Interspecific Hybrid Ancestry of a Plant Adaptive Radiation: Allopolyploidy of the Hawaiian Silversword Alliance (Asteraceae) Inferred from Floral Homeotic Gene Duplications

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The polyploid Hawaiian silversword alliance (Asteraceae), a spectacular example of adaptive radiation in plants, was shown previously to have descended from North American tarweeds of the Madia/Raillardiopsis group, a primarily diploid assemblage. The origin of the polyploid condition in the silversword alliance was not resolved in earlier biosystematic, cytogenetic, and molecular studies, apart from the determination that polyploidy in modern species of Madia/Raillardiopsis arose independent of that of the Hawaiian group. We determined that two floral homeotic genes, ASAP3/TM6 and ASAP1, are found in duplicate copies within members of the Hawaiian silversword alliance and appear to have arisen as a result of interspecific hybridization between two North American tarweed species. Our molecular phylogenetic analyses of the ASAP3/TM6 loci suggest that the interspecific hybridization event in the ancestry of the Hawaiian silversword alliance involved members of lineages that include Raillardiopsis muiri and perhaps Madia nutans and Raillardiopsis scabruda. The ASAP1 analysis also indicates that the two species of Raillardiopsis are among the closest North American relatives of the Hawaiian silversword alliance. Previous biosystematic evidence demonstrates the potential for allopolyploid formation between members of the two North American tarweed lineages; a vigorous hybrid between R. muiri and R. scabruda has been produced that formed viable, mostly tetraporate (diploid) pollen, in keeping with observed meiotic failure. Various genetic consequences of allopolyploidy may help to explain the phenomenal evolutionary diversification of the silversword alliance.

Introduction

Adaptive radiations are among the most spectacular processes in organismal evolution. Radiations are characterized by rapid bursts of evolutionary innovation and are associated with increased speciation rates, elevated levels of morphological diversity, and marked differentiation in ecological characteristics (Givnish 1997). Some of the most dramatic examples of adaptive radiations are found in the Hawaiian archipelago, where isolation, continued island formation, and a high diversity of environmental settings promote species radiations in both plant and animal groups.

The Hawaiian silversword alliance (Asteraceae: Heliantheae–Madiinae) is a premier example of adaptive radiation in plants (Carr 1985; Robichaux et al. 1990; Baldwin and Robichaux 1995; Baldwin 1997). The alliance comprises 30 perennial species in three endemic genera: Argyroxiphium, Dauabautia, and Wilkesia (Carr 1985, 1998a, 1998b). The species are distributed on six of the eight main islands of the Hawaiian archipelago (Kauai, Oahu, Molokai, Lanai, Maui, and Hawaii), with all but five species being single-island endemics (Carr 1985). Species in the alliance grow in a wide range of habitats, including exposed lava, dry scrub, mesic forests, wet forests, and bogs (Baldwin and Robichaux 1995; Baldwin 1997). The species also display an impressive array of morphological growth forms, including rosette plants, cushion plants, subshrubs, shrubs, trees, and lianas (Baldwin and Robichaux 1995; Baldwin 1997). Furthermore, the silversword alliance is characterized by a high level of chromosomal repatterning, with eight genomic rearrangements distinguished by reciprocal translocations and an aneuploid reduction (Carr and Kyhos 1986).

Understanding the origin of species-rich insular groups is crucial to our attempts to reconstruct evolutionary patterns of ecological and morphological change that characterize adaptive radiations (Givnish 1997). Both cpDNA and rDNA ITS studies have confirmed Carlquist’s (1959) hypothesis that the closest relatives of the Hawaiian silversword alliance can be found among the North American tarweeds (Asteraceae: Heliantheae–Madiinae) in the paraphyletic Madia/Raillardiopsis group (Baldwin et al. 1991; Baldwin 1992, 1996, 1997; Baldwin and Robichaux 1995). A major unanswered question concerns evolution of the polyploid condition found throughout the Hawaiian silversword alliance (Baldwin et al. 1991; Baldwin and Kyhos 1996; Baldwin 1997). Both cytogenetic (Carr and Kyhos 1986; Kyhos, Carr, and Baldwin 1990; Carr, Baldwin, and Kyhos 1996) and allozymic (Witter and Carr 1988) data indicate that the Hawaiian species are tetraploids (n = 13–14), in contrast to the basally diploid condition (n = 6–9) in each of the most closely related North American lineages of Madia/Raillardiopsis (Baldwin 1996). Resolution of the origin of polyploidy in the silversword alliance is important for understanding the genomic constitution of the founder species and its descendants, information that could help to identify factors that facilitated this spectacular adaptive radiation.

