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LOW ALLOZYME VARIATION IN FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) COLONIES IN HAWAII

K. L. STRONG[1] AND J. K. GRACE[2]
Department of Entomology, University of Hawaii,
Honolulu, Hawaii 96822-2271

Abstract.—Cellulose acetate gel electrophoresis was used to assess allozyme variation among Hawaiian populations of *Coptotermes formosanus* Shiraki. Twenty-nine protein loci were resolved in an initial survey. Eight of these proved to be reliable, and 13 termite colonies from the islands of Oahu and Maui were surveyed for these loci. Individual workers (pseudergates) were monomorphic for all loci across all colonies, although preliminary results from starch gel electrophoresis suggest that a very low level of polymorphism is present at one locus. This finding is consistent with previous electrophoretic studies of *C. formosanus* populations, which have found little genetic variation. Low allozyme variation may be characteristic of this species, or Hawaiian *C. formosanus* colonies may be derived from a single introduction or from multiple introductions of very closely related individuals.

Key Words.—Insecta, termite genetics, cellulose acetate gel electrophoresis

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is native to China (Kistner 1985) but has been distributed extensively by man through the tropical and subtropical regions of the world. The range of this serious economic pest now includes South Africa, Sri Lanka, Japan, Taiwan, Guam, the Midway islands, the Hawaiian islands and the United States mainland (Su & Tamashiro 1987). *Coptotermes formosanus* was collected in Honolulu, Hawaii in 1907 and recorded as established in 1913 (Zimmerman 1948); however, it may have been present as early as 1869 (Tamashiro et al. 1987). Since then, it has spread throughout the islands of Oahu and Kauai, with distributions restricted to certain seaports or areas immediately surrounding seaports on the islands of Hawaii, Maui, Molokai and Lanai (Tamashiro et al. 1987). This termite has spread slowly through the state and has been distributed mainly via the actions of man (Higa & Tamashiro 1983, Tamashiro et al. 1987). However, it has become the most important economic pest in the Hawaiian islands (Tamashiro et al. 1987). Populations of mature colonies of *C. formosanus* number in the millions of individuals (Su et al. 1984).

Demographics, foraging dynamics, and biochemical characteristics of several *C. formosanus* colonies on Oahu are monitored regularly using a trapping technique (Tamashiro et al. 1973). Aggressive interactions have been observed between members of some, but not all, of these termite colonies (Su & Haverty 1991). Such agonistic displays may represent interactions between genetically differentiated colonies (Su & Scheffrahn 1988). Allozyme electrophoresis has frequently been used to obtain estimates of insect genetic relatedness (Berlocher

[1] Current address: Division of Botany and Zoology, Australian National University, GPO Box 4, Canberra, ACT 2601, Australia.
[2] Author for reprint requests.

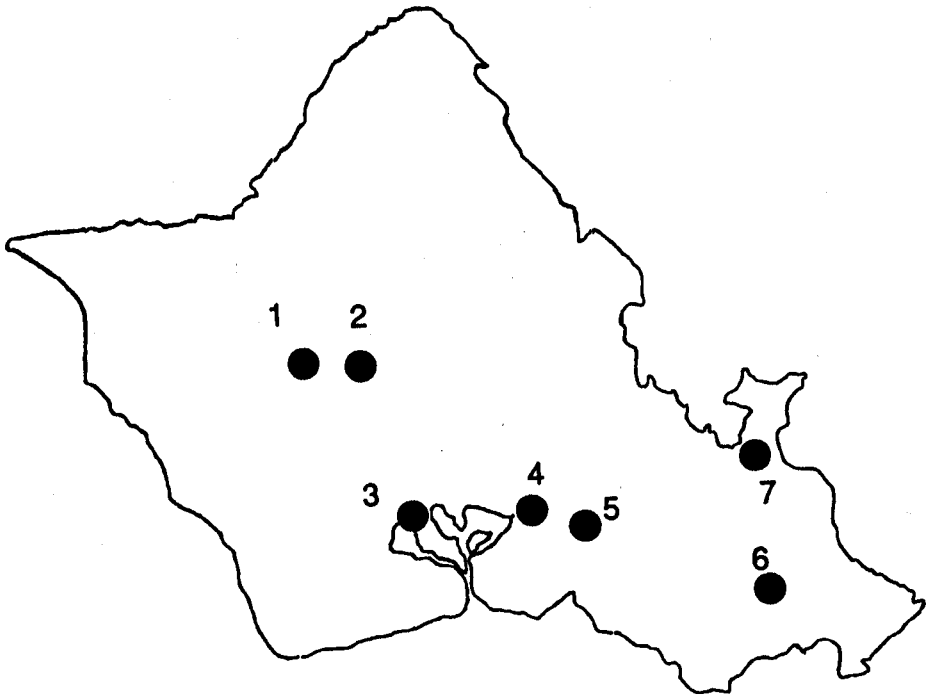

Figure 1. Locations of *Coptotermes formosanus* colonies sampled from the island of Oahu, Hawaii. An additional colony was sampled from Lahaina, Maui (not shown). 1, Poamoho Experiment Station field shed; 2, Poamoho Experiment Station (founded from laboratory stock); 3, Waipio peninsula; 4, Pearl Harbor; 5, Manana warehouses; 6, University of Hawaii at Manoa campus (six colonies); 7, Kaneohe residential yard.

