Contemporary genetic diversity and translocation plans for an endangered Hawaiian honeycreeper, the Kiwikiu (Maui Parrotbill; *Pseudonestor xanthophrys*)

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INTRODUCTION

Kiwikiu species background:

- Critically endangered Hawaiian honeycreeper
- Population ~500 individuals
- Restricted to 50 km^2 on the windward slopes of Haleakala volcano, Maui
- Highly specialized insectivore
- Only raise one offspring per year
- Juveniles have a long juvenile dependency (up to 18 months)

Recent demographics work:

- High adult survival (78%) and low juvenile survival (17%)
- Annual reproductive success 46%

Establishing a second population of Kiwikiu has been identified as a high priority recovery action by the USFWS.

Why conservation genetics?

- Are there genetic factors affecting the extinction risk for Kiwikiu to a greater magnitude than the small population size?
- Are there details in the current genetic structure and diversity that would allow management decisions to be made that could minimize future inbreeding and additional loss of genetic diversity in the population?
- How can we relate these questions on genetic diversity to plans for reintroducing Kiwikiu in a new area and establishing a second population in the future?

METHODS

We collected a total of 120 Kiwikiu blood and/or feather samples from individual Kiwikiu (92 from the east, 17 from the west and 11 in captivity) **(Figure 1)**.

mtDNA amplification

We used the control-region primers LCRL1 (5'-CGCTATGACCCTCCACGAA-3') and HCR1045 (5'-GAGACGACCTTATCCGCAAA-3') (Tarr 1995) to target a section of the mitochondrial genome (mtDNA). PCR products were sequenced by Macrogen Genomics in Seoul, Korea and by Source BioScience in Nottingham, United Kingdom.

Characterization and amplification of Microsatellite markers

Microsatellites were isolated from an enriched genomic library following procedures set out in Jones et al. (2002) by Genetic Identification Services. Sixteen loci (13 polymorphic and 3 monomorphic) were selected for further optimization. Fluorescently labeled DNA fragments were detected using an Applied Biosystems 3730 DNA Analyzer with GeneScan ROX-500 size standard (DBS Genomics, Durham, United Kingdom).

Data analysis

mtDNA

Samples were sequenced by Macrogen Inc. and Biosource Sciences. Chromatographs were edited using FinchTV (Geospiza Inc.). Dominant sequences were generated using the highest peak at each site. Sequences were aligned in ClustalX Version 2 (Larkin et al. 2005). Standard DNA polymorphism and genetic differentiation measures were calculated in DnsSP Version 4.00 (Rozas et al. 2003).

Microsatellites

Genotypes were scored using GeneMapper Software (Applied Biosystems). Heterozygosities and number of alleles per locus were calculated in GenAlEx 6 (Peakall & Smouse 2006). We tested for significant deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium in GenePop 4.0 (Raymond & Rousset 1995). We selected 12 polymorphic loci for further analyses **(Figure 2)**.

We examined subpopulation structuring using an analysis of molecular variance (AMOVA) framework in GenAlEx 6 following methods of Excoffier et al. (1992). AMOVA provided estimates of traditional *F*-Statistics (Weir and Cockerham 1984), as well as their analogues (R_{ST} and ϕ_{PT}). We calculated pairwise F_{ST} and R_{ST} for the East, West, and Captive subpopulations.

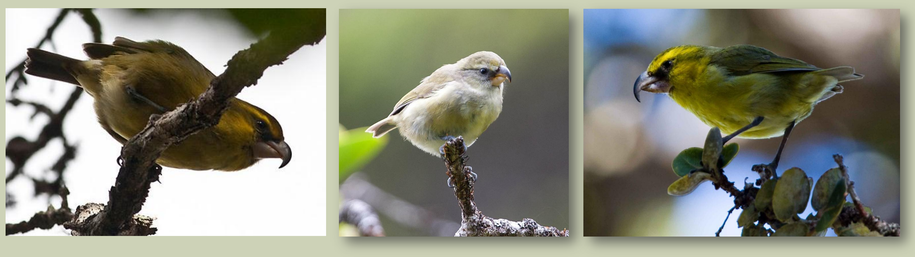

Figure 1. A total of 120 Kiwikiu were sampled between 1996-2011, including 11 individuals currently in captivity. Samples were primarily clustered east of Ko'olau gap within the Hanawi Natural Area Reserve and west of Ko'olau gap within the Waikamoi Preserve.

