

# Using Discriminant Function Analysis to Accurately Sex Maui Alauahio (*Paroreomyza montana*)

Hanna L. Mounce<sup>1</sup>, Julia C. Garvin<sup>1,2</sup>, Caitlin P. Wells<sup>1</sup>, Shane G. Dubay<sup>1</sup>, C. Dusti Becker<sup>1</sup> and David L. Leonard<sup>3</sup>

<sup>1</sup>Maui Forest Bird Recovery Project, Makawao, HI <sup>2</sup>University of Wisconsin Madison, Madison, WI  
<sup>3</sup>Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife, Honolulu, HI



## Introduction

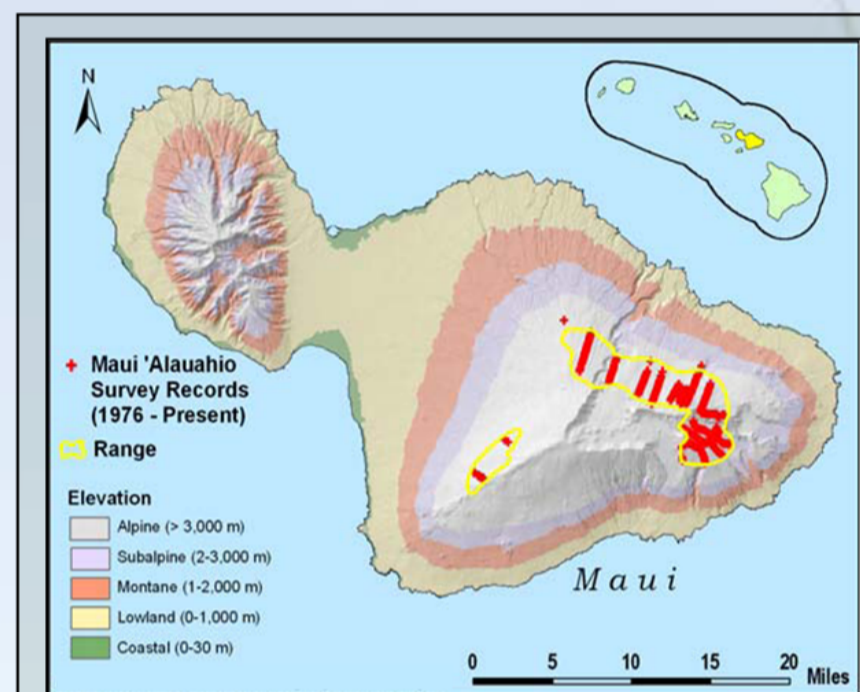
The Maui Alauahio, or Maui Creeper (*Paroreomyza montana*), is a small insectivorous honeycreeper endemic to the island of Maui. Due to habitat loss and disease, the Alauahio is restricted to two populations on east Maui (Fig. 1); the largest occurs in wet and mesic ohia-dominated forest on the windward slope and the second in exotic-dominated dry and mesic forest on the volcano's western slope. These populations provide an opportunity to examine behavioral and ecological differences resulting from habitat differences.

Plumage or morphometrics have been used to assign sex to captured Alauahio, however, recent observations suggest past assignments were not always accurate or appropriate for all individuals (e.g. tarsus-by-wing produces 57% unknown or incorrect sex assignments). Our goal was to develop an accurate method to sex Alauahio. The ability to confidently discriminate between male and female Alauahio will allow investigation of a variety of questions related to social structure, parental investment, disproportionate survival, and mating system, which may also aid in the recovery of closely related endangered honeycreeper species.

## Methods

Since 1998, Maui Alauahio have been captured as part of ongoing research in Hanawi Natural Area Reserve (NAR), on the northeastern slope of Haleakala Volcano, Maui. Mass (g), and wing, culmen, and tarsus length (mm) measurements were taken for each bird. Age was determined using plumage<sup>1</sup> for the following classes: Hatch Year (HY), Second Year (SY), After Hatch Year (AHY), and After Second Year (ASY). When possible, birds were sexed by the presence of a pronounced brood patch (BP, N = 7) or cloacal protuberance (CP, N = 31). In 2000-2002 and in 2007-2009, a small amount of blood was taken from the brachial vein of 93 individuals. Genetic sex determination was performed on all samples<sup>2</sup>, and sex was unambiguously assigned to 79 individuals. Genetic sex assignment confirmed assignment by CP/BP for all 10 individuals for which both data were available.

Discriminant function analysis is used to predict an unknown classification variable (e.g., sex) based on known quantitative variables (e.g., morphometrics). The function calculates linear combinations of the known quantitative variables (canonical variables) that explain between-class variation. We examined intersexual and inter-age differences in mass, and wing, culmen, and tarsus length of known sex birds using t-tests to determine their suitability for use in the function.



**Figure 1.** Range of Maui Alauahio showing the two disjunct populations

Data Source: Hawaii Forest Bird Interagency Database Project

## Results

All four variables (mass, wing, culmen, and tarsus) differed significantly between the sexes (Table 1) but, for the most part, not among age classes.

