



Kiwikiu

Comparing mtDNA diversity in the Kiwikiu (*Psuedonestor xanthophrys*) and the Maui Alauahio (*Paroreomyza montana*)

Peter J. Motyka¹, Hanna L. Mounce^{1,3}, David L. Leonard² and Jim Groombridge³

¹Maui Forest Bird Recovery Project, Makawao, HI 96768 ²Pacific Cooperative Studies Unit, Division of Forestry and Wildlife, Honolulu, 96813 USA

³Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury CT2 7NZ, UK



Maui Alauahio

Introduction

The endangered Kiwikiu (*Psuedonestor xanthophrys*) and Maui Alauahio (*Paroreomyza montana*) are extant insectivorous honeycreepers on the island of Maui. Both have suffered severe range contractions from habitat destruction and the compounding impacts of exotic species and diseases (Scott et al. 1986). Avian malaria has decimated low and mid-elevation bird populations and is a primary threat because of its potential rise in elevation (Benning et al. 2002).

Currently, 421 Kiwikiu and 55,262 Alauahio persist within 40-50 km² (Brinck et al. 2011). Differences in life history traits may be responsible for differences in abundance of these species, which have suffered similar range contractions and are susceptible to the same threats.

The low population number for Kiwikiu suggests that genetic factors may be increasing their risk of extinction. Populations with low diversity are susceptible to the impacts of inbreeding depression, which increases their susceptibility to diseases (Frankham et al. 2002).

The evolution of a resistance to malaria is essential for the long-term survival of Hawaii's honeycreepers, and a high genetic diversity will likely facilitate this evolution (Kilpatrick 2006; Foster et al. 2007). We investigated the mtDNA diversity in Kiwikiu and Maui Alauahio using control region sequence data.

Methods

Study Sites and Sample Collection: We sampled Kiwikiu and Maui Alauahio from the Hanawi Natural Area Reserve, The Kipahulu Valley (Haleakala National Park) and The Waikamoi Preserve (The Nature Conservancy) (Figure 1). Birds were caught with mist-nets and blood samples were collected from the brachial vein.

mtDNA Extraction and Amplification: We extracted genomic DNA from blood samples using an ammonium acetate precipitation method (Nicholls et al. 2000). We used the control-region primers LCRL1 (5'-CGCTATGACCCTCCACGAA-3') and HCR1045 (5'-GAGACGACCTTATCCGCAA-3') (Tarr 1995) for Kiwikiu and L16743 (5'-TTCTCCGAGATCTACGGCCT-3') (Tarr 1995) and CH1 (5'-CCAATAGCGCAAAAGAGCAA-3') (Marthinsen et al. 2008) for Maui Alauahio. PCR products were sequenced by MacroGen Genomics and by Source BioScience.

The first 10 DNA extractions were sequenced off both the forward and the reverse primers and showed no differences in base calls between the two. Subsequent samples were sequenced on the forward primer only. Chromatographs were edited using FinchTV (Geospiza Inc.).

Sequences were aligned in ClustalX Version 2 (Larkin et al. 2005). Samples with rare polymorphisms were sequenced twice by MacroGen Inc. and Source BioScience. Standard DNA polymorphism and genetic differentiation measures were calculated in DnaSP Version 4.00 (Rozas et al. 2003).

