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# Isolation and chromosomal localization of a germ line-specific highly repetitive DNA family in *Acricotopus lucidus* (Diptera, Chironomidae)

**Wolfgang Staiber, Irmgard Wech, Anette Preiss**

Institute of Genetics,
University of Hohenheim,
D-70593 Stuttgart, Germany

**Abstract**

In the chironomid *Acricotopus lucidus*, parts of the genome, the germ line-limited chromosomes, are eliminated from the future soma cells during early cleavage divisions. A highly repetitive, germ line-specific DNA sequence family was isolated, cloned and sequenced. The monomers of the tandemly repeated sequences range in size from 175 to 184 bp. Analysis of sequence variation allowed the further classification of the germ line-restricted repetitive DNA into two related subfamilies, A and B. Fluorescence in situ hybridization to gonial metaphases demonstrated that the sequence family is highly specific for the paracentromeric heterochromatin of the germ line-limited chromosomes. Restriction analysis of genomic soma DNA of *A. lucidus* revealed another tandem repetitive DNA sequence family with monomers of about 175 bp in length. These DNA elements are found only in the centromeric regions of all soma chromosomes and one exceptional germ line-limited chromosome by in situ hybridization to polytene soma chromosomes and gonial metaphase chromosomes. The sequences described here may be involved in recognition, distinction and behavior of soma and germ line-limited chromosomes during the complex chromosome cycle in *A. lucidus* and may be useful for the genetic and cytological analysis of the processes of elimination of the germ line-limited chromosomes in the soma and germ line.

## Introduction

The phenomenon of chromatin elimination from somatic cells during germ line − soma differentiation, first reported from *Parascaris* by Boveri (1887), has been observed in the form of the processes of chromatin diminution or chromosome elimination in various groups of animals − Protozoa, Nematoda, Crustacea, Insecta and Vertebrata (Tobler 1986).

In the chironomid *Acricotopus lucidus*, parts of the chromosome complement, the germ line-limited chromosomes (Ks, derived from Keimbahn), are also eliminated from the prospective soma cells in the course of germ line-soma segregation (Bauer and Beermann 1952; Bauer 1970). Together with the soma chromosomes (Ss) the Ks pass through a complex chromosome cycle with interesting special features:

1. The elimination of the Ks from the future somatic cells during early cleavage divisions (soma elimination).
2. The elimination of about half of the Ks during the first gonial mitoses of the primordial germ cells in newly hatched larvae (germ line elimination).
3. A compensating duplication by a monopolar movement of the Ks in the last gonial mitosis in young fourth instar larvae. In this so-called differential mitosis, all Ks move undivided to only one cell pole, whereas the Ss behave as in a normal mitosis. The cells containing both Ss and Ks develop into regular spermatocytes and oocytes, but the cells with the S set only differentiate into aberrant spermatocytes or into nurse cells.

Understanding of the function of the germ line-limited chromatin or chromosomes is still very limited (Hennig 1986). Experiments on the cecidomyids *Wachtliella persicariae* and *Mayetiola destructor* have demonstrated that the Ks are indispensable for the normal development of germ cells in both sexes (Geyer-Duszynska 1966; Bantock 1970). The Ks may bear fertility factors. It was suggested that the germ line supernumeraries in the cecidomyids have been derived from the S-complement by way of endopolyploidy (Nicklas 1960; Painter 1966). In *Sciara coprophila*, the failure of sister chromatid separation seems to be directly involved in the process of elimination of paternal X chromosomes as well as of germ line-restricted chromosomes (de Saint Phalle and Sullivan 1996).

The mechanisms for identifying and distinguishing between Ks and Ss during the complex chromosome cycle in *A. lucidus* are not known. We propose that repetitive DNA sequences located in or near the centromeric regions of Ks and Ss are involved in these processes. The aim of this investigation was the isolation, characterization and chromosomal localization of germ line- and soma-specific repetitive sequences in *A. lucidus*.

## Materials and methods

*Isolation, cloning and sequencing of the repetitive elements*

Soma DNA, isolated from larvae and prepupae of a laboratory stock of *A. lucidus*, was digested with a panel of restriction endonucleases and run in 1% agarose gels in TBE buffer (0.89 M

TRIS, 0.89 M boric acid, 20 mM EDTA). When stained with ethidium bromide, a prominent band of approximately 175 bp and, less prominent, multiples of 175 bp became visible in DraI and TaqI digests. The 175 bp DraI and TaqI monomers were excised from gels, and subcloned into EcoRV- and ClaI-digested pBluescript II KS.

Germ line DNA was isolated from testes of male prepupae. HincII digestion resulted in a germ line-specific ladder of monomer and multiples of about 180 bp, visible in Southern blots hybridized with digoxigenin-labeled germ line DNA as probe. Because of the limited amounts of germ line DNA, HincII-digested total germ line DNA was ligated into the HincII site of pBluescript II. Clones with repetitive sequences as inserts were detected by colony hybridization with labeled germ line DNA as probe.

The inserts were sequenced in both orientations on an Automated Laser Fluorescent (ALF) DNA sequencer (Pharmacia). The EMBL and GenBank databases were screened for homology to other sequences using the HUSAR computer program package of the German Cancer Research Centre, Heidelberg.

