Int. J. Plant Sci. 169(7):954–962. 2008.

1058-5893/2008/16907-0013$15.00 DOI: 10.1086/589699

THE BIOGEOGRAPHY OF *PLANTAGO OVATA* FORSSK. (PLANTAGINACEAE)

Stephen C. Meyers[1] and Aaron Liston

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, U.S.A.

Plantago ovata Forssk. (Plantaginaceae) is a species that, in North America, inhabits desert and Mediterranean habitats of the southwest United States, northwest Mexico, and the Channel Islands of California and Mexico. In the Eastern Hemisphere, *P. ovata* inhabits desert regions ranging from the Canary Islands, across northern Africa to India. Previous authors have hypothesized that *P. ovata* was introduced to North America from Asia during the Miocene or introduced anthropogenically from Europe during the eighteenth century by Spanish settlers. We examined sequence data from the chloroplast *trnL-trnF*, *trnS-trnG*, and *psbA-trnH* regions, the nuclear ribosomal internal transcribed spacer (ITS), and a putative *CYCLOIDEA*-like gene. Using a molecular clock based on an ITS calibration and a clock for plant chloroplast, we date a nonanthropogenic introduction event, from the Old World to North America, of ~200,000–650,000 yr ago. On the basis of a morphological survey of 585 specimens from throughout the world range of *P. ovata*, we suggest the recognition of four subspecific taxa. Phylogenetic analysis of chloroplast and ITS sequences supports this taxonomic treatment. Furthermore, morphological data and the *CYCLOIDEA*-like gene sequences suggest the origin of North American *P. ovata* as a result of hybridization between Old World *P. ovata* varieties.

Keywords: Plantago ovata, biogeography, hybridization, desert disjunct.

Introduction

Wide disjuncts between closely related plant taxa have been and continue to be of great interest to botanists (Thorne 1972; Wen 2001; Nie et al. 2006). For example, the North American southwest and the Mediterranean–southwest Asian regions have little overall similarity in their floras (Stebbins and Major 1965; Shmida 1985). However, *Plantago* is one of ~35 genera that have representative species in both regions (Stebbins and Day 1967; Thorne 1972). The disjunction between the two regions is ~13,000 km. Determining the timing and method by which many of these disjunctions occurred has been a subject of numerous studies (Stebbins and Major 1965; Stebbins and Day 1967; Basset and Baum 1969; Liston and Kadereit 1995; Coleman et al. 2003). Additionally, hybridization may play a role in the formation of closely related, disjunct plant taxa (Stebbins 1969). Potential examples include Hawaiian Madiinae (Barrier et al. 1999), *Microseris* (Vijverberg et al. 1999), and *Senecio mohavensis* (Coleman et al. 2003).

Plantago ovata Forssk. (Plantaginaceae) is a winter annual that primarily inhabits desert regions of the Northern Hemisphere between the twenty-sixth and thirty-sixth latitudes (fig. 1*a*). The species has two main geographic areas of distribution. In the Old World, *P. ovata* is found along the drier parts of the Mediterranean Sea and southwestern Asia, extending from the Canary Islands to western India. In the New World, the species is found in two distinct ecological areas. The first is the xeric environment of the North American Sonoran and Mojave deserts of the southwestern United States, inland Baja California, Mexico, and northwestern Mexico. Palm Springs, California, as an example, averages less than 150 mm of precipitation annually, and average midsummer temperatures are more than 40°C. The second environment is the coastal sage scrub of California and the Channel Islands of California and Mexico, which have a wetter, often foggy environment. With an average annual precipitation of 280 mm and average midsummer temperatures less than 17°C, Avalon, California, typifies this area (Western Regional Climate Center 2007).

On the basis of morphological (Rahn 1979) and molecular (Rønsted et al. 2002) phylogenies, *P. ovata* belongs to *Plantago* subgenus *Albicans* section *Albicans*. With the exception of *P. ovata*, all taxa in this section are restricted to the Mediterranean–southwest Asian region. The wide disjunction between *P. ovata* in the Western and Eastern hemispheres poses a question as to its origin and biogeography.

Stebbins and Day (1967) first addressed the biogeography of *P. ovata*. They recognized the lack of close relatives of *P. ovata* in North America and hypothesized that the species originated in the Old World, followed by an introduction event to the New World. They grew 12 accessions of *P. ovata* from seed sources from both the Old and New World. They found differences between Old and New World plants in cytology, pubescence of the bracts and rachis, the shape of the bracts, and flower color. On the basis of these differences, they divided *P. ovata* into two distinct species, *P. ovata* in the Old World and *Plantago fastigiata* in the New World. At the conclusion of their study, Stebbins and Day hypothesized an introduction event of *P. ovata* from the Old World to the New World over the Bering land bridge during the Miocene, ~20–35 million years ago.

An alternative introduction hypothesis was proposed by Bassett and Baum (1969). Using herbarium specimens, they

[1] Author for correspondence; e-mail: meyersst@science.oregonstate.edu.

Manuscript received July 2007; revised manuscript received December 2007.

