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Characterization of heterochromatin of germ line limited and soma chromosomes in *Acricotopus lucidus* (Diptera, Chironomidae) by differential banding methods

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Abstract

The distribution pattern of constitutive heterochromatin in germ line limited (=K) and soma (=S) chromosomes of *Acricotopus lucidus* was investigated by C-banding of gonial mitoses. The DNA-binding, AT- and GC-specific, fluorochromes diamidinophenylindole (DAPI) and chromomycin A_3 (CMA) indicated obvious differences in base pair compositions of K and S heterochromatin. The specific silver stainability of the two paracentromeric C-bands of each of the Ks and of the centromeric C-bands of the Ss is discussed in connexion with the different behavior of the Ks and the Ss during the complex chromosome cycle in *A. lucidus.*

Introduction

The Orthocladiinae, a subfamily of the Chironomidae, possess additional chromosomes present only in the germ line (Bauer and Beermann 1952). These germ line limited chromosomes (=Ks, K is derived from "Keimbahn" Bauer 1970), together with the soma chromosomes (=Ss), pass through a complex chromosome cycle with interesting genetic phenomena: the elimination of all Ks from the future somatic cells during early cleavage divisions (soma elimination), the elimination of about half of the Ks in the first mitosis of the primordial germ cells (germ line elimination), and the compensating doubling of the Ks in the last gonial mitosis, termed differential mitosis (for review, Beermamm 1956; White 1973).

The function of the Ks, and the reason for their different behavior from the Ss are still so far unknown (Hennig 1986). In the Cecidomyiidae, the Ks are demonstrated to be necessary for normal differentiation of the germ cells (Geyer-Duszynska 1966; Bantock 1970).

In the orthocladiid *Acricotopus lucidus*, nine different K types were identified by G-banding, which form, in different numbers and various combinations, the gonial K complements (Staiber 1988). Preliminary experiments of C-banding and X-ray induced rerrangements between Ks and Ss, with subsequent investigation of the inserted K sections in the polytene salivary gland chromosomes, showed that the Ks contain heterochromatic segments and S-homologous sections (Staiber and Thudium 1986).

In the present study it was the intention to get detailed C-banding patterns of the different K-types and the Ss, and to characterize the heterochromatic regions by differential banding and staining techniques. The use of specific DNA-binding fluorochromes as 4'-6-diamidino-2-phenylindole (DAPI) and Chromomycin A3 (CMA) permits a more precise characterization of C-positive regions, making a correlation of certain heterochromatic bands rich in AT or GC base pairs visible (Schweizer 1976). The selective digestion of the chromosomes with the restriction endonuclease AluI produces patterns of darkly and lightly stained regions representing endonuclease-resistant sections and sections susceptible to the enzyme (Mezzanotte et al. 1983; Bianchi et al. 1985). Differential silver stainability can demonstrate heterogeneity in protein composition of C-positive regions (Sudman and Greenbaum 1989).

Materials and methods

Testes of young fourth instar larvae of an *Acricotopus lucidus* laboratory stock were dissected in Firling medium, treated with hypotonic 0.5 % sodium citrate solution for 20 min. and fixed in ethanol:acetic acid (3:l) for 2 h. Slide preparations were made after the dry ice method and were then air-dried.

G-banding followed the procedure described in Staiber (1988) and C-banding was done with NaOH according to the method of Hsu (1971).

Fluorescence bandings with actinomycin D - 4'-6-diamidino-2-phenylindole (AMD-DAPI) and with chromomycin A_3 (CMA: Serva. Heidelberg) were performed according to the techniques of Schweizer (1976). Chromosome fluorescence was observed and photographed with a Zeiss Standard microscope equipped with an epifluorescence system (filter combination BP 365/FT 395/LP 397 for AMD-DAPI and G 436/FT 510/LP 520 for CMA, mercury lamp HBO 50 W). Photographs of DAPI fluorescence were taken on Agfaortho 25 film and of CMA fluorescence on Kodak T-MAX 400 film developed to 3200 ASA.

