Genetic diversity, colony genetic structure, colony identity and breeding structure of the western drywood termite, *Incisitermes minor* (Hagen).

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Abstract

The western drywood termite, *Incisitermes minor*, represents one of the most important and destructive termite species in the United States. Despite this however, and in comparison to the subterranean termites of the genus *Reticultermes*, nothing is known regarding the genetic colony structure, colony and population differentiation, and breeding structure. Understanding each of these components is essential for the development of, and the understanding of results of management strategies for control. For example, understanding the identity of and the genetic connection, or lack of, between colonies infesting a property will enable the pest control specialist to monitor over time the effectiveness of control strategies given that the molecular methods described here enable accurate identification at the colony level. Thus, using these markers it is possible to track over time change in colony infestion (i.e. if a colony is replaced by a genetically unrelated colony), colony spread (through sampling of individuals at different points in a structure), and colony breeding structure (i.e. the switch from simple families to extended, an important factor linked to the potential for rapid population expansion).

Within this study we have developed a suite of 15 microsatellite markers (small sections of the termite DNA that offer the construction of unique genetic fingerprints for individuals) for *I. minor*. This class of molecular marker is used commonly for studies in other species, including humans, to address questions such as paternity and individual identity (e.g. in crime case). The application of such markers to studies of termite biology and population structure is now common, however lacking for the drywood termites. Here we applied a selection of these markers to address three key questions relating to infestations in the urban and agricultural environment at several sites across California: 1) Colony identity, 2) Determination of breeding structure, and 3) Colony genetic structure. Results reveal that using these markers we can accurately identify cases of multiple

collections actually representing single, potentially expansive infestations by a single colony. Assuming that individuals within these expansive colonies share connections to each other and to the reproductives, such information with prove important in understanding how insecticide is transferred within a colony from a point of introduction. Colony breeding structure proved variable, with all three forms previously observed in the eastern subterranean termites also detected here (i.e. Simple families headed by a single pair of reproductives, Extended families resulting from inbreeding within the colony, and Mixed colonies potentially resulting from the fusion of two or more genetically unrelated colonies). Understanding breeding structure allows us to make predictions of colony size and age, and therefore potentially the level of damage to an infested property. Simple families are likely to be small, capable of producing relatively few workers, thus damage may be limited and restricted to a small area. Extended and mixed families however have significantly greater reproductive output, and therefore colonies may consist of considerable numbers of workers inflicting damage over a larger area within the property. Finally, understanding genetic structure within colonies allows us to understand the levels of inbreeding, an estimate linked closely to both the adaptive ability of a colony, and to the number of secondary reproductives reproducing within a colony. This estimate allows researchers to more accurately predict colony potential size and therefore again may be used to estimate the spatial scale over which damage may occur. All of these factors are especially important in a species, like *I. minor* that is cryptic within properties, thus being able to make these estimates early in treatment may enable pest operators make more informed decisions of the control strategies available.

Introduction

The western drywood termite, *Incisitermes minor* (Hagen), is recognized as one of the most economically important and destructive termite species in the United States (Su & Scheffrahn 1990). Although native from northwestern Mexico to southwestern United States, due to the ease of both intra- and inter-continental movement of infested furniture and timbers, infestations have been documented outside their native range (Hathorne *et al*. 2000; Xie *et al*. 2001; Indrayani *et al*. 2005). Given the vast economic impact of this species (Rust *et al* 1988), the understanding of population genetic structure and breeding systems is fundamental to the development of more effective management strategies.

I. Identification and characterization of 15 polymorphic microsatellite loci in the western drywood termite, *Incisitermes minor* (Hagen)

Modern molecular markers such as microsatellites, restriction fragment length polymorphisms (RFLP), and mitochondrial DNA sequencing (mtDNA) are now commonly used to address questions relating to species identification, phylogeography, life history, and dispersal behaviors of social insects (Ross 2001, Vargo & Husseneder 2009). Of these, microsatellite markers offer the greatest potential for studies relating to breeding structure and colony identification. According to Avise (2004), microsatellites are probably the most popular and powerful of the current molecular markers. Microsatellite loci are co-dominant markers, with one allele being contributed to the offspring from each of the parents, in a Mendelian fashion. Each microsatellite locus is composed of reiterated short sequences (usually of di, tri, or tetra-nucleotides [see Fig. 1 and 2 for examples]) that are tandemly arrayed at a particular chromosomal location (Hamada et al. 1984), with variation in repeat copy number often underlying a profusion of distinguished alleles within a population (Avise 2004). Additionally, they can be amplified from small quantities of tissue. The main drawback of these markers is that unless they have already been characterized for the species in question, or even a closely related species, they were, and to many still are, considered difficult, time consuming and costly to isolate. Idrayani et al. (2006) published a set of 10 polymorphic markers for *I. minor*. Of these, however, eight consisted of di-nucleotide repeats, a characteristic often

considered unfavorable due to the production of stutter bands when amplified. These stutter bands often make accurate determination of the individual genotype (i.e. homozygote [1 band] or heterozygote [2 bands] difficult to distinguish (See Fig. 1). This is not an issue with tri and tetra-nucleotide loci (See Fig. 2)

