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Patterns of Speciation in the Yucca Moths: Parallel Species Radiations within the *Tegeticula yuccasella* Species Complex

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Abstract.—The interaction between yuccas and yucca moths has been central to understanding the origin and loss of obligate mutualism and mutualism reversal. Previous systematic research using mtDNA sequence data and characters associated with genitalic morphology revealed that a widespread pollinator species in the genus *Tegeticula* was in fact a complex of pollinator species that differed in host use and the placement of eggs into yucca flowers. Within this mutualistic clade two nonpollinating "cheater" species evolved. Cheaters feed on yucca seeds but lack the tentacular mouthparts necessary for yucca pollination. Previous work suggested that the species complex formed via a rapid radiation within the last several million years. In this study, we use an expanded mtDNA sequence data set and AFLP markers to examine the phylogenetic relationships among this rapidly diverging clade of moths and compare these relationships to patterns in genitalic morphology. Topologies obtained from analyses of the mtDNA and AFLP data differed significantly. Both data sets, however, corroborated the hypothesis of a rapid species radiation and suggested that there were likely two independent species radiations. Morphological analyses based on oviposition habit produced species groupings more similar to the AFLP topology than the mtDNA topology and suggested the two radiations coincided with differences in oviposition habit. The evolution of cheating was reaffirmed to have evolved twice and the closest pollinating relative for one cheater species was identified by both mtDNA and AFLP markers. For the other cheater species, however, the closest pollinating relative remains ambiguous, and mtDNA, AFLP, and morphological data suggest this cheater species may be diverged based on host use. Much of the divergence in the species complex can be explained by geographic isolation associated with the evolution of two oviposition habits. [AFLP; coevolution; host use; mtDNA; parallel species radiation; yucca moths.]

Mutualistic interactions are part of the biotic foundation upon which many communities and ecosystems are based. These interactions can range from extremely specific relationships, such as those between endosymbionts and their hosts, to very generalized ones, such as between some plants and their pollinators (Stanton, 2003). Most of the conceptual advances in mutualism, however, have been based on a few model systems. Obligate pollination mutualisms, in particular, have served as focal systems for study. For example, obligate mutualisms between seed-eating pollinators and plants, such as yuccas and yucca moths and figs and fig wasps, have provided the empirical and theoretical basis for examining the benefits and costs, stability, and the exploitation of mutualism (Pellmyr and Huth, 1994; Anstett et al., 1996; Addicott, 1996, 1998; West et al., 1996; Herre, 1999; Herre et al., 1999; Huth and Pellmyr, 2000; Marr et al., 2001; Bronstein et al., 2003; Cook and Rasplus, 2003; Morris et al., 2003; Peng et al., 2005). Research on the ecology and evolution of these systems has affirmed that mutualism is a balanced antagonistic interaction.

Understanding how mutualisms originate and subsequently diversify requires a phylogenetic approach. The incorporation of molecular systematics into the study of obligate pollination mutualism has provided this approach and has had two important consequences. First, the use of a phylogenetic framework allows testing of hypotheses dealing with coevolution, correlated evolution, cospeciation, modes of speciation, and the evolution of cheating from within mutualistic lineages (Herre et al., 1996; Pellmyr and Leebens-Mack, 2000; Machado et al., 2001, 2005; West et al., 1996; Weiblen, 2004). Second, molecular data have shown that some of these supposedly species-poor systems are in reality complexes of cryptic species that differ little in morphology (Weiblen et al., 2001; Weiblen and Bush, 2002; Kato et al., 2003; Molbo et al., 2003). Moreover, these species complexes are sometimes the result of rapid radiations, which suggests that interactions among species may impact the rate at which these lineages diversify.

In this study, we examined the phylogenetic history of a rapid radiation within the pollinating yucca moths of the genus *Tegeticula*. Previous phylogenetic work based on mtDNA sequence data demonstrated that the geographically widespread pollinator *T. yuccasella* was, in fact, a species complex of pollinator and cheater moths (Pellmyr et al., 1996; Pellmyr and Leebens-Mack, 1999, 2000) that differed in host use and genitalic morphology. Although each of the species within the *T. yuccasella* complex as a whole were monophyletic based on the mtDNA sequence data, there was little statistical support for the species relationships within a clade containing seven pollinator species and the two cheater species. This clade originated in a burst of speciation that occurred 3.2 ± 1.8 Mya (Pellmyr and Leebens-Mack, 1999) and was concomitant with the colonization of capsular-fruited *Yucca*, the evolution of a new oviposition habit, and the evolution of cheater moth species that do not pollinate yucca flowers but still feed on yucca seeds. Most of the ecological diversity in *Tegeticula* arose during this radiation. A robust phylogeny of this group is necessary for testing hypotheses about coevolution, mutualism reversal, and cospeciation between yuccas and yucca moths. Here, we build on previous phylogenetic studies by expanding the mtDNA data set to include sequence data from another mtDNA gene, cytochrome *b*,

and adding genome-wide nuclear markers via AFLPs. AFLPs have been useful in resolving the phylogenetic relationships in other very closely related groups of organisms (Albertson et al., 1999; Parsons and Shaw, 2001; Ogden and Thorpe, 2002; Deprés et al., 2003; Mendelson and Shaw, 2005). We explore the congruence and conflict between these two data sets to examine the evolution of oviposition habit, mutualism reversal, and the diversification of the yucca moths.

The Study System

The well-known obligate pollination mutualism between yuccas and yucca moths consists of the interactions of many yucca and pollinator moth species. The yuccas (*Yucca* and *Hesperoyucca*) contain about 40 species, with *Yucca* divided into three sections based on fruit type: the spongy-fruited section *Clistocarpa*, the fleshy-fruited section *Sarcocarpa*, and the capsular-fruited section *Chaenocarpa*. Vegetative form also differs among the host plants, ranging from the tall branching tree-like form of the Joshua tree, *Y. brevifolia*, to small rosettes such as in *Y. harrimaniae*. Irrespective of fruit type or vegetative form, all yucca species are actively pollinated by at least one species of yucca moth (Pellmyr, 2003).

The pollinator yucca moths consist of two genera, *Parategeticula* and *Tegeticula*. Much of the research examining the obligate mutualism has focused on *Tegeticula*. This North American genus contains a total of fourteen described pollinator species and two derived nonpollinating cheater species (Pellmyr, 1999, 2003). The interaction between the *Tegeticula* pollinators and yuccas is highly specific, with 11 of the 14 pollinators utilizing only one yucca species (Pellmyr, 1999, 2003). All of the pollinator species actively pollinate yucca flowers using specialized mouthparts, but they differ in oviposition habit. Female yucca moths deposit eggs in yucca flowers and the resulting larvae feed on a subset of the developing yucca seeds. There are two major oviposition habits among *Tegeticula* species. Locule-ovipositing species use a long and narrow ovipositor to cut through the flower ovary wall into the locule and lay eggs next to the ovules. This is the ancestral oviposition habit for the genus (Pellmyr and Leebens-Mack, 1999). In contrast, superficially ovipositing species use a short, thick ovipositor to place eggs within or just below the tissue surface. The larva then chews into the locule to feed on the developing ovules.

Previous phylogenetic studies based on mtDNA sequence data suggested that much of the species and life history diversity within *Tegeticula* occurred in a burst of speciation (Pellmyr and Leebens-Mack, 1999, 2000). The colonization of capsular-fruited *Yucca*, the shift in oviposition habit from locule oviposition to superficial oviposition, and the evolution of cheater moths all occurred during this radiation (Pellmyr and Leebens-Mack, 1999, 2000). Within this explosion, several well-supported clades were identified, but the relationships among them were uncertain because of short internal branches. Based on the well-supported clades, Pellmyr and Leebens-Mack (2000) concluded that oviposition habit was labile, and that the evolution of cheating occurred twice within the radiation. Here, we demonstrate that the species relationships as portrayed by mtDNA contrasts with those inferred from the nuclear loci. The nuclear markers showed support for two clades of moths that differ in oviposition habit, and the two cheater species evolved from within the "superficially ovipositing" clade. Differences in moth morphology also supported the two clades identified by the nuclear loci. Furthermore, the results suggest that shifts between oviposition habits may be more phylogenetically conservative than previously indicated.

