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Breeding Patterns and Population Genetics of Eastern Subterranean Termites *Reticulitermes flavipes* in Urban Environment of Nebraska, United States

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Abstract

Reticulitermes flavipes (Kollar) has become the most destructive subterranean termite pest, on urban structures in Nebraska. In this study, we used seven microsatellite loci to infer the colony breeding system and population genetic structure among 20 infested urban structures in Nebraska. Our data revealed that 17 structures were infested by simple family colonies of *R. flavipes*, while, the remaining three were infested with mixed family colonies. The measure of population differentiation, F_{CT} value (0.459) indicated that all the 20 urban colonies (10 - 410 km apart) represented pronounced levels of genetic differentiation. The Mantel test disclosed a weak and significantly-positive correlation between genetic and geographic distance (slope = 0.0009, P = 0.001). The urban populations of *R. flavipes* in Nebraska possessed a breeding system characterized by monogamous pairs of outbred reproductives with excessive heterozygosity.

Introduction

Termite infestation of urban structures and landscapes can create very serious economic and environmental problems (Chapman & Bourke, 2001). In social insects, successful expansion of new intrusive species is largely due to a breeding system (Ross, 2001). The breeding system consists of important reproductive characteristics of social organisms, including the number of breeders in a social group, the genetic relationships of these breeders, and the extent of variation in parentage among same sex-breeders (Wade & Kalisz, 1990; Ross et al., 1993). In addition, dispersal of intrusive species into a new territory affects the propensity to monopolize a new habitat. The most likely reason is the new environment and ecology lead to a variation in the breeding system (Porter et al., 1997). Therefore, a thorough investigation of the breeding system of intrusive social insects is fundamental to further comprehend the association between their social structure, dispersal and invasion success.

Coptotermes spp. and *Reticulitermes* spp. are among well-known members of the subterranean termite species, causing numerous damages to structures and/or buildings (Curl, 2008). In general, the colony of the subterranean termite consists of a single pair of primary (winged) reproductives, which results in a simple family structure. The winged reproductives can disperse long distances, leading to a gene flow across the spatial scales. The pair of reproductives usually drop off their wings and starts a new colony (Perdereau et al., 2010). Later in the life cycle, brachypterous nymphs or workers produce secondary reproductives (neotenics) that can

replace the primary reproductives. The neotenics are unable to fly, hence, inbreeding within the colonies can occur. Colonies can be formed by multiple neotenics, resulting in extended colonies and occasionally forming spatially diffuse networks of interconnected reproductive centers (Perdereau et al., 2010; Vargo & Husseneder, 2009). For example, *R. labralis* and *R. chinensis* can produce alates that can disperse colonies in the surrounding areas for approximately 5 years and 9 years, respectively, once the colonies are established. Meanwhile, colonies headed by neotenics begin to produce alates on an average of 2.29 years (Xing et al., 2015; Ma, 1989; Lieu et al., 2002; Goodisman & Crozier 2002; Crissman et al., 2010).

A study in Massachusetts using allozyme and mitochondrial DNA (mtDNA) markers revealed a wide range of differences in the breeding structure of *R. flavipes* between two sites located 0.5 km apart (Bulmer et al., 2001). The site with rocky and poorly-drained soil consisted of a mixture of extended families (60%) and simple families (40%), where the foraging ranges were limited. Meanwhile, the site with more porous soil had equal numbers of spatially expansive extended families and mixed family colonies. In other studies, *R. flavipes* from central North Carolina identified with microsatellite markers provided consistent results concerning colony breeding structure in this region (Vargo, 2003a, b; DeHeer & Vargo, 2004). These authors reported that most colonies (75%) were simple families, while, the remaining colonies (24%) were neotenic-headed extended families, descendants of the simple families. There was also a single colony with genetically diverse individuals originating from the fusion of two distinct colonies.

