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GENETICS OF ADAPTIVE RADIATION IN HAWAIIAN AND COOK ISLANDS SPECIES OF *TETRAMOLOPIUM* (ASTERACEAE; ASTEREAE). I. NUCLEAR RFLP MARKER DIVERSITY[1]

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Thirty-three nuclear RFLP (restriction fragment length polymorphism) probes were used to study genetic diversity in Hawaiian and Cook Islands species of *Tetramolopium* for comparison with previous morphological and isozyme studies and to provide greater resolution of the events associated with adaptive radiation in the genus. Levels of RFLP diversity are greater than those reported for isozymes, yet are still low in comparison to continental species. Genetic differentiation is greatest among species in sections rather than among sections and is concordant with the hypothesis of phyletic sorting of initial variability as suggested for morphological traits. Hypothesized introgression between *T. lepidotum* and *T. filiforme* is supported, but the evidence suggests bidirectional gene flow. Systematic relationships derived from the data agree with hypotheses based on morphology in the placement of populations within their respective species and the recognition of three main lineages within Hawaii. Inclusion of the Cook Islands species, however, renders section *Tetramolopium* paraphyletic, contradicting morphological, ecological, and crossing evidence. Interpreting these results in light of evidence from previous studies, the genetic diversity and relationships seen among species and sections of Hawaiian and Cook Islands *Tetramolopium* reflect the recent and rapid evolution of this group, limited addition of new variability, and phyletic sorting.

Key words: adaptive radiation; Asteraceae; genetic diversity; Hawaii; phylogeny; restriction fragment length polymorphism (RFLP); *Tetramolopium.*

Congeneric plant species on oceanic islands are often noted for conspicuous morphological and ecological variation but low genetic differentiation (Crawford, Whitkus, and Stuessy, 1987). This recurrent pattern is explained by adaptive radiation, conceptualized by Carlquist (1974) as a rapid and recent diversification of a taxonomic group (clade) in which new, strikingly different species evolve as a consequence of successive ecological shifts (adaptation) to a diversity of new habitats. Although many plant studies have provided empirical evidence of the morphological, ecological, and genetic pattern of diversity that is the expected outcome of adaptive radiation, surprisingly little is known about processes associated with adaptation and subsequent diversification in island plant genera. A variety of questions remain unanswered that would provide the basis of further analyses. For example, do morphological and ecological traits have parallel low levels of genetic variability as isozymes in island taxa? If so, how do these traits diversify within a clade so quickly? If not, how is their genetic variability increased from the original founding population? Related questions concern the genetic basis of morphological and ecological traits, especially as related to adaptation (Orr and Coyne, 1992), and whether isozymes accurately portray genetic variability within the genome, or whether other genetic markers would provide a different pattern.

The genus *Tetramolopium* is one example of adaptive radiation in the Hawaiian Islands. The genus, composed of 11 well-defined morphological species divided into three sections, exhibits a wide range of morphological and ecological diversity, but low levels of genetic diversity as measured by isozymes (Lowrey and Crawford, 1985; Lowrey, 1986). The genus has a number of additional features that make it a model system for studying the origin and diversification of species on oceanic islands. The source area for the original colonizing population is most likely the high uplands of New Guinea where 25 additional species are found (van Royen, 1983), and the founding event is placed within the Pleistocene (Fosberg, 1948; Smith, 1977). All members of the genus are short-lived diploid perennials, able to complete a life cycle within 6 mo. Interspecific crosses among nine species in three sections are fertile through the F_2 generation (Lowrey, 1986; T. K. Lowrey and R. Whitkus, unpublished data). Phylogenetic analysis using morphology has provided a well-resolved pattern of relationships (Lowrey, 1995). In total, these data suggest that the Hawaiian group is monophyletic and of recent origin (Lowrey, 1995).

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TABLE 1. Taxa, abbreviations, and collection localities for Hawaiian and Cook Islands *Tetramolopium* used in the present study.

Taxon	Collection		
	Abbreviation	Number	Locality
Sect. *Alpinum*			
T. humile ssp. *humile*	hum3	1616	Mauna Kea, 2865 m, Hawaii
	hum27	1640	Saddle area, Hawaii
	hum70	1644	Hawaii Volcanoes Natl. Park, 2040 m, Hawaii
T. humile ssp. *haleakalae*	hal5	1618	Haleakala Crater, Kalahaku Overlook, East Maui
	hal6	1619	Haleakala Crater, 2600–2680 m, E. Maui
	hal7	1620	Haleakala Crater, 2500 m, E. Maui
	hal8	1621	Haleakala Crater, 2350 m, E. Maui
	hal9	1622	Haleakala Crater, 2200 m, E. Maui
	hal10	1623	Haleakala Crater, 2215 m, E. Maui
	hal11	1624	Puu Halalii, Haleakala Crater, E. Maui
	hal12	1625	Haleakala Crater, 2050 m, E. Maui
	hal13	1626	Haleakala Crater, 2040 m, E. Maui
	hal14	1627	Haleakala Crater, 2010 m, E. Maui
Sect. *Tetramolopium*			
T. rockii	roc15	1628	NE of Kalani, 75 m, Molokai
	roc16	1629	W of 92-15, 75 m, Molokai
	roc17	1630	top of small ridge, 30 m, Molokai
	roc18	1631	ridge W of gulch, 30 m, Molokai
	roc19	1632	ridge E beyond 92-18, Molokai
	roc20	1633	100 m from beach near Kalani, Molokai
	roc25	1638	Moomomi point, >30 m, Molokai
T. sylvae	syl21	1634	Puu Ka Pele, >20 m, Molokai
	syl22	1635	Kalaupapa Pen., >20 m, Molokai
	syl23	1636	Kalaupapa Pen., >20 m, Molokai
	syl24	1637	Kalaupapa Pen., >20 m, Molokai
T. filiforme	fil29	1642	Ohikilolo Ridge, 790 m, Oahu
	fil31	1645	Ohikilolo Ridge, 915 m, Oahu
Cook Islands	cook40	1525	Via Nauri Cave turnoff, 30 m, Mitiaro
	cook41	1525a	coastal makatea, 30 m, Mitiaro
Sect. *Sandwicense*			
T. consanguineum	con2	1615	Saddle Area, 1580 m, Hawaii
	con28	1641	Saddle Area, 1580 m, Hawaii
T. lepidotum	lep30	1643	ridge E of Puu Kaua, 850 m, Oahu
T. arenarium	are26	1639	Saddle Area, 1550 m, Hawaii

In addition to evolution in Hawaii, a secondary founding event appears to have occurred to the Cook Islands of the South Pacific. A single population occurs on the island of Mitiaro and was originally thought to be conspecific to a Hawaiian species. Additional study now indicates that the Mitiaro population represents a new species aligned with one of the derived sections of Hawaiian *Tetramolopium* (T. K. Lowrey and R. Whitkus, unpublished data). However, no explicit phylogenetic analysis has confirmed the origin of the Cook Islands species nor indicated a potential sister species.

