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POLLINATION BIOLOGY AND ADAPTIVE RADIATION OF AGAVACEAE, WITH SPECIAL EMPHASIS
ON THE GENUS AGAVE

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ABSTRACT

Agavaceae are an American family that comprises nine genera and ca. 300 species distributed in arid and semiarid environments, mainly in Mexico. The family is very successful and displays a wide array of ecological, reproductive, and morphological adaptations. Many of its members play important roles as keystone species, because they produce abundant resources during the reproductive season. In this paper we analyze the current knowledge about the pollination ecology of the different genera in the family and the role that pollination systems have played in the ecological and phylogenetic success of the group. After providing an overview of each of the genera in the family, we discuss in detail aspects of the reproductive ecology of species in the genus *Agave* s.l., which is composed of ca. 208 species and includes subgenera of *Agave* (*Agave* and *Littatea*), *Manfreda*, *Polianthes*, and *Prochnyanthes*. Finally, we describe the results of analyses to test the hypothesis that there has been an adaptive radiation in the genus *Agave*. Using chloroplast and nuclear DNA sequences we estimate the age of the Agavaceae family and the genus *Agave* to be 12–26 millions of years ago (MYA) and 10 MYA, respectively, and show that mean rates of diversification were higher in the genus *Agave* than the genus *Yucca*. The values we report for rates of diversification in *Agave* s.l. are high when compared to other radiations in plants and animals. We suggest that the desertification of North America, which started ca. 15 MYA was critical in the radiation of agaves and that the generalist pollination system of *Agave* has been more successful in generating new species than the extreme specialization of *Yucca*.

Key words: adaptive radiation, Agavaceae, *Agave*, bats, *Leptoncyteris*, pollination, reproductive ecology, *Yucca*.

THE AGAVACEAE FAMILY

Agavaceae are an American family distributed in arid to semiarid environments; the majority of species are found in Mexico. Currently nine genera and ca. 300 species are recognized in the family (see Bogler et al. 2006 and Table 1). All members of the family exhibit a similar basic structure in the arrangement of their rosette, flowers, and inflorescences. Moreover, they all share a basic chromosome number (karyotype), consisting of five large chromosomes and 25 very small ones. The only exceptions to this karyotype occur in polyploid species, which have two or more copies of the basic haploid chromosome set.

Traditional classification systems placed *Agave* and related genera as part of Amaryllidaceae based on the shared characteristic of the inferior position of the ovaries, while *Yucca* and the species related to it were classified as part of Liliaceae because their ovaries are superior. While the original classification of Agavaceae by Hutchinson (1934) included a wide diversity of species, more recent detailed morphological (Dahlgren et al. 1985; Alvarez de Zayas 1987; Hernandez 1995) and molecular studies (Eguiarte et al. 1994, 2000; Bogler and Simpson 1995, 1996; Bogler et al. 1995; Eguiarte

1995) have shown that the definition of Agavaceae should be restricted to include the genera and species found in Table 1. For a detailed account of Agavaceae and related taxa see Eguiarte et al. (2000).

Some other genera in the monocotyledons, such as *Hosta* Tratt. (Hostaceae) (Kubitzki 1998) found in Korea, China, and Japan, and some perennial bulbs from North America, such as *Chlorogalum* Kunth, *Hesperocallis* A. Gray, and *Camassia* Lindl., are now considered to be part of Hyacinthaceae, and are very closely related to Agavaceae. Detailed taxonomic studies are needed to determine if these genera should be considered part of Agavaceae or just closely related to the group (see Bogler et al. 2006).

Members of Agavaceae display a wide array of ecological, reproductive, and morphological adaptations to arid environments. The family has been important for people living in the Americas since prehistoric times, with various species providing clothes, rope, food, and beverages (both nonalcoholic and alcoholic) to humans. Currently, the family is of huge economic importance to Mexico because both tequila and mezcal are produced from *Agave* plants. In addition, fibers of significant economic importance are still de-

Table 1. Genera and species number in Agavaceae.

Genera	No. of species	No. of species in Mexico
<i>Agave</i> L.	166	125
<i>Beschorneria</i> Kunth	7	7
<i>Furcraea</i> Vent.	25	11
<i>Hesperaloe</i> Engelm.	5	5
<i>Hesperoyucca</i> (Engelm.) Baker	1	1
<i>Manfreda</i> Salisb.	28	27
<i>Polianthes</i> L.	13	13
<i>Prochnyanthes</i> S. Watson	1	1
<i>Yucca</i> L.	49	29
Total	293	217

rived from various species of the family, such as henequen and sisal from the *Agave* genus, as well as other fibers from *Yucca* and potentially from *Hesperaloe*.

GENERAL ECOLOGY OF THE FAMILY

Owing to a suite of morphological and physiological adaptations, members of Agavaceae are especially successful in arid and semiarid environments in the deserts and mountains of the Americas and play an important role as keystone species in these habitats because they produce abundant resources, mainly during the reproductive season, as will be discussed below.

While all Agavaceae species form rosettes, there is considerable variation in the extent of woodiness among species such that some are considered to be woody perennials, in particular species in *Agave* (i.e., *A. karwinskii* Zucc.), *Furcraea*, and *Yucca*, while others are completely herbaceous, producing leaves and inflorescences from a subterranean bulb, like all species in *Manfreda*, *Polianthes*, and *Prochnyanthes*. Most of the species are long-lived succulents, though leaf thickness can vary among species. Perhaps the most spectacular adaptation is in their reproductive ecology, as almost all *Agave*, *Furcraea*, and *Hesperoyucca* are monocarpic (semelparous); the rosette grows for several years, usually more than ten (see Eguiarte et al. 2000, for some estimates) and after producing a huge inflorescence, the rosette dies. However, though individual rosettes exhibit monocarpy, many species also reproduce clonally, such that a genet may survive for many generations. Though these three genera are monocarpic, all the other species (the group Striatæ of *Agave*, *Beschorneria*, *Manfreda*, *Polianthes*, *Prochnyanthes*, and *Yucca*) are polycarpic (iteroparous).

Although data on self-compatibility are scarce, most species are believed to be self-compatible. However, there is evidence that *Hesperaloe* is self-incompatible (Pellmyr and Augenstein 1997) and a few studies in *Agave* (Eguiarte et al. 2000; Molina-Freaner and Eguiarte 2003) have suggested that there may be some prezygotic barriers to reproduction although it is difficult to rule out intense inbreeding depression (Eguiarte et al. 2000; Slauson 2001).

The family displays a wide variation in pollination ecology, which we will describe in detail below; but briefly, most species are pollinated by nocturnal animals, some are pollinated mostly by moths, and in some species, hawkmoths (family Sphingidae) play an important role. *Yucca* and *Hes-*

peroyucca whipplei (Torr.) Trel. are only pollinated by *Tegeticula* and *Parategeticula* moths (Pellmyr 2003). The *Yucca*–*yucca* moth coevolution is considered, along with the fig–fig wasp interaction, as the premier example of extreme specialization and codependent pollination. Another set of species, mostly in *Agave*, is also pollinated during the night by bats, in particular by the genus *Leptonycteris* (Eguiarte et al. 2000). However, the bat pollination syndrome is “leaky” (that is, open to exploitation and usage by other visitors), since a large number of other animals is usually also involved in pollination (Proctor et al. 1996). Some other *Agave* species are primarily pollinated by bees, hummingbirds, and perching birds, in particular orioles (Ornelas et al. 2002).

Undoubtedly, pollination ecology has played an important role in the ecological and phylogenetic success of the group, but interestingly the most species-rich genera in the family, *Yucca* and *Agave*, have contrasting reproductive strategies. In *Yucca*, the pollination system is very restrictive (it includes only a set of specialized yucca moths), and involves a shift in rewards, from pollen and nectar to the developing ovules wherein the yucca moths lay their eggs. On the other hand, most species in the most diverse genus *Agave*, with ca. 166 species, have been selected to produce very large amounts of nectar and pollen, and are visited by a large coterie of pollinators, ranging from small insects to relatively large vertebrates such as perching birds and bats. While some species of *Agave* attract diverse pollinators, it has been suggested that *Agave* and bats are also an example of coevolution and mutualism (Gentry 1982; Arita and Humphrey 1988). In this paper, we analyze what is known about the pollination ecology of the different genera in Agavaceae and discuss their evolution in terms of what we know about the phylogeny and evolution of the family using molecular evolution and statistical tools.

