IRG/WP 00 - 10371

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 1 Biology

WHAT CAN DNA FINGERPRINTING, AGGRESSION TESTS AND MORPHOMETRY CONTRIBUTE TO THE IDENTIFICATION OF COLONIES OF THE FORMOSAN SUBTERRANEAN TERMITE?

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Paper prepared for the 31st Annual Meeting
Kona, Hawaii
14-19 May 2000

IRG Secretariat
SE 100 44 Stockholm
Sweden

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ABSTRACT

Multilocus DNA fingerprinting, aggression tests and morphometry were compared to evaluate their potential for the identification of colonies of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. DNA fingerprinting separates the termites from all studied collection sites. Since the genetic similarity between termites from different collection sites lies in the range of the genetic background similarity in the population, collection sites in this study represent independent colonies. No significant differences could be found in the intra- and intercolonial aggression levels. While aggression tests do not support colony identification, morphometric measurements do show differentiations between colonies. However, classification of individuals to their original colony does not reach the 100% success provided by genetic analyses. No correlation between genetic similarities and aggression levels or morphometric distances could be found. This suggests that neither aggression levels nor morphometric parameters are significantly influenced by genetic factors in this species. Genetic studies appear to be the most useful approach to the identification of colonies and the analysis of small scale population structures in *C. formosanus*.

Keywords: molecular methods, genetic similarity, population structure

INTRODUCTION

The assignment of termite infested sites (structures, aggregation traps) to specific colonies, has become essential in applied termite research. In *Coptotermes formosanus* (Rhinotermitidae, Isoptera), a severe pest species in Hawaii and many other regions, colony identification is extremely difficult. The cryptic life of this termite and its widespread colonies with subsidiary nests prevent colony identification by direct observations (e.g. Thorne et al. 1999, Tamashiro et al. 1987, Yates & Tamashiro 1990).

Behavioral bioassays and epicuticular hydrocarbons have provided somewhat ambiguous information concerning the differentiation among *Coptotermes* from different collection sites. Aggression was not correlated to cuticular hydrocarbon patterns (e.g. Su & Scheffrahn 1988, Su & Haverty 1991, Shelton & Grace 1996, Haverty et al. 1996). These results require further interpretation on a population genetic basis to assess genetic differentiation and define colony membership.

Aggression levels and hydrocarbon patterns in *C. formosanus* have not yet been linked to the population's genetic structure, because genetic markers such as alloenzymes and mitochondrial DNA did not provide sufficient polymorphism to reveal differentiation between colonies (e.g. Korman & Pashley 1991, Strong & Grace 1993, Broughton & Grace 1994, Wang & Grace 1995). In similar studies, colonies of African rhinotermitids could be identified by congruent results from multilocus DNA fingerprinting, aggression tests and morphometric measurements (Husseneder et al. 1998). These three methods were therefore applied to classify Hawaiian *C. formosanus* individuals to colonies.

Colony identification provides the basis for understanding the population structure as well as for future implementation in termite management (e.g. bait evaluation). Genetic similarities, aggression levels and morphometric distances were compared to determine if aggressive behaviour and phenotypic features are influenced by genetic factors and can therefore be used as indirect markers for description of the population structure and identification of colonies.

MATERIAL AND METHODS

Collection of termites

C. formosanus was collected in 1998 from five collection sites (aggregation traps) on the University of Hawaii campus (120-540m distance) and seven additional sites on Oahu, Hawaii (4-39km distance). For DNA analysis, workers were preserved in 95% ethyl alcohol (Quantum, California). For aggression tests, living workers were kept in petri dishes for 24h prior to the tests. For morphometric measurements soldiers were placed into 70% ethanol (Fisher Scientific, New Jersey).