Recent and rapid diversification of island species results in neutral characters that exhibit little diversity compared with features related to adaptation (i.e., unequal evolutionary rates). This expectation has been confirmed in *Tetramolopium* in that extremely low levels of genetic diversity occur within species and high average genetic identities are found among species, based on isozyme analysis (Lowrey and Crawford, 1985). The next question is whether the level of genetic diversity seen in isozyme loci is representative of the overall genome diversity. Nuclear RFLP markers in plant taxa typically have shown higher levels of diversity than that of isozymes (Gepts, 1993; Whitkus, Doebley, and Wendel, 1994). These genetic markers have the potential to provide additional information on genetic variability and phylogenetic relationships within Hawaiian *Tetramolopium.* We have been analyzing nuclear RFLP loci in an attempt to gain additional information of how genetic variation is apportioned in Hawaiian *Tetramolopium,* to produce an independent hypothesis of phylogenetic relationships, and to increase the resolution of events associated with adaptive radiation in the genus. In this study, we ask whether nuclear RFLP markers (1) show levels of genetic diversity similar to that seen previously with isozymes, (2) produce phylogenetic hypotheses concordant with morphology-based cladograms, and (3) give new insights into the radiation of Hawaiian and Cook Islands *Tetramolopium.*

MATERIALS AND METHODS

Plant material—Seeds of all taxa were collected from natural populations in Hawaii in July and August 1992 and from Mitiaro Island, Cook Islands in November 1992 (Table 1). The populations sampled represent all or most of those known for each taxon. The Cook Islands species exists as one extensive population. Collections were made from either end of the population to sample its diversity and are considered two separate populations in this study. Plants used in the analysis were grown in greenhouses at Riverside, California, and Albuquerque, New Mexico. Vouchers are on deposit at the herbarium of the University of New Mexico (UNM).

Genomic DNA isolation—Total genomic DNA was isolated from leaves and young flower buds with use of a modified cTAB procedure

(Saghai-Maroof et al., 1984). A mortar and pestle were used to grind 1–2 g of plant material in liquid nitrogen to a fine powder. Prior to thawing of the powder, 2.5 mL of extraction buffer [100 mmol/L Tris-HCl (pH 8), 2% w:v mixed alkyltrimethyl-ammonium bromide, 1.4 mol/L NaCl, 20 mmol/L EDTA (pH 8), 1% v:v β-mercaptoethanol, 1% w:v sodium bisulfite] were added, and the tissue was ground for ~1 min to form a slurry. The slurry was transferred to 15-mL polypropylene tubes and incubated at 60°C for 30 min with one mixing, followed by cooling to room temperature and extracting twice with chloroform: octanol (24:1). Genomic DNA was precipitated by mixing in an equal volume of ice-cold isopropanol, hooked on a glass pasture pipette, and transferred to 2 mL of 76% ethanol and 0.2 mol/L sodium acetate for 20 min. A second wash of 76% ethanol and 10 mmol/L ammonium acetate was performed for 2 min, followed by air drying to remove traces of alcohol before resuspension of the pellet in 200–400 μL of TE [10 mmol/L Tris-HCl (pH 8), 1 mmol/L EDTA (pH 8)]. All taxa (except *T. humile*) yielded DNA contaminated with high concentrations of polysaccharides, making it unsuitable for restriction digests. Contaminated DNAs were purified with either CsCl density gradient or caylase (CAYLA, Toulouse, France). For the CsCl density gradient, the DNA pellet was dissolved in 3.2 mL of TE, followed by 3.2 g of CsCl, and 75 μL of 6.25 mg/mL ethidium bromide. After centrifugation (4 h at 100 000 rpm at 18°C in a Beckman TLN100 rotor), the DNA band was visualized under UV illumination and removed with a 1-mL syringe and an 18-gauge needle. Ethidium bromide was removed by repeated partitioning against NaCl-saturated butanol. Two volumes of TE were added to the solution, and the DNA was precipitated with two volumes of ethanol. The precipitate was rinsed with 70% ethanol and resuspended in TE to give a final concentration of 0.5 μg/mL. The caylase procedure was modified from Rether, Delmas, and Laouedj (1993). Contaminated DNA extracts were brought up to a volume of 1 mL in water and 50 μmol/L potassium acetate (pH 5.5), 10 μmol/L EDTA (pH 8), 0.5 mg caylase M3, 0.05 mg RNase A, and incubated at 37°C for 15 h. Proteins were removed by phenol:chloroform (1:1) and chloroform: octanol (24:1) extraction. DNA was precipitated with two volumes of cold ethylene glycol monoethyl ether and pelleted by centrifuging for 5 min at 10 000 g. Pellets were washed in cold 70% ethylene glycol monoethyl ether, air dried, and resuspended in TE to give a final concentration of 0.5 μg/mL. Concentrations of DNA samples were quantified with a Hoefer (San Francisco, CA) TKO100 Mini-Fluorometer, following manufacturer's instructions.

Clones—A genomic library was constructed from an individual of *T. humile* ssp. *haleakalae.* Genomic DNA was digested with *Pst* I and ligated into pUC19 (pT7/T3α-19, GibcoBRL, Grand Island, NY) using standard procedures (Sambrook, Fritsch, and Maniatis, 1989). Ligation products were used to transform DH5αMCR competent cells (GibcoBRL). The cells were spread on LB agar plates (Sambrook, Fritsch, and Maniatis, 1989) containing 100 mg/L of ampicillin and 70 mg/L of X-gal, and incubated at 37°C overnight. Transformed colonies were picked and grown in LB with 100 mg/L of ampicillin at 37°C with agitation and stored as glycerol stocks (cells:glycerol, 1:1) at −80°C. Plasmid DNA was isolated using Wizard Minipreps DNA Purification System (Promega, Madison, WI). Low-copy number clones were selected from the genomic library by blotting 5 μL of plasmid DNA onto nylon membranes using the GibcoBRL Hybri-Dot Manifold, hybridizing the blots with ^{32}P-labeled genomic DNA, and choosing clones with weak to no signal from autoradiography as candidate clones. Probes were prepared either by gel isolation following Whitkus, Doebley, and Lee (1992) or PCR (polymerase chain reaction) amplification. Cloned DNA fragments were amplified in 50-mL reactions containing 1.25 ng plasmid DNA, 200 mmol/L each dNTP, 0.5 mmol/L M13/pUC Forward and Reverse Amplification Primers (GibcoBRL), 2 mmol/L $MgCl_2$, five units *Taq* DNA polymerase and 1X reaction buffer (Promega). Amplifications were carried out using 35 cycles of 92°C for 30 s, 55°C for 30 s, and 72°C for 500 bp/min, followed by a final step of 72°C for 13 min.

TABLE 2. List of RFLP probes used in analyses, number of patterns, heterozygous patterns, fragments, and polymorphic fragments observed across samples of Hawaiian and Cook Islands *Tetramolopium.*

Probe	Patterns	Heterozygous Patterns	Fragments	Polymorphic fragments
THH12	3	0	3	3
THH14	2	0	3	2
THH15	4	1	4	4
THH36	1	—	1	—
THH37	1	—	1	—
THH51	1	—	1	—
THH54	3	0	3	3
THH74	1	—	1	—
THH76	6	1	6	5
THH79	2	0	2	1
THH140	3	1	3	3
THH145	1	—	1	—
THH159	3	1	3	3
THH168	11	7	7	7
THH176	1	—	1	—
THH182	2	0	2	1
THH191	1	—	1	—
THH198	7	4	7	7
THH207	1	—	1	—
THH212	2	0	2	2
THH213	2	0	2	2
THH217	3	0	3	3
THH218	1	—	1	—
THH219	1	—	1	—
THH222	9	3	7	7
THH223	4	2	5	4
THH227	4	1	4	3
THH230	2	0	2	2
THH236	2	0	2	2
THH238	2	1	2	2
THH240	4	2	4	4
THH243	1	—	1	—
Helianthus rDNA	1	—	1	—

RFLP generation—RFLPs were obtained by Southern analysis using *EcoR* V and 32 *T. humile* low-copy number genomic clones and a *Helianthus* rDNA clone as probes (Table 2). Restriction endonuclease digestion, filter blotting, radioactive labeling of probes, and filter hybridization follow Whitkus, Doebley, and Lee (1992), except 5 mg of genomic DNA was used for endonuclease digestion and blotting, and filter hybridizations were performed in hybridization bottles and a hybridization oven (Robbins Scientific, Sunnyvale, CA). Filters were stripped for reuse with 0.2 mol/L NaOH for 10–15 min and neutralized with 0.2 mol/L Tris-HCl (pH 8)/0.1% SDS (sodium dodecyl sulfate) twice for 30 min.

