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Geographic Variation of Sex-linked Translocation Heterozygosity in the Termite *Kalotermes approximatus* Snyder (Insecta: Isoptera)

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Abstract. The primitive termite *Kalotermes approximatus* carries a number of reciprocal translocations (segmental interchanges) that are linked to the sex-determining mechanism in such a way that males are permanent structural heterozygotes, forming long chains or rings of chromosomes in meiosis, while females are structural homozygotes, forming only bivalents. A survey of male meiosis from collections covering nearly the whole species range in the southeastern United States reveals considerable variation in the number of translocations: males with a diploid number of 32 or 33 have meiotic chains of 11, 13, 14, 15, 16, and 17 or 19 chromosomes. The different types can be arranged in an evolutionary series of rearrangements involving translocations or Robertsonian fusions between chromosomal elements in the ring and those outside. In addition, the existence of a closed chain (ring) of 16, and of four different types of chain of 13, indicate that similar rearrangements have occurred among chain elements. The geographic pattern of these rearrangements suggests that their selection accompanied the expansion of the species northward from southern Florida sometime since the last glaciation or, alternatively, that as they arose the new translocation types successively supplanted the ancestral types, preferentially in the east-central portion of the range.

Introduction

Translocation heterozygosity as a regular feature of the breeding system is not common. The best known example is in the plant *Oenothera*, where it forms part of a complex system involving self-pollination, lethal genes, reciprocal translocations, and possibly directed segregation (Cleland, 1972). Heterozygous multiple translocations have been described as occasional variants in inbreeding populations of plants and animals (e.g., Darlington and LaCour, 1950; John and Quraishi, 1964), but only in a few instances have multiple translocations come to be maintained permanently in the heterozygous condition by being

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associated with the sex-determining mechanism. Among the most notable examples of this situation are some members of the centipede genus *Otocryptops* (Ogawa, 1954, 1961a, b), the dioecious plant *Viscum* (Barlow et al., 1978), the copepod *Mesocyclops edax* (Chinnappa and Victor, 1979), and several termite species (Syren and Luykx, 1977; Luykx and Syren, 1979; Vincke and Tilquin, 1978).

Kalotermes approximatus is one of several species of the primitive termite family Kalotermitidae in the southeastern United States that have sex-linked reciprocal translocations. About half of the male haploid complement carries translocated chromosome segments; in male meiosis, these chromosomes segregate together as a single linkage group to male-determining sperm, and thus are restricted to males.

The karyotypic survey described in this paper, covering most of the known range of the species, has uncovered ten karyotypic variants of the translocation complex in males. The variants can be arranged in an evolutionary sequence, and their geographic distribution suggests a pathway of migration during their evolution.

Materials and Methods

Whole termite colonies were collected in 1976 and 1977 from dead branches of oak, sweetgum, and magnolia in the locations listed in Table 1. In the northernmost part of the range (Cape Henry, Virginia; see Fig. 1) colonies were often found in wood of live trees. In Florida, colonies were easily located in the north central part of the state, but were not found south of Sarasota and Melbourne. The northwestern and western limits of the range of *K. approximatus* have not been precisely established. An exhaustive search starting at Atlanta, Georgia, and going south turned up just one colony (no. 54) near Tifton. Another thorough search to find the western limit of the range started at Baton Rouge, Louisiana; a single reproductive pair (not prepared for chromosome analysis) was found in Mississippi, and several colonies were found near Pensacola, Florida. In all, chromosome preparations were made from 37 colonies at various locations throughout the known range of this species.

Mitotic and meiotic chromosome spreads were provided by the gonads of alates (imagos) collected when the colonies were opened, or by the gonads of secondary reproductives that developed from immature forms within a few weeks after the colonies were subdivided. The reproductives of either kind are, in most cases, the offspring of a single founding pair; occasionally these primary reproductives themselves were found and used. From each colony separate preparations were obtained from one up to fifteen or twenty individuals.

Chromosome preparations were made by the method of Syren and Luykx (1977), or by the method of Imai et al. (1977) with the following modifications: (1) termites were injected with a solution of Colcemid (0.02–0.05%) 4 to 12 h prior to dissection; (2) gonads were dissected out in a 0.45% sodium citrate solution; and (3) slides with macerated gonads were placed in a Coplin jar containing ethanol:glacial acetic acid 3:1 for 25 min prior to final fixation in glacial acetic acid.

Observations

General

Mitotic cells in the gonads of reproductive individuals from a majority of the colonies investigated had 33 chromosomes in the male and 34 chromosomes

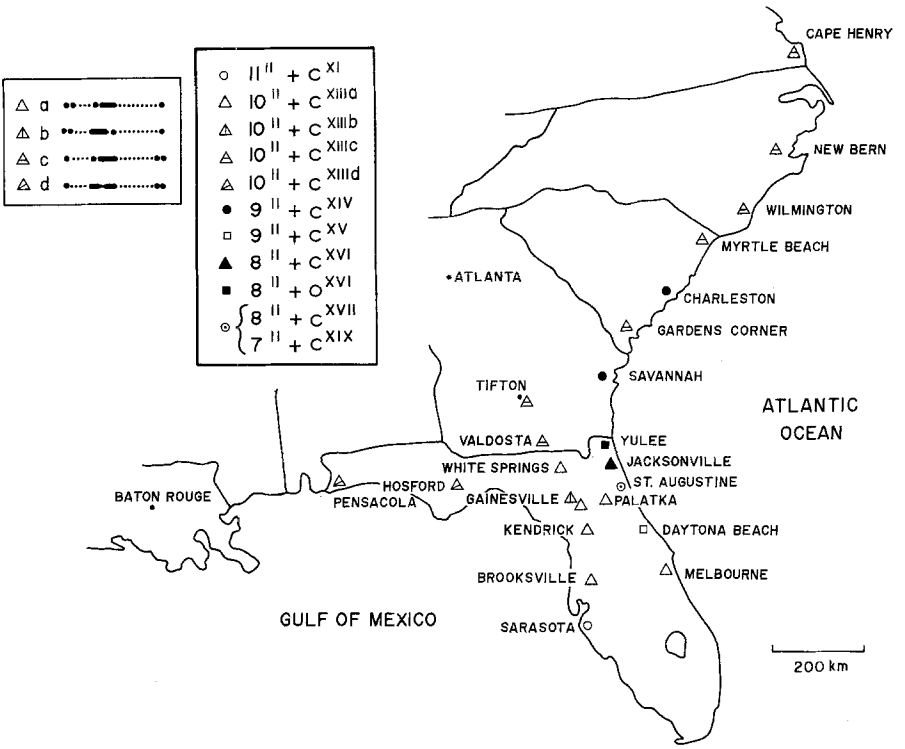


Fig. 1. Geographic distribution of meiotic chromosome arrangements in males of *Kalotermes approximatus*. Open symbols, 2n = 33; closed symbols, 2n = 32. Each symbol represents from one to four colonies examined. See Table 1 and text for details

in the female. Some colonies from northern Florida, Georgia, and South Caroline, however, had 32 chromosomes in both male and female mitosis (solid symbols in Fig. 1). Male meiotic cells in colonies from different geographic locations had from 8 to 11 bivalents; the rest of the chromosomes were associated in rings or chains from 11 to 17 chromosomes in length. Occasional cells (Fig. 5a) show a zig-zag arrangement of chromosomes at metaphase I, indicating that in the first meiotic division the chromosomes undergo regular alternate disjunction from the ring or chain. In male meiosis where 2n = 33, nearly all metaphase II cells observed had either 16 or 17 chromosomes; where 2n = 32, metaphase II cells had 16 chromosomes only. Pairing and chiasma formation appear to be normal; in early diplotene, non-terminal chiasmata can sometimes be seen in both the free bivalents and the translocation-ring or -chain, but by late diplotene all chiasmata are terminal.

