

SHORT COMMUNICATION

Absence of population-level phenotype matching in an obligate pollination mutualismW. GODSOE*†, J. B. YODER†, C. I. SMITH†¹, C. S. DRUMMOND† & O. PELLMYR†

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Keywords:codivergence;
coevolution;
insects;
plants;
pollination;
speciation.**Abstract**

Coevolution is thought to promote evolutionary change between demes that ultimately results in speciation. If this is the case, then we should expect to see similar patterns of trait matching and phenotypic divergence between populations and between species in model systems for coevolution. As measures of divergence are frequently only available at one scale (population level or taxon level), this contention is rarely tested directly. Here, we use the case of co-divergence between different varieties of Joshua tree *Yucca brevifolia* (Agavaceae) and their obligate pollinators, two yucca moths (*Tegeticula* spp. Prodoxidae), to test for trait matching between taxa and among populations. Using model selection, we show that there is trait matching between mutualists at the taxon level, but once we account for differences between taxa, there is no indication of trait matching in local populations. This result differs from similar studies in other coevolving systems. We hypothesize that this discrepancy arises because coevolution in obligate mutualisms favours divergence less strongly than coevolution in other systems, such as host–parasite interactions.

Introduction

Coevolution – reciprocal adaptation between species – has been hypothesized to promote diversification, but there is a great deal of uncertainty as to the temporal and spatial scales at which coevolution is biologically significant. Recent work has focused on documenting how coevolution promotes differentiation between populations (Brodie *et al.*, 2002; Nuismer *et al.*, 2003, 2005; Forde *et al.*, 2004; Thompson, 2005). One of the most common findings of such studies has been trait matching – correlations between the phenotypes of two interacting species. Some of the best examples of this phenomenon are in plant–insect interactions such as the strong population-level correlation between pericarp (fruit) thickness in *Camellia* and rostrum length of its major

seed predator, the weevil *Curculio camelliae* (Toju, 2008). Theory predicts that selection for phenotype matching between mutualists at the same location (population-level phenotype matching) will contribute to speciation (Kiestler *et al.*, 1984). This finding is consistent with a long-standing prediction that specialized plant pollinator interactions reduce gene flow between flowers with unlike phenotypes and so promote diversification (Grant, 1952; Fulton & Hodges, 1999; Fenster *et al.*, 2004; Sargent, 2004; Ennos, 2008). More generally, coevolution has been invoked as a cause of macroevolutionary patterns, such as speciation and diversification (Ehrlich & Raven, 1964; Pellmyr, 2003).

However, the strength of coevolutionary interactions can vary substantially over space and time (Thompson, 2005), and as a result, many authors have argued that it is unlikely to generate new species (Janzen, 1980; Machado *et al.*, 2005; Whittall & Hodges, 2007; Armbruster & Muchhala, 2009). Moreover, recent mathematical work indicates that coevolution in mutualisms can actually slow the rate of diversification (Yoder & Nuismer, in press). If selection for trait matching between populations scales up to drive species

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formation, then we should see comparable patterns of trait matching at the population level and at the taxon level. Unfortunately, whereas there are many tests for trait matching at the population level (Darwin, 1862; Brodie *et al.*, 2002; Forde *et al.*, 2004; Toju & Sota, 2006; Anderson & Johnson, 2007; Toju, 2008; Pauw *et al.*, 2009) and some examples at the taxon level (Brouat *et al.*, 2001; Weiblen & Bush, 2002; Weiblen, 2004), there are very few tests for trait matching across scales in coevolving systems (Jablonski, 2008), but see (Benkman, 1999; Benkman *et al.*, 2001). Here, we test for trait matching at both the taxon and population level in a well-documented example of co-divergence, the pollination mutualism between yuccas and yucca moths.

