Pl. Syst. Evol. 200: 61–77 (1996)

Plant Systematics and Evolution

Interspecific hybridization in natural populations of *Cyrtandra* (*Gesneriaceae*) on the Hawaiian Islands: evidence from RAPD markers

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Received March 6, 1995; in revised version July 10, 1995

Key words: *Gesneriaceae, Cyrtandra.* – Interspecific hybridization, RAPD. – Flora of Hawaii.

Abstract: Interspecific hybridization among Hawaiian species of *Cyrtandra* (*Gesneriaceae*) was investigated using randomly amplified polymorphic DNA (RAPD) markers. Thirty-three different primers were used to investigate interspecific hybridization for 17 different putative hybrids based on morphological intermediacy and sympatry with putative parental species. RAPD data provided evidence for the hybrid origin of all putative hybrid taxa examined in this analysis. However, the patterns in the hybrid taxa were not found to be completely additive of the patterns found in the parental species. Markers missing in the hybrid taxa can be attributed to polymorphism in the populations of the parental species and the dominant nature of inheritance for RAPD markers. Unique markers found within hybrid taxa require further explanation but do not necessarily indicate that the taxa are not of hybrid origin. The implications suggest that these interspecific hybridization events had, and continue to have, an effect on the adaptive radiation and conservation biology of *Cyrtandra.*

Interspecific hybridization in plants has been the focus of numerous studies over the past several decades (Anderson & Hubricht 1938, Anderson 1949, Anderson & Stebbins 1954, Baker 1951, Gillett 1972, McDade 1992). Numerous methods have been used to investigate putative hybrids, including intermediate morphology (Anderson & Hubricht 1938, Heiser 1949, Wagner & al. 1990), artificial hybridization (Byrne & Morley 1976, Roelefs 1979), micromolecular techniques (Leven 1966, McHale & Alston 1964, Flake & al. 1969, Adams & Turner 1970), chromosomal variation (Heiser 1949, Pipkin 1972), isozymes (Gallez & Gottlieb 1982, Crawford & Ornduff 1989, Werth 1991, Dickson & Weeden 1991), and more recently DNA (Rieseberg & al. 1988, Rieseberg & Brunsfeld 1992, Spooner & al. 1991, Arnold & al. 1991, Crawford & al. 1993, Cruzan & Arnold 1993).

Cyrtandra Forster & Forster is the second largest plant genus in the Hawaiian islands, comprising over 56 endemic species (Wagner & al. 1990). Hawaiian *Cyrtandra* species are characterized as perennial herbs, shrubs or small trees with rounded to angular stems, and opposite or whorled leaves. Flowers are generally perfect and occur in 1 – numerous cymes, usually in the upper leaf axils although

many species are cauliflorous. The corolla is usually bilabiate and generally white. There are five stamens with the two distal stamens being fertile and the rest staminodial. The ovary is superior and the fruit is a fleshy white berry containing numerous seeds (for a more detailed description of the genus and Hawaiian species see Wagner & al. 1990).

The taxonomy of the genus has been subject to recent revision with over 400 names synonymized to comprise the current estimate of 56 species. The extremely high number of names applied to *Cyrtandra* can be traced in part to interspecific hybrids. Wagner and Herbst (Wagner & al. 1990) identified 67 interspecific hybrids based on (1) morphological intermediacy between parents, (2) sympatry of the hybrid and both parents, and occasionally (3) decreased fertility in the hybrid. More recent field studies have revealed at least eight additional hybrid combinations that have been documented (W. Wagner, unpubl.). The 75 putative interspecific hybrids thus far identified meet at least the first two of the above criteria. For example, both *C. cordifolia* Gaud. and *C. macraei* A. Gray can be found growing sympatrically. *Cyrtandra cordifolia* has broadly cordate leaves with nonglandular hairs, flowers occur in open umbelliform cymes with a calyx that is broadly campanulate and with reflexed lobes in fruit. In contrast, *C. macraei* has broadly ovate to broadly elliptic leaves that are sparsely covered adaxially with subsessile glands, flowers occur in dense, compound umbelliform cymes and have a calyx that is subcylindrical with erect lobes in fruit. The putative hybrid has broadly ovate leaves that are not quite cordate and are sparsely covered adaxially with sessile glands. The flowers occur in a cyme that is intermediate in degree of compactness to the parental species and with a calyx that is broad and open, but not quite campanulate and lobes that are partly reflexed in fruit. The morphological characters used to determine intermediacy between parental species are currently being examined by W. L. Wagner, Smithsonian Institution, and D. R. Herbst, U. S. Dept. of Fish and Wildlife, Honolulu, Hawaii.

In many of the putative hybrids there is no decreased fertility (Luegmayr 1993) and because backcrosses could occur, fertile hybrids may have a serious impact on the population structure and maintenance of the parental species. An alternative hypothesis is that the fertile individuals with intermediate morphology are not of hybrid origin.

