

DISTINGUISHING COEVOLUTION FROM COVICARIANCE IN AN OBLIGATE POLLINATION MUTUALISM: ASYNCHRONOUS DIVERGENCE IN JOSHUA TREE AND ITS POLLINATORS

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Obligate pollination mutualisms—in which both plants and their pollinators are reliant upon one another for reproduction—represent some of the most remarkable coevolutionary interactions in the natural world. The intimacy and specificity of these interactions have led to the prediction that the plants and their pollinators may be prone to cospeciation driven by coevolution. Several studies have identified patterns of phylogenetic congruence that are consistent with this prediction, but it is difficult to determine the evolutionary process that underlies these patterns. Phylogenetic congruence might also be produced by extrinsic factors, such as a shared biogeographic history. We examine the biogeographic history of a putative case of codivergence in the obligate pollination mutualism between Joshua trees (*Yucca brevifolia*) and two sister species of pollinating yucca moths (*Tegeticula* spp.) We employ molecular phylogenetic methods and coalescent-based approaches, in combination with relaxed-clock estimates of absolute rates of molecular evolution, to analyze multi-locus sequence data from more than 30 populations of *Y. brevifolia* and its pollinators. The results indicate that the moth species diverged significantly ($p < 0.01$) more recently than their corresponding host populations, suggesting that the apparent codivergence is not an artifact of a shared biogeographic history.

KEY WORDS: Biogeography, Bouse Embayment, coevolution, cospeciation, IM, pollination, vicariance, yucca, yucca moth.

Obligate mutualisms between plants and seed-feeding insects, such as those between yuccas and yucca moths or figs and fig wasps, represent some of the most remarkable pollination systems in the natural world (Darwin 1874). Within these systems, plants rely exclusively on their specialized pollinators for reproduction, and their pollinators feed solely on the plant's developing seeds (Janzen 1979; Holland and Fleming 1999; Machado et al. 2001; Weiblen 2002; Kato et al. 2003; Pellmyr 2003; Kawakita et al. 2004). Because these organisms are entirely dependent upon one another for reproduction, and because pollinators are often

specialized on a single species of plant, biologists have predicted that these systems should promote cospeciation and parallel phylogenetic histories (Kiestler et al. 1984). However, evidence in support of this hypothesis has been limited. Although significant phylogenetic congruence has been identified in some seed-feeding pollination mutualisms, it is unclear whether these patterns reflect a history of cospeciation through coevolution per se. Within the fig–fig wasp pollination mutualism there are indications of congruence at the broadest phylogenetic scale (Weiblen 2004), but evidence for codiversification seems to be absent at the species

level (Machado et al. 2005). Similarly, recent work has identified congruence in species-level phylogenies between *Glochidion* shrubs and *Epicephala* moths (Kawakita et al. 2004), and between yuccas and yucca moths (Althoff et al., unpubl. ms.), but it is not clear in either of these systems whether congruent phylogenies represent a process of cospeciation driven by the interaction itself, rather than some common extrinsic process, such as a shared biogeographic history (Thompson 1994). A complete test of the hypothesis that pollination mutualisms themselves promote joint reproductive isolation and speciation requires a direct examination of the process of species formation. Such studies must include population genetic and phylogeographic data that could rule out alternative hypotheses, such as common vicariance events.

The Joshua tree–yucca moth pollination mutualism is ripe for testing this hypothesis. Joshua trees (*Yucca brevifolia*; family Agavaceae) are, like all yuccas, pollinated exclusively by highly specialized yucca moths (Prodoxidae; order Lepidoptera). Although *Y. brevifolia* is a well-delimited group, strongly supported by genetic data as a distinct lineage within *Yucca* (Pellmyr et al. 2007), morphological and molecular phylogenetic data have revealed that in different portions of its range *Y. brevifolia* is pollinated by two distinct sister species of yucca moths, *Tegeticula synthetica* occurring in the west, and *T. antithetica* in the east (Pellmyr and Segraves 2003). Additionally, a recent study showed that trees pollinated by the different moth species are distinct in their floral and vegetative morphology (Godsoe et al. 2008), and a new taxonomic revision designated these regional varieties as distinct species (Lenz 2007). Notably, although the eastern and western varieties of Joshua tree differ significantly in their overall gross morphology, the greatest difference between the trees is in their flowers, particularly in the size and shape of the stylar canal, through which the pollinating moths insert their blade-like apophyses during oviposition (Godsoe et al. 2008). The significant divergence in floral characters between trees pollinated by different moths, and particularly the difference in style length (the feature most directly associated with moth oviposition (Trelease 1893; Godsoe et al. 2008)), suggest that the evolution of these differences may have been caused by pollinator-mediated selection and gene flow. Several additional lines of evidence also support this conjecture. Both species of moths and both tree types co-occur in narrow contact zone on the northern edge of their range, but remain morphologically distinct in sympatry, suggesting that neither extrinsic ecological factors nor simple geographic isolation account for their morphological differences.

Although all of these observations seem to suggest a history of codivergence driven by the mutualism itself, as with other putative cases, it is difficult to rule out alternative historical processes that might have produced this pattern. In this case, a strong alternative hypothesis is that these organisms may have had a common biogeographic history. *Y. brevifolia* occurs in mid-elevation,

semidesert communities on the periphery of the Mojave Desert in California, Arizona, Utah, and Nevada (Fig. 1). The center of this range is bisected by series of deep, low-elevation, arid basins, including Death Valley, the Colorado River Valley, and the Salton Trough. These inhospitable habitats themselves represent an important barrier to dispersal for many desert organisms (Avisé et al. 1992; Lamb et al. 1995; Mulcahy et al. 2006), and geologic evidence suggest that these regions were inundated by an estuarine embayment of the Sea of Cortez, the “Bouse Embayment” (Lucchitta 1972; Lamb et al. 1995) between ca. 9 and 4 MY ago. Furthermore, in their original description of *Tegeticula antithetica*, Pellmyr and Segraves (2003) speculated that the divergence between the *T. antithetica* and its sister species, *T. synthetica*, may have been contemporaneous with the extension of the Bouse Embayment into the Mojave Desert region.