Data interpretation—A combination of multibanded patterns obtained for some probes and small population sample sizes for all probes precluded conventional locus/allele interpretation of the RFLP bands. Two interpretations of the raw data were used to alleviate bias that may be created by a single method. Our first approach assumes that a probe represents a single locus and an allele consists of the fewest, independently occurring patterns of bands across all samples. If the pattern of bands for an individual is not reducible as compared with that for the total population of samples, the individual is considered homozygous for the ''allele.'' If the banding pattern in an individual can be derived by adding the patterns from two other individuals, then the complex pattern is interpreted as heterozygous. This conservative interpretation of the data may underestimate diversity or similarities. Preliminary linkage mapping has shown that a number of RFLP probes hybridize to two or more loci (R. Whitkus, unpublished data), indicating the likeli-

TABLE 3. RFLP pattern and fragment polymorphism in Hawaiian and Cook Islands *Tetramolopium*. *N* = average number of individuals over all probes, Pp = percentage polymorphic patterns, *A* = alleles per pattern, Pt = proportion of all patterns, Up = unique patterns, Pf = percentage polymorphic fragments, Ft = proportion of all fragments, Uf = unique fragments.

Taxon	No. of pop.	*N*	Pattern				Fragment		
			Pp	*A*	Pt	Up	Pf	Ft	Uf
All species	31	113.7	63.6	2.8	1.00	—	79.5	1.00	—
Sect. *Alpinum*	12	40.6	30.3	1.5	0.52	6	27.3	0.61	4
T. humile	same								
ssp. *humile*	3	12.9	18.2	1.2	0.42	2	12.5	0.51	1
ssp. *haleakalae*	9	27.7	18.2	1.2	0.45	3	14.8	0.53	2
Sect. *Sandwicense*	4	14.7	24.2	1.3	0.47	4	11.4	0.57	3
T. consanguineum	2	8.0	3.0	1.1	0.38	2	3.4	0.45	2
T. lepidotum	1	5.3	9.1	1.1	0.39	1	8.0	0.49	0
T. arenarium	1	1.4	0.0	1.0	0.36	1	0.0	0.43	1
Sect. *Tetramolopium*	15	58.4	57.6	2.4	0.87	32	59.1	0.91	23
T. rockii	7	23.2	45.5	1.8	0.65	7	38.6	0.72	5
T. sylvae	4	16.0	30.3	1.5	0.53	2	23.9	0.65	1
T. filiforme	2	9.8	24.2	1.5	0.52	5	25.0	0.61	3
Cook Islands	2	9.4	12.1	1.2	0.42	4	9.1	0.50	3

hood of overlooked information and prompting the use of our second method of data interpretation. If the bands belong to multiple loci and are codominant, the data are comparable to single-probe, multilocus DNA fingerprinting data, with each band scored as present or absent. To avoid scoring multiple fragments of individual alleles as independent bands, any series of bands for a probe that always occurred together, across all samples, were scored as a single band. The former method of data interpretation will be heretofore referred as the ''pattern'' data and the latter as the ''fragment'' data.

Data analysis—Polymorphic indices were calculated as analogues to those for isozyme loci. These included percentage polymorphic patterns, percentage polymorphic fragments, proportion of all patterns present in a taxon (number of patterns in taxon per total number of patterns), proportion of all fragments present in a taxon (number of fragments in taxon per total number of fragments), unique patterns, unique fragments, and number of alleles per pattern. The proportions of patterns and fragments exclusively shared between taxa were calculated as the number of unique patterns or fragments shared between two taxa divided by the average number of respective polymorphic fragments or patterns in the two taxa. Diversity statistics (H_T, H_S, G_{ST}) were calculated for species, sections, and genus as in Nei (1987) for the pattern data. Similar estimates of genetic diversity were obtained for the fragment data according to Jin and Chakraborty (1994). The methods of Jin and Chakraborty (1994) also provide an estimate of the loci sampled from a fingerprint pattern. This was applied to each species for the fragment data and averaged over all species.

Pairwise distances between all populations were calculated for both methods of data interpretation. For the pattern data, the Manhattan metric was used, with each pattern allele as a character and the distances scaled to the number of probes (distance × no. alleles/no. probes). Estimates of genetic distance (Nei, 1972) were calculated for the fragment data according to Jin and Chakraborty (1994). Average linkage clustering (UPGMA) and principal coordinate analyses on distances were carried out with NTSYS-PC 1.80 (Rohlf, 1993). Neighbor-joining and Fitch-Margoliash trees were obtained with PHYLIP 3.4 (Felsenstein, 1991). Character-based cladistic analysis of species used alleles as character states of polymorphic pattern ''loci.'' Character states present, absent, or polymorphic were used from polymorphic fragments. Cladistic analyses were performed with PAUP 3.1.1 (Swofford, 1993), with characters analyzed as unordered. The most parsimonious trees were found by exhaustive searches with use of both ACCTRAN and DELTRAN transformations. Branch support was calculated by 1000 bootstrap replicates over characters and exhaustive searches. All distance- and character-based analyses were repeated with data sets that excluded *T. filiforme*, a species with a suspected hybridization history (Lowrey, 1986, 1995). In addition, *T. lepidotum* was identified as exhibiting evidence of introgression and was excluded in repeated analyses.

RESULTS

Screening seven Hawaiian and one Cook Islands species of *Tetramolopium* with 37 genomic clones resulted in 33 probes used in subsequent analyses. Four probes were excluded as a consequence of weak hybridization signal and inability to record data, or the hybridization patterns were too complex to interpret. The number of individuals surveyed per population was based on availability of sufficient DNA, individuals, and filter space. Actual numbers ranged from one to seven per population per clone, with an average of 3.7 over all clones. The number of different molecular mass-sized bands varied from one to four for both patterns and fragments. Twelve clones were monomorphic in patterns and fragments over all individuals, whereas the remaining 21 clones provided RFLPs (Table 2). Ninety-two different patterns were observed over all clones, with 1–11 patterns and 0–7 apparent heterozygous patterns recognized per clone. Eighty-eight fragments were scored, with the number of fragments varying from one to seven per clone. Assuming each clone sampled a single locus, 33 loci were surveyed via the pattern analysis. In the fragment analysis, 37.8 loci were estimated, following Jin and Chakraborty (1994).

Levels of polymorphism across taxa are equivalent between the two methods of data interpretation (Table 3). Both methods indicate that section *Tetramolopium* has the highest and section *Sandwicense* the lowest levels of polymorphism. The apparent number of alleles per locus is 2.8 over all species, ranging from 1.0 to 1.8 per species and distributed in a fashion that parallels the other polymorphism measures (Table 3).

Findings from the two methods of data interpretation show similar levels of genetic diversity within and among taxa (Table 4). Total diversity (H_T) in Hawaiian and Cook Islands taxa is 0.21. Section *Tetramolopium* has the highest diversity, followed by sections *Alpinum* and *Sandwi-*

TABLE 4. Genetic diversity in Hawaiian and Cook Islands *Tetramolopium* inferred from pattern and fragment data.

