Genome scans reveal high levels of gene flow in Hawaiian *Pittosporum*

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Abstract Species radiations in the Hawaiian Islands have resulted in closely related yet morphologically and often ecologically distinct species, many of which have highly restricted distributions. Eleven species of *Pittosporum* (Pittosporaceae) are recognized in the Hawaiian Islands, yet it remains unclear whether the morphological variation within Hawaiian *Pittosporum* is due to hybridization, genetic polymorphism, phenotypic plasticity, or some combination of these processes. In this study of Hawaiian *Pittosporum* we used a genome-wide approach employing 626 Amplified Fragment Length Polymorphisms (AFLPs) to test species boundaries and elucidate gene flow within and among species. Parsimony trees were constructed to identify clades of individuals that correspond to species, island affinity, and/or sexual system. Network analysis was used to identify the number of lineages in the dataset; results showed a lack of resolution due to reticulation. Based on Bayesian assignment tests, Hawaiian *Pittosporum* exhibited high levels of gene flow. The interspecific allelic exchange and reticulation caused non-hierarchical relationships between species and a lack of reproductive isolation rendered species boundaries permeable. This study highlights the impact of hybridization on species persistence and conservation as well as on general patterns of Hawaiian biota.

Keywords AFLP; gene flow; Hawaiian Islands; hybridization; *Pittosporum*

■ INTRODUCTION

The Hawaiian Islands have long been regarded as a natural laboratory for evolutionary biology because of their isolation from other landmasses, their chronological formation, and high ecological heterogeneity (e.g., Hillebrand, 1888; Fosberg, 1948; Wagner & Funk, 1995; Rubinoff & Schmitz, 2010). The archipelago is about 3500 km from the nearest continent, and is arranged in a line of descending age from the northwest to southeast (Carson & Clague, 1995). The chain terminates with the youngest and largest island, Hawai'i, which is less than 500,000 years old (MacDonald & al., 1983). The physical diversity and isolation of these islands has led to the evolution of an ecologically and morphologically diverse biota through multiple colonizations and rapid adaptive radiations (Carlquist, 1970; Sakai & al., 1995; Price & Wagner 2004). A majority of the Hawaiian flora is found nowhere else in the world with more than 90% of the 1030 species inhabiting the islands being endemic (Sakai & al., 1995; Wagner & al., 1999a). The Hawaiian flora has many examples of species radiations (e.g., Baldwin & Wagner, 2010), which have produced complexes of diverse, yet closely related species (e.g., *Pritchardia* palms: Hodel, 2007 and Bacon, unpub. data; silverswords: Baldwin & Sanderson, 1998; *Cyrtandra*: Cronk & al., 2005), most of which are single-island endemics and have highly restricted distributions on the island in which they occur (e.g., *Cyrtandra*: Clark & al., 2009).

The primary factors responsible for bursts of rapid evolutionary change on islands appears to be the release of species from competition, opportunity in vacant niches, and ecological variation for island immigrants (Fosberg, 1948; Carlquist, 1974; Baldwin & Wagner, 2010). Founding populations may consist of only one or several individuals, often resulting in lower levels of genetic diversity in island versus continental species (e.g., Allendorf & Luikart, 2007). Furthermore, the tendency for many plant or animal groups to undergo adaptive radiation in available habitats often leads to species that are ecologically isolated, but not significantly genetically diverged, potentially leading to extensive hybridization if ecological mating barriers are broken down (e.g., Rieseberg & Swensen, 1996). Inherent in the phyletic radiations of single or few individual colonizers is hybridization (Jorgensen & Olesen, 2001). Hybridization has contributed to the high degree of polymorphism found in island floras, and to a certain extent faunas, such as those on the Galapagos, Hawaiian, and New Zealand Islands (Carlquist, 1974). Plants can compensate for loss of genetic contact with mainland relatives by maintaining a high degree of outcrossing, of which hybridization is a heightened form (Stebbins, 1950; Barrett & al., 1996; Sakai & al., 1995).

Pittosporaceae are composed of nine genera and approximately 200 species distributed in temperate and tropical climates of the Old World, especially Australia. *Pittosporum* is the only member of the family to extend east of the Australian continent and is distributed in Pacific archipelagos including the Hawaiian Islands. The highest numbers of endemic species occur on the largest islands of the Pacific (i.e., New Caledonia and New Zealand) and the Hawaiian Islands (Haas, 1977). In the Hawaiian Islands there are 11 endemic and two naturalized species of *Pittosporum* (Wagner & al., 1999a). Distributional patterns of *Pittosporum* throughout the remote islands of the Pacific are believed to be the result of long-distance dispersal

via birds (Carlquist, 1974). Both Fosberg (1948) and Haas (1977) hypothesized based on flower and capsule morphology that Hawaiian *Pittosporum* arose from a single colonization event and further suggested that adaptive radiation into various habitats had occurred.

The name *Pittosporum* derives from the Greek *pittos*, pitch, and *sporos*, seeds, in reference to the black seeds covered with a film of viscid resin. The black seeds are conspicuous against the orange to orange-red inner surface of the dehiscing capsule valves and most likely attract birds that distribute them (Wagner & al., 1999a). In 1913, Rock observed that *P. hosmeri* was a food source for the Hawaiian endemic crow, *Corvus hawaiiensis*, which is now nearly extinct (Pimm & al., 1993; Atkinson & LaPointe, 2009).