Multilocus DNA fingerprinting

Genomic DNA from individual workers was digested with HaeIII (Roche Molecular Biochemicals, Indianapolis). The DNA fragments were separated through electrophoresis on 20 X 25cm 0.8% agarose gels at 45V for 72h, blotted onto nylon membranes (Roche), hybridized with the digoxigenated oligonucleotide probe $(GTG)_5$ (MWG-Biotech AG, Germany) and visualized by a chemiluminescence reaction (Roche). Banding patterns were scored between 4 and 23kb fragment size.

Genetic similarities between individual fingerprints were quantified using the pairwise bandsharing probability (Lynch 1991). Mean genetic similarities of termites within the same site and between different collection sites were calculated by the mean of all possible pairwise combinations. For visualization of the genetic similarities between individuals the first two principal coordinates extracted from the total matrix of genetic similarities were used as axes (NTSYS-pc, Rohlf 1990).

The data matrix for the five campus collection sites is based on 83 workers. In addition 67 individuals from seven distant collection sites were included as large scale outgroups in the correlation with aggression levels.

Aggression tests

For each experiment, two groups of five workers, each from the same or from different collection sites, were paired in petri dishes (diameter 9 cm) . The number of termites involved in aggressive behavior (biting) was scored in 1 min intervals for as long as 15 min (Kaib & Brandl 1992). The mean of these 15 scores, the aggression index, ranges from 0 (no aggression) to a maximum of 10. Each experimental pairing was repeated up to six times with a new set of termites each time and the mean aggression index (AI) was calculated. The results are based on the mean aggression levels from 31 different pairings (112 single aggression tests) of workers from the same colony, different colonies at the University of Hawaii campus and geographically isolated colonies from across Oahu.

Morphometry

Nine linear parameters of 15 soldiers from each collection site were measured according to Kaib & Brandl (1992). All nine parameters show significant differentiations of group means between the five campus colonies (d.f. 4, 70, $p<0.01$, Wilks' λ U-statistics). The data set was subjected to canonical variate analysis using collection sites as group variables. The first two canonical variates were used to visualize morphometric distances (Mahalanobis distances) between termites from different collection sites.

Statistical evaluation

Statistics, canonical variate analysis, principal coordinate analysis and graphic visualization were performed using NTSYS-pc 1.70, Exeter Software (Rohlf 1990), Sigma Plot 5.0 and SPSS 9.0 for Windows (SPSS Inc.). The classification rate of individuals was estimated by the Mahalanobis distance method (U method, Sharma 1996). Each case 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.

RESULTS AND DISCUSSION

Multilocus DNA Fingerprinting

Principal coordinate analysis of the genetic similarities visualizes the genetic relationship of the termites from the five collection sites at the University campus (figure 1a): individuals of the same collection site group together, while termites from different sites form separate clusters. Three of the five sites (G, H, P) overlap in the two dimensional projection of the multivariate space; however, they can be separated in the third dimension. The mean genetic similarity within collection sites is 0.66 (SD=0.12, N=659) and is significantly higher than between collection sites (0.29, SD=0.12, N=2247; p=0.002, two tailed U-test). Genetic similarity between collection sites approximates the population's background similarity of 0.21, i.e. the average genetic similarity between non-related individuals (Husseneder & Grace, submitted). Thus, we conclude that collection sites in this study represent independent colonies. Due to the considerable genetic differentiation between the colonies, individual termites can be assigned to their actual colony of origin in 100% of all cases.

Genetic studies on various termite species show complicated colony structures with varying degrees of inbreeding and substructure within colonies, which require sensitive methods to identify colonies unequivocally (Reilly 1987, Thompson & Hebert 1998, Husseneder et al. in press, Kaib et al. 1996, Atkinson & Adams 1997, Husseneder et al. 1998). DNA fingerprinting is a valid tool for identification of colonies and colony membership. In a natural population of the African termite species *Schedorhinotermes lamanianus,* nine collection sites (galleries) could be grouped to three colonies, one of which showed intracolonial genetic substructure (Husseneder et al. 1998). In the present study on the introduced species *C. formosanus*, termites could be assigned to collection sites which represented different colonies. Current studies use this result to provide detailed analyses of the relationships between neighboring colonies and between different collection sites within the same colony.

