

PHYLOGENETIC ORIGINS OF *LOPHOCEREUS* (CACTACEAE) AND THE SENITA CACTUS–SENITA MOTH POLLINATION MUTUALISM¹

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Recent ecological research has revealed that the Sonoran Desert columnar cactus *Lophocereus* and the pyralid moth *Upiga virescens* form an obligate pollination mutualism, a rare but important case of coevolution. To investigate the phylogenetic origins of this unusual pollination system, we used molecular sequence data to reconstruct the phylogeny of the four taxa within the genus *Lophocereus* and to determine the phylogenetic position of *Lophocereus* within the North American columnar cacti (tribe Pachycereeae). Our analysis included *Lophocereus*, six *Pachycereus* species, *Carnegiea gigantea*, and *Neobuxbaumia tetetzo* within the subtribe Pachycereinae, and *Stenocereus thurberi* as an outgroup within the Stenocereinae. Extensive screening of chloroplast and mitochondrial genomes failed to reveal sequence variation within *Lophocereus*. At a deeper phylogenetic level, however, we found strong support for the placement of *Lophocereus* within *Pachycereus* as sister group to the hummingbird-pollinated *P. marginatus*. We discuss possible hypotheses that may explain the transition from bat pollination (ancestral) to moth and hummingbird pollination in *Lophocereus* and *P. marginatus*, respectively. Additional phylogenetic analyses suggest that the genus *Pachycereus* should be expanded to include *Lophocereus*, *Carnegiea*, *Neobuxbaumia*, and perhaps other species, whereas *P. hollianus* may need to be excluded from this clade. Future study will be needed to test taxonomic distinctions within *Lophocereus*, to test for parallel cladogenesis between phylogroups within *Lophocereus* and *Upiga*, and to fully delineate the genus *Pachycereus* and relationships among genera in the Pachycereinae.

Key words: *Carnegiea*; chloroplast DNA; *Lophocereus*; mutualism; *Neobuxbaumia*; *Pachycereus*; pollination; *Stenocereus*.

Obligate pollinating “seed eater” mutualisms are systems in which the host plant is dependent upon a specific pollinator species and the pollinator is dependent upon the host plant for oviposition sites and the nourishment of its larvae. Because of their highly specialized interactions, these mutualisms have served as model systems for the study of coevolution (e.g., Wiebes, 1979; Herre, 1996; Pellmyr et al., 1996; Herre et al., 1999). Phylogenies are currently being developed for the well-known yucca–yucca moth (Brown et al., 1994; Pellmyr et al., 1996; Pellmyr and Leebens-Mack, 1999) and fig–fig wasp (Herre et al., 1996; Machado et al., 1996; Rasplus et al., 1998; Weiblen, 2000) systems to determine the extent of coevolution in these interactions. The phylogenetic origin of the recently discovered (Fleming and Holland, 1998) senita–senita moth system, the third known obligate pollinating seed-eater mutualism involving active pollination, has yet to be examined.

Senita is a columnar cactus (genus *Lophocereus*, family Cactaceae) that inhabits the Sonoran Desert of northwestern Mexico and the southwestern United States (Lindsay, 1963). Recent studies show that senita, unlike related columnar cacti that are visited by bats or birds, is night-pollinated by the moth *Upiga virescens* (family Pyralidae) (Fleming and Holland, 1998; Holland and Fleming, 1999a, b). Each stage in the life cycle of *U. virescens* is intimately associated with the host

plant. Adult senita moths (~1 cm long) rest during the day in the elongated, bristle-like spines characteristic of reproductively mature senita stems. Female moths actively pollinate and oviposit on the nocturnal white to pink flowers. The larvae feed within the developing fruit and tunnel into the stem to pupate before the damaged fruit abscise from the cactus. They overwinter within the stem, eclose, and emerge the following flowering season. The senita moth is thus both a pollinator and a seed consumer, much like yucca moths and fig wasps are on their particular hosts.

The genus *Lophocereus* presently includes two described species, the Baja California localized endemic *L. gatesii* and the more common and widespread *L. schottii*. The latter consists of three described varieties, *L. schottii* var. *australis*, *L. schottii* var. *schottii*, and *L. schottii* var. *tenuis* (Lindsay, 1963). The molecular phylogenetic relationships among these varieties and between *L. schottii* and *L. gatesii* are not known. Moreover, there are currently two conflicting hypotheses concerning the evolutionary origin of *Lophocereus* within the North American columnar cacti (tribe Pachycereeae). In one scheme, Buxbaum (1961) allied *Lophocereus* with the bat-pollinated *Carnegiea gigantea* in the Stenocereinae, a subtribe exhibiting floral adaptations to a variety of pollinators, including bats, birds, and insects. In the other scheme, Gibson and Horak (1978) placed *Lophocereus* in the subtribe Pachycereinae, which is almost entirely bat pollinated. Furthermore, Gibson and Horak identified the hummingbird-pollinated *Pachycereus marginatus* as sister to *Lophocereus*, which would make the otherwise bat-pollinated genus *Pachycereus* a paraphyletic group. Although the inclusion of *Lophocereus* into *Pachycereus*' synonym has been adopted in more recent taxonomic treatments of the Cactaceae (e.g., Hunt and Taylor, 1990; Barthlott and Hunt, 1993; Anderson, 2001), the exact

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phylogenetic position of *Lophocereus* within the tribe Pachycereeae has yet to be determined. The resolution of this issue has important implications not only for the classification of *Lophocereus* and *Pachycereus*, but also for understanding the transition to moth pollination in *Lophocereus*.

