

## Multiple origins of the yucca–yucca moth association

(pollination/mutualism/coevolution)

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**ABSTRACT** The association of species of yucca and their pollinating moths is considered one of the two classic cases of obligate mutualism between floral hosts and their pollinators. The system involves the active collection of pollen by females of two prodoxid moth genera and the subsequent purposeful placement of the pollen on conspecific stigmas of species of *Yucca*. Yuccas essentially depend on the moths for pollination and the moths require *Yucca* ovaries for oviposition. Because of the specificity involved, it has been assumed that the association arose once, although it has been suggested that within the prodoxid moths as a whole, pollinators have arisen from seed predators more than once. We show, by using phylogenies generated from three molecular data sets, that the supposed restriction of the yucca moths and their allies to the Agavaceae is an artifact caused by an incorrect circumscription of this family. In addition we provide evidence that *Yucca* is not monophyletic, leading to the conclusion that the modern yucca–yucca moth relationship developed independently more than once by colonization of a new host.

The *Yucca*–yucca moth [*Parategeticula* and *Tegeticula* spp. (Prodoxidae)] interrelationship is one of the classic examples of a tight mutualism in pollination biology. The relationship has been called “a complete, unbreakable and unshakable tie-up between plant and pollinating insect” (1) and the “quintessential example” of an obligate mutualism (2). In the simplest form of the story, the 40-odd species of *Yucca* (Agavaceae) are believed to be virtually dependent on the activities of four species of yucca moths (three *Tegeticula* and one *Parategeticula*) for sexual reproduction. The yucca moths are in turn obligately dependent on either *Yucca* seeds (*Tegeticula*) or degenerating ovules enclosed in cysts (*Parategeticula*) for their larval development.

The behavior of the moths involved in the mutualism is unique in the Lepidoptera. Typically, a fertilized female moth enters a large creamy-white to pinkish *Yucca* flower and actively gathers a mass of the sticky pollen with her maxillary tentacles, specialized appendages formed by modifications of the maxillary palps. The moth leaves the flower carrying the pollen pressed with her tentacles and forelegs against her thorax and flies to another flower of (generally) the same *Yucca* species in which she then oviposits in one of its three carpels. She subsequently crawls up the style and smears some of the pollen from her load onto the stigma (*Yucca whipplei*) or forces it into the stigmatic cavity (all other species of *Yucca*). The moth can repeat the process of oviposition followed by pollination several times within a flower (3, 4). The eggs hatch and the larvae feed on the developing seeds or ovarian tissue, crawl out of the fruit, and then drop to pupate in the soil. This pollination system, referred to as brood place pollination, is rare (5, 6) presumably because the balance it imposes between parasitism and mutualism is a delicate one (7).

*Yucca* is currently placed by most authors in the Agavaceae (Table 1 and refs. 8–10), a family of monocotyledons containing 18 genera with woody or tree-like stems and stiff leaves (cf. *Agave*, *Beaucarnea*, *Cordylina*, *Dracaena*, *Furcraea*, *Nolina*, *Sansevieria*, and *Yucca*). The monophyly of this family has been debated with various genera historically treated in a variety of ways (11). At one time, *Agave* was placed in the Amaryllidaceae because of its inferior ovary, whereas *Yucca* was placed in the Liliaceae with species that had superior ovaries. The discovery that a group of genera had a peculiar chromosome arrangement of five large and 25 small chromosomes (cf. *Agave*, *Hesperaloë*, *Yucca*, and *Hosta*; refs. 12 and 13) led to a reevaluation of the importance of ovary position as an important familial character. Dahlgren *et al.* (12) proposed a system in which the genera placed in the Agavaceae of Cronquist (8) are dispersed among several smaller families (Table 1). A recent study using *rbcl* sequences (14) suggested that the Agavaceae *sensu* Cronquist may be biphyletic and that the system proposed by Dahlgren *et al.* (12) presents a more accurate classification.

