Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae)

(speciation/selection/perennial growth/woodiness/founding populations)

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ABSTRACT   Numerous island-inhabiting species of predominantly herbaceous angiosperm genera are woody shrubs or trees. Such "insular woodiness" is strongly manifested in the genus *Echium*, in which the continental species of circummediterranean distribution are herbaceous, whereas endemic species of islands along the Atlantic coast of north Africa are woody perennial shrubs. The history of 37 *Echium* species was traced with 70 kb of noncoding DNA determined from both chloroplast and nuclear genomes. In all, 239 polymorphic positions with 137 informative sites, in addition to 27 informative indels, were found. Island-dwelling *Echium* species are shown to descend from herbaceous continental ancestors via a single island colonization event that occurred <20 million years ago. Founding colonization appears to have taken place on the Canary Islands, from which the Madeira and Cape Verde archipelagos were invaded. Colonization of island habitats correlates with a recent origin of perennial woodiness from herbaceous habit and was furthermore accompanied by intense speciation, which brought forth remarkable diversity of forms among contemporary island endemics. We argue that the origin of insular woodiness involved response to counterselection of inbreeding depression in founding island colonies.

That island-dwelling species of numerous, predominantly herbaceous genera of flowering plants tend to exhibit a perennial and woody growth form was evident to Darwin (1), Wallace (2), and many others since. Carlquist (3) coined the term "insular woodiness" to designate the phenomenon (which also is observed in highland biota; refs. 4 and 5) and found evidence that wood morphology and development in such plants differ from that of common woody tissues. The insular woody habit occurs in several angiosperm families, among them Boraginaceae, Asteraceae, Campanulaceae, Lobeliaceae, and Euphorbiaceae, and was suggested to have evolved several times independently from the herbaceous as an adaptation to climatic (primarily release from seasonality) and other factors (e.g., absence of large herbivores; ref. 3). According to the opposing relict hypothesis, which draws support from morphological, cytological, and paleogeographical data, the woody habit of island forms is considered ancestral to the herbaceous habit (6–8). Whereas polarity of evolution for perennial woodiness in island species has been heavily debated (2–8), problems concerning the underlying evolutionary mechanisms through which it arose have remained largely intractable.

An excellent case example of insular woodiness (Fig. 1) is found in *Echium* L. (Boraginaceae). Its size and distribution are particularly well-suited to study the phenomenon in several respects. First, 27 species of *Echium* are endemic to the Canary, Madeira, and Cape Verde archipelagos of the Macronesian islands, whereas the remaining (≈30) congeners are found in circummediterranean-west Asian floras. Their ende-

mism is strict; no island species occur naturally in continental floras and vice versa. Second, insular woodiness is very strongly pronounced in the genus; all but two of the island species (*Echium bonnetii* and *Echium pitardii*) are woody perennials, whereas the continental species are annual to perennial herbs (or very rarely subshrubs). Third, the ages of the islands on which the woody species occur are known (9–14), so that the evolutionary process can be correlated to geologic time. Island species of *Echium* reveal a degree of morphological diversity comparable to that found in the remaining (≈2000) species of the family. This is reflected in woody habits of the perennial island species: candelabra shrubs and monocarpic rosette trees, the large vegetative rosettes of which are formed over several vegetation periods and produce single inflorescences of several thousand flowers prior to fruiting and death. The herbaceous continental species reveal much less diversity in form and preferentially inbreed, whereas the island species are preferential outbreeders (15, 16). The Macaronesian species inhabit virtually all characteristic vegetation zones of the islands: coastal rocks, cliffs, semidesert habitats, the laurel forest region, and the volcanic subalpine zone.

Wishing to better understand the evolution of perennial woody habits in *Echium* and the phylogenetic process behind diversity of its island species, we have inferred the phylogeny of 37 *Echium* species—including most of the island inhabitants—and four outgroup representatives by amplifying, sequencing, and analyzing noncoding DNA regions from both their chloroplast and nuclear genomes. The data reveal that perennial, woody Macaronesian *Echium* species arose <20 million years ago (mya) from herbaceous continental antecedants and that all contemporary island dwellers can be traced to a single founding colonization event emanating from continental forms. Evidence that the origin of insular woodiness in *Echium* was crucial to colonization-related diversification of island forms supports and extends Wallace's suggestion (2) that reproduction rather than adaptation is ultimately causal to perennial woody habit in island species.

