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Abstract Genetic markers are a powerful tool to investigate the breeding structure and population genetics of subterranean termites. In this study, 10 - 20 subterranean termite workers, *Reticulitermes flavipes* (Kollar) were collected from colonies at 8 sites, separated by at least 200 m. Ten workers from each site were genotyped at 7 microsatellite loci. The data revealed that all 7 microsatellites were polymorphic with up to 6 alleles per locus. The frequency of most the common allele ranged from 0.11 - 0.60. Observed patterns of genetic variation within colonies revealed that most were the result of fusions of multiple colonies. Few colonies exhibited genetic variation consistent with a simple family headed by a single pair of reproductives. The analyses of F-statistics and relatedness coefficients indicated that the colonies were often inbred, suggesting they contained neotenic reproductives.

Key Words subterranean termites, *Reticulitermes flavipes*, molecular genetics, colony breeding structure

In the United States, subterranean termites are widely distributed and cause economic damage to buildings/structures up to US $1.2 billion annually (Curl 2008). Subterranean termites in the genus *Reticulitermes* spp. exhibit cryptic lifestyles, complex colony structures and specialized caste systems. The subterranean colonies can be connected to many underground galleries from several foraging sites (Thorne and Breisch 2001). Some researchers have used nests, feeding sites and behavioral pairing assays of aggression to characterize the colony affinity of a given nest or group of workers occupying a food source (Long and Thorne 2006). In addition, dye indicators and mark-release-recapture techniques were used to determine colony boundaries and foraging range (Su et al. 1984, Forschler and Townsend 1996, Thorne et al. 1996, Abdul Hafiz et al. 2007).

Molecular genetic markers have been used to understand the field population dynamics of subterranean termites (Husseneder et al. 2003, Thorne et al.1999, Vargo 2003a, b). These molecular techniques led to the estimation of genetic identity, diversity, and ancestry of termites. Microsatellite markers and the Polymerase Chain Reaction (PCR) offer a quick and accurate means of DNA fingerprinting and high resolution genetic identification.

The breeding structure and colony relatedness in subterranean termites are poorly understood. Termite colonies are principally established from a single pair of primary

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reproductives (1 queen and 1 king), usually known as simple family. However, several studies have revealed that more than 2 functional reproductives can coexist within colonies. Multiple reproductives can develop within a colony by secondary reproduction, resulting in extended-family colony (Lenz and Barrett 1982, Myles 1999). Furthermore, colonies headed by unrelated reproductives can fuse and develop a mixed colony (Clement 1981, Jenkins et al. 1999, Bulmer et al. 2001, Matsuura and Nishida 2001, DeHeer and Vargo, 2004, 2008, DeHeer and Kamble 2008, Perdereau et al. 2010). DeHeer and Vargo (2004) provided the first evidence that 2 independent colonies of *Reticulitermes* spp. can fuse over the years. Although genetic structure of subterranean termite colonies has been studied in several regions of the United States (Bulmer et al. 2001, Bulmer and Traniello 2002, Vargo 2003a, b, DeHeer and Vargo 2004, 2006, 2008, Vargo and Carlson 2006, Vargo et al. 2006a, DeHeer and Kamble 2008, Parman and Vargo 2008), specific data are lacking on *R. flavipes* (Kollar) populations in the Midwest and Great Plains, which experience dramatic annual fluctuations in temperature. This study was undertaken to determine the colony genetic and breeding structure of *R. flavipes* from natural populations in Nebraska using 7 microsatellite markers and fragment analysis methods.

Materials and Methods

Field collection of subterranean termites. Subterranean termite workers were collected from logs at 8 locations within forested sections of Wilderness Park, Lincoln, NE. Termites were identified as *R. flavipes* according to Husen et al. (2006). A minimum of 20 termite workers were extracted from each location and placed into glass vials containing 95% ethanol, stored at -20]C until further analysis. The location of each feeding site was recorded using a hand-held GPS unit (SporTrak™ Map, Thales Navigation, Santa Clara, CA).

