Chromosoma (Berl) (1988) 96:112–118

CHROMOSOMA

Chromosome 9 of *Ellobius lutescens* is the X chromosome

Walther Vogel[1], Peter Steinbach[1], Mahmoud Djalali[1], Karl Mehnert[1], Sher Ali[2], and Jörg Thomas Epplen[2]

[1] Abteilung Klinische Genetik der Universität Ulm, Oberer Eselsberg, M25, D-7900 Ulm, Federal Republic of Germany
[2] Junior Cancer Research Unit, Max-Planck-Institut für Immunobiologie, D-7800 Freiburg, Federal Republic of Germany

Abstract. *Ellobius lutescens* carries an apparently identical karyotype ($2n=17$) in both sexes. On the basis of indirect evidence the unpaired chromosome 9 has been considered to represent the X chromosome of this species. We have obtained data to substantiate this view by four different techniques. After fusion of HPRT$^-$ RAG cells with *E. lutescens* fibroblasts we demonstrated that the enzymes HPRT and G6PD are localized on the presumptive X chromosome. By analysis of pachytene figures after silver staining we showed by electron microscopy that the single chromosome exhibits the typical features of an X chromosome in male meiosis. Hybridization of $(GATA)_4$ and $(GACA)_4$ oligonucleotide probes to *E. lutescens* DNA revealed several distinct bands in the high molecular weight range some of which appeared to be specific for the individual but not for the sex of the animal. Hybridization in situ of the $(GATA)_4$ probe on metaphase spreads of *E. lutescens* did not highlight any particular chromosome segment but showed a significant deficit of these sequences in chromosome 9. These observations are discussed with respect to their bearing on X chromosome determination. Finally it is concluded that *E. lutescens* should be an ideal tool for testing candidate genes assumed to be involved in primary sex determination.

Introduction

Ellobius lutescens is a species of mull vole with a distinctive karyotype with respect to chromosomal sex determination. Since the earliest cytogenetic investigations (Matthey 1953, 1958) it has been known to possess eight pairs of homologous chromosomes and a single one in both sexes.

Studies of male and female meiosis (Matthey 1958, 1964; Castro-Sierra and Wolf 1968) and analysis of chromosome replication by autoradiography (Schmid 1967; Castro-Sierra and Wolf 1967) have suggested that the single chromosome (no. 9) represents the X chromosome. This hypothesis, however, would not allow *E. lutescens* to generate predominantly balanced offspring without complicated meiotic mechanisms. Though such a mechanism had been found in *Microtus oregoni* (Ohno 1963), it has not been possible to obtain corresponding evidence for *E. lutescens*. Hence, only accidental findings remain as a basis for explanation, e.g., the association of the sex vesicle with an autosomal bivalent has been interpreted as indicating autosomal integration of the Y chromosome (Castro-Sierra and Wolf 1968).

The analysis of banding patterns has yielded differing results on chromosomal sex determination (de la Maza and Sawyer 1976; Wolf et al. 1979). Nevertheless, both studies suggested the integration of morphologically visible correlates of sex chromosomes into one homologue of pair no. 1. Recently it has become clear that the differences between both chromosomes 1 do not represent a chromosomal basis for sex determination in *E. lutescens* (Djalali et al. 1986). Thus the coincidence of variant no. 1 chromosomes with the sex of their carriers seems fortuitous.

It has generally been accepted that chromosome 9 of *E. lutescens* represents one X chromosome in this species. The evidence, however, is rather indirect:

The DNA content of this chromosome comprises roughly 5% of the haploid genome (Ohno et al. 1964) which corresponds well with the mammalian X (Castro-Sierra and Wolf 1967).

The single chromosome is included in the sex vesicle during pachytene of male meiosis as is usual for mammalian sex chromosomes (Matthey 1958; Castro-Sierra and Wolf 1968).

There is no example of a completely unpaired mammalian autosome, but this is the common situation with the mammalian X chromosomes in male mammals.

The particular chromosome constitution of *E. lutescens* and the contradictory results of banding analyses necessitate particular caution before accepting assumptions and hypotheses. Therefore, we tried to characterize chromosome 9 of *E. lutescens* by several techniques to confirm that it is, indeed, the X chromosome. A priori we considered four techniques as potentially informative either about the identity of the X chromosome or chromosomal sex determination in this species: (i) Somatic cell genetics might allow the assignation of X linked enzymes to chromosome 9 of *E. lutescens*. (ii) Silver staining of the synaptonemal complexes should demonstrate any pairing of the smallest chromosome with an autosomal bivalent. (iii) Sex-specific simple repeated sequences of snakes (the so-called Bkm DNA) show sex-specific restriction fragments in several animals (for review see Epplen and Ohno 1987) including other rodent species. This might also apply to *E. lutescens* and thus – in conjunction with in situ hybridization – indicate a chromosomal segment involved in sex determination. (iv) When Bkm DNA, or its main component the GATA/GACA se-

Offprint requests to: W. Vogel

quences, is used for hybridization in situ the labelling is concentrated on the heterochromatic sex chromosome in mice (Singh and Jones 1982). Unequal distribution of these sequences in *E. lutescens* might help to understand chromosomal sex determination.

