

# Genetic Differentiation of *Coptotermes acinaciformis* Populations (Isoptera: Rhinotermitidae) by Esterase Patterns

by

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## ABSTRACT

Geographic populations of *Coptotermes acinaciformis* (Froggatt) in different regions of Australia are morphologically indistinguishable but have different nesting behaviors. Using allozyme electrophoresis, we were able to distinguish between subterranean and mound-building populations of *C. acinaciformis* on the basis of differences in their esterase patterns. These results are in agreement with previous reports of qualitative differences in their cuticular hydrocarbon profiles, although we also distinguished two subgroups within the subterranean-nesting population. The species *Coptotermes frenchi* differed from *C. acinaciformis* in having no detectable sorbital dehydrogenase activity.

## INTRODUCTION

*Coptotermes acinaciformis* (Isoptera: Rhinotermitidae) is a widely distributed Australian species or species complex with a wide tolerance for climatic conditions that is especially prevalent in urban areas (Watson 1988). Different *C. acinaciformis* populations demonstrate considerable variability in their nesting behaviors (Brown *et al.* 1990). The populations dwelling in northern tropical areas and northern parts of Queensland, are mound-builders, while populations occurring elsewhere make their nests underground, in tree stumps, in wooden poles, or in trunks of dead or living trees. It has been suggested that these different nesting behaviors may be attributable to food or micro-environment dependence (Gay & Calaby 1970).

Brown *et al.* (1990) investigated four of six Australian *Coptotermes* species, *C. lacteus* (Froggatt), *C. acinaciformis* (Froggatt), *C. frenchi* (Hill) and *Coptotermes* sp. P., and found that these four species could be differentiated morphologically and by cuticular hydrocarbon analysis. Brown *et al.* (1990) reported qualitative differences in hydrocarbon profiles between mound-building and subterranean *C. acinaciformis*, strongly suggesting that *C. acinaciformis* should be designated as a

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species complex containing at least two species. Quantitative differences in hydrocarbon proportions were also found within groups of mound-builders and subterraneans, but no qualitative differences were noted within these two habitat groups.

Although patterns of cuticular hydrocarbons in termites are considered to be under genetic control (Haverty *et al.* 1988), Korman *et al.* (1991) were not able to establish a correlation between the results of cuticular hydrocarbon analyses of *Zootermopsis* spp. and genetic analyses using allozyme electrophoresis. In the present study, we applied allozyme analysis to determine whether genetic differences exist between groups of *C. acinaciformis* with different nesting behaviors (mound-building or subterranean). We also examined how any allozyme differences between the two groups correlated with the hydrocarbon variation reported by Brown *et al.* (1990).

### MATERIALS AND METHODS

Thirteen *Coptotermes acinaciformis* colonies, comprising 3 geographically separate groups, were collected from Australia in 1996 with the help of the Division of Forestry and Forest Products, CSIRO, Melbourne. Of these, eight colonies were from Townsville (Leichardt Creek, Queensland), three colonies from Darwin (Northern Territory), and two from Walpeup (Victoria). Groups from Townsville and from Darwin are mound dwelling, while the group from Walpeup is subterranean (see Table 1 for details). Two of the three locations (Darwin and Townsville) studied here were the same locations examined by Brown *et al.* (1990). The group from Walpeup has the same nesting behavior (subterranean) as the group from New South Wales tested by Brown *et al.* (1990). Samples from these colonies were used to perform a pilot study in 1996.

In 1998, with the help of Mr. Berhan Ahmed (Div. of Forestry and Forest Products, CSIRO, Melbourne), twelve more *C. acinaciformis* colonies and one *C. frenchi* colony were obtained. Of the twelve *C. acinaciformis* colonies, seven were subterraneans collected from Victoria and South Australia (one of the seven was from Walpeup, Victoria, the same location as two of the colonies collected in 1996). The other five colonies were mound-builders collected from Darwin, the same location as three of the colonies collected in 1996. The one colony of *C. frenchi* was collected from a eucalyptus tree in Victoria (Table 1).

### Sample Preparation

Ten individual workers of *C. acinaciformis* from each colony were chosen randomly and each individual was homogenized inside a microcentrifuge tube with 60  $\mu$ l Tris-HCL pH 8.0 buffer on an ice bath. Microtubes with homogenate samples were centrifuged at 11,000x g for

Table 1. Collection data for *C. acinaciformis* and *C. frenchi* from Australia.