METHODS

Five individuals from each of the studied *Tegeticula* species were included in the analyses (Table 1). A new species, which we have given the working name 'T. californía' was identified based on this study. Individuals were chosen to maximize coverage of the host range and geographic range of each moth species (Fig. 1). We used *T. treculeanella* as the outgroup based on previous work by Pellmyr and Leebens-Mack (1999, 2000). We also included *T. mojavella* in the analyses to further polarize the phylogeny. This pollinator is more closely related to the ingroup than *T. treculeanella*, and we wanted to be certain that the ingroup remained monophyletic with additional mtDNA sequence and AFLP data. 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 a modified protocol of Harrison et al. (1987) or the IsoQuick DNA Extraction kit (Orca Research Inc., Bothell, WA). For larvae, the entire specimen was extracted.

Sequencing Protocol

We sequenced two regions of the mtDNA for each of the taxa. The first region was 2104 contiguous bp that included the 3′ end of cytochrome oxidase I, the intervening tRNA lysine, and the 5′ end of cytochrome oxidase II. We also sequenced 919 contiguous bp of the cytochrome *b* gene. The cytochrome oxidase region was amplified with four pairs of PCR primers that produced overlapping regions of sequence. The primer pairs were 1461F-2302R, 2231F-3020R, 2638F-3306R, and 3252F-3771R and the numbers refer to the nucleotide positions in the *Drosophila yakuba* mtDNA genome (Clary and Wolstenholme, 1985). The cytochrome *b* region was amplified with two pairs of overlapping PCR primers: cytB1-cytB2 and cytB3-cytB4. (Primer sequences are available upon request from the authors.) The thermal cycler profile for amplification of both regions 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, and 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

TABLE 1. Host use and geographic localities of *Tegeticula* species studied. Five individuals per species were sampled. Ovip. Site is oviposition site, L = oviposition into ovary locule, S = superficial oviposition into ovary wall. The two cheater species oviposit into fruit rather than flowers.

Moth sp. (ovip. site)	Host	Collection site	Lat/long	Genbank nos.	
Pollinators				COI-II	cytb
T. treculeanella (L)	*Y. treculeana*	TX. Laguna Atascosa NWR	26.25, −97.35	DQ075465	DQ075521
		TX. Big Bend National Park	29.4083, −103.1416	DQ075466	DQ075522
	Y. filifera	MEX. SLP. Pozos de Santa Clara	23.2502, −100.5474	DQ075468	DQ075524
	Y. decipiens	MEX. Durango. WSW Durango	23.9865, −104.747	DQ075467	DQ075523
	Y. filifera	MEX. Hidalgo. San Vicente	19.9974, −98.7005	DQ075469	DQ075524
T. mojavella (L)	*Y. schidigera*	CA. Morongo Valley	34.0469, −116.5808	DQ075471–72	DQ075531–32
		CA. Mountain Pass	35.472, −115.5458	DQ075473	DQ075533
		CA. Cedar Canyon	35.1646, −115.4442	DQ075474	DQ075534
		CA. Boulevard	32.6666, −116.2833	DQ075475	DQ075535
T. rostratella (L)	*Y. rostrata*	TX. Black Gap WMA	29.55, −102.1166	DQ075478–80	DQ075538–40
		MEX. Coah. Cuatro Cienegas	26.83, −102.1509	DQ075476	DQ075536
		MEX. Coah. NE San Pedro	26.113, −102.7435	DQ075477	DQ075537
T. baccatella (L)	*Y. baccata*	NM. Jornada LTER	32.5318, −106.8060	DQ075481–82	DQ075541–42
		NV. Searchlight	35.5063, −115.1392	DQ075484	DQ075544
		AZ. Tucson Mountains	32.2312, −111.0949	DQ075485	DQ075545
		NM. Taos. Las Petacas	36.3821, −105.5232	DQ075483	DQ075543
T. altiplanella (L)	*Y. harrimaniae*	UT. S Moab	38.2791, −109.375	DQ075486	DQ075546
	Y. angustissima	AZ. Peach Springs	35.561, −113.4226	DQ075489	DQ075549
		NM. Los Lunas	34.8061, −106.7327	DQ075490	DQ075550
	Y. kanabensis	UT. Hwy 89 N Kanab	37.1793, −112.6352	DQ075487	DQ075547
	Y. baileyi	AZ. St Johns	34.6666, −109.65	DQ075488	DQ075548
T. yuccasella (L)	*Y. rupicola*	TX. W Harper	30.3, −99.55	DQ075507	DQ075567
	Y. glauca	WY. S Devil's Tower	44.33, −104.9100	DQ075506	DQ075566
	Y. filamentosa	TN. Vine	36.0446, −86.3706	DQ075505	DQ075570
	Y. reverchoni	TX. Sonora	30.5, −100.375	DQ075508	DQ075568
	Y. campestris	TX. Royalty	31.3723, −102.8671	DQ075509	DQ075569
'T. california' (L)	*Y. schidigera*	CA. Torrey Pines State Park	32.9212, −117.2568	DQ075470	DQ075526–30
T. elatella (S)	*Y. elata*	TX. Big Bend National Park	29.4083, −103.1416	DQ075491	DQ075555
		AZ. Sierra Vista	31.6264, −110.1739	DQ075492−95	DQ075551–54
T. superficiella (S)	*Y. utahensis*	UT. St George	37.1406, −113.6107	DQ075496	DQ075556
		UT. Snow Canyon	37.2166, −113.6458	DQ075498–500	DQ075558–60
	Y. kanabensis	UT. N Kanab	37.1793, −112.6352	DQ075497	DQ075557
T. cassandra (S)	*Y. filamentosa*	FL. Inverness	28.9833, −82.4166	DQ075514	DQ075574
		FL. Gold Head SP	29.8782, −81.9268	DQ075511	DQ075571
		FL. Ocala NF Big Scrub Cmpgrd	29.0486, −81.6899	DQ075512	DQ075572
		FL. Placid Lakes	27.1533, −81.3762	DQ075515	DQ075575
		GA. Ludowici	31.708, −81.7423	DQ075513	DQ075573
Cheaters					
T. corruptrix (L)	*Y. glauca*	WY. S Devil's Tower	44.33, −104.9100	DQ075516	DQ075576
	Y. elata	AZ. Willcox	32.2239, −109.8809	DQ075519	DQ075579
	Y. torreyi	TX. Big Bend National Park	29.4, −103.1750	DQ07517	DQ075577
	Y. arizonica	AZ. I-10 exit 281	32.0074, −110.6893	DQ075520	DQ075580
	Y. schidigera	CA Pinyon Flat	32.575, −116.4666	DQ075518	DQ075578
T. intermedia (S)	*Y. angustissima*	NM. Los Lunas	34.8061, −106.7327	DQ075501	DQ075561
	Y. glauca	KS. Fowler	37.4583, −100.1583	DQ075502	DQ075562
	Y. constricta	TX. Jct Rte 290 × I-10	30.2916, −99.5291	DQ075504	DQ075564
	Y. filamentosa	OH. Georgesville	39.8908, −83.2219	DQ075503	DQ075563
		TN. Vine	36.0446, −86.3706	DQ075505	DQ075565

sequencing buffer. Sequencing products were cleaned using Centri-Sep sephadex columns (Princeton Separations, Inc.), lyophilized, and resuspended in 2.5 μl of formamide and loading dye from Applied Biosystems. Products were sequenced on an ABI 377 automated DNA sequencer and analyzed using Sequencher 3.1 (Gene Codes Corp., Ann Arbor, MI).

