Chromosome Research (2007) 15:735–753
DOI: 10.1007/s10577-007-1158-6

Karyology, mitochondrial DNA and the phylogeny of Australian termites

Silvia Bergamaschi[1]*, Tracy Z. Dawes-Gromadzki[2], Valerio Scali[1], Mario Marini[1] & Barbara Mantovani[1]
[1]*Dipartimento Biologia Evoluzionistica Sperimentale, Via Selmi 3, 40126, Bologna, Italy; Tel: +39-051-2094169; Fax: +39-051-2094286; E-mail: bergamas@alma.unibo.it;* [2]*CSIRO Sustainable Ecosystem, Tropical Ecosystem Research Center, PMB 44, Winnellie, NT 0822, Australia*
* *Correspondence*

Received 26 March 2007. Received in revised form and accepted for publication by Herbert Macgregor 3 May 2007

Key words: COII, isoptera, karyotype, parsimony, phylogeny, translocation

Abstract

A comprehensive karyological characterization of 20 Australian and three European species of Isoptera, together with a mitochondrial gene analysis is presented. Higher termites appear karyotypically very uniform, while lower termites are highly variable. The differences in chromosome number are explained through Robertsonian changes or multiple translocation events. An ancestral acrocentric karyotype can be suggested as the most primitive one. In Kalotermitidae chromosomal repatterning has repeatedly arisen with the X0-male type possibly representing a XY-derived condition. This argues against a simple origin of termites from cockroaches. The fixed chromosome number of Rhinotermitidae and Termitidae (2n = 42, XY/XX) may be explained with the non-random nature of chromosomal evolution. A sex-linked multivalent, either with a ring or a chain structure, is found in the majority of species. Phylogenetic analyses on COII sequences recognize Mastotermitidae as the basal lineage and define the Rhinotermitidae+Termitidae cluster with a good bootstrap support. Kalotermitidae fail to be joined in a single cluster in agreement with the detected chromosomal variability. On the other hand, the karyotypic conservation of the Termitidae family contrasts with the polytomy evidenced at the subfamily level.

Introduction

Termites are an ancient order of social insects whose origins date back to the early Cretaceous period, more than 120 million years ago.

It has long been accepted that termites appeared closely related to cockroaches and mantids, and classified in the same monophyletic superorder (Dictyoptera). New research has shed light on the details of termite evolution (Thorne & Carpenter 1992, Lo *et al*. 2000, 2003, Kjer 2004) and there is now strong evidence that termites have actually evolved from wood-feeding cockroaches. Among them, *Cryptocercus* spp. should be the closest living relatives of termites, sharing similar morphology, social features and endosymbiotic bacteria with the primitive termite *Mastotermes darwiniensis* (family Mastotermitidae). On the other hand, cytological investigations have shown that *Cryptocercus* spp. chromosome numbers range from 2n = 17–21

Electronic Supplementary Material
The online version of this article (doi:10.1007/s10577-007-1158-6) contains supplementary material, which is available to authorized users.

(Palearctic species) to 2n = 37–47 (North American and North Pacific species), suggesting a possible genome duplication or reduction within this genus (Lo *et al.* 2006a,b), while *M. darwiniensis* has a chromosome number of 2n = 98 (Bedo 1987). These quite different karyological traits do not point to any definite pattern of chromosomal evolution. Molecular phylogenetic relationships among termite families were first highlighted by Kambhampati *et al.* (1996). These authors considered the mitochondrial 16SRNA gene fragment to demonstrate that the most ancient taxon is represented by Mastotermitidae and that the Kalotermitidae family is possibly basal to a Rhinotermitidae–Termitidae group. Subsequent molecular work based on mitochondrial COII or 16S genes were employed to clarify the relationships within Rhinotermitidae, Termitidae (Miura *et al.* 1998, 2000, Austin *et al.* 2004, Ohkuma *et al.* 2004) and Kalotermitidae families (Thompson *et al.* 2000).

Data on chromosome numbers of Isoptera (Vincke & Tilquin 1978, Luykx & Syren 1979, Luykx 1990) showed that higher termites (Rhinotermitidae, Termitidae) are karyotypically uniform, with most species sharing a diploid number of 42. Lower termites (Kalotermitidae, Termopsidae) appear more variable, with diploid numbers ranging from 28 to 56. Nevertheless, a detailed karyotype analysis describing chromosome pairs has been achieved only for a few species (such as *Reticulitermes lucifugus* Rossi and *Kalotermes flavicollis* Fabr.; Fontana 1982, 1991), mainly because termite chromosomes are small, relatively numerous and with few distinguishing features.

A karyological peculiarity of termites is the wide occurrence of meiotic chromosome multivalents in the male sex. A segmental interchange complex of 11–17 chromosomes was first observed in *Incisitermes schwarzi* and *Kalotermes approximatus* males (Syren & Luykx 1977) followed by the report of a sex-linked ring of four chromosomes in the male meiosis of 21 African species of Termitidae (Vincke & Tilquin 1978). Subsequently, Luykx & Syren (1979) observed sex-linked translocations in nine species of lower termites, while Fontana (1980) found comparable interchange multivalents of four chromosomes in the males of five populations of *Reticulitermes lucifugus*. Since these complexes are restricted to males, they may imply the existence of a multiple sex chromosome system with males heterozygous for a specific series of translocations, involving a different number of chromosomes. These interchange complexes seem to have arisen independently many times, and may take the form of a ring or a chain of chromosomes (Luykx 1990). They vary within and between isopteran species.

There are good reasons to assume that the chains were initiated by a rearrangement event involving an original X chromosome and an autosome. Further translocations produced what is effectively a multiple XY sex-determining mechanism (Rowell 1985). It seems that all chromosomes behave like Xs and Ys during their segregation in male meiosis, but it is not clear how many of them are involved in sex determination (Santos & Luykx 1985). Sex-determining genes might be located in only one of the chromosomes involved in the complex, the other chromosomes containing genes that have become sex-linked only by virtue of the translocation. The fact that part of the genome has become sex-linked by means of such translocations suggests that there may be many loci (such as Acp-1 and Est-3 in *Incisitermes schwarzi*, see Santos & Luykx 1985), widely distributed over the whole genome, upon which male–female differential selection is acting.

Meiotic multivalents of four or more elements resulting from chromosome translocations are uncommon, but are found, with different features, in plants (*Viscum* and *Oenothera* spp., Cleland 1972, Barlow *et al.* 1978), in invertebrates (*Otocryptos* spp., centipede; Ogawa 1954; and *Delena cancerides,* the huntsman spider, Rowell 1985) and in monotremes (*Ornithorhynchus anatinus*, platypus, and *Tachyglossus aculeatus*, short-beaked echidna, Bick & Sharman 1975, Wrigley & Graves 1988, Gruetzner *et al.* 2004).

The reason for the widely spaced occurrence in the Isoptera of this otherwise rare phenomenon is still unclear. A possible explanation includes heterozygote advantage in the face of the high rate of inbreeding, an advantage for eusocial species associated with patterns of relatedness or uniformity engendered by sex-linked translocation heterozygosity (Rowell 1985, 1986). On the other hand it seems unlikely that the multiple sex chromosomes of termites played a main role in the evolution of their sociality (Lacy 1980, Luykx 1990, Thorne *et al.* 2003). This system probably arose sporadically and relatively recently, perhaps as a result of the reproductive pattern accompanying eusocial behaviour (Bartz 1979).

The assumption that these chromosome interchanges are lacking in females is only partially supported by direct evidence, because only a low number of studies have taken account of female meiosis. In particular, Vincke & Tilquin (1978) first, and later Luykx & Syren (1979), claimed that translocation complexes were absent in females of American and African species, respectively. On the other hand, if females are also translocation heterozygotes, one would expect male offspring with different translocated chromosomes (Syren & Luykx 1981). Given that all the males from the same colony show the same meiotic chromosome configuration, with a typical chain or ring, it seems likely that only males are translocation heterozygotes (Gruetzner *et al*. 2006). However, Fontana (1980) observed a polymorphic situation in an Italian colony of *Reticulitermes lucifugus*, with some males showing regular bivalents and others with a chain of four or six multivalents. This paper presents the karyological characterization of 20 Australian and three European species of Isoptera, with 20 taxa being analysed for the first time. Particular attention is given to the presence and sex distribution of multivalents in male and female meiosis. The karyological approach was preceded by a molecular analysis on COII mitochondrial gene in order to support proposed taxonomic relationships.

Material and methods

Samplings

Samples were field collected during 2005 in North Australia and in central–south Europe (Table 1). Australian termites were identified through the morphological keys of Hill (1942) and Miller (1991), and also through comparison with the CSIRO termite reference collection (CSIRO Entomology, Canberra).

Samplings include the only member of the family Mastotermitidae (*Mastotermes darwiniensis*), three species of the family Kalotermitidae, four species of the family Rhinotermitidae and 15 species of the family Termitidae. Twenty out of the 23 taxa molecularly analysed here are karyotyped for the first time. For each sample, alate, secondary reproductives and nymphs were maintained for the karyological investigation if possible, while workers and soldiers were immediately preserved in absolute ethanol for molecular analyses.

Molecular analyses

Samples were conserved in absolute ethanol and then used for DNA extraction and amplification of the COII mitochondrial gene through polymerase chain reaction (PCR).

Total DNA was extracted following the method of Doyle & Doyle (1987) from one to four samples per species. To avoid symbiont DNA contamination, only the head was used as source of tissue. PCR amplification was performed in 50 μl reactions using the Invitrogen kit, with recombinant Taq DNA polymerase. Thermal cycling was done in a GeneAmp PCR System 2400 (Applied Biosystem) programmable thermal cycler. The conditions for amplification were as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing 48/52°C for 30 s, extension 72°C for 2 min, and final extension at 72°C for 7 min. Primers for PCR amplification and sequencing were: 5′-CAG ATA AGT GCA TTG GAT TT-3′ (modified A-tLeu, 3022-3042 in *D.Yakuba*)/ 5′-GTT TAA GAG ACC AGT ACT TG-3′ (named B-tLys [Liu & Beckenbach 1992, Simon *et al*. 1994]; 3804–3784 in *Drosophila yakuba*). Amplicons were purified using the Wizard PCR prep DNA clear-up System (Promega), and both strands were sequenced with the DNA sequencing kit (BigDye terminator cycle sequencing, Applied Biosystem) in an ABI Prism 310 Genetic Analyzer. The nucleotide sequences of the newly analysed specimens have been submitted to the GenBank under following accession numbers: EF442695, EF442696, EF442697, EF442698, EF442699, EF442700, EF442701, EF442702, EF442703, EF442704, EF442705, EF442706, EF442707, EF442708, EF442709, EF442710, EF442711, EF442712, EF442713, EF442714, EF442715, EF442716, EF442717, EF442718, EF442719, EF442720. Alignments performed with the Clustal algorithm of the Sequence Navigator program (version 1.0.1, Applied Biosystem Inc.) were also checked by sight. Nucleotide substitutions matrices were determined using MEGA3 (Kumar *et al*. 2004).

