Chromosome Number in *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Observation of cells from the ovaries and testes of primary kings and queens, and dealates, indicated that the haploid chromosome number in *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is 21. This is comparable to reports of chromosome numbers in *Reticulitermes* species. No permanent interchange complexes were observed in *C. formosanus*, possibly because of relatively limited inbreeding in *C. formosanus* colonies in comparison to termite species in which such complexes have been found.

INTRODUCTION

In sexually reproducing organisms, each somatic cell (any cell exclusive of sex cells) contains one set of chromosomes inherited from a maternal parent and a comparable set of chromosomes from a paternal parent (Stansfield 1983). Each species has a characteristic number of chromosomes, a unique feature which reflects the species position in the phylogenetic scheme of classification (Imai *et al.* 1977). Karyotypes (ideograms of the somatic chromosome complement of a cell) of different species have been widely used to study the evolution and geographic distributions of animals (Imai et al. 1977). The numbers of chromosomes in Isoptera have been studied since 1905 (Stevens 1905, Benkert 1930, Banerjee 1961, Vincke & Tilquin 1978, Luykx & Syren 1979, Luykx 1990a).

Recent interest in the chromosomes of termites has focused on their abnormal sex chromosome system and its possible role in the evolution of eusocial behavior (Luykx 1990a). Syren & Luykx (1977) reported that *Incisitermes schwarzi* (Banks) and *Kalotermes approximatus* (Snyder) possess the most extensive complex of permanent reciprocal translocations (segmental interchange complex) found in any animal. A reciprocal translocation is a segmental exchange that involves two non-homologous chromosomes (Stansfield 1983). Translocation heterozygotes have several distinct manifestations: 1) semi-sterility due to

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adjacent disjunction; 2) some genes on non-homologous chromosomes will no longer appear to be assorting independently; and 3) position effects (Stansfield 1983). When a reciprocal translocation involves more than two non-homologous chromosomes, a complex of reciprocal translocation may be formed. This kind of complex (involving all 7 of its chromosome pairs) also occurs rarely in self-fertilized plants, (e.g., *Oenothera*) (Stansfield 1983). In *I. schwarzi*, Syren & Luykx (1977) found that complexes of reciprocal translocation involved more than half of the chromosome set and were only present in males. Also, they found that the number of chromosomes in a complex exhibited geographic variation, even among members of the same species. Their results indicated that this permanent interchange complex was sex-linked. Vincke and Tilquin (1978) reported the same phenomenon in species of the family Termitidae. Generally, animals have one or two type of sex chromosomes that determine not only their sex, but physiology, behavior and morphology that are different from the opposite sex. For this reason, the distinct patterns of complexes of reciprocal translocation can be used to trace the geographic distribution of a given termite species (Luykx & Syren 1979, Fontana & Amorelli 1977).

The kalotermitid species *I. schwarzi, I. milleri, I. snyderi, Calcaritermes nearcticus, Cryptotermes brevis, Cryptotermes cavifrons, Kalotermes approximatus, Neotermes castaneus,* and *Neotermes jouteli* from Florida have been examined for interchange complexes by several researchers using the methodology of Luykx and Syren (1979). All of the aforementioned species as well as *Reticulitermes lucifugus* (Rossi) from Italy were found to have interchange complexes, and these complexes were only present in males (Luykx & Syren 1979, Fontana & Amorelli 1977). The complex of reciprocal translocation might result from inbreeding of termites (Syren & Luykx 1977). Cytogenetic studies on *Z. angusticollis* (Stevens 1905), *R. flavipes* (Benkert 1930), *R.. lucifugus, R. santonensis* from France (Clément 1977), *Schedorhinotermes lamanianus* Sjostedt (Renoux 1975), and *Odontotermes redemanni* (Banerjee 1961) revealed no interchange complexes in these species, although inbreeding events are common in some species (Clément 1981). To date, chromosome number and structure in species of *Coptotermes* Wasmann have not been reported.

The objective of the present study was to provide basic cytological information on the chromosome complement of *C. formosanus.* A secondary objective was to determine if the chromosome number of *C. formosanus* is different from that of other species in the family Rhinotermitidae. Lastly, the possible presence of an interchange com-

plex in *C. formosanus* was investigated.

MATERIALS AND METHODS

Coptotermes formosanus alates were collected (before swarming) from colonies in wood traps installed at the Pearl City Urban Garden Center (Honolulu) and on the Manoa campus of the University of Hawaii. Alates were anesthetized with carbon dioxide, and their sexes were determined using a stereoscopic microscope, based on the descriptions of Higa (1981). Paired males and females were placed in plastic tubes with sawdust. Six months to one year later, successful pairs, with brood, were transferred, still within the plastic "nesting" tubes, into 1-gal. cans containing scrap wood (Douglas-fir). Males and females used in this study were two to three year-old kings and queens removed from these laboratory colonies, although dealates of both sexes collected directly from the field were also examined.

