

Patterns of Genetic Structure Among Populations of an Oligophagous Pollinating *Yucca* Moth (*Tegeticula yuccasella*)

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Abstract

Plant-insect associations have served as models for investigations of coevolution and the influence of biotic interactions on diversification. The pollination association between yuccas and yucca moths is a classic example of an obligate mutualism often suggested to have been affected by coevolution. Recent work has shown high host specificity in pollinating yucca moths, and here we use *Tegeticula yuccasella*, the species with the widest diet breadth, to ask how host specificity and isolation by distance contribute to specialization. Isolation by distance at a regional scale was observed in nucleotide variation within the mitochondrial gene cytochrome oxidase I (COI) ($r = .294$; $P = .003$). Host-related genetic structure ($F_{ct} = 0.08$) was found to be slightly lower than the level of structure observed between eastern and western moth populations ($F_{ct} = 0.096$). However, 56% of the COI haplotypes sampled from moths on *Yucca filamentosa*, mapped to a host-specific clade in the haplotype network. Taken together, these results suggest that differentiation among *T. yuccasella* populations on alternative hosts is slight, but gene flow is influenced by both host association and geographic distance.

Coevolution between plant-feeding insects and their hosts is influenced by a complex interaction of geographic structure, phenological variation, and host specialization (Thompson 1994, 1999). The relative importance of these factors and their interactions in the diversification of herbivorous insect lineages varies greatly. Although comparative studies have identified the influence of coevolving host chemistry and herbivore physiology on herbivore diversification (Becerra 1997; Ehrlich and Raven 1964; Farrell 1998; Mitter et al. 1988), several lines of evidence have shown that the evolutionary pressures contributing to host-related diversification of phytophagous insects vary with the natural history of particular plant-insect interactions (Berlocher and Feder 2002; Mopper and Strauss 1998).

Much recent work on the mechanisms leading to host-related herbivore diversification has been presented in the context of the longstanding debate between proponents of allopatric and those of sympatric speciation (e.g., Bush 1969; Funk 1998; Groman and Pellmyr 2000; Mayr 1963; Nason et al. 2002; many others reviewed in Berlocher and Feder 2002). With mounting evidence for the occurrence of sympatric speciation, attention is shifting to the evolutionary mechanisms that lead to the divergence of sympatric populations (Via 2001). Mechanisms that promote direct selection on host-related assortative mating, such as variation in host phenology or host-related variation in performance, have been shown to be important precursors to host-race formation.

At the same time, allopatry has been recast as one of many possible mechanisms for assortative mating (Berlocher and Feder 2002; Kirkpatrick and Ravigné 2002). In this context, we are forced to recognize that the distinction between allopatry and sympatry can be difficult in highly vagile organisms. Individuals that are geographically separated at one point in time may migrate to the same population and mate. The scale of dispersal must be understood in order to equate geographic separation with assortative mating. Indirect, marker-based estimation of gene flow and the scale of effective isolation by distance can provide a measure of the degree to which geographic structure influences mating patterns over an organism's lifetime.

In many instances, geographic, phenological, and chemical variation in host populations are confounded, so it is difficult to tease apart the relative degree to which each factor influences assortative mating and the likelihood of host-race formation. Well-designed experiments can certainly allow investigators to assess each of these factors and their interactions (Funk 1998). However, not all host-herbivore systems are amenable to experimental manipulation. With the proper sampling design, observations of genetic structure can be used to infer the relative importance of geographic barriers and host-related factors influencing race formation.

Here we ask how two factors, host specificity and geographic distance, interact in molding the genetic structure in *Tegeticula yuccasella*, a member of the pollinating yucca moth complex. Yucca moths and yuccas constitute one of the classical examples of obligate, potentially coevolved mutualism (Pellmyr 2003; Riley 1892). In this system, the female moth gathers pollen, oviposits into yucca ovaries, and then actively deposits pollen on the stigma, thus securing pollination (Pellmyr and Balcázar-Lara 2002; Pellmyr and Krenn 2002; Riley 1873, 1892). The emerging larvae later feed on a portion of the developing yucca seeds. Recent genetic and morphological studies of the genus *Tegeticula* led to the description of a complex of 13 species, previously considered one species (Addicott 1996; Pellmyr 1999; Pellmyr et al. 1996; Pellmyr and Leebens-Mack 2000). Most pollinating *Tegeticula* species use one or two host species, but *T. yuccasella* uses at least seven hosts across its rather wide geographic range (Pellmyr 1999, 2003). Only the non-pollinating species *T. intermedia* and *T. corruptrix*, with five and six hosts, respectively, have host ranges approaching that of *T. yuccasella*. Although geographic variation among *T. yuccasella* populations in integument color and wing shape has been observed, measures of genitalic characters do not show evidence of structured variation (Pellmyr 1999).

