The influence of interaction type and feeding location on the phylogeographic structure of the yucca moth community associated with *Hesperoyucca whipplei*

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Abstract

The interactions between herbivorous insects and their host plants have been central in generating diversification in both groups. We used a community of four yucca moth species, monophagous on the host plant *Hesperoyucca whipplei* (Agavaceae), to examine how the type of interaction and where insects feed within a plant influence phylogeographic structure of herbivorous insects. These four species included two fruit-feeders, one mutualistic and one commensalistic, and two commensalistic stalk-feeders. Surveys based on mtDNA cytochrome oxidase I sequence data demonstrated that the moth species differed in phylogeographic history. Populations of the mutualist pollinator, *Tegeticula maculata*, exhibited the most subdivision in comparison to the three commensal *Prodoxus* species (both genera in Lepidoptera, Prodoxidae). Feeding location was also correlated with differences in phylogeographic history through its influence on population sizes and the probability of gene flow. The results suggest that both the outcome of interactions and where insects feed may influence population structure.
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1. Introduction

The process of specialization in species interactions is one of the driving forces in diversification, particularly for herbivorous insects. Studies at both the micro- and macro-evolutionary levels have demonstrated that insect adaptation to host plant species can lead to divergence (Funk et al., 2002). At the microevolutionary level, ecological specialization to different host plant species leads to genetic differentiation and speciation, sometimes in sympatry (Abrahamson et al., 2003; Berlocher and Feder, 2002; Funk, 1998; Nosil et al., 2002; Sword et al., 2005). At the macroevolutionary level, insect lineages may specialize and phylogenetically track plant lineages (Farrell, 1998; Janz and Nylin, 1998; Jousselin et al., 2006; Kergoat et al., 2005; Percy et al., 2004). In some cases, coevolution between insects and plants may be responsible for generating diversification via cospeciation between lineages (Kato et al., 2003; Ronsted et al., 2005; Weiblen, 2004).

The relationships between insects and plants represent a multitude of interaction types along a continuum from antagonism to commensalism to mutualism, and a diversity of life habits from parasites to grazers to predators (Thompson, 1982). Not all interactions between insects and plants, however, lead to specialization and diversification of lineages. One approach for understanding the conditions in which specialization is linked to diversification is to examine the insect community on a given set of plant species. Such a comparative framework may lead to predictions about patterns of specialization and diversification. For

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example, Stireman et al. (2005) examined the genetic structure of nine herbivore species that feed on the goldenrods *Solidago altissima* and *S. gigantea* (Asteraceae). Four of the nine species exhibited genetic structure consistent with divergence due to host plant specialization. Based on the life habits of these nine herbivores, Stireman et al. (2005) proposed that greater intimacy with plant tissues predisposes insect taxa to specialization and differentiation. Internal feeders such as miners and gallers are more likely to exhibit host-associated divergence than external feeders. This study demonstrates the importance of community phylogenetics in understanding evolutionary patterns within herbivorous insects communities.

Here, we take a similar community approach by examining the patterns of phylogenetic structure within the specialist yucca moths that feed on the host plant *Hesperoyucca whipplei*. We were interested in how differences in the type of interaction (mutualism or commensalism) and where an insect feeds within a plant may influence phylogenetic structure. For example, mutualistic seed-eating pollinators experience selective pressures associated with not only feeding on the plant, but also moving plant gametes. Variation among populations in plant traits such as floral architecture and phenology may drive local adaptation in pollinator populations and restrict gene flow. Commensalist species, however, do not experience the same selective pressures as mutualists, may not be as locally adapted and, hence, individuals may be more likely to colonize different plant populations. Additionally, where insects feed within a plant also may have consequences for genetic subdivision. Insects that oviposit and feed in more ephemeral plant parts such as flowers or fruit have a more limited window of opportunity to find suitable oviposition sites in comparison to species that feed on other, less ephemeral plant parts such as shoots and leaves. Thus, fruit-feeding species may be more genetically subdivided because the opportunity for gene flow among populations may be restricted in comparison to species that feed in shoots or leaves.

