Genetic Relationship of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) Populations From the United States and China

by

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ABSTRACT

Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae) samples were obtained from 36 colonies representing six geographic populations: Hawaii (Oahu, Maui, Hawaii, Kauai); Lake Charles, Louisiana; New Orleans, Louisiana; Florida; Guangzhou, China; and Hong Kong. Using allozyme electrophoresis, we resolved 20 loci from 13 enzyme systems. Five of these loci were polymorphic: Sdh, Mdh-1, Mdh-3, Me-2 and Xdh. Four of these five polymorphic loci had two alleles representing both slow and fast moving bands, while Sdh had three alleles. Genetic distances generated by pairwise comparisons separated the six *C. formosanus* populations into two large groups; with Hawaii, Lake Charles, Guangzhou and Hong Kong in one group, and New Orleans and Florida in the second. The distance between the two groups was sufficient to possibly represent subspecific variation. Our results suggest that there have been at least two introductions of *C. formosanus* to the United States, with one probably originating in southern China, while the origin of the other introduction remains unknown. Ours is the first study of enzymatic polymorphism in introduced *C. formosanus* in the United States to include samples from China.

INTRODUCTION

Although *Coptotermes formosanus* Shiraki is believed to originate in mainland China (Kistner 1985), it is currently dispersed world wide, including Japan, Hawaii, Sri Lanka, South Africa, and the U.S. mainland. Researchers believe that *C. formosanus* was introduced into Hawaii over 100 years ago (Su & Tamashiro 1987). In the mainland United States, more recent introductions have led to the establishment of this species in the southern states, extending west into Texas, and more recently near San Diego, California (Atkinson *et al.* 1993).

Haverty *et al.* (1990) compared the cuticular hydrocarbon profiles of *C. formosanus* populations in Hawaii; New Orleans, Louisiana; Lake

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Charles, Louisiana; and Florida. Their results indicated that it was not likely that the Hawaiian population was the source of the three mainland introductions. Although the genetic variation among these four populations, as indicated by allozyme electrophoresis, was very low, Korman & Pashley (1991) concluded that is likely that more than two introductions of *C. formosanus* to the mainland U.S. have occurred. To date, very little genetic variation has been found within the Hawaiian population, either using cellulose acetate gel electrophoresis (Strong & Grace 1993) or mitochondrial DNA (Broughton & Grace 1994), which suggested that either a single introduction or multiple introductions from the same source may have occurred in Hawaii. Using a very large number of individual samples from each colony, Haverty *et al.* (1996) reported minor quantitative variation in cuticular hydrocarbon proportions among Hawaiian *C. formosanus* colonies, but the amount of variation was too little to define whether multiple termite introductions had indeed occurred in Hawaii.

None of the studies to date of *C. formosanus* enzyme polymorphism, mitochondrial DNA, or hydrocarbon profiles have included material from Asia. Without more information on *C. formosanus* from its origin in China, it is difficult to make further inferences or conclusions about the introduction of this pest to the United States. Therefore, a primary objective of the present study was to use allozyme electrophoresis to determine the genetic relationship among populations of *C. formosanus* from two areas in China and four areas of the United States, as well as to determine whether sufficient variation existed among *C. formosanus* from different parts of the Hawaiian Islands to suggest that multiple introductions may have occurred from different populations.

MATERIALS AND METHODS

Sample collection

A total of 36 *Coptotermes formosanus* colonies of *C. formosanus* were collected representing six populations: 13 colonies from the Hawaiian population in which 6 colonies were from the island of Oahu, 1 from Kauai, 3 from Maui and 3 from Hawaii; 4 colonies from New Orleans, Louisiana; 4 colonies from Lake Charles, Louisiana; 6 colonies from Florida; 6 from Hong Kong; and 3 from Guangzhou, China. All samples were refrigerated at -70^{0}C until they were analyzed.

Electrophoresis

The accuracy of enzymatic population genetics studies depends upon the number of polymorphic loci found, with five to six of such loci generally considered necessary (Richardson *et al.* 1986). We analyzed

thirteen enzyme systems in individual samples selected randomly from the 36 C. *formosanus* colonies. These enzyme systems were esterase, malate dehydrogenase, lactate dehydrogenase, sorbital dehydrogenase, malic enzyme, xanthine dehydrogenase, alcohol dehydrogenase, octanol dehydrogenase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, alpha-glycerophosphate dehydrogenase, isocitrate dehydrogenase and pyruvate kinase. From these thirteen enzyme systems, twenty loci were resolved.