These considerations suggest that the common distribution of the two *Y. brevifolia* varieties with their respective pollinators, as well as the apparent phenotype matching between floral styles and pollinator ovipositors, might be an artifact of a shared history of vicariance and allopatry, rather than joint divergence mediated by coevolution. In principle, one might be able to distinguish between these alternatives by using phylogeographic data, but in this system gene flow in the Joshua tree is confounded with pollinator dispersal. As a result, both covicariance and cospeciation driven by the interaction could give rise to shared phylogeographic patterns.

This problem can be overcome, however, by examining uniparentally inherited plastid markers, such as the chloroplast. Because the chloroplast genome is maternally inherited in yuccas (i.e., chloroplasts are not transmitted in pollen), the chloroplast provides a source of phylogeographic data that is entirely independent of pollinator dispersal. If apparent cospeciation in this system were an artifact of a shared biogeographic history (which should affect the whole genome equally), then phylogeographic patterns in the chloroplast data should match those seen in the moths. However, if codivergence were driven by natural selection associated with the pollination mutualism itself, and did not involve a shared biogeographic history, then the phylogeographic patterns in the maternally inherited chloroplast genome would not necessarily match that of the moths. Incongruence in phylogeographic histories would be particularly likely if natural selection acting on one partner only became strong enough to promote divergence after the other partner had already evolved divergent morphologies.

To test the hypothesis that apparent codivergence in the Joshua tree/yucca moth mutualism is an artifact of a common biogeographic history rather than codivergence driven by natural selection, we here examine phylogeographic data from *Y. brevifolia* and its two pollinators, with a particular focus on evaluating the degree of isolation and timing of divergence between

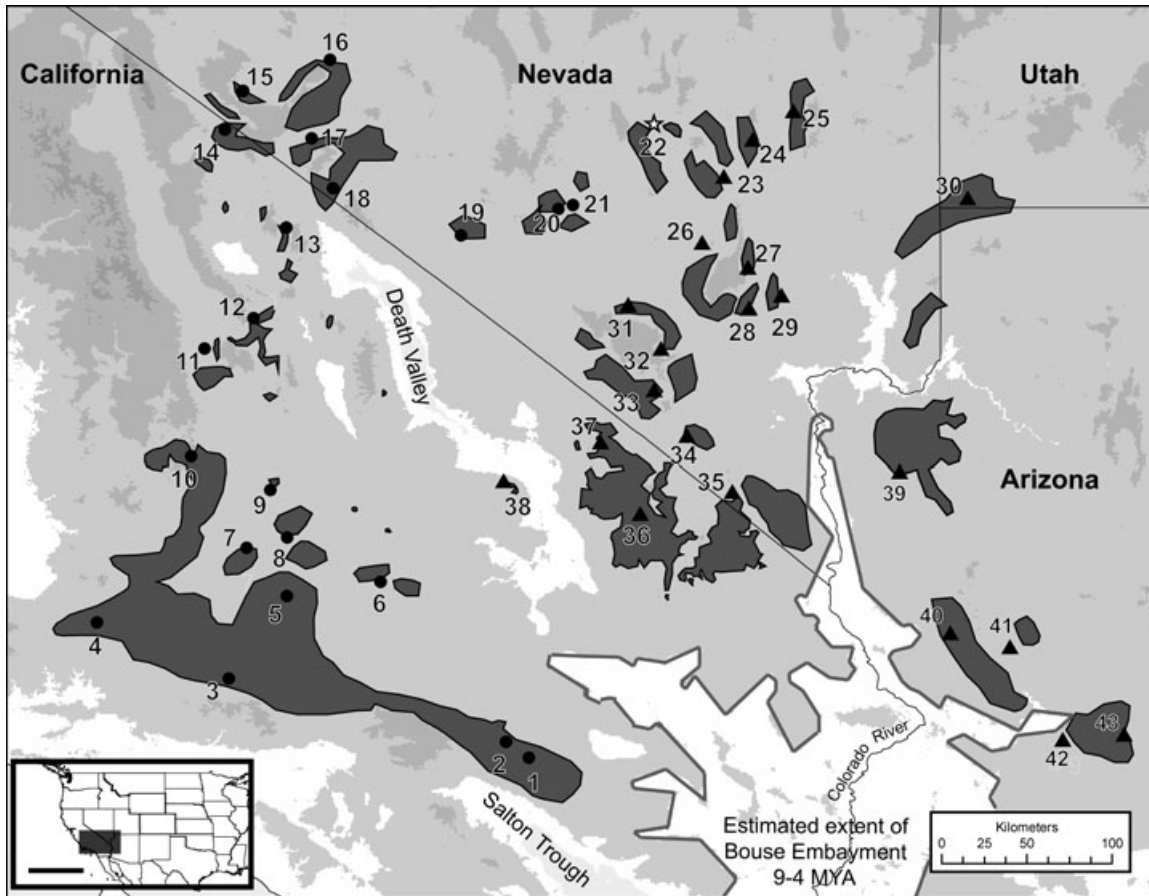


Figure 1. Distribution of *Y. brevifolia* and study sites. The Joshua tree's current range, shown as dark-gray polygons, is based on range maps in Cole et al. (2003) and Rowlands (1978). The range data have been verified through extensive ground-truthing by Godsoe et al. (2008); some false presence records contained in source maps have been removed, and new presences have been added; population boundaries are approximate. Study sites are shown as numbered points; populations pollinated by *Tegeticula synthetica* are indicated by circles, those pollinated by *T. antithetica* by triangles. The one population in which both species co-occur (site 22, Tikaboo Valley, Nevada) is indicated by a star. Background shading indicates elevation, based on the USGS GTOPO30 Digital Elevation Models (DEM) data, and has a resolution of 30 arc sec (~1 km). The lightest (white) shading indicates areas below 475 m that may represent current or past barriers to dispersal. Areas below sea level in Death Valley and the Salton Trough are shown in off-white shading. The estimated maximum extent of the Bouse Embayment, an estuarine or lacustrine body of water that inundated low-elevation areas in the Colorado River Valley between 9 and 4 MY ago (Spencer and Patchett 1997), is shown as a thick, jagged gray line. (Inset: map location; inset scale bar=1200 km).

these two groups. We present mitochondrial and nuclear DNA sequence data from more than 200 individual moths, along with chloroplast DNA sequence data from more than 70 individual trees. We use Bayesian phylogenetic analyses and relaxed molecular clock methods to produce estimates of rates of molecular evolution, profiling across post-burn-in trees to account for phylogenetic uncertainty that could affect these estimates. Finally, we combine these substitution rate estimates with coalescent-based population-genetic analyses to infer rates of gene flow and divergence times in both the plants and their pollinators. These analyses indicate that the divergence time between the two moth species significantly postdates both the geographic isolation of eastern and western Joshua-tree populations ($P = 0.006$) and is much

younger than the Bouse Embayment. This result argues against a shared biogeographic history, and suggests that the patterns of codivergence in this system may have been driven by reciprocal adaptation or by pollinators tracking independent changes in their hosts.