We report here the isolation of orthologs of two Arabidopsis floral homeotic genes, APETALA1 and...
APETALA3/TM6, from members of the Hawaiian silversword alliance and the North American tarweeds. PCR-based sampling indicates that these floral regulatory genes are each present in two copies in the Hawaiian species, but in only one copy in the North American tarweeds examined. Our results suggest that the Hawaiian silversword alliance is an allopolyploid group that descended from a hybrid between members of extant tarweed lineages.

Materials and Methods
Sampling and Nucleic Acid Isolation

Genomic DNA samples were isolated from young leaf tissue collected from 10 species of the Hawaiian silversword alliance and 7 species of North American tarweeds. The Hawaiian silversword alliance species were selected to represent each of the four major lineages in the silversword alliance as previously identified from rDNA ITS trees (Baldwin and Robichaux 1995; Baldwin 1996). Four North American species (Madia bolanderi, Madia nutans, Raillardiopsis scabrida, and Raillardiopsis muirii) were chosen to represent each of the four major lineages in the *Madia/Raillardiopsis* group as also identified from rDNA ITS trees (Baldwin 1996). Three other North American tarweed species (*Adenothamus validus, Raillardella pringlei*, and *Osmadenia tenella*) are known to fall outside the clade comprising *Madia/Raillardiopsis* and the silversword alliance (Baldwin 1996) and were included to serve as the outgroup in the analyses. Genomic DNA was isolated from leaf tissue of *Argyroxyphium sandwicense* subsp. *macrocephalum* using a modified CTAB protocol that reduced the amount of pectin and secondary-product contamination (Friar, Robichaux, and Mount 1996). For all of the North American species and the remaining Hawaiian species, genomic DNA was isolated using the methods of Palmer et al. (1986) or Doyle and Doyle (1987) and further purified on CsCl gradients. Total floral RNA was isolated from immature and mature capitula of *A. sandwicense* subsp. *macrocephalum* using a phenol/SDS extraction procedure (Ausubel et al. 1992).

Isolation of the *Argyroxyphium sandwicense* AP3/TM6 and *API* Genes

MADS-box loci can be readily identified using sets of degenerate PCR primers that specifically amplify MADS-box sequences (Rounsley, Ditta, and Yanofsky 1995). The isolation of MADS-box sequences from *A. sandwicense* subsp. *macrocephalum* by this method allowed us to obtain sequence information that permitted the design of 5′ nonoverlapping, nested PCR primers specific to the *ASAP3/TM6* and *ASAP1* genes. These gene-specific primers were used to isolate cDNAs utilizing the PCR-based rapid amplification of cDNA ends (RACE) protocol (Frohman, Dush, and Martin 1988). First-strand cDNA was synthesized from *A. sandwicense* subsp. *macrocephalum* floral RNA with AMV reverse transcriptase following standard protocols (Boehringer Mannheim), and amplified cDNA was cloned using the TA procedure (In Vitrogen) and sequenced.

PCR primers to amplify *ASAP3/TM6* and *ASAP1* genomic regions were designed based on the cDNA sequences. The primers *ASAP3*-2 and *ASAP3*-3R allowed PCR amplification of a region spanning exons 1–4 of the *ASAP3/TM6* gene. Primers were designed to isolate both size-differentiated copies of this gene (1.1 kb vs. 1.4 kb) in the Hawaiian silversword alliance species. For *ASAP1*, primers *ASAP1*3X and *ASAP1*8XR were designed to isolate both A and B copies of this gene; the length difference between the duplicate copies was too small, however, for reliable size differentiation. To isolate the two copies of *ASAP1*, gene-specific primer pairs (*ASAP1*-F1A/*ASAP1*-RB and *ASAP1*-AF/*ASAP1*-R) were constructed to amplify sequences from exons 3–8 of different duplicate copies of the gene within the Hawaiian species. The primers were used in PCR amplifications using the error-correcting rTth polymerase formulation (Perkin-Elmer) in standard buffer with 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 4 min. The nucleotide error rate for this formulation is less than 1 bp in 7 kb of sequence (unpublished observations). In experiments designed to determine whether other copies of these loci are present, an annealing temperature 5–10°C below primer melting temperature was used. PCR-amplified DNA was cloned using the TA cloning kit (In Vitrogen) and sequenced using automated sequencers (Iowa State University Sequencing Facility, NCSU DNA Sequencing Facility). Sequencing was done with nested primers, with multiple sequencing reactions conducted for divergent sequences. All sequence changes were rechecked visually against sequencing chromatograms and are deposited in GenBank (accession numbers AF147210–AF147258).