The populations of *R. flavipes* show a variation in the breeding structure at both small and large spatial scales, depending upon ecological conditions. In the United States, the colony breeding structures of *R. flavipes* are classified into two types, type 1 and type 2. Populations of type 1 comprise a majority of simple families (>50%), with some extended families headed by a few neotenics. Meanwhile, introduced populations and one population in the New Orleans, putatively a population from French, are classified as type 2. Two populations i.e. MF Massachusetts and LN Nebraska are intermediate where extended and/ or mixed families are predominant and headed by hundreds of neotenics, particularly the Massachusetts population. Both populations also have a substantial proportion of simple-family colonies (Clement, 1981; Jenkins et al., 1999; Bulmer et al., 2001; Matsuura & Nishida, 2001; DeHeer & Vargo, 2004, 2008; DeHeer & Kamble, 2008; Perdereau et al., 2010; Perdereau et al., 2015; Ab Majid et al., 2013). While a large body of work on genetic structure of subterranean termite colonies in North America has been published, specific data on the population genetic of *R. flavipes* associated with seasonal temperature fluctuations in the Midwest of the United States are, lacking (Bulmer et al., 2001; Bulmer & Traniello, 2002; Vargo, 2003a, b; DeHeer & Vargo, 2004, 2006, 2008; Vargo & Carlson, 2006; Vargo et al., 2006b; DeHeer & Kamble 2008; Parman & Vargo, 2008). Porter et al. (1997) and Tsutsui et al. (2003) reported that a new environmental condition can lead to an alteration in the breeding system due to ecological conditions such as the absence of competitors or parasites, or genetics changes due to behavioral traits associated with dispersal behavior. Furthermore, climatic variables (mean annual temperature and seasonality) are also strongly affecting the inbreeding in *R. flavipes.* Non-climatic variables, including the availability of wood substrate and soil composition are among other inbreeding factors as well, however, are more evident in *R. grassei*. These results indicated that termite breeding structure was shaped by local environmental factors and that the species can vary in their responses to these factors (Vargo et al., 2013). In China, *Reticulitermes chinensis* Snyder, an important pest of trees and buildings from the genus *Reticulitermes* were also genotyped at eight microsatellite loci. Analysis of genetic clusters showed that the two subpopulations in Chongqing city were headed by outbred unrelated pairs (Huang et al., 2013). Their study also showed that dispersal by primary reproductives was relatively limited due to the short range mating flight or due to the frequent colony reproduction**.**

In recent years, molecular markers have become unequivocal identification tools for large numbers of termite colonies, particularly in investigating colony foraging areas, population dynamics, as well as colony breeding structure (Husseneder et al., 2003). In this study, we used seven microsatellite markers to determine the colony genetic and breeding structures of *R. flavipes* sampled from 20 infested buildings in the urban habitats of Nebraska. The phylogeny of *R. flavipes* colonies in the Nebraska ecosystem was also discussed in this study.

Materials and Methods

Termites

Samples of *R. flavipes* were collected from 20 infested buildings in Nebraska (Fig 1, Table 1) during 2010 - 2011. The addresses and geographic location of termite feeding sites were recorded using a hand-held Magellan® GPS unit (SporTrak™ Map, Thales Navigation, Huntington Beach, CA). Several termite samples from the infested buildings were provided by the Pest Control Operators (PCOs). Distances between the sites were in the range of 10 to 410 km as measured by the Google Earth 7 (Google Inc., 2013). All workers from each collection point were stored in 95% ethanol and at -20^{o} C immediately after the collection prior to DNA extraction procedures. All termites were morphologically identified as *R. flavipes* according to Husen et al. (2006).

