Theor. Appl. Genet. 63, 289–293 (1982)

THEORETICAL AND APPLIED GENETICS

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C-band Morphology of T(8;9)/8;9 in *Blattella germanica*

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Summary. Centromere position and each arm of the T(8;9)/8;9 quadrivalent at late pachytene can be recognized by C-banding. The chromosome 9 breakpoint lies immediately adjacent to the centromeric C-band; that of 8, in the general region of the centromere but the relationship to centromeric bands was not determined since the latter stain very faintly. Chromosome 9 differs from no. 8 in the presence of a complex series of intercalary bands, heavy centromeric bands and, overall, a larger amount of C-band material. Possible implications of these differences with respect to chromosome breakability and the nature and distribution of mutant loci are noted. Earlier identification of adjacent-1 and adjacent-2 metaphase I ring orientations, made on the basis of orientation pattern, was confirmed.

Key words: Centromere – C-band – Chromosome breakability – *Blattella* – Cockreach

Introduction

Ring quadrivalents at metaphse I in interchange heterozygotes of *Blattella germanica* (L.) show consistent patterns among four orientation configurations, namely, adjacent-1, alternate-1, adjacent-2, and alternate-2 (Cochran 1976, 1977; Ross and Cochran 1977). In stocks where centromere-breakpoint positions were determinable from standard orcein squash preparations, alternate-1 and adjacent-2 were numerically balanced and represented the two smallest groups. The two large groups, adjacent-1 and alternate-2, were balanced if disjunction was random (total alternate approximately equal to that adjacent orientations). Otherwise, directed alternate disjunction reflected a predominance of alternate-2 over adjacent-1.

Ratios similar to the above also occur in interchanges in which breakpoints lying in the general region of the centromeres make it difficult to distinguish Type I (adjacent-1 and alternate-1) from Type II (adjacent-2 and alternate-2) by conventional techniques (Cochran 1977). One such interchange is T(8;9)/8;9. Four metaphase I ring configurations that occurred in a ratio of 2 : 1 : 1 : 2 were identified tentatively as adjacent-1: alternate-1: adjacent-2: alternate-2 on basis of this orientation pattern. Directed alternate disjunction, as evidenced by a mean of 62% alternate configurations, was due to an excess of the alternate orientation thought to represent alternate-2. C-banding was used to obtain a more positive identification of orientation configurations and to study the C-band morphology of T(8;9)/8;9 quadrivalents. The results of these studies, including observations on early pachytene and prophase II cells, are presented herein.

Materials and Methods

C-band morphology was studied from a series of 3rd to 4th instar male nymphs. They were drawn from two backcross systems, one using the closely linked chromosome 8 marker, black-body (Bl), and the other a chromosome 9 marker, ruby-eye (*ru*). Translocation heterozygotes were identified by body color indicative of *Bl* hybrids or by normal eye color. Males of these stocks are characterized by the "normal" disjunction frequency for T(8;9)/9;8 males, i.e., 60–62% directed alternate disjunction (Cochran and Ross 1974) and by the underlying orientation pattern described by Cochran (1977) and Ross and Cochran (1977).

The C-band procedure was a modification based on the schedule developed by Webb (1976) (Keil, unpubl.). In brief, squash preparations were made of testes in 15% acetic acid. The cover slips were removed after freezing in liquid nitrogen. These squash preparations were fixed three times with 3 : 1 methanol : acetic acid and aged for 24 hr at 65 °C and 3–4 days at room temperature. The slides were placed in 0.2 N HCl (10 min) followed by rinsing three times in distilled water. The slides were treated with saturated $Ba(OH)_2$ (30 min), rinsed in 70% ethyl alcohol, and rinsed with three changes of distilled water. The slides were next placed in 2×SSC at 62° –65 °C for 1 h, and washed with phosphate buffer (pH 6.8). The preparations were stained with a 10%

0040-5752/82/0063/0289/$ 01.00

solution of Leishman's stain (Sigma) in phosphate buffer for 100–120 min or longer, depending on the individual preparations. Excess stain was removed carefully in 70% and 95% ethyl alcohol. Cover slips were mounted with Dermount.