DNA extraction. Genomic DNA was extracted from each of 10 worker heads from each feeding site using a Qiagen DNeasy Kit (QIAGEN USA, Valencia, CA). The manufacturer's protocols were followed except that treatments with Proteinase K solution and RNase were omitted and DNA was eluted in 80 µL of 1X TE solution. The concentration of DNA in each extract was quantified using a nanodrop Spectrophotometer (Nanodrop Technologies, Inc. Wilmington, DE, USA)

Microsatellite genotyping. Each termite worker was genotyped at 7 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). For each microsatellite marker, the forward primer was labeled with 1 of 3 WellRED Fluorescent labels (D2, D3, and D4) for running on the Beckman CEQ 8,000 (SIGMA-Proligo, The Woodlands, TX, USA). The PCR reactions were set up in 96-well plates in 15-µL reaction mixtures containing 10X PCR buffer, 50 mM $MgCl_2$, 10 mM Dntp mix, 0.025 µM forward primer 0.025 µM reverse primer, 100 units Taq DNA polymerase (Invitrogen) 2.0 ng DNA template. All loci were amplified using a PCR thermal cycler program with an initial denaturation step 95]C (30s), followed by 35 cycles at 95]C (30s), 54]C (30s), and 72]C (30s). The reaction was terminated with one cycle 72]C (5 min) and then held at 4]C until removed from the PCR thermal cycler. Fragments were separated and sized by capillary electrophoresis using a Beckman CEQ 8,000 Genetic Analyzer in conjunction with 400 bp size standard. Data were analyzed and hand-scored using CEQ 8,000 Fragment Analysis Software version 8.0, and a subset of samples for each locus was confirmed by a second human reader (Fig. 1).

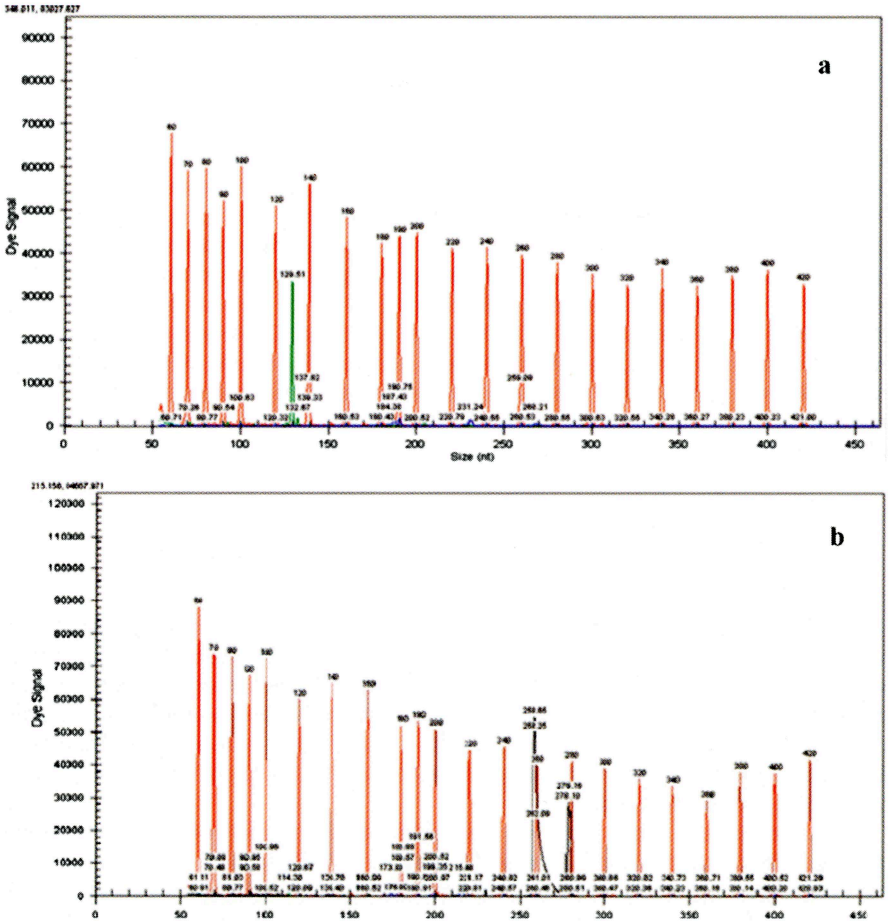

Fig. 1. Representative electropherograms after capillary electrophoresis separation of the fragments amplified by PCR. Alleles of *Reticulitermes flavipes* are showed in (a) blue and green, and (b) black peaks, whereas the red peaks are for the molecular weight size standard by WellRED dyes.

