Chromosoma (Berl) (1988) 97:231–234

CHROMOSOMA

G-banding of germ line limited chromosomes in *Acricotopus lucidus* (Diptera, Chironomidae)

Wolfgang Staiber
Institut für Allgemeine Genetik, Universität Hohenheim. Garbenstrasse 30, D-7000 Stuttgart 70, Federal Republic of Germany

Abstract. The germ line limited (K) chromosomes of *Acricotopus lucidus* (Diptera, Chironomidae) were stained for G-banding on gonial mitoses, along with the somatic (S) chromosomes. Nine different types of K chromosomes could be distinguished by the G-banding pattern and other cytological criteria. Various combinations of K chromosomes were found in the complements of different individuals and cells: some Ks were missing and others were present up to as many as five times. No two animals were completely alike in the composition of their gonial chromosome complement. Thus none of the different K types can be essential. These results are discussed in view of the complex chromosome cycle of the Orthocladiinae.

Introduction

In *Acricotopus lucidus* (Chironomidae, Orthocladiinae) the presence of additional chromosomes limited to the germ line, and the complex chromosome cycle that these "Keimbahn" chromosomes (Ks) and the somatic chromosomes (Ss) pass through, were first reported by Bauer and Beermann (1952). The mean number of Ks differs from species to species with up to 80 or so in *Cardiocladius* (Beermann 1956), and close to 0 in *Cricotopus silvestris* (W. Beermann, personal communication). Germ line limited chromosomes are also found in other Dipteran families, such as the Sciaridis and Cecidomyids. For the latter, an attempt has been made to interpret the germ line supernumeraries as descendants of the S complement by way of endopolyploidy. Here, as in the Orthocladiinae, the lack of cytological detail has so far precluded access to information on the evolution of the K chromosomes. Thus, the successful application of banding techniques in *Acricotopus* is of particular interest.

Materials and methods

Gonads of young fourth instar larvae (size 4.5–5.5 mm) of a laboratory stock of *A. lucidus*, derived from one egg batch, deposited in July 1985, were dissected in Firling medium, treated with hypotonic solution (0.5% sodium citrate) for 10–20 min and fixed in 3:1 ethanol: acetic acid. The preparations, made by the dry ice method, were air-dried and stored for 1–2 weeks. They were then heated to 100° C for 1 h 20 min. Cooled slides were placed in 0.1% trypsin solution (trypsin 1:250, Difco) for 2 min, stained in 3% Giemsa in Sörensen's phosphate buffer, pH 6.9 for 30 min, and rinsed three times in distilled water. The air-dried preparations were mounted in Euparal and were photographed using a Planapo 63/1.4 objective (Zeiss).

Results and discussion

In the present study, metaphases mainly from the last, differential gonial mitoses were examined. Subsequent to the elimination of about half the Ks in the first division of the primordial germ cells, all of their descendants, i.e. the gonial cells in both sexes, retain the reduced complement up until the last division before meiosis. This division is differential with respect to the distribution of daughter K chromosomes at anaphase, which in contrast to the

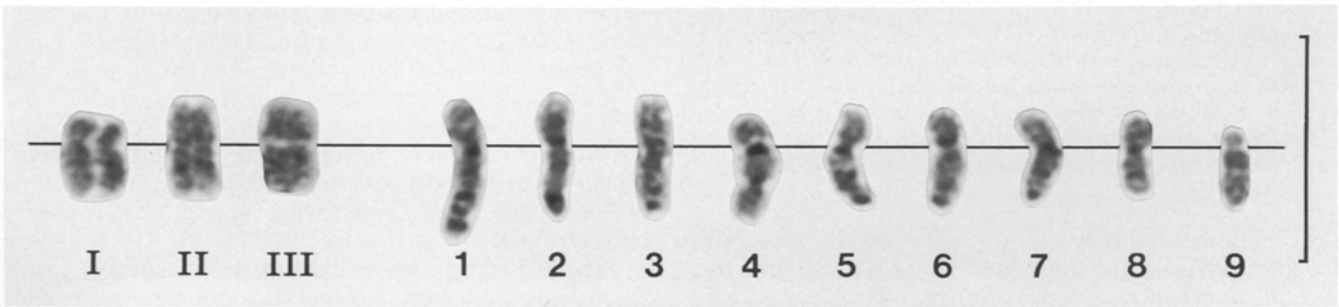

Fig. 1. The G-banded somatic chromosomes (Ss) and germ line limited chromosomes (Ks) from male gonial mitoses of *Acricotopus lucidus*. The short chromosome arms are oriented towards the top. *I, II, III* SI, SII, SIII. *1, 2, ...,9* K1, K2, ..., K9. Bar represents 10 μm

