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A test of host-associated differentiation across the 'parasite continuum' in the tri-trophic interaction among yuccas, bogus yucca moths, and parasitoids

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Abstract

Parasitic taxa span an antagonistic continuum, with some parasites inflicting no fitness costs to some that kill the host after feeding. Host-associated differentiation is postulated as a major process facilitating speciation in many parasitic taxa. Here, I examined the importance of host-associated differentiation in a parasitoid wasp that develops on yucca moths in the genus *Prodoxus*. *Prodoxus* are specialists on *Yucca*, and moth speciation is closely tied to differences in microhabitat use within a plant and among host plant species. Parasitoids in the genus *Eusandalum* have been reared from *Prodoxus* species distributed across *Yucca*. Estimates of host-use patterns obtained through rearings of adult wasps were combined with surveys of mitochondrial DNA cytochrome oxidase I sequence data and amplified fragment length polymorphism markers to determine if populations of *Eusandalum* were genetically structured based on host use. *Eusandalum* populations were genetically structured based on geographical distance rather than moth host species, microhabitats within plants, or *Yucca* species. The results are contrary to the patterns observed in the host genus *Prodoxus*. Although parasitoids exhibit parasite-like characteristics, these results suggest that *Eusandalum* may be best viewed as a predator. Female wasps are able to utilize any moth species present at a given locality, and there is little likelihood that host specialization may facilitate population subdivision and speciation.

Keywords: host use, parasitic taxa, phylogeography, population structure, speciation, yucca moths

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Introduction

Most of the earth's biodiversity is due to plant-feeding insects and their parasitoids. These two groups of taxa represent the ends of a parasitism continuum. Many plant-feeding insects can be considered parasites because individuals obtain all of the nutrients necessary for development from single plants (e.g. Price 1980). Eggs are laid on an individual plant, the larva completes its development on that plant, and, in most cases, the plant is not killed. Parasitoids have a similar life habit with the notable exception that the host insect is eventually killed as a parasitoid larva completes its development. Given the similarity of life habits between the two groups, similar mechanisms may be responsible for driving their immense diversification.

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Studies of plant-feeding insects have demonstrated that adaptation and specialization to different plant species and resource partitioning within a plant are central to generating diversification at all hierarchical levels including local population differentiation, host race formation, sibling species complexes, and radiations of insect lineages through evolutionary time (Mitter *et al*. 1988; Mopper & Strauss 1998; Berlocher & Feder 2002; Funk *et al*. 2002; Nosil *et al*. 2002; Eubanks *et al*. 2003; Funk *et al*. 2006; Joy & Crespi 2007; Lozier *et al*. 2007). Recent research has called attention to the major importance of host-associated differentiation in the evolution of many plant-feeding insects (Abrahamson *et al*. 2003; Stireman *et al*. 2005; Funk *et al*. 2006; Tilmon 2008). From scores of studies on host-use patterns and genetic differentiation, it is clear that ecological speciation via resource use is a major factor in explaining herbivorous insect diversity.

The enormous diversity of plant-feeding insects has in turn facilitated the diversification of parasitoids that use them as hosts. Almost every plant-feeding insect species is

attacked by at least one parasitoid species (Godfray 1994). Research on patterns of host use in parasitoids has demonstrated that parasitoids cover a broad range from utilization of single host species, groups of unrelated species within a specific microhabitat, or groups of related species throughout a habitat (Waage & Greathead 1986; Sheehan & Hawkins 1991; Godfray *et al*. 1994; Stireman & Singer 2003). In contrast to research on herbivorous insects, there is a comparable lack of studies linking host use with genetic differentiation and speciation in parasitoids and other natural enemies of herbivorous insects (Blair & Abrahamson 2008). A handful of studies have demonstrated that in some cases, populations of parasitoid species can be genetically structured based on host use and that previously recognized parasitoid species can be complexes of sibling species utilizing different host species (Jager & Menken 1994; Atanassova *et al*. 1998; Vaughn & Antolin 1998; Morehead *et al*. 2001; Kankare *et al*. 2005; Smith *et al*. 2006; Stireman *et al*. 2006; Marussich & Machado 2007). In other cases, no such pattern is observed (Cronin & Abrahamson 2001; Baer *et al*. 2004). Additional studies that examine genetic structure in relation to host-use patterns are needed to assess the overall importance of host-associated differentiation in generating diversification in parasitoid taxa.

In this study, I examined a well-characterized herbivorous insect–plant system in which differences in host plant use have been central to patterns of insect speciation, and tested whether there are similar patterns of host use and genetic differentiation for an associated parasitoid genus. I tested if host-use patterns are linked to the genetic structure in the yucca moth parasitoid *Eusandalum* that attacks at least nine species of internally feeding yucca moths in the genus *Prodoxus*. *Prodoxus* contains a species complex of extreme specialists on yuccas (Davis 1967; Pellmyr *et al*. 2006), and, hence, provides a species-rich template of specialist insect herbivores that could facilitate host-associated differentiation in *Eusandalum*. I used a combination of insect rearings to determine host use and phylogenetic and population genetic approaches to assess the pattern in mitochondrial DNA (mtDNA) and nuclear molecular markers to answer the following questions: (i) Are populations of *Eusandalum* genetically structured based on within-plant microhabitats, moth species, or yucca species utilized? (ii) If so, has this structuring lead to phylogenetic patterns in host use? (iii) Is the pattern of genetic structuring indicative of ongoing host-associated differentiation? (iv) If not, what factor best explains the pattern of host use and genetic structure?