For allozyme examination, five to twenty individuals were randomly chosen from each colony. The guts were carefully removed from the bodies with forceps in order to reduce the effects from protozoan fauna. The whole bodies of termites without guts were then homogenized in micro centrifuge tubes filled with 60µl of 0.05 M Tris-HCl buffer or Tris-glycine buffer in an ice bath. Then, the homogenates were centrifuged at 10,000xg for 10 minutes at 4°C.

Four kinds of running buffers, 0.01M Tris-glycine buffer, 0.1M Tris-citrate buffer, 0.13 M Tris-EDTA borate buffer and 0.015M Tris-maleate buffer (Richardson *et al.* 1986) were tested for all enzymes, and we determined that only 0.01M Tris-glycine buffer and 0.1M Tris-citrate buffer were useful for *C. formosanus* samples. Gels were made from polyacrylamide. The gel concentrations and stain recipes were based on Naruse (1988), with some modifications. Supernatants were loaded in each gel well, and electrophoresis was run under conditions of 130-150 volts for 120-180 minutes, with the running period depending upon the enzyme (Table 1).

By observing the zymogram on the gels stained actively for each enzyme, data for loci were recorded according to gel patterns and characteristics of the different enzymes (Richardson *et al.* 1986). These data were used to define the genotypes of different individuals.

Data analysis

Five polymorphic loci (Sdh, Mdh-1, Mdh-3, Me-2 and Xdh) were determined from examination of the thirteen enzyme systems (from which a total of twenty loci were detected). Four of these five polymorphic loci had two alleles representing both slow and fast moving bands, while Sdh had three alleles. Est-2A and Est-2B were the only markers which were found to be fixed at zero in some populations and at 1 in others (either present or absent).

To ensure that the samples collected represented a single termite population, each colony used in this study was first tested to see if it obeyed Mendelian rules by comparing observed genotype data with that expected using Chi-square tests at all polymorphic loci. The genotypes

Table 1. Enzyme systems analyzed in *C. formosanus* and conditions for electrophoresis.

Enzyme	Abr.	No. loci	Gel conc.	Gel buffer*	Duration of electrophoresis (min)
Esterase	Est	3	7%	TG	120
Malate dehydrogenase	MDH	3	7%	TG TC	150
Lectate dehydrogenase	Ldh	1	6%	TG	180
Malic enzyme	ME	2	7%	TC	150
Xanthine dehydrogenase	Xdh	1	7%	TG	180
Alcohol dehydrogenase	Adh	1	7%	TG	120
Octanol dehydrogenase	Odh	1	7%	TG	120
Phosphoglucomutase	Pgm	1	7%	TG	120
Sorbital dehydrogenase	Sdh	1	6%	TC	180
6-phosphogluconate dehydrogenase	6-Gdh	1	7%	TG	120
Alpha-glycerophosphate dehydrogenase	α-Gpdh	1	7%	TC	120
Isocitrate dehydrogenase	Idh	2	7%	TC	120
Pyruvate kinase	PK	1	7%	TG	150
TOTAL	13	20			

* TG = tris-glycine buffer, TC = tris-citrate buffer

of reproductives were inferred from the genotypes of their offspring in the colony, since back cross tests with termites are not practical. Gene frequencies were calculated for each of the six *C. formosanus* populations, for each of the five polymorphic loci.

Hardy-Weinberg equilibrium was tested using Chi-square tests to determine if any of the five polymorphic loci deviated from Hardy-Weinberg equilibrium across the six populations.

We presumed that the colonies taken from each geographic area without obvious barriers represented a single population. For the Hawaiian population, we assumed that all termite colonies from the four different islands shared the same gene pool, and tested this assumption by testing for Hardy-Weinberg equilibrium using Chi-square tests for each polymorphic locus.

