

POLLINATION BIOLOGY OF *ASTRAGALUS PHOENIX* (FABACEAE)
WITH NOTES ON THE NATURAL HISTORY OF ITS POLLINATOR,
ANTHOPHORA PORTERAE (HYMENOPTERA: APIDAE)

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ABSTRACT.—*Astragalus* (Fabaceae) is a broadly distributed, diverse, and economically important group of plants. Given the number of species and its distribution, it is not surprising that there are many species that are highly restricted and endangered. Among these is the Ash Meadows milkvetch, *Astragalus phoenix*. Here we investigate the breeding biology of *As. phoenix*. Our data show that *As. phoenix* is xenogamous and that *Anthophora porterae* (Hymenoptera: Apidae) is the most likely pollinator. We also noticed *Apis mellifera* visiting *As. phoenix*, though it appears unlikely that *Ap. mellifera* contributes significantly to the reproductive success of *As. phoenix*. We located a nesting aggregation of *An. porterae* and offer a description of its nest architecture.

RESUMEN.—*Astragalus* (Fabaceae) es un grupo de plantas con amplia distribución, muy diverso y de gran importancia económica. Debido a la cantidad de especies y a su distribución, no es sorprendente que muchas especies estén restringidas y en peligro de extinción. Entre dichas especies se encuentran *Astragalus phoenix*, cuya reproducción estudiamos en esta investigación. La información que obtuvimos muestra que *As. phoenix* es una especie alógama y que lo más probable es que *Anthophora porterae* (Hymenoptera: Apidae) sea el polinizador. Observamos que *Apis mellifera* visitó a *As. phoenix*, aunque parece poco probable que contribuya significativamente al éxito reproductivo de *As. phoenix*. Localizamos un conjunto de nidos de *An. porterae*, y ofrecemos una descripción de la estructura de sus nidos.

Astragalus (Fabaceae), known as milkvetch, is a large genus with a global distribution (Frodin 2004). *Astragalus* is most diverse in montane regions, such as the Andes Mountains, western North America, and the Sino-Himalayan region (Allen and Allen 1981, Isely 1998). The global impact of *Astragalus* is 2-fold. Firstly, the family Fabaceae is second only to the grasses in economic importance (Watrous and Cane 2011). It is an important source of ground cover, forage for livestock, and food crops (reviewed in Allen and Allen 1981), although some species known as “loco-weeds” are toxic to livestock (e.g., James et al. 1970). Secondly, *Astragalus* has ecological importance as forage for many native and introduced pollinators, particularly bees (Green and Bohart 1975, Krombein et al. 1979, Clement et al. 2006). Additionally, *Astragalus* is currently targeted as a species of importance for rehabilitating poorly managed rangeland (Shaw et al. 2005).

Astragalus also occupies a unique position in the taxonomy of plants. It is the most species rich of all plant genera, with approximately 3200 described species (Frodin 2004).

Given the relative size and distribution of this genus, it is not surprising that there are many geographically restricted and endangered species of *Astragalus* (Baskin et al. 1972, Sugden 1985, Karron 1987, 1989, Gallardo et al. 1993, Geer and Tepedino 1993, Geer et al. 1995, Allphin et al. 2005, Tepedino 2005). Among these is the Ash Meadows milkvetch, *Astragalus phoenix* Barneby.

Astragalus phoenix appears to be restricted to Ash Meadows National Wildlife Refuge, which is located east of California’s Death Valley and represents a relatively rare habitat, the desert wetlands (Haller et al. 1992). A series of ephemeral streams and natural springs and a high water table keep much of Ash Meadows’ soils wet throughout the year and cause salts to precipitate and form a thick crust over much of the soil. Given the rarity of this habitat, it is not surprising that Ash Meadows houses many endemic and endangered organisms, including the Devil’s Hole pupfish, *Cyprinodon diabolis* (Wales), which is the vertebrate with the most restricted habitat in the world (Knight and Clemmer 1987). Indeed, Ash Meadows has a greater number of

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endemic species than do similar habitats that also occur within the Mojave Desert (Haller et al. 1992). Among these endemic species are many rare plants, which some argue warrant government protection, including the threatened species *As. phoenix* (Beatley 1977a, 1977b, 1977c).