Taxon	Pattern			Fragment		
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
All sections	0.205	0.145	0.293	0.212	0.129	0.170
All species	0.205	0.067	0.673	0.212	0.069	0.558
Sect. *Alpinum*	0.083	—	—	0.089	—	—
T. humile	0.083	0.055	0.337	0.089	0.052	0.239
ssp. *humile*	0.044			0.050		
ssp. *haleakalae*	0.065			0.054		
Sect. *Sandwicense*	0.078	0.020	0.744	0.091	0.067	0.725
T. consanguineum	0.010			0.010		
T. lepidotum	0.029			0.033		
T. arenarium	—			—		
Sect. *Tetramolopium*	0.204	0.088	0.569	0.207	0.075	0.437
T. rockii	0.126			0.113		
T. sylvae	0.068			0.057		
T. filiforme	0.117			0.100		
Cook Islands	0.039			0.028		

cense, which are an order of magnitude lower. The species with the highest diversity is *T. rockii*, whereas the Cook Islands species is notable for its lower diversity compared with the other species in the section. All other species, except *T. filiforme*, exhibit diversity values of <0.1. Coefficients of gene differentiation (G_{ST}) are larger within sections than between sections. Section *Sandwicense* has the largest G_{ST} (>0.72) and section *Alpinum* the lowest value, but in the latter case subspecies are being compared rather than species.

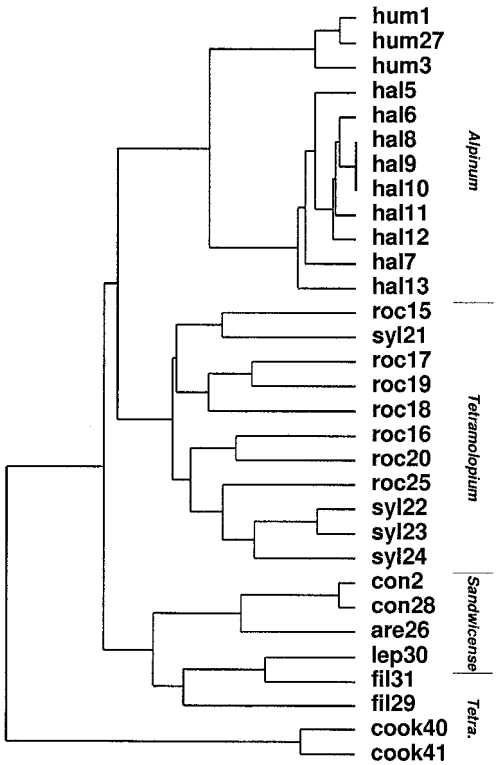

Fig. 1. Average linkage clustering of Hawaiian and Cook Islands populations of *Tetramolopium* based on RFLP pattern data. Population abbreviations are from Table 1. Sectional affiliations of populations are shown at the right.

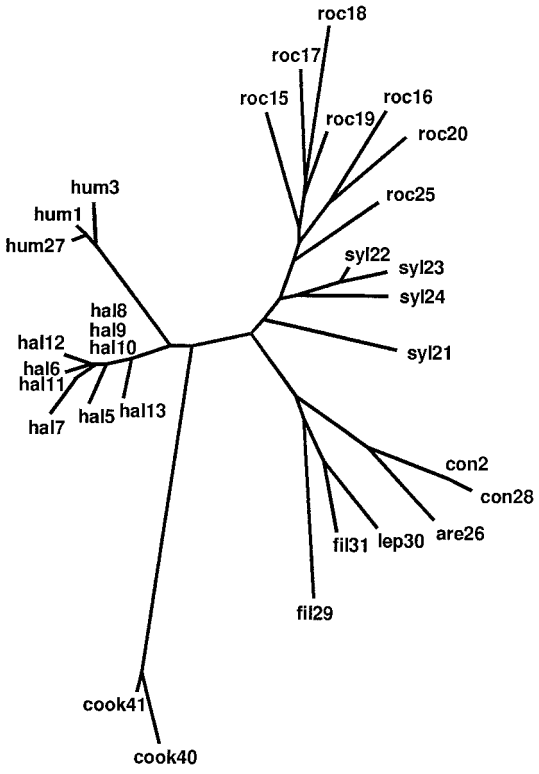

Fig. 2. Neighbor-joining analysis of Hawaiian and Cook Islands populations of *Tetramolopium* based on RFLP pattern data.

Distance-based trees of populations for both methods of data interpretation have similar topologies. Populations tend to cluster into their respective species and species within their respective sections (Figs. 1–3) with three notable exceptions: the placement of *T. filiforme* populations into the sect. *Sandwicense* cluster (Figs. 1, 2), the lack of resolution between *T. sylvae* and *T. rockii* popu-

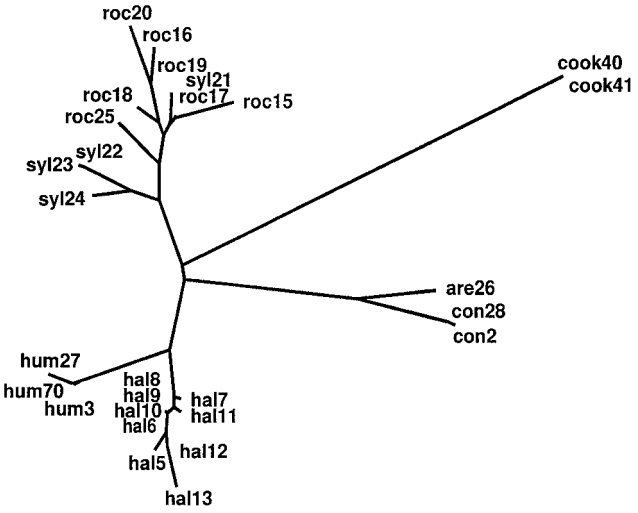

Fig. 3. Fitch-Margoliash analysis of Hawaiian and Cook Islands populations of *Tetramolopium* based on RFLP fragment data, with populations of *T. filiforme* and *T. lepidotum* removed from the analysis.

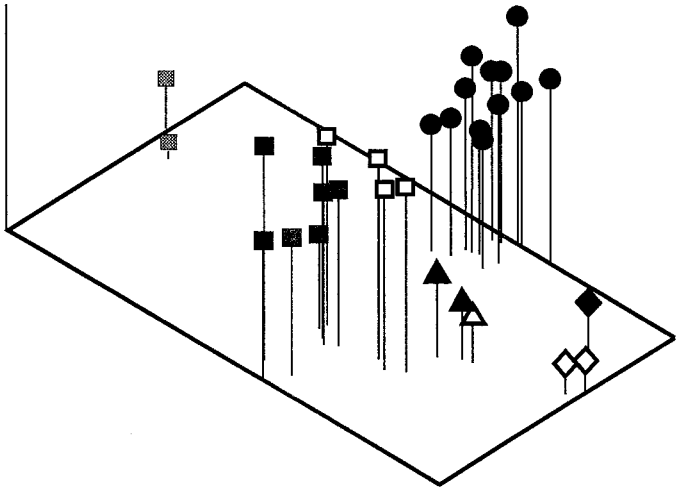

Fig. 4. Principal coordinates analysis of Hawaiian and Cook Islands populations of *Tetramolopium* based on RFLP fragment data. Symbols: open square = *T. rockii*, filled square = *T. sylvae*, shaded square = Cook Islands species, circles = *T. humile*, closed triangle = *T. filiforme*, open triangle = *T. lepidotum*, open diamond = *T. consanguineum*, closed diamond = *T. arenarium.*