The genus *Yucca* is generally considered to consist of four sections: sect. *Yucca* (= *Sarcocarpa*) (baccate fruits and recessed stigma) with about 20 species; sect. *Chaenocarpa* (capsular fruits and recessed stigma) with about 22 species; sect. *Clistocarpa* (spongy fruits and recessed stigma) with one species, *Yucca brevifolia*; and sect. *Hesperoyucca* (capsular fruit and capitate stigma) with one variable species, *Y. whipplei*. Each yucca belonging to the monotypic sections is involved in a one to one interaction with a particular yucca moth (Our Lord's Candle, *Y. whipplei*, with *Tegeticula maculata* and the Joshua Tree, *Y. brevifolia*, with *Tegeticula synthetica*), whereas the remaining 40-odd species in the other two sections of *Yucca* are all purportedly pollinated by *Tegeticula yuccasella*. Several recent studies, however, suggest that *T. yuccasella* is a complex of host races or sibling species (4, 15, 16), although no firm conclusions have been reached. In addition, two species of section *Yucca* are also pollinated by *Parategeticula pollenifera* (4).

Although recognizing the significant morphological differences within *Yucca* (dehiscent and indehiscent fruits, arborescent and acaulescent growth forms, and semelparous and iteroparous flowering), virtually all recent workers have accepted the monophyly of *Yucca* and, by implication, a single origin of the *Yucca*–yucca moth syndrome. Our phylogenetic studies based on three sets of molecular data (11, 17)‡ indicate this evolutionary picture may be incorrect. In addition, our data (11, 17, 18) indicate polyphyly of the Agavaceae as circumscribed by many authors (cf. ref. 8), thus, requiring a reevaluation of the proposed restriction to the Agavaceae of an “Agavaceae-feeding” clade (Fig. 1) of Prodoxidae (16, 19).

### MATERIALS AND METHODS

To address monophyly of both the Agavaceae and *Yucca*, we obtained data from three molecular systems: chloroplast DNA

Abbreviations: ITS, internal transcribed spacer; cpDNA, chloroplast DNA.

‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U23977–U24054).

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Table 1. Placements by various authors of angiosperm genera allied with the Agavaceae or included in Fig. 1.

Cronquist (9)	Dahlgren <i>et al.</i> (12)
Agavaceae	Agavaceae
<i>Agave</i>	<i>Agave</i>
<i>Beschorneria</i>	<i>Beschorneria</i>
<i>Furcraea</i>	<i>Furcraea</i>
<i>Hesperaloë</i>	<i>Hesperaloë</i>
<i>Manfreda</i>	<i>Manfreda</i>
<i>Polianthes</i>	<i>Polianthes</i>
<i>Prochnyanthes</i>	<i>Prochnyanthes</i>
<i>Yucca</i>	<i>Yucca</i>
	Dracaenaceae
<i>Dracaena</i>	<i>Dracaena</i>
	Nolinaceae
<i>Beaucarnea</i>	<i>Beaucarnea</i>
<i>Calibanus</i>	<i>Calibanus</i>
<i>Dasyllirion</i>	<i>Dasyllirion</i>
<i>Nolina</i>	<i>Nolina</i>
	Asteliaceae
<i>Cordylina</i>	<i>Cordylina</i>
Liliaceae	Convallariaceae
<i>Liriope</i>	<i>Liriope</i>
	Hyacinthaceae
<i>Camassia</i>	<i>Camassia</i>
	Funkiaceae
<i>Hosta</i>	<i>Hosta</i>

In his latest commentary on relationships among these genera, Cronquist (9) stated that he could conceive of arrangements different from that he advocated in 1981 but that he could not see the naturalness of the families recognized by Dahlgren *et al.* (12). As shown in Fig. 1, the molecular phylogeny largely supports the concept of separate families proposed by Dahlgren *et al.* (12).

(cpDNA) restriction site analysis and from sequencing two nuclear ribosomal DNA internal transcribed spacer (ITS) regions, ITS1 and ITS2. DNA extractions from 40 Agavaceae and Liliaceae taxa were digested with 12 restriction enzymes and sampled for restriction site variation with 25 cpDNA probes (11, 17). The cpDNA study yielded 110 (90 synapomorphic) informative restriction site characters (17). The ITS1, 5.8S, and ITS2 regions from the same taxa plus some additional outgroups were amplified by using primers 26S (5'-TTTCTTTTCCCTCCGCT-3') and 18S (5'-AAGTTCG-TAACAAGGTTTCCGTAGGTG-3') (11, 20). The same primers were used for direct double-stranded DNA sequencing in both directions, along with the additional internal sequencing primers 5.8S (5'-TGCGTTCAAAGACTCGAT-3') and its complement. Sequences were aligned by the program PILEUP of the GCG package (21) with gap penalties of 0.6 for ITS1 and 2.0 for ITS2 and a gap length penalty of 0.1 for both. The ITS data matrices were combined during this study (Fig. 1) but not combined with the cpDNA data because of the differences in outgroups and the lack of complete data for all three sets across all species.