Results

The intensity of staining varied from cell to cell within single preparations. Nevertheless, consistent banding patterns were evident. It was possible to recognize each arm of the T(8;9)/8;9 quadrivalent at late pachytene and to identify centromeric and non-centromeric C-bands.

Fig. 1a–c shows examples of diplotene quadrivalents. Figure 2 is a diagrammatic representation of the configurations, oriented as in Fig. 1. Chromosome 9 is identified by correspondence to the original description of chromosome 9 (Cochran and Ross, 1974) and by other studies of C-banded material (Keil and Ross unpubl.). Chromosome 8 is identified on the basis of its position in the quadrivalent. Arms of the quadrivalent are designated arbitrarily as a, b, c, and d. Arm a is the shortest. A heavy band at the base marks centromere position in chromosome 9 and its homologue in the translocated chromosome, T_1. Arm b is relatively long and stains most heavily in terminal portions, within which a strong sub-terminal C-band is often evident (Fig. 1a and b). It is frequently the last to separate as the quadrivalent opens into a ring configuration. Arm c stains lightly. The distal portion appears as two separate strands that rejoin terminally (Fig. 1b). This characteristic led to difficulties in the identification of chromosome 8 by length measurement in late pachytene. It was mistakenly identified as no. 11, but later corrected (Cochran and Ross, 1974; Ross and Cochran, 1981a). Centromere position in chromosome 8 and T_2 is marked by indistinct C-bands that lie near the base of arm c and that frequently appear double (Fig. 1c). Arm d is closely similar to b in length and tends to stain heavily throughout. It is marked by a complex series of small, dark, interstitial bands. Overall, chromosome 9 carries much more C-band material than does no. 8. Major C-banding on chromosome 8 is confined to the distal portion of one chromosome arm, i.e. arm b.

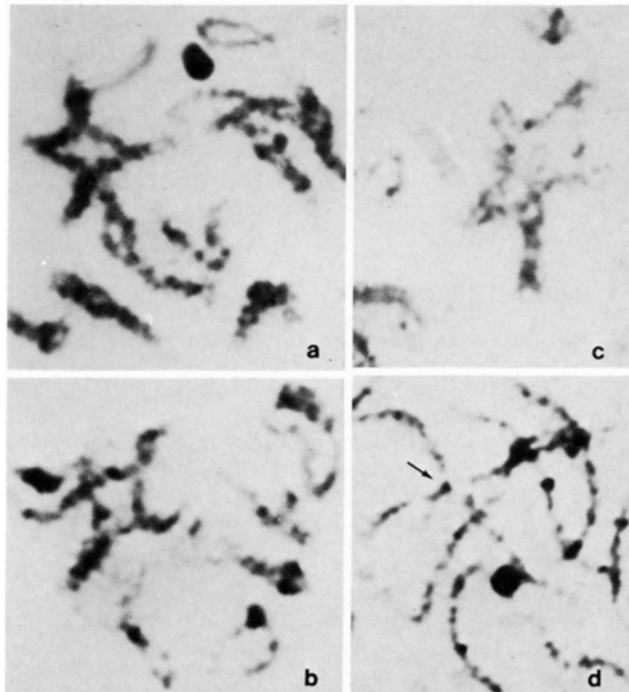

Fig. 1a–d. C-banded quadrivalents of T(8;9)/8;9 at diplotene (**a–c**) and early (**d**) pachytene, positioned as shown in Fig. 2. **a, b** Heavily stained cross configurations in which strong centromeric bands are evident at the base of the small arm at the left and a subterminal band is prominent in the uppermost arm. **c** Lightly stained preparation showing centromeric bands in the right arm. **d** Centromeric bands (arrow) in the short arm lie close to the chromosome breakpoints, i.e. the central area of asynapsis. A subterminal band marks the uppermost arm

