Open Access Theses & Dissertations

2024-05-01

Conserving One Of Hawai'i's Last Endemic Ducks: Genetics And Habitat Associations Of Koloa, Feral Mallards, And Their Hybrids

Kristi Fukunaga
University of Texas at El Paso

CONSERVING ONE OF HAWAIʻI'S LAST ENDEMIC DUCKS: GENETICS

AND HABITAT ASSOCIATIONS OF KOLOA, FERAL

MALLARDS, AND THEIR HYBRIDS

KRISTI FUKUNAGA

Master's Program in Biology

APPROVED:

_____

Philip Lavretsky, Ph.D., Chair

_____

Caitlin Wells, Ph.D.

_____

Michael Harvey, Ph.D.

_____

Elizabeth La Rue, Ph.D.

_____

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

CONSERVING ONE OF HAWAIʻIʻS LAST ENDEMIC DUCKS: GENETICS

AND HABITAT ASSOCIATIONS OF KOLOA, FERAL

MALLARDS, AND THEIR HYBRIDS

by

KRISTI FUKUNAGA, B.S.

THESIS

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

MASTER OF SCIENCE

Department of Biology

THE UNIVERSITY OF TEXAS AT EL PASO

May 2024

ACKNOWLEDGEMENTS

ABSTRACT

Increases in anthropogenic hybridization through introduced species have accelerated the loss of genetic diversity and reductions in population size of native species that are already threatened by population and genetic diversity decline. Fertile hybrids that are common among waterfowl (order Anseriformes) are especially worrisome. A prime example is the endangered Hawaiian duck (*Anas wyvilliana*; "koloa maoli"), which is the remaining endemic duck species on the main Hawaiian Islands and is threatened by genetic extinction through ongoing hybridization with feral mallards (*Anas platyrhynchos*). Of note, koloa populations are known to be strongly male-biased (3:1), and this sex bias is known to stifle population growth rates in threatened species. Sex biases is known to occur as a result of hybridization, which can generate biases in offspring sex ratios due to rising genetic incompatibilities between sex chromosomes in the heterogametic sex (i.e., female birds), also known as Haldane's rule. Alternative to Haldane's rule is the potential for post-hatch female biased mortality that can also result in a male-biased population. Here, I tested for bias in offspring sex ratios among nests representing the remaining koloa populations of Kaua'i, and the various koloa x feral mallard hybrid swarms found on the other Hawaiian Islands and investigated mitochondrial DNA (mtDNA) diversity. Although adult koloa populations are highly male-biased, I found a slight, although non-significant, female-biased sex ratio among nests on Kaua'i. Thus, I concluded that the evident male-biased adult populations must be a result of post-hatch higher female mortality. Conversely, sex ratios among feral mallard and known koloa x feral mallard hybrid nests showed signs of either a male bias or no bias in ratios, respectively. Next, I recovered all known mtDNA haplotypes from previous studies, but showed that general mitochondrial diversity continues to decline among koloa x feral mallard hybrid populations. Importantly, I not only determined that most areas outside of Kaua'i

iv

are dominated by Old World "A" mtDNA haplotypes, but that the haplotypes present across

hybrid populations are indeed due to hybridization with the game-farm mallard, a domestic

mallard breed that continues to be released world-wide. Finally, I did not find any significant

difference in habitat selection for nests amongst koloa, feral mallards, or hybrid populations.

Together, my study fills critical knowledge gaps in sex ratios and hybridization rates that will be

used in future koloa conservation efforts.

TABLE OF CONTENTS

APPENDIX A: LIST OF TABLES

APPENDIX B: LIST OF FIGURES

INTRODUCTION

Paul J. Crutzen first coined the term Anthropocene as an era where "Human activities are exerting increasing impacts on the environment on all scales, in many ways outcompeting natural processes" (Crutzen, 2006). Although as an atmospheric chemist he mostly spoke about atmospheric and geologic changes in the world, human initiated biological impacts such as habitat fragmentation, invasive species, overexploitation, and overpopulation are also altering the environment (Pievani, 2014). A prime example is invasive species, the very definition of which acknowledges that their arrival into native systems is by human assistance (Simberloff, 2013). This artificial movement assembles communities of species that have never before encountered one another, thus changing the course of how every species may exist and evolve (Otto, 2018). An invasion of an alien species can not only result in their establishment and overtaking of native species, but also in higher than natural rates of hybridization (Adavoudi & Pilot, 2021; Lavretsky et al., 2023b; Rhymer & Simberloff; 1996). Among such scenarios, the artificial influx of many domesticated species that turn feral (i.e., domestic animals surviving and reproducing in the wild) are resulting in widespread anthropogenic hybridization with their wild ancestors. This becomes a concern in the maintenance of wild native populations in different regions of changing environments, threatening many ecologically and economically vital species (Lavretsky et al., 2023b; Ottenburghs, 2021). In fact, Ottenburghs (2021) found that of 117 articles published from 2016-2020 reporting separate incidents of anthropogenic hybridization events, 53% were caused by introduction of a non-native species.

While hybridization is a source of natural evolutionary opportunities, the increased rate of hybridization occurring by anthropogenic means is cause for concern. Increases in anthropogenic hybridization can lead to outbreeding and further loss of genetic diversity and

population size of native species already threatened by population and genetic diversity decline (Adavoudi & Pilot, 2021; Allendorf et al., 2001; Moran et al., 2021; Rhymer & Simberloff, 1996). While in some cases hybridization has been used as a conservation tool, such as with the Florida panther (Johnson et al., 2010), not all hybridization occurs in a way that facilitates the continuation of a native species. Typically, for conservation purposes, 'genetic rescue' proceeds via directed interbreeding between a population of concern and an outbred population (e.g., Florida panther x Texas pumas; Johnson et al., 2010) so as to artificially increase genetic diversity in the former group. Such cases are often the result of a single pulse of interbreeding, rather than multi-generational (Chan et al., 2019) that has the potential to result in the genetic extinction of the native species of concern (Rhymer & Simberloff, 1996). The capacity for fertile hybrids creates a particular problem for waterfowl (Order Anseriformes), where interspecific hybridization is disproportionately common and often leads to viable and fertile hybrids (Ottenburghs et al., 2015). Consequently, establishing the biological relevance from anthropogenic hybridization requires an understanding of the frequency of interbreeding and the viability of hybrid offspring (Lavretsky et al., 2019; Wells et al., 2019).

Koloa Conservation Threats

A biodiversity hotspot, Hawaiʻi is now also a global extinction epicenter and known as the bird extinction capitol of the world (Banko et al., 2001). For example, Hawaiʻi used to be home to over 50 different species of honeycreepers, but today only 17 of those species are still extant, with over half of those listed as endangered or threatened species (USGS, 2022). This pattern of extinction exists for waterfowl as well. Following early human settlement, four species of geese and ducks went extinct due to humans 1600 years ago, leaving only Laysan ducks (*Anas*

laysensis), Hawaiian ducks (*Anas wyvilliana; "koloa maoli"*, koloa for short*)*, and Hawaiian geese (*Branta sandvicensis; "nēnē"*) as the remaining waterfowl (Sorenson 1999; Walther 2016). All of these are now listed as threatened or endangered species due to numerous anthropogenic factors (USFWS, n.d.). Successful translocation efforts were made for both Laysan ducks and nēnē to expand their ranges (Black 1995; Reynolds et al., 2015; USFWS 2020). In contrast, there have not been translocations that have been able to successfully establish multiple populations across the Islands for koloa. For this reason, the koloa recovery plan is a focal point for bird conservation and education in the Hawaiian Islands.

Molecular data suggests that koloa are a hybrid species. They originated from the natural hybridization of wild mallards and Laysan ducks over the last 5,000 years (Lavretsky et al. 2015), and are now the only remaining endemic duck species on the main Hawaiian Islands. Koloa were once abundant and hunted by native Hawaiians; in addition to being a valuable historic food source, koloa are an integral part of Hawaiian wetland ecosystems and culture, as the foundation of Hawaiian place names, folklore, and legends (Gomes, 2015; Gomes, 2016; Gomes, 2020; Waikōloa, N.d.; Young, 2016). However, Europeans drained many wetlands after their arrival in the 1800s to create space for sugar plantations (Engilis et al., 2002; Hart et al., 2022; Wilcox, 1997). Simultaneously, Indian mongoose were introduced across the Hawaiian Islands as a biocontrol for rats that infested the sugarcane fields on Maui, Molokaʻi, and Oʻahu (Barun et al., 2011; Engilis et al., 2002; Hart et al., 2022). Unfortunately, because koloa are ground nesting birds and did not evolve with mammalian predators, their populations were decimated by the early 1900s through this combination of habitat degradation and mongoose predation (Berger 1970; Engilis et al., 2002; Hart et al., 2022). Additionally, domestic mallards were first imported to Hawaiʻi for food and hunting beginning in the 1800s, and eventually

became commercially farmed on Oʻahu during the 1930s and 1940s; these actions eventually resulted in the establishment of multiple feral populations across all the main Hawaiian Islands (Engilis Jr et al., 2004; Engilis & Pratt, 1993; Pyle & Pyle, 2017).

