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Describing the Spatial and Social Organization of Formosan Subterranean Termite Colonies in Armstrong Park, New Orleans

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C. Husseneder¹, J. K. Grace¹, M. T. Messenger², E. L. Vargo³

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ABSTRACT

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Colonies of the Formosan subterranean termite, *Coptotermes formosanus*, possess a complicated spatial and social organization comprising interconnected foraging sites and nests containing variable numbers of reproductives. This complex colony structure may affect the distribution of bait toxicants through the colony. Due to the cryptic life of subterranean termites, their colony organization cannot be directly observed, but needs to be inferred from the genetic structure. We investigated the genetic structure of *C. formosanus* in Armstrong Park, New Orleans, employing multilocus DNA fingerprinting and microsatellite genotyping. We applied DNA fingerprinting to differentiate colonies according to their genetic profiles. We assigned termites from different sites to colonies through diagnostic bands and genetic similarities. In general, foraging areas of colonies identified by their DNA profiles correlated with results of mark-release-recapture studies. The wide intra- and intercolonial range of genetic similarities indicates a variety of social organizations as well as different proliferation strategies of colonies (budding, swarming). For more detailed analyses of colony organization we are currently using microsatellite genotyping to reveal the numbers of reproductives and degree of inbreeding within colonies. The combined information on genetic structure and social organization of colonies will tell (1) if the majority of colonies is headed by unrelated pairs (colony foundation through swarming) or by multiple related reproductives, and (2) if the presence of multiple reproductives results in a genetic substructure within colonies, which might lead to subsequent colony proliferation through budding. We also intend to apply

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¹Dept. of Plant & Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Room 310, Honolulu, HI 96822, USA

²City of New Orleans Mosquito & Termite Control Board, 6601 Stars & Stripes Blvd., New Orleans, LA 70126

³Dept. of Entomology, North Carolina State University, Raleigh, NC 27695

⁴Ft Lauderdale Research & Education Center, University of Florida, 3205 College Ave., Ft. Lauderdale, FL 33314

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genetic information to examine how termites invade areas where baiting has eliminated all prior activity.

INTRODUCTION

There is no need in the Southeastern USA to emphasize the importance of controlling the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Especially in the area of New Orleans, Louisiana, *C. formosanus* has had an enormous economic impact during the last decade. Improving management of *C. formosanus* requires a thorough understanding of the biology of this termite species.

Our work is focusing on the identification of colonies and description of their spatial and social structure, because these factors could influence distribution of bait toxicants. Colonies of *C. formosanus* show a complicated spatial and social organization. Colonies consist of widespread interconnected foraging groups and nests containing variable numbers of reproductives. The cryptic life of subterranean termites makes it difficult to affiliate workers collected from different foraging traps to colonies, as well as to estimate the number of reproductives and thus describe colony structure. However, using molecular markers to assess the distribution of genotypes and genetic differentiation among termites from different collection traps allows delimitation of colonies and sheds light on their organization. This paper summarizes preliminary results of work currently in progress in Louis Armstrong Park, New Orleans.

RESULTS AND DISCUSSION

In order to describe colonies of *C. formosanus* and their organization in Armstrong Park, New Orleans, we have employed multilocus DNA fingerprinting and microsatellite genotyping (Husseneder & Grace 2001a,b; Vargo & Henderson 2000, Husseneder *et al.* 2002). We analyzed termite material from 14 foraging areas previously outlined by mark-release-recapture studies (M. Messenger, unpublished).

First, we applied DNA fingerprinting to test if termites from collection traps within the foraging areas delimited by mark-release-recapture belonged to single colonies, and if these colonies could be differentiated using genetic markers. Pairwise genetic similarities (Lynch 1990) between individuals from the same collection trap were approximately normally distributed and did not show any evidence for mixing of genetically distinct groups. Genetic similarities within single collection traps lay in the same magnitude as genetic similarities between termites from different collection traps within the same foraging area

(0.63, SD=0.08, genetic similarity) (0.35, SD=0.14, genetic profiles approaches identified 3 foraging areas clearly different confirm that foraging traps correspond to collection traps. Different

Secondly, we tested if colonies within the same foraging traps to their collection traps from different colonies if their presence to different collection traps individuals can be identified by genetic bands for colonies from known colonies from different colonies containing each colony from different collection traps bands can be assigned to the termites collection traps. Individual termites from the same collection traps 100% because they were distinguished from the other individuals of the colony cannot be assigned to a specific collection trap overcome by genetic markers.