MATERIALS AND METHODS

Twenty-four of the 27 island *Echium* species, in addition to 13 continental species predominantly of west Mediterranean flora and four confamilial outgroup representatives, were investigated (Table 1). The outgroup representatives investigated were *L. fruticosus* Buek, *Cerinthe minor* L., *Cerinthe major* L., and *Lithodora diffusa* (Lag.) I. M. Johnston. Material was collected either in the field or from various botanical gardens and herbariums. All details of sources for all individual accessions are available from the authors upon request.

FIG. 1. *Echium creticum* (*Left*) and *Echium simplex* (*Right*), representatives of the herbaceous continental and woody island species, respectively, found in this genus of circummediterranean and Macaronesian (Canary, Madeira, and Cape Verde archipelagos) distribution. *E. creticum* is annual to biennial, whereas *E. simplex*, an endemic of the Anaga mountains (Tenerife), is a monocarpic rosette tree—i.e., forms a perennial vegetative rosette and flowers only once in life, producing several thousand propagules and dies.

DNA was isolated from leaf material (fresh, dried, or preserved in 70% ethanol) as described (21), extracted with phenol/chloroform, and purified by ultrafiltration in Microcon 100 devices (Amicon). Amplification was performed in 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 2 mM MgCl$_2$, 1 mM of each dNTP, and 1 μM of each primer containing 0.02 units/μl *Taq* polymerase (Perkin–Elmer) for 35 cycles of 1 min at 93°C, 1 min at 50°C, and 2 min at 72°C. An aliquot of the double-stranded product was used as template for asymmetric PCR in which one of the primers was diluted 100-fold. Single-stranded templates were purified by ultrafiltration (as above) and sequenced as described (22). Primers used for amplification and sequencing of chloroplast $trnT_{UGU}$–$trnL_{UAA}$ spacer, $trnL_{UAA}$ intron, and $trnL_{UAA}$–$trnF_{GAA}$ spacer were those described in ref. 23; additional internal sequencing primers were 5′-GAATAGCTAATTTCAAATAA-3′ and 5′-ATAG-GAGACTCTTATGTTGG-3′. Primers used for amplification and sequencing of the nuclear 18S–5.8S ITS1 region of rDNA were taken from the literature (24), and an additional internal sequencing primer, 5′-GTCGTAACAAGGTTTCTGTAG-3′, was used. Sequences were aligned visually; phylogenetic analyses were performed with maximum parsimony (DNAPARS of PHYLIP version 3.53c; ref. 25) and neighbor-joining (26) using the Kimura distance (27). All sequences reported here have been deposited in the GenBank data base under the accession nos. L43165–L43324.

RESULTS

Minimum and maximum sequence lengths observed for the three chloroplast DNA (cpDNA) regions within *Echium* are 611/684 bp ($trnT_{UGU}$–$trnL_{UAA}$ spacer), 477/488 bp ($trnL_{UAA}$ intron), and 349/358 bp ($trnL_{UAA}$–$trnF_{GAA}$ spacer); the length of the nuclear 18S–5.8S ITS1 region varies from 230 to 260 bp. The alignment of all four markers contains 239 polymorphic positions, 137 phylogenetically informative positions, and 27

$trnL_{UAA}$-$trnF_{GAA}$ Spacer

Continental species	...TTACACAAGTCTTGTATGATA...
Island species	...TTACA---------TATGATA...

$trnL_{UAA}$ Intron

Continental species	...GACT-----GCTTTGG...
except *E. sabulicola*	...GCCTGCTTTGCTTTGG...
E. parviflorum	...GCCTGCTTTGCTTTGG...
Island species	...GACTGCTTTGCTTTGG...

