MOLECULAR SYSTEMATICS OF *TETRAPLASANDRA*, *MUNROIDENDRON* AND *REYNOLDSIA SANDWICENSIS* (*ARALIACEAE*) AND THE EVOLUTION OF SUPERIOR OVARIES IN *TETRAPLASANDRA*

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Systematic relationships within the Hawaiian group *Tetraplasandra* A. Gray, *Munroidendron* Sherff and *Reynoldsia sandwicensis* A. Gray are investigated here based on parsimony analysis of nucleotide sequences from the internal transcribed spacer (ITS 1 and 2) regions and the non-transcribed spacer of 5S (5S-NTS) nuclear ribosomal DNA. Results indicate that *Tetraplasandra*, *Munroidendron* and *Reynoldsia sandwicensis* form a single monophyletic group, and that *Reynoldsia* A. Gray is paraphyletic. Both data sets support combining *Reynoldsia sandwicensis* with *Munroidendron* and treating it as distinct from *Reynoldsia* species outside Hawaii. Both data sets support two major clades: a *Munroidendron*/*Reynoldsia sandwicensis* clade and a *Tetraplasandra* clade, but they are discordant in relation to the monophyly of *Tetraplasandra*. 5S-NTS results support treating *Tetraplasandra*, *Munroidendron* and *Reynoldsia sandwicensis* as a single genus, whereas ITS results suggest that *Tetraplasandra* represents a distinct genus from *Reynoldsia sandwicensis* and *Munroidendron*. *Tetraplasandra gymnocarpa* (Hillebr.) Sherff is the only member of the *Araliaceae* with a completely superior ovary, and in *T. kavaiensis* (H. Mann) Sherff the ovary is partially superior. Together, they form a well-supported clade within the Hawaiian complex, confirming the hypothesis that in *Tetraplasandra* superior ovaries evolved in Hawaii from an epigynous ancestor.

Keywords. 5S-NTS, *Gastonia*, Hawaii, hypogyny, internal transcribed spacer.

INTRODUCTION

Tetraplasandra A. Gray, *Munroidendron* Sherff and *Reynoldsia sandwicensis* A. Gray are Hawaiian members of a complex of closely related genera in the *Araliaceae* that also includes *Gastonia* Lam. and the remaining species of *Reynoldsia* A. Gray (Philipson, 1970). Generic limits in the *Tetraplasandra* and *Polyscias* J. R. Forst. & G. Forst. complex have been hazy, and phylogenetic relationships among and within the genera have been uncertain. Recent ITS data (Plunkett *et al.*, 2001) suggest that the *Tetraplasandra* complex is one subclade nested within a large and complex clade of *Polyscias*. All the species in this group are small to large trees with exstipulate, imparipinnate leaves and unjointed pedicels. The species are indigenous to East Africa, the islands of the Mascarenes, Malesia and Polynesia. *Tetraplasandra*, as

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circumscribed by Lowry (1990), is a small genus endemic to the Hawaiian islands, and the monotypic genus *Munroidendron* is also a Hawaiian endemic. *Reynoldsia* is a Polynesian genus of five species (P. Lowry, pers. comm.), with one species endemic to Hawaii and four species distributed in Samoa, the Marquesas Islands and the Society Islands. *Gastonia* comprises approximately 11 species distributed in East Africa, Malesia and the islands of the Indian Ocean (Philipson, 1970).

Systematic relationships within the *Tetraplasandra* complex are unclear. Generic delimitations are poorly understood, and clear resolution of the genera has been obtained only by combining morphological characteristics with geographic distribution (Philipson, 1970). As pointed out by Philipson, *Reynoldsia* is particularly difficult to separate from *Tetraplasandra*. In general, species of *Reynoldsia* have leaflets with dentate or subdentate to subentire margins (Brown, 1935) and as many stamens as petals (Brown, 1935; Lowry, 1990). In contrast, species of *Tetraplasandra* have leaflets with entire margins, and most species, except those with hypogynous flowers, have 4–6 times as many stamens as petals (Lowry, 1990). Generic limits become blurred in those species of *Reynoldsia* with subentire leaflet margins, however, these species usually have more carpels than any species of *Tetraplasandra* (Philipson, 1970). *Munroidendron racemosum* (C. Forbes) Sherff was originally described as *Tetraplasandra racemosa* C. Forbes (Forbes, 1917). However, based on its pendulous, racemose inflorescence, Sherff (1952) treated the taxon as a monotypic genus. *Tetraplasandra* and *Reynoldsia* have umbellate or paniculate, racemose-umbellate inflorescences. Lowry (1990) suggested that *Munroidendron* is probably derived from either *Tetraplasandra* or *Reynoldsia*. He concurred with Stone (1967) that without more detailed investigations of this group, the placement of *Munroidendron* into *Tetraplasandra* or *Reynoldsia* could not be justified.

