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X-ray induced rearrangements between germ-line limited and soma chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae)

W. Staiber and D. Thudium

Institut für Allgemeine Genetik,
Universität Hohenheim,
Garbenstrasse 30, 7000 Stuttgart 70, FRG

Abstract

The germ-line limited chromosomes (Ks) [K being derived from 'Keimbahn' (Bauer, 1970)] of *Acricotopus lucidus* were studied in gonial and differential mitosis. After C-banding the soma chromosomes (Ss) are stained only at their centromeric regions whereas the Ks exhibit centromeric, intercalary and terminal heterochromatin. By X-raying sperms it was attempted to transfer K sections on or into Ss in order to bring finally S-linked K sections to polytenization in the salivary glands, and to obtain more knowledge about the structure of Ks. Seven F_1-larvae were detected with K-S-rearrangements: four with insertions of heterochromatic segments, two with insertions of sections with S-homologous banding pattern and one with a translocated K chromosome part, which consists of S-homologous euchromatic sections as well as of an intercalary and a terminal heterochromatic segment. The present results strongly suggest that the Ks of *A. lucidus* are derived from the Ss by rearrangements and by formation and accumulation of repetitive sequences.

Introduction

Bauer and Beermann (1952a) established that in Orthocladiinae, a subfamily of Chironomidae, the germ-line cells contain, in addition to the regular chromosomes of the soma (Ss), a varying number of so called 'Keimbahn'-chromosomes (Ks). During the early cleavage divisions, the Ks are eliminated from the prospective soma cells. For bisexual Orthocladiinae, this complex cycle of divisions and eliminations of Ks and Ss was described in detail by Bauer and Beermann (1952b) and Bauer (1970).

To get insight into structural details of Ks, Bauer (1970) X-rayed newly hatched females of *Smittia parthenogenetica*, a parthenogenetic species of Orthocladiinae, with the aim of transferring parts of the Ks on or into the Ss, avoiding thereby the soma-elimination and achieving their polytenization. Two cases of heterochromatin insertions into Ss could be identified by Bauer as K sections.

In the present investigation we studied the Ks of *Acricotopus lucidus*, a bisexual species of Orthocladiinae, by C-banding technique and tried, like Bauer, to get X-ray induced K-S-rearrangements.

Material and methods

Animals of different inbred lines of *Acricotopus lucidus* Staeger were used for the experiments. The larvae were reared at 10-12°C under conditions described by Panitz (1964).

For C-banding the method of Hsu (1971), modified, was followed. The air-dried squash preparations from gonads of male and female larvae of different ages (previously fixed in ethanol acetic acid, 3:1, for 15 min, squashed after the dry ice method) were treated with 2 x SSC, adjusted to pH of 12 by NaOH, for 1 min, then rinsed three times in 2 x SSC for 5 min each time and incubated in 6 x SSC at 65 °C for 24 h. The slides were rinsed twice in 70% ethanol and twice in 95% ethanol, successively, air-dried and stained for 15 min in 10% Giemsa (Chroma, Stuttgart) in 0.01 M phosphate buffer, pH 7. The air-dried preparations were mounted in Euparal.

Male imagines were exposed to various doses from 1 500 R to 5 000 R, applied with a Seifert X- ray machine 'Eresco 200' (200kV, 5 mA). The X-rayed males were bred to non-irradiated females. The salivary glands of the F_1-larvae were fixed in ethanol-acetic acid (3:1, v/v) for 2 h and stained with carmine-acetic acid and then with orcein-acetic acid-lactic acid, according to the method described by Beermann (1952).

Results

A great variation in number of the Ks was observed in the different inbred lines of *A. lucidus*. In gonial mitosis, differential mitosis and meiotic stages, besides the Ss (n = 3), haploid from 4 to 19 Ks were found. On an average, the Ks were about twice as large as the Ss. Only one inbred line exhibited one K that was smaller than the Ss.

