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Sex Chromosome Replication and Sex Chromatin in *Akodon azarae (Rodentia Cricetidae)*

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Summary. Sex chromosome length, sex chromatin area and the pattern of sex chromosome replication were determined in the field mouse *Akodon azarae* (Rodentia Cricetidae). Among the animals studied a group of spontaneously deleted females was included. The complement in deleted females was 38Xx, being the x a grossly deleted X-chromosome.

Length of sex chromosomes expressed as percentage of the haploid set was: X-chromosome 7.89% (±1.18), x-chromosome 1.47% (±0.20), Y-chromosome 1.15% (±0.29). Area of sex chromatin expressed as percentage of the haploid genome was 7.28% (±1.18) in XX females and 3.26% (±0.73) in Xx females. According to these data it was assumed that: (a) sex chromatin was formed by all those sex chromosome material in excess of the 5% of the haploid set; (b) the x-chromosome in Xx females was always involved in sex chromatin formation.

Time-sequence of sex chromosome replication was as follows: (a) at the beginning of the S period one X-chromosome starts replication early while the other X, the x and the Y-chromosomes are the last to initiate DNA synthesis; (b) in the intermediate stage of the S period sex chromosomes replicate in the same way as autosomes; (c) in late and final stages of the S period both sex chromosomes are late replicating in their whole extension. It is concluded that the pattern of sex chromosome replication at the beginning of the S period may be more informative than the pattern at the end of the S phase to distinguish between hetero-and euchromatin in the sex genome of *Akodon azarae*.

Dosage compensation by inactivation of one X-chromosome occurs early in the embryonic life of placental mammals. Thus, from that moments onwards one X-chromosome in the female is the last to finish DNA synthesis and gives the sex chromatin corpuscle in the interphase nuclei. Which of the X-chromosomes is selected for inactivation is a matter of random for any single female cell. However, once a given X-chromosome is inactivated it remains so in all descendents of the cell.

In some human females with structurally abnormal X-chromosomes the anomalous X is always late replicating and responsible for the sex chromatin body in the interphase cells. Although in those cases random has changed into preferential X-inactivation this exception to the rule remains to be proved in other mammals than human beings.

It has been lately reported that the field mouse *Akodon azarae* has easily identifiable X-chromosomes. Moreover, deletion of X-chromosomes spontaneously occurs in natural populations of this species (BIANCHI and CONTRERAS 1967). Therefore, an investigation aimed at determining the pattern of sex chromosome replication in males and in deleted and non-deleted females of *Akodon azarae* was undertaken.

Material and Methods

Thirty-one *Akodon azarae* collected in the vicinity of Brusquitas brook (Province of Buenos Aires) were studied. Specimens were deposited in the Mastozoological collection of the Facultad de Ciencias Exactas y Naturales de Buenos Aires.

H3-thymidine (2.5 μc per gr. of body weight; specific activity 6.8 C/mMole) was injected intraperitoneally. Mice were killed at 1, 3, 5, 8, 10, 12 and 15 hours after the injection. One half ml. of 0.04% colchicine solution was intraperitoneally given 3 hours before sacrificing the mice. One male, one deleted and one non-deleted female were studied in each TdH3 labeling-time; thus, among the 31 animals only 21 were selected for extensive analysis of the sex chromosome labeling pattern. Techniques for bone marrow chromosome spreads and for autoradiography have been previously reported (BIANCHI et *al.* 1964, BIANCHI and MOLINA 1966). Fifty well spread metaphases in each animal and a total of 642 autoradiograms were analysed. Exposure time for autoradiograms was 90 days.

Liver cells for sex chromatin studies were prepared as described elsewhere (BIANCHI and CONTRERAS). Two hundred cells were scored in each specimen.