The yucca–bogus yucca moth–yucca moth parasitoid system

The genus *Yucca* (Agavaceae) is distributed from Central America north to Canada and is comprised of roughly 35–40 species (Hess & Robbins 2002; Pellmyr *et al*. 2007). The main centre of diversity is in Mexico and the southwestern USA. *Yucca* species can be grouped into three monophyletic lineages that correspond to fruit type — the capsular-fruited Chaenocarpa, the fleshy-fruited Sarcocarpa, and the spongy-fruited Clistocarpa. All yucca species flower by producing an inflorescence stalk with hundreds of flowers that are visited by obligate seed-eating pollinators in the yucca moth genera *Tegeticula* or *Parategeticula* (Lepidoptera: Prodoxidae) (Davis 1967; Powell 1992; Pellmyr 2003).

The inflorescence stalks and fruits serve as a larval food source for the internally feeding bogus yucca moths in the genus *Prodoxus*. Sixteen *Prodoxus* species feed on yuccas (Pellmyr *et al*. 2006). These species form two lineages — a fruit-feeding lineage within fleshy-fruited yuccas, and a second lineage that includes stalk feeders within fleshy-fruited and capsular-fruited yuccas, a stalk feeder and fruit feeder that feed within the single spongy-fruited species, *Yucca brevifolia*, and a leaf feeder (Fig. 1). Female *Prodoxus* oviposit into their respective substrate during flowering of their host plant species. The larvae feed and diapause within the plant tissue, in some cases for up to 30 years (Powell 2001). Inflorescence stalks and fruits can remain on the plant for several years. In a subsequent year, larvae pupate and emerge as adults. Depending upon the yucca species, there may be both a stalk-feeding species and a fruit-feeding species present. Larvae of *Prodoxus* species differ in size after completing development and in how deep they feed within the plant tissue (Fig. 2). These two characteristics are important for parasitoid host use because the size of the host insect determines the size of the parasitoid adult, which ultimately determines the length of the ovipositor and host choice (Henry *et al*. 2006). Females that are smaller in size may not be able to reach larvae that are feeding deeper within plant tissue (e.g. Craig 2007).

The parasitoid *Eusandalum* (Hymenoptera: Eupelmidae) is a solitary ectoparasitoid that is currently known to utilize 11 species of yucca-feeding *Prodoxus* that span the phylogenetic lineages of yuccas and their associated fruit-feeding and stalk-feeding moth species (Force & Thompson 1984; Powell 1984). Adult females search inflorescence stalks and fruits, during and up to 6 months after the fruiting period of *Yucca*, using their antennae to locate potential host insects below the plant surface (D. M. Althoff, personal observation.). The genus *Eusandalum* occurs worldwide and other species have been reared from wood-boring beetles (Gibson 1989). The current phylogenetic and taxonomic position of the *Eusandalum* species that use *Prodoxus* is unclear and will require extensive additional systematic research.

Methods

Sampling

During 2002 to 2004, parasitoids were sampled across the phylogenetic and life-history breadth of *Prodoxus* moth