DNA Extraction

Genomic DNA was individually extracted from heads of ten workers per site using a Qiagen DNeasy Kit (QIAGEN, Gaithersburg, MD, USA). The manufacturer's protocols were

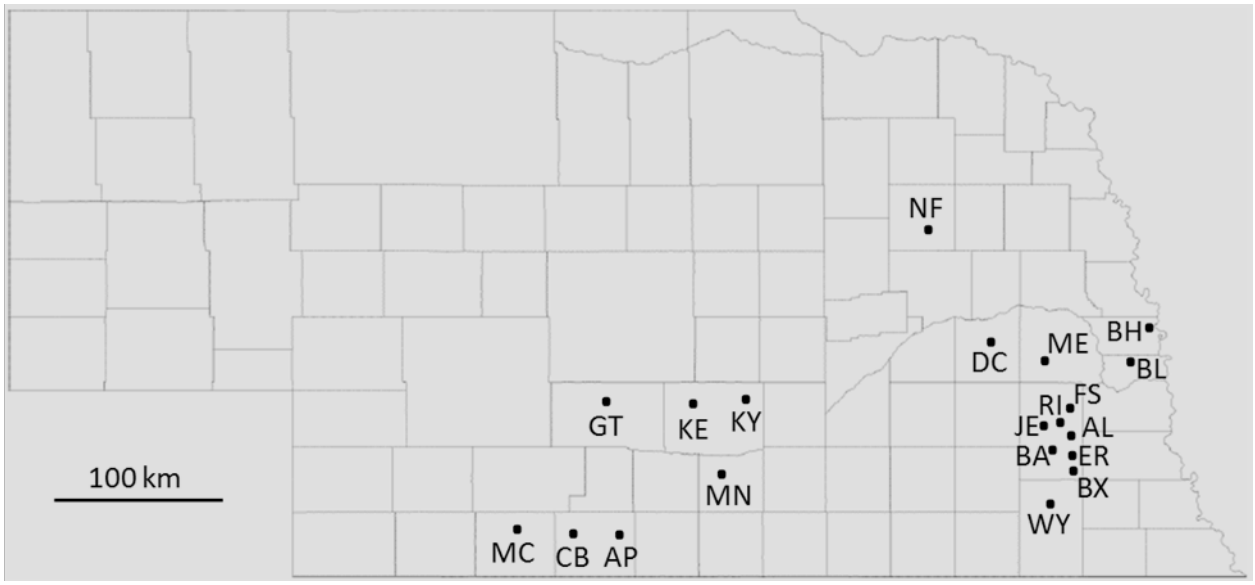

Fig 1. Sampling sites for *Reticulitermes flavipes* in various counties of Nebraska. AL = Lincoln; AP = Arapahoe; BA = Lincoln; BH = Omaha; BL = Bellevue; BX = Lincoln; CB = Cambridge; DC = David City; ER = Lincoln; FS = Lincoln; GT = Gothenburg; JE = Lincoln; KE = Kearney; KY = Kearney; MC = McCook; ME = Mead; MN = Minden; NF = Norfolk ; RI = Lincoln; WY = Wymore

followed except that treatments with Proteinase K solution and RNAs were omitted. DNA was eluted in 80 µl of 1X TE solution. The DNA concentration was quantified using an ND 1000 Spectrophotometer (Nanodrop Technologies, Inc. Wilmington, DE, USA).

Microsatellite Genotyping

We genotyped 10 termite workers per site at seven microsatellite loci: *Rf 1 - 3, Rf 5 – 10, Rf 6 – 1, Rf 11 – 1, Rf 1 – 2, Rf 15 – 2* and *Rf 21 – 1* (Vargo 2000). The PCR reactions were setup in 96 well plates in 15 µl reaction mixtures containing 1.5 µl of 10X of PCR buffer, 1.2 µl $MgCl_2$, 0.75 µl dNTPs mixture, 1.5 µl forward primer 1.5 µl reversed primer, 0.15 µl Taq DNA polymerase, 1 µl DNA template and 7.4 µl ddH_2O. All loci were amplified using PCR thermal cycler program with an initial denaturation step at 95˚C for 30 sec, followed by 35 cycles at 95˚C (30 sec), 54˚C (30 sec), and 72˚C (30 sec). The reaction was concluded with one cycle 72˚C (5 min) and then cooled to and kept at 4˚C until removed from the PCR thermal cycler. Fragments were separated by capillary electrophoresis using a Beckman CEQ 8000 Genetic Analyzer (Beckman Coulter, Fulleeton CA, USA). Data were visualized and hand-scored using CEQ 8000 Fragment Analysis Software version 8.0.