Astragalus phoenix was discovered by C.A. Purpus in 1898, collected again in 1966 by A. Cronquist, and described by Barneby (1970). *Astragalus phoenix* is a long-lived, low-growing, mat-forming perennial composed of spreading branches that form large mounds (Knight and Clemmer 1987). Each branch may terminate in 1, 2, or 3 flowers. The flowers are 2–2.5 cm long, pink-purple shortly after bloom, and purple once the flower begins to fade (Knight and Clemmer 1987). The fruit is small (rarely more than 2 cm long and 1 cm wide); entirely covered in thick, grayish pubescence; and contains approximately 30 seeds (Knight and Clemmer 1987). *Astragalus phoenix* is officially listed as “threatened” and is restricted to the hard alkaline soils in Ash Meadows (Linhart and Grant 1996); it is commonly associated with *Distichlis spicata* (L.) Greene (saltgrass), *Atriplex confertifolia* (Torr. and Frém.) S. Watson (shadscale saltbush), *Mentzelia leucophylla* Brandege (Ash Meadows blazingstar), another plant endemic to Ash Meadows), and *Isocoma acradenia* (Greene) Green var. *acradenia* (alkali goldenbush; Knight and Clemmer 1987).

Currently, very little is known about the breeding biology of *As. phoenix*. A recent review of the breeding biology of *Astragalus* showed that there is significant variability across the genus ranging from autogamous to xenogamous (Watrous and Cane 2011). Further, this review showed that there is little relationship between the rarity of the plant and its degree of autogamy (Watrous and Cane 2011). It should be mentioned, however, that the breeding biology is known for <1% of the genus. It should also be noted that there is some variation within plant species in the degree of self compatibility and that breeding categories (autogamous, geitonogamous, xenogamous) may be described better as points along a continuum rather than as discrete classes.

There is some precedent, however, to suggest that *As. phoenix* will show some degree of autogamy. Some authors argue that

geographically restricted species, such as *As. phoenix*, are likely to be self compatible because propagules that are dispersed long distances stand a better chance of generating sexually viable populations if they are capable of self fertilization (Baker 1955, 1967, Allard et al. 1968, Jain 1976, Lande and Schemske 1985). Additionally, if the population size of the rare species fluctuates significantly, self compatibility will be favored when the population size is small (Baker 1955, Stebbins 1957, Baker 1967, Allard et al. 1968, Jain 1976, Lande and Schemske 1985, Schemske and Lande 1985). Interestingly, *As. phoenix* does not fit either of these criteria; the only known populations of *As. phoenix* are in Ash Meadows, suggesting that recent dispersal events are unlikely. Secondly, *As. phoenix* is a perennial plant, and there seems to be high survivorship between seasons. Consequently, there is little yearly variation in population size.

The origins of *As. phoenix* rarity are unclear. Nevertheless, it may be unlikely that *As. phoenix* will become more common if reproductive success is at least partially dependent on pollinator services. Previous work on *Astragalus* has shown that restricted species receive less pollinator attention than sympatric, widespread congeners (Karron 1987). Other studies have shown, however, that sympatry between rare and broadly distributed species may facilitate pollination of rare plants because little fidelity is shown by the pollinators between plant species (Geer et al. 1995).

This study addresses questions regarding the reproductive biology of *As. phoenix* and the nesting biology of insects that pollinate it. Namely, we tested the ability of *As. phoenix* for autogamy through a series of pollinator exclusion experiments. Secondly, we observed the insects that visit *As. phoenix*. Based on behavioral and pollen load analyses, we determined which of the insects offers pollination services and deduced natural history information concerning these pollinators. Finally, we describe the nesting biology of insects that offer pollination services to *As. phoenix*.