Hawaiian *Pittosporum* forms a morphologically complex assemblage with many overlapping character states (Gemmill & al., 2002; e.g., leaf size and shape; fruit size, shape, and extent of sculpturing). Whereas most Hawaiian *Pittosporum* species have functionally unisexual flowers and are dioecious, at least one member, *P. confertiflorum*, presumably has both unisexual and bisexual flowers within a single population (Wagner & al., 1999a). Based on floral sexuality and other morphological similarities, Haas (1977) hypothesized two alternative scenarios for the single long-distance dispersal event from the South Pacific into the Hawaiian Islands. Specifically, two endemic Fijian species (*P. rhytidocarpum*, *P. oligodontum* Gillespie) with bisexual flowers and one endemic dioecious Tongan species (*P. yunckeri*) were suggested as possible progenitors of the Hawaiian complex and as most closely related to the Hawaiian species *P. confertiflorum* and *P. terminalioides*, respectively (Haas, 1977; Wagner & al., 1999a). Based on morphological data, Cayzer & al. (2000) identified a sister relationship between another Fijian species, *P. brackenridgei*, and Hawaiian *P. confertiflorum*. More recently, two studies (Gemmill & al., 2002; Chandler & al., 2007) identified a highly supported Hawai'i + Fiji + Tonga clade based on ITS data (100% and 98% bootstrap support respectively) and a monophyletic Hawaiian group (both studies had 98% parsimony bootstrap support), although Gemmill & al. (2002) detected no sequence divergence among Hawaiian species.

Genome-wide scans from random markers, such as AFLPs, have proven useful in many studies of Hawaiian biota from crickets (Mendelson & al., 2004) to mints (Lindqvist & al., 2003). The goals of the present study were to use AFLP loci to identify the biogeographic origin(s) and the process of subsequent range expansion of Hawaiian *Pittosporum* and to define species boundaries between currently recognized lineages. We examined whether lineages identified using AFLPs correspond to currently recognized species, to island distribution, and/or sexual system (dioecious or monoecious). We aimed to understand the processes of hybridization, genetic polymorphism, phenotypic plasticity, or a combination thereof, and how they might have formed the morphological and ecological variation present in Hawaiian *Pittosporum*. We hypothesized that *Pittosporum* species would exhibit gene flow due to the observed morphological heterogeneity in the genus and because morphological intermediates have been observed in natural populations of Hawaiian *Pittosporum*.

■ MATERIALS AND METHODS

AFLP screening. — AFLP markers are ideal for situations where there is no a priori sequence information (in our case, no variable sequence information; Gemmill & al., 2002). For this study leaf material was collected from 33 individuals from 10 species on seven islands and was preserved in silica gel (Appendix). DNA was extracted using DNeasy Kits (Qiagen, Valencia, California, U.S.A.). We followed the AFLP protocol from Vos & al. (1995) and used the selective primer sets Mse + CAG with Eco + ACA, + ACG, and + AGC, Mse + CTA with Eco + ACA and + AGC, and Mse + CTG with Eco + ACG, + AGC, + AGG, and + ACA to amplify AFLP fragments. Fluorescently labeled products from the selective amplification with internal size standards (GeneScan-500 ROX, Applied Biosystems, Foster City, California, U.S.A.) were analyzed on an ABI 3100 sequencer (Applied Biosystems). AFLP data analysis was performed using GeneScan v.3.1 (Applied Biosystems) to normalize and size fragments. Genotyper v.3.7 (Applied Biosystems) was used to compare all samples, define loci and output the data matrices. A locus was defined as any single fragment with minimum amplitude of 300 fluorescent units occurring in at least one accession. Loci were scored between 75 and 500 bp. All samples were scored for the absence or presence of an allele at each locus, and pairs of loci with overlapping positions were discarded. Between four and six randomly chosen individuals per locus were rerun to ensure reproducibility in the dataset.

Tree building analysis of AFLP dataset. — AFLPs have been advocated as a phylogenetically informative marker and a review on the potential for these data to reconstruct evolutionary relationships showed ITS and AFLP trees to be largely congruent (Koopman, 2005). AFLPs have disadvantages associated with homology assessments and homoplasy introduced by the multiple, independent ways the absence of an AFLP band can arise (e.g., Simmons & al., 2007). Despite these issues, there are many advantages to using AFLPs for phylogenetic analyses. Because the AFLP method samples unlinked multiple loci from across different genomes, sampling dominant markers may be more efficient than sampling other characters, suggesting that it may be more informative and reliable than morphological characters (e.g., Meudt & Clark, 2007). These advantages, particularly for closely related organisms, have recently led to phylogenetic reconstructions of recent species radiations (Pelser & al., 2003; Bussel & al., 2005; Ellis & al., 2006; Savolainen & al., 2006; Barluenga & al., 2008; Meudt & al., 2009). Furthermore, empirical studies that have examined the tree-like properties of AFLP datasets have been encouraging of the practice (Perrie & al., 2003; Koopman, 2005; Killian & al., 2007, Meudt & al., 2009).

AFLP data generated from multiple primer combinations were combined together into one large matrix and subject to both maximum parsimony and network analysis; the data matrix is available upon request. Equally weighted parsimony tree searches were conducted for each data matrix using 2000 random addition tree-bisection-reconnection searches in PAUP* v.4.0b10 (Swofford, 2001) with a maximum of ten trees held per replicate. To estimate branch support, parsimony

jackknife (JK) analyses (Farris & al., 1996) were conducted using PAUP* with a removal probability set to approximately e^{-1} (36.7879%), and a "jac" resampling emulated. One thousand jackknife replicates were performed with 100 random addition TBR searches (each with a minimum of ten trees held) per replicate. Evolutionary relationships are most often represented as phylogenetic trees, the justification being that evolution is usually a branching tree-like process. However, in certain cases, the data in question do not exhibit tree-like behavior, in which case they can contain a number of conflicting phylogenetic signals (Huson & Bryant, 2006). It has been suggested that network methods provide better tools for representing and quantifying the conflicting uncertainty that processes such as reticulation can introduce into data (Huson & Bryant, 2006). We used the Neighbor-Net method (Bryant & Moulton, 2003) in SplitsTree v.4.10 (Huson & Bryant, 2006) to construct a distance-based network for the AFLP dataset with the Jaccard coefficient (Jaccard, 1901). We chose to use the Jaccard coefficient because it is restricted to shared band presences rather than shared absences.