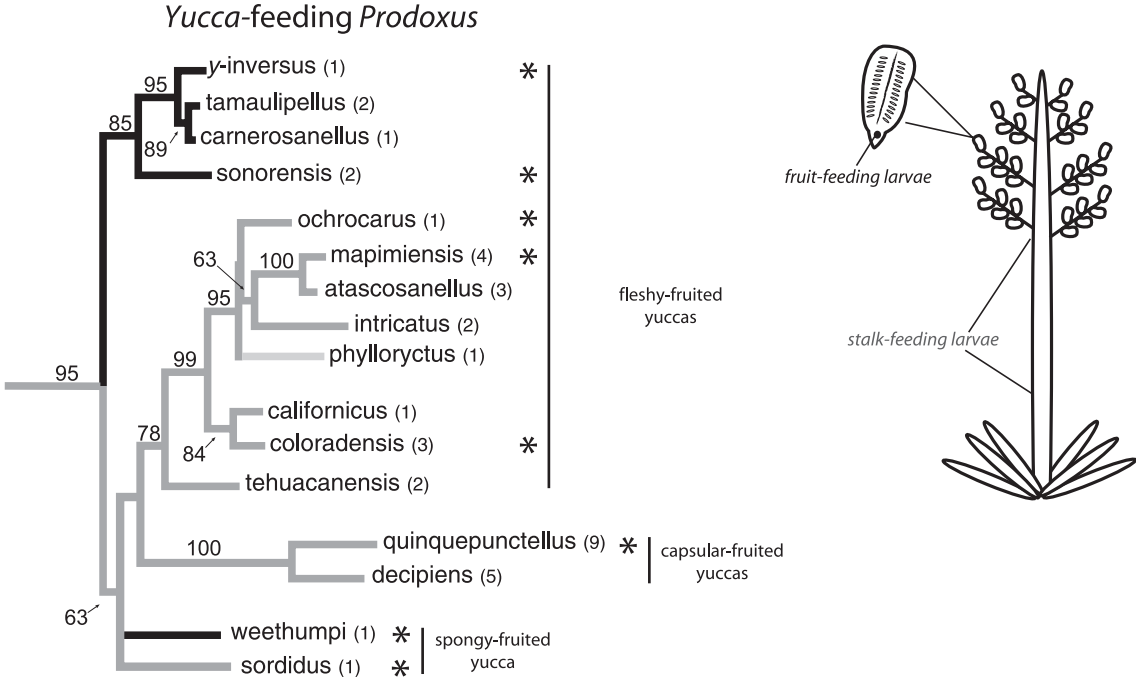

Fig. 1 Maximum-likelihood mtDNA phylogeny of *Prodoxus* moths utilized by the parasitoid *Eusandalum* (after Pellmyr *et al*. 2006). Gray branches indicate moth species that feed within the inflorescence stalk and dark branches indicate species that feed within the fruit. The light gray branch for *Prodoxus phylloryctus* indicates feeding within leaf tissue. Bootstrap values are given along branches and numbers after moth species names indicate the number of *Yucca* species utilized. Asterisks represent moth species from which *Eusandalum* was surveyed for this study. Schematic indicates where larvae feed within *Yucca* plants.

species, and the geographical range of the tri-trophic interaction (Figs 1 and 3). Inflorescence stalks and fruits were collected from the sites listed in Table 1 and placed into rearing cages composed of plastic PVC pipe frames and fine screening made from bridal veil material. For each yucca species at a site, the stalks and fruits were placed in separate cages. The stalk and fruit cages were placed in an environmental growth chamber with temperature and light settings cycled throughout the year to mimic desert conditions. Cages were checked daily and newly emerged wasps were frozen at –80 °C until used in the molecular analyses.

Because of the haploid–diploid nature of hymenopterans, only male wasps were used in the molecular analyses. Use of males facilitates the estimation of allele frequencies from amplified fragment length polymorphism (AFLP) data. Total genomic DNA was extracted using Isoquick DNA Isolation Kits (Orca Research Inc.). We used polymerase chain reaction (PCR) with the primers COIF — 5′-TWGATACWGAGCT-TAYTTTAC-3′ and COIR — 5′-CCHAYWGTAAATATAT-GRTGWGC-3′ to amplify a partial section of the mtDNA cytochrome oxidase I gene. PCR was conducted in 30-μL reaction volumes containing 50 mM KCl, 10 mM Tris (pH = 9.0), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 mM of each primer, 1 U of Promega *Taq* polymerase, and approximately 10 ng of genomic DNA. The thermal cycler profile was one cycle at 94 °C for 2 min, 35 cycles at 94 °C for 45 s, 47 °C for 45 s, 72 °C for 2 min, and a final extension at 72 °C for 5 min. PCR products were cleaned using QIAGEN PCR purification columns (QIAGEN Inc.). Dye terminator reactions were carried out following the dye terminator protocol (Applied Biosystems) with the exception that one-fourth reactions were conducted with the addition of a buffer (1 M tris-acid, 1 M magnesium chloride, pH 9.0). Dye terminator reactions were cycled 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 using Centri-sep Sephadex columns (Princeton Separations), and both forward and reverse strands were sequenced with an ABI 3730 DNA analyser (Applied Biosystems). Forward and reverse sequences for each individual were combined into contigs using SEQUENCHER 4.6 (Gene Codes Corporation). The consensus sequence for each individual was then aligned by eye in PAUP* version 4.0b10 (Swofford 2002).

AFLP protocol

AFLP markers were generated for all of the individuals used in mtDNA sequencing. We used a protocol developed by M. Gitzendanner (personal communication) that was

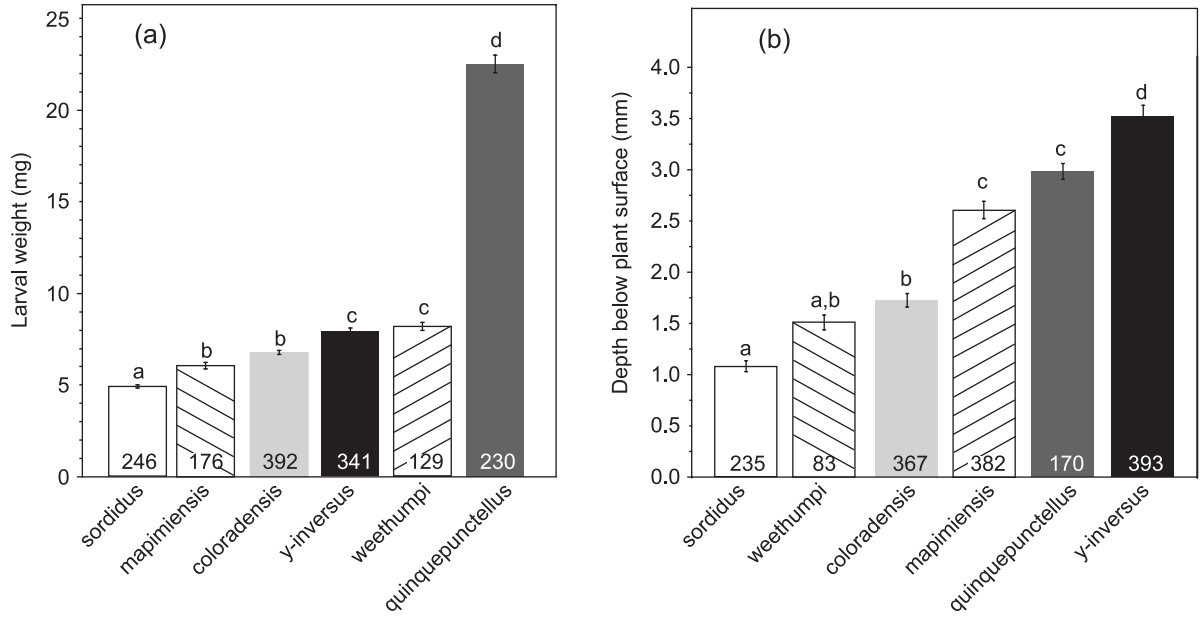

Fig. 2 Variation in the larval weight (a) and distance below plant surface (b) for *Prodoxus* species utilized by the parasitoid *Eusandalum*. Different letters above standard error bars indicate statistical differences of at least $P < 0.05$. Numbers within bars are number of larvae sampled.

