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EXAMINING GENETIC STRUCTURE IN A BOGUS YUCCA MOTH: A SEQUENTIAL APPROACH TO PHYLOGEOGRAPHY

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Abstract.—Understanding the phylogeography of a species requires not only elucidating patterns of genetic structure among populations, but also identifying the possible evolutionary events creating that structure. The use of a single phylogeographic test or analysis, however, usually provides a picture of genetic structure without revealing the possible underlying evolutionary causes. We used current analytical techniques in a sequential approach to examine genetic structure and its underlying causes in the bogus yucca moth *Prodoxus decipiens* (Lepidoptera: Prodoxidae). Both historical biogeography and recent human transplantations of the moth's host plants provided a priori expectations of the pattern of genetic structure and its underlying causes. We evaluated these expectations by using a progression of phylogenetic, demographic, and population genetic analyses of mtDNA sequence data from 476 individuals distributed across 25 populations that encompassed the range of *P. decipiens*. The combination of these analyses revealed that much of the genetic structure has evolved more recently than suggested by historical biogeography, has been influenced by changes in demography, and can be best explained by long distance dispersal and isolation by distance. We suggest that performing a suite of analyses that focus on different temporal scales may be an effective approach to investigating the patterns and causes of genetic structure within species.

Key words.—Biogeography, isolation by distance, mismatch, nested clade, population structure, yucca moth.

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Avise (2000) defines phylogeography as ''. . . a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages'' (p. 35). Phylogeography is the melding of biogeography and the evolutionary history of a lineage or groups of lineages. Early investigations used the methodology of phylogenetics to relate evolutionary history to the geographic distribution of populations within a lineage. The resulting phylogenetic tree of haplotypes was overlain on the current geographic structure of populations to make inferences about both historical and current patterns of population subdivision. This approach has and continues to work well for lineages that have been separated for long periods of time. In such lineages, the accumulation of genetic divergence translates into a phylogenetic signal that may correspond well with geographic separation.

As an increasing number of studies have shown, the methodology of phylogenetics often lacks resolving power and may even obscure the evolutionary relationships of lineages that are relatively recent or have experienced demographic variation such as population bottlenecks or expansions (Crandall et al. 1994; Crandall and Templeton 1996; Smouse 1998). For these lineages the use of a bifurcating tree, whether parsimony or distance-based, may be misleading, especially when the ancestral haplotypes are extant. The use of haplotype networks will more accurately portray the true evolutionary history of a lineage (Smouse 1998; Posada and Crandall 2001). Instead of a series of bifurcations, a network of ancestral interior and descendant tip haplotypes is produced, and it is possible to have closed loops among haplotypes that represent uncertainty about the pattern of haplotype relatedness. Templeton et al. (1995) have incorporated the use of haplotype networks into a nested clade analysis to examine historical and current processes governing phylogeographic structure within a lineage. In some cases, lineages may be so recent or may have gone through a series of demographic changes such that there may be little phylogenetic signal among geographically structured haplotypes because population separation precedes coalescence events. For such lineages, the use of analyses based on the coalescent process may be informative in determining the demographic factors shaping variation within and among populations (Hudson 1990), and analyses that partition molecular variation among populations and groups of populations must be used (e.g., AMOVA, Excoffier et al. 1992) to examine potential patterns of geographic structure.

For the evolutionary biologist interested in the phylogeography of a particular taxon or group of taxa, the choices for phylogeographic analyses seem daunting. The challenge then is to determine which method(s) may be the most appropriate to use given the data at hand. How do we optimize the analytical process to make the most out of the data? Bernatchez (2001) recommended using a combination of approaches that examine haplotype relatedness and demographic history. In a case study of brown trout, he concluded that the combined approach worked well in elucidating not only geographic structure, but also the evolutionary history producing that structure.

Here, we emulate Bernatchez's recommendation and extend it by combining a series of analyses that focus on genetic patterns at different temporal scales to study the phylogeography of the bogus yucca moth *Prodoxus decipiens* (Prodoxidae). The evolutionary history of *P. decipiens* has been influenced by historical biogeography and more recent population phenomena that provide us with a priori expectations with which to evaluate the effectiveness of each separate analysis and the analyses combined. We took a sequential approach starting first with phylogenetic analysis, moving to more demographically based and population genetic analyses as necessary. The latter analyses will likely detect the same

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geographic structure determined by a phylogenetic approach, but phylogenetic methods are unlikely to detect structuring if recent demographic changes have occurred. Each analysis in turn, however, adds more information and furthers the interpretation of genetic structure. We outline this approach in more detail as a means of guidance for studying the phylogeography of a species. We stress that there is no one analysis or technique that is as powerful or provides more information as a combination of analyses.

Outline and Rationale of Sequential Approach

The sequential approach we used was a three-step progression, starting with the examination of genetic structure in the distant past and working forward in evolutionary time. The first step we took was to use phylogenetic methods to estimate patterns of relatedness among haplotypes. These methods focus on the historical relationships of gene lineages. Although the methodology may sometimes be inappropriate for intraspecific studies (Smouse 1998; Posada and Crandall 2001), phylogenetic methods have worked well for many phylogeographic studies and can be viewed as the baseline approach that allows comparisons with previous studies. A result of monophyly of haplotypes from each population or region may be enough to determine the phylogeography, and further steps may be unnecessary. Additionally, the resulting haplotype tree can be converted into a haplotype network for use in a nested clade analysis to make inferences about the processes governing geographic structure (Templeton et al. 1995; Templeton 1998). The advantage of this method is that it represents a bridge between phylogenetic and population genetic perspectives. It can potentially tease apart historical and recent influences on a species' phylogeography, based on haplotype relatedness and geographic distributions. If there is little to no phylogenetic signal and the resulting tree/network resembles a starburst with no concordance with geography, this information is still useful because it suggests that the proliferation of haplotypes is relatively recent (Slatkin and Hudson 1991). Additional analyses, then, are needed to statistically confirm this finding and examine recent patterns of population structure.

The second step in the sequence is to incorporate analyses of demographic history (reviewed in Emerson et al. 2001) such as mismatch distributions (Slatkin and Hudson 1991; Bertorelle and Slatkin 1995; Rogers 1995; Rogers and Harpending 1995; Schnieder and Excoffier 1999) and surveys of haplotype and nucleotide diversity (Grant and Bowen 1998). These analyses provide information about the processes causing observed patterns of genetic variation. If the haplotype tree generated in step one is a starburst, analyses of mismatch distributions provide a statistical means to confirm that the proliferation of haplotypes is recent and due to population genetic bottlenecks and/or population expansions. In addition, comparisons of haplotype and nucleotide diversity can also provide insight into the historical demography of a lineage (Grant and Bowen 1998). For example, a large amount of haplotype diversity, but low nucleotide diversity, is consistent with a population bottleneck and rapid population growth. Although these analyses provide inferences about demographic history, they do not necessarily examine patterns of geographic structure.