No observations on female meiosis have been made in *Kalotermes approximatus*. But it can be concluded that temales do not carry the translocated chromosomes (and therefore cannot transmit them to their male offspring), because there was no indication that males *homozygous* for the translocations occur. Every male observed in every colony collected was a translocation heterozygote, carrying a large ring or chain in meiosis. Thus the translocated chromosomes

Table 1. Mitotic and meiotic chromosome numbers in *Kalotermes approximatus*. Male mitotic numbers in parentheses have not been observed directly, but are inferred from meiotic figures. O=ring multivalent; C=chain multivalent. The letters [a], [b], [c], [d] designate the different types of chain of thirteen (see text)

Location	Colony no.	Males		Females
		Meiosis	Mitosis	Mitosis
Florida				
Sarasota	33		33	34
Sarasota	36	$11^{II}+C^{XI}$	33	34
Sarasota	181	$11^{II}+C^{XI}$	(33)	
Sarasota	186	$11^{II}+C^{XI}$	(33)	
Melbourne	49	$10^{II}+C^{XIIIa}$	33	34
Melbourne	59	$10^{II}+C^{XIIIa}$	(33)	
Brooksville	42	$10^{II}+C^{XIIIa}$	33	34
Daytona Beach	50	$9^{II}+C^{XV}$	33	
Kendrick	47	$10^{II}+C^{XIIIa}$	33	34
Kendrick	48	$10^{II}+C^{XIII\,a}$	33	34
Gainesville	37	$10^{II}+C^{XIIIa}$	33	34
Gainesville	39	$10^{II}+C^{XIIIa}$	(33)	34
Gainesville	45	$10^{II}+C^{XIIIb}$	(33)	
Gainesville	46	$10^{II}+C^{XIIIb}$	33	34
Gainesville	125	$10^{II}+C^{XIIIa}$	33	
Palatka	140	$10^{II}+C^{XIIIa}$	(33)	
St. Augustine	58	$8^{II}+C^{XVII}$, $7^{II}+C^{XIX}$	33	34
St. Augustine	143	$8^{II}+C^{XVII}$, $7^{II}+C^{XIX}$	33	34
Jacksonville	52	$8^{II}+C^{XVI}$	32	32
Yulee	21	$8^{II}+O^{XVI}$	32	32
Yulee	53	$8^{II}+O^{XVI}$	32	
White Springs	57	$10^{II}+C^{XIIIa}$	(33)	
Hosford	132	$10^{II}+C^{XIIId}$	33	
Pensacola	129	$10^{II}+C^{XIIId}$	(33)	
Georgia				
Valdosta	56	$10^{II}+C^{XIIIc}$	33	
Tifton	54	$10^{II}+C^{XIIIc}$	33	34
Savannah	171	$9^{II}+C^{XIV}$	(32)	
South Carolina				
Gardens Corner	169	$10^{II}+C^{XIIIc}$	(33)	
Charleston	167	$9^{II}+C^{XIV}$	32	
Myrtle Beach	165	$10^{II}+C^{XIIIc}$	33	
Myrtle Beach	166	$10^{II}+C^{XIIIc}$	33	
North Carolina				
Wilmington	163	$10^{II}+C^{XIIIc}$	33	34
New Bern	160	$10^{II}+C^{XIIIc}$	(33)	
New Bern	161	$10^{II}+C^{XIIIc}$	33	
New Bern	162	$10^{II}+C^{XIIIc}$	33	
Virginia				
Cape Henry	155	$10^{II}+C^{XIIIc}$	(33)	
Cape Henry	156	$10^{II}+C^{XIIIc}$	33	

appear to be restricted to males, and are transmitted only to male offspring, as a group of multiple "Y-chromosomes." This conclusion is in accord with observations on meiosis in both sexes in the related termite *Incisitermes schwarzi* (Syren and Luykx, 1977; Luykx and Syren, 1979); there males have large translocation rings in meiosis, while females have only bivalents.

The karyotypes summarized in Table 1 are described in more detail below.

Chain of 11, with 11 Bivalents

This meiotic chromosome arrangement, the smallest chain that has been found in this species so far, occurs in the vicinity of Sarasota, Florida.

The diploid mitotic chromosome numbers are 33 for males and 34 for females. The largest chromosome in the male karyotype is a single submetacentric chromosome with a secondary constriction in the middle of the long arm (Fig. 2). This chromosome is not found in the female karyotype. Male meiotic cells have 11 bivalents and a chain of 11 chromosomes (Fig. 3). Among the free bivalents there are two large metacentric pairs; all of the other large metacentries are incorporated into the chain. The large distinctive submetacentric is located in position no. 4 (counting from the nearest end) in the chain of 11. It is oriented so that its short arm faces the near end of the chain. At this end is a small metacentric chromosome (position no. 1); at the opposite end (position no. 11) is a small chromosome with no visible constriction, possibly one of the small acrocentrics. From the number of chromosomes in the chain, and from the position of the large submetacentric chromosome, it can be concluded that the chromosomes at positions 1, 3, 5, 7, 9, and 11 in the chain are segregated to a 17-chromosome, female-determining sperm, while the chromosomes at positions 2, 4, 6, 8, and 10 are segregated to a 16-chromosome, male-determining sperm.

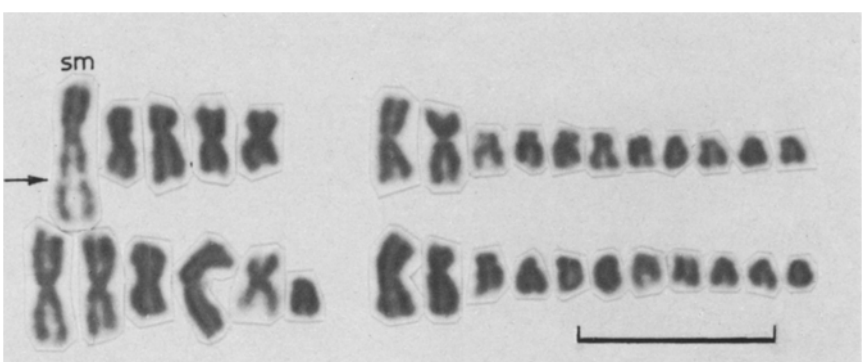


Fig. 2. Mitotic chromosomes of male, colony 186, Sarasota, Fla. The left-hand part of the figure contains the 11 chromosomes that are presumed to make up the meiotic translocation chain: the distinctive large submetacentric (*sm*) with a secondary constriction (*arrow*), all but four of the large metacentrics, one small metacentric and one small acrocentric chromosome. *Top row*, paternal set; *bottom row*, maternal set. Bar = 10 μm in this figure and Figures 3–10

No differences have been found among the four colonies collected from this area.

Chain of 13, with 10 Bivalents

Mitotic cells have 33 chromosomes in the male, 34 chromosomes in the female. With the exception of the colonies collected at Hosford and Pensacola, a single large submetacentric is evident in the male karyotypes (Fig. 4), and, as in the chain of 11 described in the previous section, this chromosome occupies position no. 4 from one end of the chain in male meiosis. (In the colonies from Hosford and Pensacola the largest chromosome is a metacentric; two large subtelocentrics also occur in the chromosome set of the male.) In all the colonies with a chain of 13 and 10 free bivalents, among the latter is a single large bivalent composed of metacentrics; all the other large metacentrics and submetacentrics are incorporated into the chain.

Although 13 is the most common and widespread number of chromosomes in the translocation-chain in male meiosis, the chromosome arrangements that fall into this category do not form a homogeneous group. At least four different types of chain of 13 can be discerned in colonies from different geographic locales. The different types vary most conspicuously in the kind of chromosomes found at the ends of the chain, and in the position and orientation of the large submetacentric or subtelocentric chromosome. The different types are described below, illustrated in Fig. 5, and summarized in the small insert in Fig. 1.

