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14

Molecular Evolution, Adaptive Radiation, and Geographic Speciation in *Cyanea* (Campanulaceae, Lobelioideae)

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Cyanea, the largest of the seven genera of native Hawaiian lobelioids, is of considerable evolutionary interest for four reasons. First, it is the largest genus of plants endemic to the Hawaiian Islands, with 55 species comprising 5.7% of the native flora (Wagner et al., 1990; Lammers, 1992). Second, *Cyanea* and the three other lobelioid genera with fleshy fruits (*Clermontia, Delissea,* and *Rollandia*) are considered by many to be the largest group of Hawaiian plants to have evolved from a single immigrant, encompassing some 98 species (Rock, 1919; Carlquist, 1965, 1980; Lammers, 1990a, 1992).

Third, *Cyanea* has undergone a striking series of adaptive radiations in growth form, leaf size and shape, and floral morphology (Carlquist, 1965, 1974, 1980). *Cyanea* varies in height from 1 to 14 m and includes treelets, shrubs, trees, and even one vinelike species; most are unbranched and occur in mesic and wet forests (1,000 to 2,000 m elevation). *Cyanea* leaves vary from simple to doubly compound and range from 0.3 to 25 cm in width and up to 100 cm in length (Lammers, 1990a). Together with most Hawaiian lobelioids, *Cyanea* appears to have coevolved with honeycreepers (Drepanidinae) and honeyeaters (Meliphagidae), native birds that served as pollinators; *Cyanea* shows an unusually wide interspecific range in corolla tube length, from 15 to 85 mm (Carlquist, 1980; Lammers and Freeman, 1986; Lammers, 1990a).

Finally, *Cyanea* shows a remarkable degree of endemism. Not only are all its species—like those of five of the other six genera of native

lobelioids—restricted to the Hawaiian archipelago, but many are endemic to single islands or portions of islands (Table 14.1), each of known geologic age (Clague and Dalrymple, 1987) and greatly isolated from the nearest areas supporting other lobelioids (Wimmer, 1943; Lammers, 1985). *Cyanea*—and the endemic Hawaiian lobelioids generally—thus provide superb material for studies of adaptive radiation and geographic speciation and for estimates of the time required for the diversification of a species-rich island clade.

We have begun an intensive study of molecular evolution in the native Hawaiian lobelioids, with a focus on *Cyanea* and related genera with fleshy, bird-dispersed fruits. Our aim is to derive a phylogeny based on restriction site variation in chloroplast DNA (cpDNA) and to use this independently derived phylogeny as a basis for interpreting patterns of adaptive radiation and geographic speciation. An independently derived phylogeny is particularly crucial for studies of adaptive radiation. Although adaptive radiation is probably the most important concept bridging ecology and evolutionary biology, very few cases have been rigorously analyzed (Givnish, 1987; Skinner, 1988; Carr et al., 1989; Baldwin et al., 1990, 1991; Sytsma et al., 1991). The fundamental problem is that, in almost every case, the very characters whose radiation is being studied (e.g., beak size and shape) are also used to classify the organisms in question, so that the exercise can become circular, chasing morphological traits down evolutionary pathways determined, at least in part, by the traits themselves.

Many studies have demonstrated that variation in cpDNA restriction sites can provide a powerful tool for inferring relationships among plant species, genera, and even families (e.g., Sytsma and Gottlieb, 1986; Jansen and Palmer, 1987, 1988; Palmer et al., 1988; Wendel, 1989; Baldwin et al., 1990; Olmstead et al., 1990, 1992; Sytsma, 1990; Baldwin, 1992; Downie and Palmer, 1992a; Doyle et al., 1992; Jansen et al., 1992; Sytsma and Smith, 1992; Wendel and Albert, 1992). Analyses based on cpDNA restriction site variation show significantly less homoplasy (convergence or parallelism, likely to distort inferences of phylogeny) than do those based on morphology (Givnish and Sytsma, 1992; contra Sanderson and Donoghue, 1989, and Donoghue and Sanderson, 1992). The large number of independent characters sampled in cpDNA analyses, their low level of homoplasy for a given number of taxa, and the conserved architecture of the chloroplast genome ensure that restriction sites can provide detailed and precise data on phylogenetic relationships

TABLE 14.1. Distribution of Species of *Cyanea* and Related Lobelioids in the Hawaiian Archipelago

Species	Kaua'i	W O'ahu	E O'ahu	Moloka'i	Lana'i	W Maui	E Maui	Hawai'i
Cyanea aculeatiflora Rock							•	
C. acuminata (Gaud.) Hillebr.		•						
C. angustifolia (Cham.) Hillebr.		•	•	•	•	•		
C. arborea Hillebr.							†	
C. asarifolia St. John	V							
C. asplenifolia (H. Mann) Hillebr.						†		
C. comata Hillebr.							†	
C. copelandii Rock							•	†
C. coriacea (A. Gray) Hillebr.	•							
C. degeneriana E. Wimm.								•
C. dunbarii Rock				V				
C. eleeleensis (St. John) Lammers	V							
C. elliptica (Rock) Lammers					•	•	•	
C. fauriei H. Lév.	•							
C. fissa (H. Mann) Hillebr.	•							
C. giffardii Rock								†
C. glabra (E. Wimm.) St. John							•	
C. grimesiana Gaud.		•	•	•	•			†
C. hamatiflora Rock							•	V
C. hardyi Rock	•							
C. hirtella (H. Mann) Hillebr.	•							
C. horrida (Rock) Degener & Hosaka							•	
Cyanea kolekolensis (St. John) Lammers	V							
C. kunthiana Hillebr.						•	•	
C. leptostegia A. Gray	•							
C. linearifolia Rock	V							
C. lobata H. Mann					†	†		
C. longissima (Rock) St. John							†	
C. macrostegia Hillebr.					•	•	•	
C. mannii (Brigham) Hillebr.				•				
C. marksii Rock								V
C. mceldowneyi Rock							V	
C. membranacea Rock		•						
C. obtusa (A. Gray) Hillebr.						†	†	
C. pilosa A. Gray								•
C. pinnatifida (Cham.) E. Wimm.		†	V					
C. platyphylla (A. Gray) Hillebr.								V
C. pohaku Lammers							†	
C. procera Hillebr.				V				
C. profuga C. Forbes				†				
C. pycnocarpa (Hillebr.) E. Wimm.								†
C. quercifolia (Hillebr.) E. Wimm.							†	
C. recta (Wawra) Hillebr.	V							
C. remyi Rock	V							
C. scabra Hillebr.						•		
C. shipmanii Rock								†
C. solanacea Hillebr.				•		?		
C. solenocalyx Hillebr.				V				
C. spathulata (Hillebr.) A. Heller	•							
C. stictophylla Rock								V

(*Continued*)

Notes: Extant populations are indicated by •; endangered populations by V; extinct populations by †; and possible, but not definitely documented, occurrence by ?.

TABLE 14.1. (*Continued*)

(*Continued*)

TABLE 14.1. *(Continued)*

Species	Kaua'i	W O'ahu	E O'ahu	Moloka'i	Lana'i	W Maui	E Maui	Hawai'i
Cyanea superba (Cham.) A. Gray		†	V					
C. sylvestris A. Heller	•							
C. tritomantha A. Gray								•
C. truncata (Rock) Rock		V						
C. undulata C. Forbes	V							
Clermontia arborescens (H. Mann) Hillebr.				•	•	•	•	
C. calophylla E. Wimm.								•
C. clermontioides (Gaud.) A. Heller							•	
C. drepanomorpha Rock							•	
C. fauriei H. Lév.	•	•	•					
C. grandiflora Gaud.				•	•	•	•	
C. hawaiiensis (Hillebr.) Rock								•
C. kakeana Meyen		•	•	•		•	•	
C. kohalae Rock								•
C. lindseyana Rock							•	†
C. micrantha (Hillebr.) Rock					†	•		
C. montis-loa Rock								•
C. multiflora Hillebr.		†				†		
C. oblongifolia Gaud.		•	•	•	†	•	†	
C. pallida Hillebr.				•				
C. parviflora Gaud. ex A. Gray								•
C. peleana Rock							†	V
C. persicifolia Gaud.		•	•					
C. pyrularia Hillebr.								V
C. samuelii C. Forbes							•	
C. tuberculata C. Forbes							V	
C. waimeae Rock								•
Delissea fallax Hillebr.								†
D. laciniata Hillebr.			†					
D. lauliana Lammers			†					

(Continued)

TABLE 14.1. *(Continued)*

Species	Kaua'i	W O'ahu	E O'ahu	Moloka'i	Lana'i	W Maui	E Maui	Hawai'i
Delissea parviflora Hillebr.								†
D. rhytidosperma H. Mann	V							
D. rivularis (Rock) E. Wimm.	V							
D. sinuata Hillebr.		†			†			
D. subcordata Gaud.		•	†					
D. undulata Gaud.	†	†				†		V
Rollandia angustifolia (Hillebr.) Rock			•					
R. crispa Gaud.			•					
R. humboldtiana Gaud.		•						
R. lanceolata Gaud.		•	•					
R. longiflora Wawra		•	•					
R. parvifolia C. Forbes	†							
R. purpurellifolia Rock		†						
R. st.-johnii Hosaka			•					
Brighamia insignis A. Gray	•							
B. rockii St. John				•	†			

Sources: Data compiled from Rock (1919); Lammers (1990a, 1992); personal communications with L. Cuddihy, R. Hobdy, J. Obata, and L. Mehroff; and personal observations of the authors.

(Palmer and Stein, 1986; Palmer et al., 1988; Clegg et al., 1990; Sytsma, 1990).

Attention must be paid to instances in which the chloroplast genome is involved as a single genetic unit in hybridization or introgression events (see Rieseberg and Brunsfeld, 1992) or in which the chloroplast genome exhibits rearrangements (Downie and Palmer, 1992b). The first is probably not an issue, as reports of hybridization in *Cyanea* are rare (Rock, 1919; Lammers, 1990a). The latter is of some concern, as some Lobelioideae show several nested rearrangements of the chloroplast genome (Knox et al., 1993). However, these rearrangements have been precisely mapped relative to the small *Nicotiana* cpDNA probes that were used in this study (see below), thus permitting unambiguous interpretation of restriction fragment patterns. Any analysis based on cpDNA variation must consider the possibility that sorting of maternal plastid lineages from a polymorphic ancestor (Neigel and Avise, 1986) might lead to a phylogeny at odds with the actual phylogeny. Lineage sorting, however, seems unlikely to be important in Hawaiian plant groups, given the small population sizes and frequent genetic bottlenecks associated with the repeated colonization of new islands.

This chapter presents a progress report on our studies of cpDNA evolution, adaptive radiation, and speciation in *Cyanea*. First, we summarize the natural history of *Cyanea* and the other fleshy-fruited genera. We then present the results of a preliminary analysis of relationships among the fleshy-fruited genera and 24 of the 45 extant species of *Cyanea* and explore their evolutionary implications. Finally, we discuss the factors that may have caused greater speciation and greater exposure to extinction in *Cyanea* than in the closely related genus *Clermontia*, and the consequent need for a vigorous program to preserve the remaining, often highly endangered species of *Cyanea*.

NATURAL HISTORY OF THE FLESHY-FRUITED HAWAIIAN LOBELIOIDS

Among the four fleshy-fruited (baccate) genera, *Cyanea* is by far the most species-rich and diverse in habit (Table 14.1). Species of *Cyanea* are generally unbranched trees or treelets of mesic and wet forest interiors (Figure 14.1A to E). A few species (e.g., *C. coriacea, C. hardyi*) are sparsely branched shrubs of forest openings and edges, often in areas with lower rainfall (Rock, 1919); one (*C. copelandii*) is vinelike. *Cyanea* flowers are tubular, often strongly curved, and white to purple in color (occasionally pink, yellow, or greenish); their floral tube is cut only about halfway to the base (Figure 14.1B). *Cyanea* fruits are orange or purplish in color and generally less than 15 mm in diameter. Species differ dramatically in stature. For example, *C. degeneriana* (found in wet forests on Hawai'i) is a subherbaceous treelet usually less than 1 m tall (Figure 14.1A); *C. mceldowneyi* of Maui grows up to 2 m (Figure 14.1C); *C. tritomantha* of Hawai'i is taller still, with a massive trunk and palmlike growth form, up to 3 m (Figure 14.1D); and a few gigantic species, such as *C. hamatiflora* of Maui and Hawai'i or *C. leptostegia* of Kaua'i, can achieve heights up to 14 m (Figure 14.1E).

Leaves of different *Cyanea* species can differ dramatically in size, from 3 mm wide in *C. linearifolia* to more than 25 cm wide and up to 1 m in length in some of the palmlike species (e.g., *C. aculeatiflora, C. hamatiflora, C. leptostegia,* and *C. macrostegia*) (Rock, 1919; Carlquist, 1965, 1980; Lammers, 1990a). Although most species have leaves with entire or minutely toothed margins, some are coarsely toothed, lobed, deeply divided, or pinnately compound (Figure 14.2). One species (*C. shipmanii* of Hawai'i) even has doubly pinnately compound leaves that resemble those of tree ferns. Most species with undivided leaves have tapered or rounded leaf bases, but one cliff-dwelling species (*C. asarifolia* of Kaua'i) has leaves with cordate bases, like those of many bole-climbing vines with erect petioles and horizontal leaves (Givnish and Vermeij, 1976; Givnish, 1986). Most species with nonentire leaf margins show marked differences between juvenile and adult leaves, with the juvenile foliage being more deeply divided (Figures 14.2 and 14.3) (Carlquist, 1965, 1974, 1980; Lammers, 1990a,b).

The juvenile foliage or stems of some species are also armed with thornlike prickles (conical outgrowths of ground tissue) (Carlquist, 1962), up to 1 cm in length (Figure 14.4). Given the absence of native terrestrial mammals and reptiles that could browse or graze vegetation near the ground, what is the significance of such prickles? Carlquist (1962, 1965, 1974, 1980) suggested that they may have defended lobelioids against herbivory by native tree snails (Succinidae). This seems unlikely, however, given that these snails have never been observed to consume lobelioid leaves and, like achatinellid snails (the largest group of terrestrial mollusks native to the Hawaiian Islands), usually graze instead on fungi (especially epiphyllic fungi) (Henshaw, 1912–14; Carlquist, 1974; S. Carlquist, unpubl.; M. G. Hadfield, unpubl.).

