

Research

First Recorded Observations of Pollination and Oviposition Behavior in *Tegeticula antithetica* (Lepidoptera: Prodoxidae) Suggest a Functional Basis for Coevolution With Joshua Tree (*Yucca*) Hosts

William S Cole Jr., Alexander S. James, and Christopher Irwin Smith¹

Department of Biology, Willamette University, 900 State St., Salem, Oregon, 97301 OR (wstaddencole@gmail.com; ajames@willamette.edu; csmith@willamette.edu), and ¹Corresponding author, e-mail: csmith@willamette.edu

Subject Editor: Lee Dyer

Received 11 September 2016; Editorial decision 4 February 2017

Abstract

Yucca moths (*Tegeticula* spp.) are the exclusive pollinators of Joshua trees (*Yucca brevifolia* s. l.). The moths actively pollinate the Joshua tree flowers and lay their eggs in the style. Recent studies have revealed that the plants commonly known as Joshua trees include two distinct, sister-species of plant: *Yucca brevifolia* Engelm. and *Yucca jaegeriana* McKelvey, each pollinated by two sister-species of yucca moth *Tegeticula synthetica* Riley and *Tegeticula antithetica* Pellmyr, respectively. A number of studies have argued that the moths have coevolved with their hosts, producing a pattern of phenotype matching between moth ovipositor length and floral style length. However, the only known descriptions of yucca moth pollination and oviposition behavior on Joshua trees are observations of *T. synthetica* made in 1893. The behavior of *T. antithetica* has never been observed before. We produced the first video recordings of the behavior of *T. antithetica*, and measured the points of oviposition and egg placement within the floral style. We found a number of differences between the behaviors of *T. antithetica* and *T. synthetica*, which appear to be a consequence of differences in floral morphology between *Y. jaegeriana* and *Y. brevifolia*. We also found that variation in floral style length strongly influences the placement of eggs within the flower, which may explain patterns of phenotype matching described previously. However, unlike in other yucca moths, we find that the mode of oviposition is unlikely to wound the floral ovules, and thus that oviposition by *T. antithetica* is unlikely to prompt floral abscission.

Key words: behavior, coevolution, oviposition, pollination, yucca-moth

Joshua trees (*Yucca brevifolia* Engelm. and *Yucca jaegeriana* McKelvey) are woody monocots endemic to the Mojave Desert of North America. Growing up to 10 m in height, Joshua trees stand out among the low shrubs and cacti that typify the Mojave Desert landscape. Their long, bent branches, tipped with short rosettes of spiny leaves, have inspired strong reactions in many visitors to the American West. The early American explorer John C. Fremont described them as “The most repulsive tree in the vegetable kingdom,” (McKelvey 1938, Rowlands 1978), whereas the 19th-century botanist, William Trelease, considered flowering Joshua trees to be the “most attractive of all the yuccas” (Trelease 1893). Finally, like most yuccas, Joshua trees produce no nectar to attract pollinators, and instead are pollinated exclusively by yucca moths (Lepidoptera: Prodoxidae) (Trelease 1893, Pellmyr and Segraves 2003, Smith et al. 2008). The moths in turn reproduce solely by laying their eggs in yucca flowers. The high levels of mutual specificity have made Joshua trees and yucca moths a model system for studies of plant and pollinator coevolution.

Pollination and oviposition behavior has been extensively studied in many yucca moths (see, for example: Riley 1892, Addicott and Tyre 1995, and Huth and Pellmyr 1999). Female moths collect pollen using uniquely derived, tentacle-like appendages (“tentacles” hereafter) that arise from the maxillary palps (Pellmyr 1999). They drag their tentacles across the anthers to remove pollen, which they then ball up and store between the tentacles and the underside of their heads (Pellmyr 2003). The moths next move to another flower (sometimes on the same plant (Marr et al. 2000)) and deposit a number of eggs into the developing flower using their hardened, partially-fused posterior apophyses (“ovipositor” hereafter). The moths typically lay their eggs in contact with the developing ovules, though the location and mode of oviposition varies across species (Pellmyr 2003); moths associated with Joshua trees oviposit into the style (Trelease 1893). Finally, the moths actively pollinate the plant using their tentacles to press pollen onto the stigma (Pellmyr 2003).