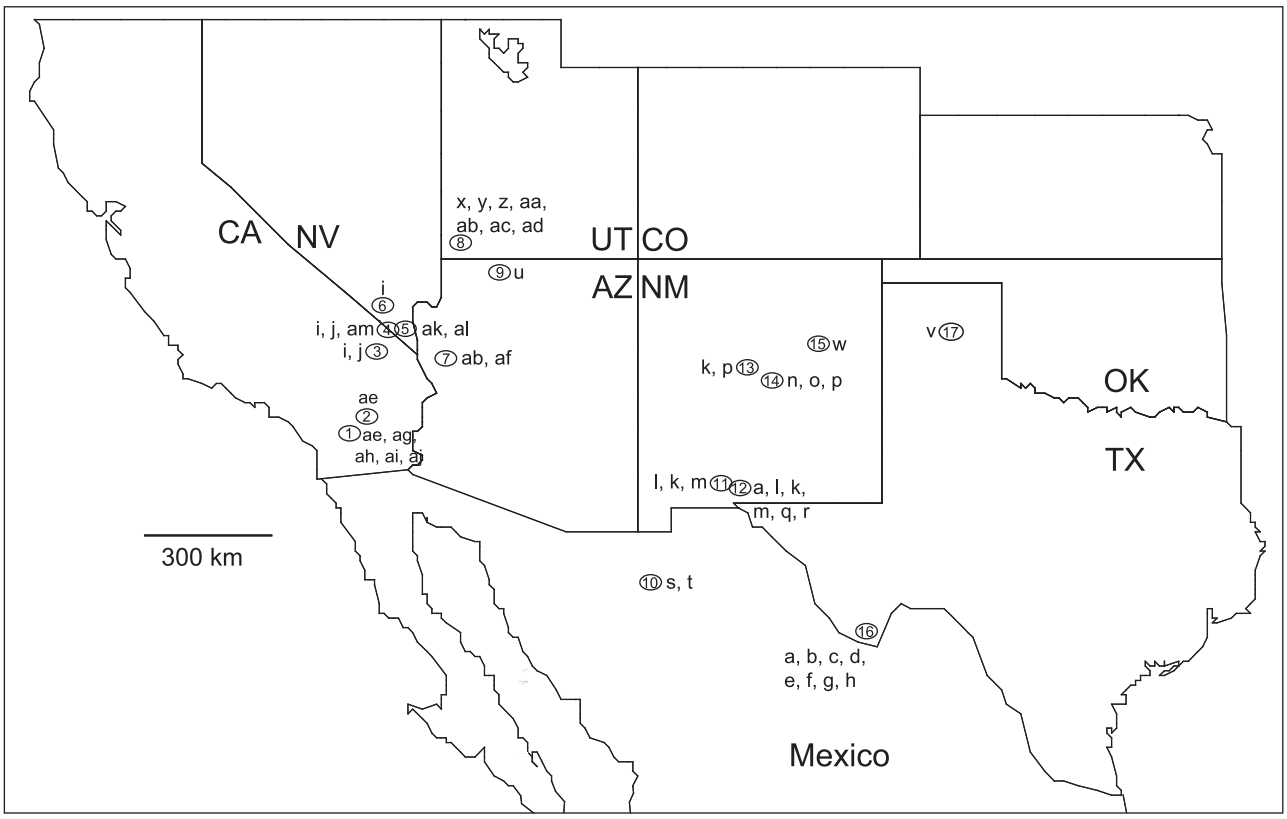

Fig. 3 Localities sampled for the parasitoid *Eusandalum* that utilizes *Prodoxus* moths feeding on yuccas. Numbers in circles correspond to Table 1 and lowercase letters represent mtDNA haplotypes found at each site.

Table 1 Locality, host-use information, and sample sizes for the parasitoid *Eusandalum (Hymenoptera: Eupelmidae)*

