

Lack of Mitochondrial DNA Variation in an Introduced Population of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

by

Richard E. Broughton¹ & J. Kenneth Grace²

ABSTRACT

Dispersal and colonization of natural populations may involve very small numbers of individuals and consequently result in severe reductions in genetic variation. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has been introduced from China to many tropical and subtropical localities world wide. Because it is a severe economic pest, great value has been placed on understanding the biology of this species. In order to quantify the amount of genetic variation present and possibly identify colony diagnostic characters, a mitochondrial DNA restriction site survey was performed on an introduced population in Hawaii. No mitochondrial DNA variation was found in any of the *C. formosanus* colonies examined. We attribute this to a population bottleneck resulting from a very limited number of introductions to Hawaii.

INTRODUCTION

The activities of humans may greatly alter the distribution of natural populations. Human assisted range expansion, whether intentional or not, may have significant effects on recipient localities; often with deleterious results. Colonization events involving small numbers of individuals may also greatly effect the genetic structure of invading populations. Such population bottlenecks may reduce genetic variation and significantly alter allele frequencies (Carson 1968; Templeton 1980). The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a prime example of human aided colonization. This species is native to China (Kistner 1985) but it has been inadvertently distributed to many tropical and subtropical regions of the world including Hawaii. Dispersed populations of *C. formosanus* provide a model for the study of introduced species.

Although it was first officially collected in Honolulu in 1907 (Swezey 1914), *C. formosanus* may have been introduced there as much as 40 years earlier (Tamashiro *et al.* 1987). This species has since spread to

¹Department of Zoology, Arizona State University, Tempe, AZ 85287-1501

²Department of Entomology, University of Hawaii, 3050 Maile Way, Honolulu, HI 96822-2271

all the major Hawaiian islands, with colonies found in greatest numbers on Oahu and Kauai. Mature colonies of *C. formosanus* may contain millions of individuals (Su *et al.* 1984) which are capable of causing extensive damage to wooden structures and crops such as sugarcane (Roonwal 1970). As *C. formosanus* has become Hawaii's greatest economic pest (Tamashiro *et al.* 1987), considerable effort has been directed at understanding the biology of this termite (Tamashiro & Su 1987). An area of particular interest is the genetic structure of *C. formosanus* populations and colonies.

Studies of agonistic behavior have revealed aggressive interactions among several *C. formosanus* colonies on Oahu but a general lack of aggression among colonies of this species from Florida (Su & Haverty 1991). These behavioral differences may reflect different genetic relationships among colonies, with the lack of aggression among Florida colonies due to the colonies being very closely related. It is thought that *C. formosanus* has been introduced into Florida only once (Su & Haverty 1991). In fact, two Florida colonies are known to have fused forming a larger colony, suggesting they are genetically similar (Su & Scheffrahn 1988). Conversely, it has been hypothesized that *C. formosanus* may have been reintroduced numerous times to Hawaii (Tamashiro *et al.* 1987) resulting in greater genetic diversity in this population.

To assess the level of genetic variation present in Hawaiian *C. formosanus* we conducted a restriction site survey of mitochondrial DNA (mtDNA). In addition, we hoped mtDNA would provide molecular markers suitable for distinguishing individuals from various colonies. This information would be useful in the study of colony and population dynamics and may provide insights on the number of introductions to Hawaii and the spread of these termites through the state.

MATERIALS AND METHODS

Colony sampling. The mtDNAs of 10 different colonies of *C. formosanus* were examined, including 9 samples from Oahu and 1 from Maui. These colonies have also been surveyed for allozyme variation (Strong & Grace 1993), and include those evaluated for intercolony agonistic behavior (Su & Haverty 1993). The Oahu colonies include 1 from the Poamoho Experiment Station, 1 from a sugarcane field on the Waipio peninsula, 1 from a residential yard in Kaneohe, and 5 from the Manoa campus of the University of Hawaii. The remaining sample was collected from fence posts in Lahaina, Maui and represents a recent introduction to that part of the island. Live termite workers (pseudergates) were frozen on dry ice and stored at -80°C . until use for mtDNA isolation.

MtDNA analysis. MtDNA isolation and restriction enzyme analysis

were conducted as described by Dowling *et al.* (1990) with only minor modification. MtDNA was isolated by cesium chloride-propidium iodide ultracentrifugation of mitochondrial enriched cell lysates obtained from approximately 50 individuals of each colony ground together (only 20 individuals were available for the Lahaina colony). Each colony mtDNA sample was digested with 16 restriction enzymes as described by the supplier (Promega Corp.). Restriction enzymes surveyed included *Apal*, *BamHI*, *BclI*, *BglII*, *BstEII*, *DraI*, *EcoRI*, *EcoRV*, *HinDIII*, *NcoI*, *NdeI*, *NheI*, *PvuII*, *SacI*, *SpeI*, and *XhoI*. Restriction fragments were end-labeled with $\alpha^{32}\text{P}$ labeled nucleotides, electrophoresed on 1% agarose and 4% polyacrylamide gels and visualized by autoradiography.

