UNIVERSITY OF CALIFORNIA, SANTA CRUZ

NECTAR BY NIGHT: NECTAR PATTERNS OF A TRIOECIOUS BAT POLLINATED CACTUS IN BAJA CALIFORNIA SUR, MEXICO

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ABSTRACT: The Cardón cactus (*Pachycereus pringlei)* is a columnar member of the Family Cactacea, growing ubiquitously along the peninsula of Baja California. It produces large, white night-blooming flowers from April - June, on plants of three sexes (trioecy): female (producing ovules but no pollen), male (producing pollen but no ovules), and hermaphrodite (producing both ovules and pollen). The flowers produce an abundance of nectar and attract the Lesser long-nosed bat (*Leptonycteris yerbabuenae)* during its breeding months. *L. yerbabuenae* is a migratory nectar feeding bat, endangered in the United States and an obligate pollinator of the Cardón cactus. I studied the nectar secretion schedules of female and hermaphrodite Cardón cactus in response to simulated bat visitation. I found a difference in nectar secretion between the sexes with females producing significantly more nectar, and a response to the nectar production after repeated removal of nectar, which could indicate that early foraging bats are more successful in areas of high bat activity. This project was made possible by the Norris Center for Natural History Grant.

KEYWORDS: Baja California, trioecious, nectar, Cactaceae, bats, desert

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Student Signature	*Date*

Background

<u>Study species: The Cardón Cactus (*Pachycereus pringlei)*</u>

The Cardón Cactus (*Pachycereus pringlei*), is a columnar cactus that grows along the Baja Peninsula. It grows up to 11 meters in height. typically has many branches with 11 - 15 accordian-like ribs, lined on the ridges with spines (Britton & Rose 1909). To photosynthesize, it uses CAM, temporally separating the fixation of CO_2 and the Calvin Cycle to minimize water-loss by opening stomata only at night (Nobel 2002). CO_2 uptake peaks are at night, between 2100 and 0600 hours, and the photosynthetic rates 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, when temperatures are lowest. Titratable acidity, indicative of CAM activity, increases from December to June, implying increased CAM activity during dryer months (Franco-Vizcaíno et al. 1990).

The flowers of plants of Cactaceae emerge from areoles, specialized shoots that possess nodes (Anderson, 2001). *Pachycereus pringlei* is one of two species in Cactaceae that is trioecious, with relatively high population frequencies of female (no pollen, ovules), male (no ovules, pollen), and hermaphroditic (both pollen and ovules) flowers (Fleming et al., 1994). There is geographic variation in the breeding system along the range, with different proportions of the three sexes in different populations. This has been suggested to be correlated with maternity roosts of *Leptonycteris yerbabuenae*, because monoecious plants are more dependent on pollinators (Fleming et al., 1998). *P. pringlei* flowers develop at the top 2 meters of the stems from April - June in funnelform or bell shapes, 6-8 cm long. The petals are fleshy and white, as is typical of bat pollinated flowers, and covered in brown hairs, growing from the pericarpels and floral tubes around the inferior ovary, which contains many ovules. The flower has a single white style, terminating in many stigma and many white stamen, with small anthers (Britton & Rose, 1909).

<u>Nectar</u>

Floral nectaries function to reward animals for providing the mobility of pollen dispersal that plants alone lack (Pacini & Nicolson, 2007). The composition of nectar is similar in most plants: an aromatic mixture of water (which may be important in arid climates in particular), ions, carbohydrates, amino acids, and proteins (Raguso, 2004).

Nectar is produced in one of two ways: from stored starch within the nectary (which is necessary in flowers that are open at night) or directly from photosynthesis (Pacini et al. 2003). Benefits to nectar secretion by stored starch include a high production and sugar concentration rate, and nectar production independent of climatic conditions, ie. sunlight. However, this also means the nectaries are less immediately respondent to their outside conditions (Pacini & Nepi, 2007). In measuring nectary activity, the standing crop (the quantity of nectar in a flower at a particular time) and the secretion rate must be determined (Corbet, 2003). The standing crop is dependent on various factors, including the secretion rate (rate of change of solute content of nectar), reabsorption (rate of solute loss from unvisited flowers or in rare cases pollinated flowers), and removal (the amount of nectar removed by pollinators) (Burquez & Corbet, 1991). A flower will benefit from keeping its nectar volume as low as possible, reducing its own costs while simultaneously attracting enough visitors (Harder et al. 2001). On the other hand, a pollinator's goal is to visit as few flowers as possible, and to find flowers with the greatest volume of nectar. Specialized flowers will produce the amount of nectar specific to their mutualists (Willmer, 2003). Finally, a flower can also resorb nectar back into its nectary, to either reduce its water and carbon costs after a failure in pollination or in the event of successful pollination.