The last step is to use population genetic analyses that examine recent population structure. This is especially important if there is little phylogenetic signal in the data. Populations may still exhibit significant genetic structure, just not at the level of haplotype relatedness. For sequence data, analyses that use both haplotype divergence and the frequency of haplotypes within and among populations should be favored (Excoffier et al. 1992; Slatkin 1993). Although there may be little sequence divergence, the distribution of that divergence within and among populations may provide insights into genetic structure. Analyses such as isolation by distance and AMOVA may be particularly useful for examining recent geographic structure. Detection of isolation by distance suggests that geographic distance significantly influences genetic structure and populations are structured at some geographic scale. Analysis of molecular variance permits the testing of a priori hierarchical patterns of geographic structure and provides a way to determine the geographic scale of genetic structure. Both types of analyses rely on the calculation of F-statistics or an analog. Taken together, the above analyses in this sequential approach move from testing deeper phylogenetic splits to inferring recent patterns of population structure. We demonstrate the utility of this approach in examining the phylogeography of the bogus yucca moth *P. decipiens*.

Background on Prodoxus decipiens

Prodoxus decipiens Riley (Lepidoptera: Prodoxidae) is a moth that feeds on several species of capsular-fruited yuccas (Agavaceae) and one species of fleshy-fruited yucca. This moth is a close relative of the pollinating yucca moths, but its larvae feed within the inflorescence stalk rather than on developing seeds (Riley 1881; Davis 1967). *Prodoxus decipiens* occurs from the eastern coast of the United States west to the Edwards Plateau in central Texas (Althoff et al. 2001), and is the only *Prodoxus* species that has extended its range into the eastern United States. Its sister species *P. quinquepunctellus* occurs on the Edwards Plateau, north along the High Plains and west of the Mexican Highlands subprovince of the southwestern United States. Prior work suggested that *P. decipiens* and *P. quinquepunctellus* likely diverged in central Texas, and *P. decipiens* has since colonized host plants along the Gulf Coast and Florida (Althoff et al. 2001). *Prodoxus decipiens* has also extended its range into the interior eastern United States as a result of recent colonizations of *Yucca filamentosa* that have been transplanted by humans within the last 200 years.

The historical biogeography of the Gulf Coast region and the recent introduction of host plants provide us with two a priori expectations of the phylogeographic structure of *P. decipiens* (Fig. 1). Based on the well-documented historical biogeography of the Gulf Coast region we would expect a genetic division between populations in Texas and Florida. From the late Miocene through the early Pleistocene, Florida was connected to the western United States via a habitat corridor that extended along the Gulf Coast (Webb 1990). This corridor provided an interchange of animals and plants,

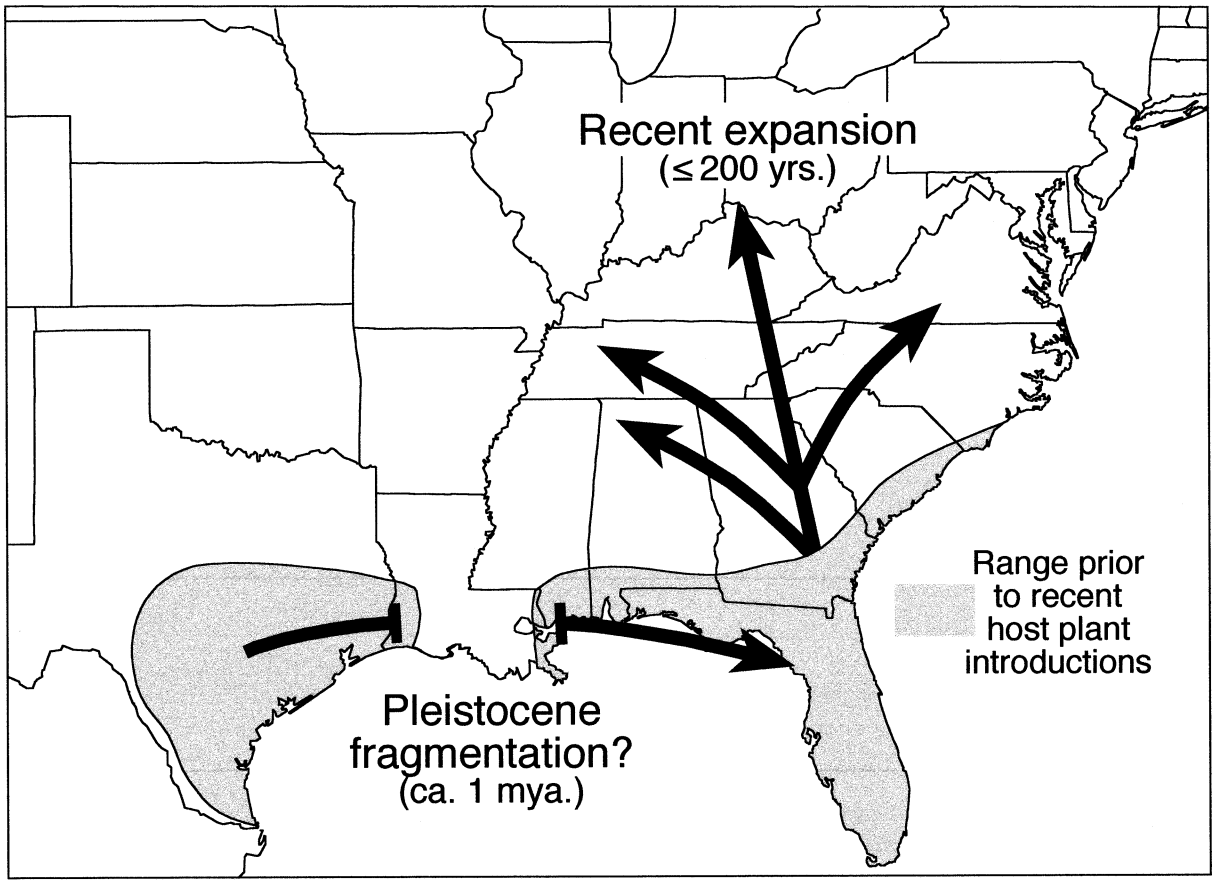

FIG. 1. A priori expectations of the phylogeographic structure of *Prodoxus decipiens*. This moth and its host were part of a rich community of western species that expanded eastward along the Gulf Coast into Florida as a habitat corridor originated and persisted between 12–3 mya. Paleontological data show that this Gulf Coast habitat corridor was closed ~1 Mya, potentially isolating populations east and west of this closure. Transplantations of the host plant *Yucca filamentosa* by settlers within the last 200 years into the interior eastern United States must have preceded moth range expansion in this area as there were no yucca species present previously.