Microsatellite Data Analysis

Summary of population statistics (allelic diversity, expected and observed heterozygosity) was calculated using the FSTAT 2.9.3.2 (Goudet 2001). Exact tests of genotypic differentiation were performed using the web-based GENEPOP (Goudet et al.1996, Raymond and Rousset, 1995 [http://wbiomed.curtin.edu.au/genepop/index.html]). When two independent samples of workers were drawn from the same colony, we are sampling from the distribution of genotypes within that colony. Conversely, when two samples of workers are drawn from two different colonies, we are sampling from two different distributions of genotypes. This is true, regardless of the specific breeding pattern of colonies involved. Therefore, if we test the differences in genotype frequencies between two workers, we expect the test to be significant if they come from different colonies, and non-significant if they come from the same colony.

Table 1. Collection sites for subterranean termites, *Reticulitermes flavipes* in Nebraska.

Population Abbreviation	City	County	Collection Date
AP	Arapahoe	Furnas	12 June 2011
BL	Bellevue	Sarpy	12 June 2011
CB	Cambridge	Furnas	12 June 2011
DC	David City	Butler	12 June 2011
GT	Gothenburg	Dawson	17 June 2011
KE	Kearney	Buffalo	20 July 2011
KY	Kearney	Buffalo	20 July 2011
AL	Lincoln	Lancaster	30 June 2011
BA	Lincoln	Lancaster	30 June 2010
BX	Lincoln	Lancaster	30 June 2010
ER	Lincoln	Lancaster	30 June 2010
FS	Lincoln	Lancaster	30 June 2010
JE	Lincoln	Lancaster	30 June 2010
RI	Lincoln	Lancaster	30 June 2010
MC	McCook	Red Willow	13 June 2011
ME	Mead	Saunders	21 July 2011
MN	Minden	Kearney	22 July 2011
NF	Norfolk	Madison	22 July 2011
BH	Omaha	Douglas	1 July 2011
WY	Wymore	Gage	22 July 2011

Colony Breeding Pattern

Breeding Pattern was classified using techniques of Vargo (2003a) and DeHeer and Vargo (2004). Individual workers from the same colony were grouped together to determine the simplest breeding system that could be invoked to explain the genotype distribution within each colony. Colonies were considered as simple-families when genotypes and the frequencies of genotypes did not significantly differ from the assumed mother and father (primary reproductives) under simple Mendelian patterns of inheritance (Vargo, 2003b). Colonies with five or more alleles at least one locus were regarded as mixed colonies headed by more than one pair of primary reproductives. The breeding structure could not be resolved unambiguously in the case of colonies that did not fit the expected genotype frequencies for progeny of a simple family and that had four or few alleles at all loci.. This is due to the fact that an extended family colony that contains secondary reproductives and a mixed colony in which the kings and queens happen to share the same four or fewer alleles cannot be distinguished clearly.

