Mammalian X-Chromosomes: A New Kind of Composite-Type X in the Vole *Arvicola scherman exitus* Miller

A large proportion of cytogenetically investigated eutherian mammals possess an X-chromosome measuring approximately 5% of the haploid ($nA + X$) metaphasic complement. This was pointed out by OHNO et al.[1] when they were comparing X-chromosomes of different mammals. It has long been known that there are exceptions to this 5% rule, namely in the sense that some species exhibit much longer X-chromosomes, e.g. the X's described in the rodents *Mesocricetus auratus* (HUSTED et al.[2]), *Microtus agrestis* (MATTHEY[3]) and *Microtus oregoni* (MATTHEY[4]) were named duplicate-, triplicate- and quadruplicate-type X-chromosomes by OHNO et al.[1]. The common finding in these species is, however, that the functional X-part again comprises around 5% of ($nA + X$) and that the additional chromatin on these X's is heterochromatic (OHNO et al.[1]). Some cytological properties of this 'structural' or 'constitutive' heterochromatin were clarified by SCHMID et al.[5] and SCHMID[6].

Among the *Microtinae* there are also types of X's with relatively short segments of structural heterochromatin inserted, e.g. in *Microtus pennsylvanicus* (SCHMID[6]) the X comprises 7.3% of the haploid set.

More unusual X's have been described in the order *Artiodactyla*: in the reindeer (FRACCARO et al., personal communication), the Situnga and the Blackbuck (WURSTER and BENIRSCHKE[7]).

A very unusual type of X, deviating in several respects from any known X-chromosome, was recently discovered by us in the vole *Arvicola scherman exitus* Miller.

Materials and methods. The animals were trapped in Henggart and in Witikon, 2 communities in the Canton of Zurich, separated by approximately 20 miles. Karyotype studies were made in cells of primary kidney epithelial cultures, as described previously (SCHMID[6]). In addition, fibroblast cultures from 1 animal of each sex were investigated. Labelling studies with 0.5 μC/ml H^3-TdR were carried out 4–5 days after setting up the kidney cultures. The isotope was added $5^1/_2$ h before termination. The autoradiographic technique was the same as described previously (SCHMID[6,8]).

Results. The karyotype was studied in 2 male and 6 female animals. The diploid number $2n = 36$ is in agreement with the findings of MATTHEY and RENAUD[9].

Figure 1 shows a complete karyotype of a female. The sex chromosomes of the male are shown in Figure 2. Chromosome pairs 8–15, the X and the Y are morphologically identifiable. The Y-chromosome is unusually long for a mammal, almost $^2/_3$ as long as the X. On the autoradiographs it is late replicating along its entire length (Figure 2). The relative length of the X-chromosome, determined from 5 karyotypes, is 6.80% of ($nA + X$).

Autoradiographs were made on 2 males and 4 females (1 male and 2 females from each locality). The labelling patterns described below were essentially identical, irrespective of the geographical origin.

The isocyclic X in the male and the female shows the same replication pattern: there is a late replicating segment in the proximal third of the long arm (Figures 1 and 2). The allocyclic X exhibits a most unusual replication pattern: it has the same late replicating segment in the proximal third of the long arm. In addition, the entire short arm and the distal third of the long arm are late replicating. The intermediate third of the long arm is, however, not late replicating (Figures 1 and 2). The autosomes show a moderate amount of relatively short late-replicating segments (Figure 1).

Conclusions and discussion. The X-chromosome of *A. scherman exitus* is, with 6.80% of ($nA + X$), neither a 'normal-type' X nor a 'duplicate-type' X, for the latter it

