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Phylogeographic Structure in the Bogus Yucca Moth *Prodoxus quinquepunctellus* (Prodoxidae): Comparisons with Coexisting Pollinator Yucca Moths

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The pollination mutualism between yucca moths and yuccas highlights the potential importance of host plant specificity in insect diversification. Historically, one pollinator moth species, *Tegeticula yuccasella*, was believed to pollinate most yuccas. Recent phylogenetic studies have revealed that it is a complex of at least 13 distinct species, eight of which are specific to one yucca species. Moths in the closely related genus *Prodoxus* also specialize on yuccas, but they do not pollinate and their larvae feed on different plant parts. Previous research demonstrated that the geographically widespread *Prodoxus quinquepunctellus* can rapidly specialize to its host plants and may harbor hidden species diversity. We examined the phylogeographic structure of *P. quinquepunctellus* across its range to compare patterns of diversification with six coexisting pollinator yucca moth species. Morphometric and mtDNA cytochrome oxidase I sequence data indicated that *P. quinquepunctellus* as currently described contains two species. There was a deep division between moth populations in the eastern and the western United States, with limited sympatry in central Texas; these clades are considered separate species and are redescribed as *P. decipiens* and *P. quinquepunctellus* (sensu stricto), respectively. Sequence data also showed a lesser division within *P. quinquepunctellus s.s.* between the western populations on the Colorado Plateau and those elsewhere. The divergence among the three emerging lineages corresponded with major biogeographic provinces, whereas AMOVA indicated that host plant specialization has been relatively unimportant in diversification. In comparison, the six pollinator species comprise three lineages, one eastern and two western. A pollinator species endemic to the Colorado Plateau has evolved in both of the western lineages. The east–west division and the separate evolution of two Colorado Plateau pollinator species suggest that similar biogeographic factors have influenced diversification in both *Tegeticula* and *Prodoxus*. For the pollinators, however, each lineage has produced a monophagous species, a pattern not seen in *P. quinquepunctellus*.

INTRODUCTION

Host plant use has been implicated as a major factor in the diversification of phytophagous insects (Ehrlich and Raven, 1964; Mitter *et al.*, 1988; Farrell, 1998). In particular, adaptations to different host plant species can drive local population differentiation, the formation of host races, and ultimately speciation (Bush, 1994; Abrahamson and Weiss, 1998; Carroll *et al.*, 1997, 1998; Funk, 1998; Feder, 1998; Filchak *et al.*, 1999; Via, 1999). The central premise to this mode of diversification is that specialization promotes host specificity that isolates insect lineages, either in sympatry or in allopatry. The conditions under which specialization leads to diversification, however, are not always clear-cut. Many factors influence specialization, such as host plant chemistry (Keese, 1998; Kopf *et al.*, 1998; Hagele and Rowell-Rahier, 2000), ecological availability (reviewed in Thompson, 1994), and enemy-free space (Holt and Lawton, 1993; Brown *et al.*, 1995; Feder, 1995; Hopkins and Dixon, 1997; Keese, 1997; Gratton and Welter, 1999), but not all may be important in promoting diversification. The key issue, then, is to understand the circumstances in which specialization leads to diversification. This issue may be best approached by use of a comparative framework. In particular, ecological and phylogenetic comparisons among specialist taxa that share host plants may provide insights into when specialization is important in driving diversification.

The interaction between yuccas and yucca moths is one system in which independent, specialist lineages utilize the same host plant species. Moths in the genera *Tegeticula* and *Prodoxus* (Prodoxidae) are both highly specific to yuccas. *Tegeticula* are pollinators of

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yucca species (Agavaceae), and yuccas serve as the sole host plants for the moths. Although this interaction has been cited as a classic example of obligate mutualism, only in the past few years has the true species diversity and host specificity of the moths been recognized. Earlier studies described one moth species, *T. yuccasella,* as the pollinator of approximately 30 species of yuccas across most of North America (Riley, 1892; Davis, 1967). The occurrence of host races or species complexes was postulated, but conclusive data were lacking (Riley, 1892; Busck, 1947; Davis, 1967; Miles, 1983; Powell, 1985, 1992). Pellmyr *et al.* (1996) used molecular data to demonstrate that *T. yuccasella* is a complex of species that included pollinators and nonpollinating "cheaters" that oviposit into developing fruit. On the basis of both molecular and morphological data, Pellmyr (1999) divided *T. yuccasella* into 13 species, including 11 pollinators and two cheaters. Eight of the pollinator species interact with only one yucca species, highlighting how host specificity may influence patterns of diversification in *Tegeticula*.

Members of the genus *Prodoxus,* known as the bogus yucca moths, are the sister group to the pollinators and are also specialists on yuccas. *Prodoxus* females, however, do not pollinate flowers, and the developing larvae feed on vegetative parts of reproductive structures rather than directly on seeds. One geographically widespread *Prodoxus* species, *P. quinquepunctellus,* feeds within the inflorescence stems of approximately 14 species of capsular-fruited yuccas and 1 fleshy-fruited species. On these plants, *P. quinquepunctellus* co-occurring with six pollinator species from three lineages within the *T. yuccasella* complex. Thus, the distribution of suitable food patches is identical for both *P. quinquepunctellus* and the six *Tegeticula* pollinators. Together, these taxa provide the opportunity to compare patterns of diversification in cooccurring specialist lineages.

We examined the phylogeographic structure of *P. quinquepunctellus* with the intent of understanding its differentiation relative to its coexisting pollinator species. As with the traditionally recognized *T. yuccasella,* previous research has suggested that *P. quinquepunctellus* may be a complex of species. Riley (1892) and Davis (1967) reported variation in the degree of forewing spotting among populations of *P. quinquepunctellus* in the eastern and western United States (Fig. 1). Riley (1892) noted that specimens east of the Mississippi River had completely white forewings, whereas those west of the river had one to five or more black spots on the forewings. Davis (1967) reported a similar pattern, but stated that both spotted and all-white moths coexist on plants farther west. Busck (1947) reported differences in female reproductive morphology that also supported an east–west division of populations. Recently, Groman and Pellmyr (2000) used allozyme data to document the presence of population structure in *P. quinquepunctellus* from the eastern United States as a result of recent, rapid host plant specialization. Populations that colonized an introduced yucca showed shifts of ovipositor morphology and moth emergence patterns. Taken together, these studies on morphology and host plant specialization in *P. quinquepunctellus* suggest that this widespread species may be a complex of species or at the least harbors extensive population genetic structure.

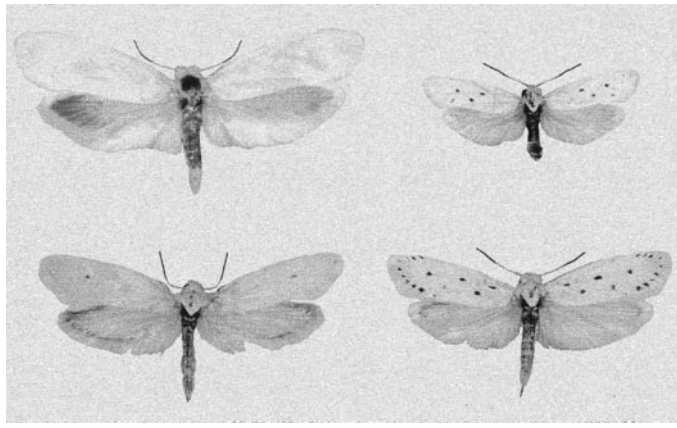

FIG. 1. Wing pattern variation in the traditionally recognized *Prodoxus quinquepunctellus*. (Top-left) Female, Jefferson, TX. (Top right) Male, Black Gap, TX. (Bottom left) Female, Hwy 98 Kanab, UT. (Bottom right) Female, Big Bend National Park, TX.