To establish the evolutionary origins of *Lophocereus* and its unusual pollination system, a phylogeny of the tribe and subtribe in which senita occurs is needed. Understanding the systematics of columnar cacti is complicated by the adaptive convergence of vegetative characters in response to xeric desert environments. Many morphological characters (such as leaves) are reduced to minimize transpirational water loss and show morphological homoplasy (Cota and Wallace, 1997). Floral characters, too, may exhibit homoplasy as a result of adaptation to a common guild of floral visitors, such as bats, which are the predominant pollinators of many columnar cacti. Phylogenetic inference is further hindered by the absence of a good fossil record for the Cactaceae. As a consequence, the generic nomenclature of columnar cacti as well as the grouping of genera into tribes and subtribes has been revised extensively in the past and is still being resolved (Gibson et al., 1986; Wallace, 1995; Cornejo and Simpson, 1997; Arias, Terrazas, and Cameron, 2001).

The main goals of this study were to use molecular sequence data to investigate the relationships among taxa within the genus *Lophocereus* and to establish the sister group and phylogenetic origins of *Lophocereus* within the North American columnar cacti (Pachycereeae). Because molecular data are less likely than morphological characters to exhibit homoplasy in response to convergent environmental selection, sequence analysis is a particularly effective method for resolving higher taxonomic relationships among cacti (Wallace, 1995; Cota and Wallace, 1997). In this paper, we first analyze the varietal and sister species relationships within the genus *Lophocereus*. We then conduct a broader molecular phylogenetic examination of the origin of *Lophocereus* within the North American columnar cacti. The sister group relationship of *Lophocereus* to *P. marginatus* was first determined, leading to the analysis of an expanded set of taxa including all six described *Pachycereus* species. To test the paraphyly of *Pachycereus* and the monophyly of *Pachycereus* and *Lophocereus*, we also included *Carnegiea gigantea* and *Neobuxbaumia tetetzo*, two closely related species within the subtribe Pachycereinae (sensu Gibson and Horak 1978). *Stenocereus thurberi*, a member of the subtribe Stenocereinae, was used as an outgroup based on the chloroplast restriction fragment length polymorphism (RFLP) analysis of Cota and Wallace (1997). The inclusion of these species allowed us to resolve conflicting hypotheses regarding the phylogenetic origin of *Lophocereus* and to speculate on the historical origin of its current pollination mutualism with *Uptiga virescens*.

MATERIALS AND METHODS

Current taxonomic treatments—*Taxonomy of Lophocereus*—Based on morphological characters, such as differences in branching pattern, height, rib number, and spacing of areoles, Lindsay (1963) delimited two species and three varieties of *Lophocereus*. Within *L. schottii*, the widespread variety *L. schottii* var. *schottii* is restricted to, but widely distributed within, the Sonoran Desert of northwestern Mexico and the southwestern United States. The two other varieties, *L. schottii* var. *tenuis* and *L. schottii* var. *australis*, are restricted to the coastal plain of southern Sonora/northern Sinaloa and the subtropical thornscrub of the Cape Region of southern Baja California, respectively. The second species, *L. gatesii*, is endemic to a small area of fog desert on the

Pacific coast of southern Baja west of La Paz. More recent taxonomic treatments (e.g., Anderson, 2001) recombine these two species within *Pachycereus* (but do not recognize the varieties of *L. schottii*). The phylogenetic distinctiveness and relationship among species and varieties within *Lophocereus* have yet to be examined using DNA sequence data.

Taxonomy of the genus Pachycereus—The classification of species in the genus *Pachycereus* within the subtribe Pachycereinae is contentious and has been modified extensively since the suggestion by Britton and Rose (1909) to include arborescent cacti with trichome-covered flowers and bur-like fruits. Several species have been added or removed from the genus by various taxonomists who have used different sets of morphological characters for their studies, with only *Pachycereus grandis*, *P. pecten-aboriginum*, and *P. pringlei* consistently assigned to *Pachycereus* (see Gibson et al., 1986, for review). Included in this study are the six *Pachycereus* species recognized by Gibson: *P. pringlei*, *P. pecten-aboriginum*, *P. marginatus*, *P. weberi*, *P. hollianus*, and *P. grandis*.