*Southern hybridization*

Soma and germ line DNA was digested, separated and Southern blotted overnight onto nylon membranes. Hybridization was performed at 62$^{o}$C for 16 h using digoxigenin-labeled probes in standard hybridization buffer (5xSSC, pH 7.0, 0.1% N-lauroylsarcosine, 0.02% SDS, 1% Blocking Reagent, Boehringer Mannheim). (1xSSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0.) Membranes were washed twice for 5 min each at room temperature in 2xSSC, 0.1% SDS, and finally twice, 15 min per wash, at 62$^{o}$C in 0.5xSSC, 0.5% SDS. Detection was carried out with alkaline phosphatase-conjugated sheep anti-digoxigenin antibody using NBT (nitroblue tetrazolium chloride) and BCIP (5-bromo-4-chloro- 3-indolyl phosphate) as substrate, as recommended by the supplier (Boehringer Mannheim).

*Chromosome preparation*

Testes of young 4th instar larvae of *A. lucidus* were dissected, treated with 0.5% sodium citrate solution for 10-20 min and fixed in ethanol:acetic acid (3:1). Salivary glands were isolated from prepupae and were immediately fixed in ethanol:acetic acid. Squash preparations were made in 45% acetic acid, frozen on dry ice, dehydrated in an ethanol series (70%, 80%, 95%, 5 min each) and air-dried. For in situ hybridization, the preparations were used on the same day.

*In situ hybridization*

Probes for in situ hybridization were labeled by random-priming with digoxigenin-11-dUTP and biotin-16-dUTP according to the manufacturers instructions (Boehringer Mannheim).

Digoxigenated probes were detected with alkaline phosphatase-conjugated anti-digoxigenin antibody, NBT and BCIP, with fluorescein-conjugated anti-digoxigenin antibody or with a fluorescent antibody enhancer set (Boehringer Mannheim). Detection of biotinylated probes was

performed with a mouse monoclonal anti-biotin antibody (Sigma) followed by a Texas Red-conjugated horse anti-mouse secondary antibody (Vector).

Air-dried squash preparations were stabilized with 2xSSC, pH 7.0 at 65°C for 30 min, rinsed in 2xSSC for 2 min and treated with a mixture of 400 ml 0.1 M triethanolamine, pH 8.0, and 625 µl acetic anhydride for 10 min. After two 5 min washes in 2xSSC the slides were dehydrated in an ethanol series (see above) and air-dried. Chromosomal DNA was denatured by immersing the slides in 0.07 N Na0H for 1-3 min at room temperature. Slides were then washed three times for 5 min each in 2xSSC, dehydrated in an ethanol series and air-dried.

The hybridization mixture was composed of the labeled probe, 5xSSC and 0.1% SDS (Schmidt 1996). 8-10 µl of the mixture was applied to each preparation under a sealed coverslip. Hybridization was performed overnight in a moist chamber at 58°C. Slides were washed three times for 20 min in 2xSSC at 53°C for detection with alkaline phosphatase, or twice for 5 min in 2xSSC and for 2 min in PBS at room temperature for fluorescence detection. Mitotic chromosomes were counterstained with propidium iodide (5 µg/ml) or with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, 1 µg/ml) in Vectashield (Vector) and polytene chromosomes were stained with 5% Giemsa (Merck) in Sörensen's phosphate buffer, pH 7.0 for 10 min.

Microphotographs were taken with an Axiophot (Zeiss) equipped with an epifluorescence system and a Plan-Neofluar 100/1,3 on Fujichrome 400 film or with a confocal laser scanning microscope (MRC 1024, Biorad) on Kodak Ektachrome Elite 100 film.

EMBL nucleotide sequence database accession numbers: Y11728 (AlSo3), Y11729 (AlKe1) and Y11730 (AlKe6)

**hier weiter**

**Results**

A useful approach for obtaining repetitive DNA involves digestion of genomic DNA with different restriction endonucleases, separation of the fragments by agarose gel electrophoresis, and subsequent staining with ethidium bromide. A bright band against a background smear indicates the presence of a repetitive element, either tandemly arranged, containing a single recognition site, or interspersed and containing two sites.