The yucca moth community on *H. whipplei* contains four species that differ in how they interact with the plant and where they feed within the plant. One species, *Tegeticula maculata*, is a mutualistic seed-eating pollinator that feeds on seeds within fruits, and the three other species in the genus *Prodoxus* are commensalists (Davis, 1967; Powell and Mackie, 1966). One of the *Prodoxus* species feeds within the fruit, like *T. maculata*, whereas the other two are internal stalk-feeders and show vertical resource partitioning (Fig. 1; Powell and Mackie, 1966). This simple community provides the opportunity to examine how interaction type (mutualism vs. commensalism) and feeding location (fruit vs. stalk) may correlate with phylogenetic structure within species. The relationship between *H. whipplei* and its four yucca moth specialists is especially well suited for a comparative approach for three reasons. First, the moths are monophagous on *H. whipplei*—adults mate and larvae feed exclusively on *H. whipplei*. Second, the four species have experienced the same biogeographic processes throughout their evolutionary history, which may reach back to an origin of the association 41.7 ± 11.1 mya (Pellmyr and Leebens-Mack, 1999). Finally, the three *Prodoxus* species form a monophyletic clade and appear to have speciated nearly simultaneously (Pellmyr et al., 2006). Thus, differences in the phylogenetic structure among the species are likely to be determined by life history attributes or other ecological attributes, rather than by differences in when they colonized the host plant or the age of the lineages.
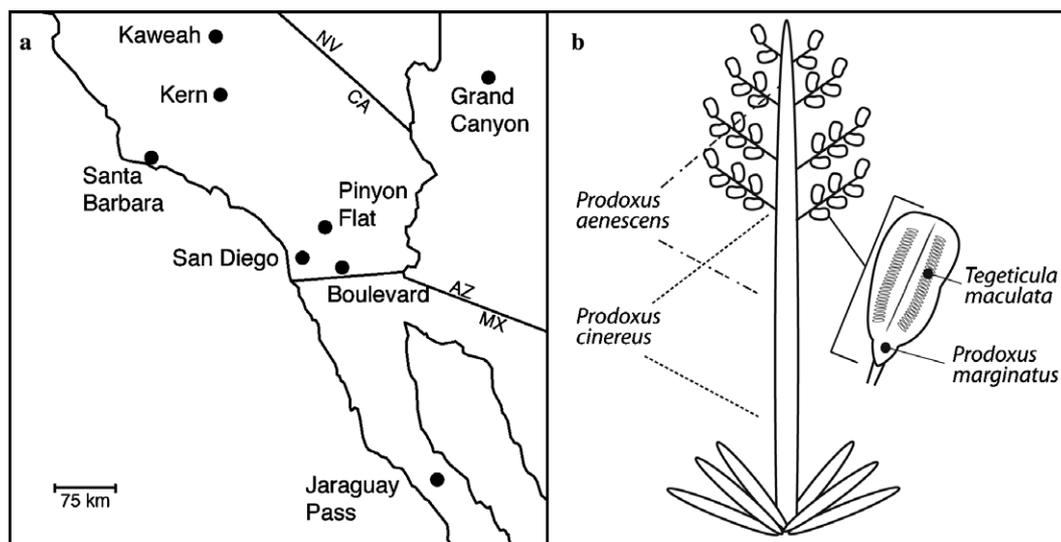

Fig. 1. (a) Map of localities sampled for the yucca moth community associated with *Hesperoyucca whipplei*. (b) Schematic showing feeding locations for the yucca moths. The commensals *Prodoxus aenescens* and *P. cinereus* feed within the inflorescence stalk. The mutualist pollinator *Tegeticula maculata* feeds on seeds within the fruit, whereas the commensal *P. marginatus* feeds in the vegetative parts of the fruit, especially near the attachment of the pedicel. All four species co-occur on the same plants.

Segraves and Pellmyr (2001) demonstrated that populations of the pollinator moth *T. maculata* were phylogenetically structured based on historical biogeography. We build on that study by elucidating the phylogenetic structure among populations within three moth species in the genus *Prodoxus*. In particular, we were interested in comparing the patterns of phylogenetic structure to address the following two questions: (1) Do mutualistic and commensalistic species have different phylogenetic structures? (2) Do fruit-feeding species exhibit different phylogenetic structure than stalk-feeding species?