Koloa historically occurred across Kauaʻi, Niʻihau, Oʻahu, Maui, Molokaʻi, and Hawaiʻi, but by 1962 fewer than 500 koloa existed on Kauaʻi and Niʻihau (Engilis et al., 2002), resulting in their federal listing under the Endangered Species Act in 1967 (Hart et al., 2022). As part of recovery actions, koloa were captive-reared and reintroduced onto Oʻahu, Hawaiʻi, and Maui until 1989 (Hart et al., 2022; Wells et al., 2019). However, hybridization between reintroduced koloa and the burgeoning feral mallard populations on those islands produced fertile offspring (Browne et al., 1993; Fowler et al., 2009; Wells et al., 2019). Recent population genetic studies confirmed that pure koloa persist mainly on Kauaʻi, with populations on other islands existing only as koloa x feral mallard hybrid swarms (Wells et al., 2019). Without immediate efforts, koloa will continue to persist on only Kauaʻi and nearby Niʻihau with continued risk of extinction due to its existence in a single population and the potential for hybridization with feral mallards (Mālama Hawaiʻi & USFWS, 2009; Wells et al., 2019). Therefore, the Koloa Recovery Implementation Group (KRIG) comprising of federal, state, University, and NGO personnel determined that understanding the genetic consequences of these koloa x feral mallard hybrid populations is required to guide future active management.

Statement of Problem

According to KRIG, there is a pressing need to research and simulate genetic outcomes under multiple active management scenarios throughout Hawaiʻi to guide the establishment of additional koloa populations. Recently, an analytical model that incorporates starting genetic

ancestry and various life-history traits (i.e., clutch size, age to maturity) to optimize management strategies was developed as a decision-making tool towards reversing consequences of hybridization across the Hawaiian Islands (Hernandez et al. 2023). In brief, the developed R-based program simulates time (in generations) to reverse the genetic constitution of a specific hybrid population towards genetically pure koloa as set by management goals and under differing management strategies (i.e., number of koloa input individuals, feral mallard and hybrid individual removal effort) (Hernández et al., 2023). This model will ultimately be used to simulate an optimal strategy for each possible wetland that could support koloa across the Hawaiian Islands.

Incomplete information on life-history traits including clutch sex ratios of koloa, mallards, and hybrids potentially bias models and lead to erroneous predictions. Sex ratios, particularly in small populations, can have strong effects on birth rate. Female-dominant populations can cause higher birth rates than male-dominant populations (Johnson, 1994). Depending on the species, sex ratios may also be influenced by the species's survival strategy, it's environment, and population dispersal (Johnson 1994; Lenz et al., 2007; Steifetten & Dale 2006). While koloa adults are known to have a 3:1 (male:female) sex ratio, it remains unknown whether this ratio is present within clutches or is due to differences in post-hatch survival of female versus male koloa (Malachowski, 2020). Haldane's rule is an evolutionary prediction that states that when two divergent lineages interbreed, the heterogametic sex – in this case females (ZW) – will be less viable due to chromosomal incompatibilities (Haldane, 1922). Following this rule, the hybrid origin of koloa (Lavretsky et al., 2015) may drive a disproportionate male to female ratio starting at hatch. If this is the case, any management efforts taken with koloa will need to account for a naturally male-biased sex ratio. Alternatively, a balanced offspring sex

5

ratio at hatch is also possible because, even though they had a hybrid origin, koloa have existed

for thousands of years and incompatibilities may have been removed from the population over

that time (Lavretsky et al., 2014). Moreover, sex ratios of feral mallard and koloa x feral mallard

hybrid clutches are entirely unknown. It is possible that sex ratios among contemporary hybrids

may be impacted by Haldane's Rule, perhaps even more strongly than within koloa, because they

are a newly formed hybrid swarm. Even in mallards, sex ratios are poorly known. Mallard sex

ratios have mostly been examined within wild individuals, where there was found to be between

an adult 1:1 or 2:1 male to female ratio (Eygenraam, 1957; Ferguson et al., 1981; Ohde et al.,

1983). However, Hawai'i's mallard population is of game-farm origin (Lavretsky et al., 2023b).

One study on game-farm mallards found adult populations have 1:1 sex-ratios (Söderquist et al.,

2021), but clutch data and more adult data on sex ratios are needed.

In addition to establishing sex-ratios of nesting waterfowl, I aim to further understand

koloa ancestry dynamics across the Hawaiian Islands. To do this, I examine mitochondrial DNA

(mtDNA), which has been used previously to assess maternal ancestry of koloa and hybrids, as

well as other species of birds (Fowler et al., 2009; Hutchison et al.,1974; Shields & Helm-

Bychowski, 1988; Wells et al., 2019). Comparing our mtDNA data to previous data allows us to

see if mtDNA proportions remain stable over time. This is helpful input for the analytical tool

being used for translocation and to also validate that these populations are hybrid swarms and

thus will generally not change in their proportions. Moreover, these analyses will also determine

whether the eggs collected in the population known to have pure koloa on Kaua'i are still at least

maternally koloa. In previous studies using mtDNA, all koloa had one of five different

haplotypes all falling within the "New World" B haplogroup, while all mallards and most

hybrids sampled in Hawai'i belonged to the "Old World" A haplogroup (Fowler et al., 2009;

Wells et al., 2019). Note that the presence of "Old World" mtDNA haplotypes in North America has been linked to the release and subsequent introgression of game-farm mallard ancestry into wild populations (Lavretsky et al. 2019, 2020, 2023b). The influx of OW A haplotypes in Hawai'i suggests mating to have proceeded between feral female mallards and koloa males. Given that these studies were done with samples from nearly a decade ago, my study provides a more contemporary update to earlier work by Fowler et al. (2009) and Wells et al. (2019).

In addition to describing mtDNA diversity, clutches with complete mtDNA ancestry will allow me to determine whether koloa, mallards, and/or hybrid females engage in nest parasitism. It could potentially be important to discover whether or not koloa engage in nest parasitism for understanding general life-history traits of these birds and hybridization dynamics that may be unknowingly occurring in the form of parasitism. For instance, if both koloa and mallards have the potential to parasitize other nests and are allowed to exist in the same population they could parasitize one another. Nest parasitism in hybridizing populations could result in unwanted successes of mallards within koloa clutches or unknown koloa in mallard clutches. This may warrant examination of each individual in newly formed clutches in areas where koloa are being translocated and/or managed, regardless of the parent species. To examine this, eggs from the same nest but with differing maternal lineage (based on mtDNA) can be safely assumed to be from an unrelated parasitizing female. This will be the first assessment of nest parasitism of koloa. Although interspecific nest parasitism is rare in *Anas* ducks, recent molecular work with American black ducks (*Anas rubripes*) showed that those females did engage in nest parasitism of related and unrelated females (Lavretsky et al., 2023a).

Finally, I will be using location, vegetation, and environmental data to see how each species uses the landscape and if there is any overlap of habitat preference during nesting

7

between species. This information will potentially be used for habitat prioritization in the recovery of koloa. It is unknown what nesting habitat hybrids use, so this work could give insight on how to manage them in areas where koloa are desired.

Objectives

My overarching goal is to understand the molecular consequences from anthropogenic hybridization as it relates to the conservation of the koloa. To do this, my first objective is to examine sex ratios of koloa, hybrids, and feral mallard populations across the Hawaiian Islands, and how this may impact populations. To achieve this objective, I am going to answer 1) is there a skewed sex ratio in koloa at hatch, as one might expect given the skewed adult sex ratio? 2) Is there a skewed sex ratio in contemporary koloa-mallard hybrids as one might expect under Haldane's rule? And 3) is there a skewed sex ratio in feral mallards as seen in wild mallard populations? For my second objective, I will characterize and update mitochondrial ancestries across islands, as well as look for nesting parasitism, and examine any potential environmental factors that may play a role in nesting site selection between species. To achieve this objective, I am going to answer: 1) have feral mallard mitochondrial haplotypes swamped koloa haplotypes on Oʻahu? 2) Do koloa, feral mallards, and/or their hybrids engage in alternative breeding strategies (e.g., nest parasitism)? And 3) are there differences in environmental variables selected between species, what are those variables, and how are they different between species?

METHODS

Study Site

The Hawaiian Archipelago is the most isolated archipelago, consisting of a chain of 137 isolated islands in the middle of the Pacific Ocean. The main Hawaiian Islands comprise of eight islands – Niʻihau, Kauaʻi, Oʻahu, Molokaʻi, Lanaʻi, Kahoʻolawe, Maui, and Hawaiʻi – with all but Kahoʻolawe being inhabited by humans. My study focuses on eight study sites on Maui, Oʻahu, and Kauaʻi (Figure 1). Part or whole eggs were collected from one site on Kauaʻi (Hanalei National Wildlife Refuge), eight locations on Oʻahu (James Campbell National Wildlife Refuge, Pearl Harbor National Wildlife Refuge Honouliuli Unit, Kākoʻo ʻŌiwi, Hāmākua Wildlife Sanctuary, Pouhala Marsh Wildlife Sanctuary, Paikō Lagoon Wildlife Sanctuary, Kawainui Wildlife Sanctuary, and Waiawa Kai, Puʻuloa), and two sites on Maui (The Tropical Plantations and Keālia Ponds National Wildlife Refuge). According to previous genetic work koloa are only found on Kauaʻi, domestic mallards were found at The Tropical Plantation, and all other locations were assumed to be various hybrid forms based on Wells et al. (2019). Study sites were chosen based on ease of collection, visitor frequency, and whether conservation efforts were already taking place.

Nest collection

Nest collections occurred until ≥30 nests per location were attained for statistical support as determined from early power analyses (Wells *personal communication*). Along with federal, state, and nonprofit organization staff members, I collected eggshells, viable eggs, and/or nonviable eggs from spring 2021 to spring 2023, with a total of ~1,300 eggs across 168 nests collected on Oʻahu (N nests = 114), Maui (N nests = 30), and Kauaʻi (N nests = 24) (Figure 1).