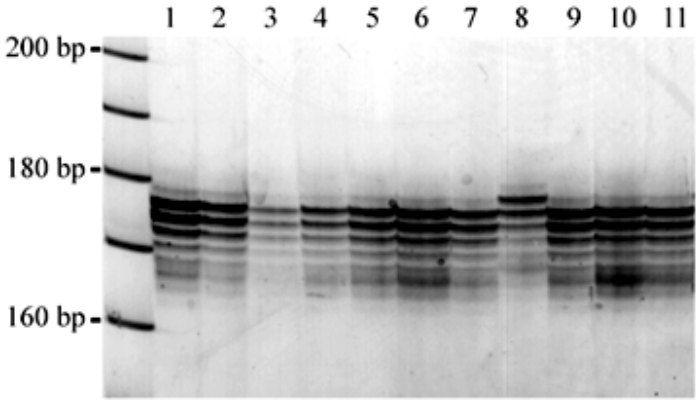

Fig. 1. Example di-nucleotide microsatellite marker. Gel image portrays 11 individuals (1 – 11) and a marker lane (left)

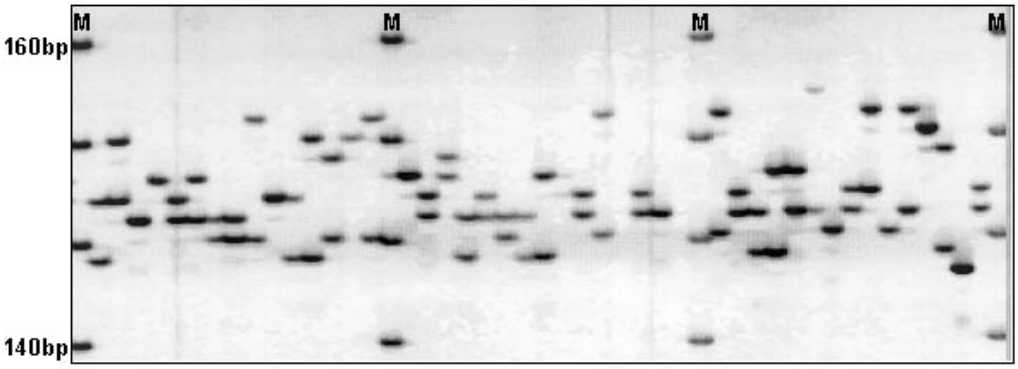

Fig. 2. Example tetra-nucleotide microsatellite marker. Gel image portrays 45 (3 groups of 15) samples and 4 size standard ladders (M).

Given the lack of suitable tri- and tetra-nucleotide microsatellite markers for *I. minor*, we developed an enriched library from which we isolated and characterized 15 polymorphic markers (Booth et al. 2008). Of these, five consisted of tri-nucleotide repeats, eight of tetra-nucleotide repeats, and only two di-nucleotide repeat motifs. Initial screening of 30 individuals, representing six individuals from a total of five

geographically separated locations, revealed that the number of unique alleles per microsatellite locus ranged from three to fifteen. Observed heterozygosity, calculated from 23 individuals collected within a single location ranged from 0.050 to 0.866. Preliminary results confirmed these markers yielded sufficient within and between colony/population polymorphism for the resolution of patterns of dispersal, gene flow and colony breeding structure in *I. minor* (Booth et al. 2008).

II. Breeding structure and colony identification

Introduction

Termite colonies are considered to fall into three categories. When reproduction within the colony is controlled by a single king and queen, the colony is referred to as a simple family. Following the death of one or both primary reproductives, the colony may undergo several rounds of inbreeding as a result of reproduction by the secondary neotenics. This is referred to as an extended family. Finally, colonies have been shown to fuse (Deheer & Vargo 2004, 2008; Johns et al. 2009). In these rare events, workers within a colony exhibit genetic ancestry to more than one colony. These are referred to as fused or mixed family colonies.

