A SEX-LINKED ESTERASE LOCUS AND TRANSLOCATION HETEROZYGOSITY IN A TERMITE

PETER LUYKX

Laboratory for Quantitative Biology, Department of Biology, University of Miami, Coral Gables, Florida 33124, U.S.A.

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SUMMARY

Slow vs. fast alleles at an esterase locus show a sex-linked pattern of inheritance in colonies of the termite *Incisitermes schwarzi*. As might be expected where a gene has only recently been brought into sex-linkage by means of a translocation between an autosome and a sex-chromosome, males as well as females can be heterozygous; males are therefore "holozygous", not hemizygous, for this gene. The rate of exchange of this gene between X and Y chromosomes is 2/266 or less. In the founding reproductives (male or female), X chromosomes may have either the fast or the slow allele; in males, only Y chromosomes carrying the fast allele have been found. This linkage disequilibrium may be due to a founder effect, to "hitch-hiking" of the gene with the translocation, or to a sex difference in the fitness of gene combinations at or near this locus.

1. INTRODUCTION

SEVERAL species of the primitive termite family Kalotermitidae carry extensive sex-linked chromosomal translocations in heterozygous condition in the male (Syren and Luykx, 1977; Luykx and Syren, 1979). This condition has arisen by successive exchanges or centric fusions between sex chromosomes and autosomes. This process brings many loci, originally autosomal, into sex-linkage. In some species, including *Incisitermes schwarzi*, up to half the chromosomes in the diploid set are in effect sex chromosomes—males have multiple X's and multiple Y's, while females have a double set of X's. The geographic variation found in the chromosome arrangements in some of these species suggests that the translocations or fusions are of relatively recent origin, so that one would expect many of the sex-linked loci to be "holozygous" rather than hemizygous in the male. The results presented in this paper show that this is so for *Est-3*, the first sex-linked allozyme locus to be described in a termite, and in addition they show that the two common alleles at this locus are in marked disequilibrium with regard to X- vs. Y-linkage.

2. MATERIALS AND METHODS

Whole colonies of *Incisitermes schwarzi* were collected around Miami, Florida, and maintained in the laboratory. The founding (primary) reproductive pair (king and queen) and their offspring (workers, soldiers, nymphs, and alates) were prepared for electrophoresis by homogenizing them individually in two drops of diluted buffer. The homogenates were either soaked up on wicks of filter paper and run on starch gels, or mixed with sucrose solution and run on vertical polyacrylamide slab gels. (No important differences were found between the results with the two methods

of gel electrophoresis.) Gels were stained with Fast Blue RR salt and one or a mixture of two of the following substrates: α -naphthyl acetate, β -naphthyl acetate, α -naphthyl propionate, β -naphthyl propionate and β -naphthyl butyrate. There were no obvious differences in the activity of the esterase toward any of these substrates.

Only colonies that were headed by primary reproductives were used for this study. Those colonies in which one or both of the primary reproductives had been replaced by secondary reproductives (themselves the offspring of the primary reproductives) were excluded. (In nature, these latter colonies make up about 25 per cent of all colonies in this species.) In a number of colonies the primary reproductives were not examined, but in all these cases their genotypes could be inferred from the esterase phenotypes of their offspring.

In all, eleven informative colonies have been examined.

3. RESULTS

Four anodally migrating esterases, named EST-1, -2, -3, and -4 in order of increasing rate of migration, were observed. Only EST-3 showed variation in the colonies studied. Both the slow (s) and the fast (f) forms of EST-3 appeared as double bands; individual animals with both forms had four bands altogether (fig. 1). No differences were found between the slow and fast forms in their heat sensitivity nor in their activity toward the different substrates.

FIG. 1.—Tracing of EST-3 region of polyacrylamide gel slab, colony PL20. The arrow indicates the anodal direction of migration. K = king, Q = queen (primary reproductives); all other individuals are offspring.

The EST-3 phenotypes found in the different colonies are given in table 1, along with the genotypes of the founding reproductives. The pattern of transmission is clearly one of sex-linkage. Furthermore, since male offspring may express both forms of the esterase (e.g., in colonies PL30, PD11, and others), they are not hemizygous in the expression of this gene; the Y-linked allele is expressed as well as the X-linked allele.

