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Describing the spatial and social organization of Formosan subterranean termite colonies in Armstrong Park, New Orleans

by

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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, LA, *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 sites and nests containing variable numbers of reproductives. The cryptic life of subterranean termites makes it difficult to affiliate foraging sites to colonies as well as to estimate the number of reproductives and thus describe colony structure. However, the use of molecular markers to assess the distribution of genotypes and genetic differentiation among termites from different foraging sites allows us to delineate colonies and to shed light on their organization. This paper summarizes preliminary results of work currently in progress in Louis Armstrong Park, New Orleans, LA.

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

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First, we applied DNA fingerprinting to determine whether termites from collection traps within foraging areas delimited by mark-release-recapture belong to single colonies and whether colonies can be differentiated using genetic markers. Genetic similarities (Lynch 1990) within collection traps lie in the same magnitude as genetic similarities between termites from different collection traps within the same foraging area (0.63, SD=0.08, 22 traps). This indicates that there is no genetic differentiation between collection traps within the same foraging area. However, genetic similarities between termites from different foraging areas are significantly lower than between termites from different collection traps within the same foraging area (0.35, SD=0.14, 43 pairs of traps), showing genetic differentiation between foraging areas. 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. The termites occupying different foraging areas represent genetically distinct colonies.

Secondly, having outlined colonies we investigated with 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 ($\geq$ 95% bandsharing) or to different colonies if their genetic profiles are different. Furthermore, individuals can be assigned to colonies by two methods: diagnostic bands for colony membership and genetic similarity to termites from known colonies. The genetic profile derived from pools of ten termites of each of the six investigated colonies contains on the average 3-4 diagnostic bands distinguishing each colony from the others. Individual termites that possess these bands can be assigned to one of these colonies. On average, 77% of the termites could be assigned to their colony on the basis of one or more diagnostic bands. Individual assignment by diagnostic bands is not 100% because individuals of a colony share on the average only around 63% of their bands (see previous paragraph). Cases in which a colony's profile differs from others 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. Based on discriminant functions each termite was classified to the nearest group (Husseneder and 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 outlined by assigning collection traps and in most cases even single individuals to colonies.

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 "tag" colonies prior to elimination for re-invasion studies. When termites re-appear in the same location after elimination, comparison of genetic profiles can tell us if they are remnants from 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 genetic structuring 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 different collection traps belonging to one colony. However, this genetic differentiation is much smaller than the differentiation between colonies. We would expect such a pattern in a colony with multiple inter-connected satellite nests. Future research is required to investigate genetic substructure within colonies because such structuring might 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 at least one colony in the park, suggesting the need for additional research on colony social and spatial organization.

REFERENCES

Husseneder, C., and J. K. Grace. 2000. What can DNA fingerprinting, aggression tests and morphometry contribute to the identification of colonies of the Formosan subterranean termite? IRG/WP 00-10371, 8pp.

Husseneder, C., and J. K. Grace. 2001a. Similarity is relative: The hierarchy of genetic similarities in the Formosan subterranean termite (Isoptera: Rhinotermitidae) in Hawaii. Environmental Entomology 30: 262-266.

Husseneder, C., and J. K. Grace. 2001b. Evaluation of DNA fingerprinting, aggression tests and morphometry as tools for colony identification of the Formosan subterranean termite. Journal of Insect Behavior 14: 173-186.

Husseneder, C., Vargo E. L., and J. K Grace. 2001. Multilocus DNA Fingerprinting and Microsatellite Genotyping: Complementary Molecular Approaches to Investigating Colony and Population Genetic Structure in Subterranean Termites. Sociobiology. *In press*.

Kaib, M., Husseneder, C., Epplen, C., Epplen, J. T., and R. Brandl. 1996. Kin-biased foraging in a termite. Proceedings of the Royal Society of London B 263: 1527-1532.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Molecular Biology & Evolution 7: 478-484.

Vargo, E. L., and G. Henderson. 2000. Identification of polymorphic microsatellite loci in the Formosan subterranean termite *Coptotermes formosanus* Shiraki. Molecular Ecology 9: 1935-1938.