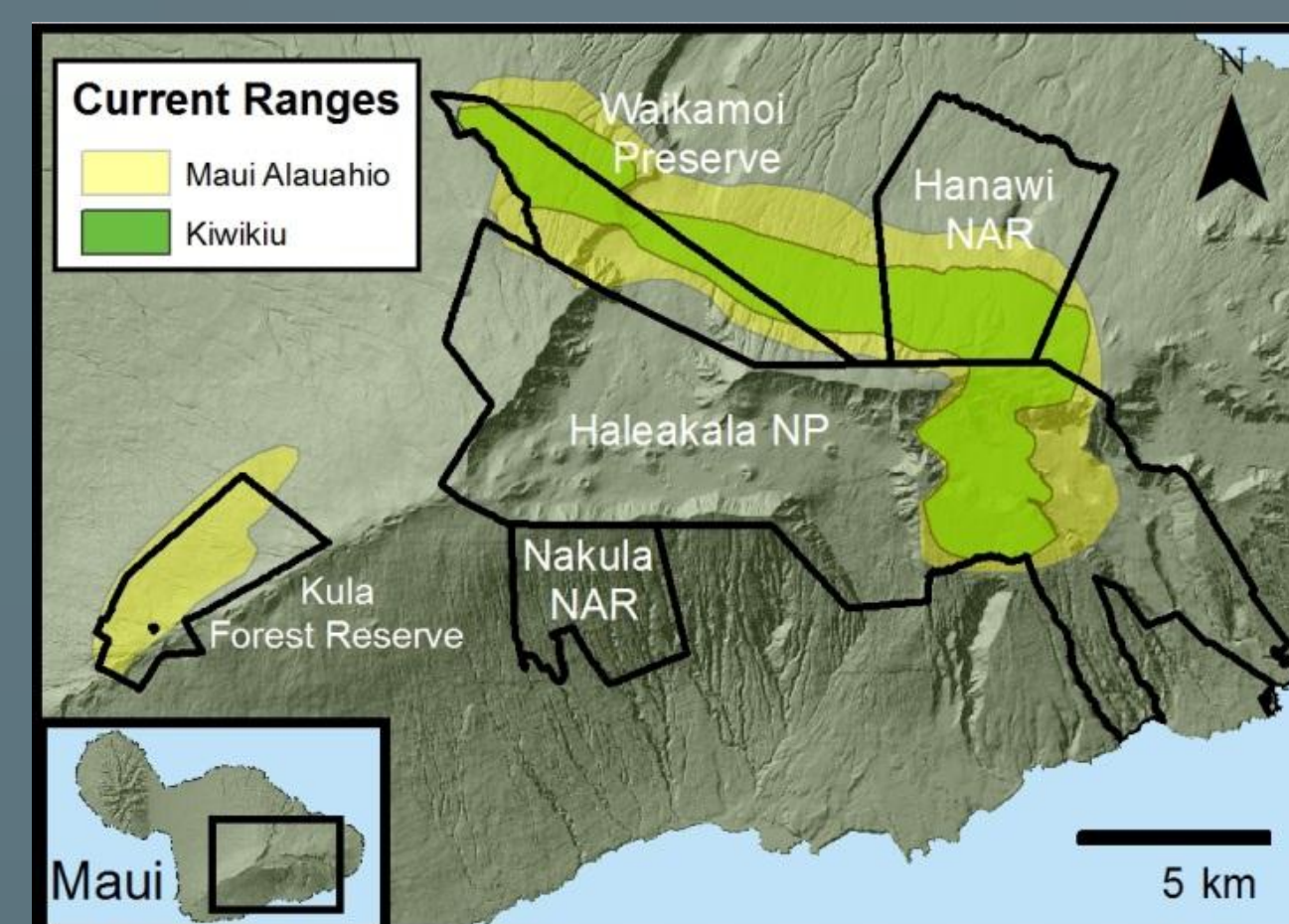


Figure 1: Current ranges of Kiwikiu (pop. 421, 209-674 with 95% CI) and Maui Alauahio (pop. 55,262, 52,729-57,921 95% CI) overlaying East Maui's protected areas.