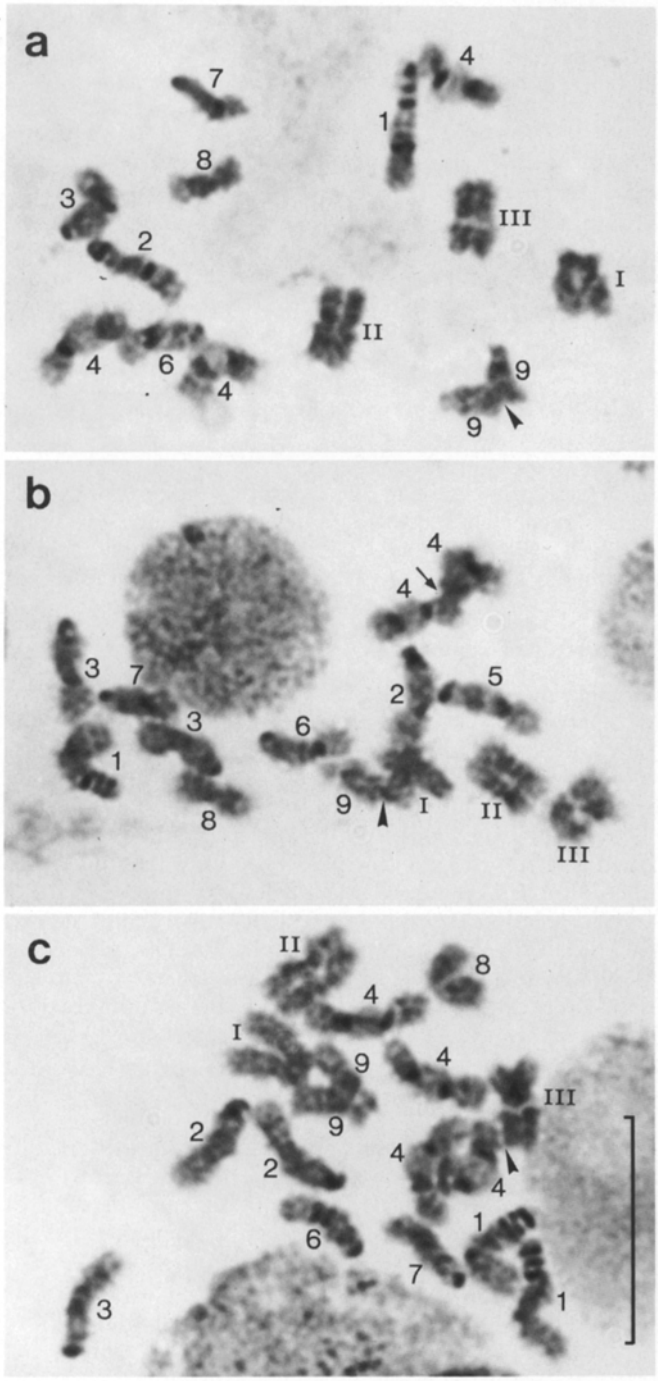

Fig. 2a–c. G-banded metaphases of spermatogonial mitoses. **a** From animal no. 8 with 11 Ks; 8 different K types are present. The homologous Ss are paired. *Arrowhead* indicates partial pairing of the K9 chromosomes. **b** From animal no. 20 with 11 Ks. All 9 K types are present. *Arrowhead* and *arrow* indicate partial pairing of K9 with SI and the partial pairing of the homologous K4s. **c** From animal no. 1 with 14 Ks. *Arrowhead* indicates partial pairing of K4 with SIII. Bar represents 10 μm

S chromosomes all move to the same spindle pole. The daughter cell with the duplicated set of Ks will give rise to a functional spermatocyte, or oocyte, where each duplicated K represents an "auto-bivalent".