The obligate mutualism between Joshua tree (*Yucca brevifolia* Engelm.; Agavaceae) and its associated yucca moth pollinators (*Tegeticula synthetica* and *Tegeticula antithetica*; Lepidoptera: Prodoxidae) is a model system of coevolution and therefore a logical case to test for a link between phenotype matching at different scales. Interactions between these mutualists strongly shape their individual fitness. The female moth cuts through the style of a flower with her blade-like ovipositor, deposits an egg and then applies pollen to the flower's stigmatic surface (Riley, 1873; Pellmyr, 2003). The larva consumes a fraction of the developing seeds. Previous studies have demonstrated substantial evolutionary divergence in both Joshua tree and its pollinators. The moths that pollinate *Y. brevifolia* are two morphologically distinct, parapatrically distributed sister species: *T. synthetica* (Riley) in the western part of the range and *T. antithetica* Pellmyr in the east. The two pollinator species diverged approximately 1.1 million years ago and serve as exclusive pollinators for Joshua tree (Pellmyr & Segraves, 2003; Smith *et al.*, 2008a). *Yucca brevifolia* may also be divided into two taxa: variety *brevifolia*, which occurs in the western part of the species range and is pollinated by *Tegeticula synthetica*, and var. *jaegeriana* that is pollinated by *T. antithetica* in the east (Rowlands, 1978; Godsoe *et al.*, 2008). These varieties may represent separate species (Lenz, 2007); though, in the absence of data from nuclear markers, we refer to them as varieties of *Y. brevifolia* (Godsoe *et al.*, 2008).

We have strong *a priori* reasons to expect trait matching between *Y. brevifolia* and its pollinators (Godsoe *et al.*, 2008). Although there are examples of phenotype matching in organisms ranging from viruses to vertebrates (Brodie *et al.*, 2002; Forde *et al.*, 2004), many of the best examples are from plant–insect interactions (Darwin, 1862; Weiblen & Bush, 2002; Anderson & Johnson, 2007; Toju, 2008). In many of these systems, an insect must pollinate or oviposit through some part of a plant, as is the case in *Y. brevifolia*, where a moth oviposits by extending her blade-like ovipositor through the style of a Joshua tree flower. There is already strong evidence for phenotype matching at the taxon level, as moths with long ovipositors (*T. synthetica*) use plants with

long styles (var. *brevifolia*), whereas moths with short ovipositors (*T. antithetica*) use plants with short styles (var. *jaegeriana*; Godsoe *et al.*, 2008).

Several lines of evidence suggest that this correlation between the lengths of the moth ovipositor and the floral style is the result of strong selective pressure. Experiments on *Yucca filamentosa* have shown that damage to the developing ovules increases the probability of floral abscission (Marr & Pellmyr, 2003), and we hypothesize that moths with overlong ovipositors do more damage than is necessary to place eggs. We expect pollinators with longer ovipositors to drill deeper, and this mechanism should favour trait matching in both partners as abscission kills a fraction of the yucca's developing seeds as well as any resident moth larvae. It is difficult to directly observe oviposition on *Y. brevifolia*, but in the zone of sympatry in Tikaboo Valley, Nevada, where the two pollinators and the two tree varieties come into contact, there is evidence of selection for phenotype matching. In this region, adult moths of each species visit both tree varieties. However, *T. antithetica* is far more likely to produce larvae on its native host (var. *jaegeriana*), and *T. synthetica* is far more likely to produce larvae on its native host (var. *brevifolia*) (Smith *et al.*, 2009). Where they do produce larvae on foreign hosts, there are fewer larvae per clutch.

Although there is a clear taxon-level correlation between the ovipositor length and the ovary wall thickness at the point of oviposition, we know far less about co-divergence among individual populations in this system. Previous analyses of population-level variation in *Y. brevifolia* focused exclusively on vegetative traits (Rowlands, 1978). Rowlands (1978) further hypothesized that many of the population-level morphological differences observed in *Y. brevifolia* are a response to differences in climate. As adaptations to the abiotic environment can produce phenotype matching in the absence of coevolution (Nuismer *et al.*, 2010), it is important to determine whether population-level differences in *Y. brevifolia* reflect climatic variation. Likewise, there have been insufficient data to investigate population-level divergence in the pollinators of Joshua tree. By combining moth morphological data from across the range of the Joshua tree with existing data on tree morphology (Godsoe *et al.*, 2008), we are here able to test for population-level trait matching for the first time in the *Yucca*–yucca moth mutualism.

