

Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): evidence from a cpDNA phylogeny

Thomas B. Patterson and Thomas J. Givnish

Department of Botany, University of Wisconsin, Madison WI 53706 USA

Summary

Author for correspondence:

Thomas J. Givnish

Tel: +1 608 262 5718

Fax: +1 608 262 7509

Email: givnish@facstaff.wisc.edu

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- We developed a molecular phylogeny for *Calochortus* (Liliaceae) to reconstruct historical patterns of evolution.
- Three cpDNA segments were sequenced and analyzed using parsimony.
- We identified seven major, geographically cohesive clades centered mainly in the California Floristic Province. Section *Calochortus* is monophyletic; section *Mariposa*, paraphyletic; and section *Cyclobothra*, polyphyletic. *Calochortus* arose in the Coast Ranges, which were uplifted 3–5 million yr ago. Three of the four major floral syndromes evolved at least twice, associated with particular environments. Serpentine tolerance evolved at least seven times.
- We argue that limited dispersal led to the narrow endemism of individual species, the geographic cohesion of clades, and parallel radiations in habitat preference, floral morphology, and serpentine tolerance. Chromosomal evolution allowed *Calochortus* to ‘double-up’ its regional radiations, preventing crosses between pairs of clades with overlapping ranges. Floral evolution in *Calochortus* is an example of consequent radiation, with selection for local diversification in habitat driving secondary specialization of flowers on the range of pollinators and abiotic conditions within each habitat, rather than selection to partition pollinators within habitats driving adaptive radiation.

Key words: adaptive radiation, California Floristic Province, *Calochortus*, convergence, consequent radiation, floral syndromes, geographic cohesion, parallel evolution.

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Introduction

To us, Verne Grant holds special interest because of the importance he consistently placed on adaptive radiation – especially in his landmark study of the phlox family with his wife Karen (Grant & Grant, 1965) – and on how selection for specialization on unusual substrates and different pollinators can lead to reproductive isolation, speciation, and the genesis of plant diversity. This paper re-examines some of these same issues using molecular systematics, and reports research we have just begun on the remarkable genus *Calochortus* (Liliaceae *sensu* Bremer *et al.*, 2003), previously the subject of a classic biosystematic study by Marion Ownbey (1940) using the traditional tools of morphology, cytology, and geography.

Calochortus is a large genus (67 spp.) of bulbous geophytes with flowers of extraordinary beauty and variety. It has a center of diversity in California and ranges north to British Columbia, west to the Dakotas, and south to Mexico and Guatemala (Ownbey, 1940; Munz & Keck, 1959; Nesom, 1983; Fiedler & Ness, 1993; Callahan, 2001). *Calochortus* has undergone striking radiations in flower morphology, habitat, and substrate preference, and most species are endemic to small geographic areas (Ownbey, 1940; Hill, 1973; Raven & Axelrod, 1978; Jokerst, 1981; Fiedler, 1985; Fredericks, 1989; Ness *et al.*, 1990; Fiedler & Ness, 1993; Dilley *et al.*, 2000). Floral syndromes include mariposas with large, brightly colored, tulip-like blossoms with conspicuous spots of contrasting hue; cat’s ears with smaller, spreading petals densely

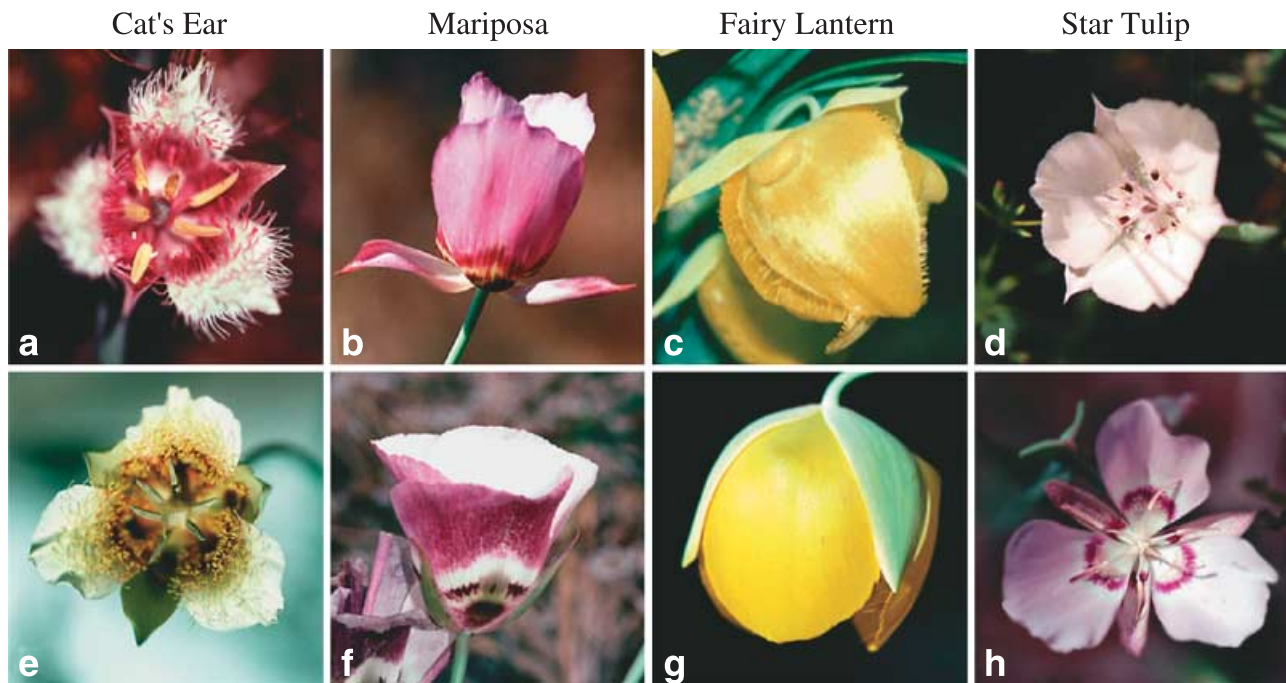


Fig. 1 Floral syndromes in *Calochortus*, exemplified by species in different cpDNA clades (see Fig. 2). Cat's-ear: (a) *C. elegans* (Pacific North-west) and (e) *C. ghiesbreghtii* (Central Mexico). Mariposa: (b) *C. greenii* (Pacific North-west) and (f) *C. venustus* (Coast Range-Sierra Nevada). Fairy lantern: (c) *C. pulchellus* (Bay Area) and (g) *C. balsensis* (Central Mexico). Star tulip: (d) *C. umbellatus* (Bay Area) and (h) *C. nudus* (Pacific North-west).

covered with trichomes; star tulips with spreading, glabrous petals; and fairy lanterns with bell- or globe-shaped, often nodding flowers (Fig. 1). Species inhabit deserts, grasslands, chaparral, meadows, vernal pools, springs, montane woodlands, and forest understories. One quarter of the taxa occur on or are restricted to serpentine substrates (Fiedler, 1985; Raven & Axelrod, 1978; Kruckeberg, 1986; Kruckeberg, 1992); nearly as many are federally endangered or extinct (Skinner & Pavlik, 1994).

