

Multivalent formation and pairing behavior of germ line limited chromosomes in male meiosis of *Acricotopus lucidus* (Diptera, Chironomidae)

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Abstract

The pairing behavior of the germ line limited chromosomes of *Acricotopus lucidus* was investigated in male meiosis using G-banding. Each of the nine different types of limited chromosomes can be absent or can be present in metaphase I with two or four homologous chromosomes, one type even with up to 10. Usually the homologues form bivalents, but frequently quadrivalents and also hexavalents consisting of the same chromosome type were observed. In some cases multivalents composed of different limited chromosomes occurred. This resulted probably from pairing and crossing-over between hom(oe)ologous segments in otherwise nonhomologous chromosomes. The observations are discussed in relation to origin and diversity of the germ line limited chromosomes.

Key words: germ line limited chromosomes, multivalent formation, male meiosis, *Acricotopus lucidus*.

Introduction

The Orthoclaadiinae, a subfamily of the Chironomidae, are characterized by the presence of chromosomes limited to the germ line (K chromosomes; K derived from “Keimbahn“ (Bauer 1970) and by a complex chromosome cycle that these chromosomes pass through, in contrast with the soma chromosomes (S chromosomes (Bauer and Beermann 1952)). One speciality of this chromosome cycle, which proceeds in both sexes in the same manner (for a review see Beermann 1956; Bauer 1970), is the last gonial mitosis, termed differential mitosis. In contrast to the Ss, which behave as in a normal mitosis, all Ks move undivided to one cell pole. This leads to a duplication of the Ks prior to meiosis, compensating for the elimination of about half the Ks during the first division of the primary germ cells. The cells containing the Ks differentiate into functional spermatocytes and oocytes, whereas the cells with the somatic set only develop into nurse cells in the female and will give rise to degenerating spermatocytes in the male. The elimination of the Ks from the prospective soma cells takes place during early cleavage divisions.

The successful G-banding of Ks in gonial metaphases was reported previously for the orthoclaiid *Acricotopus lucidus* (Staiber 1988), leading to the identification of nine different K types that form, in various combinations, the gonial K complements. In this study, the investigation is extended to meiotic stages, especially metaphase I of spermatocytes, to study the pairing behavior of the Ks with the intention of getting additional information about the origin and diversity of the Ks and their relationship to each other

Materials and methods

Testes were taken from *A. lucidus* larvae in transitional stages from 4th larval instar to prepupa (4.LS/PP, see Fig. 1 in Staiber and Behnke 1985). The larvae were characterized by light-orange eye stripes visible through the head capsule, indicating the developing imaginal eyes.

Treatment of gonads and G-banding of squash preparations were carried out as described previously (Staiber 1988).

Results and discussion

The composition of meiotic K complements of *A. lucidus* analyzed in male metaphases I (MIs) reflects and confirms the results of investigations of gonial K complements (Staiber 1988). Nine different K types were found. Each K type can be absent or present (as a result of the duplication of the Ks in the differential gonial mitosis) as even-numbered multiples of their number in gonial K complements with two or four homologues. The special germ line limited chromosome K4 can even be present with 6, 8 (Fig. 1), or 10 homologues.