We used the genetic structure detected with mtDNA sequence variation in cytochrome oxidase I in combination with morphometric data to determine whether distinct species are present within the traditionally recognized *P. quinquepunctellus*. We tested three nonmutually exclusive hypotheses regarding the pattern of differentiation that may be detected in *P. quinquepunctellus*: (1) the pattern of differentiation in molecular and morphometric data is consistent with an east–west division of moth populations as noted by earlier studies; (2) *P. quinquepunctellus* has diverged as a result of specialization to different host plant species (ecologically-based divergence); if correct, moth populations that utilize each yucca species will be monophyletic; and (3) historical biogeographic factors such as range expansion and contraction due to climate shifts and the formation of geographic barriers can explain the pattern of differentiation (vicariance-based divergence).

We demonstrate that there are three distinct monophyletic lineages within *P. quinquepunctellus*. Both morphology and molecular data support an east–west division of populations, but mtDNA haplotype relatedness also shows another division among populations in the western United States. The formation of these three groups is best explained by vicariance-based divergence rather than by ecologically-based divergence. We compare the genetic structure in *P. quinquepunctellus* with the patterns found in coexisting pollinators of the *T. yuccasella* complex.

METHODS

We collected 285 adult moths and larvae between 1993 and 1999 from 49 sites throughout the United States (Table 1; Fig. 2). These sites were chosen to encompass the extant geographic range of *P. quinquepunctellus*. Adults were collected in the field while they were resting in flowers or ovipositing into the inflorescence stalk. Stalks containing diapausing larvae were also collected and placed into 1-mm mesh cages in an environmental chamber at Vanderbilt University to allow adults to eclose. The stalks were held in diapause at 4/6°C (16 h:8 h) for 90 days during the winter, and the temperature was then raised over a 2-week period to 28/24°C (16 h:8 h) to trigger emergence over a period of several weeks.

Molecular Methods

Prior to DNA extraction, the head, wings, and genitalia were removed from adults and kept as vouchers. Total genomic DNA from the remaining thorax and abdomen was extracted a modified protocol of Harrison *et al.* (1987). For larvae, the entire individual was used. We used PCR to amplify the 3′ end of cytochrome oxidase I and transfer RNA leucine of the mitochondrial DNA. Each 30-μl reaction volume contained 50 mM KCl, 10 mM Tris (pH 9.0), 1.67 mM $MgCl_2$, 0.2 mM dNTPs, 0.33 mM each primer, 0.033 units of Promega B *Taq* polymerase, and 100 ng of genomic DNA. Primer sequences were 2231F: (5′-CCAGGATTTGGTATAAATTTC-3′) and 3020R: (5′-GTAATGGATTTAAGCCCCT-3′). Numbers refer to nucleotide positions in the *Drosophila yakuba* complete mtDNA sequence (Clary and Wolstenholme, 1985). The thermal cycler profile was 1 cycle at 95°C for 2 min, 35 cycles at 95°C for 1 min, 52°C for 1 min, 72°C for 1 min 30 s, and 1 cycle at 72°C for 10 min. PCR products were cleaned with the QIAquick PCR purification kit (Qiagen, Valencia, CA) and both forward and reverse strands were sequenced on an ABI 377 Automated DNA sequencer (PE Applied Biosystems, Foster City, CA). Sequencing products were generated with cleaned PCR product, BigDye terminator cycle sequencing mix (PE Applied Biosystems), and 4 pmol of one of the original PCR primers. The thermal cycler profile was 1 cycle at 96°C for 2 min, 25 cycles at 96°C for 30 s, 50°C for 30 s, and 60°C for 4 min. Sequencing products were cleaned with Centri-sep Sephadex columns (Princeton Separations, Adelphia, NJ). Forward and reverse sequences for each individual were checked with Sequencher 3.1 (Gene Codes Corp., Ann Arbor, MI). The consensus sequence for each individual was then aligned by eye in PAUP* version 4.0b4a (Swofford, 2000). There were no insertions or deletions.

Sequence data were analyzed with parsimony and maximum-likelihood algorithms in PAUP* version 4.0b4a (Swofford, 2000). We used Fitch parsimony, a heuristic search with simple taxon addition, and TBR branch swapping for the parsimony analysis. Based on prior phylogenetic analyses of the Prodoxidae (Pellmyr and Leebens-Mack, 1999), we used the HKY85 (Hasegawa *et al.*, 1985) model of evolution for the maximum-likelihood analyses, and the closely related *P. coloradensis* Riley was used as the outgroup for all analyses. Both parsimony and likelihood analyses revealed three major monophyletic groups. Bootstrap support was estimated for each of these three major monophyletic groups by random choice of five taxa from each group and performance of 100 bootstrap replicates. This procedure was repeated 50 times, and the bootstrap values were added together and divided by 5000 (total number or replicates). Bootstrap analyses with all taxa were prohibitively time consuming because of low phylogenetic signal among taxa within each of the major monophyletic groups. For each major monophyletic clade, we used analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) to determine whether populations were structured by host plant species attacked. We used the squared number of substitutions among haplotypes as the distance matrix for each group and specified population groupings based on host plant species used.

Morphometric Measurements

We measured a suite of male and female reproductive traits from sites selected to represent the major monophyletic lineages identified by the phylogenetic analyses. Moths were examined from the following nine sites: Buxton, NC; Cincinnati, OH; Jefferson, TX; McLean, TX; Comanche, TX; Sierra Blanca, TX; Guernsey, MT; Winona, AZ; and NW Kanab, UT. The traits were chosen based on previous work by Riley (1892), Busck (1947), Davis (1967), and Groman and Pellmyr (2000). In males, we measured forewing length from base to apex, number of black dots on the forewing, and length of the aedeagus (the male intromittent organ). In females, we measured forewing length from base to apex, number of black dots on the forewing, length of posterior apophyses (part of ovipositor that cuts into stalk tissue), ovipositor height, number of dorsal teeth on ovipositor, and diameter of the signum (a star-shaped structure located in the bursa that is used to break open spermatophores). Abdomens were cut from the thorax and boiled in 10% KOH for 5 min to remove scales and adipose tissue. Measurements were done with an Olympus SZ-PT dissecting scope fitted with an ocular micrometer. All length measurement variables were checked for normality and equal variances among sites before performing nested ANCOVAs with forewing length as the covariate to control for allometric effects. The number of teeth on the ovipositor and dots on the forewing were analyzed with the Kruskal–Wallis nonparametric test. All sta-