Taxonomy of the tribe Pachycereeae—Classification of cacti in the tribe Pachycereeae has changed extensively in the past (see Gibson et al., 1986, for review). We summarize here the two predominant hypotheses regarding the phylogenetic relationships of North American columnar cacti. Buxbaum (1961) conducted morphological and geographical analyses of cacti using as characters stem triterpenes, funicular pigments, and seed morphology. In his final phylogeny of genera within the Pachycereeae, the four subtribes Myrtillocactinae, Pachycereinae, Pterocereinae, and Stenocereinae were recognized. In contrast, based on analyses of stem chemistry, funiculus pigmentation, seed morphology, growth form, rib morphology and anatomy, flower morphology, and presence and levels of mucilage, Gibson and coworkers recognized only the two subtribes Stenocereinae and Pachycereinae in the Pachycereeae (Gibson and Horak, 1978). In their treatment (as well as in more recent classifications), the Stenocereinae largely subsume Buxbaum's Myrtillocactinae, Pterocereinae, and Stenocereinae. Buxbaum (1961) suggested that *Lophocereus* is closely related to the bat-pollinated *Carnegiea gigantea* in the subtribe Stenocereinae, a subtribe exhibiting floral adaptations to a variety of pollinators. Gibson and Horak (1978), in contrast, placed *Lophocereus* in the Pachycereinae, a subtribe that is almost entirely bat pollinated. The placement of *Lophocereus* within these subtribes has important implications for understanding the origins of its highly derived floral biology associated with the senita moth.

Tissue source and DNA extraction—To reconstruct phylogenies, we extracted DNA from seeds, flower buds, or stem sections of *L. gatesii*, *L. schottii* var. *australis*, *L. schottii* var. *schottii*, *L. schottii* var. *tenuis*, and *Pachycereus pecten-aboriginum* and from flower buds of *P. pringlei*, *S. thurberi*, and *C. gigantea* in the Sonoran Desert (Baja California and Sonora, Mexico). Seeds of *P. grandis* originated from La Noria (Puebla, Mexico). Voucher specimens for *Lophocereus* species and varieties, *P. pecten-aboriginum*, and *P. grandis* have been deposited in the Herbarium of the University of Iowa. Material from *P. pringlei*, *S. thurberi*, and *C. gigantea* is available upon request from the authors. *Pachycereus marginatus* and *P. weberi* seedlings were purchased from Mesa Garden (Belen, New Mexico, USA).

Total genomic DNA was extracted from seedlings, stem sections, or flower buds using a protocol based on the hexadecyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Modifications were implemented to optimize DNA extraction from the polysaccharide-rich cactus tissues as described previously (Hartmann, Nason, and Bhattacharya, 2001). For initial amplification of the *trnC-trnD* intergenic regions, we used purified chloroplast-DNA as template, which was isolated by CsCl gradient ultracentrifugation based on a protocol for extraction of chloroplast genomes of kelp (Fain, Druehl, and Baillie, 1988). DNA preparations from *P. hollianus* and *N. tetetzo* were a gift from R. Wallace (Iowa State University) and were used directly as template for the polymerase chain reactions (PCRs).

Polymerase chain reaction and sequence analysis—The three chloroplast intergenic spacers, *trnL-trnE*, *trnC-trnD*, and *trnS-trnM*, were PCR-amplified,

TABLE 1. Accession numbers of the chloroplast sequences determined in this study.^a

Species	Sequenced intergenic region		
	<i>trnL-trnF</i>	<i>trnC-trnD</i>	<i>trnS-trnM</i>
<i>Lophocereus schottii</i> var. <i>schottii</i>	GBAN-AY048819	GBAN-AY048839	GBAN-AY048829
<i>Pachycereus grandis</i>	GBAN-AY048824	GBAN-AY048844	GBAN-AY048834
<i>Pachycereus hollianus</i>	GBAN-AY048826	GBAN-AY048846	GBAN-AY048835
<i>Pachycereus marginatus</i>	GBAN-AY048821	GBAN-AY048841	GBAN-AY048830
<i>Pachycereus pecten-aboriginum</i>	GBAN-AY048823	GBAN-AY048843	GBAN-AY048833
<i>Pachycereus pringlei</i>	GBAN-AY048822	GBAN-AY048842	GBAN-AY048832
<i>Pachycereus weberi</i>	GBAN-AY048820	GBAN-AY048840	GBAN-AY048831
<i>Carnegiea gigantea</i>	GBAN-AY048825	GBAN-AY048845	GBAN-AY048837
<i>Neobuxbaumia tetetzo</i>	GBAN-AY048828	GBAN-AY048848	GBAN-AY048836
<i>Stenocereus thurberi</i>	GBAN-AY048827	GBAN-AY048847	GBAN-AY048838

^a The prefix GBAN- has been added to each accession number to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

sequenced, and used as phylogenetic markers for *L. schottii* var. *schottii*, *L. schottii* var. *australis*, and *L. gatesii* as well as for *S. thurberi*, *C. gigantea*, *N. tetetzo*, and the six *Pachycereus* species. The PCRs were carried out with either *Taq* polymerase (Fisher Scientific, Pittsburgh, Pennsylvania, USA) or *Biolase* DNA polymerase (Biolone, Randolph, Massachusetts, USA) in 50- μ L reaction volumes. The template DNA was first denatured at 94°C for 10 min and then subjected to 35 cycles of the following PCR program: 94°C for 1 min, annealing for 1 min, extension at 72°C. Annealing temperatures and extension times were specific for each intergenic fragment as detailed below. The final cycles included an extension of 10 min at 72°C. The PCR products were gel-purified using glassmilk (GeneClean Kit, Qbiogene, Carlsbad, California, USA) according to the manufacturer's instructions and either directly sequenced or first cloned into pGEM-T (Promega, Madison, Wisconsin, USA). Sequencing reactions used a dye termination sequencing protocol and were carried out on a 373 A Fluorescent Automated Sequencer (Perkin Elmer-Applied Biosystems, Wellesley, Massachusetts, USA).