Type a. (Fig. 5a). (Brooksville, Melbourne, Kendrick, Gainesville, Palatka, White Springs (Florida)). The large submetacentric occupies position no. 4 from one end of the chain, with its short arm facing the near end of the chain. At this end (no. 1) is a small metacentric. At the opposite end (no. 13) is

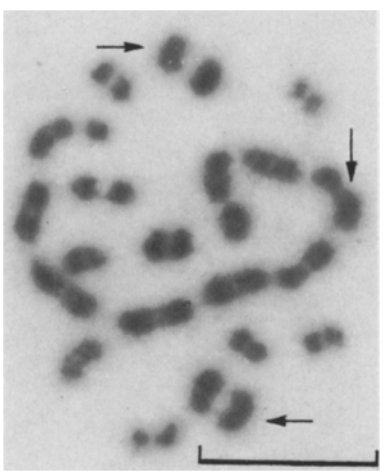


Fig. 3. Meiotic chromosomes of male, colony 186, Sarasota, Fla., 11 bivalents and chain of 11 chromosomes. *Large arrow*, submetacentric (*sm*) chromosome. *Small arrows*, metacentric bivalents

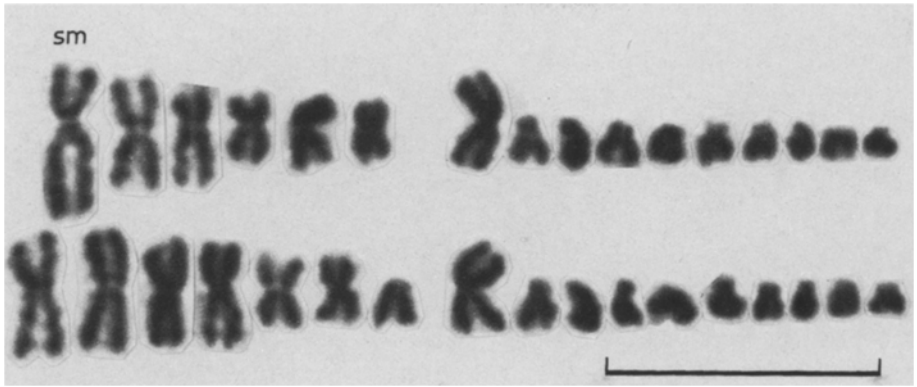


Fig. 4. Mitotic chromosomes of male, colony 163, Wilmington, N. C. The left-hand group contains the chromosomes presumed to make up the meiotic chain of 13 (type c); *sm*, the distinctive large submetacentric, restricted to males

a very small chromosome with no obvious constriction, probably an acrocentric. (In colony 140 from Palatka, however, this chromosome looks like a tiny metacentric in some cells. It is not certain whether this is really an additional variant type, or is due to unusually clear chromosome preparations in material from this colony.)

Of the five colonies collected in the vicinity of Gainesville, three (nos. 37, 39, and 125) had this chromosome arrangement; the other two (nos. 45 and 46) had an arrangement described as type b below.

Type b. (Fig. 5b). (Gainesville, Florida, colony nos. 45 and 46). The chromosome arrangement found in males of these two colonies was the same as in type a, except that the orientation of the large submetacentric in position no. 4 appears to be reversed: the short arm faces the far end of the chain. This type of chain of 13 was found only in these two colonies and nowhere else.

Type c. (Fig. 5c). (Valdosta, Tifton (Georgia); Garden's Corner, Myrtle Beach (South Carolina); Wilmington, New Bern (North Carolina); Cape Henry (Virginia)). As with type a, the large submetacentric is in position no. 4, with its short arm facing no. 1. In this type, however, the positions of the small chromosomes at the ends of the chain appear to be reversed: no. 1 is a small acrocentric, and no. 13 is a small metacentric.

Type d. (Fig. 5d). (Hosford, Pensacola (Florida)). Chains of 13 from these locations appear to differ considerably from the other types, and are difficult to homologize precisely with them. If the smallest chromosome, at one end of the chain, is designated no. 1, then no. 4, as in the other types, is the largest chromosome in the chain, but is a metacentric. Large subtelocentrics (arrows, Fig. 5d) occur at positions 8 and 10, with their short arms facing each other across no. 9.

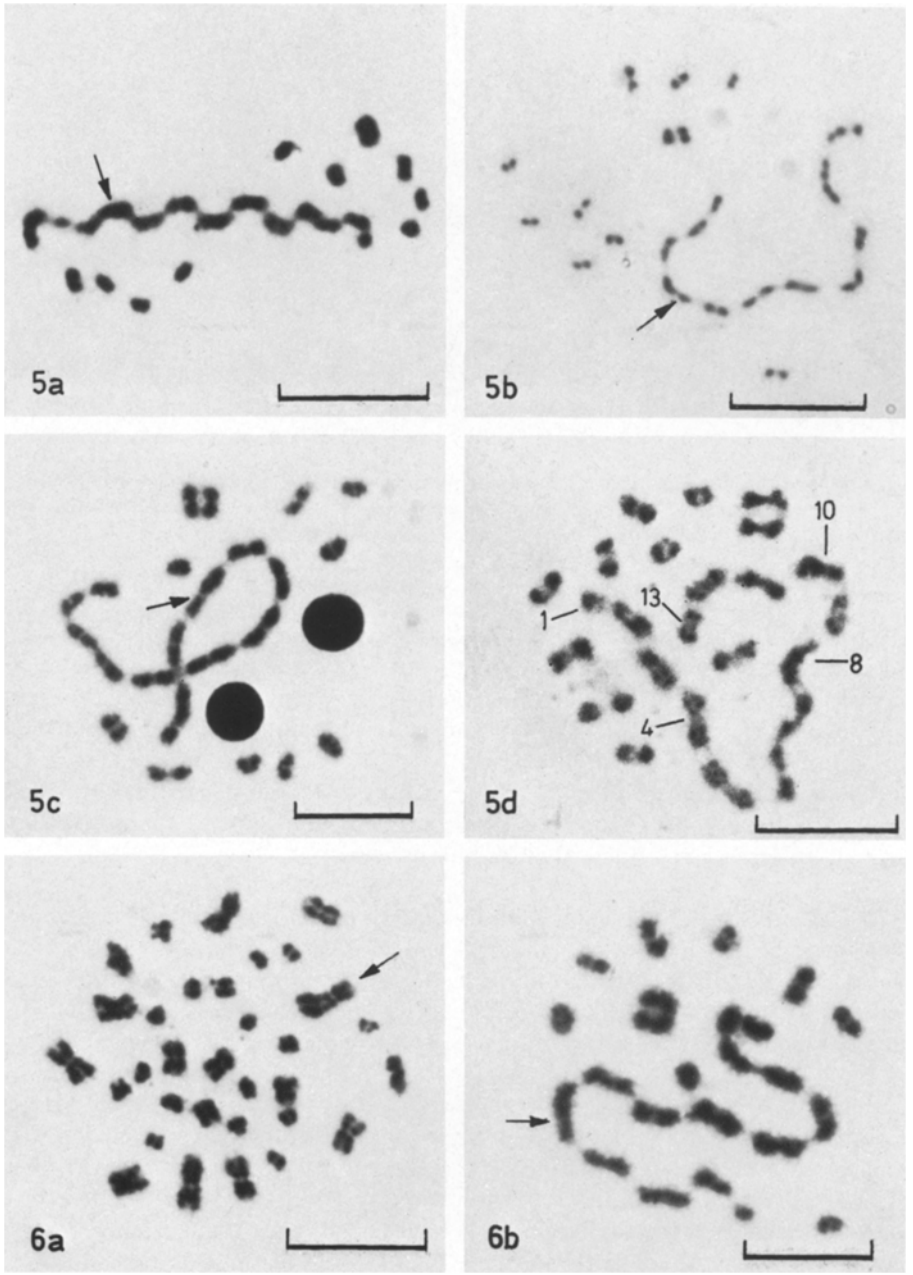


Fig. 5. Male meiotic chromosomes, 10 bivalents and chain of 13. Note the single large free bivalent in each case. *Arrows,* the large submetacentric or telocentric chromosomes. See text for further description. **a** Type a, Kendrick, Florida (colony 48) (from Luykx and Syren, 1979, Fig. 7a); **b** type b, Gainesville, Florida (colony 46); **c** type c, Myrtle Beach, S. C. (colony 166); **d** type d, Hosford, Florida (colony 132); unlike the other chains of 13, this one has a metacentric, not a submetacentric, at position no. 4

Fig. 6. Chromosomes of male, colony 167, Charleston, South Carolina. **a** Mitosis; **b** meiosis. *Arrow,* large submetacentric chromosome in position no. 5 from one end of the chain of 14

Chain of 14, with 9 Bivalents

The mitotic chromosome number is 32 for both males and females. As in the chromosome arrangements described above, the largest element of the male karyotype is a distinctive submetacentric (Fig. 6a). In male meiosis (Fig. 6b) this chromosome is in the 5th position from one end of the chain, with its short arm facing that end. At that end (position no. 1) is a very small chromosome, probably an acrocentric, while at the opposite end (position no. 14) is a small submetacentric. The chain is thus most closely related to the type c variant of the chain of 13, which occurs in the same geographic area (Georgia and South Carolina). Just as in the $10^{II}+C^{XIII}$ type of meiosis, the $9^{II}+C^{XIV}$ type has one large free bivalent composed of metacentric chromosomes.