Colony identity. Allelic diversity, expected and observed heterozygosity were calculated using Fstat 2.9.3.2 (Goudet 2001). Exact tests of genotypic differentiation were performed using GENEPOP on the Web (Goudet et al. 1996) http://wbiomed.curtin.edu.au/genepop/index.html) to determine if termites from different collection points belonged to the same colony or not. When 2 independent samples of workers are drawn from the same colony, we are sampling from the distribution of genotypes within that colony. Conversely, when 2 samples of workers are drawn from 2 different colonies, we are sampling from 2 different distributions of genotypes. This this is true regardless of the specific breeding structure of colonies involved. Therefore, if we test for differences in genotype frequencies between 2 samples of workers, we expect the

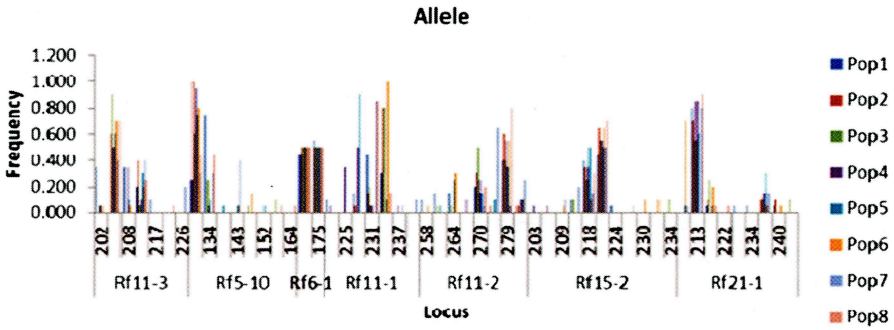

Fig. 2. Allele frequency at seven microsatellite locus within 8 colonies of *Reticulitermes flavipes*.

test to be significant if they come from different colonies and nonsignificant if they come from the same colony.

Classification of breeding structure. Breeding structure was classified using the techniques of Vargo (2003a) and DeHeer and Vargo (2004). Individuals from the same colony were grouped together to determine the simplest breeding system that could be invoked to explain the genotype distributions within each colony. If colonies consisted of workers whose genotypes could be reconstructed by assuming a single mother and father, and the frequencies of the observed genotypes did not differ significantly from those expected under simple Mendelian patterns of inheritance for this hypothetical pair (using a G-test summed over all loci, e.g., Vargo 2003b), then the colony was classified as a simple-family colony, headed by the original colony-founding pair of reproductives. Colonies that had 5 or more alleles at least 1 locus could be unambiguously identified as mixed colonies headed by more than 1 pair of primary reproductives. In the case of colonies that do not fit the expected genotype frequencies for progeny of a simple family and that had 4 or few alleles at all loci the breeding structure could not be resolved unambiguously. This is because it is not possible to distinguish between an extended family colony that contains secondary reproductive and a mixed colony in which the kings and queens happen to share the same 4 (or fewer alleles).

F-Statistics and relatedness coefficients. To determine specific genetic structure of subterranean termite colonies, F-statistics and relatedness coefficients were computed using the program FSTAT v. 2.9.3.2 (Goudet 2001). 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 coefficients was tested by permuting alleles among individuals. The overall inbreeding coefficient (F_{IT})] reflects the deficiency of heterozygotes because of nonrandom mating within populations from 8 sampling locations. F_{CT} estimates the amount of genetic differentiation (based on allele frequency differences) among colonies. 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. F_{IC} provides information on the number of reproductive and relatedness

among them. It is expected to be negative in simple families headed by a pair of reproductives (Thorne et al. 1999, Bulmer et al. 2001, Copren 2007, Vargo and Carlson 2006, Vargo et al. 2006a, b, Parman and Vargo 2008). For simple families, F_{IC} is expected to be strongly negative, F_{IC} values should approach zero with increasing number of reproductive within colonies and to become positive if there is assertive mating among multiple reproductive within colonies or there is mixing of individuals from different colonies (Crozier and Pamilo 1996, Thorne et al. 1999, DeHeer et al. 2005, DeHeer and Vargo 2008, Perdereau et al. 2010).

