Genetic Analysis of Termite Colonies in Wisconsin

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ABSTRACT The objective of this study was to document current areas of subterranean termite activity in Wisconsin and to evaluate genetic characteristics of these northern, peripheral colonies. Here, amplified fragment-length polymorphism was used to characterize levels of inbreeding, expected heterozygosity, and percent polymorphism within colonies as well as genetic structure among populations sampled. Genetic analysis revealed two species of termites occur in Wisconsin, *Reticulitermes flavipes* (Kollar) and *Reticulitermes tibialis* Banks, both found in the southern half of the state. Colonies of both species in Wisconsin are thought to represent the northern boundary of their current distributions. Measurements of within colony genetic variation showed the proportion of polymorphic loci to be between 52.9–63.9% and expected heterozygosity to range from 0.122–0.189. Consistent with geographical isolation, strong intercolony genetic differences were observed, with over 50% of F_{ST} values above 0.25 and the remaining showing moderate levels of genetic differentiation. Combined with low levels of inbreeding in most collection locations (F_{IS} 0.042–0.123), we hypothesize termites were introduced numerous times in the state, likely by anthropogenic means. We discuss the potential effects of these genetic characteristics on successful colony establishment of termites along the northern boundary compared with termites in the core region of their distribution.

KEY WORDS *Reticulitermes flavipes* (Kollar), *Reticulitermes tibialis* Banks, amplified fragment-length polymorphism, GENELAND, genetic population structure

Worldwide, an estimated US$22 billion is spent annually on termite control and repair costs, with 80% of this cost thought to be associated with subterranean termites specifically (Rust and Su 2012). In North America, much of this damage is caused by two subterranean termite species, the widely distributed, eastern subterranean termite, *Reticulitermes flavipes* (Kollar), and the invasive species, *Coptotermes formosanus* Shiraki, which is distributed along the southern United States in the Gulf coast region (Austin et al. 2006). Colonies of *R. flavipes* are most prevalent in the warmer, southeastern region of North America, although their range does extend to a number of locations further north. Currently, it is thought that termite colonies in Wisconsin are located along the northern edge for the known distribution of this species. North of this range, cold winters and deep soil frost lines are thought to impede colony establishment (Esenther 1961, 1969).

Although subterranean termites are widely distributed in North America, characteristics of these colonies have been shown to vary by geographic location in a number of studies (Esenther 1969, Raffoul et al. 2011, Vargo 2003, Vargo and Husseneder 2009). In particular, termite colonies located along their northern range have shown differences in reproduction and dispersal compared to colonies from the southern United States. For example, colonies along their northern range are thought to grow primarily by means of secondary reproductives and subsequent budding (Esenther 1969, Raffoul et al. 2011), which is underground colony expansion or isolation of parts of a larger colony, rather than nuptial alate flights. This phenomenon tends to limit the spread of termites geographically, and creates large, long-lived, super colonies in northern regions compared with their counterparts further south (Esenther 1969, 1980; Grace et al. 1989).

Differences in colony establishment can be reflected in genetic trends that can be seen at the colony level. A number of studies have attempted to elucidate mechanisms of colony formation (e.g., alate flights vs. introduction and budding) and subsequent colony structure in termites using genetic techniques (e.g., microsatellites, amplified fragment-length polymorphisms [AFLP], T-RFLP; Reilly 1987; Wang and Grace 1995; Husseneder et al. 1998; Thompson and Hebert 1998; Forschler and Jenkins 1999; Bulmer et al. 2001; García et al. 2002; Austin et al. 2005, 2006; Vargo and Husseneder 2009; Scaduto et al. 2012). These studies have shown significant variation in patterns of colony establishment and reproduction, not only among species, but also within a single species (Bulmer et al. 2001). In areas where termite swarms are common, an unrelated alate pair can establish new colonies with subsequent

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reproduction by their descendants or by secondary reproductives formed within the colony. In more northern areas, especially where colonies may be established by anthropogenic movement of infested material, populations are thought to grow by differentiation of workers into secondary reproductives (Esenther 1969, 1980). Despite evidence that termite colonies in the northern range differ from southern colonies, few studies have focused specifically on these northern colonies of *R. flavipes* particularly in terms of colony distribution and development, and degrees of genetic variation and differentiation (e.g., gene flow).