Although yucca moth behavior follows this general description across the group, there are differences in the details of oviposition

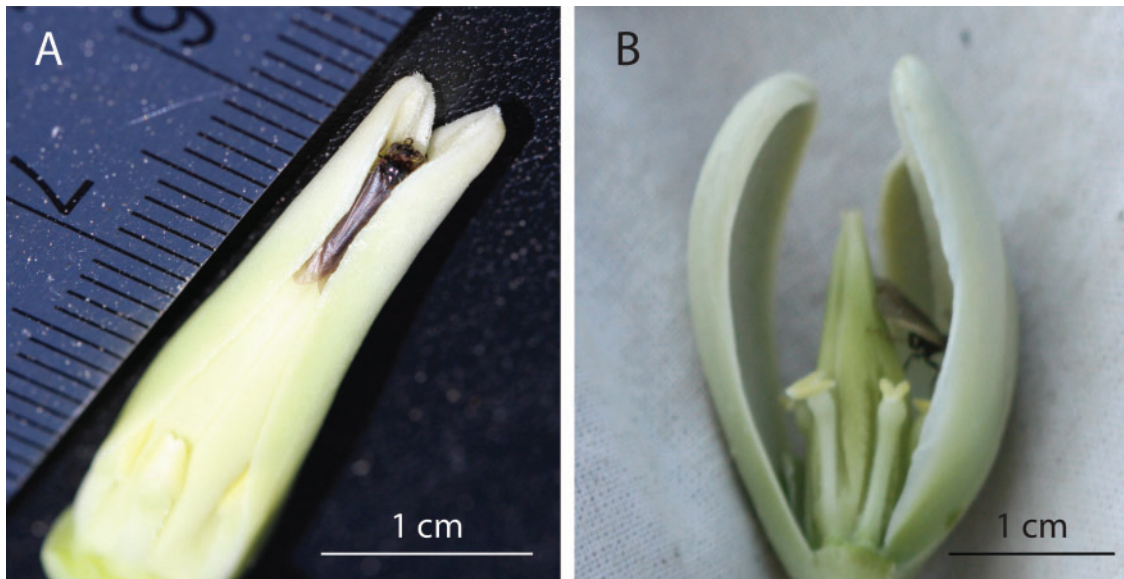


Fig. 1. Figure comparing *Y. jaegeriana* (A) and *Y. brevifolia* (B) flowers used for oviposition by yucca moths. In both photos, outer sepals have been removed to permit a view inside the flower. There are marked differences in the maturity of the flowers, and especially the extent of petal separation from the pistil. Note the two images differ slightly in scale; in panel B, the moth is attempting to escape, but its ovipositor has become caught inside the style.

and pollination in particular species, and there have been very few observations of pollination behavior in moths associated with Joshua trees. Indeed, we are not aware of any documented observations since Trelease's original (1893) description of pollination behavior in *Tegeticula synthetica* Riley (See Supp. Appendix A [online only]). The rarity of direct observations of Joshua tree pollination may be due in part to the structure and development of the flowers. Unlike other species of *Yucca*, Joshua tree flowers are almost completely closed during their receptive stage. The flowers are borne in tightly-packed inflorescences, with up to 200 flowers on a ~30-cm-long peduncle, so the petals are often pressed together by other nearby flowers. In addition, the flowers are receptive to pollination very early in development when the buds have barely begun to open and pistils are almost entirely obscured by the tightly closed petals (Trelease 1893). As a result, it is extremely difficult to observe the behavior of the Joshua tree pollinators without disturbing them (Trelease 1893).

Recent discoveries about the pollination biology of Joshua trees highlight the need for additional studies of yucca moth behavior. Pellmyr and Segraves (2003) reported that Joshua trees are associated with two distinct species of yucca moth: the previously recognized *T. synthetica* and a newly described, cryptic sister species, *Tegeticula antithetica* Pellmyr. The two moths carry deeply divergent mitochondrial haplotypes (Pellmyr and Segraves 2003), and subsequent work has confirmed that the species are genetically distinct across both the nuclear and mitochondrial genomes (Smith et al. 2008, 2009). Compared with its sister species, *T. antithetica* is smaller overall, has a smaller ovipositor, and differs in wing patterning and both male and female genital morphology (Pellmyr and Segraves 2003). In addition, several lines of evidence suggest that trees pollinated by each moth may be distinct species. Based on morphological differences between the two, Lenz (2007) recommended that trees pollinated by *T. antithetica* be recognized as a distinct species, *Y. jaegeriana*. *Yucca brevifolia* is pollinated by *T. synthetica*. Subsequent genetic work suggests that the two tree species are strongly genetically differentiated (Royer et al. 2016). However, there have been no published observations of pollination behavior

or oviposition in *T. antithetica*. Trelease (1893) based his description solely on observations made in Hesperia, CA, an area where only *T. synthetica* and *Y. brevifolia* occur.