To date no published data are available regarding the genetic identification of breeding structure, colony identification and colony genetic structure in *I. minor*. An early study by Harvey (1934) described the reproductive life history as follows: Upon king/queen bond formation, and following the excavation of a royal chamber, a queen will lay approximately two to five eggs within the first year, eight to 15 within the second, and fecundity will increase with each successive year until reaching a peak after 10 to 12 years. At five years a colony may consist of the primary reproductives, 20 soldiers and 500 nymphs. A colony at 10 years of age may contain one or more secondary (neotenic) brachypterous reproductives in addition to the primaries. Harvey (1934) acknowledged that these secondary reproductives may supplement egg production, however noted that the existence of more than one pair of functional reproductives, even in older, larger colonies was unlikely. Additional reproductives identified in *I. schwarzi* colonies were considered to result from the fusion of different

established colonies (Luykx 1986). In contrast, molecular evidence of breeding structure within the subterranean termites (Rhinotermitidae) [See Vargo and Husseneder (2009) for review], has revealed that secondary reproductives are both common and play a significant role in egg production resulting in extended family breeding structure in this group. Korb & Schneider (2007) report extended families comprised 16% of colonies in the Australian drywood termite *Cryptotermes secundus* (Hill), while 25% of colonies were the result of fusion events; however no information regarding the details of this study are provided.

The objective of this study was to determine the levels of genetic diversity within geographically separate colonies of *I. minor* using a set of species specific microsatellite markers, thus evaluating the power this marker set offers for future genetic studies of *I. minor*. Within two landscapes (Urban vs. Agricultural) we used these markers to examine the colony genetic structure, identity, and breeding structure.

Materials and Methods

Sample Collection and microsatellite screening

A total of 23 colonies were sampled from 11 distinct collection sites in California. Collection sites were split into two environment classes, urban and agricultural. Ten sites were classified as urban. These were as follows: North Hollywood Hills (n = 5), Oakland (n = 1), Los Angeles (n = 2), Santa Cruz (n = 1), Granada Hills (n = 1), New Port Beach (n = 1), Riverside (n = 2), Lakeview (n = 1), La Jolla (n = 1) and Cypress (n = 1). Agricultural samples were collected within a vineyard near Fresno CA (n = 7). From each distinct collection genomic DNA was extracted from 10 to 20 workers using the DNeasy Tissue Kit (Qiagen, Valencia, CA). These individuals were genotyped at five polymorphic microsatellite loci (*DW*-11, *DW*-12, *DW*-27, *DW*-39, and *DW*-46) following the method described in Booth et al (2008). These loci were selected from the suite of 15 described earlier based on the level of polymorphism and observed heterozygosity.

Colony identity

In order to determine colony identity genotypic differentiation was tested between colony pairs following the permutation methods implemented in FSTAT (Goudet 2001). Additionally log-likelihood (*G*) based exact tests of genotypic differentiation (Goudet et al. 1996) were performed on each pair of sample colonies, as implemented by the program GENEPOP ON THE WEB (option 3, suboption 4; Raymond and Rousset, htpp://wbiomed.curtin.edu/genepop/index.html). Using this program individual locus based estimates of significance were calculated with overall significance determined for each pair-wise comparison after Bonferroni correction based on the number of loci screened. Default parameters within this program were followed.

Classification of Breeding Structure

Colony breeding structure was classified following Vargo (2003), and DeHeer and Vargo (2004). Under this classification colonies are placed into one of three groups. Simple families are those headed by a single pair of primary reproductives. Within these families the genotypes of workers follow those expected for a single pair of parents (i.e. maximum of 4 alleles and no greater than 4 genotypic classes) and if the ratios do not differ significantly from those expected under simple Mendelian patterns of inheritance. The significance of these ratios is determined through a *G*-test performed per locus then summed across all loci. Extended families differ from simple families in the number of reproductives, but like simple families will have no more than four alleles per locus. Under this system the genotypes of the workers are inconsistent with a single pair of reproductives, identified by the presence of more than four genotypic classes at one or more loci, or if the genotypes are consistent with a single pair of reproductives but the *G*-value deviate significantly from those expected under a simple family. Mixed family colonies result from the fusion of two or more colonies and will be evident by greater than four alleles at one or more loci (See Fig. 3 for a diagrammatic explanation). The power to detect mixed family colonies depends on the variability present in the markers (i.e. As detection of a mixed family is dependent on the detection of greater than four alleles at a given locus, loci screened must exhibit 5 or more alleles within the population of interest).

a) Simple Family

Parental genotypes (diploid) – (♂)*AB* x (♀)*CD*

Possible gametes (haploid egg and sperm) – *A*, *B*, *C*, *D*.

