

# Evolution of antagonistic and mutualistic traits in the yucca-yucca moth obligate pollination mutualism

David M. Althoff  | Kari A. Segraves

Department of Biology, Syracuse University, Syracuse, New York, USA

## Correspondence

David M. Althoff, Department of Biology, Syracuse University, Syracuse, NY, USA.  
Email: dmalthof@syr.edu

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## Abstract

Species interactions shape the evolution of traits, life histories and the pattern of speciation. What is less clear is whether certain types of species interaction are more or less likely to lead to phenotypic divergence among species. We used the brood pollination mutualism between yuccas and yucca moths to test how mutualistic (pollination) and antagonistic (oviposition) traits differ in the propensity to increase phenotypic divergence among pollinator moths. We measured traits of the tentacular mouthparts, structures used by females to actively pollinate flowers, as well as ovipositor traits to examine differences in the rate of evolution of these two suites of traits among pollinator species. Morphological analyses revealed two distinct groups of moths based on ovipositor morphology, but no such groupings were identified for tentacle morphology, even for moths that pollinated distantly related yuccas. In addition, ovipositor traits evolved at significantly faster rates than tentacular traits. These results support theoretical work suggesting that antagonism is more likely than mutualism to lead to phenotypic divergence.

## KEYWORDS

adaptation, coevolution, evolutionary rates, pollination, specialization

## 1 | INTRODUCTION

Species interactions may influence local adaptation and population differentiation that leads to the genesis of new species (Althoff et al., 2014; Benkman et al., 2010; Clayton et al., 2015; Hembry et al., 2014; Zeng & Wiens, 2020). This idea has been a central tenet explaining the diversity of life in general (Douglas, 2010), as well as major groups of interacting organisms such as plants and insects (Ehrlich & Raven, 1964; Grimaldi, 1999; Suchan & Alvarez, 2015). Testing the role of species interactions in causing lineage diversification remains an active and developing field as research at both micro- and macro-evolutionary scales is needed to understand how interactions influence the evolution of phenotypic differentiation that translates into reproductive isolation within interacting lineages (Althoff et al., 2014; Chomicki et al., 2019; Gillespie et al.,

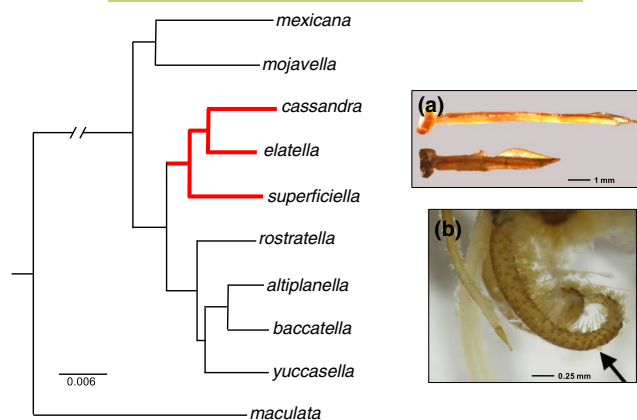
2020; Harmon et al., 2019; Hembry & Weber, 2020; Hembry et al., 2014).

The importance of species interactions in speciation may depend on whether the type of interaction generates selection favouring the creation and maintenance of phenotypic differentiation. Yoder and Nuismer (2010) used a modelling approach to show that antagonisms are likely to select for continual changes in phenotypes as prey and hosts evolve. Prey and hosts continually evolve to evade and defend against their predators or parasites, and predators and parasites respond by evolving new phenotypes to overcome those defences. In contrast, interactions such as mutualism that have long been assumed to be important in lineage diversification may be less likely to generate phenotypic differentiation that could drive speciation. Instead, mutualism is thought to lead to phenotype matching such that there is stabilizing selection to maintain matching mutualistic traits in both mutualist partners (Yoder & Nuismer, 2010).

For example, floral morphology within the Malpighiaceae has been conserved due to interactions with oil-bee pollinators (Davis et al., 2014) and the entrance size to domatia in ant-plants is relatively unchanged for plants evolving with specialized ant species (Chomicki & Renner, 2017). Two recent reviews, however, suggest that mutualism could promote species diversification. In the first review, Chomicki et al. (2019) suggested that mutualism could lead to higher diversification depending on a number of factors such as specificity, partner dependence and environmental context. Similarly, Zeng and Wiens (2020) showed that interactions such as mutualism in which there is a positive effect on fitness are more likely to increase diversification at macroevolutionary scales. Although the exact mechanisms remain unclear, both of these studies suggest that mutualism could promote phenotypic differentiation and speciation.

