

Esterase Differences Among *Coptotermes formosanus* (Isoptera: Rhinotermitidae) Populations

by

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ABSTRACT

Polyacrylamide gel electrophoresis with *Coptotermes formosanus* workers revealed five esterase loci. One of these bands (Est-1) was shown in previous work to be attributable to symbiotic gut protozoa. Four esterase inhibitors were applied in order to clarify the gel pattern. One of these, Paraoxon, selectively blocked Est-4 and Est-5. Color differences indicated that there were two loci present at the Est-2 position: Est-2A which is sensitive to alpha-naphthyl acetate substrate, and Est-2B which is sensitive to beta-naphthyl acetate substrate. *Coptotermes formosanus* populations from Guangzhou, Hong Kong, Hawaii and Lake Charles (Louisiana) have the Est-2A locus; while populations from New Orleans and Florida have the Est-2B locus. These results suggest that there have been at least two introductions of *C formosanus* to the United States, that the two populations in different parts of Louisiana have remained genetically isolated, and that the New Orleans and Florida populations are closely related.

INTRODUCTION

Enzymes with many loci, such as esterases, have proven useful due to their variability in investigations using protein electrophoresis to identify genetic markers among insect species or populations (e.g., Clement 1981, 1984; Saul *et al.* 1976; Wang *et al.* 1992; Perring *et al.* 1993; Korman *et al.* 1991). However, because esterases are coded by many loci and the products of these loci are sometimes strongly expressed, the stained bands on electrophoretic gels may overlap, providing little useful genetic information (Saul *et al.* 1976). To simplify and clarify these esterase patterns, specific inhibitors to block the expression of specific loci have been applied. Such treatments were helpful in revealing the genetic structure of European *Reticulitermes* species (Clement 1984) and *Aedes aegypti* (Saul *et al.* 1976).

In earlier research on esterases of *Coptotermes formosanus* (Wang & Grace 2000), we identified at least five esterase loci, each with one or

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two alleles. Unfortunately, the esterase gel patterns of *C. formosanus* were not adequate to generate useful genetic information because some of the products of these esterase loci were so strongly expressed that we could not determine which band was produced by which loci.

In the present study, four esterase inhibitors previously used in the genetic study of *Aedes aegypti* (Saul *et al.* 1976) were applied to clarify the gel patterns of *C. formosanus*. The objectives of this study were to determine which of these four inhibitors provided the clearest banding pattern, and whether unique loci existed that could be used to distinguish among different *C. formosanus* populations.

MATERIALS AND METHODS

Samples of thirty-six *C. formosanus* colonies were obtained from six locations: thirteen colonies from Hawaii, of which six were from the island of Oahu, one from Kauai, three from Maui and three from Hawaii; four colonies from New Orleans, Louisiana; four colonies from Lake Charles, Louisiana; six colonies from Florida; six from Hong Kong; and three from Guangzhou, China. Samples were refrigerated at -70°C prior to use.

Ten worker (pseudergate) individuals were randomly selected from each colony and were prepared for electrophoresis. Procedures for sample preparation and electrophoresis were the same as described by Wang & Grace (2000). A 0.1M phosphate buffer of pH 6.5 with alpha- and beta-naphthyl acetate substrates was used as a basic incubation mixture. Four inhibitors (Table 1) were added separately into portions of each sample in the basic incubation mixture, then stained with Fast Blue BB salt.

RESULTS AND DISCUSSION

All gels containing *C. formosanus* samples with only the basic incubation mixture added and stained with Fast Blue BB salt showed five band zones, and all band zones appeared fixed. These band zones

Table 1. Inhibitors used to clarify esterase gel patterns of *Coptotermes formosanus*.

Type of esterase	Inhibitor
Cholinesterase	Eserine 2×10^{-6} M
A	p-hydroxy mercuribenzoate 10^{-4} M
B	diethyl-p-nitrophenyl phosphate 10^{-5} M (Paraoxon)*
C	P-phenyl proprionic acid 10^{-2} M

*Paraoxon has an effect on the gel patterns produced by homogenate of *C formosanus*.

were labeled Est-1, Est-2, Est-3, Est-4 and Est-5, according to their mobility from the origin.