AFLP Protocol

We also generated AFLP markers on an additional set of samples representing each of the species. Initial AFLP runs with the individuals used for mtDNA sequencing produced small numbers of fragments and had low repeatability. This was an artefact of low-quality nuclear DNA, even though mtDNA amplification and sequencing was successful. Because many of the samples were extracted 5+ years ago, the nuclear DNA had degraded, even though the mtDNA had remained stable. We extracted DNA using the IsoQuick DNA Extraction kit (Orca Research Inc.) from an additional set of samples representing each of the species to obtain high quality DNA for the AFLP procedure. As for sequencing, individuals were chosen to maximize coverage of the host range and geographic range of each moth species. None of the individuals sequenced were used in the AFLP analyses. We used a modified protocol of the Applied Biosystems Plant Genome kit developed

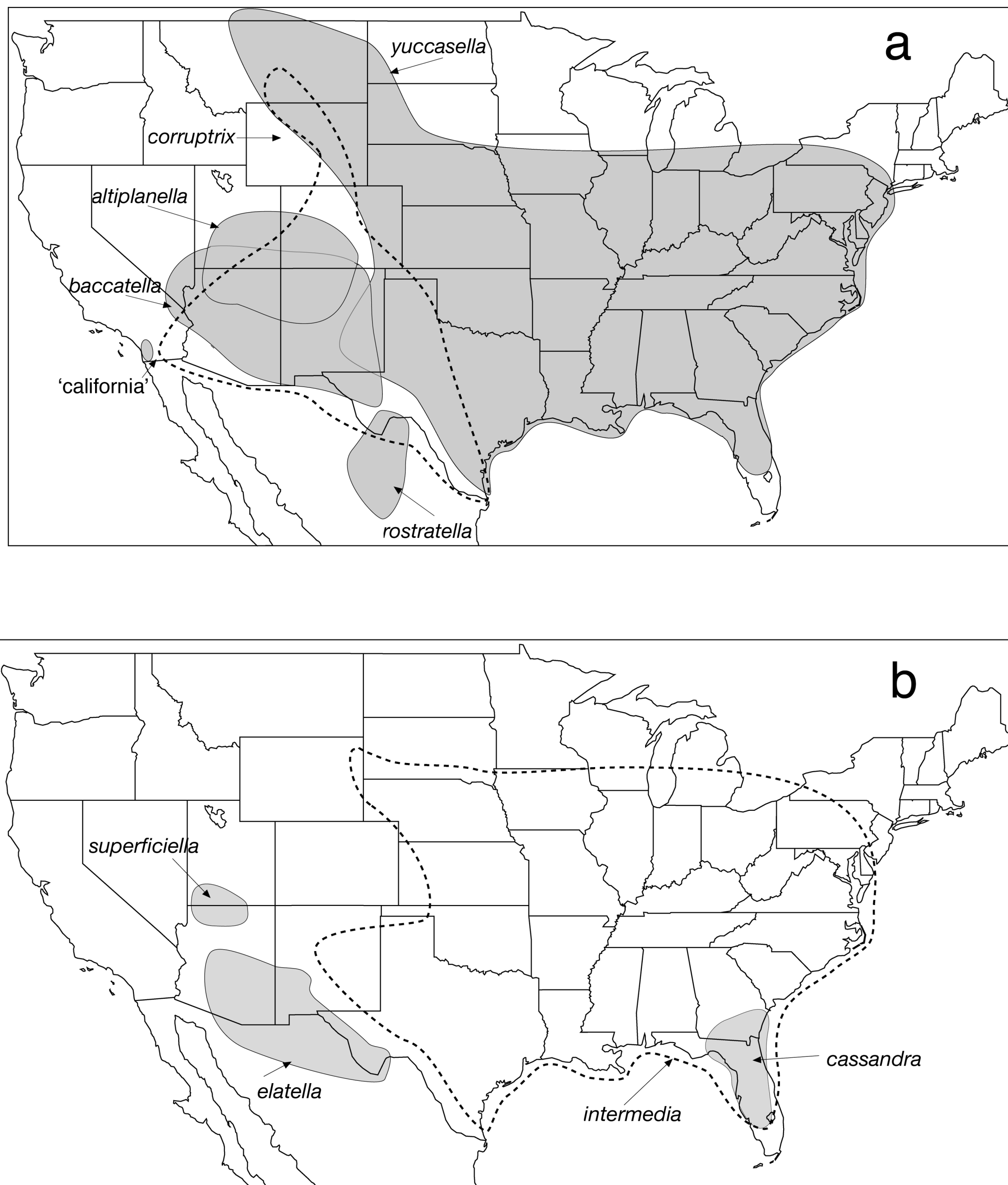

FIGURE 1. Maps showing the geographic distribution of the 10 *Tegeticula* species in the burst of speciation in the *T. yuccasella* species complex. The distributions outlined by dashed lines are for the two cheater species that oviposit into fruit. (a) Distribution of the six locule-ovipositing species (eggs laid next to developing *Yucca* ovules in flowers, except *T. corruptrix*) (b) Distribution of the four superficially ovipositing species (eggs laid in the ovary wall of *Yucca* flowers, expect *T. intermedia*).

by M. Gitzendanner (personal communication) to conduct the AFLP analysis. Restriction and ligation reactions were carried out in a single step. Genomic DNA was digested for three hours at 37°C with 5 units of *Eco*RI (Promega, Madison, WI), 0.5 units of *Mse*I (New England Biolabs, Beverly, MA) in 11 μl reaction volumes containing 0.15 units of T4 DNA ligase (Promega), 1 $\times$ T4 Ligase buffer (Promega), 0.05 M NaCl, 0.55 μg BSA, 4.5 μM *Mse*I adapter (5′-GACGATGAGTCCTGAG-3′ and 5′-TACTCAGGACTCAT-3′), and 0.45 μM *Eco*RI adapter (5′-CTCGTAGACTGCGTACC-3′ and 5′-AATTGGTACGCAGTCTAC-3′). Reactions were diluted by a factor of 20 in 1 $\times$ $TE_{0.1}$ (20 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), and followed by two rounds of selective amplification. The first selective amplification was conducted in 20-μl reaction volumes containing 4 μl of the diluted restriction-ligation reaction, 1 unit *Taq* DNA polymerase (Sigma Chemical Co., St. Louis, MO), 10 $\times$

PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 3.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.3 μM *Eco*RI +1 selective primer (5′-GACTGCGTACCAATTCA-3′), and 0.3 μM *Mse*I +1 selective primer (5′-GACGATGAGTCCTGAGTAAC-3′). Reactions were heated to 72°C for 2 min, then cycled 20 times at 94°C for 30 s, 56°C for 30 s, 72°C for 120 s, and then held at 60°C for 30 min. These reactions were diluted by a factor of 14 in 1 $\times TE_{0.1}$ and used in the final selective amplification step.

The final amplification was performed in 10-μl reactions containing 2.5 μl dilute +1 PCR product, 0.5 units Amplitaq Gold DNA polymerase (Applied Biosystems), 1 × Amplitaq PCR Buffer (Applied Biosystems), 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.05 μM of each *Eco*RI +3 primer (5′-[5-TET] GACTGCGTACCAATTCAAC-3′; 5′-[6-FAM] GACTGCGTACCAATTCACA-3′; [5-HEX]GACTGCGTACCAATTCAAG), and 0.125 μM *Mse*I +3 primer (5′-GACGATGAGTCCTGAGTAACAG-3′). The *Eco*RI +3 primers were fluorescently labeled for visualization on an ABI 377 sequencer. Because each primer was labeled with a different wavelength of dye, single reactions contained three primer combinations (i.e., each *Eco*RI +3 primer with the *Mse*I +3 primer). The reactions were held at 94°C for 2 min, then cycled 10 times starting at 94°C for 30 s, 65°C for 30 s, 72°C for 2 min, with a reduction in the annealing temperature by 1°C per cycle. Reactions were then cycled 36 times at 94°C for 30 s, 56°C for 30 s, 72°C for 120 s, followed by a 30-min 60°C hold. A total of 1.2 μl of loading dye (63% deionized formamide, 27% ABI blue loading dye, and 10% Tamra 500 size standard) from Applied Biosystems plus 1.5 μl of the +3 PCR product was loaded onto a 5% acrylamide gel. Electrophoresis was conducted on an ABI 377 sequencer using the GeneScan2400 run parameters. Fragment sizes were assigned using the Tamra 500 size standard and the GeneScan software version 3.1.2 (Applied Biosystems). We used Genotyper 2.5 (Applied Biosystems) to develop a set of AFLP markers to score for all individuals. Scoring of AFLP markers was conducted in two steps. First, we screened every individual for markers whose peak fluorescence was 300 fluorescent units or above. Each marker greater than 300 fluorescent units was added to a marker database. We then rescreened with this composite marker database to determine which subset of markers was present in each individual. A cutoff threshold of 25 fluorescent units or above was used for scoring the presence of a marker in this second step. The default detection setting on the GeneScan data collection software (Applied Biosystems) is arbitrarily set at 50 fluorescent units. Because there is always variation in overall fluorescent intensities across runs, we chose a lower threshold to increase the likelihood that a band would be scored as present. That is, we wanted to reduce the chance of missing the presence of a band because of variation in fluorescence intensities that occur due to electrophoresis conditions. This procedure would not bias the pattern of relatedness because all samples would be scored with the same threshold value.