Gastonia species usually have as many stamens as petals and entire leaflet margins. The genus is distinguished by the radiating stigmatic branches of the fruit. Based on the latter character state, several species originally described as *Tetraplasandra* from Malesia and the western Pacific are now referred to *Gastonia* (Philipson, 1970).

Previous systematic treatments of *Tetraplasandra* emphasized the importance of stamen number relative to petal number (Sherff, 1955; Philipson, 1970; Lowry; 1990). *Tetraplasandra hawaiensis* A. Gray has 4 times as many stamens as petals, and *T. waialealae* Rock and *T. waimeae* Wawra have 4–6 times as many stamens as petals. *Tetraplasandra gymnocarpa* (Hillebr.) Sherff and *T. kavaiensis* (H. Mann) Sherff have as many stamens as petals, and *T. oahuensis* (A. Gray) Harms, a widespread polymorphic species, has 1–6 times as many stamens as petals. *Tetraplasandra oahuensis*, as well as both Kauai endemics, *T. waialealae* and *T. waimeae*, are glabrous and have compound-umbel inflorescences. On the other hand, *Tetraplasandra hawaiensis* (Molokai, Lanai, Maui and Hawaii), *T. kavaiensis* (Kauai, Oahu, Lanai, Maui and Hawaii) and *T. gymnocarpa*, a rare Oahu endemic, are densely tomentose and have paniculate, racemose-umbellate inflorescences (Sherff, 1955; see Fig. 4 in Philipson, 1970; Lowry, 1990). The two Kauai endemics have the largest fruits of the genus (Carlquist, 1966) and exhibit floral characters consistent with bird pollination, most notably numerous stamens, large quantities of nectar and inferior ovaries (Grant, 1950; Lowry, 1990). *Araliaceae* usually have inferior ovaries, and *Tetraplasandra gymnocarpa* and *T. kavaiensis* are exceptional in having superior or partially superior ovaries respectively (Eyde & Tseng, 1969).

Character optimization experiments using phylogenetic models based on DNA sequence data show that hypogyny is the plesiomorphic condition in angiosperms. The transition from hypogyny to epigyny has occurred many times independently in most major clades of angiosperms; reversals are relatively infrequent (Gustafsson & Albert, 1999). As pointed out by Gustafsson & Albert (1999), the *Apiales* are one of the few major clades that are basally epigynous. Based on the results of *mat*K and *rbc*L sequence data, *Tetraplasandra* is nested well within the *Apiales* (Plunkett *et al.*, 1997). The only fully hypogynous species within the *Apiaceae–Araliaceae* is *Tetraplasandra gymnocarpa* (Eyde & Tseng, 1969). Eyde & Tseng (1969) suggested two possible factors that could have influenced the evolutionary development of hypogyny in *Tetraplasandra gymnocarpa*: (i) separation of the plants from its natural predators by dispersal to an isolated island environment would remove selective pressure against superior ovary mutants, and (ii) selection for increased outcrossing, as superior ovaries in *Tetraplasandra* presumably inhibit self-pollination.

This is the first phylogenetic study focusing on the *Tetraplasandra* complex. We have undertaken a cladistic analysis of the Hawaiian taxa *Tetraplasandra*, *Munroidendron* and *Reynoldsia sandwicensis* A. Gray based on DNA sequences of the internal transcribed spacer (ITS 1 and 2) regions of 18S–26S nuclear ribosomal DNA and the non-transcribed spacer of 5S (5S-NTS) nuclear rDNA. We chose ITS and the more rapidly evolving 5S-NTS because they are valuable for addressing phylogenetic questions at the generic and species level (Sastri *et al.*, 1992; Baldwin *et al.*, 1995; Kellogg & Appels, 1995; Cronn *et al.*, 1996; Soltis & Soltis, 1998). Our primary objectives are to test the generic limits of *Tetraplasandra*, *Munroidendron* and *Reynoldsia*, to test the monophyly of the Hawaiian taxa, and, with a cladistic approach, to examine the evolution of superior ovaries within *Tetraplasandra*.