Methods

To test the hypothesis that divergence between the two *Y. brevifolia* varieties and their respective pollinators was driven by a common vicariance mechanism, leaf tissue and moths were collected from more than 30 populations across the current range of *Y. brevifolia*. PCR and automated cycle sequencing were used to produce DNA

sequence data for two mitochondrial genes (COI, ND5) and one nuclear gene (EF1 α) from the moths, and five nonprotein-coding chloroplast regions (trnT-trnL intergenic spacer, trnL, trnL intron, trnL-F intergenic spacer, clpP intron 2) from the trees. These markers have had proven utility in previous studies (Shaw et al. 2005; Smith et al. 2008). Heterozygous genotypes in the nuclear (EF1 α) dataset were resolved using PHASE version 2.1.2 (Stephens et al. 2001). Rates of recombination among these haplotypes relative to the mutation rate were estimated in LAMARC version 2.1 (Kuhner 2006). The final moth DNA dataset included mtDNA sequences (COI +ND5) from 223 individuals (114 *T. antithetica* and 109 *T. synthetica*) and nuclear (EF1 α) DNA sequence data from 163 individuals (85 *T. antithetica* and 78 *T. synthetica*) from 42 populations, as well as five additional outgroups sampled from across the Prodoxidae. The final *Y. brevifolia* cpDNA dataset included 77 sequences from 33 populations, and 10 outgroups sampled—in order of increasing phylogenetic distance—from five other species within the genus *Yucca*, three other genera within family Agavaceae, one other family (Orchidaceae) within the order Asparagales, and one other order (Acorales) within the Monocotyledonae. Phylogenetic relationships between species and genealogical relationships between alleles for each genome (mitochondrial, nuclear, or chloroplast) were inferred using MrBayes version 3.12 (Huelsenbeck and Ronquist 2001).

Rates of molecular evolution for each gene region were estimated from the topologies inferred by MrBayes using r8s version 1.71 (Sanderson 2002), constraining the ages of key nodes in the trees based on published fossil age- and molecular clock-estimates. Divergence times in the chloroplast DNA topologies were calibrated using the age of the Asparagales and the age of the Agavaceae as constraints. The age of the common ancestor of the Asparagales was constrained to be between 93 MY and 120 MY following the constraints used by Ramirez et al. (2007). The age of the common ancestor of the Agavaceae sensu stricto (*Hesperoyucca* + *Hesperaloe* + *Agave* + *Yucca*) was constrained to be between 14 MY (the oldest known fossil from the group) (Tidwell and Parker 1990) and 32 MY (the upper range of age estimates based on a previous relaxed clock estimate (Smith et al. 2008)). Divergence times in the moth mtDNA and nuclear gene trees were calibrated by setting the age of the *Yucca*-feeding prodoxids (*Prodoxus* + *Parategeticula* + *Tegeticula*) to be 29.91 MY (Althoff et al., unpubl. ms.). To account for phylogenetic uncertainty when estimating locus-specific substitution rates, rate estimates were profiled across a sample of the post-burn-in trees estimated by MrBayes.

Adult moths were identified to species based on differences in wing coloration, external male genitalia, and overall body size described by Pellmyr and Segraves (2003). Joshua tree varieties were identified based on tree morphology, using a multivariate

analysis of variance (MANOVA) and a measure of classification accuracy based on a logistic regression model described in Godsoe et al. (2008). Rates of gene flow and divergence times between moth species and Joshua tree varieties were estimated using IM (Hey and Nielsen 2004). The mutation rates estimated in r8s were used to produce parameter estimates scaled in real time. Parameter estimates were averaged across three independent runs, after assessing convergence between runs. Independent runs were assumed to have converged when the correlation in the posterior probabilities of parameter point estimates between runs exceeded 0.9.

Wright (1978) showed that a single migrant per generation is sufficient to prevent divergence by genetic drift. To test the hypothesis that moth species and Joshua tree varieties were reproductively isolated from one another, we multiplied the coalescent-scaled, asymmetric migration rates estimated by IM by the geometric mean of the per-locus mutation rate and the effective population size to produce the posterior distribution of Nm , the number of haplotypes moving between populations per generation. We then summed the posterior probabilities of parameter point estimates for values of $Nm \geq 1$, to determine probability that migration has been sufficient to prevent divergence.

To test the hypothesis that the moths and the trees diverged at the same time, we summed under the credibility curve of the estimated divergence times to calculate the probability that the trees diverged contemporaneously with, or more recently than the moths. To test for common biogeographic patterns in the two partners, we filtered post burn in trees from the cpDNA data to calculate the probability that populations of *Y. brevifolia* pollinated by different species of moths are reciprocally monophyletic, and filtered post-burn-in trees from the moths' nuclear and mitochondrial datasets to calculate the probability that the moths reflect the same phylogeographic patterns found in the cpDNA.

More details about laboratory and analytical procedures, as well as collection locality data and GenBank accession numbers are provided as Supporting material.