Advances in molecular based technologies have created the ability to use genetic markers to examine the genetic population structure in termites. The primary objective of this study was to document all known areas of termite activity in Wisconsin and to evaluate the degree of genetic variation and differentiation among samples from these colonies. Based on current field observations and published reports, we hypothesized that the isolated nature and lack of winged reproductives has potential to create high levels of inbreeding within termite colonies in turn, reducing intracolony genetic diversity while increasing among-colony genetic differences. Studying termite populations along their northern range will provide insight into colony formation and persistence in a colder climate. Additionally, characteristics within these peripheral populations will provide information to better evaluate environmental factors that influence or control population dispersal, which may become important if rising global temperatures allow termites to continue expanding their northern range.

Materials and Methods

An extensive survey of termite populations throughout Wisconsin was conducted using pest control operators, extension agencies and field observations. Termites were collected from 12 of the 18 known active colonies, which included two separate colonies in both La Crosse and Oshkosh. A sample from Mississippi (Harrison County, MS) was also collected to serve as a genetic out-group to represent a termite population from the core area of subterranean termite activity. At least 20 specimens were collected from each site from woody debris or active shelter tubes and stored in 100% ethanol. A total of eight workers (~3rd or 4th instar) were then chosen from each collection location for DNA extraction.

Prior to DNA extraction, termite abdomens were removed to eliminate genetic material from gut microbiota. DNA was extracted from individual termites ($n = 8$ per sample) and purified using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers protocol for DNA isolation from animal tissues. Purified DNA was concentrated by KoAC precipitation, washed with 100% ethanol at 5°C overnight, and then rehydrated with 30 µl of 10 mM Tris-HCl (pH 8.0). DNA quality was checked qualitatively by 1% agarose gel electrophoresis. DNA concentration was estimated on a BioRad SmartSpec Plus spectrophotometer (BioRad Laboratories, Hercules, CA). Each sample was diluted to 50 ng/µl in Tris buffer (pH 8.0) and electrophoresed a second time to visually confirm the uniformity of each dilution.

Genetic fingerprints were generated with AFLP (Vos et al. 1995) following modified protocols described in Berres (2003). First, 200 ng of DNA was digested to completion with 20 U EcoRI (5′- G|AATTC-3′) and 10 U AseI (5′- AT|TAAT-3′). Following heat inactivation at 65°C for 20 min, double-stranded adaptor sequences EcoRI-AD and BfaI-AD (75 pmoles each) with overhangs complementary to the digested ends were ligated overnight at 16°C with 400U T4 DNA ligase. Ligated samples were diluted 1:4 with sterile 10 mM Tris-HCl (pH 8.0).

Preselective PCR amplifications were performed in 50 µl volumes (1X GoTaq Flexi Buffer, 1.5 mM MgCl2, 0.05 mM dNTP, 2% freshly prepared/deionized formamide, 1.25U Taq DNA Pol I) containing 10 µl diluted ligation mixture and 15 pmoles each of primers EcoRI+G (5′-GACTGCGTACCAATTCG-3′) and AseI+G (5′- GATGAGTCCTGAGTAATG-3′). Thermocycling consisted of one cycle of 72°C for 2 min, an initial denature at 94°C for 1 min followed by 25 cycles each of 94°C for 50 s, 56°C anneal for 1 min, and 72°C extension for 2 min. Preselective amplification products were normally diluted 1:19 in sterile 10 mM Tris-HCl (pH 8.0) but those with lower amplification (determined qualitatively by agarose gel electrophoresis) were diluted 1:9. This extra step was included so that the selective PCR would start with similar template concentrations.

Selective PCR amplification was performed in 25 µl volumes (1X GoTaq Flexi Buffer, 2 mM MgCl2, 0.3 mM dNTP, 2% freshly prepared/deionized formamide, 0.625U Taq DNA Pol I) containing 5 µl diluted preamplification mixture, 5 pmoles HPLC-purified primer EcoRI+GG (5′- GACTGCGTACCAATTCGG-3′) labeled with 6-carboxyfluorescein (6-FAM), and 25 pmoles AseI + GT (5′-GATGAGTCCTGAGTAATGT-3′). Thermocycling consisted of an initial 94°C denature for 1 min followed by 10 cycles of a 1-min annealing touchdown (1°C decrease each cycle) from 65°C to 56°C each with a 72°C extension for 2 min. The selective amplification was completed with 18 cycles of 95°C for 50 s, 56°C for 1 min, and 72°C for 2 min.

