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Evolution

Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae)

(insular evolution/adaptive radiation/biogeography/long-distance dispersal/phylogenetics)

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ABSTRACT **Chloroplast DNA restriction-site comparisons were made among 24 species of the Hawaiian silversword alliance (*Argyroxiphium*, *Dubautia*, and *Wilkesia*) and 7 species of North American perennial tarweeds in *Adenothamnus*, *Madia*, *Raillardella*, and *Raillardiopsis* (Asteraceae–Madiinae). These data and results from intergeneric hybridization indicated surprisingly close genetic affinity of the monophyletic Hawaiian group to two diploid species of montane perennial herbs in California, *Madia bolanderi* and *Raillardiopsis muirii*. Of 117 restriction-site mutations shared among a subset of two or more accessions, more than one-fifth (25 mutations) separated the silversword alliance, *M. bolanderi*, and *Raillardiopsis* from *Adenothamnus* and *Raillardella*. An additional 10 mutations distinguished the silversword alliance, *M. bolanderi*, and *R. muirii* from *Adenothamnus*, *Raillardella*, and *Raillardiopsis scabrida*. Phylogenetic analyses of these data and production of vigorous hybrids of the combinations *Dubautia laevigata* × *R. muirii* and (*Dubautia knudsenii* × *Dubautia laxa*) × *M. bolanderi* reinforce and refine Carlquist's hypothesis [Carlquist, S. (1959) *Aliso* 4, 171–236] that the Hawaiian silversword alliance arose from American tarweeds. Ultimate origin of silversword alliance chloroplast DNA from within the Californian-endemic paraphyletic genus *Raillardiopsis* was supported with high bootstrap confidence. Geologic considerations and the distribution of sporophytic self-incompatibility among these species demonstrate that the tarweed ancestor of the silverswords overcame (*i*) a dispersal barrier of at least 3900 km of open ocean and (*ii*) the breeding barrier of self-incompatibility.**

Molecular and experimental data on the continental ancestry of insular organisms are lacking. Precise resolution of the origins of island species by modern approaches promises insights on genetic change accompanying adaptive radiation, timing of insular as opposed to continental speciation, and mechanisms and limits of long-distance dispersal. Among Hawaiian plants, these aspects of phytogeography and evolution can be most readily addressed in the well-studied silversword alliance (Asteraceae), an autochthonous and highly diversified assemblage of plants with demonstrated anatomical affinity to a narrowly endemic American plant group (1).

The Hawaiian silversword alliance (Asteraceae) has been regarded "the most outstanding example of adaptive radiation among Hawaiian angiosperms" (2). This woody group comprises 28 species in three genera (*Argyroxiphium*, *Dubautia*, and *Wilkesia*), including trees, shrubs, subshrubs, rosette plants, cushion plants, and a vine (3). These taxa display great diversity in morphological, anatomical, and ecophysiological traits and collectively span habitats ranging from some of the wettest recorded on earth to extreme desert-like environments (3). Despite this enormous diversity, structural (4, 5), biosystematic (6), allozymic (7), and chloroplast DNA (cpDNA) (8) data indicate the silversword alliance originated from a single colonizing species.

Carlquist (1) presented convincing anatomical evidence indicating taxonomic alignment of the Hawaiian silversword alliance with the almost exclusively herbaceous American Madiinae or tarweeds. Gray (9) earlier suggested such affinity for *Argyroxiphium*, which was disputed by Keck (10) based on presumed morphological dissimilarities and the magnitude of the oceanic barrier to migration. Herein, we compare cpDNA restriction sites between the silversword alliance and several North American perennial tarweeds to further evaluate this relationship and its implications for adaptive radiation and long-distance dispersal. Our focus on cpDNA was based primarily on its utility for assessing phylogeny in higher plants, including Asteraceae (11), and its ease of evolutionary analysis compared with other plant DNA.

MATERIALS AND METHODS

We examined one or two populations of 24 silversword alliance species, seven perennial North American tarweed species (Fig. 1), and four Heliantheae *sensu lato* species outside both groups (ref. 12; Fig. 2). American tarweed taxa were chosen from among 99 Madiinae species based on perenniality, other phenotypic similarities to the Hawaiian assemblage (1, 12), and a preliminary cpDNA restriction site analysis of all Madiinae genera (B.G.B., unpublished results).