Admixture and genetic structure using Bayesian analysis. — Assignment methods are a powerful way to ascertain the population or species membership of individuals or groups of individuals. Traditional population genetic models characterize long-term genetic processes, whereas assignment methods approach more contemporary events (Manel & al., 2005), such as recent island colonization. In addition, assignment methods allow direct assessment of admixture between individuals by partitioning population contributions (e.g., Willing & al., 2010). The assumptions of the method are that all potential source populations are defined in advance, sampled randomly, and are in Hardy-Weinberg and linkage equilibrium.

To examine the most likely number of distinct genetic clusters (K) and the level of admixture in our *Pittosporum* dataset, we used the Bayesian clustering method implemented in STRUCTURE (Pritchard & al., 2000) adjusted for dominant markers (Falush & al., 2007). We also used STRUCTURE to identify potential zones of introgression between *Pittosporum* species. STRUCTURE infers the number of clusters (populations or species; K) by minimizing deviations from Hardy-Weinberg proportions and linkage equilibrium within populations. We performed 20 runs for each K, from K = 1–10 (Evanno & al., 2005) and calculated the most likely number of populations in the dataset [ln P(D), mean L(K)] across runs for each K (e.g., Waples & Gaggiotti, 2006) where MCMC chains were run drawing 300,000 samples after an a priori defined burn-in of 200,000 iterations. Typically, the published value of K is taken to be the highest ln P(D) (Pritchard & al., 2000). However, Pritchard & Wen (2003) warned that incremental increases in ln P(D) with an increasing K can lead to overestimation of K. Therefore, we chose between the two highest values of mean ln P(D) [K = 4 or 5] by calculating ΔK (the mean L″(K): the second order of change of L(K), averaged over 20 simulations, divided by the standard deviation; Evanno & al., 2005). In simulations, ΔK has been shown to be better at identifying the correct number of clusters than Ln(K) (Evanno & al., 2005).

RESULTS

***Pittosporum* trees and networks. —** The nine primer combinations yielded a total of 626 AFLP loci, 483 of which were polymorphic. The parsimony analysis of the AFLP dataset identified a complex picture for identifying Hawaiian *Pittosporum* origin and island colonization (Fig. 1). Low phylogenetic signal and JK support not withstanding; a monophyletic Hawaiian group was not resolved in conflict with previous molecular work (Fig. 1; Gemmill & al., 2002; Chandler & al., 2007). Two well-supported species groups were resolved, *P. yunckeri* and *P. terminalioides*, but all other species were largely unresolved even with a large sampling of loci. The network estimated using Neighbor-Net (Fig. 2) did not resolve species groups according to current species boundaries, island, or sexual system. The resulting network shows a lack of groupings and high levels of reticulation between sampled individuals. The colors in the network indicated that each edge leading to all of the individuals sampled in the study showed no

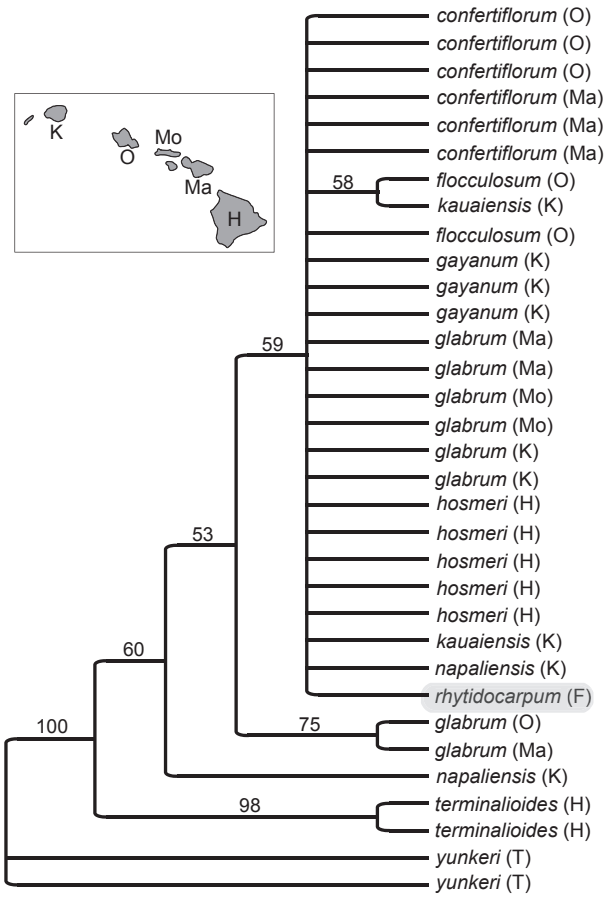

Fig. 1. Jackknife maximum parsimony analyses showing the potential contaminant individual, Fijian *P. rhytidocarpum* in gray. Each individual's collection locality is indicated in brackets after the species name: F, Fiji; H, Hawai'i; K, Kaua'i; M, Maui; Mo, Moloka'i; O, O'ahu; T, Tonga) and can also be referenced to the map inset of the Hawaiian Islands.

intuitive grouping based on island affiliation except for the two well resolved species in the parsimony analysis, *P. yunckeri* and *P. terminalioides* (Fig. 2).