Species	Localities	No. of colonies collected	Dates of collections
<i>C. acinaciformis</i>	Townsville, Queensland	8 <sup>1</sup>	July 1996
	Darwin, Northern Territory	8 <sup>1</sup>	July 1996, January 1998
	Walpeup, Victoria	3 <sup>2</sup>	July 1996, January 1998
	Adelaide and Melbourne, South Australia	6 <sup>2</sup>	January 1998
	Victoria	1 <sup>3</sup>	January 1998
<i>C. frenchi</i>			

<sup>1</sup> mound-builders<sup>2</sup> subterranean nests<sup>3</sup> nesting in eucalyptus tree

ten minutes at 4°C. Supernatants from the samples were then loaded singly into gel wells.

The electrode buffer, gel, and staining recipes followed the procedures applied by Wong & Grace (in press) to study allozyme patterns of protozoan symbionts of *C. formosanus*. Supernatants were loaded into wells of gels made from 7% concentrated polyacrylamide. Gels with samples were run vertically in a chamber of Tris-Glycine buffer at 4°C and 150 constant volts for about two hrs. When the blue front running marker was close to the gel edge, the electrical supply was turned off. Gels were removed from the glass plate and rinsed with distilled water several times. Finally, the gels were incubated in a mixture of alpha and beta naphthyl substrates dissolved in acetone and 0.1M phosphate buffer (pH 6.5) at 30°C for 20-30 min. After incubation, the gels were washed with distilled water several times, then stained with Fast Blue BB Salt (Sigma product) for 10 to 20 minutes, until bands were visible.

Two enzyme systems (esterase and sorbital dehydrogenase) in ten individual workers from each colony collected in 1996, and five individual workers from each colony collected in 1998 were analyzed. Genotypes for each colony at each locus were recorded.

### Procedures

In a pilot study, five esterase loci were found to be present in each sample of *C. acinaciformis* (Fig. 1). Three of the loci, Est-2, Est-4 and Est-5, were polymorphic in some colonies. Samples from Walpeup (subterranean nests) showed dimeric gel patterns at the Est-2 locus (Fig. 2), while samples from Townsville and Darwin (both mound-builders) had similar monomeric gel patterns at the Est-2 locus (Fig. 3).

