Evolution of Insular Pacific Pittosporum (Pittosporaceae): Origin of the Hawaiian Radiation

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We investigated the origin of Hawaiian Pittosporum and their relationship to other South Pacific Pittosporum species using internal transcribed spacer sequences of nuclear ribosomal DNA. We performed both maximum-parsimony and maximum-likelihood analyses, which produced congruent results. Sequence divergence was 0.0% between Hawaiian members of Pittosporum. These taxa formed a strongly supported clade, suggesting a single colonization event followed by phyletic radiation. Sister to the Hawaiian clade were two South Pacific species, P. yunckeri from Tonga and P. rhytidocarpum from Fiji. This result presents convincing evidence for a South Pacific origin of Hawaiian Pittosporum. Our results also identify a monophyletic group comprising three species representing the Fijian Province and East Polynesia, two introductions onto New Caledonia, and at least one (but possibly two) introduction(s) onto New Zealand. Whether the New Zealand taxa form a monophyletic group is unclear from these data. Previous morphologically based hypotheses, however, suggest the presence of four different lineages occupying New Zealand. The nonmonophyly of the New Caledonian species was not surprising based on the extent of their morphological diversity. Although this latter result is not strongly supported, these species are morphologically complex and are currently the subject of taxonomic revision and molecular systematic analyses.

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INTRODUCTION

Before the colonization of the Hawaiian Islands by the Polynesians 1600 years ago (Kirch, 1982), the Hawaiian flora evolved in isolation for at least 40 million years, producing one of the more distinctive biotas in the world. Of the approximately 1000 species of native Hawaiian flowering plants, 89% are found only on these islands (Wagner et al., 1990; Sakai et al., 1995), representing the highest degree of endemism for any known flora. The insular nature of the flora is due to various factors, including a limited number of original colonizers, radiation in a unique insular environment isolated from congeners, diverse geographic origins, and the unbalanced representation of certain groups relative to their nearest mainland congeners (Fosberg, 1948; Wagner et al., 1990). The Hawaiian flora also is characterized by striking examples of phyletic radiations, which produced complexes of diverse, yet closely related species, most of which are single-island endemics. These features make the Hawaiian archipelago an excellent locality for the study of patterns and processes of organismal evolution (e.g., Simon, 1987).

Considering the importance of the Hawaiian flora, Fosberg (1948) noted that “Especially little has been written specifically on the relationships of Hawaiian genera and species to their relatives elsewhere.” Since the time that this observation was made, a number of studies have addressed evolutionary relationships among Hawaiian relatives. The most celebrated of these are the works on the silversword alliance (Asteraceae: Baldwin, 1992; Baldwin et al., 1990; Baldwin and Robichaux, 1995; Carr et al., 1989; Witter, 1990), Hawaiian Drosophila (Drosophilidae; e.g., Carson and Kaneshiro, 1976), and the Hawaiian honeycreepers (Fringillidae; Johanson et al., 1989; Tarr and Fleischer, 1995; Fleischer et al., 1998). However, the origin and diversification of many taxa remain unclear. One contribution to our understanding of evolutionary patterns and processes within the Hawaiian Islands is the recent collaborative publication on contemporary cladistic research of Hawaiian organisms by Wagner and Funk (1995). This work emphasizes the value of the utilization of insular systems for the study of evolution.
and is a practical approach to extend to the study of archipelagoes of the South Pacific.

The angiosperm genus Pittosporum is the only member of the Pittosporaceae (Asteridae) that extends beyond the Australian continent to the east and occupies archipelagoes in the South Pacific and the Hawaiian Islands (Fig. 1). In addition to Australia, the primary center of diversity, species of Pittosporum occur throughout tropical and warm temperate regions of New Zealand (which also has cool temperate and sub-Antarctic representation), Africa, Asia, and Malesia (Haas, 1977). The highest levels of endemism, however, occur on the larger islands of the Pacific (i.e., New Caledonia and New Zealand) and the Hawaiian Islands (Haas, 1977; Wagner et al., 1990). Distributional patterns of Pittosporum throughout the remote islands of the Pacific are believed to be the result of long-distance dispersal of the black, resin-covered seeds via birds (Carlquist, 1974). Once established, species radiations are common within Pittosporum, as demonstrated by the large numbers of insular endemic taxa in the Hawaiian Islands (11 species; Wagner et al., 1990), New Zealand (26 species; Allan, 1961), and New Caledonia (ca. 50 species; J.-M. Veillon, ORSTOM, pers. comm.). Of the 11 Hawaiian species, 7 are single-island endemics. These are found on the islands of Kaua‘i (3 spp.), O‘ahu (1 sp.), Moloka‘i (1 sp.), and Hawai‘i (2 spp.). Other South Pacific islands that have endemic species include the Austral (also known as Tubuai; about 7 spp.), Fiji (3 spp.), Vanuatu (2 spp.), Samoa (1 sp.), Tonga (1 sp.), and Society (2 spp.) archipelagoes.