Nectaries of *Pachycereus pringlei*

The floral nectaries of *P. pringlei* are situated deep within the corolla tube, at the base of the stamens. This structure, along with a deep corolla and a relatively large amount of nectar produced: (1.7 ml at peak: Fleming et al. 1996) are all adaptations to slow the movement of air and minimize evaporation in an arid climate. The densely bushy stamen could also contribute to the protection of nectar (Scobell & Scott, 2002). The peak nectar secretion rate in *P. pringlei* has been found to be between the hours of 20:00 and 24:00, with a peak sucrose concentration (a disaccharide of glucose and fructose) at 28%, two hours after flower opening (Fleming et al., 1996). As a plant producing nectar from stored starch supply in a CAM system and not directly from photosynthesis, the production rate and sucrose content are likely to be high (Pacini et al., 2013). However, in the high temperature and water variability of a desert climate year to year the nectar production schedules and content are likely to vary (Fleming, 1994).

The Pollinators

The visitation of bats to flowers, either as obligate or facultative mutualists, is a common occurrence in the Neotropical ecozone and known as chiropterophily (Wilmer 2003). As nocturnal flying mammals and some of the largest pollinators, bats are associated with high reward flowers, including agaves and cacti of North American deserts. The characteristics of obligate nectar feeding bats are a long tongue and snout to reach deep into the nectaries and fine hair to collect, carry, and deposit pollen.

The lesser long-nosed bat (*Leptonycteris yerbabuenae*) is a migratory nectar feeding bat, native to the arid lands of North America and an "obligate cactophile" of *P. pringlei* (Arizmendi et al. 2002). With maternity roosts along the Baja California peninsula, *L. yerbabuenae* depends on the nectar of the cardón in April and May during the breeding season. The pallid bat (*Antrozous pallidus)* is a primarily insectivorous bat and a facultative pollinator of the cardón (Frick et al. 2009). With different natural histories and diets, the nectar feeding tactics of the two species vary: the lesser long-nosed is seen hovering gracefully alongside flowers and quickly tonguing the nectar out, while the pallid bat, with a short snout and tongue, uses its forearms grab hold of the flower and bury its face deep inside the corolla (Frick et. al 2009). *L. yerbabuenae* has been found to feed throughout the night, from just after sunset to before sunrise, regardless of other cactophile activity. In times and areas of high *L. yerbabuenae* activity, *A. pallidus* displays plasticity in its foraging habits by feeding on Cardón nectar just after sunset and not throughout the night, as it does when *L. yerbabuenae* is not present (Chavez et. al 2016). As the lesser long-nosed bat populations are at risk in the Baja California peninsula and endangered in the United States (Cole & Wilson, 2006), their resource availability is of particular interest during breeding season (April - June). I am interested in investigating the relationship, if any exists, between the nectar secretion schedule of the Cardón and bat visitation by *L. yerbabuenae*, and if there is a difference between responses by plant sex.

Questions

Is there a difference in nectar secretion between female and hermaphroditic plants?

Does the amount of available cardón nectar respond to nectar removal?
Do nectaries of female and hermaphroditic flowers respond to being pollinated by changing nectar output?

Methods and Site

The Cardón dominate the southern half (Baja California Sur), which is considered a neotropic ecozone of desert mountains. Precipitation varies considerably both yearly and by region, however the southern two-thirds of the peninsula are classified as desert with less than 25cm of rain per year. Loreto receives 19.2 cm on average annually, with most storms in the late summer and early fall. The Gulf Coast Desert Subregion, which extends in a narrow strip along the east coast of the southern half of the peninsula, is dominated by tree species Jatropha (Euphorbiaceae) and Torote *(Bursera microphylla)*. Common bushes and cactus species include Candelillo (*Euphorbia cerifera*), Cholla (*Cylindropuntia* sp.), Creosote bush (*Larrea tridentata)*, Palo Blanco (*Mariosousa willardiana*), and Ocotillo (*Fouquieria splendens*). This region falls east of the Sierra de Guadalupe and Sierra la Giganta ranges, which rise from sea level to over 1500 meters (Roberts, 1989).