A number of lines of evidence suggest that the morphological differences between *T. antithetica* and *T. synthetica* may reflect coevolution with their host plants. Although Joshua trees pollinated by each moth differ in a number of morphological features (Royer et al. 2016, Lenz 2007, Godsoe et al. 2008), statistically they are much more divergent in floral morphology than they are in vegetative morphology (Godsoe et al. 2008). Trees pollinated by *T. synthetica* have nearly spherical flowers with shorter, wider petals that curve over the tip of the pistils (Fig. 1), whereas trees pollinated by *T. antithetica* have elongate flowers with narrow petals that wrap around the pistil forming a corolla tube (Lenz 2007). In addition, trees pollinated by *T. synthetica* have a significantly longer style than those pollinated by *T. antithetica* (Godsoe et al. 2008). The style lengths of *Y. brevifolia* and *Y. jaegeriana* match the body lengths of *T. synthetica* and *T. antithetica* (cf. Pellmyr and Segraves 2003, Godsoe et al. 2008), and variation in style length across Joshua tree populations is correlated with differences in moth ovipositor length and body size (Yoder et al. 2013). Although this correlation is strongly driven by differences between species (Godsoe et al. 2010), weaker correlations exist within species, and trait mismatching appears to be the result of dispersal and gene flow between populations (Yoder et al. 2013).

Observational studies also suggest that natural selection may favor phenotype matching. At one site in central Nevada, Tikaboo Valley, both moth species and their respective hosts occur in sympatry. Here, both moths visit both species of Joshua tree, but show marked decreases in reproductive success on their nonpreferred hosts (Smith et al. 2009). *Tegeticula antithetica* oviposits into the flowers of *Y. brevifolia*, but produces significantly fewer surviving larvae per clutch than it does when ovipositing onto its preferred host (Smith et al. 2009). Smith et al. (2009) speculated the relatively shorter ovipositors of *T. antithetica* might cause the moths to deposit their eggs further from the ovules when ovipositing on the relatively longer styles of *Y. brevifolia*. As a result, the larvae might have lower survival. In contrast, although *T. synthetica* visits *Y. jaegeriana*, it does

not appear to successfully rear larvae from *Y. jaegeriana*'s flowers. Smith et al (2009) argued that this observation might also be a consequence of trait mismatching. Previous studies of *Tegeticula yuccasella*'s oviposition behavior on *Yucca filamentosa* showed that wounding of ovules during oviposition can prompt floral abscission (Marr and Pellmyr 2003). Therefore, the absence of *T. synthetica* larvae emerging from *Y. jaegeriana* fruits could indicate that *T. synthetica*'s longer ovipositor is more likely to cause ovule wounding when ovipositing into flowers with short styles. This in turn might prompt floral abscission and the death of the developing larvae. Smith et al (2009) therefore argued that moths experience selection for ovipositors that are neither too long nor too short for their local hosts.

Although the existing data are consistent with such selection for trait matching, alternative explanations are also possible. The observed pattern of trait matching could be the product of abiotic selection (for example, environmental differences across the trees' range might favor both larger body size in the moths and thicker ovary walls in the plants; Nuissner et al. 2010). The pattern of trait matching could also be simply a chance outcome of evolutionary history, given that the pattern is largely driven by differences between species (Godsoe et al. 2010). Likewise, the lower reproductive success of moths ovipositing on their nonnative hosts could be the result of other differences between *Y. brevifolia* and *Y. jaegeriana* that covary with style length.

The functional relationship between moth and floral traits might become clearer with a better understanding of the behaviors of the moths. For example, more information about how and where female moths insert their ovipositors could reveal whether moths are likely to cause ovule wounding during oviposition. Similarly, understanding precisely where the moths place their eggs might reveal whether variation in style length is likely to affect larval survival. Finally, identifying differences in behavior between *T. synthetica* and *T. antithetica* might reveal additional potential coadaptations with their hosts. Indeed, studies of *Greya* moths on *Lithophragma* showed that moths from different populations have evolved different oviposition behaviors in response to local differences in floral morphology (Thompson et al. 2013).

Here, we present the first description of pollination and oviposition behaviors of *T. antithetica* on its native host, *Y. jaegeriana*. We compare these behaviors with Trelease's (1893) written observations of *T. synthetica* on *Y. brevifolia*, examine the effects of variation in style length on moth oviposition, and discuss the potential impacts on moth fitness and floral abscission rates.

Materials and Methods

Study Location

Field observations were conducted on 21–26 March 2015, and 23–24 March 2016, in Tikaboo Valley, in Lincoln County, NV. Both *T. synthetica* and *T. antithetica* occur in sympatry here, along with their respective hosts and hybrids between them. Thus, to avoid uncertainties about tree species identity, we used existing hybrid zone maps (Royer et al. 2016) and data about the distribution of the two moth species (C. I. S. unpublished data) to identify locally allopatric areas where only *Y. jaegeriana* and *T. antithetica* occur.

Behavioral Observations

As previous work (C. I. S., personal observations) suggests that *T. antithetica* is primarily day-active, we conducted observations between 9 a.m. and 6 p.m. We searched for moths on trees with a large number of open inflorescences, noting the moths' overall

pattern of behavior and the condition of flowers selected for oviposition. We identified moths that were engaged in oviposition and pollination by searching for flowers in which the top of a moth's head was visible at the opening of the corolla tube. We then removed the flower from the inflorescence using a pocketknife to cut through the pedicel, and peeled off the three outer sepals and one of the inner petals, creating a window into the corolla tube. Although we did not make detailed observations of moths in unmanipulated flowers, handling the flowers and removing petals in general did not seem to affect moth behavior. However, removing more than one inner three petals would prompt the moth to leave the flower.