The homogeneities of gene frequencies for each of the five polymorphic loci among the six localities were tested with Chi-square tests for any one locus. If the null hypothesis of no genetic variation among the six populations was rejected, then the alternative hypothesis that there was significant genetic variation at the five polymorphic loci among the six populations was accepted. If significant variation was found at any locus, we then carried out genetic identity and genetic distance

measures between each pair of populations.

RESULTS

Five of twenty loci (25%) were polymorphic (Sdh, Mdh-1, Mdh-3, Me-2 and Xdh), while the remaining fifteen loci were found to be fixed. However, as described by Wang & Grace (2000), two of the fifteen fixed loci (Est-2A and Est-2B) were found in only some of the six populations. Est-2A was only found in the populations from Hawaii, Lake Charles, Hong Kong and Guangzhou. Est-2B was only found in the populations from New Orleans and Florida (Wang & Grace 2000).

Termites are eusocial insects with populations composed of multiple colonies inhabiting a given area, each with its own set of primary reproductives. It is reasonable to recognize a colony as the basic unit within a termite population. Thus, methods of sampling, and calculating gene frequencies in termites may differ from those applied to solitary insects. If colonies are considered the basic units of a population, than samples should be taken from a number of colonies within a given area to represent the population as a whole.

Results obtained from Chi-square tests for every colony from every geographic region through all polymorphic loci indicated no significant differences between observed and expected frequencies, indicating that no colony departed from the Mendelian rule. Thus, each colony was considered independent.

Results obtained using Chi-square tests for six populations on five polymorphic loci to test if any of the sampled populations diverged from Hardy-Weinberg expectation were variable (Table 2). Our null hypothesis was that each population sampled did not diverge significantly from the Hardy-Weinberg expectation at each polymorphic locus. The results indicate that there is no evidence to reject this hypothesis in the populations from New Orleans, Lake Charles, Florida, Hong Kong and Guangzhou. However, the results of Chi-square tests obtained through combined data from the four Hawaiian islands provided sufficient evidence to reject the hypothesis that there was no significant divergence from the Hardy-Weinberg equilibrium at polymorphic loci Sdh, Mdh-1 and Mdh-3. Tests for homogeneity of the four islands at five polymorphic loci were also carried out, with results indicating that samples from the four Hawaii sets were heterogeneous (Table 3). Therefore, the assumption that *C. formosanus* on the four Hawaiian Islands belonged to a single population was rejected. In other words, the combined data from the four islands may not represent a single population of *C. formosanus*.

Based on the results of the test and the results from previous

Table 2. Results of Chi-square tests with six populations of *C. formosanus* at five polymorphic loci.

	Locus	X^2	*P*	df	n
Hawaii[1]					
	Sdh	33.15	<0.001	5	232
	Mdh-1	6.94	<0.03	2	130
	Mdh-3	33.41	<0.001	2	130
	Me-2	0.624	N.S.	2	130
	Xdh	3.8	N.S.	2	130
New Orleans					
	Sdh	9	N.S.	5	35
	Mdh-1	4.5	N.S.	2	40
	Mdh-3	-[2]	-[2]	2	40
	Me	0.164	N.S.	2	40
	Xdh	2.48	N.S.	2	40
Lake Charles					
	Sdh	4.48	N.S.	5	40
	Mdh-1	2.5	N.S.	2	40
	Mdh-3	0.021	N.S.	2	40
	Me	4.91	N.S.	2	40
	Xdh	0.711	N.S.	2	40
Florida					
	Sdh	1.23	N.S.	5	75
	Mdh-1	2.69	N.S.	2	60
	Mdh-3	3.8	N.S.	2	60
	Me	2.27	N.S.	2	60
	Xdh	0.258	N.S.	2	60
Hong Kong					
	Sdh	8.5	N.S.	5	60
	Mdh-1	1.14	N.S.	2	60
	Mdh-3	1.07	N.S.	2	60
	Me	0.067	N.S.	2	60
	Xdh	-[3]	-[3]	2	60
Guangzhou					
	Sdh	2.23	N.S.	5	30
	Mdh-1	0.027	N.S.	2	30
	Mdh-3	0.55	N.S.	2	30
	Me	1.17	N.S.	2	30
	Xdh	0.54	N.S.	2	30

[1]only data collected from the island of Oahu were used
[2]fixed at F allele
[3]fixed at S allele

population genetic studies (Korman & Pashley 1991, Strong & Grace 1993, Broughton & Grace 1994), we hypothesized that the population of *C. formosanus* from Oahu would not diverge from Hardy-Weinberg expectation at any of the polymorphic loci. Apart from the Sdh locus, for which there was strong evidence to show significant deviation from

Table 3. Analysis of *C. formosanus* data for homogeneity between sample sets from four of the Hawaiian Islands using Chi-squared tests.