All gels with termite samples incubated with the basic incubation mixture and either eserine or *p*-hydroxy mercuribenzoate or beta-phenyl proprionic acid (stained with Fast Blue BB salt) also showed the same five band zones as the samples to which no inhibitors were added.

All gels with the basic incubation mixture and Paraoxon (stained with Fast Blue BB salt) showed only three band zones. The products of the Est-4 and Est-5 loci were blocked by this inhibitor (meaning that bands for these two loci did not appear), so that only Est-1, Est-2, and Est-3 were visible. Of these three band zones (Fig. 1), we previously identified

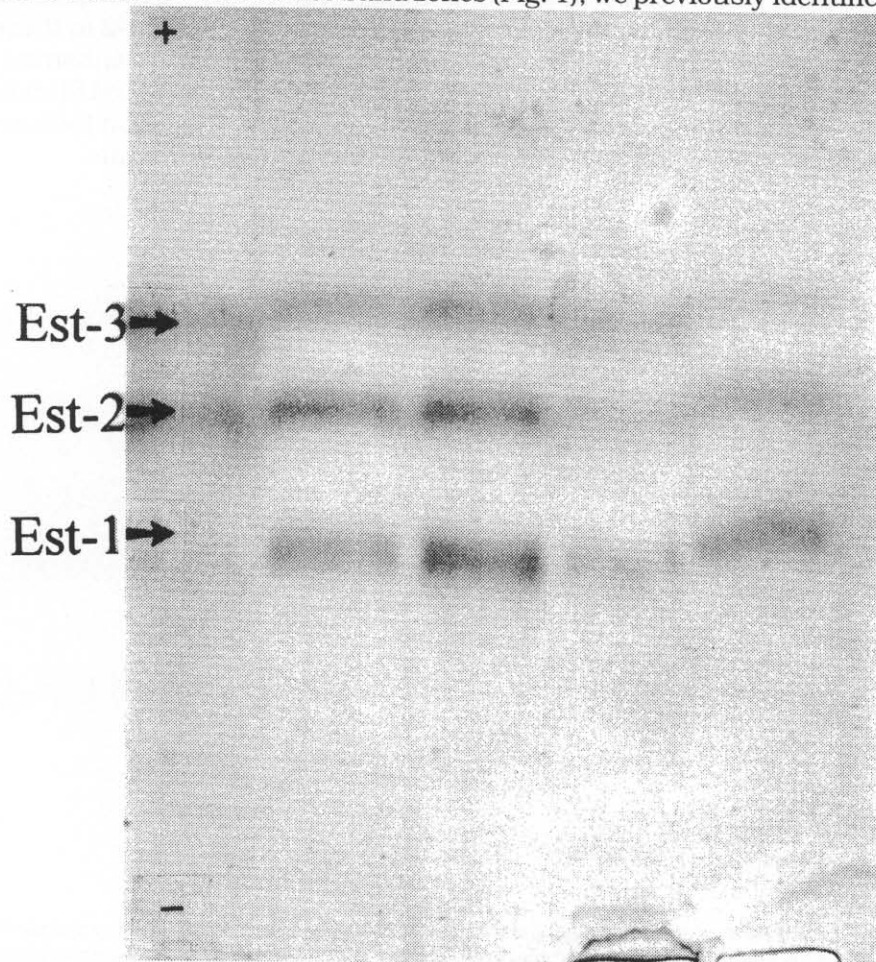


Fig. 1. Esterase gel pattern of *Coptotermes formosanus* after treatment with Paraoxon, an inhibitor that blocks the products contributed by Est-4 and Est-5.

the Est-1 band as produced by symbiotic gut protozoans of *C. formosanus* (Wang & Grace 2000). Thus, only two loci of *C. formosanus* were retained after the use of Paraaxon.