If coevolution drives diversification in this system, there should be strong phenotypic divergence between tree varieties and moth species at both the population and taxon level. In addition, the phenotypic variation in each partner should be best explained by the phenotype of the mutualists with which it interacts, rather than the taxon of its mutualist or the local climate. To test the first hypothesis, we generated linear mixed-effects models for the moth and tree data sets and estimated the proportion of variation attributable to taxon- and population-level effects. To investigate the second hypothesis, we

compared the fit of four competing regression models for moth and tree phenotypes at the population level. Specifically, if there is strong selection for population-level phenotype matching in this system, the phenotype of one mutualist should be strongly correlated with the phenotype of its partner at the same location. Alternatively, phenotype matching may only occur between taxa. If this is the case, then the phenotype of one partner should depend only on the taxon of its mutualist and information on the phenotype of its mutualist from the same population will not improve our model (Fig. 1). Finally, the phenotype of one mutualist may be a consequence of the abiotic environment, or a combination of the taxon to which its mutualist belongs and the phenotype of this mutualist. Our analyses eliminate the possibility of strong population-level phenotype matching and demonstrate that most of the variation in style and ovipositor length is attributable to taxon-level effects, not population-level trait matching or environmental effects.

Materials and methods

Data collection

Given the distinctiveness and stature of the Joshua tree, there are exceptionally detailed records from across its range (Rowlands, 1978; Godsoe *et al.*, 2009). We surveyed every accessible population of *Y. brevifolia*. During 2005–2007, we collected flowers from up to ten trees per site. We carefully examined inflorescences and collected any moths that were present. For sites where no moths were found during this 3-year period, we used moths previously collected from the same site. We excluded records from the contact zone between moth species and tree varieties in Tikaboo Valley, Nevada, as it is difficult to assign trees at this site to one variety without introducing circularity to our analysis. From this data set, we collected matching records of moths and ovipositors in 23 populations (Fig. 2; Table S1).

Following protocols in Godsoe *et al.*'s (2008) study, we stored flowers in 70% ethanol and dissected them in the laboratory to measure the length of the stylar canal. We removed moth abdomens and incubated them at room temperature in a 10% KOH solution for 24 h to digest soft tissues that obscure the chitinous structures of interest. To measure moth ovipositor length, we dissected and then photographed each specimen using an Olympus microscope fitted with a micrometer and a Nikon Coolpix 4500 digital camera. We then used the resulting images to measure ovipositor length (here defined as the length from the tip of the ovipositor to the end of the posterior apophyses) in TPS-dig (Rohlf, 2001), using 10 anchor points.

We used the 19 bioclimatic variables (summaries of temperature and precipitation throughout the year) described in Hijmans *et al.*'s (2005) study to investigate