References

- Baker, H., and P.E. Baker. 2000. Maui Alauahio (*Paroreomyza montana*). In A. Poole and F. Gill (editors), The birds of North America, No. 504. Birds of North America Inc., Philadelphia.
- Benning, T. L., D. LaPointe, C.T. Atkinson, and P.M. Vitousek. 2002. Interactions of climate change with biological invasions and land use in the Hawaiian Islands: Modeling the fate of endemic birds using a geographic information system. *Proceedings of the National Academy of Sciences (USA)* 99: 14246-14249.
- Brinck, K.W., R.J. Camp, P.M. Goresen, D.L. Leonard, H.L. Mounce, K.J. Iknayan, and E.H. Paxton. In press. 2011. Kiwikiu (Maui Parrotbill) and Maui Alauahio abundance estimates and the effect of sampling effort on power to detect a trend. Hawaii Cooperative Studies Unit Technical Report HCSU-XXX. University of Hawaii at Hilo.
- Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, New York.
- Foster, J. T., B. L. Woodworth, L. E. Eggert, P.J. Hart, D. Palmer, D. C. Duffy, and R. C. Fleischer. 2007. Genetic structure and evolved malaria resistance in Hawaiian honeycreepers. *Molecular Ecology* 16: 4738-4746.
- Kilpatrick, A.M. 2006. Facilitating the evolution of resistance to avian malaria in Hawaiian birds. *Biological Conservation* 128: 475-485.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23:2947-2948.
- Lerner, H. R. L., M. Meyer, H. F. James, M. Hofreiter, and R. C. Fleischer. 2011. Multicloc resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology* 21: 1838-1844.
- Lovette, I. J. 2004. Mitochondrial dating and Mixed Support for the "2% Rule" in Birds. *The Auk* 121: 106.
- Marthinsen, G., L. Wennerberg, J.T. Lilfeld. 2008. Low support for separate species within the redpoll complex (*Carduelis flammea-hornemanni-cabaret*) from analyses of mtDNA and microsatellite markers. *Molecular Phylogenetics and Evolution* 47: 1005-1017.
- Nicholls, J. A., M.C. Double, D.M. Rowell, and R.D. Magrath. 2000. The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). *Journal of Avian Biology* 31:165-176.
- Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X. & Rozas, R. 2003. DnaSP: DNA polymorphism analyses by the coalescent and other analyses. *Bioinformatics* 19: 2496-2497.
- Scott, J. M., S. Mountaintopping, F. L. Ramsey, and C. B. Kepler. 1986. Forest bird communities of the Hawaiian Islands: The dynamics, ecology, and conservation. *Studies in Avian Biology* 9. Cooper Ornithological Society, Allen Press, Lawrence, KS.
- Simon, J. C., P.E. Baker, and H. Baker. 1997. Maui Parrotbill (*Psuedonestor xanthophrys*). In A. Poole and F. Gill (editors), The birds of North America Inc. and Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington DC.
- Tarr, C.L. 1995. Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. *Molecular Ecology* 4: 527-529.
- U.S. Fish and Wildlife Service. 2006. Revised recovery plan for Hawaiian forest birds. U.S. Fish and Wildlife Service, Region 1, Portland, OR.