PHYLOGENY OF THE FAMILY

Members of Agavaceae have been the object of several phylogenetic studies, both morphological (Alvarez de Zayas 1987; Hernández 1995) and molecular, using either chloroplast *rbcL* sequences (Eguiarte et al. 1994; Eguiarte 1995), restriction enzymes analyses of the chloroplast genome (Bogler and Simpson 1995), or ITS nuclear sequences (Bogler and Simpson 1995, 1996; Bogler et al. 1995; Eguiarte et al. 2000). The basic relationships among the main groups have been found to be congruent in the majority of the studies, and are shown in Fig. 1 (see also Bogler et al. 2006). In contrast, it has been very difficult to resolve the phylogeny within most genera and in *Agave* s.l. (which includes the genera *Agave* s.s., *Manfreda*, *Polianthes*, and *Prochnyanthes*), because little genetic variation is found at the sequence level in chloroplast and single copy nuclear markers. This is testimony to the relatively recent origin of the group, as we will explain later. Given the recent origins of many species in *Agave* s.l., the phylogenetic relationship among the species may be unresolvable because of either insufficient time for lineage sorting or hybridization and introgression, which also seem to be important within genera (Gentry 1982; Clary and Simpson 1995; Valverde et al. 1996).

The consensus tree of the phylogenetic relationship in

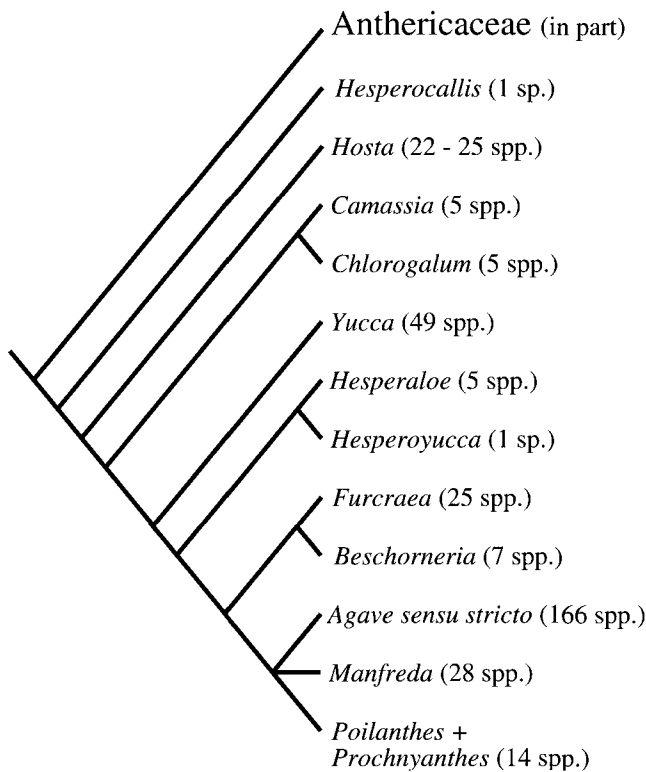


Fig. 1.—Agavaceae phylogeny.

Agavaceae, as depicted in Fig. 1, indicates that *Yucca* is the sister group of the remainder of the family. In most phylogenies, *Furcraea* and *Beschorneria* form a group, and they are a sister group to *Agave* s.l., which includes the subgenera of *Agave* (*Agave* and *Littaea* (Tagl.) Baker), and the herbaceous genera—*Manfreda*, *Poilanthes*, and *Prochnyanthes*—that represent the more derived lineages in the family.

ECOLOGY, NATURAL HISTORY, AND POLLINATION ECOLOGY OF EACH GENUS

Yucca

This is the second largest genus of the family (after *Agave*), with 49 species. It has the most northern distribution of any genus in the family, and various species can withstand cold climates extending as far north as the border between the USA and Canada and into the Midwest of the USA (Fig. 2A).

The genus is divided into three groups based on the structure of the fruit:

(a) *Clistocarpa* is defined by the presence of spongy fruits, but contains only one species, *Y. brevifolia* Engelm. (the Joshua tree), which is found in the deserts of southern California, Nevada, and a small section in Arizona.

(b) *Sarcocarpa* includes ca. 25 species with fleshy fruits (dates). These species are found throughout the southeastern USA and most of Mexico (except in sections of the west coast and the Yucatan Peninsula, where no *Yucca* species are found), and into the Lacandonian rain forest.

(c) *Chaenocarpa* contains ca. 25 species with dry fruits. They have a primarily northern distribution reaching up to

Canada and are widely distributed through the mid- and southern USA and northern Mexico.

Species of *Yucca* are long-lived perennials, usually with a trunk; some species grow very tall and become almost treelike with branches. The flowers of *Yucca* produce little or no nectar and are believed to be pollinated only by specialized moths of the genus *Tegeticula* and *Parategeticula* (but see Pellmyr 2003, for an extensive discussion). Originally, only three species of *Tegeticula* were documented (one that pollinated *Y. brevifolia*, another for *Hesperoyucca whipplei*, and another for the remaining *Yucca* species), but recently several new species of *Tegeticula* and *Parategeticula* have been recognized (Pellmyr 2003). Despite this greater diversity of yucca moths, all species are thought to behave in similar ways. Yucca moths actively collect pollen from several yucca plants, thereby pollinating the flowers, and then ovipositing in the developing ovules. Their larvae feed on the developing ovules, but rather than sacrificing future generations of *Yucca*, there is strong evidence that the ovules do not develop into seeds if there is an absence of yucca moths (Pellmyr 2003). In some *Yucca* species, cheater yucca moths have evolved that do not facilitate pollination, but only oviposit within the flowers and kill the seeds. Pellmyr and Leebens-Mack (1999) have examined in detail the molecular evolution and phylogenetics of *Tegeticula* moths, and estimated that they evolved ca. 40 MYA based on molecular clock analyses of mitochondrial genes in the moths. The fossil species *Protoyucca shadishii* Tidwell & Parker, from the Middle Miocene, has been interpreted as being close to the genus, because of its similarity to *Y. brevifolia* (Tidwell and Parker 1990).

Hesperoyucca

This genus includes only one species, *H. whipplei* (formerly known as *Yucca whipplei*, but it has been suggested that it is sufficiently distinct as to warrant generic rank), from California, Arizona, and northwestern Mexico (Fig. 2B). This trunkless yucca produces a compact *Agave*-like inflorescence and interestingly dies after reproduction, i.e., is monocarpic like most *Agave* species. It is also pollinated by a species of yucca moth, but has a relatively disjunct distribution that warrants further study—in particular, the Mexican populations in the middle of Baja California. Given the phylogenetic position of *H. whipplei*, which is more closely related to the genus *Hesperaloe* than to other *Yucca* species (Fig. 1), Bogler et al. (1995) argued that yucca moth pollination was either lost in the *Hesperaloe* genus or evolved twice, once in *Yucca* and again in *Hesperoyucca*.

Hesperaloe

This small genus, with five species, is closely related to *Yucca* (Fig. 1). The genus is restricted to northern Mexico and southern Texas (Fig. 2C). The plants are perennial, iteroparous, but without a trunk. Hawkmoths have been suggested to be the most important pollinators for the majority of species in the genus, but Pellmyr and Augenstein (1997) demonstrated that hummingbirds pollinate *Hesperaloe parviflora* J. M. Coulter, while Engard (1980) reported bat visitation and large amounts of diluted nectar in *H. nocturna* Gentry. Experimental hand-pollinations by Pellmyr and Au-