Calochortus raises many questions at the interface of ecology, evolution, and biogeography:

- Are species with similar floral morphology each other's closest relatives, or have such syndromes arisen more than once?
- Do closely related species have neighboring geographic distributions?
- Where did the genus originate?
- Has the ability to tolerate serpentine substrates evolved once or many times?

We believe that answers to these questions hinge on *Calochortus* having relatively heavy seeds that lack obvious adaptations for long-distance dispersal. Limited seed dispersal should promote local speciation and result in closely related species having peripatric ranges (Givnish, 1997), especially in highly dissected landscapes like those over much of *Calochortus*' range. Species in different, geographically restricted clades should undergo parallel radiations in flower morphology as they invade habitats that favor different floral syndromes, and

evolve serpentine tolerance as they invade areas with exposed serpentine.

To test these hypotheses, we present a molecular phylogeny for *Calochortus* based on sequence variation in rapidly evolving segments of chloroplast DNA. We use this phylogeny to do the following: test the monophyly of the three sections and 12 subsections erected by Ownbey (1940) based on morphology and chromosome number; determine if individual clades display geographic cohesion, with closely related species having nearby ranges; infer how many times each floral syndrome has evolved; assess the minimum number of origins of serpentine tolerance, and the extent to which tolerant taxa arose from wider-ranging, intolerant species; and trace the evolution of chromosome number.

Methods

We included 72 ingroup taxa, including all but five extant species of *Calochortus*, and using *Tricyrtis latifolia* as the outgroup (Tables S1 and S2). We previously identified East Asian *Tricyrtis* as sister to *Calochortus* based on *rbcL* and *ndhF* sequence variation, and inferred that *Calochortus* began to differentiate roughly 7 million yr ago (Patterson & Givnish, 2002).

Total DNA was isolated from frozen or silica-gel dried leaf tissue following Givnish *et al.* (2000). Three rapidly evolving regions of the chloroplast genome (*trnT-trnE*, *psbA-trnH*, *rpl16*) were amplified using standard primers (Taberlet *et al.*,

1991; Jordan *et al.*, 1996; Sang *et al.*, 1997). Complete sequences were obtained for the two spacers for all species. Roughly 500 bp near the center of *rpl16* were sequenced for 35 taxa stratified across Ownbey's subsections. Amplification and sequencing reactions followed Patterson & Givnish (2002). Sequences were read on an ABI Prism™ 377 automated DNA sequencer. Using Sequencher™ 3.0 (Gene Codes Corp., Ann Arbor, MI, USA), trace-files were examined for biases and possible errors, and corrected using standard procedures. We generated reverse-strand sequences for several taxa to see if they would improve sequence accuracy. These data confirmed the original single-strand sequences, suggesting that it was not necessary to obtain data from both strands for all taxa. Indels were aligned manually to minimize independent evolutionary events using local parsimony, then scored and entered as single characters regardless of length (Baum *et al.*, 1994). DNA regions that could not be aligned unambiguously were excluded from analysis. Aligned sequences were deposited in GenBank (Tables S1 and S2). Nuclear ribosomal ITS sequences were obtained for several taxa, but could not be aligned with each other (except in section *Calochortus*) or the outgroup (Patterson, 1998), and so will not be reported here. Portions of the cpDNA data set (271 of 2333 nucleotide characters) which could not be aligned unambiguously were excluded; a total of 60 indel characters were scored.

Phylogenetic analyses were conducted using PAUP* 4.0b8 (Swofford, 2001). Preliminary analyses including all taxa under Fitch parsimony generated more than 400 000 trees before computer memory was exhausted. We thus adapted the approach of Catalan *et al.* (1997) to identify the strict consensus tree implied by the data matrix. One hundred replicate searches were conducted with TBR branch swapping, using random stepwise-addition to generate a starting tree for each search and holding no more than 100 trees per replicate. The strict consensus of these trees was then used as a negative constraint in 1000 replicate TBR searches, each seeded by an independent random-addition tree, with 20 trees held per search. If all the resulting trees were longer than the initial consensus tree, it was taken as evidence that a complete TBR search would yield the same tree. Bootstrap values (Felsenstein, 1985) for resolved nodes were generated by conducting TBR searches on 1000 random re-samplings of the data matrix, holding up to 1000 trees per replicate.

A second set of parsimony analyses used 27 taxa as place-holders, with two to five species (all with *rpl16* sequenced) included for each of the major clades identified by the first analysis. One thousand complete TBR searches, each seeded by a random stepwise-addition tree, were conducted to locate the shortest tree(s) and multiple islands of such trees if they existed. Bootstrap values and decay indices (Bremer, 1988) were calculated using a search strategy similar to that used for the first set of searches.

Subsectional alignment, base chromosome number, floral syndrome, habitat type, geographic distribution, and serpen-

tine tolerance were tabulated (Tables S1 and S2) and mapped onto the cpDNA phylogeny using parsimony in MacClade 4.0 (Maddison & Maddison, 1992). Chromosomal counts were derived from Ownbey (1940), Beal & Ownbey (1943), Cave (1970), Ness *et al.* (1990). Floral syndromes included fairy lantern, star tulip, cat's-ear, and mariposa. Habitat-types were categorized to emphasize exposure to sunlight: open habitats, including grasslands, meadows, chaparral, and deserts; montane woodlands, with *c.* 50% canopy coverage or less; and forest understories, with > 50% canopy coverage. Species distributions and chromosome numbers were obtained from Ownbey (1940) monograph and more recent accounts (Nesom, 1983; Fredericks, 1989; Fiedler & Ness, 1993). Species were considered serpentine-tolerant if they occurred, in whole or in part, on serpentine. Geographic distributions were atomized into the principal mountain ranges and phytogeographic regions occupied, including the Coast Ranges, Sierra Nevada, Siskiyou/Klamath, Transverse Ranges (incorporating the San Gabriel, San Bernadino, and San Jacinto Mountains), Peninsular Ranges, and the San Diego coast (including fringes of the southern Coast Ranges), all in California and northernmost Baja; the Cascades, and the Great Basin/south-western deserts/Rocky Mountains, extending beyond the California Floristic Province; and central Mexico. Geographic distributions were derived from Ownbey (1940), Nesom (1983), Fredericks (1989), and Fiedler & Ness (1993), with additions from the CalFlora website (<http://www.calflora.org/>)