For restriction endonuclease digestion with AluI, freshly prepared slides were covered with 1.6 U of AluI (Sigma) in 100 µl reaction buffer (50 mM Tris-HCl, 10 mM $MgCI_2$, 4 mM spermidine trihydrochloride. pH 8.0; Penna et al. 1989) and were incubated in a moist chamber

for 4-5 h at 37°C. Staining was performed with 2 % Giemsa (Merck) in Sörensen's phosphate buffer, pH 6.9. for 25 min.

Differential silver-staining was obtained using a modified method of Howell and Black (1980). Colloidal developer (2 g gelatine in 100 ml deionized water and 1 ml pure formic acid) and $AgN0_3$-solution (4g $AgN0_3$ in 8 ml deionized water) were mixed in a ratio of 3:4. Freshly prepared slides were covered with the mixture and a coverslip, and were stained on a slide-warmer at 60°C for 3-5 min. Airdried preparations were mounted in glycerol and were then photographed. Subsequently the preparations were destained with "Farmer's" reducer (Tetenal, 1.1 g in 250 ml A. dest., for 30 s; Mayer et al. 1987), air-dried, and G-banded.

Results

C-banding

Differential patterns of small and large heterochromatic segments were detected by C-banding in the Ks of *A. lucidus* (Fig. la, Fig. 2). Each of the nine K types exhibits two paracentromeric C-bands. In K1-K8 they are very prominent, whereas in K9 they are less obvious. Additionally, K1 has, in the long arm, a conspicuous triplet of large subterminal C-bands. K2 and K3 show duplets of subterminal and terminal C-bands in their long arms. The subterminal band of K3 is smaller than that of K2. The chromosomes K5, K6 and K7 exhibit small terminal and interstitial C-positive bands. The main C-bands of the Ks are also to be seen after G-banding (Fig. 2).

In contrast to the Ks, each of the three Ss shows only one obvious centromeric C-band, which corresponds to the large heterochromatic block representing the centromeric region in the polytene salivary gland chromosome (Staiber and Behnke 1985). To facilitate the recognition of chromosomes and the comparison of banding patterns, a set of G-banded Ks and Ss is shown in Fig. 2.

AMD-DAPI staining

The AMD-DAPI fluorescence patterns of the Ks correspond to their C-banding patterns, i.e., all C-positive bands fluoresce brightly (Fig. lb and Fig. 2). This indicates the AT-richness of the K heterochromatin. In contrast to the very similar C-staining intensity of the paracentromeric, subterminal, and terminal bands in K1, K2 and K3, the fluorescence of the paracentromeric bands of these chromosomes is less intense when compared with the very bright fluorescence of the subterminal and terminal bands. This implies differential AT content. In the three Ss, no positive AMD-DAPI fluorescence band were observed. This is in contrast to the C-banding pattern, where the Ss exhibit clear centromeric heterochromatin block.

CMA staining

No bright CMA fluorescence bands were observed in the Ks. However, in the regions of the C-bands, negative fluorescence bands visible as clear nonfluorescent sections on the chromosomes

were apparent after CMA staining. These fluorescence gaps are especially clear in the regions of the large paracentromeric, subterminal and terminal C-bands of K1 and K2. In Fig. 2 the negative fluorescence bands of K1 are marked with arrowheads. The gaps are also present in the regions of the smaller C-bands, but less clearly to be recognized. For the Ss, in the regions of the centromeric C-bands. no positive CMA fluorescence could be determined.

