GENETIC VARIATION IN THE EASTERN SUBTERRANEAN TERMITE *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

A Thesis

by

LUCILLE H. BENAVIDES

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

December 2004

Major Subject: Entomology

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Approved as to style and content by:

Roger Gold (Co-Chair of Committee)	Anthony Cognato (Co-Chair of Committee)
Jorge Cruz-Reyes (Member)	Kevin Heinz (Head of Department)

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ABSTRACT

Genetic Variation in the Eastern Subterranean Termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). (December 2004)

Lucille H. Benavides, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Roger Gold
Dr. Anthony Cognato

The eastern subterranean termite, *Reticulitermes flavipes*, is the most widely dispersed termite in North America. The genus *Reticulitermes spp*. is responsible for 80% of total termite damage caused to urban structures each year. Little is known about the genetic structure of termites, particularly at the colony level. Evidence for what genetically defines a termite colony is a hotly debated topic in current literature due to the implications such findings would have regarding current lawsuits against pest control operations. Information on termite genetic structure is sparse.

In this study, the genetic variation and gene flow among Texas populations of *R. flavipes* at the statewide level and city level was examined. A 324-337 base pairs segment of the mtDNA, AT-rich region was a polymerase chain reaction amplified from 104 different termite specimens from 12 Texas cities. The DNA extracts were then subjected to PCR amplification using specific primers and it was then sequenced. Using the sequence data and appropriate statistical measures it was found that, at the statewide level, nucleotide and haplotypic diversity is low. Gene flow was found to be low on a statewide basis. At the city level nucleotide and haplotypic diversity was high. The findings of this study provide insights into termite genetic structure.

DEDICATION

This thesis is dedicated to my mother and father, whose
love for their daughters and sacrifice ensured that we would have the opportunities they did not. For this I am truly grateful. I always aim to make you proud.

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INTRODUCTION

Subterranean termites are the single greatest economic insect pests of structures in the United States. Annually, 80% of the 2.5 billion dollars spent each year to control termites in the United States is spent specifically on subterranean termite control (Viscardi 2003). Not only is this a problem in the United States, but in Europe the cost to control subterranean termites is expected to exceed one billion euros by 2005 (Szalanski et al. 2003). Among subterranean termites, those in the family Rhinotermitidae are the most economically important structural pests. Within this family is the genus Reticulitermes, commonly known as the subterranean termites due to their close association with the soil. The species *Reticulitermes flavipes* (Kollar), eastern subterranean termite, is the most abundant naturally residing termite species in Texas (Szalanski et al. 2003). The distribution of *R. flavipes* is vast having occupied each of the United States except Alaska.

Termites are social insects that are small, lightly pigmented, insects that gain their nutrients from digesting cellulose found in wood. It has been hypothesized that termites evolved from a primitive cockroach-like ancestor some 200 million years ago. All termites live eusocially in polytomous colonies. Sometimes called “white ants” termites feed on wood and other cellulose products. *Reticulitermes spp*. are considered lower termites and have symbiotic microorganisms in their hindgut that aid in the digestion of cellulose.

This thesis follows the format of the Journal of Economic Entomology.

These microorganisms are passed from individual to individual via the exchange of proctodial liquids. Termites feed on wood, cast skins of other termites, as well as dead individuals.

Within the family Rhinotermitidae the colony castes can be divided into pseudergate (workers), soldier and reproductives (Macom 1999). Within the reproductive castes there are primary, secondary, and tertiary. Primary reproductives, also called imagoes, are the founding termites of a colony. Primary reproductives are alate organisms, possessing mature wings that engaged in the mating flight. The mating flight occurs once the colony has matured and the required environmental conditions have been met, this can take up to 7 years depending on the species (Henderson et al. 2004). During the mating flight, alate termites emerge from the underground colony, fly into the air, and form pairs of male and female termites. After this initial flight, the termites reach the ground where they drop their wings, search out a nest site, and mate. The founding pair is the king and queen of the termite colony. Within one year, depending on species, there are only about 30 individuals produced (Henderson et al. 2004).

Sometimes in a termite colony it is necessary to replace one or both of the primary reproductives. Replacement reproductives are formed as a response to the death or loss of a primary king or queen. Supplementary reproductives may emerge even though a primary reproductive queen or king is still present (Thorne 1996). Supplementary and replacement reproductives may also populate satellite colonies, which are colonies that arise as a result of a group of workers becoming separated from the parent colony (Miller 2002).