Genetic Structure and Relatedness

The breeding patterns and genetic differentiation among colonies were further examined with hierarchical F statistics classification using techniques of Vargo (2003a), DeHeer and Vargo (2004). The FSTAT 2.9.3.2 (Goudet, 2001) were also used for all genetic structure analyses. F-statistics followed the notation of Thorne et al. (1999), with the subscripts I, C and T representing the individual, colony, and total components of genetic variation, respectively. The 95% confidence intervals were obtained by bootstrapping over loci 10, 000 times, and the significance of the estimates (coefficient) was further tested by permuting alleles among individuals (Thorne et al., 1999; Bulmer et al., 2001; Copren, 2007; Vargo & Carlson, 2006; Vargo et al., 2006a, b; Parman & Vargo, 2008). F_{IC} is a colony-level inbreeding coefficient which is perhaps the most useful measure as it varies with the number of reproductives as well as their spatial distribution within colonies. The number of reproductives and their mating patterns within a social group is reflected by F_{IC} values (Crozier & Pamilo, 1996; Thorne et al., 1999; DeHeer et al., 2005; DeHeer & Vargo, 2008; Pedereau et al., 2010). For simple families, F_{IC} is expected to be strongly negative.F_{IC} values should approach zero with increasing number of reproductives within colonies, and to become positive if there is assortive mating among multiple reproductives within colonies or there is mixing of individuals from different colonies (Thorne et al., 1999; DeHeer et al., 2005). The F_{IT} is analogous to the inbreeding coefficient F_{IS}, and thus, measures deviation from random mating in the population (the T subscript is synonymous with S because there is no subpopulation structure in this analysis). F_{CT} is comparable to the F_{ST} measure of differentiation among colonies which is very similar to relatedness (R) (Thorne et al., 1999; DeHeer et al., 2005; DeHeer & Vargo, 2008; Pedereau et al., 2010). Genetic relatedness among workers was estimated for each colony and averaged over colonies of the same site using the computer program FSTAT v. 2.9.3.2 (Goudet, 2001).

The correlation between genetic and geographic distances was carried out using the Mantel test command in GENALEX6.4 (Peakall & Smouse, 2005). The Mantel test was executed for matrix correspondence at permutation of 999 so as to test the isolation-by-distance effect.

The unweighted Pair-Group Method with Arithmetic Average Clustering (UPGMA)

We used the program TFPGA (Miller, 1997) to visualize the cluster of genetic similarity among the 20 subterranean termite colonies. The genetic distances were calculated using allelic frequencies and the dendogram was constructed using Nei's unbiased minimum distance (Nei, 1978) and 1,000 permutations of bootstrapping.

Results

Colony Diversity

Allelic diversity ranged between 1 and 6 alleles per locus, with an average of 2.293. The mean percentage of polymorphic loci was 85%. The observed heterozygosity (Ho) (0.690) was higher than the expected heterozygosity (He) (0.416) (Table 2).

Colony Breeding Pattern

There was a strong and highly significant differentiation among the *R. flavipes* and among the pairwise sample points ($P < 0.0001$). Therefore, all 20 sample sites represented different colonies. Based on the genotypes at the seven microsatellite loci and Mendel's laws, 17 samples were classified as simple family colonies, and the remaining three samples i.e. AP (Arapahoe), CB (Cambridge), and BA (Lincoln) were identified as mixed family colonies (Table 2).

Colony Genetic Structure

The genetic differentiation between the simple and mixed family colonies was evaluated by the F statistics and relatedness (Table 2). The inbreeding coefficient F_{IS} (0.099, $P < 0.001$) and the relatedness R (0.846) in the simple family colonies were higher than those of in the mixed family colonies ($F_{IS} = 0.077$, $P = 0.089$; R = 0.667), indicating that the individuals in the simple family colonies had a higher genetic similarity. The colony-level inbreeding coefficient F_{IC} value (-0.6840) in the simple family colonies was negatively higher than that of the mixed family colonies (-0.440), suggesting fewer reproductives involved in the simple family colonies. The genetic differentiation F_{CT} among the simple family colonies was 0.465 ($P < 0.001$), higher than that of

Table 2. Statistics of microsatellite loci and inferred breeding patterns in the 20 colonies of *Reticulitermes flavipes* collected from infested urban structure in Nebraska.