Total DNA was isolated from fresh leaves by CsCl centrifugation (12, 13). DNAs were digested with each of 16 restriction endonucleases (Fig. 1). cpDNA fragments, resolved on 1.25 to 4.0% agarose gels, were analyzed by Southern blot hybridization with ^{32}P-labeled cpDNA probes and autoradiography (12). The cpDNA molecule was examined throughout using a *Sac* I *Lactuca* cpDNA library (14) and a *Pst* I 9.0-kilobase *Petunia* cpDNA clone (35). Restriction site comparisons were made from fragment profiles relative to molecular weight markers. Length mutations were discriminated from closely spaced restriction sites by analyzing those cpDNA regions with various enzymes. Nucleotide sequence divergence was calculated from restriction site data according to Nei and Li (15).

Restriction site mutations common to a subset of two or more samples were analyzed by Wagner parsimony (ref. 16; see Fig. 2) and Dollo parsimony (17) to create phylogenetic trees requiring the fewest evolutionary steps. Because of the unresolved position of Madiinae in Heliantheae *sensu lato*, evolutionary direction of restriction site mutations was designated only where identity existed among all four He-

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Abbreviation: cpDNA, chloroplast DNA.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	---	32	73	82	76	71	77	78	81	80	81	72	72	72	80	79	79	77	79	77	75	75	75	73	72
2	0.68	---	69	84	78	69	75	78	81	80	79	70	72	70	76	75	77	75	77	73	73	73	73	73	72
3	1.58	1.49	---	45	39	32	38	41	44	43	44	33	33	35	43	40	44	42	44	40	40	44	38	38	39
4	1.78	1.83	0.96	---	10	25	31	32	33	32	33	28	26	26	32	33	33	31	33	31	29	31	31	29	30
5	1.65	1.69	0.83	0.21	---	19	25	26	29	28	29	22	20	20	28	27	27	25	27	25	23	27	25	23	24
6	1.53	1.49	0.68	0.53	0.40	---	18	21	24	23	24	13	15	15	25	24	24	22	24	20	20	24	18	18	19
7	1.67	1.62	0.80	0.65	0.53	0.38	---	5	12	11	12	9	9	9	19	18	18	16	18	14	14	18	12	12	13
8	1.69	1.69	0.87	0.68	0.55	0.44	0.10	---	13	12	13	12	10	10	20	19	19	17	19	17	15	19	15	13	14
9	1.76	1.76	0.94	0.70	0.61	0.50	0.25	0.27	---	1	4	15	13	13	21	22	22	20	20	20	18	22	18	16	15
10	1.74	1.74	0.91	0.68	0.59	0.48	0.23	0.25	0.02	---	3	14	12	12	20	21	21	19	19	19	17	21	17	15	14
11	1.76	1.71	0.94	0.70	0.61	0.50	0.25	0.27	0.08	0.06	---	15	13	13	21	22	22	20	20	20	18	22	18	16	15
12	1.56	1.51	0.70	0.59	0.46	0.27	0.19	0.25	0.31	0.29	0.31	---	4	6	14	13	15	13	15	11	11	15	9	9	10
13	1.56	1.56	0.70	0.55	0.42	0.31	0.19	0.21	0.27	0.25	0.27	0.08	---	4	12	11	13	11	13	11	9	13	9	7	8
14	1.56	1.51	0.74	0.55	0.42	0.31	0.19	0.21	0.27	0.25	0.27	0.13	0.08	---	12	11	13	11	13	11	9	13	9	7	8
15	1.74	1.65	0.91	0.68	0.59	0.53	0.40	0.42	0.44	0.42	0.44	0.29	0.25	0.25	---	3	9	9	11	9	7	11	19	17	16
16	1.71	1.62	0.85	0.70	0.57	0.50	0.38	0.40	0.46	0.44	0.46	0.27	0.23	0.23	0.06	---	8	8	10	8	6	10	18	16	17
17	1.71	1.67	0.94	0.70	0.57	0.50	0.38	0.40	0.46	0.44	0.46	0.31	0.27	0.27	0.19	0.17	---	2	4	6	6	10	18	16	17
18	1.67	1.62	0.89	0.65	0.53	0.46	0.33	0.36	0.42	0.40	0.42	0.27	0.23	0.23	0.19	0.17	0.04	---	2	4	4	8	16	14	15
19	1.71	1.67	0.94	0.70	0.57	0.50	0.38	0.40	0.42	0.40	0.42	0.31	0.27	0.27	0.23	0.21	0.08	0.04	---	6	6	10	18	16	17
20	1.67	1.58	0.85	0.65	0.53	0.42	0.29	0.36	0.42	0.40	0.42	0.23	0.23	0.23	0.19	0.17	0.13	0.08	0.13	---	4	8	14	14	15
21	1.62	1.58	0.85	0.61	0.48	0.42	0.29	0.31	0.38	0.36	0.38	0.23	0.19	0.19	0.15	0.13	0.13	0.08	0.13	0.08	---	4	14	12	13
22	1.62	1.58	0.94	0.65	0.57	0.50	0.38	0.40	0.46	0.44	0.46	0.31	0.27	0.27	0.23	0.21	0.21	0.17	0.21	0.17	0.08	---	18	16	17
23	1.62	1.58	0.80	0.65	0.53	0.38	0.25	0.31	0.38	0.36	0.38	0.19	0.19	0.19	0.40	0.38	0.38	0.33	0.38	0.29	0.29	0.38	---	2	9
24	1.58	1.58	0.80	0.61	0.48	0.38	0.25	0.27	0.33	0.31	0.33	0.19	0.15	0.15	0.36	0.33	0.33	0.29	0.33	0.29	0.25	0.33	0.04	---	7
25	1.56	1.56	0.83	0.63	0.50	0.40	0.27	0.29	0.31	0.29	0.31	0.21	0.17	0.17	0.33	0.36	0.36	0.31	0.36	0.31	0.27	0.36	0.19	0.15	---

