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Further chromosomal studies on *Ellobius lutescens*: Heteromorphism of chromosome No. 1 is not associated with sex determination

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Summary. Twelve animals of the species *Ellobius lutescens* from two generations were studied with various chromosomal banding techniques. This species carries 17 chromosomes in both sexes. In preceding studies chromosomal sex determination was assigned to different structural variants of chromosome No. 1. In the present study, no definite chromosomal basis for sex determination was found.
Key words. Ellobius lutescens; sex chromosomes; chromosome heteromorphism; sex determination.

The chromosomes of the Persian vole *Ellobius lutescens* Th. (Rodentia Microtinae) were first examined by Matthey[1]. He found an identical karyotype of 2n = 17 chromosomes in somatic and germ cells of both sexes. The smallest chromosome, No. 9, remains unpaired during meiosis in males and females[1]. This chromosome represents 5% of the haploid chromosome complement as does the X-chromosome in most mammals[2]. But chromosome 9 reveals no differences between the two sexes if replication or banding analyses are performed[2,3]. With the aid of banding techniques, two independent samples of *Ellobius lutescens* have been studied[3],[4]. In both of these studies, a correlation between a chromosome No. 1 heteromorphism and sex was observed. However, the heteromorphisms described in these studies were different.

The heteromorphism observed by de la Maza and Sawyer[4] consists of three structurally divergent chromosomes No. 1. The first type of chromosome 1 was found in females as well as in males. The second type was male specific, and the third type was female specific in this sample. The male heteromorphic No. 1 was interpreted as containing material corresponding to a Y-chromosome, and determining male differentiation.

In the study by Wolf et al.[3] identical banding patterns were observed on both chromosomes No. 1 in the male. One of the female chromosomes No. 1 differed from the other by a marked elongation of a proximal band in the long arm. It was proposed that the elongated region of chromosome 1 in females contains a gene which determines femaleness in a dominant way.

At present, our knowledge about naturally occurring chromosome polymorphisms in wild populations of *Ellobius lutescens* is rather limited. Therefore, the interpretations of the results from the small series studied may be premature. The only common feature described in the aforementioned publications is an association between polymorphism of chromosome pair No. 1 and sex. In this study a chromosomal segregation analysis was undertaken on several animals caught in Iran and on their descendants born in this laboratory.

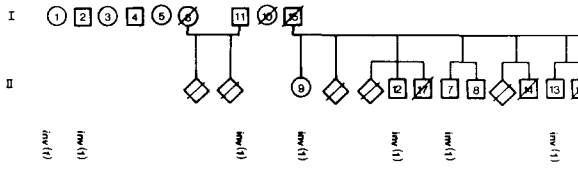

Figure 1. Schematic representation of the *Ellobius lutescens* specimens studied. Laboratory numbers were assigned to the animals according to their date of death. I = animals obtained from Iran, II = offspring gained by breeding. Males and females from our original animal sample (I) sent to us from Iran as well as from breeding (II) were observed to be heterozygous or homozygous for chromosome No. 1 variants. ∅ or ⊠ not karyotyped; inv (1) below the animal symbol means this animal was heterozygous for chromosome No. 1. All other animals were homozygous.

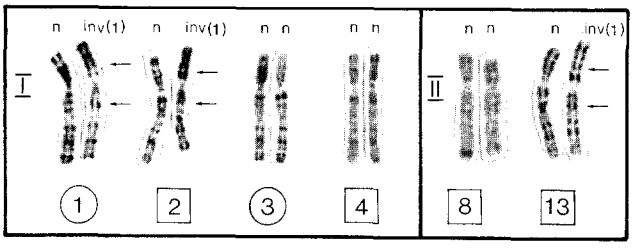

Figure 2. Chromosomes No. 1 of *Ellobius lutescens* with RBG-patterns after fluorescence plus Giemsa staining. Homozygous (n, n) and heterozygous (n, inv (1)) animals were observed in both sexes in generation I (from Iran) and in male offspring (II).

Materials and methods. Six females and four males of *Ellobius lutescens* were obtained from the Pasteur Institute, Teheran, with the help of Dr D. Farhoud, University of Teheran. Five of these animals died within the first two weeks after arrival (animals No. 1 to 5, fig. 1). A sixth animal (No. 10) died later. The remaining two pairs gave rise to several litters of small size (fig. 1), but attempts to rear a third generation were not successful.

Fibroblast cultures were established post mortem according to standard methods[5]. The sex of the animals was verified by histological examination of the gonads. The chromosomes were analyzed from at least twenty mitoses for each animal by Q-bands (QFQ), G-bands (GAG) and replication patterns (RBG). Silver staining of the nucleolus organizer regions (NOR) (Ag-NOR)[6] was also carried out for each individual (fig. 4).

