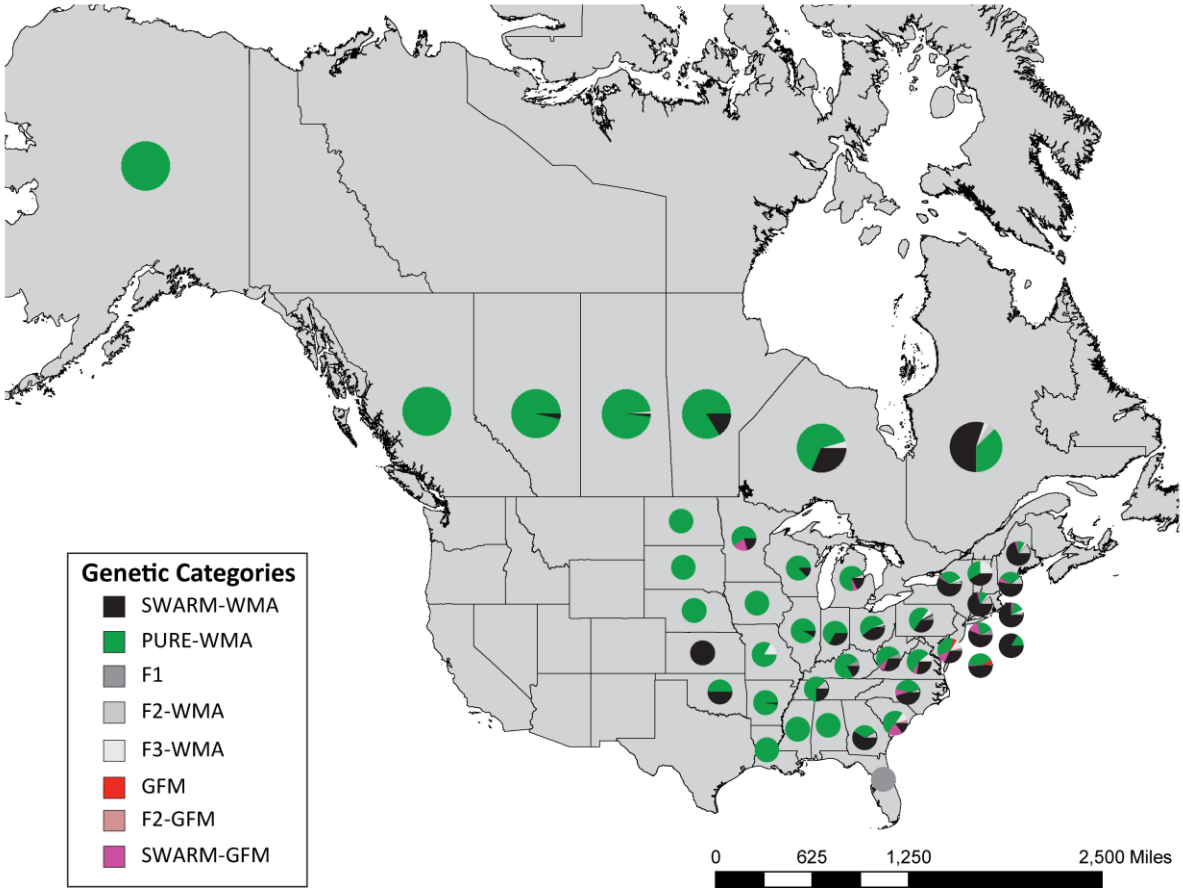


Figure 4.2. Proportion of samples from each state or province assigned to hybrid backcross generations (i.e., Wild mallard F1 – F3, Game-farm mallard F1 – F2) or to the backcrossed hybrid swarm (i.e., hybrids of unknown generation breeding with one another), with assignments based on simulated proportions (see Figure 4.3C).

Given the variance in interspecific assignment among North American samples (Figure 4.1), formal assignment across samples were based on simulated indices (Figure 4.3). Simulations at a K population model of two, assignment probabilities to wild or game-farm mallard clusters plateaued at ~99% in the F5 and F3 generations, respectively (Figure 4.3). While I find predictable declines in interspecific assignment with each generation of backcrossing in mallards (Lavretsky, DaCosta, et al., 2019; Lavretsky, Janzen, et al., 2019), game-farm mallards returned to 'purity' in relatively few generations (Figure 4.3). Regardless, individual lineages become genetically indistinguishable from their respective parent population by the third generation of backcrossing in game-farm mallards and the fifth generation in wild mallards (Figure 4.3); which is concordant to previous work in other ducks (Lavretsky, Janzen, et al., 2019; Lavretsky et al., 2016).

Using simulated indices, a total of 148 (N_{Summer} = 33; N_{Winter} = 115) pure wild mallards were recovered in my dataset (~10%; Figure 4.3D). Only 17 samples had assignment probabilities that fell within the expectations of F1 hybrids (~1%). A total of 125 and seven individuals were classified as hybrid backcross generations towards wild (i.e., F2-F5) and game-farm mallards (i.e., F2), respectively (Figure 4.3). Finally, the remaining 1,118 (79%) samples did not fall within simulated ranges across hybrid generations (N = 1,073 wild backcrosses; N = 45 game-farm backcrosses) and were therefore considered to be part of a hybrid swarm (i.e., offspring resulting from hybrid x hybrid mate-pairings of unknown generation). Within the Atlantic flyway, I recovered 734 (~98%) samples with significant game-farm ancestry (i.e., did not fall within pure mallard indices), which is higher than previous estimates based on samples collected from 2009 – 2010 (~92%, Lavretsky, Janzen, et al., 2019). Mapping assignment probabilities across North American states and provinces demarcated that hybrids were concentrated in the Northeastern part of the U.S., with a westward decline (Figure 4.2).

Mitochondrial DNA haplotype structure

A 600 base pair region of overlapping mtDNA COI was sequenced for a total of 1,368 samples (of 1,393). A total of 770 (56%) samples collected in wild settings had an OW A haplotype, however, only 25% (N = 37) of pure mallards did. Alternatively, all (N = 30) game-farm backcrosses had an OW A haplotype, while less than two thirds (721/1,206, 60%) of all hybrid samples did. Additionally, haplotype frequencies varied by flyway, with NW B haplotypes increasing in frequency in the western flyways (Figure 4.1B). Specifically, the Atlantic and Mississippi flyways had 70% and 45% of samples with OW A haplotypes, respectively. Western flyways (i.e., central, and pacific) had the fewest percentage of OW A haplotypes (23%). Finally, the majority of F1 hybrid individuals had an OW A haplotype (71%), however, both haplotypes were present.

Genotype-environment association modelling

The mean R^2 value for GF models of pure wild (R^2 = 0.149) and game-farm hybrid (R^2 = 0.090) mallards were both greater than the 95% quartile for randomized datasets (Supplementary Materials Figure S4.3), indicating that models are representing true genotype-environment associations. A total of 1,067/6,713 (16%) and 861/10,994 (8%) SNPs with a positive R^2 value were identified in wild and game-farm mallard models, respectively (Supplementary Materials Figure S4.3). Finally, the combined wild x game-farm model had a mean R^2 greater than that of the combined randomized data (R^2 = 0.119; SNPs with positive R^2 = 1,985).

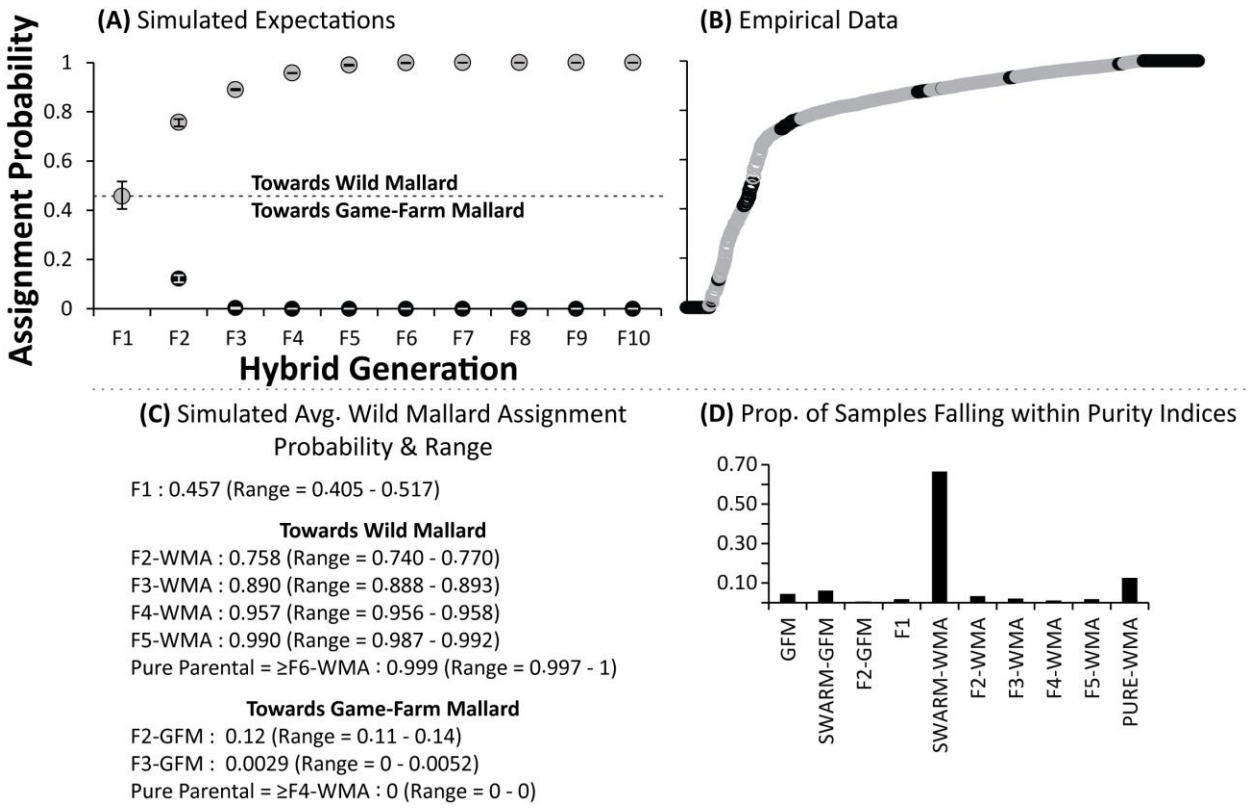


Figure 4.3. (A) Simulation based hybrid indices (F1 – F10) and (B) empirical assignment probabilities using a K of 2 for wild and game-farm mallards sampled throughout North America. (C) Range of simulated assignment probabilities for pure parental populations and hybrid backcross generations. (i.e., Wild mallard F1 – F5, Game-farm mallard F1 – F3), and (D) the proportion of samples falling within these hybrid indices.

Four of the top five most predictive environmental variables identified in pure wild mallards were related to temperature (Bio5, Bio3, Bio8) and precipitation (Bio15), with elevation being important as well (Supplementary Materials Figure S4.4). Additionally, three of these top five environmental variables were related to seasonal changes, suggesting that mallard genotypes are responding to seasonal as opposed to annual weather patterns. GEAs of hybrid individuals were similarly being influenced by a mix of temperature (Bio2, Bio 5, Bio6, Bio8) and precipitation (Bio14), with four of these being related to seasonal factors (Supplementary Materials Figure S4.4). Finally, while the top two most important environmental variables in both groups were related to annual weather patterns, pure wild mallards were more strongly affected by summer conditions as opposed to game-farm hybrids which were dictated by winter conditions (Supplementary Materials Figure S4.4).

Graphing principal components of GF outputs identified the primary adaptive range for wild mallards having minimal variability and that current allele frequencies cover a broadly adaptive gradient (Supplementary Materials Figure S4.5). This can be seen in the GEA map projected across North America, as turnover was relatively homogenous, and the only region with any kind of significant turnover was the Intermountain-west and Rocky Mountains south into the central highlands of Mexico (Figure 4.4A). Alternatively, hybrid individuals show significant turnover across larger regions of North America (Figure 4.4B). Specifically, sample sites plotted in PCA space show some partitioning between flyways, with significant genotypic turnover between groups (Supplementary Materials Figure S4.5). When hybrid GEAs were mapped across North America, the current adaptive range is extremely limited within the northeast of the U.S. Additionally, there is a large band of high genomic turnover that starts along the southern border

of Canada (Figure 4.4B). This region is largely homogenous, and only central flyway sample sites fall within this space in PCA (Supplementary Materials Figure S4.5).

Finally, genetic offset between contemporary and future climate conditions (2070 rcp2.6 & rcp8.5) differed between wild and game-farm mallards (Figure 4.5). First, wild mallards show the most significant vulnerability to changing environmental conditions occurring along the southern edge of the Boreal Forest (Figure 4.5A). Additionally, increased turnover throughout the southeast suggests that mallards may become maladaptive in the region. Importantly, local adaptation to their core breeding grounds of the central Canadian prairies is not a major threat, as offset was minimal in this region. Conversely, hybrid individuals show the most offset along the southern Canadian border where the region of high genotypic turnover fell in contemporary models (Figure 4.5B). Adaptive variation currently concentrated in the northeast U.S. is likely to be more widely adaptive across the Mississippi and Central flyways under future environmental conditions. Overall, this model identifies a significant northern shift in adaptive space of hybrid individuals.

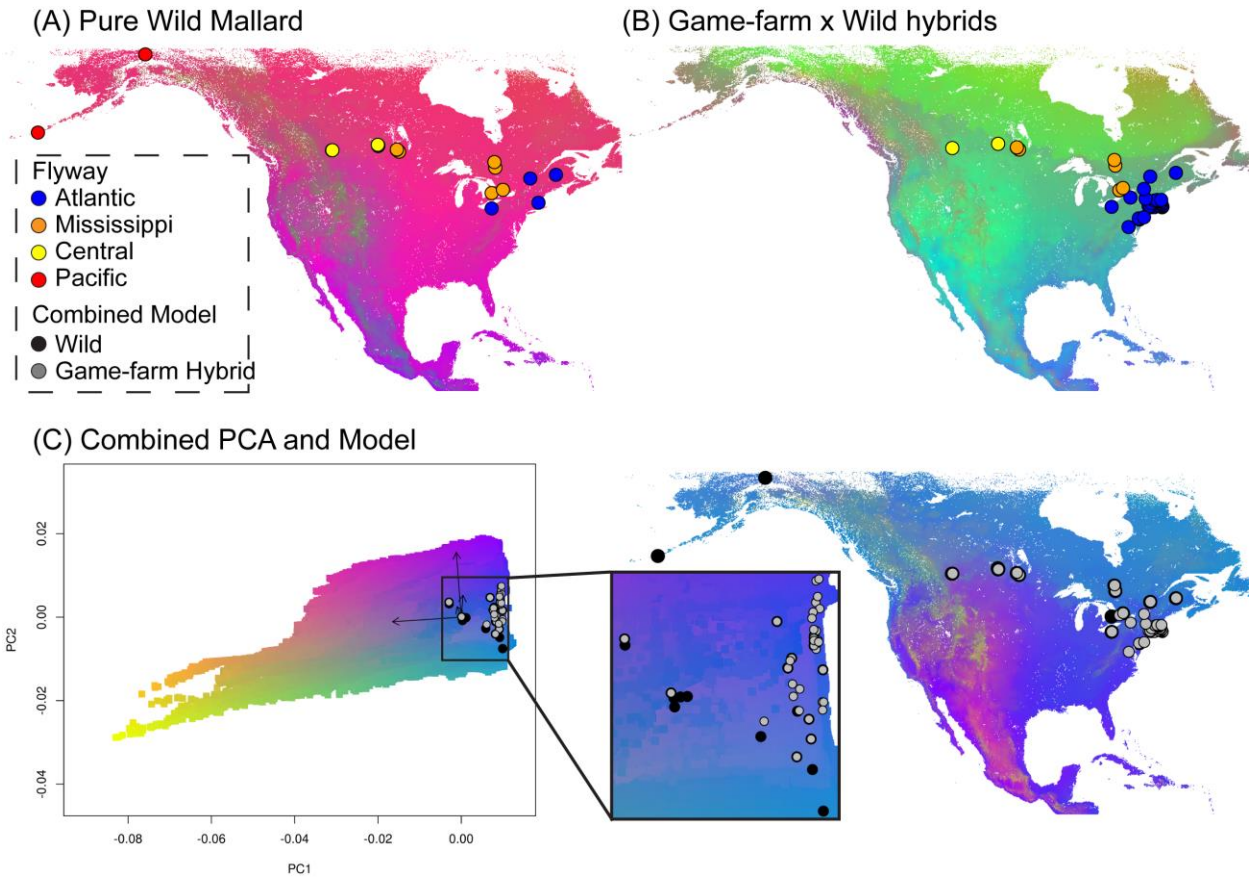


Figure 4.4. Genotype-environment association models from gradientForest (GF) mapped across North America for (A) wild mallards, (B) game-farm mallard hybrids, and (C) the combined GF PCA and model across North America.

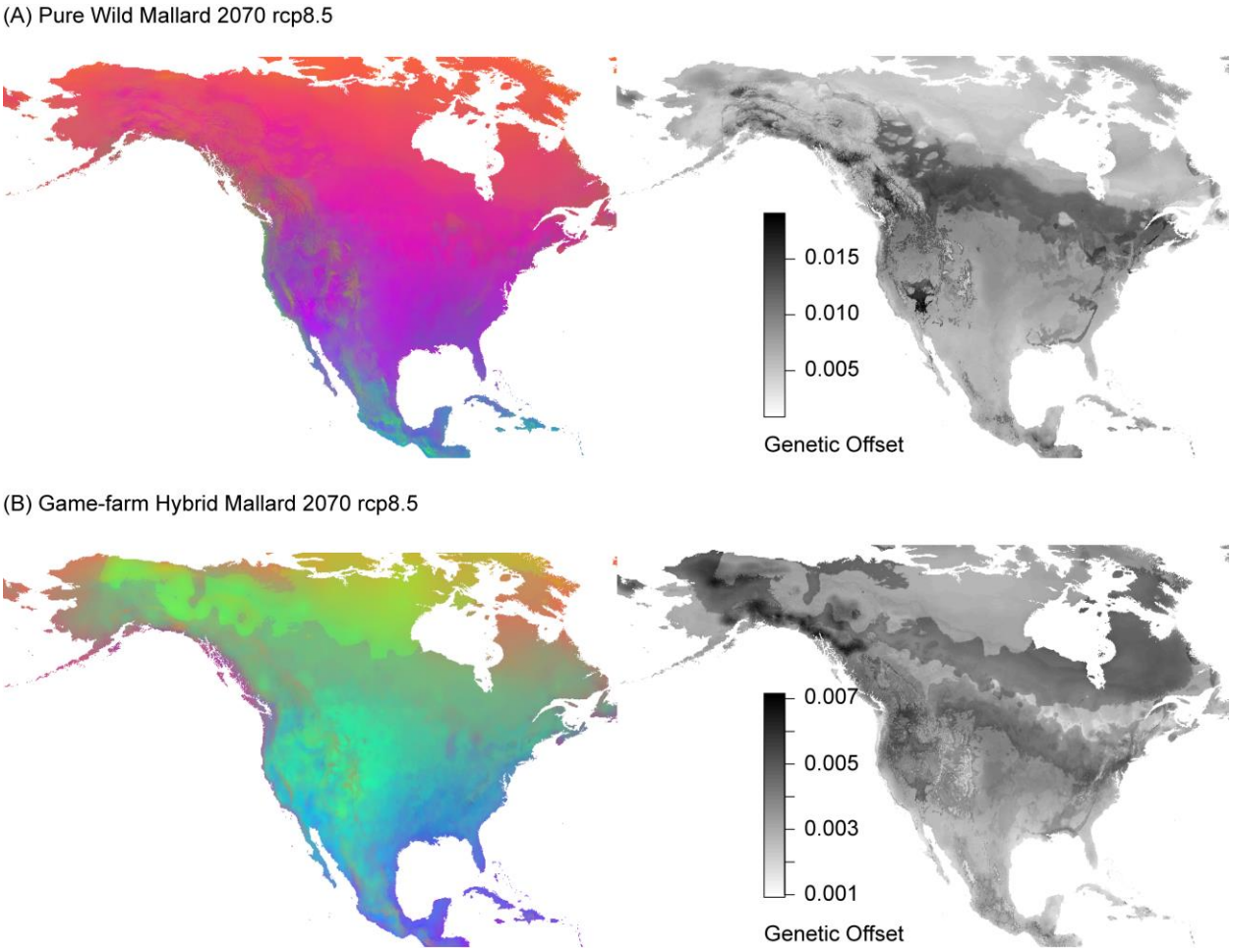


Figure 4.5. (A) Wild and (B) game-farm mallard genotype-environment association models from gradientForest (GF) based on only the top five most predictive temperature and precipitation variables. Associations are modelled across future environmental data for 2070 under the most extreme (rcp8.5) projections of climate change, with genomic offset calculated from the Euclidean distance between models based on contemporary and future climate conditions mapped across North America.

DISCUSSION

Widespread hybridization and the formation of domestic x wild hybrid swarms across North America

Population-level sampling of North America's wild mallards revealed that eastern populations are essentially a hybrid swarm, and that the frequency of hybridization between wild and game-farm mallards has increased and expanded westward over the last decade (Lavretsky, Janzen, et al., 2019). First, the prevalence of game-farm ancestry varies between flyways, with the highest proportion being in the Atlantic flyway, where ~98% of samples collected were early generation hybrids (i.e., 8% = F1-F3) or hybrid swarm individuals (79%). I note that that the ~2% of Atlantic flyway mallards identified as wild North American is a 4-fold decrease from a decade ago (Lavretsky, Janzen, et al., 2019; Lavretsky et al., 2020), supporting that these populations constitute a hybrid swarm today. Importantly, the presence of early-generation hybrids in my dataset further confirms that wild and game-farm mallards continue interbreed readily in a wild setting (Lavretsky et al., 2020; Lawson, Williams, Lavretsky, Howell, & Fuller, 2021), with the highest proportion predictably concentrated in areas where some of the largest release programs exist (e.g., New Jersey; Figure 4.2). Together, these data not only support that annual hybridization is being sustained by ongoing game-farm mallard releases, but that the genetic contribution of game-farm ancestry decreases with distance away from these same release sites. The predictable association of early-generation hybrids and game-farm release sites illustrates the genetic consequences of continued releases and the resulting introgression of putatively maladaptive domestically-derived traits.

Traits deemed beneficial in a captive setting are often detrimental in the wild; therefore, pervasive introgression into wild populations is expected to reduce adaptiveness as deleterious

alleles are continuously introduced (Page, Gibson, Meyer, & Chapman, 2019). Additionally, the domestication process naturally results in successive bottlenecking, increasing the genetic load and potential for inbreeding within wild populations (Kirkpatrick & Jarne, 2000). While lower overall survival and differences in habitat preferences (i.e., urban vs. non-urban) was previously thought to act as a reproductive barrier between game-farm and wild mallards (Heusmann, 1991), I demonstrate the inaccuracy of such ideas. Moreover, while I would generally expect purifying selection to purge maladaptive alleles from wild populations, in this case, selection is unable to keep pace with the perpetual release of game-farm birds. Going forward, research would benefit from full genome sequencing to gain a more nuanced understanding at how hybridization between game-farm and wild mallards affects genomic architecture, as well as how selection acts on introgressed regions harboring maladaptive alleles. Additionally, as anthropogenic hybridization increases in frequency across wild organisms, determining whether a particular hybrid backcross recapitulates the adaptive landscape of wild populations will be critical, particularly in scenarios where genetic swamping is a conservation threat. For example, I demonstrate that genetic sampling of thousands of wild mallards provided a dataset of individuals with varying degrees of wild and game-farm ancestry that could be used to establish generational hybrid indices, which show backcrosses can essentially become wild while maintaining some degree of game-farm derived neutral variation. Moreover, I predict that genomic region(s) harboring strongly maladaptive traits are likely to be lost in early backcross generations (i.e., ≥F3), and that game-farm derived (nearly-) neutral variation may never be lost (Page et al., 2019). Thus, comparing the genomes of late-generation backcrosses will help to determine whether the genomes of wild x game-farm complexes are comprised of small portions of (nearly-) neutral game-farm derived variation that persists within the genome.

Wild and game-farm mallards occupy different adaptive breeding ranges

Not only do I provide evidence of continuous introgressive hybridization between released game-farm and wild mallards at a landscape level, but I formally demonstrate that such interactions are resulting in decreased adaptiveness for North America's wild populations (Figure 4.4). Models of summer wild mallard GEAs are fairly homogenous across the majority of North America, with the only significant turnover occurring throughout the Intermountain West and the Rocky Mountains (a region where mallards are rare to begin with; Figure 4.4A). This lack of genotypic turnover in response to the environment corresponds with the mallard's highly adaptive nature (Baldassarre, 2014). Specifically, their generalist qualities are exemplified by their ability to adjust behavior and feeding preferences based on risk in order to take advantage of breeding habitats in both intact native and more unconventional man-made habitats (Figley & VanDruff, 1982). Alternatively, models for domestic x wild hybrids show large regions of rapid genotypic turnover, suggesting that current patterns of genetic variation restrict their adaptive breeding (summer) range to the Northeast (Figure 4.4B). I posit that turnover in adaptive space among western flyways is the result of backcrossing into wild mallard populations, which acts to re-introduce adaptive wild gene complexes. Additionally, I find significant turnover in GEA space for hybrids along the Canadian border, indicating these birds may lack the adaptive variation necessary to thrive in northern latitude environments. In fact, hybrid GEAs were most strongly affected by winter temperatures (Bio5, Bio8); and therefore, I contend that harsh winter conditions throughout Canada are unfavorable and result in lower fitness. Physiological differences in game-farm versus wild mallards includes smaller body sizes (Kaminski & Essig, 1992), decreased migratory activity (Brakhage, 1953; Söderquist, Gunnarsson, & Elmberg, 2013), and lower feeding efficiency

(Dubovsky & Kaminski, 1994; Palumbo, Petrie, Schummer, Rubin, & Bonner, 2019), all of which are known to reduce winter survival and reproductive fitness in colder environments (Stanton, Soutiere, & Lancia, 1992). Moreover, while hybrids overlap in a small portion of the combined GEA space (Figure 4.4C), there is significant turnover in the transition zones between the core ranges of wild and hybrid individuals (i.e., along the Mississippi flyway and the southern Canadian border; Figure 4.4), suggesting that genotypic turnover between these groups reflects adaptive differences. These findings are analogous to game-farm mallard releases in Europe, which have profoundly augmented the genetic integrity and lowered the overall fitness of Eurasian wild mallard populations (Champagnon, Guillemain, Elmberg, Folkesson, & Gauthier-Clerc, 2010; Champagnon et al., 2012, 2016; Söderquist et al., 2017). Overall, game-farm releases throughout Europe and North America exemplify the potential genetic consequences of supplemental stocking programs, with Europe acting as a forewarning against indefinite supplemental stocking programs, which are now needed to artificially maintain population stability (Söderquist, Norrström, Elmberg, Guillemain, & Gunnarsson, 2014).

More generally, these results appear to be the predictable outcome of most stocking programs where introgression of domestically-derived variation into wild populations often results in declining fecundity and/or survival (Bolstad et al., 2017; Champagnon et al., 2012; Harbicht, Wilson, & Fraser, 2014; Kidd et al., 2009). Here, I demonstrate that even the largest and widespread taxa (i.e., mallards have a consensus population size of ~14 million in North America; Arnold, Afton, Anteau, Koons, & Nicolai, 2017) are susceptible to maladaptation caused by continued stocking practices. Thus, I warn that continued introgression from game-farm mallards could indeed threaten local wild populations of mallards.

Future climate models suggest expanded breeding potential of domestic x wild hybrids

Incorporating adaptive potential is an essential next step when attempting to explore how standing genetic diversity of taxa will respond to future climatic conditions (Rellstab, 2021). Towards this end, GF increases the capacity of researchers to model genomic vulnerability (Bay et al., 2018; also referred to as genetic offset; Fitzpatrick & Keller, 2015), providing an estimate for the likelihood of current genotypes becoming maladaptive under future environmental conditions. For wild mallards, I show that climate change primarily threatens habitat around North America's boreal forests, with limited vulnerability found throughout the central Canadian prairies (Figure 4.5) where ~90% of mallards currently breed (Baldassarre, 2014). These results are concordant with previous work highlighting the Canadian Prairies as one of few regions predicted to see wetland growth due to increased spring precipitation (Adde et al., 2020; Zhang et al., 2020). However, models do not account for land-use changes, which may be significant over the coming decades as wetlands continue to be drained at a rapid pace for agriculture (Brown, Zhang, Comeau, & Bedard-Haughn, 2017). Alternatively, models of genetic offset for hybrids indicates future environmental conditions may be better suited for their expansion into the central Canadian prairies (Figure 4.5B). Specifically, under the most extreme projected climate model (i.e., rcp8.5), regions with the largest genotypic turnover for hybrid individuals is shifted northward, and thus suggests, that environmentally adaptive space for hybrids will expand in the future. Together, I contend that warming temperatures may have limited impacts on the breeding potential of wild individuals, while simultaneously acting to expand adaptive breeding habitat for hybrid individuals. Therefore, I warn that despite the projected stability of wild populations, the potential for hybrids to expand their adaptive breeding range into the prairie pothole region should remain a serious conservation concern.