We then placed the flower on its side on a microscope stand and recorded the moth's movements and behavior using an AM413TA Dino-Lite Pro digital microscope and DinoCapture 2.0 software running on a Dell tablet PC. Recordings were completed either in the shade or in the back of a parked car. We recorded each moth's behavior continuously until the moth either left the flower or died. We completed observations of 29 moths, and obtained video recordings of 14 of these. We recorded pollination behavior from seven moths. Each moth typically completed multiple pollination events per flower, so we were able to record a total of 18 pollination events (an average of 2.54 ± 1.51 pollination events per moth).

We measured the length of each pollination event, and the time between pollination events (i.e., time spent ovipositing and any period of inactivity), and calculated the means. Two of the 18 recorded events were omitted from calculations, one because recording started after the event had already begun, and the other because the moth's tentacles did not reach the stigma. We used linear regression to determine whether the length of pollination events affected the length of time between pollination events; the duration of pollination events was log-transformed prior to analysis to produce a normal distribution.

Floral Measurements

After completing behavioral observations, we used a pocketknife to split each pistil in half along one groove between the carpels, exposing the ovules, the stylar canal, and any eggs that the moth had deposited. We photographed 26 dissected pistils using a Dino-Lite microscope as above, with a metric ruler included for reference. When present, the eggs were clearly visible in photos, so no additional staining or treatment was necessary.

We took measurements from the resulting images using ImageJ v. 1.48v digital measuring software. For all flowers, we measured the length of the style, which we defined as the distance from the tip of the stigma to the first ovules, following Godsoe et al (2008). To evaluate how variation in style length influences egg placement, we determined the location of the eggs relative to the stigma and the ovules. We counted the eggs (if present), and measured the following: the distance from the stigma to the top egg (i.e., the egg sitting at the highest point in the stylar canal), the distance from the top egg to the ovules, the distance from the bottom egg (i.e., the egg at the lowest point in the stylar canal) to the stigma, and the distance from the bottom egg to the ovules. We also calculated the distance from the middle of the egg mass (the midpoint between the top and bottom eggs) to both the stigma and the ovules (These distances represent the range of distances that a first-instar larva would have to crawl before reaching the ovules on which they will ultimately feed, and thus may affect rates of larval survival). Last, for the 12 flowers in which there was a discernible oviposition point (a clearly visible hole in the groove between carpels), we photographed the exterior

of the flower and then used ImageJ to measure the distance from the top of the stigma to the ovipositor insertion point.

To evaluate the impact of style length on where moths insert their ovipositors, we used a linear regression to compare the point of oviposition with style length. In addition, to evaluate the impact of style length on egg placement, we used linear regressions to compare style length with the distance between the ovules and the middle of the egg mass. Similarly, we compared style length with the distance between the stigma and the middle of the egg mass. Prior to completing the linear regressions, all variables were tested for normality using an Anderson–Darling Normality test in the R package *norstest*. All regressions were tested for heteroscedasticity using a Goldfeld–Quandt test implemented in the R package *lmtest*. No tests were significant. All statistical analyses were completed in R v. 3.0.2 (R Core Team 2013).

Results

Overall Pattern of Behavior

Moths were active from the late morning into the late afternoon. Moths crawled rapidly around the outside of inflorescences and crawled into open flowers, where they typically remained for a few seconds up to several minutes, before moving to another flower. The moths appeared to enter any open flower, but were only ever observed pollinating and ovipositing into the very youngest flowers in which the anthers were not yet completely developed. The young flowers used for pollination and oviposition had petals that were still tightly wrapped around the pistil, and the tips of the petals had just begun to curl outwards revealing a small opening into the corolla tube *ca.* 2–3 mm in width (Fig. 1). When they encountered a flower in this condition, female moths entered the corolla tube head-first, but then crawled out, repositioned themselves, and then crawled backward, abdomen-first, into the flower. Moths sometimes remained in these young flowers for over an hour.

Once inside the flower, the moths repeatedly alternated between oviposition and pollination, separated by periods of apparent inactivity. We were unable to observe the moths' first action upon backing into the corolla tube. Pollination was a visibly distinct behavior, and the moths repositioned themselves just prior to, and immediately after pollination. It was not feasible to track a moth's movements after leaving the flower, so it is unclear how often moths moved between flowers on the same plant or moved between plants.