Replacement and supplementary reproductives occur in three forms: adultoid, nymphoid, and ergatoid. Adultoid reproductives are derived from mature alates and may replace members of the founding pair if death or infertility occurs. Nymphoid reproductives are those replacement and or supplemental reproductives that are derived from the nymph stage, commonly called secondary reproductives. Nymphoid reproductives are brachypterous, and possess short wing buds that do not cover the abdomen (Miller 1969). Ergatoid reproductives are neotenic reproductives derived from psuedergates/workers (Thompson et al.1920), and are referred to as tertiary reproductives. Ergatoid reproductives are apterous, lacking either mature wings or external wing buds.

The soldier caste is the least abundant of all termite castes. Their numbers are regulated through a series of pheromones produced by other soldiers or the reproductives. The soldier termite, depending on species, is the only sterile caste in the colony (Henderson et al. 2004). They are blind, wingless, and have an enlarged, scleroterized head capsule that is used to protect the colony from invading ants and foreign termites.

Pseudergates represent the largest portion of the colony. These workers carry out many of the duties involved in colony maintenance, including feeding of the colony members, construction of the nest, and foraging for food. The youngest members of this caste also perform tasks within the nest such as feeding, grooming, and caring for the young. The more mature workers perform the hazardous work such as foraging for food and nest building. All members of the pseudergate caste are immature, but posses the ability to mature into neotenic reproductives. Worker termites are soft-bodied and blind,

and posses highly scleroterized mouthparts which are used for the maceration of wood, tunnel building and colony maintenance.

Three forms of termite colonies exits, including immature, mature, and secondary. By definition, an immature colony has not staged a mating flight, or produced reproductives (Krishna 1969). A mature colony is a colony that has staged a mating flight and has produced reproductive individuals (Krishna 1969). Finally, a secondary colony is a colony that formed as a result of a group of termites becoming isolated from the parent colony in which reproduction is carried out by replacement reproductives (Krishna 1969)

Subterranean termites colonies are composed of individuals that live in nests or feeding sites connected by subterranean passageways (Bulmer et al. 2001). Worker population estimates for such colonies range from 60,000 to 1,000,000 individuals (Miller 2002). The tunneling patterns in these colonies are divided into a network of branches that systematically cover foraging areas (Su et al. 2003). Above ground foraging structures called mud tubes are constructed on above ground substrates, and are a characteristic of subterranean termites (Borror et al.1992). The two most important functions for these tunnels are to serve as protection for workers, while foraging and to maintain a high relative humidity within the colony (Weesner 1965). It has been shown that high relative humidity is positively correlated with increased termite tunneling which leads to increased colony distribution (Su et al. 2003).

While much is known about the distribution, economic importance, and caste system of the termite, little is known about their ecology and social evolution. Several factors affect our ability to thoroughly study termite ecology and social evolution. The lack of a reliable definition of a colony is one of them. In early literature, a colony was

defined as a group of individuals (of the same species) consisting of functional reproductives, workers, soldiers and immature individuals (Krishna 1969). More recently, a working definition of a colony, is those termites known to occupy the same space in time has been used (Jenkins et al. 1999). The cryptic nature and amorphous colony boundaries of termites has hindered the study of colony design and organization. Colony budding and alate dispersal also blur the boundaries among colonies because the relatedness of neighboring colonies cannot be predicted by geographic proximity (Bulmer et al. 2001). This combination of traits and uncertain colony boundaries has created ambiguity in the characterization of colony genetic structure (Deheer et al. 2004). The affects of these problems are greater in the family Rhinotermitidae, in which colonies of many species frequently move nests sites and have highly mobile patterns of foraging (Deheer et al. 2004).

Many attempts have been made to characterize a termite colony, including nest-mate recognition assays, which have not yielded conclusive evidence of colony boundary (Jenkins et al.1999). The *R. flavipes* species is not a highly territorial termite, and thus may not exhibit aggression to invading termites. Mark recapture and release studies have also been used to establish subterranean termite colony associations (Thorne 1998). This method uses the appearance of one marked individual in a separate, non-release-site inspection port as verification of the related use of feeding sites by a single subterranean termite colony (Jenkins et al. 1999).