Amplified selective PCR products were purified over Sephadex G75 and stored at −80°C. Two µl of purified product was combined with 12.3 µl formamide and 0.7 µl Geneflo 625 (mobility standard, CHIMERx Molecular Biology Products) for electrophoresis on an ABI 3730xl DNA Analyzer.

DAx 8.0 (Van Mierlo Software, The Netherlands) was used to visualize AFLP fingerprints and markers ranging from 50 to 625 base pairs were scored as present or absent. The method of Zhivotovsky (1999), implemented in the software package AFLP-SURV (Vekemans 2002, Vekemans et al. 2002), was used to calculate the magnitude of polymorphism (percent polymorphic loci), expected heterozygosity (H_e), and pairwise F_{ST}. Calculation of pairwise F_{ST} included F_{IS} values that were calculated from AFLP data directly (Chybicki et al. 2011). To test for statistically significant

population differentiation, a 95% confidence interval was constructed given the hypothesis that $F_{ST} = 0$ by permuting individuals across populations (1,000 replicates). In addition, IBDWS (Jensen et al. 2005) was used to test for a pattern of isolation by distance (IBD) among termite collection locations. The number of distinct genetic clusters among the collection locations was estimated using a Voronoi tessellation procedure allowing for correlated allele frequencies in the program GENELAND v4.0.3 (Guillot et al. 2005a,b; Guillot and Santos 2010). Randomly seeded replicates ($n = 1{,}000$) with variable K (1–20) were subjected to 1,000,000 iterations of the Markov Chain Monte Carlo (MCMC) algorithm. The presence of genetic clusters was evaluated with and without spatial data (i.e.,physical distance between sampling locations).

Individuals may exhibit a tendency for membership in more than one cluster, so replicate runs can vary slightly. Consistent clustering patterns provide support for individual membership in each cluster. We first performed a diagnostic to accept or reject the null hypothesis that the MCMC chain from each run was from a stationary distribution (Heidelberger and Welch 1981, 1983). For those replicates meeting the statistical stationary criterion, the pairwise cumulative proportion of same-cluster membership for individuals from the 1,000 independent MCMC runs was calculated. Relationships among individuals were then visualized by a topology created by applying an agglomerative hierarchical clustering procedure to estimate the unweighted average distance between individuals (UPGMA). These topologies do not depict phylogenetic relationships or population genetic structure *per se*, but rather overall relationships between individuals based on shared membership in a common cluster as determined by a specified GENELAND model. The mean posterior probability of cluster membership ranges from zero (individuals always occurred in the same genetic cluster) to one (individuals always occurred in a different genetic cluster).

Results and Discussion

As few studies have focused on characteristics of subterranean termites along their northern limits, a major goal of this work was to highlight differences that might be observed in these colonies compared with southern areas where subterranean termites are common. Based on what is known about distribution and reproduction in *R. flavipes* across its range, we developed hypotheses of characteristics that may be expected to differ in peripheral colonies, such as those found along their northern boundary (Wisconsin; this study) at least when compared with their core range (southern United States; Marschalek and Berres 2014). For the purpose of this study, colony characteristics from the Mississippi site were used for comparison, as they represent samples from a core region of subterranean termite activity. Overall, we hypothesized that Wisconsin termite colonies would show higher levels of genetic differentiation among colonies and lower levels of genetic variation within colonies, a result which has been shown in other work evaluating genetic characteristics of peripheral populations (Eckert et al. 2008, Marschalek and Berres 2014). Specific hypotheses are presented in Table 1 and are discussed relative to the main objectives of this study.

