Multilocus DNA Fingerprinting and Microsatellite Genotyping: Complementary Molecular Approaches to Investigating Colony and Population Genetic Structure in Subterranean Termites

by

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ABSTRACT

A variety of molecular techniques are being increasingly used in basic and applied termite research. Each method is best suited for investigating genetic structure at a particular level of organization. The use of multiple techniques simultaneously allows for analysis of both fine scale and large scale genetic structure. We provide an example of such an approach in research in progress in which we are employing multilocus DNA fingerprinting and microsatellite genotyping to investigate the population and colony genetic structure of the severe termite pest *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) in Hawaii. Comprehensive knowledge of the genetic structure of termite populations will provide insight into colony social and spatial organization as well as dispersal patterns and will thus facilitate remedial and regulatory control efforts.

INTRODUCTION

Subterranean termites such as *Coptotermes* spp. and *Reticulitermes* spp. are severe economic pests in the United States and worldwide. Efforts to manage these termites are directed at two levels: (1) regulatory measures need to be implemented efficiently to prevent new introductions and further spread of termite populations; and (2) remedial control techniques, such as baiting systems, must be continuously improved in order to suppress and eliminate existing colonies. Progress on both levels strongly hinges on a solid information base on the population biology of subterranean termites; e.g., source of introductions and ways of spread throughout a region, as well as colony genetic structure. Colonies of subterranean termites, for example, possess a complicated spatial and social organization comprising interconnected foraging sites and nests containing a single pair or

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multiple reproductives. During their life cycle colonies may proliferate by budding and swarming, thus creating an extensive network of interrelated colonies, which may or may not exchange individuals. This complex colony structure may affect the distribution of bait toxicants through the colony.

Due to the cryptic nesting and feeding habits of subterranean termites, many important features of their population biology and colony structure cannot be directly observed (Thorne *et al.* 1999). However, the underlying processes can be inferred from the distribution of genotypes within and among groups on different levels of organization; i.e., from substructure within a colony to relationships among populations on a global scale. The use of genetic markers, including analysis of alloenzymes, mitochondrial DNA, and genomic DNA, to examine population and colony structure in termites is an expanding field (e.g. Clément 1986; Reilly 1987; Korman & Pashley 1991; Strong & Grace 1993; Broughton & Grace 1994; Kaib *et al.* 1996; Atkinson & Adams 1997; Thompson & Hebert 1998a, b; Husseneder 1998; Husseneder *et al.* 1998; Jenkins *et al.* 1998; Thorne *et al.* 1999; Wang & Grace 2000). However, no single molecular genetic method is likely to be sufficient by itself to investigate genetic structure over the entire range of colony and population organization in subterranean termite species. A more powerful and comprehensive approach involves combining different types of molecular markers to investigate genetic structure at several levels of colony and population structure. In our work we have begun to combine two of the most potent methods for the analysis of genomic variation in termites, multilocus DNA fingerprinting (Jeffreys *et al.* 1985, 1991; applied in termites: Kaib *et al.* 1996, Husseneder *et al.* 1998, Husseneder *et al.* 1999) and microsatellite genotyping (e.g. Tautz 1989; applied in termites: Vargo 2000, Vargo & Henderson 2000, Kaib *et al.* 2000, Thompson *et al.* 2000).

The purpose of this paper is to describe the principle features of both methods and their usefulness in characterizing certain aspects of genetic structure in subterranean termites. In this paper we focus on the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), however, similar studies are being conducted on various *Reticulitermes* spp. (Vargo *et al.* unpublished). The Formosan subterranean termite is one of the most destructive pests in the world (Tamashiro & Su 1987). Presumed to have originated in China, this termite now enjoys worldwide distribution and is established in more than 13 states in the USA, including Hawaii. As an introduced species in Hawaii *C. formosanus* is likely to show limited genetic variability and was therefore chosen as a prime example for the

power and sensitivity of multilocus fingerprinting and microsatellite genotyping. We will review previous work on the colony and population structure of *C. formosanus* in Hawaii based on multilocus DNA fingerprinting and present preliminary data from microsatellite analysis.