These techniques should allow a decision on whether chromosome 9 of *E. lutescens* is indeed the X chromosome and possibly give further insight into chromosomal sex determination in this species.

Materials and methods

Animals. The animals studied were a kind gift of Dr. Farhoud, Institut Pasteur, Teheran. The cytogenetic characterization of the animals studied here has recently been reported elsewhere (Djalali et al. 1986).

Fibroblast cultures were established and maintained as described (Wolf 1974). These were used for somatic cell genetics, cytogenetics, and for preparation of DNA.

Somatic cell hybrids were derived in hypoxanthine, aminopterin, thymine, glycine (HAT) medium (Szybalski et al. 1962) by fusing an established mouse line (RAG) deficient in hypoxanthine phosphoribosyl transferase (HPRT) to normal fibroblasts from a male *E. lutescens*. The RAG cells were pretreated with thioguanine to remove HPRT-positive revertants. After cell fusion mediated by polyethylene glycol (Pontecorvo 1976) the cells were plated into 75 cm^2 tissue culture flasks (ca. 1.5×10^6 cells per flask). After maintenance in HAT medium a number of clones were isolated within 2 months and grown in the same medium for several further months with *E. lutescens* chromosomes slowly segregating. To select true hybrid clones cellulose acetate gel electrophoresis of glucose-6-phosphate dehydrogenase (G6PD) isoenzymes was done according to standard methods. After electrophoretic separation the enzyme was stained with thiazolyl blue (MTT).

For cytogenetic evaluation the hybrid clones were harvested and prepared as usual. The slides were stained with 25 µg/ml Hoechst 33258 to allow for a clear discrimination of mouse chromosomes showing brightly fluorescing C-bands and *E. lutescens* chromosomes showing Q-bands but no C-bands.

DNA preparation from *E. lutescens* fibroblasts and gel electrophoresis procedures were performed essentially according to Maniatis et al. (1982). The DNAs of the different *E. lutescens* specimens were digested with several restriction enzymes (AluI, HaeIII, MboI, TaqI, HpaII) using the conditions recommended by the supplier. DNA samples were electrophoresed in 0.8% agarose gels, photographed after ethidium bromide staining under UV and dry-blotted (Tsao et al. 1983). The $(GATA)_4$ and $(GACA)_4$ oligonucleotides were radiolabelled using ^{32}P-ATP and T4 polynucleotide kinase as described by Miyada et al. (1985). Hybridization and washing were carried out according to Schäfer et al. (1986). The hybridized gels were exposed for 12 h without intensifier screen.

In situ hybridization. Chromosomes of the different *E. lutescens* specimens were obtained from fibroblast cultures by standard techniques (Wolf 1974). Slides were treated with 100 µg/ml RNase A (Boehringer) for 1 h at 37° C, rinsed in 2 × SSC and dried in an ethanol series. After DNA denaturation (70% formamide, 2 × SSC at 70° C for 2 min), hybridization was carried out in situ under coverslips in 5 × SSPE, 0.5% SDS, 50 µg/ml *Escherichia coli* DNA and 2.5×10^6 cpm/ml of the $(GATA)_4$ probe for 3 h at 35° C. Slides were washed 3 times for at least 15 min each in 6 × SSC on ice and 1 min at the hybridization temperature and subsequently dried. Slides were coated with NTB3 emulsion (Kodak) and exposed for 4 to 8 days at −70° C. Individual metaphases were photographed after G-banding according to standard procedures (Nesbitt and Francke 1973). As a control for the hybridization specificity of the $(GATA)_4$ probe, the length-matched oligonucleotide 5′-AATTCCGTATCGATGC was used. This sequence is not present in the genomes of the mouse and *E. lutescens*. Hybridization, washing and exposure were carried out as outlined above except that the hybridization temperature was 41° C for the latter probe because of the differing base composition.

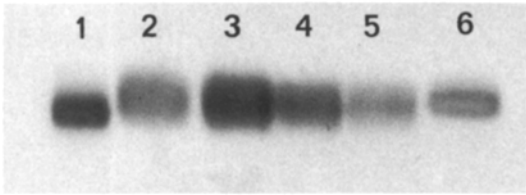

Fig. 1. Glucose-6-phosphate dehydrogenase (G6PD) patterns on a cellulose acetate gel. *Lane 1*, RAG; *Lanes 2–5*, hybrid (RAG × *Ellobius*) clones 12, 13, 14, and 16 showing the heteropolymer pattern; *lane 6*, fibroblasts of *E. lutescens*

Meiotic studies. One male of *E. lutescens* was used for meiotic preparations according to Dresser and Moses (1980). The silver staining of synaptonemal complexes (SCs) was performed according to Howell and Black (1980). For electron microscopy analysis a Philips EM301 was used.