Results

Phylogenetic analyses of the chloroplast data revealed support for the monophyly of *Yucca* (81% posterior probability), and for the monophyly of *Y. brevifolia* (99% posterior probability) (Fig. 2). As in previous analyses of cpDNA data from the Agavaceae, here we found strong support (100%) for *Agave* as the sister to *Yucca*. Within *Y. brevifolia* there was strong support (96–99%) for the monophyly of three geographically clustered populations, including a group that spans the Colorado River Valley to unite populations from Arizona with some southern-California localities. There was, however, no evidence for the monophyly of trees pollinated by either moth species, and two of the well-supported clades

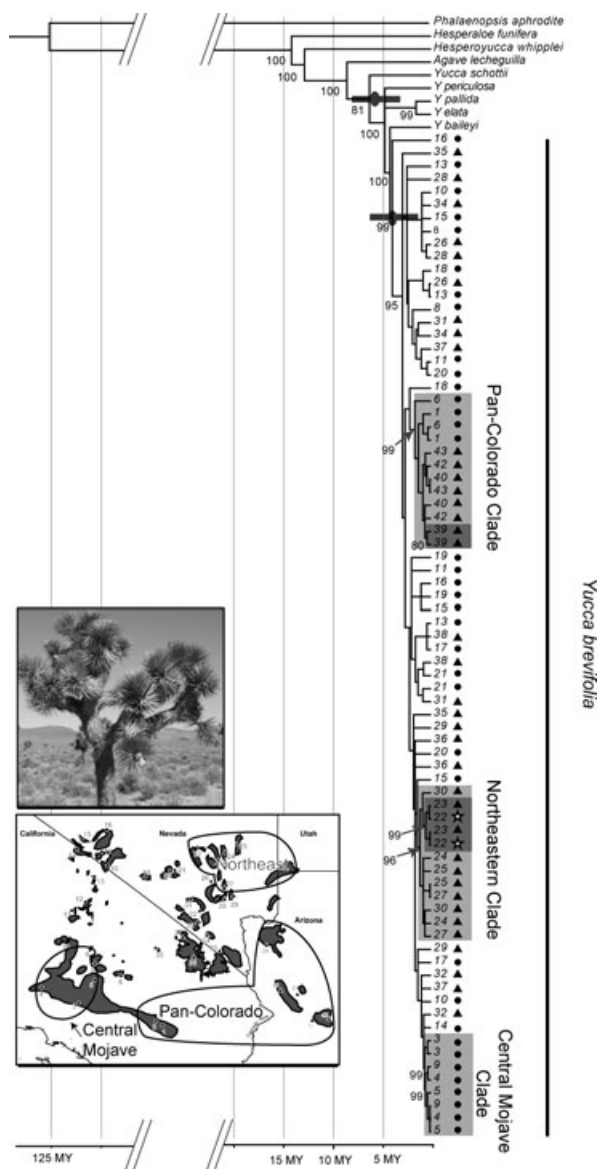


Figure 2. A chronogram of cpDNA sequence data from *Y. brevifolia* and selected taxa from across the Asparagales inferred using penalized likelihood. *Acorus calamus*, used to root the tree, was pruned in the r8s analysis and is therefore omitted from this figure. The topology underlying the chronogram was selected at random from post-burn-in trees. The root of the in-group tree was constrained to be between 125 and 105 MY old (Ramirez et al. 2007); the age of the Agavaceae sensu stricto (all taxa except *Phalaenopsis aphrodite*) was constrained to be between 32 and 14 MY old (Tidwell and Parker 1990; Smith et al. 2008). Taxon names indicate the population from which samples were obtained. Symbols beside taxon names indicate the species of pollinator associated with that population: *T. synthetica*, circles; *T. antithetica*, triangles; both species, stars. Shading indicates geographically structured clades within *Y. brevifolia*. Internal node labels show clade posterior probabilities estimated in MrBayes; unlabelled internal nodes have <50% posterior probability. Error bars on internal nodes show the mean and standard deviation of clade ages profiled across 100 post-burn-in trees.

within *Y. brevifolia* include both *T. antithetica*- and *T. synthetica*-pollinated populations.

Analyses of mitochondrial and nuclear sequence data from the moths (Fig. 3) identified strong support (100% in mtDNA; 99% in nuclear data) for the monophyly of the pollinating moths, and for the monophyly of the Joshua tree pollinators (*T. synthetica* + *T. antithetica*). However, nuclear and mitochondrial datasets differed in the inferred relationship between *T. synthetica* and *T. antithetica*; whereas the mtDNA found these species to be reciprocally monophyletic, in the topology inferred from EF1- α (not shown) *T. synthetica* was nested within a paraphyletic *T. antithetica*.

MUTATION RATE ESTIMATES

Mutation rates estimated in r8s for the *Yucca* cpDNA data were approximately 0.0008 substitutions/site/MY and 0.1 insertions/deletions per locus per million years (Table 1). In the moth mitochondrial genome (Table 2), substitution rates were approximately 0.009 substitutions/site/MY for the COI gene, and varied between ~ 0.035 and ~ 0.057 substitutions/site/MY for ND5. The moth nuclear data (EF1 α dataset) produced surprisingly high substitution rate estimates that varied between ~ 0.018 and ~ 0.06 substitutions/site/MY. The rate estimate for both the EF1 α and ND5 datasets exhibit substantial rate heterogeneity across the trees, suggesting that these lineage-specific rates should not be applied incautiously in other systems.

DIVERGENCE TIME AND MIGRATION RATE ESTIMATES

The maximum likelihood estimates of the asymmetric migration rates between western and eastern populations of *Y. brevifolia*, and between *T. antithetica* and *T. synthetica* (Table 3 and Fig. 4), were all low, consistent with a history of isolation. However, based on the posterior distribution of Nm, the asymmetric rate of chloroplast gene flow from western (*T. synthetica*-pollinated) trees into populations of eastern (*T. antithetica*-pollinated) trees was not significantly less than one, which may suggest a history of introgression, or chloroplast capture.

The maximum likelihood estimate of the time to divergence between *T. antithetica* and *T. synthetica* was ~ 1.14 MY (Fig. 5). There was no single maximum likelihood estimate of the divergence times between eastern and western populations of *Y. brevifolia*; although divergence times of less than ~ 1.8 MY can be rejected, all those above ~ 5 MY are about equally probable. (Note, however, that the chronograms estimated in r8s suggest that the time to common ancestry of cpDNA haplotypes is not likely to be greater than ~ 7 MY).