FIG. 2. Phylogenetic distribution of two indels found in *Echium* DNA sequences.

informative indels (alignment available from the authors upon request). The $trnL_{UAA}$ intron shows least variability among all regions investigated (Table 2); average divergence of the nuclear ITS1 is 4- to 20-fold higher than that of the cpDNA spacers, depending on the species group under consideration (Table 2). Nuclear divergence within the island species is remarkably lower than within their continental congeners, ranging from 0.02–0.2% to 1.9% for cpDNA markers and ITS1, respectively (Table 2).

We found several length polymorphisms (indels) in the chloroplast data set, two of which are either unique to the island species investigated or to the latter and two of the continental species, *E. parviflorum* and *E. sabulicola* (Fig. 2). Neighbor-joining and parsimony analyses of the combined data set of all four markers (Fig. 3) are totally consistent with the indel distribution, providing unequivocal support for the common descent of all island species investigated. Within the continental species, the two coastal plants *E. parviflorum* and *E. sabulicola* are more closely related to the Macaronesian species than to continental relatives, and represent the sister group to the former. *L. fruticosus* was originally studied as an outgroup representative, since morphological evidence had suggested that this South African genus is closely related to *Echium*. Yet *L. fruticosus* and all other members of that genus investigated so far proved to be ingroups (unpublished data) due to the unexpectedly deep outlying position of the west Asian herb *E. russicum* (Fig. 3); the genus *Echium* as previously defined is paraphyletic and therefore under revision (unpublished data). Trees constructed from each of the four markers individually were generally consistent with the topology in Fig. 3, yet provided low bootstrap support for internal branches due to the small number of sites sampled in each (data not shown). Neighbor-joining and parsimony trees constructed from the combined cpDNA data (61 informative sites, 22 informative indels) were completely congruent at the 80% bootstrap proportion interval both with nuclear ITS1 trees (data not shown) and with Fig. 3. Congruence between supported regions of the parsimony and neighbor-joining topologies is consistent with the finding that, in computer simulations under the given sequence parameters for our data (reasonably large number of nucleotides sampled per species and low overall sequence divergence), both methods perform efficiently (29, 30). A maximum likelihood tree constructed with DNAML of PHYLIP also found the same topology as shown in Fig. 3, with the exception of differences in branching order within island species, and all branches supported at the 80% bootstrap proportion level in Fig. 3 were significantly positive in the maximum likelihood tree at the $P = 0.01$ level. Using *Lobostemon* as the outgroup, the relative rate test was performed as described (31) for concatenated nuclear and chloroplast sequences; no difference in substitution rate for any pair of ingroup *Echium* species was found that was significant at the $P = 0.05$ level.

DISCUSSION

One Mainland-to-Island Invasion Followed by Speciation. Phylogeny correlates with biogeography in *Echium*, as shown

Table 1. Geographic distribution and habit of investigated species

Species	Habit	Distribution
Echium albicans Lag. & Rodr.	Annual-perennial herb*	S Spain
E. asperrimum Lam.	Biannual herb	W-med
E. creticum L.[†]	Annual-biannual herb	W-med
E. horridum Batt.	Annual herb	SW-med
E. italicum L.	Biannual herb	NNE-med
E. lusitanicum L.	Perennial herb	Iber
E. parviflorum Moench.	Annual-biannual herb	Circummed
E. plantagineum L.[‡]	Annual herb	Circummed[§]
E. rosulatum Lange[¶]	Biannual-perennial herb*	Iber
E. russicum Gmel.	Biannual herb	pont-pan
E. sabulicola Pomel	Annual-perennial herb*	W-med
E. tuberculatum Hoffm. & Link[‖]	Biannual herb	W-med
E. vulgare L.	Biannual herb	Eu, W Asia
E. aculeatum Poiret	Candelabra tree	Can-C, -G, -H, -T
E. auberianum Webb & Berth.	Monocarpic herb**	Can-T
E. brevirame Sprague & Hutch.	Candelabra tree	Can-P
E. bonnetii Coincy	Annual herb	Can-C, -F, -T
E. callithyrsum Webb ex Bolle	Candelabra tree	Can-C
E. decaisnai Webb & Berth.	Candelabra tree	Can-C, -F, -L
E. giganteum L.f.	Candelabra tree	Can-T
E. handiense Svent.	Candelabra tree	Can-F
E. hierrense Webb ex Bolle	Candelabra tree	Can-H
E. leucophaeum Webb	Candelabra tree	Can-T
E. onosmifolium Webb & Berth.	Candelabra tree	Can-C
E. pininana Webb & Berth.	Monocarpic rosette-tree	Can-P
E. pitardii A. Chev.	Annual herb	Can-L
E. simplex DC.	Monocarpic rosette-tree	Can-T
E. strictum L.f.	Candelabra tree	Can-C, -G, -H, -P, -T
E. triste Svent.	Annual-perennial herb*	Can-C, -G, -T
E. virescens DC.	Candelabra tree	Can-T
E. webbii Coincy	Candelabra tree	Can-P
E. wildpretii Pears.	Monocarpic rosette-tree	Can-P, -T
E. hypertropicum Webb	Candelabra tree	Cape V-A, -F, -R, -T
E. stenosiphon Webb	Candelabra tree	Cape V-A, -L, -N, -V
E. vulcanorum A. Chev.	Candelabra tree	Cape V-F
E. candicans L.f.	Candelabra tree	Mad-M, -Po
E. nervosum Ait.	Candelabra tree	Mad-M, -Po, -D
Lobostemon fruticosus Buek	Shrub	S Africa