Givnish et al. (1994) proposed an alternative explanation: Prickles near ground level protected *Cyanea* stems and leaves from browsing by large native terrestrial birds, the eight species of large flightless geese and gooselike moa-nalos (derived from mallardlike ducks), which were extirpated by the Polynesians sometime in the past 1,600 years (Olson and James, 1982a). Subfossil remains of these large browsers have recently been discovered in lava tubes and calcified sand dunes (Olson and James, 1982b, 1991). *Cyanea* may have been particularly sensitive to damage by these browsers, given its unbranched habit and its tender, poorly defended foliage and stems that pigs and goats avidly eat today (Loope and Scowcroft, 1985; L. Cuddihy et al., unpubl.). Several groups of birds (e.g., moas on New Zealand, elephant birds on Madagascar, *Sylviornis* on

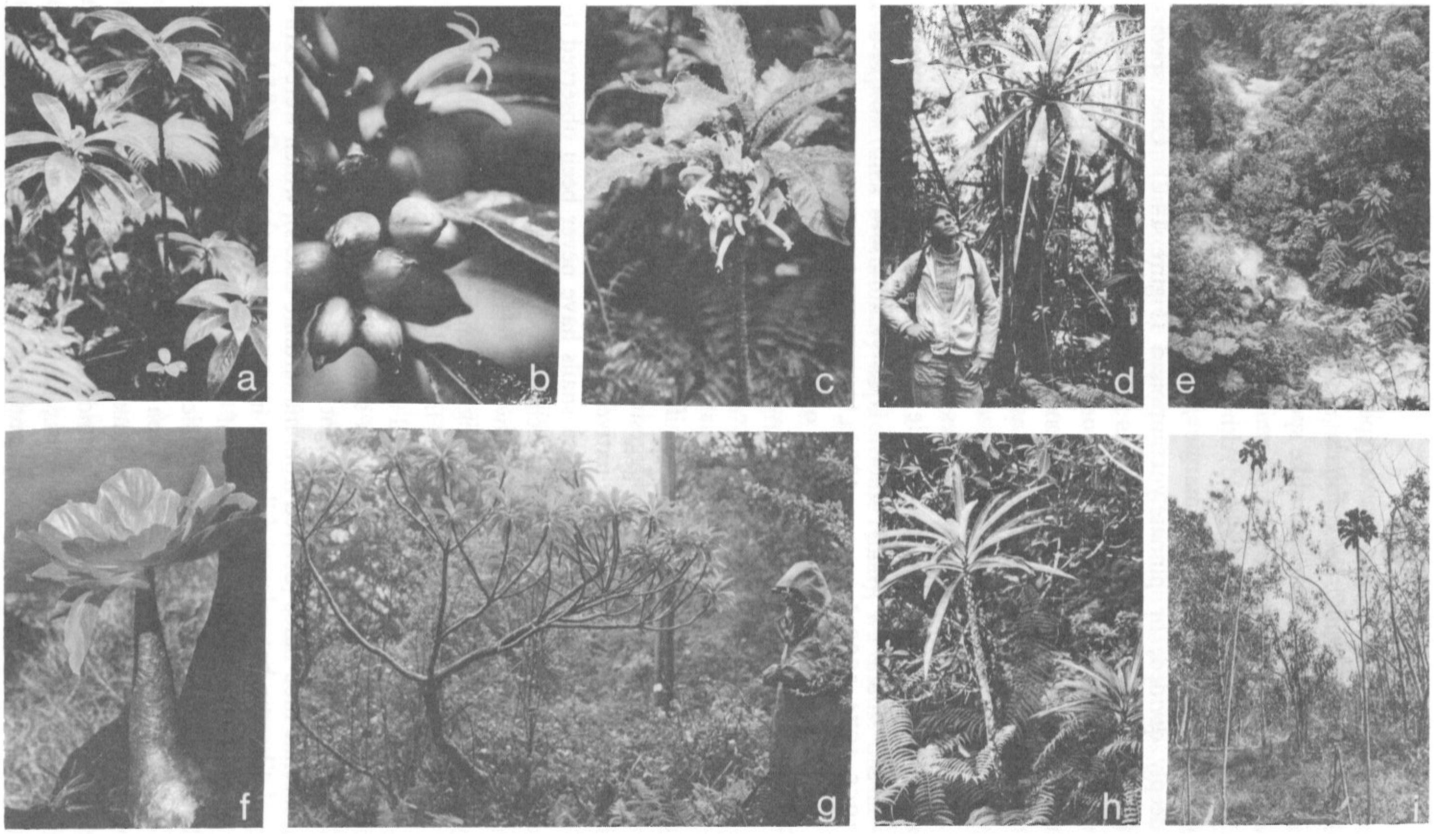


FIGURE 14.1. Habit of representative species of *Cyanea* and allied genera. (**a** and **b**) C. *degeneriana*, ʻOlaʻa Forest Reserve, Hawaiʻi, ca. 0.8 m tall: (a) habit; (b) close-up of flowers (ca. 20 mm long) and orange fruits. (**c**) C. *mceldowneyi*, Waikamoi stream area, Maui, ca. 1 m tall. (**d**) C. *tritomantha*, Desolation Trail, Hawaiʻi, with K. J. Sytsma. (**e**) C. *hamatiflora* (emergent rosette tree with narrow leaves on far side of stream near middle of photograph; note human figures in stream for scale), Waikamoi Flume, Maui. (**f**) *Brighamia insignis*, Kauaʻi (from Carlquist, 1980; reproduced courtesy of the National Tropical Botanical Garden). (**g**) *Clermontia kakeana*, ʻIao Valley, Maui, with R. Hobdy. (**h**) *Rollandia angustifolia*, Oʻahu. (**i**) *Delissea undulata*, Hawaiʻi (from Rock, 1919; reproduced courtesy of Bishop Museum Press, Bishop Museum, Honolulu, Hawaii).

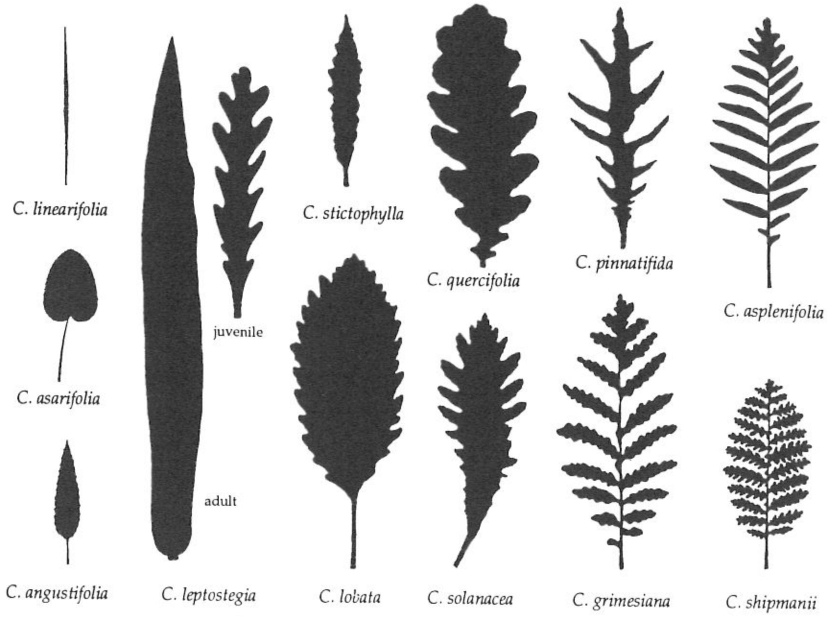


FIGURE 14.2. Interspecific variation in leaf size and shape within *Cyanea* (redrawn from Carlquist, 1965; Lammers, 1990b; and authors' photographs).

New Caledonia, and possibly dodos, solitaires, or rails on the Mascarene Islands) have been able to disperse to oceanic islands inaccessible to nonflying mammals, where they then evolved into terrestrial (and often flightless) browsers and grazers, the insular equivalents of antelopes or cervids (Carlquist, 1965; Balouet, 1984; Balouet and Olson, 1989; Olson and James, 1991). Although prickles are rare in floras of such bird-dominated islands, the densely divaricate branching patterns of the juveniles of several New Zealand trees (55 species in 21 families) apparently played a similar role as a mechanical defense against browsing by moas (Carlquist, 1974; Atkinson and Greenwood, 1989).

The corollas of *Cyanea* differ dramatically in length (15 to 80 mm) and coloration (Figure 14.5). The average corolla length of species on Kaua'i is significantly less than that on younger islands (Figure 14.6), perhaps reflecting an escalating evolutionary competition for progressively more specialized pollinators. As noted by Rock (1919) and others, the strong curvature of the flowers of most *Cyanea* (and other lobelioids except *Brighamia*) is strongly reminiscent of the curved bills of the *'I'iwi* (*Vestiaria coccinea*) and other nectarivorous honeycreepers. Research by

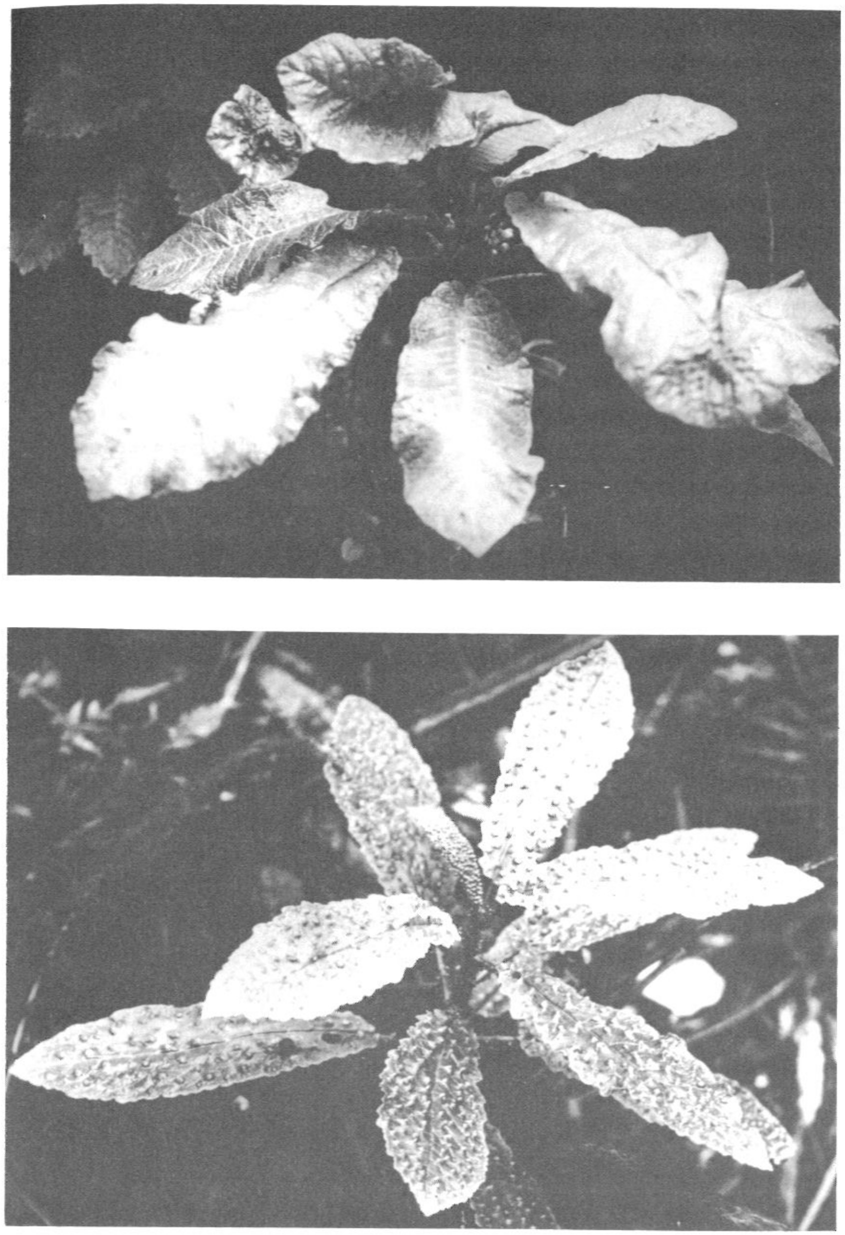


FIGURE 14.3. Examples of juvenile–adult leaf dimorphism in *Cyanea*. (**Above**) *C. solenocalyx* (Kamakou Reserve, The Nature Conservancy, Moloka'i), with dentate, nearly orbiculate juvenile leaves in shadows *at upper left*, adult leaves *below and to right*. (**Below**) *C. tritomantha* seedling, showing thornlike prickles scattered over leaf surfaces (see smooth adult foliage in Figure 14.1D).

FIGURE 14.4. Juvenile shoots of *Cyanea solanacea* on Moloka‘i (Kamakou Reserve), showing dense aggregation of thornlike prickles (ca. 1 cm in length) and deeply lobed juvenile foliage. These shoots are sprouts from an adult axis (visible at the bottom of the photograph) that had been mechanically damaged by feral pigs, a recently introduced alien herbivore. Note the loss of prickles toward the tip of the juvenile shoots, signaling the beginning of the shift toward adult morphology.

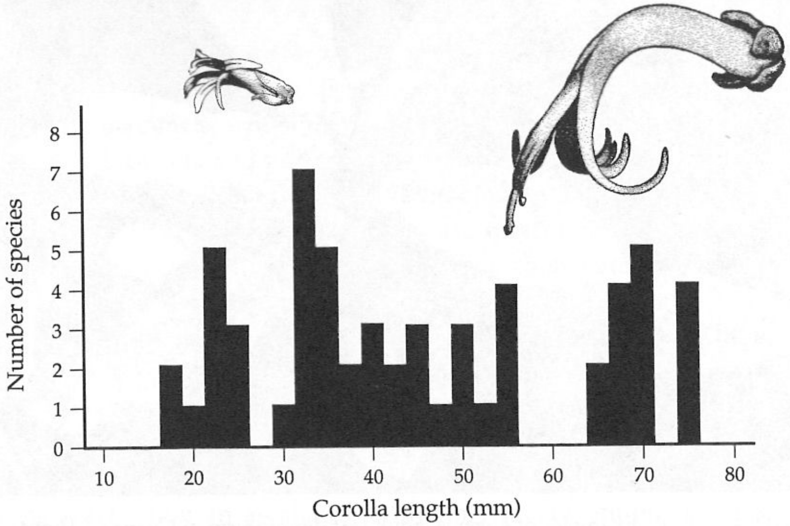


FIGURE 14.5. Interspecific variation in typical corolla tube length in *Cyanea* and *Rollandia;* values are means of maximum and minimum lengths reported by Lammers (1990a). Representative flowers shown are those of *C. fissa* from Kaua‘i (*left*) and *C. superba* from O‘ahu.

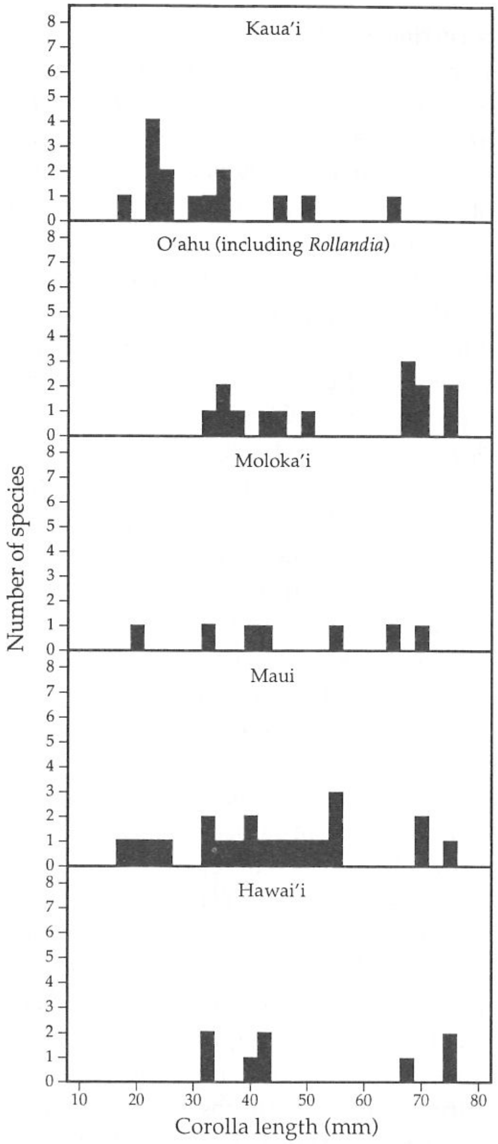


FIGURE 14.6. Distribution of corolla tube lengths in species of *Cyanea* and *Rollandia* native to each of the six high islands in the Hawaiian archipelago. The average length of corollas on Kaua‘i is significantly less ($P < .05$; two-tailed t tests) than that seen on the other islands.