Following G-banding, the Ss and Ks exhibit characteristic staining patterns, allowing reliable karyotyping (Fig. 1) from well-spread metaphases. Because of the close somatic pairing of the homologues, the Ss ($n=3$) can be easily distinguished from the Ks (Fig. 2a–c). SI is submetacentric, SII and SIII are nearly metacentric and are approximately the same size. SIII carries the only nucleolus of the S set, as described by Mechelke (1953) for the polytene salivary gland chromosomes. Definitive discrimination of SII and SIII was only possible after sequential Ag-staining of the nucleolus organizer regions (NORs) and G-banding (Mayr et al. 1987) carried out on ganglion metaphases. The NOR is located within the weakly stained section in the middle of SIII.

The number of chromosomes found in the K complement of male gonial mitoses ranges from 6 to 16 (Table 1), with the majority of K complements having 9–12 Ks. The intra-individual variation in number of Ks (i.e. within one gonad and between the gonads of the same animal) never exceeds 2 Ks (e.g. nos. 22, 23). The variation between animals of the same batch of eggs ranged up to 4 Ks (nos. 3 and 4).

The 9 different types of Ks in various combinations form the K complement (Table 1). Each of the Ks has a typical appearance, e.g. K1 with a triplet of thick dark bands in the terminal region of the long arm, K2 with a thick and a small hood-like dark band on the end of the long arm or K4 with a puffed section in the middle of the chromosome. Altogether, some 80 dark G-bands could be identified on the 9 K types and about 20 on the 3 Ss.

Because of the hypotonic treatment of the gonads prior to fixation, effecting a swelling of the chromosomes which varied depending on the duration of treatment, it is not appropriate to record the absolute lenghts of the metaphase Ss and Ks. In the metaphase in Figure 2b, where all K types are present, and also in Figure 1, the sum of the lengths of the nine different K types is about four times that of the three Ss.

As can be seen in Table 1, K complements were as a minimum formed by combinations of 5 different K types (nos. 4, 27). Maximally all 9 K types were present (nos. 12, 20 see Fig. 2b). Mainly 6–8 different K types were found but, more rarely, either 5 different or all 9 K types. None of the 31 larvae analysed agreed with any other in the composition of the gonial K complements. Each animal had a specific combination(s) of K types.

Table 1 demonstrates that some Ks were found more frequently than others, e.g. K2, K3, K4, K9 in 30 larvae against K5 or K7 in only 10 and 17 larvae respectively of the 31 larvae (28♂, 3♀). Each K type can be absent from the K complement, but it can also be present once or twice. Differing from the others, K3 and K4 can be present three times (Fig. 2a) and K4 even four (Fig. 2c) or five times (nos. 16, 19, 22) per K complement. Comparing all K types, the above mentioned intra-individual variation in number of Ks was caused most frequently by the variation of K4.

If a K type was present twice in the K complement, a spatial association of the homologues was often observed similar to that of the K1s and K2s in Figure 2c. Pairing of homologues, or at least partial pairing, was frequently observed in K4s and K9s (see Fig. 2a, b). K9s can reach a stage of pairing close to that of the somatically paired Ss. With K4, the pairing is limited to the terminal regions of both the short and the long chromosome arm. K4 and K9 are the only Ks which show specific pairing with Ss. K4 pairs partially with SIII (Fig.2c) and K9 pairs with