1984). Similarly, this technique has been used to investigate the origin of introductions of *C. formosanus* to the United States mainland and the possibility of cryptic species within the range of *C. formosanus* in the United States (Korman & Pashley 1991).

Valuable information on the population structure and size of rhinotermitid colonies has been collected in previous studies of allozyme variation (Clement 1981, Reilly 1988). Our electrophoretic investigation was initiated to find allozyme markers useful in distinguishing among different *C. formosanus* colonies. Such markers could be used to determine the number and sources of termite introductions to Hawaii, and possibly to trace the spread of this termite throughout the state. We also wished to determine if the agonistic encounters between *C. formosanus* colonies recorded, by Su & Haverty (1991), were correlated with allozyme differences.

Materials and Methods

Termite collections.—Formosan subterranean termite workers (pseudergates, or undifferentiated individuals older than the third instar) were collected from 13 locations, representing 13 different colonies, on the Hawaiian islands of Oahu and Maui (Fig. 1). These included the colonies used by Su & Haverty (1991) in their study of intercolony agonistic interactions (N.-Y. Su, personal communi-

Table 1. Presumptive enzyme loci and buffer systems used in electrophoretic survey of Hawaiian *Coptotermes formosanus*.

Locus	Abbrev.	E.C. number	Buffer system[b]	No. of loci
Aconitase[a]	Acon	4.2.1.3	B	2
Glucose phosphate isomerase[a]	Gpi	5.3.1.9	A	1
B-hydroxybutyrate dehydrogenase	Hbdh	1.1.1.30	C	1
Aldehyde oxidase	Ao	1.2.3.1	A	1
Adenylate kinase[a]	Ak	2.7.4.3	A	2
Alcohol dehydrogenase	Adh	1.1.1.1	A	1
Amino aspartate transferase	Aat	2.6.1.1	C	1
Fructose 1,6-diphosphate dehydrogenase	Fdp	3.1.3.11	C	3
Hexokinase	Hk	2.7.1.1	D	2
Isocitrate dehydrogenase	Idh	1.1.1.42	B	1
Phosphoglucomutase	Pgm	2.7.5.1	B	2
Glucose 6-phosphate dehydrogenase	G6pd	1.1.1.49	D	1
Malic enzyme	Me	1.1.1.40	B	2
Malate dehydrogenase[a]	Mdh	1.1.1.37	B	2
6-phosphogluconate dehydrogenase	6Pgd	1.1.1.44	C	1
Fumerase hydratase	Fh	4.2.1.2	B	1
Esterase[a]	Est	3.1.1.1	A	2
Mannose 6-phosphate isomerase	Mpi	5.3.1.8	B	2
Glyceraldehyde 3-phosphate dehydrogenase	Gapd	1.2.1.12	A	1

[a] Reliable loci chosen for comparison among all termite colonies.

[b] Buffer systems: A, 100 mM tris maleate (pH 7.8); B, 100 mM tris citrate (pH 8.2); C, 100 mM phosphate (pH 7.0); D, 100 mM tris borate EDTA (pH 8.9).

cation). Six of the colonies are located on the Manoa campus of the University of Hawaii, and are monitored monthly using the trapping technique described by Tamashiro et al. (1973). Two other Oahu colonies that are regularly monitored are located at the Poamoho Experiment Station: one of these was originally founded from unknown laboratory stock in 1979 (M. Tamashiro, personal communication), but the other is adventitious in the vicinity of a field shed. The other two Oahu colonies subject to monitoring are located (1) in a sugarcane field on the Waipio peninsula, and (2) in a residential yard in Kaneohe. Two other collections were made on Oahu from infested wood found on the ground within the U.S. Navy's Pearl Harbor and Manana warehouse facilities. A single collection from fence posts in Lahaina, Maui, represents a recent introduction to that part of the island. Samples from each of these 13 colony sources were maintained alive in the laboratory, or snap frozen in liquid nitrogen, until prepared for electrophoresis.