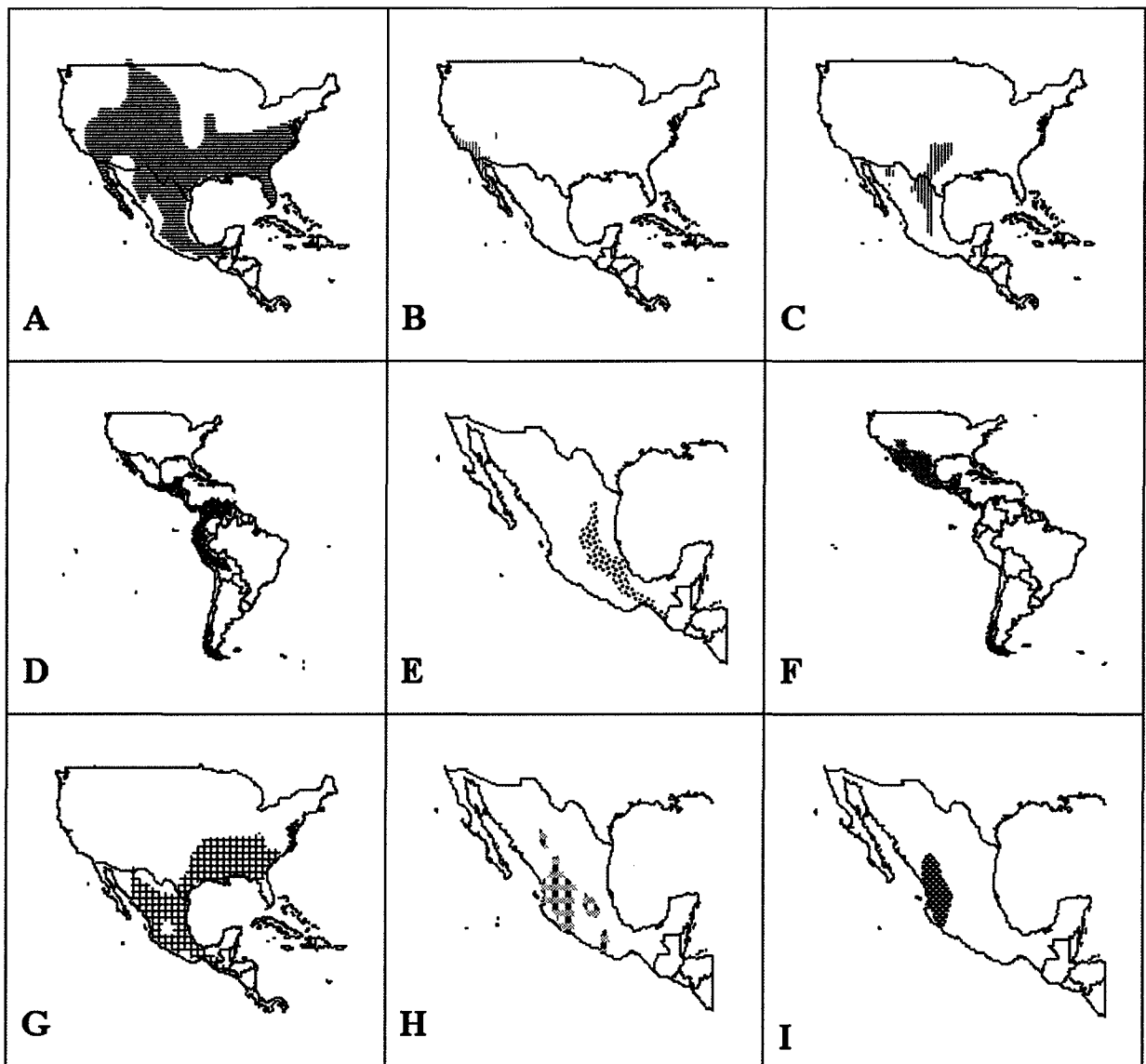


Fig. 2.—Distribution maps of the genera in Agavaceae. A. *Yucca*. B. *Hesperoyucca*. C. *Hesperaloe*. D. *Furcraea*. E. *Beschorneria*. F. *Agave* s.s. G. *Manfreda*. H. *Polianthes*. I. *Prochnyanthes*.

genstein (1997) indicated that *H. parviflora* might be self-incompatible. *Hesperaloe* species produce large numbers of flowers, but have a low fruit set (0.5–4.0%), so they appear to be obligate outcrossers.

Furcraea

This genus includes 25 species with its southernmost range reaching as far south as the Andes mountains (García-Mendoza 1999) (Fig. 2D). The genus is divided in two subgenera: *Roetzlia* Baker and *Furcraea*. Subgenus *Roetzlia* includes four species distributed from central Mexico to Guatemala, and has seedlings with one long cotyledon. The adults form large trunks with toothed leaves. Subgenus *Furcraea* has 21 species found from Mexico to Bolivia. Plants in this subgenus form rosettes with or without a trunk, bearing toothed or entire leaves and the seedlings have a short cotyledon.

Mexico is probably the center of origin for the genus and

contains 13 species (52%), nine of which are endemic. The rosettes in this genus are very large, similar to some of the largest in *Agave*, and some species have very thick, rigid, and spiny leaves, confused with those on agaves, while others have soft, nonspiny leaves, similar to those of some *Yucca*. All of the species are monocarpic, and most species display a trunk when mature (García-Mendoza 2001). Their inflorescences are massive, and measure from 3–12 m, but have a very different structure from the inflorescences of other genera. Inflorescences can be lax or dense. Dense inflorescences are divided into many branches and flowers. *Furcraea longaeva* Karw. & Zucc. can have up to 100 primary branches, from 30–50 secondary branches, and possess more than 58,000 flowers. In contrast, lax inflorescences, such as those in *F. quicheensis* Trel., possess from 40–80 branches and ca. 3000 flowers (García-Mendoza 2001). The flowers are large, radially symmetrical, and bell-shaped with pale coloration (white to pale green). Their sweetly fragrant

flowers open and release pollen and nectar at dusk and during the night. Given these characteristics, the flowers are suspected to be moth pollinated; moreover, the nectar is secreted in the mouth of the nectary and appears as droplets as in other insect-pollinated plants (Faegri and van der Pijl 1979; Percival 1979). However, there have been few observations of pollination in the field or in botanical gardens and no moth visitations have been documented (Eguiarte et al. 2000; A. García-Mendoza pers. obs.). Hummingbirds have been observed visiting *Furcraea* during the day, although no nectar or pollen production was observed during these times (A. García-Mendoza pers. obs.). Many of the species in the genus have small, clonal populations, often occurring on mountain tops, and fruit set is very low; non-pollinated flowers can develop into bulbils that are dispersed around the maternal plant, and may contribute to the formation of clonal populations. Each plant can produce thousands of bulbils: *F. macdougallii* Matuda produces more than 15,000 bulbils that persist on the inflorescence even after the plant has died (García-Mendoza 2001). It is believed that the species propagates primarily by bulbil production. Of the 25 species of *Furcraea*, 23 produce bulbils, but 15 can only reproduce in this way. Even if the inflorescences produce many flowers, they can generate hundreds of bulbils, but only 4–30 fruits (García-Mendoza 2001). It is believed that the treelike form of the genus is ancestral and that species have become progressively more herbaceous. Concomitant with the decrease in overall size, has been a decrease in the complexity of the inflorescence (García-Mendoza 2001).

Beschorneria

This is another small and poorly understood genus, with only seven species found in the Sierra Madre Oriental and central Mexico (Fig. 2E). The plants have soft leaves, few spines, and some species may produce a trunk and are found in rocky habitats, from 1900–3400 m. Hummingbirds have been suggested as the primary pollinators for most species, given the reddish color and the tubular shape of the flowers, but at least some species may be hawkmoth pollinated. All species are considered to be polycarpic, but fieldwork and detailed studies are needed in all the species.

Agave *Sensu Stricto*

This is the largest genus in the family, with ca. 166 species. Most of the species are found in Mexico (ca. 125 species). Of the remaining species, some are found in the southwestern USA, with ca. 15 species, mostly from Arizona, 12 in the Antilles (Alvarez de Zayas 1987; Rogers 2000), and the rest in Central America and northern South America (i.e., Colombia, Peru, and Venezuela) (Fig. 2F). The highest diversity of *Agave* occurs in central Mexico, in the Tehuacán-Cuicatlán Valley, the canyon “Barranca de Meztlán,” the Sierra Madre Occidental, and the Chihuahuan Desert regions (García-Mendoza 2002; Tambutti 2002). This area is called Megamexico 3 (Rzedowski 1993) and may be the center of origin for the genus. An important fact is that most species of *Agave* have narrow endemic ranges, and are typically only found in a few specific habitats and mountain ranges. For instance, García-Mendoza (1995) and Tambutti (2002) estimated that most Mexican *Agave* s.s. species are only found

in three or less squares of one degree of latitude per one degree of longitude. As each square represents ca. 12,100 km², the total spatial distribution of most species is found in areas of less than 36,100 km², the equivalent to a square of 190 × 190 km. Obviously, a species never covers the entire area and its real distribution is much smaller. There are some exceptions, species such as *A. cerulata* Trel. in Baja California, *A. angustifolia* in all of Mexico, and *A. lecheguilla* and *A. scabra* (Ortega) McVaugh in the Chihuahuan Desert not only have large distributions, but also are composed of several million individuals (Gentry 1982).

Agave has been traditionally divided in two subgenera (Berger 1921; Gentry 1982), defined on the basis of the inflorescence type: subgen. *Littaea*, which has an unbranched spike or racemose inflorescence, containing ca. 53 species with a more restricted distribution, primarily in Mexico, and subgen. *Agave*, whose species possess branched (paniculate) inflorescences, i.e., large umbelliferous aggregates of flowers (Gentry 1982) with ca. 102 species (see Fig. 2F). *Agave* subgen. *Littaea* was proposed to represent the ancestral form of the genus (Gentry 1982), although recent data suggest that subgen. *Agave* is paraphyletic (Bogler and Simpson 1996; Eguiarte et al. 2000; Good-Ávila et al. submitted). Also, it must be remembered that the genus *Agave* is paraphyletic, because within the *Agave* clade are found the genera *Manfreda*, *Polianthes*, and *Prochnyanthes*. Because it is currently unclear whether paraphyly at the molecular level is caused by insufficient time for lineage sorting to have occurred, we retain the traditional genera.

Manfreda

This is also a poorly understood genus, with ca. 28 species, most of which occur in Mexico (Fig. 2G), *M. brachystachya* (Cav.) Rose (= *M. scabra* Ortega) is found from central Mexico down to Central America, and *M. virginica* (L.) Salisb. ex Rose is found in the southeastern USA. *Manfreda virginica* grows on limestone outcrops, in juniper glades, and in open woods from West Virginia to Illinois, and south to Florida and Texas.

