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Using A Genetic Marker (MDH-1) to Study Genetic Structure in Colonies of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

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Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki is considered to be native to China (Kistner 1985). Because the species attacks wooden houses and living trees, it is recognized as a very serious economic pest throughout the world, especially in tropical and subtropical areas.

To date no good genetic marker has been found to facilitate the study of *C. formosanus* genetics. To establish a clear and reliable genetic or cytological marker, it is very important to include sympatric and allopatric species. This paper reports results obtained using polyacrylamide gel and cytological methods:

- a genetic marker (MDH-1) was established;
- genetic structures of five colonies from the Manoa campus of University of Hawaii were analyzed by using the MDH-1 locus;
- the number of chromosomes of Hawaiian *C. formosanus* was determined.

Materials and Methods

Sample and preparation for electrophoresis. Fifteen individuals were randomly sampled from each of five colonies at the UHM campus; labeled as Gilmore, Pope, Publication, Miller, and Hale Halawai Colony. The guts were removed from some individuals to determine the effects produced by protozoans. Each individual was placed into a microtube to which 50 μ l 0.05M pH 8.5 Tris-HCl was added and homogenized in an ice bath. The homogenate was centrifuged for 10 minutes at 11,000 RPM and 4°C. Only the supernatant was analyzed. Samples consisted of three workers and three soldiers with guts, and three workers and three soldiers without guts that were run on the same gel.

Cytological Procedure. Male and female alates (winged reproductives) were collected and paired during the swarming season. Each pair was held in a vial that contained a sawdust layer covered with vermiculite. All vials were held in an unlighted incubator set at 28°C. Chromosome preparation followed Luykx's protocol (1990). Ovaries were used in this study since they illustrate all stages of meiosis. Meiotic

bivalent stained with Giemsa were examined under the compound microscope (using oil emersion).

Results

Gel patterns of workers and soldiers. Three dyed bands can be observed in the samples of workers with guts. However, only two dyed bands can be seen in the samples of soldiers with guts and the samples of workers and soldiers without guts. The number of protozoans harbored in *C. formosanus* varies with the caste member. Lai (1983) reported that *C. formosanus* soldiers had only 10% of the protozoa complement found in workers, which could explain the absence of the third band observed in workers with guts.

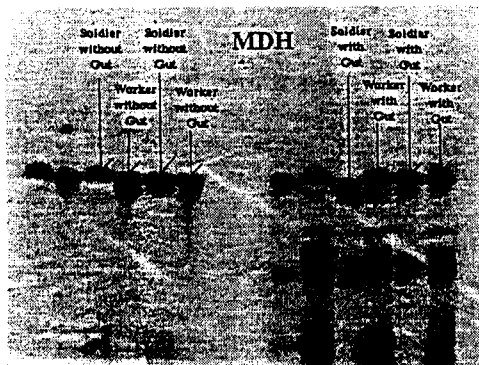


Figure 1. Gel patterns among workers and soldiers, with and without guts.

Genetic structure of colonies. Each colony from the Manoa campus was considered an independent family, and mark-release-recapture studies indicate clear separation between colonies. Since a termite colony is created by a male and female, our data can be used to infer the genotypes of the founding pairs. Only the Miller colony was found to have no significant difference between observed and expected individuals. We can conclude that the founding pair of this colony is still occupying the colony and no inbreeding has yet occurred within the colony. On the other hand, Pub., Pope, and Gil. showed significant differences between observed and expected individuals.

Table 1. Genotype numbers and frequencies of MDH-1 from five *C. formosanus* colonies.

Colony	Genotype			Allele #		Gene %	
	FF	FS	SS	F	S	F	S
Pub.	1	4	10	6	24	0.2	0.8
Mill.	0	7	8	7	23	0.23	0.77
Pope	0	1	14	1	29	0.03	0.97
Gil.	2	3	10	7	23	0.23	0.77
Hale	0	0	15	0	30	0	1

The changes in these colonies might be brought about by genetic drift from inbreeding (Wang et al. 1992). The inbreeding coefficient (F) can be used to determine the level of inbreeding in different colonies. The Pope colony had the highest inbreeding coefficient, suggesting that this is an old colony.

Table 2. Colony inbreeding coefficients (F).

Colony	F value
Pub.	0.46
Mill.	0.06
Pope	0.86
Gil.	0.60

Cytological results. The chromosome number for *Coptotermes formosanus* is $n = 21$. This result is the same as that of the typical haploid chromosome number for the family Rhinotermitidae (personal communication from Dr. P. Luykx).



Figure 2. Meiotic bivalent in *Coptotermes formosanus*.

Discussion

Different castes of *C. formosanus* contain different numbers and kinds of the protozoans in their guts (Lai et al. 1983). In order to get a clear electrophoretic gel pattern we have to consider the effects of the protozoans. Our results indicate that workers with guts show two more MDH bands than soldiers with or without guts or workers without guts. We may have observed this result because the soldier has one tenth the number of protozoans of a worker. Further research will determine if protozoans contribute the band in samples containing workers with guts.

Using MDH-1 as a genetic marker, we determined that *C. formosanus* colonies on the UHM campus have different genetic structures. The inbreeding coefficient F in each colony reflects the level of inbreeding. The inbreeding level in the Pope colony is the highest, suggesting that this is a relatively old colony containing active supplementary reproductives. The F value of the Miller colony is not significant and indicates that secondary reproductives have not yet become active in the colony. The F value of the Hale colony is not available at this time since we could not accurately differentiate between inbreeding and the genotype of founding pairs. This will require the use of individuals with known genotypes. Although the studies with the MDH-1 genetic marker are just beginning, and the sample size and localities of our samples are limited, our results promise to give us some insight into the age of termite colonies and the genetic drift of a *C. formosanus* population, and help to determine the pattern of termite colonization.

Literature cited

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