METHODS

We identified 2 populations of *As. phoenix* at Ash Meadows National Wildlife Refuge and constructed cages over 12 plants in each population. Cages were made of nylon mesh

material to prevent potential pollinators from interacting with the flowers. We also identified 12 plants that were approximately the same size and covered them in wire-mesh cages to prevent herbivory by rabbits. We then used fruit production in these plants to test for autogamy in *As. phoenix*. We also spent alternating periods observing the behavior of *As. phoenix* and collecting insects that visited *As. phoenix* in these populations. These observations and collections were made between February and April 2008 and 2009. We also conducted surveys for nesting sites for the insects that successfully pollinate *As. phoenix*. Once we located nests, we then removed the pollen provision from the legs of the bees returning to their nests. We also excavated nests and collected sealed pollen provisions.

Study Site

We conducted this study at Ash Meadows National Wildlife Refuge, which is located near Pahrump, Nevada, approximately 100 km west of Las Vegas, Nevada, and adjacent to Death Valley National Park. We located 2 populations of *As. phoenix* that consisted of at least 50 individuals. One population of *As. phoenix* was located on the north side of Ash Meadows along an unnamed road that diverged from Ash Meadows Road (population center: zone 11S, 4027916.4 N, 565036.6 E). We will refer to this population as the Bill Copeland population. The second of our study sites was located east of the intersection of South Spring Meadows Road and Devils Hole Road (population center: zone 11S, 4029324.2 N, 563350.0 E). We will refer to this population as the Collin's Ranch population. Both of these populations were located in predominantly alkali shrub/scrub habitat, though the Collin's Ranch area was naturally inundated with water. We also located large populations of 3 other rare plants at the Collin's Ranch site, namely *Calochortus striatus* Parish (alkali mariposa lily), *Enceliopsis nudicaulis* (A. Gray) A. Nelson var. *corrugata* Cronquist (naked-stem sunray), and *Ivesia kingii* S. Watson var. *eremica* (Coville) Ertter (Ash Meadows ivesia).

Plant Breeding Experiment

To test if *As. phoenix* is capable of autogamous reproduction, we constructed cages over 12 plants within the Bill Copeland and Collin's Ranch populations. The plants that

we selected consisted of mats that were at least 20 cm in diameter. We constructed cages from pliable wood rods and tricot (Fig. 1). We placed these cages over the entire plant, preventing pollinators from interacting with any of the flowers on the plants. We did not test whether *As. phoenix* is capable of geitonogamous reproduction because, when we attempted to manipulate the flowers, the flowers eventually aborted. Therefore, we tested only whether plants require insect-mediated pollination services or whether they are capable of producing fruits without insect visitation. In each population, we also identified 12 plants of approximately the same dimensions as the pollinator-excluded plants to compare the relative amount of fruit set. Ash Meadows, however, has a productive jackrabbit (*Lepus californicus* [Gray]) population. To prevent the rabbits from eating the plants, flowers, or fruits of *As. phoenix*, we constructed cages from a wire-mesh material. The 2-cm mesh size was large enough to allow insects to freely visit the plants, yet small enough to prevent rabbits from accessing the plants.

Observations and Collections

Pollinator observations were interspersed with collection periods; we observed pollinator behavior and collected floral visitors in alternating 15-min sessions. We observed the behavior of insects located within a 1-m² area, which commonly included more than one *As. phoenix* plant. We collected insects in a manner that minimized damage to the plants. If a bee was physically contacting the flower, we gently placed an insect net over the top of the plant and waited for the bee to fly up into the net. If a bee was flying near the plant (i.e., the bee had completed collecting the floral rewards and was leaving the plant), we gently swept our nets across the plants in an attempt to capture the insects. We then transferred the insects into a jar containing cyanide and quickly dispatched them. The insects were then transported to the insect museum at Utah State University where they were mounted and identified.

During the observation periods, we noted which part of the insect contacted the stamens and stigma, how many flowers a single bee visited per plant, how many plants were visited during the foraging trip, and if pollen or nectar were being collected.