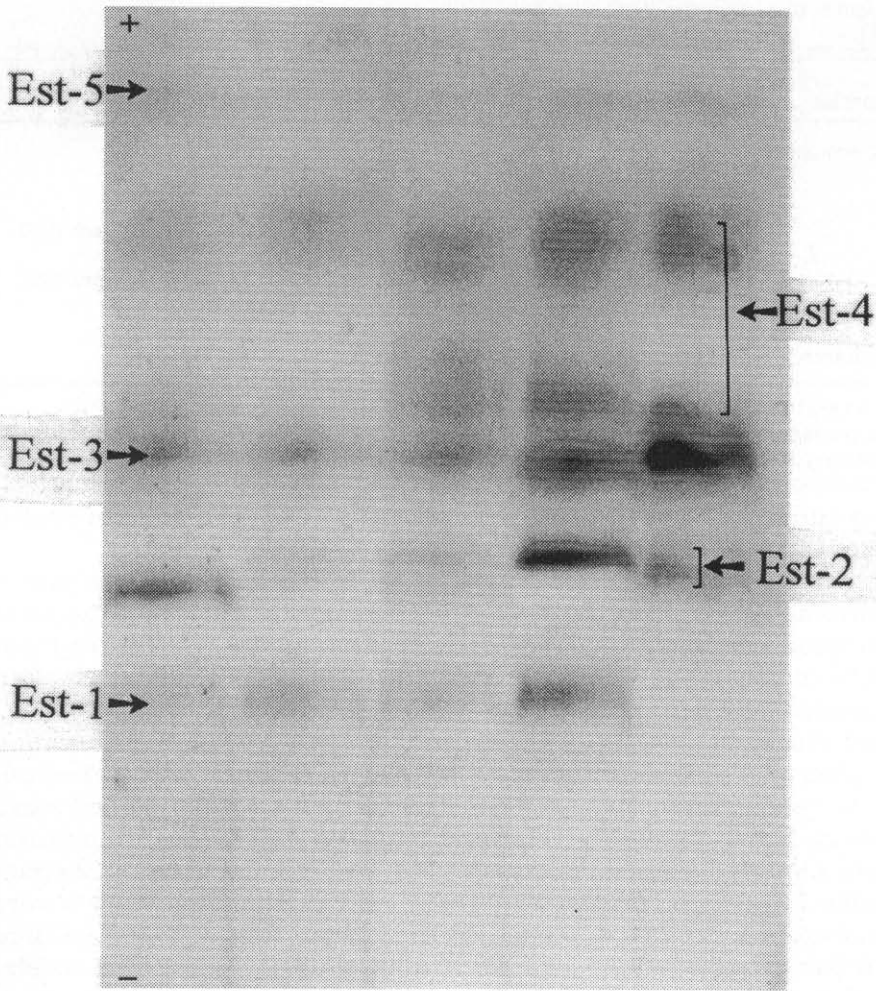


Fig. 1. Five esterase loci are shown in gel pattern of *Coptotermes acinaciformis*.

Thus, we designated the locus of Est-2 found in the samples with subterranean behavior as Est-2A, and the locus of Est-2 found in the samples with mound-building behavior as Est-2B. Possible explanations for these differences in Est-2 would include: (a) Est-2A and Est-2B are different loci; (b) Est-2A and Est-2B are the same locus with three alleles, but have only one allele in common; or (c) Est-2A and Est-2B are the same locus (or were the same locus in the past) with four alleles (or more) and each group has two unique alleles. However, we assumed that Est-2A and Est-2B were two different loci because the zymograph on the

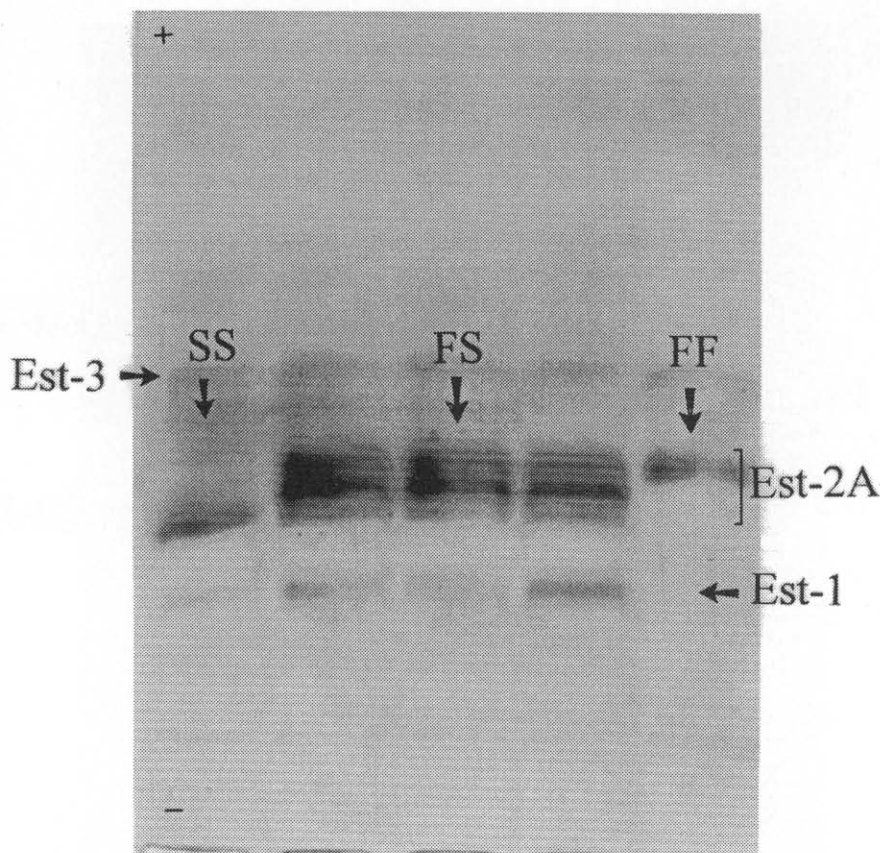


Fig. 2. Dimeric pattern is apparent at the Esterase 2 locus (Est-2A) in subterranean-nesting populations of *C. acinaciformis* from Walpeup, Victoria.

gels demonstrated by samples from the group with mound-building behavior was different from that demonstrated by samples from the group with subterranean behavior, and both appeared at the same Est-2 position. Although other possible explanations such as protein disassociation could not be excluded without performing back-cross tests, the assumption that Est-2A and Est-2B are two independent loci could be tested via Chi-square tests (Richardson 1986).