Phylogenetic Analyses

Nucleotide sequences were aligned visually. Phylogenetic analyses were conducted using maximum-parsimony techniques implemented in PAUP*4.0d5 (Swofford 1998). Both substitution and insertion/deletion (indel) differences were used and weighted equally in the analyses, with the indels separately coded (as additional characters) to reflect nonindependence of continuous gaps. Parsimony analyses were conducted using the heuristic search procedure, with random taxon addition (10 replicates), tree bisection-reconnection branch swapping, and MULPars in effect. Clade support was estimated by parsimony analysis of 500 bootstrap replicates of the data set using the search procedures outlined above.

Interspecific Hybridizations

In addition to a hybrid combination previously reported between *R. muirii* and *R. scabrida* (Baldwin 1989; Kyhos, Carr, and Baldwin 1990), hybrids were produced in the combinations *Madia madioides × R. scabrida* and *Dubautia knudsenii × (M. madioides × R. scabrida)* (unpublished data). Cross-pollinations were performed by rubbing the stylar surfaces of pollen-shedding capitula together. Full cypselae from crosses were hydrated overnight on wet filter paper, and F1 embryos were surgically excised and germinated on wet filter pa-
per. The F₁ plants were grown to flowering maturity under cool, low-humidity greenhouse conditions at the University of California–Berkeley. Pollen fertility and pollen morphology of hybrid plants were examined by staining with cotton blue in lactophenol. Chromosomal association at meiotic metaphase I was examined, using phase microscopy, in squashed microsporocytes stained with acetocarmine mixed with Hoyer’s solution.

Results and Discussion
Floral Homeotic Genes as Sources of Characters for Plant Phylogeny Reconstruction

In recent years, the molecular genetics of floral and inflorescence development have been the subject of intense study (Riechmann and Meyerowitz 1997). Much of this work has been carried out on Arabidopsis thaliana (Brassicaceae), in which several genes that control flower development have been identified (Riechmann and Meyerowitz 1997). The loci are referred to as floral homeotic genes, with mutational lesions in the genes resulting in the formation of aberrant organ types in flowers.

Molecular studies have revealed that most of the floral homeotic genes isolated to date belong to the MADS-box regulatory gene family of transcriptional activators (Ma, Yanofsky, and Meyerowitz 1991; Purugganan et al. 1995; Purugganan 1997) and appears to be an ortholog of the Arabidopsis AP1 group of genes (Purugganan et al. 1995; Riechmann and Meyerowitz 1997). Many of the duplicates that resulted in the growth of this gene family appear to have occurred fairly early in the evolutionary history of the vascular plants (Purugganan et al. 1995; Riechmann and Meyerowitz 1997). Many of the loci observed thus far, with variable regions that are evolving at 3.7 × 10⁻⁹ nonsynonymous substitutions per site per year (Purugganan et al. 1995). This rate is comparable to the mean rate for synonymous substitutions for other plant nuclear genes (4.7 × 10⁻⁹ substitutions per site per year) (Purugganan et al. 1995). The rapid evolution of the MADS-box floral homeotic genes, including their introns, makes them attractive as potential sources of new molecular characters for plant phylogenetic investigations at lower taxonomic levels.

AP3/TM6 and AP1 Orthologs in the Hawaiian Silversword Alliance

Homologs of the Arabidopsis floral homeotic genes AP3 and AP1 were successfully isolated from developing flowers of A. sandwicense subsp. macrocephalum utilizing RT-PCR techniques. The isolated AP3 homolog cDNA is 837 bp long and contains a long open reading frame encoding a putative protein of 226 amino acids (aa) that encodes a MADS domain (see fig. 1). The protein shows strong similarity to the Arabidopsis AP3 and Antirrhinum DEFICIENS proteins, with overall similarities of 84% and 88%, respectively, at the peptide level. Phylogenetic analysis indicates that the isolated gene is a member of the AP3 group of genes (Purugganan et al. 1995; Purugganan 1997) and appears to be an ortholog of the tomato TM6 locus (Kramer, Dorit, and Irish 1998). We refer to the gene as ASAP3/TM6.