MATERIALS AND METHODS

Plant material and DNA extraction

DNAs from 17 accessions of *Tetraplasandra*, five of *Reynoldsia*, three of *Munroidendron*, and one of *Gastonia* (Table 1) were isolated from fresh leaves dried in silica gel or from herbarium material using c.1.0cm^2 of tissue. DNA extraction, PCR amplification, cycle-sequencing reactions and DNA sequencing were performed as described by Struwe *et al.* (1998) with minor exceptions. ITS 1 and 2 regions were amplified using the forward primer (TATGCTTAAAYTCAGCGGGT) and the reverse primer (AACAAGGTTTCC-GTAGGTGA) modified from Baldwin (1992) and the following PCR program: hold 97°C, 50s; 97°C, 50s; 53°C, 50s; 72°C, 1min, 50s; repeat 26 cycles; extend 72°C, 7min; hold 4°C. 5S-NTS regions were amplified using the forward primer (TGGGAAGTCCTYGTGTTGCA) and the reverse primer (KTMGYGCTGGTATGATCGCA) as described by Cox *et al.* (1992) and the following PCR program: hold 94°C, 2min; 94°C, 1min; 60°C, 1min; 72°C, 1min;

TABLE 1. Taxa sequenced for 5S-NTS rDNA and/or ITS regions

				GenBank accession	
Taxon	5S-NTS	ITS	Collection and vouchers	5S-NTS	ITS
TETRAPLASANDRA					
T. hawaiensis	x	x	Mauna Kea State Park, Hawaii (HLA), *T. Motley* 1822, NY	AF368485	AF368478
T. hawaiensis	x	x	Ka-iholena Valley, Lanai, *T. Motley* 1825, NY	AF368486	AF368479
T. gymnocarpa	x		Honouliuli, Oahu, *W. Takeuchi* 3862, NY	AF368490	
T. gymnocarpa	x	x	Hawaii-loa, Oahu, *T. Motley* 1816, NY	AF368491	AF368473
T. kavaiensis	x		Haleakala E Maui, *P. Higashiro* 9261a, PTBG	AF368492	
T. kavaiensis		x	Puu-pane, Oahu (HLA), *T. Motley* 1824, NY		AF368472
T. oahuensis	x		Wai-ka-moi, E Maui, *T. Motley* 1865, NY	AF368482	
T. oahuensis		x	Hanaula W Maui, *T. Motley* 1896, NY		AF368481
T. oahuensis	x		Kewela Gulch, Molokai, *T. Motley* 1982, NY	AF368483	
T. oahuensis	x		Waihole, Oahu (HLA), *T. Motley* 1826, NY	AF368484	
T. oahuensis		x	Pupu-kea, Oahu, *T. Motley* 1836, NY		AF368480
T. waialealae	x		Kauai, *W. Takeuchi* 3699, PTBG	AF368493	
T. waialealae	x	x	Kokee State Park, Kauai, *A. Costello* 190, NY	AF368488	AF368477
T. waialealae		x	Kokee State Park, Kauai, *A. Costello* 191, NY		AF368476
T. waimeae	x	x	Kokee State Park, Kauai, *A. Costello* 189, NY	AF368495	AF368474
T. waimeae	x	x	Kokee State Park, Kauai, *A. Costello* 187, NY	AF368489	AF368475
T. lydgatei	x		Hawaii loa, Oahu, *T. Motley* 1809, NY	AF368496	
MUNROIDENDRON					
M. racemosum	x		Nounou Mt, Kauai (NTBG), *A. Costello* 237, NY	AF368497	
M. racemosum		x	Nounou Mt, Kauai (NTBG), *T. Motley* 1830, NY		AF368470
M. racemosum		x	Nounou Mt, Kauai (NTBG), *A. Costello* 238, NY		AF368471

TABLE 1. (Continued.)

				GenBank accession	
Taxon	5S-NTS	ITS	Collection and vouchers	5S-NTS	ITS
REYNOLDSIA					
R. sandwicensis	x		Ulupalakua-kaupo, E Maui, *E. L. Little Jr* 31103, NY	AF368487	
R. sandwicensis		x	Kona, Hawaii, *T. Motley* 2093, NY		AF368469
R. sandwicensis		x	Kona, Hawaii, *T. Motley* 2094, NY		AF368468
R. verrucosa	x		Mt Marau, Tahiti, *T. Motley* 2066, NY	AF368499	
R. verrucosa	x	x	Mt Marau, Tahiti, *T. Motley* 2068, NY	AF368498	AF368467
GASTONIA					
G. rodriguesiana	x	x	Rodrigues Island, *D. Lorence* 7765, BISH	AF368494	AF368466

repeat 27 cyles; extend 72°C, 4min, hold 4°C. The same primers were used for cycle-sequencing reactions. Sequences were edited using the software program Sequencher 3.0 (GeneCodes).

