POPULATION GENETICS OF THE PECAN WEEVIL, *Curculio caryae* HORN (COLEOPTERA: CURCULIONIDAE), INFERRED FROM MITOCHONDRIAL NUCLEOTIDE DATA

A Thesis

by

GLENÉ MYNHARDT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Anthony I. Cognato
	Marvin K. Harris
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ABSTRACT

Population Genetics of the Pecan Weevil, *Curculio caryae* Horn (Coleoptera: Curculionidae), Inferred from Mitochondrial Nucleotide Data.

(August 2006)

Glené Mynhardt, B.S., The University of Texas at Austin

Co-Chairs of Advisory Committee: Dr. Anthony I. Cognato
Dr. Marvin K. Harris

The pecan weevil, *Curculio caryae* Horn, is an obligate nut feeder of all North American hickory (*Carya*) and a key pest of the pecan, *C. illinoinensis* Koch. This study investigated population structure of the pecan weevil. Gene flow and genetic variation was estimated for 90 pecan weevil specimens sampled from the entire *Carya* range. Cladistic and nested clade analyses, as well as an analysis of molecular variance (AMOVA) of mitochondrial DNA cytochrome oxidase I (mtDNA COI) were performed. The data indicate *C. caryae* diverged from its sister species, *C. nasicus* approximately 4.3 million years before present (mybp). Six-hundred and forty equally parsimonious trees of 31 haplotypes demonstrated high genetic diversity across all pecan weevil samples, and significant regional subdivision. Three clades recovered in the parsimony and nested clade analyses were strongly associated with western, eastern and central localities sampled within *C. caryae*'s range. The current distribution of *C. caryae* and population structure were explained by past glaciation events. Lineage divergence between the western and eastern populations occurred during the Pleistocene (approx. 1.1 million years ago), and a more recent divergence occurred between *C. caryae* populations east and west of the Appalachian mountain range (870,000 yrs. ago). Haplotypes were segregated by region, but further sampling is necessary to test for gene flow among these regions.

DEDICATION

To my parents

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CHAPTER I

INTRODUCTION

The pecan weevil (PW), *Curculio caryae* (Horn), is one of 345 known species representing a large group of seed feeding weevils. Little work has been conducted on the genus as a whole, although relatively thorough studies have been conducted on the species north of Mexico. Currently, there are seven species groups of North American *Curculio*, with 27 valid species (Chittenden 1926, Gibson 1969). The PW can be easily distinguished from other *Curculio* species by several rostrum characters and a large femoral tooth (Gibson 1969).

The PW is the only *Curculio* species that feeds on hickory (*Carya*). It is an obligate nut feeder of all 13 (Harris 1983) or 14 (Ring et al. 1991) North American *Carya* (hickory) species (Juglandaceae). It is likely distributed throughout the entire hickory range (Fig. 1), which extends from Mexico northward along the Mississippi River and its tributaries and east to the Atlantic Ocean (Gibson 1969). The PW has been collected from all 13 (Harris 1979) or 14 *Carya* species (Ring et al. 1991); however, detailed knowledge of its distribution is only known for those populations associated with pecan (Harris 1979).

The pecan, *Carya illinoinensis* Koch is the most important horticultural crop in the United States (Brison 1974). More than 200 million pounds of pecan are produced in the US annually (Harris 1983), which makes this crop an attractive one, not just for humans, but for an entire suite of pests including birds, squirrels, pathogens, nematodes, competing weeds, and arthropods. The PW is one of the most serious (key) pests of pecan, causing millions of dollars in losses to this host each year (Barry 1947). This economic importance has resulted in intensive study of this insect and its biology. Adult PWs typically emerge from the soil during late August

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This thesis follows the style of Annals of the Entomological Society of America.

Fig. 1. Distribution of *C. caryae* haplotypes across sampled *Carya* range. Small grey circles denote sampling sites of *C. caryae* specimens. Legend refers to five most common haplotypes. Unique and singleton haplotypes are represented as letters in the pie charts. The size of each pie chart represents the relative number of sampled individuals.

to September. After successful mating, females chew through the pecan shuck and shell to the kernel, creating separate cavities to lay an average of three eggs in each nut infested. Approximately 42 days later, larvae emerge from the nut, burrow 4-6 inches (10-15 cm) beneath the soil surface, create an earthen cell, and remain there for two or three years (Harris & Dean 1997, Harp 1970). Ninety percent of a PW population pupates after one year and weevils

emerge as adults after two years, while the remaining 10% pupate after two years and emerge as adults after three years (Harp 1970, Harp & Van Cleave 1976).