Results

The complete data matrix produced a strict consensus tree 592 steps in length, with a consistency index $CI = 0.80$ ($CI' = 0.65$ excluding autapomorphies) and seven major, well-supported clades (Fig. 2). Of 2393 characters, 415 were variable and 180 were informative. Searches restricted to 27 taxa produced a single shortest tree 428 steps long, with $CI = 0.88$ and $CI' = 0.76$ (Fig. 3); relationships were consistent with those in the all-taxa analysis. Support for the major clades was high, with bootstrap values exceeding 98% and decay values = 4 steps in the place-holder study (with the exception of the Bay Area clade), and bootstrap values exceeding 70% in the all-taxa analysis. Support for relationships within the major clades was sometimes weak in the latter (Fig. 2), reflecting the absence of *rpl16* data for half the taxa, and inclusion of more species closely related to each other.

Each of the major clades exhibited a strong pattern of geographic cohesion, with their constituent species generally being found in close geographic proximity to each other (Figs 2, 4a). Ownbey's section (§) *Calochortus* is monophyletic, including our Pacific North-west clade (centered on Oregon, Washington, and Idaho) and Bay Area clade (centered on San Francisco Bay and nearby Coast Ranges, with a few taxa in the nearby Sierra Nevada). Ownbey's section

Calochortus cpDNA phylogeny

Ownbey's subsections and sections

- Pulchelli*
- Eleganti*
- Nudi*
- Nitidi*
- Gunnisoniani*
- Nuttalliani*
- Macrocarpi*
- Venusti*
- Mexican §§'s*
- Weediani*

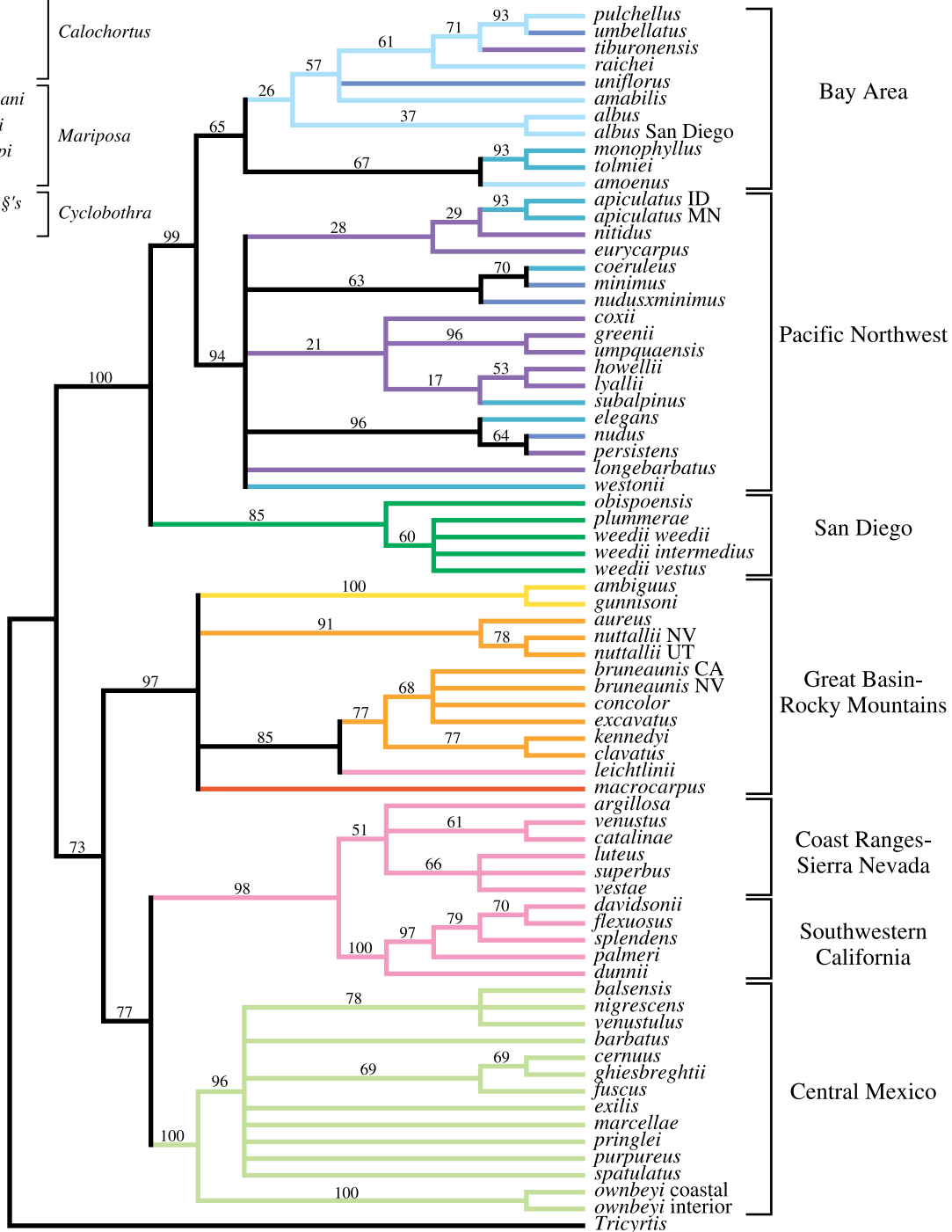


Fig. 2 Strict consensus cpDNA phylogeny of *Calochortus*. Major clades are indicated by brackets. Bootstrap support is indicated above each node. Membership of current taxa and inferred ancestors in the subsections recognized by Ownbey (1940) is overlaid using parsimony in color (black, equivocal).