MATERIALS AND METHODS

Both multilocus DNA fingerprinting and microsatellite genotyping detect genetic variance of simple, repeated tandem sequences 1-6 base pairs in length, such as $(CA)_n$ or $(GTG)_n$ (e.g. Krawczak & Schmidtke 1994; Hoy 1994, pp. 390ff). These markers, known as short tandem repeats (STR's), simple sequence repeats (SSR's) or microsatellites, are dispersed in non-coding regions across the eukaryotic genome. They are highly polymorphic due to variation in the number of repeat units (e.g. Karp *et al.* 1998, pp.195ff).

Although relying on the same genetic regions, DNA fingerprinting and microsatellite genotyping result in different genetic patterns (Fig. 1a). Consequently, these patterns contain different yet corresponding information, which can be used in a complementary way to address colony as well as population structure. These differences arise from the method of detection used by each technique. DNA fingerprinting is based on Southern Blot techniques (e.g. Karp *et al.* 1998, pp.101ff) in which digested and electrophoretically separated DNA fragments containing the target regions are visualized by hybridization with oligonucleotide probes. Either the probe itself is radio- or fluoro-labeled, or it carries an antigen (e.g. digoxigenin), which is recognized by a monospecific antibody in an immunoblot (Fig. 1a). The antibody is conjugated with alkaline phosphatase, which catalyzes a color or light reaction depending on the substrate used. This procedure yields an individual specific barcode-like banding pattern consisting of multiple loci with dominant marker bands that follow Mendelian inheritance (i.e., on the average half of the bands are derived from each parent). These DNA "fingerprints" usually show high somatic and germ line stability (e.g. Karp *et al.* 1998, p. 101). The proportion of shared bands reflects the genetic similarity between individuals within and among collection sites (Lynch 1990, 1991). In addition, a genetic profile can be derived from the sum of all existing bands of termites from the same collection site. Given sufficient genetic differentiation in the population, the genetic profile contains diagnostic bands shared only by members of this collection site, distinguishing this particular profile from genetic profiles of other sites (Fig. 1a). So far, even analyses on a small scale in introduced populations that are presumed to have reduced genetic variation, showed sufficient genetic differentiation between neighbor-

ing colonies to use this approach (Husseneder & Grace, unpublished).

The main advantage of DNA fingerprinting is its high sensitivity even in systems with low genetic variability, such as within termite colonies (Kaib *et al.* 1996, Husseneder *et al.* 1998, 1999). This strong resolution power is achieved by detecting a combination of genetic variability caused by restriction fragment length polymorphism (RFLP's) and variable numbers of tandem repeats (VNTR's). The disadvantage of this high resolution is limited applicability of the method for population analyses on larger geographic scales. The genetic similarity noted between populations rapidly decreases toward zero (Husseneder & Grace 2001a). Another drawback arises because the multilocus pattern consists of "dominant" bands that do not allow allelic interpretation. Thus, established procedures in population genetics (e.g. *F*-Statistics, coefficients of relatedness) cannot be applied directly to results from DNA fingerprints. The equations of Lynch (1990, 1991), however, provide a good approximation for calculating population statistics and relatedness from genetic similarity of fingerprint patterns (Husseneder *et al.* 1998, 1999; Husseneder & Grace 2001a).