Primers "e" (5'-GGTTCAAGTCCCTCTTCCC-3') and "f" (5' ATTTGAACCTGGTGACACGAG-3') (Taberlet et al., 1991) were used to amplify the *trnL-trnF* intergenic region of approximately 450 base pairs (bp). The PCR cycle used an annealing temperature of 55°C and an extension time of 1 min. The PCR primer "f" was used as a sequencing primer for all taxa. Because we were unable to amplify the *trnC-trnD* intergenic region from total genomic DNA of senita, we initially used purified chloroplast DNA of *L. s. schottii* as template. Primers *trnC* (5'-CAAGTTCAAATCCGGGTGTC-3') and *trnD* (5'-GGGATTGTAGTTCAATTGGT-3') (Demesure, Sodzi, and Petit, 1995) were used to amplify this region of approximately 3 kilobases (kb). The fragment was partially sequenced from both ends using the PCR primers as sequencing primers. Sequences were used to design internal primers CD-2F and CD-2R for subsequent PCR and sequencing reactions. Using primer *trnC* in combination with the internal primer CD-2R (5'-GGTACCCTGACAAAATAC-3'), we were able to amplify about 2.5 kb of the *trnC-trnD* intergenic region from total genomic DNA of the remaining 11 taxa. Sequencing reactions were done using primers *trnC* and CD-2F (5'-GGATTATTGAATTGC TTC-3'). Primers *trnS* (5'-GAGAGAGAGGGGATTTCGAACC-3') and *trnM* (5'-CATAACCT-TGAGGTCACGGG-3') (Demesure, Sodzi, and Petit, 1995) were used to amplify the *trnS-trnM* intergenic region of approximate size 1.5 kb. The PCR cycle used an annealing temperature of 62°C and an extension time of 2 min. The *trnM* PCR primer was used as a sequencing primer. Sequences determined in this study have been deposited in the EMBL/GenBank/DBJ databases (see Table 1).

Phylogenetic analyses—Sequences were aligned manually using the program SeqApp v1.9a (Gilbert, 1992). Alignments for the individual cpDNA sequences were 435 bp for *trnL-F*, 1149 bp for *trnC-D*, and 375 bp for *trnS-M*. The alignment of the concatenated sequences using all three markers for the 11 cactus taxa was 1960 bp in length. The cpDNA phylogeny was outgroup rooted with sequences from *S. thurberi*. We used the separate sequences as well as the concatenated sequences for phylogenetic reconstructions using

the maximum parsimony, neighbor-joining, maximum likelihood, and quartet puzzling methods to infer a phylogeny. Gaps were included, but treated as missing data, and all phylogenetic methods were implemented using the computer program PAUP* (Swofford, 2001). The unweighted maximum parsimony method was done using a heuristic search of ten replicates with random stepwise addition and tree bisection-reconnection (TBR) and the MulTrees option. One thousand bootstrap replicates were analyzed with this method. Hierarchical likelihood ratio tests were done to estimate the best-fit model for the cpDNA data set (MODELTEST v3.06; Posada and Crandall, 1998). This procedure showed that the F81 (Felsenstein, 1981) model incorporating the proportion of invariant sites (F81 + I) best fit our data. The F81 + I model was used to calculate the distance matrix for neighbor-joining tree-building. The bootstrap method (1000 replicates) was used to determine the support for nodes in this tree. The F81 + I model was then used to do the heuristic maximum likelihood (ML) analysis. Starting trees were obtained with stepwise addition (randomly drawn, ten rounds) and rearranged with tree bisection-reconnection. One hundred bootstrap replicates were analyzed using these settings. Quartet puzzling (10000 steps) was done using the maximum likelihood optimality criterion of PAUP* under the F81 + I model (Swofford, 2001).

Phylogenetic signal—To determine the structure of our data, we used the skewness of tree length distribution (g1 value) as an indication of strength of phylogenetic signal (Hillis and Huelsenbeck, 1992). We completed an exhaustive search using the 1960 bp alignment of the concatenated sequences. The distribution of the lengths of the trees was significantly skewed with a g1 value of -1.110391 ($P < 0.01$), indicating that a strong nonrandom structure was present in our data matrix.

Constrained topologies—Because parts of our phylogeny are not consistent with existing taxonomic schemes (Buxbaum, 1961; Gibson and Horak, 1978), we wanted to test whether the topologies predicted by Buxbaum and by Gibson and Horak are significantly less likely than our best tree given the sequence data. We therefore constructed alternative tree topologies reflecting these two classifications using the computer program MacClade v3.04 (Maddison and Maddison, 1992). The likelihoods of these two constrained phylogenies were compared to the likelihood of our best maximum likelihood tree using the Shimodaira-Hasegawa nonparametric bootstrap test (10000 replications; Shimodaira and Hasegawa, 1999).