Empirically testing whether mutualistic or antagonistic interactions are more likely to be important in lineage diversification would ideally be done using interacting lineages in which the overall interaction includes both mutualistic and antagonistic aspects, rather than making comparisons among lineages that differ in the type of interaction. Brood pollination mutualisms such as the yucca-yucca moth interaction are ideal in this regard because the insects are simultaneously mutualists and antagonists; adult females pollinate flowers (mutualism) and lay eggs near the developing seeds on which the larvae feed (parasitism) (Hembry & Althoff, 2016). Yucca species are pollinated by one or two pollinator moth species at a locale and the moths lay eggs into the pistil of the flower (see Rentsch and Leebens-Mack (2014) for one documented exception). Pollination is accomplished by female moths that have novel mouthparts called ‘maxillary tentacles’ that are used to scrape pollen from the anthers and subsequently pollinate the flowers by pushing pollen into the stigmatic cup (Davis, 1967; Pellmyr & Krenn, 2002). Thus, within females, there is both a key mutualistic trait, the tentacles, and a key antagonistic trait, the ovipositor, that facilitate the overall interaction. For the moths, there was a burst of speciation that occurred approximately 3.5 Mya, leading to the evolution of at least 11 species (Althoff et al., 2006; Darwell et al., 2016; Pellmyr & Leebens-Mack, 1999, 2000) that was coupled with changes in ovipositor morphology (Althoff, 2014; Althoff et al., 2006; Pellmyr, 1999). There was a shift from species that had long, thin ovipositors to place eggs within locules next to the seeds (deep, locule oviposition), to species with short, thick ovipositors to place eggs just under the tissue surface in a shallow well (shallow, superficial oviposition) (Pellmyr, 1999). Changes in ovipositor morphology likely arose as a way for moths to circumvent selective abscission of flowers that contain moth eggs (Addicott & Bao, 1999; Marr & Pellmyr, 2003). These differences in ovipositor morphology may also be important in causing reproductive isolation among moth species because the moths have only a single opening that is used for both mating and oviposition (Althoff, 2014).

In contrast with ovipositor morphology, we have yet to examine species-level variation in moth tentacle morphology. Because moth fitness is directly tied to seed production within flowers, we predict that selection for effective pollination will be high. Moreover, there are a number of aspects of tentacle morphology that could change in response to floral evolution. For example, tentacle length may be an



**FIGURE 1** Maximum likelihood-based mtDNA phylogeny of yucca moth species used to compare changes in the evolution of (a) antagonistic (ovipositor morphology) and (b) mutualistic (tentacle morphology) traits used in the overall interaction of yuccas and yucca moths. Representative pictures of the two ovipositor types and tentacular mouthparts used for pollen collection and pollination. Representative ovipositor for moth species with locule oviposition that deposit eggs through pistil wall and next to plant ovules (top) and moth species with superficial oviposition that deposit eggs just below the surface of the pistil or style wall (bottom). Arrow indicates tentacle. Heavy red branches indicate species with superficial oviposition

important metric that determines whether a moth can successfully deposit pollen onto the receptive surfaces within the stigmatic cup. Furthermore, the length of the hairs along the tentacle that hold the pollen may also evolve with respect to pollen traits. Mismatches between moth tentacle morphology and flowers of yucca species could lead to reduced pollination and increased larval death. This may create selection to preferentially visit natal yucca species and promote reproductive isolation in the moths on the basis of pollination ability.

We tested whether mutualistic or antagonistic traits are more likely to vary over the recent burst of speciation in the yucca moths. We combined previously published data on ovipositor morphology with new data on tentacle morphology to examine the rate of evolution of both structures in a representative subset of yucca moths that include both older and younger lineages and that differ in how females place eggs within flowers (Figure 1). Our goal was to test whether mutualistic or antagonistic traits exhibit greater phenotypic variation across species and whether there are differences in the rate of evolution for antagonistic and mutualistic traits.