FIG. 1. Pairwise comparisons of cpDNA nucleotide sequence divergence among the Hawaiian silversword alliance and North American tarweeds. Numbers of mutations separating cpDNAs from ≈875 restriction sites sampled in each appear in upper right of matrix. Nucleotide sequence divergence values (p = numbers of substitutions per base position) are in lower left as $100 \times p$. Restriction enzymes used were *Bgl* II, *Bst*NI, *Dra* I, *Eco*RI, *Eco*RV, *Hae* II, *Hinc*II, *Hind*III, *Hpa* II, *Hph* I, *Nde* I, *Nsi* I, *Sca* I, *Ssp* I, *Xba* I, and *Xmn* I. Numbers 1–25 across the top and down the left side refer to samples. Sample abbreviations: 1 = *Raillardella argentea* (A. Gray) A. Gray, *Raillardella pringlei* E. Greene, *Raillardella scaposa* (A. Gray) A. Gray; 2 = *Adenothamnus validus* (Brandegee) Keck; 3 = *Raillardiopsis scabrida* (Eastwood) Rydberg; 4 = *Raillardiopsis muirii* (A. Gray) Rydberg [Sierra Nevada]; 5 = *R. muirii* (A. Gray) Rydberg [Santa Lucia Range]; 6 = *Madia bolanderi* (A. Gray) A. Gray; 7 = *Wilkesia gymnoxiphium* A. Gray; 8 = *Wilkesia hobdyi* H. St. John; 9 = *Dubautia knudsenii* Hillebrand; 10 = *Dubautia imbricata* H. St. John and G. Carr, *Dubautia pauciflorula* H. St. John and G. Carr, *Dubautia raillardioides* Hillebrand; 11 = *Dubautia paleata* A. Gray; 12 = *Argyroxiphium sandwicense* A. P. de Candolle; 13 = *Argyroxiphium grayanum* (Hillebrand) Degener [East Maui]; 14 = *Argyroxiphium caliginis* C. Forbes, *Argyroxiphium grayanum* (Hillebrand) Degener [West Maui]; 15 = *Dubautia plantaginea* Gaudichaud-Beaupré; 16 = *Dubautia laxa* Hooker and Arnott; 17 = *Dubautia ciliolata* (A. P. de Candolle) Keck, *Dubautia scabra* (A. P. de Candolle) Keck; 18 = *Dubautia linearis* (Gaudichaud-Beaupré) Keck; 19 = *Dubautia arborea* (A. Gray) Keck; 20 = *Dubautia menziesii* (A. Gray) Keck, *Dubautia platyphylla* (A. Gray) Keck, *Dubautia reticulata* (Sherff) Keck; 21 = *Dubautia herbstobatae* G. Carr; 22 = *Dubautia sherffiana* Fosberg; 23 = *Dubautia microcephala* Skottsberg; 24 = *Dubautia laevigata* A. Gray; 25 = *Dubautia latifolia* (A. Gray) Keck.

liantheae *sensu lato* out-group species relative to one of the two in-group states. Evolutionary relationships were also assessed strictly from genetic distances using the Fitch–Margoliash algorithm (ref. 18; see Fig. 2).