Colony code	Breeding pattern	Number of alleles per locus							Mean of allele/locus	Ho	He
		Rf 11-3	Rf 5-10	Rf 6-1	Rf 11-1	Rf 11-2	Rf 15-2	Rf 21-1			
DC	Simple	1	1	2	2	3	2	2	1.857	0.714	0.388
BH	Simple	2	1	2	2	1	2	2	1.714	0.557	0.311
BL	Simple	3	2	4	2	2	2	2	2.429	0.900	0.524
FS	Simple	2	2	4	2	3	2	2	2.429	0.729	0.449
AL	Simple	2	3	3	4	2	2	4	2.857	0.629	0.499
AP	Mixed	2	3	6	3	4	3	3	3.429	0.786	0.520
MC	Simple	2	2	2	1	2	2	2	1.857	0.786	0.416
CB	Mixed	2	2	3	6	2	2	1	2.571	0.529	0.377
KY	Simple	4	1	2	2	3	3	4	2.714	0.686	0.459
KE	Simple	2	3	4	3	3	2	2	2.714	0.871	0.515
NF	Simple	2	1	2	4	2	2	2	2.143	0.657	0.366
WY	Simple	2	2	4	2	1	1	1	1.857	0.300	0.205
ER	Simple	3	2	2	2	2	1	1	1.857	0.586	0.349
GT	Simple	3	1	2	2	2	3	1	2.000	0.557	0.334
MN	Simple	1	2	2	2	3	2	3	2.143	0.857	0.446
RI	Simple	1	2	2	3	3	3	3	2.429	0.857	0.464
JE	Simple	2	1	2	2	2	2	2	1.857	0.786	0.411
BX	Simple	2	1	2	3	1	2	2	1.857	0.443	0.288
BA	Mixed	5	3	3	2	2	2	2	2.714	0.743	0.491
ME	Simple	2	3	2	2	3	1	4	2.429	0.826	0.459
Average		2.3	1.9	2.8	2.4	2.3	2.1	2.3	2.293	0.690	0.416

the mixed family colonies (0.359; $P < 0.001$), revealing more genetic separation among the simple family colonies. Although the 95% confident intervals were overlapped (Table 2), all estimations displayed the same trends in genetic differentiation between the simple and mixed family colonies, supporting our classification of the colony breeding patterns in this study.

We found the total inbreeding coefficient F_{IT} (0.111, $P < 0.001$) and overall relatedness R (0.826) were significant and high. The F_{CT} value (0.459) was also significant ($P < 0.01$) (Table 3), strongly suggesting that the genetic structure was shaped by non-random mating among the studied colonies. On the other hand, the Mantel test gave a weak but positive correlation between genetic and geographic distances ($Y = 0.0009X + 10.782$, $P = 0.001$), indicating a subordinate distance effect on the genetic differentiation among the studied colonies.

AUPGMA dendrogram is presented in Fig 2. Several colonies from nearby sites (e.g., JE and RI) or neighboring counties (e.g., KY and GT) were clustered together as well as a few colonies from far distances such as AP, ME, MC, MN, JE, and RI. These showed that the effect of geographic distance was sporadic and minor, consistent with the conclusion that the genetic structure among the termite colonies was mainly influenced by their breeding patterns as revealed by the relatedness, F-statistics and Mantel test.

Table 3. *F*-statisticsand Relatedness coefficient: *Reticulitermes flavipes* worker relatedness estimates (*r* value) from 20 infested urban structures. Confidence intervals of 95% are shown in parentheses and the sample size n refers to the number of colonies studied in each population). *P*-values were estimated by permutations.

Colony Location	F_{IT}	*P*-value	F_{CT}	*P*-value	F_{IC}	*P*-value	R
Empirical values							
All colonies (n=20)	0.111	0.000	0.459	0.000	-0.642	0.000	0.826
(CI)	(-0.014 − 0.22)		(0.401 − 0.51)		(-0.721 − -0.545)		(0.791 − 0.863)
Simple family colonies (n=17)	0.099	0.000	0.465	0.000	-0.684	0.000	0.846
(CI)	(-0.027 − 0.201)		(0.405 − 0.516)		(-0.751 − -0.605)		(0.816 − 0.877)
Mixed family colonies (n=3)	0.077	0.089	0.359	0.000	-0.440	0.000	0.667
(CI)	(-0.108 − 0.252)		(0.268 − 0.444)		(-0.627 − -0.219)		(0.535 − 0.774)

