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Evolution

Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia (Asteraceae)*

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ABSTRACT **Identification of the progenitors of plants endemic to oceanic islands often is complicated by extreme morphological divergence between island and continental taxa. This is especially true for the Hawaiian Islands, which are 3,900 km from any continental source. We examine the origin of *Hesperomannia*, a genus of three species endemic to Hawaii that always have been placed in the tribe Mutisieae of the sunflower family. Phylogenetic analyses of representatives from all tribes in this family using the chloroplast gene *ndhF* (where ndhF is the ND5 protein of chloroplast NADH dehydrogenase) indicate that *Hesperomannia* belongs to the tribe Vernonieae. Phylogenetic comparisons within the Vernonieae using sequences of both *ndhF* and the internal transcribed spacer regions of nuclear ribosomal DNA reveal that *Hesperomannia* is sister to African species of *Vernonia*. Long-distance dispersal northeastward from Africa to southeast Asia and across the many Pacific Ocean island chains is the most likely explanation for this unusual biogeographic connection. The 17- to 26-million-year divergence time between African *Vernonia* and *Hesperomannia* estimated by the DNA sequences predates the age of the eight existing Hawaiian Islands. These estimates are consistent with an hypothesis that the progenitor of *Hesperomannia* arrived at one of the low islands of the Hawaiian-Emperor chain between the late Oligocene and mid-Miocene when these islands were above sea level. Subsequent to its arrival the southeast Pacific island chains served as steppingstones for dispersal to the existing Hawaiian Islands.**

The Hawaiian Islands are 3,900 km from the closest continent and are one of the most remote land masses in the world. Despite this distance the islands have an exceptionally diverse flora. Estimates of endemism for major archipelagos show that Hawaii has the highest known (90%) compared with 40% for the Galapagos and 25% for the Canaries (1). It is estimated that the 1,000 species of native Hawaiian flowering plants originated from 270–280 progenitors (2), all as waif elements brought by long-distance dispersal. Identification of specific progenitors and source regions is difficult because of the disharmonic nature of the flora and number of possible source areas. Suggested floristic affinities include Malaysia, North America, northern South America, Australia, New Zealand, and pantropical or southern South America (2–4). Recent molecular phylogenetic studies of Hawaiian endemic plants have provided many new insights into the origin and evolution of the flora, including the identification of continental relatives from western North America (5, 6) and New Guinea (7) and both single (5, 8) and multiple colonizations (9) of the archipelago. The existence of a chain of Hawaiian islands for the past 70 million years (myr) adds to the complexity of determining the origin of the endemic flora. The affinities of the flora to disparate geographic regions and the large distances to source areas suggests that the biogeographic histories of endemic Hawaiian plants are more complicated than those of plants of other volcanic oceanic archipelagos.

We have been examining phylogenetic relationships of the Hawaiian endemic genus *Hesperomannia*, a member of the flowering plant family Asteraceae, one of the largest families in the Hawaiian Islands (2). Previous workers considered this small genus of three species to be a close relative of several South American genera of the tribe Mutisieae (10–13). *Hesperomannia* was considered basal in the tribe, and its position was used to support the thesis that the Asteraceae originated in South America (12). Our DNA phylogenies disagree with all previous studies and clearly indicate that *Hesperomannia* is closely allied to African members of the tribe Vernonieae. This result confirms a source area for the highly endemic and morphologically distinctive Hawaiian biota.

MATERIALS AND METHODS

DNA Sequencing. Phylogenetic analyses were conducted by using sequences of the chloroplast gene *ndh*F (ndhF is the ND5 protein of chloroplast NADH dehydrogenase) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. Total genomic DNA was isolated from fresh leaves (14), followed by purification in cesium chloride/ethidium bromide gradients. DNA extractions from herbarium specimens and silica-dried leaves were performed (15). Amplifications were performed in 50 μl with 1 μl of unquantified genomic DNA, 20 pmol of primer, 1× Tfl polymerase buffer, 0.5 units of Tfl polymerase (Epicentre Technologies, Madison, WI), 10 mM of $MgCl_2$, and 0.2 mM of each dNTP. The first thermal cycle consisted of 3 min denaturation at 94°C, 1 min of annealing at 50°C, and 1 min and 20 sec of polymerization at 72°C. This cycle was followed by 34 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min and 20 sec, followed by a final extension cycle of 72°C for 7 min. The amplified DNA was purified by electrophoresis in a 1% agarose gel and the GeneClean II system (Bio 101). The purified PCR product was sequenced by the snap-chill method (16) using SEQUENASE version 2.0 (United States Biochemical product no. 70770) and ^{35}S-dATP labeling.