Aggression

Workers from the same colony never show aggression (table 1). The overall level of aggression is comparatively low ranging between 0.0 and 2.1. No significant differences are found between intra- and intercolonial pairings (χ^2=3.66; df=2; p=0.16, Kruskal-Wallis ANOVA). Even aggression tests between geographically isolated colonies across Oahu do not yield significantly higher aggression than within colonies. Contrary to the results in most termite species which show aggressive discrimination of non-colony mates (e.g. Thorne & Haverty 1991, Shelton & Grace 1996, Husseneder et al. 1998), aggression tests can not be used for the determination of colony membership in *C. formosanus* (Delaplane 1991, Su & Haverty 1991).

Lack of aggression between colonies is rather uncommon in the majority of the studied termite species. It may be triggered by a high genetic relatedness between colonies or an environment where monopolizing and defending resources is obviously of little importance (e.g. Clement 1986, Grace 1996, reviewed in Thorne & Haverty 1991, Shelton & Grace 1996). The influence of genetic factors is discussed below.

Table 1: Mean aggression levels between groups of workers in small-scale (University campus) and large-scale (island of Oahu) comparison.

AI: Mean aggression index of all intra- and intercolonial pairings of workers; SD: Standard deviation; N: Number of pairings (each consists of 3-5 repeated tests).

Pairings of workers	AI	SD	N
intracolonial	0.00	0.00	8
intercolonial (small scale)	0.15	0.32	10
intercolonial (large scale)	0.31	0.61	13

Morphometry

The first two of the four canonical discriminant functions combine 86% of the total variance and are used to visualize the morphometric distances between individual termites from the campus colonies (figure 1b). The plot of the individuals shows considerable scatter: Patterns of A, G, H and P members overlap while termites from colony M are separated. On the average, in 79% of all cases the predicted group membership coincides with the actual colony of origin. Compared to the 100% successful classifications based on genetic similarities, morphometry is not an accurate predictor of colony membership in *C. formosanus*.

Morphometry has been a successful method for determining colony structure in other termite species. Kaib & Brandl (1992) inferred spatial dynamics in the colony structure of *Schedorhinotermes lamanianus* using morphometric measurements and aggression tests. In a later study on the same species congruent results of morphometry and DNA fingerprinting allowed grouping of collection sites to colonies (Husseneder et al. 1998). Due to the considerable variance of morphometric distances within groups, however, no attempt was made to assign single individuals to collection sites based on morphometric data alone.