Distribution data were taken from the literature (17–20). S, south; W, west; SW, southwest; NNE, north northeast; med, Mediterranean; Circummed, circummediterranean; Iber, Iberian peninsula; pont-pan, pontic-pannonic; Eu, Europe; Can-C, -F, -G, -H, -L, -P, and -T, Canary Islands-Gran Canoria, -Fuerteventura, -Gomera, -Hierro, -Lanzarote, -La Palma, and -Tenerife, respectively; Cape V-A, -F, -L, -N, -R, -T, and -V, Cape Verde archipelago-Santo Antão, -Fogo, -Santa Luzia, -São Nicolau, -Brava, -São Tiago, and -São Vincente, respectively; and Mad-M, -Po, and -D, Madeira Islands-Madeira, -Porto Santo, and -Desertas, respectively.
*Rarely subshrubs. In the last monograph of the genus (18), species names are given as [†]*E. spinescens* Medikus, [‡]*E. lycopsis* Grufb., [¶]*E. gaditanum* Boiss., and [‖]*E. hoffmannseggii* Litard.
[§]*E. plantagineum* occurs worldwide through human distribution.
**E. auberianum* rarely buds again after flowering and is thus not strictly monocarpic.

in Fig. 3. The congruence that we observed between the cpDNA (maternally inherited in *Echium*; ref. 32) and nuclear DNA trees indicate that pollen introgression from continental to island species (or vice versa) has not played a detectable role in *Echium* evolution, a point which cannot be established through analysis of cpDNA alone. The data provide convincing support for the view that island species of *Echium* are derived descendants of continental ancestors. The molecular phylogeny based on four markers from two compartments is consistent with the indel distribution (Fig. 2), indicating a single mainland-to-island invasion event in the history of this genus followed by accelerated speciation among island colonizers, rather than multiple invasions (18). Notably, the Canary, Madeira, and Cape Verde archipelagos, though separated by several thousand kilometers of ocean, have all been colonized by descendants of the same initial invading population. Under the relict hypothesis (6), the conspicuous diversity among Macaronesian *Echium* species would suggest that

these may be older than and ancestral to their morphologically less diverse herbaceous continental congeners, but the molecular data clearly indicate that the converse is true (Fig. 3). Since the island species of *Echium* are obviously successful long-range colonizers, it is curious that no continental *Echium* species studied is a descendant of the island inhabitants—i.e., no fruitful reinvasion of the mainland has occurred within our sample.