Based on this pilot study and the previous study carried out with cuticular hydrocarbons by Brown *et al.* (1990), we formed the hypothesis that *C. acinaciformis* is a species complex containing at least two species or subspecies. To more rigorously test this hypothesis, twelve additional colonies were examined in 1998. In addition to esterase, we used sorbital dehydrogenase to test all samples, which included samples of *C. frenchi*.

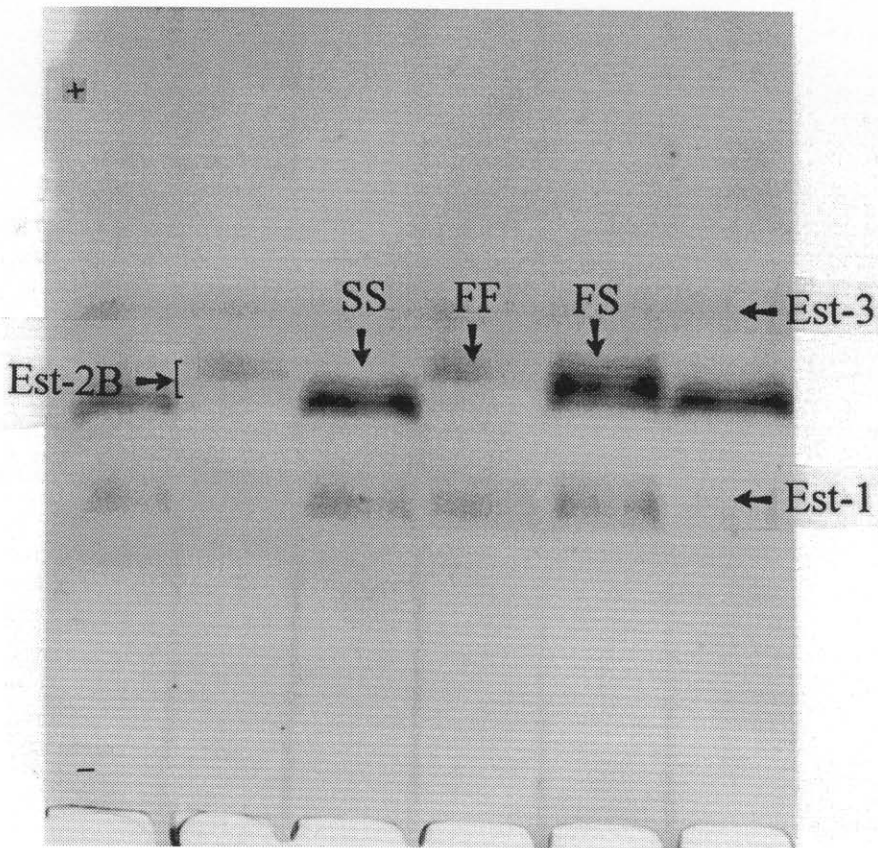


Fig. 3. Esterase 2 locus (Est-2B) is monomeric in mound-building *C. acinaciformis*.

Chi-square tests were performed for each colony at each polymorphic locus to see if the colony conformed to Mendelian rules before gene frequencies of populations were calculated. The independence of Est-2A and Est-2B were tested with a Chi-square test.

Homogeneity tests for different groups at each polymorphic esterase locus were carried out using observed S (Slow) and F (Fast) allele numbers obtained from counting observed genotypes recorded for different localities. The observed numbers of different genotypes from different localities for each polymorphic esterase locus are listed in Table 2. Because Est-2A and Est-2B were not found in the same group, and Est-5 was fixed at the F allele within the mound-building group but polymorphic within the subterranean group, only the Est-4 locus was polymorphic in both groups. Thus, homogeneity between the two groups was tested with Est-4 and Est-5.

Table 2. Observed numbers of different genotypes at four polymorphic esterase loci in *C. acinaciformis* collected from four localities in Australia.