Despite detailed knowledge of its life history, little is known regarding the genetics of PW. Phenotypic and genetic variation is often observed in insect populations with wide geographic and/or host distributions (Avise 2004, Ehrlich & Raven 1969, Roderick 1996). Although PW populations do not exhibit overt phenotypic variation, genetic variation among weevil populations is expected because of the PW's wide geographic and host distribution. Differences in vegetative traits and phenology occur across the range of pecan trees (Wood et al. 1998, Sparks 1990) and PWs exhibit preferences for certain early maturing cultivars of pecan (Calcote & Hyder 1981). This behavior suggests that the weevils choose to oviposit in certain phenotypic variants or varieties of pecan and may explain the weevil's patchy distribution within the range of pecan (Harris 1979). In addition, movement of the PW may also influence population genetic patterns. For example, recent PW infestations of pecan in Florida and Georgia are believed to have originated from native hickories (*Carya aquatica* Nutt., *C. glabra* Mill., *C. ovata* Mill.; Harris 1979). The above observations suggest a potential for genetic variation among PW populations.

Study of genetic patterns of and potential gene flow among PW populations will increase knowledge of the weevil's biology and may explain the current and historical patterns that influence its destructive potential. Thus, this study's findings may help to better understand pest populations of these weevils as demonstrated in *Anthonomus grandis*, the boll weevil (Kim & Sappington 2004); *Dendroctonus valens*, red turpentine beetle (Cognato et al. 2005); *Diabrotica indecimpunctata howardi*, southern corn root borer (Szalanski & Owens 2003); and *Pissodes strobi*, white pine weevil (Laffin et al. 2004).

Mitochondrial genes are particularly useful for intraspecific studies, because of the

Table 1. Sample locality and *C. caryae* haplotype information. Haplotype diversity (*h*) is reported for each region that includes samples from respective localities.

Region	Included Counties (Co.), States	Host	Coordinates (dd)	Haplotype (N)	*h*
North-central (IL, IN, MO)	Woodford Co., IL	*C. tomentosa*	32.65376N -83.75963W	C(6), Z(1)	0.87
	Champaign Co., IL	*C. ovata*	40.41376N -88.35005W	B(1), C(1), E(5), V(1)	
	Vigo Co., IN	*C. laciniosa*	39.46504N -87.45002W	B(3), C(1), AA(1), I(2)	
	Chariton Co., MO	*C. illinoinensis*	39.42336N -93.13048W	F(1), P(1), W(1), X(1),Y(1)	
Eastern (CT, SC, GA, FL)	Hartford Co., CT	n/a	41.78232N -72.61203W	B(1)	0.89
	Saluda Co., SC	*C. illinoinensis*	33.84542N -81.66177W	B(1), H(3), R(1)	
	Peach Co., GA	*C. illinoinensis*	32.65376N -83.75963W	B(3), S(1), T(1), U(1), BB(1), CC(1)	
	Jefferson Co., FL	*C. illinoinensis*	30.54520N -83.87016W	B(1), Q(1)	
Western (KS, AR, OK, TX, LA, NM)	Cherokee Co., KS	*C. illinoinensis*	37.16923N -94.84412W	A(3), F(1), EE(1)	0.67
	Johnson Co, AR	*C. illinoinensis*	35.47147N -93.46657W	A(1), F(1), K(1)	
	Kay Co., OK	*C. illinoinensis*	36.70698N -97.08559W	A(7), L(1), M(1)	
	Johnston Co., OK	*C. illinoinensis*	34.24093N -96.54917W	A(5), O(1), N(1)	
	Bell Co., TX	*C. illinoinensis*	30.98629N -97.35861W	A(5), D(2)	
	Dawson Co., TX	*C. illinoinensis*	32.73760N -101.95099W	D(5), G(3)	
	Caddo Co., LA	*C. illinoinensis*	32.51737N -93.80379W	A(3), F(1), J(1), DD(1)	
	Dona Ana Co., NM	*C. illinoinensis*	32.11176N -106.66250W	A(2)	

relatively fast nucleotide mutation rate, high copy number, and amino acid conservation (Morlais & Severson 2002, Simon et al. 1994). Also, maternal inheritance of the region is not subject to recombination, thus the short coalescence time of mtDNA lineages allows for the resolution of intraspecific and lower taxonomic-level phylogenies (Avise 2004). Cytochrome oxidase *c* I (COI) is the largest subunit of the cytochrome oxidase complex that transports electrons and

translocates proteins across the cell membrane (Clary & Wolstenholme 1985). This gene has been used extensively for similar intraspecific studies of other weevil species (e.g., Cognato et al. 2003, Cognato et al. 2005, Kim & Sappington 2004).