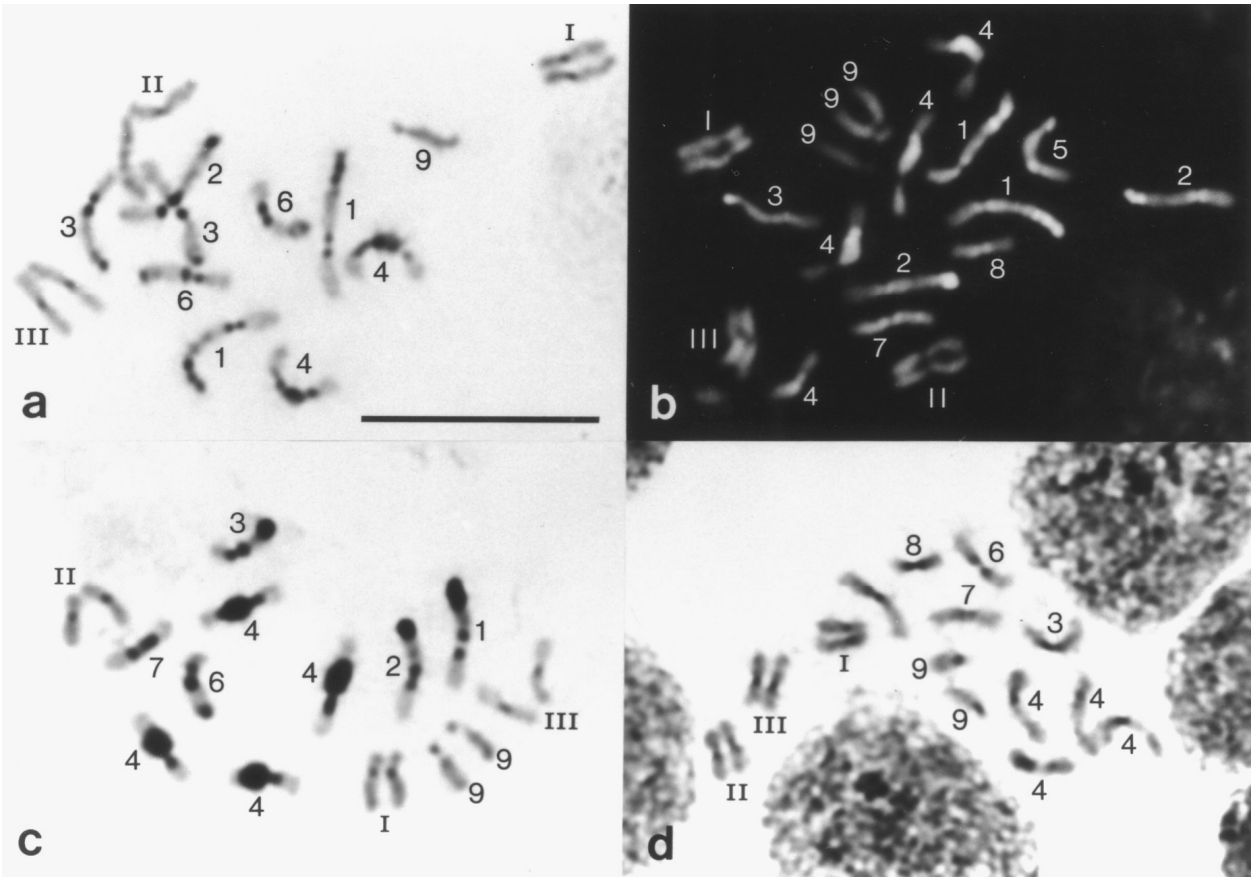

Fig. l a-d . Gonial metaphases of *A.lucidus* **a** C-banded. **b** AMD-DAPI fluorescence. **c** Digested with the restriction endonuclease AluI. **d** Silver-stained. I-III, somatically paired soma chromosomes. 1-9, germ line limited chromosomes K1-K9. Bar =10 µm

AluI digestion

The banding pattern of the Ks received after digestion with AluI and subsequent Giemsa-staining is identical to the C-banding pattern, even though the staining of all bands was frequently more intense than after C-banding (Fig. lc. Fig. 3). The same applies to the Ss, which consequently exhibit dark bands in their centromeric regions.