My work was conducted 6 km southeast of Loreto, BCS at (25°57'21.2"N 111°22'39.3"W) on eight nights between April 13th and 25th. The closest *Leptonycteris yerbabuenae* roost to the site is on Isla Carmen, 20 km east of Loreto. The cave is a maternity roost, occupied by pregnant females, new mothers, pre-volant, and volant juveniles from April to June. Plants were chosen based on number of open flowers, sex, and accessibility by ladder. No male plants grew in the area, and were thus discounted from the study due to lack of accessibility. Nectar was taken from 168 flowers from 26 individuals. Flowers were generally positioned on the south-facing side of the plant, but some were sampled growing directly from the top, and rarely from the north-facing side. Hermaphroditic and female flowers were bagged with bridal veil netting and rubber bands before opening at 20:00, and nectar samples were taken with a 3 ml syringe and needle every two hours from 21:00 to 5:00. Hand pollination was done at 21:00 with a paintbrush, and nectar was collected every two hours starting at 23:00. To test for the effects of repeated nectar removal on nectar production, I used two different techniques. The first was to remove nectar every two hours from the same flowers (totaling five collections) simulating repeated bat visitations and replicating Fleming et. al 1996. The second method was to remove nectar at 21:00, and then remove nectar just once more every two hours from different flowers, simulating a single visit early in the night. With the exception of the repeated nectar removals, nectar was taken from each flower just once to eliminate confounding "repeated visit" effects on nectaries. Nectar was not replaced when removed. Hermaphroditic flowers tended to secrete so much nectar by the end of the night that the pollen created a slide for it to spill out and make collection impossible. I created string slings to prop up the flowers with thumbtacks and eliminate spillage. On three nights, I set up motion detecting infrared cameras with monitors and watched for *L. yerbabuenae* visitation. After a visitation, I removed nectar from visited flower and an unvisited flower on the same plant to find the amount *L. yerbabuenae* takes.

Results

<u>Flowering Phenology</u>

The Cardón at my study site near Loreto bloomed throughout April, opening shortly after sunset, and closing by 12:00 the following day. The number of arms and blooms on each plant varied widely. In standing crop, both hermaphrodites and females followed the same general pattern.

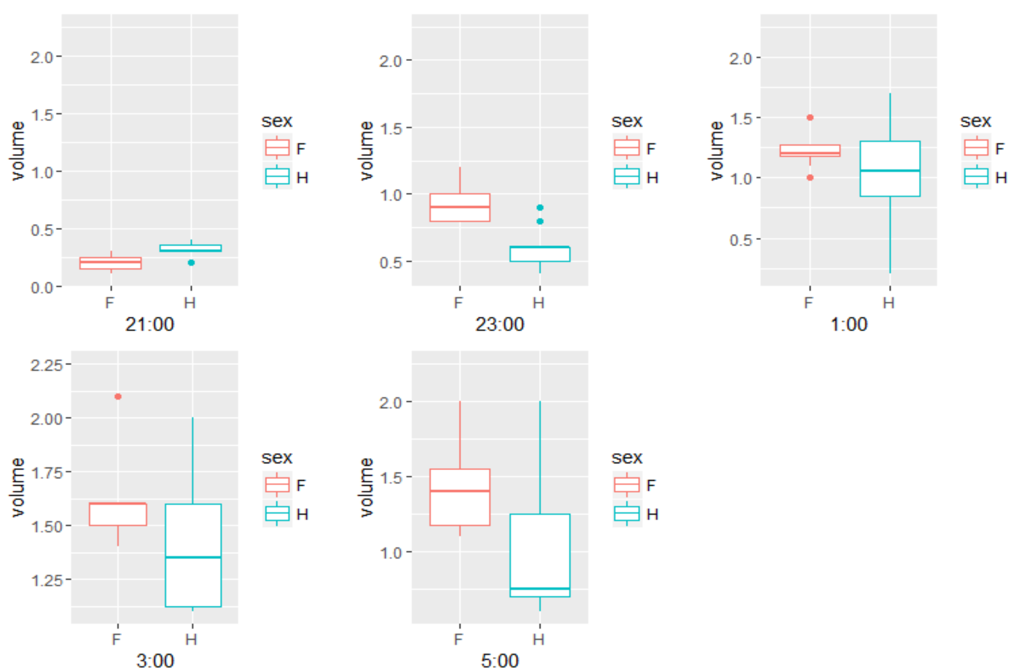

Fig.1: nectar volume (ml) by hour for standing crop

There is a significant difference between females and hermaphroditic nectar production in standing crop: the volume of nectar standing in a flower without visitation or pollination. With the exception of 21:00, females produce more nectar than hermaphrodites (Fig. 1).