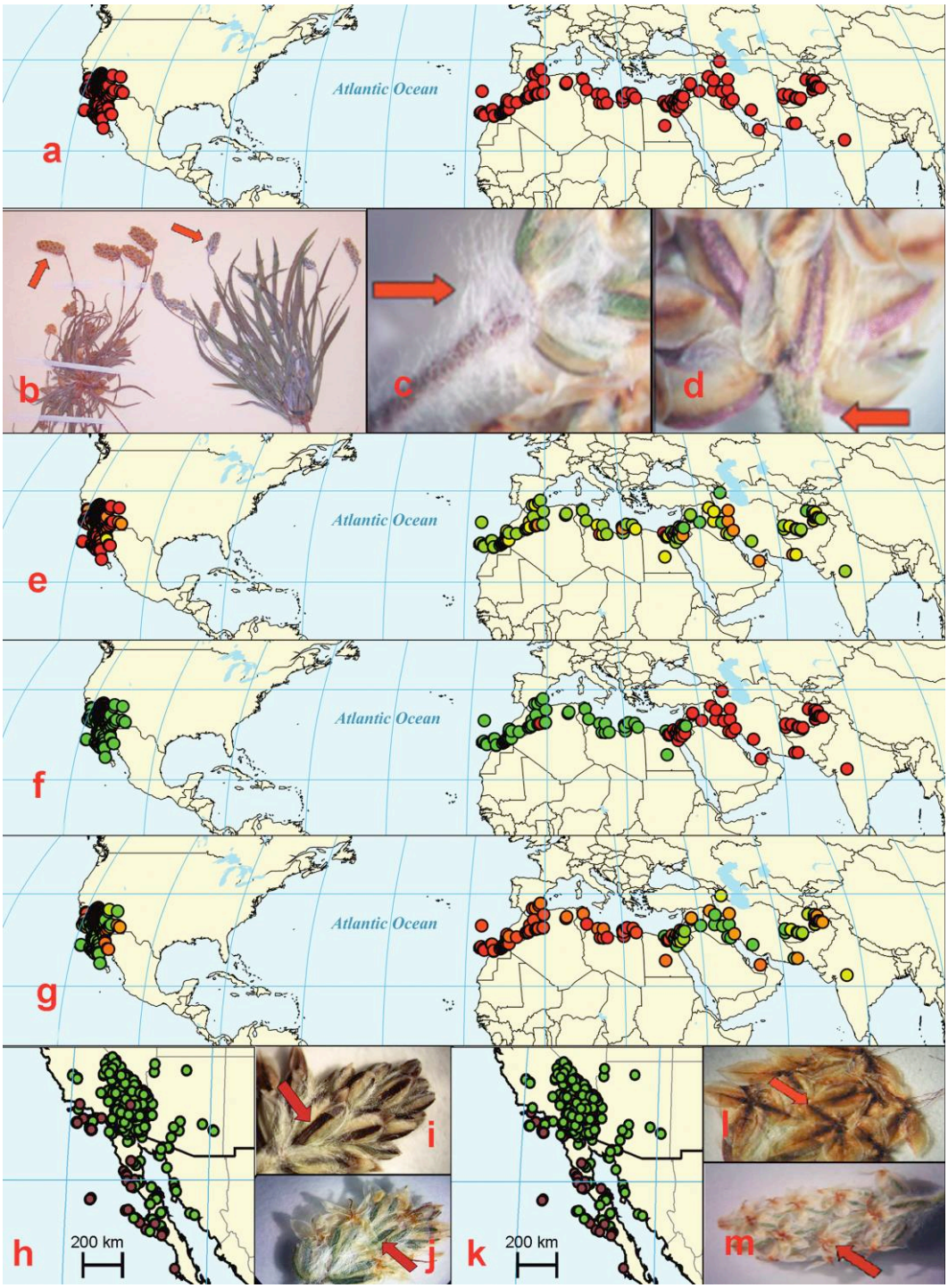

Fig. 1 *a*, Locations of 585 specimens surveyed from throughout entire world range of *Plantago ovata*. *b*, Wide spike typical of Asian and African plants (left); narrow spike typical of North American plants (right). *c*, Long trichomes typical of North American and African plants. *d*, Short trichomes typical of Asian plants. *e*, Spike widths mapped as a gradient from 6.75 mm (red) to 9.95 mm (green). *f*, Trichome lengths map. Green circles indicate specimens with long trichomes (>1/3 length of bracts); red circles indicate specimens with short trichomes (<1/3 length of bracts). *g*, Corolla lobe length/width ratio mapped as a gradient from a low ratio (from 0.9; green) to a high ratio (to 1.7; red). *h*, Bract midrib color map. Green circles indicate specimens with green bract midribs; brown circles indicate specimens with brown bract midribs. *i*, Brown bract midrib typical of North American island/coastal plants. *j*, Green bract midrib typical of North American inland/desert plants. *k*, Corolla midrib color map. Green circles indicate specimens without a prominent reddish-brown midrib. Brown circles indicate specimens with a prominent reddish-brown midrib. *l*, Prominent reddish-brown midrib typical of North American island/coastal plants. *m*, Lack of prominent reddish-brown midrib typical of North American inland/desert plants.

reexamined the same morphological characters surveyed by Stebbins and Day (1967). In addition, they also examined pollen grain morphology. Bassett and Baum failed to find, in their opinion, enough significant morphological differences between Old and New World plants to justify separating plants from the two regions into distinct taxa. On the basis of these observations, they judged *P. ovata* and *P. fastigiata* to be conspecific. Moreover, they proposed that *P. ovata* was introduced anthropogenically from the Old World to the North American Southwest by Spanish settlers during the eighteenth and nineteenth centuries. Dried seeds of *Plantago* species have and continue to be used as an aid to relieve constipation and treat severe diarrhea (Hoffman 1998; Foster and Duke 1999). The anthropogenic introduction hypothesis, proposed by Bassett and Baum, is implicitly accepted by modern floras (Wallace 1985; Beauchamp 1986; Dempster 1993; Junak et al. 1995).

The last major study of *P. ovata* before our research was conducted by Rahn (1979). Unlike the previous studies, which were qualitative and did not include an explicit analysis of morphology, Rahn surveyed morphological characters of 391 herbarium specimens from throughout the range of *P. ovata*. Rahn found a statistical difference in corolla length and width as well as spike width between Old and New World plants. In addition, a statistical difference was found in corolla length/width ratios between African plants and those of Asia and the New World. Rahn, however, did not consider these differences sufficient to separate the regional populations of *P. ovata* into separate species or subspecific taxa. He did suggest that the morphological differences between Old and New World *P. ovata* were not pronounced enough to fit the hypothesis of Stebbins and Day (1967) unless evolution within *P. ovata* had been very slow. Likewise, Rahn concluded that the differences between Old and New World *P. ovata* were too distinct to support the theory of a recent anthropogenic introduction event, unless evolution within the species was extremely fast.

The goal of our study was to resolve the conflicting opinions of previous studies concerning the taxonomy, evolution, and biogeography within *P. ovata*. To do so, we analyzed both morphological and molecular sequence data obtained from specimens throughout the world range of *P. ovata*.

The DNA sequences included the nuclear ribosomal internal transcribed spacer region (nrITS) and three noncoding chloroplast regions. The rate and pattern of nrITS sequence mutation can be appropriate for resolving relationships within plant species (Hillis and Dixon 1991; Hamby and Zimmer 1992; Doi et al. 2002). To supplement the nrITS data set, we sequenced the noncoding chloroplast *psbA-trnH*, *trnS-trnG*, and *trnT-trnL* spacer regions. In addition to nuclear and chloroplast sequences, we obtained sequences of the *coxI* mitochondrial gene. We chose to sequence the *coxI* gene as a result of findings by Palmer et al. (2000) of highly accelerated substitution rates in the mitochondrial genome of several *Plantago* species.