TABLE 1

Site Localities and Haplotypes Sampled for *Prodoxus quinquepunctellus*

Eastern lineage

Site No.	Site name	Yucca species used	Latitude	Longitude	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Hatteras, NC	*aloifolia*	35 13 09 N	75 41 26 W	7																				
2	Ocracoke, NC	*aloifolia*	35 06 52 N	75 58 53 W	4																				
3	Buxton, NC	*aloifolia*	35 16 03 N	75 32 34 W	6	1																			
4	Tybee Island, GA	*aloifolia*	32 00 00 N	80 50 45 W	6		1	1																	
5	Pawley's Island, SC	*aloifolia*	33 25 59 N	79 07 18 W	7																				
6	Nags Head, NC	*filamentosa*	35 56 28 N	75 37 28 W	4				2	1															
7	Buxton, NC	*filamentosa*	35 16 03 N	75 32 34 W	7																				
8	Cincinnati, OH	*filamentosa*	39 06 23 N	84 30 36 W							4														
9	Georgesville, OH	*filamentosa*	39 53 27 N	83 13 19 W	1						1														
10	Vine, TN	*filamentosa*	36 01 52 N	86 21 28 W	4							1	1												
11	Goldbond, VA	*filamentosa*	37 22 48 N	80 30 40 W	4						1		1												
12	Surf City, NC	*filamentosa*	34 25 27 N	77 32 47 W	6									1											
13	Dudley, GA	*filamentosa*	32 32 32 N	83 04 14 W	2								1		3	1									
14	Camp Meeting Rock, GA	*filamentosa*	33 18 19 N	85 07 30 W	6																				
15	Columbus, MS	*filamentosa*	33 30 00 N	88 24 00 W	5												1								
16	Shreveport, LA	*filamentosa*	32 34 00 N	93 36 00 W														5							
17	Jefferson, TX	*glauca* var *arkansana*	32 45 00 N	93 39 00 W	1		1											1	2	1					
18	Sarita, TX	*glauca* var *arkansana*	27 13 00 N	97 47 00 W														1			2				
19	Destin, FL	*filamentosa*	30 23 00 N	86 28 00 W	2								1		1							2			
20	McKethan Lake, FL	*filamentosa*	28 38 00 N	82 20 00 W	1																	1	5		
21	Ocala, FL	*filamentosa*	29 08 00 N	81 31 00 W	5																		3		
22	Lake Placid, FL	*filamentosa*	27 14 00 N	81 24 00 W	3																		3		
23	Harper, TX	*constricta*	30 17 00 N	99 14 00 W	1																1			2	2
24	Santa Anna, TX	*glauca*	31 44 00 N	99 19 00 W														1						4	1
25	Lawrence, KS	*filamentosa*	38 57 00 N	95 15 00 W	1																				
34	Brownwood, TX	*glauca*	31 35 00 N	99 03 00 W																				2	

Western lineage

Site No.	Site name	Yucca species used	Latitude	Longitude	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AR	AS	AU	AV	AW	AX	AY	A1	A2	A3	A4
26	Ft. Stockton, TX	*rostrata*	30 57 31 N	102 34 45 W	1	4	1																						
27	Columbus, MT	*glauca*	45 37 58 N	108 55 12 W		3		2	1																				
28	Guernsey, WY	*glauca*	42 12 42 N	104 31 00 W		5																							
29	Ogallala, NB	*glauca*	41 21 33 N	101 38 47 W		1				4	1																		
30	McLean, TX	*glauca*	35 58 42 N	100 53 14 W		3						3																	
31	Cuervo, NM	*glauca*	35 02 00 N	104 23 30 W				1				1	1	1	1	1													
32	Wickett, TX	*glauca* (*campestris*)	31 33 00 N	103 00 00 W		3						2																	
33	Kyle, TX	*rupicola*	30 31 00 N	97 56 00 W		2						2					1	1											
34	Brownwood, TX	*glauca*	31 35 00 N	99 03 00 W		2													2										
35	Black Gap, TX	*rostrata*	29 33 00 N	102 57 00 W		4		1												1									
36	Comanche, TX	*pallida*	31 53 25 N	98 30 45 W		2						3								1									
37	Los Lunas, NM	*baileyi* var *intermedia*	34 46 45 N	106 58 00 W		3															1								
38	Magdalena, NM	*elata*	34 03 33 N	106 53 27 W		1	4															1							
39	Las Cruces, NM	*elata*	32 18 28 N	106 42 30 W		1		1															1	1	1				
40	Sierra Blanca, TX	*elata*	31 10 00 N	105 21 30 W		1						4														1			
41	Cottonwood, AZ	*elata*	34 37 00 N	111 51 30 W																							1	1	1

Colorado Plateau lineage

Site No.	Site name	Yucca species used	Latitude	Longitude	BA	BB	BC	BD	BE	BF	BI	BJ	BK	BL	BM	BN	BO
42	Hwy 89 NW Kanab, UT	*angustissima* var *kanabensis*	37 02 06 N	112 44 26 W	3	3											
43	Valdez, CO	*baileyi*	37 46 30 N	104 40 30 W			1										
44	Winona, AZ	*baileyi*	35 13 45 N	111 25 30 W				2	2	1							
45	Moab, UT	*baileyi*	38 34 30 N	109 32 51 W					4		2						
46	Wilson Arch, UT	*harrimaniae*	38 16 21 N	109 22 14 W					4			1					
47	I-70, mile 102, UT	*harrimaniae*	38 49 00 N	111 08 00 W					1								
48	St. Johns, AZ	*baileyi*	34 39 30 N	109 39 00 W				1	3				1				
49	Peach Springs, AZ	*angustissima* var *angustissima*	35 33 00 N	113 25 36 W										4	1	1	1
37	Los Lunas, NM	*baileyi* var *intermedia*	34 46 45 N	106 58 00 W					1						1		

Note. Site numbers correspond to numbers in Fig. 2.