Genetic relatedness among workers was estimated for each colony and averaged over colonies of the same site. The standard errors of the means were obtained by jackknifing over colonies. For the allelic frequencies and the average relatedness estimates, colonies were weighed equally.

Results

Colony identity and boundaries. Termites collected from 8 sites were micromorphologically identified as *R. flavipes* according to Husen et al. (2006). Of the 10 termite workers screened per location, 2 - 6 microsatellite alleles (mean 3.17) were detected per locus (Table 1, Fig. 2). Expected heterozygosity within colonies ranged from 0.59 - 0.72 (mean 0.6), and observed heterozygosity ranged from 0.31 - 0.99 (mean 0.55) (Table 1). The distance between collection points within colonies was up to 200 - 500 m apart. There was strong and highly significant differentiation among the *R. flavipes* sample points ($p < 0.0001$). Therefore, all the 8 sample points represented different colonies.

Breeding structure of colonies. Of the 8 colonies, 2 colonies (25%) yielded worker genotypes consistent with those expected under a single pair of reproductive. No more than 4 alleles and no more than 4 genotypic classes were detected, all segregating with Mendelian ratios expected for a single pair of reproductives. Five colonies (62.5%) exhibited greater than 4 alleles per locus, thus providing evidence that colony fusion occurred (mixed colony genetic structure). These colonies were headed by more than 2 unrelated reproductives and consequently were classified as mixed family colonies (Table 1). One colony (12.5%) had no more than 4 alleles per locus but exhibited greater than 4 genotypic classes and could therefore either have been a mixed colony or an extended family colony with secondary reproductives.

Genetic structure and relatedness of colonies. The overall measure of inbreeding, F_{IT}, was significantly greater than zero (Table 2), indicating a general deficit of heterozygosity compared with the expectations under Hardy-Weinberg genotypic equilibrium.

The colonies classified as being simple families had a significant, negative F_{IC} (-0.296) indicating an excess of heterozygotes compared with a panmictic population with the same allele frequencies. This is consistent with the expected value of F_{IC} for a simple family (-0.209 to -0.33) (Bulmer et al. 2001, Vargo and Husseneder 2009). Furthermore, simple family colonies were genetically different with positive F_{CT} value (F_{CT}=0.195, 95% C.I -0.004 - 0.398) (Table 2)

Mixed family colonies also had a negative F_{IC} (-0.106) indicating an excess of heterozygosity similar to simple family colonies. Average relatedness values within simple family and mixed colonies were also similar (Table 2). A high level of genetic differentiation among the colonies classified as mixed family colonies (F_{CT}=0.245) (Table 2).

Table 1. Variability of microsatellite loci and basic summary statistics for 8 colonies of *Reticulitermes flavipes* collected from Wilderness Park, Lincoln, NE.

		No of alleles detected per locus/per sample							
Colony	**Family Structure**	Rf 11 - 3	Rf 5 - 10	Rf 6 - 1	Rf 11 - 1	Rf 11 - 2	Rf 15 - 2	Rf 21 - 1	Mean number of alleles
1	Mixed	4	2	2	4	5	4	5	3.7
2	Simple	2	1	2	3	4	2	4	2.5
3	unclassified	3	4	2	2	4	4	4	3.28
4	Mixed	4	2	2	5	4	4	2	3.28
5	Mixed	3	5	2	2	6	2	4	3.43
6	Mixed	4	3	2	1	4	5	4	3.29
7	Mixed	3	3	2	3	4	5	3	3.28
8	Simple	3	4	2	2	2	2	3	2.57
Total		3.25	3	2	2.75	4.125	3.5	3.625	3.17
H_e		0.629	0.536	0.503	0.705	0.722	0.587	0.551	0.6
H_o		0.588	0.338	0.988	0.313	0.625	0.575	0.413	0.55