Amplifications of the ITS region used primers ITS5 and ITS4 (17) as modified by Downie and Katz-Downie (18). Complementary strands of the ITS region were sequenced by using primers P2, P3, P4, and P5 (17). For tribal level analyses, the chloroplast gene *ndhF* was amplified by using two primers, −52 and +607, and sequenced with 12 forward primers (19). For intergeneric comparisons within the Vernonieae, only the highly variable 3′ end of *ndhF* was sequenced.

Abbreviations: ITS, internal transcribed spacer; myr, million years; ndhF, ND5 protein of chloroplast NADH dehydrogenase.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF092584–AF092607 for *ndhF* and AF092608–AF092637 for ITS).

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DNA sequences were aligned manually in MACCLADE (20) for both *ndhF* and ITS data. The ITS sequences also were aligned by using CLUSTAL V (21) followed by minor manual corrections. The boundaries of ITS-1 and ITS-2 were determined by comparing the aligned sequence with published sequences from other Asteraceae (22). MACCLADE was used to calculate transition/transversion ratios from the most parsimonious trees.

Phylogenetic Analyses. Phylogenetic analyses were conducted by using three data sets. The aligned sequences for all data sets were deposited in TreeBASE (http://herbaria.harvard.edu/treebase/). The first data set examined the tribal position of *Hesperomannia* and it included the entire *ndhF* gene (2,235 bp) for 50 representative genera from eight tribes and all three subfamilies of Asteraceae (see ref. 23 for voucher information). Three genera of the subfamily Barnadesioideae were used as outgroups (19, 24). The two other data sets were designed to examine the position of *Hesperomannia* within the Vernonieae (Table 1). One data set included the 3′ end of *ndhF* (804 bp) for two of the three species of *Hesperomannia*, 19 worldwide representatives of Vernonieae, and three genera of Liabeae as outgroups. The other used the ITS region and sampled the same two taxa of *Hesperomannia*, 16 representatives of Vernonieae from North and South America, Asia, and Africa, and one outgroup from the Liabeae. Sampling was not identical for these two data sets because it was not possible to amplify both *ndhF* and ITS for all available taxa. Phylogenetic analyses were performed for all three data sets by using PAUP (25) with all character changes unweighted. Parsimony analyses used a heuristic search with 100 random taxon additions, ACCTRAN, MULPARS, TBR, and STEEPEST DESCENT options.

Bootstrap analyses (26) were performed to assess the relative support for monophyletic groups. Bootstrap values were calculated from 100 replicates by using a Heuristic search and simple addition.

Sequence Divergence and Relative-Rate Tests. Comparisons of sequence divergence and the relative rate tests involved 10 taxa from the major lineages of the ITS and *ndhF* trees. Pairwise distances for coding regions of *ndhF* were calculated for synonymous differences by using the complete-deletion option for gaps and missing data because of different evolutionary rates at the different codon positions. Pairwise distance comparisons of noncoding regions (ITS sequences) were calculated with the transition/transversion ratio set to 1.03 by using the pairwise-deletion option for gaps and missing data. The DNADIST program of PHYLIP (27) was used to calculate nucleotide sequence divergence under Kimura's two-parameter model (28) and with Jukes and Cantor's one-parameter method (29) for 534 bp of ITS and 804 bp of *ndhF*, respectively. To assess rate homogeneity between different lineages, a relative-rate test (30, 31) was performed. *Vernonia populifolia* was used as the reference taxon for both tests because this lineage was sister to the rest of the taxa. Standard errors for estimated relative rate differences between the lineages were calculated according to Li and Tanimura (31) and Kimura (28), and were used for significance tests to assess whether a molecular clock could be rejected. The average rate of nucleotide substitutions between two clades was calculated by using percentage change per site per unit time.

RESULTS

Tribal Position of *Hesperomannia*. To investigate the tribal position of *Hesperomannia*, we compared the entire chloroplast gene *ndhF* from 50 representative genera of all of the currently recognized tribes of the Asteraceae, with more extensive sampling from the tribe Mutisieae. A total of 2,235 bp was examined, which included 707 variable sites and 347 informative sites. Parsimony analysis identified 180 trees with 1,587 steps, a consistency index of 0.59, and a retention index of 0.56. The *ndhF* tree indicated that *Hesperomannia* was not part of the tribe Mutisieae, but in fact nested deeply within the Vernonieae, a distantly related tribe of the subfamily Cichorioideae (Fig. 1). Monophyly of the group containing *Hesperomannia* was strongly supported with high bootstrap value (97%) and 11 synapomorphic changes. *Hesperomannia* was sister to *Vernonia* with 100% bootstrap support. Furthermore, a 9-bp indel at coordinate 1,717 provided additional support for the placement of *Hesperomannia* in the Vernonieae.