Phylogenetic analysis

Edited sequences were aligned using Sequencher 3.0 with hand adjustments. The ITS data matrix contains 16 taxa and 637 characters and the 5S-NTS data matrix contains 18 taxa and 237 characters. All characters were coded as unordered with equal weight and were analysed using maximum parsimony criterion (Fitch, 1971) with PAUP 4.0 software (Swofford, 1998). Gaps were treated as missing data. Branch and bound searches were performed using the following settings: sequence addition, simple; initial upper bound, computed stepwise; initial max trees, 200 (auto increased by 100); multrees option in effect, and branches collapsed if maximum branch length was zero. Internal branch support was examined by bootstrapping (1000 replicates, full heuristic search, retaining groups with frequency >50%) and with parsimony jackknifing (Farris *et al.*, 1996). Jackknifing was accomplished using the Xac application (J. S. Farris, unpublished program) with 1000 replicates, SPR branch swapping, and five random entry orders per replicate.

Anatomical sectioning

Flower buds of *Tetraplasandra oahuensis* (*T. Motley* 1835, NY) and *T. gymnocarpa* (*T. Motley* 1816, NY) were fixed in a solution of 50% ethanol, 5% glacial acetic acid and 3.7% formaldehyde. Sectioning, staining and mounting were performed as detailed in Struwe *et al.* (1997).

RESULTS

ITS 1 and 2

In the ITS analysis, *Gastonia rodriguesiana* Marais, a species endemic to the Mascarenes, was treated as the outgroup based on morphology (Philipson, 1970) and *mat*K sequence data (Plunkett *et al.*, 1997). One most parsimonious tree of 89 steps was found; this had a consistency index (CI) of 0.89 and a retention index (RI) of 0.95, with 39 characters being parsimony informative (Fig. 1). The ITS tree indicates that the Hawaiian taxa are a monophyletic sister group to *Reynoldsia verrucosa*, a species endemic to the Society Islands. Within Hawaii, two major clades are identified: a *Munroidendron*/*Reynoldsia* clade and a *Tetraplasandra* clade. Both clades are strongly supported by bootstrap and jackknife analyses (>98%). *Reynoldsia sandwicensis* shares a strongly supported clade (100%) with *Munroidendron*, suggesting that *Reynoldsia* is a paraphyletic genus. Within the *Tetraplasandra* clade, three clades are resolved: a *T. hawaiensis* lineage sister to a *T. gymnocarpa*/*T. kavaiensis* lineage, and a *T. oahuensis*/*T. waialealae*/*T. waimeae* lineage. A clade comprising both species with superior ovaries, *Tetraplasandra gymnocarpa* and *T. kavaiensis*, is nested within the Hawaiian taxa. This clade has strong bootstrap and jackknife support (97%). The three species *Tetraplasandra oahuensis*, *T. waialealae* and *T. waimeae* form a strongly supported clade that is sister to *T. gymnocarpa*/*T. kavaiensis*. The ITS data does not resolve relationships among *Tetraplasandra oahuensis*, *T. waialealae* and *T. waimeae*.