To examine *C. formosanus* chromosomes, male and female pairs were removed from the plastic rearing tubes and dissected (dealates of both sexes collected directly from the field were dissected using the same methods). Testes and ovaries of the kings and queens (or dealates), respectively, were removed by a ventral dissection of the abdomen using the procedure described by Luykx (1990b). The testes or ovaries were then placed on a microscope slide in hypotonic salt solution (0.45% sodium citrate), and cut or pulled apart. After 10-20 min in the solution, the testes or ovaries of the individual were transferred to a clean portion of the slide and excess hypotonic solution was removed with forceps. Ten drops of Fixative I (Table 1) were added to the slide, which was angled to facilitate fast coverage by the fixative solution. The gonadal tissues were macerated using a dissecting needle, and several drops of Fixative II (Table 1) were added to the center of the macerated tissues. The slides were then placed in a Coplin jar containing Fixative III (Table 1) for 5-10 min, after which they were removed from the jar and drops of Fixative IV (Table 1) were immediately added. The slides were allowed to air dry, then stained with Giemsa solution (Harleco Azure B type,

Table 1. Solutions and fixative liquids for cytological analysis, after Luykx (1990b).

Fixative liquids	HAc[a]	EtOH[a]	DW[a]	Total:
Fixative 1	1.5 ml	1.5 ml	2.0 ml	5 ml
Fixative 2	2.0	2.0	0	4
Fixative 3	10	30	0	40
Fixative 4	100%	0	one drop	0

[a] Solutions: HAc = glacial acetic acid; EtOH = absolute ethanol; DW = distilled water

diluted 1: 40 distilled water). After 10 min, each slide was briefly rinsed with water, and the tissues were covered with a cover slip. Slides were examined under a microscope to determine the stage of meiosis. Different stages were observed, and chromosome numbers within cells of the testes and ovaries were counted. The process was replicated with a total of 20 individuals of each sex.

RESULTS

Chromosomes observed in the testes cells indicated that most of the cells were in the diplotene stage (diplonema) of the first meiotic division. All cells observed, except one, contained 21 bivalent chromosomes at the diplotene stage, and no multivalent chromosomes were present (Fig. 1). Ovarial cells also appeared to contain 21 bivalent chromosomes although most chromosomes within the ovarial cells were not clearly distinguishable. One testes cell appeared to exhibit unpaired chromosomes.

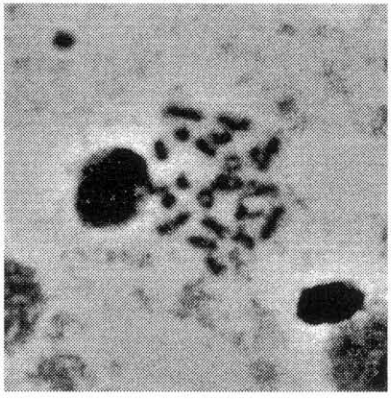

Fig 1. Chromosomes of *Coptotermes formosanus* at diplotene stage of the first meiotic division. The haploid chromosome number is n = 21.

DISCUSSION

Coptotermes formosanus males and females each had a haploid chromosome number of 21 (Fig. 1). Although this is the first report of chromosome number in *C. formosanus*, *Reticulitermes* species (Rhinotermitidae) have been reported to have the same chromosome number (Benkert 1930, Fontana & Amorelli 1977) and this may be the typical haploid chromosome number for the Rhinotermitidae.

The clearest structures for chromosome counts were the cells from testes of actively functioning primary kings (paired with a queen that was laying eggs). Chromosome counts using either female alates or

primary queens were less reliable.

No evidence was found to indicate that interchange complexes of chromosomes are present in either *C. formosanus* sex. These results concur with analyses of *Reticulitermes* species from France, but differ from *R. lucifugus* from Italy (Fontana & Amorelli 1977). According to Syren & Luykx (1977), the existence of permanent interchange complexes of chromosomes in termites is probably the result of inbreeding, such as inbreeding between siblings establishing new colonies or related secondary reproductives. Inbreeding may be a common event during flight and colony initiation by *Incisitermes schwarzi* and *Kalotermes approximatus* (Syren & Luykx 1977), and secondary reproductives are certainly common in colonies of many *Reticulitermes* spp. (Weesner 1956). However, the lifetime of *C. formosanus* kings and queens is relatively long (Li *et al.* 1994, Grace *et al.* 1995), and reports of multiple queens and kings or neotenics in *C. formosanus* colonies containing a primary queen are relatively rare. This would serve to decrease the opportunity for inbreeding by secondary reproductives within the colony, and limited inbreeding could explain why permanent interchange complexes of chromosomes would rarely occur in *C. formosanus*.

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