EXPLICIT TESTS OF BIOGEOGRAPHIC HYPOTHESES

Both the phylogenetic and population-genetic analyses offer strong evidence against a common biogeographic history for

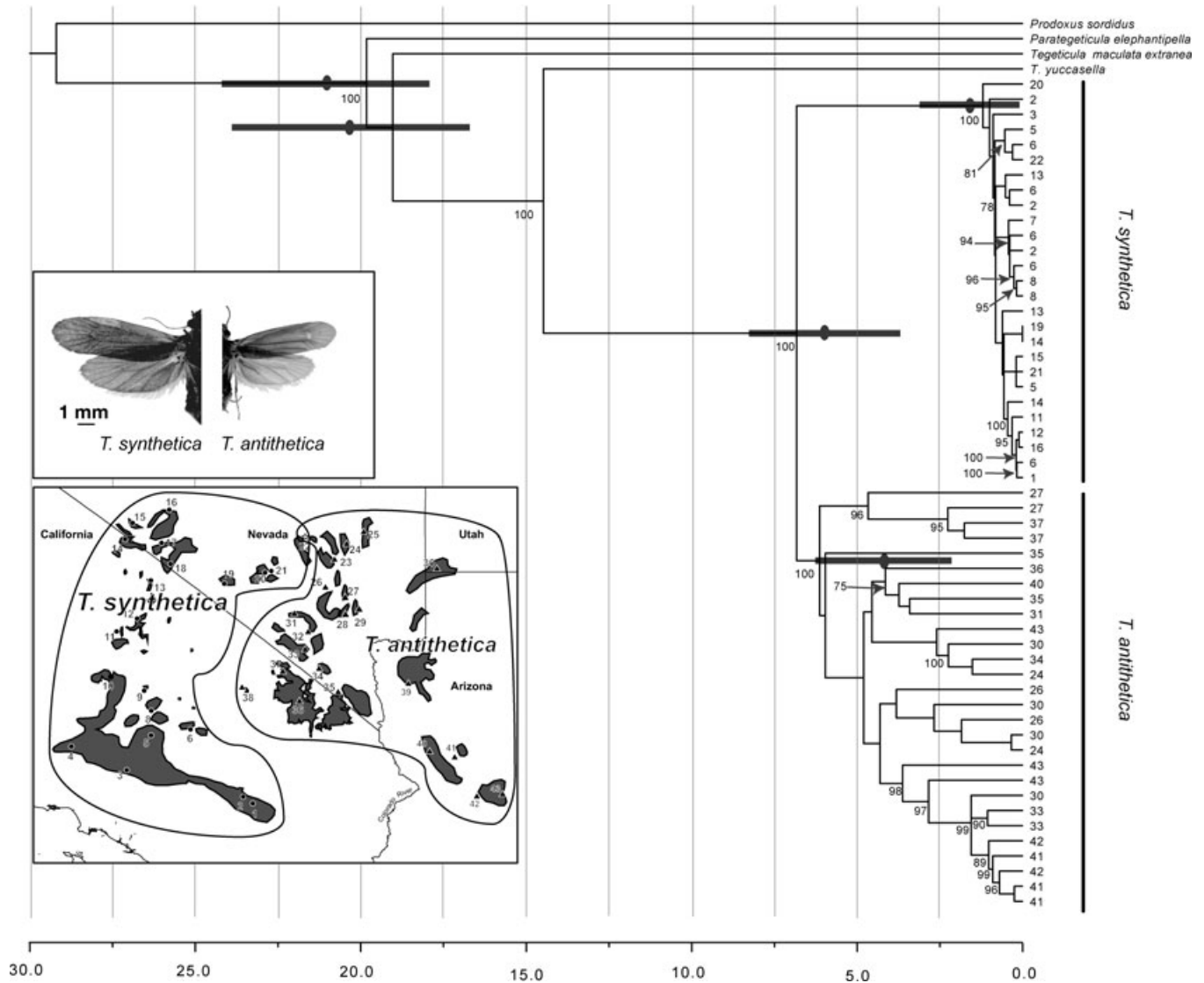


Figure 3. A chronogram of subsampled mtDNA sequence data from the two pollinating yucca moths associated with Joshua tree and from selected species within the yucca-feeding members of the Prodoxidae, estimated using penalized likelihood. *Lampronia rubiella*, used to root the tree, was pruned in the r8s analysis and is therefore omitted from this figure. Sequence data represent a random sample of 25% of the total sequences for these taxa. The topology underlying the chronogram was selected at random from post-burn-in trees; branch lengths are from the COI partition. The root of the in-group tree was assigned a fixed age of 29.19 MY (Althoff et al., unpubl. ms.). Taxon labels indicate the population from which samples were obtained. Internal node labels show clade posterior probabilities estimated in MrBayes; unlabelled nodes have <50% posterior probability. Error bars on internal nodes show the mean and standard deviation of clade ages profiled across 100 post-burn-in trees. The mismatch in some nodes between the average ages and the age shown in this tree reflect the fact that this is one randomly selected topology, not a consensus tree.

Joshua trees and their pollinators. Of the 1,352 post-burn-in trees from the MrBayes analysis of the cpDNA data, none were compatible with either the monophyly of trees pollinated by *T. antithetica*, or the monophyly of trees pollinated by *T. synthetica*. Similarly, none of the 17,800 post-burn-in trees for the moth EF1 α DNA sequence data, nor any of the 4,655 post-burn-in trees for moth mtDNA sequence data were compatible with the monophyly of the

three biogeographic groups (Central Mojave, Northeastern, Pan-Colorado) identified in the plant cpDNA sequence data. Finally, the divergence time estimates produced by IM suggest significant differences in the relative timing of isolation between the two moth species and the Joshua tree populations they pollinate. Although IM failed to identify a single maximum likelihood estimate for the time to divergence between tree populations, the probability

Table 1. Diversity indices for plant chloroplast data: segregating sites (S), number of singletons (η_1), average pair-wise differences per site (π) and the estimated mutation rate per site, per million years ($S/S/MY$) for sequence data; number of variable loci (N) and mutation rate per locus per million years for indels ($M/L/MY$). Mutation rates were estimated in *r8s*, averaging across branches and profiling across 100 post-burn-in trees inferred in MrBayes.

	Sequence data			
	S	η_1	π	$S/S/MY$
<i>Yucca</i>	32	24	0.0008	0.00085+/-0.00016
<i>Y. brevifolia</i>	11	6	0.0005	0.00076+/-0.00029
	Indels			
	N		$M/L/MY$	
<i>Yucca</i>		9	0.12+/-0.31	
<i>Y. brevifolia</i>		3	0.11+/-0.28	

that these populations diverged contemporaneous with, or more recently than, their moth pollinators is less than one percent ($P = 0.006$).