and many taxa of semiarid habitats that were endemic to the High Plains, Texas, and colonized Florida. During the mid-Pleistocene, this corridor was broken by the expansion of the Mississippi wetlands, changes in sea levels as a result of glacial cycles, and loss of semiarid habitats along the Gulf Coast (Webb 1990; Graham 1999). The absence of host plants and moths in middle and southern Louisiana suggests this break has continued through recent times (D. Althoff, K. Seagraves, and O. Pellmyr, unpubl. data). Populations of *P. decipiens* in Texas and Florida potentially have been separated for approximately one million years. Patterns of phylogenetic structure for other plant and animal groups exhibit an east-west division along the Gulf Coast (Wiley and Mayden 1985; Shapiro 1998; Gill et al. 1993; Gould and Jansen 1999; Burbrink et al. 2000). Given this scenario, we expected that phylogenetic analyses would reveal a division in relatedness between haplotypes from Texas and the eastern United States populations, and the nested clade analysis would detect historical fragmentation for these populations. We also expected that Texas should be the area with the greatest haplotype and nucleotide diversity, followed next by Florida, and then more interior populations. This expectation was based on the hypothesis that *P. decipiens* originated in Texas and subsequently moved eastward along the Gulf Coast. Populations in Texas should be the oldest, and have the greatest probability of evolving and maintaining haplotype and nucleotide diversity.

The interior, eastern United States populations of *P. decipiens* represent a recent expansion onto host plant populations transplanted by early settlers. Although *Yucca filamentosa* is a native of semiarid scrub habitat, it has been planted extensively throughout the more mesic interior of the eastern United States within the last 200 years (Trelease 1902, Pammel 1925). Specimens in the Smithsonian Institution Museum of Natural History collections establish *P. decipiens* in Missouri, Ohio, and Pennsylvania by the 1880s (O. Pellmyr, unpubl. data). Similarly, the fleshy fruited yucca *Y. aloifolia* has been naturalized along the Gulf and Atlantic Coasts in the last 500 years, and *P. decipiens* has incorporated this species as a host in some areas (Riley 1880; Groman and Pellmyr 2000). Based on the recent expansion of *P. decipiens* onto transplanted host plants populations, we expected little phylogenetic structure among haplotypes from populations in the interior United States. These haplotypes should be a genetic subset of the more ancestral haplotypes located in Florida, and geographic distance might have a strong influence on genetic structure. The nested clade analysis should be able to detect both these patterns. We also expect that

TABLE 1. Locality information and number of individuals sampled for populations of *Prodoxus decipiens.* Site numbers correspond to those in Figure 2.

Site #	Site name	*n*	*Yucca* species used	Latitude	Longitude
1	Eglin, FL	20	*filamentosa*	30°43′15″N	86°44′18″W
2	Goldhead State Park, FL	20	*filamentosa*	29°49′55″N	81°57′11″W
3	Lake Placid, FL	20	*filamentosa*	27°14′00″N	81°24′00″W
4	Ocala, FL	20	*filamentosa*	29°08′00″N	81°31′00″W
5	Perry, FL	20	*filamentosa*	30°07′02″N	83°34′55″W
6	Torreya State Park, FL	20	*filamentosa*	30°34′08″N	84°56′52″W
7	Camp Meeting Rock, GA	12	*filamentosa*	33°18′19″N	85°07′30″W
8	Ludowici, GA	16	*filamentosa*	31°42′28″N	81°44′33″W
9	Pawley's Island, SC	20	*filamentosa*	33°25′59″N	79°07′18″W
10	Nags Head, NC	20	*filamentosa*	35°56′28″N	75°37′28″W
11	Buxton, NC	17	*filamentosa/aloifolia*	35°16′03″N	75°32′34″W
12	Hatteras, NC	21	*filamentosa*	35°13′09″N	75°41′26″W
13	Goldbond, VA	20	*filamentosa*	37°22′48″N	80°30′40″W
14	Vine, TN	18	*filamentosa*	36°01′52″N	86°21′28″W
15	Wilmington, OH	20	*filamentosa*	39°26′43″N	83°49′43″W
16	Hinckley, OH	20	*filamentosa*	41°14′18″N	81°44′43″W
17	Mt Olive, MS	20	*filamentosa*	31°21′18″N	90°56′07″W
18	Jefferson, TX	21	*glauca var arkansana*	32°45′00″N	93°39′00″W
19	Tyler, TX	20	*glauca var arkansana*	32°23′36″N	95°18′29″W
20	Kirby State Forest, TX	20	*glauca var arkansana*	30°34′35″N	94°24′44″W
21	Silsbee, TX	10	*glauca var arkansana*	30°20′56″N	94°10′40″W
22	Sarita, TX	20	*glauca var arkansana*	27°13′00″N	97°47′00″W
23	Brownwood, TX	16	*glauca*	31°35′00″N	99°03′00″W
24	Brady, TX	20	*constricta*	31°08′06″N	99°20′05″W
25	Harper, TX	25	*constricta*	30°17′59″N	99°14′38″W

patterns of molecular variation would indicate significant structuring between Florida populations and those in the eastern United States. Understanding geographic structure for eastern populations will require the use of population genetic analyses such as isolation by distance and AMOVA.

METHODS

We collected 476 adult moths and larvae between 1993 and 2000 from 25 sites along transects that represent the putative historical and recent patterns of range expansion of *P. decipiens*. (Table 1; Fig. 2). These sites include a subset of the sites used by Althoff et al. (2001), the addition of eight new sites, and at least a threefold increase in the number of individuals sampled from each site. Adults were collected in the field while they were resting in flowers or ovipositing into the inflorescence stalk. Stalks containing diapausing larvae were also collected and placed in 1-mm mesh cages in an environmental chamber at Vanderbilt University to allow adults to eclose. Larvae in stalks were held in diapause at 4°/6°C (16h:8h) for 90 days during the winter, and the temperature was then raised over a two-week period to 28°/24°C (16h:8h) to trigger emergence. Larvae that had not pupated were dissected from the stalks.

Molecular methods

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 individual was used. We used PCR to amplify the 3′ end of Cytochrome Oxidase I and transfer RNA Leucine of the mitochondrial DNA. Each 30 μl reaction volume contained 50 mM KCl, 10 mM Tris (pH = 9.0), 1.67 mM $MgCl_2$, 0.2 mM dNTPs, 0.33 mM of each primer, 0.033 units of Promega B Taq polymerase, and 100 ng of genomic DNA. Primers sequences were 2231F: (5′-CCAGGATTTGGTAT AAATTTC-3′) and 3020R: (5′-GTAATGGATTTAAGCCC CT-3′). The thermal cycler profile was one cycle at 95°C for 2 min, 35 cycles at 95°C for 1 min, 52°C for 1 min, 72°C for 1 min 30 sec, and one cycle at 72°C for ten minutes. PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and both forward and reverse strands were sequenced on an ABI 377 Automated DNA sequencer (PE Applied Biosystems, Foster City, CA). Sequencing products were generated using cleaned PCR product, BigDye terminator cycle sequencing mix (PE Applied Biosystems, Foster City, CA) and four pmoles of one of the original PCR primers. The thermal cycler profile was one cycle at 96°C for 2 min., 25 cycles at 96°C for 30 sec, 50°C for 30 sec and 60°C for 4 min. Sequencing products were cleaned using Centri-sep Sephadex columns (Princeton Separations, Adelphia, NJ). Forward and reverse sequences for each individual were checked using Sequencher 3.1 (Gene Codes Corporation, Ann Arbor, MI). The consensus sequence for each individual was then aligned by eye in PAUP* version 4.0b8 (Swofford 2001). There were no insertions or deletions and all sequences were easily aligned.