Previous molecular assessment across Hawaiian Islands showed that only feral mallards and feral mallard x koloa hybrids exist everywhere outside of Kaua'i (Wells et al., 2019). Because koloa are an endangered protected species, only post-hatch shells or inviable eggs were collected on Kaua'i, whereas viable and inviable eggs and eggshells were taken from Maui and O'ahu nests. Locations of collected eggs or eggshells were recorded, as well as identification of vegetation species used as nesting material for a subset of nests. Collected eggs and eggshells were shipped to the University of Texas at El Paso in Lavretsky's lab and held at -80°C.

DNA analysis

DNA extraction

Membrane tissue was pulled from all eggs, as well as yolk or tissues and organs from the developing fetus if available. Genomic DNA was extracted for each sample using a DNeasy Blood & Tissue kit following the manufacturer's protocols (Qiagen, Valencia, CA, USA). Samples were run through gel electrophoresis with a 1% agarose gel to visualize amplification success and DNA quality based on a presence of a high molecular band. I attempted DNA extractions up to three-times before conceding failure.

Molecular Sexing

Molecular assessment of sex followed optimized PCR-based methods targeting a CHD gene found on both sex chromosomes of birds (Çakmak et al., 2017). In short, I used primers from Çakmak et al. (2017), but used optimized PCR reactions and thermocycler conditions as described in Lavretsky et al. (2023a). Each PCR reaction included 1.5 μL of template DNA, 2x GoTaq Green Master Mix (Promega), and 1.0 nM of each primer, totaling 15 μL in volume. An

Eppendorf Mastercycler (ep*gradient*) thermocycler was then used following a touch-down protocol that included an initial denaturation at 94°C for four minutes, followed by a single 94°C cycle for 30 seconds, before annealing for another 45 seconds starting at 57°C decreasing by one degree each cycle to 50°C, and a final 45 second extension at 72°C. The touch-down protocol was followed by 30 cycles of 30 seconds at 94°C, 45 seconds at 50°C, and 45 seconds at 72°C, with a final extension at 72°C for five minutes. PCR products were loaded on a 4% agarose gel, with successful amplifications expected to show one band for the homogametic sex (i.e., male) and two for the heterogametic sex (i.e., female). Each sample was run through PCR and gel electrophoresis three times in order to confirm the correct sex for the sample.

Mitochondrial DNA

Mitochondrial DNA was assessed across all samples with either degraded or undegraded DNA. I targeted the mtDNA control region using optimized methods as described in Lavretsky et al. (2014). In short, I amplified the mtDNA control region using an optimized touch-down PCR protocol on a 15 µL PCR solutions with L78 and H774 primers to sequence ~650 bp of the mtDNA control region. Once again, the PCR reaction included of 1.5 µL of template DNA, 2x GoTaq Green Master Mix (Promega), and 1.0 nM of each primer, totaling 15 µL in volume. The Eppendorf Mastercycler (ep*gradient*) thermocycler was then used for (1) initial denaturation for 7 minutes at 94 °C, then (2) 45 cycles of DNA denaturing for 20 seconds at 94 °C, primer annealing at 52 °C for 20 seconds, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplification was then verified using gel electrophoresis with a 1% agarose gel and was sent for sequencing to a separate lab with a 3130XL Genetic Analyzer at the University of Texas El Paso Border Biomedical Research Center's Genomic Analysis Core Facility. Raw

Sanger sequences were aligned and edited using Sequencer v4.8 (Gene Codes, Inc., Ann Arbor, MI, USA), a haplotype network was visualized using PopArt (Leigh & Bryant 2015), and ArcGIS Pro was used to geographically map out the haplotypes.

Spatial analysis

Habitat parameters and nest location data of different haplotypes were used to examine how genetic identity was associated with nest habitat selection. Habitat variables (i.e. rainfall, moisture, elevation, and land cover) were pulled and analyzed from the State of Hawai'i's Office of Planning (https://opendata.hawaii.gov/organization/office-of-planning). Using the land cover layer, the proximity to water and to urban environment were calculated with the ArcGIS near analysis tool, which measured the distances between the polygons of the urban or water layers and the nest points. To examine habitat preferences for each species, I ran principal component analyses with all the habitat variables to see if they clustered by haplotypes. Lastly, examining the nest sizes for each location, an ANOVA was run to see if there was differences between sites which may indicate differences in resource availability (Decker et al., 2012). This information may be used for habitat prioritization for koloa if differences are found in habitat selected for nesting.

RESULTS

Molecular Success

I attempted DNA extraction for a total of 1,270 samples and obtained high-molecular weight DNA for 409 eggs, degraded but usable DNA for 415 eggs, and no visible DNA for the remaining 446 eggs; resulting in 824 useable samples (Table 1). Success differed among the types of egg sample (membrane, yolk, or fetus tissue) used in extraction, with fetus tissue samples being the most successful (99% success), whereas DNA extraction form membrane and yolk had successes of only ~50/60% (Table 2).

Sex ratios

I was able to successfully sex 431 of 824 (~52%) useable samples from Maui (N = 51), Oʻahu (N =330), and Kauaʻi (N = 50; Table 3). Again, successful PCR to molecularly sex samples depended on tissue type, with fetus tissue being most successful (96% of samples), followed by egg membrane tissue (38% of samples), and only 32% of yolk samples being successful (Table 4). I found a slight but insignificant (Fisher's Exact Test: males = 20, total = 50, p-value = 0.42) 2:3 female bias among koloa nests, no bias for koloa x feral mallard hybrid populations (Fisher's Exact Test: males = 172, total = 339, p-value = 0.82), and a high and significant (Fisher's Exact Test: males = 37, total = 43, p-value = 0.00040) 6:1 male bias among feral mallard populations (Table 3). Thus, whereas koloa and hybrid populations do not have a significant sex ratio skew from 1:1 at nesting, there is significant increases in male eggs among nesting feral mallard populations.

Mitochondrial DNA

I successfully sequenced mtDNA for 670 of 824 samples (81%). Complete nest data was found for 39 of 148 nests, one being from mallards on Maui, 29 being from multiple sites on Oʻahu, and nine from Kauaʻi. Prior to analyzing sequences, 619 comparative mtDNA sequences for wild North American mallards, known game-farm mallards, koloa, Laysan ducks, and park ducks (a.k.a Khaki Campbell's) were also aligned and served as references in my analyses (Fowler et al., 2009; Lavretsky et al., 2023b; Wells et al., 2019). As expected, all Laysan duck sequences comprised a single and divergent haplogroup, which is now absent on the main Hawaiian Islands (Lavretsky et al. 2014, 2015; Figure 2). I recovered OW A mtDNA haplotypes across Oʻahu (66 nests) and all of Maui (11 nests), and these grouped with reference game-farm mallards and associated haplotypes that are now found world-wide due to their release and subsequent hybridization with endemic populations (Lavretsky et al., 2023b). In fact, the most common haplotype found was haplotype 1, which is the most dominant game-farm haplotype in other parts of the world (Lavretsky et al., 2023b; Wells et al., 2019) (Figure 3). Other individuals found on Oʻahu were from another OW A haplotype (haplotype 9), with the exception of seven nests that were NW B haplotypes (Figure 3). Haplotype 9 was only found in the northern part of Oʻahu and did not match any other sequences on the other islands or reference sets (Figure 3). I compared haplotype 9 sequence to the entire National Center for Biotechnology Information (NCBI) BLAST database and recovered 100% sequence identify with published game-farm mallards (*Anas platyrhynchos*), koloa x mallard hybrids (*Anas platyrhynchos x Anas wyvilliana*), and Pacific black ducks (*Anas superciliosa superciliosa*) from Hawaiʻi, China, and New Zealand. In addition, the mtDNA NW B haplotype 15 in Oʻahu's Honouliuli and Pouhala regions

14

were not recovered in any of the reference sets nor in the NCBI database (Figures 2-3), suggesting that this is a previously undesribed haplotype.

Next, mapping mtDNA haplotype diversity geographically recovered NW B and OW A haplotypes dominating most sites on and outside Kauaʻi, respectively (Figure 3). While koloa from Kauaʻi indeed carried NW B mtDNA haplotypes, the majority of the nests on Kauaʻi had haplotype 10, whereas the NW haplotypes found on Oʻahu were predominately haplotype 13 (Figures 2-3). One and two nests at Hāmākua and James Campbell National Wildlife Refuge on Oʻahu, respectively, carried the same haplotypes as those found on Kauaʻi. (Figure 3). On Kauaʻi, I only found one egg carrying an OW A mtDNA haplotype across the 118 eggs sequenced, and 51 samples carried NW B mtDNA haplotypes representing eight nests outside of Kauaʻi (Figure 3). I also note that there were no significant differences in OW A versus NW B mtDNA haplotype ratios when comparing mtDNA haplotype diversity sampled here versus those reported from a decade ago by Wells et al. (2019) (Table 6-7). In general, OW A mtDNA diversity continues to be overwhelmingly represented on all islands outside of Kauaʻi, supporting how translocation efforts of the 1980s failed to account for the feral mallard populations that resulted in eventual genetic swamping of those establishing populations (Wells et al. 2019).