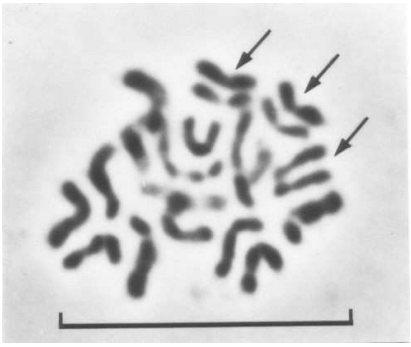

Fig. 1. Spermatogonial mitosis of a male third instar larva. The S chromosomes show somatic pairing and lie together in a group, see arrows. Bar represents 10 µm.

The SI-chromosome is submetacentric (arm ratio 1:2), the SII- and the SIII-chromosomes are nearly metacentric (arrows in Fig. 1). In metaphases SII and SIII cannot be distinguished with certainty from each other. In the K complement, all transition stages from subtelocentric to submetacentric chromosomes could be observed, except for metacentric Ks which could not be found.

During differential mitosis it is easy to distinguish the Ss from the Ks. The homologous Ss show somatic pairing (somewhat closer than during gonial mitosis) and still lie in the equatorial plate, whereas all Ks are already moving to or are already grouped around one single pole (Fig. 2). In both sexes the differential mitosis proceeds in the same manner.

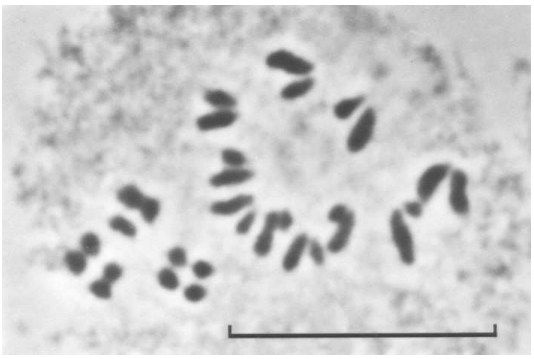

Fig. 2. Differential mitosis of a male fourth instar larva. The S chromosomes still lie together in the equatorial plate, while all K chromosomes are already moving poleward, but only to one pole. Bar represents 10 µm.

After C-banding the Ss were only stained within the range of the centromeric regions (Fig. 3a, b). In contrast to this, the Ks showed an intensive staining, which is not uniform over the whole length of the chromosomes, but concentrates mainly, but not exclusively, in the centromeric and terminal regions. According to the C-banding pattern, the Ks contain much more heterochromatin than the Ss, but the Ks also have distinct, euchromatic regions.

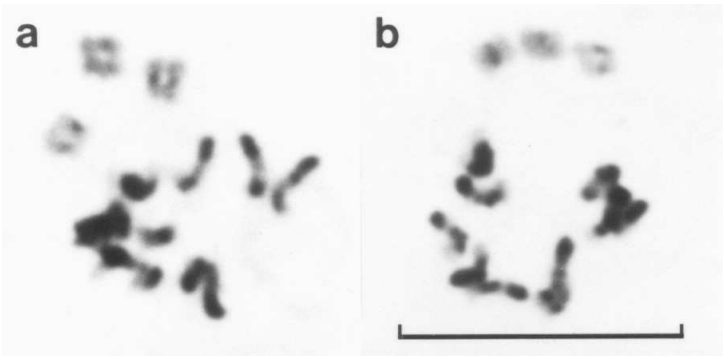

Fig. 3. C-banding: (a, b) male differential mitoses. The S chromosomes are only stained at the centromeric regions. In contrast to this the K chromosomes exhibit high amounts of heterochromatin in the form of intercalary and great terminal segments. Bar represents 10 µm.

In the male sex of *A. lucidus* the meiosis is finished before pupation, and male imagines as used for the X-raying contain only spermatozoa, no spermatogonia or meiotic stages. So, through irradiation only rearrangements within the haploid set of Ss and Ks could be induced.