RESULTS AND DISCUSSION

Restriction Site Polymorphism. Genetic sampling encompassed about 875 restriction sites or 4828 nucleotides per cpDNA, representing 3.7% of sequences in each cpDNA, excluding one copy of the inverted repeat. Restriction site mutations common to two or more silversword alliance or North American tarweed taxa comprised 117 of 167 mutations (12). More than one-fifth (25 or 21.4%) of these shared mutations separated all of the Hawaiian species, *Raillardiopsis*, and *M. bolanderi* from *Adenothamnus* and *Raillardella*. An additional 10 mutations separated all of the Hawaiian species, *R. muirii*, and *M. bolanderi* from *Adenothamnus*, *Raillardella*, and *R. scabrida*. Six of 61 Hawaiian restriction site mutations united all of the silversword alliance, separating it from the continental tarweeds (12).¶

Genetic distances in cpDNA (p values; Fig. 1; see Fig. 2) were surprisingly inconsistent in their correlation with taxonomic rank and geography. For example, 12 Hawaiian species exhibited less cpDNA nucleotide sequence divergence from either Californian *M. bolanderi* or one population of Californian *R. muirii* (0.27–0.44%) than the maximum cpDNA divergence within the Hawaiian genus *Dubautia* (0.46%; Fig. 1). In fact, cpDNA sequence divergence between populations of *R. muirii* (0.21%) was similar to that between Californian *M. bolanderi* and the Hawaiian silversword *A. sandwicense* (0.27%). The significance of these genetic distance comparisons is unclear, however, because molecular divergence in cpDNA among the Hawaiian species was not in accord with a cpDNA molecular "clock." This irregular cpDNA evolution was indicated by discrepancies between genetic distance trees of these data using algorithms that differ principally in their assumption of whether clock-like evolution has occurred (8, 12).

Phylogenetic Analyses of cpDNA Data. Wagner parsimony revealed a highly resolved lineage including *Raillardiopsis*, *M. bolanderi*, and the Hawaiian taxa, placing the silversword alliance in closest relationship to two Californian montane tarweeds, *R. muirii*, and *M. bolanderi*, with >99% bootstrap confidence (Fig. 2). Dollo parsimony and Fitch–Margoliash genetic distance analyses yielded topologically congruent results, with one exception: Dollo parsimony (DOLLOP program of PHYLIP, see Fig. 2) placed the silversword alliance as the sister group of a clade comprising both *M. bolanderi* and *R. muirii* rather than *R. muirii* alone. Restriction site comparisons with the four outgroup species indicated the Hawaiian assemblage was derived from American tarweeds (i.e., from within the subtribe Madiinae).

Intergeneric Hybridization. These data led us to attempt controlled hybridization of each of the Californian species *R. muirii* and *M. bolanderi* to available flowering specimens of silversword alliance taxa at the University of California, Davis. The first cross attempted, between *R. muirii* (pollen parent; $n = 8$) and Kauaian *D. laevigata* (pistillate parent; n

¶A complete list of the restriction site mutations examined in this study can be obtained from B.G.B. upon request.