Table 1. Composition of K complements in gonial mitoses of *Acricotopus lucidus*

Sex	Animal no.	Gonad	No. Ks	No. of K types per K complement									No. metaphases	Notes
				K1	K2	K3	K4	K5	K6	K7	K8	K9		
♂	1	A	12	1	2	1	3	–	1	1	1	2	12	[a]
		B	13	2	2	1	3	–	1	1	1	2	5	
			14	2	2	1	4	–	1	1	1	2	4	
	2		11	1	2	2	3	–	–	1	–	2	1	[a]
			12	1	2	2	3	–	1	1	–	2	5	
	3	A	11	1	2	1	2	–	1	–	2	2	4	[b]
			12	1	2	1	3	–	1	–	2	2	4	
		B	12	1	2	1	3	–	1	–	2	2	7	
	4		8	1	1	3	–	–	1	–	2	–	7	[b]
			9	1	1	3	–	–	1	–	2	1	5	
	5		8	2	1	1	1	–	–	–	1	2	5	[c]
	6	A	10	2	1	2	2	–	2	–	1	–	7	[c]
		B	10	2	1	2	2	–	2	–	1	–	13	
	7		12	1	1	1	4	1	1	–	1	2	5	[d]
	8		11	1	1	1	3	–	1	1	1	2	6	[d]
	9		10	2	2	1	1	–	1	–	1	2	3	[e]
	10		9	–	1	1	2	–	2	–	2	1	9	[e]
			10	–	1	1	3	–	2	–	2	1	1	
	11		11	1	1	2	2	–	2	1	1	1	4	
			12	1	1	2	3	–	2	1	1	1	5	
	12		10	1	1	1	2	1	1	1	1	1	7	
	13		10	1	1	2	3	–	1	–	–	2	3	
	14		11	1	1	2	3	–	1	1	1	1	9	[f]
	15		8	1	1	1	2	1	–	–	1	1	8	
	16		12	1	2	–	4	–	2	1	1	1	2	
			13	1	2	–	5	–	2	1	1	1	1	
	17		10	2	1	1	2	1	1	1	–	1	5	
	18		9	1	1	1	2	–	1	–	2	1	1	
			10	1	1	1	2	–	1	1	2	1	3	
	19		11	1	1	2	3	1	–	1	1	1	1	
			12	1	1	2	4	1	–	1	1	1	4	
			13	1	1	2	5	1	–	1	1	1	1	
	20	A	10	1	1	2	2	1	1	1	–	1	7	
		B	11	1	1	2	2	1	1	1	1	1	3	
	21		9	–	1	1	2	1	1	–	1	2	3	
			10	–	1	1	3	1	1	–	1	2	9	
	22	A	11	–	1	2	4	–	1	2	–	1	4	
			12	–	1	2	4	–	1	2	–	2	8	
			13	–	1	2	5	–	1	2	–	2	2	
		B	11	–	1	2	3	–	1	2	–	2	3	
			12	–	1	2	4	–	1	2	–	2	5	
	23	A	9	1	1	2	2	1	–	1	–	1	5	
			10	1	1	2	3	1	–	1	–	1	6	
			11	2	1	2	3	1	–	1	–	1	2	
		B	9	1	1	2	3	1	–	–	–	1	3	
			9	1	1	3	2	1	–	–	–	1	2	
			10	1	1	3	3	1	–	–	–	1	9	
			11	2	1	3	3	1	–	–	–	1	6	
	24		11	1	–	2	3	–	2	–	2	1	7	
			12	2	–	2	3	–	2	–	2	1	4	
	25		14	2	2	2	3	–	1	1	1	2	8	
	26		12	1	2	2	2	2	–	1	1	1	9	
	27	A	6	–	1	1	1	–	1	–	–	2	11	[g]
			7	–	1	1	1	–	1	–	1	2	3	
		B	7	1	1	–	1	–	1	–	1	2	2	
			8	1	1	1	1	–	1	–	1	2	6	
	28		15	2	2	3	3	–	1	1	1	2	8	
			16	2	2	3	4	–	1	1	1	2	6	
♀	29		10	1	2	1	1	1	1	–	2	1	4	[e]
	30		9	–	1	1	2	–	1	1	1	2	5	[g]
	31		8	1	1	1	3	–	1	–	–	1	8	[f]
No. of animals the K type was present				27	30	30	30	10	26	17	25	30		

[a], [b], [c], [d], [e], [f], [g] Animals from the same egg-mass

SI as well, either partially (Fig. 2b; see also Fig. 3 in Staiber 1987), or over the whole length. In the latter case, some specific K9 and SI G-bands are exactly paired. In very few cases homologous K1s and K8s were found in close pairing.

G-banding of female gonial mitoses yielded less satisfactory results than that of male ones. Therefore, K complements of only three female larvae could be analysed (nos. 29–31). In comparing the K complements of males and females, the nine different K types found in spermatogonial mitoses were also present in oogonial mitoses. No special K type was present in only one of the sexes.

It seems to be an advantage for the bisexual *Acricotopus* to maintain a multitude of K types in the germ line, and one can assume corresponding mechanisms that ensure this. In parthenogenetic Orthocladiines like *Smittia*, a reduction of diversity of K types in the K complement in successive generations would be the result of a supposed random loss of K types during the germ line elimination (Bauer 1970).

In *A. lucidus* the germ line elimination of Ks takes place in newly hatched first instar larvae during the first mitosis of the primary germ cells, by lagging in the equatorial plate (Fig. 2c in Bauer and Beermann 1952). Each gonad contains only one primary germ cell. In the present study up to four different K karyotypes were found in one gonad (see Table 1, e.g. nos. 19, 22, 23) so that one can presume that the daughter cells resulting from the gonial elimination mitosis frequently do not have the same K set, and that in the following gonial mitoses unequal divisions can occur.

