

Variation in the Nucleolus Organizer Regions (NORs) in Two Mole Rat Species (*Spalax leucodon* and *Spalax ehrenbergi*)

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Abstract: In this investigation, patterns of variation in the nucleolus organizer regions (NORs) in the chromosomes of *Spalax leucodon* and *Spalax ehrenbergi* were studied. The chromosomal location of the Ag-NORs was determined in two mole rat species. *S. leucodon* has 3 pairs of nucleolar chromosomes, but *S. ehrenbergi* has only 2 pairs of nucleolar chromosomes in their whole chromosome sets. In both mole rat species, the chromosomal locations of Ag-NORs were determined to be on the terminal of the short arms of the subtelocentrics.

The possibility of Robertsonian translocation and the loss of paracentric NORs in *S. ehrenbergi* were discussed from an evolutionary point of view.

Key Words: Nucleolus organizer regions (NORs), *Spalax leucodon*, *Spalax ehrenbergi*

İki Kör Fare Türünde (*Spalax leucodon* ve *Spalax ehrenbergi*) Nukleolus Organizatör Bölge Varyasyonu

Özet: Bu araştırmada, *Spalax leucodon* ve *Spalax ehrenbergi* kromozomlarında nukleolus organizatör bölge (NOR) varyasyonu araştırılmıştır. İki kör fare türünde Ag-NOR'un kromozomal yerleşimi tespit edilmiştir. *S. leucodon* 3 çift nukleolar kromozoma sahip iken *S. ehrenbergi* tüm kromozom takımında sadece 2 çift nukleolar kromozom içermektedir. Her iki kör fare türünde de, subtelosentrik kromozomların kısa kollarının terminalinde Ag-NOR yerleşimi tespit edilmiştir.

Sonuçta, *S. ehrenbergi*'de Robertsonian translokasyonun ihtimali ve paracentrik NOR kaybı evrimsel açıdan tartışılmıştır.

Anahtar Kelimeler: Nukleolus organizatör bölge (NOR), *Spalax leucodon*, *Spalax ehrenbergi*

Introduction

Extensive cytogenetic studies carried out on mole rats have revealed that the representatives of *Spalacidae* exhibit distinctive chromosomal polymorphism. Chromosomal investigations play a major role in explaining the speciation and interrelationship of *Spalax* species. To date,

approximately 40 different chromosomal forms of *Spalax* have been reported from the Balkan Peninsula, Turkey, Romania, Caucasus, Israel and North Africa (1).

Silver-staining methods have been developed for the differential staining of nucleolus organizer regions (NORs) of animal and plant chromosomes (2). Ag-staining methods to detect the metaphase chromosome sites of nucleolus organizer regions (NORs) have greatly facilitated comparative study of NOR variation within and between species (3). These techniques stain only the NORs activated at the previous mitotic phase, so that the difference in the number of NORs per cell may reflect differences in the number of active NORs (3). Each species appears to have a characteristic maximum number of chromosomes pairs with NORs, however, polymorphisms in the relative size of the NOR on homologous chromosomes and in the number of the active NOR sites per cell have been found for many species examined (3).

The present study was planned to determine the chromosomal locations of NORs and to compare variations in the NORs of chromosomes in *S. leucodon* and *S. ehrenbergi*.

Materials and Methods

This investigation was conducted in the central part of the Euphrates. *S. leucodon* and *S. ehrenbergi* are isolated by the Euphrates. During field studies, a total of 11 specimens (7 *S. leucodon* -3♂, 4♀- and 4 *S. ehrenbergi* -3♂, 1♀-) were live trapped in predetermined areas of their natural habitats on both sides of the river (Figure 1).

A direct method developed for bone marrow according to Patton's (4) "colchicine hypotonic citrate" technique was applied for the preparation of chromosomes. The NORs were stained directly on unstained slides using the technique of Howell and Black (2).