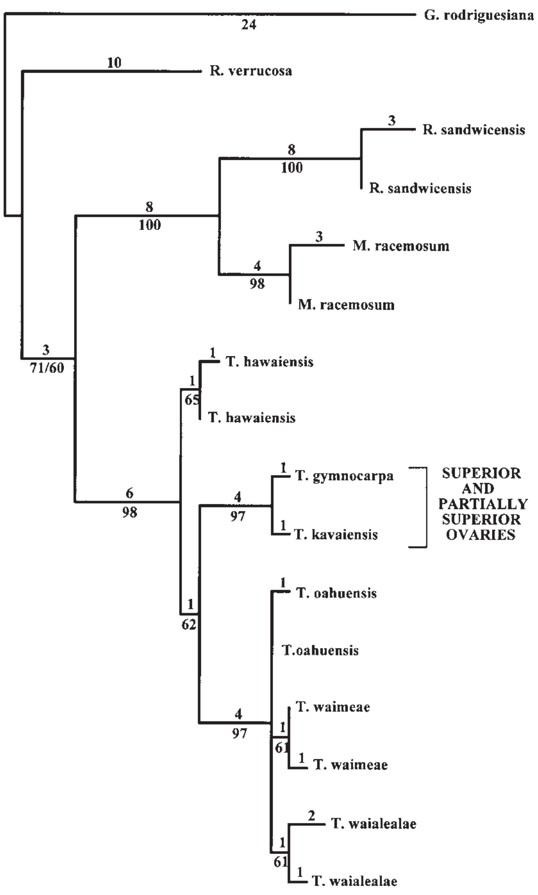

FIG. 1. Single most parsimonious tree from branch and bound parsimony analysis of ITS sequence data (89 steps; (CI) = 0.89; (RI) = 0.95). Branch lengths are proportional to number of character changes. Bootstrap and jackknife support values (reported separately if difference is >2%) are reported below each branch, respectively. Number of character changes for each branch are indicated above each branch. *Gastonia rodriguesiana* is treated as the outgroup taxon. All ingroup taxa (*Munroidendron*, *Reynoldsia* and *Tetraplasandra*) are Hawaiian endemics except for *R. verrucosa* from Tahiti.

5S-NTS

Gastonia rodriguesiana was again used as the outgroup in this analysis. The 5S-NTS analysis resulted in a single most parsimonious tree of 99 steps; this tree had a consistency index (CI) of 0.79 and a retention index (RI) of 0.90, with 52 characters being parsimony informative (Fig. 2). The topology of the 5S-NTS tree is congruent with the topology of the ITS tree, with the exception of the placement of *Tetraplasandra hawaiensis*. The Hawaiian taxa form a monophyletic group with strong bootstrap and jackknife support (100% and 92%, respectively) and are sister to *Reynoldsia verrucosa*. The major incongruence between the ITS and 5S-NTS trees

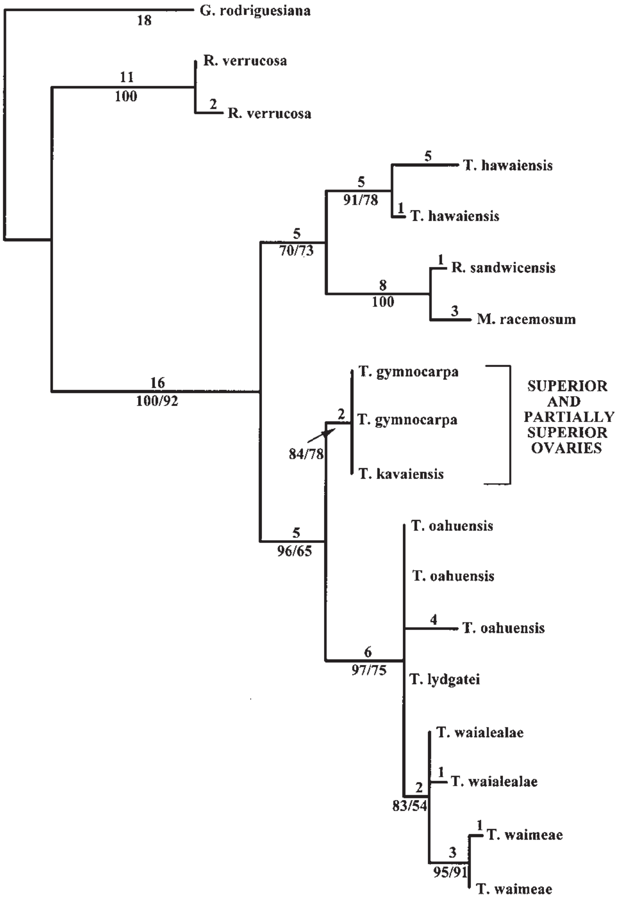

FIG. 2. Single most parsimonious tree from branch and bound parsimony analysis of 5S-NTS sequence data (99 steps, (CI) = 0.79, (RI) = 0.90). Branch lengths are proportional to number of character changes. Bootstrap and jackknife support values (reported separately if difference is >2%) are reported below each branch, respectively. Number of character changes for each branch are indicated above each branch. *Gastonia rodriguesiana* is treated as the outgroup taxon. All ingroup taxa (*Munroidendron*, *Reynoldsia* and *Tetraplasandra*) are Hawaiian endemics except for *R. verrucosa* from Tahiti.