Survey of Subterranean Termite Activity in Wisconsin. Ten new termite colonies were identified in Wisconsin compared with the nine previously known areas of termite activity listed by Esenther (1969; Fig. 1). These include colonies in Green Bay, Two Rivers, Montello, Endeavor, Grafton, Middleton, Palmyra, Monroe, Hazel Green, and Muscoda. Confirmed by morphological and genetic analysis, our study has determined that two termite species are now known to occur in the state, *R. flavipes*, which occurs in all locations surveyed with the exception of Hazel Green, which represents the only known population of *Reticulitermes tibialis* Banks in Wisconsin (Arango 2015). Termite populations were identified only in the southern half of Wisconsin, supporting the hypothesis that termites in the state represent the northern boundary of subterranean termite activity. Many colonies are thought to be quite expansive underground, likely consisting of millions of individuals, and persisting for more than 50 yrs (Esenther 1961, 1980). In a number of these cases, colonies are known from numerous areas in the same city, suggesting colony growth by budding and subsequent isolation, human movement of infested materials, or possibly short flights by alates (although this has not been reported). Locations that fit this description of many widely distributed pockets of termite activity include Janesville, Sheboygan, Milwaukee, Madison, Oshkosh, La Crosse, Kenosha, and possibly others.

Field observations of termite activity and information provided by pest control operators supports the long standing hypothesis that termites in Wisconsin are the result of multiple, independent introductions. Anthropogenic dispersal events probably occurred by transport of infested materials, particularly as termites in the state do not disperse to any measurable degree by means of winged reproductives. We hypothesize that differentiation into the alate form occurs rarely, if at all outside of swarming observed in isolated homes or structures. Cold winter temperatures and deep frost lines in the soil likely mediate this type of termite dispersal. Observations of termites during annual visits to a field site in southern Wisconsin (Janesville, WI), support this hypothesis. During the spring of 2012, small numbers of alates were found in fallen logs following a particularly warm winter, an observation that has not been repeated in subsequent years. If alate flight comprised a major form of dispersal for termites in Wisconsin, we would expect to see numerous small colonies around a known area of activity. Instead, termite colonies in Wisconsin tend to be well-isolated in a number of locations with limited spread geographically.

Characteristics of Within-Colony Genetic Variation. Intraspecific genetic variation is known to be a major factor of evolutionary change and adaptation and is likely important for the successful establishment and persistence of termite colonies along their northern

Table 1. Hypothesized and observed characteristics for termite colonies in core versus peripheral areas of termite activity

Expectations for peripheral termite populations (i.e., northern boundary)	Expectations for core termite populations (i.e., south-eastern United States)	Results from the present study
Absence of alate based dispersal methods; Colony growth from differentiation of introduced workers into neotenic or nymphal reproductives	Dispersal commonly through seasonal, synchronized alate flights; Colony development and growth originating from unrelated alate pairs	Alate development sometimes observed within structures; Major alate swarms not yet seen in the field
High inbreeding within colonies (i.e., large, positive F_{IS} values)	Low inbreeding values due to introduction from diverse gene pools, although some inbreeding may still be observed (i.e., F_{IS} values closer to zero)	Some inbreeding although lower than expected; F_{IS} values ranged from 0.042–0.123 (average $F_{IS} = 0.063$)
Expected heterozygosity values low (=high levels of homozygosity)	Expected heterozygosity values high (=low levels of homozygosity)	Lower expected heterozygosity values ($H_e = 0.122$–0.189) but comparable to the 0.159 calculated from the core, Mississippi site
High among-population genetic variation (i.e., F_{ST} values closer to one)	Low among-population genetic variation (i.e., F_{ST} values not significantly different from zero)	Mostly high among-population genetic variation; F_{ST} values indicate very strong genetic differentiation
Low levels of within-colony genetic polymorphism (smaller PPL values)	High levels of within-colony genetic polymorphism (larger PPL values)	High levels of within-colony genetic polymorphism (PPL ranged from 52.9–63.9%)
Isolated colonies without an apparent geographic pattern (i.e., mean posterior probability closer to one)	Colonies that follow a distinct geographic pattern(i.e., mean posterior probability closer to zero)	Mostly isolated colonies with mean posterior probabilities closer to one; little change between spatial and nonspatial models
No correlation between genetic and geographic distance (i.e., nonsignificant IBD pattern)	Strong correlation between genetic and geographic distance (i.e., significant IBD pattern)	Little relationship between genetic and geographic distance among WI colonies except within collections from the same city

Fig. 1. Known areas of termite activity in Wisconsin.

range. Three variables were examined to evaluate levels of within-colony genetic variation, including the proportion of polymorphic loci (PPL), expected heterozygosity (H_e), and the inbreeding coefficient (F_{IS}). Values for PPL from Wisconsin samples ranged from 52.9–63.9% (Table 2), comparable with Mississippi samples taken from the core region of subterranean termite activity (58.7%). Values for H_e from Wisconsin samples ranged from 0.122–0.189, and were also comparable with the 0.159 H_e calculated for the Mississippi samples (Table 2). The comparable levels of genetic variation between the Wisconsin peripheral populations and the Mississippi core population was not expected, as peripheral populations often have reduced genetic variation (Eckert et al. 2008).