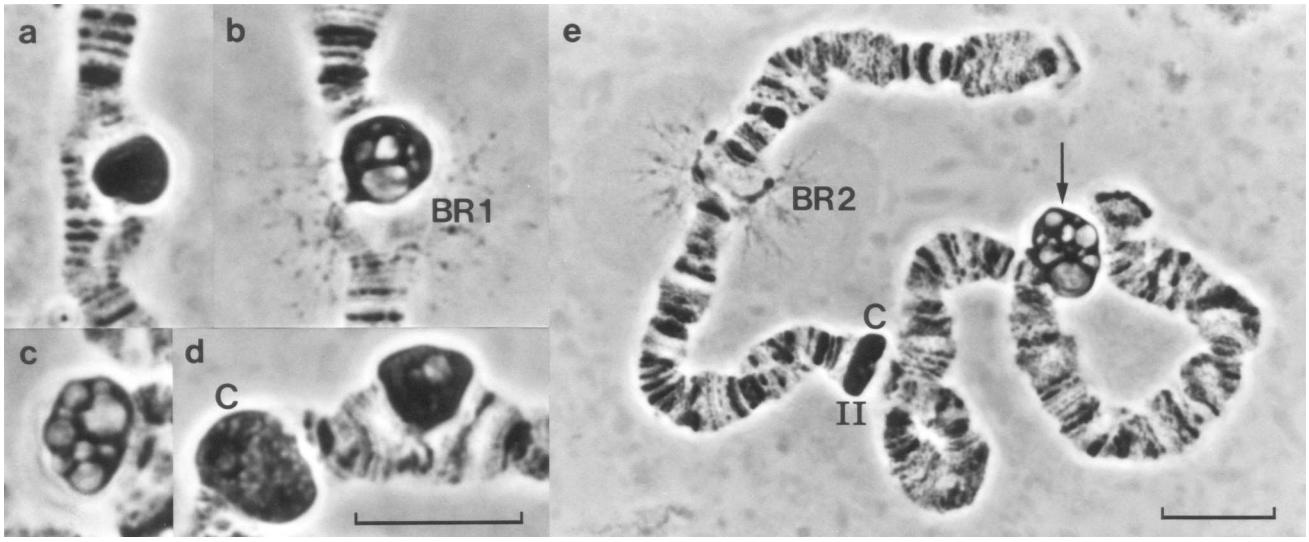

Fig. 4. X-ray induced K heterochromatin insertions: (a, b) in the left arm of SI, (a) anterior lobe, (b) main lobe with expanded BR 1; - (c) in the right arm of SI, main lobe; - (d) in the right arm of SII near the centromeric region, anterior lobe; - (e) in the right arm of SII near the BR 4-locus, main lobe. Arrow indicates the heterochromatin insertion. BR = Balbiani ring. C = centromeric region. Bars represent 10 µm.

In the X-ray experiments, the salivary gland chromosomes of 1085 F_1-larvae were checked. Of these, 216 exhibited chromosome mutations, among them 123 with interchromosomal rearrangements. Seven mutations could not be placed in the category of intra-S-rearrangements.

Four cases of insertions of heterochromatic segments:

— Insertion of a segment (length 4-6 µm, the length depending on the polytenic level of the nucleus, see below) into the short (= left) arm of SI near the Balbiani ring 1(= BR 1)-locus in section 1C68-1C84 (Fig. 4a, b; BR-pattern of *A. lucidus* see Mechelke, 1953, and Panitz, 1972; chromosome maps, Staiber and Behnke, 1985, where the bands received odd numbers and the interbands even numbers).

— Insertion of a segment (length 4.5-7 µm) into the long (= right) arm of SI near the BR 3-1ocus in section 2G56-2G60 (Fig. 4c).

— Insertion of a segment (length 4-5 µm) into the right arm of SII near the centromeric region in section 4A60-4A64 (Fig. 4d).

— Insertion of a segment (length 6-8 µm) into the right arm of SII near the BR 4-locus in the section 4E30-4E42 (Fig. 4e).

In most nuclei the heterochromatic segments are strongly vacuolized. Sometimes the segments are stuck together with the centromeric blocks. Within a salivary gland the different nuclei have not the same polytenic level. In the 4th larval stage and the prepupal stage this level ranges from 2048 to 8192 C (Speiser, 1973). In nuclei of higher polyteny the heterochromatin insertions are

larger than in nuclei of lower polyteny. This indicates, that the heterochromatin insertions replicate as euchromatin.