Phylogenetic approaches

We examined the phylogenetic relationships among sequence haplotypes by using maximum-parsimony and maximum-likelihood analyses and the most common haplotype from *P. quinquepunctellus* as the outgroup. We used Fitch

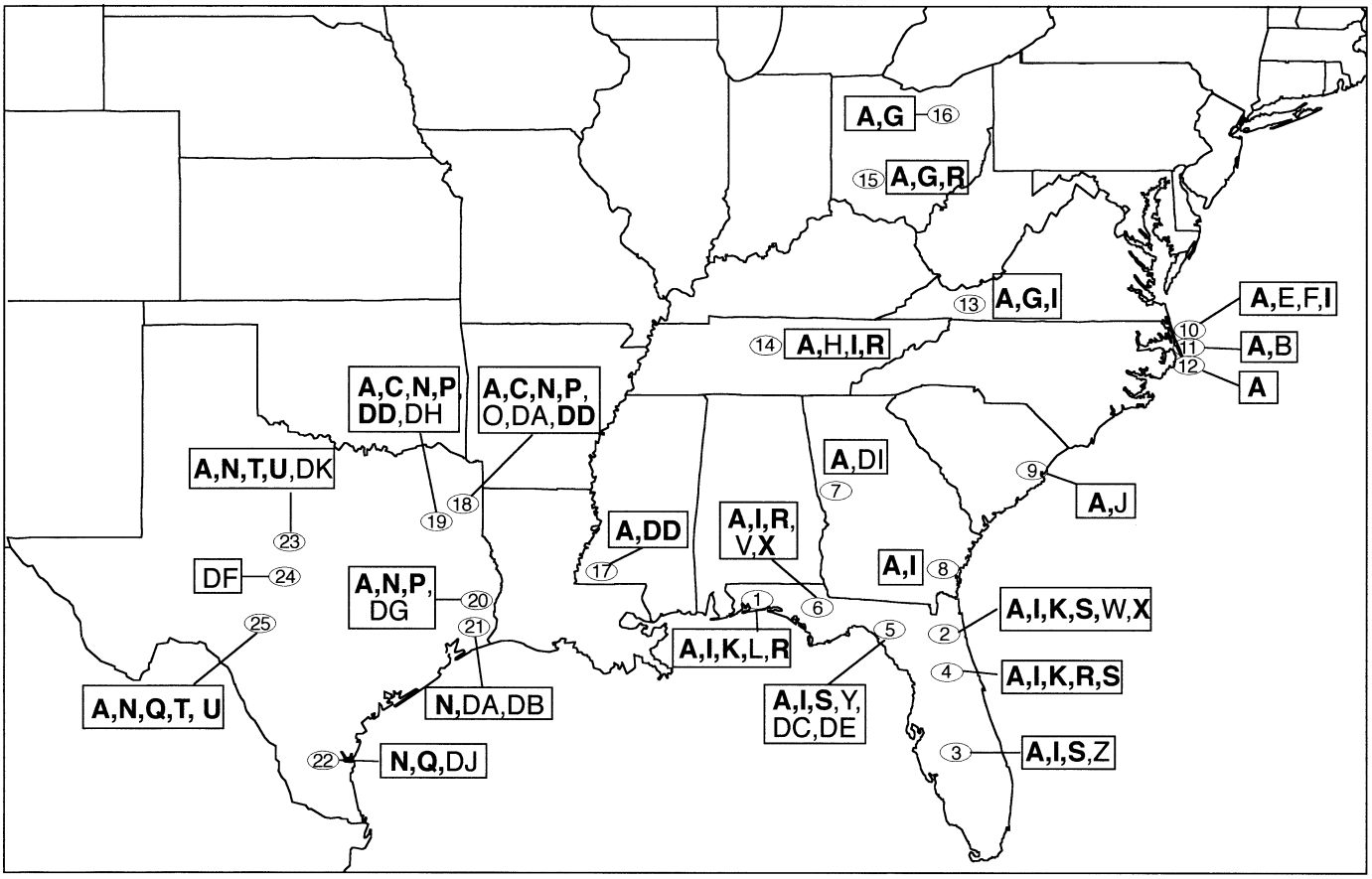

FIG. 2. Geographic distribution of the 35 unique mtDNA cytochrome oxidase I haplotypes detected for *Prodoxus decipiens*. The paucity of sites between Texas and Florida is the result of the absence of populations rather than limited sampling in this area. Bold letters represent haplotypes shared among populations, and plain letters represent haplotypes unique to that population. Visual inspection of this distribution suggests patterns that are consistent with the expectations from Figure 1. Populations in Texas and Florida exhibit high haplotype diversity. Texas populations have a unique set of haplotypes (N, P, Q, T, and U) not found in the eastern United States. Populations in the interior eastern United States have reduced haplotype diversity and contain a subset of haplotypes from Florida populations.

parsimony, a heuristic search with simple taxon addition and TBR branch swapping for the parsimony analysis. For the maximum-likelihood analysis we estimated the transition/transversion (Ti:Tv) ratio and the gamma shape parameter via maximum likelihood and used the HKY85 distance measure because of unequal base frequencies and the Ti:Tv ratio > 1 (Hasegawa et al. 1985). Support for the resulting topologies was assessed using 100 bootstrap replicates as implemented in PAUP* version 4.0b8 (Swofford 2001). We also constructed a haplotype network using TCS 1.13 (Clement et al. 2000). This computer program uses statistical parsimony to construct a network of relationships among haplotypes. The network was then used in the Nested Clade Analysis (Templeton et al. 1995) implemented in GeoDis 2.0 (Posada et al. 1999) to identify recent or historical events affecting population structure such as restricted gene flow, long distance dispersal, and patterns of population history such as historical fragmentation and range expansion.

Analyses of demographic history

All demographic analyses were implemented using Arlequin 2.0 for the Macintosh platform (Schneider et al. 2000). The Tamura measure of haplotype divergence was used for all analyses to correct for unequal nucleotide frequencies and unequal Ti:Tv rates (Tamura 1992) and the gamma value from the maximum-likelihood analysis was used. We used the mismatch analysis to examine the distribution of the frequency of pairs of individuals who differ by a certain number of nucleotide differences (Slatkin and Hudson 1991; Rogers and Harpending 1992; Bertorelle and Slatkin 1995; Rogers 1995; Schnieder and Excoffier 1999). The resulting distribution was tested against the sudden population expansion model as calculated and implemented in Arlequin 2.0. This distribution is usually unimodal for lineages that have undergone a recent bottleneck or population expansion and multimodal for a lineage whose populations are at demographic equilibrium. We also used Tajima's D to examine further the historical demography of *P. decipiens* (Tajima 1989a, b). This test for selective neutrality can be used to examine demography in that a negative value is expected under population expansion and a positive value is expected under population subdivision. We calculated haplotype and nucleotide diversity for *P. decipiens* in general as well as for populations in Texas, Florida, and eastern populations excluding Florida.