Site no.	Site name*	Latitude	Longitude	*Yucca* host plant species	*Prodoxus* host species	No. of wasps	mtDNA haplotypes
1	Pinyon Flat, CA	33.585	–116.458	*schidigera*	*coloradensis*	22	ae, ag, ah, ai, aj
2	Joshua Tree National Park, CA	33.991	–116.164	*schidigera*	*coloradensis*	19	ae
				brevifolia	*sordidus*	1	ae
3	Mojave National Preserve, CA	35.294	–115.494	*schidigera*	*coloradensis*	13	i
				brevifolia	*sordidus*	9	i, j
				baccata	*y-inversus*	1	i
4	Rte164 CA/NV border, NV	35.482	–115.21	*brevifolia*	*sordidus*	11	i, j, am
5	Searchlight, NV	35.47	–114.931	*schidigera*	*coloradensis*	7	ak, al
6	Lovell Canyon, NV	36.043	–115.719	*schidigera*	*coloradensis*	8	i
7	Yucca, AZ	34.8516	–114.146	*brevifolia*	*weethumpi*	4	ab, af
8	Shivwits, UT	37.032	–113.913	*brevifolia*	*weethumpi*	10	x, y, aa, ab, ad
				brevifolia	*sordidus*	25	x, y, z, aa, ac
9	Vermillion Cliffs, AZ	36.798	–111.679	*angustissima*	*quinquepunctellus*	12	u
10	C. Colorado, MX	30.7412	–109.971	*schottii*	*ochrocarus/sonorensis*	12	s, t
11	Jornada LTER, NM	32.475	–106.778	*baccata*	*y-inversus*	20	i, k, l, m
12	A Mountain, NM	32.265	–106.718	*torreyi*	*coloradensis*	10	a, k, l, m, q
13	Los Lunas, NM	34.779	–106.825	*intermedia*	*quinquepunctellus*	3	k, p
14	MountainAir, NM	34.5135	–106.254	*baccata*	*y-inversus*	4	n, o, p
15	Santa Rosa, NM	35.009	–104.745	*intermedia*	*quinquepunctellus*	1	w
16	Big Bend National Park, TX	29.492	–103.061	*carnerosana*	*mapimiensis*	34	a, b, c, d, e, f, g, h
				torreyi	*coloradensis*	8	a
				rostrata	*quinquepunctellus*	5	a
17	Exit 128, I-40, TX	35.18	–100.845	*glauca*	*quinquepunctellus*	7	v

AZ, Arizora; CA, California; MX, Mexico; NM, New Mexico; NV, Nevada; TX, Texas; UT, Utah.

originally modified from the Plant Genome kit (Applied Biosystems). Restriction and ligation reactions were carried out in separate steps. Genomic DNA was digested for 3 h at 37 °C with 3 U of *Eco*RI (Promega), 2.5 U of *Mse*I (New England Biolabs) in 10-μL reaction volumes containing sterile water, bovine serum albumin (BSA), and 10× enzyme buffers supplied by the manufacturers. Ligation reactions contained 1.5 U of T4 DNA ligase (Promega), 2 μL of 10× T4 ligase buffer (Promega), 9 μM *Mse*I adapter (5′-GACGATGAGTCCTGAG-3′ and 5′-TACTCAGGACTCAT-3′), 0.9 μM *Eco*RI adapter (5′-CTCGTAGACTGCGTACC-3′ and 5′-AATTGGTACGC-AGTCTAC-3′), and sterile water in 10-μL reaction volumes. The ligation reaction volumes were added directly to the restriction digests and incubated at 25 °C for 3 h. The digest/ligation reactions were diluted by a factor of 20 in 1× $TE_{0.1}$ (20 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The first selective amplification was conducted in 20-μL reaction volumes containing 4 μL of the diluted restriction–ligation reaction, 1 U *Taq* DNA polymerase (Sigma Chemical Co.), 10× 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× $TE_{0.1}$ 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 U Ampli*Taq* Gold DNA polymerase (Applied Biosystems), 1× Ampli*Taq* PCR buffer (Applied Biosystems), 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.05 μM of each *Eco*RI +4 primer 5′-(6-FAM) GACT-GCGTACCAATTCACAT-3′; (5′-PET) GACTGCGTACCAAT-TCACCT), and 0.125 μM *Mse*I +4 primer (5′-GACGAT-GAGTCCTGAGTAACTGA-3′). The *Eco*RI +4 primers were fluorescently labelled for visualization on an automated sequencer. 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 2 min followed by a 30-min 60-°C hold. One microlitre of the final amplification reaction was combined with 0.2 μL of the LIZ 500 size standard and 11 μL of HiDi formamide from Applied Biosystems and run on a ABI 3730 DNA analyser with the GENEMAPPER50_POP7_1 module file, a run voltage of 15 000, and an injection voltage of 1600 for 15 s. Fragment sizes were assigned using the Liz 500(–250) analysis method and the GENEMAPPER software version 4.0 (Applied Biosystems). GENEMAPPER was also used to score the presence and absence of fragments between 100 bp and 500 bp for

each individual. A threshold value of 50 fluorescent units was used to determine if a band was present.

Data analyses

Phylogenetic analyses. The mtDNA sequence data were analysed using maximum likelihood following the procedures and recommendations in Sullivan (2005). The best fitting model of sequence evolution was chosen 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 chosen model and parameter estimates were used in a heuristic search with random addition of taxa, 10 replicate searches, and tree-bisection–reconnection (TBR) branch swapping in PAUP 4.0b10 (Swofford 2002). One hundred replicates of the nonparametric bootstrap procedure (Felsenstein 1985) were performed on a 108-node Beowulf cluster at the University of Idaho to assess support for the nodes in the resulting tree topology. Wasps from *Yucca mixtecana* were used as the outgroup. The AFLP presence/absence data were transformed to Nei–Li distances and used in a minimum-evolution heuristic search and TBR branch swapping in PAUP 4.0b10 (Swofford 2002). One hundred replicates of the nonparametric bootstrap procedure (Felsenstein 1985) were used to assess support for the resulting nodes.

