Genome (1987) 29:702-705
DOI: 10.1139/g87-120 

# Unusual germ line limited chromosomes in *Acricotopus lucidus* (Diptera, Chironomidae)

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**Abstract**

A small supernumerary polytene chromosome was found during the last 8 years in some rare cases in larval salivary gland cells of *Acricotopus lucidus* (Diptera, Chironomidae). The chromosome may be derived from the germ line restricted parts of the genome. It consists of a short heterochromatic segment and of euchromatic sections with banding patterns homologous to sections of the short arm of soma chromosome I. When examining male meiosis, an exceptional small germ line limited chromosome was found. It is believed that this chromosome was not always recognized during soma elimination as a germ line limited chromosome, probably because of its partial homology to one of the soma chromosomes, and was then polytenized in salivary gland cells. Another germ line limited chromosome with a characteristic morphology and with a special behavior in differential gonial mitosis was found to have existed for more than 12 years in a laboratory stock. In differential gonial mitosis this special germ line limited chromosome partly pairs with the long arm of soma chromosome I. The present results strongly support the idea that the germ line limited chromosomes of *A. lucidus* are derived from the soma chromosomes, and show that chromosomes of the germ line restricted part of the genome can persist for many generations in a laboratory stock in spite of complex chromosome elimination mechanisms in primary germ cells.

**Key words**: germ line limited chromosomes, supernumerary polytene chromosomes, salivary gland, *Acricotopus lucidus*

## Introduction

During germ line - soma differentiation of the dipteran *Acricotopus lucidus* (Chironomidae, Orthocladiinae) parts of the genome, the germ line limited chromosomes (=K's, being derived from "Keimbahn"; Bauer 1970) are eliminated from the prospective soma cells (Bauer and Beermann 1952). The elimination of the K's takes place by lagging during early cleavage divisions. In many larval tissues of *A. lucidus* the homologous soma chromosomes (= S's; 2n = 6) pair and form by polytenization three giant chromosomes exhibiting, especially in the salivary gland, a distinct and reproducible banding pattern (Mechelke 1953; Staiber and Behnke 1985). A detailed description of the complex chromosome cycle of K's and S's in bisexual Orthocladiinae was reported by Bauer (1970).

Bauer (1970) X-rayed newly hatched females of the chironomid *Smittia parthenogenetica* to transfer parts of the K's into S's. Similar irradiation of sperm of *A. lucidus* resulted in the demonstration that the K's contain heterochromatic segments and S-homologous euchromatic sections (Staiber and Thudium 1986). Nevertheless, many questions as to the structure and also the function of the K's are still to be answered (for review Hennig 1986).

Over the last few years, in some rare cases, the same supernumerary chromosome has been found in larval salivary glands of *A. lucidus*. It is assumed that the supernumerary chromosome is a germ line limited chromosome, not recognized as such during soma elimination because it consists mainly of sections homologous to one of the S's.

## Materials and methods

The larval salivary glands obtained from larvae of a laboratory stock of *Acricotopus lucidus* Staeger with chromosome I constitution CF (Wobus et al. 1971) were stained as described by Staiber ande Behnke (1985).

To investigate gonial mitosis and meiosis, larval and prepupal testes, previously fixed in freshly prepared ethanol - acetic acid (3:1, v/v), were either stained in 0.5% lactoacetic orcein or, with air-dried preparations (made after the dry ice method), in 2% Giemsa in 0.01 M Sörensen phosphate buffer, ph 7.2.

## Results and discussion

A small supernumerary polytene chromosome was found in salivary gland cells of six larvae of *A. lucidus* at different times in the last years in our laboratory strain (February 1979, August 1979, November 1979, February 1980, February 1980, October 1986). Each time the chromosome appeared only in a few cells of one of the two salivary glands of a larva. The supernumerary chromosome was observed to be present in 27, 4, 4, 3, 19, and 8 cells of the some 70-80 cells of a gland. In each case the chromosome was paired with the short left arm of the polytene SI in section 1D (Figs. la and lb).