This genus is closely related to *Agave* as their flowers are very similar, although the leaves of the *Manfreda* species are soft, unlike the hard spiny leaves of most *Agave* species. *Manfreda* leaves are drought deciduous with new leaves and inflorescences produced the following year (using stored nutrients from their rhizomes) so the plants are iteroparous. There are two detailed studies of *Manfreda* species, one for *M. brachystachya* growing in central Mexico (Eguiarte and Búrquez 1987, 1988; Eguiarte et al. 2000), and another for *M. virginica* by Gromam and Pellmyr (1999). Both species are visited during the day and night, but in doing diurnal vs. nocturnal exclusion experiments, both studies demonstrated that the primary pollinators are nocturnal animals. In *M. brachystachya* the main pollinators were nectarivorous bats such as *Leptonycteris curasoae* and *Anoura geoffroyi*. Additionally, hawkmoths were observed in *M. brachystachya*, while hawkmoths and medium-sized moths were the most important pollinators in *M. virginica*. In general, it has been suggested that the genus is adapted to moth and hawkmoth pollination (Cruden et al. 1983; Eguiarte et al. 2000). Experi-

ments done by Eguiarte and Búrquez (1987) indicated that *M. brachystachya* is self-compatible.

Polianthes

This is another small genus, comprising 13 species, all from Mexico, most of which are from the State of Jalisco (Fig. 2H). This genus is closely related to *Manfreda*, but is more herbaceous, with more delicate leaves and inflorescences. Most species have whitish flowers, but *P. geminiflora* (Lex.) Rose, has reddish-orange flowers, and *P. densiflora*, yellow ones. The species *P. tuberosa* L. is widely cultivated for its flowers and fragrance (tuberose, *nardo* in Spanish). Hawkmoth pollination has been suggested for most species except for *P. geminiflora*, which has been considered to be pollinated by hummingbirds, not only for its color but also because it produces nectar in the late afternoon (Cruden et al. 1983). Verhoek (1975) demonstrated that *P. geminiflora* is self-compatible.

Prochnyanthes

This monotypic genus, *Prochnyanthes mexicana* Rose, has a relatively wide distribution in west-central Mexico, from Durango and Nayarit to Michoacán (Fig. 2I). The species is very closely related to *Polianthes* and possibly belongs to that genus. The primary difference between the two genera being that the flowers of *Prochnyanthes* have a very distinct structure with a narrow tubular corolla that widens markedly in the middle. The flowers are whitish or pale green and produce nectar with a volume and sugar concentration suggesting pollination by hawkmoths or other moths, yet the structure of the flowers is more suggestive of bee pollination (Cruden et al. 1983; Eguiarte et al. 2000).

Other Genera Closely Related to Agavaceae

Camassia.—*Camassia* bulbs produce clumps of slender green leaves and their flowers are formed in spikes. The inflorescence is usually dense and produces a stalk, which bears as many as 100 star-shaped flowers that are loosely clustered and large (4–5 cm). The flowers vary in color from white to blue or purple, but are usually pale lavender, and give off a delicate and sweet scent (McGary 2001).

Hesperocallis.—This monotypic genus, *Hesperocallis undulata* A. Gray, is found in some of the most arid regions of North America (Pires et al. 2004). Its leaves are blue-green with white margins, undulate, basal, and 20–50 cm long. Flowers are large (4–5 cm), trumpet-shaped, and white to cream with a silver or green midstripe. Their fragrance is delicate (McGary 2001).

Chlorogalum.—This genus comprises five species. *Chlorogalum purpureum* Brandege (purple amole) forms a basal rosette of typically 4–7 bright green leaves that are linear and flat at the base that produces a stem with multiple branches that supports a few bluish-purple flowers. In contrast, the common soap plant (*C. pomeridianum* Kunth) has white flowers that open in the twilight or at night. Reproduction is primarily by seed, with increased seed set apparent with insect pollination. Another species, *C. parviflorum* S. Watson, is found in dry, coastal sage scrub from central

and southern California to northern Baja California. In this species, flowers open at dawn, but last only one day and have an unpleasant aroma consistent with fly pollination (McGary 2001).

Hosta.—This genus is endemic to eastern Asia and contains approximately 22–25 species. Also known as plantain lily or funkia, several species are grown primarily for their attractive foliage, which comes in a wide range of colors including green, green variegated with white, cream or yellow, blue-green, golden yellow, and greenish yellow. Many hostas produce spikes of tubular-, trumpet- or bell-shaped flowers in different shades of purple or white, some of which are very fragrant, consistent with their pollination by bumblebees (Aden 1988; Eguiarte et al. 2000).

FLORAL BIOLOGY AND REPRODUCTION IN AGAVACEAE: *YUCCA* AND *AGAVE*

As discussed before, members of Agavaceae present a wide range of reproductive strategies. Species can vary from extreme floral specialization, such as occurs between *Yucca* and yucca moths, to apparent generalization in the case of *Agave*, especially species such as *A. marmorata* Roehl that has an impressive list of floral visitors and pollinators.

An interesting question in plant evolutionary biology is how highly specialized or generalized plant–pollinator interactions influence the evolution of reproductive structures and patterns and ultimately population structure and rates of speciation. In Agavaceae, specialized plant–pollinator interactions in the *Yucca*–yucca moth relationship appear to have evolved as a response to selection pressures mutually imposed by both plants and pollinators (Pellmyr et al. 1996), which has left a very efficient pollination syndrome even if it is open to some exploitation by cheaters. Highly specialized morphological structures or reproductive strategies have developed on both the yucca moths and yucca flowers, such as the specialized complex tentacles of the yucca moth which facilitate pollen collecting (Pellmyr and Krenn 2002) and a reduction in locule egg mortality to reduce moth damage by yucca flowers (Addicott and Bao 1999).

On the other hand, plants are expected to adopt a more generalized pollination syndrome when plant rewards are similar among different species, when traveling is costly for pollinators, and their life span is longer than flowering time in the plant species (Waser et al. 1996). Although these general plant–pollinator interactions may also be a consequence of adaptation to some “messy” visitors such as bats. The broad, evolutionary trends in plant–pollinator relationships that have evolved in Agavaceae are summarized in Pellmyr et al. (1996). Fruit production is resource limited across the family (Sutherland 1982); nocturnally opening flowers and high nectar production are common in most species, but nectar production has been lost in yuccas. Local host specificity, an important precursor for the evolution of pollination specialization, is common in moths of the Prodoxidae. Oviposition into flowers has evolved two to three times in this family, and limited larval seed destruction is widespread. Passive pollination has evolved twice within moths of the genus *Greya*, which is the sister genus to yucca moths, but active pollination has evolved only once in the ancestor of yucca moths *Tegeticula* and *Parategeticula*. Only active pol-

lination and mouth parts specialized for collecting pollen are unique to yucca moths (Pellmyr et al. 1996).

In contrast, there is variation in the floral morphology among the many species in *Agave*, but in general their flowers are robust, of pale color, varying from light green to yellow, or even red. Pollen and nectar are produced mainly at night, nectar is abundant, sugar concentration is low (between 12–25%), protein content of the pollen is high, and sometimes the flowers emulate the smell of ripening fruit. Most species are protandrous: the anthers dehisce and shed pollen prior to stigma receptivity (Howell 1972; Gentry 1982; Eguiarte et al. 2000; Slauson 2001). All of these floral traits suggest adaptation to bat pollination or chiropterophily (Faegri and van der Pijl 1966, 1979).

Systematic pollination studies in *Agave* started in the 1970s; the first studies were performed mainly in the southwestern USA and suggested that subgen. *Agave* was predominantly pollinated by bats and sometimes by hawkmoths (Howell 1972; Faegri and van der Pijl 1979; Gentry 1982). An account of the results obtained to date for other species is found in Table 2. On the other hand, Schaffer and Schaffer (1977b) suggested that agaves in subgen. *Littaea* were primarily pollinated by insects. Floral traits associated with insect pollination syndrome are indeed present in some species in subgen. *Littaea*, where floral tubes are smaller and in a horizontal position (making the insects more efficient pollinators), nectar production is less abundant, sugar concentration is high, floral color is more attractive to insects, and flowers are sweet smelling. In fact, there have been reports of species in this subgenus pollinated primarily by bees and sometimes by hummingbirds (Schaffer and Schaffer 1977b; Slauson 2001); and Eguiarte et al. (2000) suggested that hawkmoths are the most important visitors. A list of floral attributes and visitors reported in *A.* subgen. *Littaea* is found in Table 3.

Several other more detailed studies have been published recently describing the dynamic of pollination assemblages for species in both subgenera and include more data from different Mexican populations (Tables 2 and 3). Apparently the traditional view of pollination syndromes in subgen. *Agave* and *Littaea* is being challenged (see below).