Population genetic analyses. The effects of host-use patterns on population genetic structure were analysed in two ways. First, analyses of molecular variance (AMOVA) (Excoffier *et al*. 1992) were used to determine if there was significant partitioning of genetic variation among wasps that emerged from different moth host species or from different yucca species. Individuals were grouped by moth species or yucca species at each geographical locality, and the pairwise distances (generated from the maximum-likelihood analysis) between mtDNA haplotypes and the Nei–Li distances between AFLP haplotypes were used in the AMOVAS. Ten thousand permutations were performed for significance testing. The AMOVAS were performed using ARLEQUIN version 2.0. Second, partial mantel tests were also used to partition the effect of geographical distance on genetic structure. The partial mantel test allows testing of one factor such as host use while controlling another factor such as geographical distance. This is particularly important given that host species use and sampling location are confounded for *Eusandalum* populations. This analysis helps determine which factor(s) is important in genetic structure. Pairwise F_{ST} values were calculated in ARLEQUIN for the mtDNA data and AFLPSURV 1.0 (Vekemans *et al*. 2002) for the AFLP data. The program IBD version 1.52 (Bohonak 2002) was used to perform the partial mantel tests. Wasp haplotypes that shared moth or yucca hosts were coded with a 0 and those that did not were coded with a 1 in the indicator matrix.

Results

Two hundred and forty six individuals were sequenced for the mtDNA cytochrome oxidase I gene and produced 599 readable base pairs per individual that yielded 40 haplotypes (GenBank accessions EU544892–544931). There were no indels. Model-testing analyses suggested that the HKY + I model of evolution best described sequence evolution. Maximum-likelihood analyses with the resulting model and estimated parameters produced an unrooted tree that was largely consistent with geography rather than moth host species utilized (Fig. 4). Well-supported clades were present for haplotypes that occurred in southern California, the Mojave Desert area, Big Bend National Park, Texas, and the population in northern Mexico. Haplotypes at these sites were shared among individuals that feed on multiple moth host species distributed across inflorescence stalks and fruits and multiple *Yucca* species. Haplotypes from the remaining localities did not exhibit any significant phylogenetic structure as determined by nonparametric bootstrap analysis and were not correlated with host use or biogeography.

The AFLP analyses yielded 433 AFLP markers for 132 individuals. Many individuals that were used in the mtDNA analysis did not produce good quality AFLP markers because of poor DNA quality. On average, individuals had 148.48 markers scored as present. Minimum-evolution analyses on the Nei–Li distances from these 132 individuals produced a very weakly supported topology that was inconsistent with host-use patterns or biogeography (tree not shown or supplemental file). Individuals from the same locality or that utilized a particular moth species were distributed throughout the topology and there was no bootstrap support for major clades that corresponded with moth species utilized or biogeography.

Population genetic analyses of both molecular marker sets demonstrated significant genetic structure among populations of *Eusandalum* (Table 2). For the mtDNA variation, AMOVA demonstrated that most (84%) of the variation was partitioned among parasitoid populations that used the same moth species as hosts and not among populations that used different moth species. In contrast, most of the variation in AFLP markers was within populations, and approximately 11% was among populations that utilized different moth species. The AFLP analysis suggested that moth host species may exhibit a significant, but small, influence on parasitoid population structure. Partial mantel tests that corrected for geographical distance and host use, however, demonstrated that geographical distance had a significant effect in both genetic marker sets, whereas moth species or yucca species used did not (Table 3). Thus,