Recent genetic studies using micro-satellite analysis of a termite colony have provided information about colony spatial and genetic structure (Thorne 1996). In their study it was shown that 70% of the colonies exhibited simple family characteristics,

resulting from a single pair of reproductives (Deheer et al. 2004). However, colony excavations (Deheer et al. 2004) and molecular data (Bulmer et al. 2001), have demonstrated that even though colonies are characterized by a simple family structure, secondary reproductives may contribute offspring to the colony (Deheer et al. 2004). Within such colonies, the secondary reproductives appear to be present in large numbers and exhibit substantial levels of inbreeding (Bulmer et al. 2001). Based on this genetic evidence, 27% of termite colonies showed an extended family pattern based on a single founding pair.

Genetic studies that were able to assess out breeding, inbreeding, relatedness and subdivision at both the colony and population level (Bulmer et al. 2001), have provided some insight for colony definition. For example, European *Reticulitermes* (Bulmer et al. 2001) showed Mendel Ian genotypic frequencies, indicating that monogamy is the predominant mode of reproductive organization (Bulmer et al. 2001). In the same study, it was shown that inbreeding among secondary reproductives leads to a greater level of heterozygosity. This increase in heterozygosity may have lead to increased variation in breeding structure, which may have increased the genetic contrast of colony boundaries. Most importantly, there were high levels of variation in breeding patterns among *Reticulitermes* colonies within close proximity. In another study using micro-satellite analysis of a termite colony, researchers were able to gain insight into colony spatial and genetic structure (Deheer et al. 2004). In that study it was shown that 70% of the colonies exhibited what the author termed “simple family characteristics”, which were based on a single pair of reproductives giving rise to all members of the colony (Deheer et al. 2004). This information contradicted colony excavation work (Deheer et al. 2004).

Within such colonies, secondary reproductives appeared to be present in large numbers and exhibited substantial levels of inbreeding (Bulmer et al. 2001). Based on genetic evidence, 27% of termite colonies showed an extended family pattern based on a single founding pair.

Sequence data from a portion of the AT rich region of mitochondrial DNA from *R. flavipes* has been used in molecular based population genetic studies to elucidate genetic structure characteristics of the eastern subterranean termite. In 2000, Jenkins et al. used this region of mitochondrial DNA to assess the intra and inter-specific population structure of *R. flavipes* and *R. virginicus* from across Georgia (Jenkins et al. 1998). In this study, Jenkins identified individuals to colony level. Mitochondrial DNA is extremely compact and codes for relatively few genes (Lewin 2000). Mitochondrial DNA is inherited through the maternal line, singly as a non-recombining unit which reduces its effective population size to about one-fourth that of nuclear genes (Jenkins et al.1998). For this reason mitochondrial DNA is subject to a high rate of random drift. Polymorphisms, the simultaneous occurrence in the population of genomes showing allelic variations of maternal origin, are expected to sort more rapidly than ancestral polymorphisms in nuclear DNA (Jenkins et al. 1998). Thus mtDNA provides a sensitive measure of termite population subdivision that may be related to geographic proximity (Jenkins et al. 1998). The AT rich region of insect mtDNA is highly variable and is a possible source of molecular polymorphic markers for intraspecific studies (Jenkins et al. 1998). Length variation of the AT-rich region among *R. flavipes* populations suggested that nucleotide variation sufficiently differentiated *Reticulitermes* species and populations (Jenkins et al. 1998).

Foster et al. 2004 used the same region, as in the Jenkins et al. studies, to form a DNA-based system for the identification of *Reticulitermes flavipes* (Koller). In these experiments sequence data from 173 *R. flavipes*, *R. hageni*, and *R. virginicus* soldiers and pseudergates were collected from several widely dispersed Texan localities. Foster et al. found 19 different haplotypes among the 28 *R. flavipes* sampled from Texas. Among these populations nucleotide diversity was moderate (0.03 +/- 0.016 Pi). Five times greater genetic variation was revealed between populations of *R. flavipes*, *R. hageni*, and *R. virginicus* as compared to the intra population variation of *R. flavipes* (Foster et al. 2004). The haplotypic variation of *R. flavipes* was examined among Texan, Georgian, and Canadian *R. flavipes* samples. Comparisons among these *R. flavipes* haplotypes (including Georgian and Canadian haplotypes) revealed few differences (Foster et al. 2004). It was also found that Texan populations of *R. flavipes* were isolated by distance, and that there was a correlation between genetic and geographic distances (Foster et al. 2004).