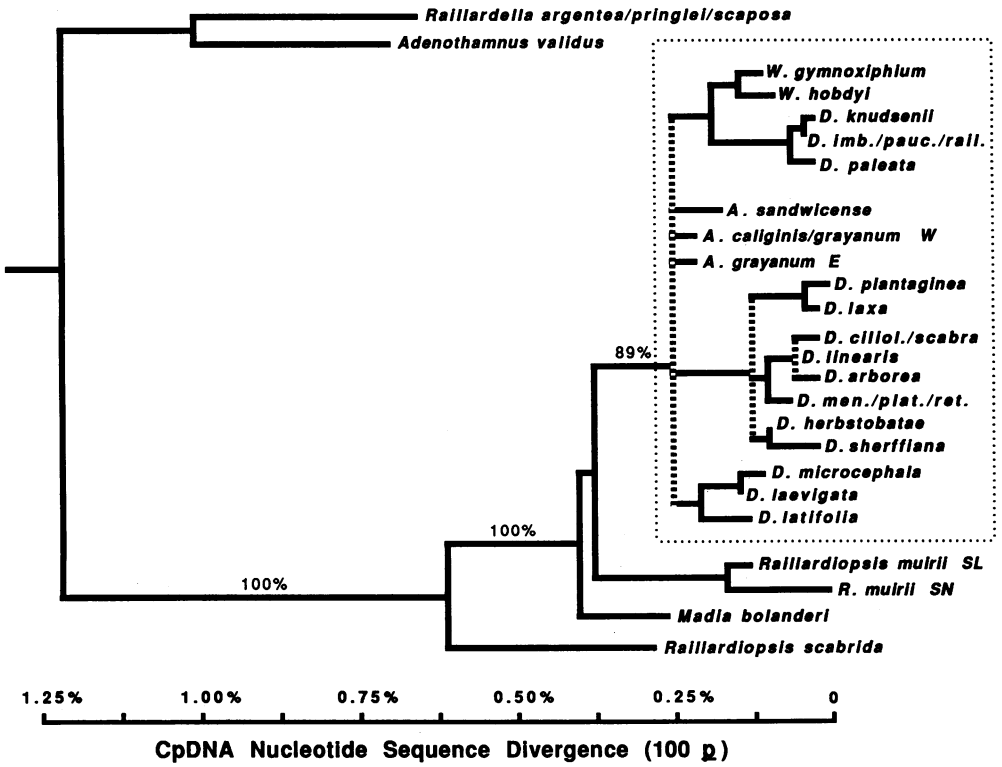

FIG. 2. cpDNA restriction site phylogeny of the Hawaiian silversword alliance (within dotted square) and related North American tarweeds. This tree is a strict consensus of the best Wagner parsimony and Fitch–Margoliash genetic distance resolutions using the PAUP, version 2.4 (BANDB and GLOBAL MULPARS options; D. L. Swofford, Illinois Natural History Survey), and PHYLIP, version 3.1 (BOOT and FITCH programs; J. Felsenstein, Univ. of Washington), statistical packages. Percentages are bootstrap confidence intervals. Genetic divergence (p) incorporates evolutionary parallelisms, unlike in Fig. 1. The tree was rooted by comparison to restriction sites of *Arnica mollis* W. J. Hooker, *Encelia densifolia* Clark and Kyhos, *Helenium bigelovii* A. Gray, and *Hulsea algida* A. Gray. *A*, *Argyroxiphium*; *D.*, *Dubautia*; *W.*, *Wilkesia*; *imb.*, *imbricata*; *pauc.*, *pauciflorula*; *rail.*, *raillardioides*; *ciliol.*, *ciliolata*; *men.*, *menziesii*; *plat.*, *platyphylla*; *ret.*, *reticulata*; W, West Maui; E, East Maui; SL, Santa Lucia Range; SN, Sierra Nevada.

= 14), succeeded as easily as if made within a single species of *Dubautia*. All of many resulting progeny from this combination were vigorous hybrids, with $2n = 22$ and a blend of phenotypic attributes of both parental species (12, 19). The second cross, between *M. bolanderi* (pollen parent; $n = 6$) and a highly fertile, intragenomic *Dubautia* hybrid, *D. plantaginea* × *D. laxa* (pistillate parent; $n = 14$), yielded two vigorous hybrids with $2n = 20$ and a mixture of morphological characteristics of each of the parents (B.G.B., G.D.C., and D.W.K., unpublished results), including ray-like corollas (*M. bolanderi* has ray florets; *Dubautia* species lack rays). The $2n = 22$ and $2n = 20$ hybrids were, as expected, highly sterile, with low modal and maximum associations of chromosomes at meiotic metaphase I (B.G.B., G.D.C., and D.W.K., unpublished results). In contrast, few intergeneric crosses have proven possible among mainland Madiinae (19, 20). Similarly, all of many attempted crosses between silversword alliance species and North American tarweed taxa other than *Raillardiopsis* and *M. bolanderi* (e.g., *A. validus*, *Madia madioides*, *Madia elegans*) repeatedly failed despite a decade and a half of effort (G.D.C. and D.W.K., unpublished results).