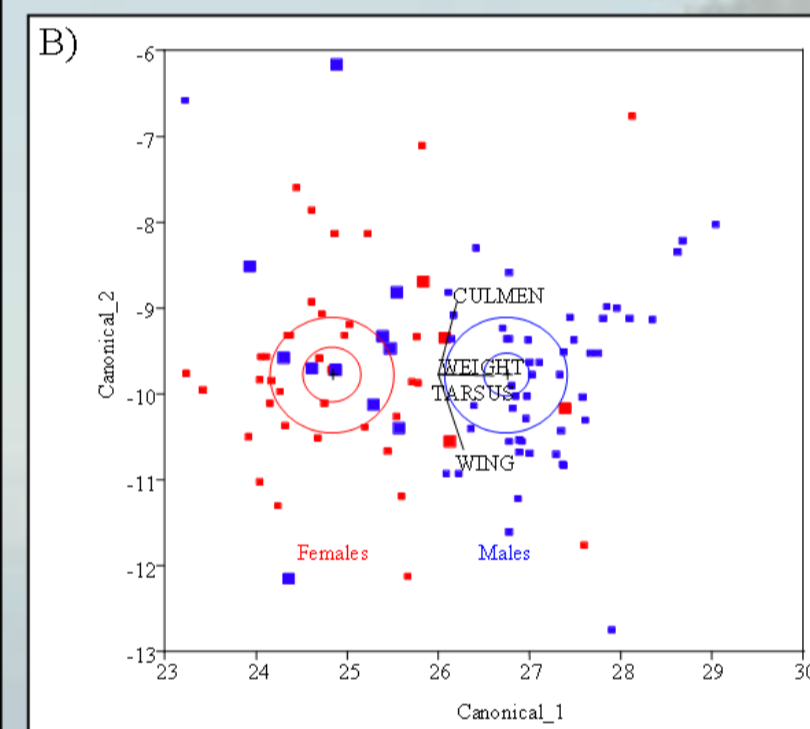
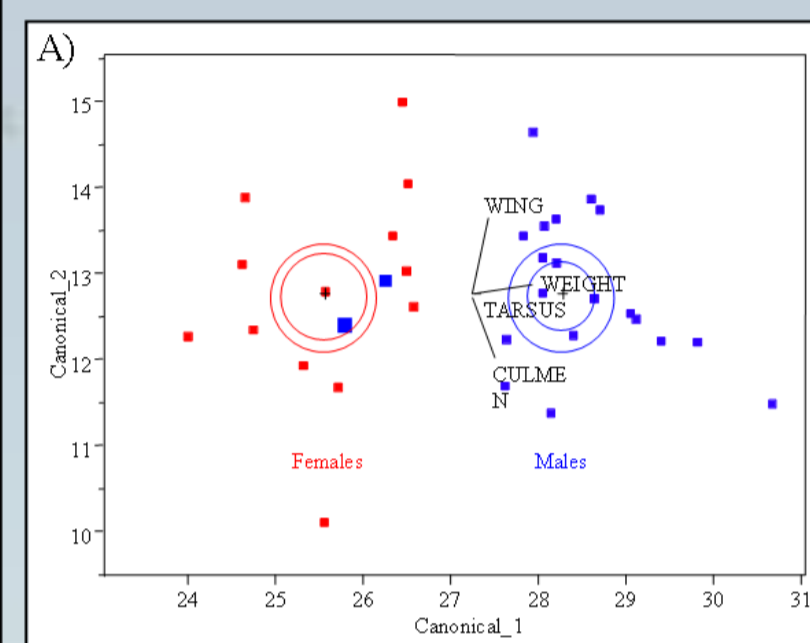
The only exception was that younger (HY and SY) birds had significantly shorter wings than older (AHY and ASY) birds ( $F_{3,100} = 4.81, p = 0.004$ ).

Most conservative function: ASY birds only, N = 34, Correct Assignment = 94.1% 13/13 females (100%) and 19/21 males (90.5%). Wilk's Lambda = 0.351,  $p < 0.0001$ . Canonical Correlation = 0.805. (Fig. 2A)

Most inclusive function: all age classes, N = 99, Correct Assignment = 84.8% 35/39 females (89.7%) and 49/60 males (81.7%). Wilk's Lambda = 0.528,  $p < 0.001$ . Canonical Correlation = 0.687. (Fig. 2B)

**Table 1.** Descriptive statistics of the measurements of 107 known-sex Alauahio. Between-sex differences in each variable as tested with a t-test are also shown.

	Males			Females			t-test	DF	p
	N	Mean	SE	N	Mean	SE			
Mass	62	14.48	0.15	40	12.63	0.15	-8.31	100	<0.0001
Wing	60	63.4	0.29	41	60.7	0.33	-6.04	99	<0.0001
Culmen	60	12.01	0.1	41	11.2	0.08	-6.03	99	<0.0001
Tarsus	60	22.7	0.09	40	21.87	0.11	-5.64	98	<0.0001



**Figure 2.** Canonical plots generated from the two discriminant functions: A) ASY birds (N = 34), B) Individuals from all age classes (N = 99). Points and multivariate means (inner circles) are displayed in the two dimensions that best separate the groups (Canonical 1 and 2). The outer circles indicate the 95% confidence limit for the theorized mean. Females are in red and males are in blue according to CP, BP, and DNA data. Large squares indicate individuals which were incorrectly assigned by the function.

## Discussion

In most Hawaiian honeycreepers, males are larger than females, but the degree of dimorphism varies<sup>3,4,5</sup>. For example, Maui Alauahio appear to be less sexually dimorphic than Maui Parrotbill, another insectivorous honeycreeper, that can be sexed with 91-93% accuracy based on wing chord alone<sup>5</sup>.

Our discriminant functions allow for relatively high levels of confidence in sex assignment based on all four variables, although there is a small degree of error comparable to other functions in the literature<sup>6,7</sup>. This error is appreciably smaller than the number of undeterminable individuals produced by sex assignment using plumage or individual morphological variables alone. Indeed, using the all-ages function on our entire banding database, we assigned sex to 281 previously unassigned individuals, equal to 51.2% of our dataset.

Using the models we developed, as well as the presence of CP/BP, we can now sex Alauahio with vastly improved confidence. This will allow us to investigate population differences and how sex is related to a variety of social and demographic questions. Additional effort will focus on reducing the error in our models and adapting them for field use.

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