Finally, examining evidence for nest parasitism, I found a single egg on Kauaʻi carrying the dominant OW A mtDNA haplotype within a nest that otherwise had NW B haplotype 10 (Table 5), suggesting that the one egg was a result of nest parasitism by a feral female mallard. This nest had six eggs, five of which were haplotype 10, and one haplotype 1, and was found in August but was not a nest that was able to be genetically sexed. In addition to this nest, another nest on Kauaʻi had eggs carrying two different NW B mtDNA haplotypes (10 and 12), suggesting koloa might engage in nest parasitism amongst each other (Table 5). I found

additional instances of nest parasitism at Oʻahu sites Hāmākua (N = 6) and Kawainui (N = 2). In all eight cases, I found a mix of different haplotypes from within the OW A mtDNA group (Table 5). In total, I found ten cases of nest parasitism out of 126 total whole nests that were able to have their mtDNA sequenced.

Spatial Analysis of Habitat

Given the lack in changing mtDNA diversity through time, I used population assignments established with nuclear DNA from Wells et al. (2019), considering Kauaʻi represented koloa only, The Tropical Plantation on Maui represented feral mallards, and with all other sites representing koloa x feral mallard hyvbrid swarms. Also note that Kākoʻo ʻŌiwi and Paikō samples were excluded as I lacked nesting GPS coordinates for these locations. Additionally, a seperate PCA was run for the Oʻahu samples to examine island-specific differences in the hybrid haplotypes found here (Figure 6).

When examining all nests across islands, PC1 accounted for 61.3% and PC2 for 28.5% of the variation found amongst the habitat variables per sample. With PC1, all the variables had approximately equal negative correlation, except water proximity with the weakest correlation, and annual rainfall with the strongest negative correlation (Table 8). In PC2, moisture and annual rainfall had a positive influence while urban proximity and water proximity had a negative influence, with water proximity as the strongest driver of variation in PC2 in general (Table 8). There was some clustering by both species and islands, which is not surprising given that this project only collected koloa on Kauaʻi, only hybrids on Oʻahu, and predominately mallards on Maui (Figure 4). For this reason it is hard to make concrete predictions as to whether variables are being more predictive of species or island. Water proximity was a predicter of nest site

selection for feral mallards and some of Oʻahu's hybrid populationss. Note that there were no predictive variables strongly associated with most hybrid populations, but some weak associations with rainfall, moisture, and water proximity (Figure 4). For koloa, I recovered strong association with moisture, annual rainfall, and urban proximity.

Next, I ran a PCA with only samples with known mtDNA haplotypes. PC1 accounted for 66% and PC2 accounted for 26% of the variation found amongst the habitat variables per sample, and PC3 and PC4 accounted for less than 10% of the variation (Table 9). With PC1, all variables except water proximity had similar positive correlations, with water proximity having the weakest correlation and annual rainfall having the strongest positive correlation (Table 9). PC2 results were similar to those of the original PCA analysis (Table 9). Koloa carrying mtDNA NW B haplotype 10 clustered in areas with higher moisture, higher annual rainfall, and greater urban proximity as recovered in the original PCA (Figure 4). Birds carrying mtDNA OW A Haplotype 1 clustered on the PC1 axis and correlation with water proximity (Figure 5). In general, mtDNA OW A haplotype 9 clustered and overlapped with birds possessing OW A haplotype 1. However, birds possessing OW A haplotypes 9 and 10 showed overlap when examining the PC2 axis, with the two correlated with moisture, annual rainfall, and urban proximity (Figure 5).

Lastly, analyzing Oʻahu alone gave the best opportunity to examine differences between nesting preferences of birds carrying NW B (koloa-like) versus OW A (feral mallard-like) mtDNA ancestry as the island carried the highest diversity of mtDNA. PC1 accounted for 41% of the variation, PC2 accounted for 37.9%, PC3 accounted for 17.7%, and PC4 accounted for 2.7%. In PC1, all but moisture had a positive direction, with urban proximity as the strongest indicator (Table 10). In PC2, all but urban proximity had a positive direction with moisture being

the strongest (Table 10). Regarless, I found no distinctive clustering among haplotype groups as seen in Figure 6, but all the locations are fairly clustered. In general, Hāmākua Marsh Wildlife Sanctuary is most strongly positive correlated with moisture and water proximity,whereas annual rainfall and water proximity is most strongly positive correlated with Kawainui Marsh Wildlife Sanctuary (Figure 6). James Campbell National Wildlife Refuge correlated strongest with urban proximity. I found no strong correlation with birds collected in Honouiluli National Wildlife Refuge and Pouhala (Figure 6). Despite finding some genotype-habitat correlations, mtDNA haplotype diversity is widely scattered in the PC space, indiciating no difference in nesting habitat selection based on mtDNA ancestry. To even further test this, I created a final PCA displaying sites and haplogroups on Oʻahu and once again found both haplogroups scattered around the graph, indicating no strong preference (Figure 7).

Clutch Size

For each location an ANOVA was run to find the average nest size of each location and see if there were significant differences between sites. Note that Pouhala, Kākoʻo ʻŌiwi, and Puʻuloa were excluded as only one nest was collected in each of these locations. Paiko and The Tropical Plantations were also combined because they share similar historical background of domesticated mallards being dumped at those sites and fed. On average, I found a clutch size of 7.76 (range = 6-9.67) across sites that is consistent with clutch sizes of koloa and mallards (Arnold et al., 2022; Swedberg, 1967; Table 11). I found that on average Oʻahu's James Campbell National Wildlife Refuge had the largest number of eggs, followed by Keālia Ponds National Wildlife Refuge, and then The Tropical Plantation on Maui (Table 11). Finally, the koloa population sampled on Kauaʻi had an average clutch size of 6, which is ~2 eggs shy of the

average of feral mallard and koloa x feral mallard populations found on the other islands (Table 11), but consistent with previous clutch size estimates for the species (Swedberg, 1967).

DISCUSSION

Here, I provide the first analysis of sex ratios and updated mtDNA ancestry of koloa, koloa x feral mallard hybrids, and feral mallard nests across Hawaiian Islands. Despite the adult population of koloa on Kauaʻi tending to be 3:1 male-biased (Malachowski et al. 2020), I did not find a significant difference from 1:1 in the sex ratio of the clutches in this population (Table 3). Given a lack of sex bias among koloa clutches, I conclude that the bias in adult populations are due to increased female mortality post-hatch. A potential reason for a higher female mortality could be due to increased vulnerability to predation during nesting and incubation, which is known to generally cause a male-bias in waterfowl populations (Eygenraam, 1957; Ferguson et al., 1981; Ohde et al., 1983). Similarly, I found an even sex-ratio for the koloa x feral mallard hybrid populations assessed on sites in Maui and Oʻahu (Table 3). Together, I did not find support that evolutionary (i.e., koloa) or contemporary (i.e., koloa x feral mallard hybrids) hybrids suffer from Haldenʻs rule as once predicted (Lavretsky et al. 2015; Wells et al. 2019). Conversely, I found a significant male bias in clutches of feral mallard populations on Maui's Tropical Plantation (Table 3). The 6:1 ratio was much more skewed than the 1:1 ratio I had expected, and while there could be a multitude of reasons, the Fisher's Exact Test does not necessarily indicate that a 6:1 ratio is correct, but more so that the mallard sex ratio is statistically likely to not be 1:1. Although, the sex disparity may be due to allelic dropout during molecular sexing, tissue material and DNA quality did not differ from other populations, and therefore, allelic dropout is unlikely a source of bias in this case. Alternatively, given the ancestry of Hawaiʻi's feral mallard populations was established from game-farm mallards (Lavretsky et al. 2023b), it is possible that their domestic history has resulted in changes in life-history traits, including clutch sex ratios (Ahmad et al., 2020, Solberg et al., 2013). Additionally, these feral

mallards are in tourist areas where they are consistnetly fed high caloric diets; and thus, sex ratios could be affected by the Trivers-Willard hypothesis that states offspring sex ratios could be biased towards the sex with higher variation in reproductive behavior (i.e., males) when parents are in good condition (Borgstede 2021; Gaulin & Robbins, 1991; Trivers & Willard, 1973). The Trivers-Willard hypothesis has been found in wild mallards before (Denk 2005). In addition, according to the Local Resource Competition theory, because mallards at the Tropical Plantation are in high density, it could also be that females are producing more males because they are the dispersing sex that would in theory leave the site and create less competition for the female in the future (Clark 1978; Gowaty 1993). Future work will require additional, and more complete clutch sampling of these and other feral mallard populations to determine whether the sex bias was a result of sampling effort in this study or truly due to some extreme selectivity for male biased clutches.

Next, I confirm that the OW A mtDNA haplotypes recovered in Hawai'i are the same as those derived from game-farm mallards released in New Zealand, mainland North American, and Eurasia (Lavretsky et al. 2023b). Mallard-like ducks of North America are characterized by two divergent haplogroups: Old World (OW) A versus New World (NW) B haplogroups (Avise et al., 1990; Fowler et al., 2009; Lavretsky et al., 2014; Wells et al., 2019; Figure 2). The origins of OW A haplotypes in North America puzzled researchers for decades, and only recently were confirmed to be the result of directly releasing game-farm mallards that are of domestic origins (Lavretsky et al. 2019, 2020, 2023b). All domestic mallards are of Eurasian descent, and thus, carry OW A haplotypes (Avise et al., 1990; Johnson & Sorenson, 1999; Kulikova et al., 2005). Although Lavretsky et al. (2023b) was able to determine that Hawai'i's feral mallards share the same game-farm mallard ancestry as in New Zealand, mainland North America, and Eurasia

based on nuclear DNA, they lacked mtDNA to confirm whether the same lineages found in the other locations existed in Hawai'i as well. Together, Lavretsky et al.'s (2023b) nuclear and my mtDNA results confirm that Hawaii's feral mallard populations originated from the release of the game-farm mallard breed like elsewhere.