To analyze the potential for hybridization between subspecific taxa, we amplified, cloned, and sequenced a putative *CYCLOIDEA* (*CYC*)-like gene. We focused on this particular gene because it is a low copy number nuclear gene (Vieira et al. 1999). Similar genes have proven useful in phylogenetic studies involving hybridization (Olsen and Schaal 1999; Sang and Zhang 1999; Small et al. 2004; Kim and Donoghue 2005).

Material and Methods

A total of 585 specimens obtained from herbaria or collected in the field from throughout the world range of *Plantago ovata* was analyzed (fig. 1*a*). We recorded all morphological characters used by previous authors, with the exception of pollen and chromosome morphology (spike width; corolla width and length; trichome length; bract and leaf pubescence presence; bract midrib, corolla, and leaf color). Additionally, spike length, plant size, seed coat color, and callous-teeth presence were surveyed on each specimen.

The principal components analysis (PCA) and statistical analysis of the morphological data were performed using Statgraphics Plus software (ver. 5.1; Manugistics, Rockville, MD). For the PCA, specimens were delineated by region (Africa, Asia, island/coastal North America, and inland/desert North America).

To estimate a phylogeny, we used 2153 base pairs (bp) of DNA sequenced from 14 specimens from throughout the world range of *P. ovata*. DNA was obtained from fresh plant material and dried herbarium specimens (table 1). We used ~50 mg of plant material to extract DNA using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA).

Primers for the mitochondrial *coxI* gene were designed from the alignment of previously published *coxI* sequences of *Plantago* specimens and yielded ~1600 bp of product. Sequences for the primers are 5′-ATCTYCAACATGCGTGG-3′ and 5′-CCAAKAAAGGTGATCC-3′. Amplifications of the *coxI* gene were performed following the protocol described by Palmer et al. (2000). All other primers were obtained from and amplified following the protocols of Taberlet et al. (1991), Liston et al. (1996), Vieira et al. (1999), and Shaw et al. (2005).

CYC-like sequences were obtained from four specimens representative of the four clades resolved in the molecular phylogeny. Twelve clones from each North American specimen and 10 clones from each Old World specimen were sequenced (44 total). A *CYC*-like sequence of *Digitalis pupurea* was obtained from GenBank (AF146865) as an outgroup.

Clones from the initially obtained *CYC* PCR reactions were constructed and amplified using a Qiagen PCR cloning plus kit (Qiagen) according to the manufacturer's instructions. Following PCR, all products were purified using QIAquick PCR purification kits (Qiagen).

Sequencing was performed by Macrogen (Kumchun-Ku, Seoul) and Northwoods DNA (Solway, MN). Sequences were aligned by eye and analyzed using BioEdit for Windows 95/98 (Hall 1999). To test whether individual chloroplast and nrITS trees should be combined and whether topologies among the trees were significantly different, Templeton and Kishino-Hasegawa tests (Templeton 1983; Kishino and Hasegawa 1989) using PAUP* were employed.

Phylogenetic analyses were conducted using PAUP* (ver. 4b10; Swofford 2002) and MrBayes (ver. 3.0b4; Huelsenbeck and Ronquist 2001). For individual and combined sequences, the most parsimonious trees were found using branch and bound maximum parsimony searches within PAUP*, employing the furthest addition sequence setting and MulTrees on. Gaps were scored as missing data. Branch support was assessed using 1000 bootstrap replicates. Modeltest 3.06 (Posada and Crandell 1998) was used to select the model rate that best fit each data set. A general-time-reversible (GTR) model incorporating a gamma

Table 1

Specimens Analyzed for DNA Sequence Data and Regions Sequenced

Location	Collector	Regions sequenced
Africa:		
Egypt; between Cairo and Alexandria	Podlech 49375 (MSB)	ch, nu
Morocco; 50 km NE of Tan-Tan	Podlech 45139 (RSA)	mi, ch, nu, *CYC*
Tunisia; Medenine	Ullman s.n. (MSB)	ch, nu
Asia:		
Afghanistan; Kunar Prov., Darrah-i-Khor	Podlech 20228 (MSB)	ch, nu
Iraq; 16 km SE of Badra	Rechinger 9218 (BSM)	ch, nu
Israel; Negev Highland, En Avdat	Danin s.n. (OSC)	mi, ch, nu, *CYC*
Inland/desert North America:		
USA; California; Riverside Co.; Thousand Palms	Meyers 179 (OSC)	mi, ch, nu, *CYC*
USA; California; San Bernardino Co.; Zzyzx	Meyers 182 (OSC)	ch, nu
USA; California; Imperial Co.; Desert Shores	Meyers 219 (OSC)	ch, nu
Mexico; Baja California Norte; Catavina	Meyers 205 (OSC)	ch, nu
Island/coastal North America:		
USA; California; Ventura Co.; San Nicolas Island	Thorne 52410 (RSA)	ch, nu
USA; California; Ventura Co.; West Anacapa Island	Blakley 5804 (SBG)	ch, nu
USA; California; Los Angeles Co.; Santa Catalina Island	Meyers 225 (OSC)	ch, nu, *CYC*
Mexico; Baja California Norte; San Quentin	Meyers 201 (OSC)	ch, nu

Note. Abbreviations: mi = mitochondrial *coxI* region; ch = chloroplast *trnT-trnL*, *trnS-trnG*, and *psbA-trnH* spacer regions; nu = nuclear ribosomal ITS region; *CYC* = *CYCLOIDEA*-like gene. For herbarium codes, see Holmgren et al. (1990). GenBank accession numbers EU036229–EU036294.

distribution was selected for each data set and used during all Bayesian searches. Bayesian searches were conducted with four simultaneous Markov chains over 2 million generations, sampling every 100 generations. All trees generated within the burn-in period (20,000) were discarded, and posterior probability confidence values were based only on trees found in the stationary phase.

To evaluate whether sequences diverged at clocklike rates, maximum likelihood trees were estimated using the GTR + γ model as implemented in PAUP*. Again using PAUP*, pairwise distances between taxa and mean nucleotide differences, adjusted for missing data, were obtained. Data from combined chloroplast sequences (2076 bp) were used in a molecular clock calculation for plant chloroplast at a rate of 2.5×10^{-9} substitutions per site per year (Wolf et al. 1987; Muse 2000; Herbert et al. 2002; GuhaMajumdar and Sears 2005). A molecular clock calculation for the nrITS sequences (605 bp) was completed using a rate of 4.27×10^{-9} substitutions per site per year. The later calculation was based on a geological calibration within the *Plantago* genus by Rønsted et al. (2002) of a dispersal event to a dateable oceanic island. Divergence dates were calculated using the following formula: pairwise distance/substitution rate $\times$ 2 = years since divergence.