The exceptional offspring in colonies PL21 (f female) and PL43 (sf female) may have arisen by crossing over between X and Y chromosomes. If so, the frequency of 2 out of a total of 266 offspring from X^sY^f males (=0.8 per cent) would correspond to the map distance between the *Est-3* locus and the translocation break point. Alternatively, these exceptional

TABLE 1.

Analysis of EST-3 phenotypes in individual colonies of Incisitermes schwarzi

Colony number	Phenotypes									Genotypes of parents Q K	
	parents Q K		Offspring								
			Females			Males					
			s	sf	f	s	sf	f			
Oleta River											
ML1	sf	sf	2	4	0	0	3	4	X^sX^f	X^sY^f	
PL20	sf	sf	2	6	0	0	2	2	X^sX^f	X^sY^f	
PL21	f	sf	0	40	1	0	0	42	X^fX^f	X^sY^f	
PL30	s	f	0	7	0	0	24	0	X^sX^s	X^fY^f	
PL70			0	0	13	0	0	13	X^fX^f	X^fY^f	
Matheson Hammock											
PL24			5	0	0	0	14	0	X^sX^s	X^sY^f	
King's Bay											
PL25			17	0	0	0	15	0	X^sX^s	X^sY^f	
PL43	s	sf	8	1	0	0	3	0	X^sX^s	X^sY^f	
PL44	s	sf	5	0	0	0	4	0	X^sX^s	X^sY^f	
PL52			10	0	0	0	7	0	X^sX^s	X^sY^f	
Black Point											
PD11	s	sf	34	0	0	0	35	0	X^sX^s	X^sY^f	

In most cases approximately equal numbers of male and female offspring were selected from each colony for electrophoresis; the numbers having each phenotype are shown. No differences were found between the different castes (workers, nymphs, alates, soldiers), so they have been added together simply as "offspring".

offspring could be the result of meiotic nondisjunction, or misclassification of the sex of the individual.

The genotypes of all the founding reproductives, and the X - and Y -allele frequencies, are summarized in table 2. The colonies around the mouth of the Oleta River, about 30 km north of the city of Miami, are significantly different from the other colonies, 20-45 km south of Miami. The X -linked fast allele (X^f) was found only at the Oleta River location, along with the X -linked slow allele (X^s); south of Miami only X^s was found ($P = 0.007$, by exact calculation). The male founding reproductives of all colonies

TABLE 2

Numbers of founding reproductives with different Est-3 genotypes (Summary of data from table 1.)

	Females			Males			
	X^sX^s	X^sX^f	X^fX^f	X^sY^s	X^fY^s	X^sY^f	X^fY^f
Oleta River	1	2	2	0	0	3	2
Other locations	6	0	0	0	0	6	0
Totals	7	2	2	0	0	9	2

Numbers of X- and Y-linked Est-3 alleles in founding reproductives.

	X^s	X^f	Y^s	Y^f
Oleta River	7	8	0	5
Other locations	18	0	0	6
Totals	25	8	0	11

examined contain only Y^f chromosomes; no Y^s chromosome has been found. These data, summarized in the last line of table 2, indicate a highly significant linkage disequilibrium ($P = 0.0001$, by exact calculation).

4. DISCUSSION

These findings provide another example of an association of a specific allele with a chromosome rearrangement. Such associations have been observed before, with both inversions (Ishii and Charlesworth, 1977; Hedrick *et al.*, 1978) and translocations (Levy and Winternheimer, 1977; Ellstrand and Levin, 1980).

The failure to find any Y chromosomes with the slow *Est-3* allele among the male primary reproductives was at first unexpected, given that X^sY^f males do occur and that chiasmata between X 's and Y 's (presumably indicative of crossing over) can occasionally be seen in male meiotic chromosomes preparations (Luykx and Syren, 1979). Chiasmata in male meiosis of *I. schwarzi* are mostly terminal, however, and may affect the *Est-3* locus only rarely. The crossover frequency of 0.8 per cent given above is a maximum estimate; because of the possibilities of meiotic non-disjunction and of misclassification of sex, the actual frequency may be very much lower. In this case it would take a great many generations for the alleles to come to equilibrium on the Y chromosomes among the descendants of any one male. With the low vagility of termites, it is conceivable that the colonies sampled in this limited survey are the descendants of one or a very few males carrying Y^f chromosomes, and that there has not been enough time for them to reach equilibrium by exchanging alleles with the X^s chromosomes in the population. If this view is correct, a wider survey should turn up some Y^s chromosomes in founding males from other locales.