1.1. Study system

1.1.1. The plant

Hesperoyucca whipplei is the sole member of its genus, which together with the small, non-moth-pollinated genus *Hesperaloe*, constitutes the sister group of the genus *Yucca* (Bogler et al., 1995). This plant is patchily distributed throughout southern California, northwestern Arizona, and north-central Baja California and Sierra Pinacate, Mexico. It exhibits variation in life history: in some populations, individuals grow as a single monocarpic rosette, whereas in others an individual plant may form clumps of rosettes which effectively makes these plants iteroparous (Powell and Mackie, 1966). Plants flower in the spring or early summer by growing a 2–5 m inflorescence stalk from the center of the rosette. Inflorescences contain one hundred to thousands of flowers which open apicad-laterad within the inflorescence (Aker, 1982; Aker and Udovic, 1981; Powell and Mackie, 1966; Udovic, 1981). Individual flowers are only open for a single day. Because rosettes die after flowering, researchers have proposed separation of monocarpic and clonal populations into separate subspecies (Haines, 1941; McKelvey, 1947; Webber, 1953), but some named entities have been raised from the seed of a single plant (DeMason, 1984).

1.1.2. The moths

The four yucca moths that utilize *H. whipplei* are members of the genera *Tegeticula* and *Prodoxus*. Adults mate within the flowers and the internally feeding larvae feed exclusively on *H. whipplei* tissue associated with the inflorescence. The species differ, however, in the way they interact with their host plants and where they feed (Fig. 1). The pollinator, *T. maculata*, actively pollinates yucca flowers and lays eggs next to developing yucca ovules in freshly opened flowers (Aker, 1982; Aker and Udovic, 1981; Davis, 1967; Pellmyr, 2003; Powell and Mackie, 1966; Richter and Weis, 1998). The larva completes its development inside fruit, where it feeds on maturing seeds. Because the moth larvae feed on only a subset of seeds, the interaction is mutualistic. Segraves and Pellmyr (2001) sampled moths from eight sites throughout the range of *T. maculata* that included both of the morphologically distinct subspecies *T. m. maculata* and *T. m. extranea*.

The other three moth species are from the genus *Prodoxus* and form a monophyletic clade within the genus (Pellmyr et al., 2006). These species do not pollinate, they feed on plant tissues other than seeds, and are likely commensalistic or slightly antagonistic with *H. whipplei* (Althoff et al., 2004; Davis, 1967; Powell and Mackie, 1966). One species, *P. marginatus*, feeds within the petiole and the tissue at the base of yucca fruits, but does not enter the locule to feed on seeds. Females visit freshly opened flowers as well as flowers that have been pollinated. The other two species, *P. aenescens* and *P. cinereus*, oviposit into the inflorescence stalk when it is fully elongated, and there is evidence of resource partitioning in that *P. aenescens* feeds in the upper portion and *P. cinereus* in the lower portion, with a small region of overlap (Fig. 1; Powell and Mackie, 1966). For all three *Prodoxus* species, there is variation from north to south in forewing and hindwing coloration (Powell and Mackie, 1966).

2. Methods

2.1. Moth collections

Mitochondrial DNA sequence data for *T. maculata* was obtained from a previously published study by Segraves and Pellmyr (2001). In order to make meaningful comparisons, we collected specimens of the three *Prodoxus* species from the same localities examined by Segraves and Pellmyr (2001): Kaweah, Tulare Co., CA (N36°32′ W118°55′), Kern Canyon, Kern Co., CA (N35°27′ W118°44′), Santa Barbara, Santa Barbara Co., CA (N34°44 W119°59′), Boulevard, San Diego Co., CA (N32°40′ W116°17′), San Diego, San Diego Co., CA (N32°53′ W117°05′), Pinyon Flat, Riverside Co., CA (N32°34′ W116°28′), Grand Canyon, Mohave Co., AZ (N35°33′ W113°20′), and Jaraguay Pass, Baja California, MX (N29°23′ W114°23′) (Fig. 1). These localities are distributed throughout the extant range of *H. whipplei*. A disjunct, small population is known from the Sierra Pinacate region of N Sonora, Mexico (Turner et al., 1995), but no samples were available from this site. The sample size was six individuals per locality. Although this number seems small, we chose to use the same sample size as Segraves and Pellmyr (2001) to reduce the possibility that differences in the phylogenetic structure were due to sampling intensity. Furthermore, based on Saunders et al. (1984) this gives a 71.4% probability (where $P = (n-1)/(n+1)$, $n =$ sample size) of sampling the deepest coalescent event. The localities included in the study were not identical across species due to a combination of differences in the ranges of the species and difficulties in obtaining specimens at some localities. A combination of field-collected adults, reared adults from stalk collections, and larvae from stalk dissections were used. Specimens were collected from 1996 to 2002 and stored at −80 °C until prepared for DNA extraction.