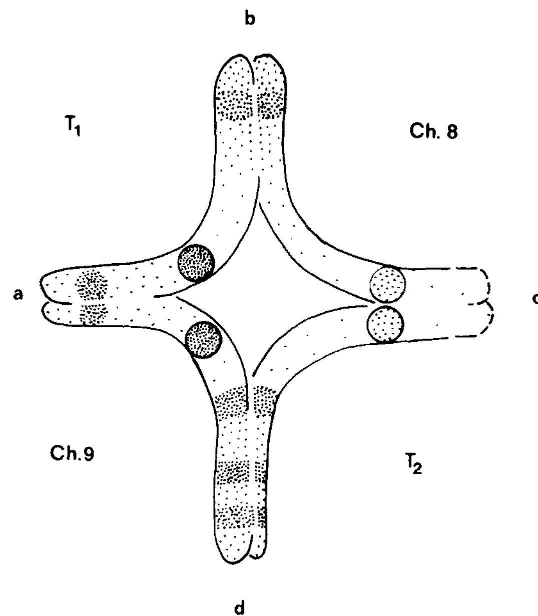

Fig. 2. Diagrammatic representation of quadrivalents shown in Fig. 1 a–c. The normal chromosomes are 8 and 9; translocated chromosomes are indicated as T_1 and T_2. Arms of the quadrivalent are designated as a–d. Circles show centromere position as determined from centromeric bands. Dark circles represents homologous centromeres of chromosome 9 in arm a; light circles, those f chromosome 8 in arm c

From Fig. 2, it is clear that when the pachytene configuration opens into a ring, the two shortest intercentromeric chromosome segments lie between each of the two pair of homologous centromeres (those separated by opening out of arms a and c). The longest segments lie between neighboring nonhomologous

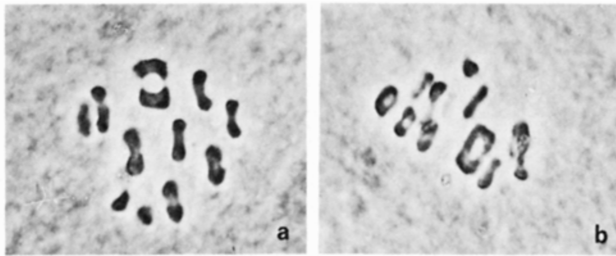

Fig. 3a and b. Metaphase I ring configurations, orcein stain. **a** Adjacent-1; **b** Adjacent-2

centromeres, for example, the segment connecting the centromere of T_1 with that of chromosome 8. A metaphase I ring in which homologous centromeres are pulled towards opposite poles (adjacent-1) would be held in a compact form with short attenuated segments lying between opposing centromeres. The long segments would lie relaxed and consequently could appear very thick. This description agrees with the configuration in Fig. 3a and confirms its earlier interpretation as adjacent-1 (Cochran 1977). Likewise, the oblong ring in Fig. 3b fits the form expected when the two longest segments are stretched by movement of two pairs of adjacent non-homologous centromeres to opposite poles (adjacent-2). The corresponding alternate configurations, derived as diagrammed by Cochran (1977), Fig. 1b), are illustrated elsewhere (Ross and Cochran 1977). Metaphase I was not a suitable stage for studies of C-banding, but it is, perhaps, worth noting that we found no intergradations between the two distinctive alternate orientations, i.e. a "zig-zag" and a "v-shaped" configuration.

A few early pachytene cells in which the quadrivalent was identified unambigously were found (Fig. 1d). A heavy C-band at the base of the short arm corresponds clearly to the centromeric band already described in arm *a* (Fig. 2). Breakpoints, as indicated by the central area of asynapsis, appear to lie closely adjacent to this band. No heteromorphism was observed that might indicate breakage within the centromeric band. Nevertheless, we cannot rule out definitively the possible translocation of a small amount of centromeric heterochromatin from chromosome 9 to chromosome 8. The position of the lighter centromeric bands of chromosome 8 seen at later stages (Fig. 1a–c) suggests the centromere is also close to the breakpoints. Absence of any lengthy interstitial segment from chromosome 8 breakage is supported by the consistent occurrence of only one point of opening in the quadrivalent, i.e., that at the center of the configuration (Fig. 1). Otherwise a secondary opening that normally marks centromere position at early diplotene should have been apparent in the arm containing the chromosome 8 centromere.