To broaden the spectrum of insight into termite biology, we combined

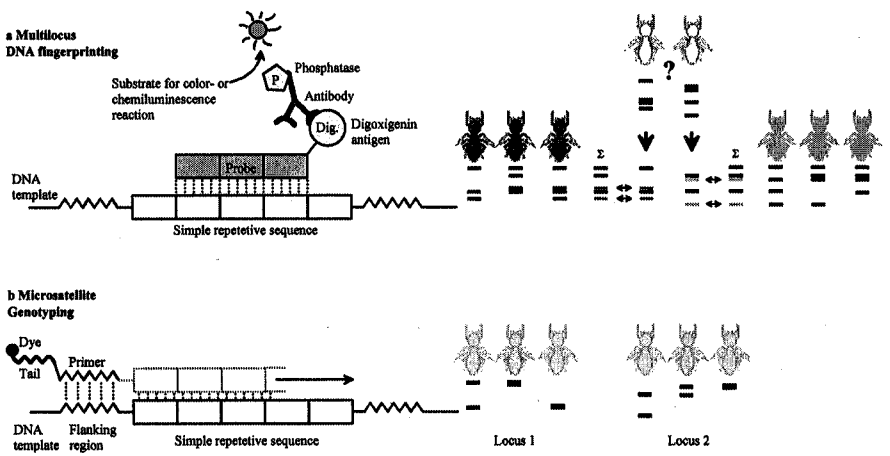

Fig. 1 Schematic representation of the method of detection of multilocus DNA fingerprinting (a) and microsatellite genotyping (b) and resulting banding patterns.
(a) Multilocus DNA fingerprinting: hybridization and immunodetection; individual fingerprints and colony profiles derived from pooled individuals (Σ). The proportion of shared bands reflects genetic similarity. Diagnostic bands that distinguish colony profiles are indicated by arrows. Termites from unknown sources (?) can be assigned to a list of possible colonies by diagnostic bands and genetic similarity.
(b) Microsatellite genotyping: PCR amplification using labeled primers; individual homo- and heterozygote genotypes at single loci. For more details on this simplified representation, see Oetting *et al.* 1995.

DNA fingerprinting with microsatellite genotyping. Microsatellite genotyping is based on PCR amplification of tandem repeats using specific primers derived from flanking regions. Primers for subterranean termites, including *C. formosanus*, were recently developed by creating and screening a genomic library (Vargo 2000, Vargo & Henderson 2000). Following a PCR reaction the products are separated electrophoretically on sequencing-length polyacrylamide gels. Different detection methods are available, including radio- or fluoro-labeled primers, using, for example, an M13 tail carrying a fluorescent dye, which is detected by laser during the gel run (Oetting *et al.* 1995; Fig. 1b). Resulting allelic patterns can be scored to a resolution of single base pair differences. The different alleles vary in length and are co-dominant, i.e. homo- and heterozygotes can be detected at each locus (Fig. 1b). Like multilocus banding patterns, microsatellite genotypes are inherited in simple Mendelian fashion, and are likely selectively neutral. Therefore, allele frequencies and genotypic distributions can be analyzed using the standard statistical procedures of population genetics (e.g. *F*-Statistics). In addition, genotypes within colonies can be tested for Mendelian distribution, thus providing information about numbers of breeding reproductives.

Using a combination of DNA fingerprinting and microsatellite genotyping techniques, a comprehensive genetic study of population biology and colony organization of *C. formosanus* in Hawaii is currently in progress. Termites from up to 19 field collection sites were collected across the island of Oahu, Hawaii, within geographical distances of 150m up to 40km to analyze colony and population genetic structure (for details on sampling of the material, see Husseneder & Grace 2001a). In addition, we compared genotypes found in the Hawaiian population to *C. formosanus* colonies from China and Japan.

RESULTS AND DISCUSSION

Results of colony identification and the hierarchy of genetic similarities of *C. formosanus* on the island of Oahu using multilocus DNA fingerprinting are published elsewhere (Husseneder & Grace 2001a, 2001b). Therefore, we will only briefly review these results in the present manuscript and refer the reader to the original papers for details. Studies based on microsatellite analysis are currently in progress and only preliminary results are reported here.

Multilocus DNA fingerprinting: Identification of colonies and colony members.

To define the colonies of *C. formosanus*, we have recently "fingerprinted" termites collected at 16 sites on the island of Oahu, Hawaii.

DNA fingerprinting characterizes each individual by a banding pattern comprising on the average 11 bands (Husseneder & Grace 2001a). The proportion of shared bands reflects the genetic similarity between individuals within and among collection sites (Lynch 1990, 1991). The genetic profile of each collection site was derived from pooled samples (Fig. 1a). By comparing these profiles, genetic differentiation between the 16 collection sites was established (Husseneder & Grace, unpublished). Comparative studies to attempt assignment of individual termites to their collection site of origin show that results based on DNA fingerprinting are more accurate than results based upon morphometry and aggression tests (Husseneder & Grace 2000, 2001b).

To determine if collection sites actually represent different colonies and to interpret genetic differences within and among these colonies, the entire hierarchy of genetic similarities of *C. formosanus* on the island of Oahu was defined by multilocus DNA fingerprinting (Husseneder & Grace 2001a). Genetic similarity between the 16 collection sites investigated in this study approached the genetic background similarity in the population; i.e., the genetic similarity measured between presumably unrelated individuals collected >3km apart (avg. 0.21). Genetic similarity between termites from the same collection site was significantly higher (avg. 0.64) than among different collection sites. Thus, collection sites in this study represent independent colonies.