2.2. DNA sequencing

We removed the head, wings, and genitalia from each adult moth to keep as a voucher. Total genomic DNA from

the remaining thorax and abdomen was extracted using the IsoQuick DNA Extraction kit (Orca Research Inc., Bothell, WA). For larvae, the entire individual was used. We amplified approximately 750 bp of the 3′-end of the cytochrome oxidase I mitochondrial gene using the primers 2231F and 3020R for each of the three *Prodoxus* species. The numbers refer to the nucleotide positions in the *Drosophila yakuba* mtDNA genome (Clary and Wolstenholme, 1985) and are available upon request from the authors. The thermal cycler profile for initial amplification was 95°C for 2 min, 35 cycles of 95 °C for 1 min 52 °C for 45 s 72 °C for 1 min 30 s, 72 °C for 10 min. Amplicons were cleaned with the Qiagen PCR purification kit (Qiagen, Valencia, CA) before being used in cycle sequencing reactions. Sequencing reactions consisted of 4 µl of DNA product, 2 µl of ABI Big Dye Terminator Sequencing Mix, 2 µl of 2 µM primer, and 2 µl of sequencing buffer. Sequencing products were cleaned using Centri-Sep Sephadex columns (Princeton Separations, Inc., Adelphia, NJ), lyophilized, and resuspended in 10 µl of HiDi formamide from Applied BioSystems, Inc. Products were sequenced on an ABI 3730 automated DNA sequencer and contigs were made with Sequencher 3.1 (Gene Codes Corp., Ann Arbor, MI). Sequence alignment was conducted by eye.

2.3. Phylogenetic analyses

The mtDNA sequence data for each of the species were analyzed using maximum likelihood with a heuristic search in PAUP 4.0b10 (Swofford, 2002). To confirm the results of Segraves and Pellmyr (2001), we reanalyzed their data set. We first determined the model of sequence evolution using DT-ModSel (Minin et al., 2003). This procedure is based on the Bayesian information criterion and incorporates relative branch-length error when choosing a model of sequence evolution. The chosen model was then used in a heuristic search with random addition of taxa, 10 replicate searches, and TBR branch swapping in PAUP 4.0b10 (Swofford, 2002). The nonparametric bootstrap procedure (Felsenstein, 1985) was used to assess support for the nodes in the resulting tree topologies.

We also used a coalescent simulation approach (Knowles and Maddison, 2002; Knowles, 2001) to determine if the evolutionary histories of the four moths were different. In this approach, we used the pattern of population subdivision from the pollinator *T. maculata* as a constraint tree in which to simulate the coalescent of haplotypes from each of the *Prodoxus* species. The population tree for *T. maculata* was chosen because it provided the best phylogenetic resolution among the four species. The gene tree for each of the *Prodoxus* species was constrained within the population tree and the degree of discord was assessed using Slatkin and Maddison's (1989). We generated the null distribution to test for significant differences of *s* using a coalescent approach with a N_e of 100,000 individuals. We estimated the values of *s* for 100 gene trees simulated under the *T. maculata* population tree. Values of *s* not contained in this

estimated distribution would indicate a greater degree of discord than expected by chance alone. One caveat of this approach is that haplotypes are not contained in multiple populations. This was violated for all three *Prodoxus* species. For haplotypes that were shared among populations, we randomly assigned the shared haplotype to one population and excluded it from the others. This procedure, in essence, would bias the *Prodoxus* pattern of subdivision towards the *T. maculata* pattern because *T. maculata* populations did not share haplotypes. The coalescent analyses were performed in Mesquite version 1.06 (Maddison and Maddison, 2005).

3. Results

We obtained 723 contiguous bp of the 3′-end of mtDNA cytochrome oxidase I for each of the *Prodoxus* species which resulted in 13 haplotypes for *P. aenescens* (GenBank Accession Nos. DQ470083–DQ470095), 12 haplotypes for *P. cinereus* (GenBank Accession No. DQ470096–DQ470107), and 30 haplotypes for *P. marginatus* (GenBank Accession Nos. DQ470108–DQ470137). Segraves and Pellmyr (2001) used 755 bp of the 5′-end of cytochrome oxidase I for *T. maculata* and detected 18 haplotypes (GenBank Accession Nos. AF182761–AF182778). The DT-ModSel analysis returned the HKY + I model for *P. aenescens*, the TrN + I model for *P. cinereus* and *P. marginatus* and the GTR + Gamma model for *T. maculata*. Uncorrected sequence divergence among the unique haplotypes in each species were: *P. aenescens* (0.14–1.80%), *P. cinereus* (0.14–2.63%), *P. marginatus* (0.14–3.50%), and *T. maculata* (0.13–1.99%). Haplotype distribution among populations within species revealed some striking differences. Populations of the stalk-feeder *P. aenescens* contained both unique haplotypes and haplotypes shared with other populations (Table 1). In contrast, populations of the fruit-feeding *P. marginatus* and *T. maculata* and the stalk-feeding *P. cinereus* were mostly composed of sets of unique alleles. Only one haplotype was shared among two or more populations (Table 1). Interestingly, all four species shared haplotypes among the San Diego and Pinyon Flat populations which suggests a common feature of population structure.