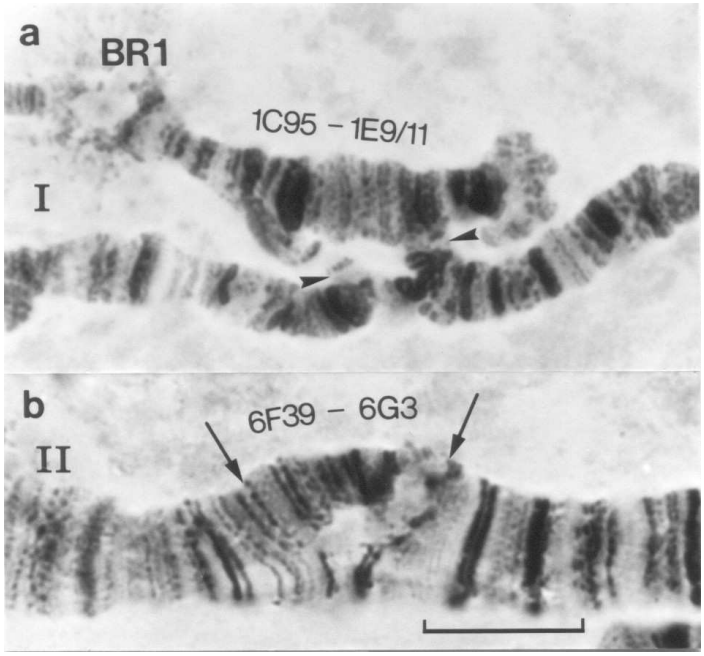

Fig. 5. X-ray induced insertions of supernumerary S-homologous sections: (a) insertion of the sequence 1C95-1E9/11 in the right arm of SI, the inserted section shows pairing with the homologous region in the left arm of SI, main lobe; - (b) insertion of the sequence 6F39-6G3 in the right arm of SII, anterior lobe. Arrows and arrowheads indicate the bounderies of the inserted sections. The chromosome in (b) has a higher polyteny than that in (a). Bars represent 10 µm.

Two cases of insertions of supernumerary euchromatic sections with a banding pattern homologous to S sections:

— Insertion of a section homologous to the S-sequence 1C95-1E9/11 (left arm of SI, 58-59 bands) into the right arm of SI in the section 2G26-2G30. Nearly in all nuclei the insertion and the homologous section in the left arm of SI are paired (Fig. 5a).

— Insertion of a section homologous to the S-sequence 6F39-6G3 (right arm of SIII, 33 bands) into the right arm of SII (break point in 4F68). In no nuclei does this insertion show pairing with the homologous section in the right arm of SIII (Fig. 5b), Both inserted sections exhibit the normal puffing pattern.

One case of a supernumary chromosome part, which is linked to the end of the right arm of the SIII (Fig. 6a), most probably as a result of a reciprocal translocation between SIII and a K chromosome - three bands of SIII are missing, 6I5, 6I7 and 6I9. So, this is an unbalanced translocation.

Beginning from the end of the right arm of SIIl the chromosome part is built as follows (Fig. 6b):

(1) A chromosome section of 14 bands, probably the bands 3C85-3D11 (left arm of SII). In no salivary gland nucleus, however, could a pairing of this section with the homologous region of SII be observed.