Although numerous techniques have been used for the identification of plant hybrids over the past several years (Anderson 1949, Heiser 1973, Rieseberg & Brunsfeld 1992), methods involving direct examination of DNA have proven to be the most conclusive and satisfactory in detecting plant hybridization (Doyle & al. 1985, Rieseberg & al. 1988, Doyle & Doyle 1988, Smith & Sytsma 1990, Spooner & al. 1991, Arnold & al. 1991, Crawford & al. 1993, Cruzan & Arnold 1993, Rieseberg & Ellstrand 1993). In this study, the recently developed randomly amplified polymorphic DNA (RAPD) primers have been used to detect genetic differences between putative parents. The choice to use RAPD markers as opposed to other sources of DNA variation was based on the low levels of genetic variation detected at the DNA level using restriction site analysis of chloroplast genes in Hawaiian *Cyrtandra* (J. Smith, unpubl.), a phenomenon demonstrated in other Hawaiian plant groups [Givnish & al. 1994 (*Campanulaceae*); Soltis & al. unpubl. (*Schiedea* Cham. & Schldl.); S. Keeley, University of Hawaii, pers.

Table 1. Hawaiian *Cyrtandra* sections grouped as lineages based on the potential number of introductions

Six introductions	Four introductions
Verticillatae St. John	*Verticillatae*
Cylindrocalyces Hillebr.	*Cylindrocalyces*
Crotonocalyces Hillebr.	*Crotonocalyces*
Macrosepalae C. B. Clarke, *Apertae* C. B. Clarke	*Macrosepalae, Apertae, Chaetocalyces*
Chaetocalyces Hillebr.	
Cyrtandra kealiae Wawra, *C. limahuliensis* St. John	

comm. (*Lipochaeta* DC.); C. Gemmil, University of Colorado, pers. comm. (*Pritchardia* Seeman & H. Wendl.)].

This study investigates seventeen of the putative primary hybrids (first and second generation hybrids) at the genetic level to determine if the plants represent (1) a genetic mixture of the two putative parents (as suggested by morphological intermediacy) or (2) distinct species that are not of hybrid origin. This information is critical as a basis for all future molecular phylogenetic work on Hawaiian *Cyrtandra*. Phylogenetic analyses will be imperative for all future evolutionary studies on this genus, and the identification of hybrids will eliminate potentially confounding results in interpreting cladistic analyses. This study will also provide a basis for investigations at the population level to determine the impact of hybridization on the integrity of parental species. This will have the greatest impact for rare species of *Cyrtandra* that are in the most serious risk of extinction by dispersal into, or replacement of, genetic material of other species.

The mode of evolution for Hawaiian *Cyrtandra* appears to be an excellent model for the evolution of invasive species. Hawaiian *Cyrtandra* species are divided into six sections, potentially representing four to six separate introductions (Wagner & al. 1990). The interspecific hybrids that have been identified to date are almost all intersectional hybrids. If we assume that the six sections represent four introductions to the Hawaiian islands (Table 1), then the pattern of hybridization would represent a mode of evolution in which each successive invasion has developed isolating mechanisms from its own wave. These isolating mechanisms are based largely on allopatry. However, sympatry with species from other sections can result in hybridization and introgression with both previous and successive waves of introductions. Further tests of this model will involve a more in depth examination of the phylogenetic history of these species to determine their appropriate sectional affiliation and pattern of diversification.

Material and methods

Plant material. The choice of plants to sample in this investigation was based on evidence from morphology (Wagner & al. 1990). Seventeen different putative hybrid taxa were sampled, many of which were represented by more than one population (Table 2). For most hybrid populations, individuals of the putative parents were collected from the same locality as the hybrid. The inclusion of parental species from the hybrid site was done to minimize the impact of intraspecific variation within the parents that could cause

Table 2. Individuals of *Cyrtandra* sampled in this analysis. Voucher specimens have been deposited at SRP unless otherwise indicated in parentheses after the voucher specimen information. Islands are abbreviated as follows: *H* Hawaii, *K* Kauai, *M* Maui, *O* O'ahu