Phylogenetic relationships were inferred through a maximum parsimony (MP) method and a maximum likelihood (ML) method using PAUP 4.0b (Swofford

Table 1. Termite species, collecting sites, COII haplotypes, diploid mitotic and haploid meiotic chromosome numbers in 23 species of Isoptera

				Chromosome number	
Taxon	Collecting place	Sample	COII haplotypes	2n	n males
Nasutitermes graveolus	C. Darwin NP – Darwin	*1*	H1	42	19 + RIV
	Mango farm – Darwin	*2*	H1		
		3	H1		
Nasutitermes triodiae	Finnis River	*1*	H2	42	/
		2	H2		
	Douglas Daly	*3*	H2		
Nasutitermes longipennis	Mary River	*1*	H3	42	19 + RIV
		2	H3		
	CSIRO – Darwin	*3*	H4		
Tumulitermes pastinator	Palmerston	*1*	H5	42	19 + RIV
	Douglas Daly	*2*	H5		
	Gunn Point	*3*	H5		
Microcerotermes nervosus	Kakadu Nat.Park.	*1*	H6	42	19 + RIV
	Reynolds River	*2*	H7		
Microcerotermes boreus	Territory of Wild Life Park	*1*	H8	42	19 + RIV
		2	H9		
Amitermes darwini	CSIRO – Darwin	*1*	H10	42	19 + RIV
	Alice Spring	*2*	H11		
Amitermes germanus	CSIRO – Darwin	*1*	H12	42	/
	Gunlom – Kakadu	*2*	H13		
Amitermes eucalipti	Home jungle – Darwin	*1*	H14	42	/
	COX peninsula	*2*	H15		
Amitermes parvus	CSIRO – Darwin	*1*	H16	42	19 + RIV
Drepanotermes septentrionalis	Gunn Point	*1*	H17	42	19 + RIV
		2	H18		
Macrognathotermes sunteri	CSIRO – Darwin	*1*	H19	42	19 + RIV
Lophotermes septentrionalis	Territory of Wild Life Park	*1*	H20	42	19 + RIV
Ephelotermes taylori	Fogg Dam	*1*	H21	42	19 + RIV
Ephelotermes melachoma	C. Darwin NP-Darwin	*1*	H22	42	19 + RIV
Heterotermes vagus	CSIRO – Darwin	*1*	H23	42	19 + RIV
		2	H23		
Coptotermes acinaciformis	Adelaide River	*1*	H24	42	19 + RIV
	Reynolds River	*2*	H25		21
Cryptotermes secundus	Mangrove forest-Batchelor	*1*	H30	(40)[a] (APEX)	(20)[a]
		2	H30		
Cryptotermes domesticus	Mandorah	*1*	H31	(29/30)[a] (APEX)	(13 + XXY)[a]
		2	H32		
Mastotermes darwiniensis	CSIRO-Darwin	*1*	H33	96	48
Retuculitermes urbis	Italy – lab. reared		H26	42	21
Reticulitermes lucifugus grassei	France – lab. reared		H27	42	19 + CIV
					21
Kalotermes flavicollis	Palermo-Italy		H28	67/68	(32 + XXY)[b]
	Croatia – lab. reared		H29	67/68	32 + XXY
Cryptocercus relictus	Russia		GeneBank	(17)[c] (APEX)	/[c]
Cryptocercus primarius	China		GeneBank	(19)[c] (APEX)	/[c]
Cryptocercus punctulatus	North America		GeneBank	(37,47)[c] (APEX)	/[c]

R = multivalent ring, C = multivalent chain
References for karyological data: [a]Lo *et al*. 2006a,b; [b]Fontana 1982; [c]Luykx 1990
/ = not determined.
Roman numbers indicate the four chromosomes involved in the multivalents.

2001) with 2000 and 100 bootstrap replicates, respectively. The best substitution model (Tamura-Nei+I+G) for ML was selected using the program Modeltest 3.06 (Posada & Crandall 1998).

European samples of *Reticulitermes* spp. and *Kalotermes flavicollis* and three species of the woodroaches *Cryptocercus* (Accession No.: DQ007645, DQ007643, DQ007642), considered to be the closest living relatives of the eusocial termites, were included to complete the phylogenetic analyses. In addition, *Periplaneta americana* sequences (Accession No.: DQ181546) were employed as an outgroup.

Cytological investigation

Mitotic and meiotic chromosome spreads were obtained from male and female gonads of alates (imagos) or from the gonads of secondary reproductives or nymphs. From each sample, separate preparations were obtained from two to 20 individuals.

Testes and ovaries were placed for 2 h in an insect saline solution plus 0.05% colchicine. A hypotonic shock was then performed for 20 min with sodium citrate 1% and the tissues were fixed for 1 h in 3:1 methanol:glacial acetic acid. Following fixation the tissues were dispersed in 60% acetic acid and cells spread by placing the slides on a hotplate. Finally the dried slides were stained with Giemsa solution (5% in phosphate buffer). Photomicrographs of metaphases were taken on Neopan Fujifilm (100 ASA) and developed in Hypam.

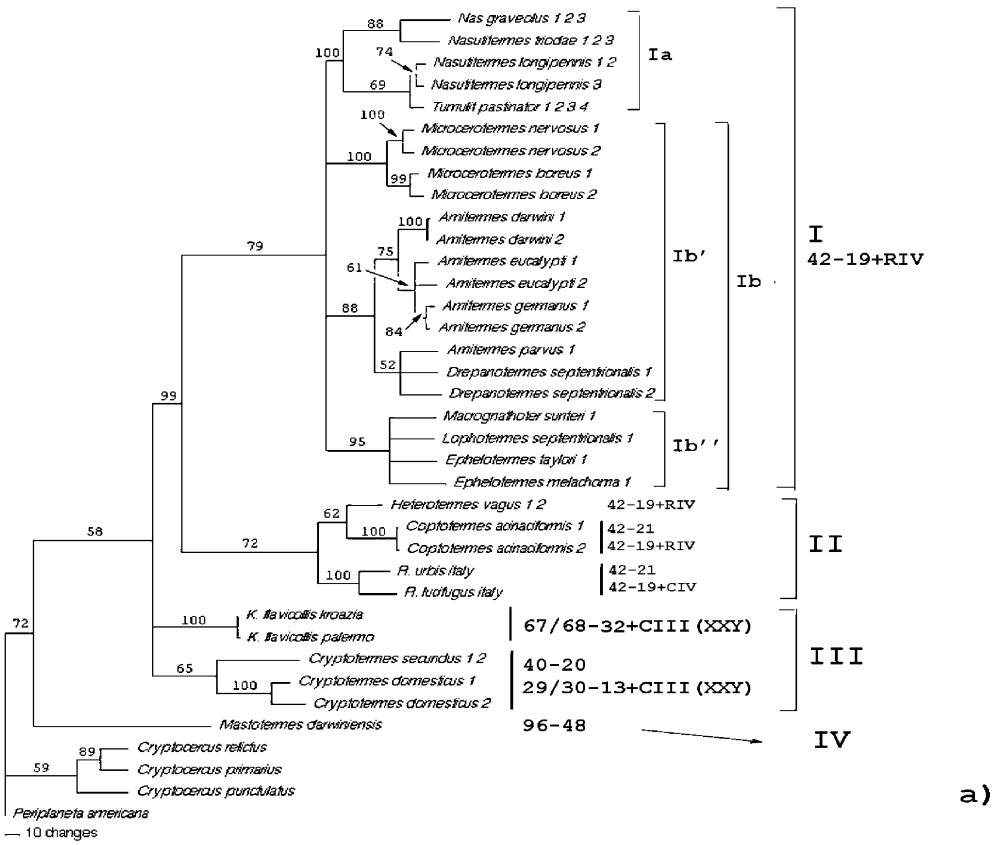
Testes generally provided cells at every stage of mitosis and meiosis. For six species meiotic figures were also obtained from females. Interpretation of sex multivalents in the first meiotic division were confirmed by examining chromosome numbers in the second meiotic division as well as in mitotic cells and by determining the diploid number of the female in the mitotically dividing follicle cells of the ovary. In each species the diploid chromosome number was confirmed by examination of between 15 and 30 male and female mitotic figures. The karyotype was obtained for 15 species by considering at least the best three mitotic plates available. Chromosome nomenclature followed Levan *et al.* (1964), with the acronyms m, sm, st and t designating metacentric, submetacentric, subtelocentric and telocentric chromosome, respectively. This classification system allows determination of the 'fundamental number' (NF, the total number of major chromosome arms in the haploid set) as the total haploid chromosome number, n, plus the haploid number of metacentrics (m) or sub-metacentrics (*sm*): $NF = n + (m + sm)$. Because it was sometimes necessary to make chromosome preparations and examine them under field conditions, no banding methods could be utilized.

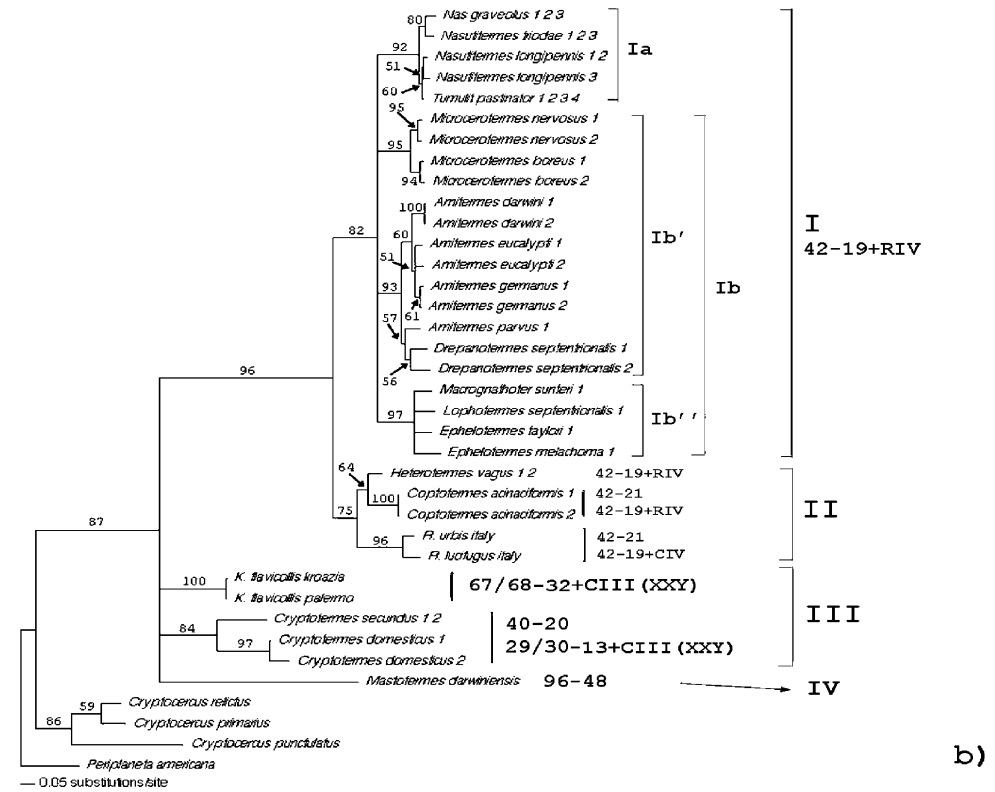
Results

Molecular analyses

A fragment of 647 bp of the COII gene, encoding for 215 amino acids, was sequenced in 38 specimens representing the 20 termite species here karyologically analysed for the first time. Nucleotide composition showed an adenine–thymine bias (A + T = 63.9%). Of 448 variable sites 413 were parsimony informative. The majority of the variation occurred at the third codon position (61%) followed by position 1 (24%) and 2 (15%). Each one of the 29 haplotypes scored for this dataset (Table 1) were sample-specific with the exception of a few haplotypes that were found in different samples of the same species: H1 for *Nasutitermes graveolus*, H2 for *N. triodiae*, H3 for *N. longipennis*, H5 for *Tumulitermes pastinator*, H23 for *Heterotermes vagus* and H30 for *Cryptotermes secundus*.