My study serves as a follow up to previous work of Foster et al. 2004. In my study I focused on Texan populations of *R.flavipes*. I used sequence data of the same 400 bp AT rich region of mtDNA of *R. flavipes* origin used in Foster et al. 2004. In his study Foster et al. stated that specimen sampling (one termite specimen per urban structure) seemed adequate for this study. My work examined haplotype diversity within each of the urban structures. Like Foster et al., I sampled a single urban structure within Texas cities. However, I increased the sample size per urban structure from 1 to 10 specimens. In his study, Foster et al. did not assess gene flow. In my study I analyzed inter-specific gene flow analysis between populations of *R. flavipes* from various cities across Texas.

I have included the intra-specific haplotypic variation within cities in Texas. I tested the hypothesis that haplotypic variation would increase with termites sampled from different urban structures within a city. I also assessed whether or not increased sampling of localities within cities had an affect on the ability to estimate gene flow over a geographic area.

MATERIALS AND METHODS

Collection and Morphological Identification of Termite Samples. The subterranean termites used in this study were selected from 611 samples collected by Dr. Bart Foster, from 28 Texas localities in the spring of 2001. From these sites, I selected 10 sample sites representing the following cities: Abilene, College Station, San Antonio, Greeneville, Beaumont, Dallas, San Angelo, Perryton, Paris, Victoria, Waco, and Tyler, Texas. Dr. Bart Foster also provided species level identification of the specimens, based on differentiation of mouthparts.

Selection of Localities. For Experiment I, I chose ten Texas cities: Tyler, Perryton, College Station, San Angelo, Abilene, Victoria, Greenville, Waco, Port Arthur, and Paris to select termites from. From each city I chose a single urban structure and selected 10 termite alate individuals collected from that structure as subjects for dissection, DNA extraction, and subsequent DNA sequencing. This gave me a total of 10 different termite DNA samples per urban structure. With ten localities or cities, I had a total of 100 different test subjects for this study. For my second experiment, I chose three additional cities: San Antonio, Dallas, and Beaumont. From each of the three cities, five termite-infested structures were selected. From each structure one termite was chosen as subject for dissection, DNA extraction, and subsequent DNA sequencing. After determining the findings of Experiment I, I found it was sufficient to sample a single termite per structure in order to assess haplotypic variation within that structure. With five different termites per city, and three different cities, I had a total of 15 different test subjects for this study.

DNA Extraction. The prothorax from each of the subject termites was dissected. Subsequently, total genomic DNA was extracted from the termites using a silica-based spin column procedure, DNeasy, Qiagen, Valencia, CA, following the manufacturer's tissue protocol. The remaining insect parts were vouchered in 0.5 µl tubes, and stored at -20º C.

Polymerase Chain Reaction. Two micro liters of extracted DNA (>50 ng / µl) from each sample was prepared for PCR. A cocktail containing 35 µl of mili-Q water, 5 µl of 10x magnesium chloride – free Promega buffer (Promega, Madison, WI), 4 µl of 25mM Promega $MgCl_2$, 1µl of 40mM dNTPs, 0.2 µl of 100 U of Promega *Taq* polymerase, and 2 µl of 5mM solution of each PCR primer. Approximately 324-337 base pairs of the mtDNA AT-rich region were amplified with primers TM-N-193 (TGGGGTATGAACCA- GTAGC) (Taylor et al. 1993) and AT-J-T1 (CACTAAGGATAATCAATTATACGTC) (Jenkins et al. 1998). PCR was conducted in a Peltier thermal cycler (PTC-200, MJR). The following PCR program was used: an initial 94º C for 120 s; 50º C for 30 s; and 72º C for 60 s; for a total of 36 cycles, followed by 72º C for 5 min.

Gel Electrophoresis. Five ul of the PCR product plus 3µl of DNA loading buffer, was then subjected to gel-electrophoresis to ensure that the proper size bands were formed. In order to prepare the gel, 0.6 g of agarose was added to 40 ml of mili-Q water. This mixture was then heated in a microwave oven for 1.5 min. to ensure a homogeneous mixture of the agarose with the mili-Q water. After allowing the solution to sit for 30 seconds, 3µl of ethidium bromide stain (10 mg/ml solution) was added. The mixture was then placed into a gel mold and allowed to sit for 30 min. The resulting 1.5% agarose gel

was then loaded with the PCR samples and allowed to run in a 1X Tris borate-EDTA buffer at 100 V for 30 min. Then using UV light, the DNA in the gel was visualized and a digital photograph of the gel was taken.