To determine the percentage of the haploid set represented by the sex chromosomes the cut out method described by OHNO and BEÇAK (1964) was employed. Ten well spread metaphases from males, ten from deleted and ten from non-deleted females were photographed. Negatives were projected at a same magnification (6000×) traced on a drawing paper and chromosomes cut out and weighted on a precision balance. The weight of the haploid set of autosomes (A) was determined as follows:

$$\text{Male A} = \frac{(\text{Total weight}) - (\text{Weight of X Y})}{2}$$

$$\text{Female A} = \frac{(\text{Total weight}) - (\text{Weight of X X})}{2}$$

Afterwards the percentage of the haploid set represented by the X, the deleted X, and the Y-chromosomes were obtained.

To determine the accuracy of the method five human metaphases and five metaphases from *Rattus norvegicus* were analysed as described above. Results obtained indicate that X-chromosome represents 5.24% of the haploid set in human beings and 6.2% in rats. In human beings the figure is coincident with those published by OHNO *et al.* (1964) using the cut out method and by the Chicago Conference (1966) using chromosome measurements. In rats the results agree with that of VRBA (1964) obtained by using chromosome measurements. Hence the cut out

method was considered accurate enough for the purposes of this work. This procedure was also employed to determine the percentage of the nuclear area represented by the sex chromatin.

Results

Sex Chromosome and Sex Chromatin Measurements — Chromosome morphology in *Akodon azarae* will only be briefly mentioned in this paper since it has been previously described (Bianchi and Contreras 1967). In males the complement is formed by 34 acrocentric, 2 small metacentric autosomes, a subterminal X-chromosome, and a dot-like Y-chromosome (Fig. 1). In females 4 varieties of complements can be found: (a) 38 chromosomes with XX sex chromosomes; (b) 38 chromosomes Xx, being the x an X-chromosome with a gross deletion in its long arm; (c) 38/37 Xx/XO mosaics; (d) 37 chromosomes with an XO complement (Fig. 1).

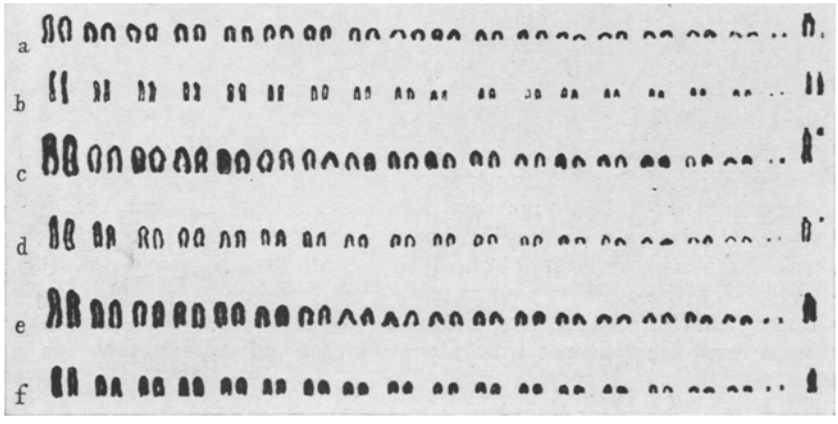

Fig. 1. Karyotypes of *Akodon azarae*: (a) male; (b) 38XX female; (c) 38$Xx_{(l)}$ female; (d) and (e) 38$Xx_{(l)}$37XO mosaic female; (f) 37XO female. 700×

Pattern of Sex Chromosome Replication — When the percentage of labeled metaphases was plotted against the labeling-times the 50% in the ascending and descending curves of labeling were found at 3 and 11 hours respectively (Fig. 5). Hence, it can be concluded that G_2 and S periods in bone marrow cells of *Akodon azarae* last 3 and 8 hours (Sisken 1964). It is also worth mentioning that in 15 hour-treatments labeled chromosomes had silver grains in only one chromatid indicating that those cells had divided twice since the moment of labeling.

