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X chromosome origin of a supernumerary-like segment in *Blatella germanica*

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Summary. An extraneous heterochromatic segment was discovered in a strain selected for a large-body trait. Derivation from the *X* chromosome is indicated by its behavior at metaphase I and association with the *X* and nucleolus in early prophase I. The segment does not pair with the *X*. Association with a mid-length bivalent is attributed to fusion of heterochromatin. Centromeric activity of small fragments, independent of, but apparently derived from, the *X*, is also reported.

Key words: *X*-chromosome – Heterochromatic fusion – *B. germanica*

Introduction

One of the best known properties of constitutive heterochromatin is its ability to fuse. A multiplicity of c-bands on meiotic chromosomes of the German cockroach, *Blattella germanica* (L.), suggest a considerable potential for chromosomal modification through fusion-breakage phenomena (Keil and Ross 1984).

The small, heterochromatic *X* chromosome is frequently connected by a thin strand of heterochromatin to c-bands on various bivalents. However, in his review of cockroach cytogenetics, White (1976) noted that he had not found any records of mechanisms of chromosomal polymorphisms based on inversions, fusions, dissociations, or extra heterochromatic segments". This is no longer the case in *B. germanica.* A recent study on a field collected strain indicated that heterochromatic fusion leading to breakage, deficiencies, and occasional integration of fragments into novel positions is indeed a mechanism capable of altering chromosome structure (Ross 1986). Whether or not this phenomenon was causally related to selection pressure sufficient to develop high-level resistance to several insecticides is a moot question, although the gross aberrations typical of the strain were not apparent in either a laboratory or a field-collected susceptible strain.

The present paper describes a second type of karyotypic modification that again implicates fusion of heterchromatin, in this instance, *X*-chromosome heterochromatin. It involves a heterochromatic segment that is carried through meiosis. The segment was discovered during study of a large-body (*Lg*) mutant (Ross, unpubl.). It shows how certain steps in the evolution of *B* chromosomes (supernumeraries) and supernumerary segments might have occurred. In their review of *B* chromosomes, Jones and Rees (1982) noted that "the origin in *B* chromosomes ...... is more a matter for conjecture than description".

Materials and methods

Male nymphs were drawn randomly from a strain selected for a large-body trait (*Lg*). Those used for study were characterized by an extraneous heterochromatic body in meiotic cells. Observations were on prophase I cells from 3rd to 4th instar nymphs, except that a few 2nd instar nymphs were used in order to examine premeiotic interphase. Cell preparations were from male testes that were squashed and stained with acetic-orcein, as described previously (Cochran and Ross 1969).

Results and discussion

The course of meiosis in individuals carrying the extra segment was essentially normal. Figure 1 shows selected stages of wild-type cells for comparison to a more extensive series of prophase I and II cells that carry the extra segment (Fig. 2). In wild-type prophase I, the *X* chromosome appears as a small heterochromatic body and is clearly the nucleolus organizer (Fig. 1a). When the extra segment is present, the *X* forms part of a complex ring that encircles the nucleolus (Fig. 2a). Subsequently, the ring separates into two unequal sections, of which the larger is the *X* (Fig. 2b, c). The smaller section is attached to the terminus of one of the autosomal bivalents, as well as

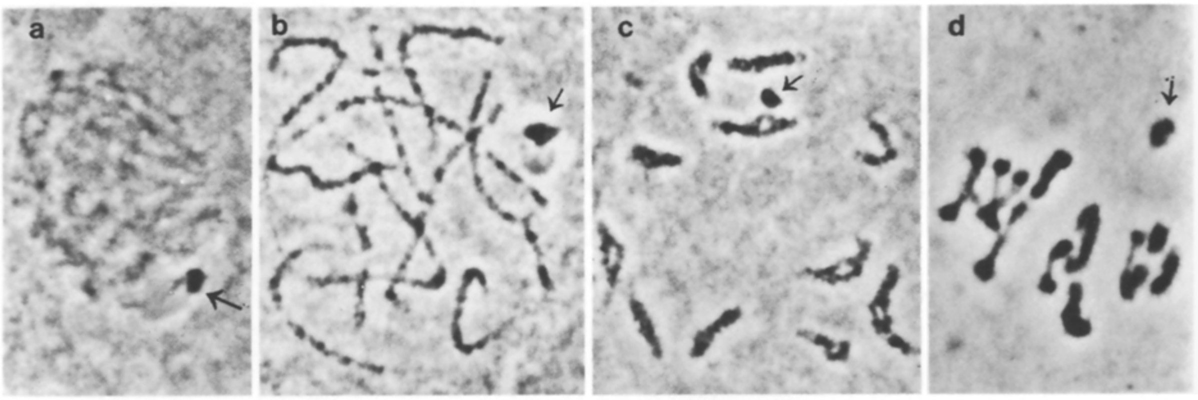

Fig. 1a–d. Leptotene-metaphase I meiocytes of wild-type *B. germanica* showing the small, heterochromatic *X* chromosome (arrow). **a** Leptotene: association of the *X* with the nucleolus indicative of nucleolar organizing activity of the *X*. **b** Pachytene: *X*-nucleolus relationship still visible. **c** Diplotene: typical attachment of the *X* to one of the bivalents. **d** Metaphase I: *X* moves precociously towards one pole