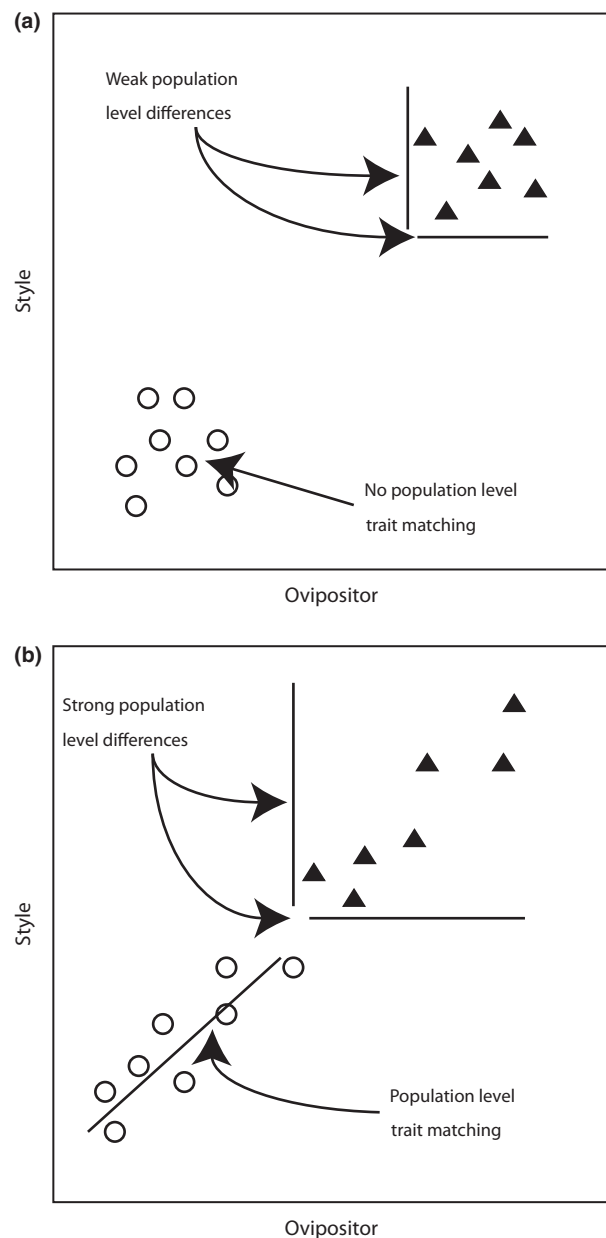


Fig. 1 An illustration of the patterns consistent with taxon-level and population-level trait matching. Each panel is a plot of style length and ovipositor length for two pairs of moth and plant species (pair 1 is white; pair two is black). Panel (a) is an example of taxon-level trait matching. Populations in each pair of taxa have similar phenotypes (small styles and ovipositors for pair 1; large styles and ovipositors for pair 2). However, selection for trait matching does not drive population-level differences. As consequence, there is no population-level trait matching (within one species, pair style length and ovipositor length are uncorrelated). In addition, a small proportion of the variation in this system is attributable to differences among populations. In panel (b), selection for trait matching drives diversification, and as a result, there is a strong population-level phenotypic correlation and substantial differences among populations.

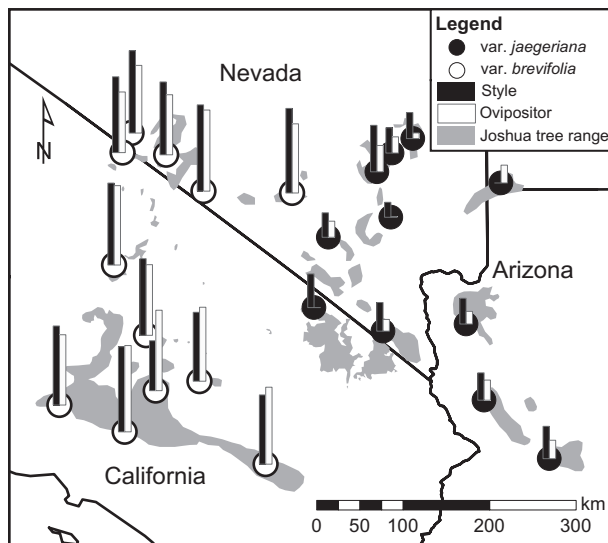


Fig. 2 Comparison of style length and ovipositor length throughout the range of *Yucca brevifolia*. Measurements have been rescaled from zero to one, by subtracting the smallest observation and then dividing by the range.

the role of climate on trait matching in this system. We summarized these data by sampling 2000 points at random from the range of *Yucca brevifolia* var. *brevifolia* and 2000 points from the range of var. *jaegeriana* and extracted principal components (see Godsoe *et al.*, 2009). This procedure reduces multicollinearity and improves the power of our tests by reducing the number of variables we must model (Myers, 1990).

Analyses

We fitted a linear mixed-effects model to determine the proportion of phenotypic variation attributable to each scale (population vs. taxon). We modelled taxon level as a fixed effect. This implies that we were specifically interested in the difference between our taxon pairs (var. *jaegeriana* vs. var. *brevifolia* in our style length analysis; *T. synthetica* vs. *T. antithetica* in our analysis of ovipositor length analysis). We then modelled population as random effect nested within taxon, implying that we were interested in how a typical population deviates from the average of all populations (within a given taxon). Comparisons of parameter estimates for this model represent an analysis of the amount of standard deviation that is attributable to differences between taxa vs. the amount of standard deviation that is attributable to differences between populations. We fit these models using the nlme package in R (R Development Core Team, 2006, Pinheiro *et al.*, 2008). It can be challenging to develop *P*-values for linear mixed-effects models. To avoid this problem, we expressed uncertainty with 95% confidence intervals (Pinheiro & Bates, 2000).