RESULTS

We amplified a 552 bp length on the control region in 85 of our individuals representing all population groups. Our overall haplotype diversity was 0.38 and frequencies of each haplotype varied per group **(Figure 3 and Figure 4)**.

We found the highest levels of heterozygosity in the east **(Figure 5 and Figure 6)**.

	Overall	Captive	East	West
Observed Heterozygosity	0.574 (0.052)	0.599 (0.099)	0.618 (0.081)	0.505 (0.096)
Expected Heterozygosity	0.534 (0.045)	0.512 (0.081)	0.605 (0.073)	0.485 (0.082)
Unbiased Expected Heterozygosity	0.550 (0.046)	0.541 (0.085)	0.609 (0.073)	0.500 (0.084)
No. of Different Alleles	4.878 (0.416)	3.818 (0.519)	6.727 (0.714)	4.090 (0.609)
No. of Effective Alleles	2.770 (0.227)	2.512 (0.295)	3.386 (0.499)	2.413 (0.319)

The highest level of sub-population differentiation was between the west and the captive
F_{IS} = -0.044 (0.037), F_{IT} = 0.014 (0.039), F_{ST} = 0.056 (0.012);
Pairwise R_{ST} East and West = 0.061, East and Captive = 0.031, West and Captive = 0.162.

Population Groups	n	Hp	Hd	± SD	π	*f* (A)	*f* (B)	*f* (C)
East	56	3	0.350	± 0.067	0.001	0.786	0.196	0.018
West	18	2	0.425	± 0.099	0.001	0.722	0.278	0.000
Captive	11	2	0.509	± 0.010	0.001	0.636	0.364	0.000
Total	85	3	0.382	± 0.050	0.001	0.753	0.235	0.012

Figure 3. Sample size (*n*), number of haplotypes (Hp), haplotype (gene) diversity (Hd), nucleotide diversity (π) and the frequencies (*f*) of haplotypes A, B and C.

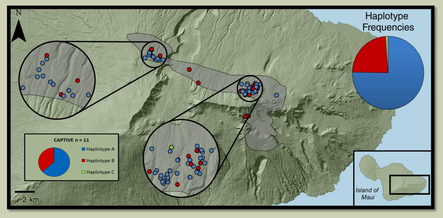

Figure 4. Locations of sampled Kiwikiu and the distribution of haplotypes across the wild population and in captivity.

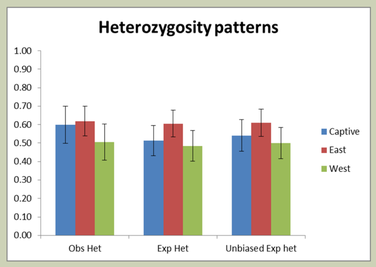

Figure 5. Observed, expected and unbiased expected heterozygosity for Kiwikiu.

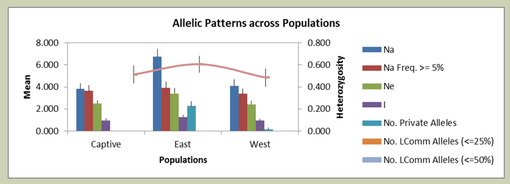

Figure 6. Number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I) and the number of alleles unique to each single population (private) and expected heterozygosity across each population of Kiwikiu.