Digestion of *A. lucidus* soma DNA with the restriction enzymes DraI and TaqI yields a 175 bp band containing monomers of a tandem repetitive DNA family as revealed by subsequent molecular characterization. The complete nucleotide sequence of nine independently cloned monomers and a consensus sequence were determined (Fig. 1). The lengths of the monomers, named AlSo1-9 (from *A. lucidus* and soma), range from 174 bp to 177 bp. AlSo1-4 represent DraI monomers and AlSo5-9, TaqI monomers. The consensus sequence of AlSo is 73% AT. Individual copies of AlSo are very similar to one another and 95%-97% identical to their consensus sequence (deletions being counted as single events).

```
                          10         20         30         40         50
consensus        AAAAAAATCC ATAACTTCTA AACGAATAGT CACATAGCAC TCATAATAGT
 AlSo1           .......... .......... .......... .......... ..........
 AlSo2           .......... .......... ...A...... .......... ..........
 AlSo3          A.........- .......... ...A...... .......... ......A...
 AlSo4           .......... .......... .......... .......... ..........
 AlSo5   .......-........T. .......... ......A.A. ........T. ..........
 AlSo6   ......A........... .......... T......... .......... ..........
 AlSo7   ..................A .........G .....G.TAC .......... ..........
 AlSo8   .................. ...TT..-.. G.....A... ..T....... ........A.
 AlSo9   .................. .........G ........A. .......... ..........
consen   CGATTTTA

                          60         70         80         90        100
consensus        ATATTCTTGT AGATAACATC AAGAGGCATT CATATTGGTA TGATTTGTGT
 AlSo1           .......... .......... ....AAA... AT........ ........C.
 AlSo2           .......... .......... .......... .......... ..........
 AlSo3           ..G....... .......... .......... .......... ........C.
 AlSo4           .......... .......... ..T......C .......... ........C.
 AlSo5           .A........ ..G....... .......... .......... ..........
 AlSo6           .......... .......... ..A....... .......... ........T.
 AlSo7           .......... .......... .......... .......... ..........
 AlSo8           .......... .......... .......... .......... ..........
 AlSo9           .....A.... .......... .......... .......... ..........

                         110        120        130        140        150
consensus        CTGTACTAAA AGATTCCATG CGGATTAAAA ATAAAAATTG CATGAAAAAG
 AlSo1           .......... .T........ .......... .......... .........A
 AlSo2           ..T....... T....T.... .......... .......... ..........
 AlSo3           .......... .......... .......... .......... ......T.G.
 AlSo4           .......... ......T... .......... .......... ..........
 AlSo5           .......... .......... .......... ..........
 AlSo6           .......... .......... .......... .C........ ........G.
 AlSo7           .......... .......... .......... .......... ........G.
 AlSo8           .......... .......... .......... .A........ ..........
 AlSo9           .......... ....-..... .......... .......... ........G.

                         160        170
consensus        CATATTTTAA TGAAGTTTTC GATTTT        bp
 AlSo1           .......... .......... .....-       175
 AlSo2           .......-.. .......... .....-       174
 AlSo3           .......... .......... .....T       176
 AlSo4           .......... .......... .....T       176
 AlSo5           .......... .........               176
 AlSo6           .......... .........               177
 AlSo7           .......... .........               177
 AlSo8           .......... .........               176
 AlSo9           .......... .........               176
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**Fig. 1**. Nucleotide sequences of cloned *Acricotopus* repetitive soma DNA compared with a derived monomer consensus sequence. AlSo1-9 represent nine independent monomers. AlSo1-4 were isolated by DraI digestion and AlSo5-9 by TaqI digestion of soma DNA. The consensus position, where identical nucleotides were present in 50% (or more) of sequences, was considered unambiguous. Dots indicate that the sequence is identical to that of the consensus and dashes indicate deletions.

A computer-assisted database search did not reveal any significant homology between the repetitive AlSo sequence family and sequences recorded in the EMBL/Gen-Bank nucleotide sequence databases.