We measured genetic structure among *T. yuccasella* populations sampled from all seven host species to test for evidence of incipient host-race formation. Because the ranges of most *T. yuccasella* hosts are not widely overlapping, observed genetic structure could be attributed to host use or geographic proximity. In order to tease these factors apart, we compared genetic structure within and between moth populations on *Yucca filamentosa* and *Yucca glauca*, the two most widespread host species (Figure 1). Nucleotide sequence data were collected for the mitochondrial locus

coding for the first subunit of cytochrome oxidase I (COI). By characterizing patterns of genetic structure within *T. yuccasella*, this study aims to improve our understanding of how the intimate interaction between yuccas and pollinating yucca moths has influenced diversification in both groups.

Materials and Methods

T. yuccasella has a range extending from the eastern contiguous United States to the Rocky Mountains, southward to the Mexican border, and northward to southernmost Canada in the Great Plains (Figure 1). A total of 170 moths were collected from 30 sites in the eastern United States (Table 1; Figure 1), covering all seven described host species (conservative host species circumscriptions taken from Pellmyr 1999). Sampling was roughly proportional to current host range, so most moths were sampled from *Y. filamentosa* (16 populations) and *Y. glauca* (7 populations; Figure 1). Most moths were collected as adults from host flowers, but some were collected as larvae as they emerged from harvested fruits (Table 1). In these instances, fruits were collected from multiple plants and brought back to the lab, where larvae emerged over 1 to 2 weeks. Genomic DNA was extracted from both moths and larvae, in accordance with the protocol of Harrison et al. (1997). Heads and genitalia of adult moths were saved as vouchers.

Polymerase chain reaction (PCR) amplifications of the mitochondrial COI were performed with modifications of the procedures described by Leebens-Mack et al. (1998). A 844-base region of the COI, corresponding to positions 1458 to 2302 in the *Drosophila yakuba* mtDNA (Clary and Wolstenholme 1985) was sequenced for the current study. Cycle sequencing reactions were performed with Thermo Sequenase Dye Terminator Sequencing chemistry according to manufacturer's protocols (Amersham Life Sciences, Arlington Heights, IL). Reaction products were run on an ABI 377 DNA sequencer. Complete sequences of complementary strands were joined in Sequencher (Gene Codes, Ann Arbor, MI). No indels were observed in manual alignments of the sequences. All distinct sequences were deposited in GenBank (accession numbers AY488807–AY488857).

Phylogeographic patterns were assessed by means of Templeton's nested clade analysis (Templeton 1998; Templeton et al. 1995). Haplotype networks and nesting structure were estimated in accordance with the procedures of Templeton et al. (1992) and Templeton and Sing (1993) as implemented in the TCS software package, version 1.13 (Clement et al. 2000). Nested clades were then used to assess geographic structure with statistical tests implemented in GeoDis, version 2.0 (Posada et al. 1999). In addition, host use was mapped onto the resulting haplotype networks in order to assess possible host-haplotype clade associations.

Following the sequential analysis of phylogeography used by Althoff and Pellmyr (2002), after cladistic analysis of haplotype variation, we evaluated demographic history and population genetic structure. The range of *T. yuccasella* has