There are no obvious differences between the chains from Savannah and those from Charleston.

Chain of 15, with 9 Bivalents

Mitotic cells from males have 33 chromosomes, the largest one being a submetacentric. In male meiosis (Fig. 7), this chromosome is probably located at position no. 4 (counting from the end of the chain with the small metacentric), with its short arm facing the near end of the chain. At the opposite end of the chain there is a small acrocentric chromosome. There is no large free bivalent.

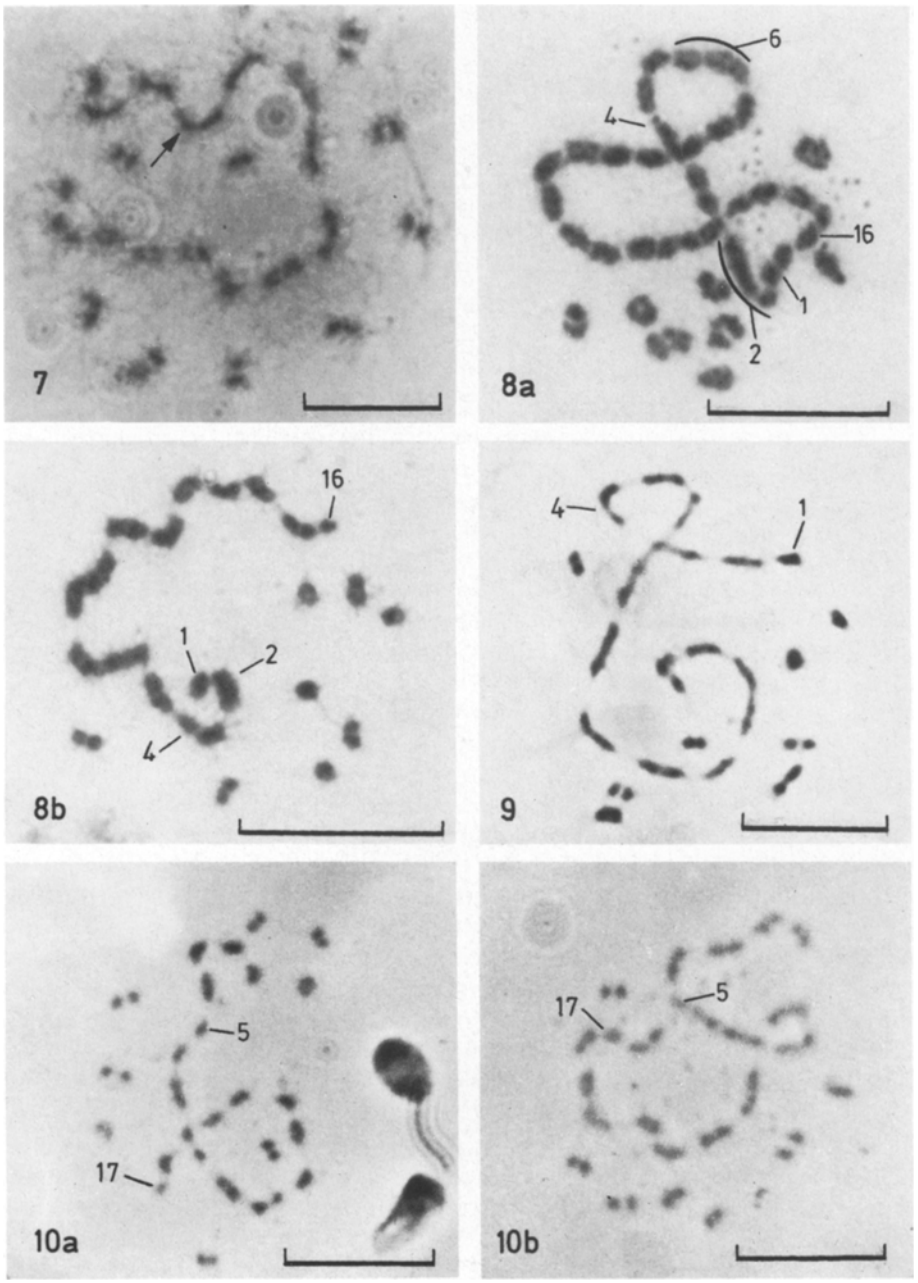
Ring of 16, with 8 Bivalents

Two colonies collected from Yulee, Florida (nos. 21 and 53), are the only ones found with males that regularly form a ring, rather than a chain, in meiosis. In these colonies the diploid mitotic chromosome number is 32 for both males and females; the male complement has two large subtelocentric chromosomes, the largest ones in the diploid chromosome set. In meiosis, one of these subtelocentrics is easily recognized by its position (designated position no. 2) in the ring (see Fig. 8a): the *alternate* positions on either side of this subtelocentric are occupied by the two smallest chromosomes in the ring. The small chromosome (at position no. 4) that is closest to the long arm of this subtelocentric is slightly larger than the other small chromosome. In this numbering system, the other large subtelocentric is located at position no. 6 in the ring. Since the diploid chromosome number is the same in males and females, it cannot be determined whether these subtelocentrics and the two smallest chromosomes in the ring (all of which segregate together in meiosis I) are equivalent to Xs or Ys, without more careful analysis of the male and female karyotypes.

In about 40% of the spermatocytes, the chiasma between the smallest chromosome (no. 16) and the adjacent chromosome (no. 1) has failed, so that a chain of chromosomes, rather than a ring, is formed in these cells (Fig. 8b).

Chain of 16, with 8 Bivalents

This type of meiotic chromosome arrangement has been found only in one colony, no. 52, collected from Jacksonville, Florida. The chromosome at one



Figs. 7–9. Fig. 7. Chromosomes of male, colony 50, Daytona Beach, Florida. Chain of 15 chromosomes, with 9 bivalents. *Arrow,* large submetacentric chromosome. **Fig. 8a and b.** Chromosomes in male meiosis, colony 21, Yulee, Florida. **a** Ring of 16 chromosomes, with 8 bivalents; chromosomes are numbered for ease of description (see text); **b** chain of 16 chromosomes, probably due to failure of chiasma between chromosomes at positions 1 and 16. **Fig. 9.** Male meiosis, colony 52, Jacksonville, Florida. Chain of 16 chromosomes, with 8 bivalents. Chromosomes are arbitrarily numbered according to the description in the text

Fig. 10a and b. Male meiosis, colony 58, St. Augustine, Florida. **a** Chain of 17 chromosomes, with 8 bivalents, seen in 85–90% of cells; **b** chain of 19 chromosomes, with 7 bivalents, seen in 10–15% of cells. Numbering according to description in text

end of the chain (arbitrarily designated no. 1) is a medium-sized metacentric; the chromosome at the opposite end (no. 16) is the smallest in the chain. Chromosome no. 4 in the chain is the largest, a distinctive submetacentric, with its short arm facing the nearest end of the chain (Fig. 9). In 1 to 2 percent of the cells, the translocation complex takes the form of a ring, but the very low frequency suggests that this may be due to no more than the fortuitous positioning of the ends of the chain during squashing.

It is clear that this chain of 16 represents a chromosome arrangement quite distinct from the ring of sixteen described in the previous section, and is not simply a chain resulting from chiasma failure in that ring. The largest and the smallest chromosome in the two types are not in homologous positions. For example, in the ring described in the previous section, the largest chromosome (at position no. 2) is flanked (at positions no. 4 and no. 16) by two very small chromosomes – while in the chain described in this section there is only one very small chromosome, and it is located in a position further removed from the largest chromosome.

Chain of 17, with 8 Bivalents

The male meiotic configuration of 8 bivalents and a chain of 17 chromosomes has been found in two colonies, nos. 58 and 143, collected from St. Augustine, Florida. Chromosome numbers from mitotic cells are 33 for males, and probably 34 for females (based on counts from a few cells). Unlike most of the other male karyotypes, this one has no distinctive large submetacentric or subtelocentric chromosome. However, the chain of 17 (Fig. 10a) does have two distinctive chromosomes, a very small metacentric chromosome at position no. 5, and a very small chromosome at position no. 17 (at one end of the chain). (Since these chromosomes are at odd-numbered positions in the chain, they must be segregated to sperm carrying a total of 17 chromosomes; i.e., to female-determining sperm.)