Tamura-Nei distances (gamma distribution shape parameter = 0.4906, Table 2 [provided Electronic Supplementary Material]) for Australian haplotypes of the same species ranged from 0.004 (*Amitermes darwini* H10 *vs* H11) to 0.120 (*Drepanotermes septentrionalis* H17 *vs* H18). Interspecific distances within families were conserved for Kalotermitidae (0.319–0.388) and Rhinotermitidae (0.141–0.145) whereas a huge range of variation was observed in Termitidae (0.031–0.303). Comparing the Australian haplotypes with the European ones from GeneBank, we observed a lower differentiation within Rhinotermitidae (0.228–0.294), than within Kalotermitidae (0.376–0.408). Interspecific distances between families ranged from 0.216 (*H. vagus* H23 vs *N. longipennis* H4) to 0.885 (*Mastotermes darwiniensis* H33 vs *Microcerotermes boreus* H9). The total number of


Nas graveolus 1 2 3
Nasutitermes triodae 1 2 3
Nasutitermes longipennis 1 2
Nasutitermes longipennis 3
Tumulitermes pastinator 1 2 3 4
Ia
Microcerotermes nervosus 1
Microcerotermes nervosus 2
Microcerotermes boreus 1
Microcerotermes boreus 2
Amitermes darwini 1
Amitermes darwini 2
Amitermes eucalypti 1
Amitermes eucalypti 2
Amitermes germanus 1
Amitermes germanus 2
Amitermes parvus 1
Drepanotermes septentrionalis 1
Drepanotermes septentrionalis 2
Ib'
Macrognathoter sunteri 1
Lophotermes septentrionalis 1
Ephelotermes taylori 1
Ephelotermes melachoma 1
Ib''
Ib
I
42-19+RIV
Heterotermes vagus 1 2
42-19+RIV
Coptotermes acinaciformis 1
Coptotermes acinaciformis 2
42-21
42-19+RIV
R. urbis italy
R. lucifugus italy
42-21
42-19+CIV
II
K. flavicollis kroazia
K. flavicollis palermo
67/68-32+CIII(XXY)
Cryptotermes secundus 1 2
Cryptotermes domesticus 1
Cryptotermes domesticus 2
40-20
29/30-13+CIII(XXY)
III
Mastotermes darwiniensis
96-48
IV
Cryptocercus relictus
Cryptocercus primarius
Cryptocercus punctulatus
Periplaneta americana
10 changes
a)


Nas graveolus 1 2 3
Nasutitermes triodae 1 2 3
Nasutitermes longipennis 1 2
Nasutitermes longipennis 3
Tumulitermes pastinator 1 2 3 4
Ia
Microcerotermes nervosus 1
Microcerotermes nervosus 2
Microcerotermes boreus 1
Microcerotermes boreus 2
Amitermes darwini 1
Amitermes darwini 2
Amitermes eucalypti 1
Amitermes eucalypti 2
Amitermes germanus 1
Amitermes germanus 2
Amitermes parvus 1
Drepanotermes septentrionalis 1
Drepanotermes septentrionalis 2
Ib'
Macrognathoter sunteri 1
Lophotermes septentrionalis 1
Ephelotermes taylori 1
Ephelotermes melachoma 1
Ib''
Ib
I
42-19+RIV
Heterotermes vagus 1 2
42-19+RIV
Coptotermes acinaciformis 1
Coptotermes acinaciformis 2
42-21
42-19+RIV
R. urbis italy
R. lucifugus italy
42-21
42-19+CIV
II
K. flavicollis kroazia
K. flavicollis palermo
67/68-32+CIII(XXY)
Cryptotermes secundus 1 2
Cryptotermes domesticus 1
Cryptotermes domesticus 2
40-20
29/30-13+CIII(XXY)
III
Mastotermes darwiniensis
96-48
IV
Cryptocercus relictus
Cryptocercus primarius
Cryptocercus punctulatus
Periplaneta americana
0.05 substitutions/site
b)

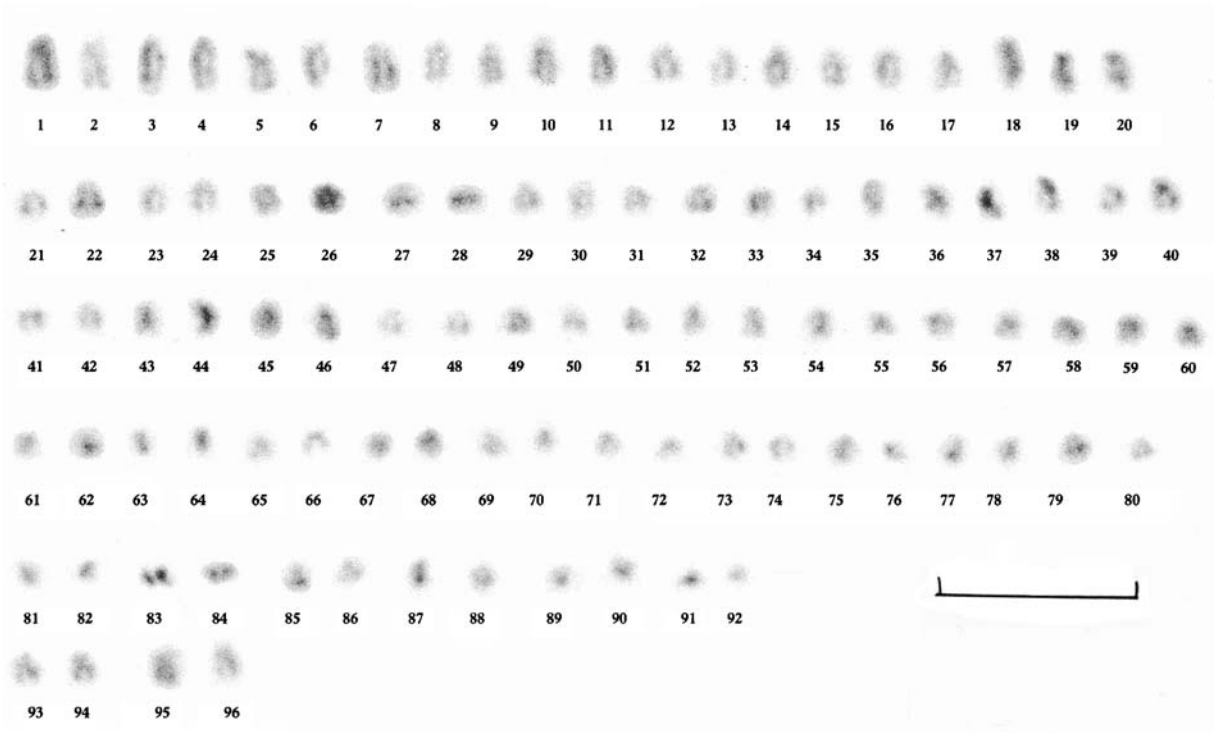


Figure 2. Giemsa-stained karyotype of *Mastotermes darwiniensis*, female; 93-96: metacentrics. Bar = 10 μm

substitutions presented the same ranges and trends with respect to Tamura-Nei distances (available from the authors).

In both MP and ML elaborations the haplotypes of Termitidae (Figure 1; group I) and Rhinotermitidae (Figure 1; group II) families, constitute two well-defined clusters, with a further subdivision of Termitidae into subfamilies, even if polytomically related. *Kalotermes flavicollis* and *Cryptotermes* spp. sequences never join in a single Kalotermitidae cluster (Figure 1; group III). Moreover, in the ML tree they are polytomically related to *M. darwiniensis* (Figure 1; group IV), this taxon appearing basal to the Isoptera taxa here considered only in the MP tree.

Karyological analysis

Standard karyotypes were not easy to construct and relate to each other from an evolutionary point of view, because all the karyotypes are basically similar. However, chromosome number and size, as well as number, position and type of secondary constrictions, and the features of corresponding meiotic plates, including translocation complexes, could be described in 23 species.

Figure 1. (**a**) Maximum parsimony tree based on COII sequences (647 bp), TL: 1481, CI: 0.390, RI: 0.646, RC: 0.252, HI: 0.610. Values above branches indicate bootstrap support on 1000 trials. Vertical bars and numbers on the right side of the tree represent families and subfamilies according to the generally accepted classification of termites (Kambhampati & Eggleton 2000). Larger roman numbers correspond to: I, Termitidae family; II, Rhinotermitidae family; III, Kalotermitidae family; IV, Mastotermitidae family; Ia, Nasutitermitinae subfamily; Ib, Termitinae subfamily; Ib′, *Amitermes* – group; Ib″, *Termes* – group. Nodes with less than 50% support were collapsed to form polytomies. The scale bar indicates the number of changes. Diploid mitotic and meiotic chromosome numbers are reported in the corresponding vertical bar. R=multivalent ring, C=multivalent chain. (**b**) Maximum-likelihood tree based on COII gene (647 bp), -lnL = 6823, 78177. Values above branches indicate bootstrap support as percentage of 100 trials. The scale bar indicates expected nucleotide substitutions per site. Vertical bars and numbers represent families–subfamilies designation and mitotic–meiotic configurations as described in the MP tree.

Karyotype Analysis

Mastotermitidae: Mastotermes darwiniensis, 2n = 96, NF = 50

Eight individuals from two different colonies were examined. In this species it was possible to pair with certainty only those chromosomes that had clear distinguishing features; the others were arranged as far as was possible according to decreasing size (Figure 2). Karyotypes had a predominance of telocentric (t) chromosomes and two pairs (93–94, 95–96) of metacentric (m) or submetacentric (sm) elements. No satellite-bearing chromosomes were observed.