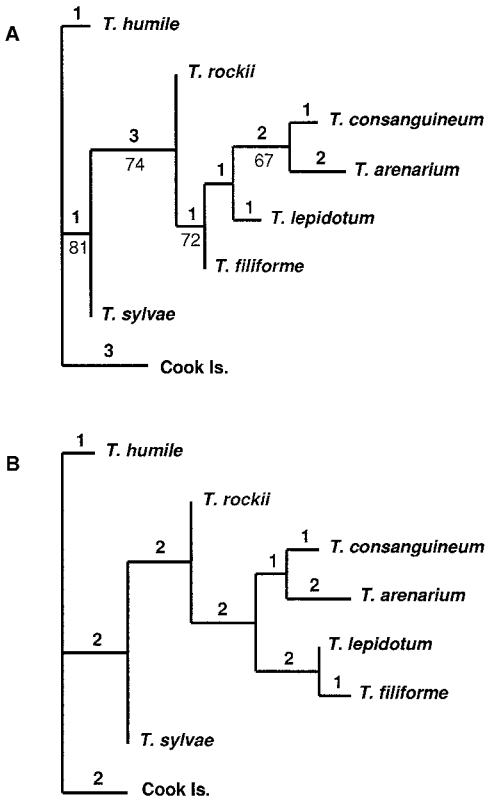

Fig. 5. The two equally parsimonious trees of RFLP pattern data for Hawaiian and Cook Islands species of *Tetramolopium.* Tree length = 92, CI = 0.989, RI = 0.900. Trees are unrooted. Values above branches are the number of character state changes, numbers below branches are the bootstrap support values from 1000 replicates. (A) Tree with *T. filiforme* basal to sect. *Sandwicense* (*T. lepidotum, T. consanguineum,* and *T. arenarium*). (B) Tree with *T. lepidotum* and *T. filiforme* forming a sister group to remainder of sect. *Sandwicense* (*T. consanguineum* and *T. arenarium*). Both trees indicate that sect. *Tetramolopium* is paraphyletic (see text for details).

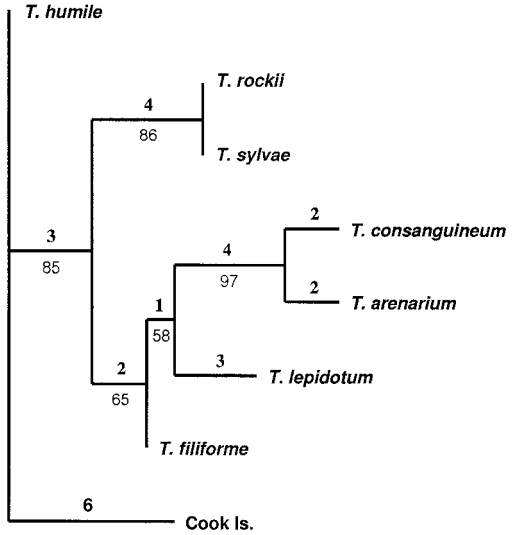

Fig. 6. The shortest parsimonious tree of RFLP fragment data for Hawaiian and Cook Islands species of *Tetramolopium.* Tree length = 127, CI = 0.992, RI = 0.923. Tree is unrooted. Values above branches are the number of character state changes, numbers below branches are the bootstrap support values from 1000 replicates. Hawaiian members of sect. *Tetramolopium* without hybridization history (*T. rockii* and *T. sylvae*) form a monophyletic group. Section is paraphyletic with inclusion of Cook Islands species.

lations (Figs. 1, 3), and the Cook Islands populations placed outside of the sect. *Tetramolopium* group (Figs. 1, 2), despite the morphological evidence that ties this species to the section (Lowrey, 1995). The only analysis that places the Cook Islands populations with the species of sect. *Tetramolopium* is the Fitch-Margoliash fragment analysis with the populations of *T. lepidotum* and *T. filiforme* removed from the analysis. Other distance-based methods do not change the placement of the Cook Islands populations when *T. lepidotum* and *T. filiforme* populations were removed. In UPGMA trees, the Cook Islands populations are the most distant from all other clusters (Fig. 1), and all other trees place these populations near the branch leading to *T. humile* or sect. *Sandwicense* (except as noted above).

Principal coordinates analyses of distances from both methods of data interpretation show the same relationships among populations as seen in the distance tree analyses. The Cook Islands populations do not show obvious affinity to any section or species in Hawaii, populations of *T. rockii* and *T. sylvae* exhibit a close association, and populations of *T. humile* form a well-differentiated group (Fig. 4). Additionally, populations of *T. filiforme* and *T. lepidotum* occur in an intermediate position between sections *Tetramolopium* and *Sandwicense.*

Character-based cladistic analyses of the species were carried out with the 21 polymorphic patterns and 70 polymorphic fragments. In the pattern analysis, 15 characters were phylogenetically informative and produced two equally parsimonious trees of 93 steps (Fig. 5). For the fragment data, 43 characters were phylogenetically informative and gave a single, most parsimonious tree of 127 steps (Fig. 6). Tree topology was not affected by ACCTRAN or DELTRAN optimization. In the fragment analysis (Fig. 6) and one tree of the pattern analysis (Fig.

TABLE 5. Proportion of shared exclusive RFLP patterns (below diagonal) and fragments (above diagonal) for Hawaiian and Cook Islands taxa of *Tetramolopium.*

	A	B	C	D	E	F	G	H	I	J
A. *Alpinum*	—	0.22	0.05	0.03	0.05	0.00	0.03	0.00	0.00	0.00
B. *Tetramolopium*	0.21	—	0.17	0.18	0.04	0.14	0.17	0.00	0.11	0.00
C. *T. rockii*	0.02	0.21	—	0.07	0.02	0.00	0.00	0.00	0.00	0.00
D. *T. sylvae*	0.03	0.17	0.09	—	0.00	0.06	0.03	0.00	0.00	0.00
E. *T. filiforme*	0.03	0.10	0.10	0.00	—	0.00	0.03	0.00	0.03	0.00
F. Cook Islands	0.03	0.11	0.03	0.06	0.00	—	0.00	0.00	0.00	0.00
G. *Sandwicense*	0.00	0.16	0.00	0.00	0.06	0.00	—	0.00	0.00	0.00
H. *T. consanguineum*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—	0.00	0.00
I. *T. lepidotum*	0.00	0.02	0.00	0.00	0.03	0.00	0.00	0.00	—	0.00
J. *T. arenarium*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—

5A), sect. *Sandwicense* is a monophyletic clade, whereas a second pattern tree shows *T. lepidotum* and *T. filiforme* as a sister group to the rest of sect. *Sandwicense* (Fig. 5B). The fragment tree supports the monophyly of the Hawaiian members of sect. *Tetramolopium* (Fig. 6), but the pattern trees show the group of taxa to be paraphyletic and include sect. *Sandwicense* (Fig. 5). As seen in the distance-based trees, sect. *Alpinum,* the Cook Islands species, and the clade containing sections *Tetramolopium* and *Sandwicense* form an unresolved trichotomy, again calling into question the placement of the Cook Islands species in sect. *Tetramolopium.*

In all cladistic analyses, the branches are supported by few character state changes. A maximum of three character states support clades in the pattern analysis and four in the fragment analysis. The pattern data produce 19 trees one step longer and the fragment data produce six trees one step longer. Despite the apparent weak phylogenetic information in both data sets, the consistency and retention indices are high, with values of 0.99 and 0.90, respectively, for the pattern analysis, and 0.99 and 0.92, respectively, for the fragment analysis. Bootstrap support values are 65% or higher for consistent branches in both analyses. Weak support (58%) is obtained for the branch leading to sect. *Sandwicense* in the fragment tree. This is a branch that collapses in the pattern trees to produce a trichotomy of *T. filiforme, T. lepidotum,* and the clade of *T. consanguineum* and *T. arenarium* in a consensus tree (not shown). The topology of trees remain even after the removal of *T. filiforme* or both *T. filiforme* and *T. lepidotum* (not shown), indicating the support of the data for the trees generated.