The intent of this study is to provide a foundation for future research on the PW in order to better understand PW biology, dispersal and the influence of current and historical environment on the evolution of PW. This endeavor has three objectives. First, a parsimony-based phylogenetic analysis mitochondrial gene sequence data is used to determine relationships among individuals and populations of PW. Second, an analysis of molecular variance (AMOVA, Excoffier et al. 1992) quantifies the genetic diversity and estimates gene flow among and within PW populations based on the diversity and frequency of haplotypes among populations. Third, nested clade analysis associates geographic location and clades by discriminating between current population processes and historical occurrences that may have influenced the current structure of populations (Templeton 1998). Together, data from these procedures test the hypothesis that PW populations are randomly distributed throughout the weevil's range with no geographic associations.

CHAPTER II

MATERIALS AND METHODS

Molecular Protocol and Specimens

Pecan growers and extension agents collected all specimens used in this study. Ideally, eight to ten beetles were sampled from 16 localities within the *Carya* range (Table 1), but in some cases only one or two individuals were available for a locality. Specimens were delivered in 70-95% ethyl alcohol (EtOH) and comprised of either, or both, larvae and adults. They were stored at -20°C until needed. Specimens were vouchered (Cur #1 – Cur #168) in the Texas A&M University, Department of Entomology insect collection.

A small shaving of the dorsum was used for DNA extraction from larval beetle specimens. A coxa or thoracic tissue was used for adult specimens. The remains of the specimens were placed in separate labeled vials and stored at -80°C. Genomic DNA was extracted from each individual using a DNeasy Tissue Kit (Qiagen Incorporated, Valencia, CA). The manufacturer's animal tissue protocol was followed, except the incubation period was increased to ~20 hours for the adult specimens in order to maximize the yield of DNA.

A region of 752 nucleotides of mitochondrial protein coding gene cytochrome *c* oxidase I (COI) was amplified via the polymerase chain reaction (PCR) using primers "Jerry" (5' CAACATTTATTTTGATTTTTTGG 3'; location 2183 within the *Drosophila yukuba* COI) and "Pat" (5' ATCCATTACATATAATCTGCCATA 3'; location 3014 in tRNA region flanking COI). These two primers have been successfully used to amplify beetle DNA (Simon et al. 1994, Cognato et al. 2003). Each PCR reaction contained: 35 µl ddH_2O, 5 µl 10x *Taq*DNA polymerase buffer (Promega, Madison, WI), 4 µl 25mM Promega $MgCl_2$, 1 µl 40 mM deoxynucleotide triphosphates (dNTPs), 2 µl of each Promega *Taq*DNA polymerase, and 1 µl of DNA template. A negative control, which contained all the ingredients as the above mixture

except DNA template, was also included. The PCR was performed on a thermal cycler (MJ Research, Waltham, MA) under the following conditions: one cycle for 2 minutes at 95ºC, 34 cycles for 1 min. at 95ºC, 0.75 min. at 45ºC, 1 min. at 72ºC, and a final elongation cycle of 5 min. at 72ºC.

DNA amplicons were visualized via electrophoresis. 5.0 µl of PCR product from each individual was mixed with 2.00 µl 5X loading buffer (Applied Biosystems, Foster City, CA). The 7 µl mixture was applied to an ethidium bromide stained 1.5% agarose gel at 100 volts for 30 minutes. The gel was then visualized under an ultraviolet light source and photographed. A 100 kb ladder was used to estimate the relative size and concentration of amplified DNA.

Unincorporated dNTPs and oligonucleotides were removed from PCR product with a Qiaquick PCR Purification Kit (Qiagen Incorporated, Valencia, CA) and were directly sequenced on an automated sequencer after a BigDye® Terminator v.1.1 (Applied Biosystems, Foster City, CA) cycle sequencing kit and sequenced on an ABI 3100 (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. Primers used in PCR were used to sequence sense and antisense strands for all individuals.

