

SHORT COMMUNICATION

Genetic variation in an apomictic grass, *Heteropogon contortus*, in the Hawaiian Islands

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to assess genetic variation within and among Hawaiian populations of an apomictic grass, *Heteropogon contortus* (pili grass). From among 56 individuals sampled from six populations on O'ahu and Hawai'i, 55 unique genotypes were detected using 33 polymorphic markers. This lack of uniformity among individuals may indicate frequent sexual reproduction in these populations. Analysis of molecular variance (AMOVA) revealed significant variation among populations (30.2%), but higher levels of variation within populations (68.1%). Cluster analysis revealed a high degree of clustering for most populations, but populations from different islands did not cluster together. The presence of among-population differentiation but lack of between-island differentiation may suggest that *H. contortus* was an early Polynesian introduction to the Hawaiian Islands.

Keywords: apomict, genetic variation, Hawai'i, *Heteropogon contortus*, population differentiation, RAPD markers

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Introduction

Plants that reproduce by apomixis develop asexual embryos that are derived from diploid cells. Apomicts are predicted to have low levels of within-population genetic variation due to founder effects and a lack of recombination in offspring, whereas high levels of differentiation among populations are expected due to limited gene flow via pollen and divergence of populations over time due to selection, drift, and accumulation of new mutations (Baker 1959; Levin & Kerster 1971; Marshall & Weir 1979; Loveless & Hamrick 1984). Apomictic species with highly restricted ranges, such as *Antennaria soliceps*, may possess essentially only one genotype (Bayer & Minish 1993). Conversely, high levels of genetic diversity have been reported in more widespread apomicts such as *Chondrilla juncea* (Chaboudez 1994), and *Taraxacum officinale* (Lyman & Ellstrand 1984). Diversity may be especially high in apomictic species complexes derived from multiple, independent hybridization events, such as the *Antennaria rosea* complex (Bayer 1990, 1991).

In general, genetic variation in apomictic populations has been attributed to occasional sexual reproduction, multiple evolutionary origins from sexual ancestors, and mutations (Ellstrand & Roose 1987). Populations of species that reproduce primarily asexually commonly have intermediate levels of diversity, with most genotypes restricted to one or a few populations (Ellstrand & Roose 1987).

Heteropogon contortus (L.) Beauv. ex Roem and Schult. is a perennial grass with a widespread distribution throughout the tropics and subtropics (Tothill 1968). It has been reported by Emery & Brown (1958) to be an obligate aposporous apomict. Apomixis in Australian *H. contortus* was confirmed by progeny testing in uniform gardens (Tothill 1970). In the Hawaiian Islands, *H. contortus*, pili grass, has often been assumed to be indigenous (Hitchcock 1922), but it could have been an early Polynesian introduction (Degener & Degener 1968; Wagner *et al.* 1990). Pili grass has cultural value as one of the preferred thatch materials of the early Hawaiians (Degener & Degener 1968). Although *Heteropogon* grasslands were once prevalent in arid, leeward portions of the Hawaiian Islands (Egler 1947; Hitchcock 1922), these grasslands have declined, especially over the past 30 years (Wagner *et al.*