Table 2. F-statistics and relatedness: *Reticulitermes flavipes* worker relatedness estimates (r) in natural colonies (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 (Wilderness Park, Lincoln, NE)	F_{IT}	P-value	F_{CT}	P-value	F_{IC}	P-value	R
Empirical values							
All colonies ($n = 8$)	0.166	0.0003	0.215	0	–0.126	0.0004	0.385
(SE)	(-0.246 - 0.377)		(0.094 - 0.353)		(-0.428 - 0.069)		(0.212 - 0.543)
Simple family colonies 2010 ($n = 2$)	–0.043	0.5758	0.195	0	–0.296	0.0005	0.408
(SE)	(-0.467 - 0.303)		(-0.004 - 0.398)		(-0.635 - 0.048)		(-0.012 - 0.683)
Mixed family colonies 2010 ($n = 5$)	0.165	0	0.245	0	–0.106	0.0095	0.421
(SE)	(-0.189 - 0.421)		(0.111 - 0.392)		(-0.412 - 0.124)		(0.236 - 0.589)

Discussion

These data provide new information on the breeding system of *R. flavipes* from a natural population in the Midwest region of the U.S. with extreme subzero temperatures in winter. Our findings provide an insight into composition of colonies in natural population. The *R. flavipes* from North Carolina contained approximately 75% of colonies consisting of simple families, about 25% contained low numbers of neotenic reproductive descended from simple families to mixed families (Vargo 2003a, b, DeHeer and Vargo 2004). Meanwhile, *R. flavipes* colonies in Massachusetts and Nebraska located at the Northern and Western part of the United States, had a majority of colonies containing many neotenics, with Massachusetts colonies mostly 33% are simple families and about 10% are mixed where the majority of colonies are composed of a single pair reproductive and their worker/soldier progeny. These are colonies that have yet to produce neotenic reproductive or those that contain neotenics that have not yet produced a progeny (DeHeer and Kamble 2008, Bulmer et al. 2001). In *Coptotermes* spp., studies showed that most introduced species have variety of colony breeding structures. The proportion of simple families varies from nearly 100% in 2 Japanese populations to no simple families present in a population from the native range in Southern China (Husseneder et al. 2008). Our data indicate that 25% of the colonies we examined consist of simple families. A negative F_{IC} value suggested that these colonies have an excess heterozugosity and low number of reproductive compared with the study conducted by DeHeer and Kamble (2008), which previously showed very high inbreeding in the colonies (positive F_{IC} value).

Mixed colonies, or colony fusion was evident based on our data, although our data showed a high proportion of mixed colonies (62.5%), the incidence was lower than the study conducted by DeHeer and Kamble (2008), where they found majority of colonies were mixed colonies and no simple family colonies. In addition, Korb and Schneider (2007) also reported a record of mixed colonies representing 25% of those sampled. Mixed family colonies have been recorded in a number of termite species, including *R. flavipes* (DeHeer and Vargo 2004, DeHeer and Kamble 2008), *R. grassei* Clement (Clement 1981, Clement et al. 2001), *Mastotermes darwiniensis* Froggatt (Goodisman and Crozier 2002), *Macrotermes michaelseni* Sjostedt (Hacker et al. 2005), and *Zootermopsis nevadensis* Hagen (Aldrich and Kambhampati 2007).

Studies from the laboratory and field indicate that the presence of multiple unrelated groups of reproductives in fused colonies of *R.)avipes* basically have shorter life, and over time reproduction in fused colonies is only limited to individuals from just 1 of the original source colonies (Deheer and Vargo 2008, Fisher et al. 2004). Clement (1981) and Clement et al. (2001) suggest that a breakdown of nest mate recognition results in colonies with overlapping of foraging areas in close proximity to each other. The variation in the tendency to form mixed colonies among species is not well understood, but geographic variation in inbreeding depression may play a role in determining colony breeding structure in certain species (Brandl et al. 2001, 2004, Kaib et al. 2001).