The analysis of the replicating behavior of sex chromosomes allows to distinguish three different patterns of metaphase labeling. The first pattern was recognized by the existence of one unlabeled chromosome and the remaining complement completely labeled or labeled in more than half of their chromosomes. The unlabeled chromosome was one X in XX females, the $x_{(l)}$ in $Xx_{(l)}$ females, and the

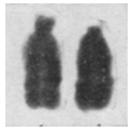

Fig. 2. X-chromosomes from an $Xx_{(s)}$ deleted female. Notice the deletion in the short arm of one of the X-chromosomes. 2000×

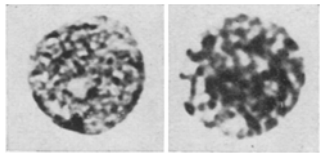

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Fig. 3. Liver cell from an XX female showing the sex chromatin body. 1200×

Fig. 4. Liver cell from an $Xx_{(l)}$ female showing the sex chromatin body. 1200×

In the present report over a total of 24 females analysed 12 were XX, 8 Xx, 2 Xx/XO and 1 XO. The remaining female showed a deletion in the short arm of the X-chromosome not previously described (Fig. 2). Long arm and short arm deleted X-chromosomes will hereafter be represented as $x_{(l)}$ and $x_{(s)}$ respectively.

When the percentage of the haploid set represented by the sex chromosomes was calculated it was found that X-chromosomes comprised 7.89% (±1.18) whereas $x_{(l)}$- and Y-chromosomes comprised 1.47 (±0.20) and 1.15% (±0.29) respectively.

In the investigation of the sex chromatin only those cells with an heterochromatic corpuscle attached to the nuclear membrane were considered as sex chromatin positive. This criterion was taken to avoid difficulties in distinguishing the sex corpuscle from other heterochromatic masses.

Sex chromatin was positive in 28% to 40% of liver cells in XX females. In $Xx_{(l)}$ females the Barr body was found in 4% to 12% of cells and in males it was negative. Furthermore, the Barr body was much larger in XX than in $Xx_{(l)}$ females (Figs. 3, 4). In the former animals the sex corpuscle measured 7.28% (±1.18) of the haploid nuclear area. In the latter ones sex chromatin comprised 3.26% (±0,73) of the haploid nuclear area.

Y in males. The other X in deleted and non-deleted females and in males was always among the labeled chromosomes (Fig. 6, 7, 8). Metaphases with this pattern were mainly observed in 10 and 12 hour-treatments; hence, they were considered as representatives of the initial stage of the S period. Over 642 autoradiograms 26% belonged to this group.

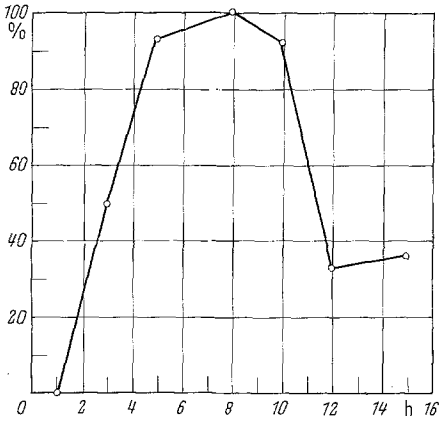

Fig. 5. Percentage of labeled metaphases in each one of the TdH3 treatments performed. The ordinate represents the percentage of labeled metaphases. The abscissa indicates the different labelings performed

The second group was formed by 48% of cells that showed labeling of the whole complement. Taking into account that these metaphases chiefly appeared in 10 and 8 hour-treatments it was assumed that they belonged to an intermediate stage of the S period. No characteristic pattern of sex chromosome replication was noticed in this group of cells.

Metaphases with the third pattern of labeling were found in 5 and 3 hour-treatments. The 36% of autoradiograms forming this group exhibited clumps of silver grains located over small chromosome areas and extense unlabeled regions of the complement. If unlabeled regions comprised less than half of the complement cells were considered to belong to late stages of the S period. When more than half of the genome was unlabeled it was assumed that thymidine-H3 had entered the cells during the final phase of the S period. The most outstanding observation in this group was the fact that both sex chromosomes in males and in deleted and non-deleted females were completely labeled and late replicating in most metaphases. However, in a small number of cells one of the X's showed a small unlabeled band located in the paracentromeric part of the long arm. This region represented no more than one sixth of the total chromosome length (Figs. 9, 10, 11, 12, 13, 14, 15, 16 17).