The phylogenetic program PAUP (22) was used to search for the most parsimonious trees of the various data sets with the heuristic option due to the large size of the matrices. Support for the branch nodes was evaluated by performing 100 bootstrap replications sampling with replacement (23).

## RESULTS

All of the molecular data provide strong support for the monophyly of an Agavaceae clade (e.g., *Hesperaloë*, *Yucca*, and *Agave* and their close allies; Fig. 1) as circumscribed by Dahlgren *et al.* (12). *Hosta* is clearly related to these taxa as indicated by its possession of the distinctive karyotype feature. The bootstrap value for this clade was 98 in the cpDNA

analysis and 97 for the combined ITS1 and ITS2 analysis (Fig. 1). Taxa such as *Nolina* and *Dasyllirion* placed by Hutchinson (10) in the Agavaceae and retained there by Cronquist (8) clearly belong in a separate clade that includes Nolinaceae, Dracaenaceae, and Convallariaceae (*Liriope*, *Maianthemum*, *Polygonatum*, etc; Fig. 1) as defined by Dahlgren *et al.* (12). The bootstrap support for this well-defined clade was 100% in the cpDNA analysis and 98% in the combined ITS study (Fig. 1).

Our studies also provide firm support for a *Y. whipplei* + *Hesperaloë* clade distinct from other *Yucca* species (considered here as *Yucca* s. str.). The cpDNA data firmly placed *Y. whipplei* as the sister of *Hesperaloë* (bootstrap value of 87) and this clade as part of a larger one consisting of the rest of *Yucca* plus *Agave* and its relatives. This same clustering of *Y. whipplei* with *Hesperaloë* was found by Hansen (24) in his cpDNA study of *Yucca* using restriction fragment data from a portion of the chloroplast genome. The data from the combined ITS sequences also placed *Y. whipplei* in a clade with *Hesperaloë* (bootstrap value 99; Fig. 1) separate from the clade containing the other species of *Yucca*. The ITS data indicate that *Camassia*, a genus of herbaceous bulbous species assigned to the Hyacinthaceae by Dahlgren *et al.* (12), is closely related to *Yucca* and the Agavaceae. In addition to the similarities found in the ITS nucleotide sequences, *Yucca* s. str. shares a 28-bp deletion in ITS2 with *Camassia* that was not found in *Y. whipplei*, *Hesperaloë*, or any other Agavaceae.

## DISCUSSION

The finding that the Agavaceae (*sensu* Cronquist) is not monophyletic has implications for the evolution of the *Yucca*-*yucca* moth mutualism. Recent phylogenetic studies of the Prodoxidae using morphological characters (16) and mtDNA data (19) suggested that restriction to the Agavaceae (*sensu* Cronquist) as host plants and single-phase larval feeding were the only synapomorphies linking the so-called "yucca moth" genera (*Tegeticula*, *Parategeticula*, *Agavenema*, *Prodoxus*, and *Mesepiola*) and distinguishing them from *Greya*, a prodoxid genus whose larvae feed on fruits of some Ranunculaceae, Saxifragaceae, and Apiaceae. *Mesepiola* differs from the other yucca moths (25-27) in its apparent consumption of *Nolina* and *Dasyllirion* (28). Because our data show that *Nolina* and *Dasyllirion* are not members of the Agavaceae (Fig. 1), we must change our concepts of host relationships among the yucca moths to include a wider range in the Asparagales *sensu* Dahlgren or perhaps to reevaluate the relationships of *Mesepiola*. We do not suggest that seed feeding arose twice within the prodoxids but, rather, that the association of *Mesepiola* with the Nolinaceae is the result of a separate colonization of some species of this family that are structurally similar to robust-rosette members of the Agavaceae *sensu* Dahlgren.