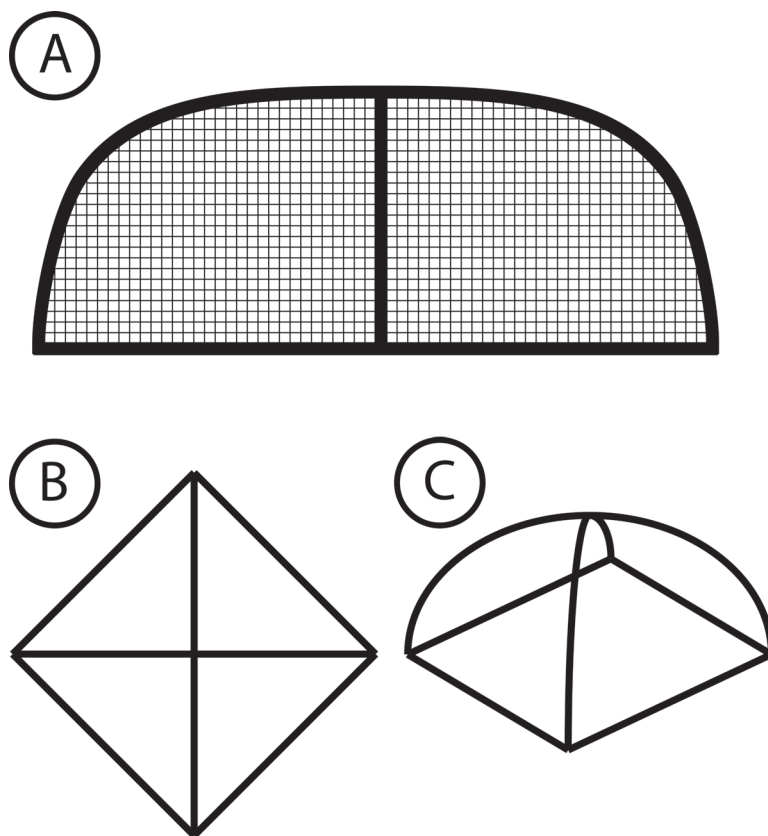


Fig. 1. Pollinator exclusion cage. These cages consisted of 6 pieces of pliable wood. Four pieces were used to construct a square base, and the remaining 2 were crossed diagonally above the base and to support the tricot: A, side view of trap; B, top view of trap without tricot; C, oblique view of trap without tricot.

Nest Surveys and Excavation

We surveyed extensively for nests of the insects that we confirmed were pollinating *As. phoenix*. We focused our attention on the area within 300 m of the plant populations. We also extensively surveyed the dune system that runs through the refuge. From our surveys, we were able to locate a single aggregation of *Anthophora porterae* Cockerell, 1900 (Hymenoptera: Apidae), which is the insect most likely to offer pollination services to *As. phoenix*. Because of the rarity of this plant, we excavated only 3 nests, removing a single completed cell from each nest. To excavate these nests, we poured hydrated plaster of paris into the nest entrance hole. Plaster was poured into the nest only after the provisioning bee was seen leaving the nest. We waited for the plaster to dry (approximately 30 min) before excavation. To excavate the nest, we then gently removed the

soil around the nest. When we reached the end of the nest, we located completed nest cells by using a knife blade to remove the soil around the rigid outer walls of the completed cells.

Pollen Analysis

To identify the pollen that we removed from the insects that visited *As. phoenix*, we made a pollen reference library that included samples of *As. phoenix* pollen and pollen from other concurrently blooming plants. To make this library, we collected anthers from *As. phoenix* and concurrently blooming plants and then transported them to the Department of Biology Insect Collection at Utah State University. We dried the anthers for approximately 24 h and transferred the pollen to a glycerin jelly on a microscope slide and dyed the pollen with a basic fuchsine stain (Kearns and Inouye 1993).

We collected insects returning to their nests and compared pollen collected from their bodies with pollen in the reference library. To identify the pollen on the insects, we removed the pollen from their bodies, placed it on microscope slides, and dyed it with the same fuchsine stain. Before removing pollen from the body of an insect, we first placed the insect in a relaxing chamber that contained paradichlorobenzene to decrease the probability that the insects would be damaged by fungus. We removed the pollen from the body of the insects with a small amount of glycerin jelly infused with fuchsine stain on a wood probe. We touched this matrix to the head, mesosoma, legs, and metasoma of each insect we collected. We made a concerted effort to remove all of the pollen from the pollen baskets of the legs of the bees. The pollen on the body of the insects adhered to the jelly matrix, and we then applied this matrix to a microscope slide. The matrix melted and dried onto a slide placed on a slide warmer, and we applied a coverslip to it.