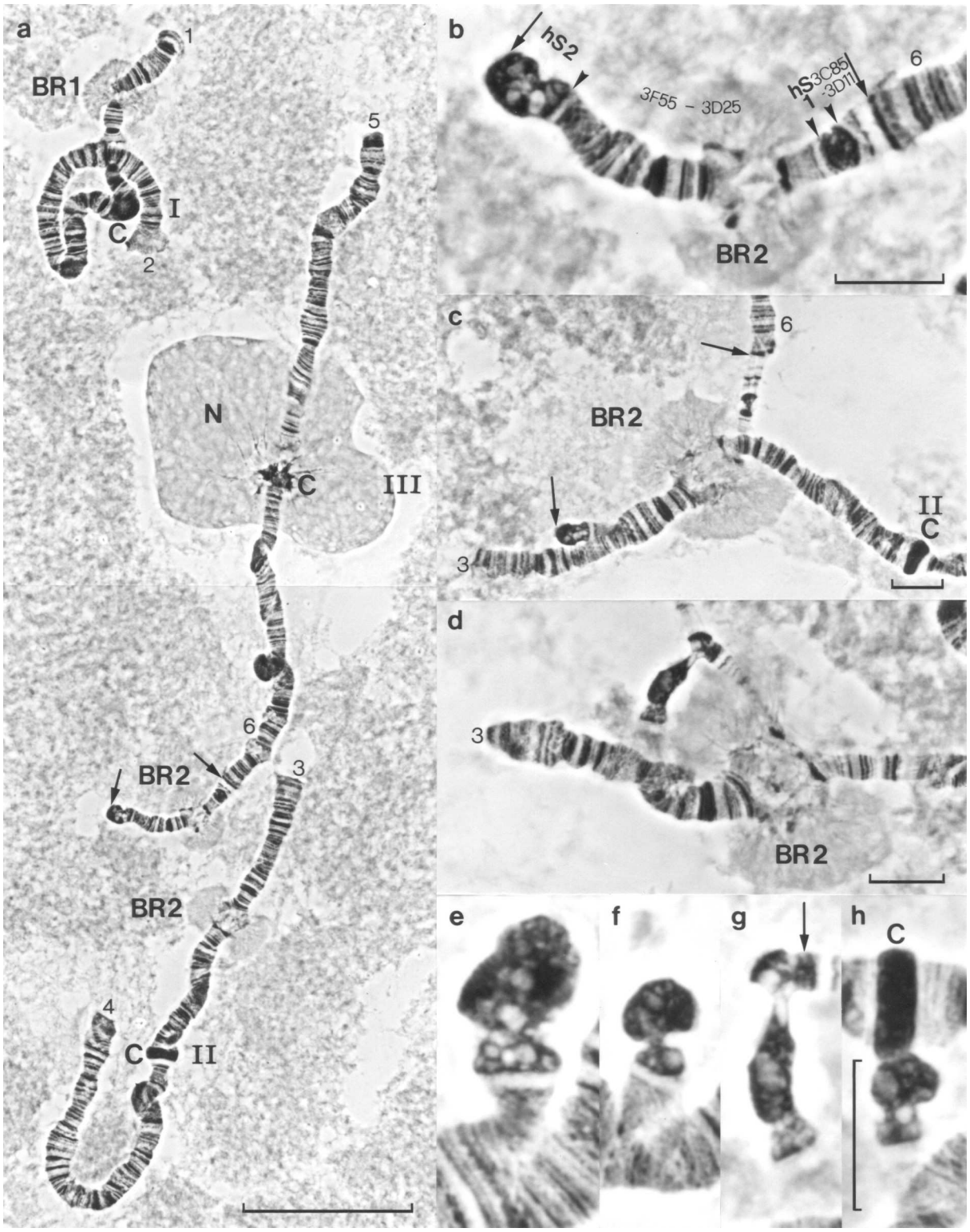

Fig. 6. X-ray induced translocation of a part of a K chromosome on the SIII chromosome: (a) Polytene S set of *Acricotopus lucidus* with the supernumerary K chromosome part (segment between the arrows), main lobe of the salivary gland. The main lobe-specific BR 2 is twice present; - (b) polytene K chromosome part of (a) at a higher magnification. Arrowheads indicate the boundaries of the eu- and heterochromatic sections; - (c) the euchromatic sections with the bands 3D25-3F55 show pairing with the homologous region of the left arm of SII. Arrows indicate the extent of the K chromosome part; - (d) hS 1 and hS 2 are stuck together; - (e, f) hS 2 in a nucleus with higher (e) and in one with lower (f) polyteny. The segment is strongly vacuolated and shows a characteristic bipartition; - (g) hS 1 and hS 2 of (d) at a higher

magnification. The arrow indicates banding structure in hS 1; - (h) hS 2 and the centromeric region of SI are stuck together. (e)-(h) have the same magnification, hS 1 = intercalary and hS 2 = terminal heterochromatin segment. N = nucleolus. 1 = short left arm of SI, 2 = long right arm of SI, 3 = short left arm of SII etc. (a) Bar represents 50 µm. (b-h) Bars represent 10 µm.