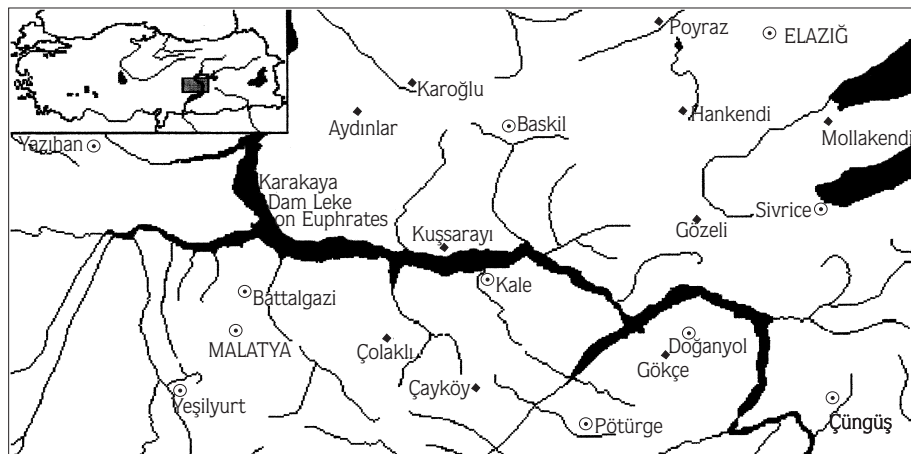


Figure 1. Showing the study area

Results

We determined *Spalax leucodon* to be located on the west side of the Euphrates district of Malatya, and *Spalax ehrenbergi* to be distributed on the east side of the river (district of Elaziğ). These data are consistent with previous studies (5, 6, 7).

In *Spalax leucodon*, the karyotype consists of 30 chromosome pairs and the NORs were terminally located on 3 pairs of large subtelocentrics (Figure 2). All of the individuals displayed a consistent number of Ag-NORs per cell. Heteromorphism for NOR size was not observed.

In *Spalax ehrenbergi*, the karyotype consists of 26 chromosome pairs. The chromosomal position of the Ag-NORs was found to be terminal on 2 pairs of the large subtelocentric (Figure 3). Heteromorphism for NOR size on two homologous chromosomes was not observed. Ag-NORs were not found on the sex chromosomes of *S. leucodon* and *S. ehrenbergi*.

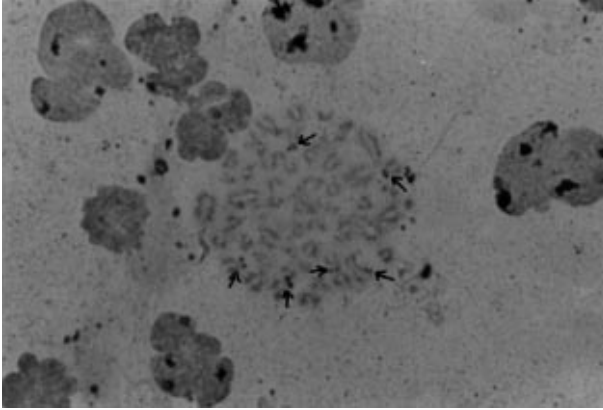


Figure 2. Silver-stained metaphase of *Spalax leucodon*. Chromosomal NORs are indicated by arrows.

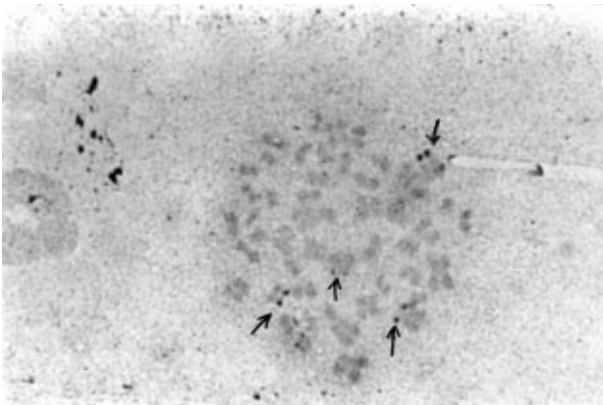


Figure 3. Silver-stained metaphase of *Spalax ehrenbergi*. Chromosomal NORs are indicated by arrows.

Discussion

The karyotypes of *S. leucodon* and *S. ehrenbergi* were reported by Yüksel (5), Gülkaç and Yüksel (7). According to these studies, *S. leucodon* has 60 chromosomes. This species includes 9 pairs of large to small subtelocentrics, 20 pairs of telocentrics, a large submetacentric X and a large subtelocentric Y chromosome in the whole chromosome set (5, 7).