Bayesian clustering analysis. — In the STRUCTURE analysis, the number of populations (K) with the highest mean ln P(D) was 5 (ln P(D) = –8972.175). The mean ln P(D) was only 17.545 lower for K = 4 but the ΔK was higher for K = 5, then K = 4 (ΔK = 20.97 and 3.77, respectively; Fig. 3). Therefore, we chose K = 5 as the most biologically relevant value for the number of *Pittosporum* species clusters (sensu Evanno & al., 2005; Fig. 4). In the resulting bar plot from the K = 5 STRUCTURE analysis, it is apparent that all predefined "species", which are denoted by the individuals plotted within the vertical black bars, share genetic affinity. Two species, *P. terminalioides* and *P. yunckeri*, show the least effect of gene flow as seen by their respective homogenous clusters (Fig. 4).

■ DISCUSSION

The main objectives of this study were to test the origin(s) of the Hawaiian *Pittosporum*, species boundaries, patterns of floral sexuality, and levels of admixture and gene flow between species. AFLP genome scans have been widely demonstrated to represent a powerful means of discriminating between closely related individuals both within (e.g., Savolainen & al., 2006) and among species (e.g., Bacon & Bailey, 2006). The results based on parsimony, Neighbor-Net, and STRUCTURE analysis presented here reveal a much more complex assignment of individuals to groups than by only their recognized taxonomic units (Fig. 4). Characterized by variable morphology and breeding system, our evaluation of the gross ecology of the Hawaiian species shows that they diverged ecologically across the archipelago, with exploitation of a diverse range of habitats from sea level to 2200 m, including coastal areas and beaches, dry lava fields, and from xeric to rainforest (Table 1). The dispersal ability of Hawaiian *Pittosporum* is highly based on fruit and capsule morphology and by the flight distance of its primary dispersal vector (the endemic crow; Dinets, 2004). High dispersal ability enables increased propagule movement and the potential for species to come into contact with one another, greatly affecting population dynamics.

Origins of the Hawaiian *Pittosporum* lineage. — Our results support a close relationship between the Hawaiian lineages and that of the Fijian *P. rhytidocarpum* and Tongan *P. yunckeri* (Figs. 1, 2 and 4), which corroborates previous results based on

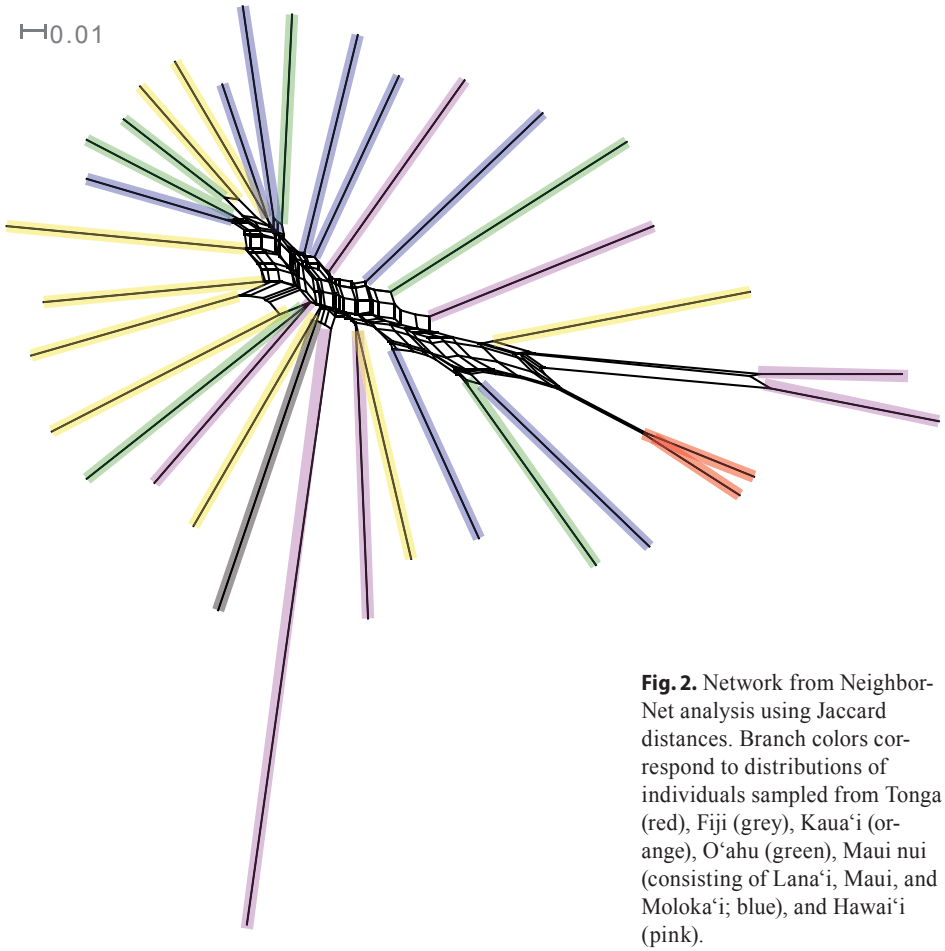

Fig. 2. Network from Neighbor-Net analysis using Jaccard distances. Branch colors correspond to distributions of individuals sampled from Tonga (red), Fiji (grey), Kaua'i (orange), O'ahu (green), Maui nui (consisting of Lana'i, Maui, and Moloka'i; blue), and Hawai'i (pink).