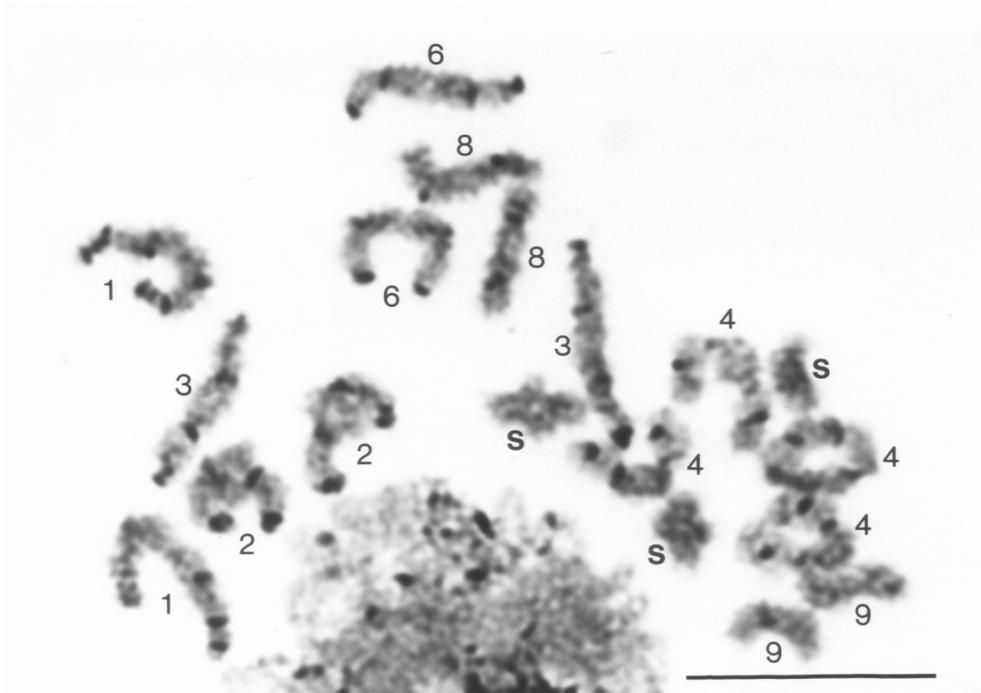


Fig. 1. G-banded spermatocyte metaphase I of *Acricotopus lucidus* with 3 bivalents of the soma chromosomes (S) and 16 bivalents of germ line limited chromosomes (K). 1, 2, ..., 9 indicate K1, K2, ..., K9. Bar represents 10 μm

No differences between the G-banding patterns of the Ks in MI and in gonial metaphases were observed. The chromosomes K1 - K4, K8, and K9 were easily identified in all MI configurations by their patterns of conspicuous bands and their characteristic morphology (Fig. 1), whereas chromosomes K5, K6, and K7 were more difficult to identify. The three bivalents of the soma chromosomes (Ss) could not be distinguished from each other in MIs because cross-like pairing configurations, resulting from interstitial chiasmata formation, frequently disaggregate the G-banding patterns (Fig. 1). In many MIs the S bivalents were observed lying together in a group. The same observation applied to homologous K bivalents (see Fig. 1, e.g., K2, K4, K8, K9).

K bivalents more frequently showed one terminal chiasma rather than two chiasmata. Therefore in MI, rod-like bivalents were more frequently observed than ring bivalents. K bivalents also occurred without chiasmata, only paired. The three larger subacrocentric K types, K1 - K3 (for morphology and G-banding pattern of the different K types see Fig. 1 in Staiber 1988), exhibited chiasmata mainly in the short chromosome arms on the side opposite to the characteristic thick dark G-bands in the long arm (Fig. 1). These bands are also C-band positive and therefore assumed to be heterochromatic (W. Staiber, unpublished).

K chromosomes present only once in the gonial complement form, as a consequence of premeiotic duplication, a bivalent at MI with their genetically identical copy. This type of bivalent was termed an autobivalent by Håkansson and Levan (1957).

A chromosome of a K type present twice before differential mitosis has one identical and two homologous but not necessarily identical partners. Assuming random meiotic pairing, this K type can form an autobivalent or a bivalent with one of its homologues. Because there are no

morphological differences between identical and homologous chromosomes, it is not possible to decide whether there are pairing preferences or even whether the chromatids of the Ks after the last gonial mitosis stay together with only autobivalents being formed. In larval development of *A. lucidus*, the time between duplication of the Ks in differential mitosis and the time when the spermatocytes reach MI is about 150 h.

Pairing preferences for identical over homologous chromosomes were found in MIs of spontaneous tetraploid tubules of testes of the grasshopper *Euchorthippus pulvinatus* (Giraldez and Santos 1981; Santos et al. 1983) characterized by heterozygous C-banding patterns for one chromosome pair.