Results

Somatic cell genetics

Under the conditions used the electrophoretic mobility of the *E. lutescens* G6PD is slightly faster than that of the mouse G6PD (Fig. 1). Among 16 isolated clones examined there were 11 showing the interspecific heteropolymer G6PD band (Fig. 1), whereas 5 clones had apparently lost the *E. lutescens* G6PD. Cytogenetic analysis of the 11 proven hybrid clones (Fig. 2) revealed the panel shown in Table 1, (5 clones deficient for *E. lutescens* G6PD were excluded from cytogenetic analysis). Chromosome 9 was the only one retained in all hybrid clones accompanied by different other chromosomes of *E. lutescens*, most frequently chromosome 3. This was absent only from clones 10 and 19. The hybrids had been maintained in HAT medium and the mouse parent was deficient for HPRT. Therefore, an active gene for HPRT – required for survival of the hybrid cells – is located on chromosome 9 of *E. lutescens*.

After cytogenetic evaluation the hybrid clones 11, 13, 14, 16, and 19 were grown for one to three further passages and again tested for G6PD by gel electrophoresis. The pres-

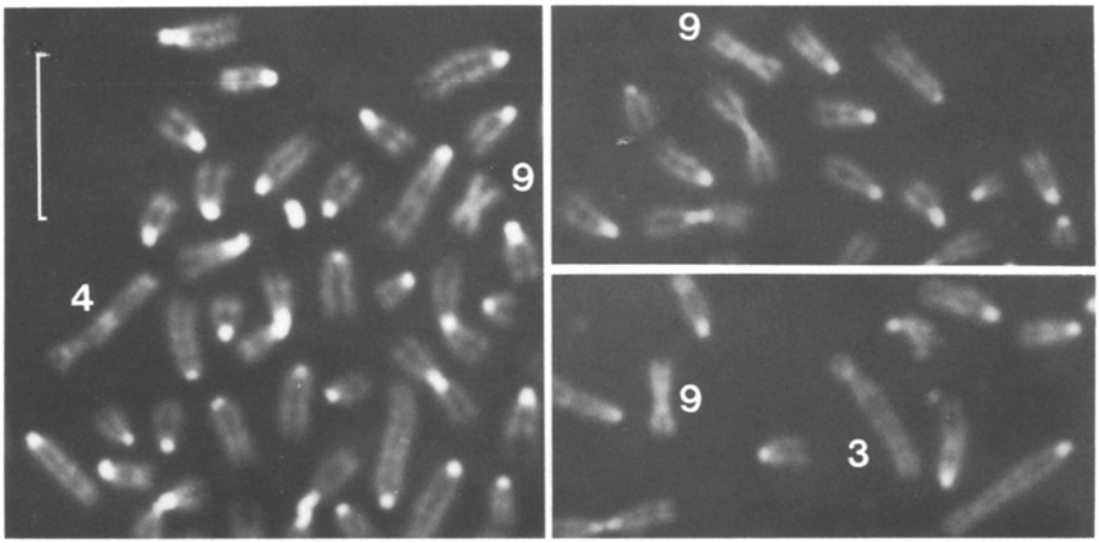

Fig. 2. Partial metaphases of RAG × *Ellobius lutescens* hybrid cells stained by Hoechst 33258. Note the bright fluorescence of the mouse C-bands and clear banding patterns on *Ellobius* chromosomes. Bar represents 10 μm

Table 1. Hybrid clone panel: *Ellobius* chromosomes retained in *Ellobius* × RAG hybrid clones showing activity of *Ellobius* HPRT and G6PD

Hybrid done no.	*Ellobius* chromosomes						
	3	4	5	7	8	9	mar
6	+	+	−	−	+	+	−
8	+	−	−	−	−	+	−
9	+	−	−	−	−	+	+
10	−	+	−	−	−	+	−
11	+	−	−	−	−	+	−
13	+	−	−	−	+	+	−
14	+	−	−	−	−	+	−
15	+	−	−	−	−	+	−
16	+	−	−	−	−	+	+
19	−	+	+	+	−	+	−

The presence of *Ellobius* G6PD was tested again after cytogenetic evaluation in clones *11, 13, 14, 16,* and *19*. Only chromosome *9* was retained in all hybrid clones. Chromosomes *1, 2* and *6* were not observed in any of the clones. *mar* unidentified marker chromosome

ence of *E. lutescens* G6PD at the time of the cytogenetic analysis was confirmed. Therefore, in *E. lutescens* not only the gene for HPRT but also the G6PD gene is linked to chromosome 9.