Discussion

Both the phylogeographic data and the divergence time estimation suggest that a history of covariance between *Y. brevifolia* and its pollinators can be statistically rejected. Eastern and western populations of *Y. brevifolia* appear to have diverged at least 5 MY ago, an age that is not inconsistent with the hypothesis that these populations were isolated from one another by the Bouse Embayment (9–4 MY ago), or some other contemporaneous vicariance event. However, the time to divergence between the two moth species that pollinate these trees is significantly younger, with an age of ~1.14 MY being best supported by the data.

Although the recent divergence in the moths is, at first glance, surprising—particularly given the relatively deep time to common ancestry in the mtDNA sequence data (about 5.7 MY, see Fig. 3)—we suspect that this finding is not in error for two reasons. First, the time to common ancestry of gene sequences drawn from two isolated populations must necessarily be older than the time to divergence between them (Edwards and Beerli 2000). Second, very large estimates of the effective population sizes for the extant and ancestral moth species (roughly 200,000) and the paraphyly of *T. antithetica* in the *EF1 α* data both suggest that the average time to coalescence in the ancestral population should be relatively

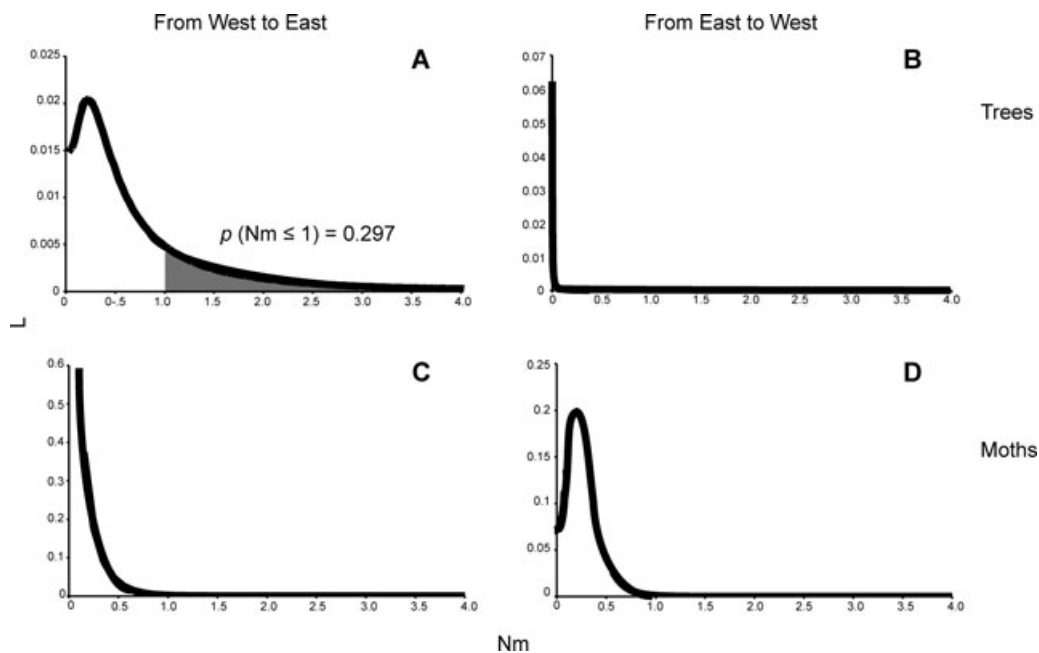


Figure 4. Posterior distribution of asymmetric gene flow estimates in *Y. brevifolia* (A, B) and their pollinating yucca moths (C, D). Gene flow estimates are expressed as Nm , the product of the effective population size and the migration rate, giving the average number of migrants per generation. A) Chloroplast gene flow from populations of western (*T. synthetica*-pollinated) trees into populations of eastern (*T. antithetica*-pollinated) trees. $P(Nm \geq 1) = 0.297$ B) Chloroplast gene flow from populations of eastern (*T. antithetica*-pollinated) trees into populations of western (*T. synthetica*-pollinated) trees. $P(Nm \geq 1) \ll 0.0001$. C) Gene flow from *T. synthetica* into *T. antithetica*. $P(Nm \geq 1) = 0.0016$. D) Gene flow from *T. antithetica* into *T. synthetica*. $P(Nm \geq 1) = 0.0024$. Note: Y-axes are not to scale.