Taxon	Locality	Voucher
C. cordifolia Gaud.	O, Manoa	Smith 2848
	O, Palolo	Smith & al. 2880
	O, Palolo	Smith & al. 2890
	O, Wailupe	Smith & Lau 2897
C. cordifolia × *C. laxiflora*	O, Maunawili	Smith 2874 (4 individuals)
C. cordifolia × *C. macraei*	O, Palolo	Smith & al. 2885
	O, Wailupe	Smith & Lau 2904
	O, Wailupe	Smith & Lau 2905
	O, Wailupe	Smith & Lau 2906
C. cordifolia × *C. garnotiana*	O, Wailupe	Smith & Lau 2896
C. cordifolia × *C. grandiflora*	O, Wailupe	Smith & Lau 2899
C. cordifolia × *C. paludosa*	O, Palolo	Smith & al. 2883
	O, Palolo	Smith & al. 2887
C. cordifolia × *C. sandwicensis*	O, Manoa	Kiehn 920907 1/8 (WU)
	O, Manoa	Kiehn 920907 1/9 (WU)
C. garnotiana Gaud.	O, Wailupe	Smith & Lau 2894
C. grandiflora Gaud.	O, Manoa	Smith 2844 (5 individuals)
	O, Manoa	Smith 2845
	O, Wailupe	Smith & Lau 2900
C. grandiflora × *C. sandwicensis*	O, Manoa	Smith 2846 (5 individuals)
	O, Manoa	Smith 2847
C. grayana Hillebr.	M, Hana'ula	Wagner 6472 (US)
C. grayana × *C. oxybapha*	M, Hana'ula	Wagner 6476 (US)
C. hawaiensis C. B. Clarke	H, Manuka	Wagner 6753 (US)
	M, Hana'ula	Wagner 6473 (US)
	O, Waiahole	Smith & Lau 2855 (5 individuals)
	O, Waiahole	Smith & Lau 2862
	O, Waiahole	Smith & Lau 2872
C. hawaiensis × *C. kaulantha*	O, Waiahole	Smith & Lau 2854 (3 individuals)
	O, Waiahole	Smith & Lau 2856
	O, Waiahole	Smith & Lau 2860
	O, Waiahole	Smith & Lau 2865
C. hawaiensis × *C. laxiflora*	O, Waiahole	Smith & Lau 2857
	O, Waiahole	Smith & Lau 2859
C. hawaiensis × *C. menziesii*	H, Manuka	Wagner 6754 (US)
C. kauaiensis Wawra	K, Kokee	Kiehn 920830 (WU)
	K, Kokee	Kiehn 920825-3/4 (WU)
	K, Kokee	Kiehn 920825-3/3 (WU)
C. kauaiensis × *C. longifolia*	K, Kokee	Kiehn 920825-3/2 (WU)
C. kaulantha St. John & Storey	O, Waiahole	Smith & Lau 2871 (5 individuals)
C. laxiflora H. Mann	O, Waiahole	Smith & Lau 2858 (5 individuals)
	O, Maunawili	Smith 2878
C. laxiflora × *C. sandwicensis*	O, Maunawili	Smith 2873
C. longifolia (Wawra) Hillebr. ex C. B. Clarke	K, Kokee	Kiehn 920825–2/1 (WU)
	K, Wahiawa	Wagner 6449 (US)
C. lysiosepala (A. Gray) C. B. Clarke	H, Wright Rd., Volcano Village	Wagner 6752 (US)

Table 2 (continued)

Taxon	Locality	Voucher
	H, Stainback hwy.	SMITH 2910
	H, Stainback hwy.	SMITH 2914
C. lysiosepala × *C. paludosa*	H, Stainback hwy.	SMITH 2920
C. lysiosepala × *C. platyphylla*	H, Stainback hwy.	SMITH 2909
C. macraei A. GRAY	O, Wailupe	SMITH & LAU 2895
	O, Wailupe	SMITH & LAU 2902
C. menziesii HOOK. & ARNOTT	H, Kealaekekua Ranch	WAGNER 6763 (US)
C. oxybapha W. L. WAGNER & HERBST	M, Hana'ula	WAGNER 6475 (US)
C. paludosa GAUD.	H, Stainback hwy.	WAGNER 6749 (US)
	O, Palolo	SMITH & al. 2884
	H, Stainback Hwy.	SMITH 2913
C. paludosa × *C. platyphylla*	H, Stainback hwy.	WAGNER 6751 (US)
	H, Stainback hwy.	WAGNER 6750 (US)
C. platyphylla A. GRAY	M, Waikapu	SMITH 2186
	H, Stainback hwy.	SMITH 2186
	H, Stainback hwy.	WAGNER 6746 (US)
C. sandwicensis (H. LÉV.) ST. JOHN & STOREY	O, Manoa	KIEHN 920907 1/11 (WU)
	O, Manoa	SMITH 2851 (5 individuals)

confusion in interpretation of bands in the hybrid (SOLTIS & al. 1992). Whenever possible, up to five individuals were sampled for each parent/hybrid combination. In many instances the populations were very small (sometimes only a single individual) and limited the number of individuals that could be sampled. This smaller sampling poses some limitations to the level of analysis (see discussion) but is unavoidable for many of the populations. To avoid the possibility of using parents that may have already undergone introgression, individuals of the parental species also were sampled from sites where each species occurs singly. These individuals represented the species with a minimal chance of introgression, and therefore, served as checks that introgression had not occurred within the parents. The possibility of backcrosses to the parents also was checked by comparing parent individuals collected from different sites. A list of the species and putative hybrids, collection localities, and voucher information are presented in Table 2. The status of the plants (parent species or hybrid) was determined in the field on a morphological basis and verified with vouchers.