We used linear regression to model the relationship between the traits of one mutualist and the following predictor variables: the mean trait value for the other mutualist (ovipositor length or style length) within that population, the pair of taxa in the population (the pair of either var. *brevifolia* and *T. synthetica* or var. *jaegeriana* and *T. antithetica*) and climate principal component 1 (a contrast between temperature and precipitation). Principal component 1 represents a surrogate for elevation and a summary of 49% of the variation in the 19 bioclimatic variables in our study area (Godsoe *et al.*, 2009). We did not include principal components 2 and 3 that account for an additional 39% of the variation in this region as these components vary primarily along the edges of the range of *Y. brevifolia* (Godsoe *et al.*, 2009).

To assess the importance of our explanatory variables, we performed model selection (Burnham & Anderson, 2002) using the Akaike information criterion corrected for small sample sizes (AIC_c). In our analyses, a model with AIC_c scores two units smaller than another was deemed superior and so retained. We determined whether the traits of one mutualist in a population were explained by the phenotype of the other mutualist in the same location, the set of taxa in that location or both variables. In addition, we tested a model that included taxa, phenotype and climatic PC1. We investigated the effects of individual variables by computing partial *F*-tests in a model including the trait and species of the other mutualist as explanatory variables.

Finally, we performed a power analysis to determine whether we would be reasonably likely to find evidence of trait matching at the population level given that there are also differences between taxa. We simulated two moth taxa and two tree taxa. The mean trait value for each taxon was set to the means of one of the taxa from our original data set. For each of these taxa, we simulated 10 populations by selecting trait values at random from normal distributions with standard deviations derived from the empirical data set. We altered proportion of variation in style length attributable to ovipositor length (R^2) from 0 to 0.99. In turn, we simulated 500 replicated data sets for each R^2 value and tested whether a model for style length that included ovipositor length and taxon would be favoured over a model that only included taxon. We then estimated the power by calculating the proportion of tests that favour a model including ovipositor length by at least two AIC_c units for each simulated R^2 value (see Figure S1).

Results

Consistent with previously published results, both floral styles and moth ovipositors differed significantly between taxa. However, in the more extensive intraspecific data analysed here, only a small proportion of standard deviation was attributable to differences among

Table 1 Support for competing models of tree phenotype (style length) or moth phenotype (ovipositor length).

Model	AICc scores
<i>Yucca brevifolia</i>	
Style ~ ovipositor	57.63003
Style ~ taxa	52.49171
Style ~ ovipositor + taxa	54.92941
Style ~ ovipositor + taxa + PC1	56.28138
<i>Tegeticula</i> spp.	
Ovipositor ~ style	-0.09
Ovipositor ~ taxa	-23.97
Ovipositor ~ style + taxa	-21.53
Ovipositor ~ style + taxa + PC1	-20.40

populations (*Tegeticula* spp. taxon: 0.961, 95% CI: 0.83–1.09, population: 0.087, 95% CI 0.041–0.186; *Y. brevifolia* taxon: 3.29, 95% CI: 2.77–3.81, population 0.47, 95% CI 0.26–0.83).

The best predictor of either mutualist's phenotype was the taxon to which its partner belonged. A model of *Y. brevifolia* style length using moth species provided strong predictions ($R^2 = 0.8543$) and AIC_c scores at least two units lower (better) than any other competing model (Table 1). A model of *Tegeticula* ovipositor lengths including *Y. brevifolia* taxon identity had AIC_c scores at least two units lower than any other model (Table 1) and an R^2 of 0.932. Partial F-tests corroborated these results: for either mutualist, a term for the taxa of its partner was significant, whereas the term for the phenotype of its partner was not (Table 2). However, the inclusion of climate principal component 1 did not markedly improve the fit of models of either style or ovipositor length (Table 1).

In our power analyses, model selection correctly inferred that the phenotype of the other mutualist mattered 58% of the time assuming a population-level R^2 of 0.25. When the population-level correlation between phenotypic traits is as strong as 0.49, model selection correctly inferred that mutualist phenotype matters 91% of the time (see Fig. S2 for the full power curve).