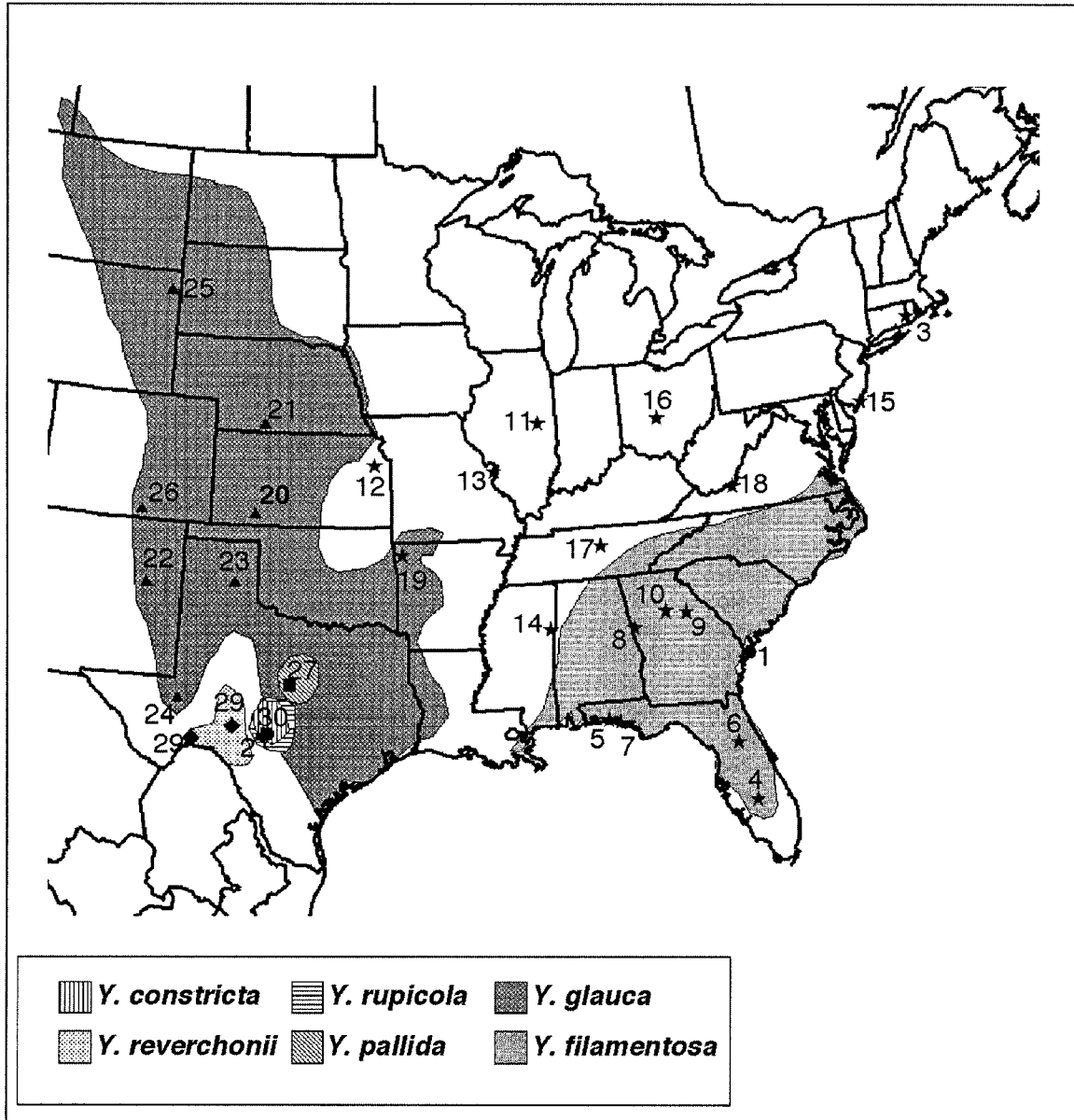


Figure 1. Estimated pre-European settlement host range distributions (Great Plains Flora Association 1977; McKelvey 1947; Pellmyr 2003) and sampling localities for *T. yuccasella*. *Y. aloifolia* currently has a coastal distribution, but its pre-European settlement range is unknown (Pellmyr 2003). Sampled host *Yucca* species are given in Table 1 and indicated here as *Y. filamentosa* (★), *Y. glauca* (▲), *Y. aloifolia* (●), *Y. constricta* (+), *Y. rupicola* (*), *Y. reverchonii* (◆), and *Y. pallida* (■).

expanded over the last 200 years as yuccas have been planted as ornamentals outside the historical range limits for the genus (Figure 1; Pellmyr 2003). Founder events associated with rapid range expansion are expected to reduce nucleotide diversity ($\pi = \sum_{i \neq j} p_i p_j d_{ij}$, where p is haplotype frequency and d_{ij} is the corrected genetic distance between haplotypes i and j) relative to haplotype diversity ($H = [n/(n-1)] * (1 - \sum p_i^2)$, where n is the sample size; Schneider et al. 2000). Tamura's correction of pairwise genetic distances, d_{ij} , was used to

account for transition bias and the skewed nucleotide frequencies observed in mtDNA sequences.