The isolated A. sandwicense subsp. macrocephalum AP1 (or ASAP1) cDNA is 839 bp long, with an open reading frame encoding a putative protein of 231 aa and possessing a 57-aa MADS domain (see fig. 1). The encoded protein shows strong similarity (80%–85%) to the Arabidopsis AP1 and Antirrhinum SQUAMOSA proteins, and phylogenetic analysis confirms that ASAP1 is the ortholog of the Arabidopsis AP1 locus (results not shown).

Duplicate Copies of the Floral Homeotic Genes in the Hawaiian Silversword Alliance

Using primers designed from cDNA sequences, ASAP3/TM6 genomic sequences spanning exons 1–4 were amplified by PCR. Two bands of 1.1 and 1.4 kb, were obtained from species in the Hawaiian silversword alliance, suggesting that ASAP3/TM6 is present in two distinct copies in each species. In contrast, only one PCR band was amplified from each North American tarweed species examined. The 1.1-kb fragment from the Hawaiian species is designated ASAP3/TM6-A, and the larger 1.4-kb product is ASAP3/TM6-B. We successfully isolated both gene copies of ASAP3/TM6 from nine Hawaiian silversword alliance species, but only ASAP3/TM6-A from Dubautia plantaginea. The two gene copies from A. sandwicense subsp. macrocephalum are minimally divergent from one another (93% similarity). The introns of ASAP3/TM6-A and ASAP3/TM6-B have
diverged by 20 indels ranging in size from 1 to 369 bp (see fig. 2). In contrast, orthologous sequences within the ASAP3/TM6-A and ASAP3/TM6-B gene groups in the Hawaiian taxa are 99% and 97%–98% similar, respectively.

The ASAP1 gene was also amplified using primers designed from cDNA sequences and (like ASAP3/TM6) was present in duplicate copies in the genomes of the Hawaiian species. PCR amplification of exons 3–8 of ASAP1 yielded two copies that were both approximately 1.8 kb in length. In contrast, PCR amplification produced only one gene copy in each of the North American species examined. The duplicate copies of the gene in the Hawaiian species, which differ only slightly in size, are designated ASAP1-A and ASAP1-B. We successfully isolated both copies from nearly all Hawaiian silversword alliance species, with the exception of the B copy from Dubautia laevigata and Dubautia latifolia. We also isolated the ASAP1 loci from a representative of each of the four major lineages within the North American Madia/Raillardiopsis group, as well as from O. tenella. As with ASAP3/TM6-A and ASAP3/TM6-B, the two gene copies of ASAP1 from A. sandwicensis subsp. macrocephalum show 93% similarity. The two gene copies are differentiated by 30 indels that range in size from 1 to 61 bp (see fig. 2). Orthologous sequences within the ASAP1-A and ASAP1-B gene groups in the Hawaiian taxa were 98%–99% and 95%–96% similar, respectively.

The presence of two distinct ASAP3/TM6 and ASAP1 genes in the Hawaiian species is not unexpected—as mentioned above, both cytogenetic (Carr and Kyhos 1986; Kyhos, Carr, and Baldwin 1990; Carr, Baldwin, and Kyhos 1996) and allozyme (Witter and Carr 1988) studies indicate that the closest related North American tarweed lineages (n = 13, 14), in contrast to the basally diploid condition of each of the most closely related North American tarweed lineages (n = 6–9) (Baldwin 1996). We utilized a permissive PCR-based approach to examine whether the members of the North American Madia/Raillardiopsis group possessed only a single gene copy of these two floral homeotic loci. A PCR-based approach was necessitated by the difficulties in conducting DNA blot analyses with these species, which appear to have both large genomes and high methylation levels (unpublished data).