DNA sequences were manually edited using the program Sequencher 4.1.1 (Gene Codes Corporation, Ann Arbor, MI). A cladistic analysis was performed using the parsimony-based program, PAUP (Swofford 1998). Reference sequences from each population were deposited in GenBank, nucleotide sequence database: http://www.ncbi.nlm.nih.gov/Genbank/ GenbankSearch.html

Population Genetic Measures

Phylogenetic Analysis

The phylogenetic relationship of 90 *Curculio caryae* mtDNA COI sequences was estimated using PAUP (Swofford 1998). *Curculio nasicus* was chosen as the outgroup because it is the sister group to *C. caryae* (Hughes and Vogler 2004). A heuristic search was performed under a maximum parsimony criterion using the tree bisection reconnection (TBR) algorithm with 1000 random addition sequence replicates. The remaining settings were left as default. All characters were equally weighted and unordered. Bootstrap proportions were determined with 1000 replicates and default PAUP settings.

Analysis of Molecular Variance and Estimates of Gene Flow

The degree of genetic variation was measured with an analysis of molecular variance (AMOVA (Excoffier et al. 1992) using DnaSP (DNA Sequence Polymorphism) version 4.0 Software (Rozas et al. 2003). AMOVA formulated a matrix of squared-distances between all pairs of haplotypes, constructing a hierarchical analysis of molecular variance. Although directly based on the standard analysis of variance, the AMOVA allowed for the application of nucleotide evolutionary models. Gene flow and genetic differentiation were estimated based on Wright's F_{ST}. Nucleotide (N_{ST}) and haplotype (G_{ST}) differentiation were calculated as pairwise comparisons between each region (Table 1) using DnaSP's "Gene Flow" module.

In addition, different components of variation, including within population (WP), among-population or within group (AP or WG), and among-group (AG) were employed in each analysis. Three regions (Table 2) were created combining populations into larger groups to prevent the analysis to be skewed by those populations with a few individuals; for example, as few as one or two individuals were available for Connecticut and Florida.

Table 2. Measures of nucleotide (N_{ST}) and haplotype (G_{ST}) differentiation among populations of *C. caryae*.

Geographic comparison	Number of populations	Number of haplotypes	N_{ST}	G_{ST}
All	15*	31	0.567	0.132
North/Central	4	12	0.329	0.237
Eastern	3	9	0.100	0.094
Western	7	12	0.076	0.170
North/Central - Western	12	24	0.120	0.636
North/Central - Eastern	8	21	0.244	0.243
Western/Eastern	11	22	0.711	0.103

*Excluded CT population due to low sample size (n=1)

Nested Clade Analysis

Nested clade analysis (NCA) was used to associate geographic location and haplotype diversity and to hypothesize the historical events that influence the associations (Templeton et al. 1995, Templeton 1998). A haplotype network, which was produced via statistical parsimony analysis (TCS v.1.13, Clement et al. 2000), was used to define a nested series of clades. These nested clades were used to estimate the association between geographic distribution and genetic diversity and discriminated between recurrent gene flow and historical events at the population level (Geodis v.2.4, Posada et al. 2000) by performing statistical tests of random associations between geographic and nested clade associations.

Table 3. Summary of demographic events deduced from inferences based on significant nested clade values.