to the *X*. By diplotene, the larger section appears as a typical *X;* the smaller is clearly an extraneous segment (Fig. 2d, e). The segment is firmly attached to the terminus of a half-bivalent that, from its relative length, probably belongs to chromosome 5 or 6 (Cochran and Ross 1969). A faint connection to the *X* may or may not be discernable. Complete separation, as in Fig. 2e, may have been due to the squashing technique. At this time, the segment bears a striking resemblance to the well known knobs on maize chromosomes and supernumerary segments in grasshoppers (see Jones and Rees 1982). Connection to the *X* is again clear at metaphase I. The segment is pulled along with the *X* as it moves precociously towards one pole (Fig. 2f). Connection of the *X* to a bivalent via the segment apparently restricts movement of the *X*, as it remains closer to the main body of chromosomes than is normally the case (compare Fig. 1d to Fig. 2f and g). Contrarily, the connection does not disturb the alignment of bivalents on the metaphase plate. Observations on prophase II cells confirm the *X*-chromosome-like behavior of the segment at metaphase I. The segment occurs only in cells that also carry the *X*. Surprisingly, only the autosomal connection is apparently retained in prophase II, although the *X* acquires a small extra section (Fig. 2h, i).

The segment often appeared to be double in prophase II cells (Fig. 2h). This observation, coupled with reoccurrence of the trait in several generations of selection for *Lg*, is strong evidence that the segment progresses through anaphase II and is transmitted from one generation to the next.

Examination of premeiotic interphase from several 2nd instar nymphs indicated that the extra segment had nucleolar organizer activity. Two nucleoli were frequently present, but apparently fuse into a single nucleolus early in prophase I (Fig. 3a). Normally a single nucleolus is present (Fig. 3b) (Cave 1976).

Figure 4 shows an unusual type of aberration in cells where the extra segment was apparently attached to the *X*. Fragments that were completely separate from the *X* also behaved like the *X* at metaphase I. Why separate fragments should move to the same pole as the *X* is puzzling. Perhaps this observation was fortuitous. Only a few examples were seen. Alternatively, the explanation may lie in kinetechore (centromere)-centrosome interactions that Rieder and Borisy (1981) demonstrated to occur during spindle formation. Possibly univalents or, more specifically, the *X* and its derivatives, mono-orient and remain in this relationship to a particular centrosome. Centromeric activity of the fragments is also of interest. The *X* is almost certainly one of the five small metacentric chromosomes figured by Cohen and Roth (1970) and, indeed, it is clearly metacentric in the diploid karyotype (Ross, unpubl.). How, then, did centric fragments become separated from the *X*? The answer may lie in *X* chromosome structure. Although it appears as a solid mass in prophase I, it shows a complex structure in premeiotic interphase cells (Fig. 3b). Sections that might be separable are evident. The occurrence of fragments with centromeric activity is also noteworthy from the point-of-view of the evolution of *B* chromosomes. No direct evidence has been available on how *B*'s might have acquired centromeric activity. Apparently this might be accomplished through fragmentation of the *X*. Perhaps the *X* carries potentially multiple centromeres.

Integration of *B* chromosomes into the autosomal complement might explain the origin of supernumerary segments (Jones and Rees 1982). The segment described here suggests a more direct origin from breakage of the *X*. If its association with an autosome was retained, but that with the *X* lost, it would appear to be a typical supernumerary segment of unknown origin. Indeed, association with the *X* at diplotene, but not during metaphase I, would probably be attributed to "stickiness", as in the case of a supernumerary segment