RESULTS

Relationships within *Lophocereus*—The 1960 bp of concatenated sequence failed to show any polymorphism between *Lophocereus* species or varieties of *L. schottii*. We also sequenced an additional 1000 bp of chloroplast intergenic region and screened about 21 kb of chloroplast and mitochondrial noncoding fragments by RFLP analysis (Southern blotting and

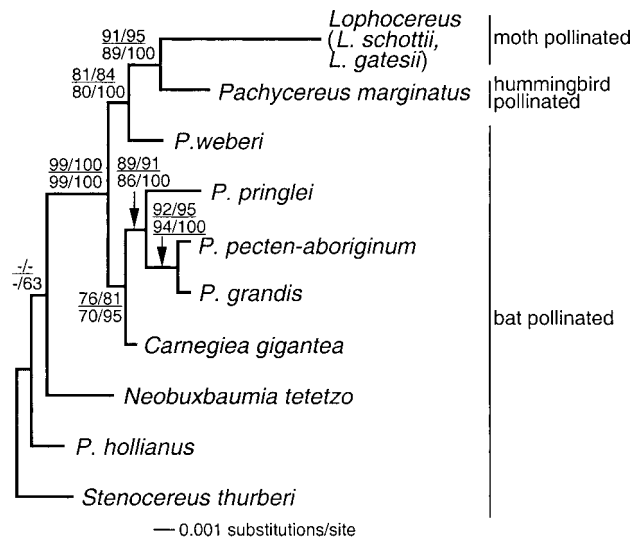


Fig. 1. Maximum likelihood tree inferred from comparisons of chloroplast intergenic regions. A total of 1960 nucleotides (19 parsimony-informative sites) were used in the analysis. Results of bootstrap analysis (1000 replications) are shown at the nodes. Values to the left and right of the slashmarks and above and below the line correspond to maximum likelihood, neighbor-joining, maximum parsimony, and quartet puzzling values, respectively. Bootstrap values below 60% are indicated by dashes. The pollination syndrome associated with each species is indicated.

PCR-RFLP; S. Hartmann, unpublished data) without finding evidence for sequence variation within *Lophocereus*. Thus, for the purposes of the following analyses, we treat the genus *Lophocereus* as a single taxon.

Phylogenetic origins of *Lophocereus*—The number of polymorphic, informative sites for alignments involving all 11 cactus species was 6 for *trnT-L*, 8 for *trnC-D*, 5 for *trnS-fM*, and 19 for the concatenated sequence. Using the combined data set for tree reconstructions, we obtained the same topology using maximum likelihood, neighbor-joining, and quartet puzzling methods (Fig. 1). In an exhaustive maximum parsimony search, two trees of equal lengths were found that differed only in the positions of *N. tetetzo* and *P. hollianus*, which were switched. Trees constructed using the three sequences separately were consistent as shown in Fig. 1, such as the monophyly of the clade containing *P. pringlei*, *P. pecten-aboriginum*, *P. grandis*, and *C. gigantea*, and the sister taxa relationship of *Lophocereus* and *P. marginatus* and of *P. pecten-aboriginum* and *P. grandis*. The concatenated sequences, however, gave higher resolution, reflected in an increase in bootstrap values for nodes in the tree, and we hereafter limit our discussion to the concatenated sequences.

Constrained topologies—We constructed alternative tree topologies shown in Fig. 2B (according to Gibson and Horak, 1978) and Fig. 2C (according to Buxbaum, 1961). Maximum likelihood values and results of the Shimodaira-Hasegawa test for these alternative topologies are given in Table 2. Our results showed that the likelihood of a constrained tree according to the classification scheme of Buxbaum (1961) was lower than that of the tree with a topology suggested by Gibson and Horak (1978). Both constrained trees were, however, signifi-