(2) A bipartite, heterochromatic segment (hS 1), length 2.5-5 µm, whose length depends on the polytenic level of the nucleus. One part of the segment is swollen and vacuolated in all cells. The other is usually swollen too, but in a few cells this part also shows banding (two bands; see Fig. 6g, arrow).

(3) A chromosome section with the bands 3D25-3F55 (left arm of SII, 116 bands). This section contains the BR 2-locus. In most of the nuclei, the section is paired with the homologous region of SII. In the main and side lobe cells with pairing, an enlarged BR 2 is expanded (Fig. 6c, d). In cells without pairing, two distinct BRs 2 are expanded (Fig. 6a).

(4) A terminal, also bipartite, heterochromatic segment (hS 2), length 5-10 µm, whose length depends on the polytenic level of the nucleus. Both parts are always swollen and strongly vacuolated. There is a tendency for the segment to stick together with hS 1 (Fig. 6d, g) or with one of the centromeric blocks (Fig. 6h). The bipartition of this segment into a smaller and a larger part occurs through something like a constriction. The increasing size of the terminal heterochromatin segment with the increasing polyteny of the nucleus (compare Fig. 6e with Fig. 6f) indicates that hS 2 is moving through replication cycles as the euchromatic sections do. The vacuolization within both heterochromatin segments is not uniform. There are very large, as well as very small, vacuoles. The chromatin between the vacuoles seems to be homogenous.

In all salivary gland cells the polyteny of the supernumerary chromosome part is about half that of the polyteny of the somatic paired Ss. In contrast to the rest of the S-chromosomal regions, the gene loci of sections 3C85 - 3D11 and 3D25- 3F55 are present in three instead of in two doses. Puffs, which are normally active in the homologous regions of SII, are also active in these two K sections. In this regard, the expansion of the BR 2, which is specific to the main and side lobe of the salivary gland, is most conspicuous. The normal puffing pattern of the S complement is not visibly affected by the presence of the supernumerary chromosome part.

Discussion

As detected by C-banding the Ks of *A. lucidus* contain high amounts of heterochromatin, above all in the form of terminal, but also of intercalary, segments. They also exhibit euchromatic sections. Naturally with metaphasic Ks, it cannot be determined whether these euchromatic sections are homologous and/or non-homologous (= K-specific) to S sections.

The question as to the exact structure of the Ks caused Bauer (1970) to make X-ray experiments with the aim of transferring parts of Ks on or into Ss, and to get, thereby, their polytenization. Bauer obtained two insertions of heterochromatin which could only be derived from Ks. Whether the Ks contain euchromatin, he could not answer.

In our X-ray experiments with *A. lucidus*, beside larvae with intra-S-rearrangements, larvae with heterochromatin insertions and with insertions of supernumerary S-homologous sections were found, as well as a larva with a supernumerary chromosome part that consisted of both euchromatic S-homologous sections and heterochromatic segments.

Several factors strongly suggest that supernumerary segments are parts of K chromosomes: since male imagines, as used for irradiation, contain exclusively spermatozoa, rearrangements can only be induced between chromosomes of the haploid set of the Ss and the Ks. Thus supernumerary S-homologous sections must derive from Ks. Large heterochromatin segments, similar to those present in the supernumerary segments, are only found in the centromeric regions of the salivary gland chromosomes. But in the present cases, the centromeric blocks of the three Ss show normal size; no loss of heterochromatin can be ascertained.