<i>C. acinaciformis</i>		Numbers of different genotypes at each loci											
		Est-2A			Est-2B			Est-4			Est-5		
Locale	Number of Colonies	FF	FS	SS	FF	FS	SS	FF	FS	SS	FF	FS	SS
South Australia <sup>2</sup>	6	2	4	24	0	0	0	14	12	4	19	7	4
Walpeup	3	2	14	9	0	0	0	2	13	10	6	14	5
Townsville	8	0	0	0	18	43	19	35	38	7	80	0	0
Darwin	8	0	0	0	11	28	16	24	23	8	55	0	0

<sup>2</sup>localities include Adelaide and Melbourne, South Australia

We also tested the hypotheses that there were no genetic differences among the termites collected from different localities within the mound-building groups and there were no differences within the subterranean groups. These two hypotheses were tested with Chi-square tests by using pooled numbers of S and F alleles at each polymorphic locus within different groups respectively. The *P*-value used to determine if the hypothesis was accepted or rejected was obtained by using summarized  $X^2$  values and degrees of freedom for all esterase polymorphic loci within a group. If genetic differences were found within a group, additional tests were carried out to see if each subgroup which was composed of several colonies from the same location was in Hardy-Weinberg equilibrium.

In addition to the esterase system, sorbital dehydrogenase analysis was applied to all samples from the two groups of *C. acinaciformis* and *C. frenchi* because this enzyme has been found to be polymorphic in other termite species, such as *C. formosanus* (Wang 1998), and *Hodotermopsis japonica* (Wang *et al.* 1992).

## RESULTS

Polymorphic Est-2A found in termites with subterranean behavior and Est-2B found in termites with mound-building behavior were independent ( $X^2=2.08$ ;  $df=4$ ;  $P=0.7$ ). Thus, the two groups of *C. acinaciformis* with different nesting behaviors could be differentiated genetically using Est-2A and Est-2B. Both Est-2A and Est-2B were found to be polymorphic. We also found that the Est-5 locus was fixed at the F allele in the termites with mound-building behavior, but was variable in the termites with subterranean behavior. This suggests that the two groups are genetically isolated.

The test result of homogeneities between the two different nesting

groups at Est-4 and Est-5 loci (polymorphic loci common in both groups) also suggests that the two groups are heterogeneous ( $X^2=113.64$ ;  $df=2$ ;  $P<0.001$ ).

The test results of homogeneities for the two subgroups with mound-building behavior from Townsville and Darwin at Est-2B and Est-4 showed that there were no genetic differences between them ( $X^2=0.657$ ;  $df=2$ ; N.S.). However, the test results of homogeneities for the subgroups with subterranean behavior from South Australian locations and Walpeup at three different polymorphic esterase loci (Est-2A, Est-4 and Est-5; see Table 3) indicated that the two subgroups from different localities were heterogeneous. This indicates that there are significant genetic differences between the sample groups with the same nesting behavior from Walpeup and South Australian locations.

To summarize the results given above, Table 4 shows gene frequencies of Est-2A, Est-2B, Est-4 and Est-5 for each of the subgroups in different nesting groups. This indicates that *C. acinaciformis* can be separated into two groups using Est-2A and Est-2B loci: one group containing two populations with subterranean behavior which have only the Est-2A locus, and a second group consisting of one population having only the Est-2B locus.

The results of Chi-square tests to examine if each of the three subgroups were in Hardy-Weinberg equilibrium indicated that one of the two subgroups with subterranean behavior (from Walpeup) and the

Table 3. Results of Chi-square tests for homogeneities between the two subgroups of *C. acinaciformis* with subterranean behavior from Walpeup and South Australia at Est-2A, Est-4 and Est-5.

Esterase loci	$X^2$ value	P-value	df
Est-2A	7.76	0.005	1
Est- 4	11.60	0.001	1
Est- 5	6.30	0.012	1

Table 4. Gene frequencies of Est-2A, Est-2B, Est-4 and Est-5 in three different subgroups of *C. acinaciformis* with two different nesting behaviors from Australia.