Gene flow between wild and domestic conspecifics has a complex context-dependent effect on fitness, which should be taken into consideration during the conservation decision-making process (Gering et al., 2019). In the case of mallards, extensive introgression from domestic conspecifics is a real conservation concern, as an expanded game-farm mallard breeding range under future climate conditions would undoubtedly increase the rate of hybridization between these two groups and threaten the genetic integrity of core mallard breeding populations. However, given that hybrid backcrosses become essentially 'genetically wild' within ~4 generations of backcrossing, stricter limits on private game-farm releases and hybrid culling strategies are likely to be extremely effective in protecting North American wild mallards. Alternatively, natural selection in the wild will not necessarily be acting against domestic-derived traits (Page et al., 2019), as deleterious allele combinations can be easily broken up or limited to only a few genomic regions. This has resulted in many wild populations continuing to harbor domestic genes with no serious ill effect on fitness (e.g., wolves (*Canis lupus*), Monzón, Kays, & Dykhuizen, 2014; wild boars (*Sus scrofa*), Goedbloed et al., 2013; and coyotes (*C. latrans*), Redford, Adams, & Mace, 2013). More broadly, I caution that while the GEA analyses discussed here are a useful tool for predicting the potential threats of game-farm introgression, the effects of selection are variable throughout the genome and may not always respond as predicted. Therefore, I argue that conservation decision makers working with a variety of taxa facing threats from hybridization with domestic conspecifics should consider the potential threats on a case-by-case basis.

Advancements and Conclusions

Captive-bred (re)stocking programs are often used in an attempt to supplement or re-establish populations of species with high economic value (such as the mallard; Heusmann &

Sauer, 1997; Rueness et al., 2017). However, without any clear effort put into identifying conservation threats that make supplemental stocking necessary to begin with, these programs can go on indefinitely. And in some cases, as native populations continue to decline, supplemental stocking quickly turns into a re-establishment program. Moreover, the released captive-bred individuals can become the driving force behind the rapid extirpation of the native taxa as extensive introgression may act to swamp out locally adaptive alleles or even introduce maladaptive traits. Here, I conclude that the introduction of game-farm mallards with imprecise conservation outcomes is now threatening the genetic integrity and future of a widespread and economically important species. In particular, while pure mallard populations remain in more western North America, the incidence of hybridization among eastern mallards has increased to the point that this population can now be considered a hybrid swarm. Moreover, models of genotypic turnover support the hypothesis that such extensive anthropogenic introgressive hybridization has indeed resulted in a more adaptively constrained mallard population; and which, at least partially explains current declines being experienced in eastern North America. In general, I posit that purifying selection working against the introgression of maladaptive traits is unable to keep pace with annual game-farm mallard introductions, requiring a careful reconsideration of private game-farm releases in North America.

More broadly, genotype-environment interactions can be complex and have a myriad of effects on local adaptation and/or rates of gene flow, and I argue that these types of broad evolutionary investigations should not be overlooked in terms of conservation. While the usefulness of population-level genetics has been established, managers and biologists could benefit from a landscape genomics approach, which I show provides important insight into threats that future climate change may pose. Furthermore, as reciprocal transplant experiments are often

impractical or impossible in non-model animal systems, GEA analyses modelling differences in adaptive space will be critical for understanding the threats that environmental changes pose to species of interest. While advancements are still being made to increase the capacity to sequence and analyze whole genomes, this kind of data remains largely unattainable for population- and landscape-level sampling; and thus, outlined analytical steps using reduced representation sequencing coupled with landscape-level sampling efforts remain a powerful combination to gain insight into population interactions today and into the future.

ACKNOWLEDGEMENTS

I am thankful to M. Schummer, all participating U.S. Fish and Wildlife and US state agency as well as Canadian Wildlife Service individuals, Delta Waterfowl, and Winous Point Marsh Conservancy for providing samples and funding. I am grateful to Ducks Unlimited for the Richard H. G. Bonnycastle Fellowship in Wetland and Waterfowl Biology, to Ducks Unlimited Canada Institute for Wetland and Waterfowl Research for the Waterfowl Research Foundation Graduate Fellowship, the National Science Foundation (NSF-DEB 2010704 award), and to the University of Texas at El Paso for providing financial support throughout this study.

REFERENCES

Adde, A., Stralberg, D., Logan, T., Lepage, C., Cumming, S., & Darveau, M. (2020). Projected effects of climate change on the distribution and abundance of breeding waterfowl in Eastern Canada. *Climatic Change*, *162*(4), 2339–2358. doi:10.1007/s10584-020-02829-9

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. doi:10.1186/1471-2105-12-

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. doi:10.1101/gr.094052.109

Alisauskas, R. T., Arnold, T. W., Leafloor, J. O., Otis, D. L., & Sedinger, J. S. (2014). Lincoln estimates of mallard (*Anas platyrhynchos*) abundance in North America. *Ecology and Evolution*, *4*(2), 132–143. doi:10.1002/ece3.906

Angeloni, F., Wagemaker, N., Vergeer, P., & Ouborg, J. (2012). Genomic toolboxes for conservation biologists. *Evolutionary Applications*, *5*(2), 130–143. doi:10.1111/j.1752-4571.2011.00217.x

Ankney, C. D., Dennis, D. G., Wishard, L. N., & Seeb, J. E. (1986). Low Genic Variation between Black Ducks and Mallards. *The Auk*, *103*(4), 701–709. doi:10.2307/4087181

Araki, H., Cooper, B., & Blouin, M. S. (2007). Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, *318*(5847), 100–103. doi:10.1126/science.1145621

Arnold, T. W., Afton, A. D., Anteau, M. J., Koons, D. N., & Nicolai, C. A. (2017). Temporal Variation in Survival and Recovery Rates of Lesser Scaup: A Response. *The Journal of Wildlife Management*. doi:10.1002/jwmg.21315

Avise, J. C., Ankney, C. D., & Nelson, W. S. (1990). Mitochondrial gene trees and the evolutionary relationships of Mallard and Black ducks. *Evolution*, *44*(4), 1109–1119. doi:10.1111/j.1558-5646.1990.tb03829.x

Baldassarre, G. A. (2014). *Ducks, Geese, and Swans of North America*. Baltimore, Maryland: Johns Hopkins University Press.

Bandelt, H. J., Forster, P., & Rohl, A. (1999). Median-joining networks for inferring intraspecific

phylogenies. *Molecular Biology and Evolution*, *16*(1), 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Bay, R. A., Harrigan, R. J., Underwood, V. Le, Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359*(6371), 83–86. doi:10.1126/science.aan4380

Blanchet, S., Páez, D. J., Bernatchez, L., & Dodson, J. J. (2008). An integrated comparison of captive-bred and wild Atlantic salmon (Salmo salar): Implications for supportive breeding programs. *Biological Conservation*, *141*(8), 1989–1999. doi:10.1016/J.BIOCON.2008.05.014

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. doi:10.1093/bioinformatics/btu170

Bolstad, G. H., Hindar, K., Robertsen, G., Jonsson, B., Sægrov, H., Diserud, O. H., … Karlsson, S. (2017). Gene flow from domesticated escapes alters the life history of wild Atlantic salmon. *Nature Ecology and Evolution*, *1*(5), 124. doi:10.1038/s41559-017-0124

Brakhage, G. K. (1953). Migration and Mortality of Ducks Hand-Reared and Wild-Trapped at Delta, Manitoba. *The Journal of Wildlife Management*, *17*(4), 477. doi:10.2307/3797053

Brown, R., Zhang, Z., Comeau, L. P., & Bedard-Haughn, A. (2017). Effects of drainage duration on mineral wetland soils in a Prairie Pothole agroecosystem. *Soil and Tillage Research*, *168*, 187–197. doi:10.1016/j.still.2016.12.015

Champagnon, J., Crochet, P.-A., Kreisinger, J., Čížková, D., Gauthier-Clerc, M., Massez, G., … Guillemain, M. (2013). Assessing the genetic impact of massive restocking on wild mallard. *Animal Conservation*, *16*(3), 295–305. doi:10.1111/j.1469-1795.2012.00600.x

Champagnon, J., Guillemain, M., Elmberg, J., Folkesson, K., & Gauthier-Clerc, M. (2010).

Changes in Mallard Anas platyrhynchos bill morphology after 30 years of supplemental stocking. *Bird Study*, *57*(3), 344–351. doi:10.1080/00063657.2010.486028

Champagnon, J., Guillemain, M., Elmberg, J., Massez, G., Cavallo, F., & Gauthier-Clerc, M. (2012). Low survival after release into the wild: assessing "the burden of captivity" on Mallard physiology and behaviour. *European Journal of Wildlife Research*, *58*(1), 255–267. doi:10.1007/s10344-011-0573-3

Champagnon, J., Legagneux, P., Souchay, G., Inchausti, P., Bretagnolle, V., Bourguemestre, F., … Guillemain, M. (2016). Robust estimation of survival and contribution of captive-bred Mallards (Anas platyrhynchos) to a wild population in a large-scale release programme. *Ibis*, *158*(2), 343–352. doi:10.1111/ibi.12341

Chapin III, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., … Díaz, S. (2000). Consequences of changing biodiversity. *Nature*, *405*(6783), 234–242. doi:10.1038/35012241

Cheng, K. M., Shoffner, R. N., Phillips, R. E., & Lee, F. B. (1979). Mate preference in wild and domesticated (game-farm) mallards: II. Pairing success. *Animal Behaviour*, *27*, 417–425. doi:10.1016/0003-3472(79)90177-5

DaCosta, J. M., & Sorenson, M. D. (2014). Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol. *PLoS ONE*, *9*(9), e106713. doi:10.1371/journal.pone.0106713

Dubovsky, J. A., & Kaminski, R. M. (1994). Potential Reproductive Consequences of Winter-Diet Restriction in Mallards. *The Journal of Wildlife Management*, *58*(4), 786. doi:10.2307/3809693

Edmands, S. (1999). Heterosis and outbreeding depression in interpopulation crosses spanning a

wide range of divergence. *Evolution*, *53*(6), 1757–1768. doi:10.1111/j.1558-5646.1999.tb04560.x

Ellis, N., Smith, S. J., & Roland Pitcher, C. (2012). Gradient forests: Calculating importance gradients on physical predictors. *Ecology*, *93*(1), 156–168. doi:10.1890/11-0252.1

Ellis, T., Hughes, R. N., & Howell, B. R. (2002). Artificial dietary regime may impair subsequent foraging behaviour of hatchery-reared turbot released into the natural environment. *Journal of Fish Biology*, *61*(1), 252–264. doi:10.1111/j.1095-8649.2002.tb01750.x

Evans, K., Smith, M., Burger, L., Chambers, R., Houston, A., & Carlisle, R. (2017). Release of Pen-reared Bobwhites: Potential Consequences to the Genetic Integrity of Resident Wild Populations. In *National Quail Symposium Proceedings* (Vol. 6).

Figley, W. K., & VanDruff, L. W. (1982). The Ecology of Urban Mallards. *Wildlife Monographs*, *81*, 3–39.

Fitzpatrick, M. C., & Keller, S. R. (2015). Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters*, *18*(1), 1–16. doi:10.1111/ele.12376

Ford, M. J. (2002). Selection in Captivity during Supportive Breeding May Reduce Fitness in the Wild. *Conservation Biology*, *16*(3), 815–825. doi:10.1046/j.1523-1739.2002.00257.x

Forester, B. R., Jones, M. R., Joost, S., Landguth, E. L., & Lasky, J. R. (2016). Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. *Molecular Ecology*, *25*(1), 104–120. doi:10.1111/mec.13476

Francis, R. M. (2017). POPHELPER: an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, *17*(1), 27–32. doi:10.1111/1755-0998.12509

Garner, B. A., Hand, B. K., Amish, S. J., Bernatchez, L., Foster, J. T., Miller, K. M., … Luikart, G. (2016, February 1). Genomics in Conservation: Case Studies and Bridging the Gap between Data and Application. *Trends in Ecology and Evolution*. Elsevier Ltd. doi:10.1016/j.tree.2015.10.009

Gering, E., Incorvaia, D., Henriksen, R., Conner, J., Getty, T., & Wright, D. (2019, December 1). Getting Back to Nature: Feralization in Animals and Plants. *Trends in Ecology and Evolution*. Elsevier Ltd. doi:10.1016/j.tree.2019.07.018

Goedbloed, D. J., van Hooft, P., Megens, H. J., Langenbeck, K., Lutz, W., Crooijmans, R. P. M. A., … Prins, H. H. T. (2013). Reintroductions and genetic introgression from domestic pigs have shaped the genetic population structure of Northwest European wild boar. *BMC Genetics*, *14*(1), 2–10. doi:10.1186/1471-2156-14-43

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. (1989). Translocation as a Species Conservation Tool: Status and Strategy. *Science*, *245*(4917), 477–480. doi:10.1126/science.245.4917.477

Hanson, H. C., Rogers, M., & Rogers, E. S. (1949). Waterfowl of the forested portions of the Canadian pre-Cambrian shield and the Palaeozoic Basin. *Ottawa Field-Naturalists Club*.

Harbicht, A., Wilson, C. C., & Fraser, D. J. (2014). Does human-induced hybridization have long-term genetic effects? Empirical testing with domesticated, wild and hybridized fish populations. *Evolutionary Applications*, *7*(10), 1180–1191. doi:10.1111/eva.12199

Hepp, G. R., Novak, J. M., Scribner, K. T., & Stangel, P. W. (1988). Genetic Distance and Hybridization of Black Ducks and Mallards : A Morph of a Different Color? *The Auk*, *105*(4), 804–807.

Heusmann, H. W. (1974). Mallard-Black Duck Relationships in the Northeast. *Wildlife Society*

Bulletin, *2*(4), 171–177. doi:10.2307/3781222

Heusmann, H. W. (1991). The History and Status of the Mallard in the Atlantic Flyway. *Wildlife Society Bulletin*, *19*(1), 14–22. doi:10.2307/3782410

Heusmann, H. W. (2017). Why Are Numbers of Mallards (*Anas platyrhynchos*) Declining in the Northeastern United States? the Possible Role of Reduction in Winter Feeding Sites. *Wilson Journal of Ornithology*, *129*(3), 528–534. doi:10.1676/16-044.1

Heusmann, H. W., & Sauer, J. R. (1997). A Survey for Mallard Pairs in the Atlantic Flyway. *The Journal of Wildlife Management*, *61*(4), 1198. doi:10.2307/3802116

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. doi:10.1002/joc.1276

Holderegger, R., & Wagner, H. H. (2008). Landscape Genetics. *BioScience*, *58*(3), 199. doi:10.1641/B580306

Huang, Y., Li, Y., Burt, D. W., Chen, H., Zhang, Y., Qian, W., … Li, N. (2013). The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. *Nature Genetics*, *45*(7), 776–783. doi:10.1038/ng.2657

Iacolina, L., Corlatti, L., Buzan, E., Safner, T., & Šprem, N. (2019). Hybridisation in European ungulates: an overview of the current status, causes, and consequences. *Mammal Review*, *49*(1), 45–59. doi:10.1111/mam.12140

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*(14), 1801–1806. doi:10.1093/bioinformatics/btm233

Johnsgard, P. A. (1967). Sympatry Changes and Hybridization Incidence in Mallards and Black

Ducks. *American Midland Naturalist*, *77*(1), 51–63. doi:10.2307/2423425

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. doi:10.1093/bioinformatics/btn129

Kaminski, R. M., & Essig, H. W. (1992). True Metabolizable Energy Estimates for Wild and Game-Farm Mallards. *The Journal of Wildlife Management*, *56*(2), 321–324.

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x

Kidd, A. G., Bowman, J., Lesbarreres, D., & Schulte-Hostedde, A. I. (2009). Hybridization between escaped domestic and wild American mink (*Neovison vison*). *Molecular Ecology*, *18*(6), 1175–1186. doi:10.1111/j.1365-294X.2009.04100.x

Kirkpatrick, M., & Jarne, P. (2000). The effects of a bottleneck on inbreeding depression and the genetic load. *American Naturalist*, *155*(2), 154–167. doi:10.1086/303312

Kraus, R. H., Kerstens, H. H., Van Hooft, P., Crooijmans, R. P., Van Der Poel, J. J., Elmberg, J., … Groenen, M. A. (2011). Genome wide SNP discovery, analysis and evaluation in mallard (*Anas platyrhynchos*). *BMC Genomics*, *12*(1), 150. doi:10.1186/1471-2164-12-150

Laikre, L., Palmé, A., Josefsson, M., Utter, F., & Ryman, N. (2006). Release of Alien Populations in Sweden. *AMBIO: A Journal of the Human Environment*, *35*(5), 255–262. doi:10.1579/05-A-060R.1

Lavretsky, P. (2020). Population Genomics Provides Key Insights into Admixture, Speciation, and Evolution of Closely Related Ducks of the Mallard Complex. In *Population Genomics: Wildlife* (pp. 295–330). Springer, Cham. doi:10.1007/13836_2020_76

Lavretsky, P., Dacosta, J. M., Hernández-Baños, B. E., Engilis, A., Sorenson, M. D., & Peters, J. L. (2015). Speciation genomics and a role for the Z chromosome in the early stages of

divergence between Mexican ducks and mallards. *Molecular Ecology*, *24*(21), 5364–5378. doi:10.1111/mec.13402

Lavretsky, P., DaCosta, J. M., Sorenson, M. D., McCracken, K. G., & Peters, J. L. (2019). ddRAD-seq data reveal significant genome-wide population structure and divergent genomic regions that distinguish the mallard and close relatives in North America. *Molecular Ecology*, *28*(10), 2594–2609. doi:10.1111/mec.15091

Lavretsky, P., Hernández-Baños, B. E., & Peters, J. L. (2014). Rapid radiation and hybridization contribute to weak differentiation and hinder phylogenetic inferences in the New World Mallard complex (*Anas spp.*). *The Auk*, *131*, 524–538. doi:10.1642/AUK-13-164.1

Lavretsky, P., Janzen, T., & McCracken, K. G. (2019). Identifying hybrids & the genomics of hybridization: Mallards & American black ducks of Eastern North America. *Ecology and Evolution*, *9*(6), 3470–3490. doi:10.1002/ece3.4981

Lavretsky, P., McCracken, K. G., & Peters, J. L. (2014). Phylogenetics of a recent radiation in the mallards and allies (Aves: Anas): Inferences from a genomic transect and the multispecies coalescent. *Molecular Phylogenetics and Evolution*, *70*(1), 402–411. doi:10.1016/j.ympev.2013.08.008

Lavretsky, P., McInerney, N. R., Mohl, J. E., Brown, J. I., James, H. F., McCracken, K. G., & Fleischer, R. C. (2020). Assessing changes in genomic divergence following a century of human-mediated secondary contact among wild and captive-bred ducks. *Molecular Ecology*, *29*(3), 578–595. doi:10.1111/mec.15343

Lavretsky, P., Peters, J. L., Winker, K., Bahn, V., Kulikova, I., Zhuravlev, Y. N., … McCracken, K. G. (2016). Becoming pure: Identifying generational classes of admixed individuals within lesser and greater scaup populations. *Molecular Ecology*, *25*(3), 661–674.

doi:10.1111/mec.13487

Lawson, D. M., Williams, C. K., Lavretsky, P., Howell, D. L., & Fuller, J. C. (2021). Mallard–Black Duck Hybridization and Population Genetic Structure in North Carolina. *The Journal of Wildlife Management*, jwmg.22085. doi:10.1002/jwmg.22085

Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. *Nature*, *475*(7357), 493–496. doi:10.1038/nature10231

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. doi:10.1093/bioinformatics/btp352

Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*(2), 298–299. doi:10.1093/bioinformatics/btr642

Manel, S., Joost, S. S., Epperson, B. K., Holderegger, R., Storfer, A., Rosenberg, M. S., … Fortin, M.-J. J. (2010). Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*, *19*(17), 3760–3772. doi:10.1111/j.1365-294X.2010.04717.x

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, *18*(4), 189–197. doi:10.1016/S0169-5347(03)00008-9

McPhee, M. E. (2004). Generations in captivity increases behavioral variance: considerations for captive breeding and reintroduction programs. *Biological Conservation*, *115*(1), 71–77. doi:10.1016/S0006-3207(03)00095-8

Merendino, M. T., & Ankney, C. D. (1994). Habitat Use by Mallards and American Black Ducks

Breeding in Central Ontario. *The Condor*, *96*(2), 411–421. doi:10.2307/1369324

Monzón, J., Kays, R., & Dykhuizen, D. E. (2014). Assessment of coyote-wolf-dog admixture using ancestry-informative diagnostic SNPs. *Molecular Ecology*, *23*(1), 182–197. doi:10.1111/mec.12570

Osborne, C. E., Swift, B. L., & Baldassarre, G. A. (2010). Fate of captive-reared and released mallards on eastern Long Island, New York. *Human-Wildlife Interactions*, *4*(2), 266–274. doi:10.2307/24868846

Page, A., Gibson, J., Meyer, R. S., & Chapman, M. A. (2019). Eggplant Domestication: Pervasive Gene Flow, Feralization, and Transcriptomic Divergence. *Molecular Biology and Evolution*, *36*(7), 1359–1372. doi:10.1093/molbev/msz062

Palumbo, M. D., Petrie, S. A., Schummer, M., Rubin, B. D., & Bonner, S. (2019). Mallard resource selection trade-offs in a heterogeneous environment during autumn and winter. *Ecology and Evolution*, *9*(4), 1798–1808. doi:10.1002/ece3.4864

Parsons, B. G., & Pereira, D. L. (2001). Relationship between Walleye Stocking and Year-Class Strength in Three Minnesota Lakes. *North American Journal of Fisheries Management*, *21*(4), 801–808. doi:10.1577/1548-8675(2001)021<0801:RBWSAY>2.0.CO;2

Petren, K., Grant, P. R., Grant, B. R., & Keller, L. F. (2005). Comparative landscape genetics and the adaptive radiation of Darwin's finches: The role of peripheral isolation. *Molecular Ecology*, *14*(10), 2943–2957. doi:10.1111/j.1365-294X.2005.02632.x

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. doi:10.1093/molbev/msu136

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P.

C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *The American Journal of Human Genetics*, *81*(3), 559–575. doi:10.1086/519795

Redford, K. H., Adams, W., & Mace, G. M. (2013). Synthetic Biology and Conservation of Nature: Wicked Problems and Wicked Solutions. *PLoS Biology*, *11*(4), 2–5. doi:10.1371/journal.pbio.1001530

Rellstab, C. (2021, February 1). Genomics helps to predict maladaptation to climate change. *Nature Climate Change*. Nature Research. doi:10.1038/s41558-020-00964-w

Rieseberg, L. H., & Burke, J. M. (2001). The biological reality of species: Gene flow, selection, and collective evolution. *Taxon*, *50*(1), 47–67. doi:10.2307/1224511

Robert, A. (2009). Captive breeding genetics and reintroduction success. *Biological Conservation*, *142*(12), 2915–2922. doi:10.1016/J.BIOCON.2009.07.016

Rueness, E. K., Cadahia-Lorenzo, L., Hoel, K., Söderquist, P., Velle, G., & Asmyhr, M. (2017). *Assessment of the risks associated with the import and release of hand- reared mallards for hunting purposes*. Oslo, Norway.

Ruzzante, D. E. (1994). Domestication effects on aggressive and schooling behavior in fish. *Aquaculture*, *120*(1–2), 1–24. doi:10.1016/0044-8486(94)90217-8

Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007). Gene Flow and Local Adaptation in Trees. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 595–619. doi:10.1146/annurev.ecolsys.38.091206.095646

Scandura, M., Iacolina, L., & Apollonio, M. (2011). Genetic diversity in the European wild boar Sus scrofa: phylogeography, population structure and wild x domestic hybridization. *Mammal Review*, *41*(2), 125–137. doi:10.1111/j.1365-2907.2010.00182.x

Slatkin, M. (1987). Gene Flow and the Geographic Structure of Natural Populations. *Science*, *236*(4803), 787–792. doi:10.1126/science.3576198

Snell, E. A. (1986). Wetland distribution and conversion in southern Ontario. In *Wetland distribution and conversion in Southern Ontario* (Vol. 48). Ottawa: Environment Canada.

Söderquist, P., Elmberg, J., Gunnarsson, G., Thulin, C.-G., Champagnon, J., Guillemain, M., … Kraus, R. H. S. (2017). Admixture between released and wild game birds : a changing genetic landscape in European mallards (*Anas platyrhynchos*). *European Journal of Wildlife Research*, *63*(98). doi:10.1007/s10344-017-1156-8

Söderquist, P., Gunnarsson, G., & Elmberg, J. (2013). Longevity and migration distance differ between wild and hand-reared mallards *Anas platyrhynchos* in Northern Europe. *European Journal of Wildlife Research*, *59*(2), 159–166. doi:10.1007/s10344-012-0660-0

Söderquist, P., Gunnarsson, G., Elmberg, J., & Dessborn, L. (2021). Survival of wild and farmed-released mallards: the Swedish example. *European Journal of Wildlife Research*, *67*(2), 1–12. doi:10.1007/s10344-021-01465-7

Söderquist, P., Norrström, J., Elmberg, J., Guillemain, M., & Gunnarsson, G. (2014). Wild Mallards Have More "Goose-Like" Bills Than Their Ancestors: A Case of Anthropogenic Influence? *PLoS ONE*, *9*(12), e115143. doi:10.1371/journal.pone.0115143

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-Based Approach to Mitochondrial Genome Sequencing in Birds and Other Vertebrates. *Molecular Phylogenetics and Evolution*, *12*(2), 105–114. doi:10.1006/MPEV.1998.0602

Sorenson, M. D., & Fleischer, R. C. (1996). Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(26), 15239–15243.

doi:10.1073/PNAS.93.26.15239

Soutiere, E. C. (1986). Hand-reared Mallard releases on 3 private farms in Maryland. In *Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies. Vol. 40* (pp. 438–445).

Stanton, J. D., Soutiere, E. C., & Lancia, R. A. (1992). Survival and Reproduction of Game-Farm Female Mallards at Remington Farms, Maryland. *Wildlife Society Bulletin*, *20*(2), 182–188.

Tufto, J. (2017). Domestication and fitness in the wild: A multivariate view. *Evolution*, *71*(9), 2262–2270. doi:10.1111/evo.13307

USFWS. (2013). *Review of captive-reared mallard regulations on shooting preserves*. Washington D.C.

Vangestel, C., Eckert, A. J., Wegrzyn, J. L., St. Clair, J. B., & Neale, D. B. (2018). Linking phenotype, genotype and environment to unravel genetic components underlying cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii var. menziesii*). *Tree Genetics and Genomes*, *14*(1). doi:10.1007/s11295-017-1225-x

Vilá, C., & Wayne, R. K. (1999). Hybridization between Wolves and Dogs. *Conservation Biology*, *13*(1), 195–198. doi:10.1046/j.1523-1739.1999.97425.x

Waples, R. S., Zabel, R. W., Scheurell, M. D., & Sanderson, B. L. (2008). Evolutionary responses by native species to major anthropogenic changes to their ecosystems: Pacific salmon in the Columbia River hydropower system. *Molecular Ecology*, *17*(1), 84–96. doi:10.1111/j.1365-294X.2007.03510.x

Williams, G. C. (1966). *Adaptation and Natural Selection: A Critique of Some Current Evolutionary Thought*. Princeton, New Jersey: Princeton University Press.

Zhang, Z., Li, Y., Barlage, M., Chen, F., Miguez-Macho, G., Ireson, A., & Li, Z. (2020). Modeling

groundwater responses to climate change in the Prairie Pothole Region. *Hydrology and Earth System Sciences*, *24*, 655–672. doi:10.5194/hess-24-655-2020

Zhou, H., Alexander, D., & Lange, K. (2011). A quasi-Newton acceleration for high-dimensional optimization algorithms. *Statistics and Computing*, *21*(2), 261–273. doi:10.1007/s11222-009-9166-3

Appendix

SUPPLEMENTAL TABLES

Table S1.1. Sample information including scientific name based on nuclear assignment, sex, date of collection, and latitude and longitude of sampling location. Plumage scores for samples with available data are also provided.