is that *Tetraplasandra* is not monophyletic in the 5S-NTS tree. *Tetraplasandra hawaiensis* falls within, and sister to, the *Reynoldsia sandwicensis*/*Munroidendron* clade. Two major clades are supported: a *Reynoldsia*/*Munroidendron*/*Tetraplasandra hawaiensis* lineage and a lineage with the remainder of the *Tetraplasandra* species. The *Reynoldsia*/*Munroidendron*/*Tetraplasandra hawaiensis* clade has 70% bootstrap support and 73% jackknife support. In addition, as in the ITS tree, there is strong bootstrap and jackknife support for grouping *Reynoldsia sandwicensis* and *Munroidendron* together as sister taxa. Within *Tetraplasandra*, there are two well supported clades: the hypogynous species and the epigynous species. 5S-NTS sequence data show no genetic variation between *Tetraplasandra gymnocarpa* and *T. kavaiensis*, and, like the ITS data, lack sufficient nucleotide variation to resolve relationships among *T. oahuensis*, *T. waialealae* and *T. waimeae*. Nevertheless, based on pair-wise comparisons between species, the 5S-NTS data are on average more variable than ITS data.

Discussion

ITS and 5S-NTS data indicate that the Hawaiian members of the *Tetraplasandra* complex, which includes *Tetraplasandra*, *Munroidendron* and *Reynoldsia sandwicensis*, are monophyletic and appear to have evolved from a single ancestral taxon introduced in the Hawaiian islands, rather than two introductions as was suggested by Fosberg (1948) and Sakai *et al.* (1995). *Tetraplasandra gymnocarpa*, the only *Araliaceae* species with completely superior ovaries, and *T. kavaiensis*, a species with partially superior ovaries, form a well-supported clade within this monophyletic complex. The derived position of *Tetraplasandra gymnocarpa* supports Eyde & Tseng's hypothesis (1969) that hypogyny evolved within the group in Hawaii. Another interesting result of the ITS and 5S-NTS phylogenies is the apparent paraphyly of *Reynoldsia*, but this result is based on limited sampling of the genus outside the Hawaiian islands.

Because both ITS and 5S-NTS results indicate that *Tetraplasandra*, *Munroidendron* and *Reynoldsia* as a group are monophyletic, they could be regarded as a single genus, as initially pointed out by Philipson (1970). Although Philipson (1970) recognized a close relationship among members of the *Tetraplasandra* complex, including *Gastonia*, he pointed out this would be a rather large genus and recommended maintaining a subdivision into four genera. Based on fruit characters, he divided the genera into two geographic groups. *Gastonia*, with its characteristic radiating style arms and calyx without clearly defined lobes, comprises the Indian Ocean–Malesian group. *Tetraplasandra*, *Munroidendron* and *Reynoldsia*, which lack radiating stigma arms and have calyxes with regular or clearly defined lobes, form the Polynesian group. Our 5S-NTS and ITS data clearly indicate a division of the complex into two major groups: a Hawaiian clade, comprising *Tetraplasandra*, *Munroidendron* and *Reynoldsia sandwicensis* and an extra-Hawaiian clade, comprising *R. verrucosa*. Nevertheless, without further sampling of *Reynoldsia* and *Gastonia* we cannot address the issue of treating the entire complex as one monophyletic group, treating the complex as two groups (Hawaii and extra-Hawaii), or retaining the structure of the current classification.

Among the Hawaiian species, the ITS and 5S-NTS trees support two major clades: a *Munroidendron*/*Reynoldsia* lineage and a *Tetraplasandra* lineage. The topology indicates that *Munroidendron* is closely related to *Reynoldsia sandwicensis*. Since its description by Forbes (1917), *Munroidendron racemosum* has been subject to conflicting classifications. Forbes placed it in *Tetraplasandra* but, based on its unique inflorescence, Sherff (1952) removed the species from *Tetraplasandra*, treating it as a monotypic genus. The ITS data suggest placing *Munroidendron* into *Reynoldsia* (*Reynoldsia* has nomenclatural priority over *Munroidendron*). However, *Reynoldsia sandwicensis*, the type species, is morphologically different from species of *Reynoldsia* outside Hawaii. Based on its deciduousness, fewer carpels, racemose-umbellate inflorescence and preference for dry to mesic habitats, perhaps *Reynoldsia sandwicensis* should be combined with *Munroidendron* and treated as distinct from *Reynoldsia* species outside Hawaii.