Silver staining

In the Ks, silver staining technique resulted in a differential staining of the paracentromeric C-bands on the one side, and of the interstitial, subterminal and terminal C-bands on the other. In K1-K8 silver effects an intense staining of the two paracentromeric bands of each of the Ks, and

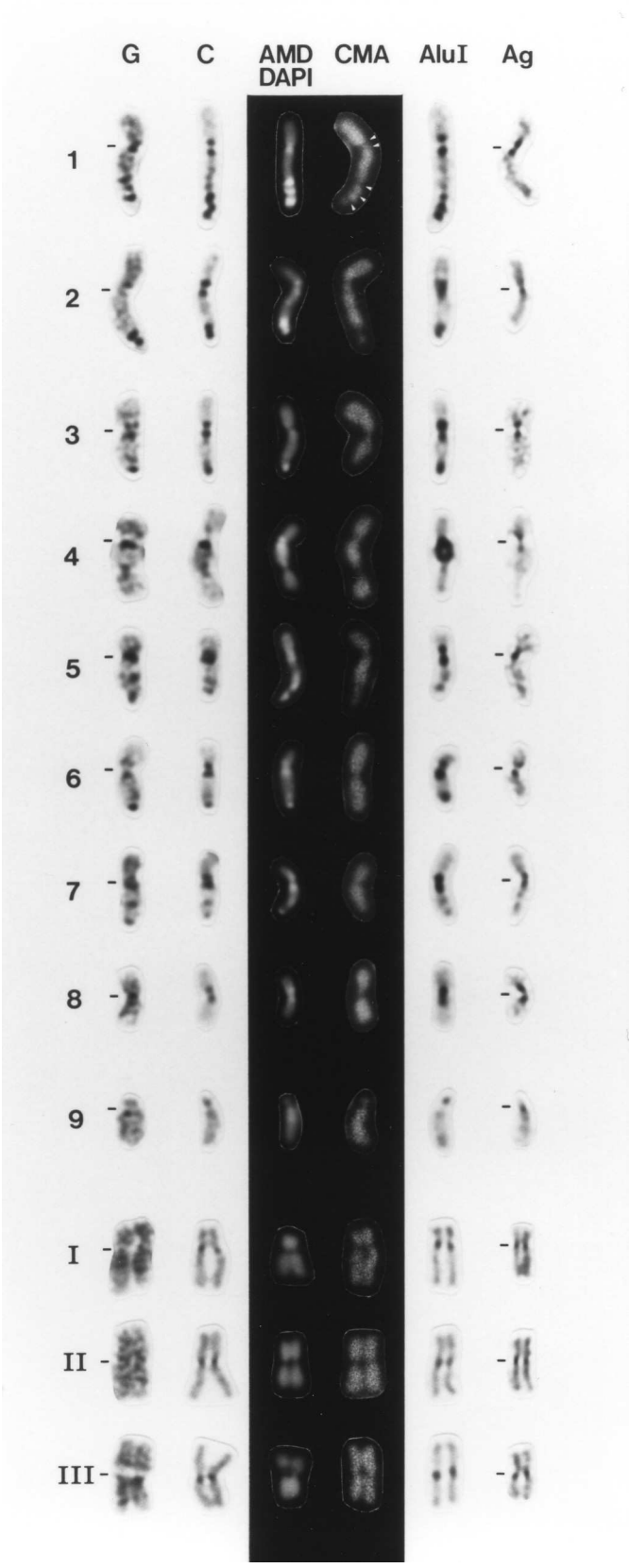

Fig. 2. Banding patterns of the germ line limited chromosomes (1-9) and the soma chromosomes (I-III) of *A. lucidus.* G-, C-, AMD-DAPI- and CMA-banded, digested with restriction endonuclease AluI, and silver (Ag)- stained. Small dashes mark positions of centromeres. Arrowheads in CMA-stained K1 indicate negative fluorescence bands.

none or a very faint staining of the other C-bands (Fig. 1d. Fig. 2). K9 shows only one, a small paracentromeric silver positiv band in the long arm. For the Ss an intense staining of their centromeric regions results from silver treatment. Compared with the other banding methods, silver staining has the disadvantage that it yields only a low number of suitable stained preparations. After destaining with Farmer's reducer, the Ks were identified by G-banding.

Discussion

C-banding demonstrates that the Ks of *A. lucidus* contain high amounts of heterochromatin in the form of paracentromeric, interstitial, subterminal and terminal C-bands. In the Ss, heterochromatin is restricted to the centromeric regions. Differences between K and S heterochromatin were observed after staining with base pair specific fluorescence dyes. Positive fluorescence of all C-bands of the Ks after AMD-DAPI staining, and negative fluorescence after CMA staining, indicate high proportions of AT base pairs of the K heterochromatin, in contrast to the centromeric S heterochromatin, which fluoresces with both dyes as do the euchromatic chromosome arms.

