



Investigating Hawaiian *Hibiscus* (Malvaceae) population genetic diversity using microsatellites



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Introduction

Investigating neutral genetic diversity using microsatellites is a useful way of tracking recent genetic changes to populations. Microsatellites target noncoding regions of the genome which exhibit higher mutation rates than coding regions since they are not subject to the same pressures of selection.¹

There are currently 5 known endemic Hawaiian *Hibiscus* species which include 9 taxa that share a single ancestor from one of the four independent colonization events of the genus.² This project will look at *H. clayi*, *H. waimeae* ssp. *waimeae*, and *H. waimeae* ssp. *hannerae*. Of these, *H. clayi* and *H. waimeae* ssp. *hannerae* are internationally listed as endangered on the IUCN Red List.³

Using microsatellite markers, the genetic diversity among species and across populations will be evaluated. Understanding endemic *Hibiscus* genetic diversity can aid in future preservation efforts.

Methods

Four microsatellite primers designed for *Hibiscus* species, **HWA-72**, **HWA-73**, **MAFLP-4**, and **H-DAT1**, were used. DNA was extracted from leaf samples taken from the island of Kaua'i using a CTAB procedure modified for the Malvaceae family (Figure 1).⁴ Primers were amplified through polymerase chain reaction and analyzed and scored on a Beckman Coulter CEQ 8000 Genetic Analysis System.

Genetic diversity was evaluated by determining the number of alleles and private alleles as well as the levels of inbreeding and heterozygosity using GenAlEX.⁵ Bayesian analysis was performed using the program Structure with 10,000 repetitions from k=2 to k=14.⁶ Structure Harvester was used to determine that k=3 was the best fit for clustering individuals, followed by k=7.⁷

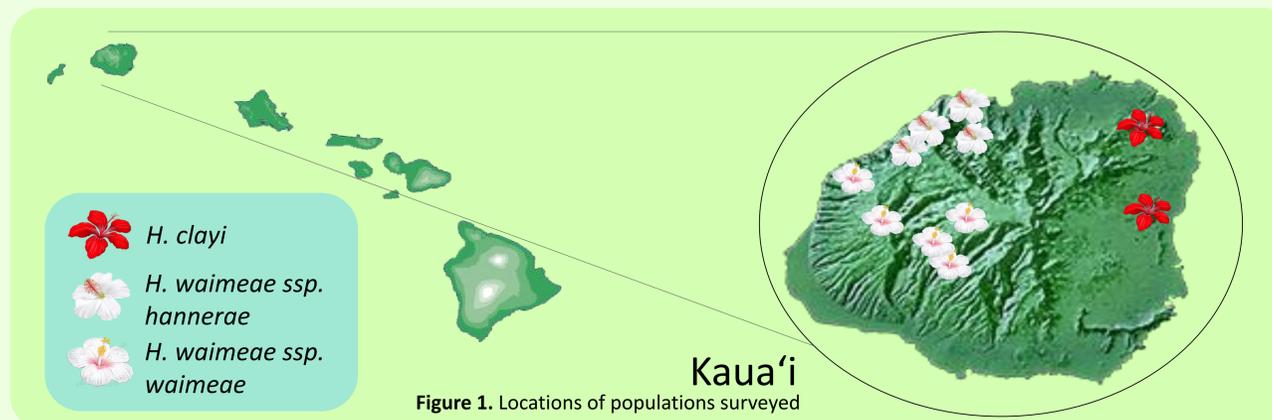


Figure 1. Locations of populations surveyed

Table 1. Genetic diversity of *Hibiscus* species across populations.