RAPD methodology. The choice of molecule to detect variation between species is critical to the level of analysis particularly when examining hybridization (SYTSMA 1990, RIESEBERG & ELLSTRAND 1993). Therefore the source of data for this study was selected based on the amount of variation observed. For the detection of hybrids, it is critical to sample from DNA that is inherited in an additive fashion, and one that will provide a sufficient number of unique markers for each of the putative species (RIESEBERG & ELLSTRAND 1993). In this investigation, we examined RAPD markers. This method has been used with success to identify individuals of hybrid origin in the *Iridaceae* (ARNOLD & al. 1991, ARNOLD 1993, CRUZAN & ARNOLD 1993), *Rosaceae* (CRAWFORD & al. 1993), *Fabaceae* (MCCOY & ECHT 1993), and *Oleaceae* (MARSOLAIS & al. 1993) and has provided useful

Table 3. Primer abbreviations and sequences

Primer	Sequence
P2′	TCTCGATGCA
P5	GCAAGTAGCT
P6	TGGTCACTGT
P17	GCAATGGCTG
P19	ACATCCCGTG
P21	AGCACCTTTC
P33	TGCTCACTGA
P34	ACCTCCAGCACTGTCA
P37	TCACGATGCA
P48	CAGCTGATCA
P51	CAGCTGAATTC
P63	CAGTCGCTT
P66	CGGCTAGGT
P67	GCTCACATC
P68	TACGCACGG
P103	CGGCCCCTGT
P104	AGGGTTCGGT
P106	ACCTCGCTCA
P115	CAGTGTGTGG
P116	TGTGGCGACT
P121	GGCACCTTTG
P128	AGGTGGTTCT
P130	ATCGGTGATG
P131	GGTTGTTGGT
P132	GGTTGGGTGA
V-1	TGACGCATGG
V-2	AGTCACTCCC
V-3	CTCCCTGCAA
V-4	CCCCTCACGA
V-6	ACGCCCAGGT
V-7	GAAGCCAGCC
V-8	GGACGGCGTT
V-10	GGACCTGCTG

information on the genetic variation found within and among populations (Brauner & al. 1992, Van Buren & al. 1994). Although RAPD variation cannot always be inferred to result from polymorphisms at specific loci, most polymorphic RAPD fragments that have been analysed genetically display simple Mendelian inheritance patterns (Williams & al. 1990) and are inherited as dominants (Williams & al. 1990, Hadrys & al. 1992, Rieseberg & Ellstrand 1993). Such patterns are critical for the detection of interspecific hybridization.

Total DNA was extracted from either fresh or frozen leaf material using the methods of Smith & al. (1992). To minimize variation between duplicate reactions, the DNA was further purified by passing the total DNA through low-melting agarose and removing only the high molecular weight DNA band for amplifications. The DNA was then amplified using thermal cycling. A series of 56 RAPD primers were surveyed and 33 were used in the analysis, each primer used singly. The sequences of these primers are listed in Table 3. Primers were selected on the basis of band intensity and reproducibility of the products.

The 33 primers used in the analysis gave consistently reproducible bands of strong intensity when stained with ethidium bromide and viewed under UV light.

Amplifications were performed with 25 µl reaction mixtures containing 1 unit of Promega *Thermus aquaticus* (*Taq*) DNA polymerse, 1 × Promega *Taq* buffer, 1.9 mM $MgCl_2$, 0.1 mM each of dATP, dCTP, dGTP, and TTP, 5 pmol of a single primer and 1 µl of the gel-purified DNA. All primers that indicated a hybrid pattern in the first amplification were re-amplified to determine the repeatability of the band patterns. Amplifications were performed in a M. J. Research thermal cycler programmed for 45 cycles of one min at 97 °C, one min at 35 °C, and two min at 72 °C. The buffer mix was added to the side of the reaction tube, and only when the thermal cycler had reached 97 °C were the tubes spun in a microcentrifuge and placed in the thermal cycler. The mixing of the template DNA and *Taq* DNA polymerase at higher temperatures resulted in more consistent results (J. Smith, unpubl.). The entire reaction mix was loaded on 1.4% agarose gels and electrophoresis performed. A ϕX174 *Hae* III digest marker was loaded on each gel to assess band size. The gels were stained with ethidium bromide and photographed under UV light.

Data analysis. Bands resulting from the RAPD amplifications were scored based on their approximate size. Bands of identical size were assumed to be homologous across the individuals sampled in this analysis. The bands that resulted from the amplification of the DNA from the putative hybrid were examined and scored as (1) common to both putative parents, (2) shared with one of the putative parents, or (3) unique to the putative hybrid. All bands that were common to both parents and the hybrid were ignored, and only bands shared with one parent species, and bands unique to the hybrid or either parent species were considered in the analysis. Bands that were unique to some, but not all, of the individuals of the parental species were not included in the analysis. Putative hybrids were classified as hybrids if the RAPD patterns were additive, or partially additive, from both of the putative parents (Doyle & Doyle 1988; Doebley 1989; Rieseberg & al. 1990 a, b; Arnold & al. 1991; Cruzan & Arnold 1993). An individual possibly could still be of hybrid origin but not between the two species found at the site of the hybrid individual. To detect this, comparisons were made of other potential parental species used in the analysis.

To examine the possibility that the bands shared between the putative hybrid and its parent species were the result of common ancestry and not hybridization, two additional pairwise comparisons were made with each of the parents as the putative hybrid and the hybrid as one of the parents. If the bands shared between the hybrid and the parents are the result of common ancestry, one would expect only slight, to no decrease in additivity when analysed in this fashion. If the bands are the result of hybridization, then there should be fewer bands shared between the two parental species and the additivity should sharply decrease when one parent is examined as the hybrid.