Gel hybridization with the oligonucleotide probes $(GATA)_4$ and $(GACA)_4$

The DNA of three male and four female specimens was probed with the $(GATA)_4$ and $(GACA)_4$ oligonucleotides. In general, the patterns showed several distinct bands in the high molecular weight range and a background smear towards smaller fragment sizes (Fig. 3). Some bands varied considerably between individual specimens indicating polymorphism of the length of restriction fragments detectable by the $(GATA)_4$ and $(GACA)_4$ oligonucleotides. Neither the presence of bands, however, nor their position allowed the detection of any sex specificity. The comparison of the patterns from a mother with those of her son and her daughter did not reveal sex-specific bands (data not shown).

Hybridization in situ

The distribution of $(GATA)_4$ elements or multiples thereof on the chromosomes of *E. lutescens* was analysed by hybridization in situ. A survey of more than 50 metaphases each from a male and a female animal clearly showed that there is no outstanding signal on any of the chromosomes (Fig. 4). A closer analysis after photography revealed a nearly even distribution of the grains over the whole chromosome set with the exception of chromosome 9 (Fig. 5). The evident reduction of grains on this chromosome indicates that this singular chromosome carries fewer $(GATA)_4$ sequences than the other pairs of chromosomes.

Synaptonemal complexes

The structure and localization of chromosome 9 (the smallest one) was studied in 68 pachytene figures. Measurements showed that the mean length of this chromosome was about two-thirds that of the smallest bivalent. It was surrounded by condensed material (Fig. 6) as is the sex vesicle in other species. The axial core of this chromosome appeared thickened and more heavily stained than the other SC's (Fig. 7). In many cells this axial element formed hairpin loops (Figs. 6 and 8a). There were up to four loops which seemed to occur in fixed positions.

An attachment of chromosome 9 to an autosomal bivalent was observed in 2 out of 68 pachytene figures. In both instances the axial element was attached to the lateral element for a short distance but formed neither an SC nor the typical structure of a trivalent (Fig. 8a, b). Furthermore, the respective autosomal bivalents were different as well as the points of attachment on chromosome 9. A terminal association of the sex vesicle to different autosomal bivalents was seen in six plates, but always without connection to the lateral and axial element. In the remaining spreads

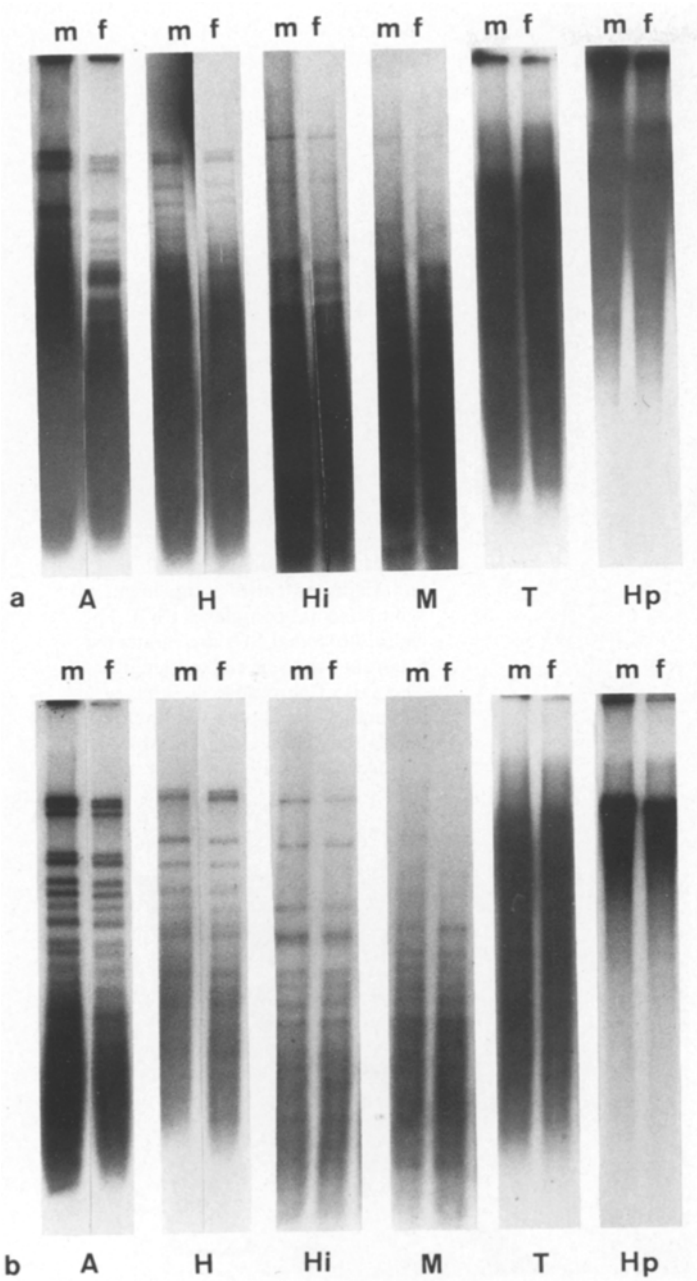

Fig. 3a, b. Gel hybridization of male (*m*) and female (*f*) *Ellobius lutescens* DNA with **a** the $(GATA)_4$ and **b** the $(GACA)_4$ oligonucleotides. Restriction enzymes are indicated at the *bottom: A* AluI; *H* HaeIII; *Hi* HinfI; *M* MboI; *T* TaqI; *Hp* HpaII. Four details see Materials and methods