RECENT ADVANCES IN THE POLLINATION
ECOLOGY OF AGAVE

Agave angustifolia and *A. subsimplex*

Molina-Freaner and Eguiarte (2003) analyzed the pollination biology of two paniculate species: *Agave angustifolia* and *A. subsimplex* in Sonora in northwestern Mexico. *Agave angustifolia* has the widest distribution, ranging from El Salvador and Honduras to central Sonora, while *A. subsimplex* has a localized distribution in the coastal regions of central Sonora and on the Island of Tiburon. *Agave angustifolia* flowers from January to late May, with a peak in March, while *A. subsimplex* flowers from early April to early June, with a peak in May. *Agave angustifolia* flowers produce more nectar (ca. 180 µL per night) than *A. subsimplex* (ca. 40 µL per night), although nectar concentration was similar in the two species (from 18–26% in *A. angustifolia*, and from 22–25% in *A. subsimplex*). Hand pollination experiments suggest that both species are self-incompatible. Pol-

Table 2. Major features in the pollination biology of paniculate agaves [*A.* subgen. *Agave*].

Species	Nectar volume	Nectar concentration	Anther dehiscence	Major visitors	Reference
<i>Agave angustifolia</i> Haw.	180 µL/night	18–26%	nocturnal	bats, birds & bees	Molina-Freaner and Eguiarte 2003
<i>A. chrysantha</i> Peebles	470 µL/night	14–18%	nocturnal	large bees, hummingbirds & hawkmoths	Slauson 2000
<i>A. havardiana</i> Trel.	?	?	?	birds & bats	Eguiarte et al. 2000
<i>A. macroacantha</i> Zucc.	110 µL/day	?	nocturnal	bats, hawkmoths & bees	Arizaga et al. 2000
<i>A. mckelveyana</i> Gentry	?	?	nocturnal	bees & wasps	Sutherland 1987
<i>A. palmieri</i> Engelm.	713 µL/night	14–19%	nocturnal	hawkmoths, large bees & hummingbirds	Slauson 2000
<i>A. salmiana</i> Otto	102 µL/2 hr	12%	nocturnal	bats & birds	Eguiarte et al. 2000
<i>A. subsimplex</i> Trel.	40 µL/night	22–25%	nocturnal	bees, moths & bats	Molina-Freaner and Eguiarte 2003

Table 3. Major features in the pollination biology of spicate agaves [*A.* subgen. *Littaea*].

Species	Nectar volume	Nectar concentration	Anther dehiscence	Major visitors	Reference
<i>Agave</i> sp.	91.5 μ L/day	13.6%	nocturnal	bees, large bees & bats	Rocha et al. 2005
<i>A. celsii albicans</i> Hook var. <i>albicans</i> (Jacobi) Gentry	132.5 μ L/day	14.7%	nocturnal	bees, large bees, bats, hawkmoths & hummingbirds	Rocha et al. 2005
<i>A. difformis</i> A. Berger	83 μ L/day	13%	nocturnal	bees, large bees & bats	Rocha et al. 2005
<i>A. garciae-mendozae</i> Galván & L. Hern.	124 μ L/day	10–15%	nocturnal	bees, large bees & bats	González 2004
<i>A. lecheguilla</i> Torr.	50–190 μ L/day	12–52%	nocturnal	hawkmoths, large bees & hummingbirds	Eguiarte et al. 2000
<i>A. parviflora</i> Torr.	0.9 μ L/day	37.4%	diurnal	large bees	Schaffer and Schaffer 1977b
<i>A. schottii</i> Engelm.	11.6 μ L/day	18%	nocturnal	bees, hawkmoths & hummingbirds	Schaffer and Schaffer 1977b
<i>A. striata</i> Zucc.	218.8 μ L/day	19.3%	diurnal	bees, large bees, bats, hawkmoths & hummingbirds	Rocha et al. 2005
<i>A. toumeyana</i> Trel.	4.6 μ L/day	62.3%	nocturnal	large bees	Schaffer and Schaffer 1977b
<i>A. xylonacantha</i> Salm-Dyck	117.6 μ L/day	24.6%	nocturnal	bees, large bees, bats, hawkmoths & hummingbirds	Rocha et al. 2005

linator exclusion experiments, direct observation, and bat capture demonstrated that *Leptonycteris curasoae* was the most effective pollinator in *A. angustifolia*, but for *A. subsimplex* diurnal (bees, in particular *Apis mellifera*) and nocturnal pollinators (mainly moths and some visits by *L. curasoae*) were equally effective. Populations of *A. angustifolia* that lie within the foraging range of their bat pollinators have high rates of sexual reproduction while populations at the limits of the foraging range exhibit significant variation in reproductive output and change from sexual to asexual reproduction (Molina-Freaner and Eguiarte 2003). We hypothesize that *A. subsimplex* is, in part, less frequently visited by *Leptonycteris* bats because its flowering time coincides with that of several local columnar cacti, which produce larger amounts of nectar per flower (i.e., *Pachycereus pringlei* Britton & Rose and *Carnegiea gigantea* (Engelm.) Britton & Rose; F. Molina-Freaner pers. obs.).

Agave marmorata: Oriole Pollination in Subgenus Agave?

Ornelas et al. (2002) studied floral biology and pollinator diversity and efficiency in this species in the Tehuacán Valley in Puebla (central Mexico). The inflorescences of *A. marmorata* are ca. 6 m in height; their flowers are bright yellow and bear small tubular corollas (14–16 mm). Pollen is released at night, as in most agaves, and most nectar production also occurs then (576.6 μ L per flower per night), even though the species produces a “considerable amount during the day” (Ornelas et al. 2002). Nectar concentration ranges widely (9–37% “Brix” or sucrose equivalents). At night *Choeronycteris mexicana*, *Leptonycteris nivalis*, and hawkmoths visit the flowers, but are not considered to be pollinators by the authors because “bats used flowers by flying to and instantaneously sitting on the perianth of the peripheral flowers, making no contact with anthers and stigmas” (Ornelas et al. 2002). During the day, hummingbirds accounted for 50.2% of the diurnal visits, and at least six species visited the flowers. In addition, nine different species of perching birds accounted for another 42.5% of the visits. Carpenter bees (*Xylocopa* sp.) accounted for the remaining 7.3% of diurnal visits. Because hummingbirds captured during the study had no visible pollen on their bodies and were observed to forage primarily along peripheral flowers, it was concluded that they were not important pollinators, while orioles fed mostly while sitting on the top and center of the umbels, coming into contact with the anthers and were observed to be dusted with pollen. For these reasons, perching birds, in particular orioles, are considered by Ornelas et al. (2002) the legitimate pollinators of *A. marmorata*. Unfortunately, no controlled diurnal vs. nocturnal pollinators experiments were performed and, for the moment, we can only conclude that orioles may be important pollinators, but more detailed experiments from more plants in diverse localities are required.

Geographic Changes in the Reproductive Ecology in Agave Subgenus Littaea

To date, the most detailed study published on the reproductive biology of a species of subgen. *Littaea* is for *A. lecheguilla* (Silva-Montellano 2001; Silva-Montellano and Eguiarte 2003a, b). *Agave lecheguilla* has a broad distribu-

tion throughout the Chihuahuan Desert, ranging from north of the Valley of Mexico City to southern Texas and New Mexico (Gentry 1982; Briones 1994). Silva-Montellano and Eguiarte (2003a) and Cadaval-Narezo (1999) studied ten populations encompassing most of the natural distribution of the species. Through a latitudinal gradient from 20°N to 32°N they studied populations at approximately every latitudinal degree. They found that the accumulated nectar volume per flower decreased along the latitudinal gradient (Silva-Montellano 2001). They also observed a latitudinal change in the shape and color of flowers: flowers in northern populations are shorter and the corolla is more splayed than in southern populations. Flower color varied from pale light green (glaucous) in the south to deep red in the north (Silva-Montellano and Eguiarte 2003a). They also identified a wide variation in the number of visits per flower and in the identity of the floral visitors, including hawkmoths, hummingbirds, and bees. On average, flowers in the south had greater rates of visitation than flowers in the north and greater fruit set, i.e., higher pollinator efficiency. During a total of 54 hours of nocturnal observation, they did not observe a single bat visit (Silva-Montellano and Eguiarte 2003a). Detailed diurnal vs. nocturnal controlled pollination experiments showed that nocturnal visitors were the most important pollinators in southern populations (moths, mainly the hawkmoth *Hyles lineata*), while in central populations the nocturnal were as important as the diurnal pollinators (mainly large bees such as *Bombus* and *Xylocopa*, as well as hummingbirds). On the other hand, genetic analyses suggested that rates of outcrossing were high in the south, intermediate in the central populations, and low in northern populations (Silva-Montellano 2001).

These studies underscore two important facets of plant reproductive ecology. First, even in a single species there can be ample variation in reproductive ecology, floral traits and the suite of pollinators that mediate them among populations. Second, although *Agave lecheguilla* appears to be adapted to nocturnal pollination, no bat visitors were found, which supported the classic hypothesis for the pollination syndrome in *A.* subgen. *Littaea*.