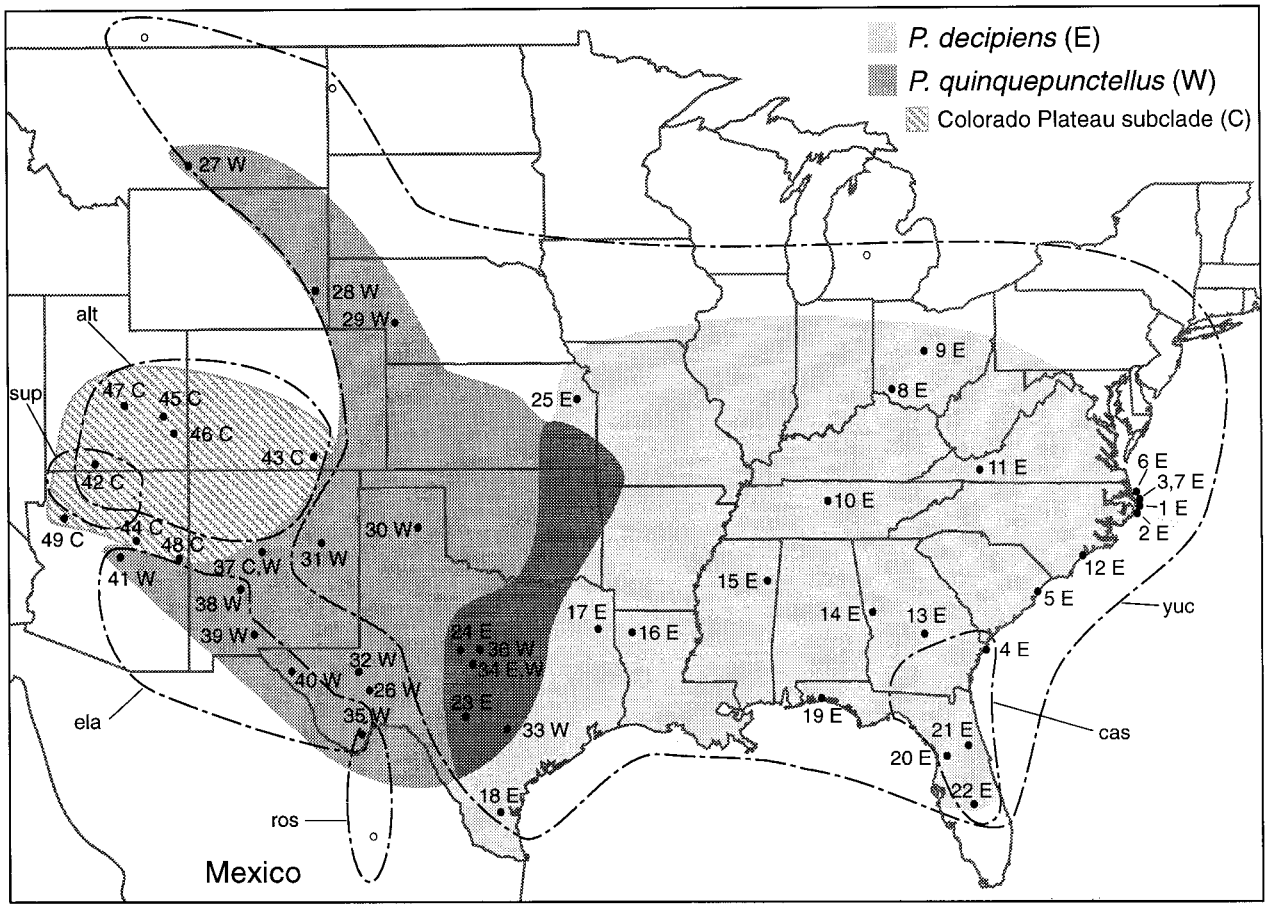

FIG. 2. Map of sites sampled for *Prodoxus quinquepunctellus*. Site numbers correspond to the names in Table 1. Capital letters next to each site represent the presence of eastern (E), western (W), or Colorado Plateau (C) mtDNA haplotypes. Shaded areas circumscribe the current ranges of each major monophyletic lineage as determined by the mtDNA COI phylogeny. Open circles represent known localities of *P. quinquepunctellus* that were not sampled in this study. Dotted lines represent known ranges of *Tegeticula* pollinators that co-occur with *P. quinquepunctellus* (from Pellmyr, 1999). Three-letter abbreviations outside of each *Tegeticula* pollinator range refer to the species name (alt, *T. altiplanella*; cas, *T. cassandra*; ela, *T. elatella*; ros, *T. rostratella*; sup, *T. superficiella*; yuc, *T. yuccasella*).

tistical tests were performed with JMP version 3.2.1 (SAS, 1998).

RESULTS

Phylogenetic Analyses

DNA sequencing produced 686 consistently readable base pairs of cytochrome oxidase I for each individual. For the ingroup, 9% (62) of sites were variable. Changes at third codon sites accounted for 42 of the variable sites, whereas changes at first codon sites accounted for 17 and second codon sites only three. Sixty unique haplotypes were detected among the 285 individuals sequenced, and these 60 sequences were deposited in GenBank under Accession Nos. AF334413–AF334472.

Both parsimony and maximum-likelihood analyses of the unique haplotypes identified two major clades of haplotypes that corresponded to a geographic division between sites in the eastern and western United States (Fig. 3). The eastern clade contained haplotypes from sites in the eastern United States westward to central Texas, whereas the western clade contained haplotypes from sites in the western United States eastward to central Texas. Within the western clade, haplotypes from sites in the Colorado Plateau Region also formed a monophyletic clade (Fig. 3). Estimates of percentage sequence divergence demonstrated that most of the sequence variation was between clades rather than within clades. Sequence divergence between the eastern and the western clades based on the Hasegawa *et al.* (1985) evolutionary model ranged from 2.7 to 3.6%. Within the western clade, divergence between haplotypes from the Colorado Plateau and the other western haplotypes ranged from 1.4 to 2.2%. In contrast, maximum sequence divergence within the three clades was 0.7% in the eastern and Colorado Plateau clades and 1.0% in the western clade.

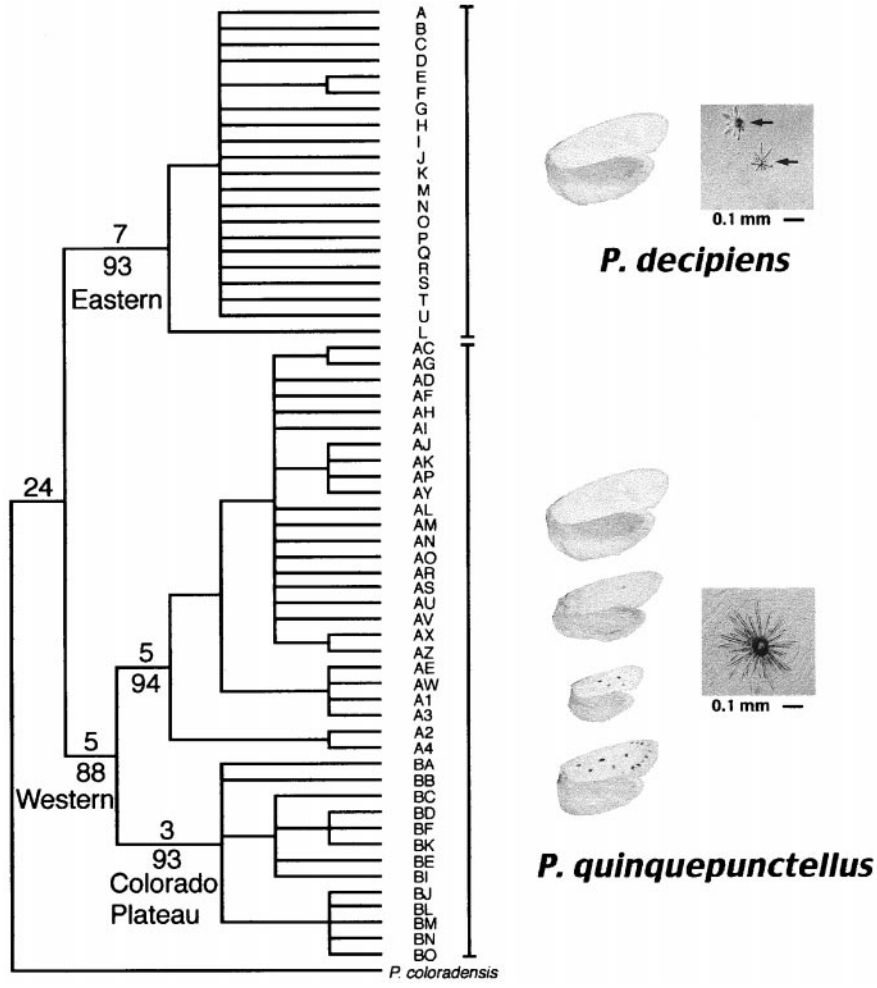

FIG. 3. Strict consensus tree of 636 most parsimonious trees of 113 steps for relationships among mtDNA haplotypes of *Prodoxus quinquepunctellus*. Maximum-likelihood analysis produced the same topology. Numbers above branches refer to branch lengths. These values were consistent across all 636 trees from the parsimony analysis and the 4 trees from the maximum-likelihood analysis. All other branch lengths were 0, 1, or 2 steps. Numbers below branches represent bootstrap values from analyses with randomly chosen subsets of taxa from each lineage (see Methods). Wing patterns, signa, and species names (based on this study; see Discussion) are provided for the eastern and western lineages. The two signa for *P. quinquepunctellus* are superimposed (signa pictures are at 20× magnification).