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[3] R. MATTHEY, Experientia *5*, 72 (1949).
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[5] W. SCHMID, D. W. SMITH and K. THEILER, Arch. Julius-Klaus-Stift. VererbForsch. *40*, 35 (1965).
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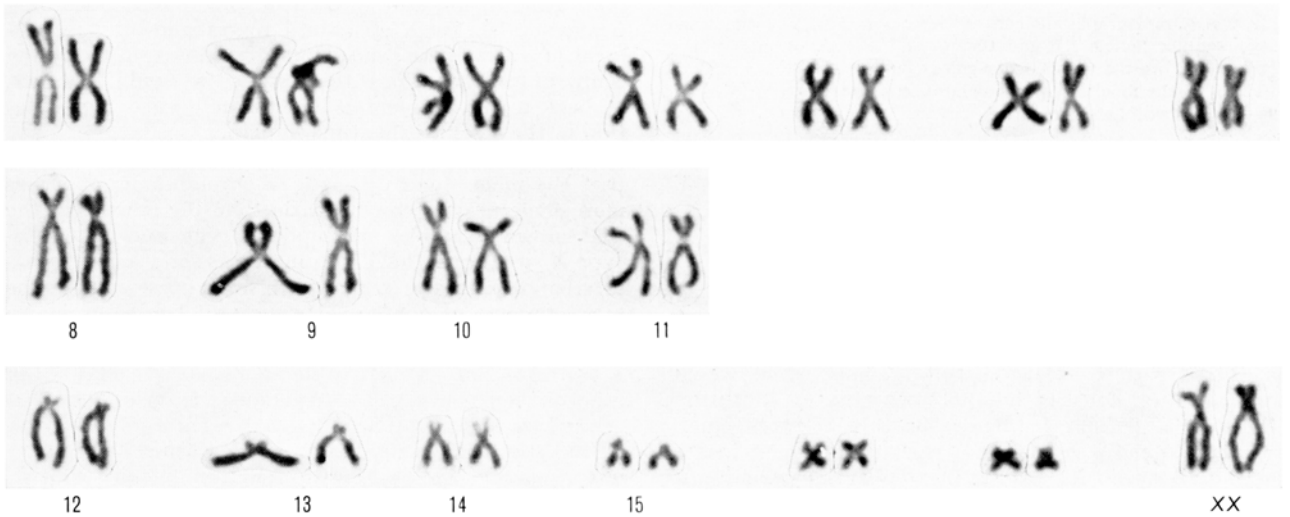
[9] R. MATTHEY and P. RENAUD, C.R.S.B., *120* (1935).

a



b

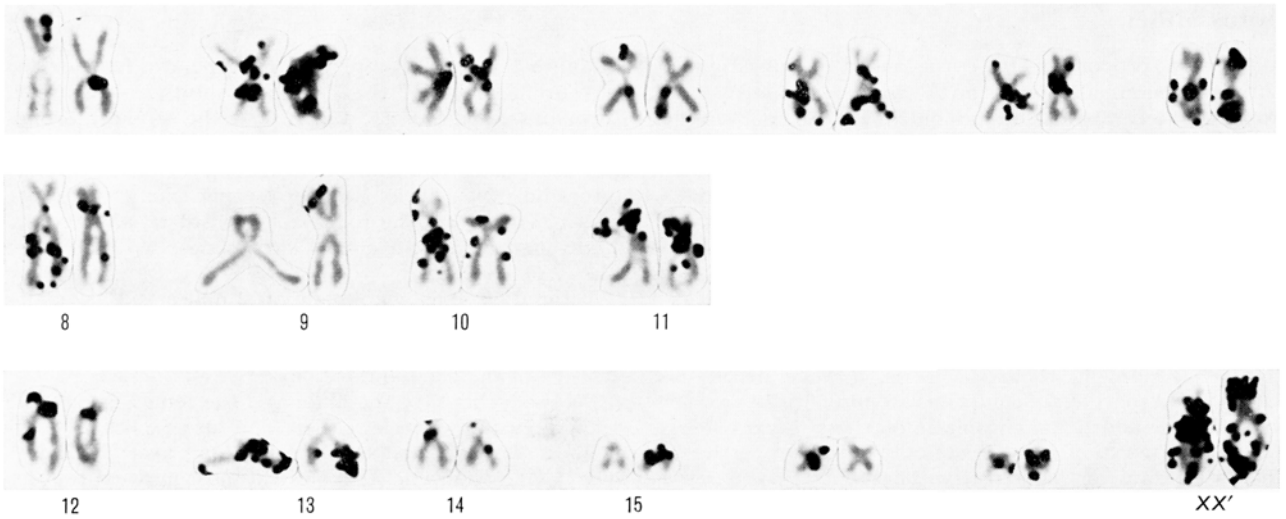


Fig. 1. Karyotype of a female *A. scherman exitus*; (a) the unlabelled chromosomes, (b) autoradiograph of the same metaphase. Chromosome pairs 8–15 and the *X*'s are identifiable by morphology. The autoradiograph obtained by continuous labelling with H^3-TdR towards the end of the S-period shows only few and short late-replicating segments in the autosomes and a characteristic pattern in the 2 *X*-chromosomes (see text). The asynchrony between the 2 structurally heterochromatic segments is, in this particular cell, very outspoken.