## 2 | METHODS

### 2.1 | Morphological measurements

We compiled data on ovipositor morphology from previously published research on *Tegeticula* (Lepidoptera: Prodoxidae) yucca moths and combined that with new measurements of tentacle morphology for eight of the 11 species within the burst of speciation 3.5 Mya (Table 1; Figure 1). This subset of species was chosen for

the following reasons. (1) All of the superficially ovipositing pollinators that lay eggs just below the floral surface (*Tegeticula cassandra*, *T. elatella* and *T. superficiella*) are included. (2) The sibling group (*T. altiplana*, *T. baccatella*, *T. rostratella* and *T. yuccasella*) to the superficially ovipositing clade and the representatives of the next most distant clade (*T. mexicana* and *T. mojavelle*) were chosen to capture the shift from the ancestral condition of locule oviposition to superficial oviposition. (3) All other yucca moth species in the genus have locule oviposition and the taxa chosen are the most phylogenetically close group of species which would minimize the effects of other factors such as genetic drift and speciation/extinction processes on determining the evolution of the mutualistic and antagonistic traits (Althoff et al., 2006; Darwell et al., 2016).

For ovipositor morphology, we focused on three traits: ovipositor length as the length of the posterior apophyses, and the height and length of the keel, a small raised area on the dorsal side of the ovipositor tip (Figure 1a). Pellmyr (1999) used these traits and others to diagnose species within the *Tegeticula* species complex. For each species, Pellmyr (1999) used five individual females taken across the range of the species except for *T. elatella* (2 individuals). These three traits also represent the major axes of variation between species that lay eggs superficially within the floral tissue versus those that deposit their eggs within the locule next to the developing ovules (Althoff et al., 2006).

For tentacle morphology, we used moths collected from larval rearings and preserved, wild-caught adults from 2000 to 2018. Similar to ovipositor morphology, we measured three traits—tentacle length, the average length of three hairs near the midpoint of the tentacle and the average length of three hairs at the tip of the tentacle (Figure 1b). We chose these traits based on the assumption that tentacle length would be important for placing pollen within the stigmatic cavity, and the basal hairs would be important for holding pollen, and the tip hairs important for transferring pollen to the stigmatic surface as a female drags her tentacles across the stigma. A combination of frozen and pinned female moths was used for these measurements. Females were placed in a humid relaxing chamber for 12–24 h before measuring. Forewing length was measured with a handheld calliper as a proxy for body size in the morphological analyses to control for size differences among individuals. The tentacles were uncurled and held in an extended position using Minutens. We photographed the tentacles next to a 5-mm ruler at 80X magnification using a Leica SP APO dissecting scope. These pictures were then examined in ImageJ (Rasband, 1997–2018) to measure the length of the tentacles by tracing along the dorsal edge of the tentacle from the base to tip. We determined hair length similarly by haphazardly selecting three typical hairs from the base to near the midpoint and three hairs at the tip. For each picture, we calibrated the measurement scale using the ruler.

## 2.2 | Analyses

Given that tentacle traits were measured on a different set of moths from Pellmyr (1999), we examined the distributions of forewing

length (proxy for body size) for the two sets of moths and the correlations of the ovipositor and tentacle traits to forewing length. If moth forewing length was significantly different between the two sample of moths and the measured traits were correlated with forewing length, this could introduce sampling bias in the analyses. For all species except for three individuals of *T. mojavelle*, one of *T. rostratella*, and one of *T. superficiella*, the moths used by Pellmyr (1999) fell within the distribution of body sizes for the individuals used for the tentacle measurements (Figure S1). We tested for differences in tentacle morphology among moth species using phylogenetic ANOVA to correct for correlations in trait changes due to phylogenetic relatedness (Garland et al., 1993). Because some of the traits were correlated, we also used principal component analyses on log-transformed trait values to test for general groupings of species based on ovipositor and tentacle morphology.