Comparing genetic similarities of individuals from field colonies (avg. 0.64) to laboratory colonies raised from sibling pairings (avg. 0.79) showed that the degree of inbreeding in field colonies is on the average rather moderate. However, degrees of inbreeding as well as number and relatedness of reproductives may vary among different colonies and will be investigated in detail using microsatellite genotyping.

We intend to use the sensitivity of DNA fingerprinting for studying intracolonial genetic structure to explore the possibility of separate breeding units within a colony, which might subsequently lead to colony proliferation by budding. Intracolonial structure was recently demonstrated in studies of the African termite *Schedorhinotermes lamanianus* Sjöstedt (Isoptera: Rhinotermitidae) by Kaib *et al.* 1996 & Husseneder *et al.* 1998. In addition, we will identify colonies by their genetic profile prior to baiting and elimination of all detectable termite activity at selected sites. In case of re-invasion of these sites, we will thus be able to determine if newly-appearing termites are (a) remnants of the same colony, (b) expanding from adjacent colonies, or (c) invading from outside the local population.

Microsatellite genotyping: Population structure and social organization of colonies.

Having identified individual termite colonies, we are currently investigating the relationship between colonies within a population. Knowledge of inbreeding within the population, and of population substructure and isolation by distance, for example, enables us to infer processes of spread throughout a geographic region. In addition, we can characterize the social organization of the colonies to investigate inbreeding on the colony level. To be able to apply standard population statistics as well as compare populations on a global scale we employed microsatellite genotyping to complement our previous fingerprinting data.

Preliminary results are based on ten polymorphic loci with two to six alleles that were analyzed in 19 termite colonies of *C. formosanus* on the island of Oahu, Hawaii. This *C. formosanus* population deviates from Hardy-Weinberg equilibrium due to homozygote excess at eight of the ten loci. Similar to previous results from DNA fingerprinting (Husseneder & Grace, unpublished), pairwise comparisons of genetic distance between colonies derived from microsatellite genotyping suggest that the deviation from Hardy-Weinberg equilibrium is not caused by population substructure nor isolation by distance (Husseneder, Vargo & Grace, unpublished). Apparently, in spite of the limited natural dispersal distance of *C. formosanus* (Tamashiro & Su 1987), gene flow around the island of Oahu is considerable, probably due to human transport. A hierarchical analysis of the variance of allelic distributions within and between colonies (Wright's *F*-Statistics, Weir & Cockerham 1984) indicates that inbreeding predominantly occurs at the level of the colony. To investigate this further, we are currently studying the social organization of *C. formosanus* colonies. To date, we have examined 7-24 individuals from each of the 19 colonies mentioned above at three loci. Seven of these colonies had Mendelian genotypes in the ratios expected in colonies headed by a single pair of reproductives. The remaining 12 colonies showed genotypes or genotypic ratios inconsistent with the assumption of a single breeding pair, thus indicating the presence of multiple reproductives. Elevated relatedness ($r>0.50$) among workers in the majority of the non-Mendelian colonies suggests the presence of inbred neotenic reproductives (Husseneder, Vargo and Grace, unpublished).

In addition, our preliminary data show clear differences in the allele distribution and frequencies of Hawaiian *C. formosanus* in comparison

to colonies from China and Japan. Alleles were found that occurred only in the populations from China or Japan, and are thus diagnostic for those populations. This degree of genetic differentiation among populations on a global scale could permit tracking of likely sources of termite introductions.

CONCLUSIONS

By applying two highly informative molecular genetic techniques, multilocus DNA fingerprinting and microsatellite genotyping, in a complementary manner, we are currently describing the genetic structure of colonies and populations of the termite pest *Coptotermes formosanus*. Enhanced understanding of spatial and social organization of termite colonies is essential for inferring the life history and evolution of these important social insects, as well as for improved colony suppression and elimination, such as through baiting systems. Knowledge of population genetic structure will also allow more accurate predictions about the nature and speed of spreading infestations. Establishing the genetic relationships among native and introduced populations may lead to identification of possible sources of introduction that will allow regulatory agencies to focus inspection and quarantine efforts more effectively.

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