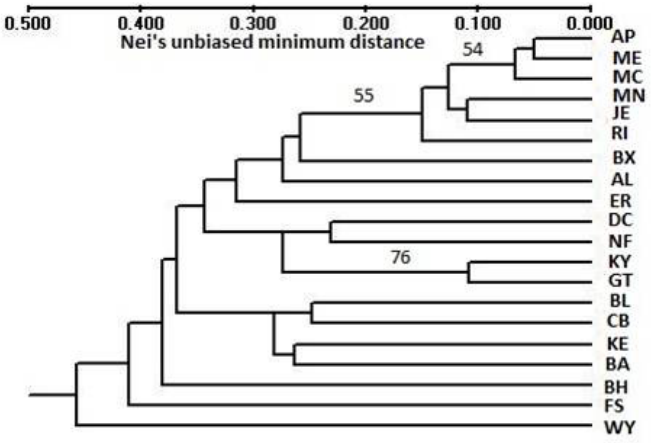

Fig 2. The UPGMA dendrogram of Nei's unbiased minimum distance (Nei,1978) over all 20 populations of *Reticulitermes flavipes* that are denoted in abbreviation. The number at the nodes indicate the bootstrap percentages > 50%. AL = Lincoln; AP = Arapahoe; BA = Lincoln; BH = Omaha; BL = Bellevue; BX = Lincoln; CB = Cambridge; DC = David City; ER = Lincoln; FS = Lincoln; GT = Gothenburg; JE = Lincoln; KE = Kearney; KY = Kearney; MC = McCook; ME = Mead; MN = Minden; NF = Norfolk ; RI = Lincoln; WY = Wymore

Discussion

Overall, the subterranean termites from 17 sites belonged to the simple family colonies, whereas the other three sites/locations (AP, CB, and PA) were found to be of mixed family colonies. No extended family was found in this study. All samples had excess heterozygositybut only the simple family colonies showed significant inbreeding (F_{IT} = 0.099, $P < 0.001$). The negative values of F_{IC} were observed in all colonies regardless of the family types. It can be assumed that colonies with spatially-separated reproductive centers were most likely absent, as this configuration can yield F_{IC} values greater than zero under a large range of conditions (Thorne et al., 1999; Nobre et al., 2008). In contrast, our data yielded negative F_{IC} values, indicating no contribution of colonies being headed by multiple neotenic reproductives inbred for several generations to the reproduction of the population studied. The presence of at least four alleles in each colony was compatible with the hypothesis that all breeders from a colony were descended from a single pair of unrelated primary reproductives.

The F statistics for the simple family colonies were consistent with monogamous reproductive pairings (Table 3) and all colonies in this category should have a single feeding site (Thorne et al., 1999; DeHeer et al., 2005; DeHeer & Vargo, 2008; Pedereau et al., 2010). The frequency of the simple families was 85% in this study. The frequency of the simple families in *R. grassei* populations from Francewas 73% (Clement, 1981; DeHeer et al., 2005). In *Coptotermes formosanus*, 90% of the colonies infesting structures in Nagasaki, Japan consisted of simple families (Vargo & Hussender, 2009). Based on the single polymorphic allozyme marker, Clement (1981) reported a high proportion of simple families of *R. santonensis* population in the La Coubre forest. In the central North Carolina, ~75% of *R. flavipes* colonies were simple families, ~ 25% contained low numbers of neotenic reproductives descended from simple families, and 1-2% were mixed families (Vargo, 2003a, b; DeHeer & Vargo 2004). In Massachusetts, most colonies possessed numerous neotenics, of which ~ 33% were simple families and ~ 10% were mixed families (Bulmer et al., 2001).

