Floral trait associations in hawkmoth-specialized and mixed pollination systems

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Article Addendum

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Datura wrightii and *Agave spp.* in the Sonoran Desert

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Variation in floral traits including odor, color and morphology, demonstrate the selective pressures imposed by specific pollinator taxa, such as insects and birds. In southern Arizona, *Manduca sexta* (Sphingidae) hawkmoths are associated with *Datura wrightii* (Solanaceae) at both the larval (herbivore) and adult (nectar feeding) stages. However during most of the summer *Manduca* feeds on "bat-adapted" *Agave* spp. (Agaveacea) flowers, and only use *Datura* when it is at peak bloom. *Manduca's* nectar-host use appears to be mediated through innate odor preferences and olfactory learning; they prefer *Datura's* "hawkmoth-adapted" traits, which facilitate the maintenance of their coevolutionary relationship, yet they are flexible enough to explore and learn to utilize novel resources, such as agave. This behavioral flexibility is likely responsible for the frequent observation of generalized, or mixed, pollination systems. Given that *Manduca* visit agave species in southern Arizona, we hypothesize that the differences in flower phenotype between two closely related agave species may be associated with the importance of hawkmoths relative to bats. The southernmost agave, *Agave palmeri* (Agavacea), exhibits floral traits typical of bat pollination, whereas the northernmost species, *Agave chrysantha* (Agavacea), exhibits mixed floral traits which appear to be adapted to insects, and to a lesser extent, bats. The differences between these agaves are likely correlated with the geographic overlap in migratory bats from Mexico and resident hawkmoth populations. Thus *D. wrightii, A. palmeri* and *A. chrysantha* populations represent a unique system in which to examine the evolution of floral traits in both specialized and mixed pollination systems associated with spatially variable pollinator assemblages.

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Floral Advertisements and Pollinator Visitation

Floral traits are signals that animal pollinators use to locate important food resources. Flower phenotypes are believed to be non-random combinations of colors, odors and morphologies hypothesized to reflect selective pressure imposed by certain classes of pollinators.[1] For example, some of the well-studied examples of floral signals come from plants with nocturnal antithesis thought to reflect adaptations for hawkmoth or bat pollination. Flowers having hawkmoth pollinated traits are white and highly reflective, emit a strong pleasant scent, and have sucrose-dominant nectar.[2-4] In contrast, bat pollinated flowers are dull yellow or cream-colored, emit a strong fetid or pungent scent, and have hexose- (fructose and glucose) dominant nectar.[5,6] Phylogenetic and morphological studies have demonstrated that these characteristics have evolved independently in many different plant families.[7] The convergence of these common floral features makes night blooming plants excellent model systems in which to examine plant-pollinator interactions and the evolution of floral traits.

In the semi-arid environments of southern Arizona USA, several hawkmoth and bat-adapted plants occur in sympatry. An example of this is Palmer's Agave (*Agave palmeri*, Agavaceae), and jimsonweed (*Datura wrightii,* Solanaceae), which exhibit bat and hawkmoth-adapted floral characters, respectively. *D. wrightii* floral traits include a sweet smelling odor composed of terpenoids (mono- and sesquiterpenoids) (79%) and benzenoids (18%), a sucrose-rich nectar, and highly reflective corollas (reflectance > 50%) that appear white to the human eye (Fig. 1A). In contrast, *A. palmeri* traits consist of a foul floral scent dominated by esters (30%), sulfur compounds (<1%), and monoterpenoids (59%), abundant hexose-rich nectar, and a pale dull coloration (reflectance < 25%) (Fig. 1B). The hawk-moth, *Manduca sexta* (Sphingidae), is an important pollinator for *D. wrightii* and has an innate attraction to *D. wrightii*'s floral odor. *M. sexta* hawkmoths also visit *A. palmeri* flowers, but only when *D. wrightii* is not locally abundant. Moths learn to utilize *A. palmeri*'s abundant nectar resources through olfactory-mediated exploration and learning.[4] The ability by pollinators to shift from using one nectar resource to another allows populations to exist when a preferred resource is scarce while likely permitting the maintenance of plant-pollinator associations.