Lammers and Freeman (1986) revealed that most lobelioids have a nectar sugar profile typical for bird pollination, except *Brighamia* (see Figure 14.1F), which seems likely to be pollinated by hawk moths.

Clermontia, with 22 species, is the second largest Hawaiian genus of lobelioids. It differs from *Cyanea* in having reduced inflorescences, possessing a repeatedly branched, shrubby habit (see Figure 14.1G), and occurring mainly in forest edges and gaps rather than shaded understories. A few species (mainly on Hawai'i) are epiphytic. The orange fruits of *Clermontia* are unusually large, 20 to 40 mm in diameter, larger than those typical of all other fleshy-fruited lobelioid genera; the pericarp (rind) is also unusually thick. The flowers are cleft all the way to the base of the corolla, suggesting that they may have excluded fewer potential pollinators than flowers of comparable size in *Cyanea*. Flowers range from less than 20 mm long in some species (e.g., *Clermontia parviflora*) to 80 mm long in others (e.g., *C. grandiflora*). Flower colors are generally pink, greenish, or whitish, but several species on Hawai'i have spectacular, wine-red corollas (e.g., *C. drepanomorpha*). In the latter and most other species of *Clermontia,* the sepals resemble the petals, giving the flowers a "doubled" appearance.

Rollandia is a small genus (six extant species, two extinct) of treelets now restricted to O'ahu, generally found in the understory of mesic to wet forests at middle elevations (see Figure 14.1H). The flowers are similar to those of *Cyanea,* but have the staminal column fused to the corolla (Lammers, 1990a). One extraordinary species (*R. st.-johnii*) is a compact, prickly treelet hugging some of the most remote and windswept ridges of east O'ahu.

Finally, *Delissea* is a relictual genus, of which only four of nine species now survive. It has an unusual growth form, with a rather narrow crown atop a tall, slender stem (see Figure 14.1I). *Delissea* species apparently occurred in rather open dry forest as well as mesic forest (Rock, 1919). The flowers differ from *Cyanea* in having one or more knoblike projections on the corolla.

Clermontia, Cyanea, Delissea, and *Rollandia* share fleshy fruits, axillary inflorescences, and a woody habit, an unusual combination among lobelioids worldwide. This has led many authors to conclude that the group is monophyletic. The most widely held view (advanced by Rock, 1919) is that their closest relative is either *Centropogon* or *Burmeistera,* both shrubs with fleshy fruits from cloud forests in Central and South America. However, Lammers (1985) proposed that certain woody species of *Pratia* section *Collensoa* from Borneo, with fleshy fruits and axillary inflorescences, may be more closely related.

With regard to the three capsular genera of Hawaiian lobelioids, Rock (1919) suggested that the closest relative of *Brighamia* may be *Sclerotheca* or *Apetahia,* two closely related genera of shrubs with capsular fruits from Tahiti, or possibly the herbaceous genus *Isotoma* from Australia. One to three additional colonization events have been proposed to account for the origin of *Trematolobelia* and the two Hawaiian sections of *Lobelia* (Rock, 1919; Skottsberg, 1928; Wimmer, 1943; Mabberley, 1974, 1975; Lammers, 1990a). Mabberley (1975) suggested that *Trematolobelia* may have been derived from the endemic Hawaiian *Lobelia* section *Galeatella* and that both are closely related to East Asian, Brazilian, or East African species of *Lobelia.*

MOLECULAR EVOLUTION AND PHYLOGENETIC RELATIONSHIPS

Methods and Taxa Studied

To date, we have surveyed cpDNA restriction site variation in 76 species using nine enzymes, examining all extant Hawaiian species of *Brighamia, Lobelia, Rollandia,* and *Trematolobelia;* 19 of 21 extant species of *Clermontia;* 2 of 4 extant species of *Delissea;* and 24 representative species of *Cyanea.* The latter group was chosen to sample variation within *Cyanea,* including 44% of all species (59% of extant species) and at least three species of each section recognized by Rock (1919). Our cladistic analysis used global parsimony (Maddison et al., 1984) using PAUP version 3.0s (Swofford, 1991), with outgroups consisting of representative species of *Burmeistera* and *Centropogon* from South America; *Pratia borneensis* Hemsl. from Borneo; *Sclerotheca jayorum* Raynal from Tahiti, and *Lobelia giberroa* Hemsl. from the East African highlands.

Total DNA was extracted from fresh and –80°C frozen leaf tissue, using either a modified cetyltrimethylammonium bromide (CTAB) procedure or CTAB applied to an organellar extract (Smith et al., 1991). DNA was cleaved using nine restriction endonucleases (*BamH* I, *Bcl* I, *Bgl* II, *Cla* I, *Dra* I, *EcoR* I, *Nci* I, *Xba* I, *Xmn* I) known to generate sites with a high rate of divergence among closely related taxa. Cleaved DNA was size-separated by electrophoresis on agarose gels, transferred to nylon filters, and probed with heterologous cpDNA clones to recognize restriction site variation through comparisons of autoradiograms using standard procedures (Sytsma and Smith, 1988). To ensure colinearity of autologous and heterologous cpDNA, we used small mapped *Nicotiana* cpDNA clones (kindly supplied by S. Downie and R. Palmer), using combinations (Table 14.2) designed to take into account the cpDNA inversions mapped in Hawaiian lobelioids and our outgroups by Knox et al. (1993).

TABLE 14.2. Coordinates (modified from Knox et al., 1993) of *Nicotiana* cpDNA Clone Combinations Used to Probe Total DNAs of Hawaiian Lobelioids

Probe	Coordinates (kb)			Region
1	86.1	→	74.2	Large single-copy region
2	74.2	→	60.9	Large single-copy region
3	53.6	→	43.4	Large single-copy region
4	43.4	→	31.9	Large single-copy region
5	31.9	→	21.9	Large single-copy region
6	21.9	→	12.3	Large single-copy region
7	12.3	→	2.8	Large single-copy region
8	53.6	→	60.7	Large single-copy region
9	111.9	→	118.6	Small single-copy region
10	118.6	→	130.6	Small single-copy region

We have now analyzed restriction site variation within the large and small single-copy regions of the chloroplast genome for the 76 species mentioned above and have begun analysis of an additional 21 species of *Cyanea*. Excluding the latter, we have detected 331 mutations thus far, of which 206 are phylogenetically informative. A complete analysis of the origins and relationships of the Hawaiian lobelioids based on this data set will be published elsewhere. Here, we will outline two results crucial to understanding adaptive radiation and speciation within *Cyanea:* monophyly and relationships of the baccate genera of Hawaiian lobelioids and relationships among the species of *Cyanea.*

Phylogeny of the Baccate Hawaiian Lobelioids

Cladistic analysis identified the baccate lobelioids as a monophyletic group, with the unexpected inclusion of the cliff succulent *Brighamia* (Figure 14.7). The cladogram shown does not incorporate the large amount of genetic variation detected within *Cyanea,* represented for the moment by a single species. This has ramifications for the position of *Rollandia* (see below), but otherwise, Figure 14.7 is fully reflective of the relationships seen among all baccate species surveyed.

One of our most surprising results is that the closest relative of *Brighamia* is *Delissea,* a genus often considered on morphological grounds to have diverged from the common ancestor of the remaining baccate genera at an early date (Rock, 1919). The association of *Brighamia* with *Delissea*—strongly supported by our molecular data—may not be so surprising, at least in hindsight: They are the only Hawaiian lobelioids that share large (ca. 1.5 mm) whitish seeds and have curiously narrow crowns for their height (see Figure 14.1F and I). In retrospect, the association of *Brighamia* with the fleshy-fruited genera may also not be that surprising; it too has axillary inflorescences and fruits that are quite fleshy early in development (Lammers, 1989) but that eventually develop into dry capsules, suggesting that *Brighamia* first gained and then secondarily lost fleshy fruits during evolution. These shared morphological characters strengthen the molecular conclusion, supported by only one cpDNA mutation, that *Brighamia-Delissea* is the sister group to the *Clermontia-Cyanea-Rollandia* clade and that their common ancestor diverged from the common ancestor of *Clermontia, Cyanea,* and *Rollandia* before the latter diverged from each other. Clearly, the decision by St.

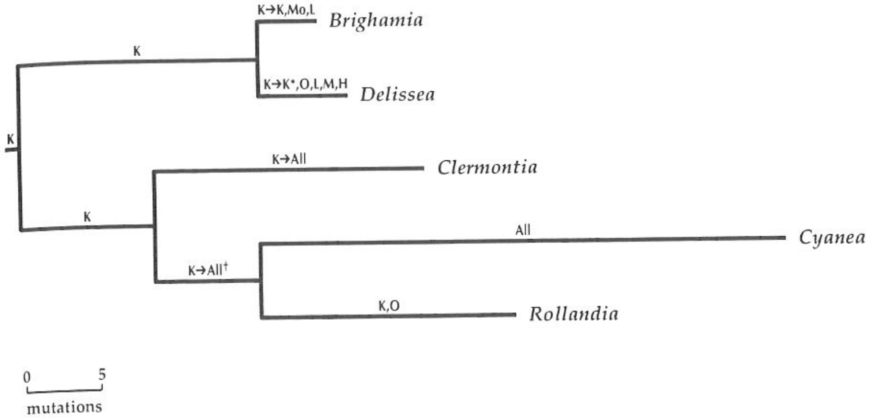


FIGURE 14.7. Phylogram showing cladistic relationships among the fleshy-fruited Hawaiian lobelioids (including *Brighamia*), based on cpDNA restriction site variation. Horizontal length of each branch is proportional to the number of mutations between taxa and inferred ancestors. Abbreviations refer to actual island distributions of current taxa and inferred island distributions of ancestral taxa (K, Kaua'i; O, O'ahu; L, Lana'i; Mo, Moloka'i; H, Hawai'i; M, Maui). Basal condition (*) for *Delissea* was inferred from distribution of subgenera; ancestral condition for *Cyanea-Rollandia* (†) was taken as *Cyanea*'s current condition, given that *Rollandia* is embedded within it (see Figure 14.8). The clade shown is a complete monophyletic sublineage from a broader analysis including *Burmeistera, Centropogon, Lobelia giberroa, Pratia,* and *Sclerotheca* as outgroups, and *Lobelia niihauensis, L. gloria-montis,* and *Trematolobelia macrostachys* as additional ingroups (see text). The consistency index for the broader analysis is 0.950 (0.833 excluding autapomorphies); PAUP version 3.0s identified a single most-parsimonious tree using the branch-and-bound search strategy and DELTRAN option.

John and Takeuchi (1987) and St. John (1987) to merge *Delissea* with *Cyanea* while retaining *Clermontia* and *Rollandia* cannot be justified on molecular grounds.

Clermontia is the sister group to *Cyanea-Rollandia* (Figure 14.7). This arrangement accords with the traditional view, based on comparative morphology, that *Rollandia* is closely related to *Cyanea* and that *Clermontia* is more distantly related (Rock, 1919; Carlquist, 1965; Lammers, 1991; see also Lammers, this volume, Chapter 15). The monophyly of the *Clermontia-Cyanea-Rollandia* clade is strongly supported by the molecular data.

Evolutionary Relationships within *Cyanea*

Within *Cyanea,* cpDNA restriction site variation among extant species is greater than that seen in any of the other Hawaiian lobelioid genera or endemic sections of *Lobelia.* Using *Brighamia insignis* and *Clermontia arborescens* as outgroups, we conducted a cladistic analysis of *Cyanea* and *Rollandia* (see Table 14.3 for voucher data). One hundred fifty variable restriction sites in this group were detected, of which 78 were phylogenetically informative (Appendix 14.1). A single most-parsimonious tree was identified using PAUP version 3.0s, with the heuristic search strategy and ACCTRAN optimization option (Figure 14.8).

Cyanea divides naturally into two clades, each defined by several synapomorphies; both are well supported by the cpDNA data, based on bootstrap and decay analyses (Figure 14.8). *Rollandia* is imbedded within the first clade. It has therefore been taxonomically submerged into *Cyanea* (Lammers et al., 1993), making it the largest angiosperm genus in the Hawaiian archipelago, with 63 species. *Rollandia* is the sister group to *C. acuminata* and *C. grimesiana* from O'ahu, suggesting an origin for *Rollandia* on O'ahu (to which all species of *Rollandia* are endemic, except the extinct *R. parvifolia* from Kaua'i). Insofar as our analysis supports an earlier origin of *Clermontia* than of *Rollandia* (see Figure 14.7), it implies either that both genera have evolved recently, if one accepts the theory of an origin for *Clermontia* on Hawai'i (Lammers, 1991; see also Lammers, this volume, Chapter 15), or that *Clermontia* arose on a now submerged or eroded island considerably older than Kaua'i and that differential dispersal, speciation, and extinction has led to its greater diversity today on Maui and Hawai'i (see further discussion below).

What is the systematic significance of our cpDNA restriction site phylogeny for relationships within *Cyanea*? The five sections of *Cyanea*

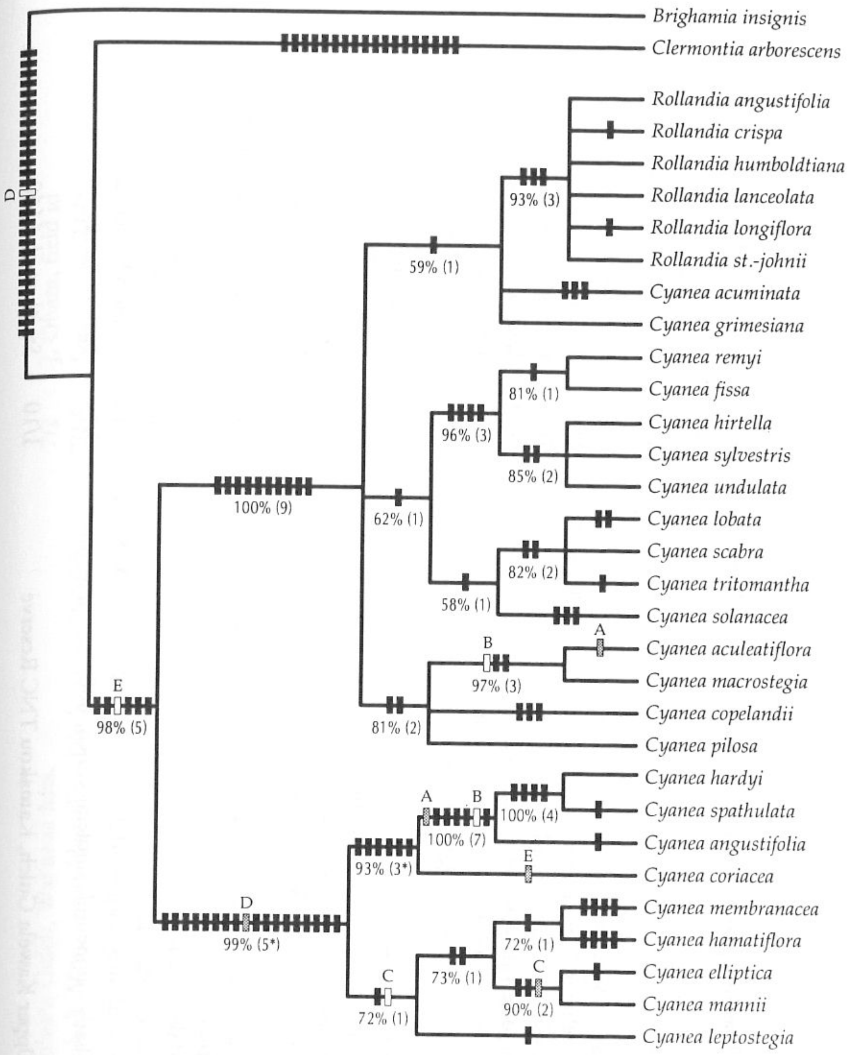


FIGURE 14.8. Cladogram showing phylogenetic relationships within *Cyanea-Rollandia,* using *Brighamia insignis* and *Clermontia arborescens* as outgroups. Consistency index = 0.974 with autapomorphies (154 steps) and 0.951 without autapomorphies (82 steps). Letters A to E identify pairs of convergent mutations. *Vertical bars* indicate synapomorphies, reflecting shared derived cpDNA restriction site gains or losses. *Hollow bars* indicate convergent site losses; *gray bars,* convergent site gains; and *solid bars,* unique site gains or losses. Bootstrap values (Felsenstein, 1985) below each node indicate the percentage of random resamplings of the cpDNA data that generate phylogenies preserving that node; decay analysis values (shown in parentheses; see Bremer, 1988) indicate the number of additional steps beyond the most-parsimonious tree needed to lose resolution at a node. Both indices reflect the relative level of support for a particular node. *Asterisks* indicate decay analysis values that are strongly affected by missing data for *C. coriacea.*