Yüksel (5) found 52 chromosomes in *S. ehrenbergi*. The whole chromosome set of this species includes 3 pairs of metacentrics, 2 pairs of submetacentrics, 6 pairs of subtelocentrics, 14 pairs of telocentrics, a large submetacentric X and a medium sized subtelocentric Y chromosome.

In *S. leucodon*, silver stained plates were compared with karyotypes given by Yüksel (5); Gülkaç ve Yüksel (7). 3 pairs of the subtelocentric nucleolar chromosomes were found to be consistent in size with st_1 , st_2 and st_3 named by Yüksel (5); Gülkaç and Yüksel (7).

In *S. ehrenbergi*, silver stained Ag-NORs karyotypes were compared with karyotypes given by Yüksel (5). 2 pairs of the subtelocentric nucleolar chromosomes were identical in size with st_1 and st_3 as named by Yüksel (5).

The Ag-NORs on homologue chromosomes do not exhibit heteromorphism in either *S. leucodon* or *S. ehrenbergi*. In both species, nucleolar chromosomes have the same morphology.

The NORs bearing chromosomes, as well as the position of NORs on the chromosomes, usually present remarkable diversity among the species (8). It seems that alterations of the NORs have occurred in close relation to the species differentiation and evolution (9). Many of the previous relationship studies of many taxa were based on variations and polymorphism of the nucleolar chromosomes (3, 9,-19).

In discussion of the karyotype evolution of *S. leucodon* and *S. ehrenbergi* from the central Eurphrates basin, Yüksel (5) concluded that the evolution of the karyotype took the course of a decrease in the diploid number of chromosomes, particularly in the number of acrocentrics and from this point of view, *S. leucodon* from Malatya seems to be the most primitive one. Through Robertsonian variation (i.e. centric fusion), two acrocentric chromosomes may fuse at the centromere to produce one biarmed chromosome. Thus, this mechanisms allows the evolution of the karyotype in such a way that the diploid number is reduced but the fundamental number is kept constant. Fundamental numbers, on the other hand, may change by pericentric inversions through which some acrocentric chromosomes may be converted into biarmed chromosomes without changing the chromosome number (5).

In *S. ehrenbergi*, a primitive subtelocentric chromosome bearing NORs (probably st_2 in *S. leucodon*) changed into a metacentric or submetacentric chromosome due to Robertsonian translocation. This translocation caused the loss of paracentric NORs parallel with a decrease in the diploid number in *Sehrenbergi*. Similarly, the loss of paracentric NORs due to Robertsonian translocation were reported in the course of chromosome evolution of four species of Microtidae by Sanches et al. (14) and South American cricetid *Graomys griseoflavus* by Zambelli and Vidal-Roja (19). In addition to Robertsonian variation, the role of pericentric inversions was discussed in the karyotype evolution of *Pagellus bogaraveo*. In relationship studies, the significance of NORs affected by pericentric inversions was mentioned by Vitturi et al. (15).

In the light of recent findings, we can say that *S. leucodon* is a primitive species compared with *S. ehrenbergi*. This presumption has been mentioned in the discussion of chromosomal evolution of *Spalax* from Turkey (5).

In the case of mole rats, natural barriers are not even necessary, in the classic sense, in as much as mole rats in peripheral situations live in small, somewhat isolated populations and their motility, in general, is very restricted. Such populations are expected to be highly inbred and gene exchange is restricted to between them. The achievement of relatively rapid fixation of new karyotypes prevents hybridization, and also often results in the evolution of pre- and post-mating reproductive isolation. Ultimately, the gene pools became more or less separated and the species level was practically reached. Thus, a change in the karyotype may affect the genome in a variety of ways involving both adaptation and speciation. It is possible to establish complete reproductive isolation due to chromosomal and ethological incompatibility.