RESULTS

No restriction site variation was found among any of the samples examined. The number of restriction fragments produced by each enzyme is as follows: *Apal* - 1, *BamHI* - 1, *BclI* - 3, *BglII* - 3, *BstEII* - 1, *DraI* - 2, *EcoRI* - 4, *EcoRV* - 2, *HinDIII* - 1, *NcoI* - 1, *NdeI* - 4, *NheI* - 1, *PvuII* - 0, *SacI* - 1, *SpeI* - 7, and *XhoI* - 0. That 9 of the 16 enzymes cut zero or once is rather unusual, however, the enzymes used tend to have G-C rich recognition sequences and the low cutting frequency may reflect a high A-T content of *C. formosanus* mtDNA. Regardless of this, mtDNA variation within the Hawaiian *C. formosanus* population is very limited.

DISCUSSION

An explanation for the low level of mtDNA variation in Hawaiian *C. formosanus* is that few reproductive individuals were likely involved in colonization. Distribution of these termites to widely dispersed geographic locations is probably exclusively through human activities. This assumption is based on the fact that *C. formosanus* alates (winged reproductives) are very poor flyers and individuals require external assistance to move more than several hundred feet (Higa & Tamashiro, 1983). Commercial shipping is the primary mode of transport with infested wooden boats, shipping containers, and wooden products themselves serving as sources of introduction.

The actual number of introductions to Hawaii is unknown but has been suggested to be small and may have involved a single event (Haverty & Grace 1993, Strong & Grace 1993). Even with several introductions, limited genetic diversity may result if all came from the same Chinese source population. Colonization events of this kind have been discussed in detail with respect to founder effect speciation

(Carson & Kaneshiro 1976; Carson & Templeton 1984; Barton 1989). Such events generally cause extreme restrictions in effective population size with a corresponding reduction in genetic variation due to sampling effects. The haploid maternal nature of mtDNA inheritance reduces its effective population size to approximately one fourth that of nuclear genes (Birky *et al.* 1983). Because of this, variation in mtDNA is affected to a greater extent by reductions in population size such as bottlenecks associated with colonization.

Such a bottleneck appears to have affected the population structure of *Drosophila mercatorum* which was introduced to Hawaii in the early 1800s (DeSalle *et al.* 1987). While this species has maintained some genetic variation at allozyme loci, mtDNA variation is very low. In addition, the population shows structuring with respect to mtDNA but appears to be panmictic for nuclear markers (Clark *et al.* 1981; DeSalle *et al.* 1987). This was attributed to the lower effective population size of mtDNA. This case appears to have strong similarities with the situation in Hawaiian *C. formosanus*. Bottleneck effects may be especially important in social insects such as termites where an introduction may involve a large number of individuals but represent only a single genetic lineage, as in the case of invasion by a single colony.

If introduction to Hawaii involved a single colony, all descendant colonies would possess identical mtDNAs barring new mutation. Assuming a first introduction less than 150 years ago and the mutation rate for invertebrate mtDNA on the order of 10^{-6} per generation (Powell *et al.* 1986; Vawter & Brown 1986), *de novo* mutation would not be expected to contribute significantly to variation in Hawaiian *C. formosanus*. Theory on founder effect speciation predicts that recently established populations may expand rapidly in the new locality but may possess allele frequencies that are significantly different from the source population, due to the bottleneck effect. Thus, we expect that mtDNA in the Hawaiian *C. formosanus* population represents a small fraction of the haplotype diversity present in natural populations in China. MtDNA analysis of Chinese populations would be informative in this regard.

The lack of variation in *C. formosanus* mtDNA is consistent with several previous studies that have found little or no genetic or phenotypic variation in this and other introduced *C. formosanus* populations. Allozyme studies of populations from Hawaii, Florida and Louisiana have shown very few genetic differences within or among populations (Korman & Pashley 1991; Strong & Grace 1993). Differences in cuticular hydrocarbon composition in these populations are also limited (Su & Haverty 1991; Haverty *et al.* 1992; Haverty & Grace

1993).

Studies on Chinese populations involving allozymes and mtDNA as well as hydrocarbon patterns would be useful in identifying the source localities for the Hawaiian population, as well as those in other geographic areas. This information is currently being developed (Grace, Haverty, Wang & Broughton, unpublished data), and should greatly improve our understanding of population and colony structure in introduced *C. formosanus* populations. Our mtDNA results, while disappointing with regard to finding colony diagnostic markers, fit the general conclusion of low genetic and phenotypic variability in introduced *C. formosanus* populations that has emerged from several recent studies on *C. formosanus* around the world.

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