Densitometric analyses of photographic negatives of ethidium bromide-stained agarose gels indicated that the DNA of the 175 bp element peak accounted for an average of about 1.5% of the total soma DNA of *A. lucidus*. Assuming a C-value of 0.12 pg in soma cells (Speiser 1973), this suggests that there are about $1.10^4$ copies of this element in the haploid soma genome of *A. lucidus*.

In situ hybridization of an AlSo3 probe to polytene salivary gland chromosomes of *A. lucidus* localized with anti-digoxigenin-alkaline phosphatase and NBT/BCIP produced strong signals on the heterochromatic centromeric blocks of all Ss (Fig. 2, I-III).

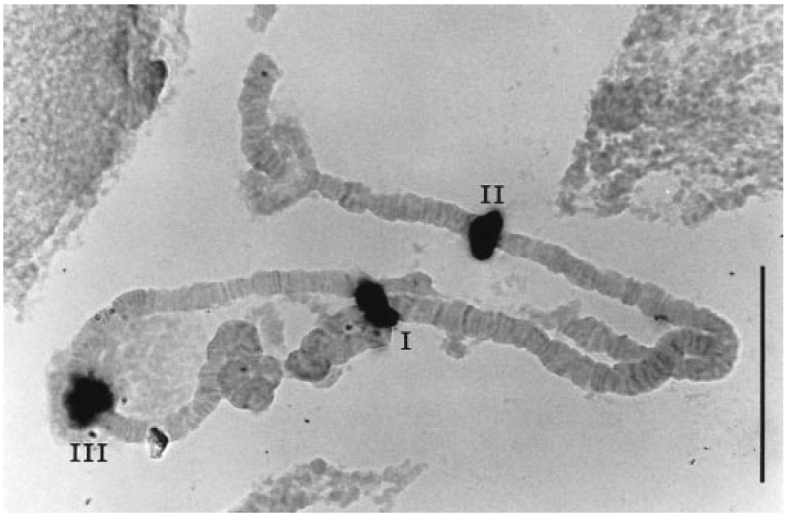


**Fig. 2**. In situ hybridization of the AlSo3 probe to polytene salivary gland chromosomes of *Acricotopus lucidus*. Strong hybridization signals are visible on the heterochromatic centromeric regions of the three Ss (I-III) after detection with alkaline phosphatase, nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Bar represents 50 µm

Fluorescence in situ hybridization (FISH) using an AlSo3 probe against gonial metaphases resulted in intense signals in the centromeric regions of the paired Ss and the germ line-limited chromosome K9 (Fig. 5d), but not in the other eight K types found in the germ line chromosome complement of *A. lucidus* (nomenclature of Ks in Staiber 1988).

In the case of a tandemly arranged organization of the AlSo, one would expect to see a ladder pattern of fragments in Southern blots of DraI-digested soma DNA hybridized with an AlSo probe because of random loss of restriction site(s) due to mutation from some monomer units. This applies for the AlSo sequence family as see in Fig. 3a. Hybridization of AlSo3 to DraI-digested soma DNA revealed a ladder of bands with the intensity of the hybridization signal decreasing regularly as the number of monomers in the oligomer increased. The ladder increased with monomeric increments to a length of more than eight oligomers. For the Southern blot in Fig. 3a, 2 µg soma DNA was digested with 20 U of DraI. After 0.5 h, one-half of the digest was frozen (lane 1), the other half incubated for 2 h, then an additional 10 U of DraI was added and the sample digested for a further 3 h (lane2). The fact that no major differences were observed between the two protocols suggests that the multimeric bands most likely are not the result of partial digestion.

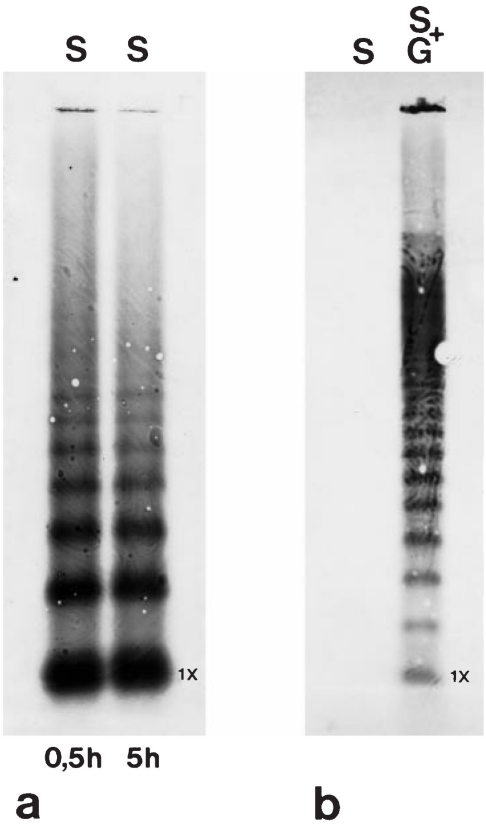


**Fig. 3. a** Southern blot of DraI-digested soma DNA (S; lane 1, 0.5 h digestion; lane 2, 5 h digestion) of *A. lucidus* hybridized with AlSo3 as probe. Band sizes are multiples of the monomer unit (1X~175 bp). **b** Germ line specificity of the AlKe sequences. Hybridization of the AlKe pentamer probe (containing the sequences AlKe4, 6, 5, 7 and 8) to a Southern blot of *A. lucidus* soma DNA (S lane 1) and soma DNA containing germ line DNA (S+G lane 2), both digested with HincII. AlKe sequences are clearly restricted to the germ line. Band sizes are multiples of the monomer repeat unit (1X ~180 bp)

For identification of germ line repetitive sequences, DNA from isolated testes of *A. lucidus* was digested with different restriction enzymes, separated, blotted and hybridized with labeled germ line DNA. A clear ladder of bands indicating a tandem repetitive sequence family with a monomer unit of about 180 bp was found in HincII digests (not shown). Because only low amounts of germ line DNA were available, rather than clone excised monomer bands from ethidium bromide-stained agarose gels, we cloned total HincII-digested germ line DNA directly into the HincII site of pBluescript.

Four clones containing germ line-specific repetitive sequences were detected by colony hybridization using digoxigenin-labeled germ line DNA versus soma DNA as probes. When the inserts were sequenced, two monomers of 183 bp, named AlKe1 and AlKe2 in Fig. 4 (AlKe is derived from *A. lucidus* and Keimbahn), one dimer of 359 bp, composed of AlKe3 and 9, and a pentamer of 897 bp, composed of AlKe4, 6, 5, 7 and 8 (in that order), were found. Analysis of sequence variation allowed a further classification of the AlKe sequences into two homology groups: subfamily A, AlKe1-5 with 183-184 bp, and subfamily B, AlKe6-9 with 175-178 bp. The subfamilies differ from each other in a section of about 70 bp (Fig. 4).

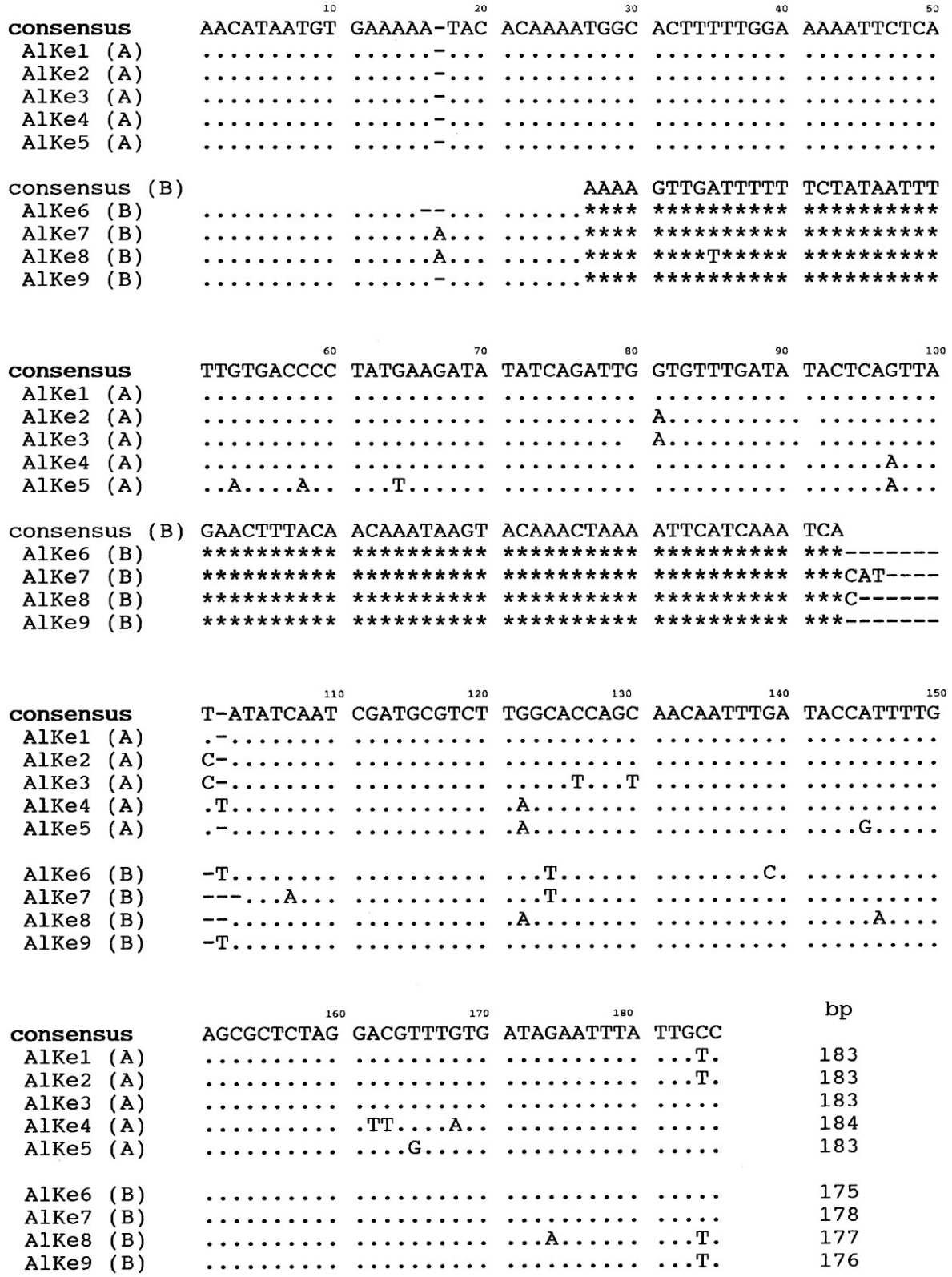