We determined the genetic similarities between individuals and subjects from the same collection traps and genetic similarities between individuals described in the same collection traps than that case. Genetic similarities to be equal to the genetic similarities assigned to the collection traps. Thus, using

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(0.63, SD=0.08, 22 traps). These values are significantly higher than genetic similarities between termites from different foraging areas (0.35, SD=0.14, 43 pairs of traps). Similarly, bandsharing between genetic profiles derived from pools of 10 termites per collection trap approaches identity within foraging areas (0.95, SD=0.03, 2-7 traps in 3 foraging areas). Among foraging areas, however, genetic profiles are clearly different (0.50, SD=0.08, 91 pairs of traps). These results confirm that foraging areas outlined by mark-release-recapture studies correspond to colonies identified by genetic similarities between individuals as well as between genetic profiles representative of collection traps. Different foraging areas represent genetically distinct colonies.

Secondly, having outlined colonies, we then tested on a subset of six colonies whether we could assign termites from different collection traps to their colony of origin. Based on the results above, pools of ten termites from different collection traps can be assigned to the same colony if their pooled genetic profiles are identical (95% bandsharing) or to different colonies if their genetic profiles are different. Furthermore, individuals can be assigned to colonies based on two methods: diagnostic bands for colony membership, and genetic similarity to termites from known colonies. The genetic profile of each of the six investigated colonies contains on the average 3-4 diagnostic bands distinguishing each colony from the others. On average termites that possess these bands can be assigned to one of these colonies. On the average, 77% of the termites could be assigned to their colony on the basis of diagnostic bands. Individual assignment by diagnostic bands, however, is not 100% because individuals of a colony share on the average only around 63% of the bands (see above). In cases where a colony's profile is distinguished from other colonies by only a few diagnostic bands, some of the colony members might lack those particular bands and thus cannot be assigned to a single colony. However, this drawback can be overcome by grouping individuals according to their genetic similarity.

We determined the pairwise genetic similarities between all individuals and subjected the data matrix to discriminant analysis. Termites from the same colony consistently grouped together due to higher genetic similarity within colonies compared to between colonies, as described in the previous paragraph. Each termite was classified to the nearest group by the discriminant functions derived from all cases other than that case. Prior probabilities of group membership were assumed to be equal (Husseneder & Grace 2000). When each termite was assigned to the genetically most similar group of termites, 100% successful assignment to the actual colony of origin was achieved. Thus, using multilocus DNA fingerprinting, termite colonies can be

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outlined by assigning collection traps and in most cases even single individuals to colonies. The power of this method is the greater when the number of potential colonies of origin is small and genetic differentiation between them is high.

In addition to assigning termites to colonies, we also intend to apply genetic information to examine how termites invade areas where baiting has eliminated all prior activity. Genetic profiles can be used to "fingerprint" colonies prior to elimination for re-invasion studies. When termites reappear in the same location after elimination, comparison of genetic profiles can tell us if they are remnants of the same colony (profiles identical to previous colony), invaders from neighboring colonies (profiles identical to another colony), or new infestations from outside the immediate area (profiles do not match any known colony).

Thirdly, we investigated the possibility of genetic differentiation occurring within colonies due to the presence of multiple reproductives. We employed microsatellite genotyping to test the genotypic distribution among 15-24 workers of the 14 colonies at 8 loci for deviation from the ratios expected for a single pair of reproductives (Mendelian ratios). Results show that about a third of the colonies are headed by multiple reproductives. These colonies showed either genotype combinations that are not possible with only a single pair of reproductives or genotypic ratios that deviated from the expectations for a single pair (Chi-square test).

The presence of multiple kings and queens may lead to asymmetries in spatial distribution within a colony, if reproductives are located in different nests or if the offspring are otherwise separated (Kaib *et al.* 1996). Preliminary data derived from multilocus DNA fingerprinting and confirmed by microsatellite genotyping suggests a slight genetic differentiation between six collection traps belonging to one colony. However, this genetic differentiation is much smaller than the differentiation between colonies. This is the pattern we would expect in a colony with multiple interconnected satellite nests. Future research is required since such a spatial structures could influence distribution of bait toxins within colonies.

In conclusion, using multilocus DNA fingerprinting, we were able to establish that foraging areas represented individual colonies and assign termites to their colony of origin. We now intend to apply this information to re-invasion studies in Armstrong Park. Using microsatellite genotyping, we found multiple reproductives within a third of the *C. formosanus* colonies. The presence of multiple reproductives resulted in a small degree of genetic differentiation between collection traps within

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at least one colony in the park, suggesting a need for additional research on colony social organization.

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