We estimated the amount of pollen collected by each insect by the following procedure. We first divided the slides of pollen into regions and randomly selected regions in which to count pollen. We then extrapolated pollen counts of the selected regions to estimate the amount of pollen on the whole slide.

We also quantified the amount of pollen in the completed provisions of the bee *An. porterae* by carefully extracting the pollen from a completed cell and adding the pollen to 100 mL of 70% EtOH. We then sonicated this solution for 180 s and added 10 mL of the homogenized solution to a HIAC/ROYCO particle counter equipped with an automatic bottle sampler (Pacific Scientific, Silver Spring, MD), which counted the amount of pollen in the sample. We used this count to extrapolate the amount of pollen in the provision.

RESULTS

Breeding Experiment

Of the 24 plants from which pollinators were excluded, all of the plants produced many flowers, but only one produced a single fruit. The fruit that this plant produced, however, was located adjacent to a tear in the tricot. It may be that a bee surreptitiously gained access to a flower through this tear and transferred pollen

to it from the flower of another plant. The 24 plants that had access to insect pollinators but were protected from herbivory by *L. californicus* produced an average of 8 fruits per plant.

Visitors and Pollinators of *An. porterae*

We observed 2 insects actively visiting the flowers of *As. phoenix*; a third insect, an unidentified syrphid fly, was found resting on the leaves and flowers, though it made no attempt to access the pollen or nectar. The first insect that we noted visiting *As. phoenix* was the European honeybee, *Apis mellifera* (Hymenoptera: Apidae). We first observed *Ap. mellifera* visiting the flowers of *As. phoenix* in early February 2009. All of the observations of *Ap. mellifera* visiting *As. phoenix* were made in late February. The results of the pollen analysis show that there was pollen on the bodies of honey bees that were visiting *As. phoenix*, though we are dubious that *Ap. mellifera* offered pollination services. We were able to recover pollen on *Ap. mellifera* only from the corbiculae; we were unable to collect *As. phoenix* pollen from the head and face of *A. mellifera*, which is the region of the body that is most likely to contact the stigma of the flower. *Apis mellifera* appeared unable to depress the keel of the flower. Also, we observed *Ap. mellifera* within populations of *As. phoenix* 4 times during the 2-year study, and during only 2 of these times did *Ap. mellifera* interact with the flowers. During its visits to *As. phoenix*, *Ap. mellifera* visited only one plant in the population and only a single flower of that plant. If *Ap. mellifera* does transfer pollen between flowers, it is likely an uncommon event.

The next insect we noted visiting *As. phoenix* was *Anthophora porterae*. We observed *An. porterae* visiting *As. phoenix* from late February to early May. *Anthophora porterae* is a large, ground-nesting bee that is known to visit the flowers of many plants (Krombein et al. 1979). The results from the pollen analysis and our observations of behavior suggest *An. porterae* is an important source of pollination services to *As. phoenix*. *Anthophora porterae* visited more than one plant of *As. phoenix* within a foraging trip in 30% of the foraging trips that we observed, and individuals consistently visited several flowers on each plant (\bar{x} = 6.203, range 1–17). When interacting with the flowers of *As. phoenix*,