From an evolutionary perspective, behavioral plasticity is likely an adaptation in response to a variable floral environment. Pollinators capable of collecting floral resources from a wide variety of plants, including those morphologically adapted to different pollinator taxa, are more likely to persist within a fluctuating environment compared with those specialized to a single, or few, partner species.[8,9] Thus it is not surprising that generalized, or mixed pollination systems, are more common than previously believed.[10]

Floral Traits of Two Agave Species in the Sonoran Desert

In the Sonoran Desert agaves are regarded as "keystone species" during summer months by providing copious amounts of nectar and pollen for a wide range of insects and vertebrates pollinators.[11-14] Two agaves in particular, *Agave palmeri* and *Agave chrysantha*, represent a unique system to explore the evolution of floral traits under selection by a diverse pollinator assemblage. While the evolutionary relationship between *Agave chrysantha* and *A. palmeri* has yet to be fully resolved using molecular techniques, the occurrence of natural hybrids and morphological studies suggest that they are closely related.[15] *Agave chrysantha* is endemic to central and southern Arizona, and is believed to be a relatively young species, whereas *Agave palmeri* is believed to be older and has a wider range that extends into Mexico.[15,16] The flowers of both agaves superficially resemble one another, possibly reflecting their shared evolutionary history: both produce abundant nectar resources (>100 µl/day), emit odor composed of monoterpenes and aliphatic compounds known to attract diverse insect species, and have floral morphologies that permit nectar access by insects, bats and birds. However, their flowers' differ from one another in

Figure 1. Contrast in floral characterisitics between *D. wrightii*, *A. palmeri* and *A. chrysantha* plants. The three plants are visited by nocturnal hawkmoths, but the floral traits in each plant differ from one another. (A) *D. wrightii* flowers. Note the bright floral reflectance of the corollas that produces a contrast with the dark foliage background. (B) *A. palmeri* umbel. Each umbel contains 8–20 flowers. *Hyles lineata* (Sphingidae), and *Cotinus mutabilis* (Scarabaeidae), are feeding from the umbel. (C) *A. chrysantha* umbel. The morphology of *A. chrysantha* closely resembles that of *A. palmeri*, but note the bright yellow corollas. (D) Principal-components analysis of the floral odor from these three plants. The *D. wrightii* flowers (blue circles) group together. In contrast, the agaves appear more variable. The odor of *A. palmeri* flowers (green squares) appears to more closely resemble that of *D. wrightii* due to the presence of monoterpenes and benzenoids in its headspace, but some *A. palmeri* flowers are also grouped in the same PCA space of *A. chrysantha* (yellow diamonds) due to their aliphatic esters. Scale bar = 5 cm. Picture images courtesy of Charles Hedgecock RBP, FBCA (ARL Division of Neurobiology, University of Arizona) and T. Beth Kinsey.

several ways. For instance, corollas of *A. chrysantha* are bright yellow-orange rather than the pale cream color of *A. palmeri* (Fig. 1C), which suggests that the former are visually more adapted to diurnal pollinators, and the latter to nocturnal pollinators.[16] *A. chrysantha* flowers produce nectar that has more than 63-times the sucrose content of *A. palmeri* (7.56 mg/flower *versus* 0.12 mg/flower, respectively), which suggest that *A. chrysantha's* nectar is under greater selection by insect pollinators.