Phylogenetic Analyses

The mtDNA sequence data were analyzed using maximum likelihood following the procedures and recommendations in Sullivan (2005). We first determined the model of sequence evolution using the DT-ModSel program (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 model was used in a heuristic search with random addition of taxa, 10 replicate searches, and TBR branch swapping in PAUP 4.0b10 (Swofford, 2002). We performed an absolute goodness of fit test (Goldman, 1993) to determine whether the model chosen by DT-ModSel was appropriate. To conduct this test, the difference in the unconstrained likelihood and the likelihood from the heuristic search was recorded. The resulting tree and parameter estimates of the model were used in Seq-Gen 1.2.7 (Rambaut and Grassly, 1997) to generate 100 replicate data sets that were used in a subsequent parametric bootstrap test. We then performed a maximum likelihood search on each replicate data set to compare the unconstrained likelihood score with the score from the heuristic search. These searches were performed on a 108-node Beowulf cluster at the University of Idaho. The difference between the unconstrained and constrained likelihoods for each replicate was used to generate a null distribution. We then compared the difference between the unconstrained likelihood and the constrained likelihood for the original data set against this distribution. Once the model was confirmed to be appropriate for the sequence data, we ran another heuristic search via maximum likelihood and used the nonparametric bootstrap procedure (Felsenstein, 1985) to assess support for the nodes in the resulting tree topology.

The presence/absence of the AFLP markers were converted to the Nei-Li distance metric (Nei and Li, 1979) in PAUP 4.0b10. We chose this measure as a means to provide a composite index of the signal from all AFLP markers rather than assessing the influence of individual markers. A heuristic search using the minimum evolution criterion was then performed, and support for nodes in the resulting topology was assessed via 100 nonparametric bootstrap replicates. Because the mtDNA and AFLP analyses yielded different topologies, we used a parametric bootstrap approach to test if the topologies were statistically different (Huelsenbeck et al., 1996). We constrained the mtDNA data set to be congruent with the AFLP topology and performed another maximum likelihood analysis. The resulting tree and parameter estimates were used in Seq-Gen 1.2.7 to generate 100 replicate data sets. The likelihood score for the unconstrained search was compared to the likelihood for the AFLP constrained search for each of the data sets to generate the null distribution. We did not conduct a combined analysis of the mtDNA sequence data and the AFLP markers because we had to use different subsets of individuals to collect each data set and the geographic localities sampled within species were not entirely consistent between the two sets of taxa. Also, there was more than

nine times the number of characters for the sequence data compared to the AFLP data which would have swamped out the phylogenetic signal from the AFLP markers.

Morphometric Measurements

Pellmyr (1999) measured 12 female and 11 male continuous morphological traits that were used in designating species within the *T. yuccasella* complex. We made the same measurements for 3 females and 2 males of 'T. california' and used data from Pellmyr (1999) for the other species. Forewing length and width were measured for both sexes. For females we also measured ovipositor traits (length of apophyses anteriores and posteriores, keel height, keel length, ovipositor tip to keel, and ovipositor height) and other reproductive traits (signum diameter, corpus bursae length, corpus bursae width, corpus bursae plus duct length). For males we measured aedeagus length and width, vinculum-saccus length, the number of spines in both pectinifers, and quantified valva shape by measuring distance from valva base to pectinifer, distance from pectinifer to valva apex, distance from valva base to apex, the valval crescent width, and valva crescent depth. We analyzed differences for both sexes among the ingroup using principal components analysis in JMP 5.0.1.2 (SAS Institute). Sample sizes for each species were 5 individuals per sex except for 'T. california,' *T. elatella* (2 females), *T. rostratella* (3 males), *T. altiplanella* (4 males), and *T. superficiella* (4 males). We used ANOVA to test if the first two principal components were significantly different between the species groupings identified by the AFLP analyses.

RESULTS

mtDNA Sequence Data

For each individual, we generated 3021 bp of mtDNA sequence data—2102 bp of cytochrome oxidase I, the intervening tRNA leucine and the 5′ end of cytochrome oxidase II, and 919 bp of cytochrome *b*. Sequences were deposited in GenBank under the accession numbers in Table 1 and the data matrix was deposited in TreeBase. There was a single nucleotide insertion for *T. altiplanella* in the tRNA leucine and an indel in the tRNA for *T. treculeanella* and *T. mojavella*. The nucleotide insertion and the indel position were not used in the phylogenetic analyses because the former was an autapomorphy for *T. altiplanella* and the latter only occurred in the outgroup taxa. The greatest sequence divergence between species in the ingroup was 2.35% (uncorrected p). DT-ModSel returned the HKY+G+I model to describe sequence evolution for the taxa. The absolute goodness-of-fit test did not reject the null hypothesis of a perfect fit between model and data (Fig. 2).

The maximum likelihood analysis of the mtDNA sequence data produced a single tree with a score of $-\ln L = 7341.001$ (Fig. 3). This tree supported monophyly for each of the moth species with the exception of the pollinator-

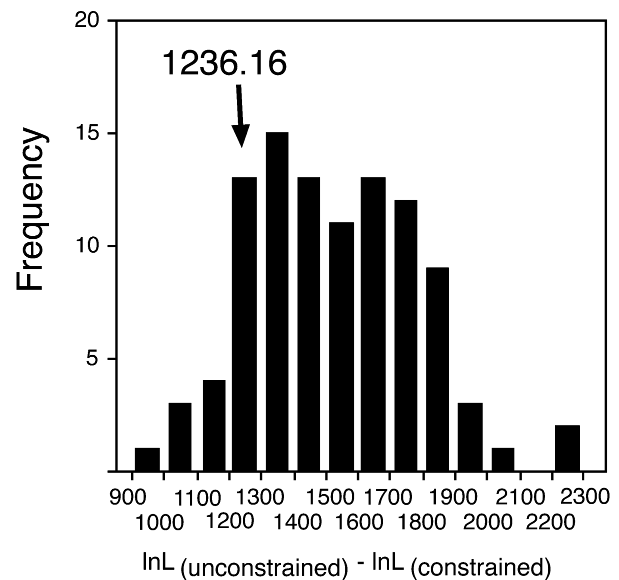

FIGURE 2. Null distribution to test for goodness of fit of model of evolution for mtDNA sequence data. Model was not rejected ($P = 0.87$). The value 1236.16 is the difference in the unconstrained and constrained likelihood values for the original data set.

cheater sister pair *T. cassandra* and *T. intermedia* (bootstrap values for species monophyly were 85 or above). There was strong bootstrap support for two major clades within the ingroup. One clade contained *T. cassandra*, *T. intermedia*, and *T. yuccasella*, and the second clade contained the other seven species. Branch lengths at the base of these two clades were much shorter than the branch lengths leading to each species.

AFLP/nDNA Data

The AFLP procedure generated 352 markers. The AFLP data matrix was deposited in TreeBase. We tested repeatability of the markers by running replicate samples on four individuals. Repeatability was 99.4%. The resulting minimum evolution tree identified two major clades of moths, but the composition of these clades was different than those inferred from the mtDNA data (Fig. 3). One clade comprised the three locule-ovipositing species, *T. altiplanella*, *T. baccatella*, and *T. rostratella*, which was found in the mtDNA analysis, but this clade also included the locule-ovipositing *T. yuccasella*. The phylogenetic relationships within this clade were well supported.

The second clade contained the three superficially ovipositing pollinators, the locule ovipositing pollinator 'T. california,' and the two cheater species. Species relationships within this clade were unresolved except for the pollinator-cheater sister pair *T. cassandra* and *T. intermedia*. The AFLP data also suggested that the locule-ovipositing cheater *T. corruptrix* may be genetically differentiated into two entities based on feeding on capsular or fleshy-fruited yuccas. A similar pattern was also seen in the mtDNA data, although *T. corruptrix* was monophyletic.