TABLE 14.3. Sources of Leaf Tissue Used in cpDNA Restriction Site Analysis of Relationships within *Cyanea* and *Rollandia*

Species	Island[a]	Location and elevation	Date[b]	Voucher or field identification[c]
Brighamia insignis	K	Kaua'i Museum, Lihu'e	1/6	*Givnish & Sytsma 3001*
Clermontia arborescens	Mo	Pepe'opae Trail, Kamakou TNC Reserve, 1,200 m	1/10	T. Givnish, field id
Cyanea aculeatiflora	M	Waikamoi Flume, E. Maui, 1,275 m	1/14	*Givnish & Sytsma 3002*
Cyanea acuminata	O	Hidden Valley, Ko'olau Mts., 425 m	6/30	J. Obata, field id
Cyanea angustifolia	O	Halawa Trail, Ko'olau Mts., 550 m	8/27	J. Obata, field id
Cyanea copelandii subsp. *haleakalaensis*	M	Lower Waikamoi Flume, E. Maui, 1,100 m	1/14	*Givnish & Sytsma 3003*
Cyanea coriacea	K	Wainiha Valley, northern coast	8/16	*Flynn 3492*
Cyanea elliptica	L	Upper Maunalei, in unnamed feeder ditch, 875 m	9/19	*Hobdy 3084*
Cyanea fissa	K	Wahiawa stream valley, 675 m	7/19	*Flynn 3460*
Cyanea grimesiana subsp. *obatae*	O	Kalua'a Gulch, Wai'anae Mts.	1/1	*Givnish & Sytsma 3004*
Cyanea hamatiflora	M	Ravine at head of Waikamoi Flume, E. Maui, 1,275 m	1/14	*Givnish & Sytsma 3005*
Cyanea hardyi	K	Anahola stream valley	1/4	*Smith 1151*
Cyanea hirtella	K	Alaka'i Swamp Trail, 1,150 m	7/20	*Smith et al. 2179*
Cyanea leptostegia	K	Koke'e State Park	1/6	*Smith et al. 1135*
Cyanea lobata	M	Juvenile near former adult population, Waikapu, W. Maui	1/14	R. Hobdy, field id
Cyanea macrostegia subsp. *gibsonii*	L	Shady ravine in Kaiholena Gulch, 800 m	9/19	*Hobdy 3088*
Cyanea mannii	Mo	Upper Kawela Gulch, Kamakou TNC Reserve	1/10	*Smith et al. 1160*
Cyanea membranacea	O	Kalua'a Gulch, Wai'anae Mts.	7/2	J. Obata, field id
Cyanea pilosa	H	Kohala Mts., upper Hamakua Ditch, above Pu'ulehua reservoir, 925 m	7/14	*Smith et al. 2145*
Cyanea remyi	K	Wahiawa stream valley, 675 m	7/19	*Givnish & Sytsma 3006*
Cyanea scabra	M	Hana'ula Trail, W. Maui, 1,025–1,125 m	1/11	R. Hobdy, field id
Cyanea solanacea	Mo	Upper Kawela Gulch, Kamakou TNC Reserve	1/10	*Givnish & Sytsma 3007*
Cyanea spathulata	K	Alaka'i Swamp Trail	1/2	*Smith et al. 1140*
Cyanea sylvestris	K	Hoary Head ridge, near Knudsen Gap	1/3	*Smith & Flynn 1146*
Cyanea tritomantha	H	Kohala Mts., Hamakua district	7/18	L. Stemmermann, field id
Cyanea undulata	K	Wahiawa stream valley, 675 m	7/19	*Smith et al. 2191*
Rollandia angustifolia	O	Halawa Trail, 550 m	8/27	*Smith 2209*
Rollandia crispa	O	Hidden Valley, Ko'olau Mts., 425 m	1/8	*Givnish 1038*
Rollandia humboldtiana	O	Moanalua Ridge, Ko'olau Mts.	1/2	J. Obata, field id
Rollandia lanceolata	O	Kalua'a Gulch, Wai'anae Mts.	1/1	J. Obata, field id
Rollandia longiflora	O	Pahole Gulch, Wai'anae Mts.	1/9	*Givnish 1039*
Rollandia st.-johnii	O	Summit crest between 'Aiea and Halawa trails, Ko'olau Mts., 850 m	8/27	*Smith et al. 2208*

[a] K, Kaua'i; Mo, Moloka'i; M, Maui; O, O'ahu; L, Lana'i; H, Hawai'i.

[b] Month/day (all in 1989).

[c] Given the unbranched habit and endangered status of several species and the fact that many well-known populations have been previously vouchered, in several instances we have relied as noted on field identification by our informants (supported by color slides in many cases). Vouchers are deposited at WIS.

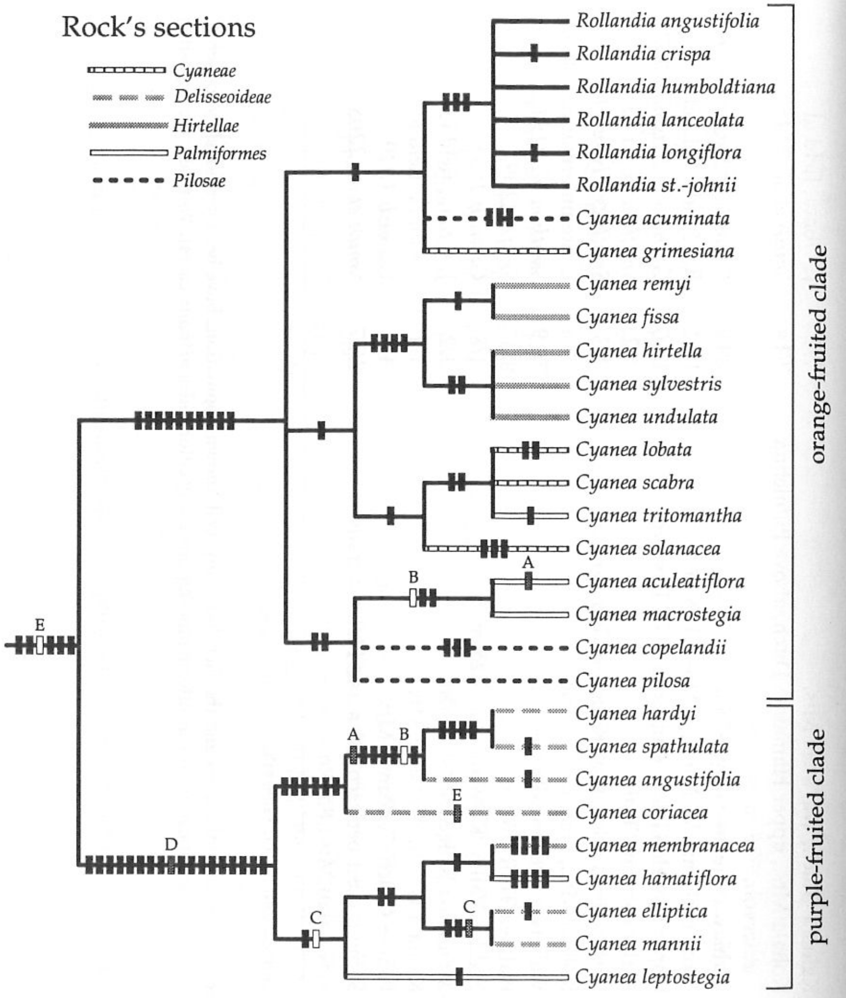


FIGURE 14.9. Morphologically defined sections of *Cyanea* (Rock, 1919) superimposed on the cpDNA phylogeny. See Figure 14.8 for explanation of symbols.

recognized by Rock (1919) on morphological grounds show only partial concordance with our molecular phylogeny (Figure 14.9). Section *Hirtellae* (unbranched treelets or small trees from Kaua'i characterized by a tomentose corolla and calyx lobes at least as long as the calyx tube) form a monophyletic group in the cpDNA analysis, provided that the recently rediscovered *C. remyi* (poorly known to Rock) is included (Figure 14.9). Section *Delisseoideae* (characterized by minute calyx lobes like those of *Delissea*) fall into the second clade shown in Figure 14.9, but two nondelisseoid, palmlike species (*C. hamatiflora, C. leptostegia*) are also included.

Palmlike species (section *Palmiformes*) appear to have arisen on at least three separate occasions and apparently do not represent a natural group. Likewise, neither section *Cyaneae* (midsized shrubs and trees with leaves that are often toothed or divided, and with smooth corollas and

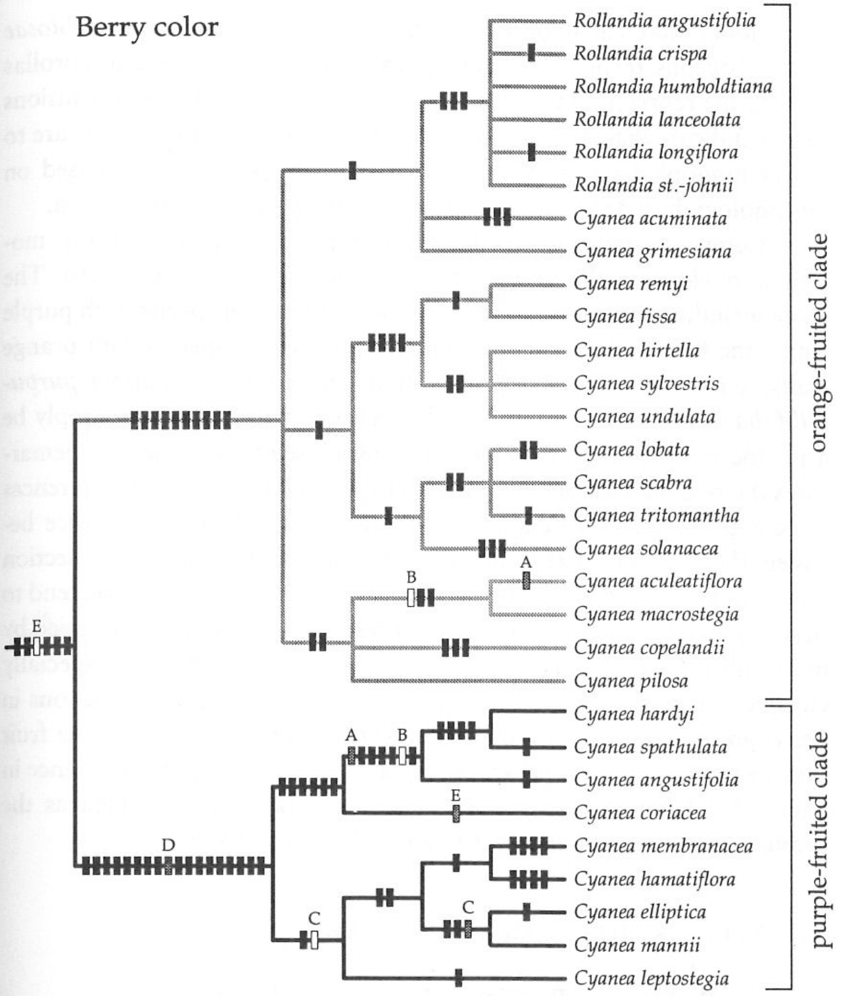


FIGURE 14.10. Berry color superimposed on phylogeny. Orange fruits, gray; purple fruits, black. *Cyanea leptostegia* has purple fruits fide Carlquist, 1974; S. Carlquist, unpubl.; T. G. Lammers, unpubl.; cf. Lammers, 1990a, and E. Knox, unpubl. See Figure 14.8 for explanation of symbols.

calyx lobes that equal or exceed the calyx tube) nor section *Pilosae* (subherbaceous treelets with similar calyx lobes and pubescent corollas and leaves) represent natural groupings (Figure 14.9). These conclusions clearly indicate that the sections of *Cyanea* must be revised if they are to reflect monophyletic groupings and that analyses of *Cyanea* based on morphology-based taxonomic schemes must be viewed with caution.

Certain morphological characters map fairly cleanly onto our molecular phylogeny; the best example is fruit color (Figure 14.10). The clade including the delisseoids is composed entirely of species with purple fruits; the first clade is composed almost entirely of species with orange fruits, with the presumed exception of the extinct *Rollandia purpurellifolia* (Lammers, 1990a). This difference in fruit color may simply be a phylogenetic quirk, a character of minimal selective value that demarcates the two main clades in *Cyanea*. However, it might reflect differences in ecology between the clades and represent an adaptive difference between them. Rock (1919) noted that many of the species of section *Delisseoideae*, most of which are sparsely branched from the base, tend to occur in forest edges or in drier, more open forests than those occupied by most other *Cyanea* species. Although purple fruits may be especially conspicuous to birds in such sites, they may tend to be inconspicuous in dense green shade under closed canopies, where a brighter, orange fruit may be more attractive. Whatever its basis, the characteristic difference in fruit color between clades makes it convenient to denote them as the *orange-fruited* and the *purple-fruited* clades, respectively.

ADAPTIVE RADIATION IN *CYANEA*

Adaptive Radiation in Relation to Flower Tube Length

We classified species of *Cyanea* and *Rollandia* into four classes based on typical corolla tube length (average of the minimum and maximum values given by Lammers, 1990a) and then superimposed flower length on phylogeny (Figure 14.11). Two patterns emerged. Perhaps the most obvious is that there is no consistent tendency for flower size to increase or decrease within a lineage (i.e., flower size is evolutionarily quite labile within *Cyanea*). This accords with some of our preliminary results (data not shown) for the genus *Clermontia*, in which the closest relative of *C. grandiflora* (with flowers among the largest in the genus) is *C. micrantha* (with flowers among the smallest).