**Fig. 4.** Comparison of the nucleotide sequences of nine cloned *Acricotopus* germ line DNA repeats, AlKe1-9, and the derived consensus sequences. *Capital letters* in parentheses reflect the monomer type (subfamily A or B). *Dots* and *stars* (only in subfamily B) indicate that the sequence is identical to that of the consensus. *Dashes* represent gaps that were introduced to improve the alignment. Base substitutions with respect to the consensus sequence are indicated

In AlKe1-5 this section varies in length from 75-76 bp and in AlKe6-9 from 67-70 bp. The consensus sequences of subfamily A and subfamily B are nearly identical from positions 1 to 26 and from positions 103 to 185. The intermediate sections between positions 26 and 103 exhibit no homology between subfamilies A and B. The AlKe sequences are AT rich: 65% of the consensus sequence in subfamily A and 70% in subfamily B. The nucleotide sequence data have been deposited in the EMBL nucleotide sequence database under accession nos. Y11729 (AlKe1), Y11730 (AlKe6) and Y11728 (AlSo3).

Restriction sites for HincII (-GTPyPuAC-) have been lost in the isolated dimer, between AlKe3 and 9 (sequence -GCCAAC-), and in the pentamer, between AlKe4 and 6, 6 and 5, 5 and 7, and 7 and 8 (-GCCAAC- in all the sequences). Therefore in positions 182-185 of the consensus the nucleotide sequence GCC is documented, which does not correspond to the restriction sequence of HincII, but GCC is present in more than 50% of the isolated repeats.

The tandemly arranged organization of the AlKe subfamily A and subfamily B sequences in the germ line DNA is reflected in the composition of the isolated dimer, arrangement A-B, and of the pentamer, arrangement A-B-A-B-B.

FISH with AlKe1 as a probe on gonial metaphases resulted in strong signals in K1-K8, but not in the Ss and not in K9 (Fig. 5a,c). Giemsa staining revealed that hybridization signals were localized to the two paracentromeric bands of heterochromatin characteristic of the germ line-limited chromosomes (Fig. 5b; Staiber 1991a). Due to the denaturation of chromosomal DNA with NaOH prior to hybridization, Giemsa staining produces a C-banding pattern (Fig. 5b), which allows the identification of the K types. No AlKe sequences could be detected on K9, even by signal amplification. In the lower metaphase in Fig. 5a,b, K9 shows partial pairing with SI and in the differential mitosis in Fig. 5c, a somewhat delayed migration to the cell pole compared with the other Ks.

To test whether the AlKe elements are germ line-specific, AlKe1 was hybridized to polytene salivary gland chromosomes of *A. lucidus*. No hybridization signals were detected on the chromosomes. As an internal hybridization control, AlKe1 was cohybridized with the single-copy clone Cla1.1, isolated from *Chironomus thummi thummi* (Kraemer and Schmidt 1993). Only the hybridization site of Cla 1.1 in the short arm of the polytene SI of *A. lucidus* could be detected (not shown).

To corroborate the observation that the AlKe sequences are restricted to the germ line and are eliminated from the soma, the AlKe pentamer (containing elements of subfamilies A and B) was labeled and hybridized to a Southern blot of HincII-digested soma DNA and soma DNA containing germ line DNA, respectively (Fig. 3b; each lane, 4.5 µg DNA, digested with 20 U of HincII for 2 h, then with an additional 20 U of HincII for a further 2.5 h). The DNA probes were obtained by dividing decapitated male prepupae into two parts, a thoracic part without and an abdominal part with the gonads. No hybridization signals and, thus, no AlKe equences were detected in soma DNA (lane 1 in Fig. 3b), but strong signals were obtained in the germ line DNA (lane 2 in Fig. 3b). A clear ladder of hybridizing bands corresponding to monomers and

oligomers of AlKe was seen. The ladder increased by monomeric increments to a length of at least 12 oligomers.

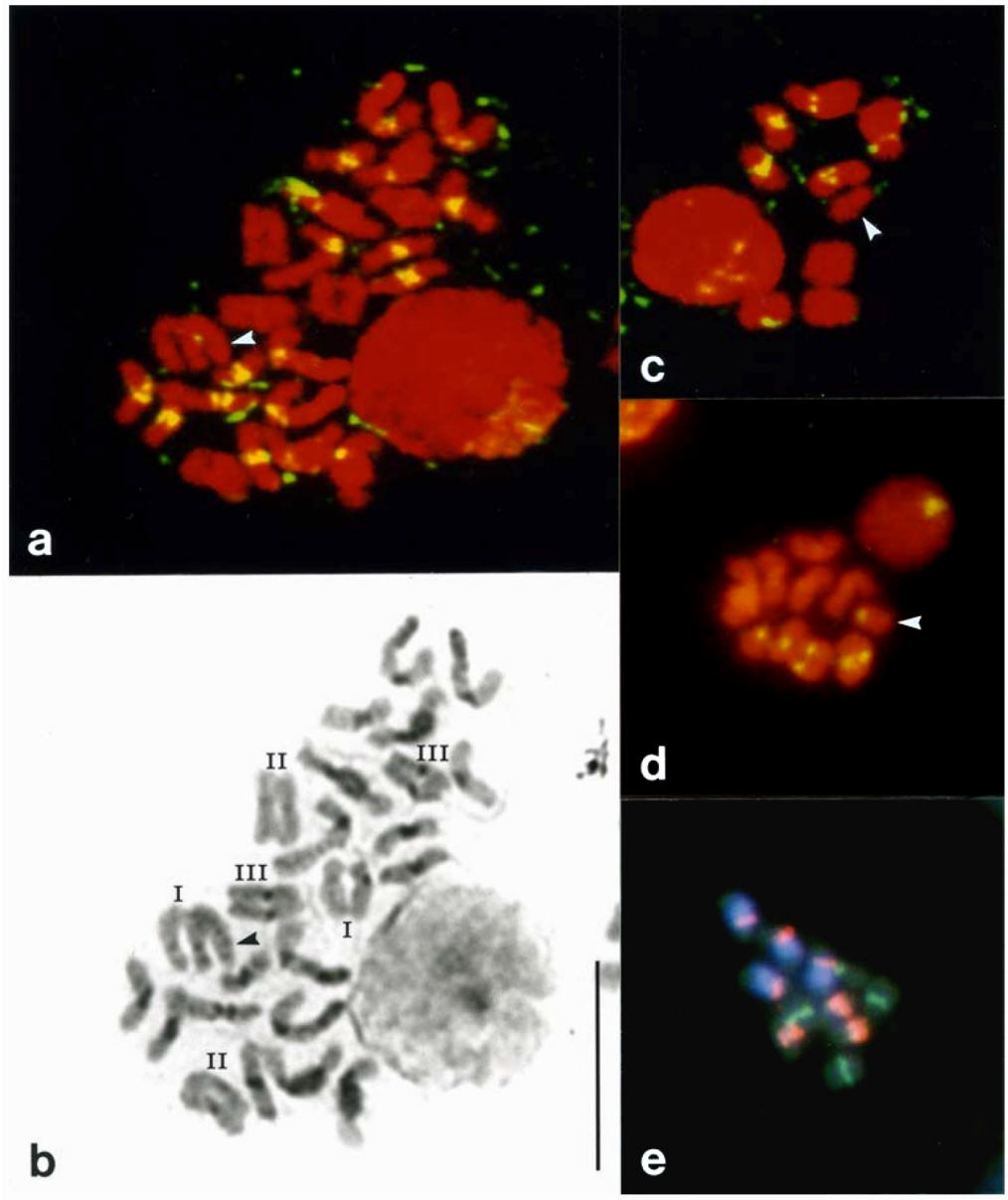


**Fig. 5a-e.** Fluorescence in situ hybridization (FISH) of AlKe and AlSo sequences to spermatogonial mitoses of *A. lucidus*. **a** FISH of the digoxigenated AlKe1 probe to two adjacent metaphases with nine (*upper*) and ten Ks (*lower* metaphase), and to an interphase nucleus. Hybridization sites are visualized with fluorescein (*yellow*). Chromosomes are counterstained with propidium iodide. In the lower metaphase K9 (*arrowhead*) is partially paired with SI. Comparison of the hybridization pattern in **a** with the C-banding pattern of the same metaphases in **b** demonstrates that AlKe1 hybridizes only to the paracentromeric C-bands of the Ks, with the exception of K9, but not on the paired Ss (I-III). Bar represents 10 µm. **c** FISH of AlKe1 on a differential mitosis. The six Ks have already moved to and grouped around one cell pole (*upper*), whereas the Ss still lie