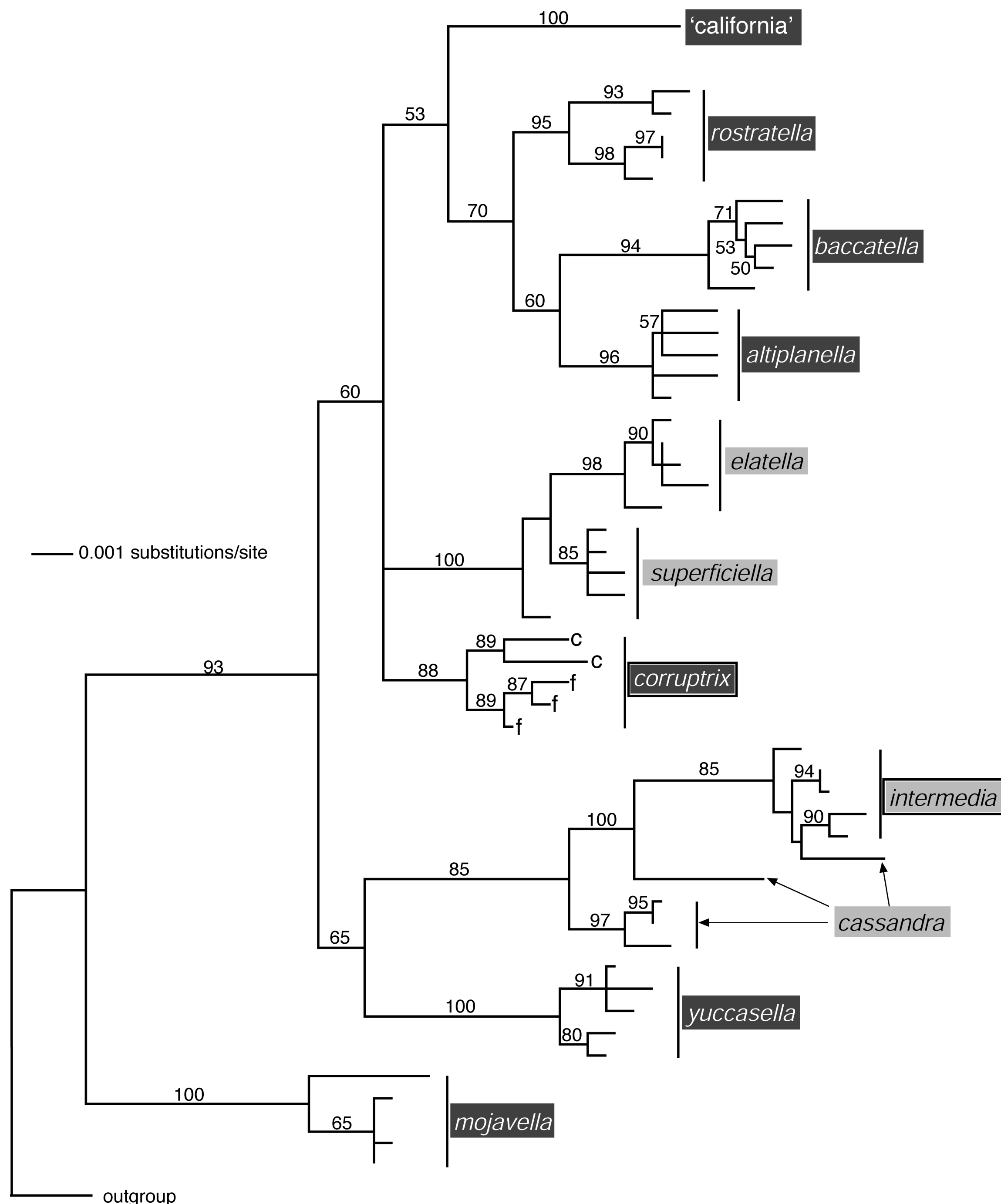

FIGURE 3. Phylogram of *Tegeticula* species based on maximum likelihood analyses of mtDNA sequence data. Bootstrap values above 50 are shown above the branches. Names in dark grey boxes denote locule-ovipositing species and those in light grey boxes superficially ovipositing species. Boxes with borders denote cheater species. Single letters after *T. corruptrix* denote capsular-fruited yucca feeders (c) and fleshy-fruited yucca feeders (f). *T. mojavella* was an additional outgroup.

Morphological Data

The PCA of both female and male morphology showed patterns among the moth species that were very similar to the AFLP topology. The four locule-ovipositing species, *T. altiplanella*, *T. baccatella*, *T. rostratella*, and *T. yuccasella*, were very similar to one another and distinct from the "superficial and cheater" species (Fig. 5). Interestingly, 'T. california' was more similar to the superficially ovipositing taxa even though it is a locule-ovipositing species. The first two principal components were significantly different between the locular and superficial and cheater clades for females (PC 1, $F_{1,49} = 7.003$,

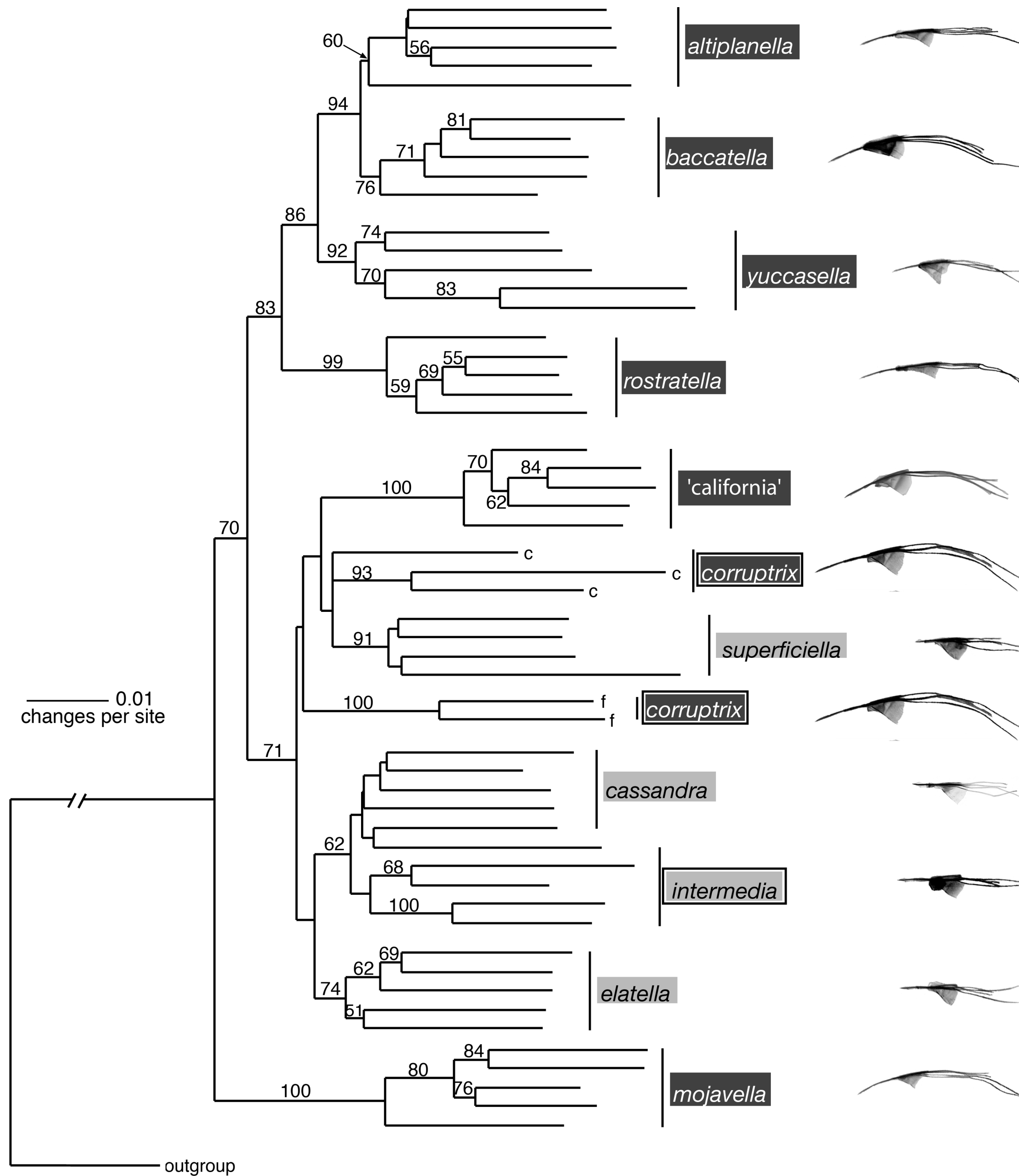

FIGURE 4. Phylogram of *Tegeticula* species based on minimum evolution analyses of Nei-Li distances calculated from 352 AFLP markers. Bootstrap values above 50 are shown above the branches. Names in light grey boxes denote superficially ovipositing species and those in dark grey boxes locule-ovipositing species. Boxes with borders denote cheater species. *T. mojavella* was an additional outgroup. Single letters after *T. corruptrix* denote capsular-fruited yucca feeders (c) and fleshy-fruited yucca feeders (f). To the right of the phylogeny are pictures of ovipositors for each species (all to the same scale). Species differences in oviposition habit corresponded with the AFLP topology. Locule-ovipositing species have longer, curved ovipositors in contrast to the shorter, straight ovipositors of superficially ovipositing species.