Species	Population	N	N _a	N _e	H _o	H _e	F
<i>H. clayi</i>	1 - Nounou	26.500	4.000	2.083	0.511	0.434	0.063
	2 - Anahola	65.250	7.000	3.180	0.419	0.559	0.210
<i>H. waimeae</i> ssp. <i>hannerae</i>	3 - Upper Limahuli	68.750	11.250	4.552	0.640	0.726	0.101
	4 - Pohakuao	6.000	2.500	2.088	0.542	0.413	-0.324
	5 - Lower Limahuli	40.250	9.000	4.335	0.572	0.650	0.072
	6 - Hanakapi'ai	43.500	8.250	4.487	0.620	0.671	0.053
<i>H. waimeae</i> ssp. <i>waimeae</i>	7 - Kawai'iiki	1.000	1.750	1.750	0.750	0.375	-1.000
	8 - Kukui Trail	12.500	7.000	3.845	0.621	0.638	-0.003
	9 - Great Escarpment	2.750	3.750	3.263	0.750	0.615	-0.236
	10 - Mahanaloa	29.500	7.500	4.089	0.627	0.663	0.016
	11 - Koai'e Canyon	20.250	9.500	5.961	0.766	0.722	-0.091

N: number of individuals; N_a: number of different alleles; N_e: number of effective alleles; H_o: observed heterozygosity = number of heterozygotes / N; H_e: expected heterozygosity; F: fixation index

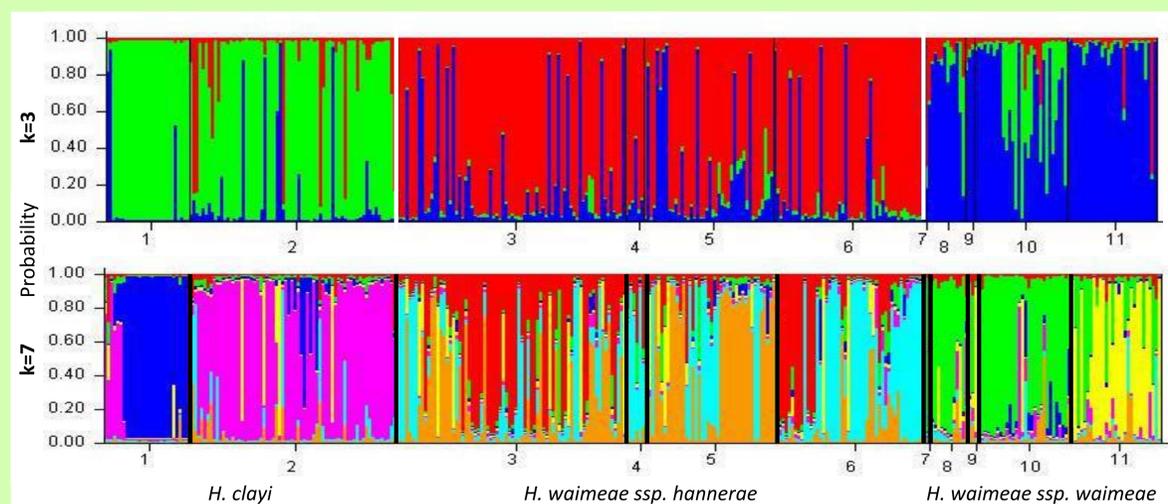


Figure 2. Structure diagrams from Bayesian analysis for *Hibiscus* populations for different k values.

Discussion

As expected, across taxa, larger populations displayed greater allelic richness (N_a) (Table 1). *H. clayi* and *H. waimeae* ssp. *hannerae* populations exhibited lower heterozygosity (H_o) and greater inbreeding levels (F), on average, than *H. waimeae* ssp. *waimeae* (Table 1). **These results indicate less diversity for small populations and rarer taxa – which could be a potential causes of endangerment.**

Unexpectedly, larger populations displayed greater inbreeding levels than smaller populations (Table 1). **Inbreeding depression in small populations could be a potential reason that this occurred.**

The structure diagram when k=3 shows that **the species are distinct**, but there is some overlap that could be due to a **limited evolutionary time for complete divergence** (Figure 2). However, when k=7 **larger genetic differences between individual populations can be seen**, which can help direct future conservation and restoration efforts (Figure 2).

Acknowledgements

Thank you to Hilary Noble and Jeremie Fant for their guidance and contributions, as well as Jennifer Pena for her assistance. Additionally, we'd like to thank NSF-REU grant DBI-1757800 for support.

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