The high degree of variability in *R. flavipes* colony structure on a small spatial scale contrasts with patterns of colony structure seen in the social Hymenoptera (Crozier and Pamilo 1996). There are cases of monogynous and polygynous colonies occurring together in some populations of the ants Formica and Myrmica, and *Solenopsis invicta* Buren, although gene flow appears to be restricted between the social forms (Pamilo et al. 1997).

In this study, we found that relatedness values for simple family colonies and mixed family colonies were almost similar. Genetic studies of other termites have shown that the degree of relatedness of the founders in monogamous colonies (simple families) varies among species and populations within the same species. Simple family colonies of *Nasutitermes corniger* Motschulsky (Atkinson and Adams 1997), *Schedorhinotermes lamanianus* Sjostedt (Husseneder et al. 1999), *R.)avipes*, *R. virginicus* and *R. hesperus* are generally headed by a pair of unrelated colony founders, whereas the reproductive pairs of *R. hageni* and *R. malletei* colonies are often related (Copren 2007, Vargo and Carlson 2006, Vargo et al. 2006b, Parman and Vargo 2008). In a French population of *R. grassei*, simple family colonies had closely-related reproductives (DeHeer et al. 2005) whereas unrelated reproductives were found in colonies of a Portuguese population (Nobre et al. 2008). Simple family colonies in Japanese populations of *C. formosanus* were headed by siblings, whereas colony founders were almost unrelated in a New Orleans population (Vargo et al. 2003, 2006a). Reproductive pairs of *C. lacteus* were related in Australia, but at a low level, which Thompson et al. (2007) suggested was due to selection for mating with distant relatives. However, there is little evidence of mate discrimination during tandem pair formation in termites. Therefore, the most likely determinant of the degree of relatedness among founders is the probability of related males and females interacting with each other during mating)ights as dictated by the distance they disperse from their originated nest.

In conclusion, the subterranean termite *R. flavipes* colonies in this study consist of variable breeding structure. High inbreeding values and relatedness suggest the *R. flavipes* colonies in our study site contain reproductive neotenics. Due to abundant food resource in the area and absence of human disturbance, the colonies can extend and fuse over time.

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References Cited

Abdul Hafiz, A. M., A. Abu Hassan, M. Z. A. Rashid and M. R. Che Salmah. 2007. Field efficacy of imidacloprid on *Globitermes sulphureus* (Isoptera: Termitidae) (subterranean termite) in Penang. Trop. Life Sci. Res. 18: 109-114.

Aldrich, B. T. and S. Kambhampati. 2007. Population structure and colony composition of two *Zootermopsis nevadensis* subspecies. Heredity 99: 443-445.

Atkinson, L. and E. S. Adams. 1997. The origins and relatedness of multiple reproductives in colonies of the termite *Nasutitermes corniger.* Proc. Biol. Sci. 264: 1131-1136.

Brandl, R., M. Hacker, R. K. N. Bagine and M. Kaib. 2001. Geographic variation of polygyny in the termite *Macrotermes michaelseni* (Sjostedt). Insectes Soc. 48: 134-137.

Brandl, R., M. Hacker, R. K. N. Bagine and M. Kaib. 2004. Yearly variation of polygyny in the termite *Macrotermes michaelseni* (Sjostedt). Insectes Soc. 51: 294-298.

Bulmer, M. S. and J. F. A. Traniello. 2002. Foraging range expansion and colony genetic organization in the subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Environ. Entomol. 31: 293-298.

Bulmer, M. S., E. S. Adams and J. F. A. Traniello. 2001. Variation in colony structure in the subterranean termite *Reticulitermes flavipes.* Behav. Ecol. Sociobiol. 49: 236-243.

Clement, J. L. 1981. Enzymatic polymorphism in the European populations of various *Reticulitermes* species (Isoptera), Pp. 49–61. *In* Howse, P.E. and J.L. Clement (eds.), Biosytematics Social Insects. Academic Press, London.

Clement, J. L., A. G. Bagnéres, P. Uva, L. Wilfert, A. Quintana, J. Reinhard and S. Dronnet. 2001. Biosystematics of *Reticulitermes* termites in Europe, morphological, chemical and molecular data. Insectes Soc. 48: 202-215.