Electrophoresis.—Cellulose acetate (Cellogel, Chemetron, Milan, Italy) horizontal gel electrophoresis was used to resolve the products of 29 presumptive enzyme loci (Table 1). This method is simple and efficient with small insects, because less than 1 μl of sample need be applied to the gel to achieve maximal stain intensity. The buffer systems and staining procedures used were those of Richardson et al. (1986). As noted in Table 1, buffer systems were: 100 mM tris maleate (pH 7.8) [A], 100 mM tris citrate (pH 8.2) [B], 100 mM phosphate (pH 7.0) [C], and 100 mM tris borate EDTA (pH 8.9) [D]. Individual termites were ground in Eppendorf centrifuge tubes using a fitted pestle and 0.1 ml of 100 mM

tris HCl (pH 8.0) buffer. Approximately 1 μl of each ground sample was applied to pre-made loading slots with a draughtsman's pen (Richardson et al. 1986).

In an initial screening, 6–21 individuals from each of the University of Hawaii *C. formosanus* colonies were assayed for each presumptive enzyme locus. Eight reliable loci were identified for comparison with the other termite colonies. All 13 colonies were then compared for these eight loci, with 4–26 individuals from each colony examined. The smaller sample sizes came from the colonies found at Pearl Harbor, Manana (Oahu), and Lahaina (Maui), where fewer individuals were collected.

Results and Discussion

Using cellulose acetate gel electrophoresis, no polymorphisms were found for any of the 29 loci (Table 1) in the 13 Hawaiian *C. formosanus* colonies surveyed, and thus no allozyme variation was identified within or between these colonies. In their survey of geographically disparate populations of *C. formosanus* from the mainland United States and Hawaii, Korman & Pashley (1991) reported that only 3 of 18 loci (16.7%) were polymorphic. They concluded that *C. formosanus* was depauperate of genetic variation, especially when compared to the approximate 40% polymorphic loci for the class Insecta as a whole (Nevo 1978). We were unable to reliably score two of the esterase loci that Korman & Pashley (1991) found to be polymorphic, and our methods did not elucidate the polymorphism reported by these authors for glucose phosphate isomerase (Gpi, Table 1). However, our preliminary results (unpublished data) with horizontal starch gel electrophoresis, using the methods of Korman & Pashley (1991), have subsequently confirmed that Gpi is polymorphic in Hawaiian *C. formosanus,* although no additional polymorphisms have been resolved.

Several studies have reported greater allozyme variation in other termite species. Korman et al. (1991) distinguished among *Zootermopsis angusticollis* (Hagen), *Z. laticeps* (Banks), and *Z. nevadensis* (Hagen) (Termopsidae) on the basis of 11 polymorphic loci. Clement (1981) found European *Reticulitermes* spp. (Rhinotermitidae) to have a high proportion of polymorphic loci (52%) and he used fixed electromorph differences to delineate different species. In contrast, allozyme electrophoresis by Reilly (1987) revealed only four polymorphic loci in *Reticulitermes flavipes* (Kollar) colonies from the southern United States, suggesting that these colonies were inbred and genetically isolated.

Cuticular hydrocarbon profiles have been used to separate colonies of *C. formosanus* from four geographically distinct areas (Florida, Hawaii, and two sites in Louisiana) (Haverty et al. 1990). In so much as cuticular hydrocarbon profiles reflect genetic phenomena, these results indicate that genetic differences may exist between *C. formosanus* colonies from disparate regions. However, Su & Haverty (1991) did not find any correlation between cuticular hydrocarbon profiles and intercolony agonistic behavior, which could also reflect genetic differences. The Hawaiian colonies studied by Su & Haverty (1991) were included in our allozyme survey and did not differ at allozyme loci. Thus, the genetic basis of these allozyme loci and of cuticular hydrocarbons appears to be unrelated to that of intercolony agonistic behavior.

Although we do not know what degree of allozyme variation is present in *C. formosanus* in China, where this species is indigenous (Kistner 1985), Hawaiian

populations may have lost variation either as a result of founder events and subsequent genetic drift, or of inbreeding. Natural movement of termites to nearby locations may take years, as demonstrated by an established colony on Maui that moved only a few kilometers in over 20 years (Tamashiro et al. 1987). Thus, the spread of *C. formosanus* throughout Hawaii was certainly aided by man via transport of partial colonies in infested wood. Inbreeding in rhinotermitids is facilitated by the common event of colony fission, or formation of new colonies by development and mating of supplementary reproductives within a large colony and subsequent "budding off" of these individuals and their offspring to form an adjacent independent colony (Weesner 1956). Asynchronous swarming of nearby termite colonies could also result in new colony formation by paired siblings, and has been suggested as a mechanism for generating inbreeding in *R. flavipes,* where winged alates captured before dispersal from different colonies are often at different stages of development (Reilly 1987).

A wider geographic sampling of *C. formosanus* colonies or alternative electrophoretic techniques may yield greater allozyme variation. However, the evidence to date from our study and that of Korman & Pashley (1991) suggests that lack of variation may be characteristic of this species and reflect an overall genetic homogeneity. Alternative, and currently equally acceptable, hypotheses are that *C. formosanus* colonies in Hawaii are derived from a single introduction, or from multiple introductions of very closely related individuals, possibly from the same geographic locale.

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