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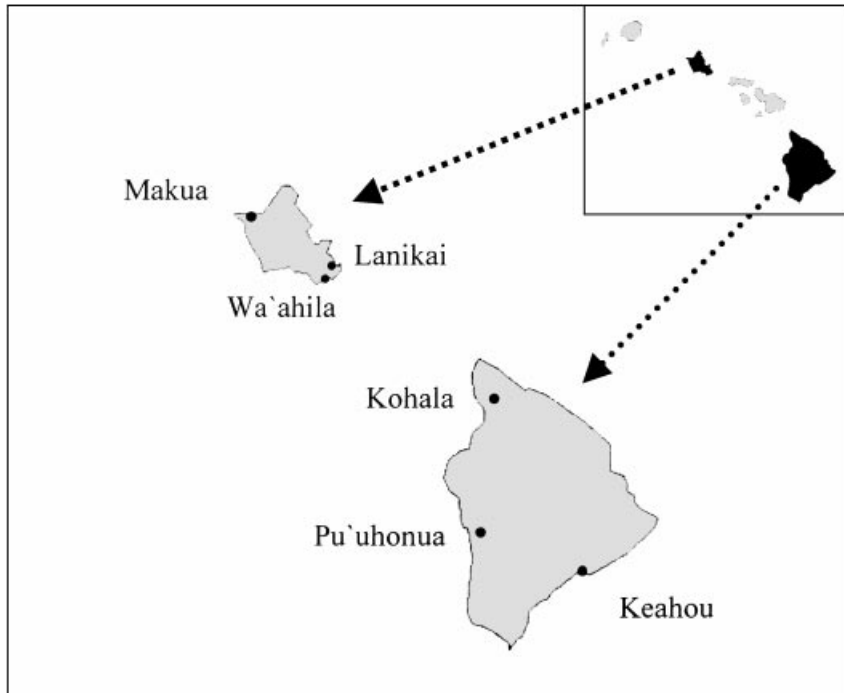


Fig. 1 Map of the Hawaiian Islands showing the locations of *Heteropogon contortus* populations sampled on O'ahu and Hawai'i.

1990; Daehler & Carino 1998). Many former *Heteropogon* grasslands have been replaced by less-diverse African grass-dominated communities that pose a greater fire hazard (Daehler & Carino 1998). Because of the cultural value of *H. contortus* and its lower potential to spark dangerous wildfires, there is increasing interest in restoring *H. contortus* grasslands.

As a species, *H. contortus* exhibits considerable morphological variation (Tothill 1968). Population variation in morphology, polyploidy, and flowering phenology have been reported (Tothill 1968, 1970; Tothill & Hacker 1976). However, little is known about variation within or among the declining Hawaiian populations. Hawaiian populations of *H. contortus* are known to vary in the degree of hairiness of the outer glume, but are otherwise morphologically uniform and similar to other Pacific island populations (Tothill 1968).

The purpose of this study was to assess genetic variation among six Hawaiian populations of *H. contortus* using random amplified polymorphic DNA (RAPD) analysis. Our prediction was that islands and populations would be highly differentiated due to divergence over time, enforced by a lack of pollen exchange between populations. We also predicted low genetic variation within populations due to population founder effects followed by production of genetically uniform offspring. DNA techniques such as RAPD analysis may reveal greater variation than isozymes and are especially useful when genetic variation is expected to be low (Bachmann 1994), but very few studies have used RAPDs to assess genetic

variation in apomictic species (Palacios & Gonzalez-Candelas 1997a, b; Schneller *et al.* 1998).

Materials and methods

Plant material

Plants were sampled from three populations on O'ahu and three populations on Hawai'i (Fig. 1). O'ahu populations were located at Kuaokala Ridge near Makua valley (Makua population), Ka'iwa Ridge near Lanikai (Lanikai population), and Wa'ahila Ridge in Honolulu (Wa'ahila population). For O'ahu, 10 plants per population were sampled. Hawai'i populations were located at Pu'uhonua o Honaunau National Historic Park (Pu'uhonua population, 10 plants sampled), Hawai'i Volcanos National Park, near Keauhou shelter (Keauhou population, eight plants sampled), and south Kohala (Kohala population, eight plants sampled). The population at Pu'uhonua was a planted population derived from a natural population located between Pu'uhonua o Honaunau National Historic Park and South Point, Hawai'i (V. Bio, Pu'uhonua National Historic Park, personal communication), while all other populations were natural and were composed of over 100 individual plants. Populations on O'ahu were all located at least 12 km apart, while populations on Hawai'i were at least 60 km apart. For all populations, plants were sampled from distinct patches each 1–5 m apart. Although additional *Heteropogon contortus* populations occur in the Hawaiian Islands, limited time and resources prevented us from

Table 1 Analysis of molecular variance (AMOVA) indicating the proportion of *Heteropogon contortus* RAPD variation among islands, among populations within islands, and within populations

Source of variation	d.f.	SSD	MSD	Variance component	% total variation	<i>P</i>
O'ahu vs. Hawai'i	1	25.38	25.38	0.104	1.62	NS
Populations within islands	4	89.64	22.41	1.938	30.23	0.001
Individuals within populations	50	218.5	4.37	4.37	68.15	0.001

sampling additional populations or more than 10 individuals per population.