Kalotermitidae: Kalotermes flavicollis, 2n = 67, NF = 38 (male) 2n = 68, NF = 38 (female)

The four studied colonies, two from Sicily (Italy) and two from Croatia, had diploid chromosome complement that could be arranged in pairs (Figures 3 and 4). Male karyotypes included at least four pairs of m and the remainder t and st chromosomes. The uneven one was t. All female complements analysed also had four pairs of m chromosomes. The numbers agree with previous data published by Fontana (1982). In all analysed karyotypes the short arms of st pairs 1–4, 11 and 12 showed satellites (Figures 3 and 4), in either homozygous or heterozygous condition. Mistaking their secondary constrictions for centromeres can lead to mismatching of chromosomes in pairs.

Rhinotermitidae: 2n = 42, FN = 25.

Coptotermes acinaciformis. The low quality of the mitotic figures obtained only allowed confirmation of the chromosome number at 2n = 42, but did not help with construction of a karyotype.

Heterotermes vagus. No evidence of odd chromosomes was found in males or females of this species. Four pairs of m chromosomes could be observed (19–21), while all other pairs were st to t (Figure 5). For both male and female complements, one satellite was detected on the short arm of the telocentric chromosome pair 12.

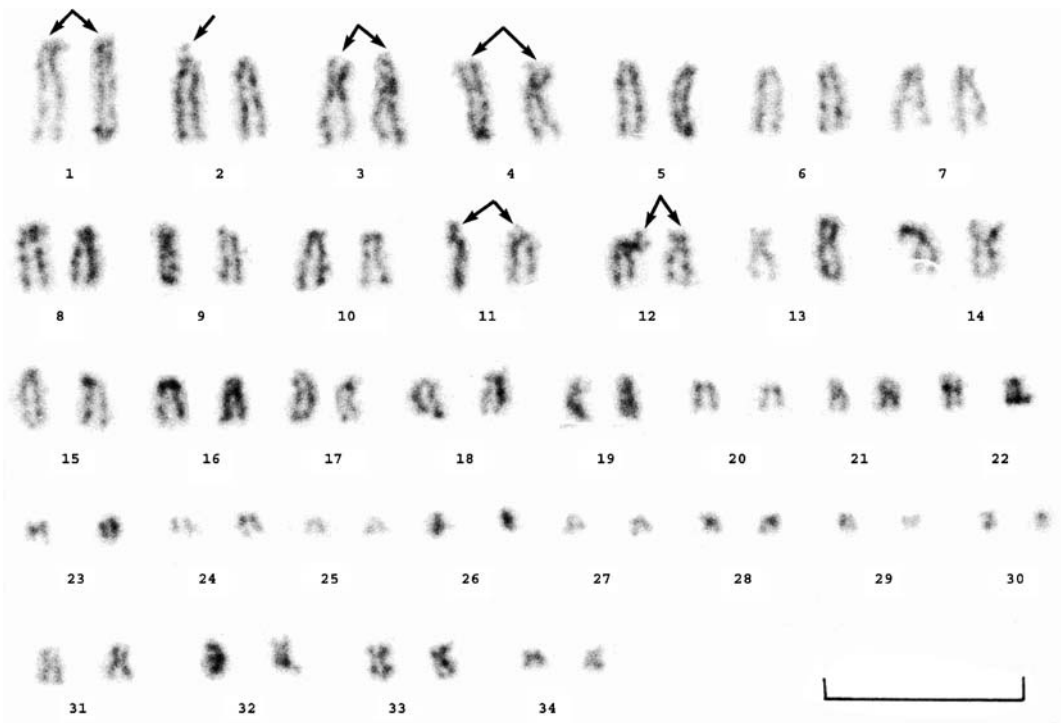


Figure 3. Giemsa-stained karyotype of *Kalotermes flavicollis*, female; 31-34: metacentrics. Arrows indicate the main secondary constrictions. Bar = 10 μm.

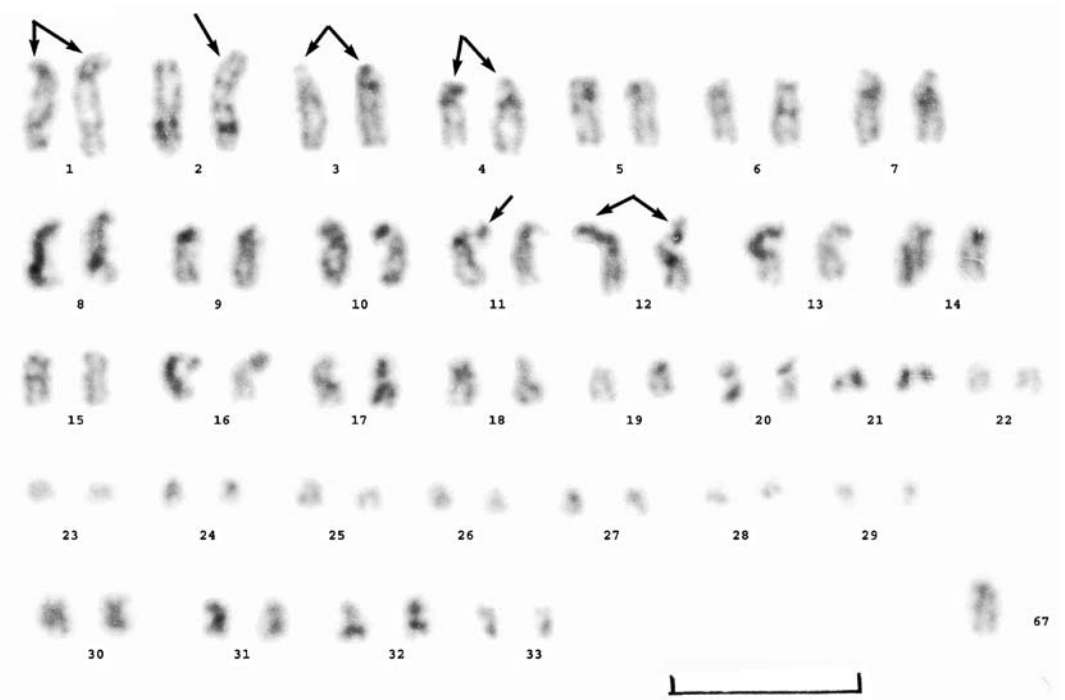


Figure 4. Giemsa-stained karyotype of *Kalotermes flavicollis,* male; 30-33: metacentrics. Arrows indicate the main secondary constrictions. Bar = 10 μm.

Reticulitermes urbis–R. lucifugus grassei. No odd chromosomes were observed in the mitotic complements of both males and females. On the other hand, four pairs of m chromosomes and one satellite on the short arms of the chromosomes 21–22 were observed (Figure 6).

Termitidae 2n = 42

A constant diploid complement and the presence of numerous satellites characterize all the members of this family. The secondary constrictions can be confused with centromeres and lead to misclassifications of chromosomes. As a consequence the exact

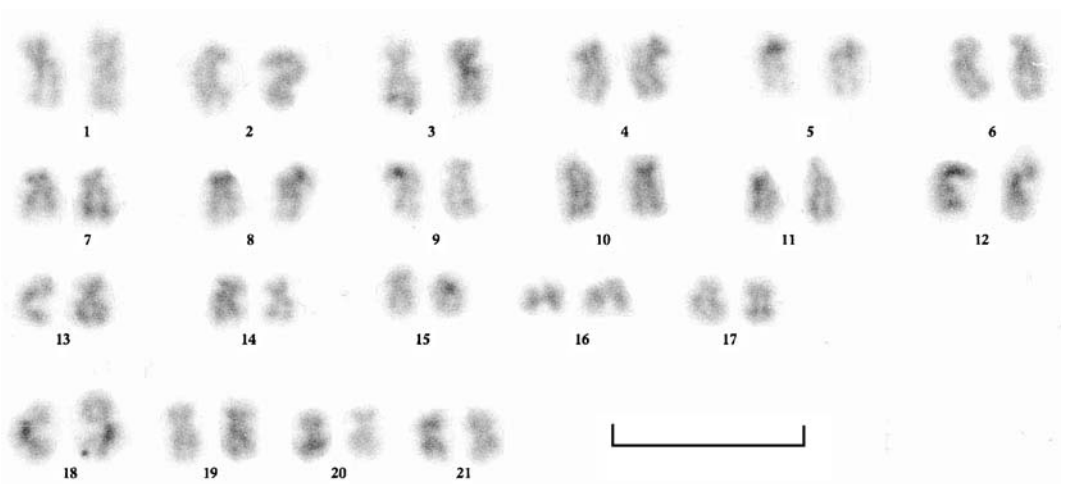


Figure 5. Giemsa-stained karyotype of *Heterotermes vagus,* male; 18-21: metacentrics. Bar = 10 μm.

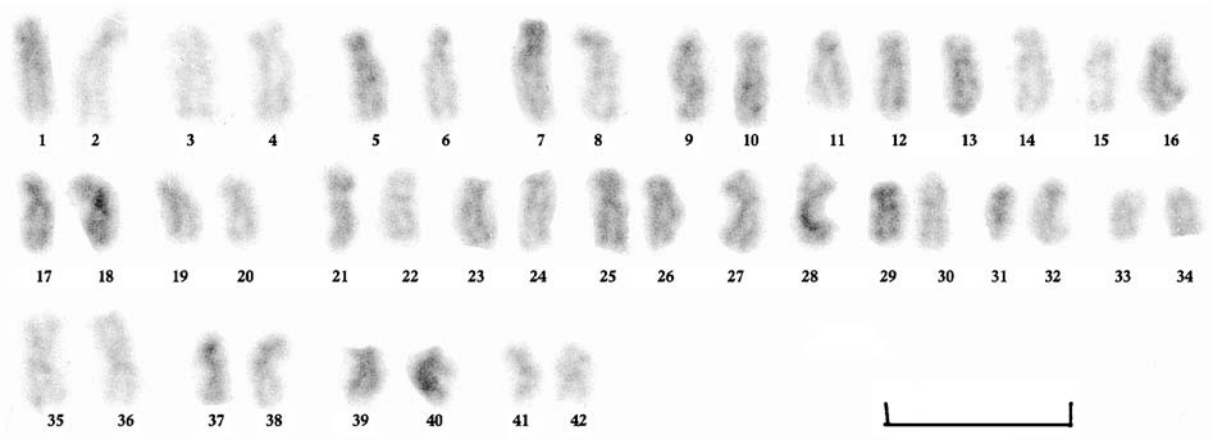


Figure 6. Giemsa-stained karyotype of *Reticulitermes urbis,* male; 35-42: metacentrics. Bar = 10 μm.

NF could not be defined. Moreover, in both sexes most chromosomes have similar size and few distinguishing features. For these reasons most chromosomes were not arranged in pairs but just according to decreasing size. The following groups were analysed:

Nasutitermitinae subfamily

The karyotypes of *Nasutitermes graveolus* (Figure 7), *N. longipennis* (Figure 8) and *Tumulitermes pastinator* showed at least four pairs of m chromosomes. Moreover, the karyotypes obtained from males and females of *N. longipennis* showed a peculiar satellite on the short arms of chromosome 8, in addition to at least another 10 smaller satellites.