Morphometric Analyses

Six of the seven morphological characters also supported a division between sites in the eastern and western United States as detected with the mtDNA sequence data. One of these traits, signum diameter, can be used as a diagnostic character to distinguish female moths from eastern and western sites. Eastern females had significantly smaller signum diameters than western females, and there was no overlap in diameter distributions (Table 2; Fig. 3). The five remaining morphological characters were also differentiated among eastern and western moths even though the character distributions overlapped between the two lineages (Table 2). Eastern moths never had spotted forewings, whereas the degree of spotting ranged from 0 to 16 spots for western moths. Eastern females had longer posterior apophyses, a higher ovipositor tip, and more teeth on the ovipositor than western females. Eastern males had a longer aedeagus than western males. Only two of the morphological characters surveyed supported a division between the Colorado Plateau populations and the remainder of the western populations. Females from the Colorado Plateau populations had a higher ovipositor tip and more teeth on the ovipositor than other western females. No male traits were significantly different between the two western clades.

Population Structure within Clades

We performed AMOVA for populations within each of the three major clades to test whether populations were further structured by host plant species attacked. For all three clades, significant ϕ_{ST} values indicated that there was population structure, but most of the

TABLE 2

Morphological Characters Measured for Each Lineage of *Prodoxus quinquepunctellus* Indicated by mtDNA phylogeny

Sex	Trait	Lineage			Test statistic
		Eastern	Western	Colorado Plateau	
Female	Sample size	18	40	25	
	Forewing length (mm)	8.88 ± 0.26	8.50 ± 0.17	8.14 ± 0.16	$F = 2.52^{ns}$
	No. of forewing dots	0	0–16	0–3	$\chi^2 = 28.21$***
	Posterior apophyses length (mm)	6.33 ± 0.16a	5.56 ± 0.12b	5.42 ± 0.12b	$F = 11.89$***
	Height of ovipositor tip (mm)	0.15 ± 0.003a	0.12 ± 0.003b	0.11 ± 0.003c	$F = 46.75$***
	Signum diameter (mm)	0.15 ± 0.01a	0.34 ± 0.01b	0.32 ± 0.01b	$F = 234.90$***
	No. of ovipositor teeth	6–11a	5–10b	4–8c	$\chi^2 = 18.44$***
Male	Sample size	30	40	20	
	Forewing length (mm)	7.07 ± 0.22	6.86 ± 0.16	6.42 ± 0.14	$F = 2.60^{ns}$
	No. of forewing dots	0	0–9	0–16	$\chi^2 = 32.30$***
	Aedeagus length (mm)	1.72 ± 0.11a	1.49 ± 0.15b	1.43 ± 0.14b	$F = 38.09$***

Note. Mean ± SE given except for the ranges for No. of forewing dots and No. of ovipositor teeth. Ranges were provided to illustrate distributions of these characters. Similar letters denote groupings based on contrasts.

*** $P < 0.001$.

haplotype variation was within populations (Table 3). Only for the eastern clade was population structure significantly influenced by host plant species use. Seventeen percent of the total variation among haplotypes in this clade was attributable to host plant species use.

DISCUSSION

As currently circumscribed, *P. quinquepunctellus* consists of two major lineages that are both genetically and morphologically distinct. Mitochondrial DNA haplotype relatedness and morphometric data demonstrated an eastern and western division of populations across the composite range. Based on the current population sampling, the eastern and western lineages appear to be mostly allopatric in distribution (Fig. 2). The eastern lineage occurs from the East and Gulf coasts of the United States into the eastern part of Texas and the Great Plains states. The western lineage primarily occurs in western Texas, the southwestern United States, and west of the Missouri River in the northern Great Plains states. The ranges are known to overlap in central Texas, with moths of the eastern and western lineages sharing *Yucca glauca* as a host species at the Brownwood site in Texas. There was further genetic structuring among populations within the western lineage. Haplotypes from the Colorado Plateau populations formed a monophyletic group, separated from the remainder of the other western populations by a considerable genetic distance. Whereas the moths from the plateau often are phenotypically distinct, we have not identified a diagnostic morphological trait that can be used to correctly discriminate between individuals from the two groups.

Based on these DNA sequence and morphological results, it is reasonable to consider the eastern and western lineages distinct species. The entities already have names. Spotted moths of the western lineage were used in describing *P. quinquepunctellus* (Chambers, 1875), whereas Riley (1880) described unspotted

TABLE 3

Analysis of Molecular Variance Results for Population Structuring Due to Host Plant Use for the Three Major Monophyletic Lineages of *Prodoxus quinquepunctellus* sensu lato

Source	*Eastern lineage*				*Western lineage w/out Colorado Plateau*				*Colorado Plateau lineage*			
	Variance	% Total	*P*	ϕ Statistics	Variance	% Total	*P*	ϕ Statistics	Variance	% Total	*P*	ϕ Statistics
Among host plant species	0.18	17.00	0.035	$\phi_{CT} = 0.17$	0.07	2.50	0.271	$\phi_{CT} = 0.025$	0.31	10.21	0.125	$\phi_{CT} = 0.102$
Among populations within host plant	0.34	31.11	0.001	$\phi_{SC} = 0.38$	0.66	23.69	0.001	$\phi_{SC} = 0.243$	0.44	14.82	0.134	$\phi_{SC} = 0.165$
Within populations	0.56	51.90	0.001	$\phi_{ST} = 0.48$	2.04	73.80	0.001	$\phi_{ST} = 0.262$	2.24	74.97	0.014	$\phi_{ST} = 0.250$

Note. Overall ϕ statistics are provided.

individuals from South Carolina as *P. decipiens*. The latter name has long been considered synonymous, but can now be revived for the eastern lineage. Redescriptions of the two species are provided in the Appendix. We refrain from giving species status to the Colorado Plateau entity at this point for two reasons. There is no unequivocal morphological character to distinguish between the two western lineages, and there are currently no data from nuclear markers to corroborate the pattern of no gene flow.

Influence of Host Plant Specialization

Given the pattern of differentiation detected by morphology and mtDNA haplotype relatedness, we examined whether host plant specialization can explain the divergence of *P. quinquepunctellus* and *P. decipiens*. Groman and Pellmyr (2000) demonstrated that rapid host plant specialization can influence population differentiation in *P. decipiens*. Moth populations attacking an introduced host, *Y. aloifolia,* had a shift in emergence time, body size, and ovipositor morphology compared to sympatric populations attacking the ancestral host *Y. filamentosa*. These differences also led to restricted gene flow among populations on each host as demonstrated by both allozyme and mtDNA sequence data. Their results indicated that differences between host plant species may serve to isolate populations of moths as they adapt to their respective host plant species.