The Timing of Island Colonization. The age of the oldest islands among the three archipelagos are known and provide the earliest possible dates of island colonization. Within the Canary Islands, eastern-most Fuerteventura and Lanzarote are the oldest and subaeric formation started ≈20 mya and ≈15 mya on these islands, respectively, as indicated by K/Ar determinations (9), whereby westernmost Hierro is the youngest (≈0.8 mya; ref. 10). A similar east-to-west gradient of Miocene to Quaternary age is found for the Cape Verde archipelago (11–13), and the younger Madeira archipelago is

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Table 2. Average divergence between taxa for sequences studied

Comparison (n)	Marker	K_{av}	SE	Mini-mum	Maxi-mum
Outgroups/Continental (36)	nITS*	303	72	227	372
	trnT/L†	34	13	18	49
	trnL/F‡	29	14	16	45
	Intron§	28	12	15	39
Outgroups/Island (63)	nITS	293	70	243	328
	trnT/L	25	11	15	31
	trnL/F	31	16	23	39
	Intron	26	11	13	34
Continental/Island (252)	nITS	97	33	68	119
	trn/L	19	10	6	32
	trnL/F	14	10	3	29
	Intron	4	5	2	7
Continental/Continental (66)	nITS	73	26	0	146
	trnT/L	17	9	0	35
	trnL/F	9	7	0	22
	Intron	6	6	0	11
Island/Island (210)	nITS	19	12	0	54
	trn/L	2	3	0	8
	trnL/F	1	3	0	3
	Intron	0.2	0.4	0	2
Cape Verde/Cape Verde (3)	nITS	3	3	0	4
Madeira/Madeira (1)	nITS	0	0	0	0

Values represent average Kimura two-parameter distance $\times 10^{-3}$ (K_{av}) between outgroup representatives (*C. minor* L., *C. major* L., and *Lithodora diffusa* Johnston, each Boraginaceae), continental (except *E. russicum*), island, Cape Verde, and Madeira *Echium* species, respectively. SE, average standard error of Kimura distance across comparisons. *n*, number of individual pairwise comparisons. Minimum and maximum values for taxon comparisons are indicated.
*Nuclear ITS1 (average, 235 sites).
†Chloroplast *trnT*$_{UGU}$–*trnL*$_{UAA}$ spacer (average, 720 sites).
‡Chloroplast *trnL*$_{UAA}$–*trnF*$_{GAA}$ spacer (average, 312 sites).
§Chloroplast *trnL*$_{UAA}$ intron (average 493 sites).

of mid-Miocene age (10–15 mya; ref. 14). Since the Macaronesian species share a single common ancestor, *Echium* island inhabitants and corresponding speciation events thus cannot be >20 million years old (9–14) and could be much younger. Using this upper bound, the substitution rate for nuclear ITS sequences within island species of *Echium* can be estimated as $\approx 1.1 \times 10^{-9}$ per site per year (at the minimum) on the basis of average divergence (Table 2) between the three earliest branching (*E. auberianum, E. pininana,* and *E. wildpretii*) and the remaining island species (63 comparisons, $K_{av} = 0.042 \pm 0.02$). This value is $\approx$4-fold lower than that found in vertebrate nuclei (31), but, conversely, if nuclear substitution rates in *Echium* and vertebrates are, in fact, similar, the island species would be much younger ($\approx$5 million years old) than the islands which they inhabit. Using the conservative rate estimate of 1.1×10^{-9} per site per year, divergence between nuclear ITS sequences indicates that the three *Echium* species endemic to the Cape Verde archipelago, *E. hypertropicum* and *E. vulcanorum* shown in Fig. 3 (and *E. stenosiphon* which differs from these in only one ITS substitution, not shown), diverged not more than 3 mya and perhaps as recently as 0.75 mya. From Table 2, the same ages can be estimated for the (only) two *Echium* endemics of the Madeira archipelago (*E. candicans, E. nervosum*).

The greatest genetic divergence within the island species is found among inhabitants of the Canary Islands, suggesting that the initial colonization and diversification occurred on this archipelago; the Madeira and Cape Verde archipelagos were apparently colonized later by perennial woody Canary Island inhabitants (Fig. 3 and Table 2). Common branches for Madeira and Cape Verde inhabitants, respectively, suggest that *Echium* speciation on these islands—like the initial invasion—again has occurred subsequent to a single founding

colonization, possibly reflecting a nascent reiteration of the initial mainland-to-island colonization/diversification process. Colonization of Madeira from the Canary Islands would involve dispersal against the northeast-to-southwest current of both prevailing winds and ocean currents surrounding all three archipelagos and would be compatible with the view that birds are responsible for long-distance seed dispersal in island species of *Echium*.