Although Kaua'i is still dominated by koloa haplotypes, feral mallard mtDNA haplotypes have swamped koloa haplotypes on O'ahu and Maui (Figure 3). Despite the NW B mtDNA haplotype 10 being the dominant haplotype recovered across sampled nests on Kaua'i, it was only recovered across two and one nest on O'ahu's James Campbell NWR and Hāmākua, respectively (Figures 2-3). I posit that the loss of this haplotype in other locations is likely due to a combination of genetic swamping by OW A haplotypes and genetic drift, or to the possibility that the nests sampled are just not fully representative of the adults present. Interestingly, I managed to recover a novel NW B mtDNA haplotype (i.e. haplotype 15, bottom solid blue haplotype in the NW group in Figure 2, listed in Figure 3). This haplotype was only found in one nest at Honouliuil and one nest at Pouhala. Lack of sampling or frequency of this haplotype in past studies may be why it was not reported before. Alternatively, this haplotype may have been recently introduced through wild mallard hybridization. Determining whether the novel haplotype is of wild mallard origin or koloa origin that has since gone extinct on Kaua'i would require additional sampling. Similarly, the OW A haplotype 9 was only found on the North Shore of O'ahu that was consistent with past studies (Wells et al. 2019), and I confirmed to have perfect sequence similarity to previously published game-farm mallards, koloa x feral mallard hybrids, and Pacific black ducks. Finally, mtDNA haplotype 13 was also found in low frequencies on Kaua'i but was the main NW B haplotype found on O'ahu, which may be due to

stochastic events during the 1980s translocation events simply capturing the haplotype in higher frequencies by chance.

Although changes in mtDNA haplogroup ratios betwen previous studies that focused on adult birds (Wells et al. 2019) as compared to this study focusing on clutches could provide a biased view of all possible individuals (breeding and non-breeding) versus breeding-only individuals, respectively, I did not find significant changes in NW B versus OW A mtDNA haplotype ratios across sampled populations (Table 6-7). I conclude that there has been a stabilization in regards to mtDNA diversity since initially being swamped by OW A mtDNA haplotypes occurring post-release, and as early as the late 1990s (Wells et al. 2019). Once again, my work further demonstrates how translocation efforts can fail if not all proximate causes for concern (i.e., hybridization with feral mallards for koloa) are properly managed (Wells et al. 2019).

Finally, I provide the first empirical evidence of inter and intra-nest parasitism among koloa, feral mallards, and their hybrids (Table 5), which is especially concerning as it could provide a mechanism for further mallard introgression on Kauaʻi. In fact, previous work found at least one hybrid per generation in Kauaʻi (Fowler et al., 2009; Wells et al., 2019). Here, I found a single case of interspecific parasitism on Kauaʻi, but note that no mallards were observed as permanent residents of Hanalei National Wildlife Refuge by staff members during the time of this study. Thus, it is possible that the case of recovered interspecific parasitism is a result of immigrating mallards that are not affecting the koloa population at a detrimental level. Nevertheless, continued monitoring is absolutely required to ensure the genetic integrity of remaining koloa. Interestingly, I report the first evidence of koloa conducting intraspecific parasitism, with a single nest possessing a mixture of two different NW B mtDNA haplotypes

(Table 5). Similarly, I found parasitism among known koloa x feral mallard hybrid populations on Oʻahu (Table 5). These results suggest a potential conservation concern in future translocation efforts where hybrids and koloa once again co-exist spatially as they may parasitize one another. And even if they only perform conspecific nest parasitism, this information might provide insight into life-history strategies that might also impact population models. It is possible that some individuals are having clutch size constraints due to external pressures and limitations, causing them to parasitize nests in order for both the remainder of their nest to survive and for the best survival chance of that singular dumped egg (Lyon et al., 2017). Moreover, parasitism may be a result of increased nest site competition, including that the parasitizing female lacking the ability or desire to complete parental care (Harvey et al., 2021).

Considerations When Working with Eggs

Only a fraction of the samples was sexed due to PCR issues. The primers used for this project were used in the past by the Lavretsky lab for a vast array of waterfowl, including koloa, and therefore I assumed primer choice was not the issue. Multiple thermocycler conditions, serial dilutions of DNA and reagents, and a PCR purification protocol were attempted to obtain sex data, but many samples did not obtain conclusive results or had a heavy male bias in what was working. Most of the samples that did not work were from degraded eggs and/or taken from less desirable parts of the egg, which could have resulted in low concentrations or no useable DNA which could be why some of the protocols resulted in inconclusive results. When I experimented with the PCR protocol, multiple cases had only one band show up in gel electrophoresis, resulting in a male identification, whereas it read as female (two bands) when using the original protocol optimized in the Lavretsky lab. The loss in a band with different protocols could be

because allelic dropout occurring as a result of PCR conditions (Tubbs et al., 2009). If this is the case, then one allele may have been preferentially amplified over the other, which would cause a male bias because of females being heterogametic. Being the homogametic sex, allelic dropout or preferential application is not concerning for males as they will be represented with a single band in gel electrophoresis. However, the same issue can be a strong bias when attempting to assess the heterogametic sex, as females in this case, require the amplification of both bands. In the end, I used the original PCR protocols in triplets as accuracy was least compromised given that confirmed females were consistently assigned female but were being identified as males in other protocols; suggesting these other protocols resulted in amplification biases. Importantly, biases in PCR amplification would result in a male-biased conclusion; but this was not the case for all but one population, giving me further confidence that the sex ratios obtained were fairly accurate (Table 3). I acknowledge that attaining sexes per clutch would have allowed for a more direct test of each clutch. However, sufficient numbers of eggs were sexed that permitted testing of sex ratio deviations at the population level, which is more directly applicable sex ratios assessed across adult populations. I also note that overall 52% success rate in sexing eggs here is much lower than the >90% success rate in previous studies (Lavretsky et al. 2023a). Thus, future studies should aim to troubleshoot and determine whether levels of degradation or other factors (i.e., humidity levels) limit success of sex identification of eggs, and whether sampling protocols can be optimized to increase PCR success rates.

Nesting Habitat Selection by Species and Haplotype

While some species and haplotypes were more associated with certain habitat variables over others (Figures 4-6; Tables 8-10), results for the Oʻahu only PCA suggest little distinctions

between habitat preference and mtDNA haplotype diversity (Figure 6; Table 10). First, the stronger correlations to variables found in all the PCA figures were clustered by island, site, and species (Figure 8). However, given that koloa x feral mallard hybrids were primarily taken from Oʻahu, koloa from Kauaʻi, and feral mallards collected on Maui, it is currently impossible to distinguish whether recovered clustering is simply due to island-specific ecological differences or whether these are due to genetic x environmental associations. Moreover, examining the PCA for just Oʻahu locations recovered all haplotypes scattered across space and environments, suggesting each mtDNA haplotype does not have a specific nesting habitat preference. In fact, these results are unsurprising as a general lack of diversifying space may simply limit the diversity and type of niche space even available for these birds. I also acknowledge that mtDNA diversity is insufficient to determine any genetic associations, and that nuclear data is required to attain the exact koloa and feral mallard ancestry present in clutches. In addition to the need of nuclear data, I am limited by the number of sites sampled outside of Oʻahu, limiting my ability to have any conclusive inference. Thus, future research into habitat selection assessments will benefit from nuclear data across additional sampling locations across islands, as well as variables unmeasurable from GIS (e.g., food source and water quality) that can be obtained from vegetative and water quality sampling throughout the year or by remote sensing.

Although I conclude a lack of nesting preferences based on mtDNA ancestry, examining clutch sizes per site could be telling on which sites may be more beneficial for ducks because the more food resources should result in more eggs (Decker et al., 2012). Towards this, I recovered National Wildlife Refuges that are actively managed for habitat quality and predators had clutches with the most number of eggs (Table 11). Importantly, my findings are encouraging given that James Campbell is the site with the most confirmed koloa mtDNA haplotypes.

Similarly, recovering large clutches in The Tropical Plantations of Maui is consistent with the high caloric feeding programs implemented there, and hinting at a major problem of mallard management in Hawaiʻi.

Koloa Cultural Significance

Understanding cultural significance of koloa is of equal importance to the biological context as they have significant ties to indigenous culture and therefore require acknowledgement and respect of their cultural place and meaning. This also gives insight into the importance of conserving the species beyond biological reasoning. Before European settlement, many Hawaiian communities used snares and nets to catch koloa, and they served as an important food source for many communities, particularly those further away from oceans for which koloa and other birds were the sole source of wild protein (e.g., Kahuku) (Gomes, 2015; Gomes, 2016). Koloa are a species associated with clean loʻi (taro fields), fishponds, and marshes, and they have been integrated in place names that express the connection to the land koloa were once prevalent in (Gomes, 2020; Waikōloa, N.d.). For example, Waikōloa translates to koloa pond (or duck water) that refers to the standing water that once hosted waterbirds including koloa (Waikōloa, N.d.). Most notable in Hawaiian legends is the story of ʻĪmaikalani, a fierce blind warrior chief of Kaʻū. It was said that he had two koloa that would alert him of the presence of his enemies by quacking at their location and guide his spears to his target with deadly accuracy. As ʻĪmaikalani was noted to have killed several chiefs in battle, in the early 1500s, when ʻUmialīloa (ʻUmi) wanted to become the sole ruler of Hawaiʻi Island, he found a warrior to kill ʻĪmaikalani. The warrior said "To kill ʻĪmaikalani, you must first kill the koloa…who are ʻĪmaikalani's guards and who give him warning of the approach of any person. Kill the birds…then you will be able to kill the guards and that is how I was able to kill him."