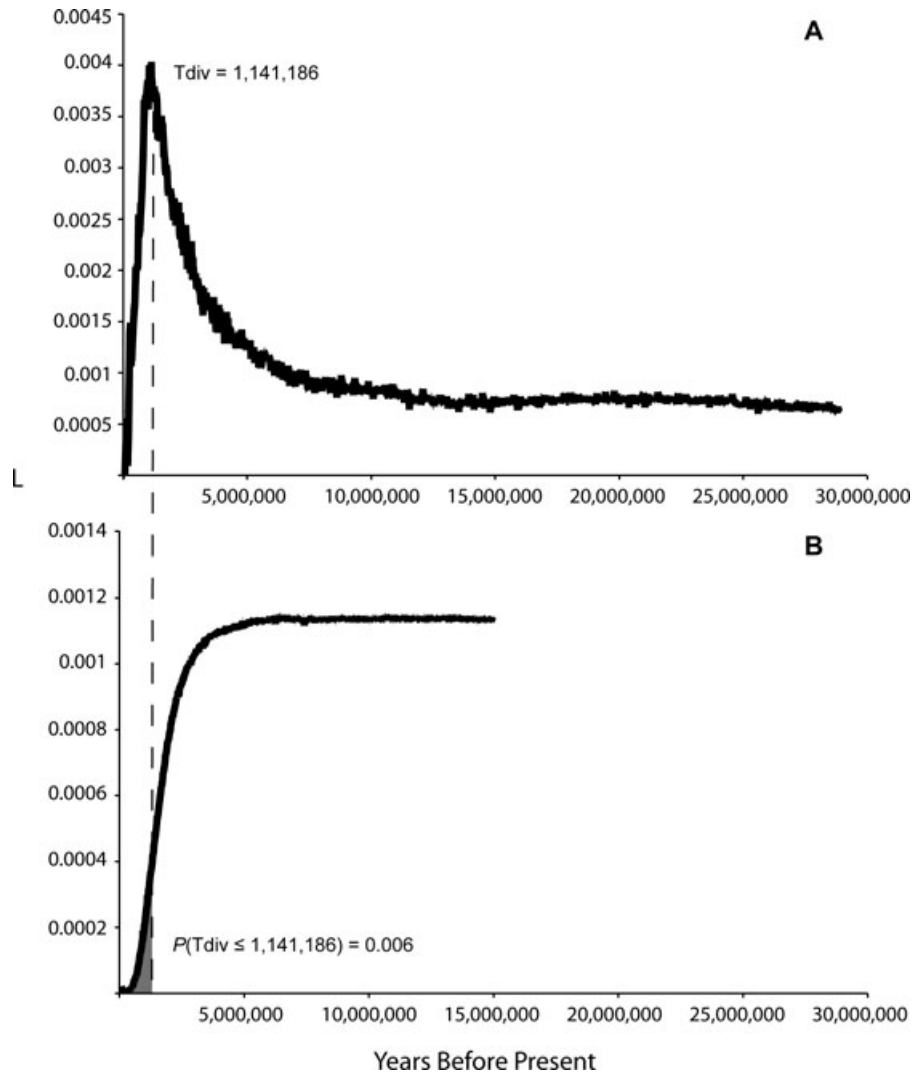


Figure 5. Posterior distribution of divergence times between *T. antithetica* and *T. synthetica* (A) and between eastern (*T. antithetica*-pollinated) and western (*T. synthetica*-pollinated) trees (B). The maximum likelihood estimate of the divergence time for the moths is ~ 1.14 MY ago, and is significantly less than the divergence time between eastern and western trees ($P < 0.01$). Note: Y-axes are not to scale.

large. We should therefore expect a large difference between the time to divergence and the time to common ancestry of all haplotypes.

Given these results, it seems likely that an ancestral population of *Y. brevifolia* was divided by an extrinsic barrier that was sufficiently strong to prevent seed dispersal between eastern and western populations, but that pollinators continued to disperse across this barrier. Only much more recently did sufficient isolation permit divergence between the moth populations to evolve. This may have come either from a secondary biogeographic event, or from the gradual development of distinct moth and floral morphologies across the range that would have disadvantaged both hybrid moths and trees produced by cross-pollination.

Of course, these data provide insight only into the relative age of reproductive isolation in *Y. brevifolia* and its pollinators,

and cannot directly inform us about the timing of anagenetic changes, such as the evolution of different floral morphology and ovipositor length. Likewise, historical data such as these cannot address the nature of natural selection acting in this system. The evidence for rapid evolution in floral morphology presented by Godsoe and colleagues (2008) argues strongly for pollinator-mediated selection as the agent underlying divergence in the trees. However, the historical sequence we infer here—an earlier divergence time in the trees than in their pollinators—could be consistent with scenarios that do not involve coevolution in the strict sense of reciprocal natural selection, such as the moths tracking independent changes in the trees' morphology. Additional ecological studies will ultimately be necessary to disentangle the relative contributions of selection on the pollinators and their hosts and to identify the evolutionary processes underlying patterns of

Table 2. Diversity indices for moth sequence data, including number of segregating sites (S), number of singletons (η_1), ratio of nonsynonymous to synonymous mutations (d_N/d_S), average pair-wise differences per site (π) and the estimated mutation rate per site, per million years (S/S/MY). Mutation rates were estimated in r8s, averaging across branches and profiling across 100 post-burn-in trees inferred in MrBayes. Clades without rate estimates were not recovered as monophyletic in the EF1 α data.

	S	η_1	d_N/d_S	π	S/S/MY
COI					
Pollinating yucca moths (<i>Tegeticula</i> and <i>Parategeticula</i>)	226	110	22/218	0.018	0.009+/-0.002
<i>Tegeticula</i>	185	86	21/174	0.017	0.009+/-0.002
Joshua tree pollinators (<i>T. synthetic</i> and <i>T. antithetica</i>)	122	41	14/112	0.016	0.009+/-0.003
<i>T. antithetica</i>	54	24	6/50	0.004	0.008+/-0.003
<i>T. synthetic</i>	68	29	10/58	0.005	0.009+/-0.003
ND5					
Pollinating yucca moths (<i>Tegeticula</i> and <i>Parategeticula</i>)	53	27	12/47	0.016	0.057+/-0.006
<i>Tegeticula</i>	40	17	9/34	0.016	0.050+/-0.008
Joshua tree pollinators (<i>T. synthetic</i> and <i>T. antithetica</i>)	29	9	5/25	0.015	0.042+/-0.010
<i>T. antithetica</i>	18	7	4/15	0.005	0.035+/-0.012
<i>T. synthetic</i>	11	5	1/10	0.005	0.037+/-0.013
EF1 α					
Pollinating yucca moths (<i>Tegeticula</i> and <i>Parategeticula</i>)	59	33	1/61	0.005	0.060+/-0.010
<i>Tegeticula</i>	42	23	1/43	0.004	–
Joshua tree pollinators (<i>T. synthetic</i> and <i>T. antithetica</i>)	23	4	0/23	0.004	0.026+/-0.006
<i>T. antithetica</i>	8	3	0/7	0.001	–
<i>T. synthetic</i>	14	1	0/15	0.003	0.018+/-0.007

phenotype matching in this system (see discussion in Gomulkiewicz et al. (2007)).