Because termite colonies in Wisconsin are thought to be the result of human movement of infested materials, the inbreeding coefficient was calculated for each sample. We hypothesized that F_{IS} levels would be high from lack of immigration or emigration from each colony. Under assumptions of random mating, F_{IS} for Wisconsin samples ranged from 0.042–0.123 and is suggestive of some amount of intracolony inbreeding, although not as high as was expected. F_{IS} for the Mississippi site was 0.043. Two collection locations, both from Oshkosh, exhibited approximately twice the level of F_{IS} compared with the other collection locations, indicating slightly higher levels of inbreeding at these two sites and may reflect smaller population sizes.

Prior to genetic analysis, we hypothesized that high levels of homozygosity would occur in Wisconsin termite colonies, as reproduction and colony growth occurs by means of differentiation of workers into secondary reproductives rather than nuptial alate flights (Table 1) (Esenther 1969). Although expected heterozygosity values were low, the amount of allelic polymorphism (PPL) and lower inbreeding coefficient values (F_{IS}) indicated a higher level of within colony genetic variation than we expected, especially when compared with the values calculated for the Mississippi site. A study by Calleri et al. (2006) used microsatellite analysis with purposely inbred and outbred members of the termite species *Zootermopsis angusticollis* and found inbred termites had a calculated F_{IS} of 0.51 where outbred termites $F_{IS} = 0.17$. Our estimates of F_{IS} based on AFLP data for Wisconsin termite populations were lower, averaging 0.063. Although the lower F_{IS} value

Table 2. Genetic differentiation among termite sampling locations

Population code	1	2	3	4	5	6	7	8	9	10	11	12	13
1	–	0.383	0.613	0.288	0.378	0.325	0.356	0.307	0.328	0.323	0.333	0.315	0.356
2	130.2	–	0.651	0.182	0.256	0.283	0.339	0.170	0.286	0.216	0.242	0.234	0.472
3	153.0	219.2	–	0.652	0.654	0.640	0.684	0.649	0.669	0.635	0.656	0.648	0.661
4	117.7	110.6	115.6	–	0.126	0.189	0.189	0.137	0.105	0.118	0.179	0.102	0.403
5	143.4	272.6	167.9	223.4	–	0.167	0.288	0.222	0.221	0.151	0.189	0.167	0.490
6	141.9	270.8	163.5	220.1	4.5	–	0.263	0.234	0.199	0.194	0.268	0.148	0.443
7	69.0	128.7	97.4	58.5	166.2	163.1	–	0.273	0.264	0.206	0.320	0.243	0.521
8	146.7	26.3	216.7	103.0	286.4	284.2	133.0	–	0.202	0.210	0.221	0.157	0.465
9	95.9	200.5	74.9	126.1	102.9	98.97	74.9	207.2	–	0.196	0.222	0.200	0.421
10	81.8	92.5	225.4	151.8	215.6	215.1	129.5	117.9	176.8	–	0.116	0.172	0.409
11	81.7	91.8	225.1	151.3	215.8	215.3	129.1	117.2	176.7	0.6	–	0.199	0.393
12	140.5	47.9	255.5	153.9	282.8	281.7	160.0	70.8	225.3	74.7	74.3	–	0.441
13	1455	1413	1328	1342	1490	1485	1396	1388	1403	1490	1489	1459	–
PPL	56.1	60	55.5	61.9	56.1	58.7	52.9	61.3	62.6	63.9	63.2	60.6	58.7
H_e (S.E. H_e)	0.166 (0.01)	0.164 (0.01)	0.147 (0.01)	0.165 (0.01)	0.145 (0.01)	0.173 (0.01)	0.122 (0.01)	0.158 (0.01)	0.161 (0.01)	0.189 (0.01)	0.161 (0.01)	0.151 (0.01)	0.159 (0.01)
F_{IS}	0.054	0.050	0.046	0.049	0.042	0.065	0.044	0.047	0.042	0.121	0.123	0.073	0.043