Termitinae subfamily

The karyotypes of the *Amitermes* group (Figures 9, 10, 11, 12) showed the highest amount of secondary constrictions of all Isopteran taxa analysed: almost all chromosomes appear to bear satellites, making the description of the karyotype particularly difficult and

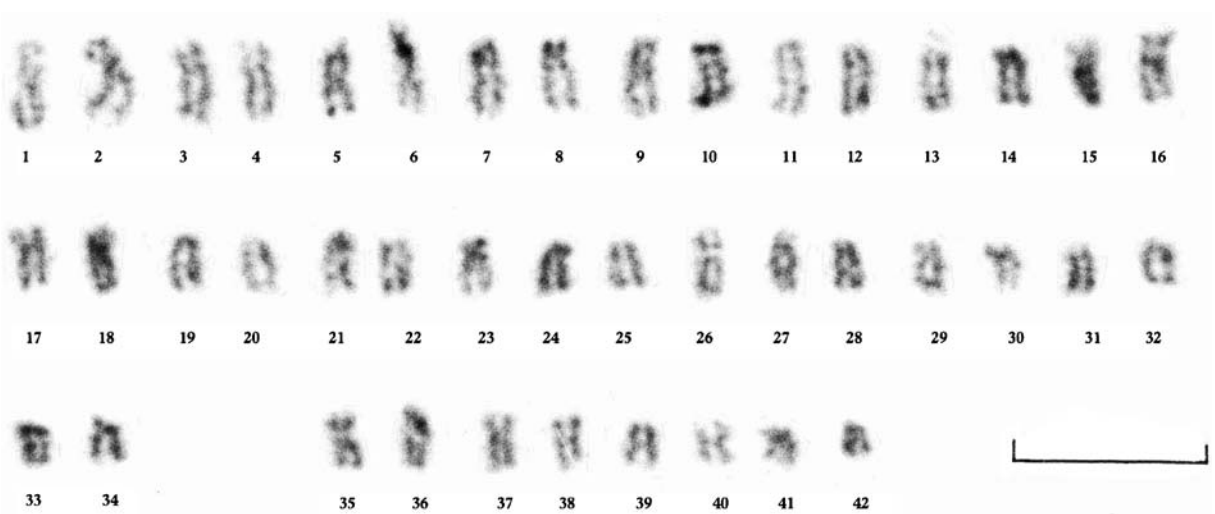


Figure 7. Giemsa-stained karyotype of *Nasutitermes graveolus,* male; 35-42: metacentrics. Bar = 10 μm.

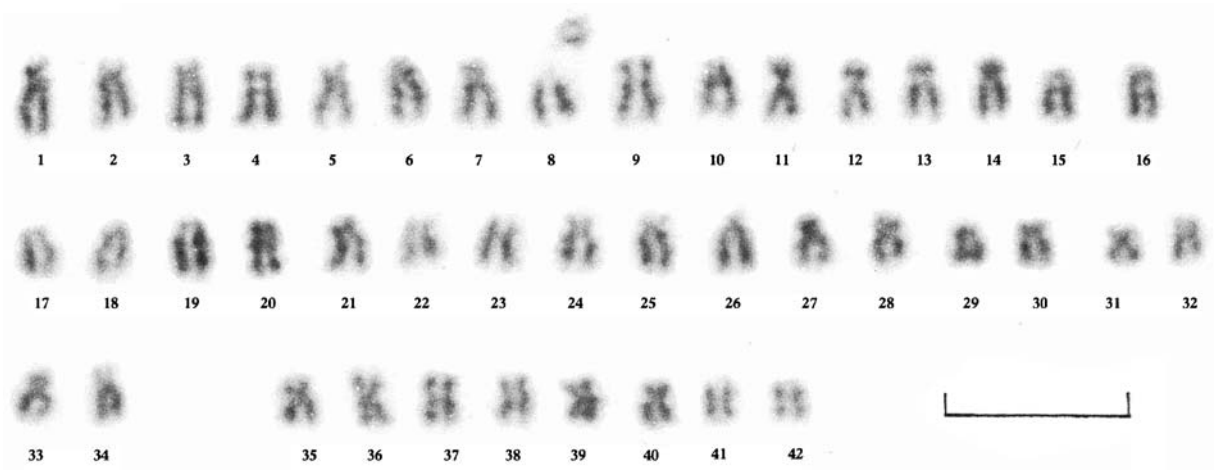


Figure 8. Giemsa-stained karyotype of *Nasutitermes longipennis,* male; 35-42: metacentrics. Bar = 10 μm.

therefore no precise FN was assignable. While it was possible to establish that the majority of the chromosomes were t to sm, it was impossible to define the exact number of the m ones. Karyotypes obtained from *Drepanotermes septentrionalis* samples (Figure 12) showed much-contracted chromosomes, and it was not possible to distinguish with certainly primary from secondary constrictions in all elements. In the *Termes* group at least three pairs of m chromosomes could be recognized. The number of satellites was lower in *Macrognathotermes* (Figure 13) than in *Ephelotermes* (Figure 14), where at least 10 chromosomes bore satellites on the short arms.

Although it must be accepted that sex chromosomes have differentiated in three out of four analysed families (see Meiotic Analysis), these were not cytologically distinguishable in any of the mitotic metaphases examined.

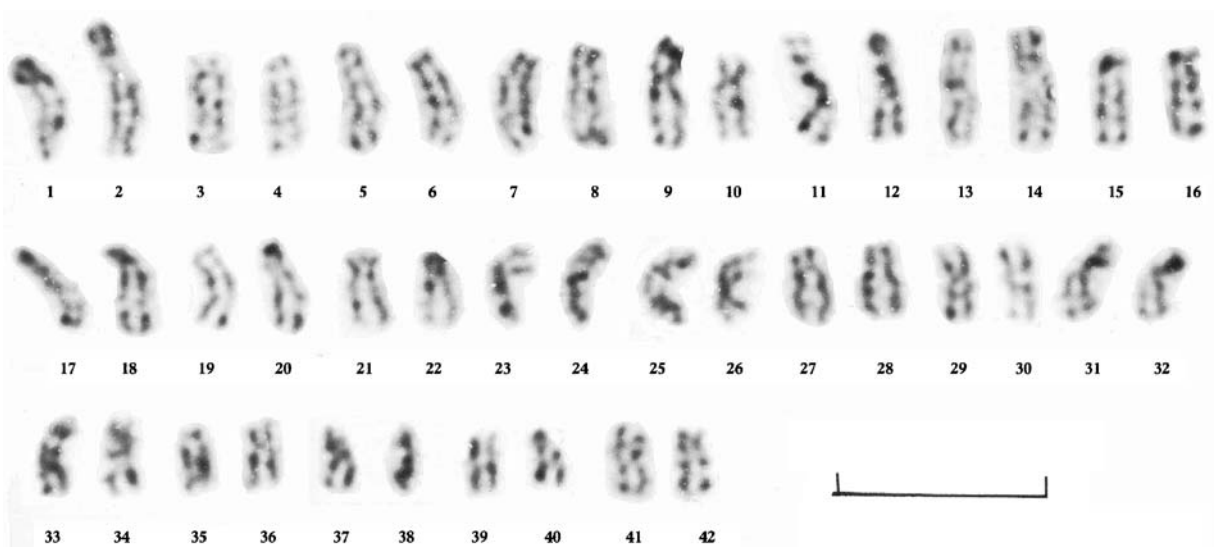


Figure 9. Giemsa-stained karyotype of *Microcerotermes nervosus,* male. Bar = 10 μm.

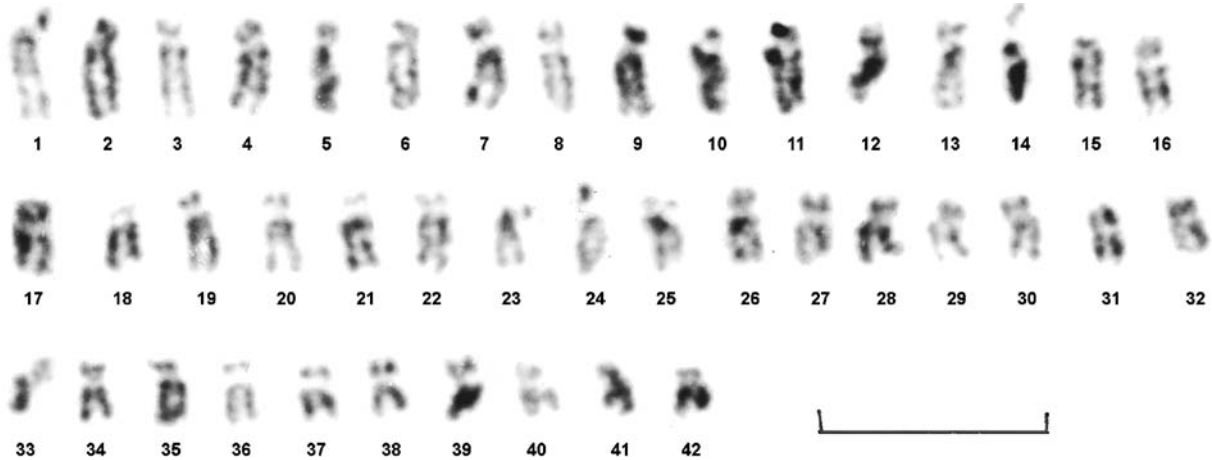


Figure 10. Giemsa-stained karyotype of *Microcerotermes boreus*, male. Bar = 10 μm.

Meiotic Analysis

Mastotermitidae

Many metaphase I divisions were found in both males and females of *M. darwiniensis*, showing that all chromosomes form regular (48) bivalents (Figure 15a). This agrees with the scoring of 96 mitotic chromosomes. Even if some bivalents were contracted or relatively small, terminal paring suggested the presence of a high number of t and st chromosomes. The lack of any chain or ring of chromosomes showed that no sex-linked translocation complex occurs in *M. darwiniensis*.

Kalotermitidae

Thirty-two regular bivalents and one trivalent were observed in each early metaphase I examined for males of *K. flavicollis*. This confirmed the diploid number of 67 (Figure 15b) and showed that a single translocation involving sex chromosomes has occurred. Unfortunately, no female meiosis could be obtained, but from mitotic counts only bivalents would be expected.