Coalescence theory suggests that after range expansion, populations will approach genetic equilibrium more rapidly in more vagile species with high rates of gene flow. The influence of population expansion on current genetic structure among *T. yuccasella* populations was assessed by comparing haplotype diversity and nucleotide diversity for the entire data set and regional subsets (Grant and Bowen

Table 1. Sample sizes, locations, and hosts species

Pop.	Location	Host	Latitude	Longitude	N _{mt}	Haplotypes
1	Tybee Island, GA	<i>aloifolia</i>	32°00'00"N	80°50'45"W	6	j(2), n(3), rr
2	S.E. Segovia, TX	<i>constricta</i>	30°17'30"N	99°31'45"W	5	k, p, r, hh, kk
3	Voluntown, CT ^a	<i>filamentosa</i>	41°34'00"N	71°51'00"W	5	x(2), aa(2), ss
4	Lake Placid, FL	<i>filamentosa</i>	27°14'00"N	81°42'00"W	1	j
5	Destin, FL	<i>filamentosa</i>	30°23'00"N	86°28'00"W	6	j(2), w, aa, gg, kk
6	Ocala Nat'l Forest, FL	<i>filamentosa</i>	29°08'00"N	81°31'00"W	6	g, l, tt(2), uu
7	Seaside, FL	<i>filamentosa</i>	30°13'48"N	86°06'36"W	6	j(2), x, aa(2), cc
8	Camp Meetingrock, GA	<i>filamentosa</i>	33°18'19"N	85°07'30"W	4	n, x, z, aa
9	Union Point, GA	<i>filamentosa</i>	33°37'12"N	83°52'12"W	5	u, v, ee, ff, kk
10	Walnut Grove, GA	<i>filamentosa</i>	33°39'00"N	83°52'12"W	6	g, l, t, aa(3)
11	Champaign, IL ^a	<i>filamentosa</i>	40°05'00"N	88°18'36"W	8	c, aa(6), bb
12	Lawrence, KS	<i>filamentosa</i>	38°57'00"N	95°15'00"W	9	a(2), c(2), n, v, kk(2)
13	Fletcher, MO ^a	<i>filamentosa</i>	38°12'00"N	90°45'00"W	11	c(3), d, s, aa(2), ba, pp(3)
14	Columbus, MS	<i>filamentosa</i>	33°30'00"N	88°24'00"W	6	g, h, m, v, x, aa
15	Ocean City, NJ ^a	<i>filamentosa</i>	39°17'24"N	74°38'24"W	6	y(6)
16	Georgesville, OH	<i>filamentosa</i>	39°53'27"N	83°13'19"W	9	c, aa(6), kk, qq
17	Vine, TN	<i>filamentosa</i>	36°01'53"N	86°21'28"W	6	n, x(2), aa(2), kk
18	Goldbond, VA ^a	<i>filamentosa</i>	37°22'48"N	80°30'40"W	5	g(2), n, aa(2)
19	Fayetteville, AR	<i>filamentosa</i>	36°03'00"N	94°12'36"W	6	c, n, kk(4)
20	Fowler, KS	<i>glauca</i>	37°22'48"N	100°11'24"W	5	e, f, n, kk, nn
21	Arapaho, NE	<i>glauca</i>	40°14'24"N	99°52'48"W	9	f(2), n(6), zz
22	Cuervo, NM	<i>glauca</i>	35°02'00"N	104°23'30"W	6	f(2), i, n(2), kk
23	McLean, TX	<i>glauca</i>	35°06'00"N	100°50'24"W	8	f, n, q, kk(4), ll
24	Royalty, TX	<i>glauca</i>	31°24'36"N	102°58'12"W	2	f, hh
25	Sundance, WY	<i>glauca</i>	44°21'00"N	104°22'48"W	4	n, o, ii, jj
26	Weston, CO	<i>glauca</i>	37°10'12"N	104°54'00"W	6	f, i, fy, ji
27	Comanche, TX	<i>pallida</i>	31°53'25"N	98°30'45"W	6	kk(5), oo
28	Dryden, TX	<i>reverchonii</i>	30°02'24"N	102°10'12"W	3	n, hh, kk
29	Sonora, TX	<i>reverchonii</i>	30°34'12"N	100°43'12"W	6	b, hh, kk(3), yy
30	W. Harper, TX	<i>rupicola</i>	30°17'30"N	99°18'30"W	2	kk, mm