Hawaiian Pittosporum, or ho‘awa, form a morphologically complex assemblage with many polymorphic, overlapping characters (e.g., leaf size and shape and fruit shape, size, and extent of sculpturing) and exhibit the greatest degree of morphological variability among the South Pacific members of this genus, including sexual expression. Whereas most Hawaiian species are believed to have functionally unisexual flowers, at least one member, P. confertiflorum, has bisexual flowers also. Based on this and general morphological similarity, Haas (1977) hypothesized that the Hawaiian species evolved from a single long-distance immigration event from the South Pacific. Two endemic Fijian species (P. rhytidocarpum and P. oligodontum) with bisexual flowers and one endemic Tongan species (P. yunckeri) with unisexual flowers have been suggested as the progenitors of the Hawaiian complex and as most closely related to the Hawaiian species P. confertiflorum and P. terminalioides, respectively (Haas, 1977; Wagner et al., 1990). Recent collections of mature capsules of P. yunckeri indicate an affinity with P. terminalioides, as both taxa have large, smooth, woody capsules (C. E. C. Gemmill, pers. obs.). The Hawaiian species are presumed to form a monophyletic lineage, although this hypothesis has not been rigorously tested.

Analyses of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) have proven particularly useful for uncovering the evolutionary and biogeographic history of a number of flowering plant lineages found in the Hawaiian Islands. Recent investigations have encompassed assessments of plant evolutionary and biogeographic relationships (Baldwin and Robichaux, 1995; Ganders et al., 2000; Soltis and Soltis, 1998), geographic provenance and dispersal patterns (Wright et al., 2000), age and rate diversification (Baldwin and Sanderson, 1998), and adaptive radiation (Baldwin, 1997). We examined the geographic origin and evolution of the insular Pacific members of Pittosporum using parsimony and maximum-likelihood analyses of nrDNA sequence variation. In particular, we investigated (a) whether the Hawaiian species are monophyletic, (b) how genetically divergent the Hawaiian members are from each other and from other Pacific Pittosporum, and (c) what the likely geographic origin of Hawaiian Pittosporum is.

MATERIALS AND METHODS

Plant Collection and DNA Isolation

Twenty-two ingroup taxa representing five South Pacific taxa, three New Zealand species, five New Caledonian species, and 9 of the 11 currently recognized Hawaiian taxa were included in this study (Table 1). The Hawaiian taxa represent the complete morphological and ecological range for Hawaiian Pittosporum. Two species, P. undulatum (Australia) and P. balfouri (Mauritius), were selected as putative outgroup species. These taxa were chosen based on preliminary phylogenetic analyses. For most taxa, total DNA was extracted from fresh leaf tissue. However, for some accessions, either herbarium or silica-dried material was used (see Table 1). DNA was isolated with a modified CTAB protocol (Doyle and Doyle, 1987) or with the Dneasy Mini Plant Kit (Qiagen).