Oviposition Behavior

We observed 14 moths' complete oviposition. Moths positioned themselves with the posterior end of the abdomen aligned with the stigma, using their metathoracic legs to brace themselves against the top of the pistil, and their pro- and mesothoracic legs to press against the petals (Fig. 2; [Supplementary Video 1](#) [online only]). While in this position, the moths rhythmically wagged their abdomens in the dorsal–ventral plane. In most cases the floral style obscured our view of the point of oviposition, but in one case we were able to cut a small window into the petal, allowing us to observe the insertion of the ovipositor. The moth used her ovipositor to probe along a groove between carpels, just below the stigma. She then punctured the style wall and pushed her ovipositor into the style using a combination of abdominal movement and extension of the ovipositor. She then retracted her ovipositor from the style wall, leaving the oviduct threaded through the hole in the style. The moth extended and retracted her ovipositor periodically, and rhythmically thrust her abdomen with the oviduct still in the style.



Fig. 2. A female yucca moth (*T. antithetica*) ovipositing on a Joshua tree (*Y. jaegeriana*). Four petals have been removed from the flower to allow a view into the corolla tube. The top of the pistil and the stigma are visible in the lower half of the photo. The moth's metathoracic legs are placed at the top of the pistil, and the tip of the abdomen extends behind the stigma. The moth's tentacles are visible on either side of her proboscis, coiled around a pollen load.

Pollination Behavior

We observed seven moths engaged in pollination. Following oviposition, each moth moved down the corolla tube, aligning her mesothorax with the tip of the stigma. She would then lower her head and uncurl and extended her proboscis, holding it away from the tentacles. Next, the moth uncurled her tentacles and extended them straight down toward the stigma with some pollen usually present on the tentacles, though most of the pollen remained adhered to the underside of the head. She then moved her head up and down, pushing the tentacles into and around the stigma (Fig. 3, [Supplementary Video 2](#) [online only]). During pollination, the moth moved her prothoracic legs up and down rapidly, scraping pollen off the tentacles and compacting it into the stigmatic cavity. Finally, the moth lifted her head, curled the tentacles, and either crawled back to an oviposition position or left the flower.

The average pollination event lasted 52 s ($n = 16$, $SD = 24$ s). The length of time between pollination events varied ($n = 9$, mean = 553 s, $SD = 360$ s). The (log transformed) duration of pollination events was correlated with the time until the next pollination event (linear regression, $df = 1$ and 6 , $R^2 = 0.542$, $F = 9.29$, $P = 0.023$), and was marginally significantly correlated with the length of time



Fig. 3. A female yucca moth (*T. antithetica*) pollinating a Joshua tree (*Y. jaegeriana*). Four petals have been removed from the flower to allow a view into the corolla tube. (A) The moth extends her tentacles with some pollen near the ends toward and into the stigma, with proboscis extended. (B) The moth quickly lowers her head, pushing her tentacles into the stigma, and then raises it again. The moth's forelegs rub against the tentacles throughout the process.

since the previous pollination event (linear regression, $df = 1$ and 7 , $R^2 = 0.3802$, $F = 591$, $P = 0.0454$). That is, longer pollination events were associated with longer periods of time between pollinations.

Floral Measurements

Statistical analyses revealed that variation in style length has a strong impact on moth oviposition (Fig. 4). The placement of the eggs within the style (Table 1) was strongly influenced by style length. The distance from the ovules to the middle of the egg mass was very strongly determined by style length (linear regression, $n = 26$, $df = 1$ and 24 ; $R^2 = 0.54$, $F = 29.83$, $P = 1.3 \times 10^{-5}$), i.e., in flowers with longer styles, moths placed their eggs further from the ovules. The distance from the stigma to the middle of the egg mass was also affected by style length, though with weaker statistical support (linear regression, $n = 26$, $df = 1$ and 24 ; $R^2 = 0.25$, $F = 9.34$, $P = 0.0054$, middle of egg mass). Finally, we found that there was no effect of style length on the point where moths insert their ovipositors (linear regression, $n = 12$, $df = 1$ and 10 ; $R^2 = -0.062$, $F = 0.36$, $P = 0.56$). Thus, moths do not appear to adjust the point at which they insert their ovipositors when interacting with long-styled flowers.

Discussion

We describe the first observations of pollination behavior in *T. antithetica*. The pattern of behavior is broadly similar to what Trelease (1893) described for its sister species, *T. synthetica*. Both species oviposit into the style, standing at the very top of the pistil and inserting the ovipositor just below the stigma. Likewise, both species appear to use the ovipositor to puncture the stylar wall, and then extend the membranous oviduct into the stylar canal. Finally, both species appear to alternate between oviposition and pollination.

However, we also noted several differences in behavior between *T. antithetica* and *T. synthetica* that appear to be due to differences in floral morphology between their hosts. Trelease described *T. synthetica* beginning its oviposition behavior by circling the pistil and then climbing to the top, standing on the pistil to oviposit. However, *Y. jaegeriana*'s petals are wrapped tightly around the pistil during the phase when the moths pollinate them. As a result, *T. antithetica*'s movement is limited by the confines of the corolla tube, preventing the moth from moving below the bottom of the style. Similarly, when transitioning from oviposition to pollination, *T. synthetica* climbs down to the base of the pistil and then back up to the stigma again before beginning pollination (Trelease 1893). In contrast, when transitioning between oviposition and pollination, *T. antithetica* simply moves up and down in the top of the corolla tube, rather than crawling to the base of the pistil.