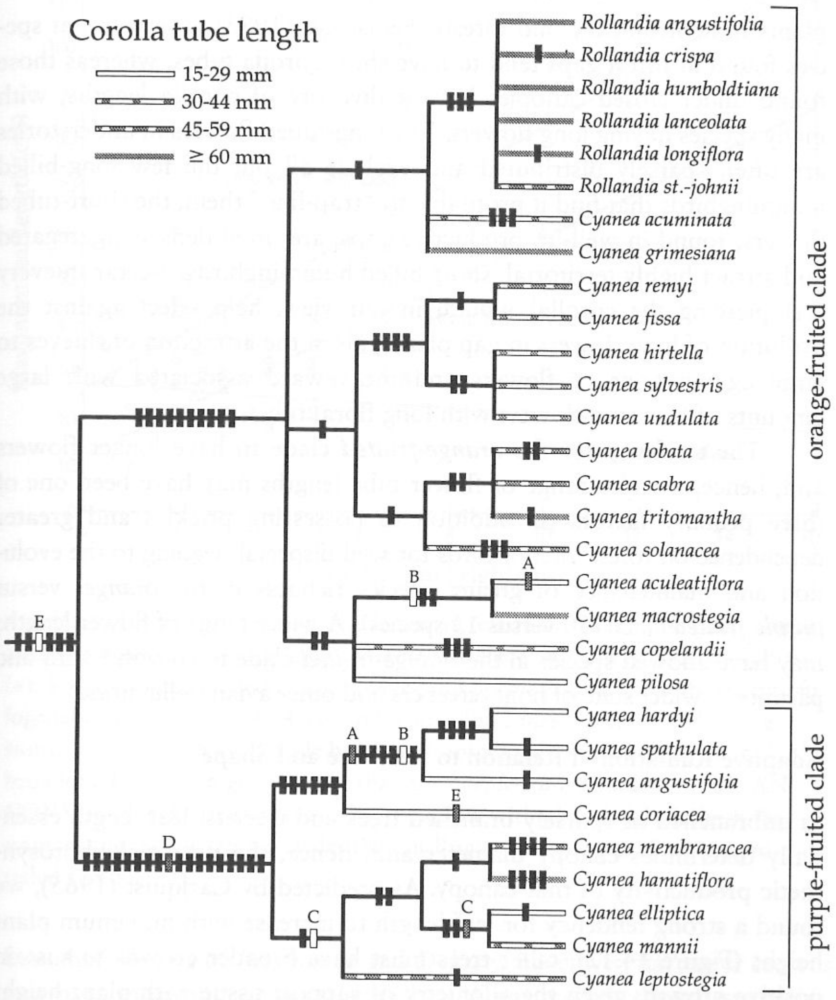


FIGURE 14.11. Corolla tube length superimposed on phylogeny. Values represent average of the minimum and maximum tube lengths reported by Lammers (1990a). See Figure 14.8 for explanation of symbols.

The *orange-fruited* clade does, however, show a greater range of variation in flower size, with a significantly greater average corolla tube length (49.5 ± 16.5 mm versus 35.7 ± 20.3 mm, $P < .05$ for a two-tailed t test, 55 d.f.). Members of this clade appear to inhabit somewhat shadier sites than those occupied by the *purple-fruited* clade (see above). This result may parallel certain findings regarding hummingbird-pollinated plants in neotropical cloud forests. Feinsinger (1983) observed that species found in forest gaps tend to have short corolla tubes, whereas those found under closed canopies have a diversity of corolla lengths, with many species having long flowers. The long-tubed flowers in understories are often sparsely distributed and exclude all but the few long-billed hummingbirds that find it profitable to "trap-line" them; the short-tubed flowers, found in well-lit, productive gaps, are often densely aggregated and attract highly territorial, short-billed hummingbirds. Nectar thievery (via piercing the corolla) would, in our view, help select against the evolution of long flowers in gap plants, given the attraction of thieves to large aggregations of flowers and the reward associated with large amounts of nectar in flowers with long floral tubes.

The tendency for the *orange-fruited* clade to have longer flowers and, hence, a wider range of flower tube lengths may have been one of three primary factors (in addition to possessing prickles and greater dependence on forest interior birds for seed dispersal) leading to the evolution and maintenance of greater species richness in the *orange-* versus *purple-fruited* clade (50 versus 13 species). A wider range of flower lengths may have allowed species in the *orange-fruited* clade to coevolve with and partition a wider suite of honeycreepers and other avian pollinators.

Adaptive Radiation in Relation to Leaf Size and Shape

In unbranched or sparsely branched trees and treelets, leaf length essentially determines canopy diameter and, hence, the potential photosynthetic productivity of that canopy. As predicted by Carlquist (1965), we found a strong tendency for leaf length to increase with maximum plant height (Figure 14.12); taller trees must have broader crowns to sustain positive growth, given the allometry of support tissue with plant height (Givnish, 1988).

Species in the *orange-fruited* clade tend to have longer leaves (and broader crowns) at a given maximum height than do species in the *purple-fruited* clade (Figure 14.12). (A more detailed analysis of this pattern that incorporates phylogenetic relationships will be conducted with T. Garland.) This accords with the view that the *purple-fruited* species generally occupy sunnier sites than do the *orange-fruited* species and thus would require less photosynthetic tissue to support growth at a given height; to the extent that these sunnier sites are also drier (Rock, 1919), the *purple-fruited* species may also simply be unable to support the transpirational load associated with broader canopies. The unbranched species of *Brighamia* and *Delissea* have even narrower crowns at a given maximum height (Figure 14.12), particularly the tallest species, in accord with their occurrence in even more open and arid habitats.

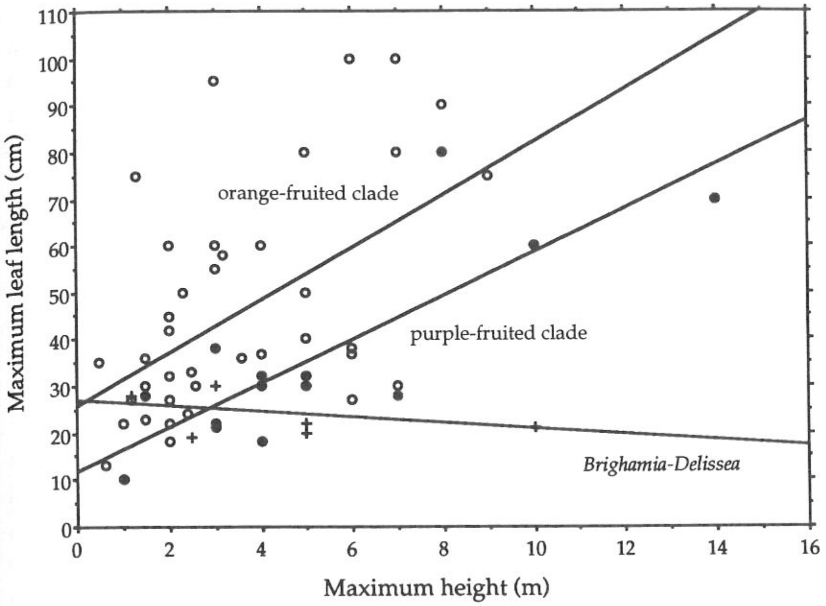


FIGURE 14.12. Maximum leaf length in relation to maximum plant height in the *orange-fruited* clade (○), *purple-fruited* clade (●), and *Brighamia-Delissea* (+). Species were assigned to the *orange-* and *purple-fruited* clades based on phylogenetic analysis (Figure 14.10) and fruit color. *Lines* represent LMS regressions. The *orange-fruited* clade has significantly longer leaves (and hence, broader crowns) at a given height than the *purple-fruited* clade ($P < .05$, ANCOVA), and leaf length increases more rapidly with plant height in both the *orange-* and *purple-fruited* clades than in *Brighamia-Delissea* ($P < .01$, two-tailed *t* tests).

Deeply toothed, lobed, or compound leaves on adult plants are restricted to the *orange-fruited* clade, based on the species surveyed to date and the berry color of the remaining taxa (Table 14.4). Such divided adult leaves are strongly associated with developmental heterophylly and vice versa, with the juvenile leaves being more deeply divided. Carlquist (1965) suggested that divided leaves may serve to spread the foliage in a thinner (and broader) presentation and thus be adaptive in shady areas. This argument does not seem compelling, as there is no obvious constraint (e.g., constant leaf tissue volume or mass) that would tie the cross-sectional thickness of leaves to area, and divided *Cyanea* leaves are

TABLE 14.4. Co-occurence of Prickles, Marked Juvenile–Adult Heterophylly, and Divided (Coarsely Toothed, Lobed, or Compound) Adult Leaves in *Cyanea* and *Rollandia*

Species	Prickles	Heterophylly	Divided adult leaves
C. aculeatiflora	×		
C. asplenifolia	×	×	×
C. grimesiana	×		×
C. horrida	×	×	×
C. leptostegia		×	
C. lobata	×	×	×
C. macrostegia[a]		×	
C. marksii	×	×	
C. mceldowneyi	×	×	
C. pinnatifida	×	×	×
C. platyphylla	×	×	
C. quercifolia	×	×	×
C. scabra	×		
C. shipmanii	×	×	×
C. solanacea	×	×	×
C. solenocalyx	×	×	
C. stictophylla	×	×	×
C. tritomantha	×	×	
C. truncata	×		
R. lanceolata	×	×	
R. longiflora	×	×	
R. st.-johnii	×		

Sources: Data compiled from Rock (1919) and Lammers (1990a, 1992).
[a] *Cyanea macrostegia* is roughly hispid but has no thornlike prickles.

generally not large enough to have a significant effect on the capture of sunflecks (Givnish et al., 1994).

We propose two alternative hypotheses to account for the significance of divided foliage in *Cyanea*. First, toothed, lobed, or deeply divided foliage in mature or juvenile stages may have evolved in *Cyanea* species exposed to low light levels, in parallel with the general tendencies for shade-adapted foliage to be thin (see review by Givnish, 1988), and for non-entire leaf margins to be associated with thin leaf cross sections, thus optimizing the area supported and supplied by leaf veins (Givnish, 1979). This hypothesis would account for the association of divided foliage with juveniles and with adults in the *orange-fruited* clade, in accord with the view that the latter is adapted to somewhat shadier conditions than the *purple-fruited* clade. It would also provide an explanation for the neotenic evolution of leaf form in *C. solanacea* and related species and races documented by Lammers (1990b).

Lammers (1990b) argued that, in a series of five species and local races occupying progressively younger terrain from Moloka‘i to eastern Hawai‘i, there is a strong tendency for leaf outlines of each taxon to be more deeply divided than those of the corresponding adults and for the leaf outlines of the adults of each species to strongly resemble those of juveniles of the preceding species in the series (Figure 14.13). If juvenile plants of forest trees and treelets are exposed to lower light levels than adults of the same species, retention of the juvenile growth form in the neotenic adults of a daughter species should make it more fit for growth in shadier microsites than the adults of the mother species, and its juveniles should have even more deeply divided foliage. The extreme endpoint of Lammers's neotenic series, the doubly compound-leaved *Cyanea shipmanii,* occurs in extremely shady ravines on Hawai‘i (Carlquist, 1980).

A second hypothesis for the evolution of divided foliage in juveniles and the orange-fruited clade would be that divided foliage served to deceive visually oriented avian browsers (now extinct) by presenting a nonlobelioid leaf outline (Givnish, 1990; Givnish et al., 1994). Fifteen of the 17 species showing marked developmental heterophylly are also armed with prickles on their juveniles leaves or stems (Table 14.4), strongly suggesting that heterophylly may indeed have been involved in defense against herbivory (Givnish et al., 1994). This hypothesis may also provide a general explanation of insular heterophylly (Friedmann and Cadet, 1976), the relatively high incidence of juvenile–adult leaf dimorphism in the floras of New Zealand, New Caledonia, Madagascar, and the Mascarene Islands; each of these isolated oceanic islands and archipelagoes was populated by various groups of flightless birds (moas, *Sylviornis,* elephant birds, and possibly dodos, solitaires, or rails, respectively) that acted as the primary terrestrial browsers (Givnish et al., 1994).

The visual defense hypothesis is compatible with the development of juvenile foliage on short stems near the ground and on mechanically wounded stems, where the plants would be visible and accessible to large browsing birds. This hypothesis is consistent with an association of divided foliage with species of the *orange-fruited* clade, because selection for antiherbivore defenses should be particularly strong in shady, unpro-

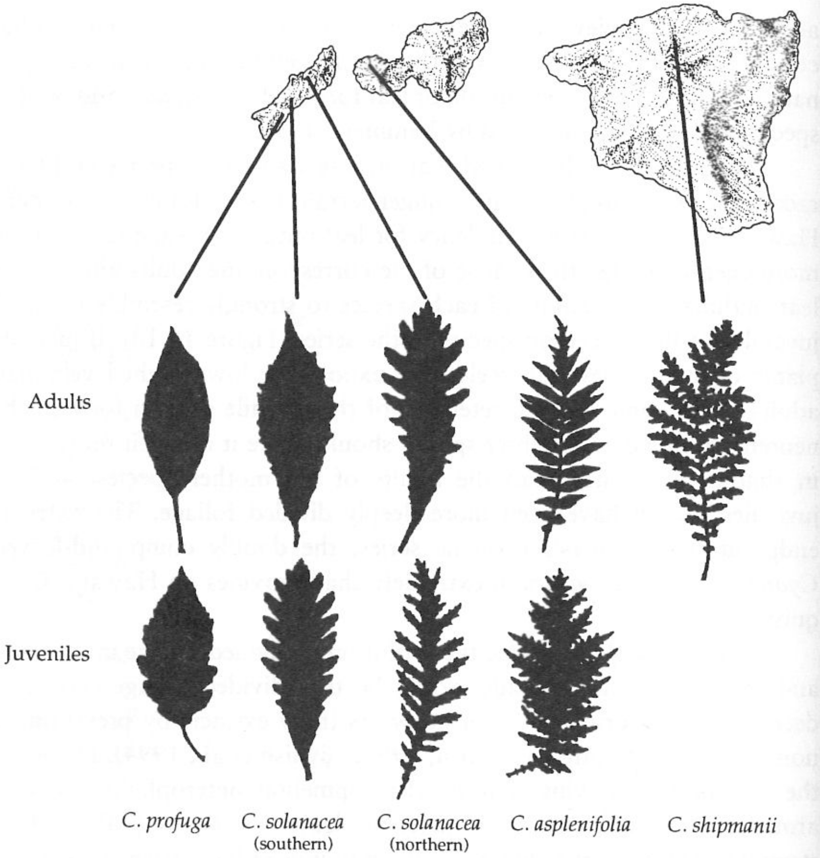


FIGURE 14.13. Apparent paedomorphosis of leaf form in a suite of species occupying a sequence of progressively younger terrains from southeastern Moloka'i to Mauna Kea on Hawai'i (redrawn from Lammers, 1990b). Note that the juvenile foliage of each species is more divided than the adult foliage of the same species and that the adult foliage of each species strongly resembles the juvenile foliage of the preceding species in the sequence.

ductive environments (Coley, 1983; Givnish, 1990). It is compatible, as well, with the production of divided foliage in shadier conditions, either because it is mechanically more efficient (see above) or because it might increase handling time or enhance visual mimicry of heavily defended tree ferns. Finally, selection for perfection of visual and mechanical defenses against herbivores could provide the selective force driving the neotenic evolution of leaf form and prickle development in the *Cyanea solanacea* clade (Figure 14.13).