The K complements can be identical in both gonads of an animal (no. 6), but they may also differ in one (nos. 3, 20) two (nos. 1, 22), three (no. 27) or four Ks (no. 23). Nevertheless, these differences are too small to assume a thoroughly random distribution of the K types during the elimination events in the two primary germ cells.

The data listed in Table 1 support the idea that there are mechanisms which are effective during germ line elimination for limiting the presence of a K type to two specimens per K complement in gonial cells. A striking exception is K4 which may be found up to five times per K complement. As stated above, each K type can be absent in a gonial K complement. But some K types were found in nearly all larvae investigated and therefore appeared about two or three times as often as other types. Comparing the presence of the four larger K types K1–K4 with the presence of the five smaller types K5–K9 in the K complements of Table 1, only one type of the former and up to three types of the latter group can be absent in a K complement. This is surely not by chance. It seems that some K types are more likely to be absent than others in the K complement.

In gonial metaphases the nine different K types together have about four times the length of the three Ss. Under the simplifying supposition that the amount of DNA increases proportionally with the length of metaphase chromosomes, the nine K types would then have fourfold as much DNA as the haploid S set. The 2*C* value of somatic cells of *A. lucidus* was determined as 0.20–0.24 pg DNA by scanning microspectrophotometry (Speiser 1973). Thus, the amount of DNA of the 9 K types may be estimated roughly as about 0.4–0.5 pg.

In contrast to ganglion metaphases, Ag-staining of NORs has not been successful to date for gonial metaphases, although in gonial interphase nuclei nucleoli can be observed. It is not possible to say whether the Ks contain active NORs or not. Kunz et al. (1970) have proved for the gall-midge *Wachtliella* that the Ks synthesize RNA during the entire oogenesis, but they found no nucleoli in the oocyte nucleus.

The observed partial pairing between individual Ks and Ss indicates homologies between these chromosomes. Such homologies in the form of S sections, which are present in the K complement, have already been established (Staiber and Thudium 1986). Within the K types, K9 occupies an exceptional position, not only because of its partial especially close pairing with SI, but also because of its special behaviour during differential mitosis, where it frequently shows delayed migration to the cell pole or even remains together with the Ss in the equatorial plate, then undergoing equal anaphase separation (Thudium 1974; Staiber 1987).

Further information about the relationship of the Ks of *A. lucidus* to each other and to the Ss, the origin of the Ks and possible patterns of K elimination, may come from G-banding of meiotic stages and of early embryonic mitoses or even of the first gonial elimination mitosis.

Acknowledgements. I am most grateful to Professor F. Mechelke for his support and to Professor W. Beermann for critical reading of the manuscript and comments. I would also like to thank Brigitta Aich and Irmgard Wech for their help in culturing the larvae.

References

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

Bauer H, Beermann W (1952) Der Chromosomencyclus der Orthocladiinen (Nematocera, Diptera). Z Naturforsch 7b:557–563

Beermann W (1956) Nuclear differentiation and functional morphology of chromosomes. Cold Spring Harbor Symp Quant Biol 21:217–230

Kunz W, Trepte H-H, Bier K (1970) On the function of the germ line chromosomes in the oogenesis of Wachtliella persicariae (Cecidomyiidae). Chromosoma 30:180–192

Mayr B, Schleger W, Auer H (1987) Frequency of Ag-stained nucleolus organizer regions in the chromosomes of cattle. J Hered 78:206–207

Mechelke F (1953) Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von Acricotopus lucidus. Chromosoma 5:511–543

Speiser C (1973) Quantitative DNS-Bestimmungen im Verlauf der Ontogenese von Acricotopus lucidus (Chironomide). Thesis, University of Hohenheim, FRG

Staiber W (1987) Unusual germ line limited chromosomes in Acricotopus lucidus (Diptera, Chironomidae). Genome 29:702–705

Staiber W, Thudium D (1986) X-ray induced rearrangements between germ-line limited and soma chromosomes of Acricotopus lucidus (Diptera, Chironomidae). Genetica 69:149–156

Thudium D (1974) Das Verhalten der Soma- und Keimbahnchromosomen bei Acricotopus lucidus Staeger (Orthocladiinae, Diptera). Thesis, University of Hohenheim, FRG

Received July 27, 1988/ in revised form August 17, 1988
Accepted by W. Beermann