Copren, K. A. 2007. Characterization of microsatellite loci in the western subterranean termite *Reticulitermes hesperus* and cross-amplication in closely related cryptic species. J. Insect Sci. 7: 17.

Crozier, R. H. and P. Pamilo. 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford Univ. Press, Oxford.

Curl, G. 2008. A strategic analysis of the U.S. structural pest control industry, Specialty Products Consultants, LLC, Mendham, NJ.

DeHeer, C.J. and S.T. Kamble. 2008. Colony genetic organization, fusion and inbreeding in *Reticulitermes flavipes* from the Midwestern U.S. Sociobiol. 51: 307-325.

DeHeer, C. J. and E. L. Vargo. 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. Mol. Ecol. 13: 431-441.

DeHeer, C. J. and E. L. Vargo. 2006. An indirect test of inbreeding depression in the termites *Reticulitermes flavipes and Reticulitermes virginicus*. Behav. Ecol. Sociobiol. 59: 753-761.

DeHeer, C. J. and E. L. Vargo. 2008. Strong mitochondrial DNA similarity but low relatedness at microsatellite loci among families within fused colonies of the termite *Reticulitermes flavipes.* Insectes Soc. 55: 190-199.

DeHeer, C. J., M. Kutnik, E. L. Vargo and A. G. Bagnères. 2005. The breeding system and population structure of the termite *Reticulitermes grassei* in Southern France. Heredity 95: 408-415.

Fisher, M. L., R. E. Gold, E. L. Vargo and A. I. Cognata. 2004. Bahavioral and genetic analysis of colony fusion in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiol. 44: 565-576.

Forschler, B.T. and M. L. Townsend. 1996. Mark-release-recapture estimates of *Reticulitermes spp.* (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, U.S.A. Environ. Entomol. 25: 952-962.

Goodisman, M. A. D. and R. H. Crozier. 2002. Population and colony genetic structure of the primitive termite *Mastotermes darwiniensis*. Evolution 56: 70-83.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). (http://www.unil.ch/izea/softwares/fstat.html).

Goudet, J., M. Raymond, T. DeMeeus and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics 144: 1933-1940.

Hacker, M., M. Kaib, R. K. N. Bagine, J. T. Epplen and R. Brandl. 2005. Unrelated queens coexist in colonies of the termite *Macrotermes michaelseni*. Mol. Ecol. 14: 1527-1532.

Husen, T. J., S. T. Kamble and J. M. Stone. 2006. A characterization of subterranean termites in Nebraska using micro-morphological and molecular techniques. Sociobiol. 48: 247-266.

Husseneder, C., R. Brandl and C. Epplen. 1999. Within-colony relatedness in a termite species: genetic roads to eusociality? Behaviour 136: 1045-1063.

Husseneder, C., E. L. Vargo and J. Grace. 2003. Molecular genetic methods: New approaches to termite biology, Pp. 358-370. *In* Goodell, B., D.D. Nicholas and T.P. Schultz (eds.), Wood deterioration and preservation: advances in our changing world. Oxford Univ. Press.

Husseneder, C., J. E. Powell, J. K. Grace, K. Matsuura and E. L. Vargo. 2008. Worker size in the Formosan subterranean termite in relation to colony breeding structure as inferred from molecular markers. Environ. Entomol. 37: 400-408.

Jenkins, T. M., C. J. Basten and R. Dean. 1999. Matriarchal genetic structure of *Reticulitermes* (Isoptera: Rhinotermitidae) populations. Sociobiol. 33: 239-263.

Kaib, M., M. Hacker and R. Brandl. 2001. Egg-laying in monogynous and polygynous colonies of termite *Macrotermes michaelseni* (Isoptera, Macrotermitidae). Insectes Soc. 48: 231-237.

Korb, J. and K. Schneider. 2007. Does kin structure explain the occurrence of workers in the lower termite *Cryptotermes secundus?* Evol. Ecol. 21: 817-828.