Adaptive Radiation in Relation to Prickles

Muricate or aculeate stems and leaves are restricted to the *orange-fruited* clade among the species surveyed (Figure 14.14); they are known from 20 species in all (Table 14.4), none of which bear purple fruit. Prickles evolved at least four times within the *orange-fruited* clade: in the *Cyanea*

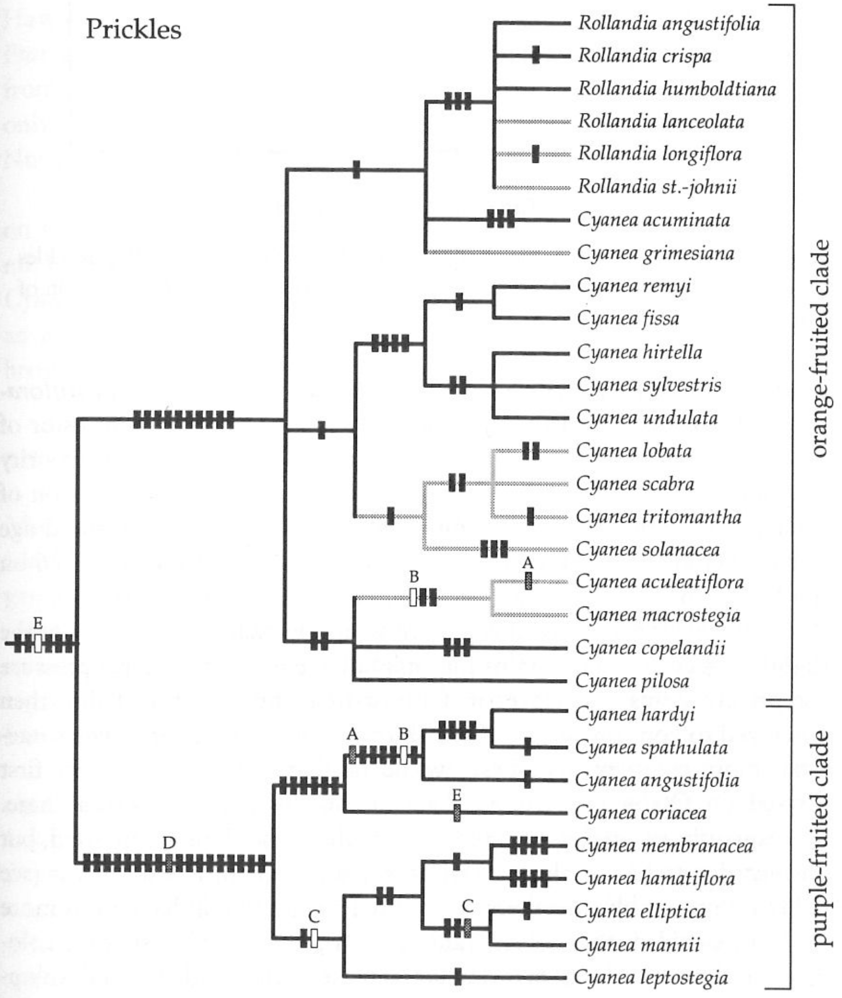


FIGURE 14.14. Possession of prickles (*gray*) superimposed on the cpDNA phylogeny of *Cyanea-Rollandia*. Prickles arose at least four times independently (indicated in *gray*), involving two origins each on O'ahu and Maui (see Figure 14.16). See Figure 14.8 for explanation of symbols.

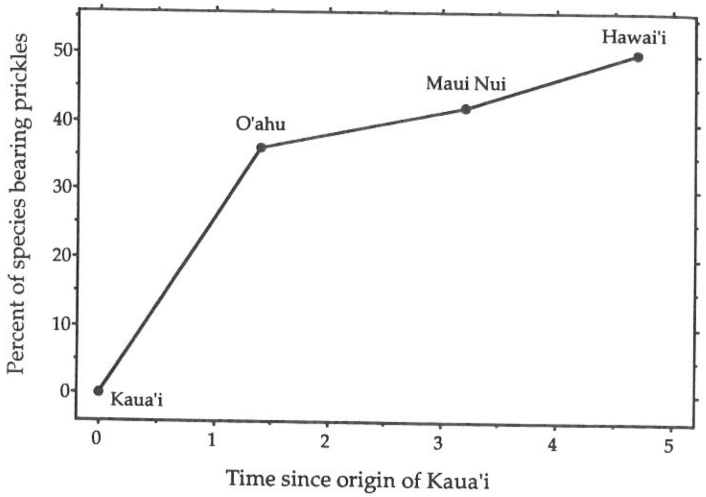


FIGURE 14.15. Percentage of species of *Cyanea-Rollandia* bearing prickles as a function of the time of island origin, in millions of years since the origin of Kaua'i.

solanacea-lobata-scabra-tritomantha subclade; in the *C. aculeatiflora-macrostegia* subclade; in *C. grimesiana;* and in the common ancestor of *Rollandia lanceolata, R. longiflora,* and *R. st.-johnii.* The great majority of prickly species occur on relatively young islands, and the fraction of such species (prickly per total) on each island decreases with island age (Givnish et al., 1994): 0 of 16 on Kaua'i, 5 of 14 on O'ahu, 11 of 26 on the Maui Nui complex, and 6 of 12 on Hawai'i (Figure 14.15).

One explanation for this pattern would be that the ancestors of the flightless geese and moa-nalos that might have exerted selective pressure for prickly stems and leaves in *Cyanea* first appeared on O'ahu, then colonized to younger islands (Givnish et al., 1994). An alternative, somewhat more complex hypothesis would be that the avian browsers first arrived on O'ahu and caused the evolution of prickly *Cyanea* there. Subsequently, the herbivores spread throughout the chain unopposed, but *Cyanea* dispersed mainly to newly formed islands to the southeast (see below). Presumably, members of prickly lineages would have been more likely to establish themselves, radiate, and produce colonists that colonized to the next island, favoring an increase in the incidence of developing prickles by species-level selection (Stanley, 1975), as well as by traditional natural selection operating within populations. Both mechanisms would require that herbivores first colonize O'ahu or at least first exert selective pressure on *Cyanea* there (Givnish et al., 1994).

In fact, one of the seven flightless avian browsers, *Chelychelynechen quassus,* occurred on Kaua'i (Olson and James, 1991). *Chelychelynechen* possessed a remarkable tortoiselike bill, unlike that of the other flightless geese and moa-nalos; we infer that it may have been more adapted to grazing than browsing. Other moa-nalos have mandibles with toothlike projections and are strongly decurved, which would increase the range of cutting forces exerted along their length (Olson and James, 1991). In the Hawaiian archipelago, the six flightless avian browsers (*Thambetochen, Ptaiochen, Geochen,* and unnamed taxa for which browsing is inferred from body size), excluding *Chelychelynechen* from Kaua'i, are known only from the younger islands, with two species each on O'ahu, the Maui Nui complex, and Hawai'i (Olson and James, 1991).

The relatively low number of species (six) in the purple-fruited clade on islands younger than Kaua'i may be because protective prickles did not evolve in this lineage. It is possible that the divided juvenile foliage of *Cyanea leptostegia,* a purple-fruited species from Kaua'i, may have arisen as a result of adaptation to shade rather than to visually orienting browsers.

PHYLOGENY IN RELATION TO GEOGRAPHY

Most species of *Cyanea* and *Rollandia* (57 of 63) are restricted to single islands, so the group provides outstanding material for the study of geographic speciation. Among the species studied thus far, there is a fairly strong association of phylogeny with geographic distribution, and some specific dispersal events—presumably a result of frugivorous birds carrying seeds internally from one island to another—are required to account for the observed pattern of diversification (Figure 14.16).

For example, within the *hardyi* sublineage of the *purple-fruited* clade, three of the four species (*Cyanea coriacea, C. hardyi, C. spathulata*) are known only from Kaua'i, whereas *C. angustifolia* is found on O'ahu, Moloka'i, Lana'i, and West Maui. For this clade, parsimony would indicate one inter-island dispersal event associated with speciation from Kaua'i to O'ahu followed by further colonization to younger islands without speciation.

The *hardyi* clade is also of interest in terms of how the species have diverged in order to coexist. All four species show very little divergence in habit, height, leaf length, or flower size (Lammers, 1990a); they essentially appear ecologically equivalent. Yet they differ dramatically in the

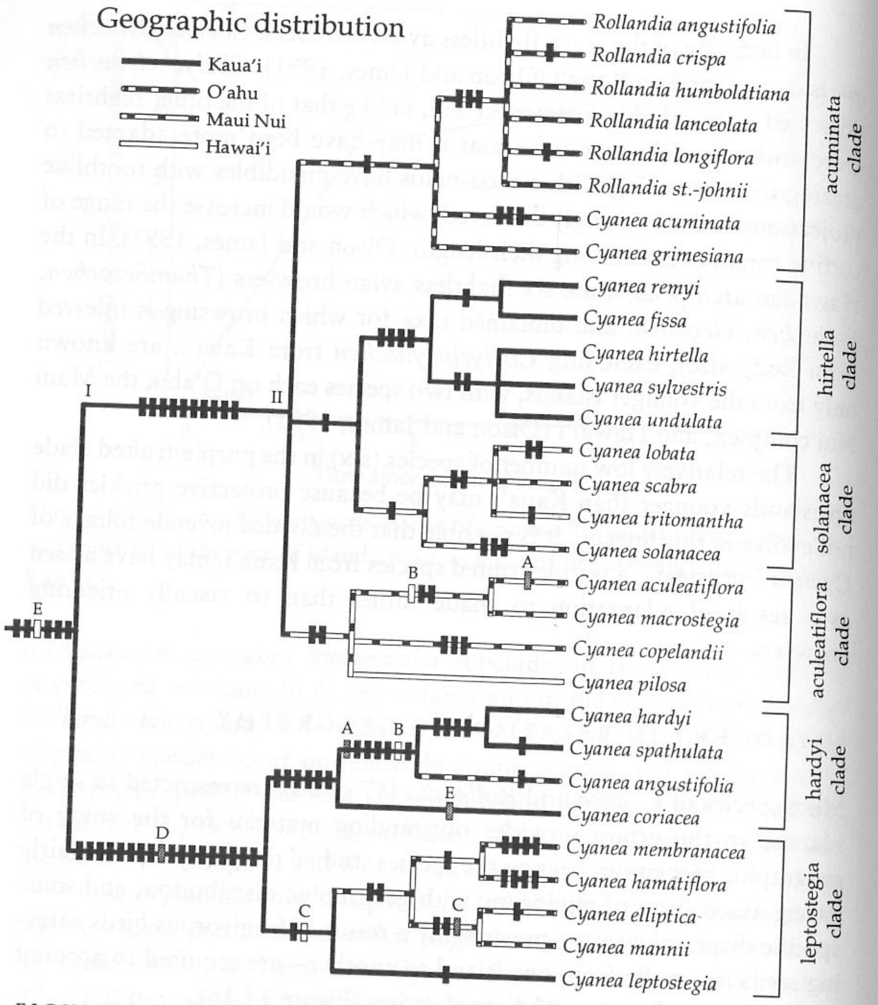


FIGURE 14.16. Geographic distribution superimposed on cpDNA phylogeny. See Figure 14.8 for explanation of symbols.

elevational ranges they occupy (Figure 14.17). The basal taxon (*Cyanea coriacea*) is restricted to low-elevation forests on Kaua'i. The next species (*C. angustifolia*) occurs from low to middle elevations on O'ahu. *Cyanea angustifolia* occupies an elevational range equivalent to that occupied by *C. coriacea* and *C. hardyi* on Kaua'i; these species partition that island elevationally, with *C. hardyi* restricted to middle elevations (300 to 700 m). Finally, *C. spathulata* occupies high-elevation habitats (700 to 1,200 m). Overall, this pattern suggests a taxon cycle (Wilson, 1961) leading from an initial invasion of low-elevation sites to progressive colonization of higher elevations, with divergence in elevational preference promoting the origin and maintenance of diversity within this clade. We do not know the phylogenetic position of *C. fauriei,* the last member of section *Delisseoideae* on Kaua'i, within the *hardyi* clade, where preliminary cpDNA data place it. However, the elevational range of *C. fauriei* straddles the gap between those of *C. coriacea* and *C. hardyi* (Lammers, 1990a). The leaves of *C. fauriei* are 4 to 9 cm wide, 6 to 12 cm wide in the low-elevation *C. coriacea,* 2.5 to 5 cm wide in the mid-elevation *C. hardyi,* and 1 to 3 cm wide in the high-elevation *C. spathulata*. This sequence of leaf width follows a typical trend of declining leaf width with increasing elevation on wet tropical mountains (Grubb, 1977; Givnish, 1987).

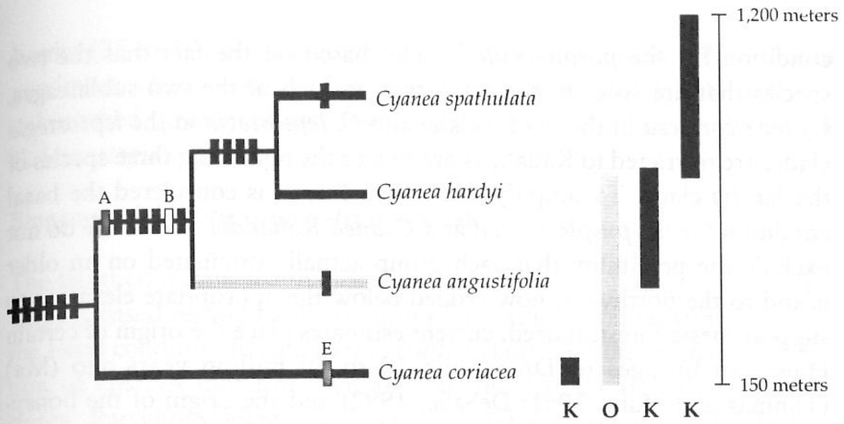


FIGURE 14.17. Elevational and geographic distribution (*right*) in relation to phylogeny within the *hardyi* clade (see text). K, Kaua'i; O, O'ahu. See Figure 14.8 for explanation of symbols.

Biogeography of the *purple-fruited* Clade

In our analysis, the basal geographic condition for the *Cyanea-Rollandia* lineage is inferred to be Kaua'i. This conclusion is based on three points: the co-occurrence of the two sister groups to the *Clermontia-Cyanea-Rollandia* clade (*Brighamia* and *Delissea*) only on Kaua'i, Ni'ihau, and Lana'i (Lammers, 1990a); the co-occurrence of the two subgenera of *Delissea* only on Kaua'i, identifying Kaua'i as the basal condition for that genus and hence for *Brighamia-Delissea;* and the co-occurrence of all five genera only on Kaua'i (see Figure 14.7). Second, Kaua'i is also the basal condition for the *purple-fruited* clade, based on the fact that the two species that are sister to the other taxa in each of the two sublineages, *Cyanea coriacea* in the *hardyi* clade and *C. leptostegia* in the *leptostegia* clade, are restricted to Kaua'i, as are two of the remaining three species of the *hardyi* clade. To simplify discussion, Kaua'i is considered the basal condition for the *purple-fruited* and *Cyanea-Rollandia* clades. We do not exclude the possibility that each group actually originated on an older island to the northwest, now eroded below the appropriate elevation to support mesic forest. Indeed, current estimates place the origin of certain Hawaiian lineages of *Drosophila* 10 to 30 million years ago (Ma) (Thomas and Hunt, 1991; DeSalle, 1992) and the origin of the honeycreepers 15 to 20 Ma (Sibley and Ahlquist, 1982; see also Tarr and Fleischer, this volume, Chapter 9), long before Kaua'i existed. The biogeography of the two sublineages within the *purple-fruited* clade can be summarized below.

Hardyi clade.—The argument given previously requires a Kaua'i-to-O'ahu dispersal event to account for the origin of *Cyanea angustifolia* on O'ahu (see Figure 14.16). Also, *C. angustifolia* most likely dispersed from O'ahu to the Maui Nui complex (see Table 14.1). Several other scenarios could account for the present-day distribution of *C. angustifolia,* but in the absence of genetic information on the non-O'ahu populations, the one proposed is the most parsimonious in terms of the number of dispersal events.