PCR Clean Up. Unincorporated dNTPs and primers were then removed from the PCR products using a Qiagen PCR clean up kit following manufacturer's protocol.

Cycle Sequencing. Following PCR clean up, the samples were cycle sequenced. A Big Dye kit (Applied Biosystems, Foster City, CA), containing fluorescently labeled dye terminator nucleotides, was used. A mixture of 8 µl of PCR grade water, 2 µl of Big Dye, 3 µl of 5X sequencing buffer, 3 µl of primers, and 2 µl of cleaned PCR product were used for cycle sequencing. Both the 5'- 3' and 3'-5' strands of the mtDNA region were sequenced in order to verify nucleotide designation at each position. After undergoing Sephadex cleaning, the samples were sequenced at Gene Technologies Laboratory (Department of Biology, Texas A&M University).

DNA Editing. Once the chromatographs were obtained, the sequences were manually edited into consensus using the program softwares Sequence Navigator version 1.0.1 (Applied Biosystems, Foster City, CA) or Sequencher™ version 4.1 (Gene Codes Corporation, Ann Arbor, MI). The resulting consensus sequences were entered in a BLAST sequence similarity search (NCBI, Bethesda, MD) to confirm the identity of the sequence fragment. The closest matching fragment was that of other *R. flavipes* specimens.

DNA Analysis. Sequences were aligned with the computer program Clustal X (Thompson et al. 1997) using alignment settings and procedure outlined by Foster et al. (2004). Sequence sites with missing data and gap positions were excluded from

haplotypic designation. A haplotype was defined as a unique sequence with a difference of at least one base pair. There are several ways to statistically analyze population structure. One important example involves "F-statistics" (Wright 1951). Wright introduced the F-statistic in 1951 as a way to describe genetic population structure in diploid organisms in terms of three allelic correlations ($F_{IT,}$ $F_{IS,}$ and F_{ST}) (Avise 2004). F_{ST} is interpreted as the proportion of genetic variation distributed among subdivided populations, as calculated by:

$$F_{ST} = (h_T - h_S) / h_T$$

In the above formula, h_S is the mean expected heterozygosity at a locus within subpopulations, and h_T is the overall expected heterozygosity given allele frequencies in the total population (Avise 2004). Values of F_{ST} can range from 0.0 (subpopulations genetically identically) to 1.0 (subpopulations fixed for different alleles) (Avise 2004). Two statistical approaches that are similar to F_{ST} are N_{ST} (Lynch and Crease 1990) and G_{ST} (Nei 1973). These latter fixation indices are better suited for haplotypic divergence (Lynch and Crease 1990) and were used for this study.

Gene flow is the transfer of genetic material between populations resulting from movements of individuals or their gametes (Avise 2004). Measures of gene flow can be measured by the statistical index N_m. N_m is the absolute number of individuals exchanged between populations per generation (Avise 2004). N_m can be inferred by measurements of G_{ST} and N_{ST} (Galacatos et al. 2002). In my study, I calculated N_m by substituting N_{ST} for F_{ST} in Wright's (1951) equation and calculating for mtDNA (Hudson et al. 1992):

$$F_{ST} = 1/(1 + 2 N_m)$$

Measures of N_m that are less than one indicate low gene flow (Galacatos et al. 2004). Measures of N_m that are greater than one indicate high levels of gene flow. (Galacatos et al. 2004). Estimates of gene flow through indirect measures can be problematic because many potential sources of error exist (Galacatos et al. 2002). However, for *R. flavipes*, as with most subjects of population genetic studies the structure of genetic variation is the only source of information about patterns of gene flow over time scales of more than a few generations (Galacatos et al. 2004). In order to measure nucleotide and haplotypic variation, G_{ST} and N_{ST} values, and gene flow DNASP version 3.99.6 (Rozas et al. 2003) was used.