Within the *Tetraplasandra* lineage, three clades are supported in the ITS tree: a *T. hawaiensis* clade, a *T. gymnocarpa* clade and a *T. oahuensis* clade. *Tetraplasandra hawaiensis* is the type species and, interestingly, it is placed as sister to the *Reynoldsia*/*Munroidendron* clade in the 5S-NTS analysis. *Tetraplasandra hawaiensis* shares characters with all three Hawaiian genera, making its placement rather ambiguous. The two accessions of *Tetraplasandra hawaiensis* are polymorphic (A) or (T) at one nucleotide site (position 164). This character is shared with *Munroidendron* as (T) and with the rest of the taxa as (A). *Tetraplasandra hawaiensis* occurs in mesic to wet forest and has a dense, golden indumentum of stellate hairs on the inflorescences, young leaves and the abaxial side of mature leaves, and thus is similar in both habitat and pubescence to *Munroidendron*. Inflorescences of *Tetraplasandra hawaiensis* are paniculate with umbellate, racemose and umbellate-racemose subordinate branches, similar to inflorescences of *Reynoldsia sandwicensis* (Gray 1854; Sherff, 1955). Stamens are numerous, usually 4 times as many as petals, as in the species of the *Tetraplasandra oahuensis* clade. A recent analysis by the authors, combining the 5S-NTS and ITS data support the monophyly of *Tetraplasandra* as shown in the ITS tree.

The two species endemic to Kauai, *T. waialealae* and *T. waimeae,* share a clade with *T. oahuensis*. As defined by Lowry (1990), *Tetraplasandra oahuensis* is the most variable species of *Araliaceae* in Hawaii. Numerous segregates of *Tetraplasandra oahuensis* have been described based mainly on the number of stamens, which greatly complicated the taxonomy of this species (e.g. Sherff, 1955). Lowry (1990) pointed out that until a detailed study can be made, it is best to treat them all as a single species. In our 5S-NTS analysis, we included *Tetraplasandra lydgatei*, a segregate of *T. oahuensis* that T. Motley (unpublished data) suggested may be sufficiently distinct to be recognized as a species. 5S-NTS shows no resolution between *Tetraplasandra oahuensis* and *T. lydgatei*. In addition, both 5S-NTS and ITS show no resolution among *Tetraplasandra oahuensis*, *T. waialealae* and *T. waimeae*. In our 5S-NTS study, we also included individuals of *T. oahuensis* collected on Oahu, Molokai and East Maui to determine whether there is substantial genetic variability among the islands. Like many Hawaiian groups, however, the large amount of morphological variation among taxa is not paralleled by a comparable amount of genetic variation, which suggests recent and rapid speciation (Ganders & Nagata, 1984; Lowrey & Crawford, 1985; Carr & Kyhos, 1986).

Tetraplasandra gymnocarpa has fully superior ovaries (Fig. 3) and shares a clade with *T. kavaiensis*, which has partially superior ovaries. *Tetraplasandra gymnocarpa*

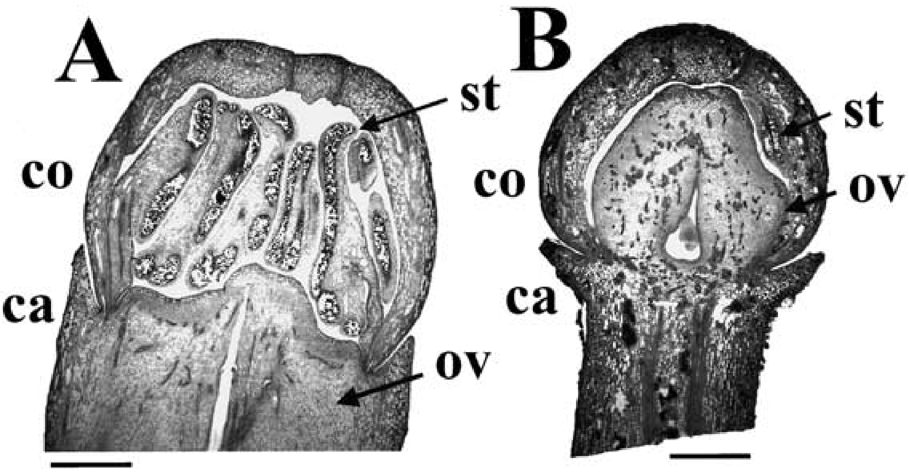

FIG. 3. Longitudinal sections of *Tetraplasandra* flower buds in late developmental stages, showing the difference between inferior ovaries and superior ovaries. (A) The epigynous *Tetraplasandra oahuensis*. (B) The hypogynous *Tetraplasandra gymnocarpa.* In *Tetraplasandra oahuensis*, the stamens are attached to the top of the ovaries. In comparison, in *Tetraplasandra gymnocarpa*, the stamens are attached to the base of the ovaries. Scale bars: 0.5mm. Abbreviations: ca, calyx; co, corolla; st, stamen; ov, ovary.