Complete autobivalent formation resulting from endomeiotic DNA duplication during early prophase was reported for the parthenogenetic stick insect *Carausius morosus* (Pijnacker and Koch 1975; Pijnacker and Harbott 1980).

As mentioned earlier, each of the 9 K types can participate in meiosis with four homologues. In addition to the formation of two bivalents, the four homologues can also form one quadrivalent or a trivalent and an univalent.

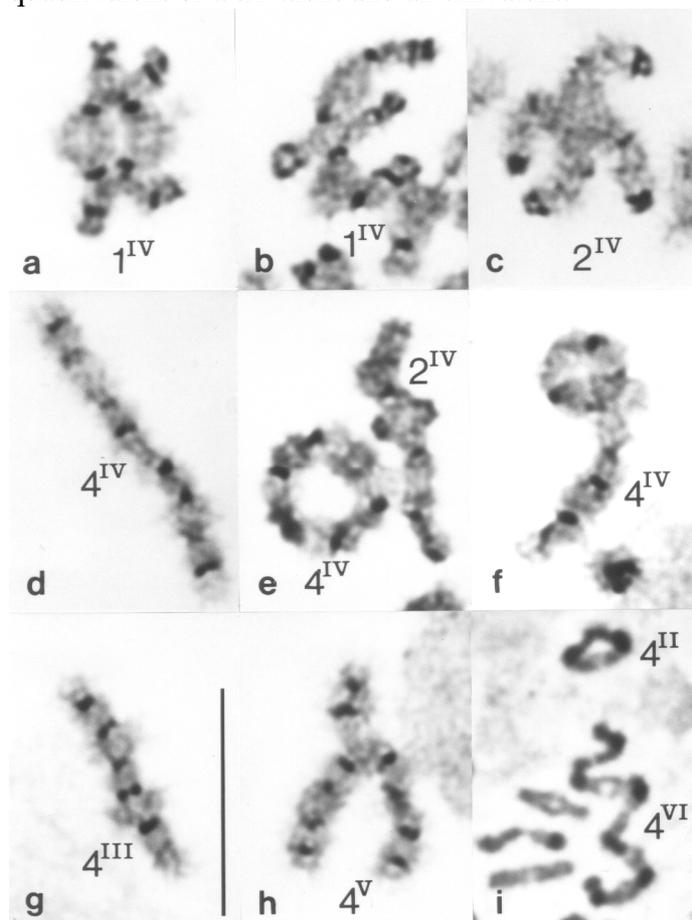


Fig. 2. Multivalents composed of homologous K chromosomes. (a-h) G-banded. (a) Ring quadrivalent (IV) of K1. Animal 5. (b) Chain IV of K1. Animal 5. (c) Cross-like IV of K2. (d) Chain IV of K4. (e) Ring IV of K4 and chain IV of K2. Animal 6. (f) “Frying pan“ IV of K4. (g) Trivalent (III) of K4. (h) Quinquivalent (V) of K4. (i) Section of an acetocarmine-stained metaphase I showing a zigzag hexavalent (VI) and a bivalent (II) of K4. Animal 1. Bar represents 10 μm .

Quadrivalent formation was observed in K1 (Figs. 2a and 2b; Tables 1 and 2), K2 (Figs. 2c and 2e), and K3 and K4 (Figs. 2d, 2e, 2f, and 3d). Trivalent formation occurred in K4 (Fig. 2g). Different types of quadrivalents were found, most frequently ring and chain quadrivalents. K4 showed the greatest spectrum of quadrivalent types (Table 3; e.g., “figure 8“ quadrivalents, “frying-pan“ quadrivalents, etc.; De Jong et al. 1989). Seven of the 11 types represented by Rieger et al. (1976, Fig. 69) appeared in MI.