Results

Initial surveys in this study were performed by amplifying regions of the chloroplast DNA (ATP-*rbc*L: Savolainen & al. 1994; *rpo*C1, *rpo*C2: Liston 1992; trnà loci: Taberlet & al. 1991), ITS regions of the nuclear ribosomal genes (Baldwin 1992, 1993; Kim & Jansen 1994), and digesting the product with restriction enzymes (Liston 1992, Rieseberg & al. 1992). This analysis was performed with the aim of identifying the pollen donor and receiver in each cross. Unfortunately, no variation was found among the species used in this analysis for any of the amplified regions or enzyme combinations used. The focus of data collection then centered on RAPD variation.

Amplification with RAPD primers (Table 2) produced unique markers for the

Table 4. RAPD bands scored for the putative hybrid taxa of *Cyrtandra*. Parents one and two are distinguished alphabetically and in no way implies direction of pollen transfer. The bands scored as unique for each parent differ for each hybrid combination as different primers were scored for each hybrid combination (see text for details). The first column provides a numerical value of the number of RAPD bands shared between the putative hybrid and the first parent over the number of bands that were found in parent one and not in parent two. The lower number includes that bands shared with the putative hybrid. The second column is the same but for parent two. The % additivity is the sum of the numerators from the first two columns divided by the sum of denominators and expressed as a percentage to reflect the degree of hybridization found in the hybrid. The final column is the number of bands that were unique to the hybrid and not found in either of the two parental species

Hybrid	Bands parent one/ unique bands	Bands parent two/ unique bands	% Additivity	Unique bands
C. cordifolia × *C. garnotiana*	11/11	1/1	100	7
C. cordifolia × *C. grandiflora*	6/6	11/12	94	1
C. cordifolia × *C. laxiflora*	6/6	5/5	100	8
C. cordifolia × *C. macraei*	3/3	3/3	100	4
C. cordifolia × *C. paludosa*	5/5	5/5	100	4
C. cordifolia × *C. sandwicensis*	1/2	1/2	50	1
C. grandiflora × *C. sandwicensis*	11/12	16/16	96	6
C. grayana × *C. oxybapha*	12/24	15/23	57	10
C. hawaiensis × *C. kaulántha*	2/2	4/4	100	0
C. hawaiensis × *C. laxiflora*	2/6	7/7	69	1
C. hawaiensis × *C. menziesii*	1/3	4/7	50	2
C. kauaiensis × *C. C. longifolia*	1/13	7/7	90	1
C. kaulantha	7/11	2/2	69	3
C. laxiflora × *C. sandwicensis*	1/2	1/1	67	0
C. lysiosepala × *C. paludosa*	2/2	2/2	100	3
C. lysiosepala × *C. platyphylla*	5/5	3/3	100	2
C. paludosa × *C. platyphylla*	12/14	4/9	69	1

parental genotypes found in each cross. Of the 56 primers used in this analysis, 33 yielded scorable data for at least one hybrid combination or individual parent. Amplifications were repeated for all primer/hybrid combinations that produced unique bands to either of the parents and thus provided data for interpreting hybrid patterns. These amplifications were reproducible for all of the bands scored in this analysis. Faint bands or bands that were not seen in repeated amplifications were not included in the analysis.

For many of the populations where more than one individual was sampled, polymorphism was found for some individual/primer combinations. If any of these bands were found in the hybrid, they were included in the analysis. Because the focus of this study is on hybridization events and not genetic variation within populations of *Cyrtandra* species, bands that were found in some individuals but not found in the hybrid were not included in the analysis.

The number of bands per putative hybrid that are shared with either parent, or unique to the hybrid are summarized in Table 4. This table also shows the portion

of bands from each parent that are found in the hybrid. Not all bands unique to either parent in the putative cross were found in the hybrid. A full data set of fragment sizes that were scored is available from the first author upon request.

All comparisons that were made with either of the parents as the putative hybrid and the hybrid as one of the parents resulted in sharp decreases in additivity. In only two instances did additivity remain at the 50% level or higher [*Cyrtandra hawaiensis* C. B. CLARKE × *C. kaulantha* ST. JOHN & STORREY with *C. kaulantha* as the hybrid (50%) and *C. cordifolia* GAUD. × *C. garnotiana* GAUD. with *C. cordifolia* as the hybrid (55%)]. It also should be noted that in these two cases when the other parent was used as the hybrid that the additivity dropped to below 50%. In all other instances the additivity ranged from five to 47%.

One species in the analysis, *Cyrtandra kaulantha* was found to be of hybrid origin between *C. hawaiensis* and *C. laxiflora* H. MANN as proposed by WAGNER & al. (1990). This species is also listed in Table 4 with its parents as *C. hawaiensis* and *C. laxiflora*. It is also interesting to note that the RAPD data for putative hybrids between *C. hawaiensis* and *C. kaulantha*, when analysed with *C. kaulantha* as the hybrid rather than one of the two parents remained at the 50% additivity level as would be expected if some of the bands shared between the hybrid and the parent were the result of common ancestry and not simply hybridization.