Table 1. Geographic origin and taxonomic placement of taxa examined

Species*	Geographic origin	Tribe/section or subsection†	Marker‡
Hesperomannia lydgatei Forbes	U.S.: Hawaii	Mutisieae	*ndhF*, ITS
H. arborescens Gray	U.S.: Hawaii	Mutisieae	*ndhF*, ITS
Gutenbergia polytrichotoma Sch. Bip.	Kenya	Vernonieae	*ndhF*, ITS
Lychnophora tomentosa Mart.	Brazil	Vernonieae	*ndhF*, ITS
Piptocarpha axillaris R. Br.	Brazil	Vernonieae	*ndhF*, ITS
Stokesia laevis L'Herit.	SE United States	Vernonieae	*ndhF*, ITS
Vernonia adoensis Sch. Bip. Ex Walp.	Zambia	Vernonieae/*Stengelia*	*ndhF*
V. alamanii DC.	Mexico	Vernonieae/*Noveboracensis*	*ndhF*, ITS
V. amygdalina Delile	Ethiopia	Vernonieae/*Strobocalyx*	*ndhF*, ITS
V. arborescens (L.) Swartz	Costa Rica	Vernonieae/*Scorpioides*	*ndhF*, ITS
V. chinensis (Lam.) Less.	China	Vernonieae/*Tephrodes*	*ndhF*, ITS
V. divergens Edgew.	India	Vernonieae/*Tephrodes*	ITS
V. fastigiata Oliv. & Hiern.	South Africa	Vernonieae/*Centrapalus*	*ndhF*
V. galamensis (Cass.) Less	Zimbabwe	Vernonieae/*Centrapalus*	*ndhF*
V. glabra Vatke	Zimbabwe	Vernonieae/*Azurae*	*ndhF*, ITS
V. jonesii B. L. Turner	Mexico	Vernonieae/*Lepidonia*	*ndhF*, ITS
V. lasiopus O. Hoffm.	Sudan	Vernonieae/*Stengelia*	*ndhF*
V. mespilifolia Less.	South Africa	Vernonieae/*Strobocalyx*	*ndhF*, ITS
V. noveboracensis (L.) Willd.	U.S.: Connecticut	Vernonieae/*Noveboracensis*	*ndhF*
V. patens H.B.K.	Guatemala	Vernonieae/*Polyanthes*	*ndhF*, ITS
V. populifolia (Lam.) Spreng.	Mauritius Islands	Vernonieae/*Distephanus*	*ndhF*, ITS
V. poskeana Vatke Hild.	Kenya	Vernonieae/*Ococephalae*	*ndhF*, ITS
V. saligna DC.	India	Vernonieae/*Tephrodes*	ITS
Liabum glabrum Adan.	Mexico	Liabeae	*ndhF*
Munnozia gigantea Ruiz & Pav.	Peru	Liabeae	*ndhF*, ITS
Sinclairia pringlei Hook. & Arn.	Mexico	Liabeae	*ndhF*

*See Keeley and Jansen (61) and Jansen and Kim (23) for all voucher information.
†Tribal classification follows Bremer (12); sectional classification of *Vernonia* follows Jones (40, 41).
‡Sequences reported in the paper have been deposited in the Genbank data base (accession nos. AF092584–AF092607 for *ndhF* and AF092608–AF092637 for ITS) and the aligned data matrices have been deposited in TreeBASE (http//herbaria.harvard.edu/treebase/).