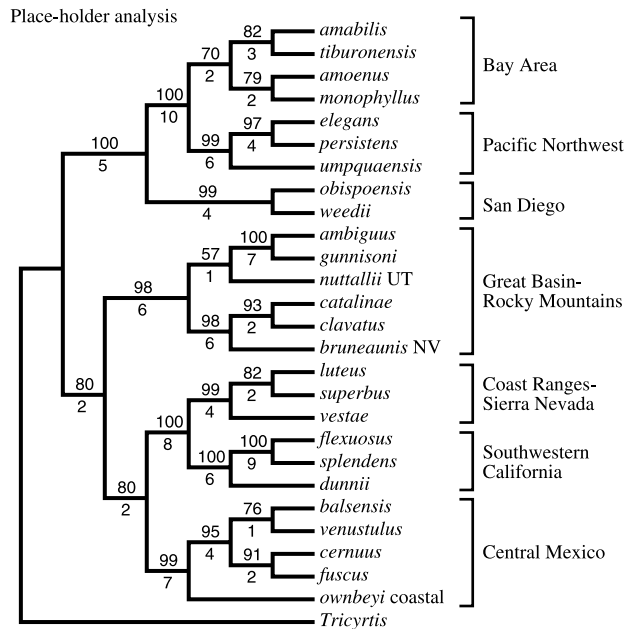


Fig. 3 Most parsimonious phylogeny for *Calochortus* placeholders (see text). Major clades are indicated by brackets. Bootstrap support is indicated above each node; Bremer support (decay value) is indicated below.

§*Cyclobothra* is polyphyletic, comprising our San Diego clade (along the southern California coast and in northern Baja California) sister to section §*Calochortus*, and our Central Mexico clade embedded in Ownbey's paraphyletic section §*Mariposa*. The latter consists of the Coast Ranges-Sierra Nevada clade (with species distributed in the mountains ringing California's Central Valley) and the Southwestern California clade (centered on the southern coast, with *Calochortus flexuosus* occurring further west), as well as the Great Basin-Rocky Mountain clade. A few taxa lie partly or wholly outside the range of the remainder of their clades (e.g. *C. westonii* and part of *C. albus*).

At a finer level of taxonomic resolution, Ownbey's 12 subsections show both concordance and discordance with the molecular data (Fig. 2). The Bay Area clade is composed mainly of subsection (§§) *Pulchelli*, with one species of §§*Eleganti* and two each of subsections §§*Nitidi* and §§*Nudi*. The Pacific Northwest Clade is a surprisingly intertwined mix of the remaining §§*Eleganti*, §§*Nitidi*, and §§*Nudi*. The San Diego clade equals §§*Weediani*. §§*Venusti* equals the Southwest California and Coast Ranges-Sierra Nevada clades, minus *C. leichtlinii*. The Great Basin-Rocky Mountain clade consists mostly of §§*Nuttaliani*, together with the one species of §§*Macrocarpi* and the two of §§*Gunnisoniani*, as well as *C. leichtlinii*. The Central Mexico clade comprises §§*Ghiesbreghtiani*, *Barbati*, and *Purpurei*.

The distributions of present-day taxa imply that *Calochortus* arose in the California Floristic Province, specifically in the Coast Ranges (Fig. 4a). These ranges – and others in California

except the ancient Siskiyou/Klamath massif – are recent features, having been uplifted in current form only during the last 3–5 million yr (Harden, 1997). These dates are close to the estimated initial divergence of *Calochortus* species from each other 7.3 ± 0.9 million yr ago (Patterson & Givnish, 2002). The distributions of individual species within the seven major clades supports the short, evocative names given to each, but also reveal a diversity of ranges, including some outside those implied by the names given to the clades. For example, the smaller of the two major lineages in the Bay Area clade is nearly restricted to the Sierra Nevada, and two species in the larger lineage also occur there, in part. We infer, however, that the ancestor of six of the 10 species in the Bay Area clade arose in the Coast Ranges (Fig. 4a); each of these species, except for some populations of *Calochortus uniflorus*, are further restricted to the northern Coast Ranges, and are indeed centered on the Bay Area (Ownbey, 1940). The Pacific Northwest clade is geographically the most diverse, with one lineage of six species restricted to the Cascades, at least two lineages and four species in the Siskiyou/Klamath region, at least two lineages and four species in the Sierra Nevada, and two lineages and four species in the Great Basin and Rocky Mountains (Fig. 4a). The inferred ancestor of the San Diego clade appears to have arisen in the Coast Ranges; present-day taxa in this group are restricted to the southern Coast Ranges, Transverse Ranges, Peninsular Ranges, and coastal areas centered on San Diego.

Seven of 10 species in the Great Basin/Rocky Mountain clade are restricted to that region, and the inferred ancestral distribution lies there or in the Coast Ranges (Fig. 4a). All members of the Coast Ranges/Sierra Nevada clade occur in the Coast Ranges, and several also have populations in the Sierra Nevada. Its inferred ancestral distribution lies in the Coast Ranges. So does that of its sister, the Southwestern California clade. The latter includes several species and/or populations restricted to the Transverse Ranges, Peninsular Ranges, and southern Coast Ranges, centering it in southwest California. All species of the Central Mexico clade occur there, with *Calochortus ghiesbreghtii* extending into Guatemala, and *C. venustulus* ranging into Chihuahua and Durango in northern Mexico (Ownbey, 1940).

The ancestral chromosome number of *Calochortus* is $x = 9$ (Fig. 4b). This condition is retained in §§*Weediani* (San Diego clade), the remaining subsections of polyphyletic §*Cyclobothra* (Central Mexican clade), and §§*Gunnisoniani* of paraphyletic §*Mariposa*. Thus, one trait that Ownbey (1940) used to define §*Cyclobothra* is plesiomorphic; the other – a fibrous-reticulate bulb coat – might also have arisen via convergence or hybridization (see below). Almost all other base chromosome numbers appear to have evolved directly from $x = 9$ (Fig. 4b).

Serpentine tolerance arose independently at least seven times, in five of our seven clades (Fig. 4c); the number of origins might fall to six if the clade including *C. elegans* were