Results

Individual parsimony trees constructed for each of the three chloroplast and nrITS regions sequenced shared a similar topology. The Templeton and Kishino-Hasegawa tests found no conflict between the separate chloroplast and nrITS data sets ($P = 0.96$ and 0.93, respectively). Likewise, the combined maximum parsimony consensus (not shown) and Bayesian majority rule consensus trees shared identical topology (fig. 2). This analysis resolves the Old World group as paraphyletic, whereas specimens from the New World are a clade. Additionally, the Old and New World samples are each subdivided. The Old World samples are separated as subgroups from Asia and Africa. Likewise, within the New World group, subgroups from the inland/desert and the island/coastal regions are apparent (fig. 2).

P values of the mean nucleotide differences between Old and New World chloroplast and nrITS sequences were calculated to be 0.0002 and 0.0014, respectively. Using the molecular clocks for both chloroplast and nrITS DNA, we date an introduction event of *Plantago ovata* from the Old World to the New World ~200,000 (chloroplast) to 650,000 yr ago (nrITS).

Of the 13 morphological characters surveyed, five delimiting characters were found (fig. 1; table 2). These characters reliably distinguish Old World plants from New World plants. The characters also distinguish regional plant populations (Africa, Asia, island/coastal North America, and inland/desert North America). The same regional plant populations formed clusters within the graphical results of the PCA (fig. 3).

North American plants share unique characters with both Asian and African plants that the Asian and African plants do not share with each other (fig. 1*b*–1*g*; table 2). Specifically, North American plants share long trichomes with African plants and a large corolla length/width ratio with Asian plants. This supports the fact that North American plant morphology may be the result of a hybridization event between Asian and African plants. Within the PCA graph, specimens from North America are found clustered between the groups of specimens from Asia and Africa (fig. 3). This result also reflects the potential for an African-Asian hybridization event.

A total of four unique cloned *CYC* sequences were found within the North American specimens. Bayesian and maximum parsimony (not shown) majority rule consensus trees constructed from those sequences display two resolved clades (fig. 4). Two of the North American sequences were identical

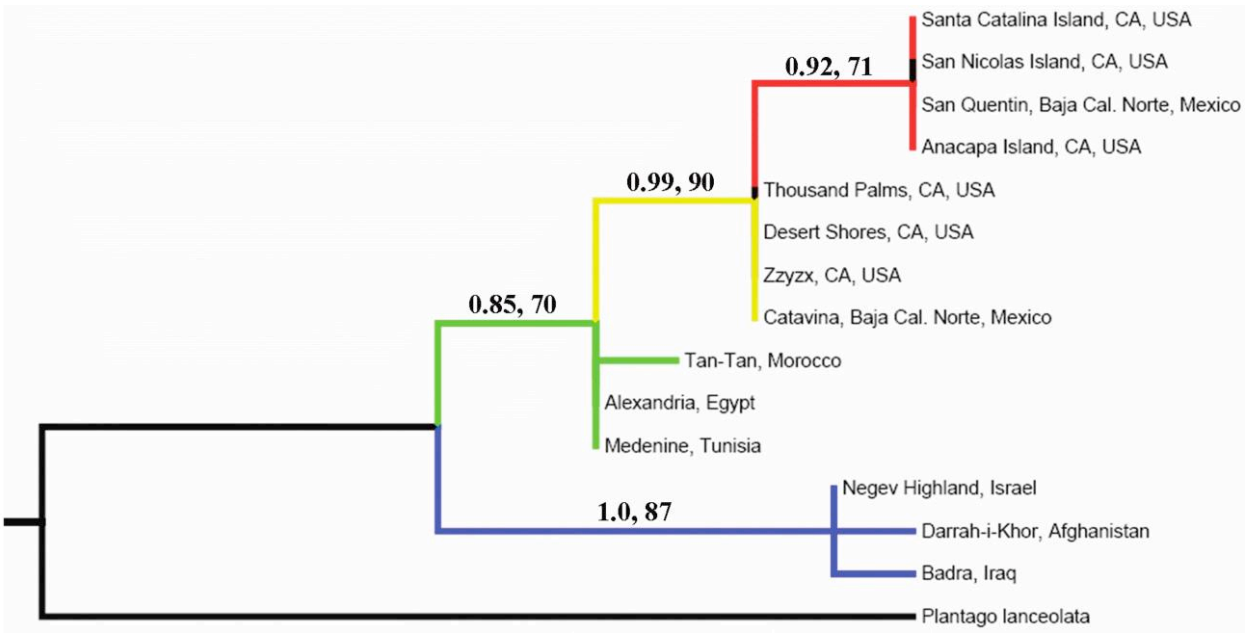

Fig. 2 Results of the Bayesian inference of phylogeny of combined nuclear and plastid sequences. Numbers above branches indicate posterior probabilities and bootstrap values, respectively. For maximum parsimony analysis, consistency index = 0.84, retention index = 0.92.

to the single Asian and African sequences found. Another two North American sequences are divergent but are contained within the two clades, respectively. This topology is consistent with the hypothesis that the North American taxa of *P. ovata* are of hybrid origin between the two Old World taxa.

Discussion

Although Palmer et al. (2000) found highly accelerated substitution rates in the mitochondrial genome of several *Plantago* species, in the specimens we compared, no sequence divergence was found. As a result, those sequences were not utilized.

Hybridization

Hybridization has been recognized as a widespread and important mode of evolution in plants, especially as a source of new plant taxa through diploid hybridization (Rieseberg 1995, 1997; Wolfe et al. 1998; Gross et al. 2003). A potential subspecific hybridization event within *Plantago ovata* has not been addressed in previous studies of the species (Stebbins and Day 1967; Bassett and Baum 1969; Rahn 1979). Although the morphological data collected by Rahn (1979) are consistent with a potential hybridization event between Asian and African *P. ovata* populations and subsequent dispersal to North America, he did not address that issue.

Typically, to analyze a hybridization event in a molecular study, nuclear DNA sequences are analyzed. While we did have nrITS sequence data, in contrast to many other plants (Sang et al. 1995; Whittall et al. 2000), nrDNA additivity was not observed in the *P. ovata* specimens sequenced.