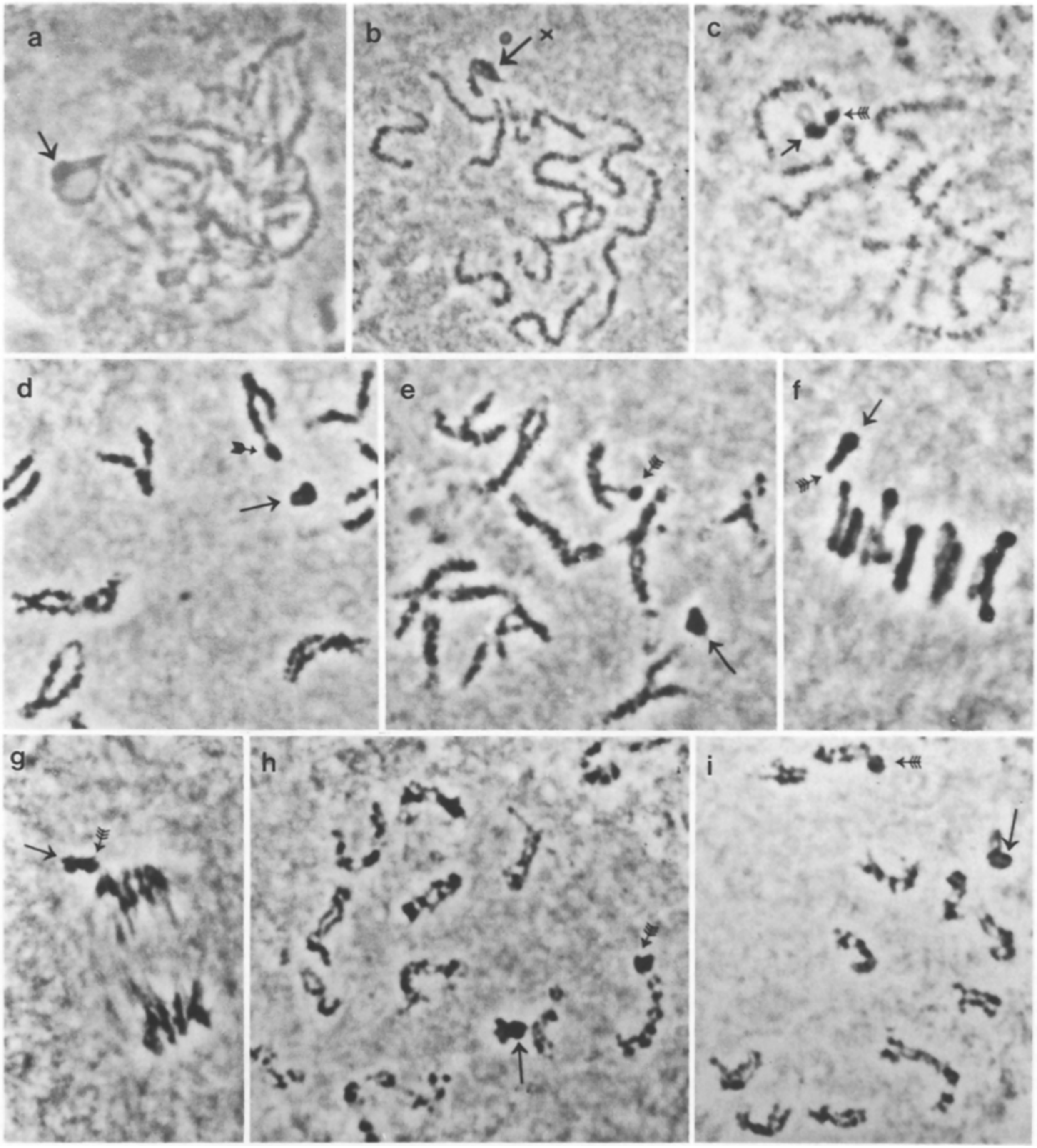

Fig. 2a–i. Leptotene-prophase II meiocytes that carry an extraneous heterochromatic segment. Notice the relationship between the segment (*feathered arrow*) and the *X* (*solid arrow*). **a** Leptotene: nucleolus encircled by a ring of heterochromatin. **b** and **c** Pachytene: separation of the ring into two sections, with connection of the smaller section to the terminus of a bivalent clearly displayed in **c, d** and **e.** Diplotene: the segment remains connected to a bivalent but is nearly (**d**) or completely separated (**e**) from the *X*. **f** and **g** Metaphase and anaphase I, respectively: the segment is pulled towards one pole along with the *X* but seems to retain its connection to a bivalent. **h** and **i** Prophase II: the segment occurs in cells that carry the *X* but is attached only to a bivalent; also, the *X* carries a small "tail" of extra material and the segment is clearly divided in **h** (*feathered arrow*)

described by Comacho and Cabrero (1982). The bivalent association appears typical of heterochromatic strands that often connect the *X* to c-bands on autosomes (Keil and Ross 1983) and that possibly reflect fusion of similar sequences of highly repetitive DNA. The association of the segment with the *X* is also more in the nature of fusion for it clearly does not pair with the *X*. Nevertheless, its behavior and nucleolar organizing activity leave no doubt as to an *X* chromosome origin. A lack of pairing of *B*'s and supernumerary segments with the *X* has been regarded as evidence of non-homology, but perhaps this assumption should be re-evaluated. Moreover, the association of the segment with the *X* and also the occurrence of the separate fragments illustrate one of the "essential" conditions in the evolution of *B*'s, that is, preclusion of pairing and chiasma formation with members of the normal chromosome complement (Jones and Rees 1982).