Expected offspring genotypes (predicted using punnett square):

	C	D
A	AC	AD
B	BC	BD

As can be seen there is a maximum of 4 parental alleles (*A,B,C,D*), and therefore no more than 4 genotypic classes (*AC,AD,BC,BD* in this example).

b) Extended Family

Parental genotypes (diploid) – derived from offspring genotypes of simple family (e.g. from example above) Parental (♂1)*AB* x (♀1)*CD*

Secondary reproductive (♀2)*BD*

Possible gametes (haploid egg and sperm) – *A*, *B*, *C*, *D*.

Expected offspring genotypes (predicted using punnett square):

	B	**C**	**D**
A	AB	AC	AD
B	BB	BC	BD

As can be seen there is a maximum of 4 parental alleles (*A,B,C,D*), and greater than 4 genotypic classes (*AB,BB,AC,AD,BC,BD* in this example).

c) Mixed Family

Parental genotypes (diploid) – Parental (♂1)*AB* x (♀1)*CD* colony A reproductives

(♂1)*EF* x (♀1)*GH* colony B reproductives

Possible gametes (haploid egg and sperm) – *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*.

Expected offspring genotypes (predicted using punnett square):

	C	**D**	**G**	**H**
A	AC	AD	AG	AH
B	BC	BD	BG	BH
E	EC	ED	EG	EH
F	FC	FD	FG	FH

As can be seen there is greater than 4 parental alleles (*A,B,C,D,E,F,G,H*), and greater than 4 genotypic classes (16 in this example).

Fig. 3. Number and ratio of genotypic classes expected under each potential family structure.

Colony genetic structure

Colony genetic structure was investigated using *F*-statistics following the method of Weir and Cockerham (1984) as implemented in the program FSTAT (Goudet 2001). Analysis was performed over all samples, among urban samples only, and among agricultural samples only. The later two methods were employed so that *F*-values used to infer breeding system were not confounded by higher-level genetic structure. 95% confidence intervals were estimated by bootstrapping over loci with 1000 replications. Those values that did not overlap zero are considered to be significant at the $\alpha = 0.5$ level. The notation of Thorne et al. (1999) and Bulmer et al. (2001) was followed in which each individual colony is considered as a sub-population and the genetic variation is partitioned among the following components: the individual (I), the colony (C), and the total (T). F_{IT} is analogous to the inbreeding coefficient and is a measure of the level of inbreeding in individuals relative to the population. F_{CT} is comparable to the F_{ST} and represents differentiation between colonies. Finally F_{IC} is the coefficient of inbreeding in individuals relative to their colony and is particularly sensitive to the numbers of reproductives present and their mating patterns within colonies. Strongly negative F_{IC} values are expected in simple families, values should approach zero with increasing numbers of reproductives within colonies and be positive with assortative mating among multiple groups of reproductives within colonies or with mixing of adults from different colonies.

Results

Summary statistics

Summary statistics for genetic variability are given in Table 1. Overall an average of 16.67 samples was screened per sample location. Between 12 and 32 alleles were detected per locus, with a mean of 19.2 per locus. Average expected heterozygosity was 0.56 and average observed heterozygosity was 0.58. Samples were then split based on environment class (Urban vs. Agricultural). Within the urban samples, between 11 and 30

Table 1. Sample location data and basic summary statistics (number of alleles per locus/sample, mean sample size, mean number of alleles, expected heterozygosity (H_E), and observed heterozygosity (H_O) for 20 colonies of *Incisitermes minor* collected in California, USA. Samples outlines in red and blue represent one colony respectively.