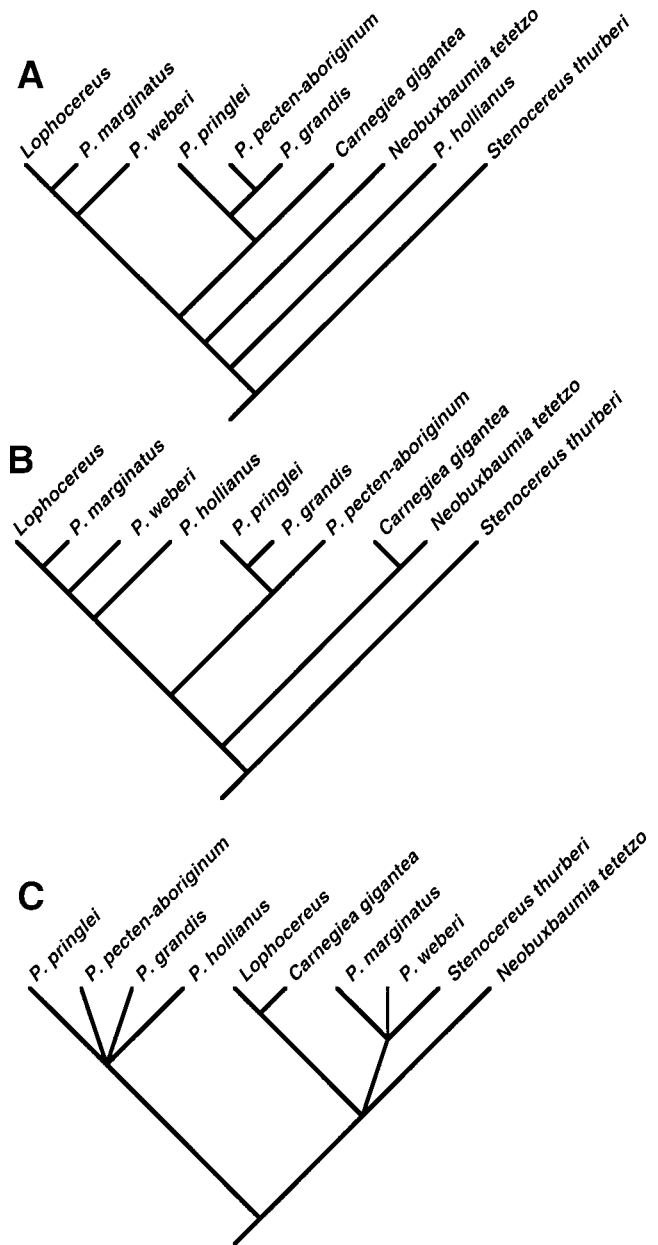


Fig. 2. Alternative classification schemes tested against our best tree. (A) Our maximum likelihood tree, same topology as in Fig. 1. (B) Topology of Cornejo and Simpson (1997) after Gibson and Horak (1978). (C) Topology suggested by Buxbaum (1961). These alternative, constrained phylogenies were significantly less likely than our best maximum likelihood tree using the Shimodaira-Hasegawa test.

TABLE 2. Results of the analysis of constrained tree topologies. Likelihood values are given for the trees shown in Fig. 2. Tree 2A is our maximum likelihood tree, tree 2B is the tree of Cornejo and Simpson (1997) after Gibson and Horak (1978), and tree 2C was constructed according to Buxbaum (1961), but using the nomenclature of Gibson and Horak (1978).

Measure	Tree		
	2A	2B	2C
Log likelihood	3162.67	3196.14	3230.54
Shimodaira-Hasegawa test: <i>P</i>	—	0.0134	0.0007

cantly “worse” than the unconstrained optimal tree shown in Figs. 1 and 2A.

DISCUSSION

Low rates of sequence divergence within *Lophocereus*—The genus *Lophocereus*, as well as the other cactus species examined in our study, appears to exhibit relatively low rates of chloroplast DNA sequence divergence compared to other plant species (see below). For example, the *trnL* intron and the *trnL-trnF* spacer in members of the African tree of the genus *Leonardoxa* were shown to contain 32 (2.51%) informative polymorphic sites in an alignment of 1275 bp (Brouat, Gielly, and McKey, 2001). In contrast, we found 0/3000 polymorphic sites between members of the genus *Lophocereus*, and only 19/1960 (0.92%) informative polymorphic sites in the ten cactus species examined. The genomic regions we assayed have been used with other species to successfully resolve evolutionary relationships at the taxonomic levels investigated here (e.g., Mayer and Soltis, 1994; Levy et al., 1996; Dumolin-Lapegue et al., 1997; Nicolosi et al., 2000). For several species, these markers have even shown sufficient variation to permit studies of intraspecific diversity (e.g., Soltis et al., 1991, 1992; El Mousadik and Petit, 1995; Maskas and Cruzan, 2000; Mohanty, Martin, and Aguinalgalde, 2000). There are more recently diverged taxa, however, that exhibit levels of chloroplast sequence variation comparable to that found here. Small et al. (1998), for example, found that five tetraploid *Gossypium* species that diverged 0.5–2 million years ago contained only four informative nucleotide substitutions (0.05%) in over 7 kb of chloroplast sequences.

Comparative studies have shown that the chloroplast genome exhibits significant rate heterogeneity both among lineages and among different DNA regions (Gaut et al., 1992). This may explain the low chloroplast sequence variation in *Lophocereus* and related columnar cacti relative to other plant taxa. The limited sequence variation could also be due to the relatively recent origin and radiation of the columnar cacti in North America. Within *Lophocereus*, sequence variation may have been further reduced as a result of genetic bottlenecks during periods of range expansion and contraction due to Pleistocene climatic change. Paleoecological analyses of plant remains preserved in packrat middens have been used to infer northward range expansion of senita from more southerly Pleistocene refugia within the last 2000–4000 yr (Peñalba and Van Devender, 1998; Van Devender, in press). Founding events coupled with the high coalescence rates of haploid genomes may, therefore, explain lack of variation within *Lophocereus*, especially in northern Baja California and Sonora.