Population-specific haplotypes are shown in bold, along with haplotype population frequencies.

^a Moth larvae sampled from yucca fruits. All other moth populations sampled as adults.

1998). Tajima's D was calculated and used to formally test for the lingering effects of range expansion ($D < 0$; Tajima 1996). Tajima's D is expected to be negative when genetic structure has been influenced by rapid range expansion.

Isolation by distance was tested in a Mantel test for correlation between genetic and geographic distance matrices. The observed correlation between geographic distance and pairwise estimates of F_{st} was compared with a null distribution generated through random permutation of one of the two distance matrices (Schneider et al. 2000). When populations are sampled on a scale comparable to dispersal distances, a significant correlation is interpreted as evidence for isolation by distance (see discussion section). In addition, geographically organized genetic structure between moth populations sampled in the eastern and western portions of the species range was assessed using analysis molecular variation (AMOVA; Excoffier et al. 1992). Geographic distances between populations in these regions ranged between 430 km and 2970 km, and, within regions, between 40 km and 1800 km. Although there is a discontinuity between native host ranges in the western and eastern United States (Figure 1), *Yucca filamentosa* has been planted widely, and naturalized populations now occur in the transition zone between the eastern deciduous forest region and the Great

Plains region (Great Plains Flora Association 1986; Pammel 1925). Two moth populations included in this analysis were sampled from *Y. filamentosa* in this area (Lawrence, KS, and Fayetteville, AR, populations 12 and 19, respectively, in Figure 1) within or slightly outside the historical range limit of *Y. glauca*. AMOVA was applied to assess genetic structure between moth populations sampled on *Y. glauca* and *Y. filamentosa* across their current ranges. All of the population genetic analyses were performed using the Arlequin software package (Schneider et al. 2000).

Results

T. yuccasella populations were found to harbor appreciable nucleotide variation in the sequenced COI segment, with 51 distinct haplotypes observed among the 170 moths. In general, the phylogenetic relationships seen in the haplotype network (Figure 2) did not correspond to geographic proximity. However, the formal nested clade analysis (sensu Templeton 1998) revealed that a number of clades showed evidence of isolation by distance. Clades 1.5, 1.9, 2.1, 2.3, and 3.1 included haplotypes or subclades with overlapping geographic ranges but restricted population distributions