A previous study has located three to four copies of *CYC*-like genes in the plant order Dipsacales (Hileman and Baum 2003). The Dipsacales and Plantaginaceae are both Asterids; therefore, numerous copies may exist in *P. ovata* as well. Although locus number in *Plantago* is unknown, our results are consistent with the presence of two *CYC*-like loci, as required by the presence of three putative alleles (sequences) in the

Table 2

Five Informative Characters That Differentiate Regional Populations of *Plantago ovata*

	Bract midrib color	Corolla midrib color/length	Trichome length	Spike width (mm)			Corolla length/width ratio		
				Average	SD	Range	Average	SD	Range
Asia	Green to brown	Variable	<1/3 length of bracts	9.95	1.40	8–14	1.42	.1	1.31–1.92
Africa	Green to brown	Variable	>1/3 length of bracts	9.72	1.10	6–13	1.12	.10	.90–1.30
Inland/desert North America	Green	None to light, <1/2 length of corolla	>1/3 length of bracts	7.00	1.10	4–10	1.43	.13	1.20–1.81
Coastal/island North America	Brown	Dark, >1/2 length of corolla	>1/3 length of bracts	6.14	1.26	2–9	1.56	.15	1.21–2.16

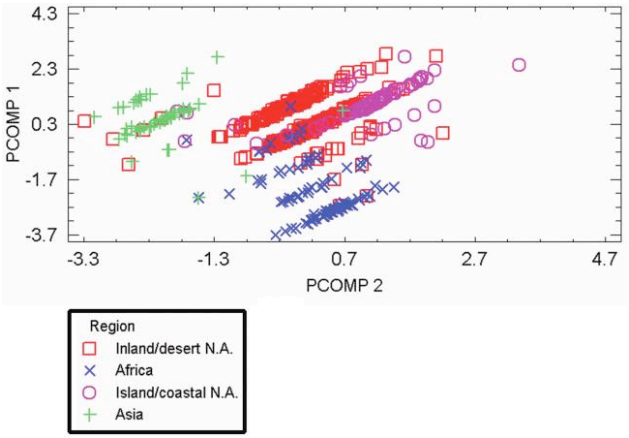

Fig. 3 Principal components analysis of morphological characters surveyed. North American specimens (squares and circles) are intermediate to African and Asian specimens. The first axis accounts for 48.6% of the variation. The second axis accounts for 39.2% of the variation.

North American island/coastal accession. Assuming that two loci are present in the Old World accessions, the single *CYC*-like sequence each possesses may be the result of interlocus concerted evolution. Alternatively, there could be a single locus in Old World *P. ovata* and a gene duplication in the derived North American clade. In either scenario, the combination of *CYC*-like sequences from the two resolved clades in both North American accessions is indicative of hybridization between Asian and African populations (fig. 4).

Molecular data from a specimen from the Sinai Peninsula, where hybrids of the African and Asian varieties may exist, would be very desirable. Unfortunately, several attempts to extract and amplify DNA from Sinai Peninsula herbarium specimens failed, likely because all specimens were more than 30 yr old. Likewise, because only eight specimens were obtained from the Sinai Peninsula, conclusions based solely on morphological data were not drawn. Although previous authors have made successful crosses between Old and New World plants, no crosses between African and Asian plants have been attempted (Stebbins and Day 1967). This may prove to be an interesting area for future study.

Long-distance nonanthropogenic dispersal following hybridization is well documented in plant taxa (Wallace and Jansen 1990; Barrier et al. 1999; Vijverberg et al. 1999). *Senecio mohavensis* ssp. *mohavensis* geographically represents a hybridization example similar to *P. ovata*. *Senecio mohavensis* ssp. *mohavensis* is a North American desert species that has been indicated to be a hybrid between the African *Senecio* taxa *Senecio flavus* and *Senecio glaucus* (Liston and Kadereit 1995; Comes and Abbott 2001). Following a tetraploid hybridization event, *S. mohavensis* ssp. *mohavensis* was apparently introduced to North America during the Pleistocene era (Liston et al. 1989; Coleman et al. 2001; Coleman et al. 2003). *Plantago ovata* provides another example of an apparent hybrid that has become successfully established after a nonanthropogenic introduction event.

Introduction into North America

We date an introduction event of *P. ovata* from the Old World to North America 200,000–650,000 yr ago, during the Pleistocene. This is ~19 million years after the date proposed by Stebbins and Day (1967). The estimated age of crown group diversification within the *Plantago* genus is 5.47 million years ago (Rønsted et al. 2002), a date nearly 15 million years after the introduction date Stebbins and Day propose.

Our introduction date is also very distant from the eighteenth- to nineteenth-century date hypothesized by Basset and Baum (1969). Our molecular dating precludes any anthropogenic introduction event. In addition, other evidence suggests that Spanish settlers would not likely have been the vehicle for the introduction of *P. ovata* into North America. While *P. ovata* has and continues to be used as a medicinal herbal remedy, its use during the eighteenth and nineteenth centuries was largely restricted to Ayurvedic practitioners in Asia, in particular, India (Jain and DeFilipps 1991).

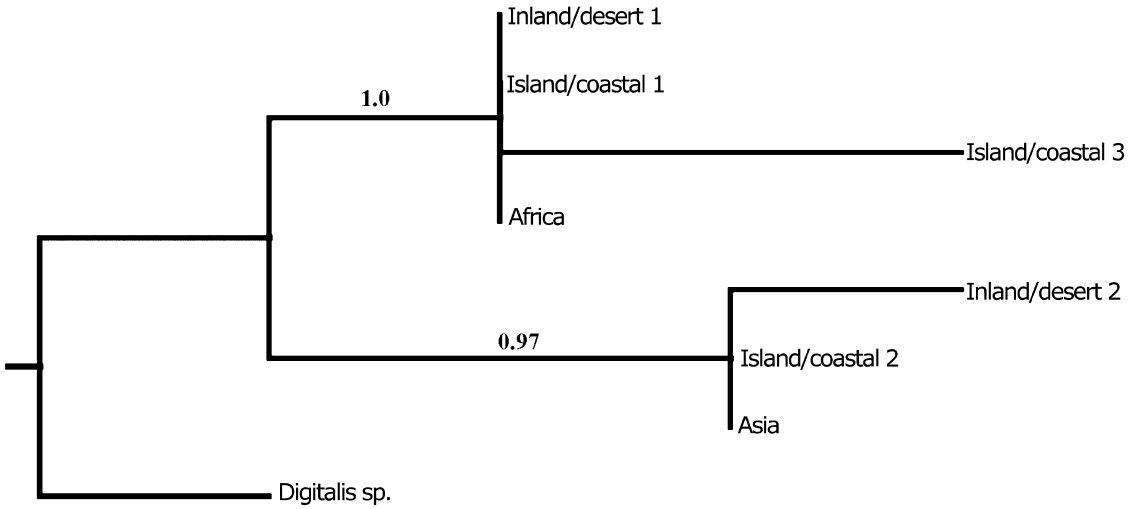

Fig. 4 Bayesian inference of phylogeny of *CYCLOIDEA*-like sequences. Posterior probabilities above branches.