Thus far, the time sequence of sex chromosome replication in bone marrow cells of males and females of *Akodon azarae* can be summarized as follows: (a) at the beginning of the S period one X-chromosome starts replication early while the other X, the $x_{(l)}$ and the Y-chromosomes are the last to initiate DNA synthesis; (b) in the intermediate stage of the S period sex chromosomes replicate in the same way as autosomes; (c) in late and final stages of the S period both sex chromosomes are late replicating in their whole extension (or almost in their whole extension). Hence it can be concluded that one of the X-chromosomes takes a longer time to replicate than the $x_{(l)}$, the Y or the other X in the case of non-deleted females.

Discussion

Although in most mammals the X-chromosome comprises about 5% of the haploid complement some rodents are endowed with X-chromosomes that

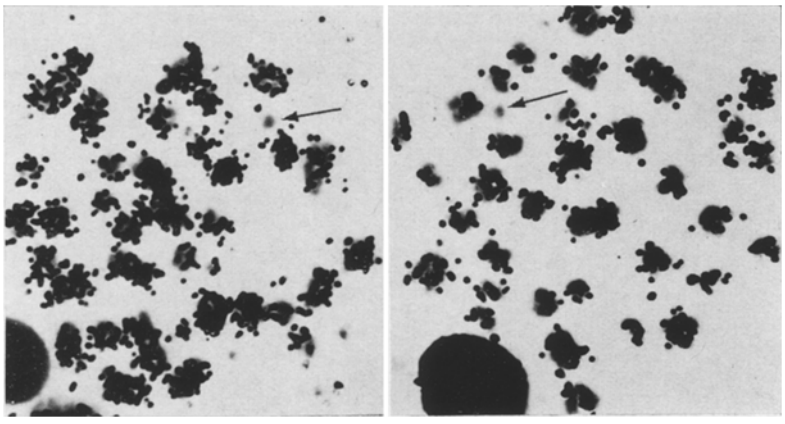

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Fig. 6. Autoradiogram from a male labeled in an initial stage of the S period. Notice all the complement labeled and the Y-chromosome unlabeled (arrow). 1200×

Fig. 7. Autoradiogram from an $Xx_{(l)}$ female labeled in an initial stage of the S period. Notice all the complement labeled and the $x_{(l)}$-chromosome unlabeled (arrow). 1200×

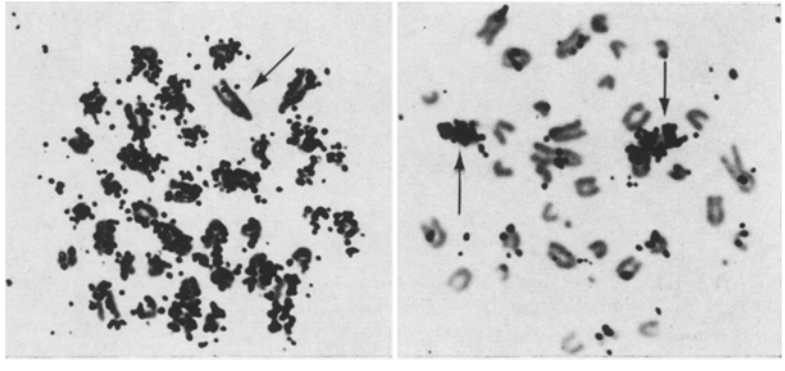

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Fig. 8. Autoradiogram from an XX female labeled in an initial stage of the S period. Notice all the complement labeled and one of the X-chromosomes unlabeled (arrow). 1200×