The question remains whether the widespread *L. schottii* and the Baja endemic *L. gatesii* are in fact distinct species. If indeed they are, the lack of observed variation between their cpDNA could reflect late divergence of these taxa, slow chloroplast sequence evolution, and/or bottlenecks in the history of the genus. Alternatively, *L. schottii* and *L. gatesii* may constitute a single species. In this case, the observed morphological differences may reflect geographical variation within a single widespread species as opposed to the existence of evolutionarily independent lineages. Climatic effects on cactus morphology have previously been documented for *Lophocereus* (Felger and Lowe, 1967) and for other columnar cacti in the Pachycereeae (Cornejo and Simpson, 1997). More detailed genetic and ecological analyses of the transition zones between

current taxonomic species and varieties will be required to resolve the phylogeny and systematics of the genus.

Phylogenetic analyses within tribe Pachycereeae—Relationships within the Pachycereinae—As shown in Fig. 1, our phylogeny strongly supported the sister relationship of *Lophocereus* and the hummingbird-pollinated *P. marginatus*. This is in agreement with Gibson and Horak (1978), who found that these two species share a very similar growth habit, external shoot morphology, and stem chemistry. In further agreement with Gibson and Horak (1978), we found that *P. weberi* is basal to the clade with *Lophocereus* and *P. marginatus*, and these three cacti are sister to a clade containing *C. gigantea*, *P. pecten-aboriginum*, *P. pringlei*, and *P. grandis*. Within this latter clade, however, our analyses indicate *P. pringlei* to be the basal species of *Pachycereus*, whereas *P. pecten-aboriginum* is basal to *P. pringlei* and *P. grandis* in the morphological phylogeny of Cornejo and Simpson (1997).

Constrained topologies—The positions of *C. gigantea* and *N. tetetzo* (or *P. hollianus*) in our phylogeny are not consistent with current taxonomic schemes (Buxbaum, 1961; Gibson and Horak, 1978; Hunt and Taylor, 1990; Barthlott and Hunt, 1993; Anderson, 2001). For example, Gibson and Horak (1978) placed *Neobuxbaumia* and *Carnegiea* in the subtribe Pachycereinae (Fig. 2B), but outside the *Pachycereus* phylad. In contrast, our data support the monophyly of *C. gigantea* and *Pachycereus* (and *Lophocereus*, Figs. 1, 2A). Thus, based on growth habit, vegetative morphology, and flower and fruit structure, *Carnegiea* appears to be more closely related to *Neobuxbaumia*, but when stem chemistry or cpDNA sequence data are used for comparison, *Carnegiea* appears to be more closely related to *Pachycereus*.

Cornejo and Simpson (1997) converted Gibson and Horak's findings into a standard phylogenetic tree format (Fig. 2B), allowing us to test the likelihood of their phylogeny by forcing its topology onto our cpDNA data set. Constraining the phylogeny so that *Carnegiea* is positioned outside of *Pachycereus* and sister to *Neobuxbaumia* results in a significantly lower maximum likelihood value (see Table 1) relative to the topology in Fig. 1. Consistent with our analysis are the results of a study using cpDNA RFLP data (Cota and Wallace, 1997), in which *Carnegiea* forms a separate clade with *Lophocereus* and *P. marginatus*, whereas *Bergerocactus emoryi*, *Lemaireocereus* (*Pachycereus*) *hollianus*, and *Neobuxbaumia euphorbioides* form a second clade within the Pachycereinae.

In the arrangement shown in Fig. 2C, we test the phylogenetic relationship published by Buxbaum (1961). Buxbaum placed *Lophocereus* and *Carnegiea* as sister taxa into the subtribe Stenocereinae and classified *P. marginatus* and *P. weberi* within *Stenocereus*. All remaining *Pachycereus* species were placed into the subtribe Pachycereinae. We used Buxbaum's phylogeny as a reference for constructing this alternative tree, though we maintained the nomenclature/classification system of Gibson and Horak. Our analysis of the tree topology constructed using Buxbaum's classification (Fig. 2C) shows that the likelihood (Table 1) of this constrained tree was lower than that of the Gibson and Horak tree and significantly lower than that of the unconstrained optimal tree shown in Figs. 1 and 2A. We conclude, therefore, that with the exception of *S. thurberi*, all species examined here are best treated as members of the subtribe *Pachycereinae*. Moreover, *Lophocereus* is very closely related and probably sister to the hummingbird-poll-

nated *P. marginatus* as suggested by Gibson and Horak (1978), and not sister to the bat-pollinated *Carnegiea*, as suggested by Buxbaum (1961). The robust phylogenetic positions of *Lophocereus* and *C. gigantea* within the *Pachycereus* phylad shows *Pachycereus* to be paraphyletic.

The phylogenetic position of *P. hollianus* and *N. tetetzo* in our tree may suggest that the genus *Pachycereus* needs to be reorganized to include *Lophocereus*, *Carnegiea*, *Neobuxbaumia*, and perhaps other species, into a monophyletic group. An alternative possibility, which is also supported by Gibson (1982), is that *P. hollianus* might not be a member of the genus *Pachycereus*. According to Gibson (1982), *P. hollianus* is the least specialized member of the *Pachycereus* group, and he speculates that it may in fact be "distinctive enough to be classified to its own genus." Furthermore, Gibson suggests that *Carnegiea* is closely related to *Neobuxbaumia* and these, in turn, to *Mitrocereus*. Clearly, further research that includes additional taxa is required to define the limits of the genus *Pachycereus* and to gain a better understanding of the relationships among genera in the Pachycereinae.