Discussion

The pattern of occurrence of the RAPD markers surveyed in this study provides evidence for widespread interspecific hybridization among natural populations of Hawaiian *Cyrtandra*. All putative hybrids examined in this study showed at least some degree of additive inheritance for RAPD markers that were unique to the putative parents (Table 4). The absolute number of shared markers varied between the different hybrids examined, but this is the result of differential RAPD sampling. Variation in the number of markers found across parental species with the primers used in this analysis did not affect the rigor of diagnosis of interspecific hybridization in any of the putative hybrids examined in this study. For example, the primers used in this study provided 47 markers unique to either *C. grayana* HILLEBR. and *C. oxybapha* W. L. WAGNER & HERBST, whereas a different set of primers provided only four markers unique to either *C. lysiosepala* (A. GRAY) C. B. CLARKE and *C. paludosa* GAUD. (Table 4). However, the putative hybrids between these pairs exhibit from 50 to 100% additivity (Table 4) regardless of the absolute number of parental markers found. In addition, incomplete additivity may reflect second generation hybrids, or backcrosses to either of the parents.

Polymorphisms within parental species. For several of the parental species examined in this analysis more than one individual was available and in some cases up to five individuals from a single population were sampled. In nearly all cases where more than one individual was sampled, polymorphism was found within the population or within the species (J. SMITH, unpubl.). Such variation is not unexpected with RAPD markers which have frequently been used for intra- and interpopulation studies (BRAUNER & al. 1992, VAN BUREN & al. 1994). However, such polymorphism clearly can have an effect on the interpretation of interspecific hybridization. The unique markers found within hybrids possibly indicate

that the hybrid's parents were not the individuals sampled in this analysis although they were from that species. It is also important to note that RAPD markers are inherited as dominants (Williams & al. 1990, Hadrys & al. 1992), and thus the parental individuals sampled in this analysis could potentially be heterozygous for any marker (and show the marker in the amplifications) yet pass the recessive form to the hybrid. Therefore, it is not surprising to find only a portion of the bands unique to each parent in the hybrid and not complete additivity.

Polymorphisms also have been detected within hybrids. The most common occurrences are where many hybrid individuals were available for analysis such as *Cyrtandra grandiflora* Gaud. × *C. sandwicensis* (H. Lév.) St. John (Table 2). Polymorphism have also been found in other hybrids where fewer individuals were sampled including the *C. hawaiensis* × *C. kaulantha* hybrid in which very little variation was found between the two parents and the hybrid (Table 4). These polymorphisms found within the hybrid individuals are further evidence that hybridization is likely to be occurring with different individuals and is not a singular isolated event.

There is also the possibility that such polymorphisms have inflated the rate of additivity seen in putative hybrid taxa since it is possible that some of the parental species-specific bands shared between the hybrid and its putative parent may be found in other individuals of the second parent not sampled in this analysis. Although every attempt was made to sample both parental species fully, the small population size (sometimes a single individual) posed serious limits on this aspect of the analysis.

Unique markers within hybrids. Although polymorphisms found within parental populations and the potential for heterozygous parents can explain the incomplete additivity found in some of the hybrids, it does not explain fully the markers that were unique to the hybrids (Table 4). Unique bands in hybrids could be simply the result of polymorphisms within the parental population that were not sampled in this analysis. However, the frequency of unique bands in the hybrids examined in this analysis (13 of the 17 sampled hybrids, Table 4) and in particular the high number of unique bands found in *Cyrtandra grayana* × *C. oxybapha* (ten, Table 4) indicate that other factors are likely to be involved.

Five possible factors could explain the unique bands. (1) RAPD markers are not necessarily inherited in an allelic fashion. The primer binding sites likely are inherited in an allelic manner, but the actual band produced by amplification could be altered by crossing-over events. As a result, two binding sites that may not have been within proximity of each other in the parental genome in order to produce amplification products could appear as such in the offspring. However, such events should not be unique to the hybrids and should appear as polymorphisms within the parental population as well. Alternatively, heteroduplex bands could be occurring in individuals that are heterozygous for two dominantly inherited RAPD loci. These heteroduplex bands appear as unique bands in hybrids (Fritsch & Rieseberg 1992).

(2) Another explanation for the unique bands in the hybrid is that there has been an accumulation of mutations within the hybrid since the hybridization event. These newer mutations are expressed as unique bands within the hybrid. The bands surveyed for this analysis exhibited a wide range in the degree of vari-

ation. Some markers were consistently present in all species in the analysis, whereas others were unique to one or few individuals (J. SMITH, unpubl.). Thus, markers unique to hybrid individuals possibly could arise as they do in individuals of the parents. This explanation is unlikely, however, since most of the hybrids examined in this study are likely to be primary hybrids (first or second generation hybrids) and the time for new mutations to have evolved is unlikely.