DNA extraction and amplification

DNA was extracted from 100 mg of fresh leaf tissue using the DNeasy plant mini kit (Qiagen, Inc., Valencia, CA, USA). RAPD amplifications were carried out in 15- μ L reaction mixtures, containing 5 ng of genomic DNA, 8.0 mM MgCl₂, 0.8 mM dNTP mixture, 0.48 mM 10-mer primer (Operon Technologies, Alameda, CA, USA), 0.8 units *Taq* DNA polymerase (Promega Corp., Madison, WI, USA), and 1 \times polymerase buffer (Promega Corp.). Amplifications were performed in a Perkin-Elmer GeneAmp PCR system with the following protocol: one cycle of 94 °C for 60 s, 36 °C for 30 s, 72 °C for 65 s; 44 cycles of 94 °C for 15 s, 40 °C for 30 s, 72 °C for 65 s, followed by 72 °C for 365 s. Amplification products were separated on 1% synergel (Research Products International Corp, Mount Prospect, IL, USA)–0.7% agarose (FMC Bioproducts, Rockland, ME, USA) gels in a 0.5 \times TPE buffer. Gels were stained with ethidium bromide, and then visualized and photographed under UV light.

Primers

A total of 30 primers from Operon (Alameda, CA, USA) primer kits OPA, OPD, OPG, OPK, and OPQ were screened with *H. contortus* DNA. Of these, 12 primers (OPK-1, OPK-4, OPK-7, OPK-11, OPK-12, OPK-13, OPK-16, OPK-17, OPQ-4, OPQ-14, OPQ-16, and OPQ-18) that provided consistent amplification products were used in the population analysis.

Analysis

Polymorphic bands were scored for presence or absence. Only reproducible, unambiguous markers were used for analysis. Genetic distance among individuals was estimated with Euclidean distance (Excoffier *et al.* 1992) and Nei & Li's (1979) distance metric using the statistical package RAPDistance version 1.4 (Armstrong *et al.* 1994). Using the software program WINAMOVA version 1.55 (Excoffier 1995), analysis of molecular variance (AMOVA) based on Euclidean distances (Excoffier *et al.* 1992) was employed

Table 2 Pairwise genetic distances between populations (Φ_{ST}). All Φ_{ST} values are significant ($P = 0.001$). *P*-values are the probability of obtaining a more extreme estimate by chance alone

	Makua	Lanikai	Wa'ahila	Pu'uhoonua	Keauhou
Lanikai	0.215				
Wa'ahila	0.297	0.191			
Pu'uhoonua	0.375	0.249	0.346		
Keauhou	0.354	0.328	0.275	0.390	
Kohala	0.278	0.367	0.299	0.456	0.318

to partition genetic variation among individuals within populations, among populations, and between islands, and to estimate pairwise genetic distances (Φ_{ST}) among the six populations. Significance testing for variance components in AMOVA was based on 1000 permutations. The resulting *P*-values are the probability of obtaining a more extreme variance component estimate by chance alone (Huff *et al.* 1993). The genetic distance matrix based on the Nei & Li (1979) distance metric was used to construct a dendrogram using the neighbour-joining method (Saitou & Nei 1987) with the computer program NJTREE (packaged with Armstrong *et al.* 1994).

Results

The 12 primers generated 46 markers, of which 33 markers were polymorphic (71.7%). Per cent polymorphic markers in each population varied, ranging from 32.6% in Pu'uhoonua to 52.2% in Lanikai. O'ahu and Hawai'i populations exhibited similar rates of polymorphism (63% and 69.5%, respectively). Of the 56 plants sampled, 55 had unique RAPD patterns; two individuals from the Keauhou population had identical banding patterns.