Sample.ID	Scientific_Name	Sex	Country	State/Province	Longitude	Latitude	Date Collected
PL3235	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	20-Jul-17
PL6004	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	25-Aug-17
PL6005	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	25-Aug-17
PL6006	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	25-Aug-17
PL6010	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	19-Sep-17
PL6011	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	19-Sep-17
PL6012	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	19-Sep-17
PL6013	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	19-Sep-17
PL6014	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	19-Sep-17
PL6017	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	22-Sep-17
PL6018	*Anas platyrhynchos domesticus*	M	United States	TX	31.820773	-106.563246	22-Sep-17
PL6019	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	22-Sep-17
PL6020	*Anas platyrhynchos domesticus*	F	United States	TX	31.820773	-106.563246	22-Sep-17
PL3200	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17

PL3201	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3202	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3203	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3204	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3205	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3206	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3207	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3208	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3210	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3211	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3212	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3213	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3214	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3215	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3216	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17
PL3217	*Anas platyrhynchos domesticus*	M	United States	KY	37.802659	-84.926963	15-Feb-17
PL3219	*Anas platyrhynchos domesticus*	F	United States	KY	37.802659	-84.926963	15-Feb-17

JIBNJ001	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ002	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ003	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ004	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ005	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ006	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ007	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ008	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ009	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ010	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ011	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ012	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ013	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ014	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ015	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ016	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ017	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17

JIBNJ018	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ019	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ020	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ021	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ022	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ023	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ024	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ025	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ026	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ027	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ028	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ029	*Anas platyrhynchos domesticus*	M	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ030	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
JIBNJ031	*Anas platyrhynchos domesticus*	F	United States	NJ	40.051487	-74.504385	2-Dec-17
UAMX1739	*Anas platyrhynchos*	M	United States	AK	52.8763	-172.8905	1-Jun-01
REW624	*Anas platyrhynchos*	M	United States	AK	67.14114	-150.35586	6-Sep-05
UAMX3170	*Anas platyrhynchos*	F	United States	AK	60.337778	-151	2002

UAMX806	*Anas platyrhynchos*	F	United States	AK	64.8	-147.7	Unk
UAMX812	*Anas platyrhynchos*	F	United States	AK	64.8	-147.7	29-Apr-99
KGM1421	*Anas platyrhynchos*	F	Canada	AB	54.5	-110.2	23-Oct-06
KGM1422	*Anas platyrhynchos*	M	Canada	AB	53.1	-111.8	22-Sep-06
KGM1423	*Anas platyrhynchos*	F	Canada	AB	54.5	-110.2	23-Oct-06
KGM1424	*Anas platyrhynchos*	M	Canada	AB	53.1	-111.8	15-Sep-06
KGM1412	*Anas platyrhynchos*	M	Canada	AB	49.9	-113.1	30-Oct-06
KGM1414	*Anas platyrhynchos*	F	Canada	AB	49.2	-113.3	28-Oct-06
KGM1429	*Anas platyrhynchos*	M	Canada	BC	49.2	-122.2	28-Oct-06
KGM1427	*Anas platyrhynchos*	F	Canada	BC	49.1	-122	30-Dec-06
CAMall01	*Anas platyrhynchos*	M	United States	CA	39.3299	-121.914	2004
CAMall05	*Anas platyrhynchos*	M	United States	CA	39.3299	-121.914	2004
CAMall12	*Anas platyrhynchos*	M	United States	CA	39.3299	-121.914	2004
CAMall17	*Anas platyrhynchos*	F	United States	CA	39.3299	-121.914	2004
PL121516	*Anas platyrhynchos*	M	United States	CA	37.287222	-120.890657	15-Dec-16
PL861	*Anas platyrhynchos*	F	United States	CT	41.3517	-72.4161	16-Oct-09
PL3220	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3221	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3222	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3223	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3224	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17

PL3225	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3226	*Anas platyrhynchos*	M	United States	KY	37.0419	-89.0896	17-Feb-17
PL3227	*Anas platyrhynchos*	F	United States	KY	37.0419	-89.0896	17-Feb-17
PL923	*Anas platyrhynchos*	F	United States	ME	43.9194	-70.4667	9-Nov-09
PL962	*Anas platyrhynchos*	F	United States	ME	44.96644167	-70.77368056	6-Oct-09
JIBCan001	*Anas platyrhynchos*	M	Canada	MB	50.19871	-98.21405	21-Oct-17
JIBCan002	*Anas platyrhynchos*	M	Canada	MB	50.19871	-98.21405	21-Oct-17
JIBCan003	*Anas platyrhynchos*	M	Canada	MB	50.19871	-98.21405	21-Oct-17
JIBCan004	*Anas platyrhynchos*	M	Canada	MB	50.19871	-98.21405	21-Oct-17
JIBCan005	*Anas platyrhynchos*	F	Canada	MB	50.19871	-98.21405	21-Oct-17
JIBCan006	*Anas platyrhynchos*	F	Canada	MB	50.19871	-98.21405	21-Oct-17
KGM1383	*Anas platyrhynchos*	F	Canada	MB	50.1	-100.4	19-Oct-06
KGM1387	*Anas platyrhynchos*	M	Canada	MB	50.1	-98.25	23-Sep-06
KGM1388	*Anas platyrhynchos*	M	Canada	MB	50.1	-98.25	9-Sep-06
KGM1390	*Anas platyrhynchos*	F	Canada	MB	50.1	-100.4	20-Oct-06
KGM1392	*Anas platyrhynchos*	M	Canada	MB	51.1	-99.8	23-Sep-06
KGM1393	*Anas platyrhynchos*	F	Canada	MB	51.1	-100.8	12-Oct-06
PL944	*Anas platyrhynchos*	F	United States	NJ	40.345	-74.48055556	13-Oct-09
PL112316	*Anas platyrhynchos*	M	United States	NM	32.953968	-107.295992	23-Nov-16
PL121317	*Anas platyrhynchos*	M	United States	NM	32.141221	-106.699413	13-Dec-17
PL824	*Anas platyrhynchos*	F	United States	NY	42.6839	-76.9572	5-Dec-09
PL832	*Anas platyrhynchos*	M	United States	NY	44.33583333	-75.91472222	1-Dec-09
PL844	*Anas platyrhynchos*	F	United States	NY	41.8528	-73.9222	26-Nov-09

PL701	*Anas platyrhynchos*	M	United States	NC	35.92055556	-79.08388889	8-Oct-09
PL262	*Anas platyrhynchos*	F	United States	ND	48.81	-103.49	19-Oct-09
PL263	*Anas platyrhynchos*	F	United States	ND	48.034722	-98.944167	17-Nov-09
PL264	*Anas platyrhynchos*	M	United States	ND	45.978889	-97.810278	16-Nov-09
PL266	*Anas platyrhynchos*	M	United States	ND	45.978889	-97.810278	15-Nov-09
KGM1395	*Anas platyrhynchos*	M	Canada	SK	52.1	-106.7	25-Oct-06
KGM1400	*Anas platyrhynchos*	F	Canada	SK	53.3	-110	9-Sep-06
KGM1404	*Anas platyrhynchos*	F	Canada	SK	52	-107	30-Oct-06
KGM1405	*Anas platyrhynchos*	F	Canada	SK	54.27	-110.8	4-Nov-06
KGM1406	*Anas platyrhynchos*	M	Canada	SK	52.1	-106.7	17-Oct-06
KGM1407	*Anas platyrhynchos*	M	Canada	SK	50.8	-104.9	10-Nov-06
PL267	*Anas platyrhynchos*	M	United States	SD	45.333611	-97.519444	4-Nov-09
PL269	*Anas platyrhynchos*	M	United States	SD	45.333611	-97.519444	2009
PL270	*Anas platyrhynchos*	M	United States	SD	44.228333	-97.308889	15-Nov-09
PL272	*Anas platyrhynchos*	F	United States	SD	45.333611	-97.519444	7-Nov-09
PL852	*Anas platyrhynchos*	F	United States	VT	44.9442	-72.2044	10-Oct-09
DICA012718	*Anas platyrhynchos*	F	United States	CA	37.198227	-120.902249	27-Jan-18
PL120516	*Anas diazi*	M	United States	CA	37.287222	-120.890657	5-Dec-16
BF3	*Anas platyrhynchos*	F	United States	NM	33.80211	-106.8784	14-Apr-17
BM11	*Anas platyrhynchos*	M	United States	NM	33.80211	-106.8784	24-Jul-17

BM12	*Anas platyrhynchos*	M	United States	NM	33.80211	-106.8784	25-Jul-17
BM13	*Anas platyrhynchos*	M	United States	NM	33.80211	-106.8784	27-Jul-17
BM7	*Anas platyrhynchos*	M	United States	NM	33.80211	-106.8784	17-Jul-17
BF6	*Anas platyrhynchos*	F	United States	NM	33.80211	-106.8784	17-Jul-17
KGM946	*Anas platyrhynchos*	M	United States	NM	33.2333	-107.317	2003
PL3243	*Anas platyrhynchos*	M	United States	TX	31.82367	-106.565	16-Aug-17
PL6030	*Anas platyrhynchos*	F	United States	TX	31.82367	-106.565	23-Feb-18
PL6027	*Anas platyrhynchos*	M	United States	TX	31.82367	-106.565	2-Feb-18
PL6031	*Anas platyrhynchos*	M	United States	TX	31.82367	-106.565	23-Feb-18
PL6032	*Anas platyrhynchos*	M	United States	TX	31.82367	-106.565	20-Apr-18
PL508	MX.FERAL	F	United States	NM	34.72	-106.8	40152
PL6015	MX.FERAL	F	United States	TX	31.82367	-106.565	43000
PL3218	*MX.FERAL*	F	United States	TX	31.82367	-106.565	14-Aug-17
PL3246	*MX.FERAL*	F	United States	TX	31.82367	-106.565	25-Aug-17
BF8	*Anas platyrhynchos*	F	United States	NM	33.80211	-106.8784	27-Jul-17
BF9	*Anas platyrhynchos*	F	United States	NM	33.80211	-106.8784	27-Jul-17
BM10	*Anas platyrhynchos x Anas diazi*	M	United States	NM	33.80211	-106.8784	20-Jul-17

BF7	*Anas platyrhynchos x Anas diazi*	F	United States	NM	33.80211	-106.8784	20-Jul-17
BF2	*Anas platyrhynchos x Anas diazi*	F	United States	NM	33.80211	-106.8784	13-Apr-17
BF1	*Anas platyrhynchos x Anas diazi*	F	United States	NM	33.80211	-106.8784	12-Apr-17
PL6016	*Anas platyrhynchos x Anas diazi*	F	United States	TX	31.82367	-106.565	22-Sep-17
PL6000	*Anas platyrhynchos x Anas diazi*	F	United States	TX	31.82367	-106.565	25-Aug-17
PL6002	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	25-Aug-17
PL6036	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	25-May-18
PL3247	*Anas platyrhynchos x Anas diazi*	F	United States	TX	31.82367	-106.565	25-Aug-17
BF5	*Anas platyrhynchos x Anas diazi*	F	United States	NM	33.80211	-106.8784	17-Jul-17
BM4	*Anas platyrhynchos x Anas diazi*	M	United States	NM	33.80211	-106.8784	10-Jul-17
PL6007	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	13-Sep-17
BM9	*Anas platyrhynchos x Anas diazi*	M	United States	NM	33.80211	-106.8784	9-Jul-17
BM5	*Anas platyrhynchos x Anas diazi*	M	United States	NM	33.80211	-106.8784	10-Jul-17
KGM968	*Anas platyrhynchos x Anas diazi*	F	United States	TX	31.7903	-106.423	2003
PL6009	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	13-Sep-17
PL3241	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	16-Aug-17
PL6024	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	19-Jan-18

PL6025	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	2-Feb-18
PL3233	*Anas platyrhynchos x Anas diazi*	M	United States	TX	31.82367	-106.565	28-Jul-17
PL3239	*Anas platyrhynchos x Anas diazi*	F	United States	TX	31.82367	-106.565	16-Aug-17
PL538	*Anas diazi*	F	United States	NM	34.02	-106.93	9-Jan-10
PL680	*Anas diazi*	F	United States	NM	34.02	-106.93	30-Oct-09
KGM927	*Anas diazi*	M	United States	NM	32.3122	-106.778	2003
PL513	*Anas diazi*	M	United States	NM	34.72	-106.8	12-Nov-09
KGM969	*Anas diazi*	M	United States	NM	33.2333	-107.317	2003
PL111117	*Anas diazi*	Unk	United States	NM	32.11799	-106.675044	11-Nov-17
PL3236	*Anas diazi*	F	United States	TX	31.82367	-106.565	14-Aug-17
BF10	*Anas diazi*	F	United States	NM	33.80211	-106.8784	11-Jan-18
PL3240	*Anas diazi*	M	United States	TX	31.82367	-106.565	16-Aug-17
LENM5	*Anas diazi*	Unk	United States	NM	32.344811	-106.857902	2017
KGM954	*Anas diazi*	M	United States	NM	32.3122	-106.778	2003
PL3229	*Anas diazi*	M	United States	TX	31.82367	-106.565	14-Aug-17
LENM6	*Anas diazi*	Unk	United States	NM	32.344811	-106.857902	2017
LENM4	*Anas diazi*	Unk	United States	NM	32.344811	-106.857902	2017

PL532	*Anas diazi*	M	United States	NM	32.3122	-106.778	13-Nov-09
PL6001	*Anas diazi*	M	United States	TX	31.82367	-106.565	25-Aug-17
BM6	*Anas diazi*	M	United States	NM	33.80211	-106.8784	13-Jul-17
KGM933	*Anas diazi*	F	United States	TX	31.7903	-106.423	2003
PL3234	*Anas diazi*	M	United States	TX	31.82367	-106.565	28-Jul-17
KGM965	*Anas diazi*	M	United States	NM	32.2611	-107.756	2003
JAT1000	*Anas diazi*	M	United States	TX	30.5377	-103.801	25-May-11
PL3245	*Anas diazi*	M	United States	TX	31.82367	-106.565	16-Aug-17
PL3230	*Anas diazi*	M	United States	TX	31.82367	-106.565	14-Aug-17
PL3231	*Anas diazi*	F	United States	TX	31.82367	-106.565	20-Jul-17
PL3232	*Anas diazi*	F	United States	TX	31.82367	-106.565	20-Jul-17
PL3228	*Anas diazi*	F	United States	TX	31.82367	-106.565	20-Jul-17
PL3237	*Anas diazi*	F	United States	TX	31.82367	-106.565	14-Aug-17
PL3244	*Anas diazi*	M	United States	TX	31.82367	-106.565	16-Aug-17
BM1	*Anas diazi*	M	United States	NM	33.80211	-106.8784	5-Apr-17
PL6029	*Anas diazi*	F	United States	TX	31.82367	-106.565	23-Feb-18
PL6023	*Anas diazi*	M	United States	TX	31.82367	-106.565	19-Jan-18

BM14	*Anas diazi*	M	United States	NM	33.80211	-106.8784	14-Dec-17
BF11	*Anas diazi*	F	United States	NM	33.80211	-106.8784	21-Feb-18
BF4	*Anas diazi*	F	United States	NM	33.80211	-106.8784	13-Jul-17
PL6028	*Anas diazi*	M	United States	TX	31.82367	-106.565	23-Feb-18
PL3242	*Anas diazi*	M	United States	TX	31.82367	-106.565	16-Aug-17
JIBTX001	*Anas diazi*	F	United States	TX	31.75706	-106.2862	10-Nov-17
BM8	*Anas diazi*	M	United States	NM	33.80211	-106.8784	20-Jul-17
PL6008	*Anas diazi*	M	United States	TX	31.82367	-106.565	13-Sep-17
PL6022	*Anas diazi*	M	United States	TX	31.82367	-106.565	13-Oct-17
PL6021	*Anas diazi*	M	United States	TX	31.82367	-106.565	23-Sep-17
BM2	*Anas diazi*	M	United States	NM	33.80211	-106.8784	12-Apr-17
BM3	*Anas diazi*	M	United States	NM	33.80211	-106.8784	13-Apr-17
PL3238	*Anas diazi*	F	United States	TX	31.82367	-106.565	16-Aug-17
PL3004	*Anas diazi*	F	Mexico	MX	28.180433	-106.902733	26-Jan-17
PL3097	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	29-Jan-17
PL3005	*Anas diazi*	M	Mexico	MX	28.180433	-106.902733	26-Jan-17
PL3007	*Anas diazi*	F	Mexico	MX	28.180433	-106.902733	26-Jan-17
PL3008	*Anas diazi*	M	Mexico	MX	28.180433	-106.902733	26-Jan-17
PL3009	*Anas diazi*	M	Mexico	MX	28.114883	-106.9887	26-Jan-17
PL3011	*Anas diazi*	F	Mexico	MX	28.114883	-106.9887	26-Jan-17
PL3012	*Anas diazi*	M	Mexico	MX	28.114883	-106.9887	26-Jan-17

PL3013	*Anas diazi*	M	Mexico	MX	28.114883	-106.9887	26-Jan-17
PL3014	*Anas diazi*	M	Mexico	MX	28.114883	-106.9887	26-Jan-17
PL3020	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3021	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3022	*Anas diazi*	F	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3023	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3024	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3025	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3026	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3027	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3028	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3029	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3030	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3031	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3035	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3036	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3037	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3038	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3039	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3040	*Anas diazi*	F	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3042	*Anas diazi*	F	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3043	*Anas diazi*	M	Mexico	MX	28.2231	-106.996217	27-Jan-17
PL3044	*Anas diazi*	M	Mexico	MX	28.2231	-106.996217	27-Jan-17
PL3045	*Anas diazi*	M	Mexico	MX	28.2231	-106.996217	27-Jan-17
PL3046	*Anas diazi*	F	Mexico	MX	28.180433	-106.902733	27-Jan-17
PL3047	*Anas diazi*	M	Mexico	MX	28.180433	-106.902733	27-Jan-17
PL3049	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3050	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3051	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3052	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3053	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3054	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3056	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3057	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17

PL3059	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3060	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3061	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3062	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3063	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3064	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3058	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3066	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3067	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3068	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3069	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	27-Jan-17
PL3077	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3078	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3079	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3080	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3081	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3082	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3083	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3084	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	28-Jan-17
PL3087	*Anas diazi*	F	Mexico	MX	28.152783	-106.983567	28-Jan-17
PL3088	*Anas diazi*	M	Mexico	MX	28.152783	-106.983567	28-Jan-17
PL3089	*Anas diazi*	M	Mexico	MX	28.152783	-106.983567	28-Jan-17
PL3096	*Anas diazi*	F	Mexico	MX	28.120683	-106.9516	29-Jan-17
PL3098	*Anas diazi*	M	Mexico	MX	28.120683	-106.9516	29-Jan-17
PL3041	*Anas diazi*	M	Mexico	MX	28.1709	-106.92745	27-Jan-17
PL3090	*Anas platyrhynchos*	F	Mexico	CI	28.15278	-106.9836	28-Jan-17
PL3100	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3101	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3102	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3103	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3104	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3108	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018
PL3109	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3110	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018

PL3111	*Anas diazi*	F	Mexico	SI	24.438402	-107.457736	2017
PL3113	*Anas diazi*	F	Mexico	SI	24.438402	-107.457736	2017
PL3114	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3115	*Anas diazi*	F	Mexico	SI	25.390542	-108.333867	2018
PL3116	*Anas diazi*	M	Mexico	SI	24.438402	-107.457736	2017
PL3117	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018
PL3118	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018
PL3119	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018
PL3121	*Anas diazi*	F	Mexico	SI	25.390542	-108.333867	2018
PL3122	*Anas diazi*	M	Mexico	SI	25.390542	-108.333867	2018
PL1000	*Anas diazi*	M	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1002	*Anas diazi*	M	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1003	*Anas diazi*	M	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1004	*Anas diazi*	M	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1005	*Anas diazi*	M	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1006	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1007	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1008	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1009	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1010	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1011	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1012	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1013	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1001	*Anas diazi*	F	Mexico	SO	27.3494	-109.988	4-Feb-12
PL1014	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1015	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1016	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1017	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1018	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1019	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1020	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1021	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1022	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1023	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12

PL1024	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1025	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1029	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1026	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1027	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1028	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1030	*Anas diazi*	F	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1031	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1032	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1034	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1036	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1037	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1038	*Anas diazi*	M	Mexico	SO	27.4459	-110.15	4-Feb-12
PL1039	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1040	*Anas diazi*	F	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1041	*Anas diazi*	F	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1042	*Anas diazi*	F	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1043	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1044	*Anas diazi*	F	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1045	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1046	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1047	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL1048	*Anas diazi*	M	Mexico	SO	26.9979	-109.897	4-Feb-12
PL3105	*Anas diazi*	M	Mexico	DG	0	0	2018
PL3106	*Anas diazi*	M	Mexico	DG	0	0	2018
PL2033	*Anas diazi*	M	Mexico	DG	24.8875	-105.073	12-Jan-13
PL2034	*Anas diazi*	F	Mexico	DG	24.8875	-105.073	12-Jan-13
PL2035	*Anas diazi*	M	Mexico	DG	24.8875	-105.073	12-Jan-13
PL2036	*Anas diazi*	F	Mexico	DG	24.8875	-105.073	12-Jan-13
PL2037	*Anas diazi*	F	Mexico	GT	21.4958	-100.659	9-Feb-13
PL2038	*Anas diazi*	M	Mexico	GT	21.4958	-100.659	9-Feb-13
MMMEDU3	*Anas diazi*	F	Mexico	ZA	23.2928	-102.701	Unk
MMMEDU6	*Anas diazi*	M	Mexico	ZA	23.2928	-102.701	Unk
MMMEDU7	*Anas diazi*	F	Mexico	ZA	23.2928	-102.701	Unk

MMMEDU9	*Anas diazi*	M	Mexico	ZA	23.2928	-102.701	Unk
PL2023	*Anas diazi*	F	Mexico	MX	19.6039	-99.7033	19-Feb-13
PL2024	*Anas diazi*	F	Mexico	MX	19.6039	-99.7033	19-Feb-13
PL2025	*Anas diazi*	F	Mexico	MX	19.6039	-99.7033	19-Feb-13
PL2026	*Anas diazi*	M	Mexico	MX	19.6039	-99.7033	19-Feb-13
PL2027	*Anas diazi*	M	Mexico	MX	19.6039	-99.7033	19-Feb-13
PL2028	*Anas diazi*	F	Mexico	MX	19.6039	-99.7033	11-Nov-13
PL2029	*Anas diazi*	F	Mexico	MX	19.6039	-99.7033	11-Nov-13
PL2030	*Anas diazi*	M	Mexico	MX	19.6039	-99.7033	11-Nov-13
PL2031	*Anas diazi*	M	Mexico	MX	19.6039	-99.7033	11-Nov-13
PL2032	*Anas diazi*	M	Mexico	MX	19.6039	-99.7033	11-Nov-13
PL2001	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2003	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2004	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2005	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2006	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2007	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2002	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2008	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2009	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2010	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2011	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2012	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2013	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2014	*Anas diazi*	F	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2015	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2016	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2017	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2018	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2019	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2020	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2021	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13
PL2022	*Anas diazi*	M	Mexico	PU	19.2596	-97.6659	18-Feb-13

Table S2.1. Individual sample information

ID	Scientific_Name	Gradient Forest Pop.	Sex	Country	State/Province	Longitude	Latitude
PL3004	*Anas diazi*	Chihuahua 1	Female	Mexico	Mexico	28.180433	-106.902733
PL3005	*Anas diazi*	Chihuahua 1	Male	Mexico	Mexico	28.180433	-106.902733
PL3007	*Anas diazi*	Chihuahua 1	Female	Mexico	Mexico	28.180433	-106.902733
PL3008	*Anas diazi*	Chihuahua 1	Male	Mexico	Mexico	28.180433	-106.902733
PL3009	*Anas diazi*	Chihuahua 2	Male	Mexico	Mexico	28.114883	-106.9887
PL3011	*Anas diazi*	Chihuahua 2	Female	Mexico	Mexico	28.114883	-106.9887
PL3012	*Anas diazi*	Chihuahua 2	Male	Mexico	Mexico	28.114883	-106.9887
PL3013	*Anas diazi*	Chihuahua 2	Male	Mexico	Mexico	28.114883	-106.9887
PL3014	*Anas diazi*	Chihuahua 2	Male	Mexico	Mexico	28.114883	-106.9887
PL3043	*Anas diazi*	Chihuahua 4	Male	Mexico	Mexico	28.2231	-106.996217
PL3044	*Anas diazi*	Chihuahua 4	Male	Mexico	Mexico	28.2231	-106.996217
PL3045	*Anas diazi*	Chihuahua 4	Male	Mexico	Mexico	28.2231	-106.996217
PL3046	*Anas diazi*	Chihuahua 5	Female	Mexico	Mexico	28.180433	-106.902733
PL3047	*Anas diazi*	Chihuahua 5	Male	Mexico	Mexico	28.180433	-106.902733
PL3049	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3050	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3051	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3052	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3053	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3054	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3056	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3057	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3059	*Anas diazi*	Chihuahua 6	Female	Mexico	Mexico	28.120683	-106.9516
PL3060	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3061	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3062	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3063	*Anas diazi*	Chihuahua 6	Female	Mexico	Mexico	28.120683	-106.9516
PL3064	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3066	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3067	*Anas diazi*	Chihuahua 6	Female	Mexico	Mexico	28.120683	-106.9516
PL3068	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3069	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3077	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3078	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3079	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3080	*Anas diazi*	Chihuahua 6	Female	Mexico	Mexico	28.120683	-106.9516
PL3081	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3082	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3083	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3084	*Anas diazi*	Chihuahua 6	Male	Mexico	Mexico	28.120683	-106.9516
PL3087	*Anas diazi*	Chihuahua 7	Female	Mexico	Mexico	28.152783	-106.983567

PL3088	*Anas diazi*	Chihuahua 7	Male	Mexico	Mexico	28.152783	-106.983567
PL3089	*Anas diazi*	Chihuahua 7	Male	Mexico	Mexico	28.152783	-106.983567
PL3096	*Anas diazi*	Chihuahua 8	Female	Mexico	Mexico	28.120683	-106.9516
PL3097	*Anas diazi*	Chihuahua 8	Female	Mexico	Mexico	28.120683	-106.9516
PL3098	*Anas diazi*	Chihuahua 8	Male	Mexico	Mexico	28.120683	-106.9516
PL3090	*Anas platyrhynchos*	NA	Female	Mexico	Chihuahua	28.15278	-106.9836
PL3020	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3021	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3022	*Anas diazi*	Chihuahua 3	Female	Mexico	Mexico	28.1709	-106.92745
PL3023	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3024	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3025	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3026	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3027	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3028	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3029	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3030	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3031	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3035	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3036	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3037	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3038	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3039	*Anas diazi*	Chihuahua 3	Male	Mexico	Mexico	28.1709	-106.92745
PL3040	*Anas diazi*	Chihuahua 3	Female	Mexico	Mexico	28.1709	-106.92745
PL3042	*Anas diazi*	Chihuahua 3	Female	Mexico	Mexico	28.1709	-106.92745
PL2033	*Anas diazi*	Durango 1	Male	Mexico	Durango	24.8875	-105.073
PL2034	*Anas diazi*	Durango 1	Female	Mexico	Durango	24.8875	-105.073
PL2035	*Anas diazi*	Durango 1	Male	Mexico	Durango	24.8875	-105.073
PL2036	*Anas diazi*	Durango 1	Female	Mexico	Durango	24.8875	-105.073
PL2037	*Anas diazi*	Guanajuato 1	Female	Mexico	Guanajuato	21.4958	-100.659
PL2038	*Anas diazi*	Guanajuato 1	Male	Mexico	Guanajuato	21.4958	-100.659
PL2023	*Anas diazi*	Mexico 1	Female	Mexico	Mexico	19.6039	-99.7033
PL2024	*Anas diazi*	Mexico 1	Female	Mexico	Mexico	19.6039	-99.7033
PL2025	*Anas diazi*	Mexico 1	Female	Mexico	Mexico	19.6039	-99.7033
PL2026	*Anas diazi*	Mexico 1	Male	Mexico	Mexico	19.6039	-99.7033
PL2027	*Anas diazi*	Mexico 1	Male	Mexico	Mexico	19.6039	-99.7033
PL2028	*Anas diazi*	Mexico 1	Female	Mexico	Mexico	19.6039	-99.7033
PL2029	*Anas diazi*	Mexico 1	Female	Mexico	Mexico	19.6039	-99.7033
PL2030	*Anas diazi*	Mexico 1	Male	Mexico	Mexico	19.6039	-99.7033
PL2031	*Anas diazi*	Mexico 1	Male	Mexico	Mexico	19.6039	-99.7033
PL2032	*Anas diazi*	Mexico 1	Male	Mexico	Mexico	19.6039	-99.7033
PL2001	*Anas diazi*	Puebla 1	Female	Mexico	Puebla	19.2596	-97.6659
PL2003	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659