Rhinotermitidae

While *R. urbis* always presented 21 regular bivalents (Figure 15c), *R. lucifugus grassei* showed different numbers among colonies. In particular, in 15 out of 20 colonies, plates with 19 bivalents and a chain of four chromosomes were observed (Figure 15d), while the other five populations showed 21 regular bivalents. A similar situation was observed in *C. acinaciformis*, where only one of the two analysed colonies presented the translocation complex in

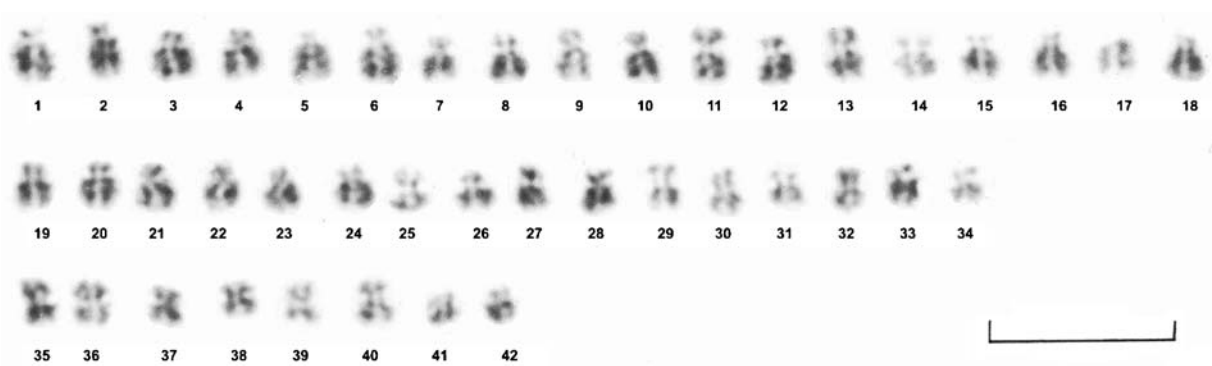


Figure 11. Giemsa-stained karyotype of *Amitermes darwini,* female. Bar = 10 μm.

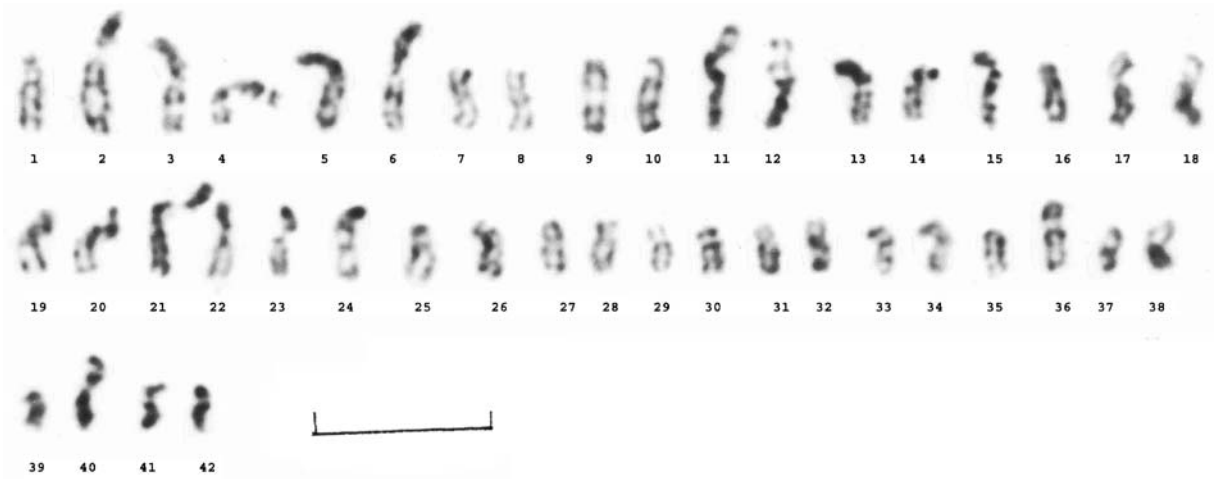


Figure 12. Giemsa-stained karyotype of *Drepanotermes septentrionalis,* male. Bar = 10 μm.

males. The same segmental interchange complex of four chromosomes in a ring besides 19 bivalents, were observed in all male meiotic first plates of *H. vagus* (Figure 15e).

Termitidae

All male members of this family showed in early metaphase I the sex-linked ring quadrivalent in addition to 19 pairs; metaphase II showed 21 chromosomes (Figures 15f–15q). On the other hand, female meiosis of *Amitermes darwini*, *Microcerotermes nervosus, M. boreus* and *Drepanotermes septentrionalis* clearly showed 21 regular bivalents.

Discussion

The taxonomic implications of the molecular analyses performed here, besides being in agreement with previously published work (see below), support the same general conclusions as the karyological data: the incongruence between MP and ML elaborations is mainly linked to the Kalotermitidae family, which represents the most variable taxon also at the chromosome level. From a strictly molecular point of view, a good agreement with current taxonomy (following Donovan *et al.* 2000, Kambhampati & Eggleton 2000, Engel & Krishna 2004) was found in

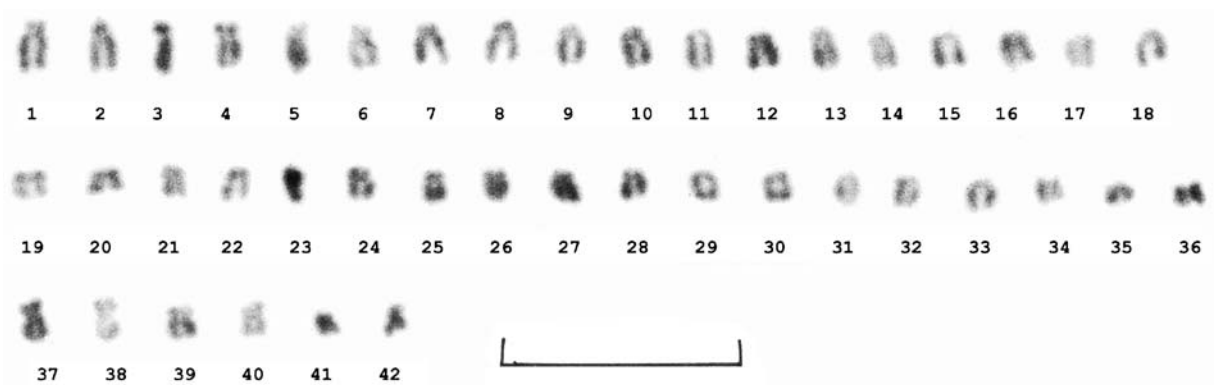


Figure 13. Giemsa-stained karyotype of *Macrognathotermes sunteri,* male; 37-42 metacenyrics. Bar = 10 μm.

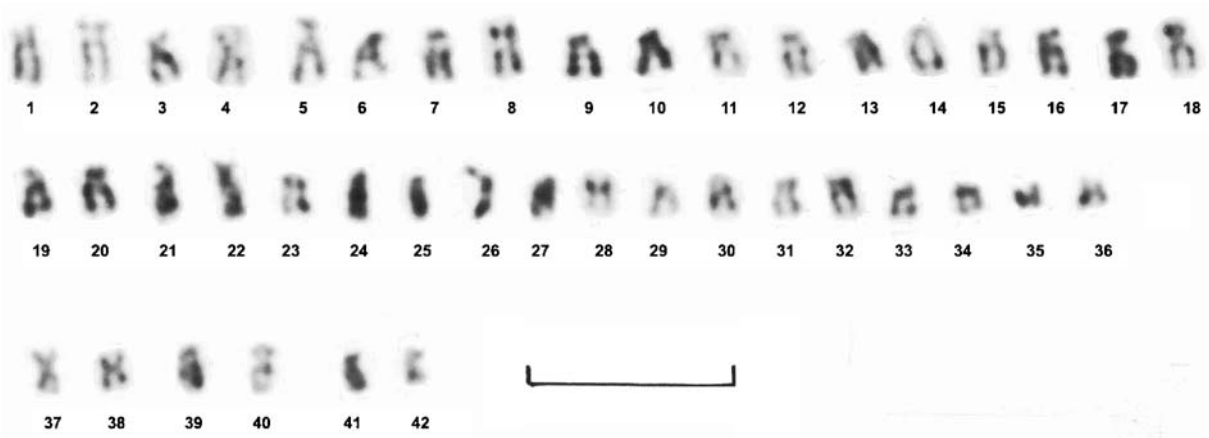


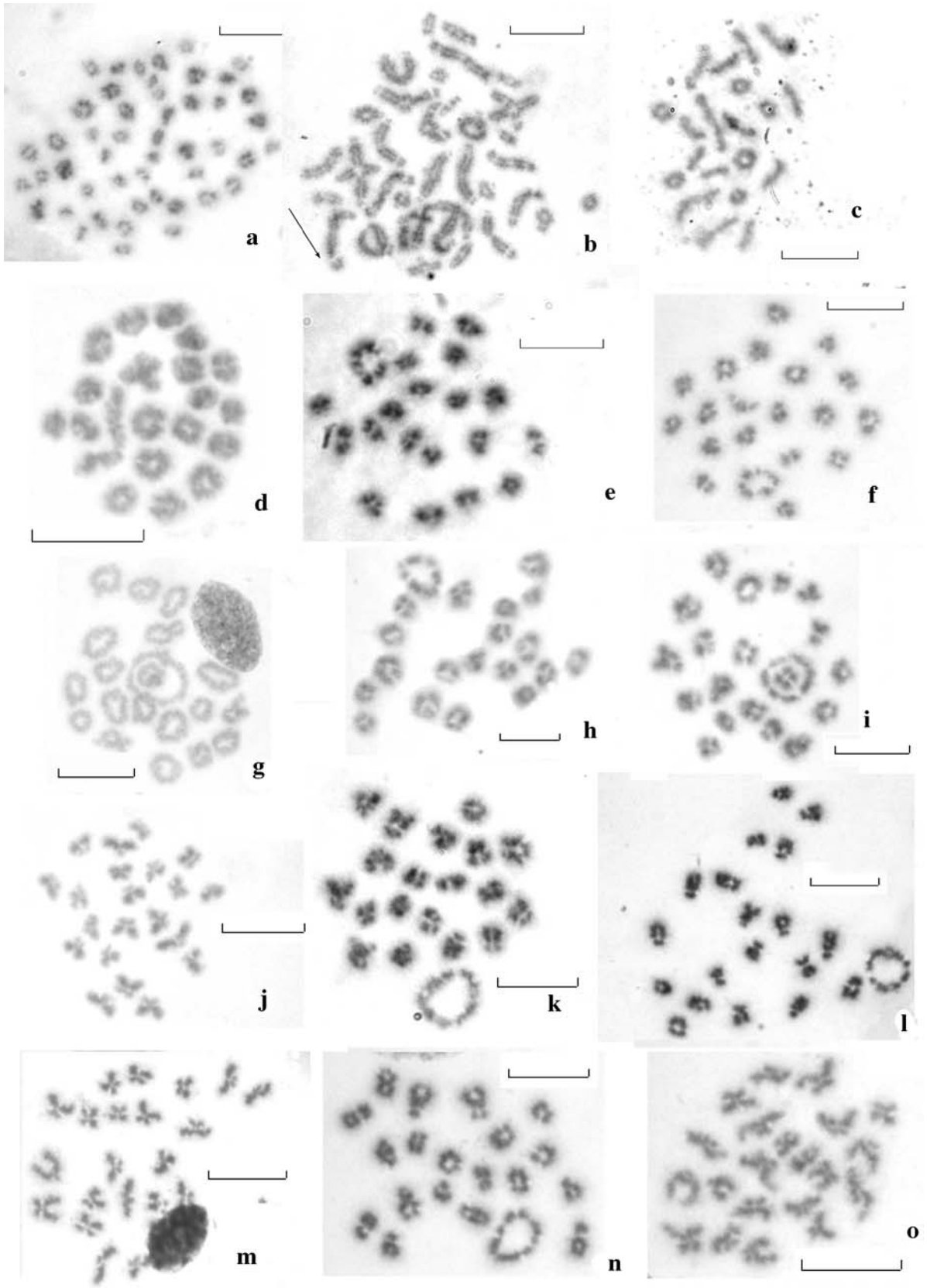
Figure 14. Giemsa-stained karyotype of *Ephelotermes melachoma,* male; 37-42: metacentrics. Bar = 10 μm.