(3) It is also possible that the hybrid is self-fertile, and the individual sampled in this analysis is from the F_2 generation. Analysis of pollen viability indicates that most of the hybrids, including some of the same individuals examined in this study, are fertile (LUEGMAYR 1993). As a result of recombination and crossing-over between the parental genomes, new primer binding site combinations could arise easily.

(4) It is possible that additional hybridization and introgression is occurring with other species that occur nearby, but were not sampled in this study. Both *Cyrtandra grayi* and *C. hawaiensis* occur in the vicinity of the *C. grayana* × *C. oxybapha* population. Although it is unlikely that there is introgression with *C. hawaiensis* since this species was sampled in this study, it is possible that many of the unique bands found in this hybrid could be attributed to *C. grayi* which was not included. This scenario is less likely for other hybrids where fewer other species occurred sympatrically. It should be noted that despite the large size of these plants, they may only be three to four years old as determined by greenhouse grown seedlings (M. KIEHN, Botanical Institute, University of Vienna, unpubl.). Therefore it is possible that the species composition of some areas may change rapidly and the lack of a sympatric parental species does not eliminate its possibility as a parent in a hybridization event.

(5) Lastly, it is possible that the unique bands are indicative of a separate evolutionary lineage for the putative hybrid, and it is not of hybrid origin. The hybrid instead may be closely related to the two parental species, and the shared bands are the result of common ancestry.

This last scenario is highly unlikely on the basis that all surveyed hybrids exhibited some degree of morphological intermediacy between the putative parents and were usually found growing sympatrically with both parents nearby. Additionally, by examining the data with the putative parents as hybrids and the hybrid as one of the parents, the rate of additivity dropped to below 50% in all comparisons, indicating that the bands shared between the putative hybrid and its parents are the result of hybridization and not common ancestry. In the two instances where the rate of additivity did not drop, one did include parents that are likely to share bands from common ancestry (*Cyrtandra hawaiensis* × *C. kaulantha, C. kaulantha* being a hybrid between *C. hawaiensis* and *C. laxiflora*). In the other instance (*C. cordifolia* × *C. garnotiana*) only one of the combinations remained above 50% additivity (*C. cordifolia* as the hybrid). With *C. garnotiana* as the hybrid, additivity dropped to 5%.

It is also unlikely that there is a close evolutionary relationship between the hybrid and the parental species such that the markers shared between the hybrid and the two parents represent evolutionary divergence. Parental species are all from different sections of Hawaiian *Cyrtandra* (Table 1) and are likely to reflect independent introductions to the islands (W. WAGNER, unpubl.). Thus the bands

shared by the hybrid and the putative parents are unlikely to represent synapomorphies and are much more likely to be the result of hybridization events.

Hybrid origin of *Cyrtandra kaulantha* and backcrosses to *C. hawaiensis*. It is clear from the RAPD data that *Cyrtandra kaulantha* is of hybrid origin between *C. hawaiensis* and *C. laxiflora* (Table 4). This hypothesis had been proposed based on morphological data alone (Wagner & al. 1990), but the presence of several novel morphological features within *C. kaulantha* and the formation of distinct populations kept Wagner & al. (1990) from listing this species as a hybrid. The presence of bands unique to both *C. hawaiensis* and *C. laxiflora* within the sampled individuals of *C. kaulantha* and the immediate presence of both parental species in the area where *C. kaulantha* was found are strong evidence that this species is indeed of hybrid origin. The presence of all three of these species along with potential backcrosses between *C. kaulantha* and at least one of its parents has created a confusing complex along the Waiahole Ditch trail on O'ahu that is beyond the scope of this study to examine fully. However, some individuals that were potentially backcrosses between *C. kaulantha* and *C. hawaiensis* were examined in this analysis (Table 2). The evidence for these individuals to be hybrids between *C. hawaiensis* and *C. kaulantha* is not strong mainly due to the large proportion of bands shared between *C. kaulantha* and *C. hawaiensis* and the few unique bands found within *C. kaulantha* (Table 4). It is also difficult to eliminate the possibility that these individuals may represent crosses between *C. hawaiensis* and *C. laxiflora* since many of the markers found within *C. kaulantha* and not *C. hawaiensis* are markers unique to *C. laxiflora*. The morphological features of these potentially backcrossed individuals do not indicate an immediate cross between *C. hawaiensis* and *C. laxiflora*, however, as they would be expected to be highly similar to individuals of *C. kaulantha*. Instead, these individuals are somewhat intermediate between *C. hawaiensis* and *C. kaulantha*. The morphological intermediacy combined with the few RAPD markers, is indicative that backcrossing is occurring between *C. kaulantha* and one of its parents, however; further investigations of this entire complex should be undertaken before any strong conclusions can be drawn.

Impact of interspecific hybridization on rare species. While the most obvious evolutionary impact of interspecific hybridization is the origin of species (Stebbins 1950), there can also be a serious impact at the population level. Hybrids may disrupt population structure by: being more successful than either of the parents (Arnold & al. 1990), being less successful than either of the parents (Whitham 1989) and, colonizing habitats that exclude the parents (Heiser 1965, Lewontin & Birch 1966). Any instance where the hybrid may fill an ecological niche that differs from the parent, the population structure will be altered.