Our analyses also strongly support a *Y. whipplei* + *Hesperaloë* clade, with most analyses indicating that this and the rest of *Yucca* form a sister clade to *Agave* and its close relatives (*Beschorneria*, *Furcraea*, *Polianthes*, *Manfreda*, and *Prochnyanthes*). From a strictly phylogenetic point of view, these data suggest that *Y. whipplei* should probably be recognized as the monotypic genus *Hesperoyucca whipplei* as suggested by Trelease (29). Whether all the members of Trelease's Yuccaceae (*Yucca*, *Clistoyucca*, *Hesperoyucca*, *Samuela*, and *Hesperaloë*) should be given generic status (cf. ref. 12) will require further studies of these taxa.

Although all species commonly included in *Yucca* s. lat. appear to be highly dependent on yucca moths for seed set (28, 30, 31), there are noteworthy differences between the floral morphology of *Y. whipplei* and that of other *Yucca* species. *Y. whipplei* has a capitate stigma with long papillae and can apparently set seed in the absence of yucca moths (32). There are a number of anecdotal reports of seed set of several *Yucca* species in the absence of yucca moths (2, 25, 32) but the deeply

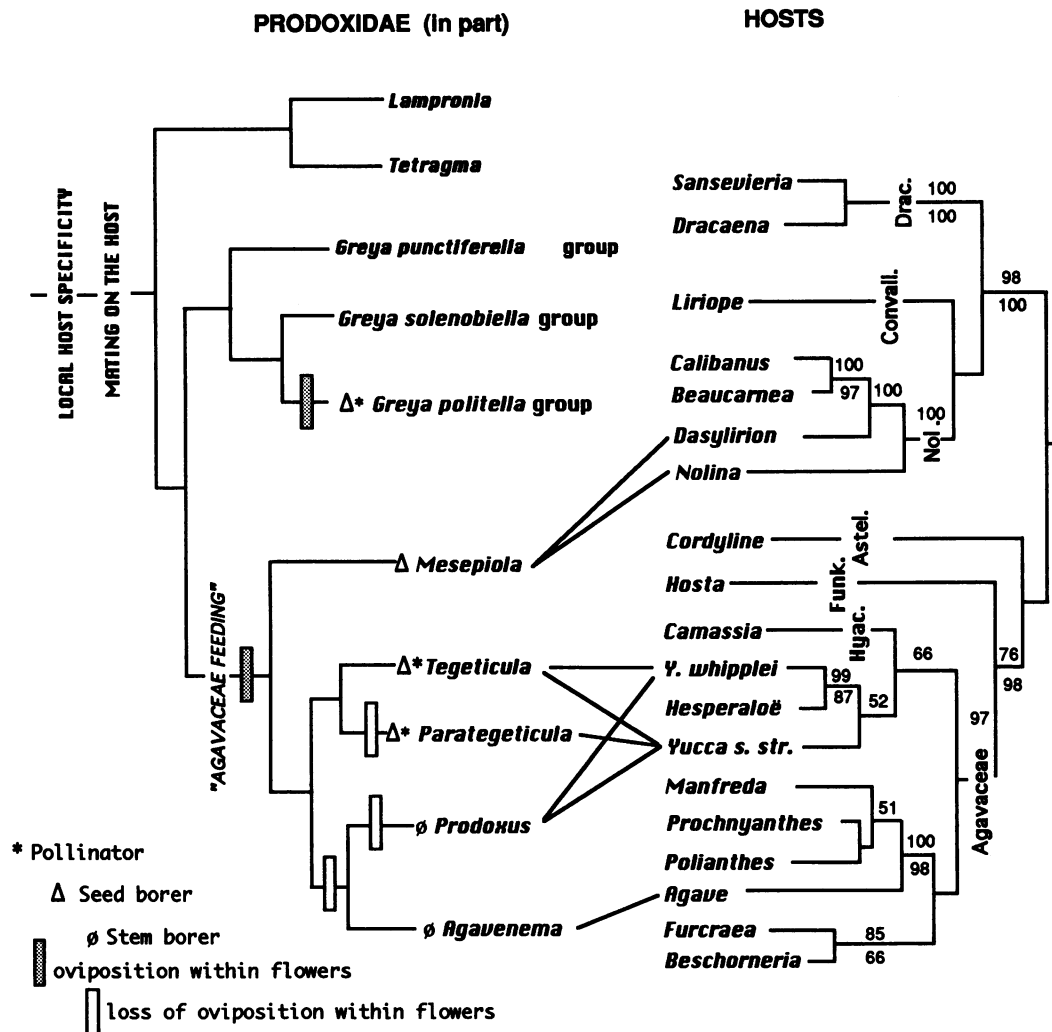


FIG. 1. Reduced phylogenies (for illustrative purposes only) of the Prodoxidae (16) and a suite of monocots (9, 12) most of which have been placed in the Agavaceae by various authors. The Prodoxidae phylogeny indicates that local host specificity and mating on the host are features that unite the family. The character "Agavaceae feeding" was considered by Pellmyr and Thompson (16) to define the yucca moths. The molecular phylogeny of Brown *et al.* (19) still recognized the existence of an Agavaceae feeding clade that consists of *Mesepiola* and its sister genera, but it placed the *Greya politella* group as a basal lineage of *Greya* and *Agavenema* within *Prodoxus*. The plant phylogeny on the right is that yielded from the combined ITS1 and ITS2 sequences. Bootstrap values >50% for the combined ITS data are given above the line and values for our cpDNA restriction site study are given below the line (17). The placements by Cronquist (9) and Dahlgren *et al.* (12) of the plant genera shown are shown in Table 1. Astel., Asteliaceae; Convall., Convallariaceae; Drac., Dracaenaceae; Funk., Funkiaceae; Hyac., Hyacinthaceae; Nol., Nolinaceae.