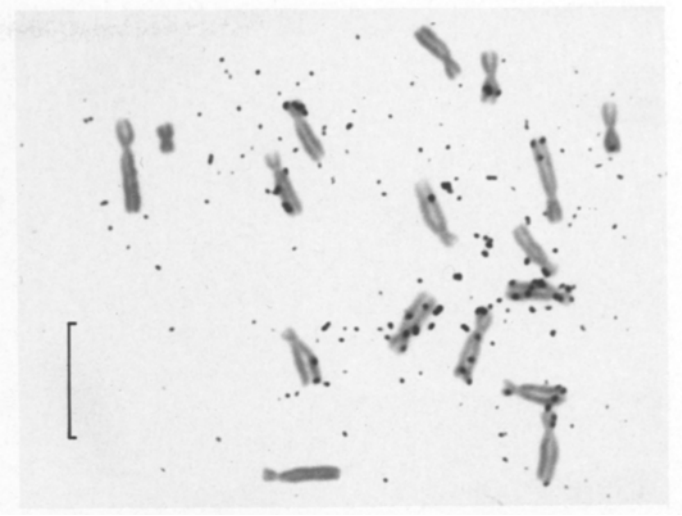

Fig. 4. Example of a metaphase of a male *Ellobius lutescens* specimen with autoradiographic detection of $(GATA)_4$ sequences after hybridization in situ. For details see text. Bar represents 10 μm

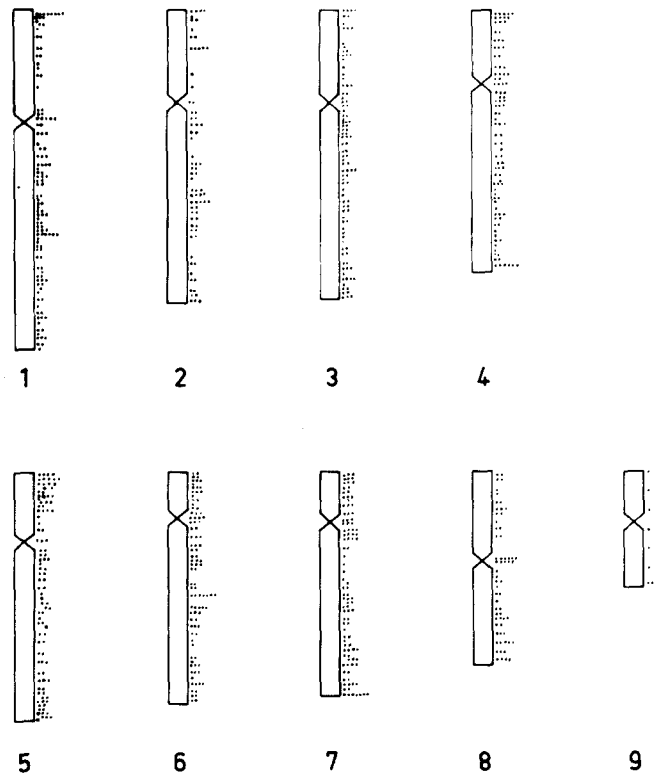

Fig. 5. Cumulative grain distribution from 28 metaphases of *Ellobius lutescens* after hybridization in situ with the $(GATA)_4$ oligonucleotide and autoradiography

the sex vesicle was separated and localized in the periphery of the pachytene figures.

Discussion

There are manifold problems with the peculiar chromosome complement of *E. lutescens*, predominantly the lack of detectable chromosomal sex determination and the unbalanced offspring expected in consequence of the odd number of chromosomes. The unpaired chromosome has always been assumed to represent the X chromosome (e.g., see Matthey 1953; Castro-Sierra and Wolf 1967). The main purpose of this study was to obtain supporting evidence for this view. There were several hints indicating that a pair of autosomes is involved in sex determination (Castro-Sierra and Wolf 1968), possibly no. 1 (De la Maza and Sawyer 1976; Wolf et al. 1979). These indications encouraged us to restudy male meiosis employing $AgNo_3$ staining and electron microscopy. Bkm DNA shows sex-specific hybridization patterns in the heterogametic sex of several ro-