PL2004	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2005	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2006	*Anas diazi*	Puebla 1	Female	Mexico	Puebla	19.2596	-97.6659
PL2008	*Anas diazi*	Puebla 1	Female	Mexico	Puebla	19.2596	-97.6659
PL2009	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2010	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2011	*Anas diazi*	Puebla 1	Female	Mexico	Puebla	19.2596	-97.6659
PL2012	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2013	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2014	*Anas diazi*	Puebla 1	Female	Mexico	Puebla	19.2596	-97.6659
PL2015	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2016	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2017	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2018	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2019	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2020	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2021	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL2022	*Anas diazi*	Puebla 1	Male	Mexico	Puebla	19.2596	-97.6659
PL3100	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3101	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3102	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3103	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3104	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3109	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3111	*Anas diazi*	Sinaloa 1	Female	Mexico	Sinaloa	24.438402	-107.457736
PL3113	*Anas diazi*	Sinaloa 1	Female	Mexico	Sinaloa	24.438402	-107.457736
PL3114	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3116	*Anas diazi*	Sinaloa 1	Male	Mexico	Sinaloa	24.438402	-107.457736
PL3108	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL3110	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL3115	*Anas diazi*	Sinaloa 2	Female	Mexico	Sinaloa	25.390542	-108.333867
PL3117	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL3118	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL3119	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL3121	*Anas diazi*	Sinaloa 2	Female	Mexico	Sinaloa	25.390542	-108.333867
PL3122	*Anas diazi*	Sinaloa 2	Male	Mexico	Sinaloa	25.390542	-108.333867
PL1000	*Anas diazi*	Sonora 1	Male	Mexico	Sonora	27.3494	-109.988
PL1002	*Anas diazi*	Sonora 1	Male	Mexico	Sonora	27.3494	-109.988
PL1003	*Anas diazi*	Sonora 1	Male	Mexico	Sonora	27.3494	-109.988
PL1004	*Anas diazi*	Sonora 1	Male	Mexico	Sonora	27.3494	-109.988
PL1005	*Anas diazi*	Sonora 1	Male	Mexico	Sonora	27.3494	-109.988
PL1006	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1007	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15

PL1008	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1009	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1010	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1011	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1012	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1013	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1014	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1015	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1016	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1017	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1018	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1019	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1020	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1021	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1022	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1023	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1024	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1025	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1026	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1027	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1028	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1030	*Anas diazi*	Sonora 2	Female	Mexico	Sonora	27.4459	-110.15
PL1031	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1032	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1034	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1036	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1037	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1038	*Anas diazi*	Sonora 2	Male	Mexico	Sonora	27.4459	-110.15
PL1039	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
PL1040	*Anas diazi*	Sonora 3	Female	Mexico	Sonora	26.9979	-109.897
PL1041	*Anas diazi*	Sonora 3	Female	Mexico	Sonora	26.9979	-109.897
PL1042	*Anas diazi*	Sonora 3	Female	Mexico	Sonora	26.9979	-109.897
PL1043	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
PL1044	*Anas diazi*	Sonora 3	Female	Mexico	Sonora	26.9979	-109.897
PL1045	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
PL1046	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
PL1047	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
PL1048	*Anas diazi*	Sonora 3	Male	Mexico	Sonora	26.9979	-109.897
JAT1000	*Anas diazi*	US 1	Male	United States	Texas	30.5377	-103.801
KGM927	*Anas diazi*	US 10	Male	United States	New Mexico	32.3122	-106.778
KGM954	*Anas diazi*	US 10	Male	United States	New Mexico	32.3122	-106.778
PL532	*Anas diazi*	US 10	Male	United States	New Mexico	32.3122	-106.778

KGM969	*Anas diazi*	US 11	Male	United States	New Mexico	33.2333	-107.317
PL513	*Anas diazi*	US 12	Male	United States	New Mexico	34.72	-106.8
PL120516	*Anas diazi*	US 13	Male	United States	California	37.287222	-120.890657
JIBTX001	*Anas diazi*	US 2	Female	United States	Texas	31.75706	-106.2862
PL111117	*Anas diazi*	US 3	Unk	United States	New Mexico	32.11799	-106.675044
PL538	*Anas diazi*	US 4	Female	United States	New Mexico	34.02	-106.93
PL680	*Anas diazi*	US 4	Female	United States	New Mexico	34.02	-106.93
LENM4	*Anas diazi*	US 5	Unk	United States	New Mexico	32.344811	-106.857902
LENM5	*Anas diazi*	US 5	Unk	United States	New Mexico	32.344811	-106.857902
LENM6	*Anas diazi*	US 5	Unk	United States	New Mexico	32.344811	-106.857902
PL3229	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3230	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3234	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3236	*Anas diazi*	US 6	Female	United States	Texas	31.82367	-106.565
PL3237	*Anas diazi*	US 6	Female	United States	Texas	31.82367	-106.565
PL3238	*Anas diazi*	US 6	Female	United States	Texas	31.82367	-106.565
PL3240	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3242	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3244	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL3245	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6001	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6008	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6021	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6022	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6023	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6028	*Anas diazi*	US 6	Male	United States	Texas	31.82367	-106.565
PL6029	*Anas diazi*	US 6	Female	United States	Texas	31.82367	-106.565
BF10	*Anas diazi*	US 7	Female	United States	New Mexico	33.80211	-106.8784
BM1	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784
BM14	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784
BM2	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784
BM3	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784
BM6	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784
BM8	*Anas diazi*	US 7	Male	United States	New Mexico	33.80211	-106.8784

KGM933	*Anas diazi*	US 8	Female	United States	Texas	31.7903	-106.423
KGM965	*Anas diazi*	US 9	Male	United States	New Mexico	32.2611	-107.756
MMMEDU3	*Anas diazi*	Zacatecas 1	Female	Mexico	Zacatacas	23.2928	-102.701
MMMEDU6	*Anas diazi*	Zacatecas 1	Male	Mexico	Zacatacas	23.2928	-102.701
MMMEDU7	*Anas diazi*	Zacatecas 1	Female	Mexico	Zacatacas	23.2928	-102.701
MMMEDU9	*Anas diazi*	Zacatecas 1	Male	Mexico	Zacatacas	23.2928	-102.701
UAMX806	*Anas platyrhynchos*	Alaska 1	Female	United States	AK	64.8	-147.7
UAMX812	*Anas platyrhynchos*	Alaska 1	Female	United States	AK	64.8	-147.7
UAMX1739	*Anas platyrhynchos*	Alaska 2	Male	United States	AK	52.8763	-172.8905
UAMX3170	*Anas platyrhynchos*	Alaska 3	Female	United States	AK	60.337778	-151
REW624	*Anas platyrhynchos*	Alaska 4	Male	United States	AK	67.14114	-150.35586
KGM1422	*Anas platyrhynchos*	Alberta 1	Male	Canada	Alberta	53.1	-111.8
KGM1424	*Anas platyrhynchos*	Alberta 1	Male	Canada	Alberta	53.1	-111.8
KGM1421	*Anas platyrhynchos*	Alberta 2	Female	Canada	Alberta	54.5	-110.2
KGM1423	*Anas platyrhynchos*	Alberta 2	Female	Canada	Alberta	54.5	-110.2
KGM1414	*Anas platyrhynchos*	Alberta 3	Female	Canada	Alberta	49.2	-113.3
KGM1412	*Anas platyrhynchos*	Alberta 4	Male	Canada	Alberta	49.9	-113.1
KGM1427	*Anas platyrhynchos*	BC 2	Female	Canada	British Columbia	49.1	-122
CAMall01	*Anas platyrhynchos*	California 1	Male	United States	California	39.3299	-121.914
CAMall05	*Anas platyrhynchos*	California 1	Male	United States	California	39.3299	-121.914
CAMall12	*Anas platyrhynchos*	California 1	Male	United States	California	39.3299	-121.914
CAMall17	*Anas platyrhynchos*	California 1	Female	United States	California	39.3299	-121.914
DICA012718	*Anas platyrhynchos*	California 2	Female	United States	California	37.198227	-120.902249
PL121516	*Anas platyrhynchos*	California 3	Male	United States	California	37.287222	-120.890657
PL3220	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3221	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3223	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3224	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3225	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3226	*Anas platyrhynchos*	Kentucky 1	Male	United States	Kentucky	37.0419	-89.0896
PL3227	*Anas platyrhynchos*	Kentucky 1	Female	United States	Kentucky	37.0419	-89.0896
JIBCan001	*Anas platyrhynchos*	Manitoba 1	Male	Canada	Manitoba	50.19871	-98.21405
JIBCan002	*Anas platyrhynchos*	Manitoba 1	Male	Canada	Manitoba	50.19871	-98.21405
JIBCan003	*Anas platyrhynchos*	Manitoba 1	Male	Canada	Manitoba	50.19871	-98.21405
JIBCan004	*Anas platyrhynchos*	Manitoba 1	Male	Canada	Manitoba	50.19871	-98.21405
JIBCan005	*Anas platyrhynchos*	Manitoba 1	Female	Canada	Manitoba	50.19871	-98.21405
JIBCan006	*Anas platyrhynchos*	Manitoba 1	Female	Canada	Manitoba	50.19871	-98.21405

KGM1383	*Anas platyrhynchos*	Manitoba 2	Female	Canada	Manitoba	50.1	-100.4
KGM1390	*Anas platyrhynchos*	Manitoba 2	Female	Canada	Manitoba	50.1	-100.4
KGM1393	*Anas platyrhynchos*	Manitoba 3	Female	Canada	Manitoba	51.1	-100.8
KGM1392	*Anas platyrhynchos*	Manitoba 4	Male	Canada	Manitoba	51.1	-99.8
KGM1388	*Anas platyrhynchos*	Manitoba 5	Male	Canada	Manitoba	50.1	-98.25
KGM1387	*Anas platyrhynchos*	Manitoba 6	Male	Canada	Manitoba	50.1	-98.25
PL264	*Anas platyrhynchos*	ND 1	Male	United States	ND	45.978889	-97.810278
PL266	*Anas platyrhynchos*	ND 1	Male	United States	ND	45.978889	-97.810278
PL263	*Anas platyrhynchos*	ND 2	Female	United States	ND	48.034722	-98.944167
PL262	*Anas platyrhynchos*	ND 3	Female	United States	ND	48.81	-103.49
BF3	*Anas platyrhynchos*	N/A	Female	United States	New Mexico	33.80211	-106.8784
BM11	*Anas platyrhynchos*	N/A	Male	United States	New Mexico	33.80211	-106.8784
BM12	*Anas platyrhynchos*	N/A	Male	United States	New Mexico	33.80211	-106.8784
BM13	*Anas platyrhynchos*	N/A	Male	United States	New Mexico	33.80211	-106.8784
BM7	*Anas platyrhynchos*	N/A	Male	United States	New Mexico	33.80211	-106.8784
PL121317	*Anas platyrhynchos*	N/A	Male	United States	NM	32.141221	-106.699413
KGM946	*Anas platyrhynchos*	N/A	Male	United States	New Mexico	33.2333	-107.317
PL112316	*Anas platyrhynchos*	N/A	Male	United States	NM	32.953968	-107.295992
PL267	*Anas platyrhynchos*	SD 1	Male	United States	SD	45.333611	-97.519444
PL269	*Anas platyrhynchos*	SD 1	Male	United States	SD	45.333611	-97.519444
PL272	*Anas platyrhynchos*	SD 1	Female	United States	SD	45.333611	-97.519444
KGM1395	*Anas platyrhynchos*	SK 1	Male	Canada	Saskatchewan	52.1	-106.7
KGM1406	*Anas platyrhynchos*	SK 1	Male	Canada	Saskatchewan	52.1	-106.7
KGM1407	*Anas platyrhynchos*	SK 2	Male	Canada	Saskatchewan	50.8	-104.9
KGM1404	*Anas platyrhynchos*	SK 3	Female	Canada	Saskatchewan	52	-107
KGM1400	*Anas platyrhynchos*	SK 4	Female	Canada	Saskatchewan	53.3	-110
KGM1405	*Anas platyrhynchos*	SK 5	Female	Canada	Saskatchewan	54.27	-110.8
PL3243	*Anas platyrhynchos*	N/A	Male	United States	Texas	31.82367	-106.565
PL6027	*Anas platyrhynchos*	N/A	Male	United States	Texas	31.82367	-106.565
PL6030	*Anas platyrhynchos*	N/A	Female	United States	Texas	31.82367	-106.565
PL6031	*Anas platyrhynchos*	N/A	Male	United States	Texas	31.82367	-106.565
PL6032	*Anas platyrhynchos*	N/A	Male	United States	Texas	31.82367	-106.565

Table S2.2. List of environmental variables used for genotype-environment association testing in GradientForest.

Variable	Description	Unit
BIO1	Mean annual temperature	°C
BIO2	Mean diurnal range	°C
BIO3	Isothermality	°C
BIO4	Temperature seasonality	°C
BIO5	Max temperature of warmest month	°C
BIO6	Min temperature of coldest month	°C
BIO7	Temperature annual range	°C
BIO8	Mean temperature of wettest quarter	°C
BIO9	Mean temperature of driest quarter	°C
BIO10	Mean temperature of warmest quarter	°C
BIO11	Mean temperature of coldest quarter	°C
BIO12	Annual precipitation	mm
BIO13	Precipitation of wettest month	mm
BIO14	Precipitation of driest month	mm
BIO15	Precipitation seasonality	mm
BIO16	Precipitation of wettest quarter	mm
BIO17	Precipitation of driest quarter	mm
BIO18	Precipitation of warmest quarter	mm
BIO19	Precipitation of coldest quarter	mm
NDVI_ANNUAL	Normalized Difference Vegetation Index Annual; MOD13A3	
NDVI_SUMMER	Normalized Difference Vegetation Index June; MOD13A3	
NDVI_WINTER	Normalized Difference Vegetation Index December; MOD13A3	
EVI_ANNUAL	Enhanced Vegetation Index Annual; MOD13A3	
EVI_SUMMER	Enhanced Vegetation Index June; MOD13A3	
EVI_WINTER	Enhanced Vegetation Index December; MOD13A3	
NPP_ANNUAL	Net Primary Productivity Annual; MOD17A2H	
SRTM	Shuttle radar topography mission; Elevation	m

Table S2.3. Log likelihood values for the five evolutionary models tested in ∂a∂i, and the scaled values of the optimum parameters identified from the geometric mean calculated across 50 individual runs from ∂a∂i.

MEDU vs. MALL ∂a∂i Evolutionary Model Optimum Parameters							
	Log-Likelihood	*Ne* Ancestral	*Ne* MEDU	*Ne* MALL	Time	Migration MEDU→MALL	Migration MALL→MEDU
Split-with-migration	**-4,931**	451,092 (± 11,032)	608,035 (± 21,590)	1,372,661 (± 97,899)	995,227 (± 25,796)	9 (± 0.27)	20 (± 0.60)
Isolation with Migration	-6,985	--	--	--	--	--	--
Neutral- No divergence	-11,093	--	--	--	--	--	--
Split without migration	-17,620	--	--	--	--	--	--
Isolation without migration	-83,332	--	--	--	--	--	--

Table S2.4. Constrained R^2 value and p-value of each Genotypic PC identified as significant for individual traits from RDA.

Phenotypic Response Variable	Aut & Z Chromosomes			Aut & Z Chromosomes Males			Aut & Z Chromosomes Female			Z Chromosome Males			Z Chromosome Females		
	Genotypic PC	R^2	P-value	Genotypic PC	R^2	P-value	Genotypic PC	R^2	P-value	Genotypic PC	R^2	P-value	Genotypic PC	R^2	P-value
PRIMARY_COVERTS	--	--	--	PC2	0.132	**0.000**	--	--	--	PC8	0.059	**0.029**	--	--	--
LESSER_COVERTS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
GREATER_COVERTS	PC1	0.052	**0.011**	PC1	0.136	**0.000**	--	--	--	PC2	0.054	**0.043**	--	--	--
SPECULUM_COLOR	PC2	0.121	**0.000**	PC5	**0.016**	0.389	PC1	0.079	0.123	PC8	0.059	**0.029**	PC1	0.246	**0.024**
TOTAL_WING	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
GREEN_HEAD	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
HINDNECK	PC6	0.053	**0.012**	--	--	--	PC4	0.223	**0.002**	--	--	--	PC1	0.246	**0.024**
BLACK_SPOTS_AROUND_BILL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TOTAL_HEAD	--	--	--	--	--	--	PC2	0.111	0.057	--	--	--	PC1	0.246	**0.024**
BACK	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SCAPULARS	PC6	0.053	**0.012**	PC1	0.136	**0.000**	--	--	--	--	--	--	--	--	--
RUMP	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
OUTER_2_RETRICES	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
MALLARD_CURL	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TOTAL_UPPERPARTS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
BELLY_PATTERN	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
BREAST_BELLY	PC6	0.053	**0.012**	PC2	0.132	**0.000**	PC4	0.111	0.057	PC1	0.008	0.604	--	--	--
FLANKS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
UNDERTAIL_COVERTS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
TOTAL_UNDERPARTS	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Table S3.1. Sample information including scientific name based on nuclear assignment, sex, date of collection, and latitude and longitude of sampling location. Plumage scores for samples with available data are also provided.

Sample.ID	Island	Lat	Long	Nuclear.ID	Spp
125371	NORTH	-37.9167	175.3	PURE.MALL	MALL
130169	NORTH	-37.9167	175.3	PURE.MALL	MALL
131361	NORTH	-37.9167	175.3	PURE.MALL	MALL
131456	NORTH	-37.9167	175.3	PURE.MALL	MALL
131460	NORTH	-37.9167	175.3	PURE.MALL	MALL
131462	NORTH	-37.9167	175.3	PURE.MALL	MALL
131501	NORTH	-37.9167	175.3	PURE.MALL	MALL
131948	NORTH	-37.2904	175.5936	PURE.MALL	MALL
143453	NORTH	-37.9167	175.3	PURE.MALL	MALL
143455	NORTH	-37.9167	175.3	PURE.MALL	MALL
143470	NORTH	-37.9167	175.3	PURE.MALL	MALL
143907	NORTH	-37.2904	175.5936	PURE.MALL	MALL
143924	NORTH	-37.2904	175.5936	PURE.MALL	MALL
153162	NORTH	-37.77949443	176.4763972	PURE.MALL	MALL
153206	NORTH	-37.77949443	176.4763972	PURE.MALL	MALL
167382	NORTH	-37.2904	175.5936	PURE.MALL	MALL
167432	NORTH	-37.2904	175.5936	PURE.MALL	MALL
167452	NORTH	-37.2904	175.5936	PURE.MALL	MALL
168162	NORTH	-37.44952191	175.0420902	PURE.MALL	MALL
172110	NORTH	-40.4	175.53	PURE.MALL	MALL
172334	NORTH	-40.4	175.53	PURE.MALL	MALL
174188	NORTH	-38.68720303	177.8949216	PURE.MALL	MALL
131467	NORTH	-37.9167	175.3	PURE.MALL	MALL
143487	NORTH	-37.9167	175.3	PURE.MALL	MALL
172325	NORTH	-40.4	175.53	PURE.MALL	MALL
172135	NORTH	-40.4	175.53	PURE.MALL	MALL
172332	NORTH	-40.4	175.53	PURE.MALL	MALL
131469	NORTH	-37.9167	175.3	PURE.MALL	MALL
143475	NORTH	-37.9167	175.3	PURE.MALL	MALL
172480	NORTH	-40.4	175.53	PURE.MALL	MALL
131474	NORTH	-37.9167	175.3	PURE.MALL	MALL
167451	NORTH	-37.2904	175.5936	PURE.MALL	MALL
131483	NORTH	-37.9167	175.3	PURE.MALL	MALL
167392	NORTH	-37.2904	175.5936	PURE.MALL	MALL
143126	NORTH	-37.2904	175.5936	PURE.MALL	MALL
167439	NORTH	-37.2904	175.5936	PURE.MALL	MALL
167426	NORTH	-37.2904	175.5936	PURE.MALL	MALL

150318	NORTH	-40.4	175.53	PURE.MALL	MALL
168312	NORTH	-37.44952191	175.0420902	PURE.MALL	MALL
143211	NORTH	-37.2904	175.5936	PURE.MALL	MALL
143479	NORTH	-37.9167	175.3	PURE.MALL	MALL
169152	NORTH	-36.46387982	174.2580166	PURE.MALL	MALL
141595	NORTH	-37.9167	175.3	PURE.MALL	MALL
168087	NORTH	-37.44952191	175.0420902	PURE.MALL	MALL
167376	NORTH	-37.2904	175.5936	PURE.MALL	MALL
172107	NORTH	-40.4	175.53	PURE.MALL	MALL
153298	NORTH	-37.77949443	176.4763972	PURE.MALL	MALL
168147	NORTH	-37.44952191	175.0420902	PURE.MALL	MALL
167127	NORTH	-37.2904	175.5936	PURE.MALL	MALL
141992	NORTH	-37.2904	175.5936	PURE.MALL	MALL
131498	NORTH	-37.9167	175.3	PURE.MALL	MALL
168534	NORTH	-37.94928117	175.2417557	PURE.MALL	MALL
172338	NORTH	-40.4	175.53	PURE.MALL	MALL
143264	NORTH	-37.2904	175.5936	PURE.MALL	MALL
143466	NORTH	-37.9167	175.3	PURE.MALL	MALL
172105	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
143687	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
167061	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
174260	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
153222	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
172231	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
172336	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
153086	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
153224	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
168279	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
172319	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
172394	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
167235	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
143462	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
167114	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
168560	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
143530	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
131458	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL

168265	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
150305	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
172133	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
168119	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
172321	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
172396	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
167446	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
168808	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
131494	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
143476	NORTH	-37.9167	175.3	F4-MALL	MALL
143557	NORTH	-37.2904	175.5936	F4-MALL	MALL
131472	NORTH	-37.9167	175.3	F4-MALL	MALL
168212	NORTH	-37.44952191	175.0420902	F4-MALL	MALL
172348	NORTH	-40.4	175.53	F4-MALL	MALL
131476	NORTH	-37.9167	175.3	F4-MALL	MALL
143456	NORTH	-37.9167	175.3	F4-MALL	MALL
131471	NORTH	-37.9167	175.3	F4-MALL	MALL
168507	NORTH	-37.94928117	175.2417557	F4-MALL	MALL
143529	NORTH	-37.2904	175.5936	F4-MALL	MALL
131466	NORTH	-37.9167	175.3	F4-MALL	MALL
167380	NORTH	-37.2904	175.5936	F4-MALL	MALL
174220	NORTH	-38.68720303	177.8949216	F4-MALL	MALL
143471	NORTH	-37.9167	175.3	F4-MALL	MALL
153182	NORTH	-37.77949443	176.4763972	F4-MALL	MALL
141728	NORTH	-37.9167	175.3	F4-MALL	MALL
167428	NORTH	-37.2904	175.5936	F4-MALL	MALL
131487	NORTH	-37.9167	175.3	F4-MALL	MALL
153261	NORTH	-37.77949443	176.4763972	F4-MALL	MALL
141972	NORTH	-37.2904	175.5936	F4-MALL	MALL
141878	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
167179	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
143472	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
170399	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
143458	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
120749	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
174310	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
153352	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL

143527	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
174171	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
143109	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
172329	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
172345	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
167500	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
146615	NORTH	-37.766172	176.474736	MALL.BACKCROSS_SWARM	MALL
143493	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
131457	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
168657	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
169157	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
172340	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
167431	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
143679	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
153359	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
167442	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
167390	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
172314	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
143459	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
168678	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
168324	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
172349	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
143227	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
168629	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
169163	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
153405	NORTH	-37.766172	176.474736	MALL.BACKCROSS_SWARM	MALL
131503	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
131499	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
116841	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
167448	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
143484	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
143994	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
168605	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
168856	NORTH	-37.94928117	175.2417557	MALL.BACKCROSS_SWARM	MALL
143461	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
143701	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
137399	NORTH	-37.766172	176.474736	MALL.BACKCROSS_SWARM	MALL

168262	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
131504	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
153287	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
174138	NORTH	-38.68720303	177.8949216	F3-MALL	MALL
168718	NORTH	-37.94928117	175.2417557	F3-MALL	MALL
143482	NORTH	-37.9167	175.3	F3-MALL	MALL
167193	NORTH	-37.2904	175.5936	F3-MALL	MALL
172218	NORTH	-40.4	175.53	F3-MALL	MALL
123506	NORTH	-37.94928117	175.2417557	F3-MALL	MALL
167186	NORTH	-37.2904	175.5936	F3-MALL	MALL
153471	NORTH	-37.766172	176.474736	F3-MALL	MALL
169001	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
167495	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
153140	NORTH	-37.77949443	176.4763972	MALL.BACKCROSS_SWARM	MALL
169092	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
143485	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
143492	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
143491	NORTH	-37.9167	175.3	MALL.BACKCROSS_SWARM	MALL
174287	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
153335	NORTH	-37.77949443	176.4763972	F2-MALL	MALL
168225	NORTH	-37.44952191	175.0420902	F2-MALL	MALL
168173	NORTH	-37.44952191	175.0420902	MALL.BACKCROSS_SWARM	MALL
152260	NORTH	-38.5969414	177.8908628	MALL.BACKCROSS_SWARM	MALL
167307	NORTH	-37.2904	175.5936	MALL.BACKCROSS_SWARM	MALL
169220	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
153484	NORTH	-37.766172	176.474736	MALL.BACKCROSS_SWARM	MALL
174302	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
174098	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
152320	NORTH	-37.766172	176.474736	MALL.BACKCROSS_SWARM	MALL
150220	NORTH	-40.4	175.53	MALL.BACKCROSS_SWARM	MALL
174164	NORTH	-38.68720303	177.8949216	MALL.BACKCROSS_SWARM	MALL
169187	NORTH	-36.46387982	174.2580166	MALL.BACKCROSS_SWARM	MALL
172012	NORTH	-40.4	175.53	F1	MALL

150411	NORTH	-40.4	175.53	F1	MALL
168074	NORTH	-37.44952191	175.0420902	F1	MALL
174265	NORTH	-38.68720303	177.8949216	F1	MALL
174153	NORTH	-38.68720303	177.8949216	F1	GRDU
174096	NORTH	-38.68720303	177.8949216	F1	GRDU
153495	NORTH	-37.766172	176.474736	F1	GRDU
168123	NORTH	-37.44952191	175.0420902	F1	GRDU
168243	NORTH	-37.44952191	175.0420902	NZGR.BACKCROSS_SWARM	GRDU
174110	NORTH	-38.68720303	177.8949216	NZGR.BACKCROSS_SWARM	GRDU
168470	NORTH	-37.44952191	175.0420902	NZGR.BACKCROSS_SWARM	GRDU
143898	NORTH	-37.2904	175.5936	NZGR.BACKCROSS_SWARM	GRDU
131963	NORTH	-37.2904	175.5936	NZGR.BACKCROSS_SWARM	GRDU
174132	NORTH	-38.68720303	177.8949216	NZGR.BACKCROSS_SWARM	GRDU
169208	NORTH	-36.46387982	174.2580166	NZGR.BACKCROSS_SWARM	GRDU
150258	NORTH	-40.4	175.53	NZGR.BACKCROSS_SWARM	GRDU
168348	NORTH	-37.44952191	175.0420902	NZGR.BACKCROSS_SWARM	GRDU
153120	NORTH	-37.77949443	176.4763972	NZGR.BACKCROSS_SWARM	GRDU
168753	NORTH	-37.94928117	175.2417557	F2-GRDU	GRDU
169325	NORTH	-36.46387982	174.2580166	F2-GRDU	GRDU
169326	NORTH	-36.46387982	174.2580166	NZGR.BACKCROSS_SWARM	GRDU
174091	NORTH	-38.5969414	177.8908628	NZGR.BACKCROSS_SWARM	GRDU
168773	NORTH	-37.94928117	175.2417557	NZGR.BACKCROSS_SWARM	GRDU
168472	NORTH	-37.44952191	175.0420902	NZGR.BACKCROSS_SWARM	GRDU
168393	NORTH	-37.44952191	175.0420902	NZGR.BACKCROSS_SWARM	GRDU
153500	NORTH	-37.766172	176.474736	NZGR.BACKCROSS_SWARM	GRDU
174146	NORTH	-38.68720303	177.8949216	NZGR.BACKCROSS_SWARM	GRDU
169010	NORTH	-36.46387982	174.2580166	NZGR.BACKCROSS_SWARM	GRDU
168193	NORTH	-37.44952191	175.0420902	F3-GRDU	GRDU
174066	NORTH	-38.5969414	177.8908628	F3-GRDU	GRDU
143566	NORTH	-37.2904	175.5936	F3-GRDU	GRDU