Family type	Population	No. of alleles detected per locus / per sample					Mean sample size	Mean number of alleles	H_E	H_O
		DW-11	*DW*-12	*DW*-27	*DW*-39	*DW*-46				
	Urban									
Simple	Lakeview	1	2	1	4	2	7.4	2	0.3	0.29
	Villa La Jolla	2	4	3	4	2	11.6	3	0.54	0.41
	Vista Real	3	X	2	3	3	18	2.75	0.57	0.78
	North Bend	1	X	3	3	3	16	2.5	0.47	0.53
	Oakland	2	2	2	3	4	20	2.6	0.55	0.68
	Granada Hills	2	3	2	4	X	19.5	2.75	0.48	0.63
	total	1.83	2.75	2.17	3.50	2.80	15.42	2.6	0.49	0.55
Extended	North Hollywood Hills #14	3	3	4	3	3	19.6	3.2	0.64	0.59
	North Hollywood Hills #24	3	3	4	3	3	19.8	3.2	0.67	0.66
	North Hollywood Hills #26	3	3	4	3	3	19.8	3.2	0.63	0.57
	North Hollywood Hills #18	2	4	2	2	4	19.8	2.8	0.56	0.7
	North Hollywood Hills #47	2	4	3	3	4	19.8	2.8	0.52	0.67
	New Port Beach	3	3	3	2	3	20	2.8	0.62	0.55
	total	2.67	3.33	3.33	2.67	3.33	19.80	3.07	0.63	0.57
Mixed	Los Angeles, Board	4	5	5	7	5	19.2	5.2	0.71	0.65
	Los Angeles, Branch	4	4	4	5	6	19.8	4.6	0.69	0.73
	Santa Cruz	2	6	6	7	5	19.8	5.2	0.63	0.58
	Riverside	2	8	3	2	5	17	4	0.51	0.66
	total	3.00	5.75	4.50	5.25	5.25	18.95	4.75	0.63	0.66
Urban mean		2.44	3.86	3.19	3.63	3.67	17.94	3.29	0.57	0.61
Total no. alleles detected		11	30	11	20	18				
	Agricultural									
Simple	B16	2	2	3	3	3	14.8	2.6	0.48	0.55
	B18	1	2	2	3	3	8	2.2	0.42	0.33
	B23	3	X	3	3	2	17.75	2.75	0.57	0.61
		2.00	2.00	2.67	3.00	2.67	13.52	2.52	0.49	0.5
Extended	B6	2	2	3	4	2	13.6	2.6	0.5	0.63
	B12	2	3	3	4	4	13.2	3.2	0.53	0.39
	B15	4	4	2	4	3	12.2	3.4	0.68	0.84
		2.67	3.00	2.67	4.00	3.00	13	3.06	0.57	0.62
Mixed	B5	3	6	2	5	2	16.8	3.6	0.55	0.39
Agricultural mean		2.43	3.17	2.57	3.71	2.71	13.76	2.91	0.53	0.53
Total no. alleles detected		5	8	6	9	8				
	Combined mean	2.43	3.65	3	3.65	3.36	16.67	3.17	0.56	0.58
Total no. alleles detected		12	32	13	20	19				

alleles were detected per locus, with a mean of 18 per locus. An average of 3.29 alleles was detected within colonies across the five loci. Expected heterozygosity within colonies ranged from 0.30 to 0.71 with an average of 0.57 and observed heterozygosity ranged from 0.29 to 0.78 with an average of 0.61. Within the agricultural samples fewer alleles were detected with between 5 and 9 alleles per locus, and a mean of 7.2 per locus. Across the five loci an average of 2.91 alleles was detected within colonies. Expected heterozygosity within colonies ranged from 0.42 to 0.68 with an average of 0.53 and observed heterozygosity ranged from 0.33 to 0.84 with an average of 0.53. Due to the small sample size within each geographic location estimates of Hardy Weinberg Equilibrium was not calculated.

Colony identity

Given the geographic separation between samples collected in the urban landscape limited samples were likely to display patterns of significant genetic similarity. Based on the results of the permutation tests implemented in FSTAT and the exact *G*-tests implemented in GENEPOP 11 of the 16 collections were considered unique colonies. The North Hollywood Hills collections represented two distinct colonies. The first colony was comprised of samples #14, 24 and 26, whereas the second colony comprised #'s 18 and 47, both with non-significant *P*-values (Table 2). Put simply, in Table 2, we can see that colonies 14, 24, and 26 share all alleles at each locus. Additionally, the frequency of each genotype produced from these alleles within each colony is found to be comparable to each other. Colonies 18 and 47 share comparable alleles and genotype frequencies at each locus, thus informing us that these two colonies actually represent a single colony. We can see that the colony comprising 14, 24, and 26 is genetically different than the colony comprised of 18 and 47, given the alternate alleles present at each Locus. For example, at locus *DW*-39, colony 14/24/26 possesses alleles 135, 138, and 165, whereas colony 18/47 possesses alleles 150 and 153. Given that these loci are ancestrally different, we can tell instantly that these colonies are genetically different. For further analysis samples #14 and #47 were selected as representative colonies for determination of breeding structure. All colonies sampled within the agricultural landscape were genetically distinct.