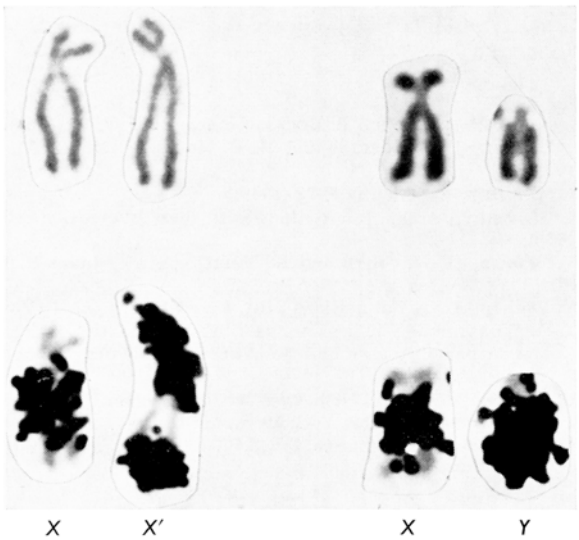


Fig. 2. Autoradiographs and control pictures of the 2 *X*-chromosomes from another female cell and the *X*- and *Y*-chromosomes from a male cell. Note the unlabelled segment in the allocyclic *X* and the division of the facultative heterochromatin into the short arm and the distal third of the long arm.

is too short. (Recently measurements on 15 female human karyotypes of the same individual were carried out in this laboratory and the *X*, which in man is considered of the 'normal' type, turned out to comprise 4.66% of $(nA + X)$.)

Both the isocyclic and the allocyclic *X* of *A. scherman* are fitted with a late-replicating segment which we call structural or constitutive heterochromatin (SCHMID[6]). There is a distinct tendency for this heterochromatic segment to show asynchrony, with the segment located on the allocyclic *X* terminating replication consistently earlier than its homologous segment (Figure 1). The functional *X*-part, as evidenced by the replication timing of its allocyclic member, is divided up into 2 regionally separated parts: the short arm and the distal third of the long arm. In the isocyclic *X*, it was noted that the functional *X*-part located in the short arm completes replication earlier than the part at the end of the long arm. This timing difference was not observed in the allocyclic state.

The most unusual feature, however, is the presence of a not-late-replicating segment in all *X*'s including the allocyclic member. If this segment were genetically active, then the dosage compensation mechanism between the 2 sexes would be disturbed, since the female receives 2 doses and the male only 1. Such a situation would be highly unusual in a mammal, but since this is apparently the rule in birds the sheer thought of it should not be too shocking for a biologist.

To produce genetic proof for such a suspicion would, however, be a formidable task. Perhaps there are simpler explanations for this unusual finding. Some 'normal-type' metacentric mammalian *X*-chromosomes, like the one of man, exhibit in the allocyclic state a relatively early-replicating region around the centromere (e.g. SCHMID[10], GIANNELLI[11]). By a structural rearrangement this segment in *A. scherman exitus* might have been shifted to the centre of the long arm. If this were so, we would, however, be back to the problem of incomplete dosage compensation in the 2 sexes; this time in man.

Another finding is unusual for mammals also; namely that the male, due to its long *Y*-chromosome, possesses more structural heterochromatin than the female. In the well-known examples of duplicate-type and triplicate-type *X* situations, the *Y*-chromosomes show a compensatory length increase, so that both sexes possess about the same amount of gonosomal structural heterochromatin (SCHMID[6]).

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The vole *A. scherman exitus*, $2n = 36$ is characterized by a most unusual sex chromosome situation as was determined by autoradiographic studies.