Fig. 9. Autoradiogram from an XX female labeled in a final stage of the S period. Notice both X-chromosomes completely labeled. 1200×

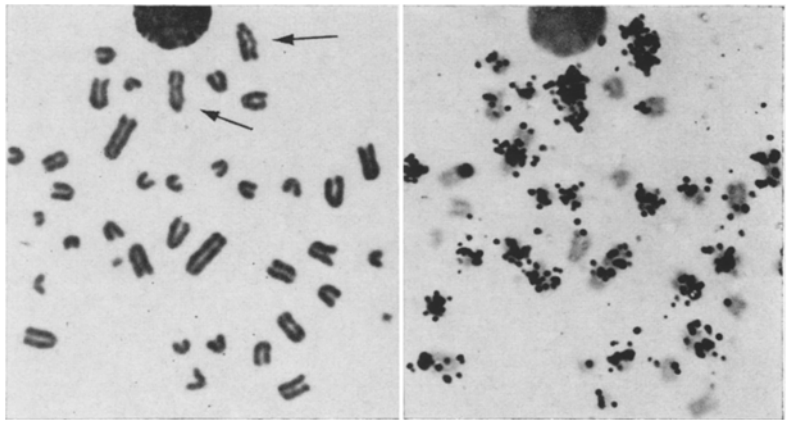

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Fig. 10 and 11. Metaphase pre-and postautoradiography from an XX female labeled in a late stage of the S period. X-chromosomes are arrowed in the metaphase preautoradiography. Arrow in the autoradiogram points out a small unlabeled segment of one X-chromosome. The remaining area of sex chromosomes is completely labeled. 1200×

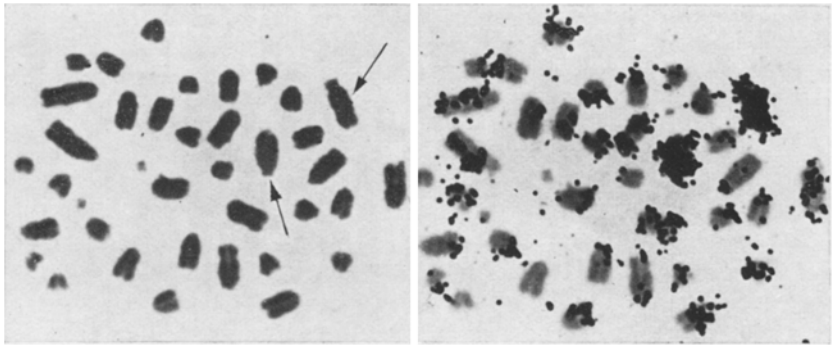

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Fig. 12 and 13. Metaphase pre-and postautoradiography from an $Xx_{(s)}$ female labeled in a late stage of the S period. Notice both X-chromosomes completely labeled (arrows). 1200×

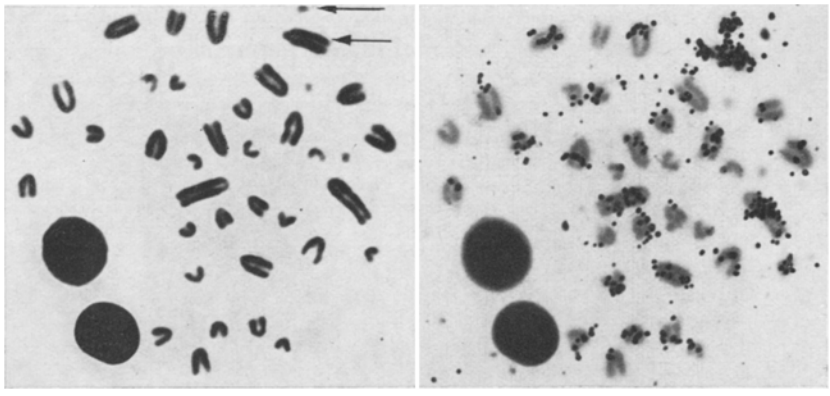

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Fig. 14 and 15. Metaphase pre-and postautoradiography from an $Xx_{(l)}$ female labeled in a late stage of the S period. X and $x_{(l)}$-chromosomes are arrowed in the metaphase preautoradiography. The arrow in the autoradiogram points out a small unlabeled segment of the X-chromosome. The remaining area of sex chromosomes is completely labeled. 1200×