in the equatorial plate. K9 (*arrowhead*) shows somewhat delayed migration to the pole compared with the other Ks. **d** FISH of a digoxigenin-labeled AlSo3 probe to a metaphase spread with six Ks. Signals can be seen on the centromeres of the Ss and K9 (*arrowhead*), but not on the other Ks. **e** Simultaneous hybridization of biotinylated AlKe1 and digoxigenin-labeled AlSo3 probes to a gonial metaphase with eight Ks. The AlSo sequences on the Ss and K9 are visualized by fluorescein (*green*), the AlKe sequences on the other Ks by Texas Red. Chromosomes are counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue). Four K4s can be identified by their more intense DAPI fluorescence

AlSo and AlKe probes can now easily be used to differentiate Ss and Ks in gonial metaphases by differential FISH. In Fig. 5e, AlKe sequences, and thus the Ks, are visualized with Texas Red, and AlSo sequences present in the Ss are detected with fluorescein. K9 behaves exceptionally, giving S-like green fluorescence in one paracentromeric C-band. However, it can be clearly identified because it is the smallest K and the only acrocentric one. The DAPI counterstain allows the identification of K4 by its conspicuously more intense blue fluorescence in the central heterochromatic part of its long chromosome arm, as seen in the gonial metaphase in Fig. 5e, where four K4s are present.

**Discussion**

The germ line-limited chromosomes (Ks) of *A. lucidus* show some striking deviations from normal mitotic behavior during their complex chromosome cycle. First, they are eliminated from the future somatic cells during early cleavage divisions. In contrast to earlier divisions, Ks no longer segregate equally but rather stay behind at the equatorial plate. Only the Ss move to the poles and the Ks are thus eliminated. Second, during the first divisions of the primary germ cells, about half of the Ks remain in the equatorial plate and are lost subsequently (germ line elimination). Third, before meiosis in the last gonial mitosis (differential mitosis), all Ks move undivided to only one cell pole. This is a monopolar movement of the Ks without separation of the sister chromatids (programmed nondisjunction), whereas the Ss segregate equally. All these events require discrimination between Ks and Ss, and we presume that DNA sequences, possibly repetitive sequences, located in or near the centromeric regions play an important role in this process.

We have isolated two repetitive DNA sequence families from *A. lucidus*. The AlSo DNA sequence family, with monomer lengths of 174-177 bp, derived from soma DNA was located by FISH to the centromeric regions of all Ss as well as to germ line-limited chromosome K9. The other, the AlKe DNA sequence family, with monomers of 183-184 bp (subfamily A) and 175-178 bp (subfamily B), is restricted to germ line cells. It was localized to the paracentromeric C-bands of all germ line-limited chromosomes, except K9. The germ line specificity of the AlKe sequences in *A. lucidus* was demonstrated by Southern blot analysis.

The AlSo sequences show no homologies to repetitive sequences localized within the centromeric regions of other chironomids, i.e. a 155 bp repeat in *Chironomus pallidivitatus* (Rovira et al. 1993) and the Cla elements (110-119 bp) of *C. thummi thummi* and *C. thummi piger* (Schmidt 1984; Hankeln et al. 1994), or to other DNA sequences deposited in the EMBL/GenBank databases.

Hybridization with AlSo3 to DraI-digested soma DNA revealed a ladder of bands with regularly decreasing intensity of signals from monomer to multimers. This strongly indicates tandem repetitive organization of the AlSo sequences in the centromeric regions of *A. lucidus* with loss of DraI restriction sites owing to mutation in some repeats. Nevertheless, the sequence

variation within the AlSo DNA family is small and the sequences seem to be conserved. Estimations of about $1x10^4$ copies of the AlSo sequences per haploid soma genome of *A. lucidus*, and strong hybridization signals in metaphase and polytene Ss, indicate that a fundamental repeat unit has been highly amplified and organized in tandem arrays.

Genomes of higher eukaryotes frequently contain high amounts of noncoding tandemly repeated satellite DNA sequences located mainly in the centromeric regions. Repetitive sequences appear to have essential structural functions at the chromosomal level (Miklos 1985; Willard 1990). Transfection experiments have suggested a role for α-satellite DNA, the centromeric satellite DNA of primate chromosomes, in centromeric function (Haaf et al. 1992). The centromere plays a central role in the process of chromosome segregation in both mitosis and meiosis.