153135	NORTH	-37.77949443	176.4763972	F3-GRDU	GRDU
131465	NORTH	-37.9167	175.3	F3-GRDU	GRDU
174083	NORTH	-38.5969414	177.8908628	F3-GRDU	GRDU
174077	NORTH	-38.5969414	177.8908628	F3-GRDU	GRDU
174245	NORTH	-38.68720303	177.8949216	F3-GRDU	GRDU
174279	NORTH	-38.68720303	177.8949216	F4-GRDU	GRDU
169020	NORTH	-36.46387982	174.2580166	F4-GRDU	GRDU
168145	NORTH	-37.44952191	175.0420902	F4-GRDU	GRDU
174115	NORTH	-38.68720303	177.8949216	F4-GRDU	GRDU
168354	NORTH	-37.44952191	175.0420902	F4-GRDU	GRDU
168395	NORTH	-37.44952191	175.0420902	F4-GRDU	GRDU
168469	NORTH	-37.44952191	175.0420902	F4-GRDU	GRDU
174089	NORTH	-38.5969414	177.8908628	F4-GRDU	GRDU
174176	NORTH	-38.68720303	177.8949216	F4-GRDU	GRDU
168305	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
153101	NORTH	-37.77949443	176.4763972	PURE.GRDU	GRDU
168088	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
168373	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
174070	NORTH	-38.5969414	177.8908628	PURE.GRDU	GRDU
152822	NORTH	-37.766172	176.474736	PURE.GRDU	GRDU
168355	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
174199	NORTH	-38.68720303	177.8949216	PURE.GRDU	GRDU
168451	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
169228	NORTH	-36.46387982	174.2580166	PURE.GRDU	GRDU
168421	NORTH	-37.44952191	175.0420902	PURE.GRDU	GRDU
152833	NORTH	-37.766172	176.474736	PURE.GRDU	GRDU
168724	NORTH	-37.94928117	175.2417557	PURE.GRDU	GRDU
169029	NORTH	-36.46387982	174.2580166	PURE.GRDU	GRDU
169191	NORTH	-36.46387982	174.2580166	PURE.GRDU	GRDU
IEE2510	SOUTH	-44.71661	171.16173	PURE.MALL	MALL

PL8063	SOUTH	-41.535928	174.083014	PURE.MALL	MALL
PL8155	SOUTH	-43.374583	171.90195	PURE.MALL	MALL
PL8224	SOUTH	-42.744369	173.259417	PURE.MALL	MALL
PL8273	SOUTH	-42.330833	171.723983	PURE.MALL	MALL
PL8302	SOUTH	-43.8208	171.6985	PURE.MALL	MALL
IEE2511	SOUTH	-44.71661	171.16173	PURE.MALL	MALL
131686	SOUTH	-46.2	168.3219	PURE.MALL	MALL
143706	SOUTH	-46.2	168.3219	PURE.MALL	MALL
PL8118	SOUTH	-44.096992	171.4691	PURE.MALL	MALL
PL8068	SOUTH	-41.535928	174.083014	PURE.MALL	MALL
PL8102	SOUTH	-44.096992	171.4691	PURE.MALL	MALL
143710	SOUTH	-46.2	168.3219	PURE.MALL	MALL
PL8189	SOUTH	-42.87442	171.03266	PURE.MALL	MALL
PL8309	SOUTH	-44.708736	170.993224	PURE.MALL	MALL
27-143743	SOUTH	-46.2	168.3219	PURE.MALL	MALL
PL8078	SOUTH	-46.4115	169.238	PURE.MALL	MALL
PL8004	SOUTH	-40.692322	172.6324	PURE.MALL	MALL
PL8069	SOUTH	-41.535928	174.083014	PURE.MALL	MALL
100036	SOUTH	-46.2184625	168.3284704	PURE.MALL	MALL
PL8225	SOUTH	-42.744369	173.259417	PURE.MALL	MALL
PL8037	SOUTH	-41.411411	173.016067	PURE.MALL	MALL
PL8292	SOUTH	-46.175206	169.137126	PURE.MALL	MALL
100008	SOUTH	-46.2184625	168.3284704	PURE.MALL	MALL
100009	SOUTH	-46.2184625	168.3284704	PURE.MALL	MALL
PL8293	SOUTH	-46.175206	169.137126	PURE.MALL	MALL
PL8109	SOUTH	-44.096992	171.4691	PURE.MALL	MALL
141564	SOUTH	-46.2	168.3219	PURE.MALL	MALL
PL8052	SOUTH	-41.535928	174.083014	PURE.MALL	MALL
27-143751	SOUTH	-46.2	168.3219	PURE.MALL	MALL
PL8074	SOUTH	-46.4115	169.238	PURE.MALL	MALL
PL8254	SOUTH	-43.100183	170.622067	PURE.MALL	MALL
PL8014	SOUTH	-41.69708	173.286005	PURE.MALL	MALL
PL8029	SOUTH	-41.795741	172.330897	MALL.BACKCROSS_SWARM	MALL
PL8310	SOUTH	-44.708736	170.993224	MALL.BACKCROSS_SWARM	MALL
PL8290	SOUTH	-46.175206	169.137126	MALL.BACKCROSS_SWARM	MALL
27-143730	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8064	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8327	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8328	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8101	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
131704	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8161	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8159	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL

PL8188	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
131731	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8248	SOUTH	-42.587778	171.15	MALL.BACKCROSS_SWARM	MALL
131707	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
143723	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8062	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
100005	SOUTH	-46.2184625	168.3284704	MALL.BACKCROSS_SWARM	MALL
131748	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8306	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
131714	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8065	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
100032	SOUTH	-46.2184625	168.3284704	MALL.BACKCROSS_SWARM	MALL
131716	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8083	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
27-143732	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
131740	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8084	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
143712	SOUTH	-46.2	168.3219	F4-MALL	MALL
PL8331	SOUTH	-44.82711	171.12996	F4-MALL	MALL
PL8307	SOUTH	-43.8208	171.6985	F4-MALL	MALL
PL8211	SOUTH	-42.87442	171.03266	F4-MALL	MALL
100016	SOUTH	-46.2184625	168.3284704	F4-MALL	MALL
131728	SOUTH	-46.2	168.3219	F4-MALL	MALL
PL8334	SOUTH	-45.899475	168.407189	F4-MALL	MALL
PL8335	SOUTH	-45.899475	168.407189	F4-MALL	MALL
131725	SOUTH	-46.2	168.3219	F4-MALL	MALL
PL8167	SOUTH	-43.374583	171.90195	F4-MALL	MALL
PL8201	SOUTH	-42.87442	171.03266	F4-MALL	MALL
PL8319	SOUTH	-44.71661	171.16173	F4-MALL	MALL
143722	SOUTH	-46.2	168.3219	F4-MALL	MALL
PL8160	SOUTH	-43.374583	171.90195	F4-MALL	MALL
PL8336	SOUTH	-45.899475	168.407189	F4-MALL	MALL
PL8075	SOUTH	-46.4115	169.238	F4-MALL	MALL
PL8157	SOUTH	-43.374583	171.90195	F4-MALL	MALL
PL8206	SOUTH	-43.37029	171.93236	F4-MALL	MALL
143708	SOUTH	-46.2	168.3219	F4-MALL	MALL
100033	SOUTH	-46.2184625	168.3284704	F4-MALL	MALL
PL8220	SOUTH	-42.744369	173.259417	F4-MALL	MALL
100004	SOUTH	-46.2184625	168.3284704	F4-MALL	MALL
PL8173	SOUTH	-42.840819	171.02014	F4-MALL	MALL
PL8202	SOUTH	-42.87442	171.03266	F4-MALL	MALL
PL8126	SOUTH	-44.297369	170.118042	F4-MALL	MALL
PL8071	SOUTH	-41.535928	174.083014	F4-MALL	MALL

PL8152	SOUTH	-43.37029	171.93236	F4-MALL	MALL
PL8200	SOUTH	-42.87442	171.03266	F4-MALL	MALL
PL8295	SOUTH	-46.175206	169.137126	F4-MALL	MALL
PL8015	SOUTH	-41.69708	173.286005	F4-MALL	MALL
PL8216	SOUTH	-46.250919	168.412931	F4-MALL	MALL
PL8330	SOUTH	-44.82711	171.12996	F4-MALL	MALL
131712	SOUTH	-46.2	168.3219	F4-MALL	MALL
PL8184	SOUTH	-43.38837	171.95016	MALL.BACKCROSS_SWARM	MALL
PL8209	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8300	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
PL8207	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8106	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
100018	SOUTH	-46.2184625	168.3284704	MALL.BACKCROSS_SWARM	MALL
27-143740	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
131701	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8239	SOUTH	-43.093167	170.391167	MALL.BACKCROSS_SWARM	MALL
27-143727	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8077	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
PL8162	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8030	SOUTH	-41.795741	172.330897	MALL.BACKCROSS_SWARM	MALL
131742	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8113	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8120	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
27-143735	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8288	SOUTH	-44.849682	171.145844	MALL.BACKCROSS_SWARM	MALL
PL8174	SOUTH	-42.840819	171.02014	MALL.BACKCROSS_SWARM	MALL
PL8103	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8108	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8117	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8215	SOUTH	-46.250919	168.412931	MALL.BACKCROSS_SWARM	MALL
PL8190	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
131733	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8151	SOUTH	-43.37029	171.93236	MALL.BACKCROSS_SWARM	MALL
PL8210	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8034	SOUTH	-41.411411	173.016067	MALL.BACKCROSS_SWARM	MALL
143720	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8104	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
27-143748	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8115	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8192	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8067	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8214	SOUTH	-46.250919	168.412931	MALL.BACKCROSS_SWARM	MALL
PL8289	SOUTH	-44.849682	171.145844	MALL.BACKCROSS_SWARM	MALL

PL8080	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
PL8169	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8107	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8318	SOUTH	-44.71661	171.16173	MALL.BACKCROSS_SWARM	MALL
PL8291	SOUTH	-46.175206	169.137126	MALL.BACKCROSS_SWARM	MALL
PL8213	SOUTH	-46.250919	168.412931	MALL.BACKCROSS_SWARM	MALL
PL8272	SOUTH	-42.330833	171.723983	MALL.BACKCROSS_SWARM	MALL
PL8247	SOUTH	-42.587778	171.15	MALL.BACKCROSS_SWARM	MALL
PL8114	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
131736	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
131658	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8038	SOUTH	-41.411411	173.016067	MALL.BACKCROSS_SWARM	MALL
PL8105	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8205	SOUTH	-43.37029	171.93236	MALL.BACKCROSS_SWARM	MALL
PL8180	SOUTH	-43.38837	171.95016	MALL.BACKCROSS_SWARM	MALL
PL8076	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
PL8196	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
IEE2518	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8112	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8204	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8066	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8183	SOUTH	-43.38837	171.95016	MALL.BACKCROSS_SWARM	MALL
PL8168	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8329	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8172	SOUTH	-42.840819	171.02014	MALL.BACKCROSS_SWARM	MALL
PL8325	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8079	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
PL8072	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
IEE2509	SOUTH	-44.71661	171.16173	MALL.BACKCROSS_SWARM	MALL
PL8156	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
100002	SOUTH	-46.2184625	168.3284704	MALL.BACKCROSS_SWARM	MALL
PL8324	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8194	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8271	SOUTH	-42.330833	171.723983	MALL.BACKCROSS_SWARM	MALL
PL8110	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8164	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
143703	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8127	SOUTH	-44.297369	170.118042	MALL.BACKCROSS_SWARM	MALL
PL8061	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8170	SOUTH	-43.38837	171.95016	MALL.BACKCROSS_SWARM	MALL
IEE2519	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8163	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8060	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL

PL8208	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
131750	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8056	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8041	SOUTH	-41.398284	173.108145	MALL.BACKCROSS_SWARM	MALL
PL8199	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
131711	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8305	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
PL8028	SOUTH	-41.795741	172.330897	F3-MALL	MALL
PL8036	SOUTH	-41.411411	173.016067	F3-MALL	MALL
PL8296	SOUTH	-46.175206	169.137126	F3-MALL	MALL
131720	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8191	SOUTH	-42.87442	171.03266	F3-MALL	MALL
27-143739	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8186	SOUTH	-43.374583	171.90195	F3-MALL	MALL
PL8332	SOUTH	-45.899475	168.407189	F3-MALL	MALL
143719	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8181	SOUTH	-43.38837	171.95016	F3-MALL	MALL
PL8253	SOUTH	-43.100183	170.622067	F3-MALL	MALL
PL8294	SOUTH	-46.175206	169.137126	F3-MALL	MALL
131732	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8153	SOUTH	-43.37029	171.93236	F3-MALL	MALL
PL8081	SOUTH	-46.4115	169.238	F3-MALL	MALL
131739	SOUTH	-46.2	168.3219	F3-MALL	MALL
27-143753	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8337	SOUTH	-45.899475	168.407189	F3-MALL	MALL
PL8128	SOUTH	-44.096992	171.4691	F3-MALL	MALL
PL8058	SOUTH	-41.535928	174.083014	F3-MALL	MALL
PL8059	SOUTH	-41.535928	174.083014	F3-MALL	MALL
PL8203	SOUTH	-42.87442	171.03266	F3-MALL	MALL
IEE2500	SOUTH	-43.37029	171.93236	F3-MALL	MALL
PL8116	SOUTH	-44.096992	171.4691	F3-MALL	MALL
PL8070	SOUTH	-41.535928	174.083014	F3-MALL	MALL
PL8007	SOUTH	-40.692322	172.6324	F3-MALL	MALL
PL8187	SOUTH	-42.87442	171.03266	F3-MALL	MALL
143717	SOUTH	-46.2	168.3219	F3-MALL	MALL
PL8082	SOUTH	-46.4115	169.238	F3-MALL	MALL
IEE2501	SOUTH	-43.38837	171.95016	MALL.BACKCROSS_SWARM	MALL
131717	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8175	SOUTH	-42.840819	171.02014	MALL.BACKCROSS_SWARM	MALL
IEE2512	SOUTH	-44.71661	171.16173	MALL.BACKCROSS_SWARM	MALL
PL8073	SOUTH	-46.4115	169.238	MALL.BACKCROSS_SWARM	MALL
27-143736	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8171	SOUTH	-42.840819	171.02014	MALL.BACKCROSS_SWARM	MALL

PL8249	SOUTH	-43.054917	170.72005	MALL.BACKCROSS_SWARM	MALL
27-143726	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
IEE2521	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8301	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
PL8027	SOUTH	-41.795741	172.330897	MALL.BACKCROSS_SWARM	MALL
PL8297	SOUTH	-46.175206	169.137126	MALL.BACKCROSS_SWARM	MALL
PL8111	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
IEE2522	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8198	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8035	SOUTH	-41.411411	173.016067	MALL.BACKCROSS_SWARM	MALL
143713	SOUTH	-46.2	168.3219	MALL.BACKCROSS_SWARM	MALL
PL8019	SOUTH	-41.69708	173.286005	MALL.BACKCROSS_SWARM	MALL
PL8195	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8158	SOUTH	-43.374583	171.90195	MALL.BACKCROSS_SWARM	MALL
PL8320	SOUTH	-44.71661	171.16173	MALL.BACKCROSS_SWARM	MALL
PL8333	SOUTH	-45.899475	168.407189	MALL.BACKCROSS_SWARM	MALL
PL8042	SOUTH	-41.398284	173.108145	MALL.BACKCROSS_SWARM	MALL
PL8234	SOUTH	-42.903	171.091833	MALL.BACKCROSS_SWARM	MALL
IEE2520	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8154	SOUTH	-42.840819	171.02014	MALL.BACKCROSS_SWARM	MALL
PL8053	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8303	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
PL8006	SOUTH	-40.692322	172.6324	MALL.BACKCROSS_SWARM	MALL
PL8304	SOUTH	-43.8208	171.6985	MALL.BACKCROSS_SWARM	MALL
PL8055	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8228	SOUTH	-42.796218	170.918425	F2-MALL	MALL
PL8010	SOUTH	-40.692322	172.6324	F2-MALL	MALL
PL8212	SOUTH	-42.87442	171.03266	MALL.BACKCROSS_SWARM	MALL
PL8236	SOUTH	-42.903	171.091833	MALL.BACKCROSS_SWARM	MALL
PL8119	SOUTH	-44.096992	171.4691	MALL.BACKCROSS_SWARM	MALL
PL8031	SOUTH	-41.795741	172.330897	MALL.BACKCROSS_SWARM	MALL
PL8326	SOUTH	-44.82711	171.12996	MALL.BACKCROSS_SWARM	MALL
PL8231	SOUTH	-42.796218	170.918425	MALL.BACKCROSS_SWARM	MALL
PL8218	SOUTH	-42.744369	173.259417	MALL.BACKCROSS_SWARM	MALL
PL8125	SOUTH	-44.297369	170.118042	MALL.BACKCROSS_SWARM	MALL
PL8051	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8240	SOUTH	-43.093167	170.391167	MALL.BACKCROSS_SWARM	MALL
PL8256	SOUTH	-41.985667	171.8875	MALL.BACKCROSS_SWARM	MALL
PL8039	SOUTH	-41.411411	173.016067	MALL.BACKCROSS_SWARM	MALL
PL8054	SOUTH	-41.535928	174.083014	MALL.BACKCROSS_SWARM	MALL
PL8121	SOUTH	-44.297369	170.118042	MALL.BACKCROSS_SWARM	MALL
PL8221	SOUTH	-42.744369	173.259417	MALL.BACKCROSS_SWARM	MALL
PL8219	SOUTH	-42.744369	173.259417	MALL.BACKCROSS_SWARM	MALL

PL8023	SOUTH	-41.795741	172.330897	F1	GRDU
PL8021	SOUTH	-41.795741	172.330897	F1	GRDU
PL8026	SOUTH	-41.795741	172.330897	F1	GRDU
PL8250	SOUTH	-43.054917	170.72005	NZGR.BACKCROSS_SWARM	GRDU
PL8182	SOUTH	-43.38837	171.95016	NZGR.BACKCROSS_SWARM	GRDU
PL8123	SOUTH	-44.297369	170.118042	NZGR.BACKCROSS_SWARM	GRDU
PL8263	SOUTH	-42.348217	171.741567	NZGR.BACKCROSS_SWARM	GRDU
PL8282	SOUTH	-42.0839	171.867944	NZGR.BACKCROSS_SWARM	GRDU
PL8281	SOUTH	-42.0839	171.867944	NZGR.BACKCROSS_SWARM	GRDU
PL8229	SOUTH	-42.796218	170.918425	NZGR.BACKCROSS_SWARM	GRDU
PL8057	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8237	SOUTH	-42.883692	171.033819	NZGR.BACKCROSS_SWARM	GRDU
PL8223	SOUTH	-42.744369	173.259417	NZGR.BACKCROSS_SWARM	GRDU
PL8222	SOUTH	-42.744369	173.259417	NZGR.BACKCROSS_SWARM	GRDU
PL8047	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8005	SOUTH	-40.692322	172.6324	NZGR.BACKCROSS_SWARM	GRDU
PL8040	SOUTH	-41.2605	173.01816	NZGR.BACKCROSS_SWARM	GRDU
PL8022	SOUTH	-41.795741	172.330897	NZGR.BACKCROSS_SWARM	GRDU
PL8286	SOUTH	-44.82711	171.12996	NZGR.BACKCROSS_SWARM	GRDU
PL8280	SOUTH	-42.0839	171.867944	NZGR.BACKCROSS_SWARM	GRDU
PL8235	SOUTH	-42.903	171.091833	NZGR.BACKCROSS_SWARM	GRDU
PL8049	SOUTH	-41.535928	174.083014	F2-GRDU	GRDU
PL8226	SOUTH	-42.744369	173.259417	F2-GRDU	GRDU
PL8032	SOUTH	-41.411411	173.016067	F2-GRDU	GRDU
PL8227	SOUTH	-42.744369	173.259417	F2-GRDU	GRDU
PL8122	SOUTH	-44.297369	170.118042	NZGR.BACKCROSS_SWARM	GRDU
PL8043	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8046	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8044	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8018	SOUTH	-41.69708	173.286005	NZGR.BACKCROSS_SWARM	GRDU
PL8000	SOUTH	-42.35535	171.745317	NZGR.BACKCROSS_SWARM	GRDU
PL8025	SOUTH	-41.795741	172.330897	NZGR.BACKCROSS_SWARM	GRDU
PL8017	SOUTH	-41.69708	173.286005	NZGR.BACKCROSS_SWARM	GRDU
PL8050	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8045	SOUTH	-41.535928	174.083014	NZGR.BACKCROSS_SWARM	GRDU
PL8003	SOUTH	-40.732319	172.6815	NZGR.BACKCROSS_SWARM	GRDU
PL8013	SOUTH	-41.775921	171.645665	NZGR.BACKCROSS_SWARM	GRDU
PL8260	SOUTH	-42.434292	171.359785	NZGR.BACKCROSS_SWARM	GRDU
PL8012	SOUTH	-41.775921	171.645665	NZGR.BACKCROSS_SWARM	GRDU
PL8176	SOUTH	-42.840819	171.02014	NZGR.BACKCROSS_SWARM	GRDU
PL8268	SOUTH	-42.313733	171.60625	F3-GRDU	GRDU
PL8274	SOUTH	-42.330833	171.723983	F3-GRDU	GRDU
PL8245	SOUTH	-42.587778	171.15	F3-GRDU	GRDU

PL8009	SOUTH	-40.692322	172.6324	F3-GRDU	GRDU
PL8016	SOUTH	-41.69708	173.286005	F3-GRDU	GRDU
PL8178	SOUTH	-42.840819	171.02014	F3-GRDU	GRDU
PL8048	SOUTH	-41.535928	174.083014	F4-GRDU	GRDU
PL8279	SOUTH	-42.330833	171.723983	F4-GRDU	GRDU
PL8246	SOUTH	-42.587778	171.15	F4-GRDU	GRDU
PL8242	SOUTH	-43.093167	170.391167	F4-GRDU	GRDU
PL8278	SOUTH	-42.330833	171.723983	F4-GRDU	GRDU
PL8001	SOUTH	-42.35535	171.745317	F4-GRDU	GRDU
PL8287	SOUTH	-44.82711	171.12996	F4-GRDU	GRDU
PL8284	SOUTH	-42.158747	171.913264	F4-GRDU	GRDU
PL8252	SOUTH	-43.100183	170.622067	GRDU	GRDU
PL8241	SOUTH	-43.093167	170.391167	GRDU	GRDU
PL8262	SOUTH	-42.348217	171.741567	GRDU	GRDU
PL8233	SOUTH	-42.903	171.091833	GRDU	GRDU
PL8277	SOUTH	-42.330833	171.723983	GRDU	GRDU
PL8033	SOUTH	-41.48083	172.839882	GRDU	GRDU
PL8261	SOUTH	-42.434292	171.359785	GRDU	GRDU
PL8020	SOUTH	-41.795741	172.330897	GRDU	GRDU
PL8269	SOUTH	-42.310533	171.706517	GRDU	GRDU
PL8275	SOUTH	-42.330833	171.723983	GRDU	GRDU
PL8002	SOUTH	-42.3125	171.602333	GRDU	GRDU
PL8257	SOUTH	-41.985667	171.8875	GRDU	GRDU
PL8265	SOUTH	-42.35535	171.745317	GRDU	GRDU
PL8238	SOUTH	-42.883692	171.033819	GRDU	GRDU
PL8255	SOUTH	-41.985667	171.8875	GRDU	GRDU
PL8024	SOUTH	-41.795741	172.330897	GRDU	GRDU
PL8276	SOUTH	-42.330833	171.723983	GRDU	GRDU
PL8266	SOUTH	-42.35535	171.745317	GRDU	GRDU
PL8011	SOUTH	-40.732319	172.6815	GRDU	GRDU
PL8177	SOUTH	-42.840819	171.02014	GRDU	GRDU
PL8179	SOUTH	-42.840819	171.02014	GRDU	GRDU
PL8230	SOUTH	-42.796218	170.918425	GRDU	GRDU
PL8232	SOUTH	-42.903	171.091833	GRDU	GRDU
PL8243	SOUTH	-43.914817	168.94825	GRDU	GRDU
PL8244	SOUTH	-43.914817	168.94825	GRDU	GRDU
PL8251	SOUTH	-43.100183	170.622067	GRDU	GRDU
PL8258	SOUTH	-41.985667	171.8875	GRDU	GRDU
PL8259	SOUTH	-42.434292	171.359785	GRDU	GRDU
PL8264	SOUTH	-42.3517	171.775567	GRDU	GRDU
PL8267	SOUTH	-42.35535	171.745317	GRDU	GRDU
PL8283	SOUTH	-42.158747	171.913264	GRDU	GRDU
PL8285	SOUTH	-42.158747	171.913264	GRDU	GRDU

Table S3.2. Assignment probabilities from ADMIXTURE based simulation for hybrid backcrosses into both parental species (F1 – F10).

	K of 2			K of 3		
	Average	Minimum	Maximum	Average	Minimum	Maximum
NZGD x NZMA - F1	0.51	0.49	0.53	0.49	0.47	0.52
F2-backcrossed-NZGD	0.78	0.77	0.78	0.76	0.75	0.77
F2-backcrossed-NZMA	0.26	0.25	0.26	0.22	0.21	0.23
F3-backcrossed-NZGD	0.91	0.91	0.92	0.89	0.88	0.90
F3-backcrossed-NZMA	0.13	0.12	0.13	0.08	0.07	0.09
F4-backcrossed-NZGD	0.99	0.98	0.99	0.97	0.96	0.97
F4-backcrossed-NZMA	0.06	0.05	0.06	0.02	0.01	0.03
F5-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.98	0.99
F5-backcrossed-NZMA	0.02	0.02	0.02	0.00	0.00	0.00
F6-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.98	0.99
F6-backcrossed-NZMA	0.01	0.01	0.01	0.00	0.00	0.00
F7-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.98	0.99
F7-backcrossed-NZMA	0.00	0.00	0.01	0.00	0.00	0.00
F8-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.98	0.99
F8-backcrossed-NZMA	0.00	0.00	0.01	0.00	0.00	0.00
F9-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.98	1.00
F9-backcrossed-NZMA	0.00	0.00	0.01	0.00	0.00	0.00
F10-backcrossed-NZGD	1.00	1.00	1.00	0.99	0.99	0.99
F10-backcrossed-NZMA	0.01	0.00	0.01	0.00	0.00	0.00

Table S3.3. List of environmental variables used for genotype-environment association testing in gradientForest.

Variable	Description	Unit
BIO1	Mean annual temperature	°C
BIO2	Mean diurnal range	°C
BIO3	Isothermality	°C
BIO4	Temperature seasonality	°C
BIO5	Max temperature of warmest month	°C
BIO6	Min temperature of coldest month	°C
BIO7	Temperature annual range	°C
BIO8	Mean temperature of wettest quarter	°C
BIO9	Mean temperature of driest quarter	°C
BIO10	Mean temperature of warmest quarter	°C
BIO11	Mean temperature of coldest quarter	°C
BIO12	Annual precipitation	mm
BIO13	Precipitation of wettest month	mm
BIO14	Precipitation of driest month	mm
BIO15	Precipitation seasonality	mm
BIO16	Precipitation of wettest quarter	mm
BIO17	Precipitation of driest quarter	mm
BIO18	Precipitation of warmest quarter	mm
BIO19	Precipitation of coldest quarter	mm
NDVI_ANNUAL	Normalized Difference Vegetation Index Annual; MOD13A3	

NDVI_SUMMER	Normalized Difference Vegetation Index June; MOD13A3	
NDVI_WINTER	Normalized Difference Vegetation Index December; MOD13A3	
EVI_ANNUAL	Enhanced Vegetation Index Annual; MOD13A3	
EVI_SUMMER	Enhanced Vegetation Index June; MOD13A3	
EVI_WINTER	Enhanced Vegetation Index December; MOD13A3	
NPP_ANNUAL	Net Primary Productivity Annual; MOD17A2H	
SRTM	Shuttle radar topography mission; Elevation	m

Table S4.1. List of environmental variables used for genotype-environment association testing in gradientForest (GF).