Sections *Alpinum* and *Tetramolopium* have the highest proportion of exclusively shared patterns (0.21) and fragments (0.22) (Table 5). Section *Sandwicense* shares no unique patterns but a small proportion of unique fragments with sect. *Alpinum* and a larger proportion of unique patterns (0.16) and fragments (0.17) with sect. *Tetramolopium* (Table 5). In comparing species pairs, the fragment data show the pairs *T. rockii–T. sylvae* and *T. sylvae*–Cook Islands sharing the largest proportions, but the pattern data indicate *T. rockii–T. sylvae, T. rockii–T. filiforme,* and *T. sylvae*–Cook Islands share the largest proportions. Examining single species, the Cook Islands species shares the largest proportion of exclusive fragments and patterns with sect. *Tetramolopium* (*T. sylvae,* specifically). The species *T. filiforme* shares the largest proportion of exclusive patterns with sect. *Tetramolopium* (*T. rockii,* specifically), followed by sect. *Sandwicense.* For the fragment data, *T. filiforme* shares approximately equal proportions with all three sections and the highest with sect. *Alpinum.* At the species level, *T. filiforme* shares fragments exclusively with *T. rockii* and *T. lepidotum.* One interesting anomaly is the sharing of exclusive fragments and patterns of *T. lepidotum* with sect. *Tetramolopium* and *T. filiforme.* The other species of sect. *Sandwicense* do not share exclusive patterns or fragments with any taxon (Table 5).

DISCUSSION

Comparison of methods of data interpretation—The method of interpreting what constitutes an RFLP locus can affect estimates of diversity (Brubaker and Wendel, 1994). The probe as locus approach has been used in several previous studies (cf. Brubaker and Wendel, 1994) but may inflate diversity measures if the actual number of loci revealed by a probe is greater than one since there would appear to be greater allelic diversity. The approach of Jin and Chakraborty (1994) was developed for multilocus probes of highly polymorphic genomic regions (VNTR, SSR), but we feel it is a general approach that can be extended to low-copy number nuclear RFLPs. In the present analysis, interpreting RFLPs as patterns or fragments made little difference in the results and a high degree of concordance is seen between the two data sets in estimated patterns of genetic polymorphism and diversity in Hawaiian and Cook Islands *Tetramolopium* (Tables 3, 4). Moreover, the actual values of the diversity estimates are similar for the two methods (Table 4). The lack of consistent over- or underestimation of one method compared with the other indicates little or no systematic bias.

Regarding the actual number of loci sampled, we evaluated the number provided by both methods with data from a mapping study of RFLPs in *Tetramolopium* (R. Whitkus, unpublished data). An estimate of the minimum number of loci revealed by 37 polymorphic genomic clones was obtained in the mapping study; nine of these probes are included in the current study. The number of loci per polymorphic probe obtained from the nine probes used in both studies did not significantly differ from the additional 28 in the mapping analysis (1.22 ± 0.66 [SD] vs. 1.29 ± 0.73, respectively), giving a combined estimate of 1.27 ($\pm$ 0.71) loci per polymorphic probe. Extending these findings to the 33 genomic clones employed in the present study, we would expect to have surveyed 38.7 loci (21 polymorphic probes $\times$ 1.27 + 12 monomorphic probes), with a range

from the standard deviation of 23.4–53.6. This large range reflects the small number of loci sampled and variability in the number of loci per polymorphic probe (1–3). Although large, the range does encompass the number of loci provided by our methods, again indicating little systematic bias between the two.

RFLP-based systematics of Tetramolopium—When used for determining systematic relationships, the RFLP results differ notably from that in Lowrey (1986, 1995). With regard to Hawaiian *Tetramolopium,* the three main clades (sections) recognized by Lowrey (1986, 1995) are consistently apparent in cluster, principal coordinates, and cladistic analyses. Only the character-based cladistic analysis of patterns shows sect. *Tetramolopium* to be paraphyletic (Fig. 5). When the Cook Islands species is added, concordance between studies breaks down in that only the Fitch-Margoliash tree of fragments that excludes *T. filiforme* and *T. lepidotum* shows monophyly of sect. *Tetramolopium* (Fig. 3), and all others fail to join the species into a cluster or clade corresponding to the section. Nonconcordance is also seen at the species level. Although most populations cluster within their respective species or subspecies, *T. rockii* and *T. sylvae* populations are weakly resolved (Figs. 1, 3, 4).

This lack of concordance of morphological and genetic marker data is a well-known feature of insular plant taxa, which typically have different rates of morphological and neutral genetic marker evolution (Crawford, Whitkus, and Stuessy, 1987). *Tetramolopium* is recognized as a very recent arrival in Hawaii (Lowrey, 1995) and probably has not had time to accumulate sufficient mutational differences at RFLP loci to match the morphological evolution evident in the group. Another explanation for the nonconcordance may be uneven sampling of taxa across studies. Previous studies did not have the Cook Islands species available for analysis. The present study included only extant, available taxa, a choice that precluded species known only from herbarium material (*T. conyzoides, T. tenerrimum*) or extant species unavailable for collection (*T. remyi, T. capillare*). Thus results may conflict as a result of inclusion of different taxa. Finally, small sample sizes within species can bias estimates of relationships by missing clade- or species-specific alleles. Population sizes in *Tetramolopium* are typically small and plants are self-compatible (Lowrey, 1986). This combination suggests that the low variability of RFLPs and isozymes are indicative of inbred populations. Increased sampling within species, when possible, would not be likely to increase the number of informative markers or change the relationships revealed by the present analysis because inbred populations carry few alleles (Hamrick and Godt, 1990). Therefore, the nonconcordance between our findings and those of previous analyses probably results from a combination of few phylogenetically informative markers and incomplete taxon sampling, but probably not severely affected by sampling within species.

RFLP variability in Tetramolopium—The level of genetic polymorphism and diversity detected by nuclear RFLPs was greater than that reported by Lowrey and Crawford (1985) for Hawaiian *Tetramolopium.* On the basis of 22 isozyme loci the authors found 27.3% polymorphic loci at the genus level, 1.4 alleles per locus, and only three alleles unique to any section (Lowrey and Crawford, 1985). These values are half or less than half of those found in the current study (Table 3). Genetic diversity was not reported in Lowrey and Crawford (1985) but has been calculated from their published data (Table 6). Total diversity (H_T) is an order of magnitude lower than that found with RFLPs, except for sect. *Sandwicense.* As found in the present study, the degree of differentiation was higher among species in sections than between sections, as measured by G_{ST}. Studies of other island plant taxa show that *Tetramolopium* has low levels of isozyme variability. In a survey of 14 isozyme studies, DeJoode and Wendel (1992) report mean species values of 25% polymorphic loci (range 0.00–0.57), 1.32 alleles per locus (range 1.0–1.93), and total diversity of 0.064 (range 0.000–0.189). Recently, Weller, Sakai, and Straub (1996) reported a mean of 43.6% polymorphic loci, 1.84 alleles per locus, and total diversity of 0.188 for Hawaiian *Schiedea* and *Alsinidendron* in the Caryophyllaceae. For comparison, mean species values for *Tetramolopium* are 7.8% polymorphic loci, 1.09 alleles per locus, and total diversity of 0.016. Thus, isozyme analysis indicates that *Tetramolopium* has some of the lowest reported values of genetic variation of island plant taxa. In a study of RFLP variation, Brubaker and Wendel (1994) compiled diversity values for wild and cultivated plant species from ten reports. Polymorphic loci ranged between 7 and 90%, alleles per locus ranged between 1.07 and 8.5, and total diversity (H_T) ranged between 0.014 and 0.684. Additional, recent studies (Zhang, Saghai Maroof, and Kleinhofs, 1993; Deu et al., 1994; Petersen, Østergård, and Giese, 1994; Velásquez and Gepts, 1994; Cui et al., 1995; Lu et al., 1996) give parallel values with polymorphic bands/loci between 38 and 100%, alleles/fragments per band/locus of 3.1–5.6, and total diversity (H_T) values between 0.38 and 0.47. Almost all of the work on RFLP diversity is based on cultivated plants and their close relatives, with one or two species studied. Our analysis of eight species of Hawaiian and Cook Islands *Tetramolopium* falls into the low range of values reported in these other studies. Therefore, although RFLP loci show higher levels of genetic variability in *Tetramolopium* than do isozymes, RFLP loci concur with isozymes in that overall genetic variability and diversity are low in the group.