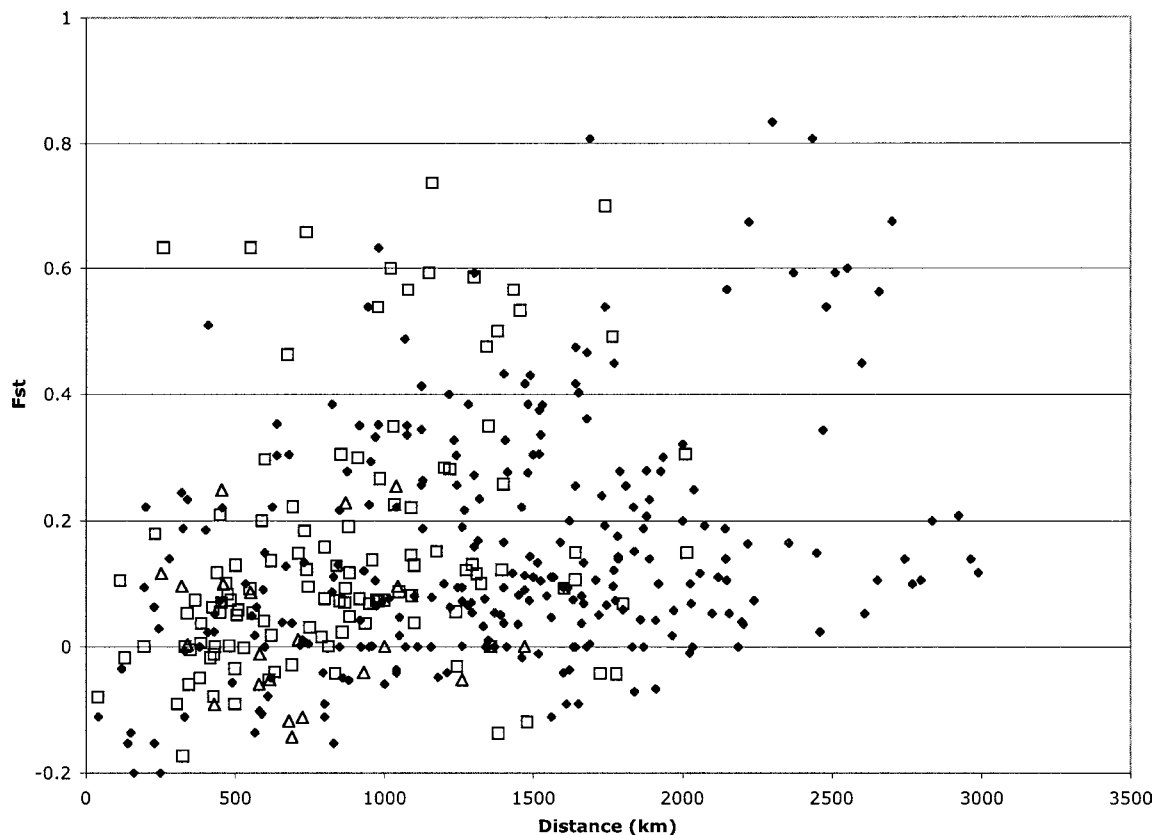


Figure 3. Isolation by distance is indicated by the weak but significant correlation ($r = .294$; $P = .003$) between F_{st} and geographic distance. Pairwise comparisons between moths on *Y. filamentosa* populations are shown as \square , and comparisons between populations on *Y. glauca* are shown as Δ . All other comparisons are represented as \blacklozenge .

Arkansas (population 19), moth populations were collected from *Y. filamentosa* populations in the contact region, geography and host species were largely confounded.

While host population explained only 8% of the observed molecular variation, a high degree of structure was observed in haplotype network for moths sampled from *Y. filamentosa* populations (Figure 2; Table 1). Two of the three most common haplotypes, n and kk, were found from multiple host populations, but the third common haplotype, aa, was sampled only from moths on *Y. filamentosa*. Fifty-six percent of the moths on *Y. filamentosa* had haplotypes that fell into a large clade (clade 3.2, Figure 2) devoid of samples from the six other host species, and consisted mostly of moths from the east (Table 1). Moreover, in the Midwest *filamentosa* moth populations included haplotypes that were not observed from other host populations in the region (Lawrence: haplotypes a, c, and v; Fayetteville: haplotype c).

Discussion

The importance of host specialization as a diversifying force within populations of herbivorous insects has been a focal

point for investigations of gene flow and local adaptation (Mopper and Strauss 1998; reviewed in Slatkin 1987), sympatric speciation (Bush 1969; Berlocher and Feder 2002; Via 2001), and coevolution (Becerra 1997; Ehrlich and Raven 1964; Farrell 1998; Mitter et al. 1988). In each of these contexts, assortative mating has been identified as a key factor in the evolution of host races and speciation. Recent models of speciation have emphasized the similarity between allopatric and sympatric speciation by treating geographic position, mating season, or mating site as alleles at an assortative mating locus (Berlocher and Feder 2002; Kirkpatrick and Ravigné 2002). For highly vagile species, however, the temporary nature of geographic position weakens the relationship between sampling location and mating history. Taking the analogy a step further, estimates of the penetrance of geographic position “alleles” can be inferred from estimates of the scale of isolation-by-distance. For example, studies of several species of flying insects detected little genetic structure across their ranges (McCauley et al. 1995; Peterson and Denno 1998), suggesting weak assortative mating between geographically separated populations. In these systems, the opportunity for diversification through host specialization may not be significantly

Table 2. Comparisons of diversity statistics calculated for all populations and population subsets of *T. yuccasella*