Although rapid host specialization may be important in structuring populations, it does not appear to have been a critical factor facilitating the divergence of *P. decipiens* and *P. quinquepunctellus* or the Colorado Plateau clade. We base this conclusion on two findings. First, within each group relatively little phylogenetic or population structuring was due to host plant species use. Only haplotypes from moths collected on *Y. baileyi* were monophyletic; however, these haplotypes were also associated with moths found on two other yucca species. Moreover, AMOVA detected a significant effect due to host plant use for just the eastern lineage, but host plant use explained only 17% of the haplotype variance (Table 3). Second, each moth lineage can also utilize yucca species attacked by another lineage. For example, eastern and western haplotypes were found on *Y. glauca* in east-central Texas and both western haplotypes and haplotypes from the Colorado Plateau clade were found on *Y. angustissima* var *intermedia* in central New Mexico. This suggests that, in areas of secondary contact, moths are able to use any locally available capsular-fruited yucca species, even those used by another lineage. In the case of the eastern lineage, there has also been a switch to feeding on a fleshy-fruited yucca species. Overall, populations may specialize on different host plant species, as indicated in the colonization of *Y. aloifolia,* but this specialization may be unimportant in maintaining isolation of populations long enough to facilitate speciation.

Influence of Historical Biogeography

The pattern of differentiation detected in *P. quinquepunctellus* sensu lato is best explained by patterns in biogeography rather than by host plant specialization. The three monophyletic lineages correspond well with major physiographic regions of North America as defined by Graham (1999). The ancestral distribution of *P. decipiens* encompasses the low Coastal Plain regions of the eastern United States that extend west to the Edwards Plateau in central Texas. Populations of *P. decipiens* north of the Gulf Coast states are the result of a recent range expansion facilitated by horticultural transplantations of *Y. filamentosa* by European settlers (Trelease, 1902; Pammel, 1925; Frack, 1982). *Prodoxus quinquepunctellus* sensu stricto occurs throughout the High Plains region of the western United States (the Edwards Plateau westward and north along the eastern edge of the Rocky Mountains) and across the Mexican Highland subprovince of the southwestern United States. The distribution and monophyly of haplotypes from the Colorado Plateau further suggest that biogeographic factors have been important in structuring differentiation.

Although the distribution and relatedness of haplotypes for each of the three lineages correspond with major biogeographic regions, it is unclear whether divergence was driven by the formation of geographic barriers marking the edges of these regions or whether the barriers predated the divergence of the lineages. We used the availability of a molecular clock for the Prodoxidae (Pellmyr and Leebens-Mack, 1999) to examine whether the formation of geographic barriers coincided with the divergence among the three lineages. We calculated moth divergence times by estimating the maximum-likelihood tree under a clock model with the prodoxid moth *Mesepiola specca* as the root and divided the node to tip distances for each of the three lineages by the node to tip distance for *M. specca*. These numbers were then multiplied by 44.1 My (from Pellmyr and Leebens-Mack, 1999) to obtain the divergence estimates. Using this protocol, we estimated the time of divergence between *P. decipiens* and *P. quinquepunctellus* to be 12.5 Mya and the divergence of the Colorado Plateau lineage within *P. quinquepunctellus* to be 8 Mya.

One potential geographic barrier separating *P. decipiens* and *P. quinquepunctellus* is the Edwards Plateau in central Texas. This geologic feature marks the boundary of the High Plains and the Gulf Coast regions of North America and has been a major influence on the biogeography of many organism groups, including plants, invertebrates, and vertebrates (Blair, 1950; Block and Zimmerman, 1991; Richardson and Gold, 1995; Davis, 1996). Current geologic reconstructions

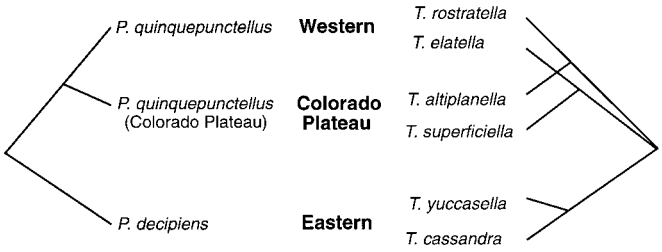

FIG. 4. Comparison of the phylogenetic patterns for *Prodoxus quinquepunctellus* sensu lato and its six cooccurring *Tegeticula* pollinator species (phylogeny for *Tegeticula* species from Pellmyr and Leebens-Mack (2000)). In the two western pollinator lineages, note the independent evolution of a Colorado Plateau species. *Tegeticula rostratella, T. elatella,* and *T. cassandra* are specific to one yucca species.

suggest that the Edwards Plateau formed during the Miocene approximately 10–20 Mya (Abbott and Woodruff, 1986) as a result of tectonic activity along the Balcones Fault that runs through central Texas. These reconstructions suggest that the formation of the Edwards Plateau coincided with or preceded the divergence of *P. decipiens* and *P. quinquepunctellus*. Within *P. quinquepunctellus,* the origination of the Colorado Plateau lineage appears to have occurred after the plateau was formed. The Colorado Plateau is estimated to have risen 10–17 Mya (Lucchitta, 1989; Christiansen and Yeates, 1992; Parsons and McCarthy, 1995; Zandt *et al.* 1995; Spencer, 1996; McQuarrie and Chase, 2000), at least two million years prior to moth population divergence. Although the error associated with both moth divergence times and the formation of the Edwards Plateau and the Colorado Plateau preclude any firm conclusions, the distribution and monophyly of the three lineages appears to be the result of biogeographic factors.

Comparison of Differentiation with Pollinator Taxa

The yucca species used by *P. decipiens* and *P. quinquepunctellus* are pollinated by six *Tegeticula* species. Three of these pollinator species, *T. cassandra, T. elatella,* and *T. rostratella,* are specific to one species of yucca. The six pollinator species represent three separate lineages within the *T. yuccasella* complex (Pellmyr and Leebens-Mack, 2000) (Fig. 4). Divergence among and within these pollinator lineages appears in part to parallel the divergence detected in *P. quinquepunctellus* sensu lato. For example, the ancestral distribution of the *T. yuccasella–T. cassandra* lineage corresponds to the ancestral Gulf Coast distribution of *P. decipiens* (Fig. 3). In the two western pollinator lineages, it appears that the Colorado Plateau has influenced pollinator divergence as it has in *P. quinquepunctellus* sensu stricto. Both *T. superficiella* and *T. altiplanella* are confined to the Colorado Plateau region. Thus, biogeographic factors have produced similar patterns of divergence for both groups of taxa. For each pollinator lineage, however, there has been the evolution of a moth species that utilizes a single yucca species. This suggests that other factors may be influencing pollinator diversification relative to *P. decipiens* and *P. quinquepunctellus*. Ultimately, as more ecological and phylogenetic data accumulate for both *Prodoxus* and *Tegeticula* we will be able to evaluate the factors determining diversification in both of these specialist taxa.