Bat Pollination in a Group of Sympatric Agave Subgenus Littaea in Central Mexico

In contrast to *Agave lecheguilla*, we have data for six species in *A.* subgen. *Littaea*, which suggest that bats, in particular *Leptonycteris curasoae* (but also *Choeronycteris mexicana* and *Glossophaga* sp.), are important pollinators. This study was carried out in the Metztlán Canyon in central Mexico, in one of the richest regions of *Agave* diversity (González 2004; Rocha et al. 2005). These species in *A.* subgen. *Littaea* have more localized distributions than *A. lecheguilla* and, in addition to having different microhabitats in the same locality (i.e., some species are found in steeper, rockier, and dryer areas than others), they exhibit diverse flowering phenologies: *A. xylonacantha* and *A. celsii* var. *albicans* (Jacobi) Gentry flower in spring; *Agave* sp. (which is currently being described) flowers in the autumn, while the remaining three species, *A. difformis*, *A. garciae-mendozae* Galván & L. Hern., and *A. striata* flower in summer at overlapping times. In addition, a different coterie of pol-

linators visits each species. The pollinators also vary between years and localities within the study area (Rocha et al. 2005). For instance, in *A. difformis* we observed that bees were the most frequent visitors in 2001, while in 2002 we recorded a higher frequency of bat visitors. In *A. garciae-mendozae* bees and bats seemed to be similarly abundant (González 2004). *Agave xylonacantha*, *A. celsii* var. *albicans*, and *A.* sp. show a similar pattern: bats are the most important pollinators, but there are minor visits from other animals. Finally, *A. striata* showed the most diverse range of pollinators and the highest overall visitation rates, including visits from honeybees, bumblebees, hawkmoths, hummingbirds, and bats. *Agave* sp., *A. xylonacantha*, *A. celsii* var. *albicans*, *A. garciae-mendozae*, and *A. difformis* are nocturnal nectar producers, whereas *A. striata* begins to produce nectar early in the afternoon. The volume and concentration of nectar were high in all species and similar to that in other bat-pollinated plants (see Table 3). Our observations suggest that species are potential competitors, as they share floral visitors. But, in order to reduce competition, plants may change their phenology (as we see in *A. xylonacantha* or in *Agave* sp.), or use different pollinator assemblages (such as *A. striata*). Alternatively, one species (*A. garciae-mendozae*) is isolated geographically (in the higher sections of the canyon). *Agave striata* and *A. difformis* share the same microhabitats, but *A. striata* relies more on asexual reproduction and its fruit set is very low (Rocha et al. 2005). These results suggest that in more complex communities with diverse but variable pollinating fauna, *Agave* species may show complex patterns of interaction with multiple pollinators.

EVOLUTION OF THE POLLINATION SYSTEMS IN AGAVE: GENERALIZATION OR COADAPTATION TO *LEPTONYCTERIS*?

These recent studies represent an important advance in our understanding of the evolution of *Agave* and underscore that, as more data accumulates, the simple patterns originally proposed for the genus will probably not hold. On the one hand, some agaves in subgen. *Littaea* may indeed be pollinated primarily by insects (either by bees or hawkmoths), while others are clearly pollinated by *Leptonycteris* bats (Table 3) as was previously shown in *Manfreda brachystachya* by Eguiarte and Búrquez (1987). On the other hand, birds, particularly perching birds and hummingbirds, may indeed be the most important pollinators in species of subgen. *Agave* (Table 2). But still, *Leptonycteris* has been reported in detailed studies (Eguiarte et al. 2000; Slauson 2001; Rocha et al. 2005) and in most cases is not only relevant, but the single most important pollinator.

We suggest the following scenario, based on what it is known of the adaptive radiation of *Agave* s.l. (see below) and about the phylogeny of Phyllostomid bats (Wetterer et al. 2000; Simmons and Wetterer 2002). Nectar feeding New World bats are estimated to have evolved no more than 15 MYA (Proctor et al. 1996) and Wilkinson and Fleming (1996), using mitochondrial sequences of the control region, estimated that *Glossophaga* and *Leptonycteris* shared a common ancestor about 2.4 MYA and that the two *Leptonycteris* species diverged ca. 1 MYA. We suggest that ca. 10–11 MYA (Eguiarte 1995; Good-Ávila et al. submitted, and see below), a moth-pollinated lineage of Agavaceae started to

specialize to a generalist Phyllostomid bat (for a review of bat adaptations to agave and cactus pollination see Howell 1972, Howell and Hodgkin 1976, and Fleming and Nassar 2002, and for a discussion of agave adaptations to bat pollination see Slauson 2001). The bats selected for a large floral display, a larger volume of nectar, and increased reproductive effort (Schaffer and Schaffer 1977a). While pollination by bats is efficient in producing high-quality outcrossed seeds, bat pollination of early agaves may have been so costly that it killed the plant: all of the modern species in subgen. *Agave* has delayed reproduction, monocarpy, and massive flowering (see also Schaffer and Schaffer, 1977a; in other species, such as some of those in Bombacaceae, columnar cacti, and *Manfreda* that are bat pollinated, the plants also display synchronous, massive flowering [Eguiarte et al. 1987; Eguiarte and Búrquez 1987; Fleming et al. 2001]). In time, the bat lineages evolved into two nectarivorous species, *Leptonycteris nivalis* in the central Mexican highlands and the more tropical *L. curasoae* (see Arita 1991), which initiated an adaptive radiation of *Agave* in response to diverse habitat selection that occurred in synchrony with a radiation of bat-pollinated columnar cacti in Mexico and northern South America (see below). As noted earlier, the bat pollination system is in general a leaky system, open to exploitation and usage by other visitors. Thus, secondarily and very recently, some *Agave* species have evolved to rely on other pollinators. These *Agave* species include *A. lecheguilla*, *A. marmorata*, the species in subgen. *Littaea* from Arizona described by Schaffer and Schaffer (1977b), and hawkmoth-, bee-, and hummingbird-pollinated species in *Manfreda*, *Polianthes*, and *Prochnyanthes* (see below). These hypotheses involve the mutual adaptation of a few bat species with a large number of *Agave* and columnar cactus species, and interactions with a large guild of possible secondary and minor pollinators, as well as nectar and pollen robbers. A clear advantage of generalization for pollination services for the animals is that they can use other plants, such as cacti, when for some reason resources in *Agave* flowers are not available.

COLUMNAR CACTI: ANOTHER PIECE IN THE PUZZLE

Bat pollination occurs mainly in the tropics, and bats are found mainly where there is a succession of suitable flowers for them all year-round (Arita 1991; Proctor et al. 1996). Phenological data from paniculate agaves and columnar cacti suggest that both groups form a nectar corridor during bat migration (Gentry 1982; Arita 1991; Fleming et al. 1993; Molina-Freaner and Eguiarte 2003). Columnar cacti belong to the tribe Pachycereae, which contains 58 species (Dávila-Aranda et al. 2002), in which 70% of the species present a bat pollination syndrome: nocturnal dehiscence, white flowers, production of high volumes of nectar and pollen, and a putrid smell (Valiente-Banuet 2002). These cacti are strictly bat pollinated in south-central Mexico, whereas bats, insects, or birds are the effective pollinators of these species in the Sonoran Desert (Fleming 2000). Pollination systems of cardon (*Pachycereus pringlei*), organ pipe (*Stenocereus thurberi* (Engelm.) Buxb.) and saguaro (*Carnegiea gigantea*) are more generalized than those of their bat-pollinated relatives. In the case of columnar cacti, diurnal visitors are not effi-

cient pollinators, probably because the stigmas of these flowers lose their receptivity during daytime or because the flowers actually close before sunrise. Valiente-Banuet et al. (1996) suggested that pollination generalization is favored along the northern distribution limits of columnar cacti because of year-to-year variation in the abundance of migratory nectar-feeding bats. It has been shown that, at least in Mexico and the southwestern USA (i.e., Megamexico 3 according to Rzedowski 1993), sexual reproduction of columnar cacti from tropical deserts depends almost exclusively on nectar-feeding bats, whereas bats and diurnal pollinators are important in extratropical deserts near or beyond the limits of distribution for *L. curasoae* (Fleming et al. 2001; Valiente-Banuet 2002). The dependence of tropical columnar cacti on *L. curasoae* has also been documented in Venezuela by Nassar et al. (1997, 2003). Previous evidence suggested that this pattern could be found in *A.* subgen. *Agave*, i.e., that within the tropics, agaves tend to be more specialized to bat pollination by *Leptonycteris* and show moderated generalization outside the tropics (see Table 2; and Arizaga et al. 2000; Slauson 2000, 2001).