Among these two esterase loci (Est-2 and Est-3) visible after Paraaxon treatment, no variation was detected (Fig. 2) except for color differences between gels containing samples from different locations. Different colors in similar banding patterns may indicate that different loci are present. The bands produced by Est-3 from all samples at the six locations were red, indicating that Est-3 was sensitive to the beta-naphthyl acetate substrate and was fixed in all populations. Samples from Guangzhou, Hong Kong, Hawaii and Lake Charles showed grey color bands at the Est-2 position indicating Est-2 in these populations was sensitive to the alpha-naphthyl acetate substrate. However, the *C. formosanus* samples from the New Orleans and Florida populations showed reddish color bands at the Est-2 position indicating they were sensitive to the beta-naphthyl acetate substrate.

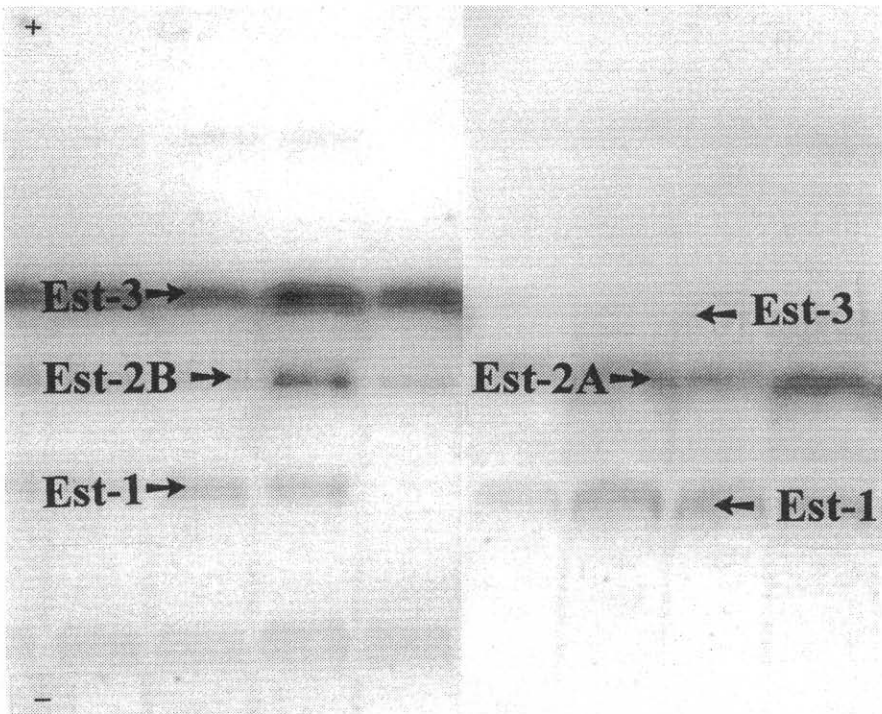


Fig. 2. Comparison of esterase gel patterns of *C. formosanus* from two different populations. Although not visible in this monochrome illustration, the Est-2A band is grey, and the Est-2B band is reddish in color. This indicates that they are two different loci, and that Est-2A is sensitive to alpha-naphthyl acetate, while Est-2B is sensitive to beta-naphthyl acetate substrate.

Based upon the color differences apparent at the Est-2 position in the *C. formosanus* samples from different locations, we concluded that there are two different loci present. We designated the alpha-naphthyl acetate sensitive locus in the Est-2 position which was present in the Guangzhou, Hong Kong, Hawaii and Lake Charles populations as Est-2A; and the beta-naphthyl acetate sensitive locus in the Est-2 position which was present in the New Orleans and Florida populations as Est-2B (Fig. 2).

These results suggest that there were at least two introductions of *C. formosanus* into the United States, each from a different source. The two Louisiana *C. formosanus* populations in Lake Charles and New Orleans appear to represent separate introductions and to have remained isolated from each other; while the population of *C. formosanus* in Florida appears to be related to the population from New Orleans. Korman & Pashley (1991) also concluded that populations from Florida and New Orleans were closely related. Further work with additional enzyme systems is in progress to elucidate the relationships among these geographically distinct termite populations.

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