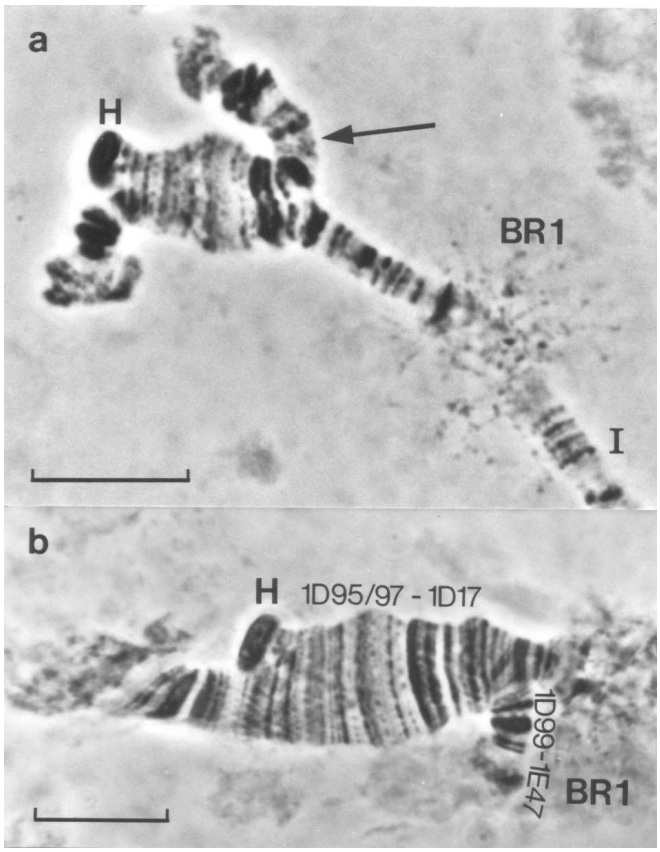


FIG. 1. (a) Supernumerary polyene chromosome (arrow), found in October 1986, which is paired with the short arm of soma chromosome I. (b) Pattern of bands of the supernumerary chromosome, found in February 1979. BR 1, Balbiani ring 1. H, heterochromatic segment. Bar = 10 µm

The chromosome consists of a heterochromatic segment of about 1.5 - 2 µm in length and of an euchromatic section of about 70 bands. Starting from the heterochromatic block there is the S-homologous section 1D95/97 - 1D17 (40-41 bands), the section in which the supernumerary chromosome is synapsed with SI; a section of about five unidentificd bands; and the S-homologous section 1D99-1E47 (25 bands, Fig. 1b*).* 1E47 corresponds to the telomeric band of the short left arm of SI (Staiber and Behnke 1985). Most probably the centromere of the supernumerary chromosome is located within the heterochromatic block. The morphology of this block is comparable with that of the centromeric regions of the polytene S's; it differs remarkably from the structure of the X-ray induced K-heterochromatin insertions (Staiber and Thudium 1986).

The supernumerary chromosome is estimated to have the same degree of polyteny as the somatically paired polytene S's. Supposing as many replication cycles as in the S's, the presumable K had to be present before polytenization in duplicate.

The supenumerary polytene chromosome does not affect the regular puffing pattern of the S complement in those cells of a salivary gland that possess the additional chromosome. Probably the S complement tolerates this genomic unbalance because it is only a small part of the S complement that is present in fourfold instead of in twofold doses. That the bands of section 1D really exist in the germ line restricted parts of the genome was experimentally established by

transferring euchromatic K sections into S's by X-raying sperms of *A. lucidus* (see Fig. *5a* in Staiber and Thudium 1986).

Supernumerary polytene chromosomes in larval salivary glands of chironomids have also been found in populations of *Chironomus plumosus* and *Chironomus melanolus* (Keyl and Hägele 1971), but these species lack germ line limited chromosomes. Furthermore, the additional chromosomes were present in all cells of soma and germ line investigated of individuals possessing supernumerary chromosomes.

In the laboratory stock in which the supernumerary chromosome of Fig. la occurred in salivary gland cells, an exceptional small K was found in different stages of spermatocyte meiosis (arrows in Figs. 2a and 2b). Its length was about one-third of one of the S's. Usually the K‘s of *A. lucidus* are about double the size of the S's. In anaphase I, beside normal equal separation, the small K is frequently observed to migrate to one of the spindle poles (Fig. 2b).