NECTAR FEEDING BATS IN MEXICO

As confirmed before, *Agave* species mainly occur within the distribution range of nectarivorous bats (although some *Agave* species in the USA are out of the range of any nectarivorous bats). Mexico has a high diversity of nectar-feeding bats—12 species (Phyllostomidae subfamily Glossophaginae): *Anoura geoffroyi*, *Choeroniscus godmani*, *Choeronycteris mexicana*, *Glossophaga soricina*, *G. morenoi*, *G. comissarisii*, *G. leachii*, *Hylonycteris underwoodi*, *Leptonycteris nivalis*, *L. curasoae*, *Lichonycteris obscura*, *Musonycteris harrisoni*, most of which are associated with tropical and subtropical dry areas (Santos and Arita 2002). The wealth of nectar-feeding bat species in Mexico reaches maximum values along the Pacific versant (the Balsas region) and decreases with latitude (Arita and Santos-del-Prado 1999; Rojas-Martínez et al. 1999). Of these species, the degree of nectarivory (and pollinivory) varies, from some occasional nectar-feeding species, such as *Glossophaga soricina* (Reid 1998) to the genus *Leptonycteris*, which depends almost exclusively on nectar and pollen. Two species of *Leptonycteris* are generally recognized, *L. curasoae* and *L. nivalis*, and both feed mainly on agaves and columnar cacti (Arita and Humphrey 1988; Rojas-Martínez et al. 1999), although in the dry forest they may feed mostly on tropical tree species, such as *Ceiba* Mill. and *Pseudobombax* Dugand (Alvarez and González-Quintero 1970; Ceballos et al. 1997). Available evidence indicates that *L. curasoae* is resident year-round in the tropics and migrates to extratropical areas. This species is locally abundant and is usually the most common bat visiting agave and columnar cacti in Mexico and Venezuela and has a widespread distribution, from Arizona to Honduras and El Salvador in central America to northern Venezuela and northeastern Colombia in South America and adjacent islands (Reid 1998; Arita and Santos del Prado 1999; Rojas-Martínez et al. 1999). The abundance of *L. curasoae* in the Sonoran Desert varies significantly within and among years (Fleming et al. 2001; Molina-Freaner et al. 2003). Bat unpredictability has been suggested as the major

ecological force behind the evolution of (perhaps secondary) generalized pollination systems in northern columnar cacti (Valiente-Banuet 2002) and we might expect the same pattern in *Agave*.

Given the similar distribution of *Agave* and *Leptonycteris* species, the dependence of many species of columnar cacti on bats and similar patterns in phenology and migration, the *Leptonycteris*–columnar cactus–*Agave* association may represent a complex but very successful case of diffuse coevolution (Gentry 1982; Arita 1991; Valiente-Banuet et al. 1996, 1997; Valiente-Banuet 2002).

ADAPTIVE RADIATION OF AGAVE SENSU LATO

Using the available molecular phylogenies (for example, see Bogler et al. 2006), it is impossible to separate subgenera of *Agave* (*Agave* and *Littaea*) from *Manfreda*, *Polianthes*, and *Prochnyanthes*. As mentioned above, we will call this large monophyletic clade *Agave* s.l. Thus, *Agave* s.l. comprises *Agave* s.s. (166 spp.), *Manfreda* (28 spp.), *Polianthes* (13), and *Prochnyanthes* (1) for a total of at least 208 species.

Our chloroplast sequences (*rbcL* [1428 bp], a region of the *trnL* intron [768 bp] and a spacer between *trnL* and *trnF* [534 bp], Good-Ávila et al. submitted) and nuclear sequences (ITS1 and ITS2 [772 bp]) show generally low levels of genetic differentiation among species of *Agave* s.l., suggestive of a very recent origin for the group (Eguiarte 1995; Eguiarte et al. 2000; Good-Ávila et al. submitted). In contrast, our population genetics studies indicate large levels of genetic variation within populations (except domesticated species) with little or no inbreeding, except for example, on the periphery of a species' range (Eguiarte et al. 2000).

As stated above, plant-pollinator interactions may influence the rates of speciation of a group (Simpson 1953; Schluter 2000). In order to examine the evidence for a radiation of the agaves, we have performed a series of analyses using molecular data as described in Good-Ávila et al. (submitted). First, we used *rbcL* sequence data (from GenBank), and performed analyses on 334 monocot sequences. With this database, we used two methods to estimate the age of the Agavaceae family and the group *Agave* s.l. First, we used the method of linearized trees following Takezaki et al. (1995). This method estimates the divergence times on a phylogenetic tree by removing all of the slow- and fast-evolving species on the phylogeny and then imposes a constant rate of molecular evolution. We constructed a minimum evolution (distance based) tree (Rzhetsky and Nei 1993) using the *rbcL* data described above and calibrated the molecular clock on the tree using a crown group age of 132 MYA for the origin of the angiosperms, as suggested from fossil data described in Brenner (1996) and Magallón and Sanderson (2001). Using this method, we estimated an age of 12.75 MYA for the origin of Agavaceae and one of 10.2 MYA for the *Agave* s.l. clade.

To calculate an estimate of the age that does not force the use of a molecular clock, we used the method of penalized likelihood developed by Sanderson (2002), which allows different evolutionary rates to be estimated on different branches of a phylogenetic tree but imposes a penalty for changing rates too quickly. To perform these analyses, we first gen-

erated a phylogeny of the monocots based on the *rbcL* data described above using maximum parsimony and then estimated the branch lengths on this tree using maximum likelihood methods, again constraining the crown age of all angiosperms to be 132 MYA but this time adding four internal calibration points based on fossil data (for details, see Good-Ávila et al. submitted). This method generated similar dates of 11.73 MYA of age for the Agavaceae family and 10.25 MYA for the extant *Agave* s.l. clade. How trustworthy are these dates? Earlier, Eguiarte (1995) estimated the age of the *Agave* family to be 14 MYA, using more basic methodology and *rbcL* sequences, and Eguiarte et al. (2000) estimated the age of *Agave* s.l. to be 8 MYA and that of *Yucca* to be 6 MYA. On the other hand, Pellmyr and Leebens-Mack (1999) examined *Yucca*–*yucca* moth coevolution, using COI and COII mtDNA molecular markers combined with biogeographic and paleontological data to calibrate their molecular clock, and suggested an age of 41.5 MYA for the origin of Agavaceae. In contrast, the yucca-like fossil found in Nevada was estimated to be ca. 14 MYA (Tidwell and Parker 1990) and Palacios and Rzedowski (1993) reported Agavaceae-like fossil pollen to be in the range of 15–25 MYA; there are also *Agave* and *Yucca* fossils from the Late Miocene in the Chihuahuan Desert (Wells 1974; Tidwell and Parker 1990). The oldest Asparagales fossils are estimated to be 37.5 MYA according to Magallón and Sanderson (2001). The possibility that rates or patterns of molecular evolution are significantly different in yucca moths than in Agavaceae is worth exploring.

There are inherent difficulties in using molecular sequence data to calibrate times of diversification, and *rbcL* data, in particular, has been criticized because it exhibits considerable variation in the rate of substitution between first and second vs. third base pair (bp) positions—the latter becoming saturated much earlier than the former (Sanderson and Doyle 2001). For this reason, we also estimated the time of origin of Agavaceae using the same methods but other genes. In particular, we generated and then analyzed 768 bp of sequence data from the chloroplast intergenic spacers of the *trnL* intron (primers “c” and “d” of Taberlet et al. 1991) in 26 species of *Agave* s.l., four species of *Yucca* and analyzed this data set along with sequences from 35 other monocots (most of these from the GenBank) using *Acorus* L. as an outgroup. We also obtained 534 bp of sequence (some from our laboratory, some from GenBank) from 77 species of the chloroplast intergenic region between *trnL* and *trnF* (using the primers “e” and “f” of Taberlet et al. 1991). Using the method of linearized trees described above, we calculated an age of 25.8 ± 2.1 MYA for the origin of the family and 10.1 ± 1.7 MYA for *Agave* s.l. using these data sets; i.e., an older age for the family than that suggested by the *rbcL* data set, but almost the same age for the group *Agave* s.l. The apparent cause for this discrepancy in age was a change in the phylogenetic position of the *Yucca* clade with respect to other members in Asparagales using the intron and intergenic spacer chloroplast data sets. In particular, the *Yucca* clade groups more closely to genera outside Agavaceae, such as *Camassia* and *Chlorogalum*. Thus, these results suggest that if *Yucca* is more closely related to *Camassia* and *Chlorogalum* then the date of origin for *Yucca* may be older than indicated by the *rbcL* data, a date of 10.0

± 2 MYA for the origin of the genus *Agave* is in agreement with other analyses.

In order to understand the tempo and mode of speciation events in *Agave* s.l. our next approach was to compare rates of speciation/cladogenesis in *Agave* s.l. to rates of speciation within *Yucca*. We calculated the absolute rates of diversification in *Agave* s.l. using two methods, one based on a Yule process with the Kendall/Moran estimator (Nee et al. 1992; Baldwin and Sanderson 1998; Good-Ávila et al. submitted). The first gives rise to an estimate of the rate of speciation in millions of years using the information inherent in the branch lengths of a phylogenetic tree and incorporates both birth and death, speciation and extinction processes into an estimator defined as (S). The second method simply uses the time of origin of a clade (T) and the number of extant species (N) to derive an estimate of the absolute rate of diversification (D) assuming no extinction.