Analysis of molecular variance (AMOVA) revealed that only 1.6% of the genetic variation was partitioned between islands (Table 1). Among-population differences within islands accounted for 30.2% of the genetic variation and 68.2% of the variation was attributable to individual differences within a population. Although genetic variation among populations was lower than variation within populations, pairwise genetic distances (Φ_{ST}) between populations were all significant ($P = 0.001$, Table 2). The

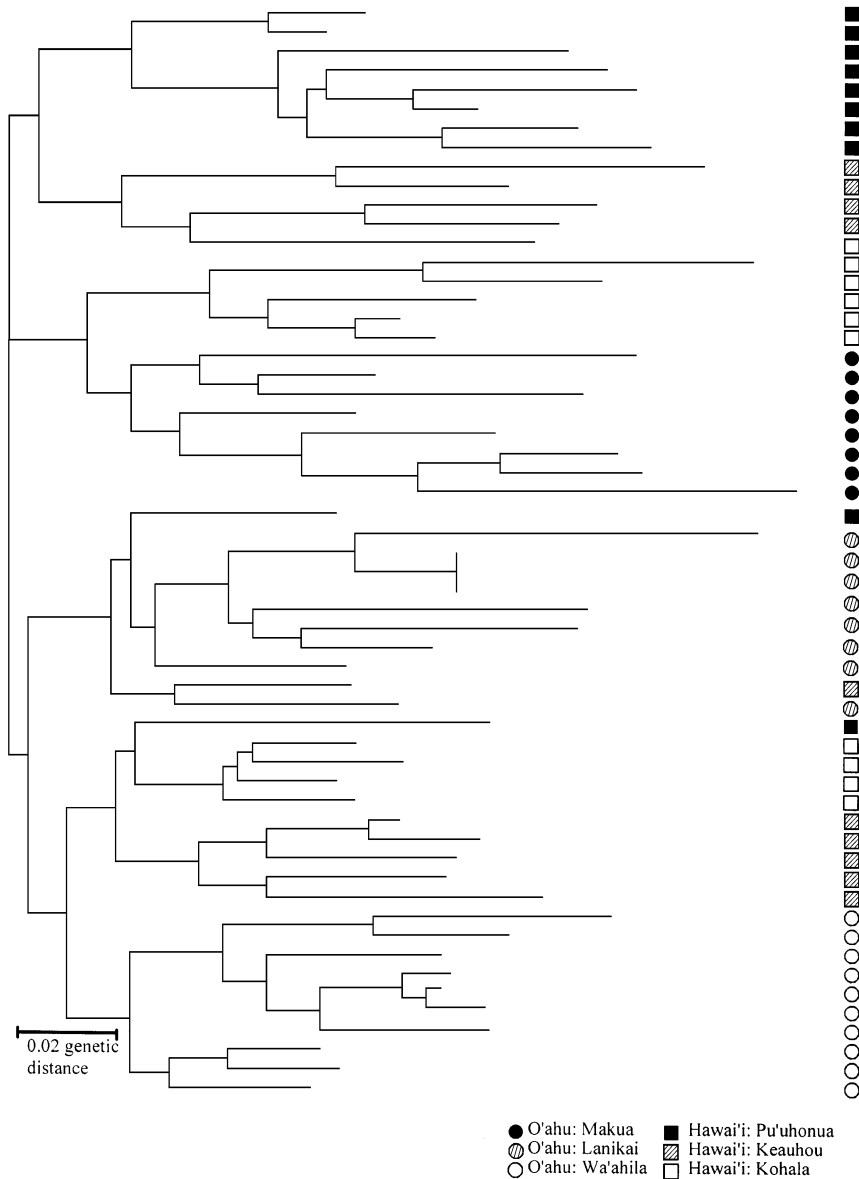


Fig. 2 Dendrogram of 56 *Heteropogon contortus* individuals sampled from six populations on two islands, constructed using the neighbour-joining method (Saitou & Nei 1987) based on the Nei & Li (1979) distance metric.

Wa'ahila and Lanikai populations are geographically the closest populations to each other, approximately 12 km apart, and these populations also had the smallest genetic distance (Table 2). Neighbour-joining analysis with all 56 individuals showed a high degree of clustering for most populations, but populations from O'ahu and Hawaii'i were interspersed within the dendrogram (Fig. 2).