We noted some differences in pollination behavior, which also appear to be the result of differences in floral morphology. Trelease described *T. synthetica* placing her head even with the stigma and uncurling her tentacles, before drawing them back and forth across the stigma and scraping pollen off the tentacles with the stigmatic notches. There appears to be insufficient space within the *Y. jaegeriana* flower to allow this behavior; rather than holding the tentacles perpendicular to the style and scraping pollen into the stigma, *T. antithetica* held her tentacles parallel to the style and pushed them down into the stigma. *Tegeticula antithetica* moths also occasionally rubbed their tarsi and forelegs against the tentacles and stigma, seemingly trying to scrape pollen off the tentacles.

Comparing the moth's behavior with variation in floral anatomy also suggests a functional basis for coevolution with their Joshua tree hosts. The placement of the eggs within the style was strongly correlated with style length ($R^2 = 0.55$, $P = 1.3 \times 10^{-5}$); that is, when ovipositing into flowers with longer styles, the moths placed their eggs further from the ovules than when ovipositing on short-styled flowers. When ovipositing into flowers with longer styles,

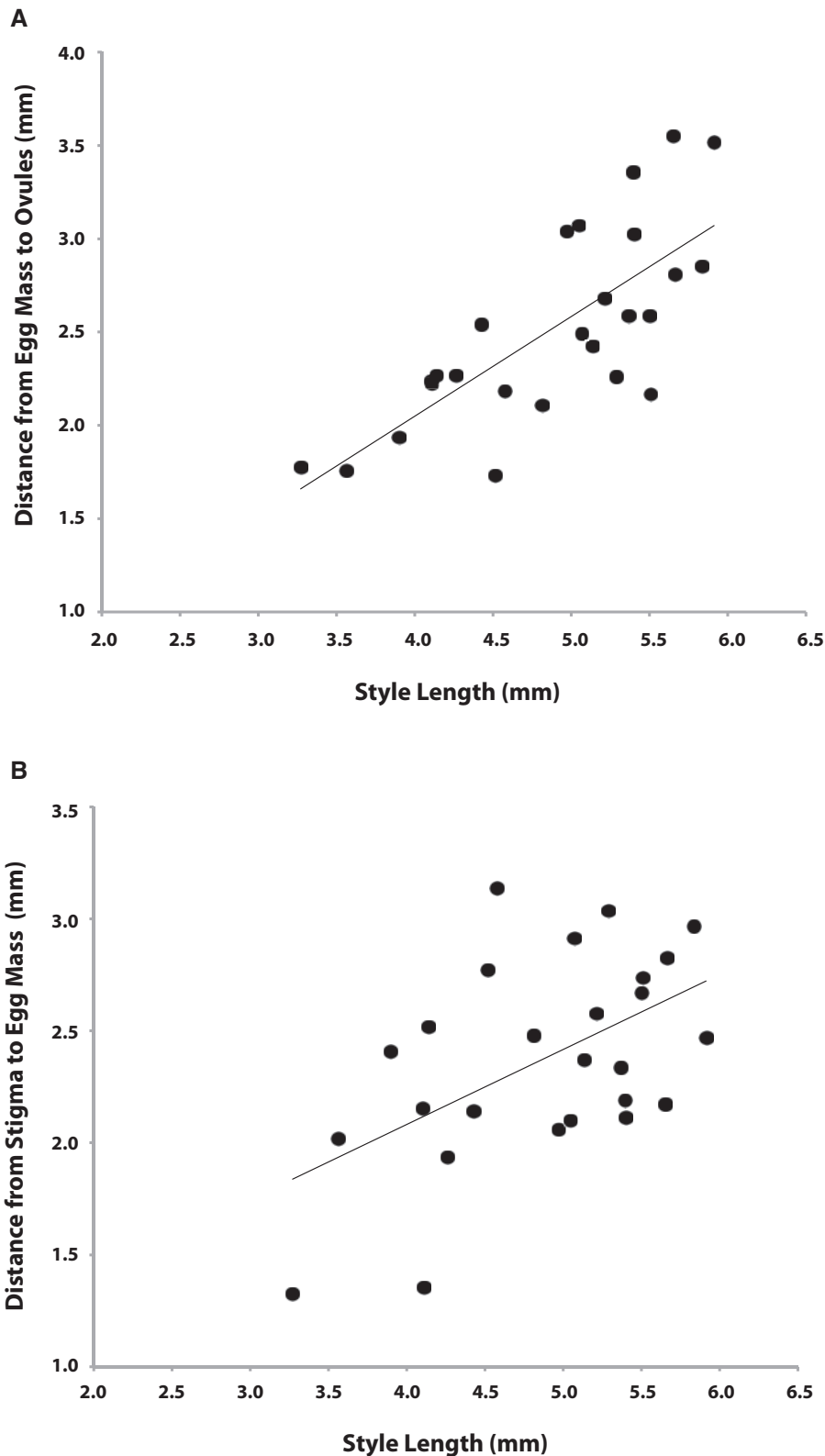


Fig. 4. Effects of style length on egg placement. Increasing style length causes moths to deposit eggs further from the ovules ($R^2 = 0.55$, $P = 1.3 \times 10^{-5}$; Panel **A**), and further from the stigma ($R^2 = 0.25$, $P = 0.0054$; Panel **B**). In both cases, measurements are from the center of the egg mass.

moths appear to partially compensate by placing their eggs further from the stigma ($R^2 = 0.25$, $P = 0.0054$), perhaps by extending the oviduct deeper into the stylar canal. However, eggs are still on average placed further from the ovules in long-styled flowers. The ovules

are the food source that the moths' offspring will use during their larval development; upon hatching the first-instar larvae must crawl down the stylar canal to reach the ovules. If placing the eggs relatively further from the ovules results in lower larval survival, this

Table 1. Flower dissection measurements shown with standard deviations

Flower measurements				
Measurement	Mean	N	SD	95% C. I.
Style length (mm)	4.788	26	0.741	4.54–5.072
Distance from stigma to ovipositor insertion point (mm)	0.817	12	0.205	0.701–0.933
Bottom egg to ovules (mm)	1.674	26	0.669	1.405–1.943
Stigma to top egg (mm)	1.707	26	0.528	1.504–1.91
Distance from middle of egg mass to ovules (mm)	2.514	26	0.518	2.315–2.713
Distance from middle of egg mass to stigma (mm)	2.374	26	0.456	2.199–2.549
No. of eggs	17	26	14	11.62–22.38

effect may explain the observed correlation between floral style length and moth ovipositor length across species and across populations (Yoder et al. 2013).