Clade	χ^2	Nested Clades	D_C	D_N	Chain of Inference	Demographic Event
1-1	0.003	I (INT) II (TIP) III (TIP) IV (TIP) V (TIP) VI (TIP) I-T	>, P = 0.395 n.s. n.s. n.s. <,P = 0.02 n.s. >, P = 0.014	n.s. n.s. n.s. n.s. n.s. n.s. n.s.	1 no, 2 yes, 3 no, 4 yes 9 yes, 10 no	Geographical sampling scheme inadequate to discriminate between fragmentation and isolation by distance
1-3	n.s.	VII (INT) VIII (TIP) I-T	n.s. n.s. n.s.	n.s. n.s. n.s.	1 no, 2 no 11 no, 17 no	Inconclusive outcome
1-7	0.024	XI (INT) XII (INT) XIII (TIP) XIV (TIP) XV (TIP) XVI (TIP) I-T	n.s. n.s. n.s. n.s. n.s. <, P = 0.001 >, P = 0.006	n.s. n.s. n.s. n.s. n.s. n.s. n.s.	1 no, 2 yes 3 no, 4 no	Restricted gene flow with isolation by distance
1-9	n.s.	XVII (TIP) XVIII (INT) I-T	n.s. n.s. n.s.	n.s. n.s. n.s.	1 yes, 19 yes 20 no	Inadequate geographic sampling
2-1	0.001	1-1 (TIP)* 1-2 (TIP) 1-3 (INT)* I-T	n.s. n.s. <, P = 0.046 n.s.	n.s. n.s. n.s. n.s.	1 no, 2 no 11 yes, 12 no	Contiguous range expansion
2-2	n.s.	1-6 (TIP) 1-7 (INT) 1-8 (TIP) 1-9 (TIP) I-T	n.s. >, P = 0.003 n.s. n.s. >, P = 0.012	n.s. >, P = 0.003 n.s. <, P = 0.009 >, P = 0.000	1 no, 2 yes, 3 no, 4 no	Restricted gene flow with isolation by distance
2-4	0.002	1-13 (TIP) 1-14 (TIP) 1-15 (TIP) 1-16 (TIP) 1-17 (INT) I-T	n.s. n.s. n.s. n.s. <, P = 0.013 >, P = 0.012	>, P = 0.006 n.s. n.s. n.s. <, P = 0.013 >, P = 0.000	1 no, 2 no 11 yes, 12 no	Contiguous range expansion
3-1	n.s.	2-1 (INT) 2-3 (TIP) I-T	>, P = 0.037 n.s. >, P = 0.021	>, P = 0.021 <, P = 0.021 >, P = 0.021	1 no, 2 yes, 3 yes, 5 no 6 (<2 clades), 7 yes	Restricted gene flow/dispersal but with some long distance dispersal
3-4	0.002	2-4 (TIP) 2-5 (TIP) 2-6 (INT) I-T	<, P = 0.002 n.s. n.s. >, P = 0.000	<, P = 0.002 >, P = 0.018 n.s. >, P = 0.000	1 yes, 19 yes 20 no	Inadequate geographic sampling
4-1	0.000	3-1 (INT) 3-2 (TIP) I-T	<, P = 0.000 <, P = 0.009 n.s.	n.s. n.s. n.s.	1 yes, 19 yes 20 no	Inadequate geographic sampling
4-2	n.s.	3-3 (INT) 3-4 (TIP) I-T	n.s. n.s. n.s.	n.s. n.s. n.s.	1 yes, 19 yes 20 no	Inadequate geographic sampling
Total Cladogram	0.000	4-1 (TIP) 4-2 (TIP) I-T	n.s. <, P = 0.019 N/A	n.s. n.s. N/A	N/A	N/A

CHAPTER III

RESULTS

Of 752 bp of mtDNA COI, 108 sites were variable, 31 sites were parsimony informative; this variation occurred most at third (65%), first (26%) and second (9%) codon positions. The mean base frequencies were as follows: A = 0.301, C = 0.161, G = 0.153 and T = 0.385. This distribution of nucleotide frequencies and variation is similar to other beetle species (e.g. Cognato et al. 1999, Cognato et al. 2003). A total of 31 haplotypes including 22 singletons, were identified for 90 *C. caryae* individuals. Nine haplotypes were shared by least two individuals. The overall haplotype diversity was high (h = 0.892), while nucleotide diversity was low (π = 0.00955). The number of haplotypes per region (north/central, eastern, and western) ranged from 10 to 12. Haplotype A was the most abundant, but was present only in the western region. Haplotype B was the second most common, but was found only in the eastern region. Similarly, haplotype C was the third most abundant, and was found only in the north/central geographic region (Fig. 2).

The phylogenetic analysis yielded 640 equally most parsimonious trees of length 130. The homoplasy indices (CI = 0.8615, RI = 0.9593, RC = 0.8262) demonstrated low homoplasy among the mtDNA characters. There are three lineages, which are closely associated with geography (Fig.1). Bootstrap values for most nodes were above 50%.