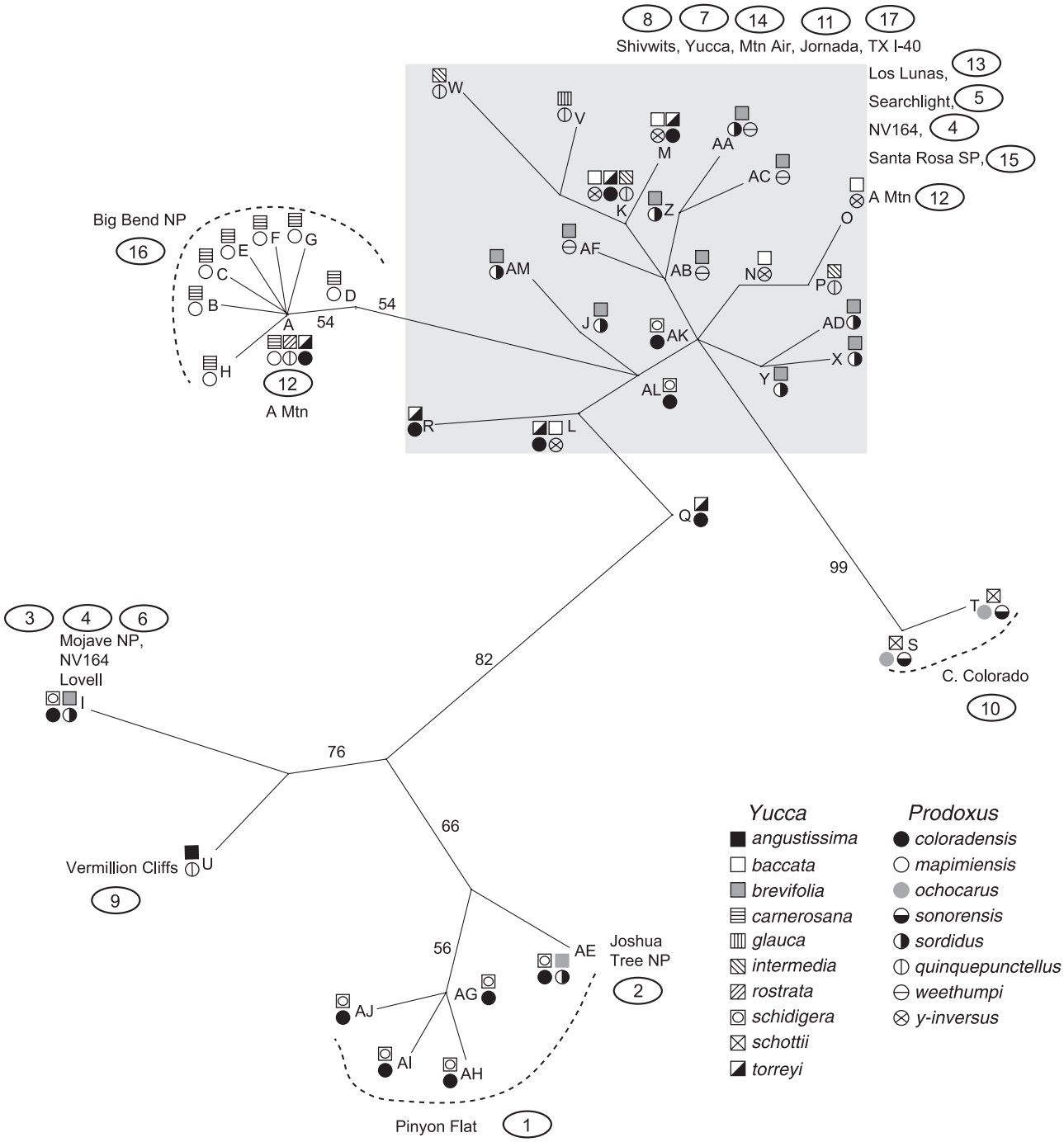

Fig. 4 Unrooted phylogram obtained from maximum-likelihood analyses of mtDNA haplotypes for *Eusandalum*. Haplotypes at node represent collapsed zero branch lengths. Numbers along branches are nonparametric bootstrap values. Site names and numbers correspond to those in Table 1.

Table 2 Analysis of molecular variance results for partitioning of genetic variation in *Eusandalum* grouped by *Prodoxus* moth host species utilized

		mtDNA			AFLP	
Source of variation	d.f.	Variance component	Percentage of variation	d.f.	Variance component	Percentage of variation
Among moth spp.	6	–0.00002	–0.32	6	0.0005**	10.74
Among populations within moth spp.	13	0.0061***	84.06	6	0.0002***	4.78
Within population	184	0.0012***	16.26	113	0.0037***	84.48
Overall F_{ST}		0.84***			0.16***	

Table 3 Results of partial Mantel tests for partitioning of genetic variation in *Eusandalum* parasitoids based on *Prodoxus* moth species utilized, geographical distance, and *Yucca* species utilized

	mtDNA		AFLP	
Correlation	*r*	*P* <	*r*	*P* <
Genetics with moth species	–0.093	0.806	–0.040	0.684
Genetics with geography	0.605	0.001	0.588	0.003
Genetics with moth species controlled for geography	–0.130	0.086	–0.108	0.856
Genetics with geography controlled for moth species	0.609	0.001	0.594	0.998

	mtDNA		AFLP	
Correlation	*r*	*P* <	*r*	*P* <
Genetics with plant species	0.045	0.226		
Genetics with geography	0.356	0.001	same as	
Genetics with plant species controlled for geography	–0.120	0.960	moth use	
Genetics with geography controlled for plant species	0.372	0.001		

geography was the overriding factor in determining the genetic structure of parasitoid populations. The partial mantel test is a more realistic test because it takes into account actual geographical distance unlike the AMOVA. For example, with the among moth species grouping in the AMOVA, differences may be driven by geography especially if groups of wasp populations that utilized different moth species are correlated with geography.

Discussion

Specialization in species interactions can be a major factor in diversification of some groups of organisms (Thompson 2004). Parasitic taxa, in particular, exhibit characteristics that suggest that parasites are especially prone to speciation via specialization to their hosts (e.g. Price 1980). There are many examples demonstrating the link between specialization in host use and species diversification in parasitic taxa (e.g. Hafner & Page 1995; Hughes *et al*. 2007). Parasitoids are included in the broad category of parasitic taxa, but are at the extreme end of the antagonistic effects of parasites on their hosts' fitness. This endpoint presents the opportunity to test the importance of host specialization and speciation in all parasitic taxa. Does a shift from commensalistic or slightly antagonistic interactions to strongly antagonistic interactions influence the link between host specialization and diversification? Comparisons among many different types of parasitic taxa are needed to fully answer this question.

For the tri-trophic interaction among *Yucca*, *Prodoxus*, and *Eusandalum*, shifts in the strength of antagonistic interactions correlate with differences in the importance of host specialization to diversification. *Prodoxus* species are extreme specialists on *Yucca* and are likely to have commensalistic or slightly antagonistic interactions with their host plants. Larvae do not feed on yucca seeds, and feeding does not appear to influence floral abscission or seed set (Althoff *et al*. 2004). Specialization in host use has been important in this genus. Use of different species of *Yucca* and different microhabitats within a plant has gone hand-in-hand with diversification (Pellmyr *et al*. 2006). Larval differences in host location within a plant, body size and depth below the plant surface suggest there is ample variation to facilitate differentiation in the parasitoids that attack these moth species.