	All (n = 170)	West (n = 60)	TX (n = 32)	FL, GA, AL (n = 39)	<i>Y. filamentosa</i> (n = 104)
Number of haplotypes	51	21	13	19	30
Haplotype diversity	0.926 (0.01)	0.862 (0.029)	0.772 (0.074)	0.929 (0.0234)	0.902 (0.02)
Nucleotide diversity (%)	0.36 (0.209)	0.279 (0.171)	0.242 (0.155)	0.329 (0.198)	0.363 (0.211)
Theta (S)	8.932 (2.304)	5.381 (1.763)	4.221 (1.602)	5.204 (1.838)	6.326 (1.847)
Tajima's D	-0.231	0.657	0.989	1.299	0.911

increased by an allopatric distribution of alternative host species. It should be noted, however, that the sample may be somewhat biased by including numerous agricultural pests. Here we assessed genetic structure among geographically separated *T. yuccasella* populations within and among largely allopatric host ranges (*Y. filamentosa* and *Y. glauca*).

Isolation by Distance

The vagility of *T. yuccasella* is evident in the scale of isolation-by-distance observed for COI (Figure 3). It may seem contradictory to associate vagility with observed isolation-by-distance, but migration rate is never totally independent of geographic distance. Estimates of gene flow for species with limited dispersal ability will decline with distances among populations in close proximity and equilibrate at a long-term average that will not change over larger distances (Peterson and Denno 1998; Porter and Geiger 1995; Slatkin and Barton 1989). Therefore, failure to detect isolation-by-distance among populations sampled over hundreds or thousands of kilometers should not necessarily be equated with long-distance dispersal. On the other hand, a species with strong dispersal ability may not exhibit genetic structure among populations separated by tens of kilometers, but isolation by distance is expected at a scale of hundreds or thousands of kilometers. In this study, the signal for isolation-by-distance observed in the nested clade analysis and the correlation between F_{st} and geographic distance (Figure 3) is strongest between populations separated by >2000 km.

The geographic patterns of genetic variation observed in this study are largely consistent with previous analyses of the bogus yucca moth, *Prodoxus decipiens* (Althoff and Pellmyr 2002), two monophagous pollinating yucca moths in the southwestern United States (Leebens-Mack et al. 1998), and estimates of moth-mediated gene flow in the host species *Y. filamentosa* (Massey and Hamrick 1998). Estimates of genetic structure among *T. yuccasella* populations across its range were slightly higher than average for vagile Lepidoptera (Table 3), but still reflect substantial gene flow ($N_e m = (1/F_{st} - 1)/2 = 1.88$). Genetic structure within region or host was found to be slightly greater than between regions or hosts, but there was significant hierarchical genetic structure (Table 3). Taken together, these patterns suggest that geographic separation is a weak reproductive barrier for yucca moth populations that are separated by less than 1000 km.

The nested clade analysis provided no clear indication of the historical biogeography of *T. yuccasella*. There was no evidence for range expansion, nor for population fragmen-

tation. Although central populations in Lawrence and Fayetteville may include a mixture of historically eastern and western haplotypes, the formal nested clade analysis did not indicate secondary contact. The analysis of haplotype diversity did reveal significantly higher levels of variation in the southeasternmost portion of the species range relative to Texas (Table 2), giving weak support for the notion that *T. yuccasella* may have originated in the southeast on *Y. filamentosa*.

Comparison of Populations on Different Hosts

A degree of divergence between moths sampled from *Y. filamentosa* and *Y. glauca* populations is clearly evident in the COI haplotype network (Figure 2) and AMOVA analysis (Table 3). Haplotypes for *filamentosa* moths were clustered in the haplotype network, and over half (56%) were restricted to a large host-specific clade (clade 3-2 in Figure 2).