Nucleotide divergence (N_{ST}) for the all three regions was 0.567 (Table 2). Similarly, haplotype divergence (G_{ST}) for all three regions was 0.132. Pairwise comparisons for N_{ST} and G_{ST} were measured for all the three assigned regions (Table 3). One population (CT) was not included in the analysis, due to low sample size (n = 1). The highest value for haplotype/gene differentiation (G_{ST}) is found between the western and north/central regions, but this estimate is probably inflated due to a high amount of haplotypes (24) among the two regions. Low gene

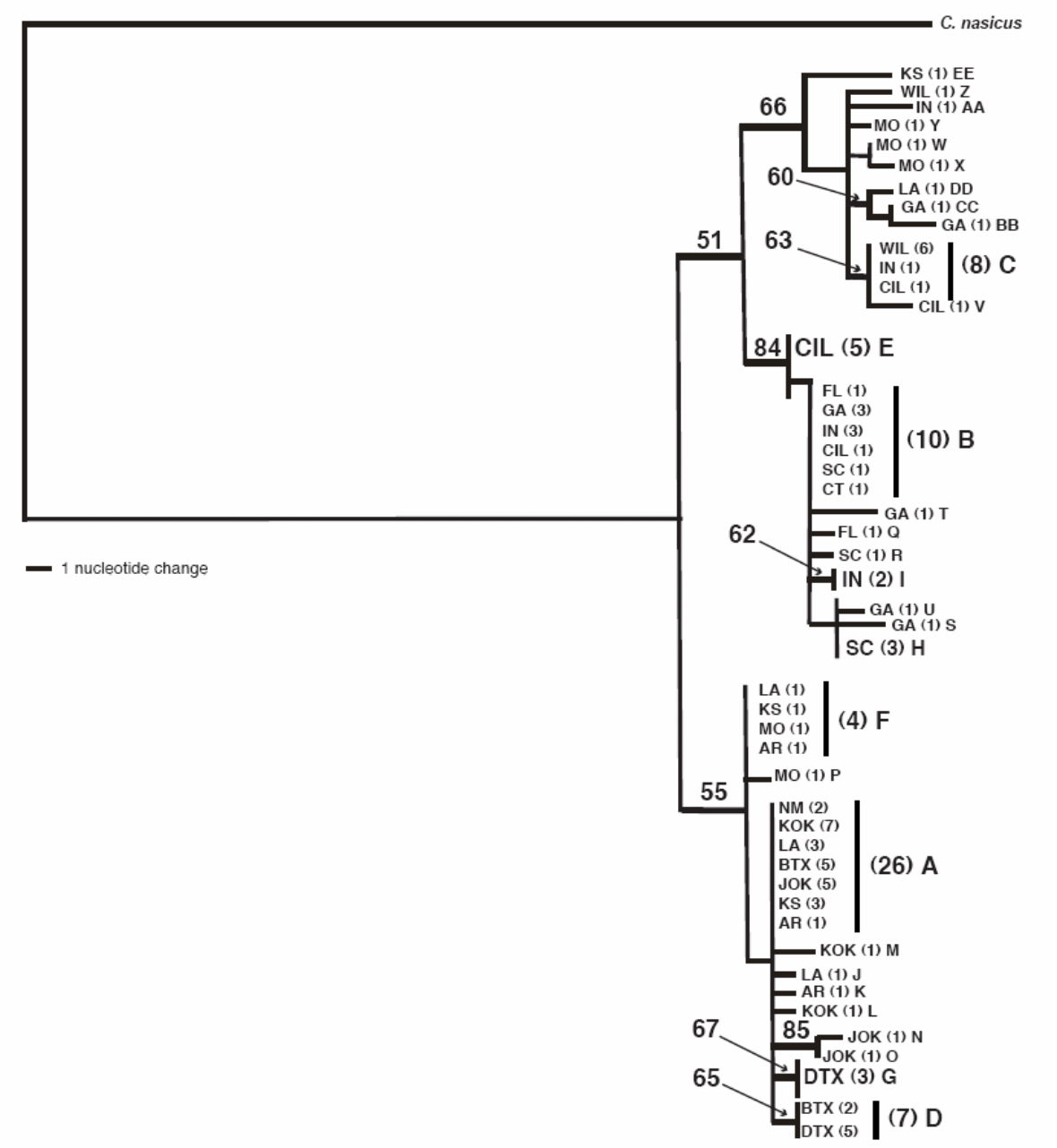

Fig. 2. One of 640 equally parsimonious trees of 90 *C. caryae* individual mtDNA COI sequences, sampled from 16 populations (locations) within the range of *Carya* species in the U.S. Numbers above bold lines are bootstrap values and refer to nodes found in the strict consensus of equally parsimonious trees. Terminal labels indicate the population; numbers in parentheses indicate number of individuals for a given haplotype; final single letters denote haplotypes (Table 1). Haplotypes that were common to more than one individual are referenced by a larger font type.