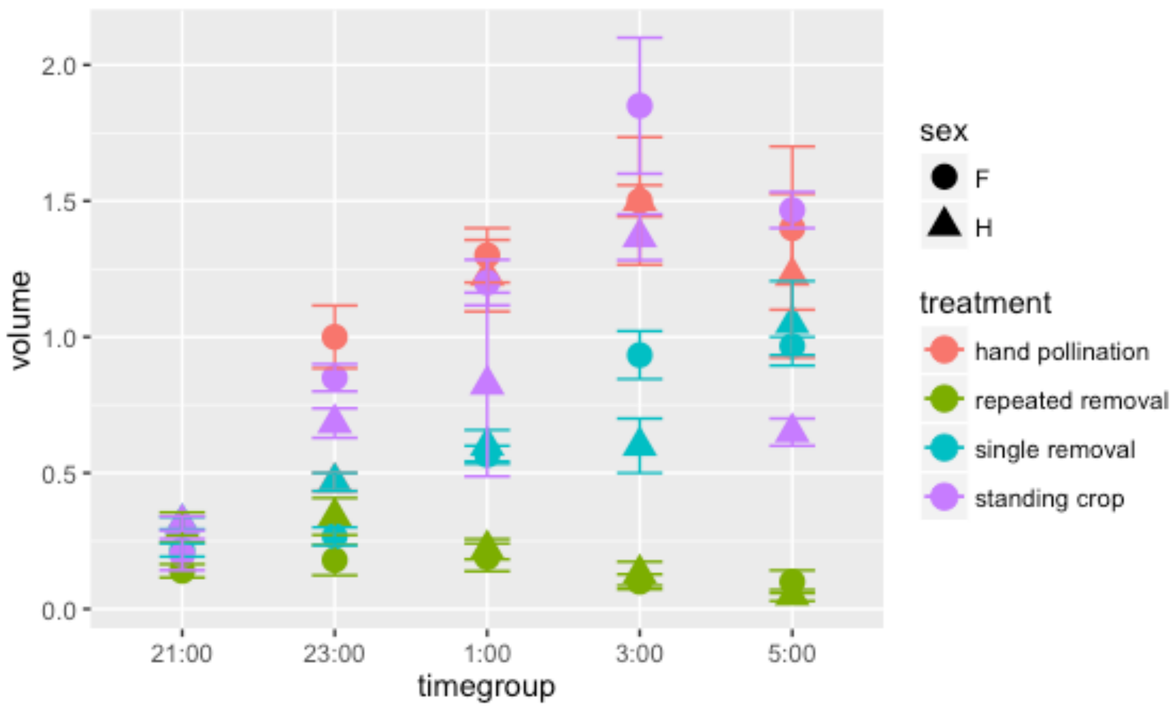

Fig. 2: nectar volume (ml) by time in all four treatments

Pollination has no effect on the volume of nectar produced. The nectar secretion pattern did not change after hand pollination at 21:00, and followed the same secretion pattern as for the standing crop. Single removal, simulating a single bat visitation early in the night, resulted in less nectar throughout the night than for the standing crop but still followed the increasing trend. No difference between plant sexes was found in either of these treatments (Fig. 2).

Repeated removal of nectar, simulating bat visitations throughout the night, results in nectaries of both sexes halting their production around 23:00, and slowly decreasing their production in the following hours. The total amount of nectar secreted from a single flower after repeated removal was not equal to the total secreted in standing crop--indicating that there was a decrease in total nectar production with repeated removal. There was no difference found between the plant sexes in this trend (Fig. 2).

With the use of the cameras, I observed three *L. yerbabuenae* visitations between the hours of 23:00 and 1:00. I found that bats entirely drain flowers of all nectar.

Discussion

Difference between nectar volumes by sex in standing crop

The difference in nectar secretion volume between sexes could be an indication of different resource allocation, "organisms have a limited supply of some critical resource...which they must generally divide between several competing functions, broadly defined as growth, maintenance, and reproduction. These functions are further assumed to be mutually exclusive, so that the allocation to one function necessarily leads to a decrease in the simultaneous allocation of other functions" (Bazzazz et. al 2000). With two sexes of the same species with the same nectar secretion pattern, the difference in volume of secretion becomes significant, and indicative of different resource strategy. Female flowers could be producing more nectar than hermaphroditic flowers because they lack the need for resources allocated to pollen production, and the associated function. Hermaphrodites must distribute limited resources between producing nectar and producing pollen.