recessed hidden stigmas of all *Yucca* species except *Y. whipplei* strongly suggest that pollen transfer would be quite difficult in the absence of the moths. Nevertheless, all detailed pollination studies have indicated that *T. maculata* is *Y. whipplei*'s primary, and usually only, pollinator (33–38). *Hesperaloë* has a more common nectar-based animal pollination system. The narrow diurnal rose-colored flowers of *Hesperaloë parviflora* are almost certainly hummingbird-pollinated, whereas the dull cream-to-green nocturnally opening flowers of *Hesperaloë nocturna* suggest pollination by nectar feeding settling moths (Noctuidae?). Both *Hesperaloë parviflora* and *Hesperaloë nocturna* produce appreciable quantities of nectar (>15  $\mu$ l/day; J.L.N., unpublished data). Our studies show nocturnal anthesis, reflexed white tepals, and copious nectar production (>500  $\mu$ l), suggesting that *Hesperaloë funifera* is bat-pollinated.

Within the clade consisting of *Y. whipplei* + *Hesperaloë*, *Yucca*, and *Agave* and its allies (Fig. 1), oviposition in the flower by prodoxid moths is found only in *Y. whipplei* and *Yucca* s. str. Most other members of this clade are pollinated by nectar (or nectar and pollen) feeding bees, bats, or birds. Our data suggest that the *Yucca*-yucca moth symbiosis probably arose when one of the seed-feeding prodoxid moths

(*Tegeticula*) evolved the ability to pollinate "purposefully" the plants upon which its larvae preyed, a development that occurred in the ancestor of the clade consisting of sections *Yucca*, *Chaenocarpa*, and *Clistocarpa*.

The demonstration that *Yucca* is paraphyletic indicates two possible origins for the famous *Yucca*-*Tegeticula* mutualism. Under a scenario retaining the classic view of a single origin of the mutualism but incorporating our phylogenetic evidence, one might envision that yucca moth pollination evolved in the ancestor of (*Y. whipplei* + *Hesperaloë*) and *Yucca* s. str. prior to the evolution of the floral specialization of *Yucca* s. str. (Fig. 2A). The yucca moth pollination syndrome could have been subsequently completely lost in *Hesperaloë* and retained in *Y. whipplei* without the development of extreme stylar and stigmatic modifications. The ancestor of *Yucca* s. str. would have later developed the highly derived specializations of the distinctly recessed stigma, clavate and often bent filaments, outward pollen presentation, little or no nectar, and nocturnal blooming. This scenario retains the traditional single origin of yucca moth pollination but would require the loss of presumably obligate yucca moth pollination in *Hesperaloë* and with it virtually all the morphological features (e.g., white bowl-