For permissive PCR, primers were designed based on the conserved regions of both A and B copies of each gene and used to amplify sequences in the Madia/Raillardiopsis species using permissive annealing temperatures (below primer melting temperature [5–10°C]). The design of these primers around conserved regions ensures the amplification of both gene copies if they are present in the North American taxa. This permissive PCR approach resulted in the detection of nine new sequences of >500 bp. Analyses of these novel amplicons against GenBank identified two of these sequences: (1) a Tylicopia-like retrotransposon and (2) a plant extensin-like gene. The other seven sequences could not be identified from database searches. No duplicates of the ASAP1 or ASAP3/TM6 genes were detected in the Madia and Raillardiopsis genomes using this permissive PCR approach. Together with the previous cytogenetic and allozyme studies, these results suggest that these floral homeotic loci are present in only single copies in the immediate North American relatives of the Hawaiian silversword alliance.

ASAP3/TM6 Gene Tree

Figure 3A depicts a gene phylogeny for the ASAP3/TM6 loci obtained from maximum-parsimony analysis. The tree demonstrates that the ASAP3/TM6-A and ASAP3/TM6-B genes from the Hawaiian silversword alliance each form a monophyletic group. The ASAP3/TM6-A sequences group together with 83% bootstrap support, and the ASAP3/TM6-B copies constitute a monophyletic group with 100% support. The phylogeny of the loci also suggests that the presence of the A and B copies in the Hawaiian species is the result of evolutionary reticulation, rather than duplication following divergence from the North American tarweeds; the A and B copies are not resolved as sister to one another,
but rather as sister to genes from different species or species groups of North American tarweeds. The ASAP3/TM6-A genes appear to be closely related to the gene in \textit{R. scabrida}, with the association showing 100% bootstrap support. In contrast, the ASAP3/TM6-B sequences appear most closely related to the sequences of \textit{R. muirii} and \textit{M. nutans}, with 89% bootstrap support. In the ASAP3/TM6 phylogeny, the \textit{R. muirii} and \textit{M. nutans} genes also group together with 89% bootstrap support.

A large number of indels differentiate the ASAP3/TM6 loci in the different species examined. A minimum of 176 indel events in the ASAP3/TM6 introns can account for the length differences observed and scored for analysis. Many of the indels are found in only one species, but several appear to be shared by multiple taxa; e.g., the indels diagnosing the ASAP3/TM6-A and ASAP3/TM6-B gene lineages in the silversword alliance. The indels range in size from 1 bp to a large deletion of 1.2 kb restricted to \textit{M. nutans}. Five microsatellite loci also contribute to some of the length variation.

Several indels provide support for distinct North American tarweed ancestries of the ASAP3/TM6-A and ASAP3/TM6-B genes (see fig. 3A). Two indels, a 4-bp insertion and a 3-bp deletion in intron sequences, unambiguously group the \textit{R. scabrida} locus with the Hawaiian ASAP3/TM6-A sequences. Moreover, at least four indels, ranging in size from 10 to 159 bp, are shared between the \textit{R. muirii} gene and the Hawaiian ASAP3/TM6-B sequences.

Length variation in ASAP3/TM6 exhibits homoplasy that partly complicates identification of the North American tarweed gene lineages most closely related to those of the Hawaiian silversword alliance. None of the four length variants shared between the Hawaiian ASAP3/TM6-B sequences and the \textit{R. muirii} gene is found in the \textit{M. nutans} sequence, although a separate 7-bp deletion is shared by all three lineages. Three indels can also be found that are shared by the ASAP3/TM6-B genes and the sequences from \textit{R. muirii} and \textit{M. bolanderi}.

**ASAP1 Gene Tree**

The ASAP1 gene provides an additional set of nuclear sequences for analysis of relationships between the Hawaiian silversword alliance and the North American tarweeds. Like the ASAP3/TM6 gene, ASAP1 was found in duplicate copies in the Hawaiian species, yet appears to be present in only one copy in the genomes of North American members of the \textit{Madia/Raillardiopsis} group. Each of the copies found in the Hawaiian species, ASAP1-A and ASAP1-B, forms a monophyletic group with 100% bootstrap support (see fig. 3B). Moreover, a larger clade containing the genes of \textit{R. scabrida} and \textit{R. muirii} with both Hawaiian ASAP1-A and ASAP1-B loci is supported by bootstrap analysis at the 87% level. Thus, the ASAP1 tree corroborates the finding from the ASAP3/TM6 phylogeny that the genes of \textit{R. scabrida} and \textit{R. muirii} are more closely related to those of the...
Hawaiian species than are the genes of other sampled species of the *Madia/Raillardiopsis* group (see fig. 3B).