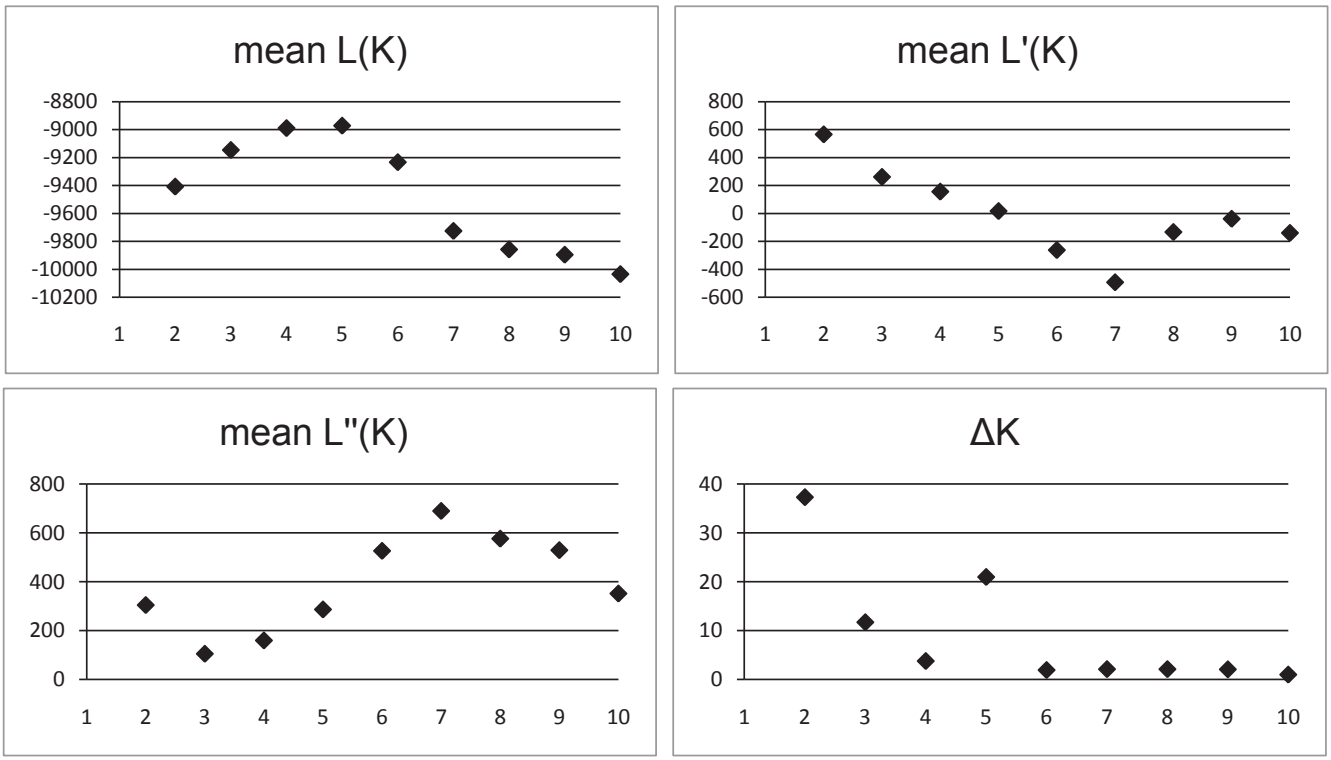

Fig. 3. Mean L(*K*) from STRUCTURE analysis showing a peak in probability at both *K* = 4 and 5, the first (L′) and second (L″) derivatives of the mean likelihood, and Δ*K* indicating that *K* = 5 is the best estimate of the number of biologically relevant species clusters in the AFLP dataset (Evanno & al., 2005).

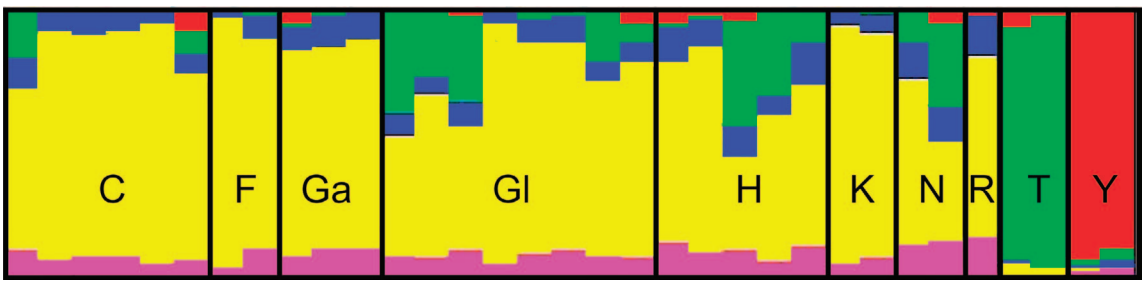

Fig. 4. Bar plot results from STRUCTURE, where *K* = 5 was the inferred number of genetic clusters based on *K* and Δ*K* comparisons. The Y axis represents the probability of membership to a cluster and each color represents one of the five *K* clusters. Species groups were defined a priori (delimited by vertical black lines) as *P. confertiflorum* (C), *P. floccolosum* (F), *P. gayanum* (Ga), *P. glabrum* (Gl), *P. hosmeri* (H), *P. kauaiense* (K), *P. napaliense* (N), *P. rhytidocarpum* (R), *P. terminalioides* (T), and *P. yunckeri* (Y).