References

1. Yüksel, E., Gülkaç, M.D.. On the karyotypes in some populations of the subterranean mole rats in the lower Euphrates-basin, Turkey. *Caryologia*, 45(2): 175-190 (1992).
2. Howell, W.M., Black, D.A.. Controlled silver-staining of nucleolus organizer region with a protective colloidal developer: a 1-step method. *Experientia*, 36: 1014-1015 (1980).
3. Thiriot-Quiévreux, C., Insua, A.. Nucleolar organizer region variation in chromosomes of three oyster species. *J. Exp. Mar. Biol. Ecol.*, 157: 33-40 (1992).
4. Patton, J.L.. Chromosome studies of certain pocket mice genus *Perognatus* (Rodentia: Heteromyidae). *J. Mammalogy*, 48: 27-37 (1967).
5. Yüksel, E.. Cytogenetic study in *Spalax* (Rodentia: Spalacidae) from Turkey. *Communications, Serie C: Biologie*, 2: 1-12 (1984).
6. Kıvanç, E.. Türkiye *Spalax*'larının Coğrafik Varyasyonu (Mammalia: Rodentia). 72 Teksir-Daktilo-Fotokopi, pp. 88, Ankara (1988).
7. Gülkaç, M.D., Yüksel, E.. Malatya yöresi köf fareleri (Rodentia: Spalacidae) üzerinde sitogenetik bir inceleme. *Doğa TU Biyol.*, 13(2): 63-71 (1989).
8. Takai, A., Ojima, Y.. Some features on nucleolus organizer regions in fish chromosomes. *Indo-Pacific Fish Biology: Proceeding of Second International Conference on Indo-Pacific Fishes, Ichthyological Society of Japan*, pp. 899-909, Tokyo (1986).
9. Oberdorff, T., Ozouf-Costaz, C., Agnese, J.F.. Chromosome banding in African catfishes: Nucleolar organizer regions in five species of the genus *Synodontis* and one of the genus *Hemisyndontis* (Pisces, Mochokidae). *Caryologia*, 43(1): 9-16 (1992).
10. Bickham, J.W., Rogers, D.S.. Structure and variation of the nucleolus organizer regions in turtles. *Genetica*, 67: 171-184 (1985).
11. Wahrman, J., Richler, C., Gamperl, L., Nevo, E.. Revisiting *Spalax*: mitotic and meiotic chromosome variability. *Israel J. Zool.*, 33: 15-38 (1992).
12. Burgos, M., Jimenez, R., Guardia, R.. Comparative study of G- and C-banded chromosomes of five species of *Microtidae* chromosomal evolution analysis. *Genome*, 30: 540-546 (1988).
13. Gold, J.R., Zoch, P.K.. Intraspecific variation in chromosomal nucleus organizer regions in *Notropis chrysocephalus* (Pisces: Cyprinidae). *Southwestern Naturalist*, 35: 211-215 (1990).

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14. Sanchez, A., Burgos, M., Jimenez, R., Guardia, R., Variable conservation of nucleolus organizer regions during karyotypic evolution in Microtidae. *Genome*, 3: 119-122 (1990).
15. Vitturi, R., Mazzalo, A., Catalano, E., Karyotype analysis, Ag-NORs and C-bands location in *Pagellus bogaraveo* (Brünnich 1768) (Pisces, Sparidae). *Biol. Zent. bl.*, 109: 223-226 (1990).
16. Almeida-Toledo, L.F., Foresti, F., Zambelli Daniel, M.F., Toledo-Filho, S.A., Nucleolar chromosome variants in *Sternopygus macrurus* (Pisces, Sternopygidae) from three Brazilian river basins. *Caryologia*, 46(1): 53-61 (1993).
17. Insua, A., Thiriot-Quévieux, C., Karyotype and nucleolus organizer regions in *Ostrea puelchana* (Bivalvia: Ostreidae). *The Veliger*, 36(3): 215-219 (1993).
18. Rodriguez-Daga, R., Amores, A., Thode, G., Karyotype and nucleolus organizer regions in *Epinephelus caninus* (Pisces, Serranidae). *Caryologia*, 46(1): 71-76 (1993).
19. Zambelli, A., Vidal-Roja, L., Loss of nucleolar organizer regions during chromosomal evolution in the South American cricetid *Graomys griseoflavus*. *Genetica*, 98: 53-57 (1996).