$P = 0.011$; PC 2, $F_{1,49} = 78.816$, $P < 0.0001$) and males (PC 1, $F_{1,49} = 87.681$, $P < 0.0001$; PC 2, $F_{1,49} = 6.871$, $P = 0.011$). In terms of differences in female morphology, PC1 and PC2 together explained 81.5% of the variation. The loadings on these two components suggested that all of the measured traits are important in explaining the variation between the two groups (Table 2). No single trait had a disproportionately large eigenvector for either principal component. For PC1, ovipositor length (apophyses posteriores and anteriores) and the other components of reproductive morphology (signum diameter, corpus bursae length, corpus bursae width,

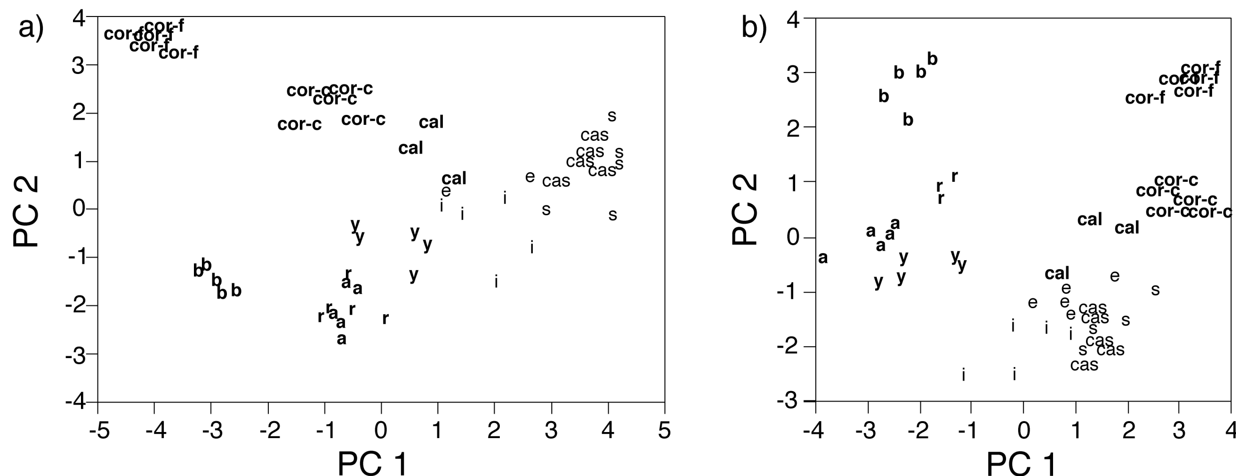

FIGURE 5. Principal component analyses of morphology for (a) female and (b) male *Tegeticula* species. Superficially ovipositing species: cas = *cassandra*; e = *elatella*; i = *intermedia*; s = *superficiella*. Locule-ovipositing species: a = *altiplanella*; b = *baccatella*; cal = 'california'; y = *yuccasella*; cor-c = *corruptrix* from capsular-fruited yuccas; cor-f = *corruptrix* from fleshy-fruited yuccas.

corpus bursae plus duct length) were contrasting. For PC2, body size and ovipositor traits were most important. Morphological differences among males were influenced by body size, aedeagus length, and valval size for PC1, and vinculum-saccus length and aedeagus length for PC2 (Table 2). The late cheater, *T. corruptrix*, exhibited two distinct morphological clusters that reflected individuals that use capsular and fleshy-fruited yuccas, respectively.

Conflict between Molecular Data Sets

Because the AFLPs and morphology presented a similar picture of moth relationships, we tested whether the mtDNA topology was in conflict with these data. We used a parametric bootstrap to test if the mtDNA topology was statistically different from the AFLP topology, by constraining the mtDNA sequence data to the AFLP topology. The likelihood of the unconstrained mtDNA tree was $-\ln L = 7341.001$ and the likelihood of the tree constrained to the AFLP topology was $-\ln L = 7355.108$. The difference of 14.107 likelihood units was well outside of the probability of finding this difference by chance (Fig. 6). Thus, the organellar DNA yielded a significantly different topology than the nuclear loci.

DISCUSSION

Species Radiation and Conflict between Data Sets

Some of the most striking cases of ecological diversity in evolutionary lineages are the result of species radiations (Schluter, 2000). Adaptation to different environments produces combinations of ecologically important traits that generate reproductive isolation among populations (McKinnon et al., 2004). In many radiations, adaptation is rapid and causes speciation events

TABLE 2. Results of principal components analyses for female and male morphology. Eigenvectors for the first two principal components are shown.

	Females			Males	
	PC1	PC2		PC1	PC2
Eigenvalues	6.299	3.482	Eigenvalues	4.625	2.943
Cumulative percent	52.50	81.51	Cumulative percent	42.04	68.80
Eigenvectors			Eigenvectors		
Forewing length	0.201	0.374	Forewing length	0.306	0.290
Forewing width	0.086	0.458	Forewing width	0.384	−0.090
Apophyses posteriores	0.361	0.207	viniculum-saccus length	0.014	0.571
Apophyses anteriores	0.370	0.162	No. teeth on pectinifers	−0.107	0.333
Ovipositor height	0.204	0.315	Valva base to pectinifer	0.357	0.116
Keel height	−0.212	0.324	Pectinifer to apex	0.403	0.009
Ovip. tip to keel	0.255	−0.323	Valva base to apex	0.401	0.158
Keel length	0.253	0.362	Valval crescent width	0.395	−0.120
Signum diameter	−0.345	0.212	Valval crescent depth	0.182	0.075
Corpus bursae length	−0.354	0.150	Aedeagus length	0.010	0.553
Corpus bursae width	−0.343	0.186	Aedeagus width	0.328	−0.326
Corpus bursae + duct	−0.327	0.197			

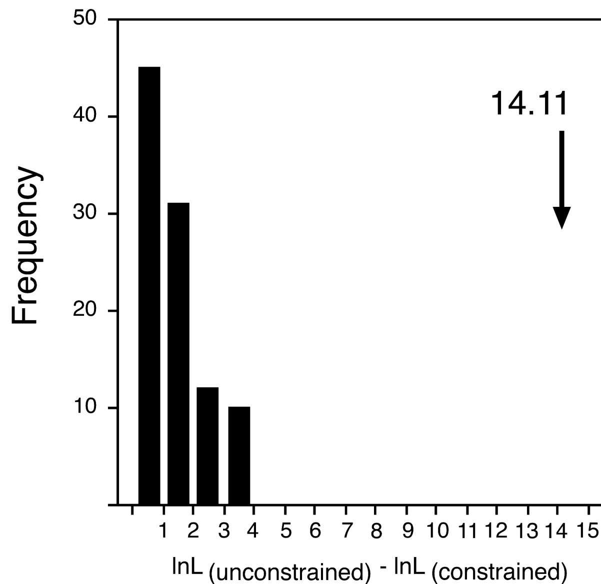

FIGURE 6. Null distribution to test whether mtDNA sequence data constrained to AFLP topology was significantly different from unconstrained mtDNA topology. The two topologies were significantly different ($P < 0.01$).

to happen in quick succession. As a consequence, resolving the phylogenetic relationships among rapidly radiating taxa is difficult, and studies that have incorporated both organellar and nuclear genes have frequently documented phylogenetic conflict (Shaw, 2002; Seehausen et al., 2003; Sullivan et al., 2004). In some cases, the combination of multiple genes still does not resolve species relationships and the resulting phylogenetic tree may contain a hard polytomy (sensu Madison, 1989).

For the yucca moths, previous phylogenetic research based on mtDNA sequence data indicated that this group of mutualists and cheaters had undergone recent and rapid diversification (Pellmyr and Leebens-Mack, 1999, 2000). In the present study, the mtDNA sequence data and AFLP nuclear markers gave somewhat differing pictures of the evolutionary relationships, but both supported the idea that speciation in this group has happened in quick succession. One phylogenetic characteristic of a species radiation is that internal branches are relatively short compared to terminal branches (e.g., Jackman et al., 1999; Fishbein and Soltis, 2004). This pattern was observed in both of the molecular data sets. For the mtDNA topology, the internal branches (i.e., branches uniting two or more species) were significantly shorter than the branches leading to terminal taxa ($F_{1,19} = 5.58$, $P = 0.0296$). The same was true for the AFLP topology ($F_{1,21} = 396.39$, $P < 0.0001$), although this result may be slightly biased by the large number of AFLP markers that were autapomorphies.