It is surprising, if one looks at the great number of 123 interchromosomal rearrangements and the great number of Ks which can be present in a sperm nucleus, that in only few cases K-S-rearrangements could be found. The reasons for the rarity of K-S-rearrangements probably are:
(1) Animals with K-S- rearrangements in the soma are very much affected in their development and have a low survival rate, for example in cases of unbalanced translocations, when too large sections of the S complement are lost.
(2) In the sperm nucleus, the Ss are more or less set off from the Ks, and the formation of intra-S-rearrangements is therefore more probable than the formation of K-S-rearrangements. The observation that in gonial mitosis the Ss often lie close together in a group (see Fig. 1), supports the assumption that such a spatial arrangement and association of the Ss may occur.

Since Heitz's investigations of the heterochromatic regions of mitotic chromosomes and the chromocenter-heterochromatin of polytene nuclei of *Drosophila* (Heitz, 1934), two types of heterochromatin have been distinguished: a compact, condensed, only rarely vacuolized heterochromatin which increases in size only a little if at all during polytenization (= α-heterochromatin); and a less condensed, mostly vacuolized heterochromatin which can show banding, and which grows during polytenization, as does the adjacent euchromatin (= β-heterochromatin). The underreplication of the α-heterochromatin of *Drosophila* during polytenization was verified by cytophotometric and cytofluorometric measurements (Rudkin, 1965; Berendes & Keyl, 1967; Lakhotia, 1984), by in situ-hybridization (Gall et al., 1971), and by EM-autoradiographic studies after ^{3}H-thymidine-labelling (Lakhotia, 1974). In the α- and β- heterochromatin of *Drosophila* especially, moderately and highly repetitive DNA-sequences are located (Rae, 1970; Gall et al., 1971; Renkavitz, 1978a, b). The less condensed, highly vacuolated structure and the increasing size of the heterochromatic segments with increasing polyteny indicate the presence of β-heterochromatin.

One of Bauer's heterochromatin insertions in *Smittia* (called 20.118; Bauer, 1970) was studied intensively by Hägele (1980). The insertion is C-banding positive, late replicating, inactive in RNA synthesis, all characteristic for heterochromatin. Moreover the insertion can be subdivided by N-banding into an N-positive part (= α-heterochromatin) and an N-negative part (= β-

heterochromatin). It cannot be excluded that the heterochromatin insertions and hS 1 and hS 2 of the translocated K chromosome part also consist of different types of heterochromatin. Unfortunately it is not possible to check this, because the F_1-larvae with the K sections were dissected in the process of study.

The expression of the BR 2 shows especially well that genes which lie in the euchromatic S-homologous sections of the translocated K chromosome part can be activated. The BR 2 represents even a specific gene activity of the main and side lobe of the salivary gland (Mechelke, 1953).

To judge by the wide range of K chromosome numbers which can be found in *A. lucidus* (haploid from 4 to 19 Ks) without any phenotypic differences or changes in fertility, the Ks seem to be subject to no strict selection.

The nearest explanation for the occurrence of additional chromosomes in the germ-line is that they have a function in germ-cell formation or maturing. Results of experiments on the Cecidomyiid *Wachtliella persicariae* support this consideration (Geyer-Duszyńska, 1966). That the Ks are really active in transcription during oogenesis of *Wachtliella* was demonstrated by Kunz et al. (1970).

Experiments of Nicklas (1959) on the Cecidomyiid *Miastor* indicate that Ks are disadvantageous for soma development and must, therefore, be eliminated from the soma nuclei. The elimination of the Ks possibly takes place because their very large content of heterochromatin (at least in *A. lucidus*) would affect genetic activities of the S complement.

Summarizing the results of the present study: The Ks of *A. lucidus* consist of euchromatic as well as of heterochromatic sections. There are euchromatic K sections, which are homologous to S sections. It cannot be excluded, that euchromatic K sections may also exist with a K-specific banding pattern. To elucidate this, further experiments are necessary. The staining pattern of the Ks after C-banding, and the structure of polytenized K sections, strongly suggest that the Ks of *A. lucidus* are derived from Ss by rearrangements and by the formation and accumulation of repetitive sequences.

Acknowledgement

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