To test for differences in the evolutionary rate among traits, we used the evolutionary rates analyses developed by Adams (2013). For our analysis, we relied on previously published phylogenies based on mtDNA, AFLPs and RADseq data (Althoff et al., 2006; Darwell et al., 2016; Pellmyr et al., 2008). The mtDNA and nuclear data produced different evolutionary relationships, only for the placement of *T. yuccasella*. The mtDNA data nested *T. yuccasella* with *T. cassandra*, whereas both nuclear datasets placed it with other moth species that place eggs next to developing ovules (locule oviposition). Given evidence of mtDNA introgression in *Tegeticula* (Segraves & Pellmyr, 2004), we constrained the maximum likelihood mtDNA phylogeny from Pellmyr et al. (2008) based on the topology from the RADseq data analysed in Darwell et al. (2016) and trimmed the tree to only include taxa for which we had morphological data. We did this for two reasons: (1) given that the nuclear dataset contained hundreds of loci scattered across the genome we felt that this provided a more robust hypothesis of the evolutionary relationships and (2) using the constrained maximum likelihood mtDNA phylogeny allowed us to have a topology with estimated branch lengths that are necessary for conducting the evolutionary rate analyses. As in previously published phylogenetic analyses, we used *T. maculata* as the outgroup (Althoff et al., 2006; Darwell et al., 2016; Pellmyr et al., 2008).

To perform the evolutionary rates test, we log-transformed the trait data to account for differences in scale. We calculated species-level means for each trait to use in the analysis (Table 1). We performed the evolutionary rates' test on three datasets: One analysis contained all of the traits and the other two analyses used one of the first two principal components describing the overall changes in ovipositor and tentacle morphology. We performed these additional tests because some of the individual traits were correlated. Confidence intervals for the rate estimates were obtained by non-parametric bootstrapping of individuals within species and recalculating species means to make 1000 replicate datasets. The trait measurements, topology and estimated branch lengths are presented in Table S1. Simulations by Revell et al. (2018) suggest small numbers of taxa ( $n = 10$ ) such as used in this study could lead to reduced power to detect differences in evolutionary rates. Thus, any significant differences in evolutionary rates among traits are likely robust.

TABLE 1 Summary of data to compare the evolutionary rate of change for tentacles and ovipositor morphology in yucca moths

Species	Ovipositor morphology			Tentacle morphology				Tip hair	
	Sample size	Oviposition habit	Wing length	Ovipositor length	Sample size	Wing length	Tentacle length		Basal hair
<i>Altiplanella</i>	Pellmyr, 1999	Locule	10.78 (0.12)	6.38 (0.03)	13	10.46 (0.25)	1.96 (0.12)	0.112 (0.004)	0.073 (0.003)
<i>Baccatella</i>	Pellmyr, 1999	Locule	11.82 (0.16)	7.84 (0.18)	10	10.92 (0.30)	2.20 (0.12)	0.118 (0.005)	0.063 (0.004)
<i>Rostratella</i>	Pellmyr, 1999	Locule	10.92 (0.22)	5.97 (0.22)	3	10.8 (0.18)	2.39 (0.06)	0.129 (0.006)	0.102 (0.009)
<i>Yuccasella</i>	Pellmyr, 1999	Locule	10.29 (0.27)	5.504 (0.15)	30	11.24 (0.36)	2.46 (0.07)	0.143 (0.007)	0.095 (0.003)
<i>Mojavella</i>	Pellmyr, 1999	Locule	10.30 (0.13)	6.83 (0.04)	9	8.57 (0.23)	1.97 (0.08)	0.088 (0.005)	0.140 (0.061)
<i>Mexicana</i>	Pellmyr, 1999	Locule	10.53 (0.20)	5.64 (0.02)	5	9.74 (0.42)	2.12 (0.15)	0.113 (0.009)	0.059 (0.002)
<i>Superficiella</i>	Pellmyr, 1999	Superficial	11.87 (0.46)	3.95 (0.10)	11	10.86 (0.26)	2.16 (0.08)	0.107 (0.005)	0.081 (0.009)
<i>Elatella</i>	Pellmyr, 1999	Superficial	11.97 (0.07)	4.76 (0.28)	19	11.03 (0.17)	2.21 (0.07)	0.123 (0.005)	0.081 (0.004)
<i>Cassandra</i>	Pellmyr, 1999	Superficial	11.34 (0.22)	4.34 (0.06)	13	10.82 (0.18)	2.11 (0.06)	0.121 (0.005)	0.086 (0.003)

Note: All values were measured in millimetres (mean [standard error]). Sample sizes used in Pellmyr (1999) were 5 individuals per species, except for *T. elatella*.