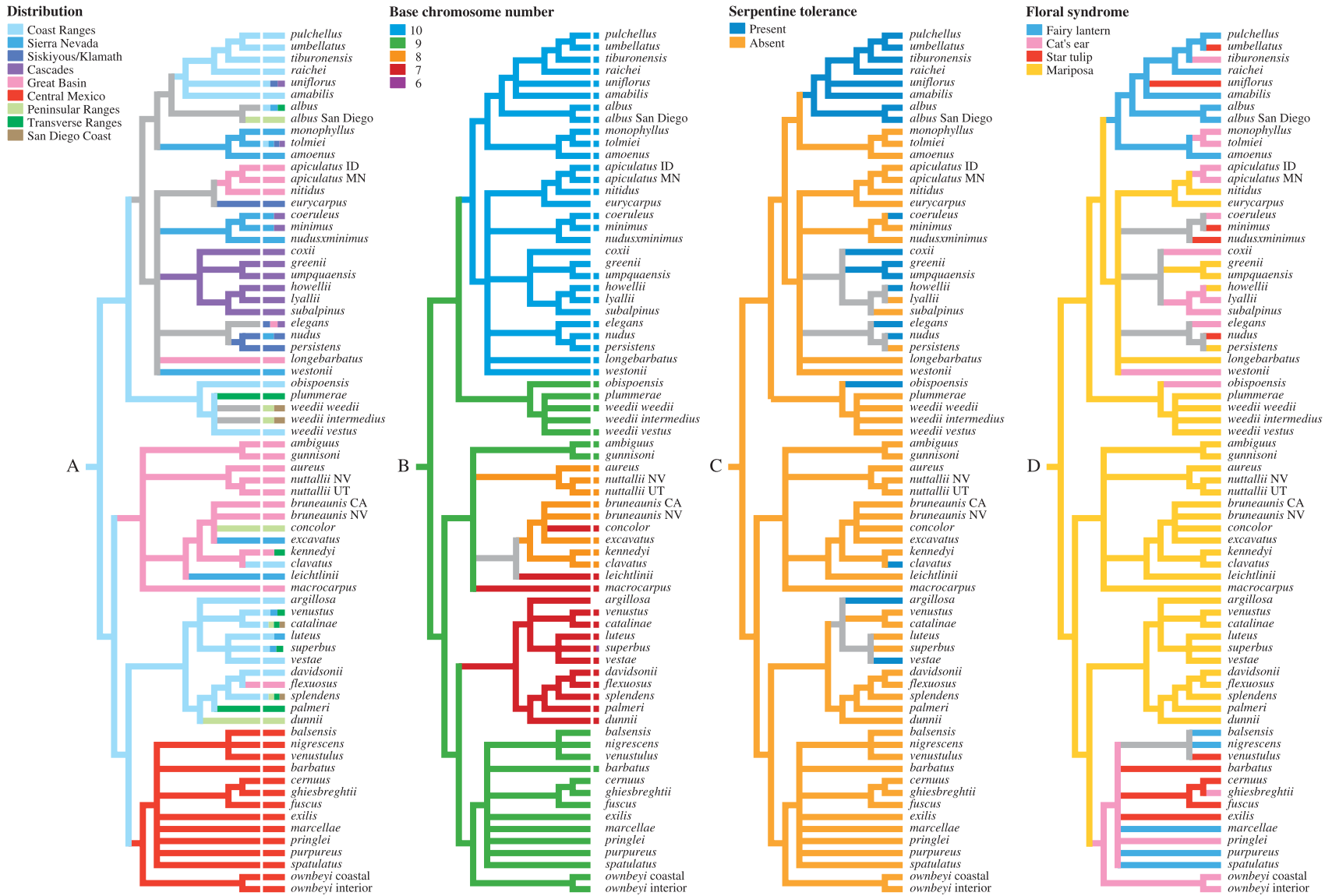


Fig. 4 Evolution of (a) geographic distribution (b) serpentine tolerance (c) base chromosome number, and (d) floral syndrome in *Calochortus*, inferred using parsimony (hollow branches, equivocal). Data boxes for a and b show polymorphisms and/or missing data. Branching sequence follows Fig. 2, but with *Tricyrtis* deleted.

ultimately to be shown to be sister to that containing *C. coxii*. Intolerance, however, is the inferred ancestral condition in all clades (Fig. 4c). The largest serpentine-tolerant lineage consists of seven species from the Bay Area clade. Serpentine tolerance evolved 5 of 7 times in the Bay Area and Pacific Northwest clades, accounting for 14 of the 18 tolerant species. These two clades occur in geographic areas (San Francisco Bay, Coastal Ranges, Siskiyou/Klamath Mountains, Sierra Nevada) with unusually large numbers of serpentine outcrops.

Three of the four main floral syndromes in *Calochortus* evolved two to six times within the genus; the fourth – mariposas, with large, brightly colored blossoms – is ancestral (Fig. 4d) and strongly associated with open habitats. Mariposa flowers occur in the three clades comprising §*Mariposa*, in the San Diego clade (except *C. obispoensis*), and in several elements of the Pacific Northwest clade. Fairy lanterns are concentrated in the Bay Area clade, but somewhat similar forms – often bearing a striking resemblance to the blooms of some *Fritillaria* (Liliaceae) and *Iridaceae* – occur in the Central Mexico clade. The cat's ear floral syndrome evolved independently in the Bay Area, Pacific Northwest, and Central Mexico clades, at least twice in each instance. The star tulip syndrome evolved independently at least twice in each of these clades as well. Species with mariposa flowers frequently occur in open, warm, dry habitats; cat's ears, in montane woodlands; star tulips, in open montane meadows or rocky sites; and fairy lanterns, in closed forest understories (Tables S1 and S2; T. J. Givnish and T. B. Patterson, unpublished).

Discussion

Taxonomic implications

Ownbey's classic biosystematic study (Ownbey, 1940) divided *Calochortus* into three sections based on single morphological traits: §*Calochortus*, with winged capsules; §*Mariposa*, with the basal leaf senescing at anthesis; and §*Cyclobothra*, with fibrous-reticulate bulb coats. Our data show that §*Calochortus* is monophyletic, §*Mariposa* is paraphyletic, and §*Cyclobothra* is polyphyletic, with the last comprising the San Diego and Central Mexico clades (Fig. 2). The Central Mexico clade confounded Ownbey's inferences, having secondarily lost basal-leaf senescence at anthesis (with a shift to relatively moist subtropical woodlands, forests, and montane meadows) and convergently gained a fibrous bulb coat similar to that in the San Diego clade and *C. tiburonensis*. The Central Mexico and San Diego clades retained the ancestral chromosome count of $x = 9$ (Fig. 4b).

At the subsectional level, our molecular phylogeny is largely consistent with Ownbey's treatment for §*Mariposa* but not §*Calochortus* (Fig. 2). Subsections of §*Mariposa* are based on relatively minor traits (e.g. presence/absence of a membrane enclosing part of the gland at the base of each petal). But subsections of §*Calochortus* are essentially based on the major floral

syndromes (Tables S1 and S2), which are associated with specific habitats and groups of pollinators (Jokerst, 1981; Dilley *et al.*, 2000; see below), and probably under strong selection.