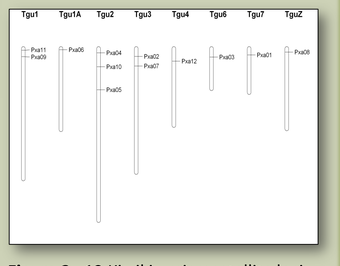

Figure 2. 12 Kiwikiu microsatellite loci mapped on the zebra finch (*Taeniopygia guttata)* genome.

DISCUSSION

Our sample sizes from the east and the west did differ, however based on the proportion of the overall Kiwikiu population estimated to be in each of these areas, the birds sampled represented approximately 20% of the western population and 22% of the eastern.

What do the genetic results tell us?

- The haplotype diversity expressed in Kiwikiu is promising compared to other island bird species that have gone through more severe bottlenecks as well as compared to other successfully translocated island endemics (ex. Nihoa Millerbird (*Acrocephalus familiaris kingi*) H_d=0.22).
- Our observed and expected heterozygosity values were very close and thus we do not suspect high levels of inbreeding or that any portion of this population was ever completely isolated from the rest.
- We were not able to clearly distinguish the number of Kiwikiu subpopulations but we know that there is some likelihood of genetic subpopulation structure above 1.

What does this mean for translocations and/or reintroductions?

- If reintroductions from captive propagation is the best option for the recovery of the species, the captive population will need to incorporate new individuals from the eastern wild population in order to address the significant differentiation between the captive flock and the east.
- If translocations from wild individuals is the best option for the recovery of the species, managers will need to incorporate both eastern and western individuals into translocation protocols.

Plans for the future:

- Since the wet, windward forest is the only suitable habitat remaining for these birds, it is possible that it is not their preferred habitat, but rather their only option. Indeed our data on nest failures has linked heavy wind and rain to lower productivity.
- The Nakula NAR on leeward Haleakala has been selected to establish a second population of Kiwikiu in the future **(Figure 7)**.
- Restoration trials for this area are currently being developed and planting and other experimental restoration efforts will being in 2013.
- We will use the contemporary genetic data we have to help design the best reintroduction plan possible for this species to succeed in this new area.
- Translocation protocols will prescribe the most optimal mix of individuals from both areas to give us the best chance of capturing at least 80% of the overall diversity available in the current population for the translocated individuals.

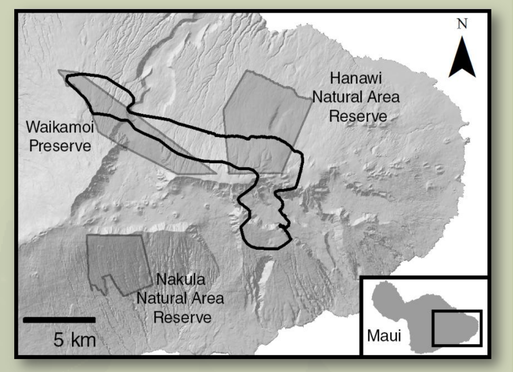

Figure 7. The current distribution of Kiwikiu covers the Hanawi NAR and the Waikamoi Preserve. The Nakula NAR has been selected as the site for establishing a second population of Kiwikiu in the future.

Acknowledgements

This study was conducted as part of a larger research effort by the Maui Forest Bird Recovery Project, Pacific Cooperative Studies Unit under the Research Corporation of the University of Hawai'i. The State of Hawai'i Division of Forestry and Wildlife and the Pacific Island Office of the U.S. Fish and Wildlife Service provided funding, oversight, scientific input, access into the Hanawi Natural Area Reserve, and the required state and federal permits. We would like to thank the following partners for logistical support and/or access to their properties: Haleakala National Park, The Nature Conservancy Maui, San Diego Zoological Society, American Bird Conservancy, Tri-Isle RC & D, Pacific Helicopters and Windward Aviation. Photo credit goes to C. Robby Kohley and Mike Neal. Samit Kundu provided technical assistance. Finally, we thank all of the seasonal biologists who assisted in the strenuous sample collection for this study.

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