Contrary to expectation, host use in *Eusandalum* was not linked with genetic structuring. Populations of *Eusandalum* were not genetically structured based on within-plant microhabitats, moth species, nor yucca species. Individuals that were reared from moth larvae in inflorescence stalks and fruits within a *Yucca* species, from different *Prodoxus* species, and from different *Yucca* species shared mtDNA haplotypes. Phylogenetic patterns of mtDNA haplotype relatedness did not correlate with host use, nor was there evidence of on-going host-associated differentiation among wasps at a given locality. Much of the genetic structure among *Eusandalum* populations can be explained by geography rather than host use (Table 2 and Fig. 4). *Eusandalum* appears to be able to utilize a suite of *Prodoxus* species distributed over several species of yuccas at a locality, and lacks corresponding genetic structure indicative of host races or restricted gene flow among parasitoids that utilize different moth species. There was no evidence of host-associated genetic differentiation within sites or across moth host species or *Yucca* species. In fact, *Eusandalum* appears to be a geographically widespread species capable of utilizing any *Prodoxus* species that it contacts.

There are several potential ecological reasons for the lack of congruence between host-use patterns and genetic structure in *Eusandalum* even though there is a strong congruence for *Prodoxus*. The phenology of host availability is significantly longer for *Eusandalum* than for *Prodoxus*. Oviposition by *Prodoxus* females is closely tied to the flowering period of their *Yucca* host plants that typically flower during 1 to 2 months in the spring or summer. *Yucca* species also flower at different times when in sympatry (Svensson *et al*. 2005). In contrast, oviposition by *Eusandalum* females can occur throughout the year whenever environmental conditions are favourable. *Prodoxus* larvae are present in the inflorescence stalks and fruits all year. In some cases, larvae remain in the diapause for many years (Davis 1967; Powell 1989). Female and male *Eusandalum* have been observed searching *Yucca* stalks and fruits during flowering and fruiting in the early spring and after this into late October (D. M. Althoff, personal observation). This prolonged activity increases the ecological opportunity for an expanded host range. Mating habits for *Prodoxus* and *Eusandalum* also differ. *Prodoxus* mate within the flowers of their *Yucca* host plants (Davis 1967). Males search flowers for females, and both sexes use the flowers as refugia during the day. In contrast, *Eusandalum* is active after the flowering period and there is not a direct mechanism for finding mates on a particular *Yucca* species. Although the mating behaviour of *Eusandalum* has not been observed in the field, preliminary laboratory trials in the absence of host plants demonstrated that males and females that emerged from different moth hosts on different *Yucca* species will readily mate (D. M. Althoff, unpublished data). The genetic results and the fact that there is no mechanism to tie mating to a particular moth host species or *Yucca* species suggests that there is no host-associated mating in *Eusandalum* that would likely fuel host-associated differentiation.

In a larger context, the results for *Eusandalum* suggest the need to continue to examine and refine expectations of host-associated differentiation in parasitoids. Specifically, further exploration of the likely importance of mechanisms that may drive host-associated differentiation in parasitoid taxa is needed. In particular, differences in oviposition strategy may have important consequences for the possibility of host-associated differentiation. Internally feeding parasitoids that allow the host to continue to develop (endoparasitic koinobiont parasitoids) may be more likely to exhibit host-associated differentiation than externally feeding parasitoids that permanently paralyze or kill the host at the time of oviposition (ectoparasitic idiobionts) (Askew & Shaw 1986; Sheehan & Hawkins 1991; Althoff 2003). Koinobiont parasitoids, in contrast to idiobiont parasitoids, have to contend with a functioning host and the host's immune system. Selection on circumventing host defences could generate specialization in host use. Comparisons of host use and genetic structure among parasitoid taxa that have different oviposition strategies but utilize the same host species would be especially revealing. On a more mechanistic level, host-associated mating significantly increases the probability that host use may drive genetic differentiation (Berlocher & Feder 2002). There is a dearth of studies examining the mating behaviour of parasitoids in relation to host use. In perhaps the first test of this idea, Cronin & Abrahamson (2001) examined the possibility of assortative mating between *Eurytoma gigantea* parasitoids that emerged from different host races of the gall maker *Eurosta solidaginis*. In the laboratory, in the absence of the host plant, there was no assortative mating among parasitoids from each of the host races. Studies on host-associated differentiation that incorporate testing for assortative mating both in the laboratory and the field, as suggested by Cronin & Abrahamson (2001), are needed to determine if host-associated mating is a potential mechanism for generating parasitoid differentiation.

In conclusion, analyses of genetic structure in the yucca moth parasitoid *Eusandalum* did not indicate a correlation between the partitioning of genetic variation among wasp populations with respect to host-use patterns. Geographical distance explains much of the genetic structure, even though host use has lead to specialization and speciation in the parasitoid's host genus, *Prodoxus*. In contrast to other studies on parasitoid taxa, there was no evidence for cascading host race formation in the *Yucca–Prodoxus–Eusandalum* tri-trophic interaction. It is clear from studies on sibling species of parasitoids that differences in host use are correlated with speciation (Vet & Janse 1984; Kraaijeveld *et al*. 1994; Lopez-Vaamonde *et al*. 2005). What still remains to be widely tested is whether this is a corollary of speciation

via other mechanisms, or whether host use is the driver of speciation. Additional studies examining this phenomenon are needed in order to determine the importance of host use as a mechanism of diversification in one of the most diverse groups of organisms on earth.

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