Subgroups	Est-2A		Est-2B		Est-4		Est-5	
	F	S	F	S	F	S	F	S
South Australia <sup>1</sup>	0.13	0.87	0.00	0.00	0.43	0.57	0.90	0.10
Walpeup <sup>1</sup>	0.36	0.64	0.00	0.00	0.34	0.66	0.52	0.48
Townsville and Darwin <sup>2</sup>	0.00	0.00	0.48	0.52	0.66	0.34	1.00	0.00

<sup>1</sup> subterraneans;

<sup>2</sup> mound-builders.

subgroup with mound-building behavior were within Hardy-Weinberg expectations at all polymorphic esterase loci, while the second subgroup with subterranean behavior (from South Australia) was not (Table 5).

All samples of *C. acinaciformis* tested with sorbital dehydrogenase

Table 5. Results of Chi-square tests for examining whether the three groups of *C. acinaciformis* are in Hardy-Weinberg equilibrium at Est-2A, Est-2B, Est-4 and Est-5 polymorphic loci, using observed and expected numbers of different genotypes at each polymorphic locus.

Locus	$\chi^2$	P	df	n
South Australia <sup>1</sup>				
Est-2A	5.75	<0.06	2	30
Est-4	16.76	<0.001	2	30
Est-5	47.26	<0.001	2	30
Walpeup				
Est-2A	1.15	N.S.	2	25
Est-4	0.62	N.S.	2	25
Est-5	0.75	N.S.	2	25
Townsville and Darwin				
Est-2B	0.39	N.S.	2	135
Est-4	0.03	N.S.	2	135
Est-5	. <sup>2</sup>	. <sup>2</sup>	2	135

<sup>1</sup> termites collected from South Australia were pooled to form the population

<sup>2</sup> population fixed at F allele

were found to have activity, but the samples of *C. frenchi* tested under the same conditions were found to have no activity.

## DISCUSSION

In this study, widely-distributed samples of *C. acinaciformis* with different nesting behaviors were examined using the esterase system. Est-1, Est-3, Est-4 and Est-5 were found in all samples. The dimeric locus Est-2A was only found in the subterranean termite samples and the monomeric locus Est-2B was only found in the mound-building samples. The results of Chi-square tests for determining independence of the two loci confirmed that Est-2A and Est-2B were independent. Although it is difficult to prove that Est-2A and Est-2B are two different loci directly without carrying out back-cross tests, this result is consistent with the observation that termites with different behaviors are not found in the same geographic regions, at least in South Australia. Thus, we were able to separate these termites with different nesting behaviors into two groups by Est-2A and Est-2B.

Based on our electrophoretic results, we discriminated one population of *C. acinaciformis* with mound-building behavior and at least two distinct populations with subterranean nesting behavior. Brown *et al.* (1990) reported that there were qualitative differences in cuticular hydrocarbons between groups with different nesting behaviors, but no qualitative differences within each group; while there were quantitative differences in cuticular hydrocarbon profiles in both. In other words, the two geographically separated populations with mound-building nesting behavior could be distinguished by quantitative variation in cuticular hydrocarbon profiles. The separation of the two nesting groups by either allozyme markers in our study or qualitative cuticular hydrocarbon profiles (Brown *et al.* 1990) was the same, but we were not able to distinguish among the subgroups of mound-builders collected from different locations using allozyme data. However, we were able to differentiate two subterranean subgroups from different sampling locations. Although congruence among data sets obtained with different methods is desirable, we would suggest that allozyme data may be more reliable for separating populations than quantitative cuticular hydrocarbon profiles, since the latter have been reported to vary to some extent seasonally and among different castes (Haverty *et al.* 1996). Of course, allozyme patterns may vary depending upon the age of the individuals and the tissue type selected for extraction (Saul *et al.* 1976), so in either case care must be taken to obtain homogenous samples of the various populations.

The two species *C. acinaciformis* and *C. frenchi* can be distinguished by morphological characters and qualitative cuticular hydrocarbon profiles (Brown *et al.* 1990), and by using sorbital dehydrogenase (Sdh). Sdh activity was found in both nesting types of *C. acinaciformis*, but not in *C. frenchi*. Comparing the sorbital dehydrogenase gel patterns of mound-builders with those of subterranean nesting *C. acinaciformis*, six genotypes were apparent in mound-builders, meaning that the Sdh locus has three alleles, while subterraneans had only one genotype of the Sdh locus. This lends further support to genetic isolation of the mound-building and subterranean nesting subgroups of *C. acinaciformis* in Australia.

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