### 3 | RESULTS

#### 3.1 | Variation in traits important to the overall interaction

After correcting for phylogenetic relationships with a phylogenetic ANOVA, none of the tentacle traits were significantly different among moth species (tentacle length  $F_{2,6} = 1.06$ ,  $p < 0.40$ ; basal hair length  $F_{2,6} = 2.81$ ,  $p < 0.14$ ; tip hair length  $F_{2,6} = 0.32$ ,  $p < 0.74$ ; Figure 2). The phylogenetically corrected ANOVA demonstrated that phylogenetic history had a major influence on tentacle traits. That is, variation in traits among species was determined more by their shared relatedness—closely related species were more similar even if pollinating different yucca species.

Because some of the morphological traits were correlated (Table 2), we used principal component analyses to examine overall morphological differences among moth species. Analysis of the ovipositor morphology based on data from Pellmyr (1999) clearly delimited the locule and superficially ovipositing moth species (Figure 3a). The length of the ovipositor and the size of the keel were the main factors separating moths into two major groups based on oviposition mode (Table 3). Locule-ovipositing species had long, thin ovipositors with a slightly raised keel. In contrast, superficially ovipositing species had ovipositors that were shorter and with a tall keel (Figure 1). For tentacle morphology, there were no clear groupings by species and individuals of species from both oviposition modes were interspersed (Figure 3b).

#### 3.2 | Evolutionary rates of traits important to overall interaction

The evolutionary rates analyses revealed that antagonistic traits on average had significantly greater rates of evolution than mutualistic traits (Figure 4; Table 4). For the antagonistic traits associated with the ovipositor, keel height had a rate that was at least 8x larger than for the ovipositor length or keel length. For the mutualistic traits associated with active pollination, the average length of hairs near the tip of the tentacles had the fastest rate and was the second-fastest rate of all the traits. Evolutionary rates analyses of PC1 and PC2 demonstrated that only the rate of evolution for PC2 differed. The rate of the PC2 for ovipositor morphology was approximately 4x faster than for the PC2 for tentacle morphology (118.6 vs. 28.8, respectively, LRT statistic = 4.17,  $p < 0.04$ ).

### 4 | DISCUSSION

The evolution of defences against natural enemies and herbivores, the colour and shape of flowers, and character displacement in traits of competing species clearly indicates that interactions are important in the evolutionary process. Interactions driving trait divergence and speciation lie at the nexus of several major evolutionary

hypotheses such as evolutionary arms races (Abrams, 1986; van Valen, 1973), escape and radiate coevolution (Ehrlich & Raven, 1964; Suchan & Alvarez, 2015) and adaptive radiations of interacting species (Clayton et al., 2015). What is less clear is whether different types of species interactions produce selection that increases phenotypic divergence and whether such selection and divergence is strong enough to promote reproductive isolation and speciation (Althoff, 2014; Hembry et al., 2014).

We examined the role of antagonistic and mutualistic traits of the pollination mutualism between yuccas and yucca moths in generating phenotypic divergence among a group of yucca moths that rapidly speciated in the last 3–5 My (Pellmyr & Leebens-Mack, 1999, 2000). Moths have distinct traits associated with the ability to deposit eggs into floral tissue and actively pollinate yucca flowers (Althoff et al., 2006; Pellmyr, 1999; Pellmyr et al., 2020; Pellmyr & Krenn, 2002; Pellmyr & Leebens-Mack, 2000). During the evolutionary history of *Tegeticula*, a recent clade of moths switched from using a long, thin ovipositor to cut into the locule and place eggs next to the plant's ovules to a short, stout ovipositor to place eggs just below the surface of the pistil tissue (Pellmyr & Leebens-Mack, 2000). The evolution of superficial oviposition allowed these moths to avoid selective floral abscission that results in larval death (Marr & Pellmyr, 2003) and resulted in an ovipositor morphology that is quite distinctive from the ovipositors of locule-ovipositing species (Figure 3a).