Results

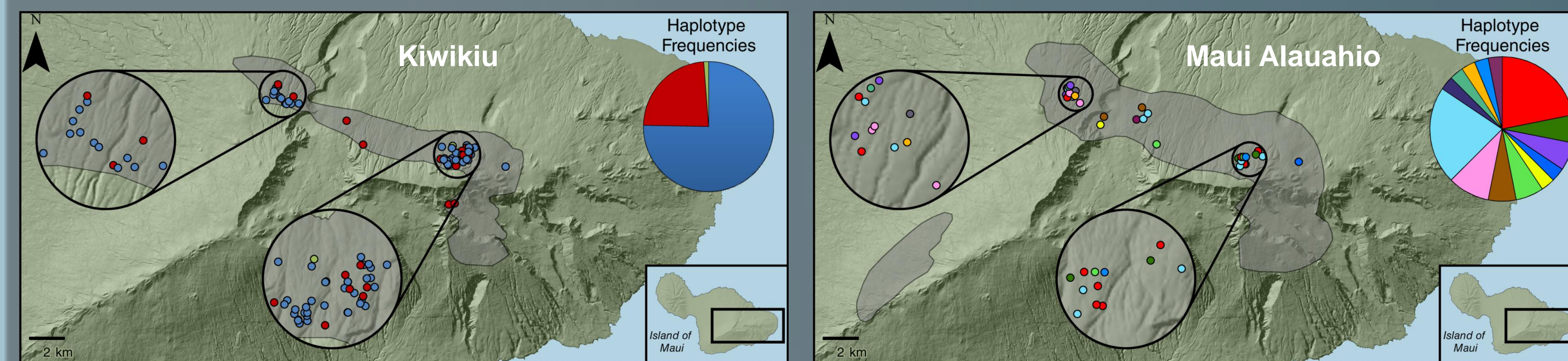


Figure 2: Locations of sampled Kiwikiu and Maui Alauahio and the distribution of unique haplotypes, each represented by a different color. The pie chart displays the proportion of the sampled birds expressing each of these haplotypes.

We sequenced 74 Kiwikiu across the population for a 667 bp length of the control region. These data defined 3 haplotypes (A, B, and C). While haplotypes A and B were found in all sections of the population, haplotype C was only found in one individual. Haplotype diversity (Hd) was 0.365, nucleotide diversity (π) 0.001.

We sequenced 32 Maui Alauahio within the range of Kiwikiu. Using a 519 bp length of the control region, these data defined 14 haplotypes. There was wide variation in base pair changes for Alauahio. (Hd) was 0.901 and (π) was 0.006.

Discussion

The Kiwikiu has lower genetic diversity than the Maui Alauahio. Although this is expected this due to their smaller population size, the high level of diversity in Alauahio and the comparison between these two species was surprising. An earlier evolutionary divergence, a lesser degree of specialization and a higher fecundity are likely responsible for the larger population and the higher level of genetic diversity in Alauahio. The small population size and low genetic diversity of Kiwikiu makes the reestablishment of a second wild population a high priority for their recovery (USFWS 2006).

In the radiation of the Hawaiian Honeycreepers, the Maui Alauahio diverged 1.18 million years earlier than Kiwikiu (Lerner et al. 2011). mtDNA is theorized to mutate about 2% sequence divergence per million years (Lovette 2004), thus this earlier divergence may contribute some diversity but does not account for the majority of the differences present.

Kiwikiu, with its powerful hooked bill, is more specialized, and does not persist in exotic forests occupied by Alauahio. This suggests a higher level of adaptability and behavioral plasticity in Alauahio. Additionally, Alauahio maintain higher densities with a higher fecundity than Kiwikiu (Simon et al. 1997, Baker and Baker 2000).

Evidence suggests that Kiwikiu have saturated their available (disease free) habitat (MFBRP unpubl.). An expansion of habitat would allow a population increase and an increase in genetic diversity over time. This may facilitate the evolution of a resistance to malaria. This resistance has been demonstrated in the Hawaii Amakihi (*Hemignathus virens*) on the island of Hawaii (Foster et al. 2007).

Restoration efforts have begun in the Nakula Natural Area Reserve (NAR) (Figure 1). Managers plan to translocate Kiwikiu, historically found island-wide, to this habitat within 5 years.

Although the impacts of founder effects will be severe for the Kiwikiu, expanding their range may be their only hope for increasing populations and genetic diversity. Currently, the Maui Alauahio has a greater potential to develop a resistance to malaria.

Acknowledgements

The Maui Forest Bird Recovery Project operates under the auspices of the Pacific Cooperative Studies Unit, Research Corporation of the University of Hawaii. Funds for this research were provided by the U.S. Fish and Wildlife Service and the Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife. Photographs were taken by Peter Motyka and C. Robby Kohley. Claire Rasin and Hannah Wickenden assisted with laboratory work at the University of Kent. We remain beholden to all the field crews of MFBRP for their hard work and dedication, without which none of this work could be completed.