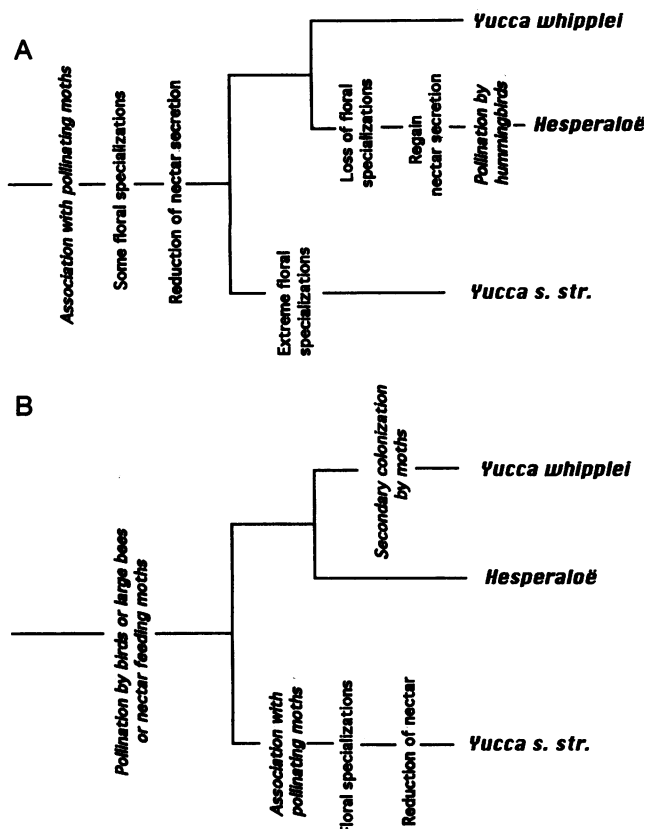


FIG. 2. Classic (A) and alternative (B) scenarios of the association of *Tegeticula* with species of *Yucca* s. str. and *Y. whipplei*. (A) The symbiotic pollination system arose in the basal ancestor of the clade containing *Yucca* s. str., *Y. whipplei*, and *Hesperaloë* and was subsequently lost in *Hesperaloë*, which now exhibits none of the floral features associated with yucca moth pollination. (B) The highly specialized *Yucca*–*yucca* moth system arose in the ancestor of the lineage that now contains only the species of *Yucca* s. str. *Y. whipplei* was secondarily colonized by a species of *Tegeticula* and subsequently developed the few morphological floral traits it possesses that are associated with yucca moth pollination.

shaped flowers, thickened filaments, pollen as the primary floral reward, odor cues for oviposition in the ovaries) associated with the yucca moth system.

Under a second scenario, the ancestor of the *Y. whipplei* + *Hesperaloë* and *Yucca* s. str. clade presumably had a more general zoophilous pollination system. The ancestor of *Y. whipplei* and *Hesperaloë* retained this system whereas the ancestor of *Yucca* s. str. switched to yucca moth pollination. Selection to reduce the costs of a traditional resource-based pollination system (small anthers, reduction or loss of nectar production, and perhaps the recessed stigma) led to the high dependence on yucca moths now seen. We suggest that a secondary yucca moth mutualism apparently arose when the ancestor of *Y. whipplei* was colonized by a yucca moth as a pollinator (Fig. 2B). Reduction or loss of nectar production and elaboration of the large cup-shaped white flowers would have resulted as convergent features evolving with increased dependence on yucca moths as pollinators. Characters such as a capitate stigma, inwardly dehiscent anthers, and diurnal blooming would be features that *Y. whipplei* would have retained from its previous pollination system. We favor this scenario because it is more parsimonious than the single-origin hypothesis.

Additional data on the systematics of *Yucca* s. str. and the *T. maculata* complex and its relationship to the other *Tegeticula*

spp. should provide additional clues about the evolution of the primitive *Yucca*–*yucca* moth association and may provide evidence of congruent phylogenetic histories between some clades within *Yucca* s. str. and those of *Tegeticula*.

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