Table 2. Microsatellite alleles and absolute numbers present at five *I. minor* samples collected within the North Hollywood Hills sampling site. Based on allele size and number present colonies 14, 24, and 26 are not genetically different and therefore represent a single colony. Based on the same principle colonies 18 and 47 represent a single colony.

Colony I.D.	Microsatellite locus									
	*DW-*11	n	*DW-*12	n	*DW-*27	n	*DW-*39	n	*DW-*46	n
North Hollywood Hills #14	292	19	205	9	318	9	135	6	144	13
	298	12	241	15	324	10	138	9	162	21
	307	9	260	16	342	10	165	25	168	4
					348	9				
North Hollywood Hills #24	292	20	205	9	318	12	135	11	144	20
	298	9	241	12	324	9	138	11	162	13
	307	11	260	17	342	7	165	18	168	7
					348	12				
North Hollywood Hills #26	292	26	205	7	318	10	135	7	144	17
	298	6	241	11	324	10	138	10	162	17
	307	8	260	20	342	10	165	23	168	6
					348	10				
North Hollywood Hills #18	298	26	245	9	318	9	150	27	148	8
	301	14	264	7	324	31	153	13	158	12
			272	12					168	12
			292	10					172	8
North Hollywood Hills #47	298	28	245	6	318	9	150	32	148	7
	301	12	264	9	324	31	153	8	158	12
			272	11					168	12
			292	14					172	7

Colony breeding structure

Following the detection that the five North Hollywood Hills collection points represented two genetic colonies a total of 20 distinct colonies were available for determination of colony breeding structure (Table one). Of the 13 urban samples, 6 colonies (46.2%) yielded worker genotypes consistent with those expected under a single pair of reproductives. No more than four genotypic classes were detected, all segregating with Mendelian ratios expected for a single pair of reproductives. Three colonies (23.1%), while possessing no more than four alleles per locus, exhibited greater than four genotypic classes (i.e. too many homozygous genotype classes), or Mendelian segregation patterns inconsistent with a single pair of reproductives. These were therefore determined to be extended families. The remaining four colonies (30.77%) exhibited greater than 4 alleles at one or more loci thus providing evidence for colony fusion (mixed colony structure). Within the seven agricultural collections three colonies (42.86%) meet the criteria of being simple families. A further three (42.86%) exhibit an excess of genotypic classes while possessing no more than four alleles at each locus, and were therefore identified as extended families. The final colony (14.28%) exhibited greater than 4 alleles at more than one locus and was determined to be a mixed family.

Overall, when colonies were combined across habitats 9 (45%) colonies were classed as simple families, 6 (30%) were extended families, and the remaining 5 (25%) were mixed family colonies.

Colony genetic structure

F-statistics and relatedness estimates are shown in Table 2. Overall, workers in both urban and agricultural populations showed signs of inbreeding (F_{IT} = 0.351, 95% C.I. 0.324 – 0.376) with relatedness values of 0.549 (0.507 – 0.597). Population within both environments appeared equally inbred – F_{IT} urban = 0.320 (0.295 – 0.343), F_{IT} agricultural = 0.319 (0.163 – 0.482) (P = 0.49 – paired t-test assuming unequal variances) and had comparable relatedness values (urban = 0.522 [0.471 – 0.588], agricultural = 0.493 [0.420 – 0.567]) (P = 0.28). Across colony type F_{IT} values were not significantly different from each other (P = <0.05). Considering the simple families only, within the urban landscape F_{IC} and r did not differ significantly from those expected from simple

Table 3. F-statistics and relatedness coefficients for worker nestmates of I. minor from urban and agricultural landscapes within California, USA, and values expected for some possible breeding systems of subterranean termites as derived from computer simulations.