The phylogenies of each of the species revealed differences in the patterns of relatedness among the mtDNA haplotypes (Fig. 2). The phylogeny for *T. maculata* was well resolved and there was bootstrap support for monophyly of three populations—Grand Canyon, Jaraguay Pass, and Kaweah. The phylogeny also revealed a split between northern and southern populations consistent with the subspecies designation within *T. maculata* (Segraves and Pellmyr, 2001). The branch leading to the haplotypes from Kaweah suggested a deep divergence of these haplotypes relative to the others. In contrast to *T. maculata*, the phylogeny of the other fruit-feeder *P. marginatus* was largely unresolved and no population was monophyletic. There was a deep divergence between two

Table 1
Distribution of mtDNA cytochrome oxidase I haplotypes for *Prodoxus marginatus*, *P. aenescens*, *P. cinereus*, and *Tegeticula maculata*

P. marginatus haplotypes

	Kaweah	Kern	Santa Barbara	Grand Canyon	Boulevard	San Diego	Pinyon Flat	Jaraguay Pass
M1	1							
M2			1					
M3			1					
M4			1					
M5	1							
M6	1							
M7			1					
M8		2						
M9		3						
M10	1							
M11				2				
M12		2						
M13					2			
M14				1				
M15						1		
M16						1		
M17							1	
M18				1				
M19					1			
M20						1		
M21		1						
M22				1				
M23				1				
M24					2	2		
M25						1		
M26					1			
M27							1	
M28							3	
M29	1							
M30	1							

P. aenescens haplotypes

	Kaweah	Kern	Santa Barbara	Grand Canyon	Boulevard	San Diego	Pinyon Flat	Jaraguay Pass
A1	4	5	3					
A2	1							
A3			1					
A4	1							
A5			1					
A6			1					
A7	1							
A8							2	
A9							1	
A10				4			1	
A11							1	
A12						6	6	
A13				2				

P. cinereus haplotypes

	Kaweah	Kern	Santa Barbara	Grand Canyon	Boulevard	San Diego	Pinyon Flat	Jaraguay Pass
C1						2	2	3
C2						2		
C3								1
C4								1
C5					2			
C6				5				
C7				1				
C8					1			
C9						1		
C10								1
C11							1	
C12		6						

T. maculata haplotypes

Site	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
Kaweah	3	1																
Kern							2	3	1									
Santa Barbara														2	1	1	1	1
Grand Canyon										4	1	1						
Boulevard						6												
San Diego						6												
Pinyon Flat			1	1		4												
Jaraguay Pass					2	4												

Not all species are represented at all localities.

haplotypes (M29 and M30) from Kaweah and the rest of the *P. marginatus* haplotypes. Other Kaweah haplotypes, however, were more closely related to haplotypes from other populations than to the M29 and M30 haplotypes. Like *P. marginatus*, the phylogeny for *P. aenescens* did not exhibit monophyly for any of the populations. Two populations of *P. cinereus*, Grand Canyon and Kern, were monophyletic. For both stalk-feeding species, there was bootstrap support for a division between haplotypes from populations in the north (Kaweah, Kern, Santa Barbara) and those in the south (San Diego, Boulevard, Pinyon Flat, Grand Canyon, Jaraguay Pass). Unlike for the two fruit feeders, the Kaweah population of *P. aenescens* was not deeply diverged from the other populations. The same comparison could not be made for *P. cinereus* because the northern-most population is at Kern.