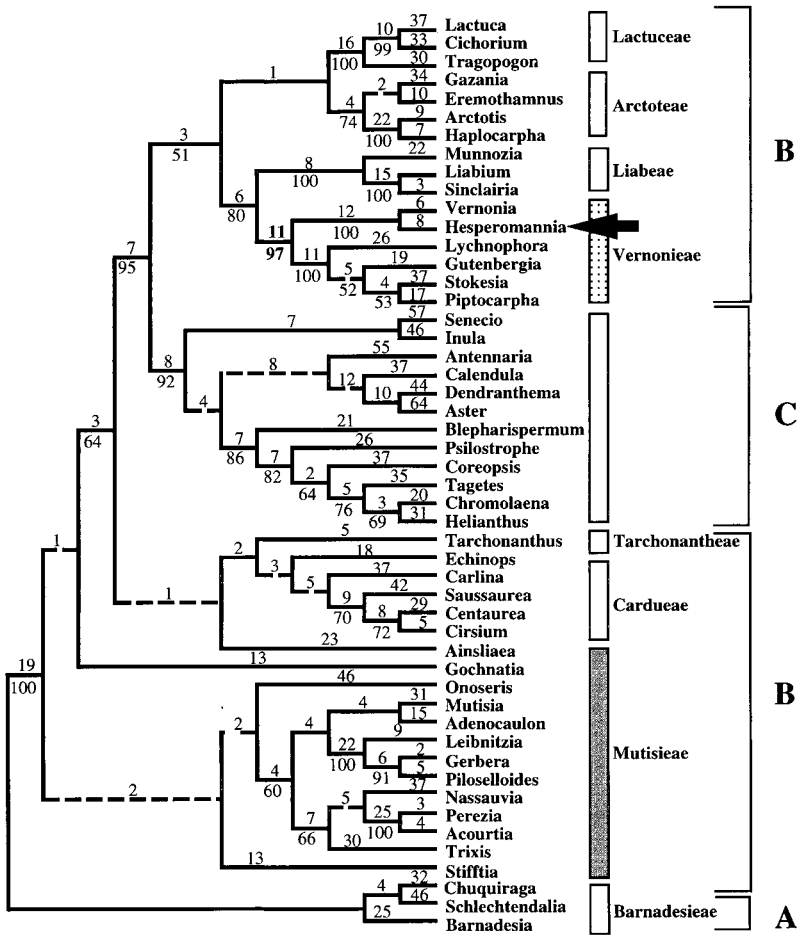

FIG. 1. *ndhF* tree of the Asteraceae showing the tribal placement of *Hesperomannia* (see arrow). This is one of 180 equally parsimonious trees with 1,587 steps, a consistency index of 0.596, and retention index of 0.564. Numbers above and below the nodes indicate numbers of supporting characters and bootstrap support, respectively. Dashed lines indicate nodes that collapse in the strict consensus tree. Brackets show tribal and subfamilial classification following Jansen and Kim (23). A, Barnadesioideae; B, Cichorioideae; C, Asteroideae.

Relationships of *Hesperomannia* Within the Vernonieae. The affinities of *Hesperomannia* with other members of the Vernonieae were examined by using two independent sequences, 804 bp of the 3′ end of *ndhF* and the ITS region of the nuclear ribosomal repeat. For *ndhF*, two species of *Hesperomannia* and five genera of Vernonieae (*Lychnophora*, *Piptocarpha*, *Stokesia*, *Gutenbergia*, and *Vernonia*) were examined, including 15 species of *Vernonia* representing a wide range of taxonomic and geographic diversity (Table 1). Three genera of the Liabeae were used as outgroups (19, 32, 33). The *ndhF* sequences included 154 variable sites, 68 of which were phylogenetically informative. Eight equally parsimonious trees of 224 steps were identified, with a consistency index of 0.81 and a retention index of 0.81. The trees indicated a strong relationship (bootstrap value of 90%) between the Hawaiian endemic species of *Hesperomannia* and four species of African *Vernonia* (Fig. 2). *Hesperomannia* was sister to two African species (*V. amygdalina* and *V. mesipilifolia*) from subsection *Strobocalyx*.

We examined the ITS region of the nuclear ribosomal repeat to test the phylogenetic hypotheses suggested by the *ndhF* tree. The ITS data set consisted of four genera (*Lychnophora*, *Stokesia*, *Gutenbergia*, and *Vernonia*), including 12 representative species of *Vernonia* from all major geographic regions, two species of *Hesperomannia*, and one outgroup (*Munnozia*) from the Liabeae (Table 1). The ITS sequences contained 456 variable sites, 351 of which were phylogenetically informative changes. A single most parsimonious tree was identified with 1,262 steps, a consistency index of 0.62, and a retention index of 0.64. The ITS tree (Fig. 3) was congruent with the *ndhF* results in strongly grouping *Hesperomannia* with the same two species of African *Vernonia* from subsection *Strobocalyx*.