The heavy centromeric bands of chromosome 9 furnished a means for distinguishing between adjacent-1 and adjacent-2 segregants among prophase II chromatids in those instances when end-to-end attachments were retained. Terminal attachments of T_1 and chromosome 8 and T_2 and chromosome 9 would occur in adjacent-1 (Fig. 2). In either case, one member of the chromatid pair would carry a chromosome 9 centromere and the other would not. Terminal attachments associated with adjacent-2 segregants would occur between T_1 and chromosome 9 and T_2 and chromosome 8 in arms a and c, respectively. Both chromatid pairs carry homologous centromeres. Fig. 4 shows examples of attached chromatids identified on this basis as to adjacent-1 or adjacent-2 segregation. The two adjacent-2 segregants are easily distinguished (Fig. 4c and d), but no clearcut differences have been found that separate those of adjacent-1. Adjacent-1 types, showing the general characteristics of that in Fig. 4a, predominated over those of adjacent-2. Random photography of cells with weldisplayed chromatid attachments showed 17 adjacent-1, but only 7 adjacent-2 segregants. Fig. 4b shows an unusual attachment that apparently reflects a non-terminal chiasma position, as well as absence of terminalization.

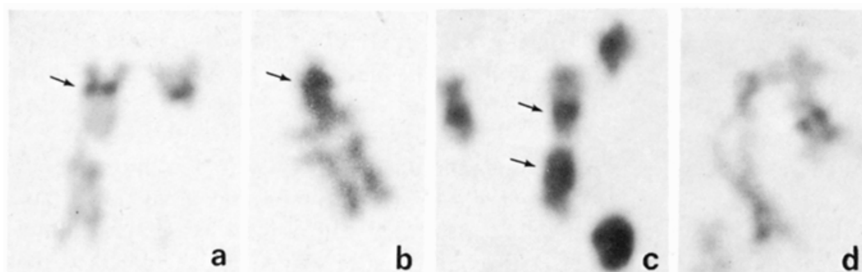

Fig. 4a – d. C-banded patterns found among attached chromatids (adjacent segregants) at prophase II. Arrows indicate heavy centromeric bands of chromosome 9. **a** Adjacent-1 segregant, identified by occurrence of chromosome 9 centromere in one pair only. **b** An unusual subterminal attachment in an adjacent-1 segregant. **c** Adjacent-2 segregant in which attachment involves the short quadrivalent arm (arm a). **d** Adjacent-2 segregant with attachment at the end of the long, trailing segment of arm c

Discussion

C-band morphology of T(8;9)/8;9 quadrivalents, described herein, has resolved the problem of Type I (alternate-1 and adjacent-1) and Type II (alternate-2 and adjacent-2) identification in this interchange heterozygote. The results support the identification of predominant orientation patterns in T(8;9)/8;9 and other interchange heterozygotes described by Cochran (1977). With this information and research reported subsequent to 1977, it is appropriate to re-examine certain aspects of orientation patterns and to comment on the striking differences between the C-band morphology of chromosomes 8 and 9.

The existence of orientation patterns common to various interchange heterozygotes in *B. germanica* is probably related to similarity of physical properties. The latter include breakage in the general region of the centromeres, confirmed here in T(8;9)/8;9 as a whole arm transfer in chromosome 9 and at least close to one in chromsome 8. In the majority of cells, quadrivalents are maintained as rings at metaphase I by a single chiasma in each chromosome arm. The chiasmata appear in terminal positions and probably represent distally localized crossover events (Keil and Ross, unpubl.). Thus, variation that might result from interstitial crossingover or chaining has played little part in the observations to date. In addition, we suggest there is little variation that might result from reorientation. Configurations at metaphase I are almost certainly final orientations. The higher frequency of the adjacent ring configuration described by Cochran (1977) and identified here by C-banding as adjacent-1 agrees with present observations of a high frequency of adjacent-1 segregants among attached prophase II chromatids. Stability of a 4:2 malorientation in a ring of six was documented in an earlier report (Cochran and Ross, 1977). In many years of study, we have not seen 3:1 or 4:0 orientations in quadrivalents. This suggests both stability and an early determination of 2:2 centromere arrangement (Rickards, 1965).