Coalescent analyses in which the gene trees of the *Prodoxus* species were constrained to the pattern of popu-
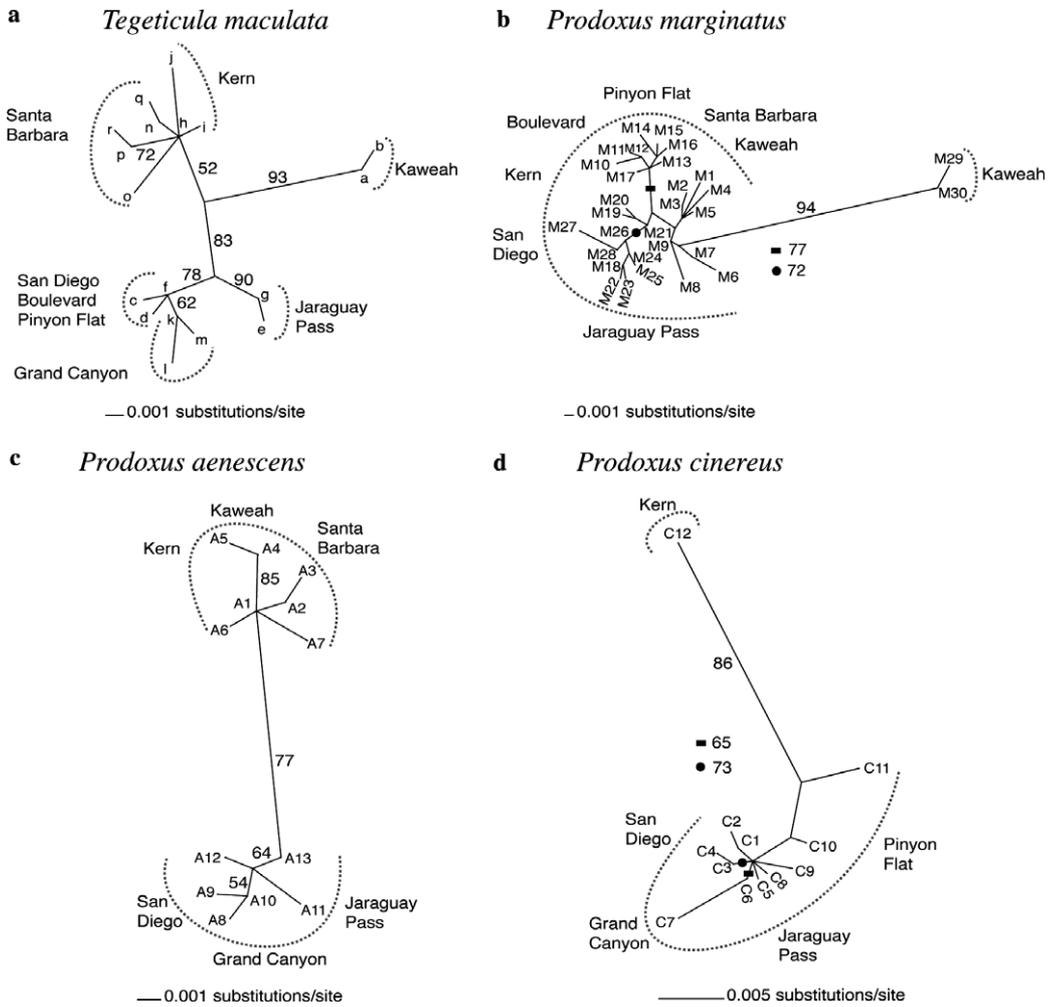
Fig. 2. Unrooted maximum likelihood phylograms for each of the yucca moth species utilizing *Hesperoyucca whipplei*. *Tegeticula maculata* and *Prodoxus marginatus* larvae feed within the fruit. *Prodoxus aenescens* and *P. cinereus* larvae feed within the inflorescence stalk. For the *Prodoxus* species, none of the haplotypes from a locality were monophyletic except for the Grand Canyon and Kern localities for *P. cinereus*. Dark circles and rectangles denote bootstrap values as reported in (b) and (d).
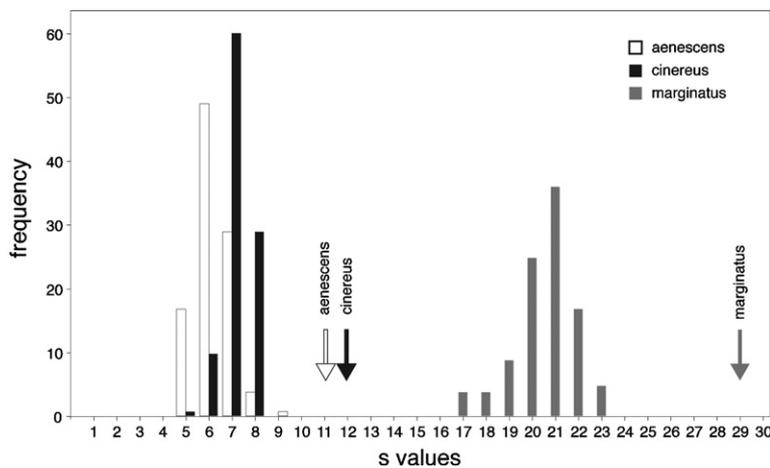
Fig. 3. Null distributions of s values generated by coalescent simulations to test whether the *Prodoxus* gene trees exhibited significant discord when contained within the population tree of *Tegeticula maculata*. The actual s values (denoted by arrows) for all three *Prodoxus* species were above their null distributions which suggests they have a different evolutionary history than *T. maculata*.