Genetic variability within species is strongly influenced by breeding system and distribution. In genetic surveys by Hamrick and Godt (1990), species with narrow or endemic distributions or mating systems that favor selfing have statistically lower levels of genetic variability than widespread or outcrossing species. All species of *Tetramolopium* are self-compatible and are found in small, isolated populations (Lowrey, 1986). There are notable differences in levels of genetic variation that are reflected by the biological attri-

TABLE 6. Genetic diversity in Hawaiian *Tetramolopium* calculated from isozyme data in Lowrey and Crawford (1985).

Taxon	H_T	H_S	G_{ST}
All sections	0.051	0.040	0.216
All species		0.009	0.824
Sect. *Alpinum*	0.002	0.002	0.000
Sect. *Sandwicense*	0.073	0.005	0.931
Sect. *Tetramolopium*	0.046	0.016	0.652

butes of the various species. Species of sect. *Sandwicense* have a limited distribution and are composed of small populations. Only a few populations of *T. consanguineum* exist today; they are restricted to the island of Hawaii and occur in numbers of fewer than 50 individuals per population (T. K. Lowrey and R. Whitkus, personal observation). Previously considered extinct, *T. arenarium* is known from two populations on the island of Hawaii, with fewer than 100 individuals known for the species (T. K. Lowrey and R. Whitkus, personal observation). Finally, *T. lepidotum* is found only on two ridges on the island of Oahu and consists of fewer than 100 individuals. The species with the most widespread distribution is *T. humile,* found over extensive areas of the alpine zone on East Maui and Hawaii (Lowrey, 1986). Additionally, this species is phylogenetically closest to the likely ancestral species of the Hawaiian group (Lowrey, 1995). Because *T. humile* has more individuals and a longer history than most other Hawaiian species of *Tetramolopium,* the increased level of variability observed in the RFLP data set is understandable. The highest levels of RFLP diversity, however, are found in sect. *Tetramolopium.* Although populations tend to be few and restricted to small geographic regions, they have some of the highest population numbers, ranging into the hundreds (T. K. Lowrey and R. Whitkus, personal observation). Additionally, plants are monoecious in this section rather than gynomonoecious as in the other sections (Lowrey, 1986, 1995). This combination of biological factors appears to account for the high levels of RFLP diversity for this section.

Adaptive radiation in Tetramolopium—The low level of genetic diversity detected in the present study supports the hypothesis of a recent origin and rapid diversification of the morphologically distinct taxa of *Tetramolopium* in the Hawaii and Cook Islands. Lowrey (1995) suggested that the pattern of geographical distribution of *Tetramolopium* resulted from the survival of a series of founder dispersal events that began with the establishment of a New Guinean *Tetramolopium* in the tropic-alpine habitat on East Maui or perhaps Maui Nui, followed by many dispersal events and adaptation to other habitats below the alpine zone. Because most RFLP diversity exists within sections rather than between sections, the pattern of diversity may reflect the steps of adaptive radiation in islands as envisioned by Carlquist (1974). The major lineages (sections) would originate via founder events that in turn allow drift to sample the initial genetic variation that existed in the generations following the founding of *Tetramolopium* on Hawaii. Although drift would reduce genetic variability over all taxa, a degree of genetic relatedness among the lineages would be maintained because they were drawn from the same pool of genetic variation. Additional founding events and drift accompanying the formation of individual species within each section would further reduce genetic variation, and increase differentiation among species. Thus, the pattern of genetic diversity seen in the RFLP data may reflect a combination of phyletic sorting of the original genetic variation, little time to accumulate new diversity, and maintenance of diversity on the basis of biological attributes of individual species. Evidence of this type of sorting is seen in the proportion of shared exclusive patterns or fragments (Table 5). Section *Alpinum* shares a greater proportion of exclusive fragments with sects. *Tetramolopium* and *Sandwicense* than with any of the constitutive species. The same is true between sects. *Alpinum* and *Tetramolopium* for the pattern data.

Lowrey (1995) envisioned phyletic sorting as an explanation for the combination of apomorphic and plesiomorphic morphological character states in sects. *Tetramolopium* and *Sandwicense,* especially those related to sex expression syndromes and the polymorphic nature of some of these characters in *T. humile.* For example, disk floret corolla color is plesiomorphic in sect. *Sandwicense* and apomorphic in sect. *Tetramolopium,* whereas number of heads per capitulum and disk floret number are apomorphic in sect. *Sandwicense* and plesiomorphic in sect. *Tetramolopium,* yet all three characters are polymorphic in sect. *Alpinum* (Lowrey, 1995). Therefore RFLP and morphological data concur with the view that evolutionary diversification in Hawaiian *Tetramolopium* resulted through phyletic sorting of existing variation following a single introduction. Since the age of Hawaiian *Tetramolopium* has been dated from the Pleistocene (Fosberg, 1948; Smith, 1977), accumulation of new genetic material most probably has been limited.

Introgression—Lowrey (1995) reported no documented evidence of natural interspecific hybridization in Hawaiian *Tetramolopium* but pointed out a number of instances in which suspected historical introgression may account for the patterns of morphological diversity shared among pairs of species. One case is the polymorphism of sex expression syndrome characters in sect. *Alpinum.* As discussed previously, this pattern may have arisen from phyletic sorting of morphological features in a polymorphic ancestral species to the present-day taxa, a pattern that is consistent with the RFLP data. Alternatively, Lowrey (1995) suggests that the polymorphic nature of *T. humile* may result from introgressive hybridization between *T. humile* ssp. *humile* and a member of sect. *Sandwicense.* The RFLP data do not support such an explanation because all distance- and character-based methods clearly show the distinct nature of the two sections, with few shared exclusive fragments and no exclusive patterns between the two sections and none between individual species.

A second case of hypothesized introgression is *T. filiforme,* which shares morphological features of both sects. *Tetramolopium* and *Sandwicense* (Lowrey, 1986, 1995). Based on the isozyme data of Lowrey and Crawford (1985), *T. filiforme* shares one low-frequency allele (*Pgi-1a*) with sect. *Alpinum,* one low-frequency allele (*6Pgdh-1b*) with sect. *Tetramolopium,* and two high-frequency alleles (*Aat-1b, Aat-2b*) with sect. *Tetramolopium.* In the RFLP data, *T. filiforme* shares one unique fragment with sect. *Sandwicense,* specifically with *T. lepidotum,* and two unique patterns with sect. *Sandwicense,* one specifically with *T. lepidotum* (data derived from Table 5). Distance and cladistic trees show that either *T. filiforme* is a sister species to *T. lepidotum,* placing both in sect. *Sandwicense,* or *T. filiforme* is basal to sect. *Sandwicense,* making sect. *Tetramolopium* paraphyletic. Thus, the morphological and genetic marker data are consistent with the hypothesis of Lowrey (1986, 1995) that *T. filiforme* may have experienced introgression from sect. *Sandwicense,* with *T. lepidotum* a likely source.