Discussion

This study revealed substantial RAPD genetic diversity in *Heteropogon contortus*, considering its apomictic mode of reproduction (Emery & Brown 1958; Tothill 1970). The level of within-population variation among Hawaiian populations of *H. contortus* is more typical of sexually

reproducing plants. For example, rates of polymorphism in RAPD markers among outcrossing and partially selfing sexual species have ranged between 45% and 100% (Huff *et al.* 1993; Liu *et al.* 1994; Bonnin *et al.* 1996), while we observed 35–52% polymorphic markers in *H. contortus*. Also, the proportion of genetic variation within populations for outcrossing sexual species ranged between 72% and 85% (Huff *et al.* 1993; Martin *et al.* 1997), comparable to the 69% we found in *H. contortus*. Tothill (1968, 1970) speculated that occasional reversion to sexuality is responsible for the worldwide variability found in *H. contortus*. Cytological evidence suggested that sexuality has occurred in some African and Australian populations (Tothill & Hacker 1976). Sexual plants have been identified in populations of apomictic buffel grass *Cenchrus*

ciliaris (Bashaw 1962), and sexual reproduction probably occurs, at least occasionally, in all apomicts (Bashaw & Hanna 1990). Almost every individual that we sampled was unique. The high levels of within-population variation found in Hawaiian populations of *H. contortus* suggest that the occurrence of sexual reproduction, either historic or ongoing, may be much higher than we expected, based on early reports of obligate apomixis (Emery & Brown 1958). Alternatively, high genetic variation may also be due to accumulation of mutations following dispersal into the Hawaiian Islands. The patterns of genetic variation found in European populations of the apomictic fern *Dryopteris remota* may have developed due to occasional spore dispersal between distant populations followed by subsequent mutations (Schneller *et al.* 1998). In our populations, most polymorphic markers were found in more than one population and on both islands, suggesting that most variation was not due to new, unique mutations arising in different populations.

The lack of differentiation between islands was surprising considering that significant population differentiation within islands was detected. This pattern of genetic differentiation is consistent with an early Polynesian introduction of *H. contortus* to the Hawaiian Islands (perhaps *c.* 1500 years ago) followed by subsequent differentiation of isolated populations through genetic drift or selection. As would be expected for an early Polynesian introduction, our populations of *H. contortus* have relatively low interpopulation distances (0.191–0.3904) when compared with another declining (but known endemic) apomict (0.515–0.656; Palacios & Gonzales-Candelas 1997b). *H. contortus* was one of the preferred thatching materials for the early Polynesians and could have been transported to the Hawaiian Islands along with other valuable plants (Degener & Degener 1968; Cuddihy & Stone 1990). Alternatively, *H. contortus* may have been present in the Hawaiian Islands long before the arrival of Polynesians, but the Polynesians may have transported plants frequently between islands, obscuring any inter-island differences. This explanation is less parsimonious because transport would also be expected among populations within islands, whereas we observed significant differences among populations within islands.

Conservation implications

In recent decades, *H. contortus* populations have declined, and there is currently much interest in restoring *H. contortus* grasslands. Populations of *H. contortus* on O'ahu and Hawai'i possess high levels of genetic variation. While most of this variation was within populations, differences among populations were detected. Significant genetic variation in plant size, growth rate, time to flowering, biomass allocation to roots and shoots have

been found among apomict genotypes of other species (Stratton 1991, 1992). As there is a possibility that the RAPD population differences we observed correlate with population differences in ecological traits or local adaptation, efforts should be made to collect local seeds or plants for use in restoration projects in order to maximize chances for local restoration success.

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Debbie Carino is a MS student working in the laboratory of Curt Daehler. The authors are interested in the conservation of declining *Heteropogon contortus* populations in the Hawaiian Islands. The survey of genetic variation was conducted to assess the genetic distinctness of remaining populations. More generally, research in their laboratory focuses on the effects of invasive species on native plants.