In this context, it is worth noting that the evolution of repetitive sequences can operate across nonhomologous chromosomes. Molecular and in situ hybridization data from *Chironomus* (Hankeln et al. 1994) and from harvest mice (*Reithrodontomys*; Hamilton et al. 1990) indicate that there is intragenomic movement of tandem repetitive sequences within chromosomes and among nonhomologous chromosomes, and that there are mechanisms for distributing and homogenizing repeats of a satellite DNA family throughout the genome.

Perhaps this is also true for the AlSo sequences in the Ss and especially for the AlKe sequences in the Ks of *A. lucidus*. The pairing of nonhomologous Ks in the formation of multivalents observed during meiosis in *A. lucidus* may well lead to such a transfer of repetitive sequences within the K complement (Staiber 1989).

Chromosome elimination also occurs in vertebrates, e.g. during early embryogenesis of various hagfish species (Cyclostomata; Kohno et al. 1986; Nakai et al. 1991). There, from 21% to 55% of the chromatin is eliminated as entire chromosomes (2-20) or parts of chromosomes from the prospective soma cells. Kubota et al. (1993) isolated two germ line-restricted, highly repeated DNA sequence families from the Japanese hagfish *Eptatretus okinoseanus*. In *E. okinoseanus* (type A) the two repetitive DNA families represent about 19% of the eliminated DNA. The sequences are located on several C-band-positive small chromosomes that are limited to the germ cells. Comparison of the germ line-restricted repetitive sequences EEEo1 (95 bp) and EEEo2 (85 bp) of *E. okinoseanus* with the germ line-restricted sequences AlKe1 (subfamily A) or AlKe 6 (subfamily B) of *A. lucidus*, revealed no homologies. AlKe1 and AlKe6 also exhibit no homologies to another highly repetitive germ line-restricted DNA sequence family (consensus sequence 121 bp) isolated from the nematode *Ascaris lumbricoides* (Müller et al. 1982) or to other sequences recorded in the EMBL/GenBank databases. There is also no homology between the AlKe and the AlSo sequences of *A.lucidus*.

The AlKe subfamilies A and B differ from each other by a section of about 70 bp ranging from position 27 to position 102 of the consensus sequence of subfamily A. No sequence homology was found when these sections of subfamilies A and B were compared. The sequences

before and after this section seem to be conserved between the subfamilies. This also holds for the sequences of the 70 bp sections of the monomers within one subfamily.

The composition of the isolated AlKe pentamer demonstrates the presence of combinations of sequences of subfamilies A and B in the tandem arrays, possibly indicating a higher-order organization in the paracentromeric heterochromatic bands. In primate α-satellite DNA, sequence variants can be organized in tandem arrays forming in part chromosome-specific subsets with clearly defined multimeric higher-order repeat units (Willard and Waye 1987; Willard 1991).

In *A. lucidus* the number of Ks ranges widely, from 6 to 16, as determined on spermatogonial metaphases (Staiber 1988), so one cannot calculate or estimate the portion of the AlKe sequences in the germ line-restricted DNA. The Ks in *A. lucidus* contain S-homologous chromosome sections and heterochromatic segments, as demonstrated on X ray-induced K-S rearrangments in the polytene salivary gland chromosomes (Staiber 1991b). The function, if there is any, of the S-homologous sequences in the Ks is unknown.

In *Sciara coprophila*, another dipteran with germ line-limited (L) chromosomes and with an extraordinary chromosome cycle, the programmed elimination of chromosomes, like the X chromosomes, paternal autosomes and L chromosomes, occurs in various stages. Also the phenomenon of programmed nondisjunction of chromosomes, i.e. of X chromosomes, was detected (for review see Gerbi 1986). In this case, the failure to complete sister chromatid separation might be the mechanism for elimination of the paternal X chromosomes during early embryonic divisions (de Saint Phalle and Sullivan 1996). No effect was observed on the separation of the centromeric regions, but there was a failure in the separation of the chromatid arms. In *S. coprophila* a cis-acting element, named Controlling Element (CE), is necessary for the elimination of the X chromosomes in the course of syncytial divisions (Crouse 1979). In X-autosome translocation the CE is able to control the separation of autosomal sister chromatids: paternally inherited translocation autosomes containing the CE are eliminated during syncytial divisions (Rieffel and Crouse 1966).