Amplification and Sequencing of the ITS Region

The ITS region comprises two internal transcribed spacers, ITS1 and ITS2, and the 5.8S subunit of the nrDNA cistron (Baldwin, 1992; Baldwin et al., 1995). The ITS region was amplified by polymerase chain reaction (PCR) with the 3' universal eukaryote primer ITS-4 (White et al., 1990) and the slightly modified “higher-plant” primer (ITS-5 HP, 5'-GGAAGGGAGAAGTCGTAACAAGG-3'; Laboratory of Molecular Systematics (LMS), Smithsonian Institution). PCR was performed in a 100-µL total volume containing 25 mM MgCl₂, 2.5 mM each dNTP, 20 µM each primer, 10.0 µL of 10 µL Taq buffer, 5 µL dimethyl sulfoxide, 1 unit of Taq polymerase, and approximately 30 ng of DNA as template with Epicenter Taq polymerase (Madison, WI). PCR conditions were as follows: 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C; followed by 10 min at 72°C. The PCR product was then digested with NdeI and SpeI, and the ITS fragments were purified using Sephadex G-50 (Amersham). The purified PCR products were then sequenced using the ABI 377 automated sequencer (Perkin-Elmer) at the University of Hawai‘i. The primers used were ITS1R (5'-GGAAGGGAGAAGTCGTAACAAGG-3'), ITS2F (5'-CCACTGGTTCAACAATGCATG-3'), and ABIITSI (5'-CCGCTCCGCTTATCTGATCGGTCAC-3').
FIG. 1. Map of the Pacific Islands showing localities of the taxa included in this study. An asterisk indicates a Hawaiian single-island endemic. Note that *P. balfouri* from the island of Mauritius off the eastern coast of Africa is not shown.
template DNA. The profile for PCR amplification included an initial denaturation cycle of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 48°C, 30 s at 72°C, and a final extension cycle of 7 min at 72°C. Samples were maintained at 4°C following amplification.

PCR products were purified from primers and dNTPs with either polyethylene glycol (PEG) or QIAquick PCR Purification kit (Qiagen). Cycle sequencing of these purified products was performed with the AmpliCycle Sequencing kit (Perkin–Elmer) and the following PCR conditions: 1 cycle of 4 min at 96°C and 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Samples were maintained at 4°C following amplification.

Cycle sequencing products were purified with a series of 95% and then 75% ethanol washes. Direct automated sequencing of double-stranded PCR products was performed with Big Dye chemistry on an ABI automated sequencer, both at the LMS as described in Wen and Zimmer (1996) and at the University of Waikato’s DNA Sequencing Unit. Two accession numbers (P. rhytidocarpum and P. yunckeri) were sequenced with FS chemistry following the manufacturer’s protocol. Sequences were aligned by eye following initial alignments of gel trace files with Sequencher 3.1.1 software (Gene Codes, Inc.). GenBank accession numbers are given in Table 1, and the aligned sequences are available from the authors.

Data Analysis

Phylogenetic analyses were conducted with maximum-parsimony (MP) and maximum-likelihood (ML) methods. Parsimony analyses were performed via the heuristic search mode of PAUP* (Swofford, 1998) with DELTRAN optimization, TBR branch swapping options, and random addition of taxa. Gaps were treated as missing data. Character states (nucleotides) were treated as unordered and trees were rooted with P. undulatum and P. balfouri. Relative support of clades was assessed in a bootstrap analysis (Felsenstein, 1985) with a heuristic search mode (CLOSEST addition, TBR swapping, MULPARS, and DELTRAN options) and 100 replicates.

For molecular sequence data, recent theoretical work (e.g., Sullivan et al., 1999) suggests that maximum-likelihood analyses also are powerful methods when used in combination with parsimony. Using a modification of the search strategy outlined by Sullivan et al. (1999), we generated a starting tree using maximum-parsimony and then four different substitution models, (1) Jukes–Cantor (Jukes and Cantor, 1969), (2) Kimura two-parameter (Kimura, 1980), (3) Hasegawa-
Kishino–Yano (Hasegawa et al., 1985), and (4) a general time-reversible model (see REV of Yang, 1994), in combination with four models of among-site rate variation, (1) all sites with equal rates, (2) proportion of sites assumed to be variable with equal rate associated at variable sites (Hasegawa et al., 1985; I), (3) rates of all sites assumed to follow a discrete approximation (Yang, 1994) of the gamma distribution (I'), and (4) some sites assumed to be invariable with Γ-distributed rates at variable sites (Gu et al., 1995). Maximum-likelihood heuristic searches were performed with the preferred model as determined by higher log likelihood values (GTR base substitution, with among-site heterogeneity and gamma rates), stepwise addition, and TBR swapping. A successive approximation approach (Farris, 1969; Swoford et al., 1996) was employed to find the best trees (as identified by log likelihood values) until the same tree was found in all iterations.