Although *P. ovata* inhabits a small portion of the southern Iberian Peninsula, it is far more likely that Spanish settlers would have used a more common *Plantago* species as an herbal medicine. For example, *Plantago major* and *Plantago lanceolata*, common in Spain and established in North America after European introduction during the eighteenth and nineteenth centuries, was and is used for the same medicinal effects as *P. ovata* (Schauenberg and Paris 1977).

Our introduction date of *P. ovata* from the Old World into North America 200,000–650,000 yr ago is similar to the introduction date of *S. mohavensis* ssp. *mohavensis* from the Old World into North America ~150,000 yr ago. These dates, however, differ greatly from other estimates of Mediterranean–North American introductions. *Datisca*, for example, was calculated to have reached western North America from Eurasia 10–50.5 million years ago (Liston et al. 1992; Liston 1997). Other examples are the introduction of *Styrax* to North America, estimated at 5–13.8 million years ago (Fritsch 1996), and *Aphanisma*, which was calculated to have arrived in North America 14.6–20.3 million years ago (Hohmann et al. 2006). Assuming that the calculated introduction dates are correct or even roughly accurate, a bimodal (Miocene-Pleistocene) series of Mediterranean–western North American introductions may have occurred. Whether the route or mechanism for each of these introductions has a similar pattern is not currently known. While other disjunctions, such as the eastern Asia–eastern North American disjunction, have been extensively studied (Wen 2001), comparatively fewer studies have researched the Mediterranean–western North American disjunction (Hohmann et al. 2006). As the biogeography of more Mediterranean–western North American disjunct taxa is investigated, patterns are likely to emerge.

The mechanism of the introduction of *P. ovata* into North America can only be speculated at this point. The seeds of *P. ovata* are not suitable for wind dispersal, but similar to the seeds of *S. mohavensis* ssp. *mohavensis*, which become mucilaginous when wet, dispersal via epizoochory is a possibility. It should be noted, however, that mucilage is thought to be connected with germination rather than dispersal (Sorensen 1986). It is also worthwhile to note that during the Pleistocene, extensive wetland habits were common in both Asia/North Africa (Cox and Moore 1993) and southwest North America (Schaffer 1993). While bird migration between Asia/North Africa and southwest North America is unknown today, ecological conditions during the Pleistocene epoch may have been more favorable for such a migration to occur.

Taxonomic Implications

Our morphological findings are similar to those of Rahn (1979), who did not suggest the recognition of subspecific taxa within *P. ovata*. However, on the basis of our survey of 585 specimens versus Rahn's 391 specimens, we propose the recognition of four subspecific taxa within *P. ovata*.

Within North America, we propose recognition of two varieties of *P. ovata*. The inland/desert variety, *P. ovata* var. *fastigiata*, is found in xeric desert habitats. *Plantago ovata* var. *fasigiata* is morphologically different from the Old World varieties by nature of its narrow spike width (fig. 1*b*, 1*e*). This variety is distinguished from the other North American variety, *P. ovata* var. *insularis*, by the green bract midrib of its mature flowers and corolla lobes with little or no dark brown/red coloration (fig. 1*b*–1*m*).

The second North American variety, *P. ovata* var. *insularis*, is found in coastal sage scrub habitats and on hilltops and basins of the Channel Islands of California and Mexico (fig. 1). Like *P. ovata* var. *fastigiata*, *P. ovata* var. *insularis* differs from the Old World varieties of *P. ovata* by its narrow spike width (fig. 1*b*, 1*e*). *Plantago ovata* var. *insularis* is easily distinguished from *P. ovata* var. *fastigiata* by brown bract midribs found on mature flowers and corolla lobes with wide dark brown/reddish coloration found on the midrib (fig. 1*b*–1*m*).

The recognition of subspecific taxa based on combined morphological and environmental distinctions is quite common. For example, *Limnanthes floccosa* ssp. *grandiflora*, found on the wetter, inner fringes of vernal pools, has large corollas and sparse pubescence, while *L. floccosa* ssp. *floccosa*, found on the drier, outer fringes of the same vernal pools, has small corollas and denser pubescence (Arroyo 1973). Our conservative recognition of the two North American *P. ovata* taxa as varieties is well within the bounds of conventional naming tradition.

Within the Old World, we propose recognition of two varieties. *Plantago ovata* var. *decumbens* is found in Asia, from the Sinai Peninsula in the east to west India. *Plantago ovata* var. *decumbens* is distinguished from the other Old World variety, *P. ovata* var. *ovata*, by a typical corolla length to width ratio greater than 1.3 (fig. 1*g*) and trichomes beneath the spike less than 1/3 the length of the bracts (fig. 1*c*, 1*d*, 1*f*).

The second Old World variety, *P. ovata* var. *ovata*, is found in the geographic area from the Canary Islands east to the Sinai Peninsula. This variety is distinguished from *P. ovata* var. *decumbens* by its corolla length to width ratio typically less than 1.3 (fig. 1*g*) and trichomes longer than 1/3 the length of the bracts (fig. 1*c*, 1*d*, 1*f*).

We have chosen to recognize these four taxa as varieties and not subspecies because, while they are morphologically distinct, the taxa within the Old and New Worlds are not separated by a limiting geographic or environmental barrier and likely intergrade. The two varieties within the Old and New Worlds could potentially be consolidated into two subspecies consisting of two Old and New World subspecies. This consolidation, however, would result in the naming of taxa with quadrinomials. These names, while accurate, are cumbersome and not likely to be broadly accepted. We do not believe that recognition of these taxa at the species rank is warranted either. Within Plantaginaceae, the splitting of taxa is traditionally conservative (Rahn 1979). While the taxa we recognize are distinct, the differences are not extensive enough to warrant naming at the species rank.

Key to Plantago ovata

1. Corolla lobe length/width ratio 0.9–1.3; Africa . . . . . *P. ovata* var. *ovata*
1. Corolla lobe length/width ratio (1.2) 1.3–1.7
2. Trichome length <1/3 length of bracts; Asia . . . . . *P. ovata* var. *decumbens*
2. Trichome length >1/3 length of bracts
3. Usually a prominent reddish-brown midrib on corolla lobes; bract midrib of mature flowers brown; coast sage scrub

of North America and Channel Islands of California and Mexico . . . . . *P. ovata* var. *insularis*

3. Corolla lobes usually without a reddish-brown midrib; bract midrib of mature flowers green; inland deserts of North America . . . . . *P. ovata* var. *fastigiata*

Plantago ovata Forssk. var. *ovata*—Fl. Aegypt. Arab.:31, 1775—Type: Forsskal 253#,[2] designated by Rahn, 1979 (C lectotype#, 249 and 250 isolectotypes#). For synonymy, see Rahn (1979).