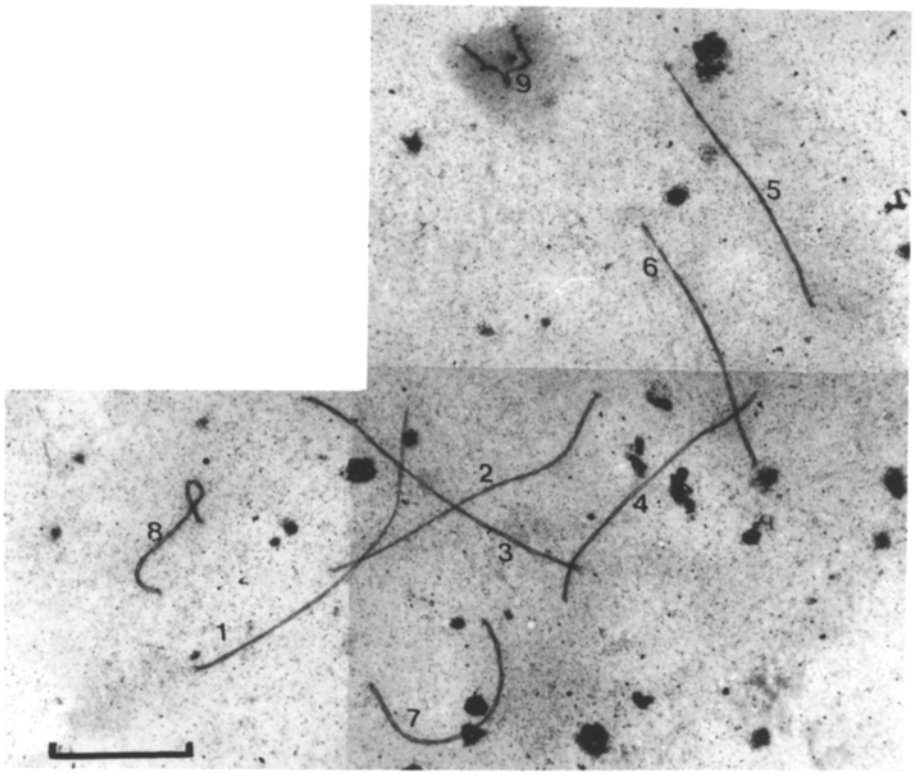

Fig. 6. Spread pachytene cell containing an entire complement of synaptonemal complexes (SC). The eight autosomal SCs are numbered according to their ranked lengths. The axis of chromosome 9 has a distinctive morphology at pachytene and is thus clearly recognizable. Bar represents 10 μm

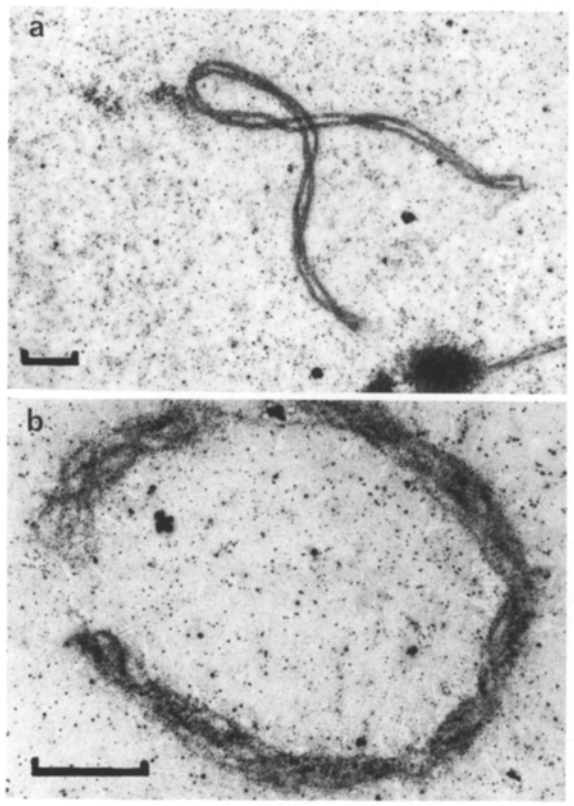

Fig. 7a, b. The synaptonemal complex (SC) of a bivalent is shown; the lateral elements appear as thin threads consisting of two parallel filamentous subunits. **b** In contrast the axial element of chromosome 9 is thickened and shows four plaited fibrils. Bars represent 1 μm