RESULTS

 ITS Sequence Variation

The lengths of ITS1 and ITS2 ranged from 171 to 207 bp (mean of 201) and from 217 to 225 bp (mean of 219), respectively. A majority of species showed a uniform length of 203 bp in the ITS1 region. However, two South Pacific taxa, P. yunckeri and P. rhytidocarpum, had notably shorter lengths (171 bp). Most species exhibited equal lengths in the less-variable ITS2 region (219 bp). The 5.8S region was of uniform length, and identical in sequence, for all taxa (168 bp). GC content ranged from 56 to 63% with an average of 59% in ITS1 and from 57 to 67% with an average of 60% in ITS2. A χ² test showed that nucleotide composition in ITS1 and ITS2 across all taxa, and between the two spacer regions: two indels (32 bases for both ITS1 and ITS2) of equal length. The numbers of variable and constant nucleotide positions for ITS1 and ITS2 were 32 and 125, and 44 and 145, respectively. Manual alignment of the sequences required 45 gaps in ITS1 and 15 in ITS2. The number of single-base indels for ITS1 was 5, whereas that of ITS2 was 3. There were three indels of 5 or more bases in the spacer regions: two indels (32 bases for both P. yunckeri and P. rhytidocarpum) in ITS1 and one indel (7 bases for all taxa except P. undulatum) in ITS2. There were no indels in the 5.8S region. Pairwise sequence divergence (uncorrected for multiple hits) across all taxa ranged from 20.1% in ITS1 to 18.0% in ITS2 with an average of 10.2% across the two spacers. Pairwise sequence divergence among all Hawaiian taxa was 0.0% (Table 2). Taxa exhibiting the least amount of sequence divergence from the Hawaiian group were P. rhytidocarpum (1.5%) and P. yunckeri (1.7%).

Phylogenetic Relationships

The parsimony analysis with gaps treated as missing data yielded two most parsimonious trees of 283 steps, a consistency index (CI) of 0.71, a retention index (RI) of 0.80, and a rescaled consistency (RC) index of 0.70. By comparison, the maximum-likelihood tree was 287 steps in length, with CI, RI, and RC equal to 0.70, 0.85, and 0.59, respectively. The strict consensus of the two MP trees is shown in Fig. 2A. The two MP trees differ in their resolution of the two P. arborescens specimens (one each from Tonga and the Cook Islands) and P. taitense either either the Cook Island P. arborescens and P. taitense were each other’s closest relatives, with P. arborescens from Tonga sister to both, or the relationships among the three samples were unresolved. In the ML tree (Fig. 2B), relationships among these three samples are similarly unresolved. In rooted analyses, P. balfouri was sister to a large clade containing all other representatives of Pittosporum. In both MP and ML unrooted analyses (preliminary analyses not shown), P. undulatum and P. balfouri were resolved as sisters to the remaining species.

The ingroup comprises several major clades. Both MP and ML analyses robustly support (100%) the placement of P. rhytidocarpum and P. yunckeri as the most closely related lineage to all Hawaiian taxa. Members of the Hawaiian group, in turn, form a strongly supported (99%) monophyletic group. However, relationships within the Hawaiian clade are unresolved. The New Caledonian species P. gatopense is sister to P. rhytidocarpum and P. yunckeri, but its placement is only weakly supported (62%). Both MP and ML analyses weakly support the monophyly of the remaining New Caledonian taxa. An unresolved clade composed of two accessions of P. arborescens (one from East Polynesia and one from the Fijian Province) and P. taitense (from East Polynesia) forms a strongly supported (100%) monophyletic group. Another clade comprising two New Zealand species (P. rigidum and P. turneri) is also strongly supported (100%) as monophyletic. However, it is unclear whether the New Zealand taxa form a monophyletic group; the position of P. rigidum and P. turneri relative to P. cornifolium is unresolved.

DISCUSSION

Origin and ITS Evolution of Hawaiian Pittosporum

Both MP and ML analyses strongly support (98 and 99%, respectively) the monophyly of Hawaiian Pittosporum (Figs. 2A and 2B). In addition, seven synapomorphic nucleotide positions (five in ITS1 and two in ITS2) identify the Hawaiian taxa as a distinct group (Fig. 3). Primary morphological features that support the monophyly of the Hawaiian taxa are the increased seed and fruit sizes and the relative woodiness of the
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 pairing Sequence Divergences (Uncorrected for Multiple Hits) for Pittosporum ITS Data

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Note. Comparisons discussed in the text are highlighted in boldface. Brackets indicate within-region comparisons. Localities for P. arborescens: C, Cook Islands; T, Tonga.
capsules in all Hawaiian species, as compared to other non-Hawaiian Pittosporum (Carlquist, 1974). Indeed, these reproductive features serve as the primary basis for distinguishing among species of Hawaiian Pittosporum, although differences in leaf texture and leaf indumentum are also useful (Wagner et al., 1990). However, the level of morphological divergence among species is modest compared to other well-known endemic species groups (e.g., the Hawaiian silversword alliance). This moderate level of morphological divergence in Hawaiian Pittosporum closely matches the low levels of molecular divergence reported here.