RESULTS

In this experiment the sequence data revealed 9 unique haplotypes for the 90 *R. flavipes* sequence samples analyzed from 9 Texas locations. Initially a total of 100 sequence samples were to be used for the data analysis, however, 10 sequence samples from Paris, Texas returned unusable results. On average one haplotype was found at each city. The highest numbers of haplotypes were identified from Abilene. Overall haplotype diversity, measured as the mean number of pair wise differences among individuals, was low (HdT = 0.078) (Table 1). Nucleotide diversity was also low (PiT: 0.0008) (Table 1).

Table 1. Measurement of haplotype numbers and nucleotide and haplotype diversity for *R. flavipes* populations of central/eastern Texas, Experiment I.

Geographic Comparison	Number of haplotypes		Haplotype Diversity (HdT)*	Nucleotide Diversity (PiT)*
College Station	1		0.000	0.0000
Greenville	1		0.000	0.0000
Abilene	3		0.380	0.0027
San Angelo	1		0.000	0.0000
Perryton	2		0.330	0.0045
Port Arthur	1		0.000	0.0000
Victoria	1		0.000	0.0000
Waco	1		0.000	0.0000
Tyler	1		0.000	0.0000
Paris**	n/a		n/a	n/a
Total	9	Mean	0.078	0.0008

*(HdT) stands for haplotype diversity total. (PiT) represents total measurement of nucleotide diversity.

** The sequence data from this locality yielded unusable results and thus could not be used for analysis.

The total estimates of G_{ST} = 0.78239 and N_{ST} = 0.74568 indicating moderate genetic structure (Table 2). Nm measures, calculated from Nst, indicate low gene flow at Nm = 0.09 (Table 2).

Table 2. Estimates of genetic population structure and gene flow within populations of *R. flavipes* from Texas, Experiment I.

Total Nst*	Total Gst*	Nm*	Gene Flow
0.746	0.782	0.09	Low/Moderate

*G_{ST} and N_{ST} are measures of haplotype diversity and nucleotide divergence that tend to be highly correlated (Lynch & Crease 1990) and are measured on a 0.0 to 1.0 scale. When G_{ST} and N_{ST} = 0, low genetic structure, G_{ST} and N_{ST} = 1, high genetic structure (Galacatos et al. 2004).
**Nm measurements are inferred from measurements of $N_{ST.}$ Nm is a measure of genetic structure and gene flow can be inferred from these measurements (Galacatos et al. 2004). When Nm is less than 1 gene flow is low. When Nm is greater than 1 gene flow is high (Galacatos et al. 2004).

For the second experiment, a total of 14 individuals were used. The single sequence from Beaumont did not yield usable results. For Experiment II, the sequence data revealed 12 different haplotypes for the 14 *R. flavipes* sequence samples tested (Table 3). On average there were four haplotypes found at each city. San Antonio and Dallas had the highest number of haplotypes each with five each (Table 3). Overall haplotype diversity, measured as the mean number of pair wise differences among individuals, was high (HdT = 0.98) (Table 3), while nucleotide diversity was low (PiT: 0.01442) (Table 3).

Table 3. Measurement of haplotype numbers and nucleotide and haplotype diversity for *R. flavipes* populations of central/eastern Texas, Experiment II.

Geographic Comparison	Number haplotypes		Haplotype Diversity (HdT)*	Nucleotide Diversity (PiT)**
San Antonio	5		1.0	0.01744
Dallas	5		1.0	0.01450
Beaumont	4		0.83	0.01108
Total	14	Mean	0.98	0.01442

*(HdT) stands for haplotype diversity total.
**(PiT) represents the total measurement of nucleotide diversity.

The total estimates of $G_{ST} = 0.025$ and $N_{ST} = 0.018$, which indicates high genetic structure (Table 4). Nm measures, calculated from Nst, indicate high gene flow at Nm = 31.91 (Table 4).

Table 4. Estimates of genetic population structure and gene flow within populations of *R. flavipes* from Texas, Experiment II.

Total Nst*	Total Gst*	Nm**	Gene Flow
0.018	0.025	31.91	High

*G_{ST} and N_{ST} are measures of haplotype diversity and nucleotide divergence that tend to be highly correlated (Lynch & Crease 1990) and are measured on a 0.0 to 1.0 scale. When G_{ST} and $N_{ST} = 0$, low genetic structure, G_{ST} and $N_{ST} = 1$, high genetic structure (Galacatos et al. 2004).
**Nm measurements are inferred from measurements of $N_{ST.}$ Nm is a measure of genetic structure and gene flow can be inferred from these measurements (Galacatos et al. 2004). When Nm is less than 1 gene flow is low. When Nm is greater than 1 gene flow is high (Galacatos et al. 2004).