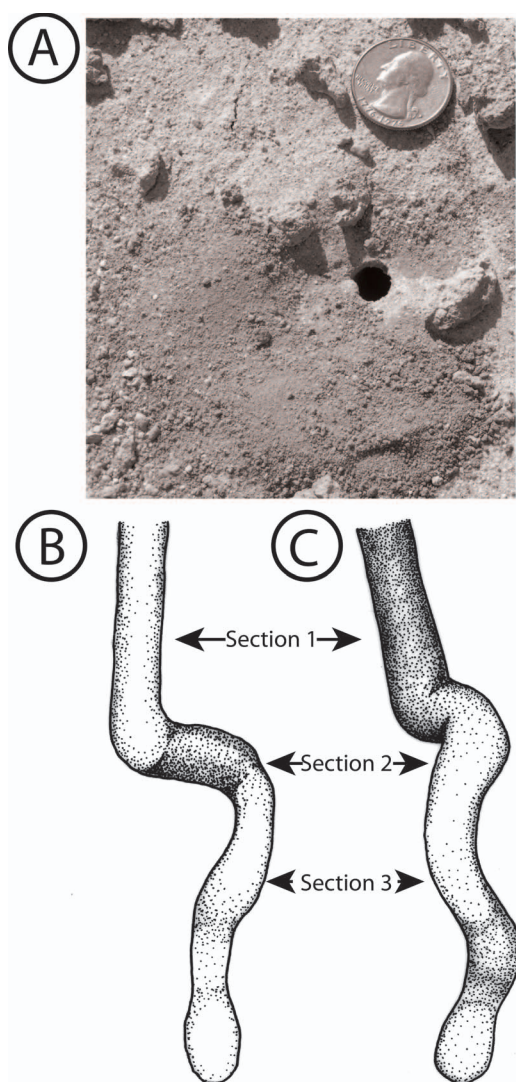


Fig. 2. Nest of *Anthophora porterae*: A, entrance hole; B, architecture of nest; C, 90° rotation of the same nest.

An. porterae depresses the keel of the flower and inserts her entire head and the anterior portion of the mesosoma into the flower to access the pollen and nectar. The pollen analysis shows that pollen was distributed across the body of the bees, including the head and face.

Nest Architecture and Nesting Biology

We located one nesting aggregation of *An. porterae* and excavated 3 nests. This nest aggregation was located in dry sandy/loamy soils approximately 150 m from the nearest

As. phoenix population. The upper 3–4 mm of soils was compacted and very hard, but the soil was much looser below this surface crust. The nest aggregation was surrounded by a shallow, dry streambed, which made the area where the aggregation was located appear uplifted. The nest aggregation consisted of 20 entrance holes, though we limited the number of excavated nests to 3 to preserve as many of the next generation's potential pollinators as possible. The entrance holes were approximately 1 cm in diameter (Fig. 2a). The nests consisted of 3 distinct sections (Fig. 2b). The first section included the entrance hole and a length of the nest that was approximately 7 cm long and oriented nearly vertical. The second section of the nest is much shorter (approximately 3 cm) and oriented approximately 90° relative to the entrance tube and almost parallel with the soil surface. The third section was oriented nearly similarly to the first and was approximately 9 cm long. This portion of the tube was not straight, though how the nest curves varied between nests, and we assumed the curvature was partially determined by soil consistency.

Pollen Analysis

Our analysis of the pollen collected from *Ap. mellifera* showed that 94% of the pollen was from *As. phoenix* and the remaining 6% from an unidentified plant. The average pollen load (pollen located across the body) consisted of 642 ($n = 2$) pollen grains. These data, however, were collected from the 2 bees that we observed visiting *As. phoenix*. Our analysis of the pollen collected from *An. porterae* showed that approximately 63% of the pollen was from *As. phoenix*. The remaining 37% was from an unknown *Cryptantha* sp. This average, however, was made across bees collected throughout the season. The pollen loads early in the season consisted almost exclusively of *As. phoenix* pollen; as the season progressed, *Cryptantha* pollen became a more significant part of the pollen load. We can anecdotally report that this shift in the relative amounts of *As. phoenix* and *Cryptantha* corresponded loosely to the floral maturation of these plants. The average pollen load of *An. porterae* consisted of 2412 ($n = 16$) pollen grains.

We quantified the amount of pollen in 3 nest provisions. The mean pollen count for all 3 provisions was 8,485,860 pollen grains

(provision 1 = 8,869,400 pollen grains, provision 2 = 10,381,430 pollen grains; and provision 3 = 6,206,750 pollen grains). Therefore, data suggest that, for each provision, *An. porterae* will need to make approximately 3500 foraging trips.