The low levels of sequence divergence in the ITS region did not allow resolution of relationships within the Hawaiian clade. We believe that this lack of resolution may be indicative of a relatively recent origin for Hawaiian Pittosporum (i.e., a hard polytomy). Alternatively, the lack of resolution may be due to a slow rate of evolution in the ITS region. Other studies employing ITS, however, do not corroborate this latter hypothesis. Indeed, most molecular phylogenetic studies involving insular Pacific taxa (e.g., P. S. Soltis et al. (unpubl.), Schiedea–Alsinidendron; Baldwin and Robichaux, 1995; Baldwin et al., 1998; silversword alliance, Wright et al., 2000, Metrosideros) find levels of ITS evolution that are similar to those reported here. Consequently, we suggest that the lack of resolution among the Hawaiian taxa is indicative of a relatively recent phyletic radiation in the Hawaiian archipelago. This view is further supported by the fact that the ITS region appears to be sufficient for resolving relationships among members of other insular Pacific clades identified in this study (e.g., the New Caledonian clade), which is consistent with these clades occupying geologically older island systems (Kroenke, 1996).

### ITS Sequence Divergence

Members of Hawaiian Pittosporum are the least genetically divergent from two other South Pacific taxa, *P. rhytidocarpum* and *P. yunckeri* (see Table 2), which show sequence divergence of 1.5 and 1.7%, respectively. *P. rhytidocarpum* and *P. yunckeri*, in turn, are highly genetically similar, with only 0.8% sequence divergence from each other. The next divergent taxon to the Hawaiian clade is *P. gatopense*, which exhibits seven times the sequence divergence observed in *P. rhytidocarpum* and *P. yunckeri* (12.2%). As cited above, these results are comparable to those observed in other studies involving Hawaiian sister groups (e.g., Baldwin and Sanderson, 1998).
Members of the New Caledonian clade differ from each other on average by 8.4% and from the Hawaiian taxa by 13.7%. Within the mixed Fijian–East Polynesia clade, sequences diverge by 1.7% overall and by 12.9% compared to the Hawaiian taxa. The two New Zealand sister taxa diverge only by 0.2%, but differ by 12.5% in their sequences from the Hawaiian taxa.

One viable alternative to use of the ITS region for the resolution of relationships among closely related taxa may involve the use of the external transcribed spacer (ETS) region of nrDNA. This region has been successfully employed to resolve relationships among closely related species of the Hawaiian silversword alliance (Asteraceae; Baldwin and Markos, 1998) and appears to show useful levels of sequence divergence in Hawaiian Alsinoideae (Caryophyllaceae; Nepokroeff et al., 2001). We are currently investigating the utility of the ETS region for the resolution of relationships among Hawaiian and South Pacific Pittosporum.

**Geographic Origin of the Hawaiian Lineage**

ITS analyses with both MP and ML methods reveal that Hawaiian Pittosporum are most closely related to Fijian *P. rhytidocarpum* and Tongan *P. yunckeri*. This finding suggests that the most likely landmass of origin for the progenitor of the Hawaiian lineage is the Fijian Province (including both Fiji and Tonga). This is supported by the monophyly of the Hawaiian taxa coupled with high bootstrap values (98 and 99%, respectively), which identify the Tongan and Fijian species as the sister group to the Hawaiian clade. The Fijian–Hawaiian biogeographic relationship is consistent with a morphological cladistic analysis of Australian Pittosporum (Cayzer et al., 2000), which identifies a sister-taxon relationship between another Fijian species, *P. brackenridgei*, and Hawaiian *P. confertiflorum* (sole Hawaiian representative in this study).