The unique morphology of *Calochortus tiburonensis* – a federally endangered species – has confounded attempts to classify it since its discovery (Hill, 1973). Our cpDNA phylogeny places it in the Bay Area clade (Fig. 2). This accords with its presence on the Tiburon peninsula, but is surprising based on morphology: no other species in §*Calochortus* has a fibrous-reticulate bulb coat and angled capsules (Ownbey, 1940; Hill, 1973). We believe that *C. tiburonensis* acquired a Bay Area chloroplast genome via introgression with formerly adjacent populations of a species from the San Diego clade (most likely *C. obispoensis*, which shares cat's ear flowers of strikingly similar yellow-orange color, but with unusually narrow petals). A nuclear DNA ITS sequence phylogeny for §*Calochortus* places *C. tiburonensis* in the Bay Area clade, sister to *C. umbellatus* (Patterson, 1998), to which it is also closely related based on cpDNA (Fig. 2) and with which it co-occurs. Introgression may have affected both cpDNA and nrDNA markers (Sytsma, 1990; Rieseberg *et al.*, 1996). The petals of *C. tiburonensis* are wide like those of white-flowered, smooth-petaled *C. umbellatus*, but bear the prominent petal hairs and distinctive pigmentation of *C. obispoensis*. All three species are serpentine endemics.

Subsection *Venusti* of §*Mariposa* splits fairly cleanly into our South-west California and Coast Ranges-Sierra Nevada clades (Fig. 2), marked by monochasial vs subumbellate inflorescences, respectively. Ownbey placed *Calochortus leichtlinii* in §§*Venusti*, but molecular data put it in the Great Basin clade, otherwise composed of §§*Nuttalliani* (Fig. 2). Hybridization may be involved in its origin, given its strong floral similarity to *C. aureus*, *C. bruneaumis*, and *C. nuttalliani* in §§*Nuttalliani*, its possession of depressed glands and seed coats with conspicuously inflated, hexagonally netted cells (typical of all §*Mariposa* except §§*Venusti*), its lack of a membrane around the gland (typical only of §§*Venusti*), and its range east of the Sierra Nevada, near where the ranges of *C. bruneaumis* and §§*Venusti* overlap. It has been impossible to date, however, to align rapidly evolving ITS sequences outside §*Calochortus* to test this idea (Patterson, 1998).

Evolutionary implications

Many aspects of the systematics, biogeography, ecology, and morphology of *Calochortus* appear driven by poor seed dispersability. Fruits are capsular and borne close to the ground, with relatively heavy, passively dispersed seeds that lack fleshiness, stick-tights, or (save in one species) wings. As a result, seed dispersal range is quite limited: Bullock (1976) found that *Calochortus* seeds moved no more than 1.4 meters downhill after a chaparral fire. The one species with winged seeds – *C. macrocarpus* – has the largest geographic range (Ownbey, 1940). Limited seed dispersal, in combination with the barriers imposed by the mountainous and highly dissected

landscapes in which *Calochortus* occurs, should promote genetic differentiation (in response to drift and/or local selection pressures) and, ultimately, speciation at very small spatial scales (Givnish, 1997). Closely related species and sister clades should have neighboring ranges. Moreover, species in different, geographically restricted clades should undergo parallel adaptive radiations in habitat, serpentine tolerance, and flower form as they each adapt to local conditions.

Biogeography Each major clade exhibits strong geographic cohesion, with species restricted to a particular region (Fig. 4a). Within the limits of resolution imposed by our data, this pattern appears to extend to individual taxa in many cases. Within the Bay Area clade, for example, many species have peripatric ranges that abut those of their closest relatives. *Calochortus monophyllus* and *C. tolmiei* are sister species, and abut where the northern Sierra Nevada and Siskiyou/Klamath Mountains intersect (Ownbey, 1940, <http://www.calflora.org/>). *C. monophyllus* grows in the northern and central Sierra, next to the range of *C. amoenus* in the southern Sierra. *C. amoenus*, in turn, is peripatric to *C. albus* in roughly the same area. The remaining species are endemic to serpentine outcrops, and most are peripatric to *C. albus* in the Bay Area: *C. amabilis* and *C. uniflorus* in the northern Coast Ranges, *C. pulchellus* on Mt. Diablo (east of the Bay) and points northwest, *C. tiburonensis* on the Tiburon peninsula in the northern part of the Bay, *C. raichei* at the Cedars in the northern Coast Ranges, and *C. umbellatus* just south of the Bay, peripatric to both *C. albus* (with which it overlaps extensively at coarser geographic scales) and *C. pulchellus* (with which it is peripatric at such scales) (Ownbey, 1940; <http://www.calflora.org/>).

Ownbey (1940) stated that his sections and subsections each had a characteristic geographic range, but his taxonomic scheme differs from our phylogeny in several respects. For example, our Bay Area clade includes species in all four subsections of § *Calochortus*. Cat's-ear and star-tulip flowers each evolved twice from fairy-lanterns in this group. Despite this dramatic range in floral syndrome and occurrence on serpentine and nonserpentine substrates, the Bay Area species all share branched stems.

The overall distribution of *Calochortus* includes nine massive mountain ranges: the Cascades, Rockies, Siskiyou/Klamath, Coast Ranges, Sierra Nevada, Transverse Range, Sierra Madre Oriental, Sierra Madre Occidental, and Eje Volcanico Transversal. Topographic complexity promotes local speciation by isolating populations in small geographic areas (Ehrlich & Raven, 1969; Cracraft, 1985). Species in relatively flat, desert and dry grassland habitats of the Intermountain Region (e.g. *C. ambiguus*, *C. bruneaunis*, *C. gunnisoni*, *C. kennedyi*, *C. macrocarpus*, *C. nuttalliani*) often have large ranges. Poor seed dispersal capacity and a dissected landscape should enhance each other's effects on isolation and geographic cohesion at various taxonomic scales.

A correlation between geography and phylogeny might also arise if introgression were extensive. Species with overlapping

or abutting ranges should introgress more frequently than others (Levin, 1981; Arnold, 1992). Rieseberg *et al.* (1991) found that chloroplast capture in *Helianthus* sect. *Helianthus* occurred most frequently among peripatric species, so that neighbors also seemed to be each other's closest relatives on a cpDNA phylogeny (Rieseberg *et al.*, 1996). Tests for introgression in *Calochortus* using chloroplast and nuclear markers should now be pursued. However, an ITS phylogeny for § *Calochortus* is largely congruent to our cpDNA phylogeny, as expected if hybridization and introgression have not been common (Patterson, 1998).

Despite narrow species ranges, poor dispersability, and geographic cohesion, some long-distance dispersal (perhaps via range shifts during glacial cycles) has no doubt occurred. The Mexican species concerned Ownbey (1940), who believed that *C. obispoensis* and other members of § *Weedii*, more than 1000 km away, were their closest relatives. Our data indicate, however, that the Southwest California, Coast Ranges, and Great Basin clades are the closest relatives of the Central Mexico clade. Among these groups, *C. ambiguus* occurs in southernmost Arizona and New Mexico, *C. flexuosus* reaches south-central Arizona, and *C. kennedyi* crosses into northern Sonora, all within 250 km of *C. venustus*, the northernmost Mexican species.