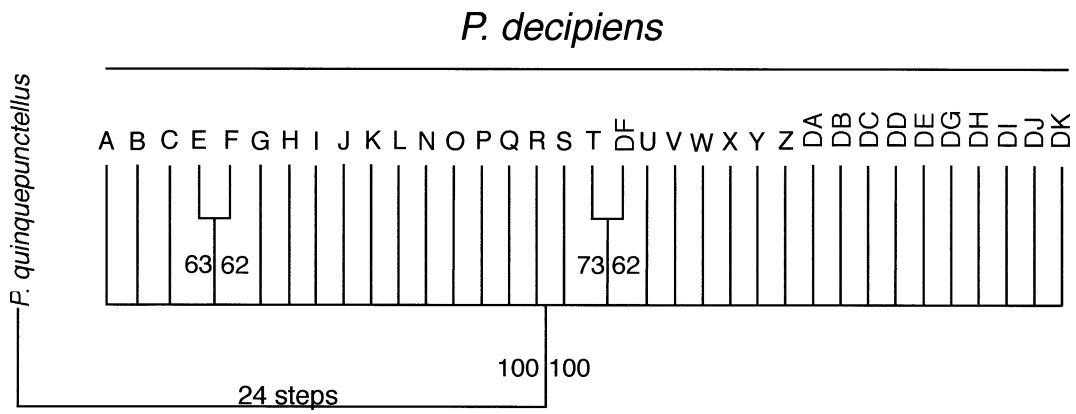

FIG. 3. Bootstrap consensus tree generated by the parsimony and maximum-likelihood analyses. Numbers to the left of the branches are bootstrap values from the parsimony analysis and numbers to the right are from the maximum-likelihood analysis. The maximum number of steps between haplotypes of *Prodoxus decipiens* was six. These analyses suggest a starburst pattern of haplotype relatedness. Note the monophyly of *P. decipiens* haplotypes and the large number of steps separating this moth species and its sister species *P. quinquepunctellus*.

For each estimation, populations within a region were combined into one. Measures of haplotype and nucleotide diversity are useful in examining the demographic history of a lineage (Grant and Bowen 1998). Centers of origin should be more diverse in haplotype and nucleotide diversity than more recently founded populations.

Analyses of population structure

To examine the possibility of population subdivision we tested for isolation by distance by plotting pairwise population F_{ST} values against geographic distance. We used Mantel tests (Mantel 1967) to test the significance of isolation by distance for all populations, populations in the eastern United States, and populations in Texas. We used Analysis of Molecular Variance (AMOVA) to examine hierarchical population structure. We used our a priori expectation of a genetic division between populations in Texas and the eastern United States to group populations into regions. We conducted the analysis between Texas populations and populations exclusively in Florida to control for the human-facilitated range expansion of *P. decipiens* into the interior eastern United States. The population at Mt. Olive, Mississippi was excluded from these analyses because it was the only geographically intermediate site that could not be placed in either region. We also tested whether eastern United States populations were subdivided into two regions—a region comprising the Florida populations and a region comprising the remaining eastern United States populations. We used Tamura's measure of haplotype divergence with the estimated gamma value from the maximum-likelihood analysis for the isolation by distance analyses and the AMOVA.

RESULTS

We sequenced 686 bp of cytochrome oxidase I for all 476 individuals and detected 35 unique haplotypes. Haplotypes A through U can be found in GenBank under Accession numbers AF334413–AF334433, and haplotypes V through DK under accession numbers AY083312–AY083327. Thirty sites were variable (4.4%), including 13 at the first codon position, two at the second codon position, and 15 at the third codon position. Nineteen of the substitutions were synonymous and eleven were nonsynonymous. Nucleotide frequencies were strongly AT biased as has been recorded for other insects (frequency of A = 0.34, C = 0.13, G = 0.12, T = 0.41). The Ti:Tv ratio estimated via maximum likelihood was 3.63 and the shape parameter of the gamma distribution was 0.0153, indicating a large number of invariant sites. Maximum-sequence divergence based on the HKY85 measure corrected with the estimated shape parameter was 0.0181. The greatest number of absolute differences among haplotypes was six.

Visual inspection of haplotype distribution among populations suggested several phylogeographic patterns (Fig. 2). First, haplotype A may be the most ancestral because it is present in 22 out of the 25 populations sampled. Second, populations in Texas had a suite of haplotypes not found in the eastern United States. Third, both Texas and Florida had more haplotypes than populations in the interior eastern United States. Finally, interior populations had a subset of the haplotypes found among Florida populations and only three unique haplotypes. Qualitatively, haplotype distributions appear to correspond well with the a priori expectations.

Patterns of phylogenetic relatedness

Parsimony and maximum-likelihood analyses produced similar patterns of haplotype relatedness. A strict consensus of 1420 equally parsimonious trees revealed only two nodes that were present in all trees, and the maximum-likelihood bootstrap analysis returned a tree identical to the parsimony strict consensus tree (Fig. 3). Only two nodes had bootstrap values above 50. The overall picture of haplotype relatedness is one of a starburst pattern, suggestive of a very recent origin of most haplotypes.

As expected based on the widespread of occurrence of haplotype A, the haplotype network revealed a central, interior position for this haplotype (Fig. 4). Eighteen of the 35 haplotypes were one mutational step from haplotype A. The interior position of this haplotype and its widespread occurrence among all populations suggests that it may be the an-