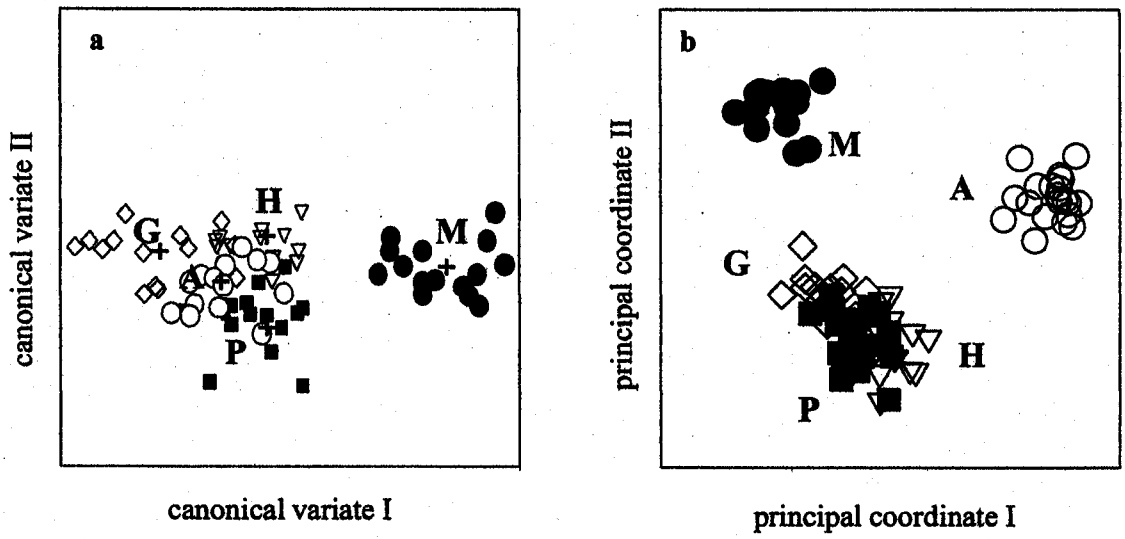

Figure 1: Ordination of morphometric distances and genetic similarities of termites from the five campus collection sites.

a Principal coordinate analysis of genetic similarities between workers. Axes combine 34% of the total variance.

b Canonical variate analysis of morphometric distances of individual soldiers. + indicates group centroids. Axes combine 86% of the total variance.

Correlation of aggression levels and morphometric distances with genetic similarities?

Aggression levels between colonies are low or absent and are not correlated to genetic similarities ($r=-0.27$; $p>0.20$, one-tailed permutation test). The lack of correlation can be explained in three ways: (1) the population is genetically homogenous (2) the colonies are highly related or (3) aggression is not a function of genetic relatedness.

With respect to each of these possible explanations: (1) Studies in the European *Reticulitermes* show that termite societies can be "open", i.e. low aggression permits exchange of individuals between neighboring sites leading to genetic uniformity (Clement 1986). However, in the Hawaiian *C. formosanus* genetic similarities within colonies (0.66) are significantly higher than between colonies (0.29). This does not support the hypothesis of genetic uniformity. (2) *C. formosanus* is an introduced species in Hawaii. Limited number of introductions and genetic bottlenecks may have resulted in a lack of genetic variation in the population, leading to a high genetic background similarity and inbreeding (see Haverty et al. 1996, Strong & Grace 1993, Broughton & Grace 1994). Contrary to these assumptions, the present study shows considerable genetic variability in the population: The population's genetic background, 0.21 (Husseneder & Grace submitted), lies in the range of natural populations (termites: Husseneder 1998; other species: Lynch 1991). Local inbreeding is unlikely due the fact that even on a small spatial scale the genetic similarities between colonies are not considerably higher than the genetic background (0.29 compared to 0.21 background similarity). (3) We therefore favor the third hypothesis. Contrary to similar studies in other termite species (e.g. Adams 1991, Husseneder et al. 1997) aggression does not appear to be evoked by low genetic relatedness in *C. formosanus*.

Although morphometry shows some differentiation between colonies, the morphometric distances are not correlated to the genetic similarities ($r=-0.02$; $p>0.20$, one-tailed permutation test). These results are in contrast to related studies, where morphometric distances proved to be a good predictor of genetic similarity (Husseneder et al. 1998).

In sum, classical approaches to colony definition, like aggression tests or morphometry, can only be applied with limited success in *C. formosanus* and are not correlated to the genetic structure. DNA fingerprinting, however, is able to show a pattern of differentiation between colonies and to identify colony members even on a small spatial scale.

ACKNOWLEDGMENTS

We thank R.J. Woodrow, C.H.M. Tome, R.J. Oshiro and S.Y. Uchima for help with field collections; K. Funes for assistance in the molecular work; and S. McCombs and S. Saul for providing facilities. We are also grateful to D.E. Oishi and J. Ziesmann for useful comments on early drafts of this manuscript. Financial support was provided by the German Academic Exchange Service (DAAD) and USDA-ARS Specific Cooperative Agreement 58-6435-8-107.

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