An alternative explanation for the geographic origin of the Hawaiian lineage is that the Hawaiian members,
along with P. rhytidocarpum and P. yunckeri, are together derived from a single common ancestor. This alternative hypothesis, however, does not alter our conclusion that the Hawaiian lineage is of South Pacific origin and is the result of a single introduction event. This conclusion is consistent with Fosberg's (1948) inference of a single colonization event of Indo-Pacific affinity based on morphology. This idea was elaborated on by Haas (1977) who specifically identified P. yunckeri and P. rhytidocarpum as the likely progenitors of the Hawaiian lineage and placed them together into the P. rhytidocarpum group. Using data gathered from capsule and flower morphology, she hypothesized a single introduction via long-distance dispersal from the South Pacific to the Hawaiian Islands. Haas (1977) and Wagner et al. (1990) also suggested that the Hawaiian endemic P. confertiflorum was closest, morphologically, to the putative ancestor of Hawaiian Pittosporum. Alternatively, Wagner et al. (1990) suggested that the morphology of P. terminalioides may be more like that of the common ancestor. Given the lack of sufficient sequence divergence among members of the Hawaiian clade, however, we can neither affirm nor reject these hypotheses.

An interesting outcome of our findings is that in unrooted MP and ML analyses the Australian species, P. undulatum, and then the Mauritian species, P. balfouri, are successive sisters to the remaining species in the ITS phylogeny. That we designate P. undulatum as a member of the outgroup, however, precludes identification of Australia as the definitive center of origin for Pittosporum. Nevertheless, the idea that Australia is a center of species diversity for Pittosporum is not new. Cooper (1956), for example, recognized nine genera of Pittosporaceae (of which Pittosporum is the largest and the only one found outside Australia), which include a total of 48 species endemic to Australia. Thus, identification of Australia as a center of origin is a viable hypothesis, but one that requires further testing. We anticipate conducting direct tests of this hypothesis by including additional Australian taxa and other members representing the full geographic distribution of Pittosporum (e.g., Africa [1 sp.], Indo-China [approx. 11 spp.], and India [5 spp.]).

Despite the fact that we cannot specifically identify a center of origin for Pittosporum, separate introductions (presumably from Australia) to remote Pacific regions (in addition to the Hawaiian introduction) are likely to have occurred. Given the lack of resolution among the New Caledonian, New Zealand, Fijian Province, and East Polynesian taxa, however, it is impossible to specify the number of introductions. One possible scenario involves a single introduction from Australia, possibly to New Zealand, followed by dispersal to the other South Pacific islands. It should be noted, however, that this model is only one among several possibilities, given the unresolved relationships of the South Pacific taxa. Nevertheless, the wide Pacific distribution of Pittosporum is suggestive of a multiple introduction pattern. Consider, for example, that in addition to the geographic distribution cited here, Pittosporum species are also found on New Hebrides (P. anatynese), Fiji (P. oligodontum and P. brackenridgei), Samoa (P. samoense), the Society Islands (P. orohenense), and the Caroline Islands (P. ferrugineum), with the latter species being the most widespread of any Pittosporum (Haas, 1977). This broad distribution was also noted by Carlquist (1974), who suggested a long-distance dispersal model (via birds) to explain the colonization of remote Pacific islands by Pittosporum species.

Relationships among non-Hawaiian taxa

The monophyly of the clade containing the two P. arborescens samples and P. taitense reflects relationships based on morphological variation observed among these taxa. Haas (1977) placed P. arborescens and P. taitense within the P. arborescens group. These taxa share many morphological features but can be distinguished by the entire leaf margin, more complex inflorescence structure, and larger fruits with more seeds in P. arborescens. It is interesting to note that the P. arborescens sample from Tonga has an ITS sequence more similar to that of P. taitense from Tahiti (0.9% sequence divergence) than it does to the other P. arborescens sample from the Cook Islands (1.7% sequence divergence) and that this sequence similarity is of the same magnitude as that between the Hawaiian taxa and P. yunckeri from Tonga. Because the specimen from the Cook Islands was once considered a separate species (P. rarotongense), species delimitations within this group should be reassessed carefully within the context of a complete revision of the insular species of the P. arborescens group.

Our analyses do not identify the New Caledonian taxa as a monophyletic group. However, the placement of New Caledonian P. gatopense as sister to the Hawaiian clade (plus its New Caledonian sister group) is not strongly supported, and the lowest pairwise sequence divergence estimate for P. gatopense is with P. undulatum (9.3%). This split also is not supported by any obvious morphological characteristics. Many of the New Caledonian species are taxonomically problematic due to insufficient sampling and morphological complexities presumably resulting from heteroblasty (i.e., two or more leaf forms on the same plant). The revision of the New Caledonian taxa currently underway (J.-M. Veillon, ORSTOM, pers. comm.) will provide an improved comparative basis for further molecular analyses of these taxa.