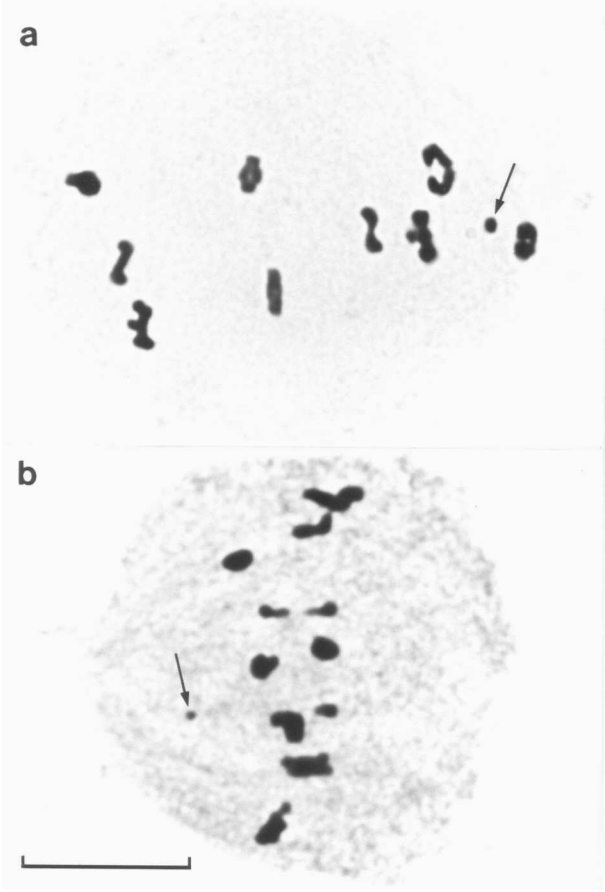


FIG. 2. (a) Spermatocyte metaphase I of *A. lucidus* with three S and seven K bivalents, among them an exceptional small K (arrow; Orcein staining). (b) Early anaphase I. The small K moves to one pole (arrow; Giemsa staining). Bar = 10 µm.

The elimination of the K's from the prospective soma cells takes place by lagging during the 5th cleavage division about 2.5 h after oviposition (Thudium 1974). lt is strongly supposed that the small K was not recognized as a K in the process of soma elimination at this early embryonic stage because it consists mainly of sections of the S complement.

The repeated occurrence of the identical polytenized K over a time of about 8 years indicates that K's can persist in the germ line of *A. lucidus* over many generations, in spite of the elimination of about one-half of the K's from the germ line during the first divisions of the primary germ cells (Bauer 1970). The same conclusion may be drawn from the observation of a special K first described by Thudium (1974), which is still present after more than 12 years in the germ line of an *A. lucidus* laboratory stock. Estimated at about six generations per year, this would amount to about 72 generation.

The special K differs from the other K's by its characteristic morphology, being acrocentric with an arm ratio of about 1:3 (Figs. 3b and 3c), and by its special behavior during differential mitosis. In differential mitosis, the last gonial mitosis, the reduction of the K's in the primary germ cells is compensated by the process that all K's move undivided to one pole, while the somatically paired S's first remain in the equatorial plate (Fig. 3a) and then make a normal equal separation. In the male sex a spermatocyte (2S- and 2K-chromosome sets) and an aberrant spermatocyte (2S-chromosome sets) are the result. In differential mitosis the special K can be observed either lying between the S's and the K's, or being associated with the S's, or partially paired with the long arm of SI (Figs. 3b and 3c). The partial pairing of the special K and SI indicates a partial homology between these chromosomes; that is, both types of chromosomes contain the same SI section(s). However, the exceptional behavior of the special K cannot be ascribed only to the presence of SI-homologous sections, because the K's also contain sections of SII as established by polytenized K-S rearrangements (Staiber and Thudium 1986). A pairing of one of the K‘s with SIl was never observed.