ITS sequences (Gemmill & al., 2002; Chandler & al., 2007) and morphological evidence presented by Haas (1977) and Wagner & al., (1999). In contrast to previous results, we did not resolve a Hawaiian clade (Fig. 1) due to the inclusion of Fijian *P. rhytidocarpum* that we interpret as indicative of contamination, either in the laboratory or the field. When the *P. rhytidocarpum* individual is excluded from the analyses, we do reconstruct a monophyletic Hawaiian group, which provides further evidence that the sample is a contaminant. The origin of most lineages in the Hawaiian flora are within the past 5 Ma (e.g., *Pritchardia* at 3.5 Ma, Bacon & al., submitted; *Schiedia* at 5–7 Ma, Frajman & al., 2009; the silversword alliance at 5.1 Ma, Baldwin & Sanderson, 1998) and it has been shown based on models of long-term landscape changes and associated shifts in dispersal and speciation, that 19 of 22 lineages of Hawaiian organisms originated following the formation of Kaua‘i (5.1 Ma; Price & Clague, 2002). No sequence divergence was detected between Hawaiian *Pittosporum* species in a study by Gemmill & al. (2002), which the authors attributed to a recent origin (i.e., a hard polytomy). Our results cannot determine the timing of colonization, but the high levels of admixture found in this study (Fig. 4) may be due to recent origin and hybridization. Alternatively, this pattern may be explained by incomplete lineage sorting and/or phenotypic plasticity (see below).

Table 1. Overview of gross ecological differences in Hawaiian *Pittosporum* species and their progenitors (derived from Haas, 1977).

Species	Distribution	Elevation range (m)	Habitat	Flowering time
P. confertiflorum	O‘ahu, Lana‘i, Maui	300–2200	Rainforest, exposed ridges	November, January, April through August
P. flocculosum	O‘ahu	300–800	Rainforest, exposed ridges	At least March and April
P. gayanum	Kaua‘i	1100–1500	Rainforest, forested ridges	July, August, November through February
P. glabrum	O‘ahu, Moloka‘i, Lana‘i, Maui	200–1200	Dry forest, dry exposed ridges, rainforest	Throughout the year
P. hosmeri	Hawai‘i	900–1500	Dry forest, lava slopes and fields	March, June, December
P. kauaiensis	Kaua‘i	1000–1200	Rainforest, exposed ridges	August, September, December through March
P. napaliensis	Kaua‘i	300–700	Rainforest	Information not available
P. terminalioides	Lana‘i, Maui, Hawai‘i	0–2100	Coastal beach, lava sloped and fields, rainforest	Throughout the year
P. rhytidocarpum	Fiji	0–1100	Wet forest, open rocky areas	Throughout the year
P. yunckeri	Tonga	3–300	Seaside limestone cliffs, exposed ledges, edge of wet forest	April, June, July

***Pittosporum* species boundaries. —** Based on genome-wide scans we were unable to tease apart all 10 ecologically and/or morphologically distinct *Pittosporum* species (Figs. 1, 2, and 4). We resolved both *P. yunckeri* and *P. terminalioides* as genetically distinct species, but all other 33 individuals tested with AFLPs showed high levels of admixture (Figs. 1, 2, and 4). *Pittosporum terminalioides* appears to have contributed significantly to the genetic diversity detected in other Hawaiian species such as *P. glabrum* and *P. napaliensis* (green bars in Fig. 4). Despite gene flow between *Pittosporum terminalioides* and other species, it appears to maintain its specific identity, potentially through a form of unilateral incongruity (e.g., Liedl & al., 1996) where pollen from one plant population (or species) is prevented from functioning on pistils from another population, while in the reciprocal cross, no incompatibility is observed. Unilateral incongruity demonstrates interspecific reproductive barriers and may explain the lack of admixture in *P. terminalioides*. *Pittosporum terminalioides* is found on Lana‘i, Maui, and Hawai‘i and despite its molecular divergence from other samples in this study (Figs. 1 and 4), is thought likely to hybridize with *P. hosmeri* and *P. hawaiiense* Hillebr.based on intermediates found on Hawai‘i (Wagner & al., 1999a).

Evolution of dioecy in the Hawaiian archipelago. — One goal of this study was to determine the patterns of floral dimorphism in Hawaiian *Pittosporum*. The evolution of sexually dimorphic flowers was suggested to occur independently in different geographical populations of *Pittosporum* (Haas, 1977). The development of dioecy has been proposed to promote outcrossing, especially on islands (Bawa, 1980; Sakai & al., 1995). *Pittosporum confertiflorum* has both unisexual and bisexual flowers and both Haas (1977) and Wagner & al. (1999) suggested that the Hawaiian *P. confertiflorum* was closest, morphologically, to the putative ancestor of Hawaiian *Pittosporum*. Our results are ambiguous as to the assertion that *P. confertiflorum* is one of the ancestral lineages of the Hawaiian clade (Figs. 1 and 2) but it may indeed represent an intermediate form in the evolution of dioecy. Although our AFLP data were unable to tease apart the relationships of some Hawaiian species and *P. rhytidocarpum*, our data together with the shared dimorphic floral traits and other morphological data (Haas, 1977; Wagner & al., 1999a) suggest they are more closely related to each other than to other species outside the clade including *P. yunckeri* (Fig. 1). The presence of dioecy in the bulk of the Hawaiian *Pittosporum* diversity may be due not only to selectively advantageous shifts in *P. confertiflorum*, but to other factors involved in breeding system evolution (Carlquist, 1966). Hypotheses on the selective forces promoting the evolution of dioecy primarily suggest that dioecy has evolved as a mechanism to avoid inbreeding depression (e.g., Bawa, 1980). Trends towards dioecy in the Hawaiian flora have been well documented (e.g., Sakai & al., 1997) and reported to be the highest of any known flora worldwide (Sakai & al., 1995). Despite the high numbers of dioecious Hawaiian plants, most are derived from dioecious progenitors and few have evolved dioecy after colonization (Sakai & al., 1995).