lation subdivision detected for *T. maculata* demonstrated significant discord for all three species (Fig. 3). The empirical values of *s* were greater than the distributions generated via coalescent simulations. This demonstrates that the evolutionary history of the *Prodoxus* species was different than that of *T. maculata*.

4. Discussion

Host-associated divergence has a major influence on the diversification of organisms, especially insect lineages (Funk et al., 2006). Multiple factors, such as host plant chemistry, phenology, and mating behavior can all facilitate host-associated divergence. Furthermore, life history characteristics such as larval feeding habits (i.e., internal vs. external feeders) may influence diversification rates by allowing resource partitioning on host taxa (Farrell and Sequeira, 2004). The results for the yucca moth community on *H. whipplei* suggest that the type of species interaction may also influence phylogenetic structure. Below, we first describe the phylogeographic patterns detected for each of the moth species, and then we discuss how the type of interaction and feeding location within a plant influence phylogenetic structure.

4.1. Overall phylogeographic patterns

Segraves and Pellmyr (2001) demonstrated that the phylogeography for the pollinating seed-eater *T. maculata* was consistent with the two morphologically defined subspecies—*T. maculata maculata* in the northern localities and *T. maculata extranea* in the southern localities. In addition, there was divergence of the Kaweah population within *T. maculata maculata* that was as old as the split between the two subspecies. The results also suggested that there was no apparent gene flow among each of the localities. Many of the haplotypes from each site formed monophyletic clades, and only one haplotype was shared among populations. The divergence between the subspecies and the Kaweah lineage was consistent with historical biogeographic boundaries as well as postglacial changes in the distribution of *H. whipplei* populations. *Hesperoyucca whipplei* was historically present in a less patchy pattern in the region depicted in Fig. 1, but has become increasingly patchily distributed due to changes in climate and human disturbance. For example, the closest extant sampled population to the Grand Canyon population is Pinyon Flat even though data from fossilized leaves in packrat middens show that historically there were other populations between these two localities (Segraves and Pellmyr, 2001).

The phylogeographic structure for each of the three *Prodoxus* species gave different pictures of the evolutionary history of these lineages (Fig. 2). For the fruit-feeder, *P. marginatus*, there was relatively little phylogenetic structure among the sampled haplotypes, and none of the populations were monophyletic. Furthermore, there was no evidence for a phylogenetic split between northern and southern populations as there was for the other species. Previous researchers (Powell and Mackie, 1966; Davis, 1967) reported ample variation in wing pattern within this species, but this variation appeared not to show any phylogenetic structure. As with *T. maculata*, there were haplotypes from the Kaweah population that were highly divergent from the rest of the haplotypes with no difference in gross morphology of the adults. The uniqueness of Kaweah haplotypes for both the fruit feeders *T. maculata* and *P. marginatus* is particularly intriguing, given that the stalk-feeder *P. aenescens* does not show this divergence.

The two stalk-feeding *Prodoxus* species exhibited phylogeographic structure that was similar to *T. maculata*. As for *T. maculata*, there was support for a phylogenetic split between the populations in the north and those in the south. In addition, *P. cinereus* exhibited monophyly of the Grand Canyon and Kern populations. For both *P. cinereus* and *P. aenescens* there were haplotypes that were shared between the San Diego and Pinyon Flat populations. *Prodoxus aenescens* also shared haplotypes among the populations in the north. The results for the stalk-feeders suggest a deep divergence among the northern and southern populations, but gene flow within each of these regions. Morphological variation in wing coloration exhibits north–south variation in both of the stalk feeders. For *P. aenescens*, adult forewing coloration is purplish-bronze in the north and dark grey in the southern populations (Powell and Mackie, 1966, personal observation). Forewing coloration in *P. cinereus* varies in the opposite direction from *P. aenescens*. Adults are a light grey in the north and a darker grey in the south, and it may be categorical or clinal. Powell and Mackie (1966) remark that in the southern populations, determining the species identity of adults can be somewhat problematic due to the close resemblance in size and wing coloration of the two species.