It is also possible that because of a very low rate of recombination, the association of the fast allele with a Y chromosome has simply persisted over many generations since the initial translocation between a sex chromosome and the autosome carrying the *Est-3* locus (the hitch-hiking effect, reviewed by Ishii and Charlesworth, 1977).

Another explanation for the failure to find Y^s chromosomes in founding males is that there are selective forces operating differentially on the two sexes. (Selection could act on the *Est-3* locus itself or, more likely, on a number of associated sex-linked loci; we can regard the *Est-3* locus as a marker for these loci.) The absence of Y^s chromosomes in this survey could be, for example, a simple result of strong selection against homozygous X^sY^s males. A more general proposition is that this marked linkage disequilibrium is a consequence of the same selective factors that are responsible for the evolution of the translocation heterozygosity characteristic of males of this species. Following a suggestion of White (1973, p. 613), Charlesworth and Charlesworth (1980) have shown by algebraic analysis and computer simulation that where there is a sex difference in allele frequency at an autosomal locus, a translocation between the autosome and a sex chromosome once having occurred will be favoured and will spread throughout the population. In effect, translocation heterozygosity in the male provides a means whereby different combinations of alleles can be efficiently maintained in the two sexes. The outcome of this selection process is permanent translocation heterozygosity in the hetero-

gametic sex, fixation of one allele (or one set of alleles, at different loci) in *Y*-bearing gametes, and fixation of the opposite allele in *X*-bearing gametes. This is precisely the pattern seen in the colonies of *I. schwarzi* south of Miami. We can imagine that the original translocation (or centric fusion) that brought the *Est-3* locus into sex-linkage was between a *Y*-chromosome and the homologue carrying the slow allele. The data presented here therefore are compatible with the model of differential selection between the two sexes, at the genic level, as the basis for translocation heterozygosity (at the chromosomal level) in the male. However, to evaluate the possibility that selection, rather than the hitch-hiking effect, is responsible for the linkage disequilibrium observed, other sex-linked genes with greater rates of exchange between *X*'s and *Y*'s will have to be found.

The Oleta River population appears to have reached fixation for the same *Y^f* allele as in the colonies further south, but it is still polymorphic at the *X*-linked locus. If selection is acting on the *Est-3* gene, this polymorphism might reflect a greater degree of outcrossing in the Oleta River population than in the southern populations. The Oleta River population is relatively large, dense, and continuous, and there is some evidence that the colonies there are also more synchronous in their swarming periods, thus providing more opportunities for inter-colony matings. The populations south of Miami, on the other hand, are less dense and more patchy in distribution, with colonies possibly less synchronous in their swarming. Matings between individuals from the same colony would be more common, and the more intense inbreeding would be expected to increase the rate of fixation of alleles under selection. These expectations are borne out by the finding of variation in the *X*-linked *Est-3* locus in the Oleta River populations, and the lack of such variation in the populations south of Miami.

In the history of chromosome evolution in this species, the translocation that first brought the *Est-3* locus into sex-linkage cannot have been the most recent one to occur. Male chromosome complements from King's Bay and more southern colonies carry six translocations (9 bivalents and a ring of 14 sex chromosomes), those from Matheson Hammock and Oleta River carry seven translocations (8 bivalents and a ring of 16 sex chromosomes) (Luykx and Syren, 1979, and unpublished observations). Yet *Est-3* is sex-linked in colonies collected from all these locations. The gene must therefore be located on a chromosome segment involved in an earlier gonosomal-autosomal translocation (or on the original pair of sex chromosomes), and the association of the *Est-3* fast allele with a *Y*-chromosome probably antedates the translocation that increased the number of sex chromosomes from 14 to 16.

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