Evolutionary Implications. Our results strongly reinforce Carlquist's hypothesis (1) that the Hawaiian silversword alliance is a monophyletic group derived from American tarweeds. In addition, our data bring greater resolution to this relationship by demonstrating that the cpDNA and at least one nuclear genome of the tetraploid Hawaiian assemblage arose from an extant, Californian lineage including diploid, montane, perennial herbs in *Raillardiopsis* and *Madia*. This unexpected finding indicates that *Raillardiopsis*, which had been considered a phenotypically conservative genus of two nearly identical species, was an ancestral genetic source for the extensive evolutionary repatterning seen in the Hawaiian silversword alliance. The apparent derivation of *M. bolanderi* from the morphologically and ecologically-disparate genus *Raillardiopsis*, a relationship also suggested by cytological data (12, 19), is evidence of inherent evolutionary plasticity in the North American ancestry of the silversword alliance. Because of uniparental inheritance of cpDNA (21) and the unknown origin of polyploidy in the Hawaiian group, a hybrid origin of the silversword alliance involving an additional extralineal tarweed or two members of the identified ancestral lineage (e.g., *Madia* and *Raillardiopsis*) remains a possibility to be assessed by molecular analyses of nuclear DNA.

Timing of the silversword alliance radiation is uncertain because of inconstant cpDNA sequence divergence within the group (8, 12). The topology of the cpDNA phylogeny (Fig. 2) does, however, indicate considerable speciation of the Californian tarweeds prior to origin of the silversword alliance. By assuming that modern North American tarweed genera were in existence by the Pliocene (22), an origin of the silversword alliance on Kauai (≈6 million years old; ref. 23) or an older extinct island is consistent with these and other available data (24).

Biogeographic Implications. Our molecular genetic and biosystematic data confirm a North American ancestry of an endemic terrestrial-organismic lineage in the Hawaiian archipelago. Two considerations demonstrate that this biogeographic relationship represents extreme long-distance dispersal of intercontinental magnitude: (*i*) No ancient islands bridged the 3900-km oceanic barrier between Hawaii and North America. In fact, the distance between North America and the Hawaiian chain was even greater in the past. North

America has been gradually displaced westward, by expansion from the mid-Atlantic ridge, toward the Hawaiian hot spot (25, 26). In contrast, the majority of Hawaiian plant introductions appears to have occurred from the Indo-Malaysian and Austral-Asian regions (2, 27). Island-hopping and propagule transport along the eastward-moving jet stream could have promoted immigration of Indo-Malaysian and Austral-Asian species to Hawaii (28). (*ii*) The silversword alliance and tarweeds are both distinctive products of geographically restricted conditions. Diverse adaptations (29) and near restriction of American tarweeds to the isolated Mediterranean climatic region of California, southern Oregon, and northern Baja California (i.e., the California floristic province; ref. 22) indicate primary radiation of these species in this area (22).

The sticky bracts and fruit appendages of the perennial tarweeds are excellent animal-mediated dispersal adaptations (30, 31). Adherence of such fruits to a migratory bird is the most probable mode of tarweed transport across the Pacific ocean to Hawaii (30, 31). North American migratory bird species, as well as accidental arrivals, are regularly sighted in the Hawaiian Islands (32).

The most exceptional aspect of Hawaiian colonization by tarweeds is that physical barriers to dispersal were compounded by self-incompatibility (i.e., the inability to reproduce sexually by self-fertilization). Sporophytic self-incompatibility is present in all three genera of the silversword alliance (33) and among most tarweeds (19), including the perennial taxa herein (12, 19). This breeding system allows sexual reproduction only between individuals bearing different S-alleles. The powerful evolutionary barrier to reconstitution of such incompatibility once lost (34) probably accounts for its absence in all other investigated Hawaiian plants (33). Establishment of the silversword ancestor in Hawaii, therefore, required introduction of at least two individuals or accumulation of S-allele mutations in a long-lived or vegetatively propagating perennial (33). The absence of self-incompatibility in the only two tarweed species that naturally occur outside North America, *Madia chilensis* and *Madia sativa* of South America, and in the North American species complex from which they are derived (20) indicates that these taxa are not directly ancestral to the silversword alliance.

Diverse lines of evidence now support an origin of the Hawaiian silversword alliance from self-incompatible tarweeds in North America. These data and Pacific basin geology force the conclusion that the silversword ancestor was transported across an unbroken oceanic expanse about 60% greater than that separating Africa and South America. This evidence reinforces the need to seriously consider extreme long-distance dispersal of intercontinental magnitude as a possible explanation for major geographic disjunctions in plants.

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