In about 10–15% of the cells from individual males, one of the "free" bivalents is associated with the chain at position no. 17. This is a frequency higher than one would expect by chance positioning of one of the bivalents during squashing. Each male therefore appears to have a mixture of two kinds of metaphase I spermatocytes, a majority with 8 bivalents and a chain of 17, and some with 7 bivalents and a chain of 19 (compare Fig. 10a and 10b). In either case, regular alternate segregation from the chain results in secondary spermatocytes with either 16 or 17 chromosomes.

The association of an extra pair of chromosomes with the chain of 17 in some cells may be the result of a small translocation between the short arm of no. 17 in the chain and the short arm of an acrocentric autosome; chiasmata might form only infrequently between the two homologous short arms, thus giving a high percentage of cells with only 17 chromosomes in the chain. Alternatively, chromosome no. 17 might be prone to some kind of non-chiasmate association with an autosomal pair. For the sake of discussion, these two colonies will be considered as having a chain of 17 chromosomes (which, in any case, is likely to have been an intermediate stage in the evolution of a chain of 19).

Discussion

The two most important findings of this cytogenetic study on a lower termite, *Kalotermes approximatus,* are that (i) in all 37 colonies investigated, covering most of the known range of the species, all the males are heterozygous for an extensive series of translocations, involving up to half the haploid chromosome set; and (ii) there is considerable geographic variation in the translocations, in a pattern that suggests a progressive evolutionary series.

Translocation Heterozygosity in Males

Since in this survey meiosis in only the male of the species was studied, how can it be asserted that females do not carry heterozygous translocations? (Observations on mitotic chromosome spreads from the two sexes in this species are usually not very informative; most of the chromosomes in the diploid set are not sufficiently different in size or centromere position to allow one to detect translocations there.) If females were also translocation heterozygotes, one would expect their male offspring to differ among themselves in their translocated chromosomes. But this was never found. Without exception the male alates or male secondary reproductives from a single colony all showed the same meiotic chromosome configuration, with the same number of chromosomes in the multivalent ring or chain. It therefore seems likely that only the males are translocation heterozygotes.

In being restricted to males, the translocated chromosomes are equivalent to a series of multiple Y chromosomes (Fig. 11). It does not seem likely, however, that all of the translocated chromosomes (and the "standard" set with which they are associated in male meiosis, equivalent to X chromosomes) are acting as sex chromosomes in a functional sense. The observations on male meiosis in colonies no. 58 and 143 (from St. Augustine, Florida) bear on this question. Males from these colonies have mixtures of two kinds of spermatocyte: a majority of cells with 8 bivalents and a chain of 17, and a significant minority (10–15%) with 7 bivalents and a chain of 19. In the latter, the association of two chromosomes with the end of the chain would be expected to direct their segregation at anaphase I in a precise manner – the chromosome at the end of the chain is segregated to a female-determining sperm cell, the adjacent chromosome to a male-determining sperm cell (assuming of course that the association is due to homologous pairing with an autosome carrying a small translocated segment). But in the former type (8 bivalents and a chain of 17) these two chromosomes comprise one of the free bivalents, and so presumably are distributed at random to the two kinds of sperm cell. Since neither of these chromosomes would be regularly distributed to offspring of one sex or the other, they could not contain important sex-determining genes or sex-restricted alleles. Therefore, even when they occur as part of the translocation chain, they are best regarded as autosomes, in a functional sense, rather than as sex-determining chromosomes. This is probably also true for most of the chromosomes in the translocation complex, since it seems unlikely that many of them carry true

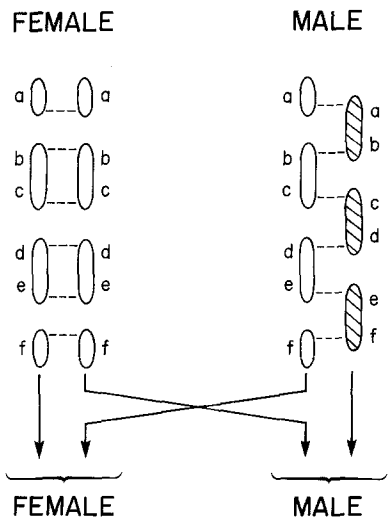


Fig. 11. Diagram showing behavior of translocated chromosomes in male meiosis and in fertilization as a series of Y chromosomes (*shaded*); letters indicate homologous segments, linked by chiasmata

sex-determining genes, in view of the large number of chromosomes involved and the variability of the translocations from different locales. Nevertheless, for convenience in the discussion that follows, they will be referred to as X and Y chromosomes because their segregation in meiosis follows that of the true sex-determining (and as yet unidentified) X and Y chromosomes, and because the general consequences of translocations between autosomes and any of the different Xs – or any of the different Ys – are the same.

Karyotype Evolution

The principal chromosomal arrangements found in male meiosis are summarized diagrammatically in Figure 12. Two kinds of translocation are apparent:

(i) Robertsonian translocation between acrocentrics (centromere fusion). The mixture of acrocentrics and metacentrics in mitotic karyotypes suggests that Robertsonian fusions have occurred frequently in the evolution of this species. Other members of the family Kalotermitidae also show the same pattern (Luykx and Syren, 1979). In addition, some of the differences in the translocation complexes from different geographic locales are best explained by assuming that Robertsonian translocations have occurred, as described below.

(ii) Reciprocal translocation involving metacentrics. As described below, the number of chromosomes involved in the translocation complex of males can increase by means of a translocation between a chromosome in the complex and a member of one of the autosomal pairs. Translocations *between* members of the complex lead to rearrangements within the complex, without an increase in the number of chromosomes in the complex. Comparison of the different meiotic configurations reveals that both of these kinds of translocation have occurred. They have sometimes been between two metacentrics, as well as be-

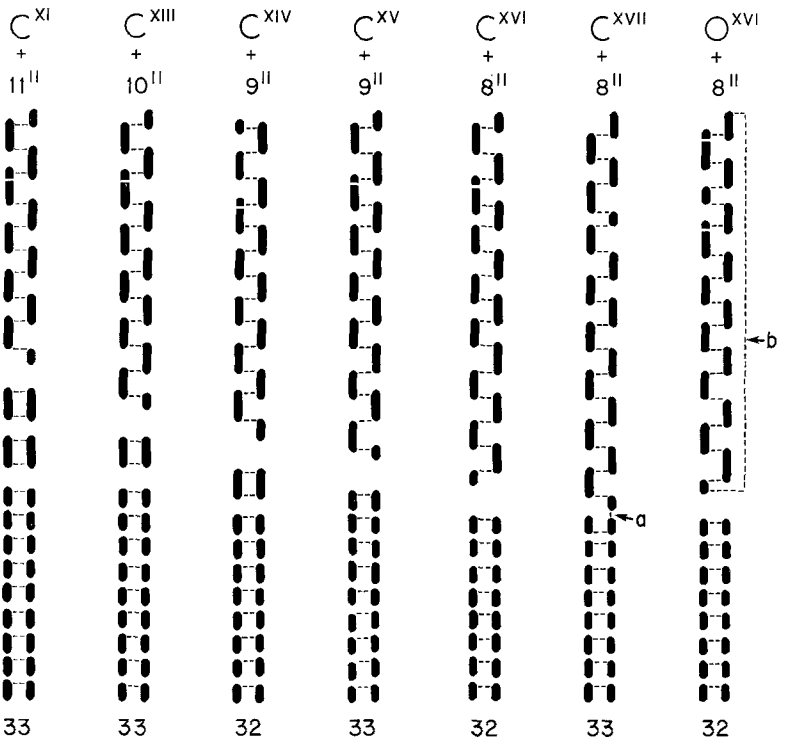


Fig. 12. Diagram of male meiotic chromosome complements in *Kalotermes approximatus*. Chromosomes of sex-lined translocation complex in zig-zag arrangement at top of each karyotype, free bivalents at bottom. *Dotted lines* represent chiasmata. In each translocation complex, Y-chromosomes are on the left side of the zig-zag chain, X-chromosomes on the right; *a* in 10–15% of the cells with a C^{XVII}, there is an association between the chain and an additional pair of chromosomes; *b* in 60% of the cells there is an association between the two end-elements in the chain, making it a ring

tween a metacentric and an acrocentric. Since the sizes of two paired chromosome arms in the translocation chain are often unequal (e.g., Fig. 5c), the exchanges must have sometimes involved only segments of chromosome arms, rather than whole arms.