F_{ST} pairwise comparisons above the diagonal and geographic distance (km) below the diagonal. Percent polymorphic loci (PPL), expected heterozygosity (H_e), standard error of H_e (S.E. H_e), and inbreeding coefficient F_{IS} listed below. Population codes: 1-Endeavor, 2-Grafton, 3-Hazel Green, 4-Janesville, 5-La Crosse (1), 6-La Crosse (2), 7-Middleton, 8-Milwaukee, 9-Muscoda, 10-Oshkosh (1), 11-Oshkosh (2), 12-Sheboygan, 13-Mississippi. (All F_{ST} values were significantly greater than zero at a 95% confidence level).

seen in this study could be due to differences in the molecular markers used and analytical analysis, it may also have a biological explanation. Lower levels of inbreeding and higher amounts of within-colony genetic variation in nearly all Wisconsin collection locations may play a role in their successful establishment in new locations. Colonies or individuals with low genetic variation to start or with high levels of inbreeding may simply be eliminated due to reduced fitness (DeHeer and Vargo 2006). It is possible that termites have evolved certain breeding mechanisms to reduce the potential of inbreeding and loss of genetic variation, although this hypothesis requires further study. It is also possible that low F_{IS}, as seen here, may be the result of initially high genetic variability in founder populations, which will eventually be reduced in future generations in the absence of out-breeding.

Characteristics of Among-Colony Genetic Variation. Three variables, F_{ST}, mean posterior probability (MPP) from GENELAND genetic clustering, and IBD, were calculated to better understand patterns of termite dispersal and degree of genetic similarity among Wisconsin sites (Table 2). To determine levels of genetic differentiation among the various termite collection locations in Wisconsin, estimates of F_{ST} were examined. All F_{ST} pairwise values were significant at a 95% confidence level and are indicative of strong genetic differences among the termite collection locations. According to categories developed by Wright (1978), F_{ST} values above 0.25 indicate very great genetic differentiation. In this study, over 50% of F_{ST} values fell in this category with the remaining samples showing moderate levels of genetic differentiation. Interestingly, lower pairwise F_{ST} values were observed between the Janesville site and a few of the other Wisconsin populations (i.e., La Crosse (1), Muscoda, and Sheboygan). The two Oshkosh and two La Crosse samples also had comparably smaller F_{ST} values. As expected, samples from Mississippi were considerably more differentiated than those sampled from within Wisconsin.

Assignment of individuals into genetic clusters using GENELAND produced similar patterns as observed in the F_{ST} calculations. Individuals from each collection location were assigned to the same genetic cluster suggesting a high level of similarity within each location as well as strong genetic uniqueness among locations (Fig. 2a and b). The spatial and nonspatial GENELAND models yielded nearly identical results. The spatial model (i.e., including geographic data—Fig. 2a) identified a modal $K = 11$ genetic clusters with a frequency of 0.67 (Fig. 3a). This model combined both La Crosse collection locations into a single cluster (MPP = 0.3) and the two Oshkosh locations as another cluster (MPP = 0.0). The nonspatial model (Fig. 2b) exhibited a modal $K = 12$ genetic clusters with a frequency of 0.39 (Fig. 3b) and more complicated genetic relationships among the collection locations. In contrast to the spatial model, the nonspatial model identified the two La Crosse locations as separate clusters