Furthermore, the difference in nectar volumes could be a result of nocturnal pollinator abundance hypothesis in relation to trioecy, as studied by Fleming et. al 1998. This hypothesis states that in areas of higher bat visitation, trioecious populations are more likely as male and female plants are more dependent on pollinator visits than hermaphroditic plants, which are self compatible (Fleming et. al 1994). As hermaphroditic flowers can rely on self-fertilization, their volume of nectar could be less important than in female flowers to attract a pollinator and ensure reproductive success. For the pollinators, visitations with varying amounts of nectar reward might encourage foraging for more hours with more visitation in order to fill caloric needs.

Pollination

The lack of change in nectar secretion schedule to pollination in both sexes indicates that the nectaries do not respond to pollination events. As the motives of the flowers are different in terms of pollinator activity, I expected their nectar secretion to show a difference. The goal of female flowers is to accrue a pollination event, and I hypothesized female flowers would halt nectar production after pollination, while hermaphrodites would continue producing nectar, attracting visitors to distribute their pollen throughout the night. However, I found no difference in the nectar secretion schedules between sexes, nor between pollinated flowers and standing crop. As discussed in the introduction, *P. pachycereus* produces nectar from stored starch, instead of directly from photosynthesis. This means the nectar is found in higher volume and sugar concentration than non CAM plants, but also that nectar production is independent of climatic conditions, and possibly non-respondent to a pollination event. With less than 24 hours to be pollinated, the flower might have success in continuing to produce nectar even after a single pollination event, especially in high bat activity areas. The reabsorption of nectar back into nectary might not be a viable strategy because flowers do not have another chance to re-secrete it. Finally, I applied only one hand-pollination event, at 21:00 in my treatment. With bat activity throughout the night, the pollination event per flower could be much greater than once per night, and the nectar secretion may only respond to repeated pollination events.

<u>Repeated removal</u>

Repeated removal of nectar, resulting in nectar production decrease and eventual depletion for both sexes, indicates that repeated visitation to an individual flower decreases the total amount of nectar secreted and amount available. The volume of nectar at the first two removals, 21:00 and 23:00, were not significantly different from the standing crop data. However, at the third removal at 1:00, the volume of nectar was largely different from an unvisited flower, by up to a milliliter. Even more dramatic is the difference in available nectar between repeatedly visited flowers and unvisited flowers at 3:00 and 5:00. By those hours, nectar averages at 0.2 and 0.1ml, respectively, for repeatedly visited flowers, and 1.6 and 1.1 ml (average of sexes) for unvisited flowers.

As *L. yerbabuenae* forage throughout the night, from 21:00 to around 5:00, regardless of other pollinator activity (Chavez et. al 2016), the amount of nectar available must vary hugely between flowers by the end of the night, depending on the population sizes of bats and open flowers. There could be more competition between bats to find flowers with nectar remaining by the end of the night, or longer flights between flowers with nectar.

Larger context

The nectar secretion change in response to repeated removal could provide an explanation for the relative visitation activity of the pollinators, *L. yerbabuenae* and *A. pallidus.* As found by Chavez et al., 2016, *A. pallidus* changes its foraging habits with the presence of high *L. yerbabuenae* activity. Instead of feeding shortly before sunrise, as the *A. pallidus* does when there is no inter-specific competition, the population will concentrate its feeding efforts shortly after sunset before switching to foraging for arthropods for the remainder of the night. This could help guarantee high nectar reward for *A. pallidus*, as they are likely to be first to open blooms and not visiting flowers that have been drained multiple times by *L. yerbabuenae*. This could also mean in times and areas of high *A. pallidus* activity, *L. yerbabuenae* could have more difficult time finding flowers still full of nectar, influencing its success during critical spring breeding months. Future research on resource availability to *L. yerbabuenae* should include the activity of *A. pallidus.*

Future research

To expand this project, I would investigate the nectar secretion patterns of male plants. I would investigate the relative *A. pallidus* and *L. yerbabuenae* activity in the site area, as well as proximity to roosts. I would repeat the pollination treatment to several pollination events throughout the night to perhaps more accurately mimic visitation throughout the night.

Lower bud will open by 21:00

Two bagged buds, will open by 21:00

Open hermaphroditic flower on cactus

Supported hermaphrodite to prevent spill

L to R: two females, two hermaphrodites

Female, no pollen on stamen

Hermaphrodite with pollen

Female (left), Hermaphrodite (right). Both with inferior ovaries and ovules. Nectary located at base of style.

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