Results. Seven males and five females were investigated during this study (figs. 1 and 2). In all cases the same chromosome number was found (2n = 17). Animals No. 3 (female), 4 (male), 5 (female), 8 (male) and 16 (female) revealed apparently identical pairs of chromosomes No. 1. The banding pattern of this chromosome was designated as the 'normal' type (fig. 2). In the other animals heteromorphism was detected in chromosome pair No. 1. This heteromorphism consists of one normal type chro-

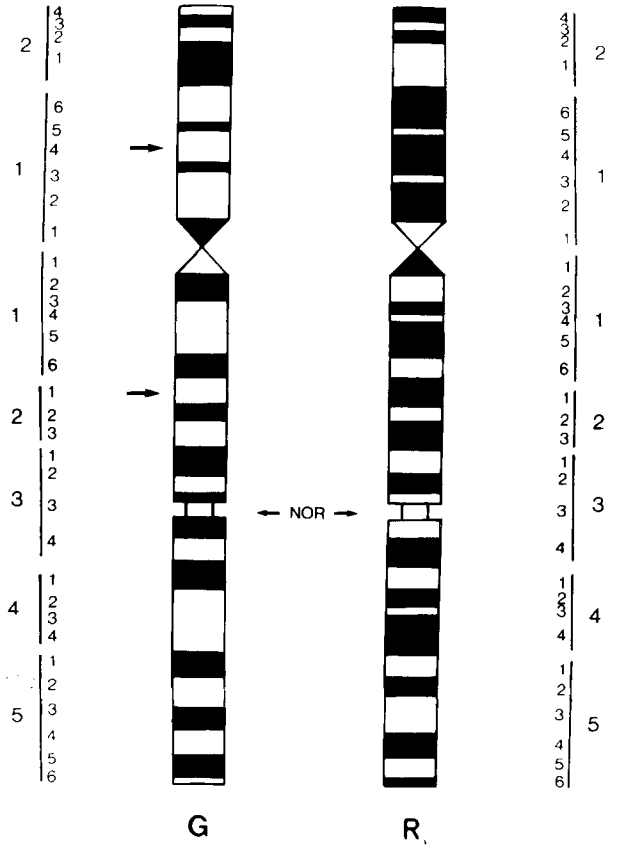

Figure 3. Schematic drawing with proposed banding nomenclature for chromosome No. 1 of *Ellobius lutescens*. Left = GTG-banding; right = RBG-banding. The arrows mark the breakpoints of the inverted chromosome No. 1 (p14; q21).

mosome No. 1 and a variant one which carries a pericentric inversion with break points in p14 and q21 (fig. 3). In none of these animals was a difference in number or size of the bands between the inverted and the non-inverted homologues observed (fig. 2). In the breeding pair (animals '15 × 16', (fig. 1)) the male was not available for cytogenetic study; the female was found to be homozygous for the normal chromosome No. 1. In the progeny, 4 animals were found to be heterozygous for inv(1) and one had two normal chromosomes No. 1. From this it can be deduced that the father was heterozygous for chromosome No. 1. During this study it became clear that the sex of the animals was independent of the presence or absence of the variant chromosome No. 1.

The nucleolus organizer regions (NOR) in this species were localized by silver staining. The pattern of NOR appeared identical with respect to location, size and number among all animals studied. Chromosomes Nos. 1 and 2 carry an interstitial NOR on the long arm whereas the remaining NORs are localized at telomeres (fig. 4).

Discussion. Earlier studies have shown a surprisingly high number of variants of chromosome No. 1 in *Ellobius lutescens*[3,4]. One of these chromosome variants (the normal one in fig. 2) is present in at least one copy in each of the animals studied so far.

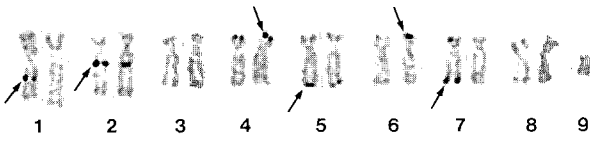

Figure 4. AG-NOR stained chromosomes. The arrow points to the NOR.

This variant is identical with type lmf described by de la Maza and Sawyer[4] and type 'a' described by Wolf et al.[3]. In our present investigation we observed only one chromosome No. 1 variant, inv(1) (p14; q21), which may be identical with type c described by Wolf et al.[3].

In both of the earlier studies, a strict association has been observed between the sex of the animals and the variants of chromosome No. 1. The findings of the present study are different. The variant found was not sex specific, and some karyotypes associated with a particular sex in the previous studies were observed in the present work associated with the opposite sex. These inconsistencies do not, however, exclude a relationship between chromosome No. 1 and sex determination in *Ellobius lutescens*, but they exclude a direct assignment of a sex determining gene to one of the structural variants of this chromosome.

Chromosomal sex determination is usually characterized by a chromosomal dimorphism which occurs in a 1:1 ratio and correlates with the sex of the animals. In the small wild population (generation I in fig. 1) analyzed here the only heteromorphism observed concerns an inversion of chromosome No. 1 of *Ellobius lutescens*. In contrast to earlier studies, however, the sex of the animals does not correlate with chromosomal type in our sample.

Nevertheless, a correlation between sex and chromosome No. 1 as suggested by these studies cannot be dismissed completely on the basis of our data. One might assume for example that sex determining factors may be localized on this chromosome and may have cosegregated with different structural variants in the samples studied earlier. This hypothesis offers an easy explanation for the diverse results obtained with small sample sizes from various regions. In any case, the problem of sex determination in *Ellobius lutescens* remains open.

The material available now may be useful for further investigations with the help of somatic cell genetics and DNA-probes specific to the sex chromosomes in order to test the role for sex determination of chromosome No. 1, its variants, and chromosome No. 9 in this species.

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