The analyses of morphology suggest that selection for dealing with the plant defence of selective abscission appeared to generate distinct morphological divergence in the ovipositor, much more so than selection on the tentacles for pollinating different yucca species. In terms of the evolution of tentacle morphology, there were no clear patterns of phenotypic divergence that paralleled the results for ovipositor morphology (Figure 3). Tentacle morphology did not differ significantly among species after we accounted for phylogeny (Figure 2). Individuals within species overlapped substantially in morphospace with individuals from other moth species, including both close and distant relatives (Figure 3b). There was also no pattern to

suggest that pollinating distantly related yucca species selected for differences in tentacle morphology. The three moth species (*T. mexicana*, *T. mojaveella* and *T. baccata*) that pollinate fleshy-fruited yuccas in the section *Sarcocarpa* overlapped in morphospace with the other species that all pollinate capsular-fruited yuccas (Figure 3).

Given the differences in the pattern of evolution in antagonistic and mutualistic morphologies, we assessed whether there were differences in the evolutionary rates of these traits. All traits exhibited some evolutionary change across the phylogeny (Figure 4). For the ovipositor traits, the height of the keel near the tip of the ovipositor had the greatest rate followed by the length of the ovipositor and then the length of the keel. For the tentacle traits, average tip hair length had the highest rate, followed by average basal hair length and then length of the tentacle. When comparing antagonistic versus mutualistic traits, antagonistic traits had, on average, higher rates than mutualistic traits (Figure 4). The evolutionary rate for the height of the keel was approximately eight times greater than the mutualistic trait with the greatest rate, average tip hair length. In terms of overall morphological changes, only PC2 showed a significant difference in rate with ovipositor shape having a rate that was four times greater than tentacle shape (118.7 vs. 28.8, respectively). Surprisingly, the overall length of the ovipositor had a relatively low evolutionary rate even though this trait is important for determining egg placement within floral tissue and was likely the focus of selection on moths to avoid selective floral abscission by yuccas (Pellmyr & Leebens-Mack, 2000). Thus, changes in the ovipositor near the tip were potentially very important for egg placement just under the floral tissue surface.

The greater rate of evolutionary change in ovipositor morphology and relatively lower rate of change in tentacle morphology correspond with results from ecological studies of the importance of these traits for host use by yucca moths (Althoff, 2014, 2016; Arteaga et al., 2020; Leebens-Mack et al., 1998). The shift in ovipositor morphology correlates with the appearance of new species of pollinator moths as well as the evolution of cheater species. In addition, changes in ovipositor morphology caused correlated changes in male

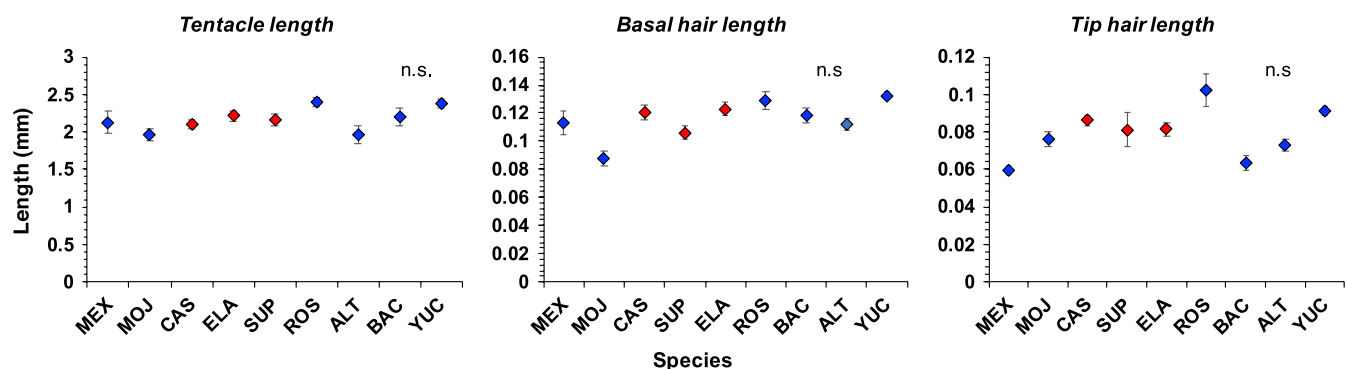


FIGURE 2 Variation in mutualistic traits used in pollination among species of *Tegeticula* pollinator moths. Taxa are arranged in same order as phylogeny in Figure 1. Species indicated with a blue diamond deposit eggs through the pistil and next to developing ovules (locule), whereas those with red diamonds deposit eggs just below the pistil or style tissue (superficial). (means and standard error bars displayed, some bars obscured by marker). Traits were compared with a phylogenetic ANOVA to account for phylogenetic relationships