Variable	Description	Unit
BIO1	Mean annual temperature	°C
BIO2	Mean diurnal range	°C
BIO3	Isothermality	°C
BIO4	Temperature seasonality	°C
BIO5	Max temperature of warmest month	°C
BIO6	Min temperature of coldest month	°C
BIO7	Temperature annual range	°C
BIO8	Mean temperature of wettest quarter	°C
BIO9	Mean temperature of driest quarter	°C
BIO10	Mean temperature of warmest quarter	°C
BIO11	Mean temperature of coldest quarter	°C
BIO12	Annual precipitation	mm
BIO13	Precipitation of wettest month	mm
BIO14	Precipitation of driest month	mm
BIO15	Precipitation seasonality	mm
BIO16	Precipitation of wettest quarter	mm
BIO17	Precipitation of driest quarter	mm
BIO18	Precipitation of warmest quarter	mm
BIO19	Precipitation of coldest quarter	mm
NDVI_ANNUAL	Normalized Difference Vegetation Index Annual; MOD13A3	
NDVI_SUMMER	Normalized Difference Vegetation Index June; MOD13A3	
NDVI_WINTER	Normalized Difference Vegetation Index December; MOD13A3	
EVI_ANNUAL	Enhanced Vegetation Index Annual; MOD13A3	
EVI_SUMMER	Enhanced Vegetation Index June; MOD13A3	
EVI_WINTER	Enhanced Vegetation Index December; MOD13A3	
NPP_ANNUAL	Net Primary Productivity Annual; MOD17A2H	
SRTM	Shuttle radar topography mission; Elevation	m

SUPPLEMENTAL FIGURES

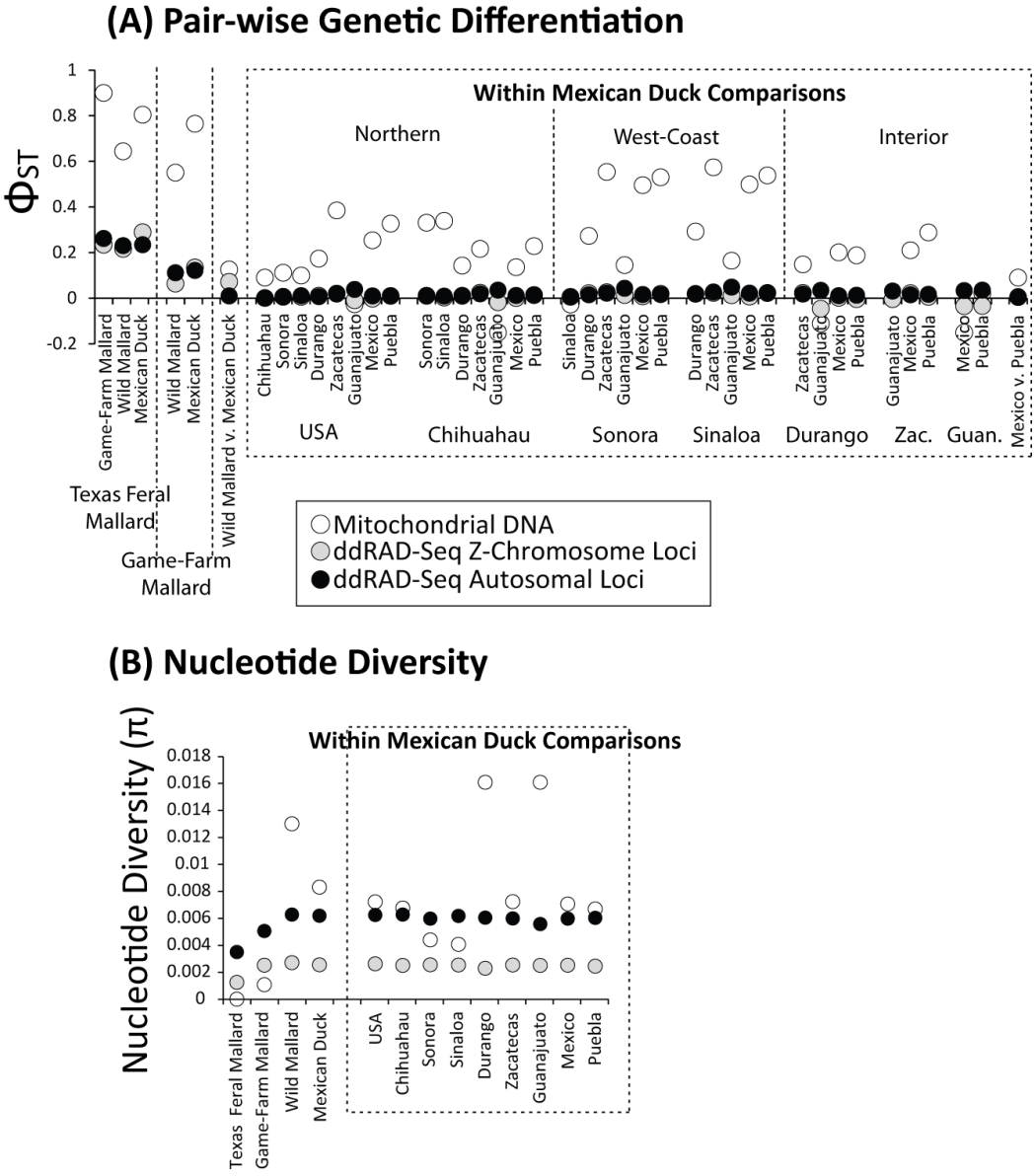


Figure S1.1. (A) Pairwise composite ΦST and (B) calculated nucleotide diversity estimated across 3,015 and 174 ddRAD-seq autosomal and Z-sex chromosome linked loci, respectively. Comparisons were done by species or domestic group, and within Mexican duck comparisons done by geographical location.

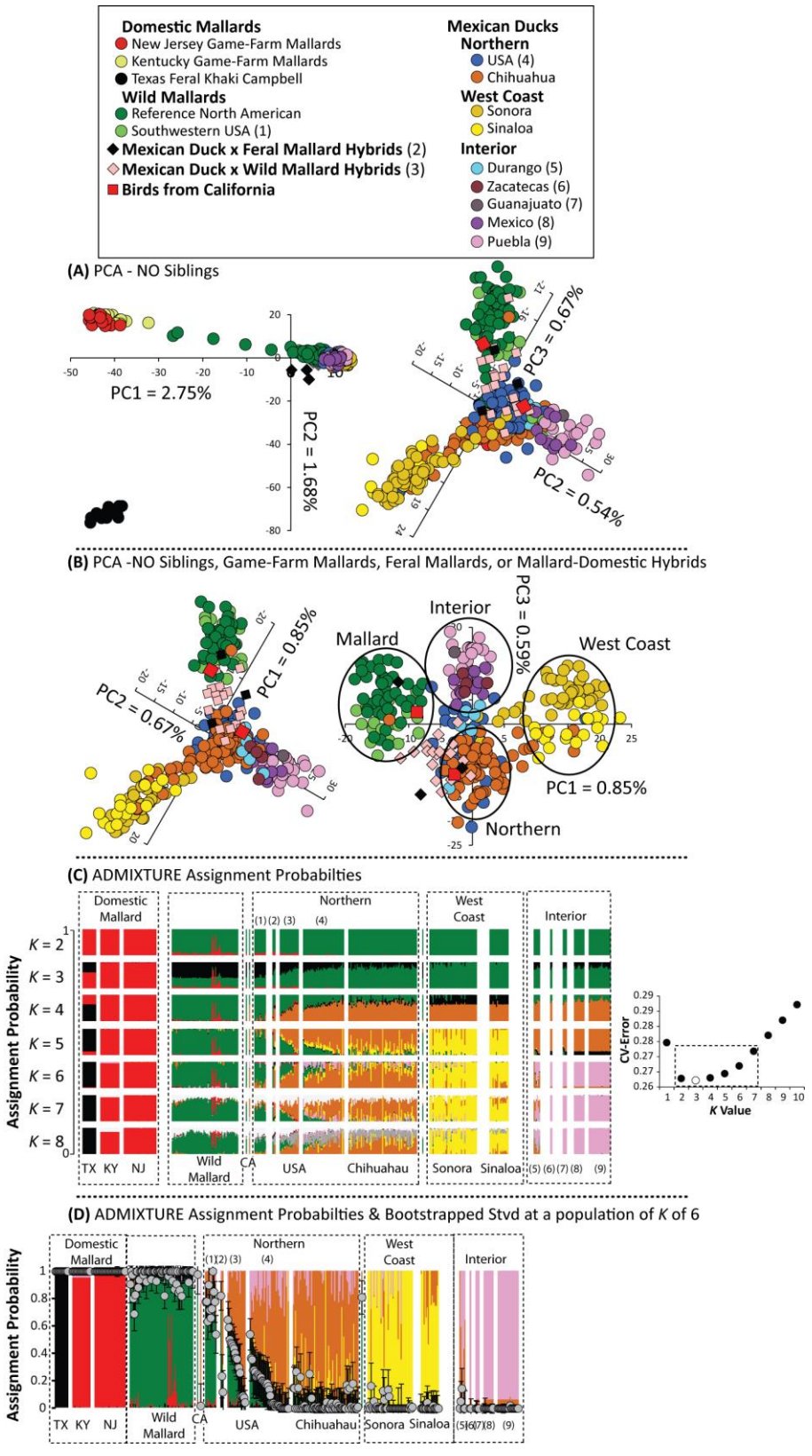

Domestic Mallards
New Jersey Game-Farm Mallards
Kentucky Game-Farm Mallards
Texas Feral Khaki Campbell
Wild Mallards
Reference North American
Southwestern USA (1)
Mexican Duck x Feral Mallard Hybrids (2)
Mexican Duck x Wild Mallard Hybrids (3)
Birds from California
Mexican Ducks
Northern
USA (4)
Chihuahua
West Coast
Sonora
Sinaloa
Interior
Durango (5)
Zacatecas (6)
Guanajuato (7)
Mexico (8)
Puebla (9)
(A) PCA - NO Siblings
PC1 = 2.75%
PC2 = 1.68%
PC3 = 0.67%
PC2 = 0.54%
(B) PCA -NO Siblings, Game-Farm Mallards, Feral Mallards, or Mallard-Domestic Hybrids
PC1 = 0.85%
PC2 = 0.67%
PC3 = 0.59%
Mallard
Interior
West Coast
Northern
PC1 = 0.85%
(C) ADMIXTURE Assignment Probabilties
Assignment Probability
K = 2
K = 3
K = 4
K = 5
K = 6
K = 7
K = 8
Domestic Mallard
Northern
West Coast
Interior
(1) (2) (3)
(4)
TX KY NJ
Wild Mallard
CA
USA
Chihuahau
Sonora
Sinaloa
(5) (6) (7) (8)
(9)
CV-Error
K Value
(D) ADMIXTURE Assignment Probabilties & Bootstrapped Stvd at a population of K of 6
Assignment Probability
Domestic Mallard
Northern
West Coast
Interior
TX KY NJ
Wild Mallard
CA
USA
Chihuahau
Sonora
Sinaloa

Figure S1.2. Population structure analyses of mallards (domestic and wild) and Mexican ducks, excluding all but one sample per identified sibling group (Supplementary Materials Figure S3), and using 12,696 independent bi-allelic ddRAD-seq autosomal SNPs. (A) PCA of all samples, identifying domestic mallards distantly clustering from wild mallards and Mexican ducks. (B) PCA excluding domestic mallards and siblings that identifies the four major clusters that Mexican ducks fall into. (C) ADMIXTURE assignment probabilities across samples for K populations of 2-8 and included respective CV-errors. (D) ADMIXTURE assignment probabilities analyzed under a K populations of 6 with overlapping bootstrapped average and standard deviation assignments to the summation of all possible mallard clusters (i.e., assignment probabilities to Feral Khaki Campbell , Game-Farm mallard, and wild mallard genetic clusters). Note that I identify mallards by origin (wild versus domestic) and Mexican ducks by geographical location in PCA, whereas assignment probabilities are colored by identified genetic clusters as estimated with ADMIXTURE for each population K value.

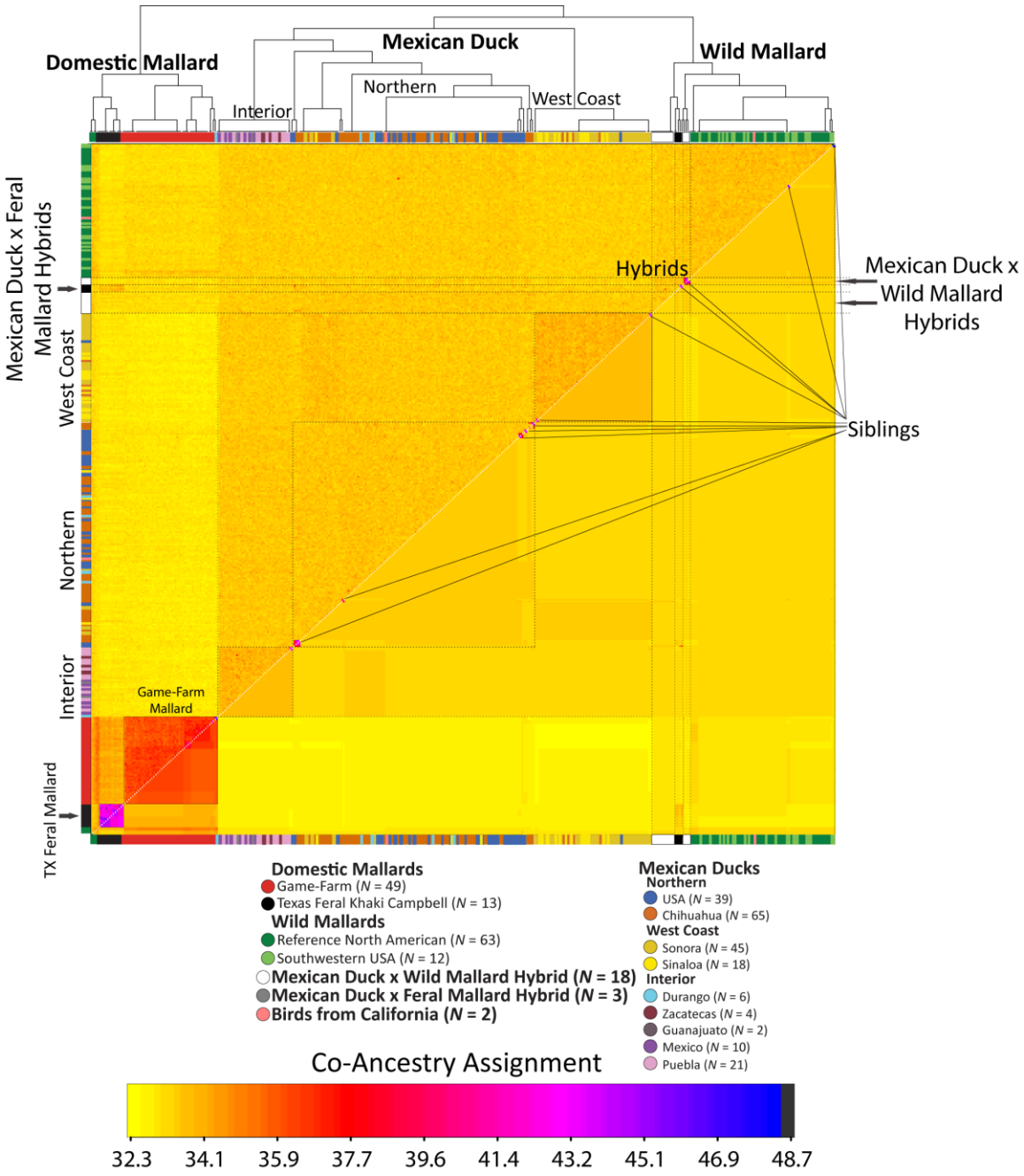


Figure S1.3. fineRADstructure individual coancestry coefficient matrix for the complete 387 sample dataset and based on 12,899 independent bi-allelic ddRAD-seq autosomal SNPs. The level of recent coancestry is color coded from low (yellow) to high (blue) is provided. I color code mallards by origin (wild versus domestic) and Mexican ducks by geographical location, as well as identify Mexican duck x (wild/feral) mallard hybrids, wild x game-farm mallard hybrids, and sibling groups.

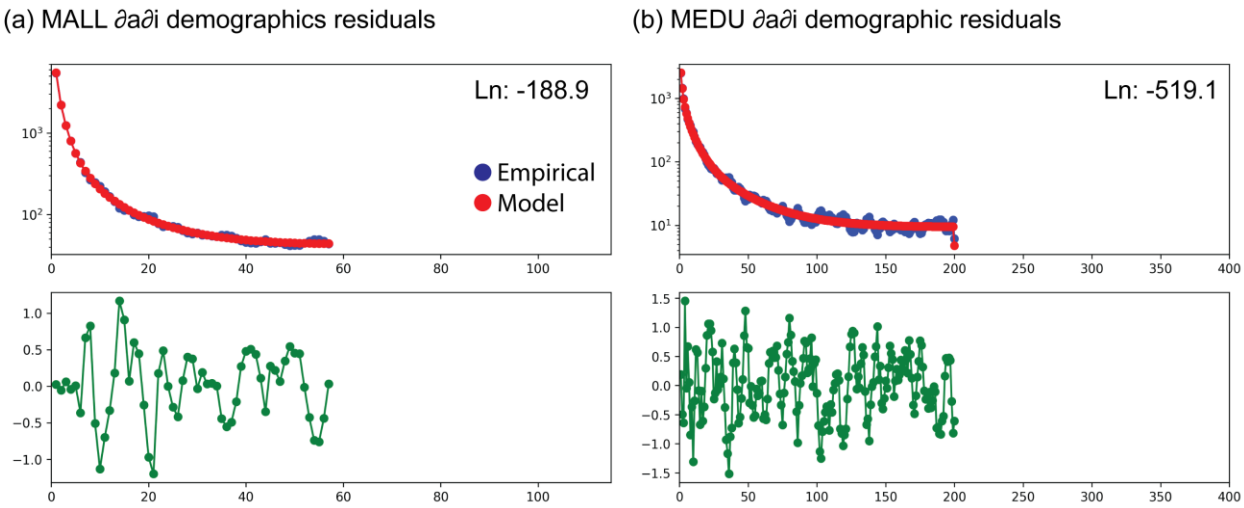


Figure S2.1. Model residuals from ∂a∂i estimated time-series demographic models.

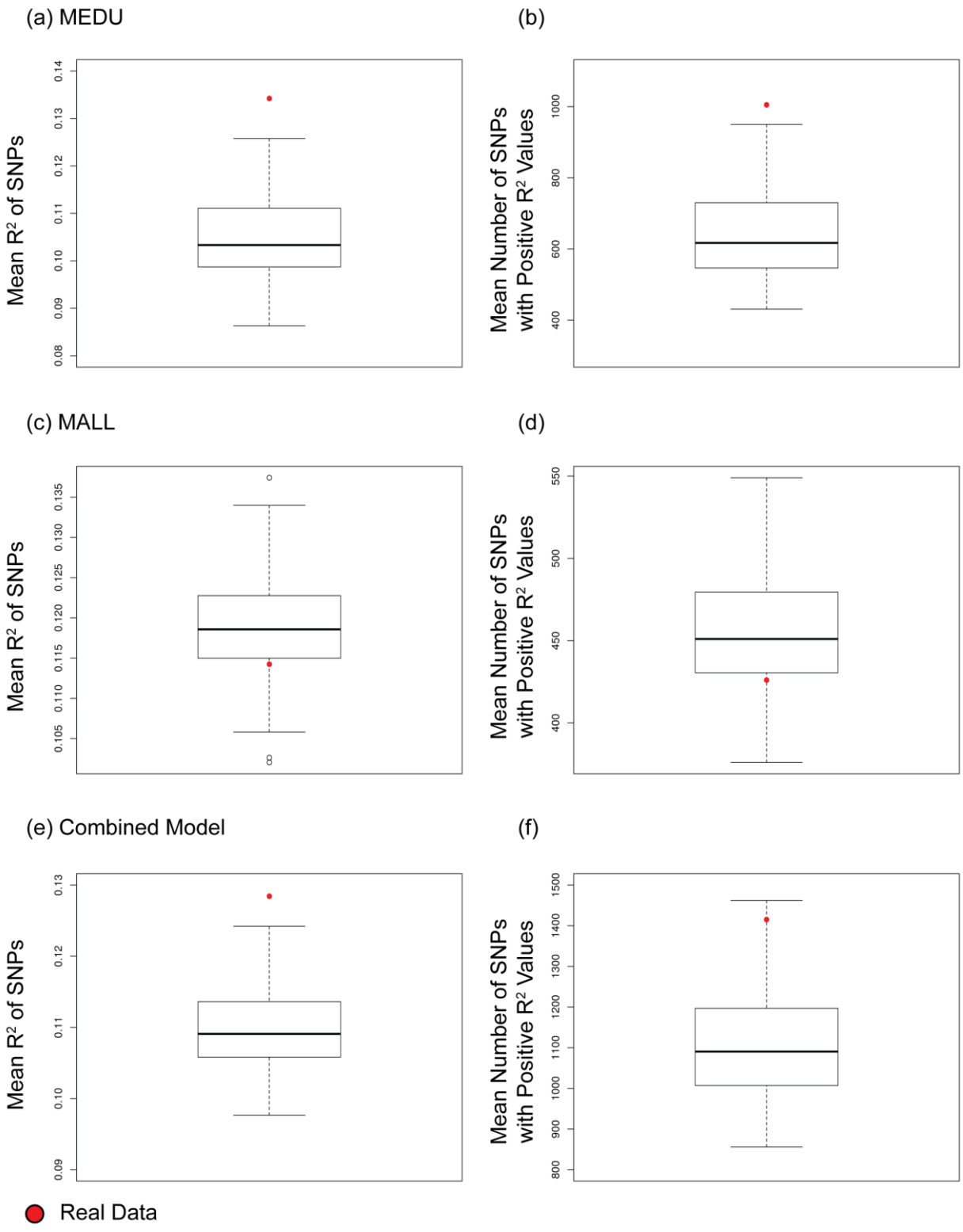


Figure S2.2. Boxplot of R^2 values from randomized datasets tested in gradientforest compared to the actual data.

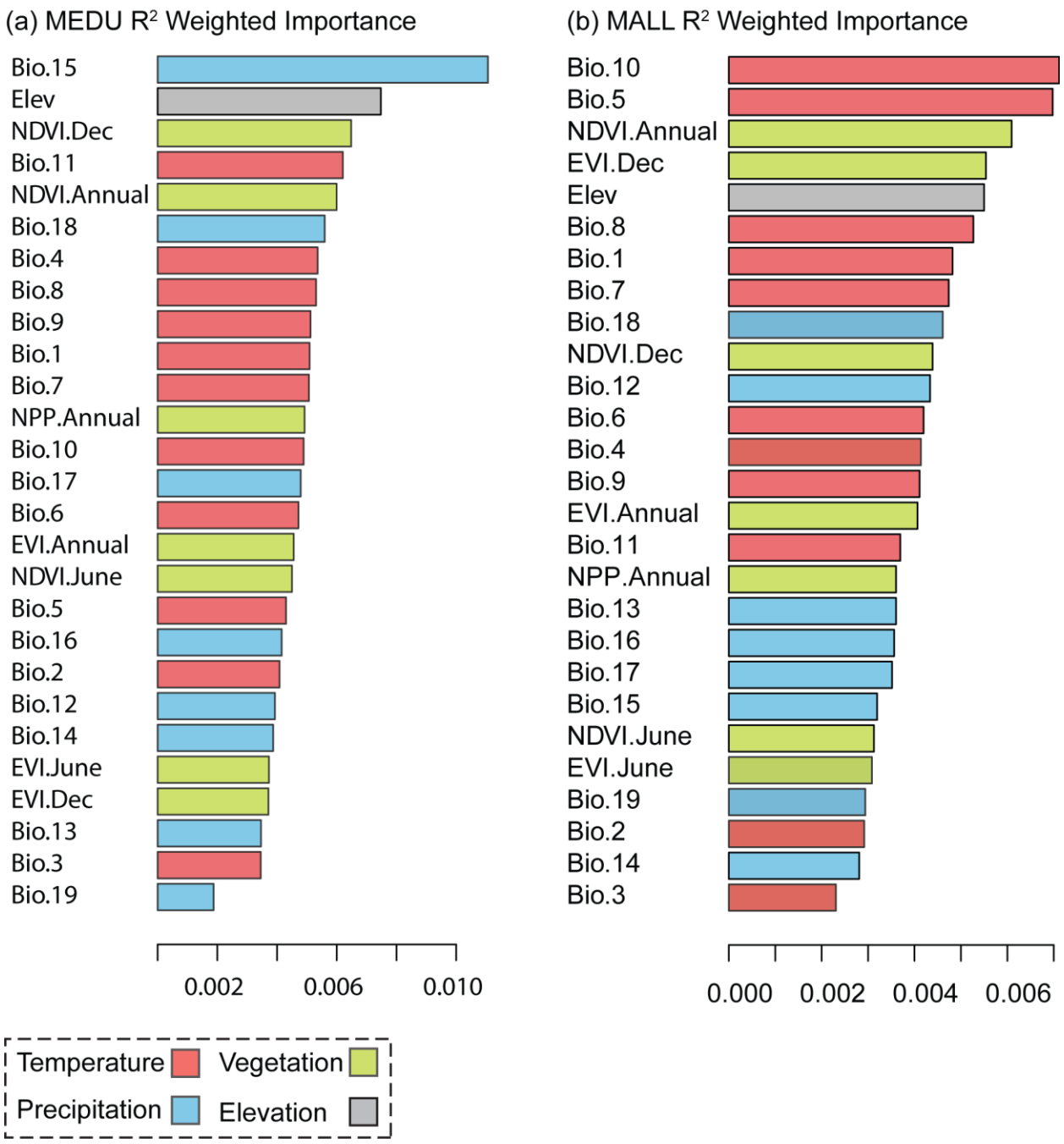


Figure S2.3. Cumulative R^2 weighted importance ranking of 27 environmental predictor variables from GradientForest.

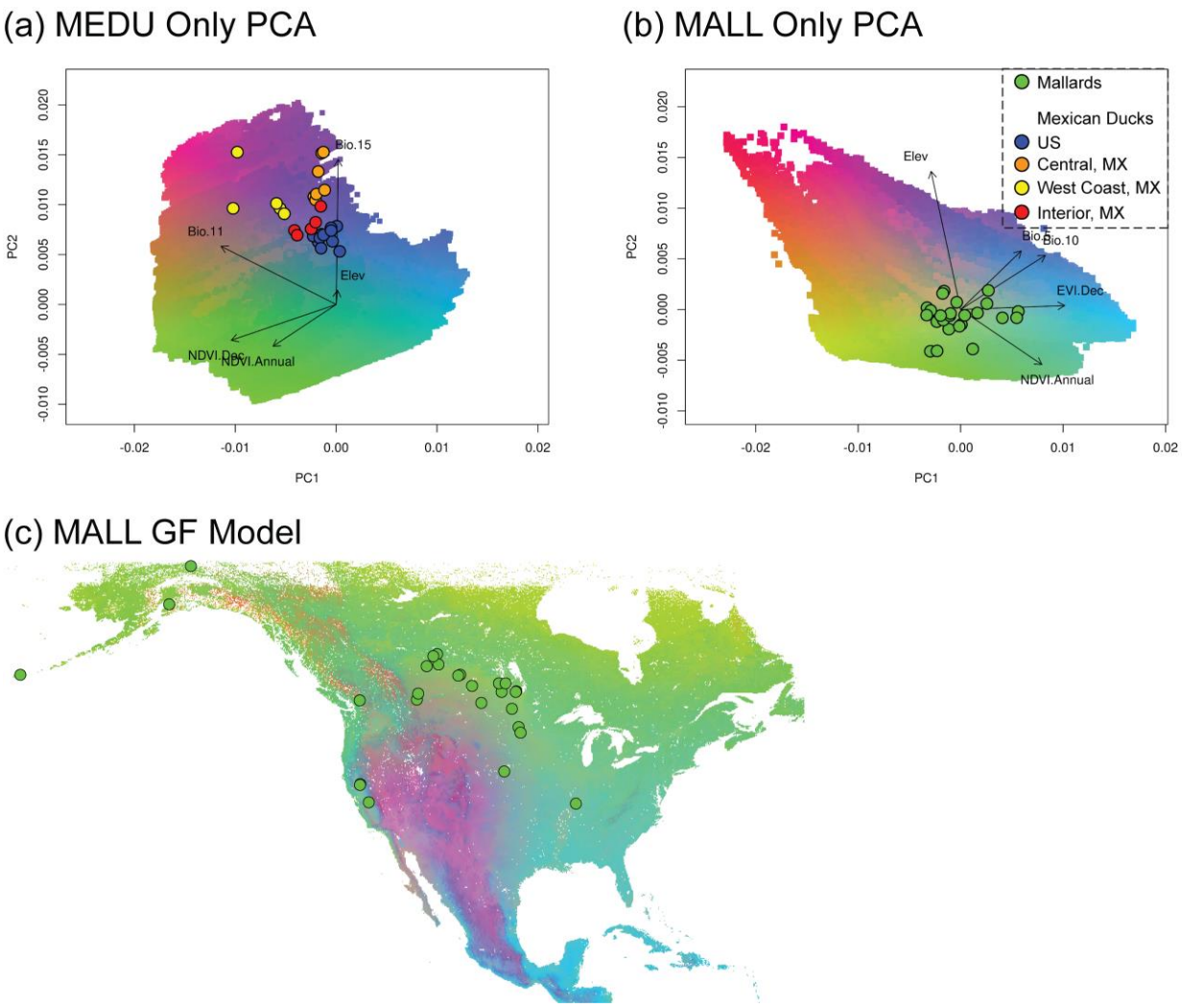


Figure S2.4. Individual Mexican duck and mallard PCA from gradientforest analyses, as well as the mallard only map projected across North America.