Plantago ovata Forssk. var. *decumbens* (Forssk.) Zohary—Palestine Journ. Bot. ser. 1:227, 1938–40. *Plantago decumbens* Forssk.,—Fl. Aegypt. Arab.:30, 1775—Types: Forssk. 254 and 259, we designate 259 as lectotype (C lectotype#, isolectotype#). For synonymy, see Zohary (1938).

Plantago ovata Forssk. var. *insularis* (Eastwood) S. Meyers & A. Liston, comb. nov.—*Plantago insularis* Eastwood, Proc. Calif Acad. Sci. 3,1:112, 1898—Type: Trask s.n. (CAS holotype!, K isotype). No synonyms exist.

Plantago ovata Forssk. var. *fastigiata* (Morris) S. Meyers & A. Liston, comb nov.—*Plantago fastigiata* Morris, Bull. Torrey Bot. Club 27:116, 1900—*Plantago insularis* Eastwood var. *fastigiata* (Morris) Jepson, Man. Fl. Pl. Calif.; 956, 1925—Type: Toumey 355a, designated by T. Ayers, 1998. We confirm this lectotypification (US lectotype#, isolectotype#).

Synonymy. *P. minima* A.M. Cunningham, Proc. Indiana Acad. Sci. 1896: 202.—*P. scariosa* Morris, Bull. Torrey Bot. Club 27: 117, 1900.—*P. insularis* Eastwood var. *scariosa* (Morris) Jepson, Man. Fl. Pl. Calif.: 956, 1925.—Types: Bailey s.n. (US paratype#), Coville & Funston 678 (US paratype#), Palmer s.n. (US lectotype#). We designate Palmer s.n. as lectotype.

P. brunnea Morris, Bull. Torrey Bot. Club 27: 115, 1900.—*P. fastigiata* Morris var. *brunnea* (Morris) Pilger, Das Planzenreich IV 269: 374, 1937.—Type: Palmer 654 (US holotype#, K,F, isotypes).

P. gooddingii Nelson & Kennedy, Muhlenbergia 3:142–143, 1908.—Type: Goodding 808a.

Acknowledgments

We would like to thank the following herbaria for the loan of specimens required for this study: ASU, CAS, FI, M, RSA, SBBG, W, and UC. We are also indebted to J. Andrew Alexander (OSC), Avinoam Danin (HUJ), and Richard Halse (OSC) for assistance with collecting plant material. We also thank two anonymous reviewers and the editor for helpful comments on the manuscript.

Literature Cited

Arroyo MTK 1973 A taximetric study of infraspecific variation in autogamous *Limnanthes floccosa* (Limnanthaceae). Brittonia 25: 177–191.

Barrier M, BG Baldwin, RH Robichaux, MD Purugganan 1999 Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. Mol Bio Evol 16:1105–1113.

Bassett IJ, BR Baum 1969 Conspecificity of *Plantago fastigiata* of North America with *P. ovata* of the Old World. Can J Bot 47:1865–1868.

Beauchamp RM 1986 A flora of San Diego County, California. Sweetwater River, National City, CA.

Coleman M, DG Forbes, RJ Abbott 2001 A new subspecies of *Senecio mohavensis* (Compositae) reveals Old-New World species disjunction. Edinb J Bot 58:389–403.

Coleman M, A Liston, JW Kadereit, RJ Abbott 2003 Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). Am J Bot 90:1446–1454.

Comes HP, RJ Abbott 2001 Molecular phylogeography, reticulation and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). Evolution 55:1943–1962.

Cox CB, PD Moore 1993 Biogeography: an ecological and evolutionary approach. Blackwell Scientific, Cambridge, MA.

Dempster LT 1993 Plantaginaceae. Pages 820–821 *in* JC Hickman, ed. The Jepson manual: higher plants of California. University of California Press, Berkeley.

Doi K, A Kaga, N Tomooka, DA Vaughan 2002 Molecular phylogeny of genus *Vigna* subgenus *Ceratotropis* based on rDNA ITS and *atp*B-*rbc*L intergenic spacer of cpDNA sequences. Genetica 114: 129–145.

Foster S, JA Duke 1999 A field guide to medicinal plants and herbs: of eastern and central North America. Houghton Mifflin, New York.

Fritsch P 1996 Isozyme analysis of intercontinental disjuncts within *Styrax* (Styracaceae): implications for the Madrean-Tethyan hypothesis. Am J Bot 83:342–355.

Gross BL, AE Schwarzbach, LH Rieseberg 2003 Origin(s) of the diploid hybrid species *Helianthus deserticola* (Asteraceae). Am J Bot 90:1708–1719.

GuhaMajumdar M, BB Sears 2005 Chloroplast DNA base substitutions: an experimental assessment. Mol Genet Genom 273:177–183.

Hall TA 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Hamby RK, EA Zimmer 1992 Ribosomal RNA as a phylogenetic tool in plant systematics. Pages 50–91 *in* PS Soltis, DE Soltis, JJ Doyle, eds. Molecular systematics of plants. Chapman & Hall, New York.

Herbert J, PM Hollingsworth, MF Gardner, RR Mill, PI Thomas 2002 Conservation genetics and phylogenetics of New Caledonian *Retrophyllum* (Podocarpaceae) species. N Z J Bot 40:175–188.

Hileman LC, DA Baum 2003 Why do paralogs persist? molecular evolution of *CYCLOIDEA* and related floral symmetry genes in Antirrhineae (Veronicaceae). Mol Bio Evol 20:591–600.

Hillis DM, MT Dixon 1991 Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–453.

Hoffman D 1998 The herbal handbook: a user's guide to medicinal herbalism. Healing Arts, Rochester, VT.

Hohmann S, JW Kadereit, G Kadereit 2006 Understanding Mediterranean-Californian disjunctions: molecular evidence from Chenopodiaceae-Betoideae. Taxon 55:67–78.

Holmgren PK, NH Holmgren, LC Barnett 1990 Index herbariorum. I. Herbaria of the world. 8th ed. New York Botanical Garden, New York.