Evolution from Herbaceous to Radiating Woody Species. Outgroup comparison to the closest continental sisters (*E. parviflorum* and *E. sabulicola*) of woody *Echium* island species strongly suggests that the ancestors of the latter were herbaceous. Although the data do not thoroughly exclude the alternative explanation that the herbaceous habit evolved from woody ancestors (6–8), that scenario appears rather unlikely, since it would have necessarily occurred independently in several different continental lineages (Fig. 3). Evolution of woody from herbaceous forms is by far the most straightforward interpretation of the data, and is congruent with similar conclusions recently drawn from cpDNA restriction mapping for the genus *Lobelia* (Lobeliaceae; ref. 33). The position of the herbaceous island inhabitants *E. bonnetii* and *E. pitardii* (which on the basis of ITS1 data alone is the immediate sister of *E. bonnetii*; data not shown) is not robustly resolved as basal or "crown" within the island phylogeny (Fig. 3). Although the data do not clearly reveal whether their herbaceous states are homologous to that of continental sisters (ancestral) or whether it represents a recent reversal from the insular woody habit, the position of *E. bonnetii* in the tree would favor the view that it has undergone very recent reversal since its divergence from a woody island ancestor.

The very low degree of genetic divergence observed between island *Echium* species (Table 2) combined with remarkable morphological diversity (Fig. 3) clearly indicates that they are still undergoing active diversification and that speciation occurs directly in conjunction with island colonization. This is congruent with conclusions recently drawn for other island genera on the basis of less comprehensive data (34, 35). Note that a prominent speciation mechanism in angiosperms, ploidy change (36, 37), can be excluded for island inhabitants of *Echium*, since they have constant chromosome number ($2n = 16$; refs. 38 and 39), whereas their continental sisters show considerable ploidy variation with deviations from $x = 8$ ($n = 8, n = 16, x = 6, x = 5, x = 7$; refs. 40 and 41). *E. parviflorum* and *E. sabulicola* are clearly close continental relatives of the island species (Figs. 2 and 3) and also possess $2n = 16$ (41), further supporting their position as the sister group to the island colonizers. Speciation of the older and cytologically more polymorphic continental *Echium* forms might thus have entailed chromosome evolution, but rapid speciation among the woody island *Echium* species clearly did not.

We have found that in *Echium* (*i*) only one initial invasion of the islands from the continent has occurred, (*ii*) the perennial woody forms are very proficient colonizers, and (*iii*) speciation occurs in conjunction with island colonization (even across ocean distances much greater than that to the continent). Taken together, these findings compellingly suggest that the critical and rate-limiting step in the evolutionary success (measured in terms of both new species and forms per unit time) of the contemporary island species was the origin of the perennial woody habit. Insular woodiness is crucial to understanding colonization-related diversity found in the genus.