Table 3. Results of analysis of molecular variance (AMOVA) among *T. yuccasella* populations on *Y. glauca* and *Y. filamentosa*

Source	Variance	Total (%)	F
A.			
East (15) versus west (14)	0.04441	9.06	$F_{CT} = 0.096^*$
Among populations within region	0.05282	11.95	$F_{SC} = 0.131^*$
Within populations	0.38464	78.99	$F_{ST} = 0.210^*$
Total	0.48187		
B.			
<i>Y. glauca</i> (7) versus <i>Y. filamentosa</i> (16)	0.03902	7.98	$F_{CT} = 0.080^*$
Among populations within host	0.06253	12.80	$F_{SC} = 0.139^*$
Within populations	0.38714	79.22	$F_{ST} = 0.208^*$
Total	0.48869		
C.			
<i>Y. filamentosa</i> north (8) versus south (7)	0.00076	0.14	$F_{CT} = 0.002$
Among populations within region	0.06717	14.84	$F_{SC} = 0.148$
Within populations	0.38469	84.99	$F_{ST} = 0.150^*$
Total	0.45261		

All F values are highly significant ($*P < .001$) for analyses of structure between populations in the east and west (A) and between populations on *Y. filamentosa* and *Y. glauca* (B), but there is not significant structure between moth populations on *Y. filamentosa* north and south of its precolonial range limit (C).

Host-related structure was also evident in the AMOVA ($P < .001$), with 8% of the total variation attributed to differences between moths on *Y. glauca* and *Y. filamentosa*.

The polyphyletic distribution of clades with moth haplotypes sampled on *Y. filamentosa* (Figure 2) may be responsible for the apparent conflict between the AMOVA analysis and the mapping of *filamentosa* moths on the haplotype network. Some of the clades dominated by *filamentosa* moths are quite divergent (e.g., clades 3.2 and 2.6). In the AMOVA, the polyphyly of these clades of *filamentosa* moth increased diversity within host and population variance, and decreased between-host variance.

The haplotype network suggests host-related diversification, but additional sampling and analysis of multiple loci may be necessary to detect host-race divergence in the AMOVA. A study of the stalk-feeding bogus yucca moth, *P. decipiens*, provided strong evidence for recent population divergence following a host range expansion from *Y. filamentosa* to *Y. aloifolia* within the last 500 years (Groman and Pellmyr 2000). The host shift was so recent and apparently rapid, however, that the clear pattern of divergence in allozyme frequencies of sympatric populations was not apparent in a smaller sample of mtDNA haplotypes. Sample sizes are larger in this study, but sampling error is still appreciable and may be influencing estimates of divergence in haplotype frequencies. In addition, only maternally inherited mitochondrial haplotypes were analyzed in this study. Although the oviposition behavior of female moths will have the most direct effect on yucca–yucca moth coevolution, data from nuclear markers could indicate whether male host specificity is resulting in restricted gene flow.

The weak genetic divergence between *T. yuccasella* populations on *Y. filamentosa* and western hosts is reminiscent of the phylogenetic split between the two bogus yucca moths *P. decipiens* and *Prodoxus quinquepunctellus* that co-occur with *T. yuccasella* on eastern and western capsular fruited yuccas (Althoff et al. 2001). A major difference in this pattern is that the estimated time of divergence in *Prodoxus* occurred about 12.5 million years ago, whereas divergence between *T. yuccasella* populations is much more recent. The estimated age of *T. yuccasella* is just 1.26 ± 0.96 million years (Pellmyr and Leebens-Mack 1999).

Yucca Moth Diversification

Although the occurrence of many single-host pollinator species (Pellmyr 1999, 2003) suggests a role for species specialization in yucca moth diversification, the ecological pressures responsible for population divergence remain obscure. Previous studies have suggested that phenology may be a more effective barrier to gene flow in yucca moths than geographic separation (Leebens-Mack et al. 1998; Miles 1983). Phenological divergence between yucca moth populations has been associated with the origin of parasitic yucca moth species (Pellmyr and Leebens-Mack 2000) and may play a role in host-race formation. However, variation in host phenology appears not to be promoting host specialization

and diversification among *T. yuccasella* populations. Much variation in flowering time in the host yuccas of *T. yuccasella* follows a latitudinal gradient, but the AMOVA of *Y. filamentosa* moth populations found no significant structure between populations in the northeast and the southeast (Table 3). In the absence of host-driven disruptive selection on phenology, it is possible that host specificity may not be adaptive in *T. yuccasella*. This hypothesis needs to be addressed in naturally or experimentally sympatric host populations. Improved understanding of the influence of host specialization on yucca moth fitness will elucidate the role of codiversification in the history of the yucca–yucca moth association.

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