The structure of K heterochromatin of *A. lucidus* was investigated on X-ray induced K insertions in the polytene salivary gland S chromosomes (Staiber and Thudium 1986) The K heterochromatin insertions were highly vacuolated. In contrast, the heterochromatic centromere regions of the polytene Ss frequently exhibit distinct banding patterns (Staiber and Behnke 1985). In an earlier investigation it was demonstrated that centromeric S bands contain sequences that could be activated to form a Balbiani ring when they are translocated into euchromatic chromosome sections (Staiber 1982).

In *A. lucidus* the whole heterochromatic segments of the paracentromeric C-bands of the Ks and of the centromeric C-bands of the Ss are intensely stainable with silver. The other C-bands of the Ks are not or only weakly colored in such selectively silver stained metaphases. The different silver stainability of centromeric and paracentromeric C-bands, compared with that of interstitial, subterminal and terminal C-bands, indicates different compositions of these heterochromatic segments. These differences may be based on different protein and/or DNA compositions. Different DNA compositions of differently located C-bands were established for the grasshopper *Atractomorpha similis*. With a cloned probe of satellite DNA, John et al. (1986) detected highly repeated DNA-sequences in subterminal and terminal C-bands, which were not present in centromeric or paracentromeric C-bands. Specific nonhistone proteins are known to be responsihle for the selective silver stainability of active NORs and of nucleoli (Howell, 1977; Buys and Osinga (1984). Most probably the same holds true for the disclosure of kinetochores by silver staining (Denton et al. 1977). Using this method Sudman and Greenbaum (1989) found on meiotic chromosomes of the cactus mouse *Peromyscus eremicus* that only the kinetochores, not the whole centromeric heterochromatin bands, were silver-stained. These authors also reported on stainability differences between mitotic and meiotic kinetochores. Positive fluorescence after AMD-DAPI and negative fluorescence after CMA staining showed AT-

richness of all C-bands of the Ks, as also of the paracentromeric bands. In contrast, the centromeric heterochromatin of the Ss exhibited no specific fluorescence. That indicates different DNA compositions. So the selective silver stainability of paracentromeric K and centromeric S heterochromatin is most probably ascribable to specific proteins.

The striking difference, all Ks with the exception of K9 having two prominent paracentromeric C-bands whereas the Ss have only one centromeric band, might raise the supposition that this is connected with the different behavior of Ks and Ss during the complex chromosome cycle in *A. lucidus,* exhibiting specifics of germ line elimination, soma elimination, and differential mitosis. During germ line elimination about half of the Ks, and during soma elimination, all Ks remain in the equatorial plate and are then eliminated, while the Ss make a normal anaphasic migration (Beermann 1956). In differential mitosis, all Ks move undivided in one direction to one cell pole only, while the Ss separate and move to both poles. Probably the two paracentromeric K heterochromatin bands serve for the recognition of a K chromosome as such and/or influence the interaction of Ks with the spindle fibers, causing the above mentioned behavior. An earlier report of the structure of a special K, which spontaneously escaped from the soma elimination and was then polytenized in the larval salivary gland (Staiber 1987), supports the idea of the importance of the two paracentromeric bands for the Ks. This special K, having only a single heterochromatic block, was therefore probably not recognized as a K during soma elimination.

The germ line limited chromosome K9 takes an exceptional position under the Ks. After C-banding the two paracentromeric bands of K9 are only weakly stained, and only one band, the paracentromeric band in the long arm, is stainable with silver, similar to the centromeric bands in the Ss. This S-like staining behavior supports the idea of an intermediate position of K9 between Ss and Ks, which was based on earlier observations of partial pairing of K9 with soma chromosome I (see Fig. 3b, c in Staiber 1987), of the sometimes delayed migration to the one cell pole in contrast to the other Ks in differential mitosis, and of the - in rare cases observed - anaphasic separation of K9 together with the Ss in differential mitosis (Thudium 1974).

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