Possible Causes of Insular Woodiness. That insular woodiness as well as candelabra shrub and rosette tree habits have arisen independently in many different angiosperm genera (3) strongly suggests that they are selected for, rather than occurring as the result of drift. But what type(s) of selection can account for the woody habit of island colonizers? If selection had entailed adaptation to constant climates as suggested (3), one would expect *Echium* inhabitants of comparable (i.e.,
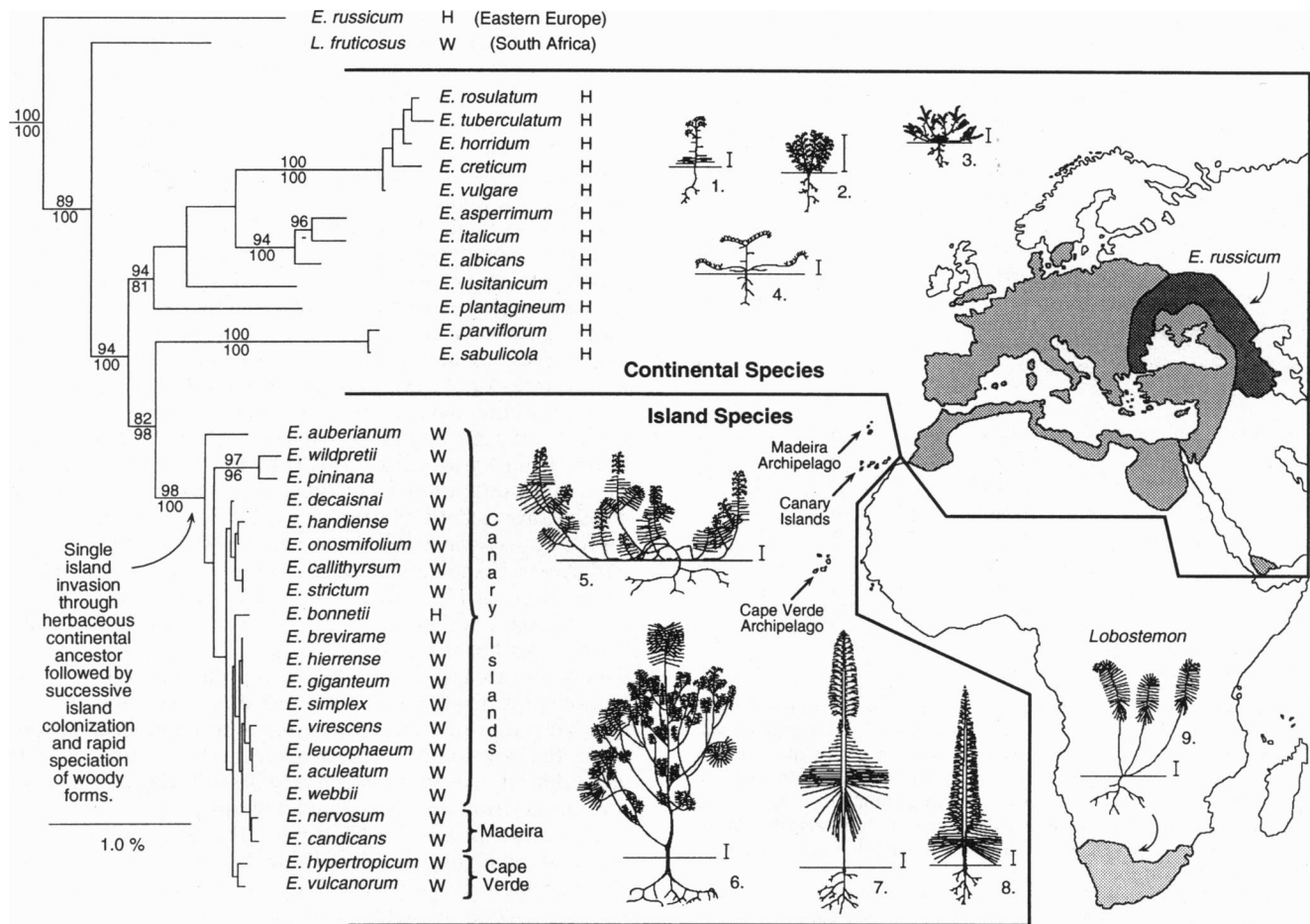
FIG. 3.   Correlation between phylogeny and biogeography in *Echium*. On the left side of the figure is a neighbor-joining tree (26) for Kimura distances (27) between combined sequences from noncoding cpDNA and nuclear DNA. The horizontal scale bar indicates 1% sequence divergence. The tree is based on a total of 1676–1790 bases per species, and the alignment contains 239 polymorphic positions, 137 informative positions, and 27 informative indels (available from the authors upon request). Numbers above branches indicate the number of times the branch was found in 100 bootstrap neighbor-joining replicates (28) using the Kimura distance, numbers below branches indicate the number of times the branch was found in 100 bootstrap parsimony replicates. Bootstrap values <80 are not indicated. H, herbaceous growth form; W, woody growth form. In the middle portion of the figure is a schematic representation of representative habits. Vertical scale bars indicate 10 cm. In the right portion of the figure is geographic distribution of species (the lighter shaded region indicates the distribution of *E. russicum*). Outgroup representatives are *C. minor* L., *C. major* L., and *Lithodora diffusa* Johnston (each Boraginaceae). Use of the unpublished sequences for these four regions from *Symphytum officinale* L., *Pulmonaria officinalis* L., *Anchusa officinalis* L., and *Brunnera macrophylla* (Adams) I. M. Johnston (all Boraginaceae) as outgroups changes neither the position of the root nor regions of the topology supported at the 80% confidence interval. The island species shown do not occur in natural mainland floras, nor do continental species naturally occur on Macaronesian islands (indicated by bold lines). Circummediterranean grey shading indicates the cumulative distribution of continental *Echium* species; distribution of *E. russicum* and *Lobostemon*, respectively, are indicated. Schematic drawings of *Echium* species are redrawn from ref. 18. (*1*) *Echium plantagineum*; (*2*) *E. humile*; (*3*) *E. rosulatum*; (*4*) *E. parviflorum*; (*5*) *E. onosmifolium*; (*6*) *E. decaisnai*; (*7*) *E. simplex*; (*8*) *E. wildpretii*; and (*9*) *Lobostemon regulareflorus*. Sequences of *E. stenosiphon*, *E. triste*, and *E. pitardii* of the Cape Verde and Canary archipelagos, respectively, were not obtained for the *trnT*$_{UGU}$–*trnL*$_{UAA}$ spacer and are thus not included in the figure, but these species are descendants of the same invasion as documented by sequence data from the remaining markers.