Lenz, M. and R. A. Barrett. 1982. Neotenic formation in field colonies of *Coptotermes lacteus* (Froggatt) in Australia, with comments on the roles of neotenics in the genus Coptotermes (Isoptera: Rhinotermitidae)..Sociobiol. 7: 47-59.

Long, C. E. and B. L. Thorne. 2006. Resource fidelity, brood distributing and foraging dynamics in laboratory colonies of *Reticulitermes flavipes* (Isoptera:Rhinotermitidae). Ethol. Ecol. Entomol. 18: 113-125.

Matsuura, K. and T. Nishida. 2001. Colony fusion in a termite: what makes a society 'open'? Insectes Soc. 48: 378-383.

Myles, T. G. 1999. Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. Sociobiol. 33: 1-43.

Nobre, T., L. Nunes and D. Bignell. 2008. Colony interactions in Reticulitermes grassei population assessed by molecular genetic methods. Insectes Soc. 55: 66-73.

Pamilo, P., P. Gertsch, P. Thoren and P. Seppa. 1997. Molecular population genetics of social insects. Annu. Rev. Ecol. Evol. Syst. 28: 1-25.

Parman, V. and E. L. Vargo. 2008. Population density, species abundance, and breeding structure of subterranean termite colonies in and around infested houses in central North Carolina. J. Econ. Entomol. 101: 1349-1359.

Perdereau, E., A. G. Bagneres and S. Dupont. 2010. High occurrence of colony fusion in a European population of the America termite *Reticulitermes flavipes.* Insectes Soc. 57: 393-402.

Su, N.Y., M. Tamashiro, J. R. Yates and M. I. Haverty. 1984. Foraging behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Environ. Entomol. 13: 1466-1470.

Thompson, G. J., M. Lenz, R. H. Crozier and B. J. Crespi. 2007. Molecular-genetic analyses of dispersal and breeding behaviour in the Australian termite *Coptotermes lacteus*: evidence for non-random mating in a swarm-dispersal mating system. Aust. J. Zool. 55: 219-227.

Thorne, B. L. and N. L. Breisch. 2001. Effects of sublethal exposue to imidacloprid on the subsequent behavior of the subterranean termite *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 492-498.

Thorne, B. L., J. F. A. Traniello, E. S. Adams and M. Bulmer. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae): a review of evidence from behavioral, ecological, and genetic studies. Ethol. Ecol. Evol. 11: 149-169.

Thorne, B. L., E. Russek-Cohen, B. T. Forschler, N. L. Breisch and J. F. A. Traniello. 1996. Evaluation of mark-release-recapture methods for estimating forager population size of subterranean termite colonies. Environ. Entomol. 25: 938-951.

Vargo, E. L. 2000. Polymorphism at nucleotide microsatellite loci in the subterranean termite, *Reticulitermes flavipes.* Mol. Ecol. 9: 817-820.

Vargo, E. L. 2003a. Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. Evolution 57: 2805-2818.

Vargo, E. L. 2003b. Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait. Environ. Entomol. 32: 1271-1282.

Vargo, E. L. and J. R. Carlson. 2006. Comparative study of breeding systems of sympatric subterranean termites (*Reticulitermes flavipes* and *R. hageni*) in Central North Carolina using two classes of molecular genetic markers. Environ. Entomol. 35: 173-187.

Vargo, E. L. and C. Husseneder. 2009. The biology of subterranean termite: Insight from molecular studies on *Reticulitermes* and *Coptotermes*. Annu. Rev. Entomol. 54: 379-403.

Vargo, E. L., C. Husseneder and J. K. Grace. 2003. Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. Mol. Ecol. 12: 2599-2608.

Vargo, E. L., C. Husseneder and D. Woodson. 2006a. Genetic analysis of colony and population genetic structure of three introduced populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the Continental United States. Environ. Entomol. 35: 151-166.

Vargo, E. L., T. R. Juba and C. J. DeHeer. 2006b. Relative abundance and comparative breeding structure of subterranean termite colonies (*Reticulitermes flavipes, Reticulitermes hageni, Reticulitermes virginicus,* and *Coptotermes formosanus)* in a South Carolina lowcountry site as revealed by molecular markers. Ann. Entomol. Soc. Am. 99: 1101-1109.