(Young, 2016). In addition, due to their long cultural importance, koloa taxonomy always used indigenous knowledge, further showcasing the time and care taken to understand their place in Hawaiian ecosystems by native people (Gomes, 2020).

CONCLUSION

This study was initiated because of the need to understand life-history traits and hybridization rates of the koloa in order for future koloa translocations to be successful. First, I successfully filled in critical knowledge gaps regarding koloa sex ratios at clutch. Specifically, I did not find a sex bias among clutches that is disparate to the 3:1 male-bias seen in adult populations (Malachowski, 2020); and therefore, management actions can be taken to mitigate increased female mortality occurring post-hatch (e.g., reduced predation). Next, I was able to showcase that mtDNA diversity has not changed since the 2010s, which is encouraging because it means that perhaps the rate of hybridization is slowing and is potentially manageable. Importantly, I was able to confirm that OW A mtDNA haplotypes that entered Hawai'i were indeed due to introduction of game-farm mallards that pose conservation concern world-wide today (Lavretsky et al. 2023b).

Together, my work fills in critical knowledge gaps regarding koloa and non-koloa life-history traits that will be integrated to re-optimize management models (Hernandez et al. 2023), which are critical for future koloa translocation efforts. Nevertheless, updating ancestry assignments based on nuclear data is critical. Moreover, given that I recovered a unique koloa mtDNA haplotype at Pouhala Marsh Wildlife Sanctuary and Honouliuli National Wildlife Refuge, expanding genetic surveying of other locations is also important when attempting to determine what koloa ancestry remains. Particularly, mountain ranges and streams where conservation folk alike have witness koloa have had no surveying done, which could lead to new insight on both their ancestry and life cycle. Most importantly, any future translocation efforts will require active control of feral mallards, predators, invasive species, as well as continued habitat restorative efforts to increase habitat availability. More generally, I highlight the

importance of understanding the genetic make up of species in order to understand life-history

traits and species interactions that may influence their ability to navigate in the wild, which in

turn, helps us understand how to manage and conserve wildlife better in our changing world.

APPENDIX A: TABLES

Table 1. DNA extraction results with total samples categorized as either degraded, high quality DNA, and failed (i.e., no visible DNA).

Locations	Degraded DNA	High molecular-weight DNA	No DNA
Keālia (hybrids)	5	12	0
Tropical Plantation (mallards)	62	69	105
Maui Total	67	81	105
Hāmākua	231	175	258
Honouliuli	11	27	14
James Campbell	6	23	0
Kākoʻo ʻŌiwi	4	1	1
Kawainui	17	21	56
Paikō	4	7	2
Pouhala	1	7	2
Waiawa Kai, Puʻuloa	4	0	0
Oʻahu Total (All location hold hybrids)	278	261	333
Kauaʻi Total (koloa)	70	67	8

Table 2. Sample specific success rates of DNA extraction.

Sample Type	Success	Not Success	Percent success
Membrane Samples	216	125	63%
Yolk Samples	370	343	52%
Tissue Samples	245	3	99%

Table 3. Sex ratio results.

Sex per Location with Species Indicated	Male	Female	Inconclusive
Keālia (hybrids)	5	3	9
Tropical Plantation (mallards)	37	6	89
Maui Total	42	9	98

Hāmākua	116	125	165
Honouliuli	11	10	17
James Campbell	18	8	3
Kākoʻo ʻŌiwi	1	2	2
Kawainui	14	8	16
Paikō	4	3	6
Pouhala	3	4	1
Waiawa Kai, Puʻuloa	1	2	1
Oʻahu Total (All location hold hybrids)	168	162	211
Hanalei	20	30	87
Kauaʻi Total (koloa)	20	30	87

Sex Ratio per Species			Fisherʻs Exact Test P-value
Koloa overall ratio (2:3)	20 (40%)	30 (60%)	0.42
Hybrid overall ratio (1:1)	173 (51%)	165 (49%)	0.82
Mallard overall ratio (6:1)	37 (86%)	6 (14%)	0.00040

Table 4. Success rates of samples being sexed, examining both the type of sample and quality.

Sample Type	Worked	Didn't Work	Percentage Success
Membrane Sample	72	120	38%
Yolk Sample	120	240	33%
Tissue Sample	239	10	96%
DNA quality			
Low molecular weighted band	29	122	19%
Degraded DNA	143	99	59%
High molecular weighted band	259	149	63%

Table 5. Cases of nest parasitism, with location, dominant haplogroup, dominant haplotype number, as well as the number and ID of the alternative mtDNA haplotype recovered in the same nest.

Location	Dominant Haplo-group	Dominant Haplotype	Region	Other Haplotypes
Kauaʻi	B	Hap_10	KNWR	1 Hap_1 (A)

Kaua'i	B	Hap_10	KNWR	2 Hap_12 (B)
O'ahu	A	Hap_1	Hāmākua	1 Hap_9 (A)
O'ahu	A	Hap_9	Hāmākua	1 Hap_1 (A)
O'ahu	A	Hap_13	Hāmākua	2 Hap_1 (A)
O'ahu	A	Hap_1	Hāmākua	1 Hap_9 (A)
O'ahu	A	Hap_9	Hāmākua	1 Hap_1 (A)
O'ahu	B	Hap_10	Hāmākua	1 Hap_1 (A)
O'ahu	A	Hap_9	Kawainui	1 Hap_1 (A)
O'ahu	A	Hap_1	Kawainui	1 Hap_8 (A)

Table 6. Per site comparison of mitochondrial haplogroups OW A and NW B ratios between overlapping sites recovered in the 2010s and here, along with the resulting significance.

Region	A:B ratios (Current)	A:B ratios (2010s)	Chi-square p-value
KNWR	0:24	3:143	0.48
Keālia Ponds	1:0	2:2	0.36
Hāmākua	66:4	57:1	0.25
JCNWR	2:1	4:9	0.25
Kawainui	7:0	1:0	N/A

Table 7. Per island comparison of mitochondrial haplogroups OW A and NW B ratios between overlapping sites recovered in the 2010s and here, along with the resulting significance.

Island	A:B ratios (Current)	A:B ratios (2010)	Chi-square p-value
Maui	1:0	2:2	0.36
O'ahu	75:5	62:10	0.11
Kaua'i	0:24	3:143	0.48

Table 8. PCA Eigenvectors for all nests across islands, with each variable denoted as positively or negatively associated.

Habitat Variables	PC1	PC2	PC3	PC4
Moisture	-0.57	0.22	-0.54	-0.57
Annual Rainfall	-0.62	0.10	-0.10	0.77
Urban Proximity	-0.52	-0.34	0.73	-0.29
Water Proximity	-0.02	-0.91	-0.42	0.05

Table 9. PCA Eigenvectors for nests across islands grouped into specific mitochondrial haplotypes, with each variable denoted as postiviely or negatively associated.

Habitat Variables	PC1	PC2	PC3	PC4
Moisture	0.56	0.26	0.61	-0.49
Annual Rainfall	0.60	0.16	0.02	0.78
Urban Proximity	0.56	-0.18	-0.72	-0.37
Water Proximity	0.15	-0.94	0.32	0.06

Table 10. PCA Eigenvectors for nests found on Oʻahu only, with each variable denoted as postiviely or negatively associated.

Habitat Variables	PC1	PC2	PC3	PC4
Moisture	-0.15	0.78	0.02	-0.61
Annual Rainfall	0.50	0.48	-0.55	0.47
Urban Proximity	0.66	0.35	-0.23	-0.62
Water Proximity	0.53	0.21	0.81	0.16

Table 11. Per location's average clutch size, along with associated ANOVA test for significance.

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
HANWR	24	144	6	2.70		
Hamakua	87	660	7.57	6.20		
Honouliuli	7	53	7.57	6.62		
JCNWR	3	29	9.67	5.33		
Kawainui	13	94	7.23	5.53		
KPWR	2	16	8.00	8.00		
Tropical Plantation and Paiko	30	250	8.33	13.68		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*		
Between Groups	91.04	6	15.17	2.16	*P-value*	*F crit*
Within Groups	1116.46	159	7.02		0.049	216
Total	1207.49	165				

APPENDIX B: FIGURES

Figure 1. Study site locations moving east to west on Kauaʻi, Oʻahu, and Maui.

Figure 2. Haplotype network reconstructed in PopArt (Leigh & Bryant 2015). The two major haplogroups (Old World A on the left, New World B on the right) and samples color coded by reference group, samples in this study, and samples sequenced referenced from previous studies. Reference sequences include those for game-farm (domesticated origins) mallards, park ducks, wild mallards, koloa, and Laysan ducks. Included is also sequences from a previous study that examined birds from Oʻahu, Maui, Kauaʻi, and Hawaiʻi Island, which are labeled as such for a side comparison with samples taken more recently in this study.

Figure 3. Per location mitochondrial (mtDNA) haplotype ratios denoted by number and haplogroup denoted by color.

Figure 4. PCA analysis of all nests across all islands. Samples are color coded by island and shaped by species as either koloa (HAWD), feral mallard (MALL), or koloa x feral mallard hybrids (hybrid).

Figure 5. PCA analysis of all nests across all islands. Samples are color coded by mtDNA haplotype and shaped by species as either koloa (HAWD), feral mallard (MALL), or koloa x feral mallard hybrids (hybrid).

Figure 6. PCA analysis of Oʻahu nests only. Samples are color coded by haplotype and shaped by specific location.