DISCUSSION

Astragalus phoenix occurs only in the Mojave Desert wetlands of the Ash Meadows National Wildlife Refuge. Prior to this study, little was known about the breeding biology of *As. phoenix* or the insects that visit it. We show that *As. phoenix* fails to produce fruit in the absence of pollinators. We also show that there are 2 insects that may provide pollination services to this plant: *Apis mellifera* and *Anthophora porterae*. If, however, *Ap. mellifera* does contribute to the reproductive success of *As. phoenix*, its contributions are rare and perhaps negligible. When interacting with the flower, *Ap. mellifera* orients her body to the side of the corolla and rarely penetrates into the flower as deeply as *An. porterae*. This posture is similar to that exhibited by *Ap. mellifera* when engaging in nectar robbing on other *Astragalus* species (Richards 1987), though we saw no evidence of nectar robbing in the limited number of *Ap. mellifera* we observed on the flowers of *As. phoenix*. The pollen analysis showed that *Ap. mellifera* collects a substantial amount of pollen from *As. phoenix* and that *Ap. mellifera* shows a high degree of fidelity to *As. phoenix*. This apparent fidelity might be misleading, however, because the bees whose pollen loads we analyzed were collected from the flowers of *As. phoenix*. These bees may not have visited other plant species prior to arriving at *As. phoenix*, but we do not know if they collected pollen from other species after leaving *As. phoenix*. We also do not know which plant species these bees visited during previous foraging trips. Finally, when we made these observations, there were very few alternative sources of forage available, so we do not know how promiscuous *Ap. mellifera* would be in the presence of alternative sources of forage.

Alternatively, *Anthophora porterae* may contribute significantly to the reproductive success of *As. phoenix*. When interacting with the flowers of *As. phoenix*, *An. porterae* inserts her entire head and the anterior portion of

her mesosoma into the flower and then pulls the pollen with her forelimbs across the ventral surface of her body. Consequently, *An. porterae* has a substantial amount of pollen located on her face, which may contact the stigma of the flowers and the hairs on the ventral surface of the mesosoma. Additionally, *An. porterae* may visit many *As. phoenix* plants and many flowers on the same plant within a single foraging trip. The pollen analysis showed that the floral fidelity of *An. porterae* to *As. phoenix* decreased over the course of the season, with the greatest fidelity early in the season and the lowest degree of fidelity when the flowers of *As. phoenix* begin to senesce. Over the course of this 2-year study, however, we did not observe *An. porterae* visiting *Cryptantha* flowers that coincidentally occurred with *As. phoenix*. Analysis of pollen load showed that *An. porterae* will visit both plant species during the same foraging trip. Combined with our observations, these data suggest that *An. porterae* shows flower fidelity within a patch of flowers, though not necessarily flower fidelity between patches.

We fortuitously located one nest aggregation. Like many other bees, *An. porterae* excavates nests in loose soils that are at least partially sand (Cane 1991). The aggregation that we studied was located on an area of soil that was slightly raised and that, presumably, is not frequently inundated with water. Excavation yielded a unique architecture of the nests of *An. porterae*. Instead of excavating a straight tube, *An. porterae* excavates a tube that turns abruptly before continuing down into the soil (Fig. 2). It is unclear what function this chamber serves. It may be where *An. porterae* "rests" overnight or during inclement weather. This chamber may also be important for defense against inundation with water or invasion from parasites.

The results of our study show that at least 2 insects visit the rare plant *Astragalus porterae*, but that it receives pollination services almost entirely from *Anthophora porterae*. This seems a unique scenario for both *As. phoenix*, which belongs to a genus that is known to support a broad range of pollinating insects, and *An. porterae*, which is known to visit a wide range of plants. Our study shows that the pollen provisions consist primarily of *As. phoenix* and *Cryptantha* pollen. Moreover, it appears as though *As. phoenix* is xenogamous. If this is

true, the health of the population of *An. porterae* will significantly influence the long term viability of *As. phoenix* populations.

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