The status of *T. lepidotum* in sect. *Sandwicense* has never been questioned. According to Lowrey (1986, 1995) the species is representative in sect. *Sandwicense* and basal to the section in his cladistic analysis (Lowrey, 1995). Our analyses also place the species basal to sect. *Sandwicense* or as a sister group to the section. Examination of shared exclusive patterns and fragments of *T. lepidotum,* however, raises a question concerning the history of this species. In the ordinations, *T. lepidotum* and *T. filiforme* are intermediate in position between sects. *Tetramolopium* and *Sandwicense* (Fig. 4). Section *Tetramolopium* and *T. lepidotum* have five uniquely shared fragments and one uniquely shared pattern (data from Table 5). These observations are not consistent with strictly unidirectional gene flow between *T. lepidotum* to *T. filiforme.* The threefold increase of observed polymorphic patterns, fragments, and total diversity seen in *T. lepidotum* in comparison with *T. consanguineum* (Tables 3, 4) is consistent with the basal position of *T. lepidotum* in sect. *Sandwicense.* These observations are also consistent with bidirectional gene flow from a past intersectional hybridization event. If *T. filiforme* is accepted as having experienced introgression with *T. lepidotum,* it is possible that a low level of gene flow occurred into *T. lepidotum.* The distribution of RFLP markers does not provide conclusive evidence for either hypothesis but does raise the possibility of a previously undetected pattern of historical events.

Origin of the Cook Islands species—The Cook Islands plants consistently form a group distinct from the Hawaiian species of *Tetramolopium* (Figs. 1–5), and this agrees with the treatment of this taxon as a distinct and undescribed species on the basis of field observations, greenhouse cultivation, and additional molecular analyses (T. K. Lowrey and R. Whitkus, unpublished data). Interestingly, our analyses give conflicting information concerning the affinity of the Cook Islands species. All analyses (except the Fitch-Margoliash tree of fragment data, which excludes species with suspected introgressive histories) indicate that sect. *Tetramolopium* is paraphyletic with the inclusion of the Cook Islands species. This result does not support the hypothesis based on morphological data of an Hawaiian origin of this species through secondary long-distance dispersal (Lowrey, 1995). Compelling RFLP evidence for the affinity of the Cook Islands species is provided from the proportion of shared exclusive patterns and fragments (Table 5). The highest proportion of shared exclusive patterns and fragments of the Cook Islands species is with sect. *Tetramolopium,* with actual numbers of five and six, respectively. Comparing individual species, *T. sylvae* shares the highest proportion of unique patterns and fragments (actual number two each).

Nonconcordance of the morphology and majority of RFLP trees with respect to the affinity of the Cook Islands species requires an evaluation of the origin of this species. Three equally parsimonious hypotheses can be developed to explain the two dispersal events required to account for the distribution of the genus on Hawaii and the Cook Islands. The Cook Islands species: (1) is the result of a secondary long-distance founding event from Hawaiian *Tetramolopium,* (2) was the original founder from New Guinea, and Hawaiian *Tetramolopium* is a derivative of a secondary long-distance founding event from the Cook Islands, or (3) is an independent founding population from New Guinea, not related to the Hawaiian group. The combination of the suite of morphological features found in the Cook Islands species, especially the apomorphic sex expression syndrome (Lowrey, 1995), full interfertility of the Cook Islands species with Hawaiian taxa but not with New Guinean taxa (T. K. Lowrey, unpublished data), and proportion of shared exclusive RFLP patterns and fragments with sect. *Tetramolopium* indicates that the Cook Islands species is a close relative of the Hawaiian taxa, leading us to question the third hypothesis. The New Guinea–Cook Islands–Hawaiian sequence is less parsimonious than the New Guinea–Hawaiian–Cook Islands sequence on the basis of ecological and morphological characters. Considering ecological requirements, all New Guinean species are alpine semishrubs, adapted to cool, moist conditions (van Royen, 1983). The Cook Islands species is typical of extant members of sect. *Tetramolopium* in its being adapted to low-elevation, coastal habitats, and dry, saline conditions (Lowrey, 1986, 1995). Dispersal from New Guinea to the Cook Islands would involve establishment in a new, extreme habitat on Mitiaro, and secondary dispersal to Hawaii would require re-invasion of the alpine habitat to produce *T. humile,* the only member of sect. *Alpinum* outside New Guinea (Lowrey, 1986, 1995). Also, morphology argues for Hawaii as the origin of the Cook Islands species. The combination of the apomorphic sex syndrome character suite recognized by Lowrey (1995), which defines sect. *Tetramolopium,* would have to be lost in the re-establishment of the sect. *Alpinum* in Hawaii. When the data are viewed as a whole, there is stronger support for the Hawaiian origin of the Cook Islands species than for any alternate hypothesis.

If the Cook Islands species is a derivative of the Hawaiian clade, then why do RFLP trees fail to consistently recognize its affinity to sect. *Tetramolopium*? According to the cladistic analysis of morphology (Lowrey, 1995), *T. tenerrimum* is a basal taxon in sect. *Tetramolopium,* and *T. capillare* and *T. remyi* form a sister group to the *T. rockii–T. sylvae* clade. The absence of three of five known species of the section from the present analysis, including the basal taxon, may have obscured the affinity of the Cook Islands species to sect. *Tetramolopium.* The *T. rockii–T. sylvae* clade may be of very recent origin. *Tetramolopium rockii* is endemic to lithified calcareous dunes on the north side of the island of Molokai (Lowrey 1986). The origin of the dunes has been dated at 15 000 yr BP (Stearns, 1973), and endemism of *T. rockii* to these dunes suggests that its progenitor colonized the area within this time (Lowrey, 1995). The three populations of *T. sylvae* (syl22, syl23, syl24) that form a cluster apart from *T. rockii* populations occur on the Kalaupapa Peninsula on the north side of the island of Molokai. The peninsula is geologically young, the result of volcanic activity in the late Pleistocene/Holocene era (Stearns, 1985). Thus, the Hawaiian taxa included in the present analysis are likely to be recent derivatives, not representative of the total diversity in sect. *Tetramolopium,* and obscure the affinity of the Cook Islands species.

Another factor that may explain the placement of the Cook Islands species is that the secondary dispersal event occurred early in the history of *Tetramolopium* on the Hawaiian islands. Cladistic analysis of the pattern data

(Fig. 5) places sect. *Sandwicense* as a derivative of sect. *Tetramolopium.* In addition, sect. *Tetramolopium* shares a larger proportion of exclusive patterns and fragments with sect. *Alpinum* than sect. *Sandwicense* (Table 5). These results may be indicative of an early diversification of *Tetramolopium* in Hawaii in the formation of sect. *Tetramolopium,* which would give an early date for the dispersal and establishment of the Cook Islands species. Thus, a combination of an early founder event for the Cook Islands species and a long period of isolation could result in a scarcity of apomorphic RFLP markers shared with Hawaiian taxa.

Conclusions—This study represents the first use of nuclear RFLP markers to study patterns of diversity in an island plant group that has undergone adaptive radiation. The markers provide an independent source of information regarding the relationships and diversification of species in the Hawaiian–Cook Islands clade of *Tetramolopium.* Although earlier results from morphological and isozyme analyses are supported by the present data, a number of new questions are raised. In addition, diversification via phyletic sorting is seen in the data, concordant with views of morphological diversification for the group. Limited sampling in numbers of populations and number of loci and taxa may contribute to the patterns observed, as well as very recent and rapid evolutionary diversification of *Tetramolopium.* Information from additional genetic markers, providing a larger sample of the genome and increased sample sizes, and markers with high phylogenetic content are currently being explored. A more complete picture of adaptive radiation in *Tetramolopium* will require multiple sources of data.

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