These data do not clearly resolve the issue of monophyly of the New Zealand taxa. The two sister taxa, P. rigidum and P. turneri, are divaricating shrubs (interwoven branches that spread at wide angles), a feature unique to the New Zealand species, whereas P. corni-
folium exhibits a regular growth form and is generally epiphytic. P. turneri is unusual and presents a more complex life history in having a divaricating juvenile form and a nondivaricating adult form (Cooper, 1956), which may be the result of hybridization between divaricating and nondivaricating species (B. D. Clarkson, pers. comm.). Previous authors (e.g., Allan, 1961; Cooper, 1956), based on morphological characters such as capsule number, presence of a papery endocarp, divaricating habit, and putative recent colonization events, have suggested that as many as four evolutionary lineages are represented in New Zealand. Despite these morphological observations, it remains unclear whether the New Zealand taxa are monophyletic on the basis of the current data set.

Comparison with Other Insular Pacific Taxa

A relevant biogeographic comparison to Pittosporum is found among the insular Pacific members of Metrocynthia (Wright et al., 2000). Using an ITS-based phylogeny, Wright et al. (2000) proposed four separate introductions into the Pacific, with a majority of taxa dispersed from New Zealand. They also suggested that colonization of remote insular regions (e.g., Polynesia) may be attributed to recent changes in wind patterns during the Pleistocene; unlike Pittosporum, Metrocynthia has small, wind-dispersed seeds. The largest clade in their consensus tree includes 11 species dispersed over several Pacific islands (e.g., New Zealand, Society, Cook, Kermadec, and Hawaiian Islands). However, with the exception of 1 New Zealand species, taxa on these islands differ by only a single base substitution, resulting in an unresolved Pacific clade. This stands in striking contrast to the ITS phylogeny of Pittosporum, which reveals a strongly supported monophyletic Hawaiian lineage and allows the identification of its South Pacific sister group.

CONCLUSIONS

Our results support earlier morphologically based hypotheses (e.g., Fosberg, 1948; Haas, 1977; Wagner et al., 1990) on the origin and derivation of Hawaiian Pittosporum. Both the MP and the ML analyses strongly support the monophyly of the Hawaiian taxa and identify two South Pacific species of the Fijian Province, P. rhytidocarpum and P. yunckeri, as the sister group (those also identified by Haas (1977) as likely ancestors of the Hawaiian taxa), thus supporting a South Pacific origin and a single colonization preceding diversification. These taxa, in turn, may represent pioneer species that migrated from New Caledonia. Of the areas sampled with multiple species (Hawaiian Islands, New Zealand, New Caledonia, and Tonga), it is only in the Hawaiian Islands that we have strong evidence for a single introduction, which may be a consequence of the remote locality of these islands. It will be of interest to see whether this pattern endures as we complete our sampling of all insular Pittosporum (e.g., P. oligodonium from Fiji and P. orohenense from Tahiti). We also suggest that the monophyly of the Hawaiian species, coupled with low levels of sequence divergence, may be indicative of a rapid speciation event following a single colonization from either Fiji or Tonga. Unfortunately, the ITS data do not allow adequate resolution for the reconstruction of relationships among members of the Hawaiian clade. These data further suggest (but do not confirm) multiple South Pacific island introductions, most likely from Australia. The tendency for Pittosporum to be dispersed by birds, coupled with its broad insular distribution, provides additional support for a multiple-introduction hypothesis. As discussed above, our sequence divergence results corroborate other studies of insular plant taxa based on ITS sequence variation. Current and future work will focus on the development of high-resolution markers such as the externally transcribed spacer of nrDNA and amplified fragment length polymorphisms (AFLP), the enhancement of our sampling base, and the completion of morphological cladistic analyses. For example, we are particularly interested in comparing phyletic patterns across the island systems of the Hawaiian Islands, New Caledonia, and New Zealand. Ultimately, these phylogenetic hypotheses should provide the necessary framework for future studies focusing on the evolution of morphological characters and breeding systems in Pittosporum.

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