Table 2 ANOVA tables for models of style length and ovipositor length, using marginal sums of squares. Test statistics on one and 20 degrees of freedom.

Coefficient	Estimate	P-value
<i>Yucca brevifolia</i>		
Intercept	4.0192	0.1711
Ovipositor	0.4692	0.6360
Species	1.3849	0.0323
<i>Tegeticula</i> spp.		
Intercept	2.202	< 0.0001
Style	0.0168	0.636

Discussion

The results presented here provide evidence for matching phenotypes between moth species and tree varieties, but little indication of trait matching at the population level. Existing models of divergence in obligate mutualisms predict gradual change with trait matching at the population level (Kiestler *et al.*, 1984). Instead, we find abrupt discontinuities between species, little variation and no indication of phenotype matching at the scale of populations. Moreover, this result differs from many previous reports of trait matching at the population level in coevolving species (Benkman *et al.*, 2001; Brodie *et al.*, 2002; Thompson & Cunningham, 2002; Anderson & Johnson, 2007; Toju, 2008).

The absence of population-level trait matching in this study may be the result of multiple processes that are not mutually exclusive (Thompson, 2005; Nuismer *et al.*, 2010). Here, we consider three possible explanations: that trait matching exists, but is difficult to detect, that trait matching is obscured by spatial variation in coevolutionary interactions and that the nature of obligate mutualisms discourages population-level trait matching.

Recent theoretical work indicates that coevolution between mutualists produces trait matching between populations, but unless reciprocal fitness effects are strong, this association may be difficult to detect (Nuismer *et al.*, 2010). Our power analyses indicate a reasonable chance to detect a correlation as small as 0.5. Although we cannot eliminate the possibility of population-level trait matching with a finite data set, we can say that this effect is, at best, weak. In so doing, we demonstrate that trait matching in *Y. brevifolia* must be different from the pattern described in other plant–insect systems. For example, Toju (2008) detected a population-level trait correlation of 0.87 ($R^2 = 0.76$) between the thickness of the pericarp in *Camellia* fruits and the length of the rostrum of their major seed predator, the weevil *C. camelliae*. Similarly Anderson & Johnson (2007) measured a tight correlation ($r = 0.83$; $R^2 = 0.69$) between tongue length of the fly *Prosoeca ganglbaueri* and the corolla length of its primary food plant *Zaluzianskya microsiphon*.

Another possibility is that coevolution acts in a complex way across the range of *Y. brevifolia* such that our global analysis obscures trait matching in a subset of populations. Thompson (2005) argued forcefully that the importance of coevolutionary interactions could vary over space if, for example, one partner is absent or unimportant in some populations. When this is the case, we should only expect trait correlations in populations with strong reciprocal interactions between species. Because it lacks explicit measures of fitness outcomes, our analysis cannot assess differences in the strength or form of coevolutionary selection across the range of *Y. brevifolia* and its pollinators. However, this obligate mutualism arguably represents one of the systems least

likely to be affected by selection mosaics (Thompson, 2005). The geographic range of moth species and tree varieties is tightly correlated, and *Y. brevifolia* only reproduces sexually through pollination by one of the two moth species. As such, there seems to be little opportunity for spatial variation in the strength of selection at different populations within one taxon. Most importantly, we have not only ruled out strong correlations among populations but also shown that only a small proportion of variation in this system is among populations. Even if there is a selection mosaic, it can only explain a small fraction of the variation we observe.

Instead, we suggest that our results represent an important difference between obligate mutualisms and other systems. Selection for trait matching in mutualisms should typically prevent rapid change in either partner. If, for example, a population of plants and pollinators are well matched, then individuals with novel phenotypes (say a pollinator with an excessively large ovipositor) will be less likely to complete their life cycle. One consequence of this pressure is that unusual phenotypes will be unlikely pass on their genes to the next generation. Given this, obligate mutualisms may be less likely to produce trait matching between populations than antagonistic interactions. In existing models, divergence in a mutualism requires some extrinsic force, such as drift or abiotic selection (Kiestler *et al.*, 1984). Unless there are substantial differences between populations, the effects of selection on trait matching may be overwhelmed by other causes of evolutionary divergence. We hypothesize that this is the case in the Joshua tree/yucca moth system. Specifically, that extrinsic evolutionary forces such as drift, selection from sources extrinsic to the mutualism and developmental plasticity result in population-level variation, but that this variation is too modest to generate population-level trait matching.