Figure 7. PCA analysis of Oʻahu nests only. Samples are color coded by haplogroup and shaped by specific location.

REFERENCES

Adavoudi, R., & Pilot, M. (2021). Consequences of hybridization in mammals: A systematic review. *Genes*, *13*(1), 50.

Ahmad, H. I., Ahmad, M. J., Jabbir, F., Ahmar, S., Ahmad, N., Elokil, A. A., & Chen, J. (2020). The domestication makeup: Evolution, survival, and challenges. *Frontiers in Ecology and Evolution*, *8*, 103.

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome research*, *19*(9), 1655-1664.

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. *Trends in ecology & evolution*, *16*(11), 613-622.

Arnold, T. W., Howerter, D. W., Devries, J. H., Joynt, B. L., Emery, R. B., & Anderson, M. G. (2002). Continuous laying and clutch-size limitation in mallards. *The Auk*, *119*(1), 261-266.

Avise, J. C., Ankney, D. C., & Nelson, W. S. (1990). Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. Evolution, 44, 1109–1119. https://doi.org/10.1111/j.1558-5646.1990. tb03829.x

Banko, P. D., David, R. E., Jacobi, J. D., & Banko, W. E. (2001). Conservation status and recovery strategies for endemic Hawaiian birds. *Studies in Avian Biology*, *22*, 359-376.

Barun, A., Hanson, C. C., Campell, K. J., & Simberloff, D. (2011). A review of small Indian mongoose management and eradications on islands.

Berger, A. J. (1970). The present status of the birds of Hawaii.

Black, J. M. (1995). The Nene Branta sandvicensis recovery initiative: research against extinction. *Ibis*, *137*, S153-S160.

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 & Evolution*, *1*(5), 1-5.

Borgstede, M. (2021). Evolutionary dynamics of the Trivers–Willard effect: A nonparametric

       approach. *Ecology and evolution*, *11*(18), 12676-12685.

Browne, R. A., Griffin, C. R., Chang, P. R., Hubley, M., & Martin, A. E. (1993). Genetic

       divergence among populations of the Hawaiian Duck, Laysan Duck, and Mallard. *The*

       Auk, *110*(1), 49-56.

Çakmak, E., Akın Pekşen, Ç., & Bilgin, C. C. (2017). Comparison of three different primer sets

       for sexing birds. *Journal of Veterinary Diagnostic Investigation*, *29*(1), 59-63.

Canu, A., Vilaça, S. T., Iacolina, L., Apollonio, M., Bertorelle, G., & Scandura, M. (2016). Lack

       of polymorphism at the MC1R wild-type allele and evidence of domestic allele

       introgression across European wild boar populations. *Mammalian Biology*, *81*(5), 477-

       479.

Chan, W. Y., Hoffmann, A. A., & van Oppen, M. J. (2019). Hybridization as a conservation

       management tool. *Conservation Letters*, *12*(5), e12652.

Clark, A. B. (1978). Sex ratio and local resource competition in a prosimian

       primate. *Science*, *201*(4351), 163-165.

Crutzen, P. J. (2006). The "anthropocene". In *Earth system science in the anthropocene* (pp. 13-

       18). Berlin, Heidelberg: Springer Berlin Heidelberg.

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), 106713.

       https://doi.org/10.1371/journal.pone.0106713

Decker, K. L., Conway, C. J., & Fontaine, J. J. (2012). Nest predation, food, and female age explain seasonal declines in clutch size. *Evolutionary Ecology*, *26*, 683-699.

Denk, A. G. (2005). *Male and female reproductive tactics in Mallards (Anas platyrhynchos L.): sperm competition and cryptic female choice*. Cuvillier Verlag.

Department of Land and Natural Resources. (2023). Laysan duck. Division of Forestry and Wildlife: Wildlife Program. https://dlnr.hawaii.gov/wildlife/birds/laysan-duck/#:~:text=The%20Laysan%20duck%20is%20a,Northwestern%20Hawaiian%20Islands%20(NWHI).

Engilis Jr, A., & Pratt, T. K. (1993). Status and population trends of Hawaii's native waterbirds, 1977-1987. *The Wilson Bulletin*, 142-158.

Engilis Jr, A., Pyle, R., & David, R. (2004). Status and occurrence of migratory birds in the Hawaiian Islands: Part 1–Anseriformes: Anatidae (Waterfowl). *Bishop Museum Occasional Paper*, *81*.

Engilis, A., Uyehara, K. J., & Giffin, J. G. (2002). *Hawaiian Duck: Anas wyvilliana*. Birds of North America, Incorporated.

Eygenraam, J. A. (1957). *The sex-ratio and the production of the Mallard, Anas platyrhynchos L*. ITBON.

Ferguson, E. L., Jorde, D. G., & Sease, J. J. (1981). Use of 35-mm color aerial photography to acquire mallard sex ratio data. *Photogrammetric Engineering and Remote Sensing*, *47*(6), 823-827.

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*, 1747-1758.

Gaulin, S. J., & Robbins, C. J. (1991). Trivers-Willard effect in contemporary North American

       society. *American Journal of Physical Anthropology*, *85*(1), 61-69.

Gomes, N. J. (2020). Reclaiming native Hawaiian knowledge represented in bird

       taxonomies. *Ethnobiology Letters*, *11*(2), 30-43.

Gomes, N. (2016). Some traditional native Hawaiian bird hunting practices on Hawai'i

       Island. *Hawaiian Journal of History*, *50*(50), 33-51.

Gomes, N. J. (2015). Meha ka Leo i ka Nahele: He Noiʻina i ka Poʻe Kāpili Manu o ke Au

       Kahiko. *University of Hawaii Hilo.*

Gowaty, P. A. (1993). Differential dispersal, local resource competition, and sex ratio variation

       in birds. *The American Naturalist*, *141*(2), 263-280.

Haldane, J. B. (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of*

       genetics, *12*(2), 101-109.

Hart, P., Tanimoto-Johnson, A., & Harriman-Pote, S. (2022). *Manu Minute: Hawaiʻi's endemic*

       duck. Hawai'i Public Radio. Retrieved March 1, 2023, from

       https://www.hawaiipublicradio.org/podcast/manu-minute/2022-08-29/manu-minute-

       hawaiis-endemic-duck

Harvey, K., Lavretsky, P., Foth, J., & Williams, C. K. (2021). Nest parasitism, promiscuity, and

       relatedness among wood ducks. *Plos one*, *16*(12), e0257105.

Heikkinen, M. E., Ruokonen, M., White, T. A., Alexander, M. M., Gündüz, İ., Dobney, K. M., ...

       & Pyhäjärvi, T. (2020). Long-term reciprocal gene flow in wild and domestic geese reveals

       complex domestication history. *G3: Genes, Genomes, Genetics*, *10*(9), 3061-3070.

Hernández, F., Brown, J. I., Kaminski, M., Harvey, M. G., & Lavretsky, P. (2021). Genomic evidence for rare hybridization and large demographic changes in the evolutionary histories of four North American dove species. *Animals*, *11*(9), 2677.

Hernández, F., Janzen, T., & Lavretsky, P. (2023). simRestore: A decision-making tool for adaptive management of the native genetic status of wild populations. *Molecular Ecology Resources*.

Hutchison III, C. A., Newbold, J. E., Potter, S. S., & Edgell, M. H. (1974). Maternal inheritance of mammalian mitochondrial DNA. *Nature*, *251*(5475), 536-538.

Jones, O. R., & Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular ecology resources*, *10*(3), 551-555.

Johnson, K. P., & Sorenson, M. D. (1999). Phylogeny and biogeography of dabbling ducks (genus: Anas): a comparison of molecular and morphological evidence. *The Auk*, *116*(3), 792-805.

Johnson, S. D. (1994). Sex Ratio and Population Stability. *Oikos*, *69*(1), 172–176. https://doi.org/10.2307/3545299

Johnson, W. E., Onorato, D. P., Roelke, M. E., Land, E. D., Cunningham, M., Belden, R. C., ... & O'Brien, S. J. (2010). Genetic restoration of the Florida panther. *Science*, *329*(5999), 1641-1645.

Kulikova, I. V., Drovetski, S. V., Gibson, D. D., Harrigan, R. J., Rohwer, S., Sorenson, M. D., ... & McCracken, K. 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., Engilis Jr, 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.

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.

Lavretsky, P., Hoyt, A., Musni, V. M., Howell, D., & Williams, C. K. (2023a). Frequency and types of alternative breeding strategies employed by nesting American black ducks in North Carolina. *Plos one*, *18*(2), e0278905.

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.

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.

Lavretsky, P., Mohl, J. E., Söderquist, P., Kraus, R. H., Schummer, M. L., & Brown, J. I. (2023b). The meaning of wild: Genetic and adaptive consequences from large-scale releases of domestic mallards. *Communications Biology*, *6*(1), 819.

Leigh, J. W., & Bryant, D. (2015). POPART: full-feature software for haplotype network construction. *Methods in ecology and evolution*, *6*(9), 1110-1116.

Leitwein, M., Gagnaire, P. A., Desmarais, E., Berrebi, P., & Guinand, B. (2018). Genomic

    consequences of a recent three-way admixture in supplemented wild brown trout

    populations revealed by local ancestry tracts. *Molecular Ecology*, *27*(17), 3466-3483.

Lenz, T. L., Jacob, A., & Wedekind, C. (2007). Manipulating sex ratio to increase population

    growth: the example of the Lesser Kestrel. *Animal Conservation*, *10*(2), 236-244.