The consistently higher frequency of adjacent orientations among Type I cells (adjacent-1 and alternate-1) versus that of alternate orientations among Type II (adjacent-2 and alternate-2) among interchanges in *B. germanica* is puzzling (Cochran 1977). Possibly the answer lies within the frequency with which particular pairs of centromeres are directed toward the same (nearest?) pole vs opposite poles (see Nicklas 1974). Other factors that might be involved include homologous centromere coorientation (Lacadena and Candela 1977), the distance covered by two centromeres at metaphase I (Arana et al. 1980), or the distance covered by two centromeres before stabilization (Vosselman 1981). However, it is difficult to relate these to the orientation patterns observed in *B. germanica*. We suspect a difference in some very early step in ring orientation. For example, suppose it consists of ring configurations first taking 2:2 centromere positions with respect to the equatorial plate? Coorientation of homologous centromeres might then favor maintenance of the initial ring configuration if homologous centromeres were on opposite sides of the plate (favoring of adjacent-1 in Type I cells), or the reverse (movement into alternate configurations) if homologous centromeres were on the same side.

An atypical orientation pattern was associated with a change of genetic background in T(8;9)/8;9 males (Ross and Cochran 1977b), and a similar change in T(3,12)/3;12 has been maintained by selection (Ross and Cochran 1979, 1981b). Although differences in chiasma position might be involved in such alterations, they are difficult to assess due to typically terminal positions. The rare occurrence of a clearly non-terminal attachment among prophase II chromatids described herein suggests these might be used to detect a shift from the more usual chiasma position. At least in T(8;9)/8;9 this could be followed one step further in C-banded preparations by distinguishing adjacent-1 and adjacent-2 segregants and, in the latter, by identifying the specific chromosome arm showing the altered position.

Chromosomes 8 and 9 are closely similar in length (Cochran and Ross 1969) and, in conventionally stained preparations, they are difficult to distinguish. C-banding revealed striking differences: chromosome 9 has a comparatively large amount of C-banded material, including heavy centromeric bands; overall, chromosome 8 stains lightly, with small, often indistinct centromeric bands. Undoubtedly this accounts for a higher breakability of chromosome 9. Chromosome 8 is known to be involved only in the T(8;9)/8;9 interchange; no. 9 is involved in single interchanges with chromosomes 7, 8 and 11, and four interchanges with chromosome 10, of which three have a similar phenotypic effect (Ross and Cochran 1975; Ross unpubl.). Preferential breakage in heterochromatic and centromeric areas is known from such diverse organisms as the tomato (Gill et al. 1980) and *Drosophila* (Jancey and Walden 1972). Added here is evidence of a similar phenomenon in *B. germanica*. Moreover, a distinctive C-band morphology opens the way for comparative studies of breakpoint position within chromosome 9. In *Drosophila*, clusters of chromosomal breakpoints have been found associated with intercalary heterochromatin, and a model was developed which indicated that the occurrence of identical breakpoints might be expected (Yoon and Richardson 1976; 1977).

Differences also occur between the distribution and nature of loci born on chromosomes 8 and 9. Those of

no. 8 (linkage group VI) are spread throughout a map distance of at least 30 and, possibly, 70 map units (depending on interpretation of notched pronotum linkage data; Ross 1973). Chromosome 9 loci (linkage group VIII) are concentrated within 4 map units and include mutants with homeotic and maternal-lethal effects (Ross and Cochran 1975; Ross and Keil 1978). Possibly extensive C-band material, presumably constitutive heterochromatin, blocks crossingover throughout most of chromosome 9, although data thus far argue against this posibility since chiasmata are apparently localized in sub-terminal to terminal positions in all bivalents (Keil and Ross unpubl.). However, a possible relationship between the organization of DNA within heterochromatin and the function of its genetic material, as seen in the prowing and notched sternite mutants on chromosome 9 of *Blattella* and the bithorax complex of *Drosophila* (Anaiev et al. 1978), is, at least, noteworthy.

Acknowledgements

We thank Donald G. Cochran for critical reading of the manuscript and Mr. David Morris for technical assistance. We also appreciate the advice of Dr. G. C. Webb concerning the C-banding procedure, especially in regard to the use of Leishman's stain. The research was supported, in part, by research grant PCM-8001620 from NSF. C.B.K. was supported by a fellowship from the Johnson's Wax Foundation and a Cunningham Fellowship from the Graduate School, VPI & SU.