TABLE 2 Trait correlations for suites of (a) antagonistic and (b) mutualistic traits in yucca moths

(a) Trait	Forewing length	Ovipositor length	Keel height	Keel length
Forewing length	-			
Ovipositor length	-0.13	-		
Keel height	<b>0.54</b>	<b>-0.73</b>	-	
Keel length	<b>0.45</b>	<b>0.33</b>	0.22	-
(b) Trait	Forewing length	Tentacle length	Avg. basal hair length	Avg. tip hair length
Forewing length	-			
Tentacle length	0.17	-		
Avg. basal hair length	<b>0.47</b>	<b>0.43</b>	-	
Avg. tip hair length	0.08	<b>0.26</b>	<b>0.36</b>	-

Note: Values in bold are statistically significant at the  $p < 0.05$  level. Forewing length was used as a proxy for body size.

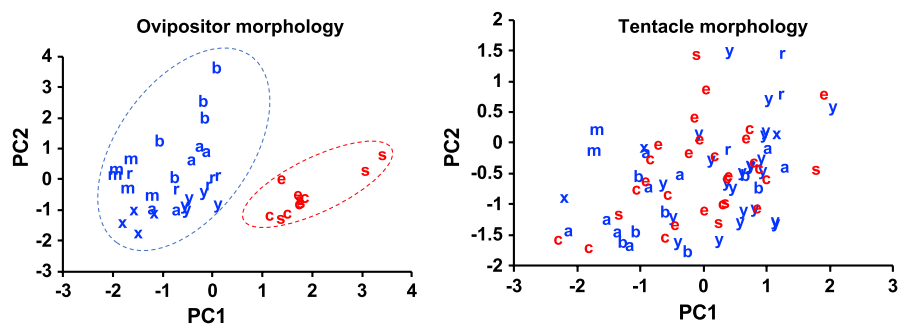


FIGURE 3 Results of principal components analyses of antagonistic (ovipositor) and mutualistic (tentacle) traits in yucca moth pollinators. Lower case letters represent species names from Figure 1 ( $x = T. mexicana$ ). There are two distinct groups based on ovipositor morphology—locule (blue) and superficial (red) oviposition strategies as indicated by 95% confidence ellipses. No such groups were detected for tentacle morphology. For ovipositor morphology, PC1 and PC2 explained 50.2% and 36.8% of variance, respectively, and for tentacle morphology, 49.7% and 20.8% of variance respectively

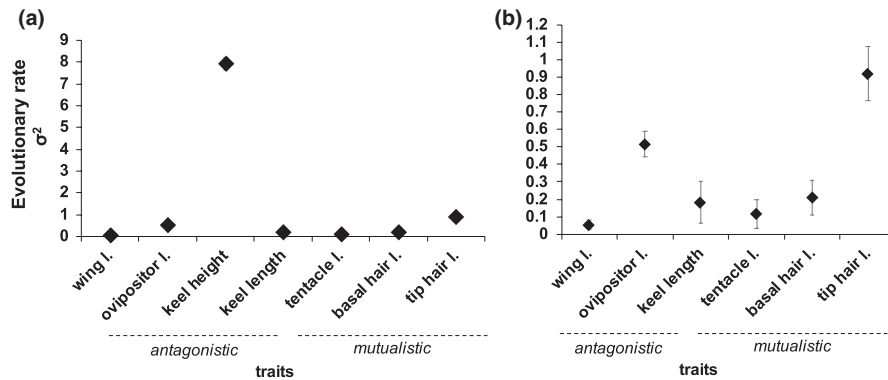
TABLE 3 Principal components loadings for antagonistic traits and mutualistic traits in yucca moths. Values in bold are statistically significant at the  $p < 0.05$  level