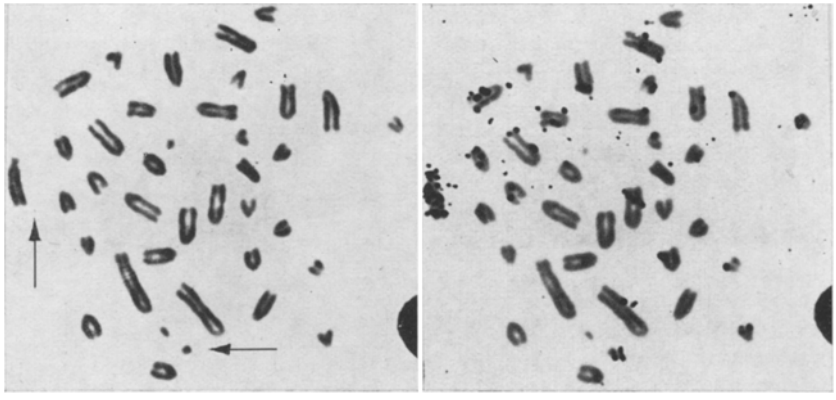

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Fig. 16 and 17. Metaphase pre-and postautoradiography from a male labeled in a final stage of the S period. Notice the X and the Y-chromosomes completely labeled (arrows). 1200×

represent 10% (duplicate type), 15% (triplicate type) or 20% (quadruplicate type) of the haploid set (Ohno *et al.* 1964). In those species, the analysis of metaphases previously labeled with TdH3 have demonstrated that all sex chromosome DNA in excess of the 5% of the haploid genome is late replicating.

In species with duplicate, triplicate, or quadruplicate X-type chromosomes sex chromatin in females as well as in males is double positive. Corpuscles in females being formed by an entire X-chromosome and by the part of the other X in excess of the 5% of the haploid set; and in males by the extra part of the X and by the entire Y which habitually is a large element (the only exception to this rule is the chinchilla in which the Y remains a minute chromosome) (Ohno 1967).

In XX females of *Akodon azarae* the X-chromosome and the sex chromatin comprise 7.9% and 7.28% of the haploid genome respectively. Hence, the first conclusion to reach that sex chromatin is formed by a single heterochromatic X-chromosome. In this species, a second sex chromatin corpuscle formed by the part of the other X-chromosome in excess of the 5% of the haploid genome has not been detected. However, its existence cannot be ruled out since the extra part is only about 2.9% and a corpuscle of such a size may be difficult to distinguish from other heterochromatic masses of the nucleus.

In $Xx_{(l)}$ females the $x_{(l)}$-chromosome and the sex chromatin are 1.47% and 3.26% of the haploid complement respectively. In these animals it seems evident that Barr bodies are formed by the entire $x_{(l)}$ plus a small part of the non-deleted X-chromosome. The low percentage of sex chromatin found (5% to 12%) is probably due to the fact that only those nuclei in which the $x_{(l)}$ and the extra part of the X are attached show an heterochromatic corpuscle large enough to be scored. Conversely, the lack of binding between the Y-chromosome and the heterochromatic part of the X may account for the absence of sex chromatin in males.

Thus far it can be concluded that *Akodon azarae* behave like the other mammals in that sex chromatin seems to be formed by all the sex chromosome material in excess of the 5% of the haploid genome. Moreover in $Xx_{(l)}$ females the deleted X-chromosome is always involved in sex chromatin formation; therefore, in deleted females of *Akodon* random X-inactivation has changed into preferential X-inactivation such as described for human beings with structurally abnormal X-chromosomes.