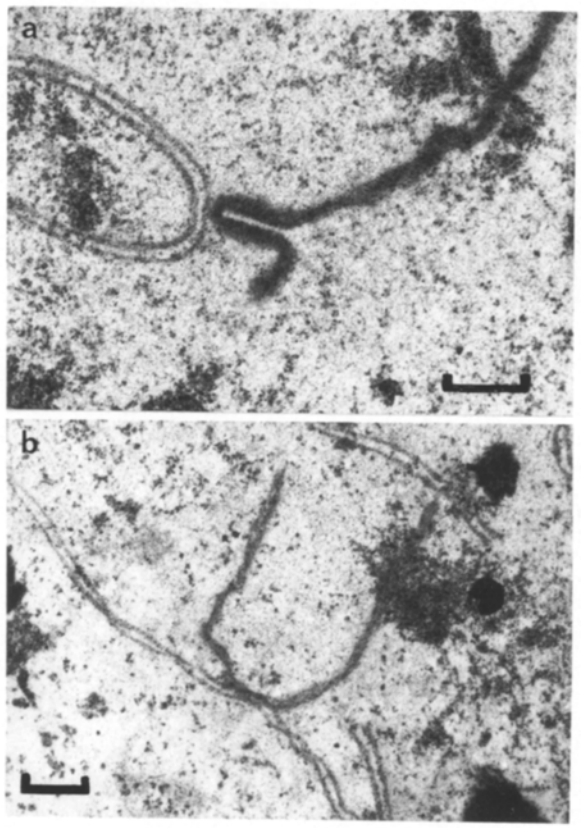

Fig. 8a, b. In two pachytene figures an association of the axial core of chromosome 9 with an autosomal bivalent was seen. In **a** the attachment point lies in the area of a hairpin loop near the end while the other (**b**) is near the centre of chromosome 9. Bars represent 1 μm

dent species. It appeared worthwhile, therefore, to hybridize *E. lutescens* DNA with these probes.

The results obtained by the four different techniques employed are fully compatible with the assumption that chromosome 9 of *E. lutescens* represents the X chromosome. Direct evidence, however, emerged only from somatic cell genetics. Since HPRT and G6PD can clearly be assigned to chromosome 9, it is obvious that this chromosome is the X chromosome. There are no known exceptions to the localization of these genes of the X chromosome in all mammalian species studied so far.

The analysis of SCs in pachytene figures revealed no discrepancy from the earlier studies with conventional staining (Matthey 1958; Castro-Sierra and Wolf 1968). The single element can now be identified as chromosome 9 by length measurements. Whereas the eight pairs of autosomes show typical bivalents the axial element of no. 9 corresponds to the X chromosome of other mammals in structure and localization. Hairpin loops like those observed here have been described in mouse and hamster (Moses 1977; Pathak and Hsu 1979). Furthermore, they have been observed in XO oocytes of mouse and man (Speed 1986).

In *E. lutescens* a frequent association between the univalent and an autosomal bivalent led to the assumption that this bivalent might be involved in sex determination (Castro-Sierra and Wolf 1968). We also saw two such associations, yet they were unspecific and no trivalent was formed. From these observations we conclude, therefore, that neither an homologous segment nor pairing and recombination between the X chromosome and an autosome is involved in the sex determination mechanism of *E. lutescens*.

GATA/GACA sequences reveal sex-specific restriction patterns in several species (Epplen and Ohno 1987). Therefore, we analysed the five males and four females available with a series of restriction enzymes which are informative in other rodents. The patterns varied without any detectable sex specificity.

Sex specificity with the aforementioned oligonucleotide probes can easily be seen in the mouse (Singh et al. 1981; Epplen et al. 1983) and has been successfully used to characterize the murine Sxr and Sxr′ genotypes (Epplen and McLaren 1987). In man the $(GATA)_4$ oligonucleotide does not detect sex-related differences directly but Y chromosomal genomic clones could be isolated by GATA sequences (Arnemann et al. 1986). Yet another situation occurs in the rodent *M. agrestis* where GATA sequences are concentrated in the constitutive heterochromatin of the X chromosome (Nanda et al., in press). Considering these heterogeneous findings in related species, it is not surprising that the oligonucleotides failed to detect sex-specific differences in *E. lutescens*. There is indirect evidence that GATA/GACA sequences spread through the eukaryotic genomes by a transposon-like mechanism (Epplen et al. 1983). The relative deficiency of GATA sequences on the X chromosome can at present be demonstrated but not explained. This is the more astonishing since no specific excision mechanisms are known for GATA/GACA elements and other repetitive DNA (Epplen and Studer 1987).

The Y chromosomes of many mammals carry chromosome-specific sequences. Among these sequences it is not easy to identify those which are responsible for primary sex determination. *E. lutescens* evidently has developed a particular sex determination mechanism and the Y chromosome has disappeared cytogenetically. Nevertheless, the genes encoding the function of primary sex determination must be present somewhere in the genome of this species. Therefore, this species should be an ideal tool for testing candidate genes assumed to be involved in primary sex determination.

Acknowledgements. The excellent technical assistance of Andrea Becker, Susanne Bauer, Margot Autenrieth, and Christine Klett is highly appreciated.