While close relatives are often close neighbors, the converse is not always true. The Bay Area and Coast Ranges/Sierra Nevada clades strongly overlap in their overall ranges, as do parts of the Pacific Northwest and Great Basin/Rocky Mountain clades, as well as the San Diego and South-western California clades. Remarkably, in each of these three cases, the overlapping clades differ from each other in base chromosome number (Fig. 4b). This previously overlooked but fundamental difference – as well as simple allopatry among most of the species pairs involved – eliminates the possibility of almost all interclade crosses.

Biogeographic inferences can be sensitive to the scale at which biogeographic regions are atomized. Within California, our atomization of mountain ranges corresponds largely to that adopted in the Jepson manual (Hickman, 1993) and associated website (<http://ucjeps.berkeley.edu/>), except that we do not divide the Coast Ranges, Sierra Nevada, and Transverse ranges into numerous subunits. This approach seemed a good compromise, given the coarse-scale atomization used outside the California Floristic Province (e.g. Great Basin/Rocky Mountains) and the presence of ecological corridors joining the subunits of the Coast Ranges, Sierra Nevada, and Transverse ranges recognized in the Jepson project. Atomizing the Coast Ranges into northern and southern subunits identifies the southern Coast Ranges as the cradle of *Calochortus*, and indicates that § *Calochortus* arose in the Sierra Nevada and underwent a shift to the northern Coast Ranges within the Bay Area clade (analysis not shown). The biogeographic reconstruction shown in Fig. 4 A is otherwise not affected. Given the low degree of phylogenetic resolution at present in the Central Mexico clade, little purpose would have been

served in atomizing distributions within this region into individual mountain ranges.

Serpentine Tolerance Serpentine tolerance arose at least 7 times in *Calochortus*, but 5 of these events occurred within the Bay Area and Pacific Northwest clades, accounting for 14 of 18 serpentine-tolerant species (Fig. 4c). These two clades occur in geographic areas with immense areas of serpentine, including the largest such outcrops in North America (Kruckeberg, 1992; Coleman & Kruckeberg, 1999). Serpentine-derived soils have unusually high levels of Mg and Fe and low levels of Si, and often have relatively high concentrations of Ni, Cu, Cr, and Co. Such heavy metals, and a low Ca:Mg ratio, are toxic to many plants (Fiedler, 1985; Kruckeberg, 1992). At least 19 angiosperm species are endemic to serpentine in the Bay Area alone, 90–100 in the north Coast Ranges, and an even larger number in the ancient Siskiyou/Klamath Mountains on the Oregon-California border (Kruckeberg, 1992; Coleman & Kruckeberg, 1999). Despite the insular nature of serpentine outcrops, 11 of 18 tolerant species reflect just two origins of serpentine tolerance, with seven from just one origin (Fig. 4C). Limited dispersal among outcrops may have favored geodaphic speciation via geographic isolation from populations of a tolerant ancestor (Kruckeberg, 1991).

Selection can lead rapidly to the evolution of heavy-metal tolerance (Wu *et al.*, 1975; Al-Hiyaly *et al.*, 1990; Bendomir & Jones, 1998). Classic examples include populations of *Festuca ovina* and *Agrostis canina* under galvanized fences evolving zinc tolerance in less than 30 yr, and tolerance to otherwise toxic concentrations of heavy metals arising in *Agrostis tenuis* in a single generation (Antonovics *et al.*, 1971). In *Mimulus guttatus*, one major gene and several minor genes control copper tolerance (Tilstone & Macnair, 1997), and it appears that the genetic mechanism underlying Cu tolerance is independent of that for tolerance to other heavy metals (Tilstone & Macnair, 2001). Tolerance to heavy metals depends on the ability to prevent such cations from being absorbed in the first place, and/or the ability to sequester them successfully after root uptake (Fitter & Hay, 1987; Clemens, 2001; Assuncao *et al.*, 2003a; Cobbett, 2003). The tissue-specific expression of specific metallothioneins and phytochelatins appears crucial for survival on metalliferous soils (Murphy & Taiz, 1995; Cobbett & Goldsbrough, 2002; Schat *et al.*, 2002; Assuncao *et al.*, 2003b; Guo *et al.*, 2003). Tolerant species are often slower growing and less competitive than intolerant plants when grown on substrates with low levels of heavy metals, which would tend to restrict dispersal of tolerant species across the intervening matrix of nonserpentine substrates. This, coupled with mortality of nontolerant plants on serpentine substrates, would result in serpentine-tolerant and -intolerant species being reproductively isolated in different habitats even if they occur in the same region.

Rajakaruna *et al.* (2003a,b) provide compelling evidence that a pair of cryptic species in the *Lasthenia californica* com-

plex have undergone parallel evolution of tolerance to serpentine, based on differences in their ability to absorb various cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) and maintain proper ionic balance of K^+/Na^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ when growing on soils unusually rich in Na and Mg. (An ability to preferentially absorb Ca^{2+} over Mg^{2+} also marks the serpentine sunflower, *Helianthus bolanderi* ssp. *exilis* (Madhok & Walker, 1969)). Such differences in ionic absorption apparently also allow tolerant races of these two species to grow on other substrates presenting severe ionic stress, including coastal bluffs and salt flats. Finally, Rajakaruna *et al.* (2003b) note that both tolerant races of *Lasthenia* show heavy allocation to sulfated flavonoids, and speculate that synthesis of such compounds help them tolerate the high soil sulfate levels found in serpentine, marine, and alkaline substrates.

Our data provide limited support for Raven & Axelrod (1978) theory of catastrophic speciation. They argued that intense droughts – especially since the advent of summer drought in California roughly 15 million yr ago (Baldwin & Sanderson, 1998) – might restrict some populations to serpentine outcrops, given the frequent occurrence of springs on such sites and the moisture-holding capacity of their fine-grained soils. This should lead to strong selection for heavy-metal tolerance, and – combined with intense competition off serpentine – lead to repeated evolution of serpentine endemics from formerly widespread species. The origin of serpentine tolerance from seemingly intolerant species seven times in *Calochortus* supports the Raven-Axelrod theory. The fact that 61% of the tolerant species form two large clades, however, does not. In the largest tolerant clade, *Calochortus albus* – a widespread species occasionally found on serpentine – is sister to a clade composed of six serpentine endemics, each of whose range it abuts or overlaps slightly. In the second largest clade of serpentine-tolerant species, four allopatric species are restricted to serpentine outcrops in a small area of the Siskiyou and Cascades in northern California and southern Oregon.