Differences between *Akodon azarae* and other mammals show up when the relationship between late replicating sex chromosome regions and sex chromatin is established. It has been demonstrated that in most mammals sex chromatin is formed by late replicating and genetically inactive parts of the

sex genome (GRUMBACH *et al.* 1962, 1963). In *Akodon azarae* the area of late replicating regions in sex chromosomes by far exceed the area of the sex chromatin body. Consequently, this fact poses a very interesting problem: there are genetically inert parts of the sex genome not included into the sex chromatin body, or oppositely, late replicating sex chromosome regions not always indicate genetic inactivity. No answer to this problem can be suggested at the present moment.

The analysis of the pattern of sex chromosome replication at the beginning of the S period showed that Y-chromosomes in males, the $x_{(l)}$-chromosome in $Xx_{(l)}$ females, and one of the X-chromosomes in XX females are the last to start replication. The pattern in males is similar to that described in human beings (BIANCHI and BIANCHI 1965), rats (BIANCHI and BIANCHI 1966) and in Chinese hamster (HSU 1964). In females analogies with other mammals cannot be established since the beginning of sex chromosome replication is still rather controversial in this sex (BIANCHI and BIANCHI 1966).

In $Xx_{(l)}$ females the $x_{(l)}$-chromosome beside to be the last in starting replication does always give rise to the sex chromatin body. In XX *Akodon* there are no clue allowing to determine which of the two X's is the one entirely involved in sex chromatin formation. However, on account of the findings in $Xx_{(l)}$ females it may be assumed that the X-chromosome that begins replication last, is also the heterochromatic one. In that case the pattern of sex chromosome replication at the beginning of the S period may be more informative than the pattern at the end of the S phase to distinguish between hetero- and euchromatin in the sex genome of *Akodon azarae*.

Zusammenfassung

An der Feldmaus *Akodon azarae* wurde die Länge der Geschlechtschromosomen, die Größe des Geschlechtschromatinkörperchens und das Muster der Geschlechtschromosomenreplikation bestimmt. Unter den untersuchten Tieren befand sich auch eine Gruppe von Weibchen mit einem spontan deletierten X-Chromosom. Der Karyotyp dieser Weibchen war 38 Xx, wobei x das stark deletierte X-Chromosom darstellt.

Die Länge der Geschlechtschromosomen wurde wie folgt, ausgedrückt in Prozent des haploiden Satzes, ermittelt: X-Chromosom 7,89% (±1,18), x-Chromosom 1,47% (±0,20), Y-Chromosom 1,15% (±0,29). Die Größe des Geschlechtschromatinkörpers, ausgedrückt in % des haploiden Genoms, betrug 7,28% (±1,18) bei XX-Weibchen und 3,26% (±0,73) bei Xx-Weibchen. Auf Grund dieser Daten wird angenommen, daß a) das Geschlechtschromatin durch das gesamte Geschlechtschromosomenmaterial, das 5% des Haploidsatzes übersteigt, gebildet wird, und b) das x-Chromosom bei den Xx-Weibchen stets an der Geschlechtschromatinbildung beteiligt ist.

Die Geschlechtschromosomenreplikation ging zeitlich wie folgt vor sich: a) zu Beginn der S-Periode beginnt ein X-Chromosom früh mit der Replikation, während das andere X-Chromosom und das x- und Y-Chromosom die DNS-Synthese als letzte beginnen; b) im intermediären Stadium der S-Periode replizieren die Geschlechtschromosomen in gleicher Weise wie die Autosomen, c) in den späten und Endstadien replizieren beide Geschlechtschromosomen in ihrer gesamten Ausdehnung spät. Das Muster der Chromosomenreplikation zu Beginn der S-Periode kann als aufschlußreicher für die Unterscheidung von Hetero- und Euchromatin im Geschlechtsgenom von *Akodon azarae* angesehen werden als das Replikations-Muster der Endphase.

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