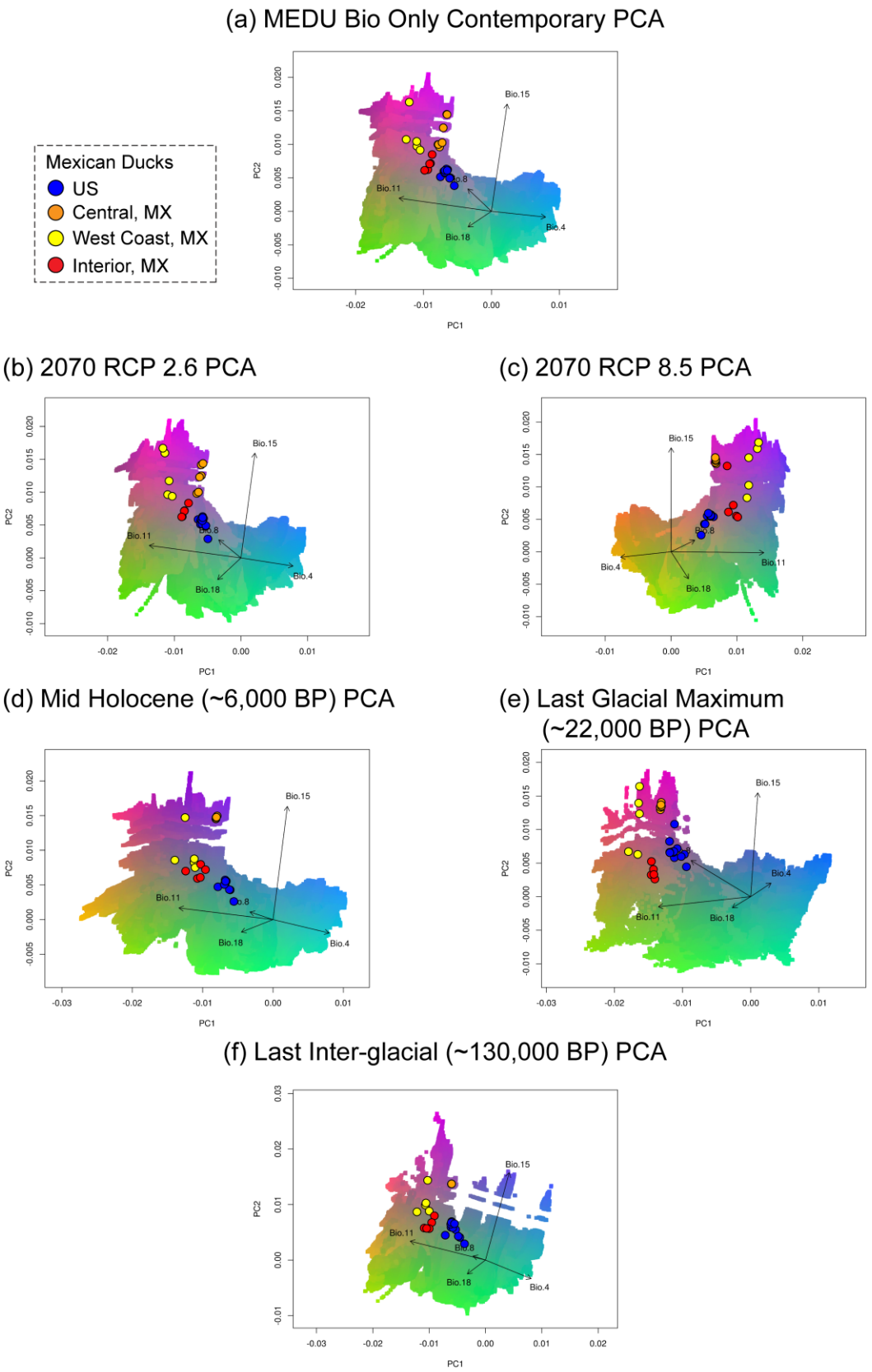


Figure S2.5. Individual PCA from gradientforest analyses across historic and future environmental conditions.

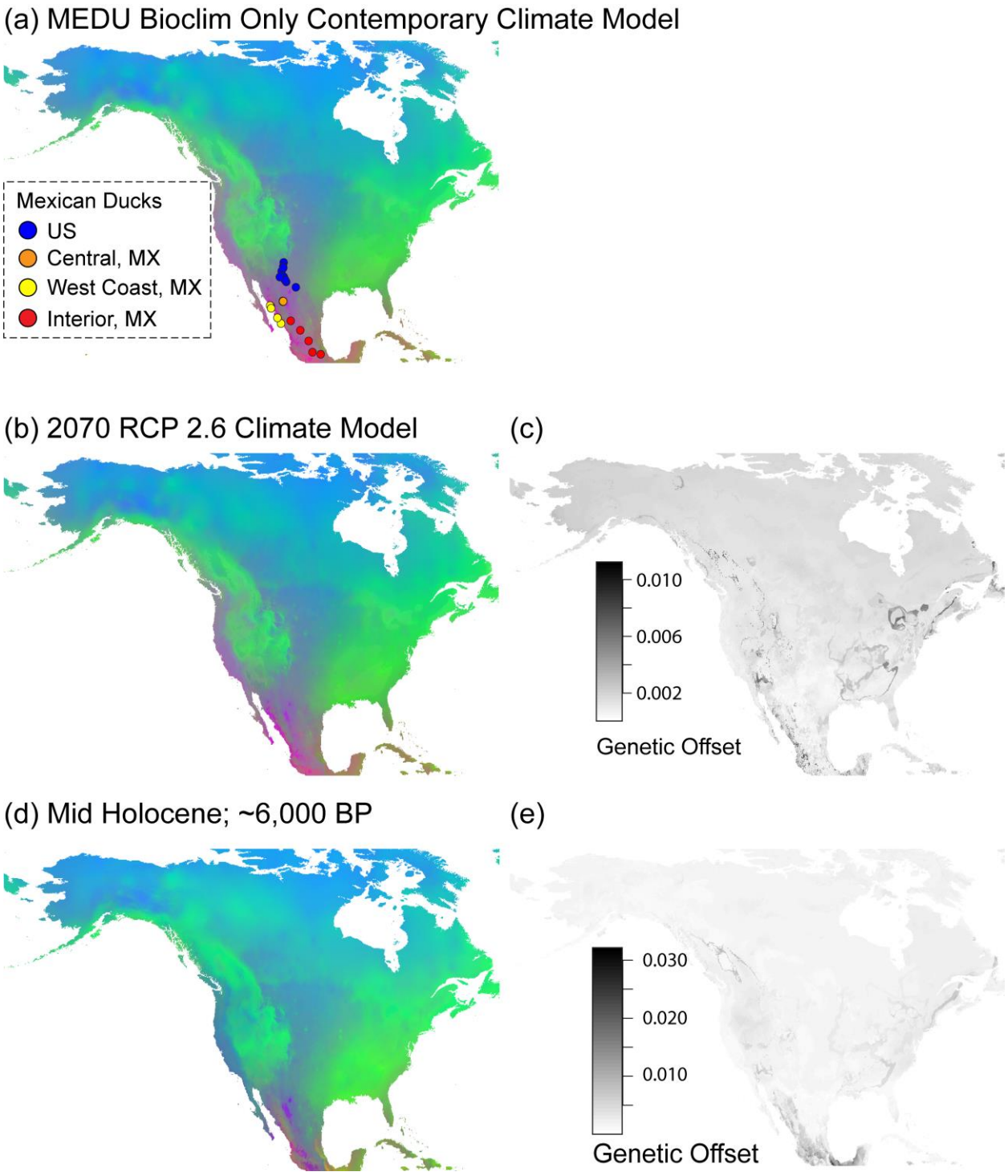


Figure S2.6. (a) Mexican duck genotype-environment association models from gradientforest (GF) based on only the top five most predictive contemporary temperature and precipitation variables. (b) GF models projected across the most mild estimates of future climate conditions (rcp 2.6) and (d) modelled environmental data from the Mid-holocene (~6,000 YBP). (c, e) Genomic offset calculated from the Euclidean distance between models based on contemporary and historic/future climate conditions mapped across North America.

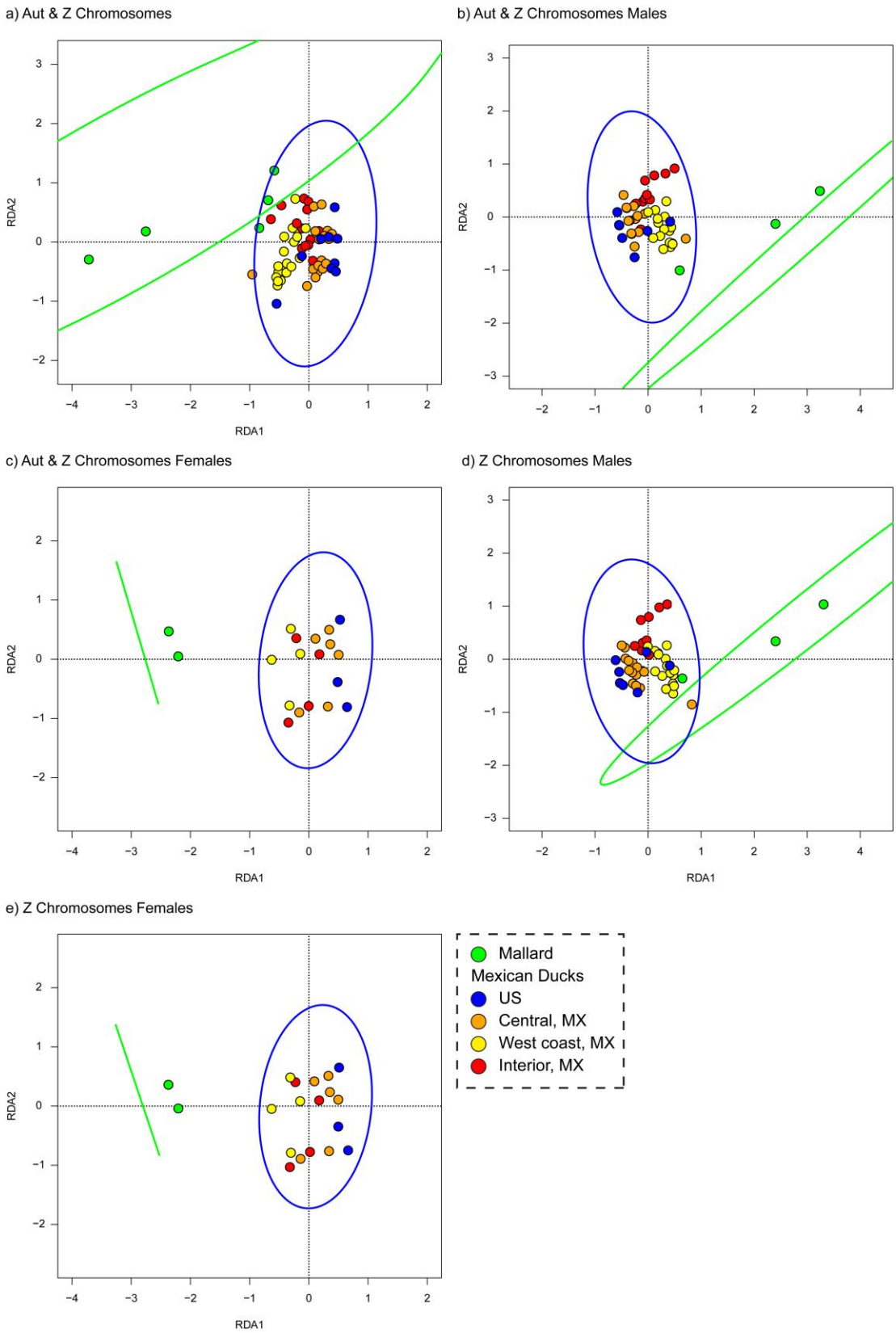


Figure S2.7. Plots of top two RDA constrained axes with the significant phenotypic traits plotted along with vectors of the Genotypic PCs that explain them.

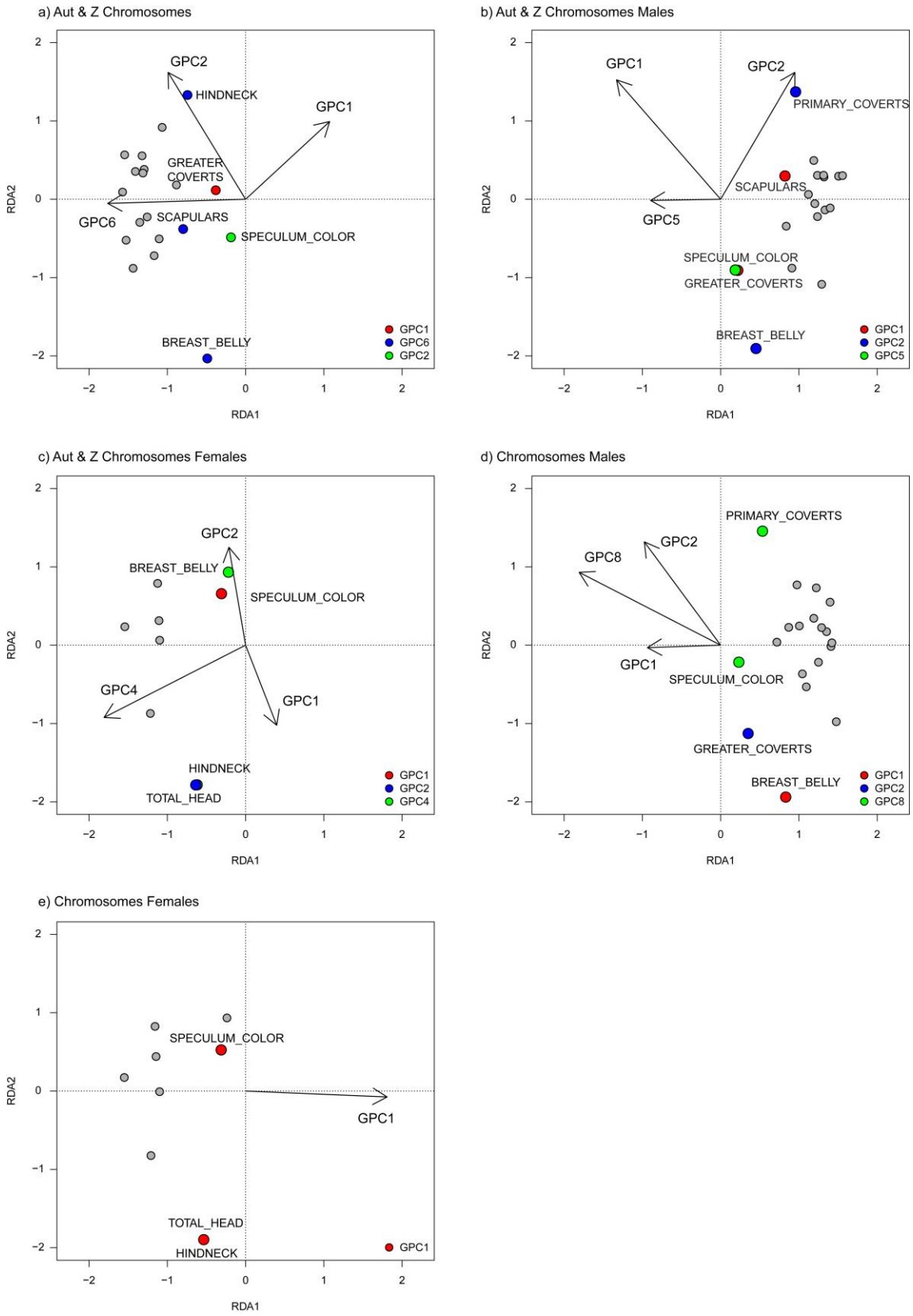


Figure S2.8. Individual samples with 95% coverage ellipse plotted along the top two RDA constrained axes.

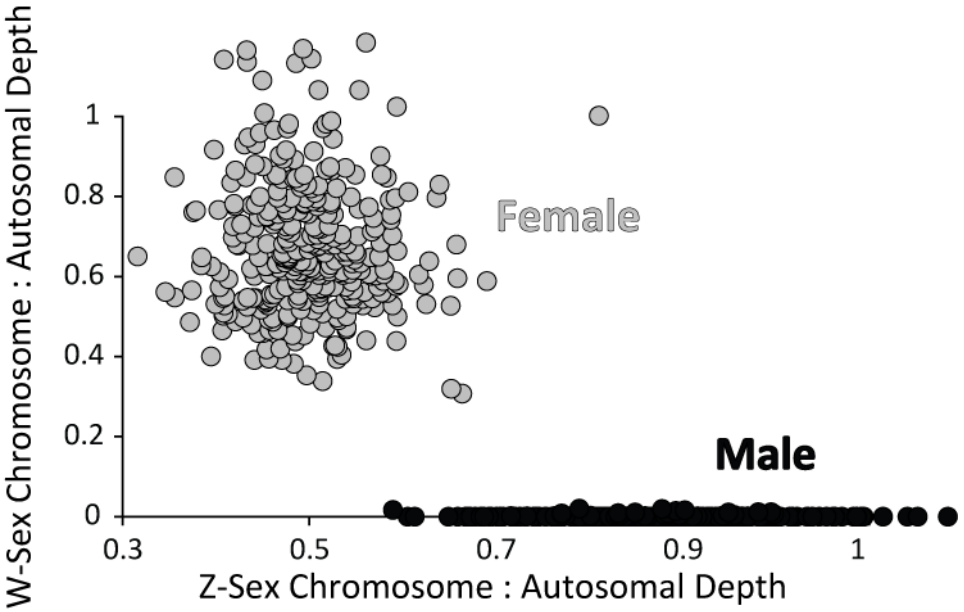


Figure S3.1. Sequencing depth ratio between Z and W-chromosome vs. autosomal ddRAD-seq loci used to determine sex for each sample.

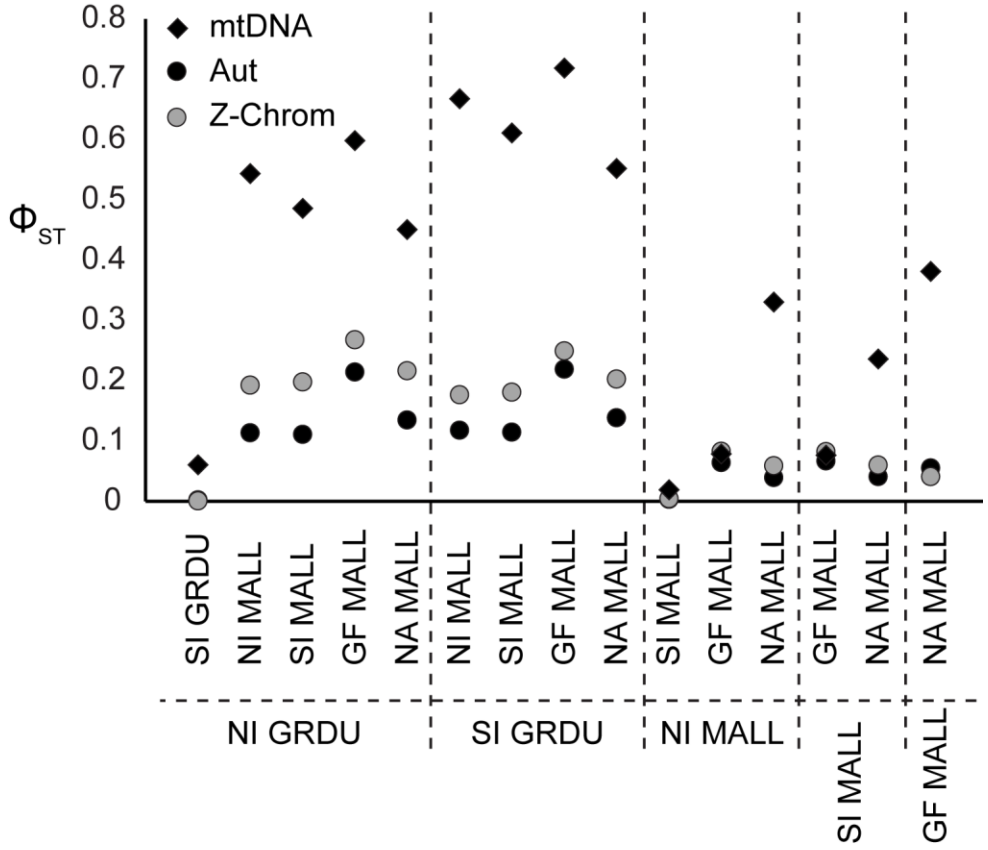
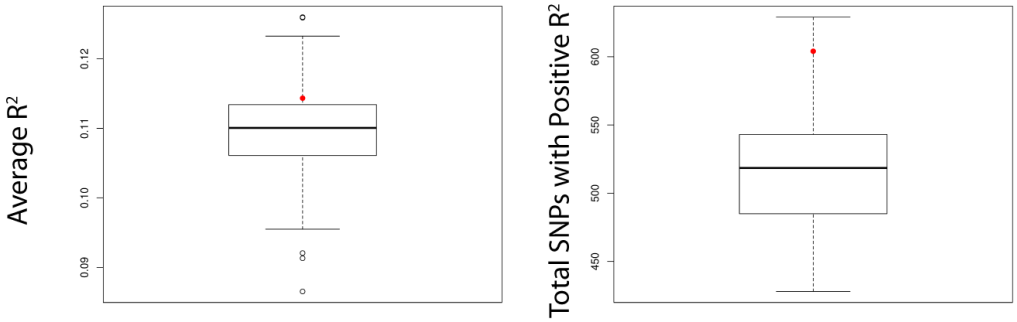
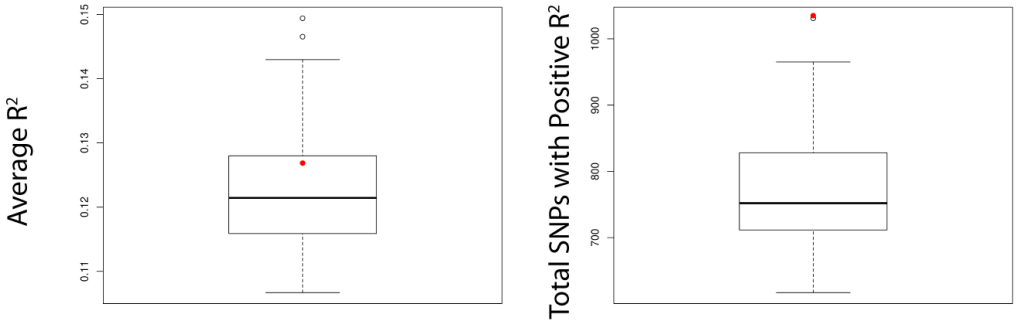


Figure S3.2. Composite pairwise Φ_{ST} estimates for Autosomal, Z-chromosome, and mtDNA loci (SI = South Island; NI = North Island; NA = North American; GRDU = grey ducks; MALL = mallard; GF MALL = game-farm mallard).

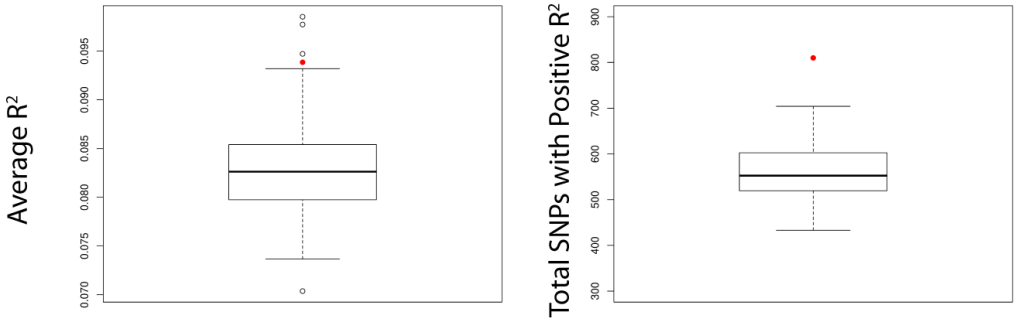
(A) Pure grey duck



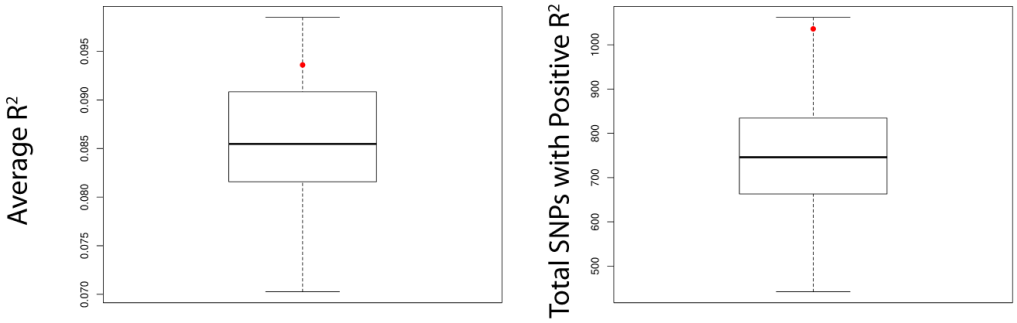
(B) Pure mallard



(C) Grey duck backcross



(D) Mallard backcross



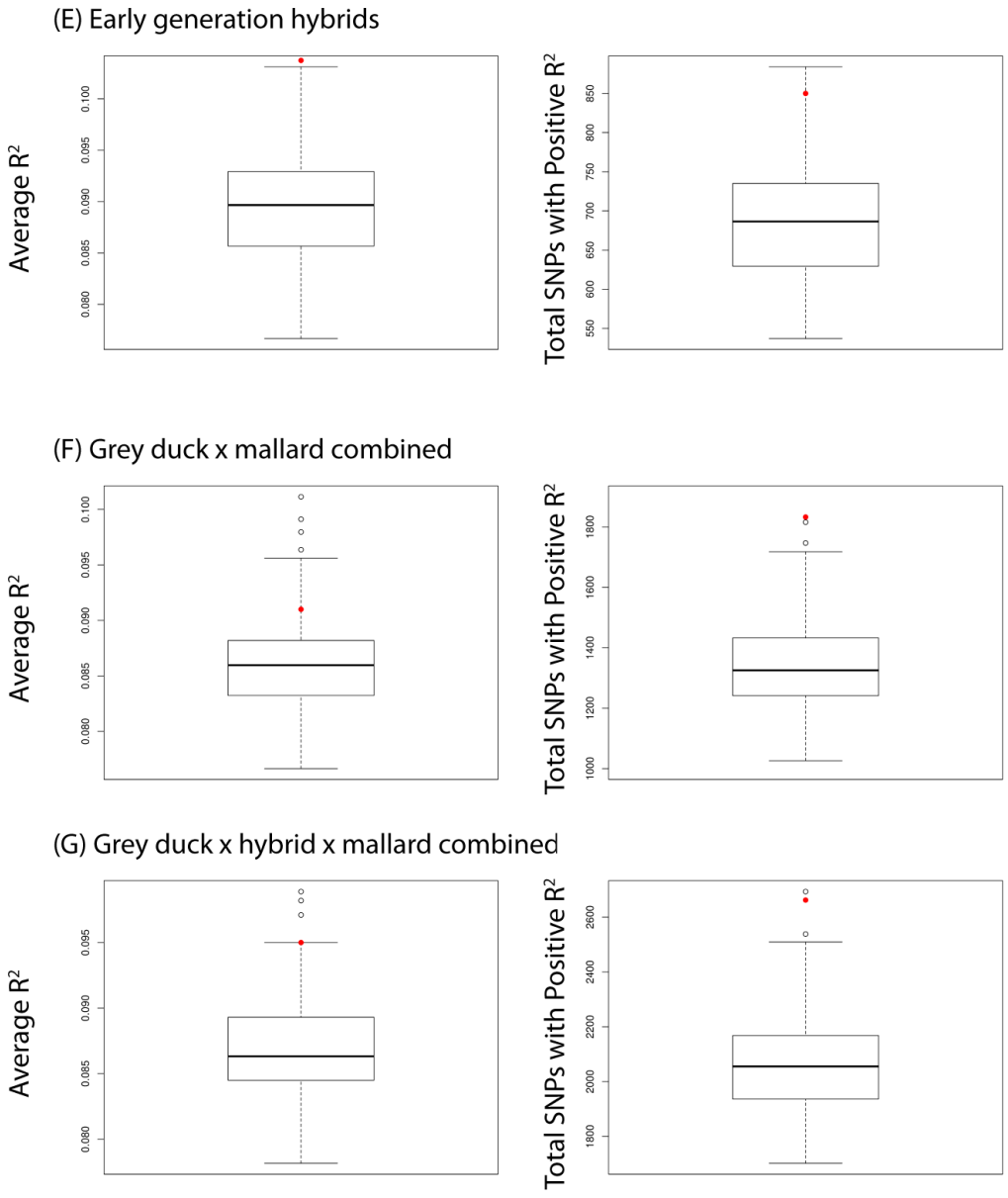


Figure S3.3. Boxplot of R^2 values from randomized datasets tested in gradientForest compared to the actual data (red dot).