Fiedler (1985) showed that three serpentine endemics (*C. obispoensis*, *C. pulchellus*, *C. tiburonensis*), one tolerant species (*C. albus*), and one taxon endemic to calcareous desert springs (*C. striatus*) all accumulate relatively high levels of Ni and Cu. She proposed that the ability to accumulate and tolerate such heavy metals may have been the ancestral condition in *Calochortus*, and that heavy-metal tolerance may simply have been lost repeatedly rather than evolving many times. Our reconstruction (Fig. 4c) does not support this interpretation, and the four species studied by both Fiedler and ourselves are closely related, serpentine-tolerant members of the Bay Area clade. But there might be some feature of *Calochortus* that underlies its repeated evolution of serpentine tolerance. We note that serpentine tolerance has evolved repeatedly in bulbous monocots in western North America, in several species of *Erythronium*, *Fritillaria*, and *Lilium* (Raven & Axelrod, 1978; Kruckeberg, 1986, 1991) of closely related *Liliaceae* (Patterson & Givnish, 2002), and in *Allium* of Asparagales.

Floral morphology, consequent evolution, parallel radiations, and species diversity Floral divergence is usually thought to evolve via selection to partition pollinators or attain reproductive isolation (Bogler *et al.*, 1995; Givnish *et al.*, 1995; Kampny, 1995; Hapeman & Inoue, 1997; Goldblatt *et al.*, 1998, 2001; Johnson *et al.*, 1998; Schemske & Bradshaw, 1999; Perret *et al.*, 2003). This was a major theme in Verne Grant's research (Grant, 1949; Grant & Grant, 1965) and evolutionary syntheses (Grant, 1963, 1977, 1994). In *Calochortus*, this schema appears to apply only in part. Different floral syndromes are indeed associated with different sets of pollinators. Jokerst (1981) found that mariposa flowers are visited by a wide range of pollinators, including beetles, bugs, and small bees; fairy lanterns are visited by specialist bees (often *Bombus*); and star tulips and cat's ears are visited by several non-specialist bees. However, in a benchmark study of 19 species, Dilley *et al.* (2000) showed that most *Calochortus* are generalists that attract many visitors, including several families of beetles lured by the conspicuous glands on the petal bases of many species. Although species differ in the proportions of different visitors they attract, these overlap enough that floral divergence in *Calochortus* is unlikely to have evolved via selection for pre-mating isolation (Dilley *et al.*, 2000).

Given that species with different floral syndromes often grow in different habitats, we propose that floral radiation in *Calochortus* mainly reflects selection for habitat divergence among species in a given area, followed by adaptation to the pollinators and abiotic conditions in different habitats, rather than direct selection for pollinator partitioning within habitats. We term such a process consequent radiation, to distinguish it from the usual process of adaptive radiation involving divergent selection on traits directly linked to competition for key resources (Givnish, 1997; Schluter, 2000).

The functional significance of the four major floral syndromes is unclear, but some possibilities seem evident. Fairy lanterns restrict access to larger or longer-tongued bees, and may be analogous to the long-tubed, exclusionary flowers pollinated by birds in forest understories of Central America and Hawaii (Givnish *et al.*, 1995). Their pendent form might also help protect stamens and pistils from heavy precipitation in coastal California and Mexico (H. McDonald, pers. comm.). In Mexico, mimicry of *Fritillaria* (Ownbey, 1940) or *Tigridia* (A. Rodriguez, pers. comm.) may also be involved. Trichomes on the inner surfaces of cat's-ear flowers may trap heat in their cool, high-elevation habitats; the highest elevations and latitudes are indeed occupied by cat's-ears (e.g. *C. apiculatus*, *C. subalpinus*) (Ownbey, 1940). Mariposas and star tulips bear visually conspicuous, brightly colored flowers adapted to open conditions with bright, wide-spectrum light (Givnish & Patterson, 2000). Mariposas, in drier environments, have petals more vertically inclined, which may reduce water loss. They also bear hairs around the gland, which may help attract a wide range of beetles. Beetles are often prominent pollinators in Mediterranean and desert climates elsewhere; the flowers

they visit are often marked by conspicuous dark or light spots on the petal bases (Dafni *et al.*, 1990; Goldblatt *et al.*, 1998; Bernhardt, 2000), similar to those seen in mariposa *Calochortus* and *Clarkia* of dry habitats in California. A large roster of potential pollinators – especially beetles with thick cuticles – may be adaptive in habitats of low and unpredictable rainfall.

Conclusions and synthesis

In any adaptive radiation, there is always a question of the extent to which adaptive diversification is the cause or consequence of speciation (Givnish, 1997). In *Calochortus*, it may well be that speciation is caused largely by two nonadaptive factors – poor dispersal leading to speciation at small spatial scales, and chromosomal evolution allowing different clades essentially to 'double up' and radiate independently in the same area without hybridizing. These factors may largely drive speciation and then facilitate parallel adaptive radiations in habitat and floral morphology, rather than adaptive radiation in habitat and floral morphology driving speciation.

Parallel radiations in flower form, habitat, and serpentine tolerance in *Calochortus* appear analogous to those recently documented in geographically isolated clades of other plants and animals, including *Cyanea* on Hawaii (Givnish, 1997), cichlids in African rift lakes (Kocher *et al.*, 1993), and *Anolis* lizards of the Greater Antilles (Losos *et al.*, 1998). In each of these cases, limited dispersal appears to have combined with divergent selection in similar landscapes to produce a series of parallel radiations. Our study is also among the first to use molecular data to evaluate adaptive radiation, geographic speciation, and time of origin in a major element of the highly diverse flora (for smaller groups, see Baldwin & Sanderson, 1998; Lee *et al.*, 2002; Calsbeek *et al.*, 2003; Kelch & Baldwin, 2003; Rajakaruna *et al.*, 2003a).

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Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/NPH/NPH951/NPH951sm.htm>

Table S1 Location, voucher/source, molecular clade, floral syndrome, geographic distribution, serpentine tolerance, and base chromosome number for *Calochortus* species sequenced in this study. Sections (§) and subsections (§§) of *Calochortus* follow Ownbey (1) and more recent publications describing new species (see text, Callahan 2001).

Table S2 GenBank accession numbers for cpDNA sequences included in this study. Sections (§) and subsections (§§) of *Calochortus* follow Ownbey (1) and more recent publications describing new species (see text).

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