In the discussion that follows, translocations between acrocentrics, with the subsequent loss of one centromere and a small amount of adjacent chromosomal material, is considered equivalent to centromere fusion. The term "metacentric" will usually be meant to include submetacentrics and subtelocentrics as well; i.e., any bi-armed chromosome both of whose arms regularly participate in meiotic pairing and chiasma formation.

The outcome of translocations between one of the sex chromosomes and one member of an autosomal pair is summarized in Figure 13. The consequences of such translocations vary depending on the combination of metacentrics and acrocentries involved, but in most cases lead to an increase in the number of sex chromosomes by one or two. Translocations with an autosomal *metacentric* always lead to an increase in the number of sex-chromosomes by two,

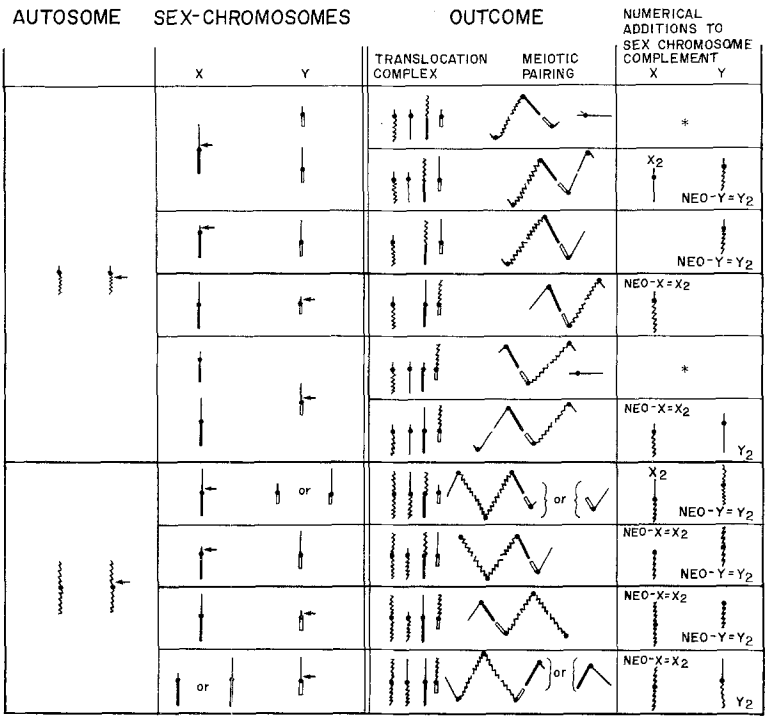


Fig. 13. Consequences of translocation between a sex-chromosome and an autosome. *Small arrows* indicate break-points. The outcome of translocations between an autosome and an X- or a Y-chromosome depends on the acrocentric or metacentric nature of the chromosomes involved. The end result in each case, addition of a neo-X, a neo-Y, or both, to the sex-chromosome complement, is shown in the right-hand column. Where a metacentric sex-chromosome is involved in a translocation with an acrocentric autosome, the normal pattern of segregation of sex-chromosome segments is disrupted; such translocations would be selected against (*asterisks*)

regardless of the nature of the original sex-chromosomes involved. Translocations between an acrocentric autosome and an acrocentric Y will increase the number of X-chromosomes by one, while translocations between an acrocentric autosome and an acrocentrix X will increase the number of Y-chromosomes by one. Translocations between an acrocentric autosome and a *metacentric* sex-chromosome (either X or Y) usually break up the original pattern of segregation of X-and Y-chromosomes, and so presumably would be selected against. (Only in the special case where all Xs and Ys are bi-armed and all arms are paired – i.e., in ring bivalents or ring multivalents – will the translocation complex survive as a unit, transformed to an open chain with an additional acrocentric at each end. Because only one of the ten different multivalents found in *K. approximatus* is a ring, and no chains having two chromosomes more than this ring have been found, this special situation probably has not played a role in the recent evolution of the different karyotypes described here.)

Some members of the family Kalotermitidae have karyotypes consisting almost exclusively of acrocentrics (Luykx and Syren, 1979); this is probably

the original ancestral condition. Sex multivalents composed primarily of metacentrics can arise by repeated Robertsonian fusions between gonosomal and autosomal acrocentrics; but they can also be built up by fusion between autosomal acrocentrics first and then incorporation of the resulting metacentrics into the sex multivalent by means of translocations with one of the sex chromosomes. This is evidently what occurred in the evolution of the C^{XI} karyotype (with two autosomal metacentric pairs) to the C^{XIII} karyotype (with one autosomal metacentric pair) to the C^{XV} karyotype (with no autosomal metacentrics).

Many of the karyotypic variations – both those involving an exchange between a sex chromosome and an autosome, and the exchanges involving two sex chromosomes, described below – could have come about by means of translocations involving either X-chromosomes or Y-chromosomes. A translocation, in a male, between an autosome and a *Y-chromosome* will immediately give, in the next generation, male progeny with an altered sex-chromosome translocation complex; a new stable male chromosome configuration will arise without any change in the chromosome constitution of females. On the other hand, if a translocation between an autosome and an *X-chromosome* occurs, in either a male or a female, then translocation-heterozygous females will appear in the next generation, and a stable cytogenetic system will be established only after female homozygotes are selected. In such an event, both sexes carry the new translocation; males are translocation-heterozygotes, and females are translocation-homozygotes.

In most cases it is not possible to decide which kind of sex-chromosome translocation has actually occurred. In a few instances, however, the evidence does favor one particular evolutionary pathway.

A translocation between an acrocentric *X-chromosome* and an acrocentric autosome is required to change an odd-numbered chain in this system to the next higher even-numbered chain; i.e., an X_1X_2Y type to an $X_1X_2Y_1Y_2$ type. This has apparently occurred twice, once in the evolution of a C^{XIII} to a C^{XIV} karyotype, and again in the evolution of a C^{XV} to a C^{XVI} karyotype. (In this second case, careful comparison of chromosome morphology in the different chains makes the alternative – the evolution of the C^{XVI} type from the C^{XIV} type – less likely.) In both cases, to generate an additional Y-chromosome to form an even-numbered chain, a Robertsonian translocation between an acrocentric autosome and the terminal acrocentric X in the original chain must have occurred.

It is probable that translocations between *Y-chromosomes* and the autosomes have also played a part in the evolution of these karyotypes, although the evidence here is not as strong. In an ancestor with XX females and XY males, a Y-autosome translocation is required to generate an odd-numbered chain with more Xs than Ys (e.g., $X_1X_1X_2X_2$ females and X_1X_2Y males), the most common kind of chain found in this survey. Such multivalents might also have originated, however, by an X-autosome translocation in an XO-male system (White, 1973). The XO-male sex-determining mechanism is characteristic of the roaches (White, 1976), considered to be ancestral to the termites. But the C^{XVII} multivalent ($X_1X_2X_3X_4X_5X_6X_7X_8X_9Y_1Y_2Y_3Y_4Y_5Y_6Y_7Y_8$) probably originated from the C^{XVI} ($X_1X_2X_3X_4X_5X_6X_7X_8Y_1Y_2Y_3Y_4Y_5Y_6Y_7Y_8$), and this would require a Robertsonian translocation between an acrocentric auto-

some and a terminal acrocentric Y. (It is less likely that the C^{XVII} was derived from a C^{XV} directly, because the latter karyotype does not contain the necessary metacentric autosome.)

Other examples in which both X-autosome and Y-autosome translocations have occurred in the same evolutionary line are provided by the studies of Matthey (1965) on different subspecies of the mouse *Mus minutoides,* of Martin and Hayman (1966) on the marsupial hare-wallaby *Lagorchestes conspicillatus* and its near relatives, and of White et al. (1973) on morabine grasshoppers.