A second characteristic of a rapid species radiation is that phylogenetic relationships within major clades may be poorly supported or unresolved even though species are monophyletic. Again, this was true for both data sets. Of the 10 species in the ingroup, 6 were monophyletic for both mtDNA and nuclear markers. The pollinator *T. superficiella* and the cheater *T. corruptrix* were both monophyletic in at least one data set. *Tegeticula superficiella* was monophyletic based on the AFLP data but not the mtDNA data, whereas the converse was true for *T. corruptrix*. Moreover, both molecular data sets suggested that there was intraspecific divergence in *T. corruptrix* based on host use of capsular and fleshy-fruited yucca species. The pollinator-cheater sister pair *T. cassandra*–*T. intermedia* was the only species pair that was not monophyletic in at least one data set. Previous research has demonstrated that these two species were closest relatives and have had limited hybridization between them (Segraves and Pellmyr, 2004); thus, a combination of incomplete lineage sorting and hybridization may explain the lack of monophyly for this clade. Taken together, the pattern of short internal branches, poor resolution at the base of clades, and species monophyly are indicative of speciation events that happened in rapid succession during the early stages of this radiation.

Although both the mtDNA and nuclear data sets supported a rapid radiation of moths, there were differences in the phylogenetic relationships within the radiation. The mitochondrial and nuclear data sets produced significantly different topologies. Both data sets supported two major clades of moths, but the species composition of these clades differed. Which molecular marker data set is presenting a more accurate estimate of the species tree is unclear. There are two methodological reasons that could result in disparate estimates of the moth phylogenies—sampling and algorithm differences. Because we had to use a different set of individuals for the mtDNA and AFLP analyses, sampling could have impacted the phylogenies. That is, data from different individuals would contain different phylogenetic signal, especially in terms of species relationships. This problem has shown to be important by Funk and Omland (2003) when species are highly polyphyletic. The monophyly of many species for both data sets, however, suggests that sampling different individuals within species would not produce different topologies. Each individual within a species would likely contain the same pattern of genetic divergence with respect to other species surveyed. Due to the lack of models for AFLP data, we analyzed the AFLP data with minimum evolution rather than maximum likelihood as for the sequence data. The difference in the search algorithm could result in different topologies. We investigated this possibility by converting the mtDNA sequence data to the HKY85 distance measure and conducting a search using minimum evolution. The minimum evolution search yielded a topology identical to the maximum likelihood search (data not shown), which suggests that there are differences in phylogenetic signal between the two data sets. Neither sampling bias nor search algorithm appears to be a likely cause of the topological differences between the marker sets.

Studies that have surveyed both organellar and nuclear markers in other rapidly radiating lineages have also demonstrated conflict in the phylogenies produced

by each data set (e.g., Shaw, 2002; Sullivan et al., 2004). There are three reasons tentatively to assume that the AFLP topology may be more accurate. First, there may not have been enough time during the early stages of the radiation for sibling taxa to obtain reciprocal monophyly for mtDNA prior to the next speciation event (i.e., incomplete lineage sorting). As a result, the distribution of the ancestral mtDNA haplotypes across the newly formed species was likely somewhat random rather than consistent with the true sequence of speciation. Once the species were formed, however, each species remained distinct and became monophyletic for the mtDNA inherited at the early stage of the radiation. Thus, species are currently monophyletic, but for ancestral mtDNA haplotypes that do not reflect the pattern of speciation. Second, hybridization with pollinators has been identified at least two times in the cheater *T. intermedia* (Segraves and Pellmyr, 2004; Segraves et al., 2005). Hybridization events, either early in the radiation or more recently, would likely significantly affect the mtDNA phylogeny (Ballard and Whitlock, 2004). For example, hybridization might result in capture of mtDNA from a distantly related moth lineage. Thus the organellar phylogeny would not be indicative of the species phylogeny. For AFLP markers, incomplete lineage sorting is also possible; however, the likelihood that the same pattern of sorting would occur across the many different markers is low. Instances of hybridization would result in a merging of the genome rather than wholesale transfer from one species to another as is possible for mtDNA. Finally, differences in genitalic morphology among moth species are more consistent with the AFLP topology than the mtDNA topology (see below).

Morphology and Life History Evolution

Differences in oviposition habit within *Tegeticula* have important consequences for our understanding of diversification within this genus. Morphology of both sexes varies with oviposition habit (Fig. 5). Females of locule-ovipositing species have a long, narrow ovipositor for depositing eggs into the locule next to developing yucca ovules or seeds (Pellmyr, 1999). In contrast, the ovipositors of superficially ovipositing females are short and wide. Analyses of morphology produced species groupings consistent with the major clades depicted by the AFLP topology. For males and females, the locule-ovipositing species *T. rostratella*, *T. altiplanella*, *T. baccatella*, and *T. yuccasella* were distinct from the superficially ovipositing species. The two largest locule-ovipositing species, *T. corruptrix* and 'T. california,' were more morphologically similar to the superficially ovipositing species as was also suggested genetically by the AFLP data. Within the cheater species *T. corruptrix* there was a strong difference in morphology associated with utilizing fleshy-fruited or capsular-fruited *Yucca* species. This distinctiveness is also mirrored in both molecular data sets; however, more data are required to test whether there is genetic differentiation partitioned by host use in this cheater species.

The number of evolutionary transitions between the two broad categories of oviposition habits differs between the phylogenies produced by the mitochondrial and nuclear data sets. For the mtDNA phylogeny, superficial oviposition evolved at least twice—once for the pollinators *T. superficiella* and *T. elatella*, and once for the pollinator-cheater sister pair, *T. cassandra* and *T. intermedia*. The AFLP topology, however, suggests a single origin for all of the superficially ovipositing species. Locule-oviposition is the ancestral condition within *Tegeticula* (Pellmyr and Leebens-Mack, 1999, 2000), which suggests that 'T. california' and *T. corruptrix* may be basal in this superficial clade, and that superficial oviposition evolved just once. Additional phylogenetic resolution is required to fully evaluate this hypothesis.

Pellmyr and Leebens-Mack (2000) used mtDNA sequence data to show that the transition from pollinating to cheating has occurred twice within the radiation, and that both cheaters evolved from superficially ovipositing pollinators. The expanded data set used in the present study corroborates two origins of cheating. For the cheater *T. intermedia*, both mtDNA and AFLP markers identified the superficially ovipositing pollinator *T. cassandra* as the closest relative. The sister species to the locule-ovipositing cheater, *T. corruptrix*, however, remains unclear. Pellmyr and Leebens-Mack (2000) suggested that the superficially ovipositing pollinators *T. superficiella* and *T. elatella* were sibling species to *T. corruptrix*. Based on the current data, we were unable to identify the closest pollinator species for this cheater.

Diversification and Coexistence of Moth Species

The differences in oviposition habit are also important in understanding species distributions and the pattern of speciation within *Tegeticula*. The AFLP and morphological data suggest that there have been two radiations of pollinator species—one locule-ovipositing and one mainly superficially ovipositing clade. Where pollinator species co-occur, the moths differ in oviposition habit. One possible explanation for this pattern is that differences in oviposition habit translate into differences in reproductive morphology. These differences may prevent interspecific hybridization and allows species differing in oviposition habit to coexist, whereas species with similar reproductive morphology would hybridize when in sympatry. In cases where species with similar oviposition habits do come in contact, hybridization has occurred. Segraves and Pellmyr (2004) and Segraves et al. (2005) have documented that the superficially ovipositing cheater *T. intermedia* has hybridized with the superficially ovipositing pollinators *T. cassandra* and *T. elatella*.

The current geographic distributions of the superficially ovipositing pollinator species are allopatric, and this pattern is true for the locule-ovipositing pollinator species with the exception of *T. baccatella* (Fig. 1). *Tegeticula baccatella*, however, uses a fleshy-fruited yucca species in contrast to the sympatric pollinators which use capsular-fruited yuccas from an entirely different *Yucca* section. Thus, host use may also serve as another means

for isolating moth lineages (Leebens-Mack et al., 1998). The phylogenetic results and the current species distributions suggest that geographic separation has been very important in facilitating speciation rather than switches between oviposition habits within moth lineages.