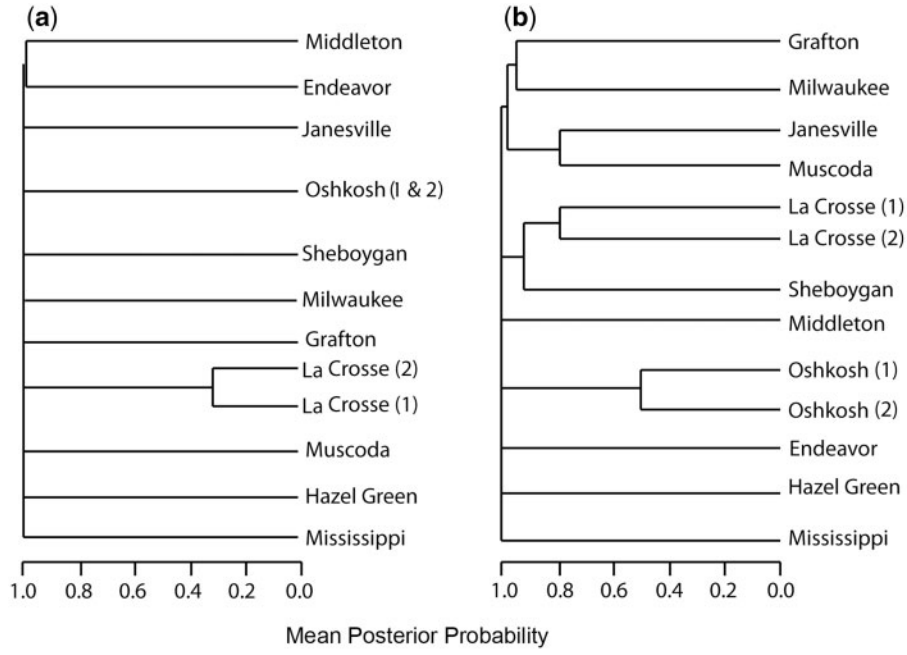

Fig. 2. UPGMA linkage among 92 individuals in genetic clusters generated with GENELAND using (a) spatial and (b) nonspatial models (1070 and 715 MCMC replicates, respectively). Branching ranges from an MPP of zero (always placed in the same cluster) to one (never placed in the same cluster).

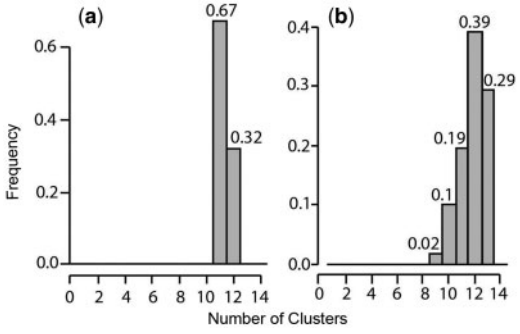

Fig. 3. Distribution of genetic clusters identified in (a) spatial and (b) nonspatial GENELAND analyses. The modal K is denoted by the highest frequency of clusters.

(MPP = 0.8) also associated with Sheboygan, but only slightly (MPP = 0.92). Weak intersite associations were also present between Janesville and Muscoda and also Grafton and Milwaukee.

The genetic data were tested to evaluate if a pattern of IBD existed among the termite collection locations. Results indicated a statistically significant pattern of IBD ($Z = 42.0461$, $r = 0.1938$, $P = 0.0040$) among all sampling locations. We considered this result inconsistent with our hypothesis of anthropogenic distribution. Further analysis showed that the significance observed between genetic and geographic distances resulted from two same-city samples. When samples from La Crosse and Oshkosh were removed, IBD was no longer statistically significant ($Z = 30.9428$, $r = 0.1530$, $P = 0.1730$). Thus, no correlation between genetic similarity and geographical location in the majority of sampling location occurs except for the La Crosse and Oshkosh samples.

Results from both the pairwise F_{ST} comparisons and GENELAND analyses supported our initial hypothesis that Wisconsin termites would show significant genetic differences among collection locations (Table 1; Figs. 2 and 3). This level of genetic variation among colonies suggests that termites were likely introduced numerous times into the state, from a variety of sources. Exceptions to this are seen in samples collected from the same city. The lower F_{ST} values between the two La Crosse colonies and two Oshkosh collection locations, for example, indicate a possible common origin of introduction or establishment of a parent colony and subsequent translocation from that location by human movement of infested materials, a hypothesis supported by the IBD data.

Overall, results from the present study highlight the need to consider termite populations near their northern limit differently than the core regions of termite activity, particularly in terms of dispersal patterns and distribution. It appears that current climatic conditions and temperatures have limited alate dispersal along a strongly demarcated northerly line. However, increasing global temperatures may alter this condition, resulting in alate formation and increased distribution of termite colonies along their northern range. This

phenomenon would have serious implications in housing markets and forest management practices and would necessitate policy changes in terms of building codes and inspection regulations.

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