Translocations between two members of a sex-chromosome chain have also occurred. Two kinds are theoretically possible – those in which the translocation break-points are symmetrically located with regard to the centromeres of the elements in the chain, and those in which the break-points are asymmetrically located. All of the latter, with one exception, lead to a dissociation of the chain into two independent multivalents, and so could not be the basis of a stable sex-multivalent system. The one exception is the case where the translocation involves two end-elements whose unpaired arms might be lost without deleterious effects. This is equivalent to a Robertsonian fusion of the two end-chromosomes, and leads to a ring having one chromosome fewer than the ancestral chain (Fig. 14, A). Such an event has apparently occurred in the evolution of a ring of 16 (in the colonies from Yulee, Florida) from an ancestral chain of 17. A similar occurrence has been described by Craddock (1975) in the evolution of a secondary X-Y ring bivalent by fusion of the Y-chromosomes in an XY_1Y_2 system in the stick insect *Didymuria violescens.*

Translocations with *symmetrical* break-points could occur between two Xs or between two Ys. (A translocation between an X and a Y seems less likely, because it would result in a redistribution of some X- and Y-segments, or even whole X- and Y-chromosomes, to the wrong sex – see Fig. 14, D.) Translocations between Xs or between Ys lead to a rearrangement of the elements of the chain, without any change in number. The chain-of-13 variants found in this study could have arisen by either X-X or Y-Y translocations; on the basis of the observations available, it is not possible to decide which.

Symmetrical translocation break-points in any two chromosome arms in a chain give a rearranged chain in which the order of chromosomes between the two break-points is reversed; if the break-points occur at sites an equal number of chromosome-arms on either side of a marker chromosome, such as a submetacentric, then the orientation of the submetacentric will be reversed with respect to the ends of the chain (Fig. 14, B). Considering the chain designated C^{XIIIa} as ancestral to the other chain-of-13 variants – as seems likely from its southern and central geographic location, and its proximity and morphological similarity to the chain of 11 – the C^{XIIIb} variant can be derived from the C^{XIIIa} by a single translocation of this kind. Similarly, a translocation with break-points equidistant from the ends of the chain would reverse the positions of the end chromosomes, with respect to a marker chromosome located between the break-points (Fig. 14, C). The C^{XIIIc} variant undoubtedly arose from the C^{XIIIa} chain in this way.

All the different sex-chromosome configurations found in *K. approximatus* can be explained as steps in a single evolutionary scheme (Fig. 15). At least eleven different translocations, each involving X- or Y-chromosomes, must be

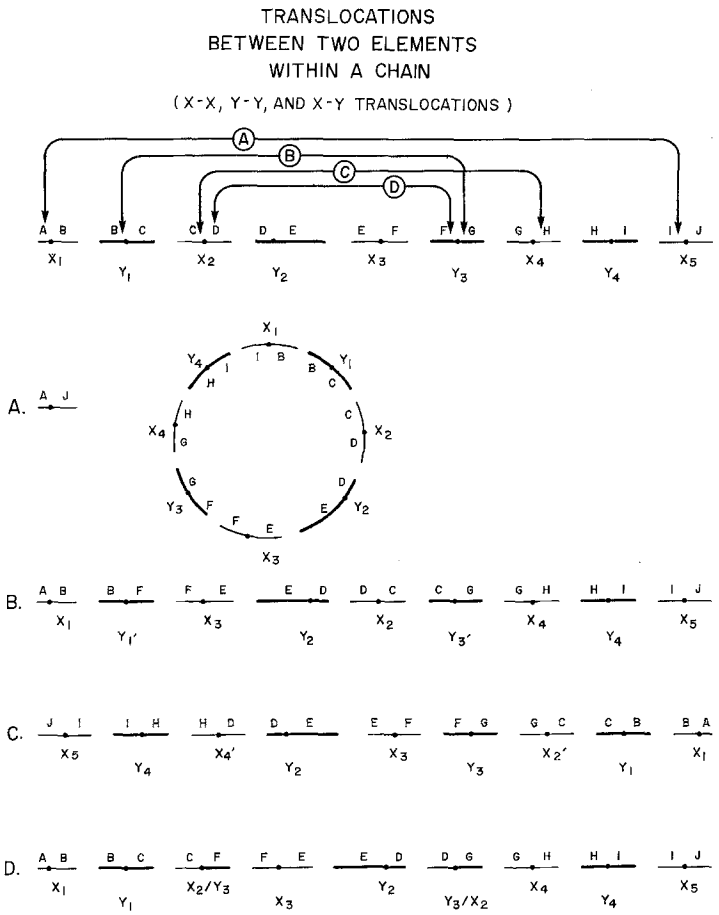


Fig. 14. Consequences of translocations between two sex-chromosomes. Arrowheads indicate break-points. *A*; a translocation between two end-chromosomes transforms the chain to a ring; *B*; two break-points equidistant from the submetacentric chromosome DE reverses polarity of DE; *C*; two break-points equidistant from the ends of the chain reverses the positions of the end-chromosomes; *D*; a translocation between an X and a Y disrupts the normal segregation of Xs and Ys

postulated to account for the different arrangements observed. This evolutionary scheme is the simplest one that accounts for all the observations. Alternative schemes, such as a parallel but separate evolution of odd- and even-numbered chains, or an increase in the number of sex-chromosomes by only one chromosome at each step, cannot be ruled out but seem unlikely on the basis of the available evidence. It is possible, of course, that more structural rearrangements have occurred in the evolution of these karyotypes than are revealed by study of the number and gross morphology of the chromosomes alone. Hidden complexities of this kind have been revealed in other translocation systems by cytological analysis of hybrids between populations; e.g., in the

lobeliaceous plant *Isotoma* (James, 1965) and in the weevil *Pissodes* (Smith, 1970).

Systems of multiple sex-chromosomes of the kind described here occur in many, but not all, species of termites. They are expected to have strong genetic effects: restriction of many alleles to males, maintenance of extensive genic heterozygosity, increased linkage in the male of many genes that in the female assort independently, increased genetic similarity of offspring to the same-sex parent and to same-sex siblings. For these reasons, it seems unlikely that this remarkable cytogenetic system has arisen by genetic drift alone; rather, it appears to be a good example of "karyotypic orthoselection" (White, 1973) within a species; i.e., repeated chromosomal rearrangements of the same kind, successively added on to a basic ancestral karyotype.

The geographic distribution of the translocation complexes is not random. Each of the higher-order complexes is found near the next lower one from which it can be derived, in a majority of cases, by a single translocation (Fig. 15). All the translocation types therefore appear to be related, and undoubtedly had a common origin. The geographic pattern may therefore reflect a migration or flow of the translocations from their point of origin, together with temporal or geographic changes in the pressure of selection leading to the establishment of new translocations. Two general models can be envisioned.

The first is illustrated in Figure 16. One can imagine that each new translocation arose and became established at the periphery of the species range (perhaps because of genetic drift, or selection related to intense inbreeding, in small isolated populations) as the range gradually extended northward from the Sarasota region in southern Florida. This ancestral population around Sarasota has persisted unchanged since its inception, but its descendants spread northward with a new translocation, and then later descendants of those spread westward into the Florida panhandle, eastward to the northeast Florida coast, and then further northward into the southeastern coastal states, as additional translocations occurred. By maintaining higher levels of heterozygosity or by strengthening the linkage of gene complexes adapted to a peripheral environment, each new translocation would confer on its bearers, spreading into a new area, an advantage over the parent population.

The species presently occupies a limited geographic range along the coastal plain of the southeastern United States, coinciding with the relatively warm and moist conditions of the southern mixed forest (beech, sweetgum, magnolia, pine, oak) in the south and the oak-hickory-pine forest along the coast in the northern part of the range. Analysis of pollen types in lake sediments (Whitehead, 1967; Watts, 1971) indicates that the colder, drier climate and the vegetation of the last glaciation were probably unsuitable for this species throughout its present range; it probably therefore spread into the areas it presently occupies sometime within the last 10,000 years, perhaps even within the last 5,000 years. Since other members of the family Kalotermitidae are abundant throughout the Caribbean Islands, southwestern United States, Mexico and Central America (Weesner, 1970), the ancestors of *K. approximatus* probably spread into the present range from the south. The distribution of