Previous studies in other yucca species have found that the plants may selectively abort flowers that received poor quality pollination (Huth and Pellmyr 2000), or that experience a large number of oviposition events (Pellmyr and Huth 1994, Wilson and Addicott 1998). This may be a mechanism that maintains the mutualism by preventing overexploitation of the seeds by yucca moths (Pellmyr and Huth 1994). Studies of *Y. filamentosa* suggest that abscission is prompted by wounding of ovules during oviposition (Marr and Pellmyr 2003). However, our observations do not suggest that *T. antithetica* injures the ovules of *Y. jaegeriana* during oviposition. The moths consistently inserted their ovipositors just below the stigma (mean = 0.817 ± 0.205 mm S.D.; *n* = 12 moths), 3.97 mm from the ovules on average (S.D. = 0.662 mm; *n* = 12 moths). In comparison, *T. antithetica*'s ovipositors are typically between 2.20 and 2.25 mm in length (Pellmyr and Segraves 2003). Ovipositing this close to the tip of style means that the moths are very unlikely to contact the ovules with their sclerotized ovipositor. Of course, it is possible that other mechanisms may exist that can prompt floral abscission in *Y. jaegeriana* and that these might prevent overexploitation by the moths, as seen in other yuccas (Pellmyr and Huth 1994, Wilson and Addicott 1998, Marr and Pellmyr 2003). It is also possible that *T. synthetica*, which has an ovipositor that is between 3.55 and 3.77 mm in total length (Pellmyr and Segraves 2003), might contact the ovules when ovipositing on particularly short-styled *Y. jaegeriana* flowers.

There are some limitations of the evidence we present here. First, we were unable to directly observe the behavior of *T. synthetica*, so all of our inferences about differences with *T. antithetica* must be based on comparisons of Trelease's (1893) written descriptions with our observations, photos, and videos. The information we have about each moth's behavior is therefore not directly comparable. Second, it has not been directly shown that the distance between the eggs and the ovules affects larval survival. Experimental or observational tests of this hypothesis are sorely needed.

Acknowledgments

Kendra Autumn, Lydia Bailey, Ross Calhoun, Austin Guimond, Nancy Hauser, Candace Fallon, Ramona Flatz, Malia Santos, Furey Stirrat, and Jackson Waite-Himmelwright assisted in the collection of field data. Ramona Flatz, David Hembry, Rob Raguso, Anne Royer, and Jeremy Yoder provided useful commentary on drafts of this manuscript. Lee Dyer and one anonymous reviewer provided constructive feedback on the manuscript. This work was supported by a grant from the National Science Foundation to CIS (DEB-1253849) and by the Willamette University Research Immersion in Evolutionary Ecology Program. We gratefully acknowledge this support.