Iberian) continental climate zones also to be woody, but they are herbaceous. In our view, this is a strong argument against the climate adaptation hypothesis. Wallace's earlier suggestion that a connection may exist between the perennial woody habit of island species and reproductive strategy deserves closer inspection. He argued that the perennial insular woody growth form primarily reflects selection for longevity (rather than for woodiness *per se*) of insect-pollinated species in an environment where insects initially should be expected to be rare (2), noting that the resulting increase in size may have provided additional advantage in niche competition among initial colonizers. Bramwell's studies of breeding behavior are generally compatible with that view, since in *Echium* species studied, the woody island forms were found to be outbreeding, setting only 0–11% fertile seed in selfings, whereas their continental sisters as well as the herbaceous island inhabitant *E. bonnetii* were preferentially inbreeding (15, 16).

But in light of our surprising findings that the island forms are rapidly speciating colonizers, their outbreeding behavior seems puzzling at first sight, since, in an extreme scenario, one could expect successful island colonizers to be able to establish populations with but a single propagule (42). Although selection for successful pollination (2) can fully account for the woodiness (longevity) and large inflorescences observed in island *Echium* species, it does not explain the requirement for cross-pollination in island inhabitants. We suggest that outbreeding is manifest among island inhabitants as the result of counterselection of inbreeding depression in selfing colonizers. This view derives strong support from the finding that natural per generation mutation rates in inbreeding herbaceous members of the Boraginaceae are sufficiently high to cause inbreeding depression in newly arisen populations (43). If outbreeding is the primary selective factor in island colonization, pollination pressure will subsequently favor rare,

large, conspicuous inflorescences among outbreeders and, as a consequence, select perennial (and therefore woody) habits capable of producing them, in agreement with Wallace's salient arguments (2). Under this view, diversity of contemporary woody *Echium* forms reflects a multiplicity of selectable developmental pathways toward longevity, rather than selection for specifically environment-adapted variants of such woody perennial habits as schematically depicted in Fig. 3. In other words, insular woodiness in *Echium* might simply betray "survival of the founders," and many differences between perennial woody habits could be nonadaptive. The proposal that avoidance of inbreeding depression may be causal to the perennial woody growth form of island species can easily account for available data in *Echium* and also for the finding that gynodioecy, a common strategy to achieve outbreeding, although observed in several island *Echium* populations, does not correlate with perennial woodiness, since it is also found in herbaceous continental *Echium* species (16, 44–46). It furthermore predicts that estimates of outcrossing rates (47, 48) in natural populations of insular woody *Echium* species should be higher than in those of herbaceous continental congeners. Outbreeding, perennial growth and the insular woody habit may reflect three manifestations attributable to a single selective pressure: the need to escape inbreeding depression in geographically isolated founding populations.

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