Hybridization. — The morphological features differentiating *Pittosporum* species are frequently controlled by ecological (Haas, 1977) rather than genetic factors (e.g., Gemmill & al., 2002), and it is generally understood that morphological intermediacy can result from evolutionary processes other than

hybridization (e.g., Rieseberg & Ellstrand, 1993). There are other viable alternative hypotheses that can explain our results in *Pittosporum* species, including phenotypic plasticity, and incomplete lineage sorting (e.g., Doyle, 1992). Numerous previous studies have shown high levels of phenotypic plasticity in Pittosporaceae, in traits such as heteroblasty (Cayzer & al., 1999), wood (Carlquist, 1981) and leaf anatomy (Wilkinson, 1992). In general, morphological studies of Pittosporaceae have been hindered by plasticity and its interpretation, largely owing to differences in environmental conditions, and considerable variation expressed within individual plants, especially at different ages (Chandler & al., 2007). Incomplete lineage sorting is another alternative hypothesis for recently derived island species because the timing of Hawaiian colonization (potentially less than 5 Ma) causes a short internal node on a phylogeny and species may have ancestral polymorphism that underwent differential fixation of alleles (Doyle, 1992). Incomplete lineage sorting can cause incongruence for the extrapolation of the species tree from the gene tree.

We hypothesize that the high levels of gene flow detected and the lack of clearly defined species based on our AFLP data can be attributed to hybridization and introgression in Hawaiian *Pittosporum* species. Evidence of hybridization and gene flow in *Pittosporum* has previously been identified based on morphological studies. Fosberg (1948) reported that polymorphic groups such as *Pittosporum* would likely reveal hybridization, although potentially not only of recent origin, but also as the products of ancient hybridizations. Wagner & al., (1999) observed that *P. terminalioides* occasionally grows sympatrically with *P. hosmeri* and perhaps *P. hawaiiense* and hybridizes with them, forming intermediates that have been collected on Hawai'i. Furthermore *P. kauaiensis* grows sympatrically with *P. glabrum* and *P. napaliensis* and all three hybridize based on intermediates found on Kaua'i. Haas (1977) also noted that the ranges of the Hawaiian species are either sympatric or essentially contiguous, which would promote hybridization based on geographic proximity alone.

Hybridization between rare and common species has two potential consequences that are important to conservation. Some degree of gene flow is a normal, evolutionarily constructive process, and not all gene pools and genotypes can be preserved. However, hybridization with and without introgression may, nevertheless, threaten a rare species' existence. In this study, four of the endemic species are considered rare according to the most recent survey (Wagner & al., 1999b), *P. napaliensis* is categorized as endangered, and *P. terminalioides* is threatened (IUCN, 2010). One outcome of hybridization is that if F_1 or later-generation hybrids can be partly sterile or have reduced vigor then rare species can be further endangered by outbreeding depression (e.g., Allendorf & Luikart, 2007); that is, rare populations may have reduced fitness due to the production of unfit hybrid individuals. In contrast, if hybrids are fertile and vigorous, hybridization may lead to genetic assimilation of the rare species by a numerically larger one (Rhymer & Simberloff, 1996). Island plants are particularly susceptible to genetic assimilation through hybridization because of small population size, the general lack of strong genetic barriers to hybridization, the colonization (and potential invasion) of islands by closely related exotics, and the increasing loss and disturbance of island habitat due to human activities (Baker, 1955; Carlquist, 1966; Rieseberg, 1991).

Despite records of hybridization between *Pittosporum* species, our data show a less intuitive pattern. Species with non-overlapping distributions, even at the island level, share a high level of genotypic information. For example, both *P. glabrum* from O'ahu and Maui and *P. napaliensis* from Kaua'i share a high number of alleles with the Hawai'i Island endemic *P. terminalioides* (Fig. 4). Hybridization between islands could occur and it has been suggested to potentially aid in the persistence of populations through bottlenecks such as dwindling land area and climatic stress (Rattenbury, 1962). Volcanic eruptions on oceanic islands could both temporarily diminish available land area and create new barriers to gene flow (Carlquist, 1974). Disturbance is more likely to lead to hybridization and genetic assimilation among island species because of small niche size, extensive sympatry, and the close genetic relationships among species (Rieseberg & al., 1989). Furthermore, hybridization can swamp out local adaptation that has evolved through time, and because of the loss of allelic variation, can cause an increase of deleterious alleles (Allendorf & Luikart, 2007).

When considering the seminal literature on Hawaiian biota, it is evident that genetic barriers among island species that have resulted from adaptive radiation range from weak to virtually absent (Carlquist, 1966). It has also been suggested that woody species, such as *Pittosporum*, characteristically develop few interspecific fertility barriers (Baker, 1955). Moreover genetic barriers would be "deleterious, for they would subdivide stock into portions each having less heterozygosity (and thus less genetic momentum for long-term survival in isolation) than the whole" (Carlquist, 1966). Lastly, most oceanic islands capable of supporting rich floras such as Hawai'i have a relatively uniform maritime climate, which prolongs the flowering times over a longer portion of the year and encourages outcrossing and hybridization (Table 1) (Carlquist, 1966). Our AFLP data, together with observations on a general pattern, point to hybridization as an important player in speciation and evolution in the Hawaiian archipelago. Clearly ecological speciation and niche differentiation has occurred between *Pittosporum* lineages, even in the presence of extensive of gene flow and hybridization, and the two forces together act as important components in the diversification of *Pittosporum* on Pacific Islands.