References Cited

- Addicott, J. F., and A. J. Tyre. 1995. Cheating in an obligate mutualism: How often do yucca moths benefit yuccas? *Oikos* 72: 382–394.
- Godsoe, W., B. Yoder, C. I. Smith, C. S. Drummond, and O. Pellmyr. 2010. Absence of population-level phenotype matching in an obligate pollination mutualism. *J. Evol. Biol.* 23: 2739–2746.
- Godsoe, W.K.W., B. Yoder, C. I. Smith, and O. Pellmyr. 2008. Coevolution and divergence in the Joshua tree/yucca moth mutualism. *Am. Nat.* 171: 816–823.
- Huth, C. J., and O. Pellmyr. 1999. Yucca moth oviposition and pollination behavior is affected by past flower visitors: Evidence for a host-marking pheromone. *Oecologia* 119: 593–599.
- Huth, C., and O. Pellmyr. 2000. Pollen-mediated selective abortion promotes evolutionary stability of mutualism between yuccas and yucca moths. *Ecology* 81: 1100–1107.
- Lenz, L. W. 2007. Reassessment of *Y. brevifolia* and recognition of *Y. jaegeriana* as a distinct species. *Aliso* 24: 97–104.
- Marr, D. L., and O. Pellmyr. 2003. Effect of pollinator-inflicted ovule damage on floral abscission in the yucca-yucca moth mutualism: The role of mechanical and chemical factors. *Oecologia* 136: 236–243.
- Marr, D. L., H. Leebens Mack, L. Elms, and O. Pellmyr. 2000. Pollen dispersal in *Yucca filamentosa* (Agavaceae): The paradox of self-pollination behavior by *Tegeticula yuccasella* (Prodoxidae). *Am. J. Bot.* 87: 670–677.
- McKelvey, S. D. 1938. Yuccas of the Southwestern United States. Arnold Arboretum, Harvard University, Jamaica Plain, MA.
- Nuismer, S., R. Gomulkiewicz, and B. Ridenhour. 2010. When is correlation coevolution? *Am. Nat.* 175: 525–537.
- Pellmyr, O. 1999. Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. *Syst. Entomol.* 24: 243–271.
- Pellmyr, O. 2003. Yuccas, yucca moths and coevolution: A review. *Ann. Missouri Bot. Gard.* 90: 35–55.
- Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372: 257–260.
- Pellmyr, O., and K. A. Segraves. 2003. Pollinator divergence within an obligate mutualism: Two yucca moth species (Lepidoptera; Prodoxidae: *Tegeticula*) on the Joshua Tree (*Yucca brevifolia*; Agavaceae). *Ann. Entomol. Soc. Am.* 96: 716–722.
- R Core Team 2013. R: A language and environment for statistical computing.
- Riley, C. V. 1892. The yucca moth and yucca pollination. *Missouri Bot. Gard. Annu. Rep.* 1892: 99–158.
- Rowlands, P. G. 1978. The vegetation dynamics of the Joshua tree (*Yucca brevifolia* Engelm.) in the southwestern United States of America. University of California, Riverside, CA.
- Royer, A., M. A. Streisfeld, and C. I. Smith. 2016. Population genomics of divergence within an obligate pollination mutualism: Selection maintains differences between Joshua tree species. *Am. J. Bot.* 103: 1730–1741.
- Smith, C. I., K. W. Godsoe, S. Tank, J. B. Yoder, and O. Pellmyr. 2008. Distinguishing coevolution from covariance in an obligate pollination

- mutualism: Asynchronous divergence in Joshua tree and its pollinators. *Evol. Int. J. Org. Evol.* 62: 2676–2687.
- Smith, C. I., S. Drummond, J. B. Yoder, W. Godsoe, and O. Pellmyr. 2009. Host specificity and reproductive success of yucca moths (*Tegeticula* spp. Lepidoptera: Prodoxidae) mirror patterns of gene flow between host plant varieties of the Joshua tree (*Yucca brevifolia*; Agavaceae). *Mol. Ecol.* 18: 5218–5229.
- Thompson, J. N., C. Schwind, P. R. Guimaraes, and M. Friberg. 2013. Diversification through multitrait evolution in a coevolving interaction. *Proc. Natl. Acad. Sci.* 2–7.
- Trelease, W. 1893. Further studies of yuccas and their pollinators. *Annu. Reports Missouri Bot. Gard.* 4: 181–226.
- Wilson, R. D., and J. F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: Is oviposition behavior responsive to selective abscission of flowers? *Oikos* 81: 109–118.
- Yoder, J. B., I. Smith, D. J. Rowley, R. Flatz, W. Godsoe, C. Drummond, and O. Pellmyr. 2013. Effects of gene flow on phenotype matching between two varieties of Joshua tree (*Yucca brevifolia*; Agavaceae) and their pollinators. *J. Evol. Biol.* 26: 1220–1233.