Unlike the ASAP3/TM6 phylogeny, however, the ASAP1 phylogeny does not provide strong resolution of the relationships among the Hawaiian ASAP1-A and ASAP1-B genes and the ASAP1 genes of *R. muirii* and *R. scabrida*. Several indels provide evidence for a lineage comprising the *R. muirii* gene and ASAP1-B sequences; three indels ranging in size from 3 to 8 bp are shared by only the *R. muirii* ASAP1 gene and the ASAP1-B sequences. However, a 9-bp insertion is shared by the *R. muirii* gene and both Hawaiian gene lineages, and two small deletions place both the *R. muirii* and *R. scabrida* ASAP1 sequences closer to the ASAP1-A lineage than to the ASAP1-B lineage. The incomplete resolution within the lineage of Hawaiian and *Raillardiopsis* genes may be due to the effects of homoplasy accompanying the high rate of molecular evolution of the ASAP1 gene, which appears to evolve faster than the ASAP3/TM6 locus (unpublished data).

A Hybrid (Allopolyploid) Ancestry of the Hawaiian Silversword Alliance

The origin of the Hawaiian silversword alliance, considered "the best example of adaptive radiation in plants" (Raven, Evert, and Eichorn 1992), was ill-defined until Carlquist (1959) suggested that the group was related to North American tarweeds. Molecular systematic investigations using both cpDNA restriction site data (Baldwin et al. 1991) and rDNA ITS sequences (Baldwin and Robichaux 1995; Baldwin 1997) have confirmed and extended Carlquist's (1959) hypothesis by showing that the Hawaiian species are phylogenetically nested within the paraphyletic *Madia/Raillardiopsis* group and originated after considerable diversification of the North American tarweeds (Baldwin 1996; Baldwin and Sanderson 1998). Previous investigations have not, however, determined whether the species of the silversword alliance are auto- or allopolyploids (see Baldwin 1997).

Our molecular phylogenetic analyses using two nuclear-encoded floral homeotic genes provide evidence that species in the Hawaiian silversword alliance are allopolyploids that descended from a hybrid between species of two extant lineages in the *Madia/Raillardiopsis* group. Based on the ASAP3/TM6 analysis, the interspecific hybridization event in the ancestry of the Hawaiian silversword alliance involved members of lineages that include *R. muirii* (and perhaps *M. nutans*) and *R. scabrida*. The ASAP1 analysis also suggests that the two species of *Raillardiopsis* are among the closest North American relatives of the silversword alliance. Moreover, the earlier finding that *R. muirii* cpDNA is more closely related to the Hawaiian cpDNAs than is *R. scabrida* cpDNA (Baldwin et al. 1991) suggests that a member of the lineage represented by *R. muirii* was the maternal parent of the hybrid ancestor of the silversword alliance (see fig. 4). The phylogenetic position of the lineage represented by *M. nutans* remains uncertain because of apparent topological conflict between the ASAP3/TM6 and ASAP1 trees. The ASAP3/TM6 tree placement of *M. nutans* and *R. muirii* as sister taxa receives support, however, from the rDNA ITS phylogeny, which is topologically congruent with the ASAP3/TM6 results (fig. 4).

Phylogenetic analyses of nuclear genes that have not undergone interlocus concerted evolution have proved crucial to our identification of a hybrid ancestry of the silversword alliance. Both cpDNA and rDNA ITS analyses, which have provided the most widely used molecular data for investigations of plant phylogeny, can sometimes prove insufficient for identifying reticulate evolution (see fig. 4). Uniparental cpDNA trees are useful for aiding resolution of hybridization events only in comparison with phylogenetic data from the nuclear genome (e.g., Baldwin 1997). Analyses of rDNA ITS sequences in the Hawaiian silversword alliance did not reveal evidence of divergent or recombinant ITS copies within species (Baldwin and Robichaux 1995; Baldwin 1997; Baldwin and Sanderson 1998), a result that may be explained by rapid, unidirectional concerted evolution following the ancient hybridization event resolved here (Hillis et al. 1991; Baldwin and Sanderson 1998). Strongly conflicting signal between cpDNA and rDNA ITS data, another possible indication of hybrid ancestry, was not found from comparisons of the two character sets using the partition homogeneity test (Farris et al. 1995), as implemented in PAUP* (Baldwin 1992; unpublished data).