Family type	F_{IT}	F_{CT}	F_{IC}	r
Urban				
Simple (n = 6)	0.308	0.427	-0.208	0.653
(95% C.I)	0.152 - 0.480	0.318 - 0.533	-0.333 to - 0.051	0.539 - 0.738
Extended (n = 3)	0.293	0.22	0.093	0.34
(95% C.I)	0.017 - 0.547	0.151 - 0.283	-0.177 - 0.378	0.270 - 0.399
Mixed (n = 4)	0.285	0.308	-0.033	0.479
(95% C.I)	0.205 - 0.355	0.221 - 0.415	-0.128 - 0.049	0.360 - 0.613
All (n = 13)	0.32	0.344	-0.037	0.522
(95% C.I)	0.295 - 0.343	0.314 - 0.392	-0.111 - 0.025	0.471 - 0.588
Agricultural				
Simple (n = 3)	0.412	0.435	-0.04	0.616
(95% C.I)	0.199 - 0.614	0.307 - 0.551	-0.312 - 0.319	0.442 - 0.753
Extended (n = 3)	0.256	0.308	-0.075	0.491
(95% C.I)	0.021 - 0.464	0.256 - 0.354	-0.321 - 0.190	0.444 - 0.546
Mixed (n = 1)	NA	NA	NA	NA
(95% C.I)				
All (n = 7)	0.319	0.325	-0.009	0.493
(95% C.I)	0.163 - 0.482	0.299 - 0.357	-0.242 - 0.230	0.420 - 0.567
Overall (n = 20)	0.351	0.370	-0.031	0.549
(95% C.I)	0.324 - 0.376	0.344 - 0.408	-0.094 - 0.022	0.507 - 0.597
Simulated breeding system				
(A) Simple-family colonies headed by outbred reproductive pairs	0	0.25	-0.33	0.5
(B) Extended-family colonies with inbreeding among neotenics				
(1) $N_f = N_m = 1$, X = 1	0.33	0.42	-0.14	0.62
(2) $N_f = N_m = 1$, X = 3	0.57	0.65	-0.22	0.82
(3) $N_f = N_m = 10$, X = 1	0.33	0.34	-0.01	0.51
(4) $N_f = N_m = 100$, X = 3	0.34	0.34	0.00	0.51
(5) $N_f = 5$, $N_m = 1$, X = 1	0.27	0.34	-0.11	0.53

families headed by unrelated reproductives. Comparable results were obtained for simple family colonies within the agricultural landscape. Considering extended families, between landscapes with the exception of estimates of *r*, values for F_{IT}, F_{CT} and F_{IC} were not significantly different than expected (P = <0.05). The high levels of inbreeding and F_{IC} values nearing zero (95% C.I. overlap zero) observed in both landscapes suggest colonies consist of numerous neotenic reproductives (Table 2, B3 through 5), or multiple groups of neotenic reproductives located in spatially separated reproductive centers within the colony. This later scenario would be expected where nest budding events have occurred with interconnected daughter nests. Due to the small sample size, and thus large 95% confidence intervals, accurate determination of the number of neotenic reproductives could not be made.

Discussion

Results presented here represent the first documentation of breeding structure, colony identification, and colony genetic structure to date for *I. minor* using highly polymorphic molecular markers. The high level of polymorphism (12 to 32 alleles per locus) exhibited at these five loci proved highly suitable for colony identification, and therefore may represent a powerful tool for future studies investigating colony survival and re-infestation events post insecticide treatment, informing the pest management professional (PMP) of the efficiency of a given treatment.

Our findings provide an insight into the composition of colonies in both the urban and agricultural landscapes. The majority of colonies (45%) are composed of a single pair of reproductives and their worker / soldier progeny. Thus, according to Harvey (1934) these probably represent colonies that are up to or just over 5 years of age. These are colonies that have yet to produce neotenic reproductives or those that contain neotenics that have not yet produced progeny. It is not uncommon to find simple families as the predominant family type within termite populations. For example, Vargo et al. (2006) detected between 48% and approximately 82% of colonies of the Formosan termite, *Coptotermes formosanus*, collected within North Carolina, South Carolina and Louisiana, to be simply families. In a study of colony organization in the subterranean termite

Reticulitermes flavipes, DeHeer and Vargo (2004) found 70% of colonies to be simple families headed by a single pair of reproductives.

In the drywood termite *I. schwarzi* Luykx (1986) suggested that additional reproductives found within a colony were the result of fusion events with different established colonies. Our results do not support this hypothesis as being consistent with *I. minor*. Across both landscapes extended families composed of multiple reproductive neotenics corresponded to 30% of the colonies sampled. These colonies all exhibited highly positive F_{IT} values and positive or nearing zero F_{IS} values. Accurate estimation of the number of reproductive neotenics within extended families based on the simulations generated by Thorne et al. (1999) and Bulmer et al. (2001) are impossible due to the small sample size and therefore the large confidence intervals. Our results, however, suggest that multiple reproductive neotenics contribute to offspring production in these colonies. If we follow the proposed age structure outlined in Harvey (1934), these extended families represent colonies that are approximately 10 years of age or older, and therefore, structural damage to a building may be extensive.