DISCUSSION AND CONCLUSIONS

A single termite sample per urban structure was sufficient to assess haplotype variation at the urban structural level. I found fewer unique haplotypes per sequence sampled (9 haplotypes for 90 sequences) than did Foster et al. 2004 (28 haplotypes for 173 sequences). I also found that *R. flavipes* populations collected at a single urban structure posses limited genetic variation based on low levels of haplotypic and nucleotide differentiation (Table 1) comparable to previous measurements (Foster et al. 2004). In Experiment I, there was a low level of inter-specific gene flow occurring between populations of *R. flavipes* from different Texas cities as corroborated by the appropriate Nst and Gst values (Table 2). The low mean number of haplotypes per locality (1; Table 1) coupled with the low levels of genetic variation pointed towards genetically homogenous populations at the urban structural level.

In Experiment II, increased intra specific sampling of termites from different urban structures within a city revealed a greater level of haplotypic and nucleotide variation (Table 3). There was also a higher ratio of unique haplotypes per sequence found, 1:1, for Experiment II.

The unique haplotype to sequence ratio of Experiment II is greater than the 1:10 ratio for Experiment I and is also greater than the 28:173 found previously (Foster et al. 2004). These results may point towards higher levels of genetic variability within a city as compared to statewide. This new data may also indicate that with increased intra specific sampling more genetic variability may be elucidated for Texas populations of *R. flavipes*. Underestimation of haplotype diversity may be occurring when only sampling a single locality within a city. In order to determine whether there is greater haplotypic and nucleotide diversity within cities as opposed to state wide, a study incorporating more intra-specific sampling of more cities across Texas should be conducted.

Finally, the data showed that there was a high level of genetic flow occurring between populations of *R. flavipes* within the same city as compared to Experiment I which showed only low levels of gene flow occurring between populations of *R. flavipes* from different cities (Table 5). However, only one haplotype was shared among two of the three cities. Thus the high gene flow estimate was likely skewed given the small sample size.

Table 5. Summary of differences between genetic structure data of *R. flavipes* populations from Experiments I and II and Foster et al. 2004.

	Experiment I	Experiment II	Foster et al. 2004
Total Number of Haplotypes	9	14	28
Total Number of Individuals	90	14	173
*Genetic Drift	LOW	HIGH	n /a
Haplotypic Diversity	LOW	HIGH	LOW
Nucleotide Diversity	LOW	HIGH	MODERATE

*Gene flow values based on Nm values as calculated from N_{ST}. N_{ST} is a measure of haplotype diversity and nucleotide divergence (Lynch & Crease 1990) and is measured on a 0.0 to 1.0 scale. When $N_{ST} = 0$, low genetic structure, $N_{ST} = 1$, high genetic structure (Galacatos et al. 2004). Nm measurements are inferred from measurements of N_{ST}. Nm is a measure of genetic structure and gene flow (Galacatos et al. 2004). When Nm is less than 1 gene flow is low. When Nm is greater than 1 gene flow is high (Galacatos et al. 2004).

Limited genetic diversity within an urban structure and sharp delineation of haplotypes between different urban structures supports the idea that, within a single urban structure, there is limited genetic variability. These findings may also serve as genetic evidence for theories previously expressed in entomological literature which debate the mechanisms of exotic termite introductions and their spread throughout a geographic region. Given that *R. flavipes* lack the ability to travel long distances due to short flight and foraging ranges it is highly unlikely termite behavior is responsible for the vast

distribution of this species. Unwitting movement of termite-infested materials resulting in the establishment of exotic populations is a well-documented phenomenon (Jenkins et al. 2002). Infested wood and wood by products can be transported from one location to another because termites do not often reveal their presence without clear signs of activity (Jenkins et al. 2002). The lack of genetic variation in *R. flavipes* across the state of Texas is consistent with the founding of colonies via a single or a few matrilineal lines. This challenges previous notions that *R. flavipes* is spread throughout Texas via repeated human transportation of a single or a few matrilineal lines.

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