In preparations with six or more homologous K4s per meiotic K complement, the K4s formed in addition to quadrivalents, quinquivalents (Fig. 2h) and hexavalents (Fig. 2i), but these configurations were rare. Hexavalents were the largest multivalents of K4s observed in MI cells containing 10 K4s, although theoretically octovalents or decavalents might be formed.

In the present investigation 66 G-banded testes preparations of 48 larvae were analyzed. In these, K multivalents occurred in 43 preparations of 31 larvae.

The centromere orientations of K4 chain quadrivalents and hexavalents were studied in acetocarmine-stained MI cells, because the hypotonic treatment of testes prior to G-banding causes a swelling of chromosomes and nucleus and thus disaggregates the centromere orientations. The characteristic morphology of K4 allows the identification of this K type even without G-banding. Of the 18 K4 chain multivalents found in animals 1-3 (Table 1), 14 exhibited alternate centromere orientations (Fig. 2i), while 4 showed no clear orientation. In animal 1 with K complements containing 8 or 10 K4s, about one-third of the MIs analyzed (12 of 34 cells) showed K4 multivalents.

Table 1. K multivalent frequencies in spermatocyte MIs of *Acricotopus lucidus*

Animal No.	No. of cells	No. of multivalents/cell						No. of multivalents composed of:	
		None*	1 III + 1 I	1 IV	2 IV	1 V + 1 I	1 VI	homologous K†	nonhomologous K†
Acetocarmine stained									
1	34	21(13-16)	1	8	2	1	1	13 (12 K4, 1 ?)	—
2	29	21(11-14)	1	7	—	—	—	8 (8 K4)	—
3	30	27(11-14)	1	1	—	—	1	3 (3 K4)	—
G-banded									
4	53	24(11)	2	27	—	—	—	26 (26 K4)	3 (3 K2/K3)
5	32	23(13-15)	—	8	—	—	1	8 (5 K1, 3 K4)	1 (K3/K4/K4)
6	24	16(10-12)	—	7	1‡	—	—	8 (3 K2, 5 K4)	1 (K2/K3)
7	21	19(13-15)	—	2	—	—	—	2 (2 K2)	—
8	22	15(13-14)	—	7	—	—	—	7 (3 K2, 4 K4)	—
9	13	12(13-14)	—	1	—	—	—	—	1 (K1/K3)
10	20	19(14-16)	—	1	—	—	—	1 (K4)	—
11	18	16(14-17)	—	2	—	—	—	2 (2 K4)	—
12	24	19(14-15)	—	5	—	—	—	3 (3 K4)	2 (K1/K2, K2/K3)
13	18	12(12-14)	—	6	—	—	—	6 (2 K1, 4 K4)	—

*“None” indicates that only bivalents were formed. The number of bivalents formed is given in parentheses.

†Composition is given in parentheses.

‡One K2 quadrivalent and one K4 quadrivalent (see Fig. 2e).

As reported previously (Table 1 in Staiber 1988) each of the *A. lucidus* larvae has different compositions of gonial K complements. Even in the two testes of one animal, or within one testis, cells with different K complements may be present. Thus it is difficult to decide (e.g., by analyzing MIIs of the same gonad) whether in metaphase I - anaphase I an equal or unequal distribution of multivalent chromosomes has occurred.

One exceptional G-banded preparation exhibited the same K complement composition of 16 Ks, among them six K4s, in all MI cells analyzed (animal 4, K complement composition: 2 K1, 2K2, 2K3, 6K4, 2K7, 2K9). In 26 MIs (of a total of 53 cells) the K4s formed chain quadrivalents (30.8%; see Table 3), ring quadrivalents (38.4%), other types of quadrivalents (23.1 %), or trivalents with univalents (7.7%). Hexavalents were not observed. In analyzing MIIs of the same preparation three K4s were always found, so that one can infer to a correct distribution of the K4 multivalent chromosomes in metaphase 1 - anaphase 1.