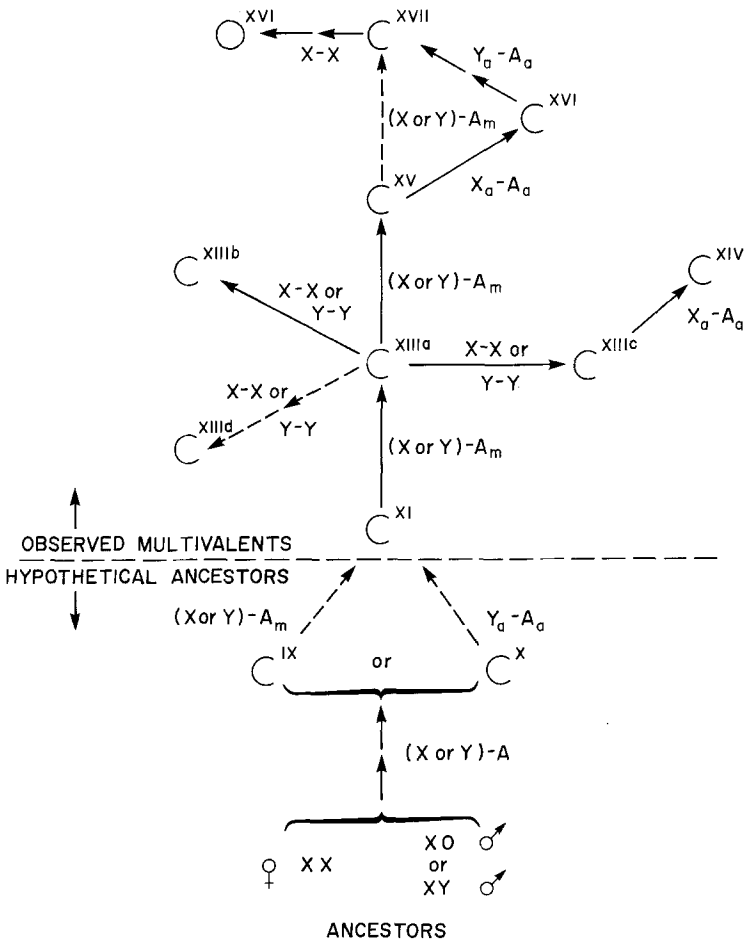


Fig. 15. Evolutionary pathway of sex-chromosome translocations in *Kalotermes approximatus*. The minimum number of translocations is shown. *Two arrows* in tandem indicate that the chromosomes of the ancestral and new types are sufficiently different to require more than a single translocation. *Dashed arrows* indicate uncertain pathways. Different kinds of translocation are designated as follows: X-A, between an X-chromosome and an autosome; X-X, between two X-chromosomes, etc. Subscripts: *a* acrocentric; *m* metacentric or submetacentric

the translocation complexes shown in Figure 16 would be consistent with the extension of the species range northward from southern Florida with the post-glacial warming of the climate. Ancestral types with lower-order translocations that may have existed further south a few thousand years ago are probably extinct.

There are some problems with this view. There is evidence (Clausen et al., 1979) for recent fluctuations in sea level, and perhaps in climate and vegetation also, suggesting that there may not have been a simple northward-moving optimal climatic zone during the spread of this species into its present range. In addition, the species is presently rather sparsely distributed in the Sarasota area; the same is true at the southern end of the range on the Florida east

Fig. 16. Map showing the evolution of sex-chromosome translocation types accompanying extension of the species range

coast (Melbourne), and in the Florida panhandle (Pensacola). Why have lower-order translocation types been preserved in these regions? Given the isolation of these populations, why haven't higher-order translocations evolved? Perhaps the ecological conditions are unfavorable for their establishment, or the numbers of individuals in these areas too small to make the occurrence of the appropriate translocations probable. Another problem is the existence of four different C^{XIII} types. The cytological evidence and the geographical distribution suggest that the C^{XIIIa} translocation complex gave rise to the other types, by means of X-X or Y-Y translocations. Yet such translocations do not bring additional autosomes into sex-linkage, and therefore would not be expected to increase the level of male heterozygosity or the number of sex-linked genes. What selective advantage would these types then offer over the ancestral C^{XIIIa} type? Perhaps some of these translocations have other genetic effects, such as position effects.

A second model, illustrated in Figure 17, is related to the idea of "stasipatric" karyotype differentiation developed by White and his co-workers from their work on morabine grasshoppers in southeastern Australia (White et al., 1977; White, 1972, 1978). In this case and a number of others (summarized by White, 1977, 1978), adjacent populations have been found to differ by one or two, or sometimes by several, chromosome rearrangements. It has been proposed that the species range may not have changed drastically over the time span within which karyotypic subdivision of the species has occurred. Instead, rearrangements

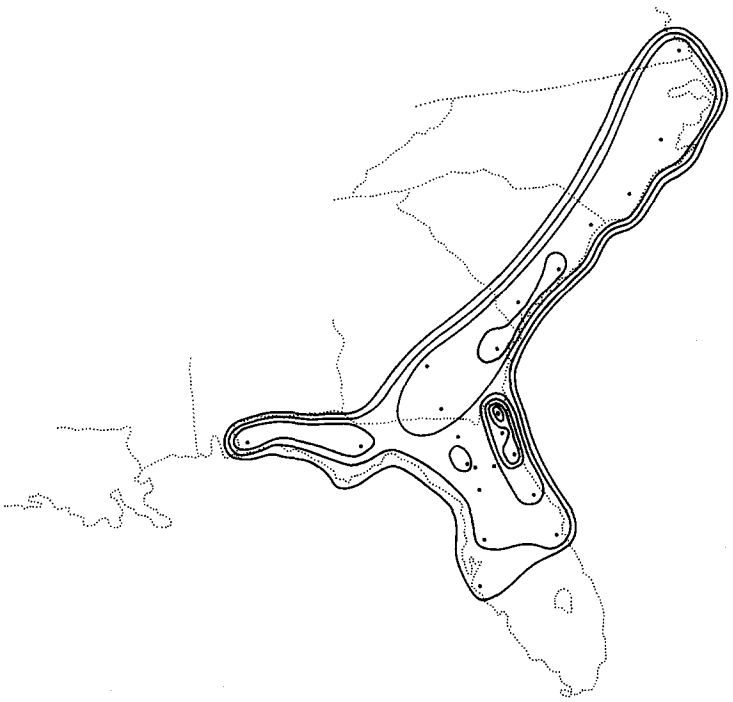


Fig. 17. Model of incipient stasipatric speciation. The map can be viewed as a contour map showing the levels of translocation heterozygosity reached in different regions by the addition of successive translocations on to the more primitive karyotypes. The largest area, covering the present range of *Kalotermes approximatus,* is assumed to have contained the most primitive translocation type found in this study. Additional translocations were added on to each immediately ancestral type, and the new type spread to the boundaries of the sub-areas within the range. Where two boundaries are closely parallel, it is assumed that the newer type has completely supplanted the immediately ancestral type. Dots indicate collection sites

may have arisen from time to time anywhere within the range, and occasionally been selected (in homozygous form) for their role in protecting local coadapted gene complexes from disruption, by virtue of the lower fecundity of the structural heterozygotes that arise when individuals from neighboring populations interbreed. Although the situation described here for *Kalotermes approximatus* appears to differ in two important respects from many of these cases – adjacent populations seem to be very closely related karyotypically, and it is structural heterozygosity (in the male) that has in fact been selected *for* – nevertheless the same basic principles may apply. Males from neighboring populations may differ by additional rearrangements not detected in this study, and females from neighboring populations may also carry different X-chromosome translocations, in homozygous condition, that would break up the regular meiotic chain formation in interpopulational hybrid males. In other respects *Kalotermes approximatus* has many of the properties that characterize other species in which stasipatric karyotype differentiation has occurred: low vagility, narrow boundaries between the different karyotypes, little morphological differentiation across

the whole species range, and the apparent absence of reinforcing behavioral barriers to mating between individuals carrying different chromosomal arrangements (Syren and Luykx, unpublished observations).

If this model is correct, it is remarkable that the successive later translocations have all occurred in a limited geographical area – from Daytona Beach to Yulee in northeastern Florida. It may be that, for some reason, ecological conditions are more favorable for the establishment of new translocation types here than elsewhere.

We have insufficient knowledge to decide between these two models. We do not really know on what basis these translocations are being selected, nor whether the ecological conditions surrounding their selection are the same now as they were in the past history of the species. They could have arisen when the species first colonized the southeastern United States, perhaps under ecological conditions rather different from the present ones. In this case we would be seeing, in the geographic pattern of the translocations, the remnants of past evolutionary events. On the other hand, it is conceivable that many of these changes have become established more recently. It is possible that the higher-order translocation complexes have arisen only since human settlement began to strongly influence the ecology of the southeastern coast, within the last 150 years. In this case we might be witnessing, in the varying karyotypes and their geographic distribution, evolution in progress.

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