Divergence Time. Sequence divergences among 10 taxa ranged from 3.8% to 68.2% and 0% to −14.3% for the ITS region and *ndhF* gene, respectively (Table 2). The high level of ITS sequence divergence was largely the result of the presence of indels and the fact that the highly conserved 5.8S coding region was not included. Relative rate tests indicated that a molecular clock was not rejected in most pairwise comparisons (Tables 3 and 4), including all those involving *Hesperomannia* and African *Vernonia*. The average sequence divergence between Hawaiian *Hesperomannia* and African *Vernonia* subsection *Strobocalyx* was 1.2 ± 0% and 20.20 ± 0.8% for *ndhF* and ITS, respectively. The average pairwise ITS sequence divergence between these two clades was about 17 times higher than the *ndhF* sequence divergence. Intrageneric divergence of *Hesperomannia* (0.38%) was about 29 times less than in *Vernonia* (10.97%). Although there has been considerable debate on rates of ITS sequence divergence (34–37), we selected the average rate of 0.78% nucleotide substitutions per myr proposed by Sang *et al.* (36) in *Dendroseris* for three reasons: (*i*) *Dendroseris* and *Hesperomannia* are in the same family (Asteraceae), (*ii*) both are endemic to oceanic islands, and (*iii*) species in both genera are woody, thus minimizing the effect of generation time on substitution rates. The divergence time between African *Vernonia* and *Hesperomannia* based on ITS sequence data was estimated to be 25.89 ± 1.03 myr ago. For *ndhF* we used the average rate of 0.05–0.07% per myr suggested by Wendel and Albert (38) and Seelanan *et al.* (39), which resulted in a divergence time of 17.14–24.0 ± 3.43 myr ago. The divergence time based on the nuclear sequence was only 1.08–1.5 times older than that estimated for *ndhF* and the two estimates are within each others' SE. We also estimated divergence times within *Hesperomannia*. The divergence times for the ITS (4.91 myr) were again older than the estimate for *ndhF* (1.81–2.54 myr).

DISCUSSION

Phylogenetic Position of *Hesperomannia*. Previous workers considered *Hesperomannia* a close relative of several South

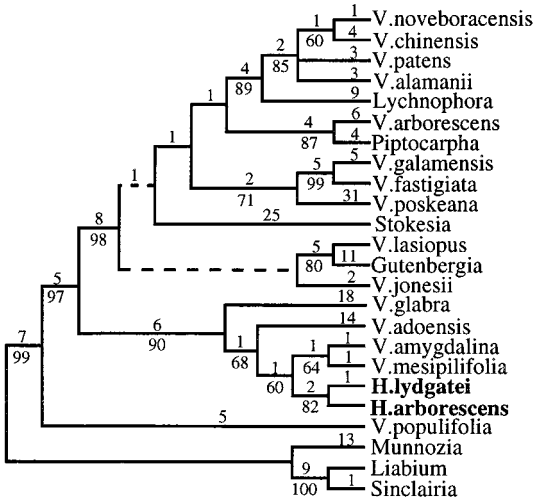

FIG. 2. *ndhF* tree of the Vernonieae showing the placement of *Hesperomannia* (bold). This is one of eight equally parsimonious trees with 224 steps, a consistency index of 0.812, and a retention index of 0.812. Numbers above the nodes indicate number of supporting characters. Numbers below the nodes indicate bootstrap values. Dashed lines indicate nodes that collapse in the strict consensus tree.

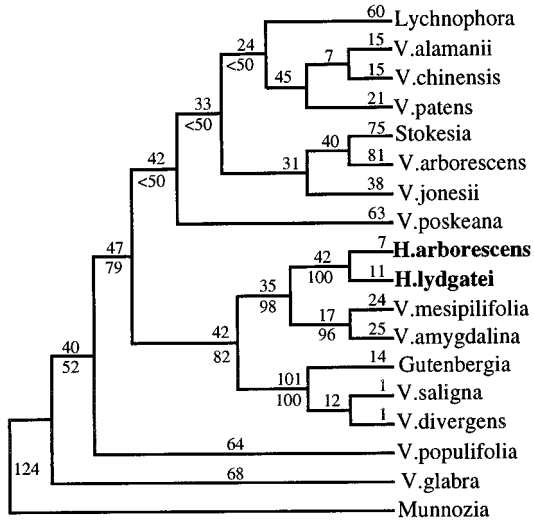

FIG. 3. ITS tree of the Vernonieae showing the placement of *Hesperomannia* (bold). This is the single most parsimonious tree with 1,262 steps, a consistency index of 0.623, and a retention index of 0.636. Numbers above the nodes indicate number of supporting characters. Numbers below the nodes indicate bootstrap values.