Even if phenotype matching in obligate mutualisms rarely causes diversification at the population level, it can still play a crucial role by maintaining divergence arising through other means. This is because co-adapted mutualists in one region will be unable to efficiently exploit potential partners in another region. In the small region where the tree varieties co-occur, we observe a strong pattern of host specialization consistent with this hypothesis. Adult moths visit both tree varieties, but moth larvae are much more likely to emerge from their native host indicating selection for moths to pollinate their native hosts (*Tegeticula synthetica* on var. *brevifolia*, *Tegeticula antithetica* on var. *jaegeriana*; Smith *et al.*, 2009). In this scenario, co-adaptation becomes an additional mechanism of reproductive isolation. If coevolution in obligate mutualisms plays a significant role maintaining differences between populations but is rarely a direct cause of diversification, we make the following predictions: (i) trait matching at the population

level should be relatively rare in obligate mutualisms; (ii) when trait matching does occur, it should be associated with reciprocal fitness effects, not adaptation to different environments; and (iii) the evolution of an obligate mutualism will not lead to increased rates of diversification. The latter prediction has already been demonstrated in comparisons of diversification rates in yuccas and close relatives that are not pollinated by obligate mutualists (Good-Avila *et al.*, 2006; Smith *et al.*, 2008b). However, in the obligate mutualism between figs and fig wasps, figs are markedly more diverse than their sister group Castilleae (Clement, 2008). Improved phylogenies and sampling of intraspecific morphological variation in the obligate mutualisms between figs and fig wasps or the obligate mutualism between *Glochidion* flowers and their *Epicephala* moth pollinators should eventually provide even stronger tests of these predictions.

The results presented here emphasize the scale-dependent nature of studies of coevolution. What appears to be a strong pattern of trait matching between taxa disappears upon a finer-scaled analysis. Detailed tests of many aspects of coevolution will require a careful dissection of the fitness consequences of interactions (Gomulkiewicz *et al.*, 2007; Nuismer *et al.*, 2010). However, some questions, including the role of coevolution in speciation, must be tested by comparing patterns across scales.

Acknowledgments

We thank the US National Park Service, US Fish and Wildlife Service, US Department of Energy, Death Valley National park, Mojave National Preserve, Desert National Wildlife Refuge Joshua Tree National Park and the Nevada Test Site. The National Science Foundation Grant DEB-0516841 provided financial support. W. Kent Ostler helped in arranging access to the Nevada Test Site and helped with data collection. Paul De Prey and David Reznick provided logistical support. Sharon Bewick provided helpful comments. W. G. was supported by a post-doctoral fellowship at the National Institute for Mathematical and Biological Synthesis, an institute sponsored by the National Science Foundation, the U.S. Department of Homeland Security, the U.S. Department of Agriculture through the National Science Foundation Award #EF-0832858, with additional support from the University of Tennessee, Knoxville. During his graduate studies, W. G. was also supported by a PGS fellowship from the Canadian National Science and Engineering Research Council with a supplement from the foundation for Orchid Research and Conservation. J. B. Y. received support from the Joshua Tree National Park Graduate Student Research Program and a Doctoral Dissertation Improvement Grant from NSF (DEB-0910223). Nicole Koeltzow and Ana Richters provided helpful comments.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 List of locations used in our analyses along with the sample size for measurements of style and ovipositor length.

Figure S1 (a) The observed relationship between style length and ovipositor length. (b) The relationship in a simulated data set when there is an effect of taxon but no relationship between style length and ovipositor length between populations. (c) The relationship in a simulated data set with an effect of taxon where 49% of the variation in style length between populations is explained by ovipositor length.

Figure S2 Graph of the probability of detecting a significant correlation between ovipositor and style lengths after accounting for differences between taxa (1 – type II error), measured against different possible correlation values.

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Received 27 April 2010; revised 7 August 2010; accepted 31 August 2010