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Appendix. List of taxa and individuals used in the AFLP analysis with voucher information (geographic origin, collector, collector number, herbarium).

Pittosporum Banks ex Gaertn.: ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Pupukea, *C.E.C. Gemmill 221-6* (WAIK). ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Hau'ula, *C.E.C. Gemmill 222-6* (WAIK). ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Loa Ridge, *C.E.C. Gemmill 223-6* (WAIK). ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, Maui, West Maui Mts., Honokawai, Kapunakea Preserve, *C.E.C. Gemmill 237-4* (WAIK). ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, Maui, West Maui Mts., Honokawai, Kapunakea Preserve, *C.E.C. Gemmill 237-5* (WAIK). ***P. confertiflorum*** A. Gray, U.S.A., Hawaiian Islands, Maui, West Maui Mts., Honokawai, Kapunakea Preserve, *C.E.C. Gemmill 237-6* (WAIK). ***P. flocculosum*** (Hillebr.) Sherff, U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Mauna Wili, *C.E.C. Gemmill 229-5* (WAIK). ***P. flocculosum*** (Hillebr.) Sherff, U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Mauna Wili, *C.E.C. Gemmill 229-5* (WAIK). ***P. gayanum*** Rock, U.S.A., Hawaiian Islands, Kaua'i, Koke'e State Park, *C.E.C. Gemmill 255-1* (WAIK). ***P. gayanum*** Rock, U.S.A., Hawaiian Islands, Kaua'i, Koke'e State Park, *C.E.C. Gemmill 255-1* (WAIK). ***P. gayanum*** Rock, U.S.A., Hawaiian Islands, Kaua'i, Koke'e State Park, *C.E.C. Gemmill 255-1* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, O'ahu, Ko'olau Mts., Hawai'i Loa Ridge, *C.E.C. Gemmill 224-2* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Maui, Pu'u Kukui, *C.E.C. Gemmill 231-4* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Maui, Pu'u Kukui, *C.E.C. Gemmill 231-8* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Maui, West Maui Mtns., Maui Pine, *C.E.C. Gemmill 241-10* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Moloka'i, Kua Gulch, *C.E.C. Gemmill 242-1* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Moloka'i, Kua Gulch, *C.E.C. Gemmill 244-4* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Kaua'i, Koke'e State Park, *C.E.C. Gemmill 246-2* (WAIK). ***P. glabrum*** Hook. & Arn., U.S.A., Hawaiian Islands, Kaua'i, Koke'e State Park, *C.E.C. Gemmill 248-1* (WAIK). ***P. hosmeri*** Rock, U.S.A., Hawaiian Islands, Hawai'i, Kohala Mtns., Pu'u-O-Umi Nature Preserve, *C.E.C. Gemmill 238-3* (WAIK). ***P. hosmeri*** Rock, U.S.A., Hawaiian Islands, Hawai'i, Kohala Mtns., Pu'u-O-Umi Nature Preserve, *C.E.C. Gemmill 238-6* (WAIK). ***P. hosmeri*** Rock, U.S.A., Hawaiian Islands, Hawai'i, Kohala Mtns., Koai'a Tree Sanctuary, *C.E.C. Gemmill 239-3* (WAIK). ***P. hosmeri*** Rock, U.S.A., Hawaiian Islands, Hawai'i, Kohala Mtns., Koai'a Tree Sanctuary, *C.E.C. Gemmill 239-4* (WAIK). ***P. hosmeri*** Rock, U.S.A., Hawaiian Islands, Hawai'i, Kohala Mtns., Koai'a Tree Sanctuary, *C.E.C. Gemmill 239-5* (WAIK). ***P. kauaiensis*** Hillbr., U.S.A., Hawaiian Islands, Kaua'i, Koke'e, Pa'ahiki Valley, *C.E.C. Gemmill 247-1* (WAIK). ***P. kauaiensis*** Hillbr., U.S.A., Hawaiian Islands, Kaua'i, Koke'e, Nu'alolo Trail, *C.E.C. Gemmill 250–1* (WAIK). ***P. napaliensis*** Sherff, U.S.A., Hawaiian Islands, Kaua'i, Na Pali, Ho'olulu, *C.E.C. Gemmill 257-1* (WAIK). ***P. napaliensis*** Sherff, U.S.A., Hawaiian Islands, Kaua'i, Na Pali, Ho'olulu, *C.E.C. Gemmill 257-1* (WAIK). ***P. rhytidocarpum*** Planch. ex A. Gray, Fiji, *A.C. Smith 4485* (US). ***P. terminalioides*** A. Gray, U.S.A., Hawaiian Islands, Hawai'i, Ka'u, *C.E.C. Gemmill 240-44* (WAIK). ***P. terminalioides*** A. Gray, U.S.A., Hawaiian Islands, Hawai'i, Ka'u, *C.E.C. Gemmill 240-46* (WAIK). ***P. yunckeri*** A.C. Smith, Kingdom of Tonga, 'Eua, *C.E.C. Gemmill 303-1* (WAIK). ***P. yunckeri*** A.C. Smith, Kingdom of Tonga, 'Eua, *C.E.C. Gemmill 303-2* (WAIK).