Interspecific hybridization will have the greatest impact on populations that are very small, most notably, rare species. The introduction of foreign genes into a small population will have an immediate, and direct impact on the population structure, as well as future evolution of rare species. Interspecific hybridization with rare species will pose the greatest threat when hybrids occur between a rare, and a more widespread species (Liston & al. 1990, Rieseberg 1991, Rieseberg & Gerber 1995). In such cases a rare gene could be completely absorbed into the greater gene pool of the common species, and the integrity of the rare species

destroyed (Rieseberg & al. 1989, Rieseberg & Gerber 1995). This is especially critical with island species where populations are in general very small and habitat loss and disturbance places additional limitations on the species (Rieseberg 1991).

Additionally, if the hybrid taxa have reduced vigor or are partly sterile the rare species may be endangered from outbreeding depression (Price & Waser 1979, Templeton 1986, Leberg 1993) where the genetic material is lost to less viable, or unfit individuals.

Although none of the species examined in this study are considered to be rare among the Hawaiian flora, all species are endemic to the Hawaiian islands and putative hybrids involving rare species of *Cyrtandra* have been identified on the basis of morphology and geographic sympatry.

Intersectional hybridization. The Hawaiian species of *Cyrtandra* are classified into six sections and are believed to represent four to six different introduction events (Table 1) to the Hawaiian islands (Wagner & al. 1990). All interspecific hybrids examined in this study are also intersectional hybrids. However, hybridization between species within a section is uncommon as observed with morphological data (Wagner & al. 1990) and subsequently has not been examined with molecular genetic techniques. The low frequency of intra-sectional hybrids is largely due to the lack of sympatry for species within a section, but may also be attributed to the difficulty in recognizing hybrid individuals from two morphologically similar species. For the species examined in this study there is only one instance where species from the same section were found growing sympatrically. These species were (excluding the interspecific hybrid species, *C. kaulantha*) *C. hawaiensis* and *C. calpidicarpa* (Rock) St. John & Storey (sect. *Verticillatae* St. John) in a gulch along the Waiahole Ditch Trail, O'ahu. In this instance the two species were separated by several hundred meters ground distance and possibly an equal distance in elevation. The two species were not in a mixed population as were individuals of *C. hawaiensis* and *C. laxiflora* (sect. *Macrosepalae* C. B. Clarke) at lower elevations in the gulch.

The implications for the low level of intrasectional hybrids are that different introductions to the islands may have resulted in separate, largely allopatric species radiations. Subsequent introductions resulted in similar radiations and consequently species occupied niches previously filled in earlier introductions. A lack of reproductive barriers between the sections allows interspecific and thus intersectional hybrids. Similar situations have been observed in historical times when human-introduced alien species have hybridized with native species in the British Isles (Abbott 1992) or with introduced *Tragopogon* L. species in northwestern Washington, USA (Novak & al. 1991). If the six sections are the result of six different introductions (Table 1), then this scenario is the most likely explanation for both the species diversity of Hawaiian *Cyrtandra* and for the extensive hybridization observed. If the six sections are the result of only four introductions (Table 1, sects *Macrosepalae, Chaetocalyces* C. B. Clarke and *Apertae* C. B. Clarke representing a single introduction), then the scenario is still valid as there are only a few interspecific hybrids among the species in these three sections. However, it should be noted that *Cyrtandra* is a large genus with a wide range of morphological characters and geographic distribution. Many of the Hawaiian species share similar

characters and it is possible that all of the Hawaiian species represent a single introduction event. Further investigations on the phylogenetic relationships of these species and sectional relationships will be necessary before any strong conclusions regarding speciation patterns can be made.

In conclusion, the RAPD data from this analysis indicate that the putative hybrids, as previously identified on the basis of morphology and geographic distribution (Wagner & al. 1990) are also genetic intermediates between the two parental species. Additivity of RAPD bands for all putative hybrid taxa is 50% or greater and the likelihood that this additivity is the result of common ancestry rather than hybridization is unlikely on the grounds that similar rates of additivity are not found if the parental species are viewed as the hybrids and the putative hybrid as one of the parents. Unique bands within hybrid taxa do not necessarily indicate a lack of hybridization but can be explained largely to sampling errors and novel bands formed in hybrid individuals.

The authors would like to thank Joel Lau of the Hawaii Nature Conservancy, Michael Kiehn of the Botanical Institute in Vienna, Clyde Imada of the Bishop Museum, and Thomas J. Givnish of the University of Wisconsin-Madison for assisting in field collections and supplying material of *Cyrtandra* for this study. The authors also thank the Hawaii Department of Land and Natural Resources for collecting permits on state land, the Bishop Museum herbarium (BISH) and National Tropical Botanical Garden, Lawa'i, Hawaii (PTBG) for use of their facilities and herbarium material and Brunella Bowditch, Michael Kiehn, Loren Rieseberg, and two anonymous reviewers for comments on the manuscript. Funding for this study was provided by a grant from the Idaho State Board of Education and NSF grant DEB-9317775 to JFS.

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Accepted July 24, 1995 by D. Giannasi