Leptostegia clade.—Given the occurrence of *Cyanea elliptica, C. hamatiflora,* and *C. mannii* on the once-interconnected islands of the Maui Nui complex (see Table 14.1), it is parsimonious to assume that they and *C. membranacea* of O'ahu share a common ancestor from Maui Nui. This implies a dispersal event from Kaua'i to the Maui Nui complex to account for the origin of the sister group to *C. leptostegia* and a subsequent colonization from the Maui Nui complex to O'ahu to account for *C. membranacea* (see Figure 14.16). Also, a subsequent dispersal of *C. hamatiflora* from Maui to Hawai'i seems quite likely, to account for the presence of *C. hamatiflora* in both East Maui and the Kona district of the island of Hawai'i (see Table 14.1) and for the subsequent origin of the (now-extinct) *C. giffardii. Cyanea hamatiflora* appears to be the closest relative of *C. giffardii* because these are the only two *Cyanea* species with purple fruits to occur on Hawai'i, and more important, *C. giffardii* shares with *C. hamatiflora* a greater number of derived morphological character states than it does with any other species in the *purple-fruited* clade, including long corolla tubes (60 to 80 mm versus 20 to 55 mm), large berries (25 to 45 mm diameter versus 6 to 12 mm), broad (14 to 15 mm maximum versus 2 to 12 mm) cuneate leaves, and a tall (5 to 10 m) unbranched growth form (these last traits are also shared with *C. leptostegia*).

Biogeography of the *orange-fruited* Clade

The basal condition for this clade is assumed to be Kaua'i (or an older island), based on the basal condition for *Cyanea-Rollandia* as a whole, the basal condition for the *purple-fruited* clade, and the relative distribution of cpDNA mutations within both the *purple-* and *orange-fruited* clades. Parsimony identifies Kaua'i as the basal condition ("I" in Figure 14.16) in the lineage defining the *orange-fruited* clade but leaves unresolved the condition at the end of that lineage ("II" in Figure 14.16), at the point where it split into several sublineages. Based on the relative distribution of mutations in the *purple-fruited* lineage before versus after dispersal from Kaua'i (see above) and in the *orange-fruited* lineage before versus after the radiation of that lineage, it is reasonable to assume that Kaua'i was the island from which that radiation took place (i.e., Kaua'i is the condition at point "II" in Figure 14.16). Given this assumption, several dispersal events are indicated for the four sublineages of the *orange-fruited* clade.

Acuminata clade.—The origin of this lineage requires a simple Kaua'i-to-O'ahu dispersal event. Subsequent dispersal events from O'ahu to the Maui Nui complex and from Maui Nui to Hawai'i would be required to account for the sparser presence of *Cyanea grimesiana* in these latter two areas.

Hirtella clade.—No inter-island dispersal is required to account for the distribution of this lineage, whose extant species are restricted to Kaua'i. However, a Kaua'i-to-O'ahu dispersal would be required to account for the origin of the extinct *Cyanea truncata* on O'ahu, which shared an unusual, broad leaf shape only with *C. remyi* of Kaua'i, a presumed close relative of the morphologically defined section *Hirtellae,* which are otherwise restricted to Kaua'i (Lammers, 1990a; T. G. Lammers, unpubl.).

Solanacea clade.—In contrast, the most-parsimonious basal condition for the *solanacea* clade is the Maui Nui complex. Thus, a Kaua'i-to-Maui Nui dispersal is required for this group's common ancestor (see Figure 14.16). A subsequent Maui Nui-to-Hawai'i dispersal is required for the origin of *Cyanea tritomantha.* If one accepts Lammers's (1990b) morphological argument that *C. shipmanii* is most closely related to the

C. solanacea-quercifolia-asplenifolia group on the Maui Nui complex, then an additional Maui Nui-to-Hawai'i dispersal event is indicated.

Aculeatiflora clade.—One dispersal event from Kaua'i to the Maui Nui complex is needed to account for the common ancestor of the *aculeatiflora* clade, followed by two dispersals from Maui Nui to Hawai'i to account for the occurrence of *Cyanea copelandii* on the island of Hawai'i and for the origin of *C. pilosa.*

Summary of Inter-island Dispersal

Figure 14.18 summarizes our best estimate regarding the minimum number of inter-island dispersal events (15) required to account for diversification within *Cyanea,* based on inferences from molecular data supplemented by comparative morphology. As more nodes in the phylogeny are resolved with additional data and species of *Cyanea* added to our analysis, the number of required dispersal events will almost surely increase. Nevertheless, the biogeographic pattern now apparent is similar in many respects to that seen in the much more intensively studied *Drosophila* and silversword alliance, with dispersal mainly proceeding from one island to the next younger island in the chain (Carson, 1983a; Carr et al., 1989; Baldwin, 1992). This pattern is generally thought to reflect the greater chance of establishment and subsequent radiation by colonists on nearby, newly formed, relatively unoccupied islands created to the southeast as the oceanic crust moves past the Hawaiian hot spot. An apparent exception to this rule is the slightly greater number (three) of events from Kaua'i to the Maui Nui complex relative to the number (two) of events from O'ahu to the Maui Nui complex, but this may be an artifact of having examined relatively few of the O'ahu taxa thus far.

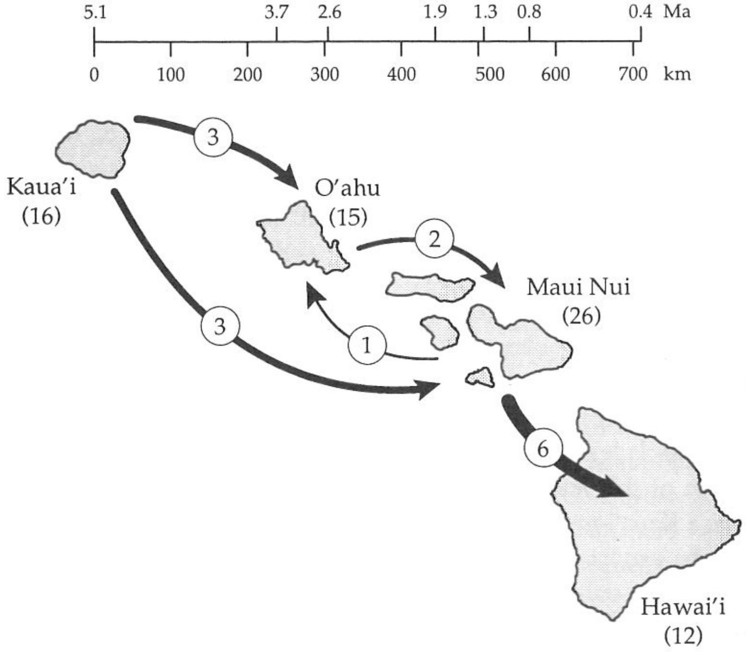


FIGURE 14.18. Minimum number of inter-island dispersal events required to account for diversification of 35 species and 40 populations of *Cyanea-Rollandia* (see text), illustrating a general tendency for dispersal from one island to the next younger island in the archipelago. Width of each arrow is proportional to the number of dispersal events between the corresponding pair of islands; the number of species found on each island or island group is indicated in parentheses.

The diversity of *Cyanea* results partly from inter-island dispersal events, isolating populations on different islands, and partly from the isolation of populations within islands by a variety of means, including (1) dispersal to new areas of moderate elevation formed by volcanism (e.g., Wai'anae and Ko'olau Mountains on O'ahu, and Pu'u Kukui and Haleakala on Maui); (2) dissection of existing landscapes by erosion, subsidence, or changes in sea level (e.g., Lana'i, Moloka'i, and Maui in the Maui Nui complex); and (3) formation of *kipuka* by lava flows (e.g., possibly *C. giffardii* on Hawai'i, which was restricted to a single *kipuka* near Glenwood). Given that we have invoked 12 (of 15) inter-island dispersal events to account for 34 species, an additional 22 speciation events within islands would be required to account for the diversity of the group considered to date.

Time and Island of Origin

At present, our molecular data are probably inadequate for a precise analysis of the time of origin of the common ancestor of *Cyanea.* However, a regression analysis of the data currently available suggests an origin on an island somewhere between Nihoa and French Frigate Shoals, 8.7 to 17.4 Ma, well before Kaua'i, the oldest high island at present, emerged approximately 5.1 Ma.

The method adopted involves estimating the average number of restriction site mutations down each lineage subsequent to an identified dispersal event, then relating that divergence to the geologic age (Clague and Dalrymple, 1987) of the younger island to calculate mutations per unit time. There are two obvious methods of relating the time of dispersal to the geologic age of the younger island. A "conservative" approach would assume that, on average, the dispersal event took place halfway through the lifetime of the younger island. This may not be biologically reasonable, however, in that colonizations early in an island's existence may be more likely to succeed than colonizations that occur later, after more competitors and predators have arrived. Thus, an "open ground" approach would assume that dispersal took place soon after the origin of the younger island. Clearly, the open ground approach will yield a time of origin twice that given by the conservative approach.

There are two Kaua'i-to-O'ahu dispersal events of a maximum age of 3.7 million years for which we have restriction site data, involving mutation subsequent to the origin of the *acuminata* clade and the clade including *Cyanea angustifolia, C. hardyi,* and *C. spathulata* (see Figure 14.16). Counting mutations down each lineage separately, summing, and dividing by the total number of species/lineages involved (11), we obtain an average of 5.6 mutations per lineage.

There were three dispersals from Kaua'i to the Maui Nui complex no more than 1.9 Ma, involving dispersal before the origins of the *solanacea* clade, the *aculeatiflora* clade, and the *Cyanea elliptica-hamatiflora-mannii-membranacea* group. These yield an average of 4.8 mutations along the lines leading to each species, subsequent to inter-island dispersal.

There was only one back-dispersal from the Maui Nui complex to O'ahu, also no more than 1.9 Ma, involving the origin of *Cyanea membranacea,* which shows three mutations since its divergence from its common ancestor with *C. hamatiflora.* There were two dispersals from the Maui Nui complex to Hawai'i no more than 0.4 Ma involving the origins of *C. pilosa* and *C. tritomantha,* which show an average of 0.5 mutations since their divergence from ancestors they share with each of their respective relatives.

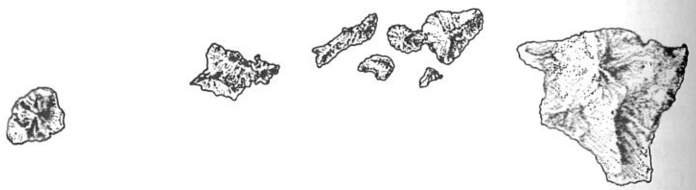
The best linear fit to these data is 3.03 and 1.52 mutations per million years under the conservative and open ground assumptions, respectively ($r^2 = 0.82$). The average number of mutations down each species lineage (counting the relatively undifferentiated *Rollandia* once) since the origin of the common ancestor of the extant species of *Cyanea* would be 26.4, yielding times of origin of 8.7 Ma and 17.4 Ma under the conservative and the open ground assumptions, respectively. The former estimate would place the origin of *Cyanea* (and its divergence from a common ancestor with *Clermontia*) when Nihoa was a high island, ca. 400 km northwest of Kaua'i; the open ground estimate points instead to an origin on French Frigate Shoals, 1,100 km northwest of Kaua'i, when they formed a high island. The fleshy-fruited clade as a whole appears to have arisen 3.3 to 6.6 million years earlier, which could have been on an island an additional 275 to 550 km further northwest, based on the 10 mutations from the origin of the fleshy-fruited clade to the divergence of *Cyanea-Rollandia* from *Clermontia* (see Figure 14.7). These estimated times of origin for *Cyanea* and the fleshy-fruited clade correspond to portions of the Hawaiian chain in which two to three hot spots were active simultaneously, presumably creating fairly extensive high islands similar to Maui Nui and Hawai'i, which were created by the double hot spot that is currently active (Clague and Dalrymple, 1987). Although the total amount of genetic divergence among extant species of *Cyanea-Rollandia* is much greater than that seen in *Brighamia, Clermontia,* or *Delissea,* the total amount of divergence down each lineage is roughly comparable (see Figure 14.7), justifying our extrapolation of mutation rates in *Cyanea-Rollandia* to the entire clade. Contrary to Lammers (this volume, Chapter 15), we believe that the low amount of genetic divergence within *Clermontia* reflects not its recent origin on Hawai'i (counterindicated by our analyses showing the extensive chloroplast DNA evolution in *Clermontia* since its divergence from *Cyanea*) but rather the repeated extinction of basal species, leaving only the current species, which show a pattern of radiation from Maui and Hawai'i.

Our estimates of the time of origin of *Cyanea* are admittedly crude but are the first such estimates for a Hawaiian plant group and among the very few for any plant group (see also Wolfe et al., 1989; Sytsma et al., 1991). They indicate that *Cyanea* (including *Rollandia*) had 8.7 to 17.4 million years to coevolve with its avian pollinators, frugivores, and (at least for the past 3.7 million years) herbivores and to undergo massive radiations in leaf form, flower morphology, growth habit, and prickliness. Given that the divergence of the *Brighamia-Delissea* clade from *Clermontia-Cyanea-Rollandia* occurred even earlier, it is clear that lobelioid coevolution with avian pollinators and frugivores has proceeded even longer, perhaps as long as 24 million years. Sibley and Ahlquist (1982) estimated from molecular evidence that the Hawaiian honeycreepers, likely to have been the most important group for both pollination and seed dispersal of the baccate lobeloids, originated at least 15 to 20 Ma (cf. Tarr and Fleischer, this volume, Chapter 9). This date is consistent with the honeycreepers coevolving at an earlier stage with the radiation of the baccate lobeloids proposed here.

SPECIES RICHNESS AND EXTINCTION

With the exception of Maui, the four largest islands support roughly the same number of species of *Cyanea* (including *Rollandia*): 15 on Kaua'i, 14 on O'ahu, 21 on Maui, and 12 on Hawai'i (Figure 14.19) (mean = 15.5 ± 3.9 species). The apparent excess of species on Maui may be partly explained in terms of the invasion of dry leeward forests, a novel habitat for *Cyanea,* by four species there. With the exception of Hawai'i, *Clermontia* generally has far fewer species (6.8 ± 4.6 species) on each of these islands, and only one-third as many species as *Cyanea* overall (see Table 14.1; Figure 14.19). Two key questions arise: Why do roughly 15 species of *Cyanea* occur on each large island? And why are there so many more species of *Cyanea* than of *Clermontia?*

The first question is an intriguing puzzle for which no definite answer can yet be given. We have explored various constraints on "community assembly," and some patterns do emerge. For example, two to four palmlike species have evolved on each large island. Species that span nearly the entire range of corolla tube lengths have arisen on each island, although the average flower size on Kaua'i is significantly less than that of species found on younger islands (see Figures 14.5 and 14.6). Yet, we have been unable to develop a model to predict exactly which combinations of growth forms, elevation ranges, and leaf and flower morphologies are likely to evolve on each island. Indeed, some growth forms or ecological roles are clearly missing from certain islands. For example, subherbaceous treelets with pubescent leaves (e.g., *Cyanea degeneriana* and *C. pilosa*) that vegetatively mimic species of *Cyrtandra* (Gesneriaceae) are found exclusively on Maui and the island of Hawai'i.