all analyses, with a corresponding subdivision in the main families of the order. Moreover, also family relationships in parsimony and likelihood analysis also mainly agree with other studies based on either morphological or molecular approaches (Kambhampati *et al*. 1996, Kambhampati & Eggleton 2000, Thompson *et al*. 2000). Mastotermitidae is clearly the basal lineage among the four families included in this study, discounting the ML analysis, where there is evidence of polytomy between *M. darwiniensis* and Kalotermitidae taxa. The uncertain position of Kalotermitidae in our study may be due to the absence of members of Hodotermitidae and Termopsidae, usually considered as basal to this family. However, Thorne & Carpenter (1992) found Mastotermitidae and Kalotermitidae to be sister families on the basis of previously published morphological, developmental and anatomical characters. The position of Kalotermitidae was found by Kambhampati *et al*. (1996) to be relatively apical among lower termites, as in our MP topology, and relatively basal in the morphological analyses (Ahmad 1950, Krishna 1970, Emerson & Krishna 1975, Thorne & Carpenter 1992). All this suggests the need for further investigation of the Kalotermitidae, which appears the most diversified family at both the molecular and the chromosomal levels. On the other hand, in our analysis Kalotermitidae always appeared to be related to the Rhinotermitidae+Termitidae group, in agreement with Kambhampati *et al*. (1996) and Noirot (1995).

Within Rhinotermitidae a clear-cut differentiation seems to have occurred between *Reticulitermes* and a group containing both *Coptotermes* and *Heterotermes* haplotypes. This splitting was also observed by Austin *et al*. (2004) in their study based on the COII gene sequences.

Termitidae monophyletism (82% ML, 79% MP) and sub-family relationships (Miura *et al*. 1998, Donovan *et al*. 2000, Ohkuma *et al*. 2004) are here also strongly supported. The Termitinae subfamily is the less clearly defined entity: in present dendrograms the relationships between the so-called *Amitermes* group and *Termes* group was in fact depicted by a polytomy. This casts doubt on the monophyly of Termitinae. Nasutitermitinae constitute a group

Figure 15. Meiotic cells from several termite species. (**a**) *Mastotermes darwiniensis*: male, metaphase I, 48 bivalents. (**b**) *Kalotermes flavicollis*: male, metaphase I, 32 bivalent and one trivalent (CIII) (arrow). (**c**) *Reticulitermes urbis*: male, metaphase I with 21 bivalents. (**d**) *Reticulitermes lucifugus grassei*: male, 19 bivalents+CIV. (**e**) *Heterotermes vagus*: 19 bivalents and a multivalent ring of four chromosomes, RIV. (**f**) *Ephelotermes taylori*: 19 bivalents + RIV. (**g**) *Macrognathotermes sunteri*: 19 bivalents + RIV. (**h**) *Microcerotermes boreus*: 19 bivalents + RIV. (**i**,**j**) *Microcerotermes nervosus*: 19 bivalents+RIV and metaphase II with 21 chromosomes. (**k**) *Amitermes darwini*: 19 bivalents + RIV. (**l**,**m**) *Nasutitermes longipennis*: metaphase I with 19 bivalents + RIV and metaphase II with 21 chromosomes. (**n**,**o**) *Nasutitermes graveolus*: metaphase I with 19 bivalents + RIV and metaphase II with 21 chromosomes. Bar = 10 μm in all the figures.


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o

strongly supported by bootstrap values, but the monophyly of genera within this subfamily remains uncertain (Bergamaschi et al. unpublished).

From a karyological point of view the data presented here generally agree with previous work in showing the uniformity of higher termites, with most species having a diploid number of 42 and a single sex-linked translocation resulting in a ring of four chromosomes in male meiosis. The Kalotermitidae, on the other hand, are highly variable, with diploid numbers ranging from 28 to 56 and with sex-linked translocations ranging from none to eight, resulting in sex chromosomes varying from a simple XY pair to 9 XY pairs (present data; Luykx 1990).

The large differences in chromosome numbers observed between lower and higher termites could be explained by several Robertsonian changes (centric fusions or fissions) or multiple translocation events (Luykx 1990), frequently involving sex chromosomes. The ancestral karyotype organization is not clear, but our observations on Mastotermitidae and Kalotermitidae suggest a set of acrocentric chromosomes as the most primitive, in agreement with Luykx's (1990) data on other lower termites. At present it is impossible to say whether a haploid complement of n = 48, such as in *Mastotermes darwiniensis*, can be considered as the ancestral one, or whether polyploidization events have occurred in this order. Robertsonian changes are recognized as a common factor in karyotype evolution in lower termites (Luykx 1990), involving more centric fusions than centric fissions among autosomes as well as between sex chromosomes and autosomes. The Kalotermitidae situation appears extremely diversified, suggesting that mutual interchanges and translocations have arisen independently many times within this family.

In none of the species examined by us could heteromorphic chromosomes be distinguished. Owing to the occurrence of multivalents in males, they appear to be the heterogametic sex, while females, always forming regular bivalents, should be the homogametic one.

Sex multivalents are common in male meiosis of termites, but they are by no means a general feature and do not appear to be concomitant with eusociality (Crozier & Luykx 1985). At variance with previous data (Bedo 1987), where *M. darwiniensis* showed a diploid number of 98 chromosomes, all the mitotic plates examined by us presented 96 chromosomes and 48 bivalents in meiotic metaphase I. As far as sex chromosome translocations are concerned, our observations on *M. darwiniensis* confirm the absence of these complexes in both male and female meiosis. On the other hand, observations on *K. flavicollis* (Kalotermitidae) agree with previous data in the existence of a male sex-determining mechanism of the X_1X_2Y type (Fontana 1982). The trivalent formed in male meiosis may be produced by a medium-sized acrocentric chromosome (X1) on one side and a small acrocentric (X2) on the other, each paired with the two limbs of an intermediate Y of the same length of X1 (Fontana 1982). On this basis the hypothesis of a possible origin by polyploidization of *K. flavicollis* (Clèment 1977) must be rejected. The particular XXY sex-determining mechanism in *K. flavicollis* males probably originates from an initial X0/XX system. Afterwards, a reciprocal translocation between the original X and one chromosome of a pair of autosomes, may have brought about the trivalent complex formation at meiosis, such as in *Cryptotermes* spp. (Luykx 1990).

Five species of *Cryptotermes* out of the 13 taxa studied by Luykx (1990) also presented an XXY trivalent at meiosis. It should be noted, however, that the Kalotermitidae show different types of sex-linked complexes. *K. approximatus* and *Incisitermes schwarzi* have reciprocal translocations involving from 11 to 19 chromosomes of the male chromosome set (2n = 32; Syren & Luykx 1977, 1981). In *Neotermes insularis* (2n = 52) a six-membered ring was found (Luykx 1990). In Rhinotermitidae different situations were also recorded, even involving populations of the same taxon. Some populations of *R. lucifugus grassei* and *C. acinaciformis* showed a chain of four chromosomes and others with only regular bivalents. On the other hand, *H. vagus* showed a quadrivalent ring in each analysed population, while *R. urbis* never presented the complex. Notably, Fontana (1980) found male meiotic plates with different features in individuals of the same Italian population of *R. lucifugus*. Fontana (1980) also reported chains involving four to eight chromosomes.

The chain conformation in *Reticulitermes* suggested that two of the four chromosomes involved in the interchange complex are uniarmed and the other two probably submetacentric (Fontana 1980). On the contrary, in all the taxa of the Termitidae family examined by us, a ring was observed: this may be the product of heterozygous reciprocal translocations

between two pairs of submetacentric or subtelocentric chromosomes.

The presence of a chain or a ring in males of most Rhinotermitidae and in all the Termitidae species examined by us and previous investigators (Vincke & Tilquin 1978) suggests that the multivalents may derive from a translocation between the Y chromosomes of an initial XY male type and one chromosome of an autosome pair. This would lead to a system of two Y and two X chromosomes (Vincke & Tilquin 1978).

The XO-male type may represent the final stage of differentiation following an initial XY system. The XO-male type is generally considered to be highly derived (Bull 1983), originating from an ancestral XY type where the evolutionary loss of the Y chromosome may have occurred. This system was also found in the Kalotermitidae *Stolotermes victoriensis* (Luykx 1990) and in cockroaches. This argues against a simple derivation of termites from cockroaches, but, in agreement with Grassè (1986), Luykx (1990) and Zompro (2005), a common ancestor with an XY/XX sex determination may be postulated. Subsequently, the cockroach lineages may have rapidly lost the Y chromosomes. Moreover, chain length variation within Kalotermitidae could indicate that these multivalents are not the result of one or a few original rearrangements in common ancestors, but their development has been ongoing in the evolution of the group. On the other hand, Rhinotermitidae and Termitidae show a fixation of the XY-male type (always involved in a reciprocal translocation with two autosomes), as the unique and stable stage of differentiation.

An apparent reduction in chromosome number seems to be involved in the evolution of Isoptera karyotypes, but the chromosomal repatterning leading to a constant number of $2n = 42$ remains unexplained. Actually, the fixed chromosome number observed here, and in the literature in all higher termites, indicates that numerical changes have not occurred during the divergence of Rhinotermitidae and Termitidae. On the other hand, species clustering in groups that share major karyotype characteristics may indicate that, if the mechanism of speciation within each group involved chromosome rearrangements, these may have been small or cryptic structural mutations that did not modify the karyotypic morphology (e.g. paracentric inversion or reciprocal translocations with segments of equal size). King (1993) tried to explain the chromosome complement stability through the non-random nature of chromosomal evolution.

On the whole, both molecular and karyological data showed a wide variability within the Kalotermitidae family: the phylogenetic relationships among its genera gave a polytomic pattern and the karyotypic features appeared extremely diversified. On the contrary, Rhinotermitidae and Termitidae families showed a high molecular and karyological affinity among genera and species, suggesting an ongoing concerted evolution.

Acknowledgements

We are greatly indebted to Luciana Dipietrangelo for her fundamental help with all the photographic techniques employed. Further thanks to Dr Ombretta Marescalchi for her useful hints in analysing karyotypes, to Dr Andrea Luchetti for precious assistance in molecular analysis and to Dr Andrew White for his accurate language revision of the manuscript.

This work was supported by Canziani and RFO funds. S.B. travel was funded by Marco Polo grants from the University of Bologna.

References

Ahmad M (1950) The phylogeny of termite genera based on imago-worker mandibles. *Bull Am Mus Nat Hist* **95**: 39–86.