The *X* comprises 6.80% of $(nA + X)$; it is of a complex composite type. The functional part is divided into 2 regionally divided sections, the short arm and the distal third of the long arm. The proximal third of the long arm is apparently structurally heterochromatic, the intermediate third is not late replicating, it may be a euchromatic segment, remaining so in the allocyclic *X* as well.

The *Y*-chromosome is unusually long ($^2/_3$ of the *X*), and completely structurally heterochromatic. Thus, the male possesses more structural heterochromatin than the female sex[12].

Zusammenfassung. Autoradiographische Untersuchungen mit H^3-Thymidin deckten bei der Wühlmaus *A. scherman exitus* ($2n = 36$) eine ungewöhnliche Geschlechtschromosomensituation auf. Das *X*-Chromosom misst 6,8% von $(nA + X)$. Sein funktioneller Anteil ist zweigeteilt, daneben verfügt es über einen strukturell heterochromatischen und einen stets euchromatisch bleibenden Anteil. Das *Y* ist für ein Säugetier aussergewöhnlich gross ($^2/_3$ des *X*).

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Studies on r_{II} Region of T_2-Phage

Considerable efforts have been made over the last decade and a half to analyse more incisively the structure and function of the gene. Many reports[1-3] have appeared about the divisibility of the gene and its subunit structure, thus demolishing the concept of the gene being the ultimate unit of heredity.

By selecting a suitably marked microorganism as an experimental tool in the study of recombination, one can subdivide the gene almost down to a single nucleotide pair[3-4]. While studying the internal structure of the gene, BENZER was able to elucidate the unit of function of the r_{II} region of the T_4 phage and has shown that r_{II} mutants of this phage fall into 2 groups r_{II} A and r_{II} B[3-6].

We report here a similar genetic analysis using mutants falling in the r_{II} region of T_2 L (LURIA, 1945) phage in order to determine to what extent the findings concerning functional unit within the r_{II} region of T_4 phage could be generalized to the r_{II} region of T_2 phage.

The strains used were *E. coli* B, *E. coli* $K_{12}S$ and *E. coli* K_{12} (λ) and bacteriophage T_2 L (wild type) which were obtained from the Microbial Genetics Research Unit, Hammersmith Hospital, London W.12.

The different cultures of *E. coli* were maintained on nutrient agar slants; phage stocks were maintained in M_9 medium[7] and stored at 4 °C. Chloroform was added to act as a preservative.

T_2 L phages were used for the isolation of '*r*' mutants and *E. coli* B as a host bacterium. 5-Bromouracil and acridine orange were used as mutagens.

Mutants in the '*r*' region were isolated by the method of LITMAN and PARDEE[7]. 5-Bromouracil (50 μg/ml) and acridine orange (4 μg/ml) were used for the isolation of mutants[8]. The percentage of mutagenesis was calculated under standard conditions for both the cases. Each mutant was isolated from a separate plaque and freed from contaminating wild type particles by replating. The isolated '*r*' mutants were further differentiated according to BENZER's scheme[9]. Some of the r_{II} mutants had a tendency to revert spontaneously to wild type, so only stable h^+r mutants[10] were selected for further study.

All T_2 r_{II} mutants were tested in possible pairs for the cistrons test as described in the case of T_4 phage[9]. The recombination test was carried out by the method of HERSHEY and ROTMAN[11] by infecting a culture of *E. coli* B with equal multiplicities of each type and incubating at 37 °C for 90 min. Recombinants were detected on *E. coli* K_{12} (λ).

The results in Table I show that acridine orange (AO) acts as a better mutagenic agent than 5-bromouracil (5-BU). The mutagenic activity of AO being $1^1/_2$ times that of 5-BU.

Table I. Mutagenic effect of 5-bromouracil and acridine orange

Mutagens	Plaque counts obtained		% Mutagenesis
	wild	mutants	
5-Bromouracil	148	190	56
	109	106	50
Acridine orange	51	149	74
	49	200	79

E. coli B grown in supplemented M_9 medium to 10^8 cells/ml. 10^8 particles of T_2 L phage were added to it and aerated for 120 min. Mutants were isolated on B + B/2L mixed indicator bacteria (2:1).

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