Evolution of pollination systems in the Pachycereae and the Pachycereus phylad—Selection pressures induced by interactions of flower and pollinator have led to reciprocal adaptations often referred to as "pollination syndromes." Knowledge of these syndromes can provide a useful tool for predicting the suite of visiting pollinators. Nonetheless, several morphological and physiological characters important for attracting pollinators are shared among flowers pollinated by New World bats, birds, and hawkmoths. Shared traits include relatively large flowers and quantities of nectar and blossoms positioned outside of the foliage where they are exposed to hovering pollinators (Faegri and van der Pijl, 1971). The flowers of night-blooming bat and hawkmoth-pollinated species often attract nectar-feeding birds at dusk and in the morning (e.g., *C. gigantea* and *S. thurberi*; Fleming, Tuttle, and Horner, 1996; Fleming et al., 2001). Similarly, day-flying hawkmoths may compete with hummingbirds for nectar at bird blossoms (Faegri and van der Pijl, 1971). These observations underscore the potential for overlap in attraction among bat, hummingbird, and hawkmoth flowers and are consistent with frequent evolutionary transitions among these pollination systems (van Helverson, 1993).

Bat pollination is widespread in the tribe Pachycereae and many species produce flowers ideally adapted for bat pollination (Gibson and Horak, 1978). In a recent survey, 42 of the 70 species in the Pachycereae were found to be exclusively or primarily bat-pollinated (Valiente-Banuet et al., 1996). Given that bat pollination is the ancestral condition, selection pressures have in several cases led to pollination by smaller insects, hummingbirds, and hawkmoths. These shifts are primarily in the subtribe *Stenocereinae* (Gibson, 1982). As indicated in Fig. 1, and given that all species and varieties of *Lophocereus* are a single species, then *Lophocereus* and its sister *P. marginatus* are the only two cacti in the *Pachycereus* phylad of which we are aware with derived, non-bat-pollination syndromes. *Pachycereus weberi*, which is sister to *Lophocereus* and *P. marginatus* in our phylogeny, is nocturnal, bat pollinated, and has funnel-form flowers that are 8–10 cm in length (Gibson and Horak, 1978).

We suggest three possible scenarios that may have led to the evolution of moth pollination in *Lophocereus* and to hummingbird pollination in *P. marginatus*. In the first, the transi-

tion from a bat-pollinated common ancestor may have occurred independently in these two lineages: the lineage leading to *P. marginatus* evolved smaller, diurnal, hummingbird-pollinated flowers, whereas in the lineage leading to *Lophocereus*, flowers remained nocturnal but experienced a reduction in size and nectar production, apparently associated with adaptation to moth-pollination. The second possibility is the sequential evolution of derived pollination syndromes in *Lophocereus* and *P. marginatus* from a bat-pollinated common ancestor. Under this hypothesis, an initial transition to hummingbird pollination would have served as a preadaptation to moth pollination in *Lophocereus*. The evolution of reduced flower size, reduced nectar production, and diurnal anthesis may have been followed by a reversal to nocturnal anthesis. Hummingbirds are known to be significant pollinators of primarily bat-pollinated species, at least in some years (Fleming, Tuttle, and Horner, 1996; Sahley, 1996) and under this scenario would have been an active selective agent on the floral biology of the bat-pollinated ancestor. The alternative hypothesis is that the transition to moth pollination occurred first and served as a preadaptation to hummingbird pollination in *P. marginatus*. Under this scenario, the evolution of reduced flower size and nectar production would have preceded the evolution of diurnal anthesis. The transition from a highly specialized obligate pollination mutualism to a more generalized hummingbird-pollinated system seems perhaps the less parsimonious of these two alternative scenarios; however, there is molecular phylogenetic support for this evolutionary sequence in *Yucca* with the hummingbird-pollinated taxon *Hesperaloe* nesting within an obligately moth (*Tegeticula*)-pollinated clade (*Yucca* including *Y. whipplei*; Bogler, Neff, and Simpson, 1995; Clary and Simpson, 1995). The third possible scenario is the evolution of hummingbird- and moth-pollinated lineages from a common ancestor with a more generalized pollination system. Under this hypothesis, a transition from bat pollination to a generalized pollination system, perhaps involving various insect and hummingbird species, served as a preadaptation for the evolution of the more specialized pollination systems in *Lophocereus* and *P. marginatus*.

In summary, our analyses failed to reveal cpDNA variation in *Lophocereus*. This result is consistent with the conspecificity of taxa within the genus, however further genetic and ecological studies will be required to resolve the phylogeny of *Lophocereus*. Our analyses strongly support the close relationship of *Lophocereus* and *P. marginatus*. These results suggest that the shift from bat pollination in the Pachycereinae likely represents a single evolutionary event and did not arise independently in *Lophocereus*.

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