4.2. Influence of interaction type and feeding location

The differences in the phylogeographic structure among the species are the result of a variety of factors such as demographic history, population size, and gene flow. The question remains as to how interaction type and feeding location can influence these factors and, in turn, shape the phylogenetic patterns. If we divide species into interaction types, we see that there are correlated phylogeographic differences. The mutualist pollinator, *T. maculata*, exhibited the highest degree of phylogeographic structure. Many populations were monophyletic, there was little evidence of gene flow, and there were deep divisions among the subspecies and populations. The genetic division also coincided with morphological differences among the subspecies (Powell and Mackie, 1966; Davis, 1967; Segraves and Pellmyr, 2001). In contrast, the three commensal species exhibited a lower degree of structure. For the stalk-feeders, there was a phylogenetic split between northern and southern populations, but there was also evidence for gene flow among populations. The commensal fruit-feeder, *P. marginatus*, exhibited the least phylogenetic structure of all four species. There was no support for a north–south split, and none of the populations were monophyletic, although current haplotype distributions suggest very little or no gene flow. The results of the coalescent analyses also demonstrated that the three commensal species had an evolutionary history that was significantly different than the mutualist pollina-

tor. There was significant discord between the gene trees for each *Prodoxus* species and the population tree for *T. maculata* (Fig. 3).

In terms of correlated differences with feeding location, the results are less clear. One expectation would be that species feeding on more ephemeral food substrates should have less opportunity for gene flow and be subdivided more than species feeding on less ephemeral substrates. For *T. maculata*, colonizing adults must arrive at new populations when freshly opened flowers are available. Adults of *P. marginatus* have a slightly larger window of opportunity because they can utilize freshly opened, pollinated flowers, and young fruit, but they rely upon *T. maculata* for fruit production. The two stalk-feeders have the greatest window of opportunity because they oviposit in the stalk throughout the flowering/early seed provisioning period and can persist even if plants do not produce fruit (Powell and Mackie, 1966). The expectation was upheld by the phylogenetic structure for *T. maculata* but not for *P. marginatus*. Among the *Prodoxus* species, the two stalk-feeding species exhibited greater phylogenetic structure than *P. marginatus*.

One overriding influence on phylogeographic structure for the moths may be associated with the population size of each of the species, rather than the type of interaction or feeding location. For example, species with small population sizes will harbor less haplotype diversity and achieve monophyly of haplotypes faster than species with larger population sizes given that gene flow is not significant. Both interaction type and feeding location are correlated with population size. If we were to order the species in terms of potential population sizes at each locality, the pollinator *T. maculata* would have the smallest, followed by *P. marginatus*, and then the two stalk-feeders. A single inflorescence stalk can harbor hundreds of stalk feeding larvae (Powell and Mackie, 1966, personal observation), whereas the densities of the pollinator and *P. marginatus* do not reach these high levels (Powell and Mackie, 1966, personal observation). *Prodoxus marginatus* have more larvae per fruit than *T. maculata* (Powell and Mackie, 1966, personal observation). As would be predicted based on relative population size alone, *T. maculata* has the greatest amount of genetic subdivision, both on evolutionary time and more recent time scales. The prediction, however, does not hold for the commensal species. *Prodoxus cinereus* and *P. aenescens* exhibited more phylogenetic structure than *P. marginatus*. Although *P. marginatus* populations appear to have no or little gene flow in recent time, there was no phylogenetic structure among the haplotypes and this species had the greatest number of haplotypes. The evolutionary history of this species is clearly different from the others.

In conclusion, phylogeographic comparisons among four species of specialist moths revealed that each of the species had a different genetic structure even though they occur at the same localities and utilize the same host plant species. Among the four species, the mutualistic pollinator exhibited the greatest phylogeographic structure. This mutualist utilizes the most ephemeral plant resource, freshly opened flowers, and has the smallest population size of the four species. The three commensal species, however, exhibited differences in phylogeographic structure that did not correlate well with plant part utilized or population size. This study demonstrated that it may be difficult to disentangle the effects of the type of interaction and feeding location from other factors that could also influence phylogenetic structure. Additional comparisons within insect communities and within clades of insects are needed to further assess the generality of the influence of species interactions and feeding location on phylogenetic structure in insect lineages.

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