APPENDIX

Redescriptions of *Prodoxus decipiens* and *P. quinquepunctellus*

Prodoxus decipiens Riley *[Prodoxus quinquepunctellus* auct.*]*

Morphology. Wingspan male 11.0–18.0 mm, female 14.5–24.5 mm. Integument amber to medium brown. *Head:* With white scales. Maxillary palp 5-segmented, without tentacle on basal segment. Labial palp 3-segmented, with prominent apical sensilla. Proboscis tan-colored, relatively long. Antenna dark brown, with white scales on basal half. *Thorax:* With white scales. Forewing length in male 4.0–8.8 mm, female 4.6–11.0 mm; dorsal surface completely white, except for a dark frontal edge on basal quarter of costa. Underside brown except for yellowish white portion overlapping hindwing. Hindwing light to medium grayish brown. Underside sparsely scaled in brownish gray, with darker area along fore edge where overlapping with forewing. Wing fringes white. *Abdomen:* With dorsal scaling white and light tan, with last two segments with white linear semierect scales forming brush. Underside white to light tan in female, light to medium tan and rarely white in male. In male, valva with white or light tan scales. *Male genitalia:* Valva mostly linear but expanded dorsally at apex, with 3–6 short spines along apicoventral margin. Aedeagus 1.21–1.96 mm long. *Female genitalia:* Posterior apophyses 3.6–7.6 mm long; ovipositor 0.06–0.19 mm high near tip, with 5–11 dorsal teeth; corpus bursae with two 0.10- to 0.22-mm signa.

Material examined and types. 404 males, 227 females (151 males, 152 females for genitalia). The species was originally described by Riley (1880) from 25 specimens collected in Bluffton, South Carolina, and elsewhere. No labeled specimens from the type material were recovered by Davis (1967), and an exhaustive examination of all prodoxid material in the USNM collections by one of us (O.P.) confirmed that no syntypes exist in USNM. For this reason, we have chosen a specimen from a nearby location as neotype. *Neotype,* male. USA. South Carolina, Charleston Co., McClellanville, Fairfield Plantation. 10 May 1981; Leg. R. W. Hodges (USNM).

Distribution and recorded hosts. The range is shown in Fig. 2. *Yucca filamentosa* (incl. *Y. louisianensis, Y. smalliana,* and *Y. flaccida*), *Y. glauca* var. *arkansana, Y. glauca, Y. constricta,* and *Y. aloifolia.*

*Prodoxus quinquepunctellus (*Chambers*)*

Morphology. Wingspan male 11.0–16.5 mm, female 11.5–21.0 mm. Integument medium dark brown. *Head:* With white scales. Maxillary palp 5-segmented, without tentacle on basal segment. Labial palp 3-segmented, with prominent apical sensilla. Proboscis tan-colored, relatively long. Antenna dark brown, with white scales on basal half. *Thorax:* With white scales. Forewing length in male 4.9–8.7 mm, female 5.0–10.4 mm; dorsal surface white or light creamy white, with 0–18 spots ranging from a single scale to having a long axis of ≤0.7 mm. As many as 8 spots can be interior, whereas the remainder are distributed at vein tips along the costa and outer edge. If only a single spot present, it is invariably in the discal field. Underside brown except for yellowish white portion overlapping hindwing. Hindwing light to medium gray. Underside sparsely scaled in brownish gray, with darker area along fore edge where overlapping with forewing. Wing fringes white. *Abdomen:* With dorsal scaling white to light tan, with last two segments with white linear semierect scales forming brush. Underside white to light tan in both sexes. In male, valva with white or light tan scales. *Male genitalia:* Valva mostly linear but expanded dorsally at apex, with 2–5 short spines along apicoventral margin. Aedeagus 1.15–1.82 mm long. *Female genitalia:* Posterior apophyses 4.0–6.8 mm long; ovipositor 0.08–0.16-mm high near tip, with 4–10 dorsal teeth; corpus bursae with two 0.27- to 0.42-mm signa.

Material examined and types. 792 males, 553 females (60 males, 58 females for genitalia). Lectotype, *Hyponomeuta 5-punctella,* from Bosque Co., Texas (MCZ). Designated by Davis (1967). A second lectotype, *Hyponomeuta paradoxica,* also designated by Davis (1967), from NE of Colorado Springs, Colorado (MCZ), has not been examined by us, but may pertain to the Colorado Plateau haplotype clade.

Distribution and recorded hosts. The range is given in Fig. 2. *Yucca pallida, Y. rostrata* (incl. *Y. rigida*), *Y. glauca, Y. angustissima* (incl. *Y. kanabensis*), *Y. baileyi* (incl. *Y. intermedia, Y. navajoa,* and *Y. standleyi*), *Y. elata,* and *Y. harrimaniae* (incl. *Y. gilbertiana* and *Y. neomexicana*).

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REFERENCES

Abbott, P. L., and Woodruff, C. M. Jr. (Eds.) (1986). "The Balcones Escarpment: Geology, Hydrology, Ecology and Social Development in Central Texas," Comet Reproduction Service, Santa Fe Springs, CA.

Abrahamson, W. G., and Weis, A. E. (1997). "Evolutionary Ecology across Three Trophic Levels: Goldenrods, Gallmakers and Natural Enemies," Monographs in Population Biology No. 29, Princeton Univ. Press, Princeton, NJ.

Blair, W. F. (1950). The biotic provinces of Texas. *Texas J. Sci.* **2:** 93–117.

Block, S. B., and Zimmerman, E. G. (1991). Allozymic variation and systematics of plains pocket gophers (*Geomys*) of south-central Texas. *Southwest. Nat.* **36:** 29–36.

Brown, J. M., Abrahamson, W. G., Packer, R. A., and Way, P. A. (1995). The role of natural enemy escape in a gallmaker host-plant shift. *Oecologia* **104:** 52–60.

Busck, A. (1947). The yucca moth. *In* "Yuccas of the Southwestern United States" (S. D. McKelvey, Ed.), Vol. 2, pp. 180–185. Arnold Arboretum, Jamaica Plain, MA.

Bush, G. L. (1994). Sympatric speciation: New wine in old bottles. *Trends Ecol. Evol.* **9:** 285–288.

Carroll, S. P., Dingle, H., and Klassen, S. P. (1997). Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. *Evolution* **51:** 1182–1188.

Carroll, S. P., Klassen, S. P., and Dingle, H. (1998). Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. *Evol. Ecol.* **12:** 955–968.

Chambers, V. L. (1875). Tineina from Texas. *Can. Entomol.* **7:** 7–12.

Christiansen, R. L., and Yeats, R. S. (1992). Post-Laramide geology of the U.S. Cordilleran region. *In* "The Cordilleran Orogen: Conterminous United States" (P. W. Burchfiel, B. C. Lipman, M. L. Zoback, and P. C. Burchfiel, Eds.) "The Geology of North America," Vol. G-3, pp. 261–406. Geol. Soc. Am., Boulder, CO.

Clary, D. O. and Wolstenholme, D. R. 1985. The mitochondrial DNA molecule of *Drosophila yakuba:* nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.* **22:** 252–271.

Davis, D. R. (1967). A revision of the moths of the subfamily Prodoxinae (Lepidoptera: Incurvariidae). *U.S. Natl. Mus. Bull.* **255:** 1–170.

Davis, J. R. (1996). The creeping water bugs (Hemiptera: Naucoridae) of Texas. *Southwest. Nat.* **41:** 1–26.