Observations of asynchronous X chromosome elimination in the nuclei of a *Sciara* embryo indicate that the decision to eliminate the X occurs at the level of the individual nucleus, and is dependent on the position of the nucleus within the embryo. De Saint Phalle and Sullivan (1996) propose the same mechanism of blocked sister chromatid separation for the elimination of the L chromosomes in *Sciara.* The authors discuss models that could explain the processes of chromosome elimination. One model suggests a maternally supplied diffusible factor, necessary for sister chromatid separation, which is titrated by the X chromosomes and also by the L chromosomes in Sciara at each division cycle. Another model involves the activation of a factor holding the sister chromatids together at a specific nuclear cycle.

Experiments on cecidomyids support the view of a maternally supplied factor involved in the process of elimination of the germ line-limited chromosomes (Geyer-Duszyńska 1966; Bantock 1970). Early embryos of *Wachtliella persicariae* and *Mayetiola destructor* were ligated or

centrifuged to delay the migration of the future germ cell nuclei to the pole plasm. This induced the elimination of the germ line-limited chromosomes also from the future germ line nuclei. As a result no mature germ cells were formed. These results indicate factor(s) present in the pole plasm preventing the elimination of these extra chromosomes during syncytial divisions. Yet such a model could not explain the elimination of about half of the Ks during germ line elimination in first instar larvae in *A. lucidus*. Instead, activation of factors holding the sister chromatids together at distinct developmental stages would fit better the observation of the germ line elimination of Ks in *A. lucidus*.

In *Sciara* the above-mentioned CE was localized adjacent to the centromere of the X chromosome. For Ss and Ks of *A. lucidus* specific tandem repetitively organized sequences were localized to the centromeric regions and to the heterochromatic sections flanking the primary constrictions. We propose that elements present in the paracentromeric heterochromatic bands of the Ks are involved in the identification of the Ks in *A. lucidus*. Perhaps these are elements like the CE in *Sciara* that also induce soma and germ line elimination of Ks by failure of sister chromatid separation during anaphase.

There is a striking difference in the distribution of the AlKe and AlSo sequences in the Ks. K1-8 contain high amounts of germ line-specific AlKe sequences, but no AlKe sequences are present in K9. K9 clearly belongs to the K clan of chromosomes, but it shows some further peculiar differences that make it more S like: partial pairing with SI, S-like pairing with its homolog in gonial mitoses, delayed migration to the cell pole compared with the other Ks, and, in rare cases, equal anaphase separation together with the Ss during differential mitosis. All Ss contain high amounts of AlSo sequences in their centromeric regions; surprisingly K9 does too, but the other Ks do not.

Thus, the intermediate K-S behavior of K9 might be explained either by the presence of high amounts of the AlSo sequences characteristic of the S-chromosomes, or by the absence of K chromosome-characteristic AlKe sequences. Perhaps K9 is an evolutionarily younger K with centromeric similarity to the Ss in the form of AlSo sequences, but which has still not accumulated AlKe sequences.

With the K- and S-specific sequences it is also possible to distinguish the chromosome types by FISH during embryonic divisions, which now allows the investigation of the behavior of Ks during soma elimination, i.e. in whole mount embryos, and possibly during germ line elimination in the gonads of newly hatched larvae.

In summary, we have characterized two major families of tandemly repeated DNA from the chironomid *A. lucidus*, one of which is specific for the germ line. These results strongly support our earlier hypothesis (Staiber and Thudium 1986), that during evolution Ks have developed from the Ss by rearrangements and by the formation and accumulation of repetitive elements - germ line-specific repetitive sequences as demonstrated in this investigation.

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