Polymorphic loci	X^2	df	*P*-value
Sdh	38.09	6	< 0.0001
Mdh-1	10.71	3	< 0.014
Mdh-3	51.98	3	< 0.0001
Me-2	28.58	3	< 0.0001
Xdh	49.99	3	< 0.0001

Hardy-Weinberg expectation (X^2=22.26, d.f.=5, p<0.0001), there were no significant differences between observed and expected genotypes at the other four polymorphic loci. The explanation for the departure of the Oahu group from Hardy-Weinberg equilibrium at Sdh remains unknown, but could possibly result from natural selection.

Chi-square test values and *P*-values for homogeneity for the six populations at the five polymorphic loci are listed in Table 4. These results show that the gene frequencies at all loci in the six populations are independent. Because the groups from different Hawaiian islands were not in Hardy-Weinberg equilibrium, the data used are for the Hawaiian population was taken from Oahu only (which was in Hardy-Weinberg equilibrium at all polymorphic loci except the Sdh locus).

Using the gene frequencies summarized in Table 5, genetic identities for all pairwise comparisons of the six populations were calculated, and Nei's genetic distance for each pairwise comparison was determined from the genetic identity values (Table 6).

The Nei's genetic distance values for each pairwise comparison from the six populations were used to draw a UPGMA phenogram (Fig. 1). The six *C. formosanus* populations were clustered into two major groups, with New Orleans and Florida in one group; and Hawaii, Lake Charles, Hong Kong and Guangzhou in another group. The average genetic

Table 4. Analysis of *C. formosanus* data for homogeneity among six populations using Chi-squared tests.[1]

Polymorphic loci	X^2	df	*P*-value
Sdh	94.92	10	< 0.0001
Mdh-1	30.33	5	< 0.0001
Mdh-3	77.98	5	< 0.0001
Me-2	52.01	5	< 0.0001
Xdh	110.11	5	< 0.0001

[1] Oahu, Hawaii; New Orleans, Louisiana; Lake Charles, Louisiana; Florida; Hong Kong; Guangzhou, China.

Table 5. Allele frequencies at 20 loci coding for 13 enzymes in 29 colonies from six *C. formosanus* populations: Hawaii (Oahu), New Orleans, Lake Charles, Florida, Hong Kong and Guangzhou.

		Localities[1]					
		USA			China		
Locus	Allele[2]	HI	FL	NO	LC	HK	GZ
Est-2A	a	1.00	0.00	0.00	1.00	1.00	1.00
Est-2B	a	0.00	1.00	1.00	0.00	0.00	0.00
Est-3	a	1.00	1.00	1.00	1.00	1.00	1.00
Ldh	a	1.00	1.00	1.00	1.00	1.00	1.00
Sdh	F	0.27	0.27	0.19	0.24	0.00	0.35
	M	0.57	0.55	0.34	0.67	0.27	0.27
	S	0.20	0.18	0.47	0.09	0.73	0.38
Mdh-1	F	0.41	0.55	0.25	0.49	0.59	0.58
	S	0.59	0.45	0.75	0.51	0.41	0.42
Mdh-2	a	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-3	F	0.46	0.67	1.00	0.57	0.48	0.45
	S	0.56	0.33	0.00	0.43	0.52	0.55
Me-1	a	1.00	1.00	1.00	1.00	1.00	1.00
Me-2	F	0.82	0.83	0.62	0.49	0.52	0.67
	S	0.18	0.17	0.38	0.51	0.48	0.33
Idh-1	a	1.00	1.00	1.00	1.00	1.00	1.00
Idh-2	a	1.00	1.00	1.00	1.00	1.00	1.00
Adh	a	1.00	1.00	1.00	1.00	1.00	1.00
Odh	a	1.00	1.00	1.00	1.00	1.00	1.00
6-Gdh	a	1.00	1.00	1.00	1.00	1.00	1.00
-Gpdh-1	a	1.00	1.00	1.00	1.00	1.00	1.00
-Gpdh-2	a	1.00	1.00	1.00	1.00	1.00	1.00
Pgm	a	1.00	1.00	1.00	1.00	1.00	1.00
Xdh	F	0.37	0.42	0.60	0.51	0.00	0.48
	S	0.67	0.58	0.40	0.49	1.00	0.52
PK	a	1.00	1.00	1.00	1.00	1.00	1.00
Total:[3]	N=29; n=397	N=6; n=167	N=6; n=60	N=4; n=40	N=4; n=40	N=6; n=60	N=3; n=30