Austin JW, Szalanski AL, Cabrera BJ (2004) Phylogenetic analysis of the subterranean termite family rhinotermitidae (Isoptera) by using the mitochondrial cytochrome oxidase II gene. *Ann Entomol Soc Am* **97**: 548–555.

Barlow BA, Wiens D, Wiens C, Busby WH, Brighton C (1978) Permanent translocation heterozigosity in *Viscum album* and *V. cruciatum*: sex association, balance lethals, sex ratio. *Heredity* **40**: 33–38.

Bartz SJ (1979) Evolution of eusociality in termites. *Proc Natl Acad Sci USA* **76**: 5764–5768.

Bedo DG (1987) Undifferentiated sex chromosomes in *Mastotermes darwiniensis* Froggatt (Isoptera, Mastotermitidae) and the evolution of eusociality in termites. *Genome* **29**: 76–79.

Bick Y, Sharman G (1975) The chromosomes of the platypus (*Ornithorhynchus*): Monotrema. *Cytobios* **14**: 17–28.

Bulmer MS, Crozier RH (2004) Duplication and diversifying among termite antifungal peptides. *Mol Biol Evol* **21**: 2256–2264.

Bull JJ (1983) *Evolution of Sex Determining Mechanisms*. Benjamin/Cummings: Menlo Park, CA

Cleland RE (1972) *Oenothera, Cytogenetics and Evolution*. New York: Academic Press.

Clèment JL (1977) Carytypes des Reticulitermes francais. *CR Acad Sci Paris* **284**: 2355–2356.

Crozier RH, Luykx P (1985) The evolution of termite eusociality is unlikely to have been based on a male-haploid analogy. *Am Naturalist* **126**: 867–869.

Donovan SE, Jones DT, Sands WA, Eggleton P (2000) Morphological phylogenetics of termites (Isoptera). *Biol J Linn Soc* **70**: 467–513.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation method for small quantities of fresh tissues. *Phytochem Bull* **19**: 11–15.

Eggleton P (2001) Termites and trees: a review of recent advances in termite phylogenetics. *Insect Soc* **48**: 187–193.

Emerson AE, Krishna K (1975) The termite family Serritermitidae (Isoptera). *Am Mus Novitates* **2570**: 1–31.

Engel MS, Krishna K (2004) Family-group names for termites (Isoptera). *Am Mus Nat Hist* **3432**: 1–9.

Fontana F (1980) Interchange complexes in Italian populations of *Reticulitermes lucifugus* Rossi (Isoptera: Rhinotermitidae). *Chromosoma* **81**: 169–175.

Fontana F (1982) Cytological analysis of the chromosome complement of *Kalotermes flavicollis* Fabr. (Isoptera Kalotermitidae). The sex determining mechanism. *Cytologia* **47**: 147–152.

Fontana F (1991) Multiple reciprocal chromosomal translocations and their role in the evolution of sociality in termites. *Ethol Ecol Evol* **1**: 15–19.

Grassè PP (1986) *Termitologia* Vol.III Masson: Paris

Gruetzner F, Ashley T, Rowell D, Marshall Graves JA (2006) How did the platypus gets its sex chromosome chain? A comparison of meiotic multiples and sex chromosomes in plants and animals. *Chromosoma* **115**: 75–88.

Gruetzner F, Rens W, Tsend-Ayush E *et al.* (2004) In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature* **432**: 913–917.

Hill GF (1942) *Termites (Isoptera) from the Australian Region*. Melbourne: CSIR.

Kambhampati S, Eggleton P (2000) Phylogenetics and taxonomy. In: Abe T, Higashi M, Bignell DE, eds. *Terminates: Evolution, Sociality, Symbiosis, Ecology*. Dordrecht: Kluwer Academic Publishing, pp. 1–23.

Kambhampati S, Kjer KM, Thorne BL (1996) Phylogenetic relationship among termite families based on DNA sequence of mitochondrial 16S ribosomal RNA gene. *Insect Mol Biol* **5**: 229–238.

King M (1993) *Species Evolution: the Role of Chromosome Changes*. Cambridge, UK: Cambridge University Press.

Kjer KM (2004) Aligned 18S and insect phylogeny. *Syst Biol* **53**(3): 506–514.

Kràl J, Musilova J, St'àhlavsky F *et al.* (2006) Evolution of the karyotype and sex chromosome system in basal clades of araneomorph spiders (Araneae: Araneomorphae). *Chromosome Res* **14**: 859–880.

Krishna K (1970) Taxonomy, phylogeny and distribution of termites. In: Krishna K, Weesner FM eds. *Biology of Termites*. New York: Academic Press, pp. 127–152.

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings Bioinformat* **5**: 150–163.

Lacy RC (1980) The evolution of eusociality in termites: a haplodiploid analogy? *Am Nat* **116**: 449–451.

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201– 220.

Liu H, Beckenbach AT (1992) Evolution of the mitochondrial cytocrome oxidase II gene among 10 orders of insects. *Mol Phylogenet Evol* **1**: 41–52.

Lo N, Bandi C, Watanabe H *et al.* (2003) Evidence for cocladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. *Mol Biol Evol* **20**: 907–913.

Lo N, Eldrige RH, Lenz M (2006a) Phylogeny of Australian Coptotermes (Isoptera: Rhinotermitidae) species inferred from mitochondrial COII sequences. *Bull Entomol Res* **96**: 433–437.

Lo N, Luykx P, Santoni R *et al.* (2006b) Molecular phylogeny of *Cryptocercus* wood-roaches based on mitochondrial COII and 16S sequences, and chromosome numbers in palearctic representatives. *Zool Sci* **23**: 393–398.

Lo N, Tokuda G, Watanabe H *et al.* (2000) Evidence for multiple gene sequences indicates that terminates evolved from wood-feeding cockroaches. *Curr Biol* **10**: 801–804.

Luykx P (1990) A cytogenetic survey of 25 species of lower termites from Australia. *Genetica* **33**: 80–88.

Luykx P, Syren RM (1979) Thecytogenetics of *Incisitermes schwarzi* and other Florida terminates. *Sociobiology*.

Macaisne N, Dutrillaux AM, Dutrillaux B (2006) Meiotic behaviour of a new complex X-Y-autosome translocation and amplified heterochromatin in *Jumnos ruckeri* (Saunders) (Coleoptera, Scarabaeidae, Cetoniinae). *Chromosome Res* **14**: 909–918.

Miller LR (1991) A revision of the Termes–Capritermes branch of the Termitinae in Australia (Isoptera: Termitidae). *Invertebr Taxon* **4**: 1147–1282.

Miller LR (1997) Systematic of the Australian Nasutitermitinae with reference to evolution within the Termitidae (Isoptera). Ph.D. thesis, Australian National University, Canberra, Australia.

Miura T, Maekawa K, Kitade O, Abe T, Matsumoto T (1998) Phylogenetic relationship among subfamilies in higher termites (Isoptera: Termitidae) based on mitochondrial COII gene sequences. *Ann Entomol Soc Am* **91**: 515–523.

Miura T, Roisin Y, Matsumoto T (2000) Molecular phylogeny and biogeography on the Nasute Termite Genus Nasutitermes (Isoptera: Termitidae) in the Pacific tropics. *Mol Phylogenet Evol* **17**: 1–10.

Noirot C (1995) The gut of termites (Isoptera). Comparative anatomy, systematics, phylogeny, lower termites. *Annals de la Societè Entomologique de France* **31**: 197–226.

Ogawa K (1954) Chromosome studies in the Myriapoda. VII. A chain-association of the multiple sex chromosomes found in *Otocryptos sexspinosus* (Say). *Cytologia* **19**: 265– 272.

Ohkuma M, Yuzawa H, Amornsak W *et al.* (2004) Molecular phylogeny of Asian Termites (Isoptera) of the families Termitidae and Rhinotermitidae based on mitochondrial COII sequences. *Mol Phylogenet Evol* **31**: 701–710.

Ozeki M, Isagi Y, Tsubota H, Jacklyn P, Bowman D (2007) Phylogeography of an Australian termite, *Amitermes laurensis* (Isoptera, Termitidae), with special reference to the variety of mound shapes. *Mol Phylogenet Evol* **42**: 236–247.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Rowell D (1985) Complex sex-linked fusion heterozigosity in the Australian huntsman spider Delena cancerides (Aranea: Sparassidae). *Chromosoma* **93**: 169–176.

Rowell DM (1986) Complex sex-linked translocation heterozygosity and its role in the evolution of social behaviour. *Can J Genet Cytol* **28**: 168–170.

Santos O, Luykx P (1985) Holozigosity for sex-linked genes in males of the termite *Incisitermes schwarzi*. *Biochem Genet* **23**: 729–739.

Schneeweiss GM, Palomeque T, Colwell AE, Weiss-Shneeweiss H (2004) Chromosome numbers and karyotype evolution in holoparasitic Orobanche (Orobanchaceae) and related genera. *Am J Bot* **91**: 439–448.

Shimodaira H, Hasegawa M (1999) Multiple comparison of log-likelihoods with application to phylogenetics inference. *Mol Biol Evol* **16**: 1114–1116.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial genes sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am* **87**: 651–701.

Swofford DL (2001) *PAUP*-Phylogenetics Analysis Using Parsimony (* and Other Methods), Ver. 4b*. Sunderland, Massachusetts: Sinauer Associates.

Syren RM, Luykx P (1977) Permanent segmental interchange complex in the termite *Incisitermes schwarzi*. *Nature* **266**: 167–168.

Syren RM, Luykx P (1981) Geographic variation of sex-linked translocation heterozigosity in the termite *Kalotermes approximates* Snyder (Insecta, Isoptera). *Chromosoma* **82**: 65–88.

Thompson GJ, Miller LR, Lenz M, Crozier RH (2000) Phylogenetic analysis and trait evolution in Australian lineages of drywood termites (Isoptera, Kalotermitidae). *Mol Phylogenet Evol* **17**: 419–429.

Thorne BL, Carpenter JM (1992) Phylogeny of the Dictyoptera. *Syst Entomol* **17**: 253–268.

Thorne BL, Breisch NL, Muscedere ML (2003) Evolution of eusociality and the soldier caste in termites: influence of intraspecific competition and accelerated inheritance. *PNAS* **100**(22): 12808–12813.

Vincke PP, Tilquin P (1978) A sex-linkedring quadrivalent in Termitidae (Isoptera). *Chromosoma* **67**: 151–156.

Watson JAL, Abbey HM (1993) *Atlas of Australian Termites*. Melbourne, Australia: CSIRO Publishing.

Wrigley JM, Graves JAM (1988) Karyotypic conservation in the mammalian order Monotremata (subclass Prototheria). *Chromosoma* **96**: 231–247.

Zompro O (2005) Inter- and intra-ordinal relationships of the Mantophasmatodea, with comments on the phylogeny of polyneopteran orders (Insecta: Polyneoptera). *Mitt Geol-Paläontol Inst Univ Hamburg Heft* **89**: 85–116.