If autobivalents were normally formed in *A. lucidus*, the multivalent formation resulting from crossovers between homologous but not identical Ks would be a possibility for reaching genetic recombination within a K type.

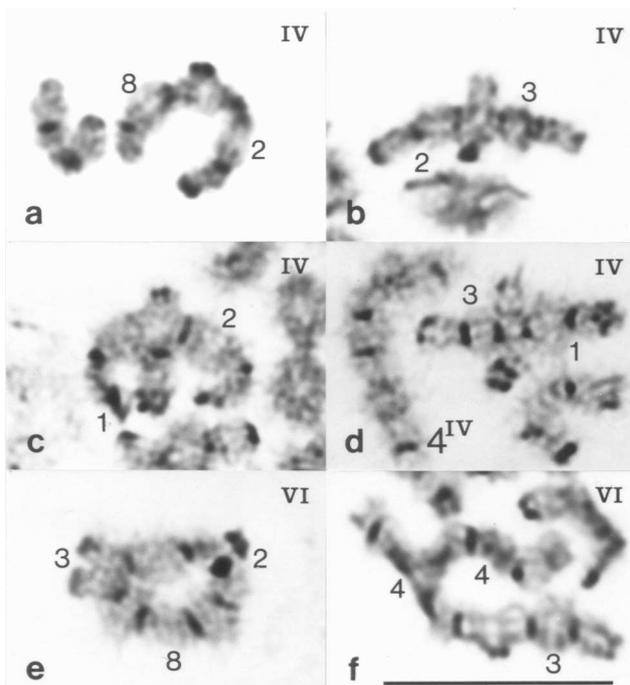


Fig. 3. G-banded multivalents composed of nonhomologous K chromosomes (a) Quadrivalent (IV) of K2 and K8. (b) IV of K2 and K3. Animal 6. (c) IV of K1 and K2. Animal 12. (d) IV of K1 and K3, by the side of it a chain IV of K4. (e) Hexavalent (VI) of K2, K3, and K8. (f) VI of K3 and K4. Animal 5. Bar represents 10 μ m.

Multivalents composed of nonhomologous Ks were found in 13 testes preparations of 12 animals. They were quadrivalents formed by different combinations of two types of Ks (Table 2; Figs. 3a -3d) and hexavalents formed by two types (Fig. 3f) or three types (Fig. 3e) of Ks.

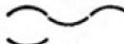
Multivalents consisting of homologous Ks as well as of nonhomologous can both occur in the same preparation (Table 1).

Table 2. Compositions of K multivalents found in spermatocyte MIs

Homologous Ks		Nonhomologous Ks	
Multivalent	Composition	Multivalent	Composition
III	K4	IV	K1/K2, K1/K3, K2/K3, K2/K8
IV	K1, K2, K3, K4	VI	K2/K3/K8, K3/K4/K4
V	K4		
VI	K4		

Multivalent formation of nonhomologous Ks was observed mostly between the four largest Ks, K1 - K4 (Tables 1 and 2). Multivalents composed of Ss and Ks were not found.

Table 3. Frequencies of K4 multivalent configurations in animal 4 exhibiting six K4s in all MI cells

Configuration		Frequency
Ring IV (Fig. 2e)		10
Chain IV (Fig. 2d)		8
“Figure 8” IV		1
“Frying-pan” IV (Fig. 2f)		3
“Cross” IV		1
“Y” IV		1
III and I (Fig. 2g)		2
Total no. of cells with K4 multivalents		26

Chiasma formation between different K types are probably the result of crossovers between homologous segments of partially homologous chromosomes. Such segments might be those sections of S homologous sequences that could be proved to be parts of the Ks (Staiber and Thudium 1986). This would support the hypothesis that the Ks derive from Ss by rearrangements.

Crossovers between nonhomologous Ks result in new K types and may be of importance for the evolution of the K type diversity. The occasional occurrence of an unusual small K type in A.

lucidus (Staiber 1987) might be interpreted as the result of such nonhomologues pairing and crossing over.

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