American genera of Mutisieae, especially *Stifftia*, *Stenopadus*, and *Wunderlichia* (10–13). Our DNA phylogenies (Figs. 1–3) disagree with all previous studies and clearly indicate that *Hesperomannia* is closely allied to Old World species of *Vernonia*. The Vernonieae includes approximately 80 genera and 1,450 species, 900–1,000 of which are in the genus *Vernonia* (40). *Vernonia* includes two subgenera, *Vernonia* from the New World and *Orbisvestus* from the Old World (41). Several features distinguish these two subgenera, including basic chromosome number, sesquiterpene lactone chemistry, and morphology of the styles and pollen. Comparison of *Hesperomannia* with members of the Vernonieae provides convincing independent support for a close phylogenetic relationship with Old World species of *Vernonia*. Both have a chromosome number of $n = 10$, pollen with a continuous micropunctate tectum and echinate exine, and long slender styles with acute or obtuse tips.

The DNA phylogenies (Figs. 2 and 3) suggest a sister group relationship between *Hesperomannia* and African species of *Vernonia* subsection *Strobocalyx*. This subsection includes 28 species, 25 of which are distributed in tropical Africa with the remaining three species in Burma, China, and India (41). Despite the limited number of species examined, we believe that the phylogenies from two independent DNA markers provide strong support for a sister group relationship between *Hesperomannia* and African species of *Vernonia* for four reasons: (*i*) our sampling includes two of the three species of *Hesperomannia* and taxa from all of the major geographic areas and 12 taxonomic sections of *Vernonia* (Table 1); (*ii*) support is very strong for both markers (Figs. 2 and 3); (*iii*) levels of sequence divergence are much lower between *Hesperomannia* and African species of *Vernonia* section *Strobocalyx* than between any species of *Vernonia* examined (Table 2); and (*iv*) *Hesperomannia* and all African species of subsection *Strobocalyx* have type A pollen, whereas two of the three remaining Old World species (from Burma and India) have type B pollen (41).

Biogeography and Evolution of the *Hesperomannia*. Despite the remarkable geographic distance between the Hawaiian Islands and the African continent, our molecular phylogenies strongly suggest a biogeographic connection between these two areas. The Hawaiian flora, which includes approximately 1,000 native species of flowering plants within 88 genera and 211 families (2), is derived exclusively from long-distance dispersal. Floristic affinities of the Hawaiian flora have been estimated to be 40.1% Indo-Pacific, 16.5% Austral, 18.3% American, 12.5% Pantropical, 2.6% Boreal, and 10.3% unknown (3, 4). The latter category includes plants that have been isolated long enough to have no apparent living relatives and probably includes some of the earliest immigrants to the Hawaiian Islands.

Support for the possibility of a biogeographic connection between Hawaii and Africa is provided by recent molecular phylogenetic investigations of the Malvaceae (39), which demonstrate a robust relationship between the East African-Madagascan genus *Gossypioides* and the Hawaiian endemic genus *Kokia*. Seelanan *et al.* (39) proposed that *Gossypium* diverged

Table 2. Nucleotide sequence divergence comparisons of the 10 representative taxa from those included in phylogenetic analyses

	Lych	Vjon	Stok	Vpos	Vchi	Hlyd	Harb	Vmes	Vamy	Vpop
Lych		0.024	0.108	0.070	0.033	0.065	0.065	0.062	0.062	0.046
Vjon	0.305		0.088	0.041	0.012	0.068	0.068	0.062	0.062	0.034
Stok	0.437	0.369		0.134	0.015	0.143	0.143	0.136	0.137	0.119
Vpos	0.423	0.352	0.527		0.034	0.103	0.103	0.096	0.096	0.080
Vchi	0.311	0.276	0.444	0.351		0.075	0.075	0.072	0.072	0.043
Hlyd	0.548	0.505	0.643	0.524	0.559		0.000*	0.012	0.012	0.046
Harb	0.520	0.496	0.637	0.539	0.558	0.038		0.012	0.012	0.046
Vmes	0.545	0.446	0.646	0.523	0.569	0.217	0.212		0.000	0.040
Vamy	0.516	0.454	0.650	0.520	0.529	0.196	0.207	0.110		0.040
Vpop	0.524	0.516	0.682	0.494	0.520	0.448	0.465	0.431	0.431	

Divergence values of the *ndhF* based on Jukes-Cantor corrected proportion using synonymous differences are above the diagonal. Divergence values of the ITS based on Kimura's two-parameter model are below the diagonal. Full names of taxa are in Table 1.