	Kaua'i	O'ahu	Moloka'i	Lana'i	Maui	Hawai'i
Cyanea s.l.	**16**	**15**	**7**	**5**	**21**	**12**
Clermontia	1	5	5	4	10	11
Delissea	4	4	0	1	1	3
Brighamia	1	0	1	0	0	0

FIGURE 14.19. Geographic variation in the number of species in each genus of the baccate lobelioid alliance in the Hawaiian archipelago.

TABLE 14.5. Summary of Primary Ecological Differences between *Cyanea* (including *Rollandia*) and *Clermontia*

	Cyanea	*Clermontia*
Habitat	Forest interiors	Edges, gaps
Growth form	Unbranched	Branched
Corolla	Cut halfway to base	Cut to base
Fruit	Small (ca. 15 mm)	Large (ca. 30 mm)
Dispersal agent	Forest interior birds?	Forest edge birds?
Inferred dispersal capacity	Low	Relatively high
Geographic distribution	Narrowly endemic	Relatively widespread
Elevational distribution	Relatively narrow	Relatively broad
Diversity	High (63 species)	Moderate (22 species)
Sensitivity to perturbation	Extinction-prone	Relatively resistant

A more satisfying explanation is available, however, for the great species richness of *Cyanea* relative to *Clermontia.* This disparity undoubtedly reflects ecological differences between the genera that shaped their evolution and that now expose them to quite different chances of extinction (Table 14.5).

Three differences seem crucial. First, *Cyanea* is a forest interior group, whereas *Clermontia* is a pioneer of forest gaps and edges. A branched growth habit allows *Clermontia* to respond opportunistically to unpredictable shifts in irradiance during early or gap-phase succession, whereas the unbranched form of *Cyanea* may be better suited to constant low light levels. It may be no accident that the only islands where there are large numbers of *Clermontia* species are Maui and Hawai'i, where frequent lava flows (and associated forest fires) now and in the recent past generated large amounts of early successional habitat. We suggest that *Clermontia* was far more common and diverse on older islands when they were several million years younger and that many species have become extinct with the loss of abundant, early successional habitats associated with volcanism at mid-elevations.

Second, the flowers of *Clermontia* are cut to the base of the corolla tube and so may have been visited by a broader range of honeycreepers and other pollinators than comparably sized *Cyanea* flowers. This difference accords with the early successional nature of the habitats occupied by most *Clermontia* and with the tendency for longer, more exclusionary bird-pollinated flowers to occur in more shaded sites, as discussed. A broader range of flower tube lengths in *Cyanea* is also consistent with a greater potential for reproductive isolation via the partitioning of nectarivores of widely differing bill lengths.

Finally, and most important, we believe that an understory group such as *Cyanea* had more limited powers of seed dispersal than that of a pioneer lineage such as *Clermontia* and that this difference has had a cascading series of effects on gene flow, extent of geographic and elevational ranges and tendency to speciate, species diversity within and among islands, and rates of extinction. We believe that *Cyanea* had lower powers of seed dispersal than *Clermontia* because their fruits were much smaller (and thus perhaps less attractive) and because *Cyanea* would have relied on forest interior birds to eat their fruits and disperse their seeds. On other islands, forest interior birds are relatively sedentary and less likely to cross water barriers and inappropriate habitat (Diamond et al., 1976). Presumably, this would have been true in the Hawaiian archipelago as well, but that is now impossible to ascertain. Little is known about avian frugivores in the Hawaiian Islands because they have been especially hard hit by extinction (Freed et al., 1987) or, as with most surviving members of the avifauna, greatly reduced in abundance and elevational range. Before the arrival of the Polynesians, frugivores would have included several species of honeycreepers, the Hawaiian Thrush and its relatives, the Hawaiian Crow and its relatives, and honeyeaters (Amadon, 1950; Freed et al., 1987; Olson and James, 1991).

Whether because of a dependence on forest interior birds or possession of less attractive fruit, a relatively low seed dispersal capacity in *Cyanea* would have reduced gene flow, increased geographic isolation, and fostered a high rate of speciation (Diamond et al., 1976). Reduced dispersal rates and high rates of speciation should, in turn, have resulted in narrower geographic and elevational ranges in *Cyanea* (and especially in the orange-fruited clade, even more strongly associated with forest interior conditions), due to differences in colonization rates and community packing. In fact, *Cyanea* does exhibit much narrower endemism than *Clermontia:* 89% of its species are restricted to single islands, compared with only 58% of *Clermontia* species. The average elevational range of *Cyanea* species is 438 ± 294 m versus 840 ± 367 m for species of *Clermontia* ($P < .0001$, two-tailed *t* test, 59 d.f.).

Finally, the narrow geographic and elevational ranges of individual *Cyanea* species, their limited dispersal, and their usual dependence on a narrower range of pollinators may have made them more susceptible to extinction. In the face of the pressures wrought by widespread habitat destruction, decimation of lobelioid pollinators and dispersers, and introduction of alien mammalian browsers, 22% of all species of *Cyanea* (including *Rollandia*) are now extinct, and 29% are currently greatly endangered, often known from only one or two individuals (see Table 14.1). By comparison, only 5% of historically known species of *Clermontia* are extinct, and 13% are currently endangered (see Table 14.1). As we would predict from the foregoing, the principal factors correlated with the likelihood of a historically known species going extinct are initial rarity (Terborgh and Winter, 1980), occurrence in areas heavily disturbed by humans, and possession of longer, more specialized flowers.

Eight of the 14 extinct species were known historically from only one site, compared with only 4 of 49 extant species ($\chi^2 = 9.61$, $P < .01$ with 1 d.f.), including the just-described *Cyanea eleeleensis* and *C. kolekolensis* and the recently rediscovered endangered *C. dunbarii* and *C. recta.* The average elevational range of the extinct species (to the extent data are available) is significantly less than that for extant species (182 m versus 481 m, $P < .01$, two-tailed *t* test with 38 d.f.). According to Rock (1919), the extinct species *C. arborea, C. comata, C. pohaku,* and *C. quercifolia* all occurred on leeward East Maui, in habitats that were heavily logged or cleared for pastures or *Eucalyptus* plantations; *C. giffardii* occurred on a single *kipuka* near Glenwood on Hawai'i that was largely cleared for cattle grazing. Finally, extinction has occurred at a significantly higher rate among species with longer flowers and is absent among species with a typical corolla tube length less than 45 mm (Table 14.6). It is not clear whether this is purely a result of the more-specialized pollination requirements of long-flowered species or whether the occurrence of many short-flowered species on Kaua'i, which has had less logging and grazing at middle elevations than other islands, has created this correlation as an artifact. The extinction rate of historically known species of *Cyanea* (including *Rollandia*) was highest on Maui (8 of 21 species, or 38%) and lowest on Kaua'i (none of the 16 species) and O'ahu (none of the 14 species). Thirteen of the 14 extinctions occurred in the *orange-fruited* clade, a higher rate (25%) than in the *purple-fruited* clade (7.7%),

TABLE 14.6. Corolla Tube Length of Extant and Extinct Species of *Cyanea*

Corolla tube length (mm)	No. of species	
	Extant	Extinct
15–29	10	0
30–44	13	0
45–59	4	7
60–74	7	5
75–89	2	1

$\chi^2 = 17.56$; $P < .002$, 4 d.f.
Data compiled from Lammers (1990a).

although the difference is not statistically significant ($P > .1$, χ^2 test with 1 d.f.).

The past extinction—and looming future loss—of large numbers of *Cyanea* species is of broad concern. Not only did this genus contribute 1 of every 15 plant species to the native flora and constitute one of the most widespread and characteristic elements of mesic and wet forest vegetation (Rock, 1919), it also played a starring role as a "keystone mutualist" (Gilbert, 1980; Terborgh, 1986) in helping create and maintain biological diversity in the Hawaiian biota.

Specifically, the evolution of *Cyanea* and other baccate lobelioids may have been interrelated with that of the avifauna that pollinated their flowers and dispersed their seeds. Lobelioids were particularly important, and *Cyanea* especially so, as nectar sources for birds with long bills and may have played a role in the evolution of the long-billed portions of the honeycreeper radiation. To the extent that *Cyanea* and other lobelioids provided a common nectar source for long-billed honeycreepers (e.g., *Drepanis, Hemignathus, Vestiaria*), they may have helped maintain not only those specialized nectarivores but also other plant groups that depend on their services (e.g., *Stenogyne* [20 species] of the Lamiaceae, *Hibiscadelphus* [6 species] of the Malvaceae), as well as the nectarivorous nitidulid beetles (Rock, 1919) frequently found in lobelioid flowers. Species of *Cyanea, Clermontia,* and *Rollandia* provide mating sites and food sources for many species of *Drosophila,* the most species-rich radiation of Hawaiian insects, and the best-studied example of insular evolution and speciation in the world (Carson, 1987b; Simon, 1987). They provided sites on which succinid snails grazed on epiphytic fungi and algae (Carlquist, 1974). Finally, certain species of *Cyanea* may have helped shape the evolution of an extinct series of flightless geese and moa-nalos, the distinctive Hawaiian contingent of terrestrial browsers.

It is a disturbing fact that, even though the Hawaiian lobelioids—and especially *Cyanea*—provide some of the best material in the world for studies of plant evolution on islands, many of them today are in great peril of extinction. They have been driven to the very brink by habitat destruction, grazing by goats, pigs, and cattle, competition with exotic plants, and loss of many avian pollinators and seed dispersers. The efforts of the National Tropical Botanical Garden, The Nature Conservancy, and the National Park Service to protect these species deserve our strongest support. To save these plants, we must immediately implement an integrated program, involving (1) fencing of relict populations to protect them from herbivores; (2) protection of endangered species from logging on public and private lands; (3) establishment of saplings of each species at arboreta; (4) continued efforts to propagate species from seed and to obtain fruits of species that are rare or thought to be extinct; and (5) widespread implementation of new advances (by G. Koob, University of Hawaii) in the cloning of nonflowering individuals via tissue culture.

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APPENDIX 14.1. Presence/Absence of cpDNA Restriction Sites in the 32 Taxa Involved in the Study of Phylogenetic Relationships within *Cyanea* and *Rollandia* (see Figure 14.8)

Restriction site descriptors: 0, absent; 1, present; ?, unknown.

Species	Restriction sites
(1) Brighamia insignis	001011001100011011010001111111100000001100000001001110010
(2) Clermontia arborescens	001110101000000101111000011100010100001110010001000011010
(3) Rollandia angustifolia	001010110100000111100001110001101010000100110010000110111
(4) Rollandia crispa	001010110100000111100001110001101010000100110010000110111
(5) Rollandia humboldtiana	001010110100000111100001110001101010000100110010000110111
(6) Rollandia lanceolata	001010110100000111100001110001101010000100110010000110111
(7) Rollandia longiflora	000010110100000111100001110001101010000100110010000110111
(8) Rollandia st-johnii	001010110100000111100001110001101010000100110010000110111
(9) Cyanea acuminata	001010111100000111100001110001101010000100110010000110111
(10) Cyanea grimesiana	0010101111000001111000011100011010100001001?0010000110111
(11) Cyanea remyi	101000111100000111100001110001101000000100110010000110111
(12) Cyanea fissa	101000111100000111100001110001101000000100110010000110111
(13) Cyanea hirtella	?????????????????????????1100????????????????????????????
(14) Cyanea sylvestris	?????????????????????????1100????????????????????????????
(15) Cyanea undulata	?????????????????????????1100????????????????????????????
(16) Cyanea lobata	001000111100100101100001110001101000100101110010000110111
(17) Cyanea scabra	001000111100000111100001110001101000100101110010000110111
(18) Cyanea tritomantha	001000111100000111100001110001101000100101110010000110111
(19) Cyanea solanacea	001000111100000111100001100001001000100100110010000110111
(20) Cyanea aculeatiflora	001010111110000111100001110001101000000100110010000110111
(21) Cyanea macrostegia	001010111110000111100001110001101000000100110010000110111
(22) Cyanea copelandii	001010111100000111101001110001101000000100110010000110111
(23) Cyanea pilosa	001010111100000111100001110001101000000100110010000110111
(24) Cyanea hardyi	0110101011000000101000110100011111000000001?0010110001111
(25) Cyanea spathulata	01101010110000001010001101000111110000000010001011000?111
(26) Cyanea angustifolia	011010101101000010100011010001111100000000100010110001111
(27) Cyanea coriacea	?????????????????????????1110????????????????????????????
(28) Cyanea membranacea	011010101100000011100001110001111101000010100010110011111
(29) Cyanea hamatiflora	011010101100000011100101110001111100000000010101011001111
(30) Cyanea mannii	011010101100000011100001110001111100000000100110110011111
(31) Cyanea elliptica	011010101100000011100001110001111100000000100110110011111
(32) Cyanea leptostegia	0110101011000000111000011100011111000000001?0010110011111

APPENDIX 14.1 (*Extended*)

Species	Restriction sites
(1)	000011000001011111000011001011000111001101111111100001100000001010011001101101100000010010
(2)	00001100000011011000001001101010101000110111110011110110101111010010000101100111010000100
(3)	00001100100001001000000000110110010010110001010110100010111011010010001101100110010010110
(4)	00001100100001001000000000110110010010110001010110100010111011010010001100100110010010110
(5)	00001100100001001000000000110110010010110001010110100010111011010010001101100110010010110
(6)	00001100100001001000000000110110010010110001010110100010111011010010001101100110010010110
(7)	00001100100001001000000000110110010010110001010110100010111011010010001101100110010010110
(8)	00001100100001001000000000110110010010110001010110100010111011010010001101100110010010110
(9)	00000100000001001000000000110110010011110001110110100010111011010010001101100110010010110
(10)	00001100000001001000000000110110010010110001110110100010111011010010001101100110010010110
(11)	00001100000001001000000000110110010010110001110110101010111011010010001001100110110011110
(12)	00001100000001001000000000110110010010110001110110101010111011010010001001100110110011110
(13)	?????????????????????????????????10010110001110110100010111011010010001001100110111011111
(14)	?????????????????????????????????10010110001110110100010111011010010001001100110111011111
(15)	?????????????????????????????????10010110001110110100010111011010010001001100110111011111
(16)	00001100000001001000000000110110010010110001110100100010111011010010001101100110010010110
(17)	00001100000001001000000000110110010010110001110100100010111011010010001101100110010010110
(18)	00001100000001001000100000110110010010110001110100100010111011010010001101100110010010110
(19)	00001100000001001000000000110110010010110001110110100010111011010000001101100110010010110
(20)	00001100000000001000000000110110010010110001100110100011111011010110001101000110010010110
(21)	00001100000000001000000000110110010010110001100110100010111011010110001101000110010010110
(22)	00001110001001001000000000110110010010110001110110100010111011010110001101000110010010110
(23)	00001100000001001000000000110110010010110001110110100010111011010110001101000110010010110
(24)	10111101010000001101001001101010010110011010111110100111101011001010101111100010010110110
(25)	10111101010000001101001001101010010110011010111110100111101011001010101111100010010110110
(26)	00111101010000001101001001101010010110011011111110100111101011000010101111100110010110110
(27)	????????????00101100001000111010010110011011111110100110101011000010001111100110010110110
(28)	0011100101000101100101000111010110110011011111110100110101010110010011101110110010010110
(29)	0011110101000101100101000111010110110001011111110100100101010010010011101110110010010110
(30)	0011110101000101100101000111010010110011111111110100110101011010010011101110110010010110
(31)	0011110101000101000101000111010010110011111111110100110101011010010011101110110010010110
(32)	0111110101000101100101000111010010110011011111110100110101010010010001101100110010010110