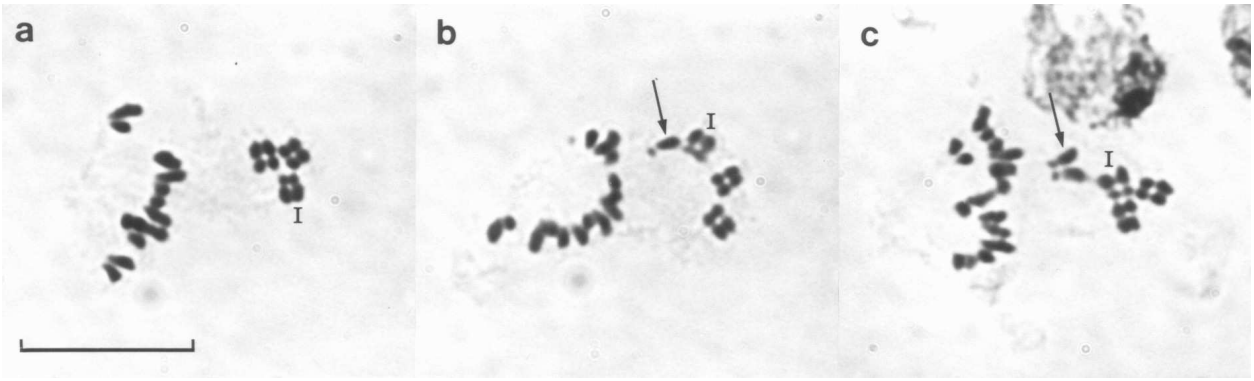


FIG. 3. Male differential mitoses of *A. lucidus.* The K's are assembled undivided around one pole, while the somatically paired S's still lie in the equatorial plate. Differential mitoses from the same testis exhibiting none (a), one (b), and a pair (c) of a special K (arrows in band c). In b and c the special K is partially paired with SI. Bar = 10 µm.

In the same testis, differential mitoses could be found, exhibiting either none, one, or a pair of the special K (Figs. 3a, 3b, and 3c). From that, one can conclude that either a nondisjunction of the special K in gonial mitoses occurred or that an elimination of both (paternal and maternal), one, or none of the special Ks took place in the primary germ cells.

Thudium (1974) reported that in differential mitoses the special K can either behave as the other K's, that is, move undivided to one pole, or it can make a normal anaphasic separation, as do the S's, so that each of the generating cells gets one chromatid. Thus, the special K takes up an intermediate position between S's and K's.

Both types of the above-mentioned K's contain rearranged SI sections. Interesting in this connection is that in natural populations of *A. lucidus* the SI especially exhibits a tendency to rearrangement (Wobus et al. 1971; Panitz 1972).

The present results support former findings resulting from X-ray experiments and C-banding (Staiber and Thudium 1986); namely, that the K's of *A. lucidus* contain S-homologous sections and that the K's may therefore be derived from the S's by rearrangements. As has been established, there are K‘s that persist in a laboratory stock for many generations, despite the elimination of about one-half of the K's from the primary germ cells during the complex chromosome cycle of *A. lucidus.* The process of soma elimination seems not to be an absolute process because mistakes do occur, and in rare cases therefore K's do pass into the soma.

**Acknowledgement**

I wish to express my thanks to Professor F. Mechelke for encouragement and support.

**References**

BAUER, H., 1970. Rearrangements between germ-line limited and somatic chromosomes in *Smittia parthenogenerica* (Chironomidae, Diptera). Chromosoma, 32: 1-10.

BAUER, H., and BEERMANN, W. 1952. Chromosomencyclus der Orthocladiinen (Nematocera, Diptera). Z. Naturforsch., 7b: 557-563.

HENNIG, W. 1986. Heterochromatin and germ line-restricted DNA. *In* Results and problems in cell differentiation. Vol. 13. Germ line - soma differentiation. *Edited by* W. Hennig. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo. pp. 175-192.

KEYL, H.-G., and HÄGELE, K. 1971. B-Chromosomen bei *Chironomus*. Chromosoma, 35: 403-417.

MECHELKE, F. 1953. Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von *Acricotopus lucidus.* Chromosoma, 5: 511-543.

PANITZ, R. 1972. Balbiani ring activities in *Acricotopus lucidus. In* Results and problems in cell differentiation. Vol. 4. Developmental studies on giant chromosomes. *Edited by* W. Beermann. Springer-Verlag Berlin, Heidelberg, New York. pp. 209-227.

STAIBER, W., and BEHNKE, E. 1985. Developmental puffing activity in the salivary gland and Malpighian tubule chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae). Chromosoma, 93: 1-16

STAIBER, W., and THUDIUM, D. 1986. X-ray induced rearrangements between germ-line limited and soma chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae). Genetica (The Hague), 69: 149-156.

THUDIUM, D. 1974. Das Verhalten von Soma- und Keimbahnchromosomen bei *Acricotopus lucidus* Staeger (Orthocladiinae, Diptera). Doctoral thesis, University of Hohenheim, Stuttgart.

WOBUS, U., SERFLING, E., BAUDISCH, W., and PANITZ, R. 1971. Chromosomale Strukturumbauten bei *Acricotopus lucidus* korreliert mit Änderungen im Proteinmuster. Biol. Zentralbl. 90: 433-442