is extremely rare, it is only found on the summit areas of the Koolau Mountains, whereas *T. kavaiensis* is common on Kauai and occurs, although rarely, on Oahu, Lanai, Maui and Hawaii (Lowry, 1990). As our results here indicate, the occurrence of superior ovaries is a derived character state in *Tetraplasandra*, indicating an evolutionary reversal from inferior to superior ovaries.

In addition to superior ovaries, *Tetraplasandra gymnocarpa* and *T. kavaiensis* have other floral characters that are considered derived within *Tetraplasandra* (Eyde & Tseng, 1969), however ancestral relative to the *Araliaceae* (Plunket *et al.*, 1996, 1997). Recent molecular cladistic analyses (Plunkett *et al.*, 1996, 1997) suggest ancestral araliad flowers had bicarpellate gynoecia and pentamerous perianths and androecia. Both superior-ovary species in *Tetraplasandra* show a reduction in the number of stamens and carpels. They have only 6–8 stamens, equal to the number of the petals, and 2–5 carpels. In contrast, the other species of *Tetraplasandra* have numerous stamens, 4–6 times as many as petals. *Tetraplasandra hawaiensis* has 7–13 carpels and *T. waialealae*, *T. waimeae* and *T. oahuensis* have 4–8 carpels. Internal floral characteristics of *Tetraplasandra gymnocarpa* and *T. kavaiensis* are also more derived than those of *T. hawaiensis* (Eyde & Tseng, 1969, 1971). In the former two species, carpel margins are sealed above the locules and the ventral vascular supply consists of one bundle per carpel. In the latter, the carpel margins are open above the locules and some of the ventral bundles are in pairs.

Assuming that inferior ovaries evolved in order to protect developing seeds from pollinators and predators (Grant, 1950), Eyde & Tseng (1969) suggested that separation of the plants from their natural predators by dispersal to an isolated island system would release the selective pressures favouring inferior ovaries. They also suggested that superior ovaries may be another means to increase outcrossing in an insular system, following long-distance dispersal as discussed in Baker's rule (Baker, 1955, 1967). Dispersal to Hawaii could have increased selective pressures promoting outcrossing and inhibiting selfing and removed selective pressures against superior-ovary mutants. Perhaps the floral autapomorphies of *Tetraplasandra gymnocarpa* and *T. kavaiensis* reflect selective pressures driven by pollinator shifts, which also appears to be the case in Hawaiian Laminaceae (Lindquist *et al.*, unpublished data). In *Tetraplasandra* the evolution of superior ovaries and a reduction in stamen and carpel numbers could correlate with pollinator shifts from birds to insects.

The structure of the ITS and 5S-NTS trees are congruent, with one exception. *Tetraplasandra* is not monophyletic in the 5S-NTS tree, because of the alternative placement of *T. hawaiensis* sister to the *Reynoldsia*/*Munroidendron* clade, rather than as sister to the other species of *Tetraplasandra*. Showing *Tetraplasandra* as paraphyletic, the 5S-NTS phylogeny suggests treating *Tetraplasandra*, *Reynoldsia sandwicensis* and *Munroidendron* as a single variable genus. The ITS phylogeny, however, supports treating *Tetraplasandra* separate from *Reynoldsia sandwicensis* and *Munroidendron*. While our present study has focused primarily on the Hawaiian taxa, further molecular research, including more species of *Reynoldsia* and *Gastonia*, should elucidate the phylogenetic relationships within the *Tetraplasandra* complex. A preliminary analysis combining the molecular data sets resolve *Tetraplasandra* as a monophyletic group; comparable with the topology of the ITS tree. Further research is also under way to include a morphological data set, to be analysed both independently and in combination with the molecular data sets. Adding a morphological data set may resolve relationships among the species of *Tetraplasandra*, in particular *T. oahuensis*, *T. waialealae* and *T. waimeae* on one hand and between *T. gymnocarpa* and *T. kavaiensis* on the other.

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