Plausibility of the Inferred Evolutionary Reticulation Based on Evidence from Artificial Hybrids

Although up to 5–6 Myr may have elapsed since the onset of diversification of the silversword alliance

![Figure 4](https://academic.oup.com/mbe/article-abstract/16/8/1105/2925504/fig-4)
Although production of synthetic allopolyploidy in Asteraceae (Baldwin 1989; Kyhos, Carr, and Baldwin 1990) is more frequent than triporate, a condition correlated with unreduced meiotic microspores, one hybrid between R. muirii and R. scabrida was unusually large and tetraporate (rather than triporate), a condition correlated with unreduced pollen viability (pollen stainability 9%) and mostly univalents at meiotic metaphase I. Despite low fertility of the plants, the ability to produce these vigorous intergeneric hybrids indicates that the Hawaiian species and their closest relatives among North American tarweeds continue to retain considerable genetic similarity after millions of years of evolutionary divergence.

Genomic Constitution of the Hawaiian Founder and Extinction Implications

Our evidence for allopolyploidy of the silversword alliance leads us to conclude that the tarweed founder, not just the most recent common ancestor, of the Hawaiian group was an allopolyploid. Otherwise, we must hypothesize (1) ancient long-distance dispersal of two (diploid) North American tarweed species to the same location in the Hawaiian Islands, (2) hybridization and formation of an allopolyploid lineage in situ, and (3) subsequent extinction of the two diploid species in Hawaii (Baldwin 1997). The alternative scenario of dispersal of an allopolyploid species from western North America is simpler, requiring only one dispersal to the Hawaiian Islands and extinction of one lineage—the North American allopolyploid group (Baldwin 1997). Although multiple polyploid lineages are known within the Hawaiian Islands and extinction of one lineage—Hawaiian allopolyploids of the same genomic constitution in North America proved to be an evolutionary dead end. A similar allopolyploid extinction in western North America following successful long-distance dispersal (to South America) appears to have occurred in the Asteraceae genus Blennosperma (Ornduff 1961).

Genetic Consequences of Allopolyploidy in the Hawaiian Silversword Alliance

The inferred hybrid constitution of the Hawaiian founder may have endowed the ancestor with more genetic variation and a greater ability to respond to selection (Jiang et al. 1998) than would be found in a diploid progenitor. Any intergeneric (allosyndetic) chromosome pairing that may have occurred in the early Hawaiian allopolyploid origin of the silversword alliance ancestor in the Hawaiian setting, despite the phenomenal evolutionary success of the silversword alliance from the North American tarweeds (Baldwin 1996; unpublished data). We conclude that the Hawaiian species originated well after divergence of the silversword alliance from the North American tarweeds (Baldwin 1996; unpublished data). We conclude that the inferred hybrid constitution of the Hawaiian founder may have promoted adaptive radiation of the silversword alliance. The presence of two divergent genomes in the colonizing ancestor of the Hawaiian group may have endowed the ancestor with more genetic variation and a greater ability to respond to selection (Jiang et al. 1998) than would be found in a diploid progenitor. Any intergeneric (allosyndetic) chromosome pairing that may have occurred in the early Hawaiian allopolyploidy.
ploids would have allowed for wide genetic segregation and a broad array of progeny phenotypes (see Grant 1975), thereby conceivably enhancing the prospects for success in diverse island habitats. The impact of such extensive recombination on diversification of the silversword alliance may have been great, particularly in light of the ecological and morphological diversity of the North American lineages implicated in the formation of the hybrid ancestor of the Hawaiian group.

In general, the increased number of different loci correlated with allopolyploid formation provides novel avenues for molecular evolutionary and phenotypic divergence (Soltis and Soltis 1995). Allopolyploidy may impart upon plants a greater degree of genetic variation (Levin 1983; Soltis and Soltis 1995), increased redundancy of developmental pathways (Gibson and Spring 1998), and potential for structural and functional divergence of duplicated loci (Ohno 1970). We conclude that a variety of genetic factors associated with allopolyploidy may have been critically important in the spectacular adaptive radiation of the Hawaiian silversword alliance.

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