*This value is 0 because it is based on synonymous substitutions and the two species of *Hesperomannia* differ by only a single nonsynonymous substitution. If sequence divergence is calculated using all substitutions the value is 0.0013.

Table 3. Evaluation of molecular clock hypothesis based on ITS sequence data between 10 representatives of the Vernonieae and two species of *Hesperomannia*

	Lych	Stok	Vjon	Vpos	Vchi	Harb	Hlyd	Vmes	Vam
Lych		ND	ND	ND	ND	ND	ND	ND	ND
Stok	0.007		ND	ND	ND	ND	ND	ND	ND
	(0.214)								
Vjon	0.001	0.008		***	ND	ND	ND	ND	ND
	(0.068)	(0.201)							
Vpos	0.004	0.008	0.036		ND	ND	ND	ND	ND
	(0.077)	(0.227)	(0.061)						
Vchi	0.001	0.008	0.001	0.004		ND	ND	ND	ND
	(0.057)	(0.215)	(0.041)	(0.066)					
Harb	0.007	0.010	0.00	0.004	0.007		ND	ND	ND
	(0.081)	(0.224)	(0.078)	(0.076)	(0.081)				
Hlyd	0.009	0.011	0.009	0.006	0.009	0.001		ND	ND
	(0.080)	(0.224)	(0.077)	(0.073)	(0.079)	(0.121)			
Vmes	0.012	0.011	0.012	0.012	0.012	0.014	0.006		ND
	(0.079)	(0.223)	(0.072)	(0.054)	(0.077)	(0.025)	(0.029)		
Vam	0.012	0.011	0.012	0.009	0.011	0.016	0.011	0.0001	
	(0.078)	(0.224)	(0.073)	(0.072)	(0.079)	(0.022)	(0.016)	(0.051)	

Relative rate test values and variances (brackets) are shown below the diagonal. Significance levels of evolutionary rate differences are shown above the diagonal; no significant difference (ND), significant at 2% level (***). Full names of taxa are in Table 1.

from the *Kokia*/*Gossypioides* clade approximately 12.5 myr ago, but that *Kokia* and *Gossypioides* diverged much more recently, in the Pliocene. Such a relationship implies long-distance dispersal, which might be either directly to Hawaii or via islands in the archipelago that now are submerged. Floristic affinities between the South Pacific island of New Caledonia and Africa (42) also indicates a biogeographic connection between these areas.

Dispersal of the ancestor of *Hesperomannia* from Africa to the Hawaiian Islands seems unlikely given that these areas are currently separated by approximately 15,000 km. Carlquist (43) suggested that birds were the most likely dispersal vector of *Hesperomannia*, an hypothesis that achene morphology appears to accommodate. The fruit is small enough to be eaten by birds, and the dry achene surface is resistant enough to be viable after passing through an animal's digestive tract. The achenes are too large and lack buoyancy for transportation over great distances by normal wind or air currents (44, 45). However, some members of *Vernonia* subsection *Strobocalyx* have a pappus of flattened bristles, which certainly would be conducive to wind dispersal. Bird migration pathways between the Pacific Oceanic Islands and Africa have not been proposed. Early workers (46–49) suggested several possible colonization routes to tropical Pacific islands. One of the main routes was from the New World. The other hypothesized route was that tropical Pacific land birds originated from the west, mainly from New Guinea southeastward to the Bismarcks, Solomons, New Hebrides, and Hawaiian Islands.

There are two likely hypotheses for long-distance dispersal of Hawaiian *Hesperomannia*. In the first, the New World served as a steppingstone from Africa during mid to late Tertiary when the Atlantic Ocean was considerably narrower than at present. This hypothesis is more or less congruent with divergence times estimated by using the two molecular markers in this study. The distance at that time may have enabled easier travel for the vector across the Atlantic Ocean. However, dispersal to Hawaii still would be difficult because there is no land along the Pacific coast of the New World except for the Galapagos, Juan Fernandez, and the few inshore islands (50). Another difficulty with this scenario is that there are few similarities between the biotas of Hawaii and South America. In the second hypothesis, the Hawaiian/African connection might be explained by long-distance dispersal via southeast Asia and the many Pacific island chains. It is likely that the biota of the tropical Pacific Oceanic Islands is derived primarily from the west (3) because numerous island chains eastward from New Guinea served as steppingstones for the transportation of propagules by birds. A possible dispersal route could have been northeastward in Africa to southeast Asia and finally to the Hawaiian Islands by using the many southeast Pacific Island chains as steppingstones. Under this scenario the possibility that the actual ancestor of the Hawaiian *Hesperomannia* already might be extinct is highly likely. It may, as pointed out by Fosberg (3), represent one of the oldest lineages in the Hawaiian Islands.