Mixed family colonies, or colony fusion, as observed in this study may prove difficult to explain. Korb & Schneider (2007) present a record of mixed colonies representing 25% of those sampled, however actual data regarding these are not available. Mixed family colonies have been recorded in a number of termite species, including *R. flavipes* (DeHeer & Vargo 2004), *R. grassei* (Clément 1981; Clément et al. 2001 but not confirmed by DeHeer et al. (2005) using microsatellite markers), *Mastotermes darwiniensis* (Goodisman & Crozier 2002), *Macrotermes michaelseni* (Hacker et al. 2005), and *Zootermopsis nevadensis* (Aldrich & Kambhampati 2007). Explanations as to how these mixed family colonies form have been broadly divided into two categories: those driven by worker foraging behaviors and those driven my alate reproductive strategies. Clément (1981) and Clément et al. (2001) documented genetic and behavioral evidence that suggests a breakdown of nest mate recognition, resulting in colonies associated in close proximity to each other overlapping in foraging area. Whether this results in eventual reproduction events between reproductives of these adjacent colonies is unlikely given that Pickens (1934) documents the immediate destruction of primary reproductives of *R. hesperus* when introduced into established

colonies. Thus in this instance colonies may not actually represent a mixing of genetic lineages within a large colony, but rather an event were colony foraging areas overlap. *M. michaelseni*, in contrast, exhibits true mixed colony composition with the foundation of colonies occurring through pleometrosis. Here, multiple unrelated female alates cooperate during colony formation and contribute to the production of sterile (workers and soldiers) (Hacker et al. 2005). Johns et al. (2009) presented evidence that colony fusion events may occur in the primitive dampwood termites, *Zootermopsis nevadensis*. Here independent colonies may be formed when pairs make use of the same piece of wood. Dampwood termites are single piece nesters; hence it is likely that eventually colony foraging areas within the wood will come into contact. At this time kings and/or queens are killed and the surviving members merge as one colony. Following encounters, members of each colony cooperate as a single social unit and replacement reproductives develop from workers of either or both colonies. Korb & Schneider (2007) describe a situation similar to that of *Z. nevadensis* in the drywood termite, *Cryptotermes secundus*, however both reproductives from one colony are killed leaving only workers from the subordinate colony. These then assist in the duties of the dominant colony. Whether, or not, secondary neotenics from the subordinate colony then mate with reproductives from the dominant colony is unknown. An alternative explanation may be that samples collected that appear genetically as being fused/mixed may actually represent distinct colonies whose activity centers or nests were proximate within the sampled wood but not actually fused. Thus, resulting in the collection of two distinct colonies inadvertently and labeling them as a single collection.

Conclusion

Given the significant economic impact of *I. minor* in the western U.S., the ability to accurately identify colonies, their breeding structure and genetic structure is of fundamental importance for the formulation of effective management strategies. We present the first results generated using highly informative microsatellite markers for this species and demonstrate the power such markers deliver in exploratory analysis of colony structure and life history. These markers may allow the following to be estimated with relative ease:

1) Colony size and age: Simple family structure may inform us that colonies are relatively small, detected in the early stages of infestation. Higher levels of inbreeding and the determination of colonies as being extended informs us that the colonies are likely to be extensive, containing numerous secondary reproductives each producing workers. Thus given the cryptic nature of this species we can now make an informed decision as to the likely extent of damage without extensive physical investigations of a structure.

2) Colony range: The treatment method employed may vary significantly depending on its range. Using these markers we can determine if colonies are contained to small regions of a property, or if they extend throughout a building. Thus, in instances of a single colony ranging throughout a structure, less environmentally intensive insecticide treatment (for example baits) may be developed to transmitted through the nest system from a single treatment point.

3) Treatment efficacy: When a structure is treated with an insecticide, resulting in apparent colony extinction, its efficacy can be determined through genetic testing. Sampling individuals prior to the treatment and post, assuming a structure becomes reinfested, will allow us to determine if the original colony was successfully exterminated and therefore determine if re-infestation occurred from an outside source, or if a fragment of the original colony survived to re-infest the structure.

These microsatellite markers represent a new powerful tool for future investigations of drywood termite population genetic structure and dynamics. The wealth of information possible through the application of these molecular markers is likely to inform PCO's of the extent of infestations, the connectivity of infestations geographically, and the success of prior treatments, thus allowing informed decisions to be made in regards to their control.

ACKNOWLEDGMENTS

The authors wish to acknowledge Michael Bollinger, Shawn Leighton, Sara Moore, and Lynette Yang for dissections of vineyard stumps and processing of termite samples for further analyses. We also thank Michael Haverty for reviewing an earlier draft of this report.

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