CONCLUSIONS

Members of the *T. yuccasella* species complex comprise a recent species radiation that includes the evolution of a new oviposition habit and two origins of cheating species. Phylogenetic analyses of mtDNA and AFLP markers provided different evolutionary histories of this radiation. Most species were monophyletic for both data sets, but the composition of major clades varied between the data sets. Morphological data corroborated the AFLP data set and together these data suggest that there have been two parallel radiations within the species complex—one involving locule-ovipositing species and the other involving superficially ovipositing species. Discrepancies between the mtDNA and AFLP data sets are potentially the result of the lack of lineage sorting of the mtDNA markers during the early phase of the radiation and/or hybridization. Differences in oviposition habit may be an important mechanism isolating species lineages within this radiation.

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REFERENCES

Addicott, J. F. 1996. Cheaters in yucca/moth mutualism. Nature 380:114–115.

Addicott, J. F. 1998. Regulation of the mutualism between yuccas and yucca moths: Population level processes. Oikos 81:119–129.

Albertson, R. C., J. A. Markert, P. D. Danley, and T.D. Kocher. 1999. Phylogeny of a rapidly evolving clade: The cichlid fishes of Lake Malawi, East Africa. Proc. Natl. Acad. Sci. USA 96:5107–5110.

Anstett, M. C., J. L. Bronstein, and M. Hossaert-McKey. 1996. Resource allocation: A conflict in the fig/fig wasp mutualism? J. Evol. Biol. 9:417–428.

Ballard, J. W., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13:729–744.

Bronstein J. L., W. G. Wilson, and W. F. Morris. 2003. Ecological dynamics of mutualist/antagonist communities. Am. Nat. 162:S24–S39.

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

Cook, J. M., and J. Rasplus. 2003. Mutualists with attitude: Coevolving fig wasps and figs. Trends Ecol. Evol. 18:241–248.

Deprés, L., L. Gielly, W. Redoutet, and P. Taberlet. 2003. Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. Mol. Phylo. Evol. 27:185–196.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

Fishbein, M., and D. E. Soltis. 2004. Further resolution of the rapid radiation of Saxfrigales (Angiosperms, Eudicots) supported by mixed-model bayesian analysis. Syst. Bot. 29:883–891.

Funk, D. J., and Omland, K. E. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annu. Rev. Ecol. Syst. 34:397–423.

Goldman, N. 1993. Statistical tests of models of DNA substitution. J. Mol. Evol. 36:182–198.

Herre, E. A. 1999. Laws governing species interactions? Encouragement and caution from figs and their associates. Pages 209–237 *in* Levels of selection in evolution (L. Keller, ed.). Princeton University Press, Princeton, New Jersey.

Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: Exploring the paths between conflict and cooperation. Trends Ecol. Evol. 14:49–53.

Herre, E. A., C. A. Machado, E. Bermingham, J. D. Nason, D. M. Windsor, S. S. McCafferty, W. V. Houten, and K. Bachmann. 1996. Molecular phylogenies of figs and their pollinator wasps. J. Biogeogr. 23:521–530.

Huelsenbeck, J. P., D. M. Hillis, and R. Jones. 1996. Parametric bootstrapping in molecular phylogenetics: Applications and performance. Pages 19-45 in Molecular zoology: Advances, strategies and protocols (J. D. Ferraris and S. R. Palumbi, eds.). John Wiley & Sons, New York.

Huth, C. J., and O. Pellmyr. 2000. Pollen-mediated selective abortion in yuccas and its consequences for the plant-pollinator mutualism. Ecology 81:1100–1107.

Jackman, T. R., A. Larson, K. De Queiroz, and J. B. Losos. 1999. Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. Syst. Biol. 48:254–288.

Kato, M., A. Takimura, and A. Kawakita. 2003. An obligate pollination mutualism and reciprocal diversification in the tree genus *Glochidion* (Euphorbiaceae). Proc. Nat. Acad. Sci. USA 100:5264–5267.

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

Machado, C. A., E. Jousselin, F. Kjellberg, S. G. Compton, and E. A. Herre. 2001. Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. Proc. R. Soc. Lond. Series B Biol. Sci. 268:1–10.

Machado, C. A., N. Robbins, M. T. P. Gilbert, and E. A. Herre. 2005. A critical review of host specificity and its coevolutionary implications in the fig/fig wasp mutualism. Proc. Natl. Acad. Sci. USA 102:6558–6565.

Maddison, W. P. 1989. Reconstructing character evolution on polytomous cladograms. Cladistics 5:365–377.

Marr, D. L., M. T. Brock, and O. Pellmyr. 2001. Coexistence of mutualists and antagonists: Exploring the impact of cheaters on the yucca-yucca moth mutualism. Oecologia 128:454–463.

McKinnon, J. S., S. Mori, B. K. Blackman, L. D. D. Kingsley, L. Jamieson, J. Chou, and D. Schluter. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.

Mendelson, T. C., and K. L. Shaw. 2005. Sexual behaviour: Rapid speciation in an arthropod. Nature 433:375–376.

Minin, V., Z. Abdo, P. Joyce, and J. Sullivan. 2003. Performance-based selection of likelihood models for phylogeny estimation. Syst. Biol. 52:674–683

Molbo, D., C. A. Machado, J. G. Sevenster, L. Keller, and E. A. Herre. 2003. Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. Proc. Natl. Acad. Sci. USA 100:5867–5872.

Morris, W. F., J. L. Bronstein, and W. G. Wilson. 2003. Three-way coexistence in obligate mutualist-exploiter interactions. Am. Nat. 161:860–875.

Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

Ogden, R., and R. S. Thorpe. 2002. The usefulness of amplified fragment length polymorphism markers for taxon discrimination across graduated fine evolutionary levels in Caribbean *Anolis* lizards. Mol. Ecol. 14:437–445.

Parsons, Y. M., and K. L. Shaw. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. Mol. Ecol. 10:1765–1772.

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. 2003. Yuccas, yucca moths and coevolution: A review. Ann. Mo. Bot. Gard. 90:35–55.

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

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

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

Peng, Y. Q., D. A. Yang, and Q. Y. Wang. 2005. Quantitative tests of interactions between pollinating and non-pollinating fig wasps of dioecious *Ficus hispida*. Ecol. Entomol. 30:70–77.

Rambaut, A. E., and N. C. Grassly. 1997. SEQ-GEN: An application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comp. Appl. Biosci. 13:235–238.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford, UK.

Seehausen, O., E. Koetsier, and W. V. Schneider. 2003. Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock. Proc. R. Soc. Lond. Series B Biol. Sci. 270:129–137.

Segraves, K. A., D. M. Althoff, and O. Pellmyr. 2005. Limiting cheaters in mutualism: Evidence from hybridization between mutualist and cheater yucca moths. Proc. R. Soc. Lond. Series B Biol. Sci. 272:2195–2201.

Segraves, K. A., and O. Pellmyr. 2004. Testing the "Out of Florida" hypothesis on the origin of cheating in the yucca-yucca moth mutualism. Evolution 58:2266–2279.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA 99:16122–16127.

Stanton, M. L. 2003. Interacting guilds: Moving beyond the pair-wise perspective on mutualisms. Am. Nat. 162:S10–S23.

Sullivan, J. 2005. Maximum-likelihood estimation of phylogeny from DNA sequence data. Methods Enzymol. 395:757–778..

Sullivan, J. P., S. Lavoue, M. E. Arnegard, and C. D. Hopkins. 2004. AFLPs resolve phylogeny and reveals mitochondrial introgression within a species flock of African electric fish (Mormyroidea: Teleostei). Evolution 58:825–841.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Weiblen, G. D. 2004. Correlated evolution in fig pollination. Syst. Biol. 53:128–139.

Weiblen, G. D., and G. L. Bush. 2002. Speciation in fig pollinators and parasites. Mol. Ecol. 11:1573–1578.

Weiblen, G. D., D. W. Yu, and S. A. West. 2001. Pollination and parasitism in functionally dioecious figs. Proc. R. Soc. Lond. Series B Biol. Sci. 268:651–659.

West, S. A., E. A. Herre, D. M. Windsor, and P. R. S. Green. 1996. The ecology and evolution of the New World non-pollinating fig wasp communities. J. Biogeogr. 23:447–358.

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