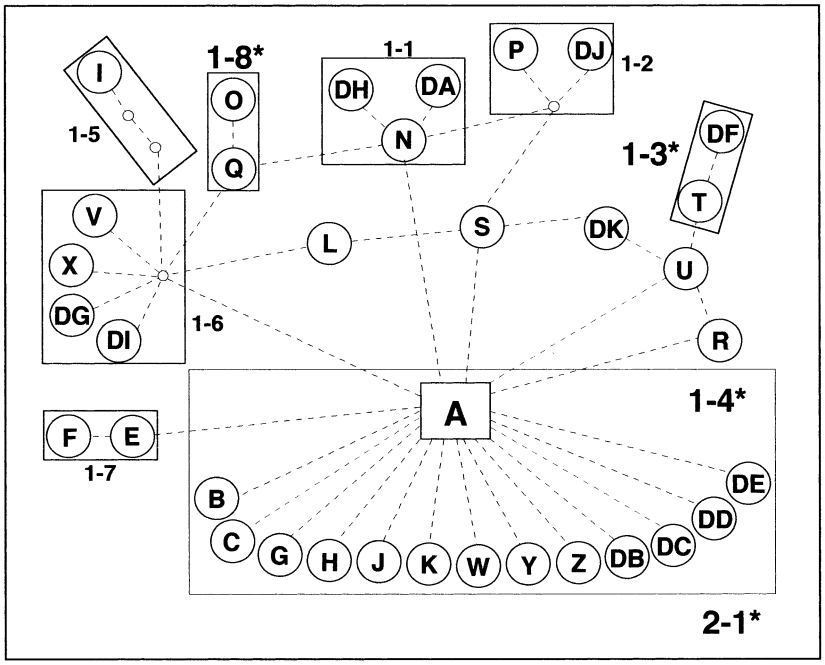

FIG. 4. Haplotype network of the 35 unique haplotypes detected for *Prodoxus decipiens*. Haplotypes not sampled or extinct are designated by small circles. Dotted lines connecting haplotypes represent one mutational step. Clade numbers with an asterisk are ones for which the nested clade analysis detected a significant geographic association. Restricted gene flow was detected in all of these clades. All haplotypes nested into clade 2-1.

cestral haplotype for *P. decipiens*. The network also revealed a number of closed loops among haplotypes from Florida and Texas (Fig. 4). Interior haplotypes in a closed loop and without tip haplotypes were not nested in any one step clade. These haplotypes were instead nested in the next higher level clade as per the suggestion of Templeton and Sing (1993) for dealing with ambiguity in haplotype relatedness. The nested clade analysis detected restricted gene flow with isolation by distance in clade 1–3, restricted gene flow with long distance dispersal for clade 1–4, and long distance colonization for clade 1–8. For clade 2-1, which includes all haplotypes except haplotype I, the analysis detected restricted gene flow with some long distance dispersal events (Fig. 5). Overall, populations of *P. decipiens* exhibited restricted gene flow. The nested clade analysis, however, did not detect historical fragmentation between Texas and Florida or a range expansion for populations in the eastern United States.

Patterns of historical demography

The relatively low amount of sequence divergence and the lack of phylogenetic signal suggest that the extant haplotypes in *P. decipiens* evolved relatively recently compared to its divergence with its sister species *P. quinquepunctellus*. The shallow divergence among *P. decipiens* haplotypes suggests that this species has experienced a series of increases and reductions in population size throughout its history. The high haplotype diversity (0.7236 ± 0.0211, mean $\pm$ SE) relative to nucleotide diversity (0.1915 (% π) $\pm$ 0. 1324) is indicative of a population bottleneck followed by rapid population growth and accumulation of mutations (Grant and Bowen 1998; Avise 2000). This is supported by a significantly negative value for Tajima's D (-1.855, $P = 0.0157$). Similarly, fit of the mismatch distribution of pairwise distances between all possible pairs of the 476 individuals to a model of sudden population expansion supported this interpretation (Fig. 6). Patterns of haplotype and nucleotide diversity also supported the a priori expectation that Texas was the center of origin of *P. decipiens*, and populations in the interior eastern United States were recently derived. Both diversity measures were highest among the Texas populations and lowest among interior populations (Table 2).

Patterns of recent population structure

Isolation by distance was detected among all populations and for populations in the eastern United States (Fig. 7). Populations in Texas, however, did not exhibit isolation by distance. This result is contrary to the detection of isolation by distance for Texas haplotypes T and DF by the nested clade analysis (Fig. 5). Incorporating both sequence divergence and haplotype frequencies among populations, the AMOVA detected significant structuring between populations in Texas and Florida (Table 3). Seventeen percent of the molecular variation is between these two groups. The AMOVA also detected significant structuring between populations in Florida and those in the rest of the eastern United States. Significant F_{ST} values from both analyses indicated overall structuring among populations (Table 3). Together, these analyses suggest that restricted gene flow is a result of geographic separation.

DISCUSSION

Avise et al. (1987) envisioned phylogeography as the bridge between population genetics and systematics/phylo-

Clade 1 - 4

Haplotypes	A	B	C	G	H	J	K	W	Y	Z	DB	DC	DD	DE
D_c	573.02 ^{S}	0	58.63 ^{S}	129.50 ^{S}	0	0 ^{S}	315.47	0	0	0	0	0	169.82 ^{S}	0
D_n	578.63 ^{S}	677.86	1138.01 ^{L}	675.08	401.6	342	527.85	410.82	429.35	754.46	1142.92	429.35	1002.76 ^{L}	429
$(I\text{-}T)_c$	459.42 ^{L}													
$(I\text{-}T)_n$	-111.32 ^{S}													
Steps in inference key	1,2,3,5,6,7, restricted gene flow/dispersal, but with some long distance dispersal													

Clade 1-3

Haplotypes	T	DF
D_c	66.99 ^{L}	0 ^{S}
D_n	74.78 ^{L}	14.47 ^{S}
$(I\text{-}T)_c$	66.99 ^{L}	
$(I\text{-}T)_n$	60.31 ^{L}	
Steps in inference key	1,2,3,4, restricted gene flow with isolation	

Clade 1-8

Haplotypes	Q	O
D_c	61.28 ^{S}	0
D_n	161.23 ^{S}	585.68 ^{L}
$(I\text{-}T)_c$	61.28	
$(I\text{-}T)_n$	-424.45 ^{S}	
Steps in inference key	1,2,11,12,13, long distance colonization	

Clade 2-1

Clades	1-4	1-1	1-2	1-3	1-5	1-6	1-7	1-8	L	R	S	U	DK
D_c	576.85 ^{S}	238.67 ^{S}	207.55 ^{S}	34.77 ^{S}	0 ^{S}	388.63	0 ^{S}	101.21 ^{S}	0	416.09 ^{S}	122.48 ^{S}	70.81 ^{S}	0 ^{S}
D_n	650.26 ^{S}	899 ^{L}	798.84	1211.61 ^{L}	1066.88 ^{L}	358.73 ^{S}	1066.88 ^{L}	1193.43 ^{L}	224.38	428.50 ^{S}	711.14	1205.22 ^{L}	1177.36 ^{L}
$(I\text{-}T)_c$	361.21 ^{L}												
$(I\text{-}T)_n$	-304.02 ^{S}												
Steps in inference key	1,2,3,5,6,7, restricted gene flow/dispersal, but with some long distance dispersal												

FIG. 5. Results of nested clade analysis for clades in which a significant geographical association was detected. Restricted gene flow was detected in each of the four clades. Haplotypes/Clades shaded in grey are interior clades. D_c is the average distance of individuals from the clade's geographical center. D_n is the average distance of individuals from the geographical center of all members of the nested clade. $(I–T)_c$ is the average distance between interior and tip clades within a given clade and $(I–T)_n$ is the average distance between interior and tip clades in the nested clade. Superscript L or S denotes significantly large distances and significantly small distances for $P < 0.05$, respectively.