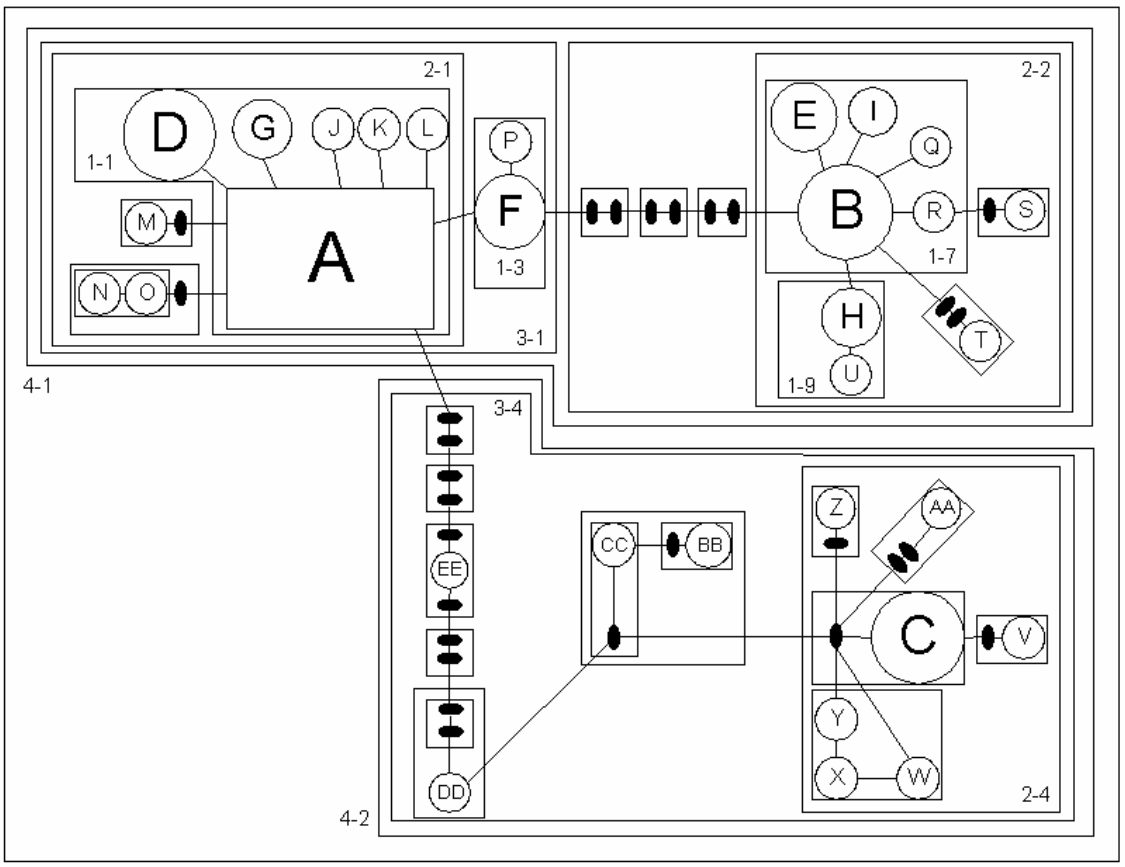

Fig. 3. Nested network of *C. caryae* haplotypes. Hollow circles and squares indicate individual haplotypes with frequencies proportional to size. Haplotype A is the most common. Black ovals represent intermediate (unsampled) haplotypes. Lines between haplotypes and intermediates denote one nucleotide change. Numbered clades (e.g. 2-4, 4-2) are significant; unnumbered clades are non-significant.

flow among all pairwise region comparisons was inferred from these analyses.

Three distinct clades were also found with the NCA (Fig. 3) but few conclusions of haplotype and geographic associations could be inferred. Lower-level clade nestings in NCA showed little geographic concordance, and more samples are necessary to determine

demographic effects that influenced the PW's current distribution. Significant non-random geographic associations were found for the western and eastern regions, except for higher-level clade nestings. Second-order nestings showed that contiguous range expansion occurred for these two regions. The north/central region, however, showed no statistical significance for non-random geographic association, which again is presumed due to a lack of samples.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

Populations of *Curculio caryae* exhibit geographic structure. Thus, the null hypothesis of random association between haplotypes and geography is rejected. These phylogenetic patterns were likely caused by events that occurred during the Pleistocene era. Based on the generalized arthropod nucleotide substitution rate for mtDNA (2.3% divergence per million years, Brower 1994), *C. caryae* diverged from its sister species, *C. nasicus* approximately 4.3 million years before present (mybp). The first split of a *C. caryae* lineage, which includes haplotypes from western populations of PW, occurred approximately 1.1 mypb. This event corresponds with one of the coldest periods during the Ice Age (Ericson and Wollin 1968).