References

Arnemann J, Jakubiczka S, Schmitke J, Schäfer R, Epplen JT (1986) Clustered GATA repeats (Bkm sequences) on the human Y-chromosome. Hum Genet 73:301–303

Castro-Sierra E, Wolf U (1967) Replication patterns of the unpaired Chromosome 9 of the rodent Ellobius lutescens TH. Cytogenetics 6:268–275

Castro-Sierra E, Wolf U (1968) Studies on the male meiosis of Ellobius lutescens Th. Cytogenetics 7:241–248

De la Maza LM, Sawyer IR (1976) The G and Q banding pattern of Ellobius lutescens. A unique case of sex determination in mammals. Can J Genet Cytol 18:497–502

Djalali M, Hameister H, Vogel W (1986) Further chromosomal studies in Ellobius lutescens: Heteromorphism of chromosome 1 is not associated with sex determination. Experientia 42:1281–1282

Dresser ME, Moses MJ (1980) Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster (Cricetulus griseus). Chromosoma 76:1–22

Epplen JT, McLaren A (1987) GACA/GATA simple repeated DNA as a means to dissect the sex-reversed (Sxr) mutation of the mouse. In: Wachtel SS (ed) Evolutionary mechanisms in sex determination. CRC Press, Boca Raton, Florida

Epplen JT, Ohno S (1987) On DNA, RNA and sex determination. In: Lau YFC (ed) Selected topics in molecular endocrinology. Oxford University Press, New York

Epplen JT, Studer R (1987) On interspersed repetitive DNA sequences in animals. In: Sharma T (ed) Advances in chromosome research, in press

Epplen JT, Cellini A, Romero S, Ohno S (1983) An attempt to approach the molecular mechanisms of primary sex determination: W- and Y-chromosomal conserved simple repetitive DNA sequences and their differential expression in mRNA. J Exp Zool 228:305–312

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective collodial developer: a 1-step method. Experientia 36:1014–1015

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Matthey R (1953) La formule chromosomique et le probleme de la determination sexuelle chez Ellobius lutescens (Rodentia-Muridae-Microtinae). Arch Julius Klaus-Stift Vererbungsforsch 28:271–280

Matthey R (1958) Un nouveau type de determination chromosomique du sex chez les mammiferes Ellobius lutescens. Th et Microtus (Chilotus) Bachm (Murides-Microtines). Experientia 14:240–241

Matthey R (1964) Etudes sur les chromosomes d'Ellobius lutescens (Mammalia-Muridae-Microtinae). II. Informations complementaires sur les divisions meiotiques. Rev Suisse Zool 71:401–410

Miyada CG, Reyes AA, Studencki AB, Wallace RB (1985) Methods of oligonucleotide hybridization. Proc Natl Acad Sci USA 82:2890–2894

Moses M (1977) Synaptonemal complex karyotyping in spermatocytes of the Chinese hamster. Chromosoma 60:99–125

Nanda J, Neitzel H, Sperling K, Studer R, Epplen JT (1988) Simple repetitive GATA/GACA sequences characterize the X-chromo-

somal heterochromatin of Microtus agrestis, european field role (rodentia cricetidea). Chromosoma, in press
Nesbitt MN, Francke U (1973) A system of nomenclature for band patterns of mouse chromosomes. Chromosoma 41:145–158
Ohno S (1963) The creeping vole (*Microtus oregoni*) as a gonosomic mosaic. I. The OY/XY constitution of the male. Cytogenetics 2:232–235
Ohno S, Jainchill J, Stenius C (1964) X-autosome ratio and the behaviour pattern of individual X-chromosomes in placental mammals. Chromosoma 15:14–30
Pathak S, Hsu TC (1979) Silver stained structures in mammalian meiotic prophase. Chromosoma 70:195–203
Pontecorvo G (1976) Production of indefinitely multiplying mammalian somatic cell hybrids by polyethylene glycol (PEG) treatment. Somatic Cell Genet 1:397–400
Schäfer R, Ali S, Epplen JT (1986) The organization of the evolutionarily conserved GATA/GACA repeats in the mouse genome. Chromosoma 93:502–510
Schmid W (1967) Heterochromatin in mammals. Arch Julius Klaus-Stift Vererbungsforsch 42:1
Singh L, Jones KW (1982) Sex reversal in the mouse (Mus musculus) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. Cell 28:111–120
Singh L, Purdom JF, Jones KW (1981) Conserved sex-chromosome associated nucleotide sequences in eukaryotes. Cold Spring Harbor Sym Quant Biol 45:805–813
Speed RM (1986) Oocyte development in XO foetuses of man and mouse: the possible role of heterologous X-chromosome pairing in germ cell survival. Chromosoma 94:115–124
Szybalski W, Szybalski EH, Ragni C (1962) Genetic studies with human cell lines. Natl Cancer Inst Monogr 7:75–89
Tsao SGS, Brunk CF, Pearlman RE (1983) Hybridization of nucleic acids directly in agarose gels. Anal Biochem 131:365–372
Wolf M, Schempp W, Vogel W (1979) Ellobius lutescens Th (Rodentia, Microtinae): Q-, R-, and replication banding patterns. Chromosome 1 polymorphism in the male and presumptive heterogamety in the female. Cytogenet Cell Genet 23:117–123
Wolf U (1974) Cell cultures from tissue explants. In: Schwarzacher HG, Wolf U (eds) Methods in human cytogenetics. Springer, Berlin Heidelberg New York

Received July 16, 1987 / in revised form September 9, 1987
Accepted by D. Schweizer