The negative values of F_{IC} in the three mixed family colonies indicated that colony fusion was uncommon if ever occurred. F_{IC} value could be positive as two or more unrelated colonies mixed (Vargo & Husseneder, 2011). In addition, the high relatedness (R > 0.5) in this study suggested that kin selection might occur in this species, especially in mixed family colonies (Crozier et al., 1987). According to Thorne (1997), the kin selection proposed for the diploid termites was the mechanism to generate similarly-skewed degrees of relatedness among sibs in comparison to the relatedness between parents and offspring. These would facilitate kin selection as the driving force toward eusociality in the termites and raised unrelated nymphs by indirect fitness and altruism in the colonies. Furthermore, relatedness in these colonies remains high enough for sterile workers to achieve some indirect fitness benefits (Goodisman & Crozier, 2002).

The correlation between genetic and geographic distances is an indication of genetic differentiation attributed to separation-by-distance even though the correlation is weak. This correlation reflects where populations at greater distances are more genetically distinct than those of populations that are geographically close. The weak correlation between genetic and geographic distances in this study could be due to inbreeding Wahlund effect, founder effect, or technical issues including null alleles and errors (Vargo & Husseneder, 2011; Pusadee et al., 2009). It occurs when colonies fuse either they are in the early stages of the fusion process or because there is no interbreeding between reproductives in the two (or more) original colonies (Perdereau et al., 2015). The presence of null alleles could lead to an overestimation of both F_{CT} and genetic distance. Errors during the allele scoring and data analyses could affect the correlation between geographic distance and genetic distance (Chapuis & Estoup, 2007). Furthermore, according to Vargo and Husseneder (2011), ecological factors could shape the colony breeding structure, especially factors that select against inbreeding. A study in the region of southeastern Nebraska with the Dissected Till Plains and the Great Plains (Fraser, 1998) demonstrated that the lands are flat, and lack of distinct geographic factors such as big mountains and lakes to impact the dispersal of *R. flavipes*.

Colony reproduction by budding, if common, should lead to a significant positive relationship between main parental colony and the satellite nest. Moreover, if both parents and offspring colonies contain large numbers of replacement

reproductives (neotenics), it may take several generations for genetic drift to allow one to detect significant genetic differentiation between these physically-separated colonies (Thorne et al., 1999; Vargo, 2003a, b). Nonetheless, no genetic data are available to support the view of budding thus far. In addition, budding in *Reticulitermes* spp. is not common. During mating flights, the reproductives disperse relatively far or they actively avoid relatives when forming tandem pairs (Bulmer & Traniello, 2002; DeHeer & Vargo, 2004; Vargo et al., 2006). In this study, colonies from two locations, namely McCook (MC) and Mead (ME), were clustered together in the UPGMA dendrogram and were inferred as simple families although both locations were > 300 km apart. (Fig 2 and Table 2). Furthermore, these two samples shared the same ecological conditions and genetic similarities, thus determining which of these two factors is of more importance for shaping the breeding structure was hardly possible. Other factors may involve such as founder effects; typically occur when a small number of individuals invade a new habitat and ultimately produce a new population. Moreover, houses built on natural populations can lead to habitat fragmentation due to human disturbance (Booth et al., 2012; Vargo et al., 2006). Vargo and Husseneder (2011) suggested that genetic structure in termite populations with dispersal andcolony breeding regime should be studied in order to gain a better understanding of the roles of genetic drift and selection that may eventually lead to speciation in the termites. However, from the above-mentioned explanations, it is common to encounter distant populations that are genetically similar, and close populations that are genetically different due to the weak correlation between genetic and geographic distance.

Given the limited data on breeding and population genetic structure, there could be an ample opportunity to study the natural selection pressures affecting termite populations and colony breeding structure. Long-term studies across Nebraska either in urban or rural areas concerning *R. flavipes* breeding structure are needed to determine its overall patterns. In addition, further studies examining other urban population in big cities e.g. Omaha and Lincoln will facilitate us to better understand the breeding structure of *R. flavipes* in the Nebraska ecosystem, mainly after the buildings have been treated for termite infestation. More studies are also required to investigate the effect of urbanization on overall breeding and genetic structures of the termites. Our study documents the initial data on the breeding and population genetic structure of *R. flavipes* from the urban environment in Nebraska.

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