During the Kansian glaciation period, it is likely that climatic changes forced pecan (Malstrom 1978) and other tree species, such as spruce (Webb and Bartlein 1992) southward. As the pecan moved south, probably into Mexico, the PW was probably forced south as well. Presently there are a few small pecan populations in Mexico but PWs have not been collected from these trees (Harris 1979). During this time period western and eastern populations were separated by an arid region that occurred along an expanded Gulf Coast shore (Althoff and Pellmyr 2002). The warmer Holocene climate likely allowed for a contiguous range expansion of PW into the north, as suggested by NCA results (Table 3). The split between the north/central and eastern clade occurred approximately 870,000 ybp. This period corresponds with an interglacial period during the Pleistocene. As glaciers retreated north, southern populations of pecans and PWs migrated northward. The Appalachian mountain range, which had formed prior to the Pleistocene, probably acted as a barrier, which likely caused a split between the two eastern lineages.

Similar Pleistocene patterns of geographic isolation have been observed for other insects that currently occupy the same range with PW, including the yucca moth (Althoff and Pellmyr 2002) and several species of katydids (Shapiro 1998). Similar patterns have also been found in vertebrates, including North American chickadees (Gill et al. 2003), fish (Wiley and Mayden 1985), and rat snakes (Burbrink et al. 2000). In all studies, an eastern/western lineage divergence of haplotypes was discovered. In addition, recent studies of the tiger salamander showed an east/west clade split, especially along the Appalachian range, which acted as a refugium during the Wisconsonian glacial period (Church et al. 2003). Similarly, the PW appears to have split into two clades associated with the eastern and western sides of the Appalachian Mountains. The clade corresponding with the western range of the pecan weevil is likely to have co-evolved with native pecan for a longer time as well.

As observed with other insects, host use may also influence the present population genetic patterns of *C. caryae* (e.g. Kelley et al. 1999). Hickory species and pecan populations are phenotypically plastic (Thompson and Grauke 1990, Sparks 1990, Wood et al. 1998) and thus create a mosaic of host variation that is distributed across the range of *C. caryae*. Although little is known of *C. caryae* life history on hickories, it exhibits preferences for certain early maturing cultivars of pecan but will oviposit on later maturing cultivars if emergence is delayed by drought (Harris & Ring 1980, Calcote & Hyder 1981). Whether this variability in oviposition behavior has evolutionary consequences is unknown. However, this behavior suggests host choice is liable to host phenotypic variation but the behavior may be more influenced by differences among hickory species as compared to pecan cultivars. Host phenotypic variation may explain the infrequent collection of weevils on other hickories and the weevil's patchy distribution within the range of pecan (Harris 1979). A patchy distribution and the low dispersal rate imply isolated breeding populations. Our population genetic patterns and estimate of low

gene flow suggest that populations are isolated (Tables 2, 3). However, untangling the effects of host variation and past geographic isolation with the present data is difficult. A few haplotypes were associated with specific hosts (Table 1). However, sample size is limited and future studies should include PW samples from multiple hickory species in order to test the significance of haplotype and host associations. Given the present data, past geographic isolation likely had the most influence on PW population genetic structure.

Conclusions

Even though our conclusions are limited, these results can help better manage PW. On a broad geographic scale, PW populations are different lineages (Fig. 1), each with potentially fixed behavioral traits. Thus pecan growers would be wise to manage PW pests based on population attributes and not on "average" species behavioral characteristics. In addition, our study provides baseline data for the DNA identification of PW populations (e.g., Foster et al. 2004). Recent PW infestations in New Mexican pecan orchards have raised much awareness for PW quarantine. It is often necessary to track the source of infestations, and the use of DNA can help elucidate such information. Based on parsimony analysis, the New Mexico specimens contain haplotype A and were likely introduced from a western population. Also, based on our data, recent PW infestations of pecan in Florida and Georgia likely originated from native hickories (*Carya aquatica* Nutt., *C. glabra* Mill., *C. ovata* Mill.; Harris 1979) and were not introduced from western populations. Again, increased sampling of haplotypes across the range of PW will undoubtedly increase the efficiency of a DNA identification system.

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