Ehrlich, P. R., and Raven, P. H. (1964). Butterflies and plants: A study in coevolution. *Evolution* **18:** 586–608.

Excoffier, L., Smouse, P. E., and Quattro, J. M. (1992). Analysis of

molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131:** 479–491.

Farrell, B. D. (1998). Inordinate fondness explained: Why are there so many beetles. *Science* **281:** 555–559.

Feder, J. L. (1995). The effects of parasitoids on sympatric host races of *Rhagoletis pomonella* (Diptera: Tephritidae). *Ecology* **76:** 801–813.

Feder, J. L. (1998). The apple maggot fly, *Rhagoletis pomonella*: Flies in the face of conventional wisdom about speciation. *In* "Endless Forms: Species and Speciation" (D. J. Howard and S. H. Berlocher, Eds.), pp. 130–144, Oxford Univ. Press, London.

Filchak, K. E., Feder, J. L., Roethele, J. B., and Stolz, U. (1999). A field test for host-plant dependent selection on larvae of the apple maggot fly, *Rhagoletis pomonella*. *Evolution* **53:** 187–200.

Frack, D. C. (1982). "A Systematic Study of Prodoxine Moths (Adelidae: Prodoxinae) and Their Hosts (Agavaceae) with Descriptions of the Subfamilies of Adelidae (*s. lat.*)," M.S. thesis, California State Polytechnic University, Pomona; CA.

Funk, D. J. (1998). Isolating a role for natural selection in speciation: Host adaptation and sexual isolation in *Neochlamis bebbianae* leaf beetles. *Evolution* **52:** 1744–1759.

Graham, A. (1999). "Late Cretaceous and Cenozoic History of North American Vegetation North of Mexico," Oxford Univ. Press, New York.

Gratton, C., and Welter, S. C. (1999). Does "enemy-free space" exist? Experimental host shifts of an herbivorous fly." *Ecology*. **80:** 773–785.

Groman, J. D., and Pellmyr, O. (2000). Rapid evolution and specialization following host colonization in a yucca moth. *J. Evol. Biol.* **13:** 223–236.

Hagele, B. F., and Rowell-Rahier, M. (2000). Choice, performance and heritability of performance of specialist and generalist insect herbivores towards cacalol and seneciphylline, two allelochemicals of *Adenostyles alpina* (Asteraceae). *J. Evol. Biol.* **13:** 131–142.

Harrison, R. G., Rand D. M., and Wheeler, W. C. (1987). Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Mol. Biol. Evol.* **4:** 144–158.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **21:** 160–174.

Hopkins, G. W., and Dixon, A. F. G. (1997). Enemy-free space and the feeding niche of an aphid. *Ecol. Entomol.* **22:** 271–274.

Holt, R. D., and Lawton, J. H. (1993). Apparent competition and enemy-free space in insect host–parasitoid communities. *Am. Nat.* **142:** 623–645.

Keese, M. C. (1997). Does escape to enemy-free space explain host specialization in two closely related leaf-feeding beetles (Coleoptera: Chrysomelidae)? *Oecologia* **112:** 81–86.

Keese, M. C. (1998). Performance of two monophagous leaf feeding beetles (Coleoptera: Chrysomelidae) on each other's host plant: Do intrinsic factors determine host plant specialization? *J. Evol. Biol.* **11:** 403–419.

Kopf, A., Rank, N. E., Roininen, H., Julkunen-Tiitto, R., Pasteels, J. M., and Tahvanainen, J. (1998). The evolution of host-plant use and sequestration in the leaf beetle genus *Phratora* (Coleoptera: Chrysomelidae). *Evolution* **52:** 517–528.

Leebens-Mack, J., Pellmyr, O., and Brock, M. (1998). Host specificity and genetic structure of two yucca moth species in a yucca hybrid zone. *Evolution* **52:** 1376–1382.

Lucchitta, I. (1989). History of the Grand Canyon of the Colorado River in Arizona. *In* "Geologic Evolution of Arizona" (J. P. Penney and S. J. Reynolds, Eds.), *Arizona Geol. Soc. Digest* **17:** 701–705.

McQuarrie, N., and Chase, C. G. (2000). Raising the Colorado Plateau. *Geology* **28:** 91–94.

Miles, N. J. (1983). Variation in host specificity in the yucca moth *Tegeticula yuccasella* (Incurvariidae): A morphometric approach. *J. Lepidopt. Soc.* **37:** 207–216.

Mitter, C., Farrell, B. D., and Wiegmann, B. (1988). The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification? *Am. Nat.* **132:** 107–128.

Pammel, L. H. (1925). The extension of the yucca moth. *Science* **61:** 414–415.

Parsons, T., and McCarthy, J. (1995). The active southwest margin of the Colorado Plateau: Uplift of mantle origin. *Geol. Soc. Am. Bull.* **107:** 701–715.

Pellmyr, O. (1999). Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. *Syst. Entomol.* **24:** 243–271.

Pellmyr, O., and Huth, C. J. (1994). Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* **372:** 257–260.

Pellmyr, O., and Leebens-Mack, J. (1999). Forty million years of mutualism: Evidence for Eocene origin of the yucca–yucca moth association. *Proc. Natl. Acad. Sci. USA* **96:** 9178–9183.

Pellmyr, O., and Leebens-Mack, J. (2000). Reversal of mutualism as a mechanism for adaptive radiation in yucca moths. *Am. Nat.* **156:** S62–S76.

Pellmyr, O., Leebens-Mack, J., and Huth, C. J. (1996). Non-mutualistic yucca moths and their evolutionary consequences. *Nature* **380:** 155–156.

Powell, J. A. (1985). Biological interrelationships of moths and *Yucca schotti*. *Univ. Calif. Publ. Entomol.* **100:** 1–93.

Powell, J. A. 1992. Interrelationships of yuccas and yucca moths. *Trends Ecol. Evol.* **7:** 10–15.

Richardson, L. R., and Gold, J. R. (1995). Evolution of the *Cyprinella lutrensis* species complex. II. Systematics and biogeography of the Edwards Plateau shiner, *Cyprinella lepida*. *Copeia* **1995:** 28–37.

Riley, C. V. (1880). The true and bogus yucca moth, with remarks on the pollination of *Yucca*. *Am. Entomol.* **3:** 141–145.

Riley, C. V. (1892). The yucca moths and *Yucca* pollination. *Annu. Rep. Missouri Bot. Gard.* **3:** 99–158.

SAS Institute (1998). JMP 3.2.1. Cary, North Carolina.

Spencer J. E. (1996). Uplift of the Colorado Plateau due to lithosphere attenuation during Laramide low-angle subduction. *J. Geophys. Res.* **101:** 13595–13609.

Swofford, D. L. 2000. "PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)", Version 4. Sinauer, Sunderland, MA.

Thompson, J. N. (1994). "The Coevolutionary Process," Univ. of Chicago Press, Chicago.

Trelease, W. (1902). The Yucceae. *Annu. Rep. Missouri Bot. Gard.* **13:** 27–133.

Via, S. (1999). Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution* **53:** 1446–1457.

Zandt, G., Meyers, S. C., and Wallace, T. C. (1995). Crust and mantle structure across the Basin and Range—Colorado Plateau boundary at 37°N latitude and implications for Cenozoic extensional mechanism. *J. Geophys. Res.* **100:** 10529–10548.