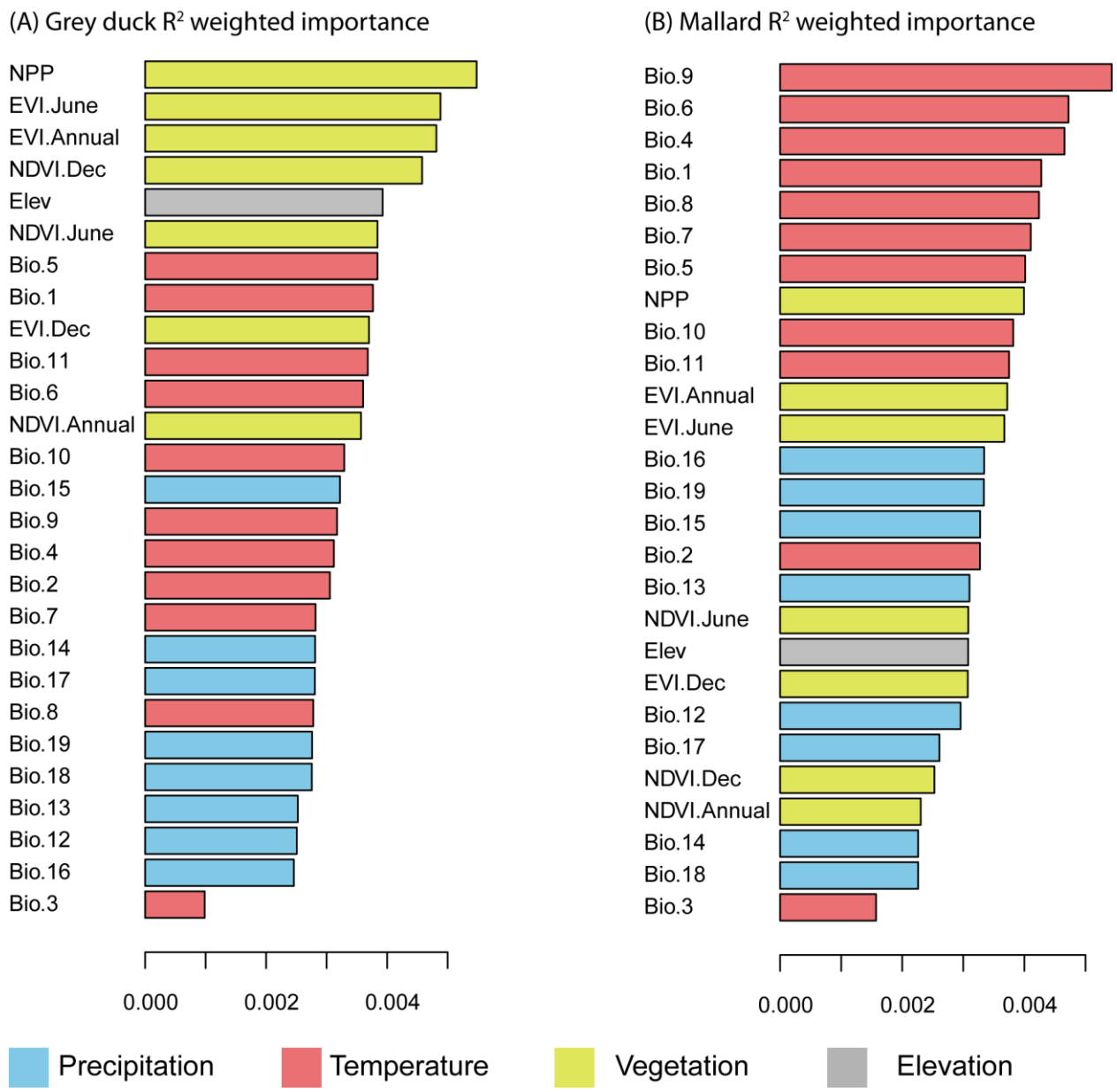


Figure S3.4. Cumulative R2 weighted importance ranking of 27 environmental predictor variables from gradientForest.

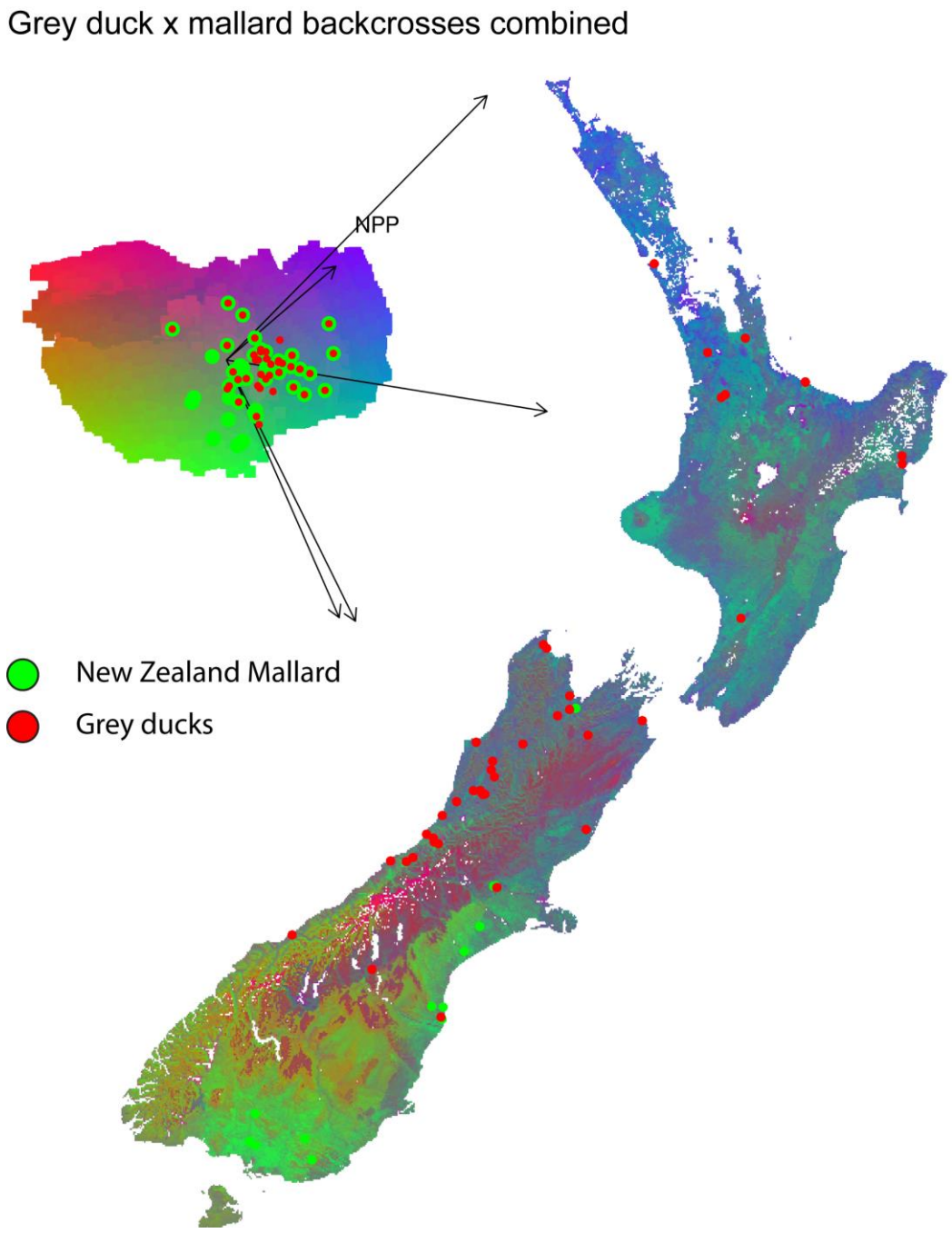


Figure S3.5. Combined model of genotype-environment associations from gradientForest for grey duck and mallard backcross projected across New Zealand.

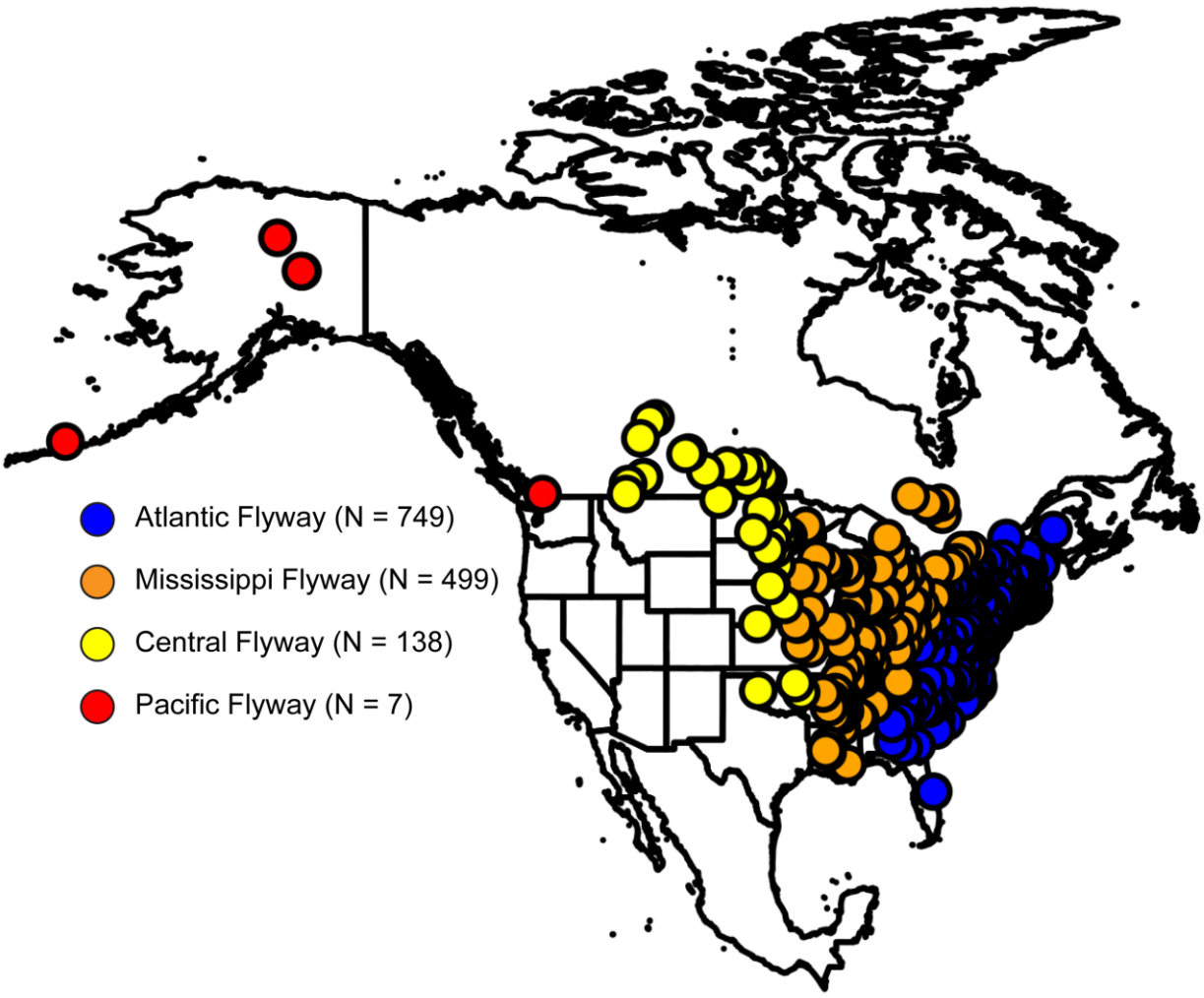


Figure S4.1. Summer and winter sample sites for mallards collected in a wild setting across North America.

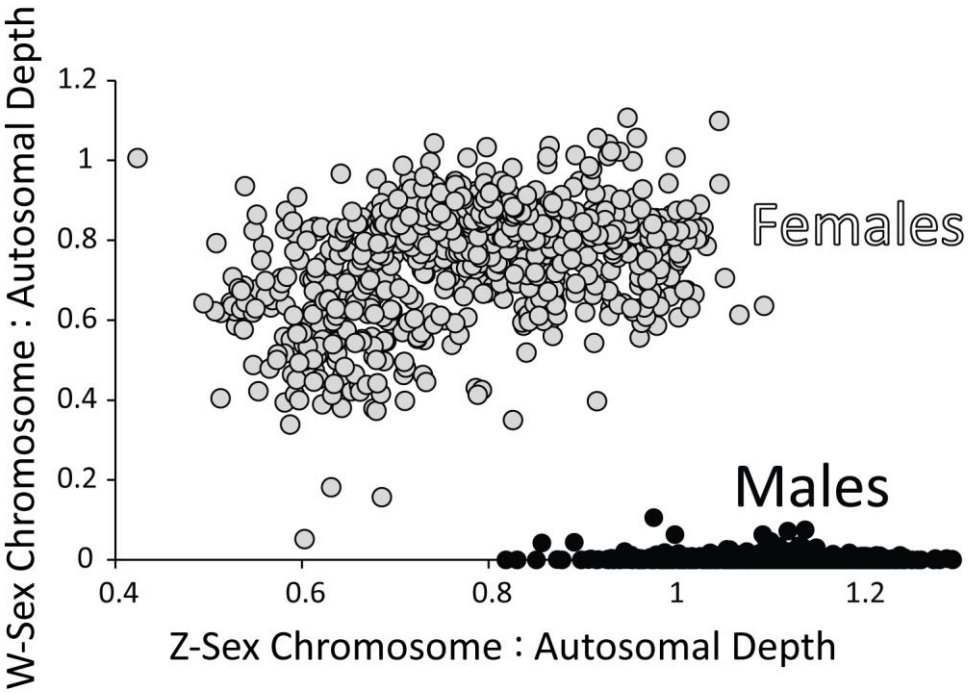


Figure S4.2. Sequencing depth ratio between Z and W-chromosome versus autosomal ddRAD-seq loci used to determine sex across mallard samples.

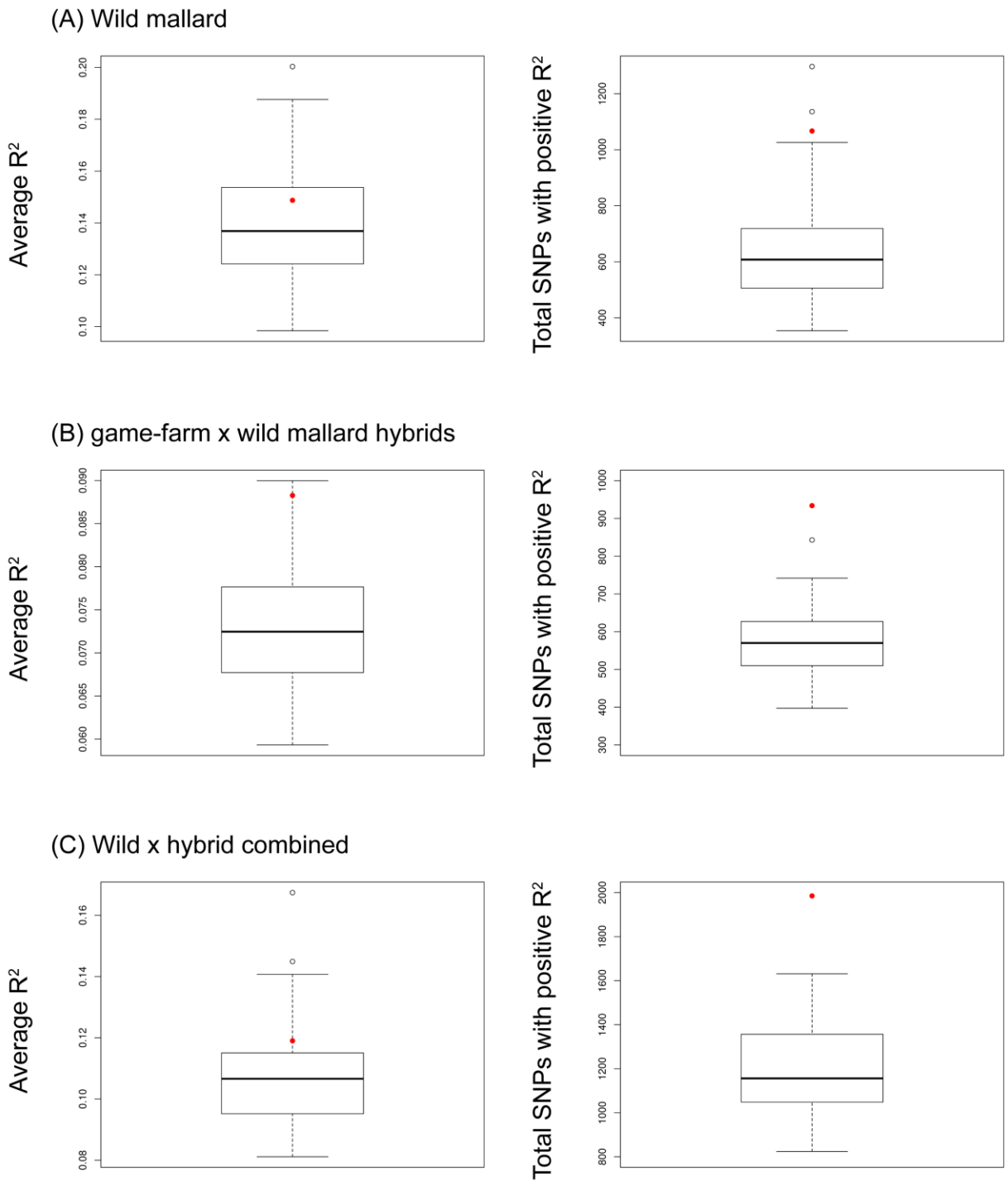


Figure S4.3. Boxplot of R^2 values from randomized datasets tested in gradientForest compared to the actual data.

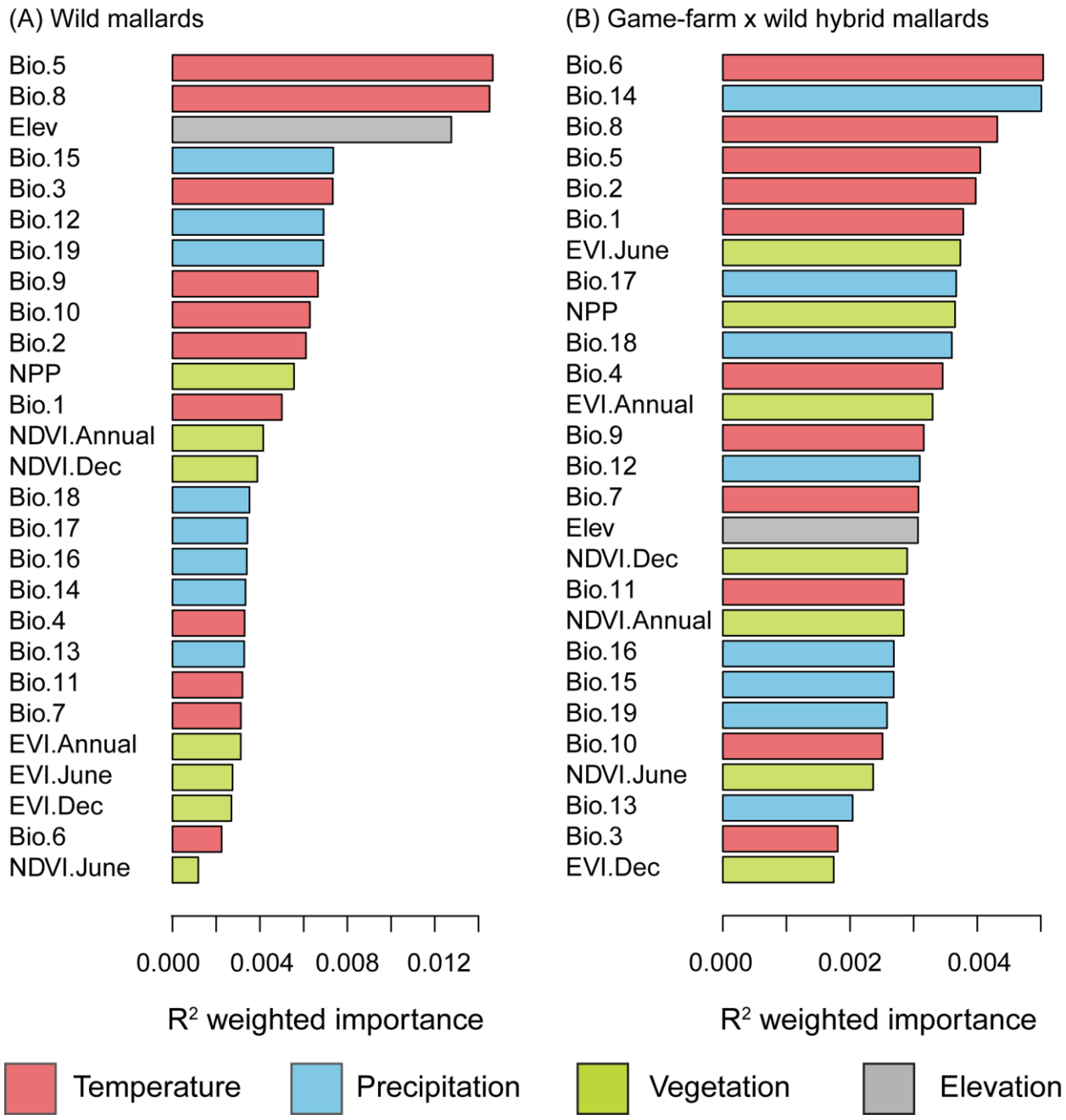


Figure S4.4. Cumulative R2 weighted importance ranking of 27 environmental predictor variables from gradientForest.

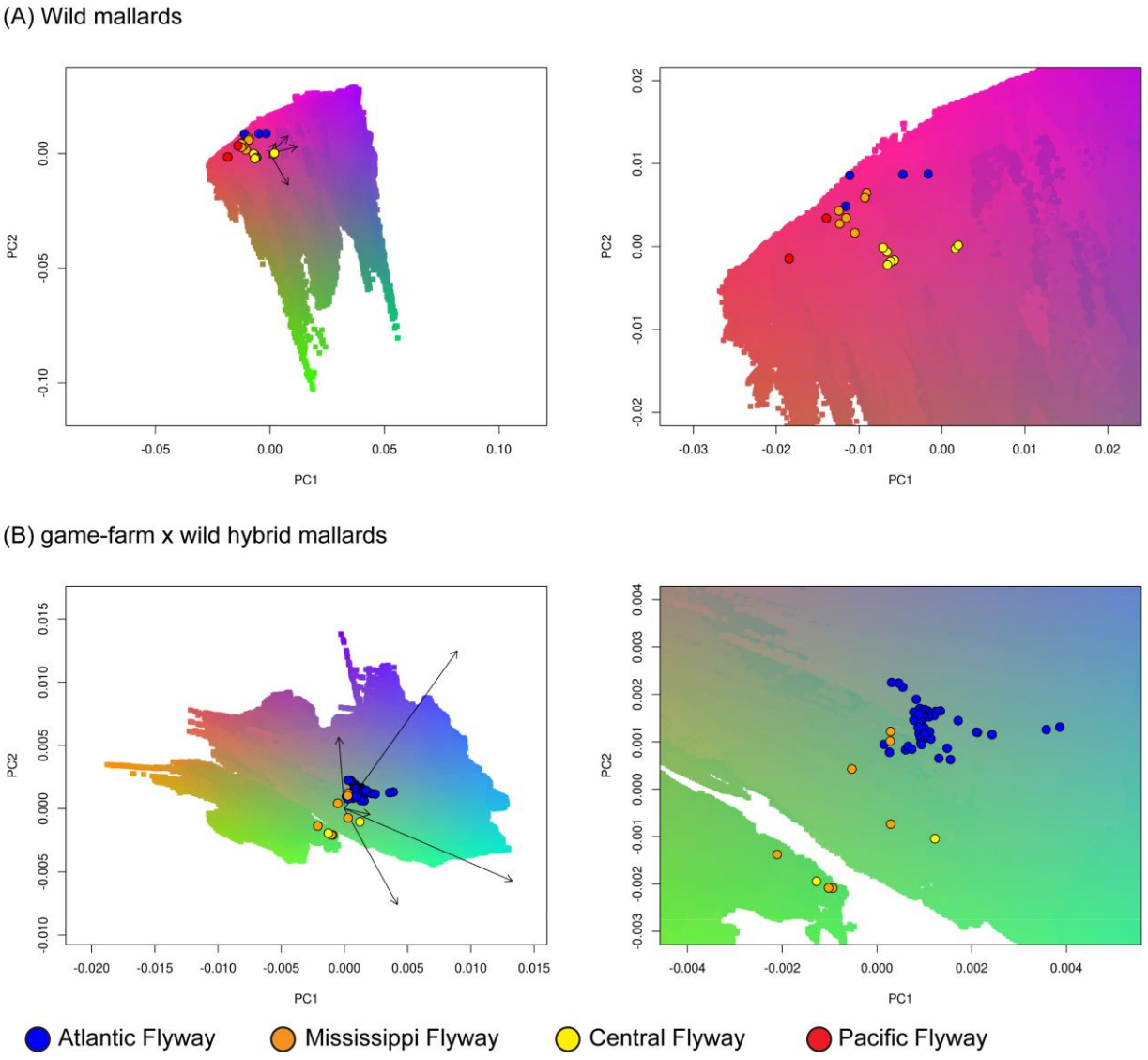


Figure S4.5. PCAs of genotype-environment association models from gradientForest (GF) for (A) wild and (B) game-farm hybrid mallards.

Vita

Joshua I. Brown was awarded a Bachelor of Science degree in Wildlife and Fisheries Sciences from Texas A&M University in 2016. He was admitted to the Ecology and Evolutionary Biology doctoral program at the University of Texas at El Paso (UTEP) that same year in 2016. He worked as a research assistant for Dr. Lavretsky's Lab through 2019, helping to establish the lab and develop undergraduate courses and lab activities. During his studies, Joshua was the recipient of multiple research grants and scholarships including the Ducks Unlimited Institute for Wetland and Waterfowl Research (IWWR) Waterfowl Research Foundation fellowship (2017-2020), the Stuart Moldaw scholarship (2019-2021), two UTEP Dodson Research Grants (2019-2020), and numerous travel scholarships. He has presented his work at eight different regional and international conferences including Evolution (2017), North American Duck Symposium 8 (2019), the Plant and Animal Genome XVIII Conference (2020), and the Northeast Natural History Conference (2021). In addition to being an invited guest lecturer for Evolution (2020) and Population Genetics (2018-2019) undergraduate courses, Joshua was invited to present his research at the seminar series for Ducks Unlimited Canada IWWR. Based on his research, Joshua has published four peer-reviewed manuscripts, and has another four in preparation or in review. Joshua volunteered as a Graduate Student Association representative for the Ecology and Evolution Department, as well as acted as a committee member for the UTEP Biology and Environmental Engineering (BEE) student organization.

Joshua is interested in using landscape genomics to inform conservation decision making as well as to investigate evolutionary history. He plans to pursue a career as a professor researching these topics.

This dissertation was typed by Joshua I. Brown.