Lyon, B. E., & Eadie, J. M. (2017). Why do birds lay eggs in conspecifics' nests?. *Avian Brood*

    Parasitism: Behaviour, Ecology, Evolution and Coevolution, 105-123.

Malachowski CP. (2020). Movement Ecology and Population Dynamics of the Endangered

    Hawaiian Duck (*Anas wyvilliana*). *Oregon State University.*

Mālama Hawaiʻi and USFWS. (2009). Koloa Maoli Communication and Outreach Plan.

Mattucci, F., Galaverni, M., Lyons, L. A., Alves, P. C., Randi, E., Velli, E., ... & Caniglia, R.

    (2019). Genomic approaches to identify hybrids and estimate admixture times in

    European wildcat populations. *Scientific Reports*, *9*(1), 1-15.

 McKinney, F. (1985). Primary and secondary male reproductive strategies of dabbling

    ducks. *Avian monogamy*, *37*, 68-82.

Moran, B. M., Payne, C., Langdon, Q., Powell, D. L., Brandvain, Y., & Schumer, M. (2021).

    The genomic consequences of hybridization. *Elife*, *10*, e69016.

Ohde, B. R., Bishop, R. A., & Dinsmore, J. J. (1983). Mallard reproduction in relation to sex

    ratios. *The Journal of Wildlife Management*, 118-126.

Ottenburghs, J. (2019). Multispecies hybridization in birds. *Avian Research*, *10*(1), 1-11.

Ottenburghs, J. (2021). The genic view of hybridization in the Anthropocene. *Evolutionary*

    Applications, *14*(10), 2342-2360.

Ottenburghs, J., Ydenberg, R. C., van Hooft, W. F., Van Wieren, S. E., & Prins, H. H. (2015).
The Avian Hybrids Project: gathering the scientific literature on avian
hybridization. *Ibis*, *157*(4), 892-894.

Otto, S. P. (2018). Adaptation, speciation and extinction in the Anthropocene. *Proceedings of the
Royal Society B*, *285*(1891), 20182047.

Pievani, T. (2014). The sixth mass extinction: Anthropocene and the human impact on
biodiversity. *Rendiconti Lincei*, *25*, 85-93.

Puigcerver, M., Sanchez-Donoso, I., Vilà, C., Sardà-Palomera, F., García-Galea, E., &
Rodríguez-Teijeiro, J. D. (2014). Decreased fitness of restocked hybrid quails prevents
fast admixture with wild European quails. *Biological conservation*, *171*, 74-81.

Reynolds, M. H., Courtot, K. N., Brinck, K. W., Rehkemper, C. L., & Hatfield, J. S. (2015).
Long-term monitoring of endangered Laysan ducks: Index validation and population
estimates 1998–2012. *Journal of Fish and Wildlife Management*, *6*(1), 92-101.

Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual
review of ecology and systematics*, *27*(1), 83-109.

Rhymer, J. M., Williams, M. J., & Braun, M. J. (1994). Mitochondrial analysis of gene flow
between New Zealand mallards (Anas platyrhynchos) and grey ducks (A.
superciliosa). *The Auk*, *111*(4), 970-978.

Shields, G. F., & Helm-Bychowski, K. M. (1988). Mitochondrial DNA of birds. In *Current
ornithology* (pp. 273-295). Boston, MA: Springer US.

Simberloff, D. (2013). *Invasive species: what everyone needs to know*. Oxford University Press.

Söderquist, P., Elmberg, J., Gunnarsson, G., Thulin, C. G., Champagnon, J., Guillemain, M., ...
& Kraus, R. H. (2017). Admixture between released and wild game birds: a changing

genetic landscape in European mallards (Anas platyrhynchos). *European Journal of Wildlife Research*, *63*(6), 1-13.

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*, 1-12.

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.

Solberg, M. F., Skaala, Ø., Nilsen, F., & Glover, K. A. (2013). Does domestication cause changes in growth reaction norms? A study of farmed, wild and hybrid Atlantic salmon families exposed to environmental stress. *PloS one*, *8*(1), e54469.

Sorenson, M. D., Cooper, A., Paxinos, E. E., Quinn, T. W., James, H. F., Olson, S. L., & Fleischer, R. C. (1999). Relationships of the extinct moa-nalos, flightless Hawaiian waterfowl, based on ancient DNA. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1434), 2187-2193.

State of Hawaii. (2023). Retrieved March 1, 2023, from

https://files.hawaii.gov/dbedt/economic/library/facts/Facts_Figures_browsable.pdf

Steifetten, Ø., & Dale, S. (2006). Viability of an endangered population of ortolan buntings: the effect of a skewed operational sex ratio. *Biological Conservation*, *132*(1), 88-97.

Swedberg, G. E. (1967). *The koloa: a preliminary report on the life history and status of the Hawaiian Duck (Anas wyvilliana)*. Wildlife Branch.

Teijeiro, J. D. (2014). Decreased fitness of restocked hybrid quails prevents fast admixture with wild European quails. *Biological conservation*, *171*, 74-81.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, *179*(4068), 90-92.

Tubbs, R., Stoler, M., & Hunt, J. L. (2009). Loss of Heterozygosity. In *Cell and tissue based molecular pathology*. essay, Churchill Livingstone/Elsevier.

Pilot, M., Greco, C., vonHoldt, B. M., Randi, E., Jędrzejewski, W., Sidorovich, V. E., ... & Wayne, R. K. (2018). Widespread, long-term admixture between grey wolves and domestic dogs across Eurasia and its implications for the conservation status of hybrids. *Evolutionary Applications*, *11*(5), 662-680.

Pyle, R. L., & Pyle, P. (2017). The birds of the Hawaiian Islands: occurrence, history, distribution, and status. *BP Bishop Museum, Honolulu, HI, USA. Version*, *2*(1).USFWS. (2012). Recovery plan for Hawaiian waterbirds.

U.S. Fish and Wildlife Service. (2020). *Endangered and Threatened Wildlife and Plants; Reclassifying the Hawaiian Goose From Endangered to Threatened With a Section 4(d) Rule*. Federal Register. Retrieved February 22, 2024, from https://www.federalregister.gov/documents/2019/12/19/2019-26548/endangered-and-threatened-wildlife-and-plants-reclassifying-the-hawaiian-goose-from-endangered-to

U.S. Fish and Wildlife Service. (n.d.). *Listed Animals.* Environmental Conservation Online System. Retrieved March 8, 2024, from https://ecos.fws.gov/ecp0/reports/ad-hoc-species-report?kingdom=I&status=SAT&mapstatus=3&fcrithab=on&fstatus=on&fspecrule=on&finvpop=on&fgroup=on&header=Listed%2BAnimals.

U.S. Geological Survey. (2022). *A Climate Change Canary in the Coal Mine - the Endangered Hawaiian Honeycreepers*. U.S. Geological Survey. Retrieved April 2, 2023,

from www.usgs.gov/news/featured-story/climate-change-canary-coal-mine-endangered-hawaiian-honeycreepers.

Waikōloa. (n.d.). Retrieved March 1, 2023, from https://www.waikoloadryforest.org/place-waikoloa-ahupuaa/#:~:text=You'll%20see%20the%20place,birds%20including%20the%20native%20Koloa.

Walther, M. (2016). *Extinct birds of Hawaiʻi*. Mutual Publishing, LLC.

Wang, M., Lu, Y., Botella, J. R., Mao, Y., Hua, K., & Zhu, J. K. (2017). Gene targeting by homology-directed repair in rice using a geminivirus-based CRISPR/Cas9 system. *Molecular plant*, *10*(7), 1007-1010.

Wells, C. P., Lavretsky, P., Sorenson, M. D., Peters, J. L., DaCosta, J. M., Turnbull, S., ... & Engilis Jr, 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.

Wilcox, C. (1997). *Sugar water: Hawaii's plantation ditches*. University of Hawaii Press.

Young, P. T. (2016). *ʻĪmaikalani*. Images of Old Hawaiʻi. Retrieved March 1, 2023, from https://imagesofoldhawaii.com/imaikalani/

CURRICULUM VITA

Kristi Fukunaga joined the University of Texas at El Paso (UTEP) in the Fall 2022 as a graduate student in Biological Sciences after working in wildlife management, research, and rehab in the Hawaiian Islands for 5 years. During her time in Hawaiʻi, she gained work and volunteer experience doing predator control, forest restoration, forest bird counts, working with captive wildlife, handling, banding, and studying an array of native Hawaiian birds, and much more. From these experiences, she learned the top issues Hawaiian birds face are habitat loss, invasive species, and disease. For this reason, an understanding of both spatial analysis and genetics were tools she wanted to gain through her masters, so she chose her studies in the Lavretsky lab to gain insight of how to use genetics in conservation and spatial science classes. Her masters research consisted of field work collecting duck eggs across Hawaiian Islands and coordinating with multiple agencies to assist before even enrolling in school while working full time as a park ranger, many hours of genetic lab work, and writing and analyzing her study system. Throughout her studies at UTEP she worked as a teaching assistant and during the summer she led a crew of technicians in American Samoa on a study of Pacific Black Ducks, later being a research assistant to help analyze and process a majority of the data collected from that project. Upon her last semester, she took a job as the Avian Malaria Research Associate with United States Geological Survey at the Pacific Island Ecosystem Research Center stationed in Hawaiʻi Volcano National Park, while finishing up her degree. Here, her work focuses on finding candidate genes and/or microbial communities that may help Hawaiian forest birds become resistant against avian malaria, the leading cause of extinction of Hawaiian honeycreepers today.