[1]HI= Hawaii (only data collected from Oahu Island were used), FL= Florida, NO = New Orleans, Louisiana, LC = Lake Charles, Louisiana, HK= Hong Kong, GZ= Guangzhou, China.
[2]a = locus fixed with only one allele; F = allele of fast mobility; S= allele of slow mobility.
[3]N = number of colonies; n = number of individual workers tested from each population.

distance within the group containing Hawaii, Lake Charles, Hong Kong and Guangzhou was 0.0262±0.01361. The genetic distance between the populations from New Orleans and Florida was 0.0280±0.000. The average genetic distance between these two major groups was 0.1337±0.0198.

Table 6. Genetic identities (above diagonal) and genetic distances (below diagonal) among populations of *C. formosanus* from the United States, Hong Kong and Guangzhou, China.

Locality	HI	FL	NO	LC	HK	GZ
HI	------	0.8937	0.8651	0.9865	0.9627	0.9874
FL	0.1124	------	0.9724	0.8882	0.8580	0.8853
NO	0.1149	0.0280	------	0.8687	0.8465	0.8680
LC	0.0136	0.1186	0.1407	------	0.9549	0.9805
HK	0.0380	0.1532	0.1666	0.0462	------	0.9732
GZ	0.0126	0.1219	0.1415	0.0197	0.0272	------

HI: Hawaii (Oahu); FL: Florida; NO: New Orleans, Louisiana; LC: Lake Charles, Louisiana; HK: Hong Kong, China; GZ: Guangzhou, China.

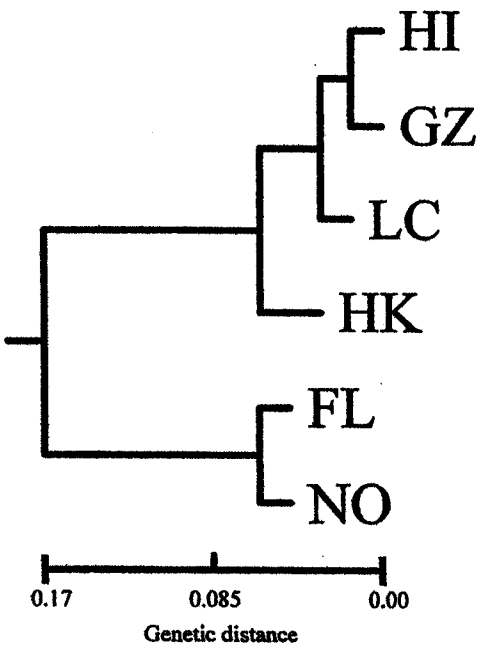

Fig. 1. Genetic relationships of six populations of *C. formosanus* from the United States and China. HI: Hawaii; FL: Florida; NO: New Orleans, Louisiana; LC: Lake Charles, Louisiana; HK: Hong Kong, China; GZ: Guangzhou, China.

DISCUSSION

Population genetic studies of Isoptera using allozymic data have shown various levels of polymorphism: 50% of loci in European *Reticulitermes* (Clement 1981), 53% of loci in *Zootermopsis* (Korman *et al.* 1991), 49% of loci in *Hodotermopsis* (Wang *et al.* 1992), 16.7% of loci within *C. formosanus* (Korman & Pashley 1991), 29.4% within *Z. nevadenis*, 52.9% within *Z. angusticollis* and 12.5% within *Z. laticeps* (Korman *et al.* 1991). Genetic data in this study showed that 25% (5 of 20 loci) in *C. formosanus*, including both populations from the United States and China, were polymorphic. This value is slightly higher than the 16.7% polymorphism found by Korman & Pashley (1991), possibly due to the use of different enzyme and buffer systems. Genetic diversity among the six *C. formosanus* populations did not exceed the highest genetic diversity level of 52.9% found within any other termite species to date. Thus, based upon enzyme polymorphism, the populations investigated in this study represent a single species.