Literature

Ananiev, E.V.; Gvozdev, V.A.; Ilyin, Yu. V.; Tchurikov, N.A.; Georgiev, G.B. (1978): Reiterated genes with varying location in intercalary heterochromatin regions of *Drosophila melanogaster* polytene chromosomes. Chromosoma **70**, 1–17

Arana, P., Santos, J.L.; Giraldez, R. (1980): Chiasma interference and centromere co-orientation in a spontaneous translocation heterozygote of *Euchorthippus pulvinatus gallicus* (Acrididae; Orthoptera). Chromosoma **78**, 327–340

Cochran, D.G. (1976): Disjunction types and their frequencies in two heterozygous reciprocal translocations of *Blattella germanica* (L.). Chromosoma **59**, 129–135

Cochran, D.G. (1977): Patterns of disjunction frequencies in heterozygous reciprocal translocations from the German cockroach. Chromosoma **62**, 191–198

Cochran, D.G.; Ross, M.H. (1969): Chromosome identification in the German cockroach. J. Hered. **60**, 87–92

Cochran, D.G.; Ross, M.H. (1974): Cytology and genetics of T(9;11) in the German cockroach and its relationship to other chromosome 9 traints. Can. J. Genet. Cytol. **16**, 639–649

Jancey, R.C.; Walden, D.B. (1980): Cytogenetic analysis of chromosomal translocations in the tomato: preferential breakage in heterochromatin. Can. J. Genet. Cytol. **22**, 333–341

Lacadena, J.R.; Candela, M. (1977): Centromere co-orientation at metaphase I in interchange heterozygotes of rye, *Secale cereale* L. Chromosoma **64**, 175–189

Nicklas, R.B. (1974): Chromosome segregation mechanisms. Genetics **78**, 205–213

Rickards, G.K. (1965): An analysis of co-orientation in mitosis and meiosis. J. Theor. Biol. **9**, 332–349

Ross, M.H. (1973): The notch-pronotum, shriveled-wing, and cubitus interruptus mutants of the German cockroach. Ann. Entomol. Soc. Am. **66**, 112–116

Ross, M.H.; Cochran, D.G. (1977): Analysis of a double reciprocal chromosome translocation in the German cockroach. J. Hered. **68**, 231–237

Ross, M.H.; Cochran, D.G. (1979): Properties of a three chromosome double translocation heterozygote in the German cockroach. J. Hered. **70**, 259–266

Ross, M.H.; Cochran, D.G. (1981a): Synthesis and properties of a double translocation heterozygote involving a stable ring-of-six interchange in the German cockroach. J. Hered. **72**, 39–44

Ross, M.H.; Cochran, D.G. (1981b): Genetics and cytogenetics of the German cockroach. In: Cytogenetics and genetics of vectors (eds. Pal, R.; Kitzmiller, J.B.), pp. 181–195. Japan: Kodansha Scientific

Ross, M.H.; Keil, C.B. (1978): Genetic variability in the German cockroach. XI. Does chromosome 9 carry remnants of a primitive gene system? J. Hered. **69**, 337–340

Vosselman, L. (1981): Meiotic segregation of five different reciprocal translocations in the Onion fly, *Hylemya antiqua* (Meigen). Chromosoma **81**, 727–738

Webb, G.C. (1976): Chromosome organization of the Australian plague locust, *Chortoicetes terminifera*. 1: Banding relationships of the normal and supernumerary chromosomes. Chromosoma **55**, 229–246

Yoon, J.S.; Richardson, R.H. (1976): Evolution of Hawaiian Drosophilidae. 2: Patterns and rates of chromosome evolution in an Antopocerus phylogeny. Genetics **83**, 827–843

Yoon, J.S.; Richardson, R.H. (1978): A mechanism of chromosomal rearrangements: the role of heterochromatin and ectopic joining. Genetics **88**, 305–316

Received March 10, 1982
Accepted July 15, 1982
Communicated by H. F. Linskens

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