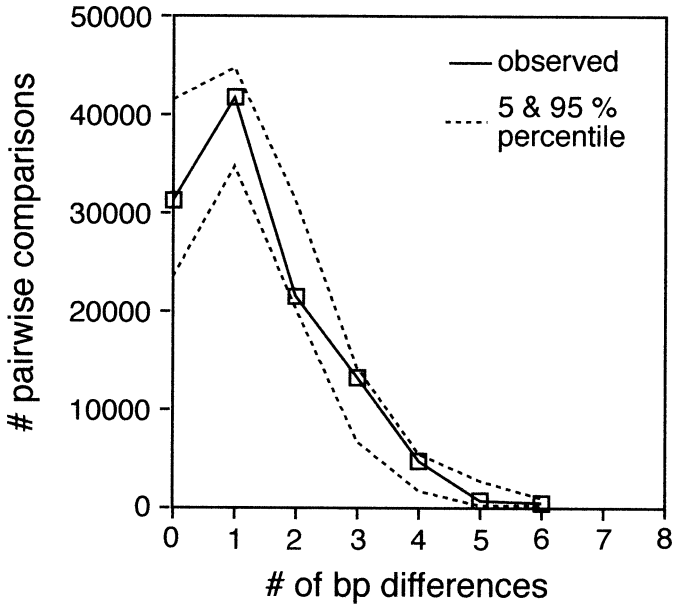

FIG. 6. Mismatch distributions for all pairwise combinations of the 476 individuals sequenced for *Prodoxus decipiens*. Observed distribution conformed to the expected distribution from a model of sudden population expansion as implemented in Arlequin 2.0 (P_{SSD} = 0.09). Dotted lines show the 95% confidence intervals for the model.

genetics. Early studies of phylogeography, however, were dominated mostly by phylogenetically oriented analyses, and it was not until the last five years or so that demographic and population genetic analyses have been incorporated. The combination of these analyses, as used here, provided a much broader interpretation of phylogeography than any one alone. We see them as complementary—phylogenetic analyses provide the evolutionary time frame, demographic analyses provide insights into historical population phenomena that may influence genetic structure, and population genetic analyses provide a contemporary view of genetic structure. We demonstrate how all three types of analyses contributed to examining the phylogeography for *P. decipiens*.

TABLE 2. Estimates of haplotype and nucleotide diversity for different population groupings of *Prodoxus decipiens.* Texas populations have the highest diversity, Florida populations the second highest, and eastern United States populations the lowest.

Population grouping	Haplotype diversity	Nucleotide diversity (%)
Texas	0.822 ± 0.024	0.315 ± 0.195
Eastern United States	0.509 ± 0.034	0.092 ± 0.080
Florida only	0.657 ± 0.043	0.124 ± 0.098
Eastern U.S. without Florida	0.424 ± 0.044	0.072 ± 0.068

Based on historical biogeography and knowledge of recent human influences, we expected to find two phylogeographic patterns. First, we expected historical fragmentation between populations in Texas and Florida. A strong phylogenetic break in genetic structure and even speciation has been reported between eastern and western Gulf Coast populations for other insects (katydids, Shapiro 1998), rat snakes (Burbrink et al. 2000), birds (chickadees, Gill et al. 1993; scrub jays, Pitelka 1951; Peterson 1993), and plants (*Spigelia*, Gould and Jansen 1999). For *P. decipiens*, haplotypes from Texas and Florida were expected to form two primarily monophyletic groups. Phylogenetic analyses, however, produced a pattern of haplotype relatedness that resembled a starburst (Fig. 3). There were no clades of haplotypes that corresponded to a division between Texas and Florida. Similarly, the nested clade analysis did not detect historical fragmentation between populations in Texas and Florida. The second expectation was a pattern of range expansion for populations in the eastern United States. For this pattern, we expected to find ancestral haplotypes in Florida and more derived haplotypes in the other eastern United States populations. Again, because of low phylogenetic signal, there were

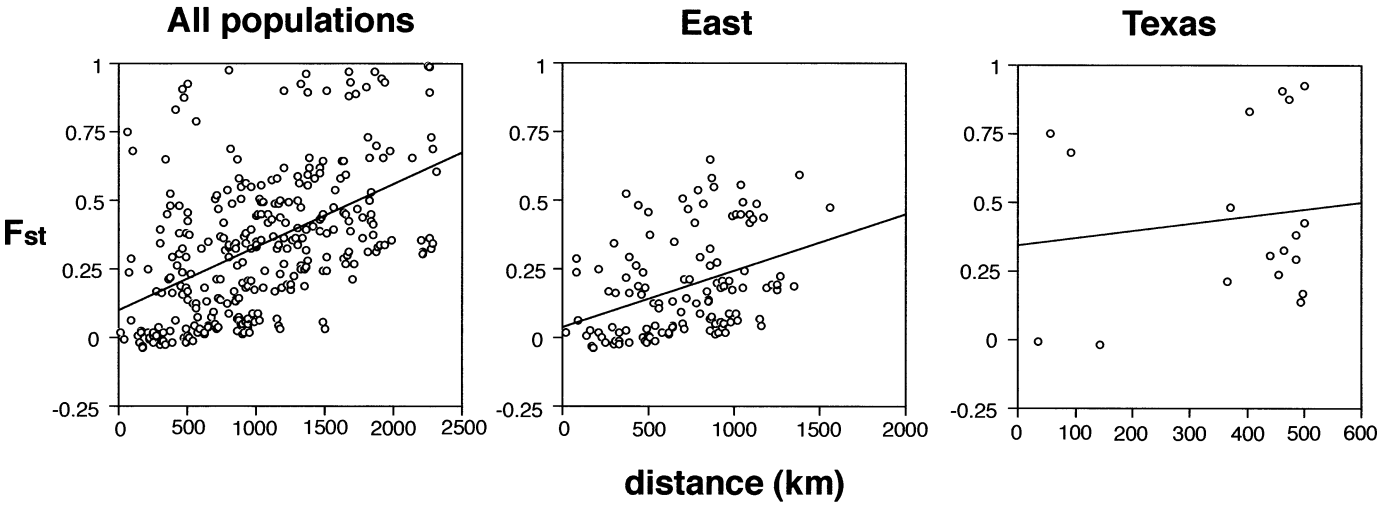

FIG. 7. Tests of isolation by distance in *Prodoxus decipiens* based on F_{ST} estimates using Tamura distance measure with a gamma of 0.02. Isolation by distance was detected for all populations and the eastern United States populations, but not for populations in Texas. Mantel test results for all populations, r = 0.48, P = 0.0001; eastern populations, r = 0.37, P = 0.005; Texas populations, r = 0.07, P = 0.273.

TABLE 3. AMOVA results for testing genetic subdivision between populations of *Prodoxus decipiens* in Texas and Florida, and Florida and the rest of the eastern United States populations. ** $P < 0.01$.