While differing in their visual display and nectar chemistry, examination of their scent profiles by principal components analysis (PCA) revealed substantial overlap in the two agave species (Fig. 1D). For instance, *A. palmeri* and *A. chrysantha* share similar percentages of
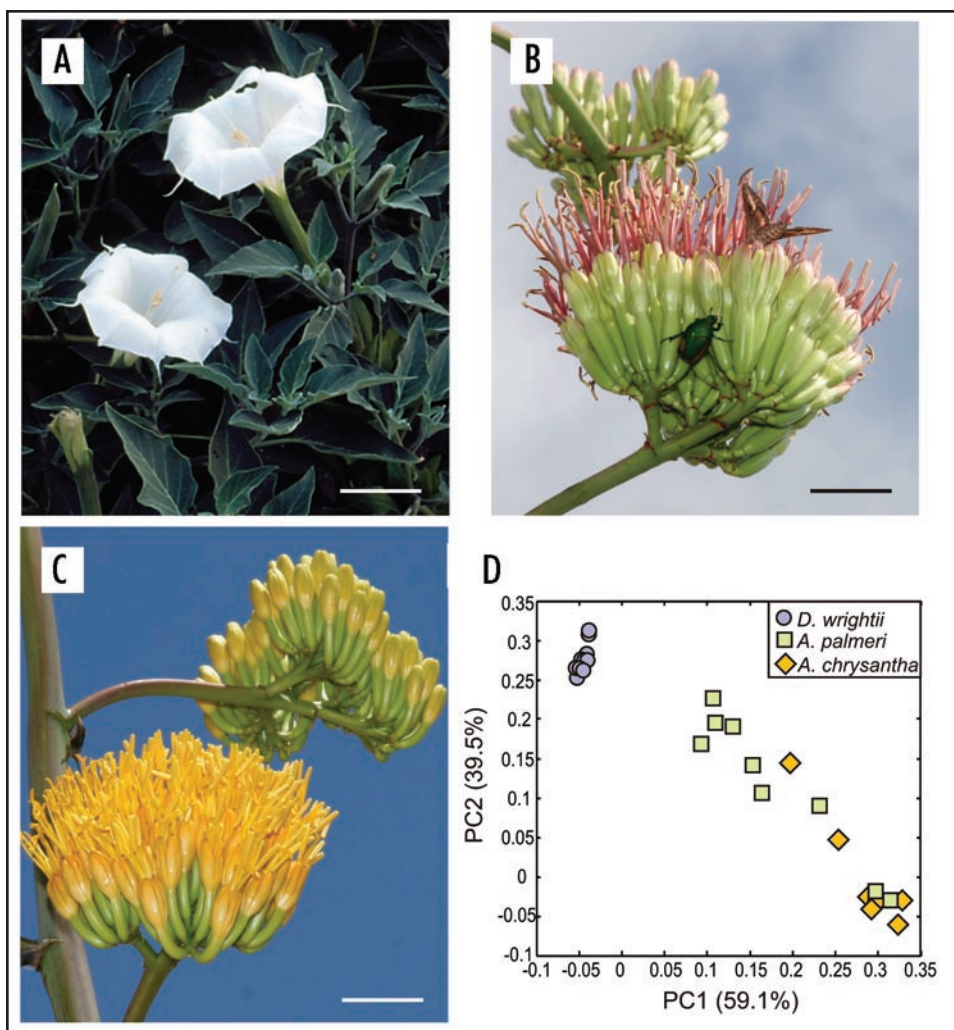
chemical odorant classes, including low levels of sulfur compounds (<1%)—a common marker of bat-adapted flowers—esters (ca. 53% and 77%, respectively), and terpenoids (ca. 38% and 15%, respectively). The contrast in floral color and nectar chemistry suggest that these two agaves are adapted to different pollinator assemblages, but the similarity in their floral odors likely reflects their shared evolutionary history.

A Unique Study System for Evaluating Pollinator-Mediated Floral Evolution

Assuming that *A. chrysantha* recently evolved from *A. palmeri*,[15] the differences in floral color, nectar and odor chemistry between the

two species are likely due to differences in visitation by migratory bats, in particular the migratory Lesser Long-nosed Bat (*Leptonycteris curasoae*). Recent work[12,14,17] suggests that the relationship between Lesser Long-nosed Bats and their nectar plants varies latitudinally, with bats and plants mutually dependent upon each other in central Mexico, but plants are less depended upon bats at the northern end of their range (i.e., Arizona, USA), presumably because bat migrations are variable from year to year. Given that the range of the Lesser Long-nosed Bat entirely overlaps with the distribution of *A. palmeri* in southern Arizona, but only overlaps the southern most portion of *A. chrysantha's* range,[14] it is likely that *A. chrysantha* evolved in the absence of bat pollination. Coincidentally, the region where *A. chrysantha* and Lesser Long-nosed Bat's ranges overlap is where *A. chrysantha* hybridizes with *A. palmeri*, suggesting that bat-mediated gene flow, or the lack thereof, is central to the evolution of *A. chrysantha's* floral characters.

The differences in floral traits likely reflect variation in the effective pollinators of these two agave species. Sulfur compounds are important mediators of bat attraction to flowers[18] and it has been suggested that shifts from bat to moth pollination reflects the reduction of sulfur compounds and the presence of terpenoids and benzenoids in a floral headspace. For instance, flowers of the plant *Browneopsis disepala* (Leguminosae) are visited by both moths and bats and displays a mixed pollination syndrome with a bat pollinated floral display and an odor profile that is dominated by aliphatics and monoterpenes but lacks the sulfur compounds.[7] These agaves represent additional examples of mixed pollination systems, wherein the odor profile and visual displays of *A. palmeri* and *A. chrysantha* indicate that one is more bat-adapted than the other. Because hawkmoths are common nocturnal visitors of these agave species,[14-16] the agaves, together with *D. wrightii* which is specialized for hawkmoth pollination, present an excellent system to examine the relationship between floral advertisements and rewards with their primary nocturnal visitors.

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