Using these methods, we calculated mean rates of diversification per million years in *Agave* s.l. to be $S = 0.32$ net speciation events per million years (species/MY) and $D = 0.51$ species/MY. We then compared diversification rates of *Agave* s.l. with those estimated in *Yucca*.

Since we do not have a detailed phylogeny of *Yucca*, we can only calculate D: using our estimates of 11.83–25 MYA for the origin of the family, and given that there are ca. 50 extant species of *Yucca* we calculate rates of D either at 0.2 or 0.15 species/MY. These values of D are less than half of our estimates in *Agave* using the same method, suggesting that the generalist resources-rich pollination system of *Agave* has been more successful generating species than the extreme form of specialized pollination found in *Yucca*.

COMPARISON TO OTHER KNOWN ADAPTIVE RADIATIONS

Are these rates of diversification high compared to other organisms? In particular, are rates of diversification in *Agave* s.l. sufficiently high to justify our assertion that it represents a notable radiation (0.32–0.51 species/MY)? Eriksson and Bremer (1992) estimated rates of diversification for different families of angiosperms and suggested a median value of 0.12 to a maximum value of 0.39 species/MY. In a similar study, Magallón and Sanderson (2001) found an average of 0.077–0.089 net speciation events per million years across angiosperms, with the highest values in Asterales estimated to be between 0.27–0.33 species/MY. In all these “average” comparisons, Agavaceae rates continue to be relatively high. A more equitable comparison may be with the Hawaiian silversword alliance, a group that has undergone one of the most impressive diversification and morphological adaptations known in plants. Baldwin and Sanderson (1998) estimated rates of diversification to be of 0.56 ± 0.17 species/MY, higher than our estimates in *Agave*, albeit the total number of species in *Agave* s.l. is far higher (208 spp. in ca. 10 MY, than the total in the silversword group, of 28 spp. in ca. 5 MY).

A longterm goal of our work in the *Agave* genus is to compare rates of diversification and the role of plant–pollinator coevolution in influencing speciation rates in different subsections or geographic regions. There has been some debate about whether overall rates of diversification are lower in plants than in animals. If this is true, this will be an im-

portant consideration when we compare rates of diversification in agaves and their pollinators. For instance, recently Webb et al. (2004) resolved the phylogeny of 37 species of Goodeidae fish in Mexico, a group that appears to have undergone an adaptive radiation with an estimated age of 16 MY, thus we can calculate $D = 0.217$ species/MY for this group. Stanley (1979) estimated rates of diversification for families of rodents to be 0.22–0.35 species/MY, with the highest values for murid rodents. Considering these estimates, the values we report for *Agave* s.l. appear to have similar or even higher values than those reported for several radiation events in animals.

While estimates of rates of diversification are useful for comparative purposes, a more interesting use of molecular phylogenies aims to infer when rates of speciation were elevated and whether periods of elevated diversification can be associated with other factors. For example, in the case of agaves, our fieldwork predicts that rates of speciation would be elevated at the same time that columnar cacti, nectarivorous bats, birds, and/or hawkmoths diversified in Mexico. Using the DNA sequence data for the *trnL* intron and the spacer between *trnL* and *trnF*, we performed preliminary analyses to test whether rates of speciation have been decelerating or accelerating toward the present using both lineage-through-time plots and the statistic γ developed by Pybus and Harvey (2000). These analyses indicated that rates of diversification were higher early in the evolution of the group *Agave* s.l. (Good-Ávila et al. submitted). In the future, we would like to test specific hypotheses concerning the patterns of speciation in both *A.* subgen. *Agave*, and *Littaea* and to correlate patterns of speciation in agaves with different pollinators or different levels of pollinator specialization.

We can conclude from this review that, at least in terms of timing and the absolute number of species, we have evidence for a radiation in *Agave* s.l. (and an impressive, though not an especially high one in *Yucca*). We are currently in the process of understanding what evolutionary forces may have contributed to these high rates of diversification, e.g., adaptation. From our field studies and from hypotheses suggested by other authors, we propose a series of factors that appear to have been instrumental in driving the evolution of the agaves, the relative importance of each of these will be addressed in future studies.

THEN, WHY SO MANY SPECIES IN AGAVE?

The desertification of North America started between 8–15 MYA, as higher temperatures and reduced availability of water occurred in all of North America in the Middle Miocene (Axelrod 1979). This desertification, coupled with intense volcanic activity, generated the complex topography that dominates present Mexico and created extensive environmental heterogeneity. The heterogeneous topology and diversity of ecological niches that resulted are central to understanding the adaptive radiation of *Agave*. Agaves are adapted to semiarid deserts, which are relatively open niches not only because they are abundant in Mexico, but also because these habitats present relatively few plant competitors. The ability to colonize these environments, coupled with remarkably heterogeneous habitats throughout Mexico, acts as a natural barrier to gene flow. Thus, promoting population

processes (genetic isolation and genetic drift) that are believed to facilitate speciation, thereby accelerating rates of speciation in *Agave* s.l. (Martinez-Palacios et al. 1999; Eguiarte et al. 2000; Navarro-Quezada et al. 2003). Furthermore, the very recent evolution of herbaceous taxa within *Agave* s.l. (*Manfreda* and *Polianthes*) has allowed the group to explore more mesic environments that have developed more recently in central Mexico.

Other authors have suggested that plant–pollinator interactions may also precipitate adaptation or diversification. For example, Fleming and Holland (1998) concluded that arid regions of North America appear to promote the evolution of obligate pollination mutualisms involving nocturnal moths in self-incompatible plants whose fruit set is resource limited. As predicted by Pellmyr et al. (1996), active pollination can evolve in a specialized pollinator in the presence of effective co-pollinators. Fleming and Holland (1998) described that a columnar cactus, *Lophocereus schottii* Britton & Rose (senita), is mainly pollinated by a specialized moth, *Upiga virescens* (Pyralidae, senita moth), and the system is similar to the *Yucca*–*yucca* moth system, as the moth oviposits into the flowers and the larvae eat a substantial portion of the developing seeds. Greater pollination reliability makes senita moths more effective pollinators although the system remains open to other pollinators such as bees, which sometimes act as redundant, but other times may also contribute to reproductive success of the plant (Holland and Fleming 2002).

In addition to the role that the interaction between *Agave* spp. and their pollinators may have had, and the processes mentioned above, there are some interesting adaptations in *Agave* s.l. that may have contributed to their high rates of speciation:

(1) Morpho-physiological adaptations: enable *Agave* to resist dry conditions (e.g., CAM photosynthesis, very succulent leaves/rosette, water storage) and colonize dry habitats (Nobel 1988). *Agave* s.s. is the most arid-tolerant of all genera in Agavaceae.

(2) Shape: the physical structure of the leaves into a rosette enables the capture of water, both because the rosette serves as a funnel and because species with thin long leaves can condense mist humidity (Martorell and Ezcurra 2002). The shape of the plant also offers protection from extreme variation in temperature (Nobel 1988).

(3) Roots: are very widespread and superficial in *Agave*; Gentry (1982) suggested that this represents an adaptation for efficient water capture.

(4) Protection against large herbivores: Janzen (1986) suggested that *Agave* is extremely well armed; having large, strong, very sharp spines, hard borders, teeth, very hard fibers, raphides (calcium oxalate crystals) (Salinas et al. 2001), and a vast array of chemical compounds that protect it from medium to large herbivores, which perhaps represents a “fossil” adaptation to the extinct Pleistocene megafauna.

(5) Efficient (albeit suicidal) reproduction: the huge inflorescences of *Agave* attract large numbers of animals, which insure animal pollination and high rates of outcrossing and gene flow. Agaves also maintain large, effective population sizes: the combination of large population size with high

outcrossing rates allows for efficient natural selection to occur.

(6) Extensive clonal propagation: allows successful genotypes to survive, and is an efficient “escape” in case the suicidal reproductive effort fails. In some conditions, clonal propagation also enables *Agave* spp. to colonize harsh environments or dominate large areas where pollinator abundance may be low or unreliable.

(7) Adaptation to microecological conditions: different species within *Agave* s.l. are adapted to contrasting conditions of soil rockiness and humidity.

(8) Diversity of life-history traits: species in *Agave* s.l. range from self-compatible almost annual herbs to long-lived completely outcrossing, nonclonal species.

This diversity of morphological and life-history strategies is sufficiently broad that it has enabled agaves to be successful in most of the environmental conditions found in Mexico. The focus of our future studies will be to understand how specific reproductive or morphological features have evolved over distinct geographic or environmental habitats and how the evolution of agaves has been influenced by their pollinators or by other plant species (such as cacti).

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