Source of variation	Texas vs. Florida		Florida vs. eastern U.S.	
	Variance components	Percent of variation	Variance components	Percent of variation
Among regions	0.171**	16.99	0.019**	5.60
Among populations within regions	0.343**	34.14	0.071**	20.59
Within populations	0.491**	48.88	0.254**	73.81
Overall F_{ST}	0.51**		0.26**	

no haplotypes from Florida that could be considered ancestral to haplotypes in the other eastern populations. The nested clade analysis detected restricted gene flow for some eastern haplotypes, but did not to detect range expansion.

Results from the phylogenetically based analyses did not support either a priori expectation. For the expectation of range expansion, this finding is not unexpected. The colonization of host plants in the interior eastern United States is most likely too recent to leave a signature on patterns of haplotype relatedness. The phylogenetic analyses and the nested clade analysis in particular, however, should have detected historical fragmentation between Texas and Florida populations if it had occurred. The nested clade analysis should be more powerful than strictly phylogenetic analyses, because it incorporates both haplotype relatedness and geographic distributions, but it still did not support this expectation. The low level of phylogenetic signal potentially influenced this analysis. The haplotype network used for the nested clade analysis revealed a number of closed loops among haplotypes (Fig. 4). To produce a conclusion of fragmentation, at least two separate clades had to be present, and there had to be a mainly nonoverlapping geographical distribution of haplotypes in those clades (Templeton et al. 1995). Visual inspection of haplotype distributions (Fig. 2) hints at this pattern, but the underlying phylogenetic relationships among haplotypes were not present. Published studies in which the nested clade analysis has been informative in detecting fragmentation or range expansion have also reported strong phylogenetic signal in the haplotype networks used (e.g., Durand et al. 1999; Bernatchez 2001; Creer et al. 2001; Hurwood and Hughes 2001; Nielson et al. 2001). We know that there is a current break in the range of *P. decipiens* across Louisiana, but this break appears to be recent enough that it has not influenced mtDNA haplotype relatedness.

Although the phylogenetic and nested clade analyses did not support the a priori expectations, the analyses did suggest that the origin of sampled haplotypes for *P. decipiens* is relatively recent (overall short branch lengths), and there is some geographic structuring among populations. The nested clade analysis detected an overall pattern of restricted gene flow with some long distance dispersal for a subset of the nested clades and the clade containing all haplotypes. One possible explanation for these results is that *P. decipiens* is a relatively young species that has recently spread across its range. We evaluated this possibility by examining the relationship of *P. decipiens* to its sister species *P. quinquepunctellus*. Two lines of evidence suggest that *P. decipiens* is not a young species. Althoff et al. (2001) have shown that the mtDNA haplotypes of each species are monophyletic, which is suggestive of an older speciation event (Avise 2000), and estimates based on a calibrated molecular clock for the Prodoxidae (Pellmyr and Leebens-Mack 1999) date the speciation event to 12.5 Mya.

Shallow divergence among haplotypes within an older species is consistent with changes in historical population demography. In particular, population bottlenecks and expansions can produce the starburst pattern of haplotype relatedness seen in *P. decipiens* (Slatkin and Hudson 1991; Rogers and Harpending 1992; Rogers 1995). The unimodal distribution of pairwise distances between haplotypes of *P. decipiens* did fit a model of sudden population expansion. This finding is corroborated by a significantly negative Tajima's D and by the high level of haplotype diversity and low level of nucleotide diversity (Grant and Bowen 1998). We can conclude, then, that *P. decipiens* has experienced demographic changes throughout its history, and that the changes have been recent enough to remove much of the phylogenetic divergence among haplotypes. This also means that in order to understand patterns of genetic structure, population genetic analyses that focus on a more recent time frame are needed.

Analyses focusing on recent genetic structure demonstrated that populations are genetically structured. The nested clade analysis detected restricted gene flow with long distance dispersal for the clade nesting all haplotypes and some lower level clades. The isolation by distance analyses supported this result, but only at larger geographic scales. Geographic distance influenced genetic similarity across the moth's entire range and also among eastern populations. Populations in Texas, however, did not exhibit isolation by distance even though this was detected by the nested clade analysis for Texas haplotypes T and DF. One reason for the lack of isolation by distance for Texas populations is that populations are separated by much shorter distances than those in the eastern United States—isolation by distance may only be found at larger geographic scales (Fig. 7). For example, a reanalysis of the eastern populations in which we excluded populations separated by greater than 600 km failed to detect isolation by distance ($r = 0.12$, $P < 0.42$). These results show that genetic structure in *P. decipiens* occurs primarily across large geographic scales, and groups of populations at smaller scales may still have substantial gene flow and be in nonequilibrium with respect to migration and drift.

The AMOVA allowed us to test for large scale patterns of genetic structure. We used the a priori expectations to designate population groupings in the analyses. Even though phylogenetic analyses and the nested clade analysis failed to detect historical fragmentation, a genetic division might still be present between Texas and Florida populations. Indeed, the AMOVA did detect significant structuring between Texas and Florida populations (Table 3). This finding coupled with the phylogenetic analyses suggested that any division between Texas and Florida populations occurred very recently. The analyses of demographic history also indicated that *P. decipiens* experienced a significant change in population size during its evolutionary history, but this change must have predated the eastward expansion of *P. decipiens* along the Gulf Coast and into the eastern United States. One possible

explanation for these results is that *P. decipiens* may have ancestrally survived in a single location, and its current distribution is likely the result of a recent process of colonization.

The AMOVA also detected significant structuring between Florida and the other eastern populations as would be expected with the recent range expansion onto human transplanted host plants. The greater haplotype and nucleotide diversities in Florida relative to the rest of the eastern United States populations also lend support to this finding. The time frame in which this pattern has emerged suggests two observations about dispersal in *P. decipiens*. First, moths appear to have the ability to travel long distances to find their host plants as evidenced by their rapid spread onto yuccas transplanted into the eastern United States. Second, it appears that the frequency of long distance dispersal events is low enough to allow a pattern of isolation by distance to develop.

Conclusions

The sequential application of phylogenetic, demographic, and population genetic analyses produced a complementary effect in understanding the phylogeography of *P. decipiens*. Taken together, the results from the analyses showed genetic structuring between populations in Texas and Florida, and between Florida and other eastern United States populations. Despite the potential power of a combined approach and a substantial sampling effort, however, we still could not differentiate among the possible underlying causes of this structure. Genetic structuring in *P. decipiens* appears most likely to have evolved relatively recent and was preceded by a bottleneck event and subsequent dispersal of *P. decipiens* across its range. Although we know that fragmentation of populations was very likely, both historically and more recently, and that range expansion has occurred, the current pattern of genetic structure is most consistent with a series of long distance dispersal events and isolation by distance rather than fragmentation and expansion. We suggest that the combination of analyses that examine both spatial and temporal patterns of genetic structure provides more information than just a single analysis, but we caution that in some cases this may still not be enough to determine the underlying evolutionary causes of genetic structure.

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