Huelsenbeck JP, F Ronquist 2001 MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

[2] The pound sign indicates that a digital photo of the specimen was examined.

Jain SK, RA DeFilipps 1991 Medicinal plants of India. Reference Publications, Algonac, RI.

Junak S, T Ayers, R Scott, D Wilken, D Young 1995 A flora of Santa Cruz Island. Santa Barbara Botanic Garden, Santa Barbara, CA.

Kim S, M Donoghue 2005 Molecular phylogeny and the role of hybridization in the diversification of *Polygonum* section *Persicaria* (Polygonaceae). XVII International Botanical Congress, Vienna.

Kishino H, M Hasegawa 1989 Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order in Hominoidea. J Mol Evol 29:170–179.

Liston A 1997 Biogeographic relationships between the Mediterranean and North American floras: insights from molecular data. Lagascalia 19:323–330.

Liston A, JW Kadereit 1995 Chloroplast DNA evidence for introgression and long distance dispersal in the desert annual *Senecio flavus* (Asteraceae). Plant Syst Evol 197:33–41.

Liston A, LH Rieseberg, TS Elias 1989 Genetic similarity is high between intercontinental disjunct species of *Senecio* (Asteraceae). Am J Bot 76:383–388.

Liston A, LH Rieseberg, MA Hanson 1992 Geographic partitioning of chloroplast DNA variation in the intercontinental disjunct genus *Datisca* (Datiscaceae). Plant Syst Evol 181:121–132.

Liston A, WA Robinson, JM Oliphant, E Alverez-Buylla 1996 Length variation in the nuclear ribosomal DNA internal transcribed spacers of non-flowering seed plants. Syst Bot 21:1–12.

Muse SV 2000 Examining rates and patterns of nucleotide substitution in plants. Plant Mol Biol 42:25–43.

Nie Z, H Sun, PM Beardsley, RG Olmstead, J Wen 2006 Evolution of biogeographic disjunction between eastern Asia and eastern North America in *Phryma* (Phrymaceae). Am J Bot 94:1343–1356.

Olsen KM, BA Schaal 1999 Evidence on the origin of Cassava: phylogeography of *Manihot esculenta*. Proc Natl Acad Sci USA 96: 5586–5591.

Palmer JD, KL Adams, Y Cho, CL Parkinson, Y Qiu, K Song 2000 Dynamic evolution of plant mitochondrial genomes: mobile genes and introns and highly variable mutation rates. Proc Natl Acad Sci USA 97:6960–6966.

Posada D, KA Crandell 1998 Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rahn K 1979 *Plantago* ser. *Ovatae*: a taxonomic revision. Bot Tidsskr 74:13–20.

Rieseberg LH 1995 The role of hybridization in evolution: old wine in new skins. Am J Bot 82:944–953.

——— 1997 Hybrid origins of plant species. Annu Rev Ecol Syst 28: 359–389.

Rønsted N, MW Chase, DC Albach, MA Bello 2002 Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trn*L-F sequence data. Bot J Linn Soc 139:323–338.

Sang T, DJ Crawford, TF Stuessy 1995 Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. Proc Natl Acad Sci USA 92:6813–6817.

Sang T, D Zhang 1999 Reconstructing hybrid speciation using sequences of low copy nuclear genes: hybrid origins of five *Paeonia* species based on Adh gene phylogenies. Syst Bot 24:148–163.

Schaffer JP 1993 California's geological history and changing landscapes. Pages 49–54 *in* JC Hickman, ed. The Jepson manual: higher plants of California. University of California Press, Berkeley.

Schauenberg P, F Paris 1977 Guide to medicinal plants. Keats, New Canaan, CT.

Shaw J, EB Lickey, JT Beck, SB Farmer, W Liu, J Miller, KC Siripun, CT Winder, EE Schilling, RL Small 2005 The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Bot 92:142–166.

Shmida A 1985 Biogeography of desert flora. Pages 23–71 *in* M Evenari, I Noy-Meir, DW Goodall, eds. Ecosystems of the world: hot deserts and arid shrublands. Vol 12. Elsevier Science, Amsterdam.

Small RL, RC Cronn, JF Wendel 2004 Use of nuclear genes for phylogeny reconstruction in plants. Aust Syst Bot 17:145–170.

Sorensen AE 1986 Seed dispersal by adhesion. Annu Rev Ecol Syst 17:443–463.

Stebbins GL 1969 The significance of hybridization for plant taxonomy and evolution. Taxon 18:26–35.

Stebbins GL, A Day 1967 Cytogenetic evidence for long continued stability in the genus *Plantago*. Evolution 21:409–428.

Stebbins GL, J Major 1965 Endemism and speciation in California flora. Ecol Monogr 35:1–35.

Swofford D 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer, Sunderland, MA.

Taberlet P, L Gielly, G Pautou, DJ Bouvet 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109.

Templeton AR 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

Thorne RF 1972 Major disjunctions in the geographic ranges of seed plants. Q Rev Biol 47:365–411.

Vieira CP, J Vieira, D Charlesworth 1999 Evolution of the *cycloidea* gene family in *Antirrhinum* and *Misopates*. Mol Biol Evol 16:1474–1483.

Vijverberg K, THM Mes, K Bachmann 1999 Chloroplast DNA evidence for the evolution of *Microseris* (Asteraceae) in Australia and New Zealand after long-distance dispersal from western North America. Am J Bot 86:1448–1463.

Wallace GD 1985 Vascular plants of the Channel Islands of southern California and Guadalupe Island, Baja California, Mexico. Natural History Museum of Los Angeles County, Contributions in Science no. 365.

Wallace RS, RK Jansen 1990 Systematic implications of chloroplast DNA variation in the genus *Microseris* (Asteraceae: Lactuceae). Syst Bot 15:606–616.

Wen J 2001 Evolution of eastern Asian–eastern North American biogeographic disjunctions: a few additional issues. Int J Plant Sci 162(suppl):S117–S122.

Western Regional Climate Center 2007 http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?caplms+sca.

Whittall J, A Liston, S Gisler, RJ Meinke 2000 Detecting nucleotide additivity from direct sequences is a SNAP: an example from *Sidalcea* (Malvaceae). Plant Biol 2:211–217.

Wolfe AD, QY Xiang, SK Kephart 1998 Diploid hybrid speciation in *Penstemon* (Scrophulariaceae). Proc Natl Acad Sci USA 95:5112–5115.

Wolfe KH, W Li, P Sharp 1987 Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc Natl Acad Sci USA 84:9054–9058.

Zohary M 1938 On the *Plantago* species of the Near East. Palest J Bot 1:225–232.