Genetic data for *C. formosanus* from Hawaii generated with combined samples from four different islands did not support the idea that *C. formosanus* living on these islands all belong to a single population. This suggests that several populations (or subpopulations) exist among the islands. Although these genetic data cannot be used to determine if there have been movements of *C. formosanus* between the islands, Chi-square tests for five polymorphic loci in the population from Maui supported the hypothesis that this population was in Hardy-Weinberg equilibrium. Chi-square tests for all polymorphic loci except Sdh in the population from Oahu indicated that this population was in Hardy-Weinberg equilibrium. However, tests of the population from Hawaii (the population from Kauai could not be tested since only one colony was available) indicated that this population was not in Hardy-Weinberg equilibrium.

Departure from Hardy-Weinberg expectation at a certain polymorphic locus may occur for several reasons. For example, the population may consist of groups of closely related colonies due to localized inbreeding, multiple introductions may have occurred, or environmental diversity on a small scale within a particular region could possibly lead to diverse paths for natural selection. Inbreeding can occur among supplementary reproductives after death of the primary queen, or as the result of alates from the same colony mating. Either of these conditions could cause a decrease in the numbers of heterozygotes in a population at a certain polymorphic locus.

In this study, we treated the colony as the basic unit of which a population is composed. The termite colony is not a clone, but is the product of a paired king and queen. A queen and king with different genotypes will produce offspring with different genotypes, which should follow Mendelian rules. The 36 *C. formosanus* colonies used in this study, with one exception (from Kauai), were found to follow Mendelian rules. However, genetic variation within a colony is not necessarily useful in distinguishing different colonies, because royal pairs with the same genotypes in different colonies could produce offspring with the same ratios of different genotypes.

The phenogram (Fig. 1) generated using genetic distance data among the six populations indicated that the populations evaluated represent two distinct groups. The genetic distance between the two groups is 0.1337±0.0198, and distances among populations within each group are 0.0262±0.01361 and 0.0280±0.000. These results indicate that the relationship between the two large groupings is basically equivalent to the subspecies level, whereas the relationship between populations within groups is at the level of local races. According to Nei (1987),

genetic distance between local races varies from 0.00 to 0.05, while inter-subspecific distance is generally greater than 0.05. Genetic distance distributions among the populations do not support the suggestion that larger geographical distances necessarily mean larger genetic distances. This is consistent with the observation that the distribution of *C. formosanus* around the world results from introductions, and subsequent founder effects, facilitated by human activity rather than from natural movement.

The degree of separation of the two major groups in the phenogram (Fig. 1) leads to the conclusion that at least two introductions of *C. formosanus* occurred in the United States, as previously hypothesized by Wang & Grace (2000) from esterase data. Our results support a hypothesis that one of the introductions of *C. formosanus* was from China, since the populations from Lake Charles and from Hawaii fall into the same larger group as the populations from Guangzhou and Hong Kong. Our conclusions are generally consistent with those of Haverty *et al.* (1990) based upon quantitative variation among cuticular hydrocarbon profiles of *C. formosanus* samples from Lake Charles, New Orleans, Florida and Hawaii; and Korman & Pashley (1991) based upon allozyme analysis of Louisiana and Florida samples. Although Lake Charles *C. formosanus* differed in cuticular lipid proportions from each of the other U.S. groups (Haverty *et al.* 1990), and New Orleans and Florida populations were reported to be similar based on enzyme polymorphism (Korman & Pashley 1991), the inclusion of termite samples from mainland China (Guangzhou) and Hong Kong in our study provides a more complete picture of the genetic relationships among these geographic populations. Genetic studies with still broader samplings of *C. formosanus* from around the world (Su & Tamashiro 1987, Wang & Grace 1999) are needed to elucidate the full pattern of distribution of this adaptable and pestiferous termite species.

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