Trait	Eigenvalues				Value	Per cent
	PC1	PC2	PC3	PC4		
Antagonistic traits						
Forewing length	<b>0.733</b>	<b>0.469</b>	0.489	<b>1</b>	2.014	50.34
Ovipositor length	<b>-0.684</b>	<b>0.691</b>	0.109	<b>2</b>	1.475	36.88
Keel length	0.310	<b>0.865</b>	<b>-0.382</b>	<b>3</b>	0.427	10.68
Keel height	<b>0.954</b>	-0.1423	-0.174	<b>4</b>	0.084	2.10
Mutualistic traits						
Forewing length	<b>0.646</b>	<b>-0.599</b>	<b>0.376</b>	<b>1</b>	1.9869	49.65
Tentacle length	<b>0.762</b>	0.134	0.234	<b>2</b>	0.832	20.79
Avg. basal hair length	<b>0.776</b>	-0.158	-0.002	<b>3</b>	0.640	16.01
Avg. tip hair length	<b>0.621</b>	<b>0.656</b>	<b>-0.803</b>	<b>4</b>	0.542	13.55

intromittent organs that could lead to reproductive isolation among moth species with different oviposition strategies (Althoff, 2014). In terms of pollination ability, studies on hybridization in yuccas suggest that moths have the ability to effectively pollinate non-natal

yucca species. Contact zones between *Y. baccata* and *Y. schidigera* in Nevada have led to hybridization of these two species, even though each has its own specialized pollinator species (Leebens-Mack et al., 1998). Similarly, hybridization of *Y. valida* and *Y. capensis* in the Baja





**FIGURE 4** Estimated evolutionary rates for antagonistic and mutualistic traits important in the interactions of yucca moths with yuccas. (a) All traits included. Keel height had a significantly higher rate of evolution compared with all other traits. (b) Same graph with keel height excluded to highlight differences among remaining traits. Significance of pairwise contrasts of rates is presented in Table 2

**TABLE 4**  $p$  values for pairwise comparisons of evolutionary rates among ovipositor and tentacle traits

Trait	Ovipositor length	Keel height	Keel length	Tentacle length	Tentacle basal hair length	Tentacle tip hair length
Ovipositor length	–					
Keel height	<b>0.0003</b>	–				
Keel length	0.125	<b>2.83E–06</b>	–			
Tentacle length	0.014	<b>1.23E–07</b>	0.302	–		
Tentacle basal hair length	0.099	<b>1.95E–06</b>	0.901	0.363	–	
Tentacle tip hair length	0.416	<b>0.003</b>	0.023	<b>0.002</b>	0.017	–

Note: Values in bold denote significance values when using Bonferroni corrections for multiple comparisons of the same trait ( $\alpha_{\text{altered}} < 0.001$ ).

California Peninsula suggests that a single pollinator species can pollinate multiple species of yuccas (Arteaga et al., 2020). For the most generalist pollinator species, *T. yuccasella*, Althoff (2016) demonstrated that a local population of this moth has the ability to effectively pollinate a wide variety of capsular-fruited yucca species never before encountered, but has drastically reduced success parasitizing these same species. This moth species is also known to pollinate the distantly related fleshy-fruited, *Y. aloifolia* (Rentsch & Leebens-Mack, 2012). Taken together, these results suggest that the possession of tentacles provides moths with the ability to successfully pollinate many yucca species. In contrast, selection on the antagonistic side of the interaction is more important in determining specialization in host use and phenotypic divergence in yucca moths.

The results for yucca moths support theoretical work showing that antagonistic interactions may be more likely to generate phenotypic divergence that could fuel speciation (Yoder & Nuismer, 2010). The relative importance of antagonism versus mutualism in generating lineage diversification, however, could change depending on the selective context. At some point during yucca moth evolution, changes in mouthpart morphology could have been faster and led to discrete phenotypes as moths evolved to be active pollinators. Thus, there may be cycles of changes in antagonistic and mutualistic traits as the focus of selection switches between them. Additional tests could be done in a number of brood pollination mutualism systems such as figs and fig wasps, leafflower and leafflower moths, and senita cactus and senita moths (Fleming & Holland, 1998; Kato et al., 2003; Ramirez, 1969) to determine the robustness of the results presented here. Similarly, research examining geographic variation

among populations adapting to different host plant species could also provide comparisons about how antagonism and mutualism drive trait change (e.g. Thompson et al., 2013).

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#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13967>.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study can be viewed at <https://doi.org/10.5061/dryad.mw6m905z0> as part of the Dryad.org database as well as in the supplemental material.

#### ORCID

David M. Althoff  <https://orcid.org/0000-0003-3199-1348>

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