Table 4. Evaluation of molecular clock hypothesis based on *ndhF* sequence data between 10 representatives of the Vernonieae and two species of *Hesperomannia*

	Lych	Stok	Vjon	Vpos	Vchi	Harb	Hlyd	Vmes	Vam
Lych		***	ND	***	ND	ND	ND	ND	ND
Stok	0.055		***	ND	***	ND	***	***	***
	(0.013)								
Vjon	0.007	0.071		***	***	ND	ND	ND	ND
	(0.018)	(0.012)							
Vpos	0.033	0.003	0.059		***	***	***	***	***
	(0.010)	(0.014)	(0.008)						
Vchi	0.005	0.059	0.024	0.043		ND	ND	ND	ND
	(0.007)	(0.013)	(0.004)	(0.009)					
Harb	0.000	0.049	0.013	0.028	0.003		ND	ND	ND
	(0.009)	(0.015)	(0.010)	(0.028)	(0.010)				
Hlyd	0.000	0.049	0.013	0.028	0.003	0.005		ND	ND
	(0.009)	(0.015)	(0.010)	(0.012)	(0.010)	(0.002)			
Vmes	0.007	0.054	0.007	0.034	0.003	0.016	0.016		ND
	(0.009)	(0.015)	(0.010)	(0.012)	(0.010)	(0.004)	(0.004)		
Vam	0.007	0.054	0.007	0.034	0.003	0.006	0.016	0.001	
	(0.009)	(0.015)	(0.010)	(0.012)	(0.010)	(0.004)	(0.004)	(0.002)	

Relative rate test values and variances (brackets) are shown below the diagonal. Significance levels of evolutionary rate differences are shown above the diagonal; no significant difference (ND), significant at 2% level (***). Full names of taxa are in Table 1.

Time and Island(s) of Origin. The Hawaiian Islands have existed as a group for at least 70 myr with spacing and location similar to that found today (51, 52). Colonization between older and younger islands is not considered to be continuous during this time period (53). Only the past 29 myr are thought to be relevant for understanding the origin of the endemic biota of Hawaii. The age of the eight current high islands ranges between 0.4 and 5.1 myr old (53–55). The divergence time (17–26 myr) between *Hesperomannia* and African *Vernonia* estimated by our *ndh*F and ITS sequence data predates the oldest high island, Kauai, on which *H. lydgatei* is found. Thus the ancestor of *Hesperomannia* is unlikely to have originated on one of the current islands. The estimated divergence times between *Hesperomannia* species (1.81–4.91 myr) is more recent than the ages of the current islands, which also supports our hypothesis that the ancestor existed on older sunken islands and arrived to the high islands recently.

The geology of the Hawaiian archipelago provides a framework to develop a scenario regarding the time and place of origin of *Hesperomannia*. Magma perforates the Pacific Ocean Basin plate, forming a series of discrete volcanoes as the plate moves slowly over the hot spot. This process has produced islands of various ages from Hawaii (0.5 myr old) to Kure atoll (29 myr) in the Hawaiian Ridge to the older Emperor Chain seamounts. As a result, a series of consecutively produced islands have been available for interisland exchange for many millions of years, much longer than previously was assumed to be the case with the extant high islands (4). Our estimated time of divergence of 17–26 myr for the Hawaiian and African clades would make any island between French Frigate Shoals and Midway atoll likely sites for initial arrival and establishment.

If the proposed scenario is correct, the dispersal of *Hesperomannia* ancestors occurred between the late Oligocene and mid Miocene when now-sunken islands in the Hawaiian archipelago were high above sea level. The estimated divergence time is consistent with the general view (56–60) that the family Asteraceae underwent explosive radiation and dispersal during or near the Oligocene/Miocene border. The extreme morphological changes subsequent to this dispersal have made it difficult to identify close relatives of *Hesperomannia*. Nevertheless, the close genetic relationship between *Hesperomannia* and *Vernonia* subsection *Strobocalyx* clearly shows ancestral connections between the Hawaiian Islands and Africa.

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