From the above, it seems that the *X* is a likely source of *B* chromosomes and heterochromatic segments in *B. germanica.* None have been reported, but this is hardly surprising in view of the lack of study on population cytogenetics.

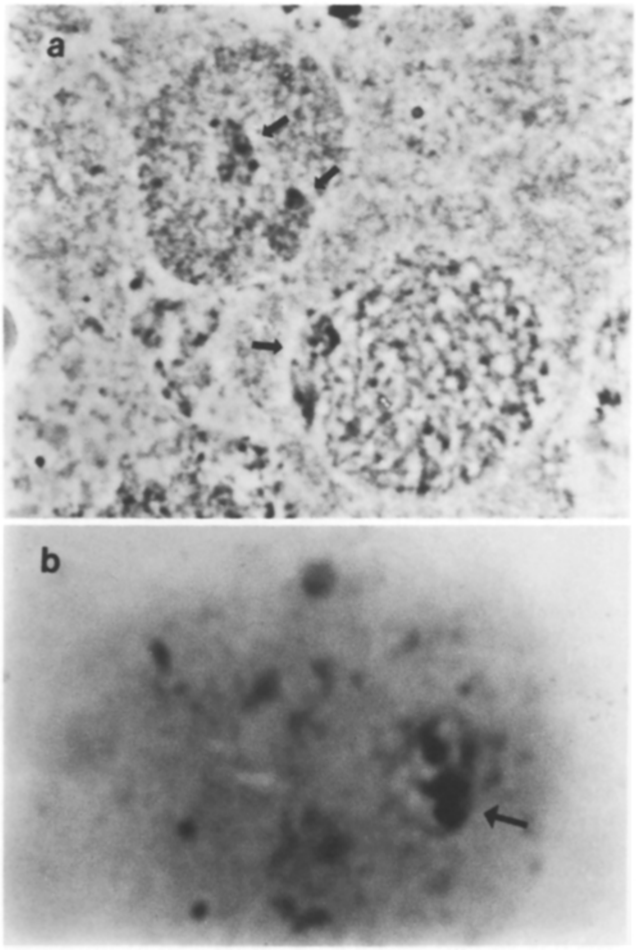

Fig. 3. a Occurrence of two nucleoli in premeiotic interphase (*upper left*) and coalescence into a single nucleolus in early leptotene (*lower right*) in cells that carry the extra segment. **b** C-banded interphase cell from wild type showing single nucleolus and complex structure of associated *X* chromosome heterochromatin

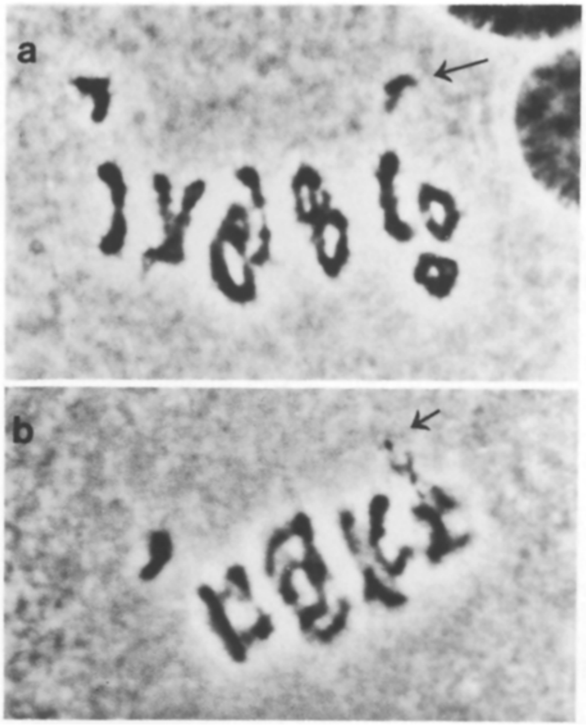

Fig. 4a, b. Movement of fragments (*arrows*) towards the same pole as the *X* but independently of the *X*. The extra segment is apparently attached to the *X*

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