REPRODUCTIVE ISOLATION BETWEEN JOSHUA TREE POPULATIONS

A somewhat surprising and unexpected result was the relatively high rate of chloroplast gene flow from western populations into eastern populations. Given the significant differences in tree growth form and floral morphology between eastern and western populations (Godsoe et al. 2008), one might have expected to see nearly complete reproductive isolation between these demes. Indeed, previous taxonomic revisions have recommended the elevation of the western and eastern tree types to varietal or even species status (McKelvey 1938; Webber 1953; Lenz 2007). The inference of ongoing gene flow is likely due to the Northeastern and Pan-Colorado haplotype clades (Fig. 2), that encompass both eastern (*T. antithetica*-pollinated) and western (*T. synthetic*-pollinated) tree types, and which are nested within a larger clade of western trees. It is possible that these shared haplo-groups can be attributed to deep coalescence, but as the IM analysis used here is specifically designed to distinguish recurrent gene flow from deep coalescence, this scenario is unlikely to explain the high migration estimates. Instead, this finding might be explained by chloroplast capture (Rieseberg and Soltis 1991; Soltis and Kuzoff 1995), driven either by selection or by pollinator-mediated (nuclear) gene flow from eastern trees into populations

of formerly western trees that retain western chloroplast haplotypes.

This unidirectional pattern of gene flow also suggests that there may be some asymmetry in the degree of plant/pollinator incompatibility between regions. Godsoe and colleagues (2008) speculated that pollinator host specificity in this system is mediated by phenotype matching between the size of the moth's ovipositor and the length of floral style, and previous work on *Y. filamentosa* suggests that the injury of plant ovules during moth oviposition may affect floral abscission rates (Pellmyr and Huth 1994, 1995). It could be that in this case the larger ovipositor of the (western) *T. synthetic* causes sufficient damage to induce floral abscission if these moths attempt to oviposit into the short-styled eastern trees, but that the smaller *T. antithetica* can successfully oviposit into (and subsequently pollinate) trees of either type.

MUTATION RATES AND DIVERGENCE TIMES

The data presented here provide new estimates of rates of molecular evolution in the chloroplast and insect mitochondrial genomes, and a first estimate of the absolute rate of sequence evolution in EF1 α for the Insecta. The mutation rates estimated here for the chloroplast data are quite similar to those previously estimated for these same gene regions across the Agavaceae (Smith et al. 2008), despite a somewhat younger calibration (105 MY) for the maximum age of the monocotyledons used in the former paper. Likewise, the age of *Yucca sensu stricto* inferred here (6.07 MY),

Table 3. IM results. Maximum likelihood estimates (ML), mean, and 95% credibility interval for time to divergence between populations (Tdiv) in millions of years, and asymmetric migration rates (Nm), expressed as number of gene copies moving between populations per generation.

	Tdiv			
	ML	Mean	lower 95%	upper 95%
Joshua trees	5.30 MY	7.32 MY	1.88 MY	14.57 MY
Yucca moths	1.14 MY	1.02 MY	0.79 MY	27.91 MY
	Nm West to East			
	ML	Mean	lower 95%	upper 95%
Joshua trees	0.223	0.456	0.027	5.497
Yucca moths	0.050	0.050	0.050	0.553
	Nm East to West			
	ML	Mean	lower 95%	upper 95%
Joshua trees	0.0001	0.007	0.0001	0.094
Yucca moths	0.166	0.233	0.033	0.632

is quite similar to the 6.41 MY estimate inferred by Smith et al. (2008).

Within the insect mitochondrial genome, previous estimates of the rate of sequence divergence (roughly twice the substitution rate if multiple hits are neglected) in COI vary between 1.5%/MY and 2.3%/MY (Brower 1994; Farrell 2001), so the current estimate falls well within this range. The overall similarity of our mutation rate and divergence time estimates to previously published studies that used different fossil calibrations suggest that our results are robust to alternative datings of particular nodes.

However, the mutation rate estimated here for the ND5 gene is considerably greater than previous estimates (e.g., Braby et al. (2005)), and although rates of evolution in EF1 α have not previously been explicitly estimated for the Insecta, the EF1 α rate estimates also seem quite high. Whereas our rate estimates vary between 1.8% and 6% per million years, extrapolation from published sequence data for other groups suggests that rates of 0.1–0.5% per million years are more typical (C. I. Smith, unpubl. data). Nevertheless, the mutation rates estimated here may not necessarily be inaccurate; a number of studies have shown that observed substitution rates may decay over time as weakly deleterious mutations are gradually purged, and as a result the substitution rate observed on a phylogenetic time scale may be as much as an order of magnitude lower than that the mutation rate visible on a microevolutionary time scale (Ho et al. 2005, 2007; Ho and Larson 2006). This “time dependency” may account for the differences between the apparent mutation rates inferred here and those typical of nuclear markers on a phylogenetic time scale; additional

analyses including more fossil-calibration points could address this hypothesis.

Given these considerations, extreme caution is clearly warranted in applying these rate estimates to other phylogenetic problems, or to divergence time estimation in other systems. Conversely, because IM uses the geometric mean of mutation rates across all loci to convert the divergence time estimates from coalescent-scaled time units into years, and because the EF1 α dataset is quite small relative to the mtDNA data, estimates of the overall divergence time between species should be robust to errors in rate estimation for this partition.

Conclusions

A small but growing number of studies have identified patterns of phylogenetic congruence between plants and pollinators in obligate pollination mutualisms. As with many biological questions, it has often been difficult to distinguish among alternative ecological and evolutionary processes that may give rise to this pattern. Particularly in the case of pollination mutualisms, in which patterns of gene flow in the plant are often entirely determined by pollinator dispersal, it is difficult to distinguish cospeciation driven by coevolutionary interactions per se, from a common biogeographic history that is often shared between codistributed organisms.

By using chloroplast DNA sequence data as a source of biogeographic information that is independent of pollinator-mediated gene flow, we identify statistically significant discord in phylogeographic patterns and divergence times between Joshua trees and their pollinators. This finding suggests that we can reject the hypothesis that apparent codivergence in this system is the product of extrinsic biogeographic factors. Instead, intrinsic features of the interaction itself may have produced this pattern. Additionally, our results suggest an asymmetry in the degree of reproductive isolation between eastern and western tree types, which may be mediated by differences in pollinator host specificity or floral abscission.

These findings suggest several important predictions. First, as information about mutation rates in the plant nuclear genome becomes available, these data should likely show divergence times more consistent with the pollinator ages, rather than the chloroplast data. Second, the smaller *T. antithetica* moth may be capable of successfully pollinating western trees, despite the differences in moth and flower phenotypes. Finally, these data suggest that there may not be intrinsic barriers to reproduction between different Joshua tree morphotypes, but that these populations are maintained as evolutionarily distinct groups by pollinator specificity.

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