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KARYOTYPE AND SEX-CHROMOSOME POLYMORPHISM IN NESOKIA INDICA, FROM IRAN. (*)

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Currently, we seem to be in the middle of a new wave of research activity, related to rat chromosome studies, with attention being focused most on *Rattus rattus,* from different localities in the world.

Recently Markvong *et al.* [1], have reviewed in detail the chromosomes of different species of rats and mice of Thailand and Mittol and co-workers have studied chromosome number and sex mechanisms of 17 species of rodents from India [2]. But no reference is being made, however, as to number and morphology of *Nesokia indica,* in the present data.

This report therefore, concerns itself with the study of chromosomes of *Nesokia indica,* and sex chromosome polymorphism among the individuals so far studied.

The species reported in this discussion were colledted by means of rat traps, from a farm within the institute,located 45 km west of Tehran, Iran. Interestingly enough, all these genetically different rats have come from an area not very much larger than 1000 square meters. The rats as outlined in this note were kindly identified by Dr. E. Etemad, Professor of Zoology, at the Veterinary School of The University of Tehran.

The method of chromosome preparations was essentially the same as those reported for hedgehogs of Iran[3], with the exception that blood lymphocyte culture practically in all cases failed to yield dividing cells, suitable for chromosome preparations. The red blood cells cultured in 5 ml vials, for some unknown reason, undergo hemolysis, which intensifies by time, al likely phenomenon responsible for the arrest of cell division, in the system. The hemolysis

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however, seems to be due to the action of heparin to which the red blood cells of this animal are sensitive (unpublished data).

Bone marrow aspirates were suspended in 5 ml aliquots of BME and then incubated in the presence of colchicine for 2–3 hours at 37°C, and centrifuged for 5 minutes at 1000 rpm. The cell pellet was suspended and kept in 0.075m K Cl solution for 40 minutes at 37°C. The cells were then recentrifuged and to the cell pellet thus obtained were added 3 ml of Ethanol – acetic acid 3/1 fiixative, the latter being changed three times, before the cells were dropped onto wet slides.

The metaphase plates obtained by this method, consistently led to compact and poorly defined condensed chromosomes, even when exposure time to Colchicine was decreased or the hypotonic treatment was extended. This method' however, is unquestionably simple, rapid, and with no need to sacrifice the animal under study. It proved thereof necessary to make use of another cell system which could lend itself to preparations, suitable for characterization and photography. This was accomplished by preparation of kidney cell culture from individuals with characteristic chromosome structures initially verified by bone marrow technique. Four to eight days after primary seeding the semi-confluent sheets were treated with colchicine for two to three hours, and the trypsinized cells were processed in the same manner as outlined for bone marrow technique.

Very well defined, and rather elongated chromosomes of distinct structure can be obtained by this method (see the karyotype), despite its being elaborate and time consuming.

The diploid number of *Nesokia indica,* in all 27 specimens studied, proved to be 42, excluding a single female rat having 2n-1=41, desingenated as 41, XO (Fig. 13, No. 5).

There was also a phenotypically different rat trapped in the same area, that was identified by Dr. Etemad as *Arvicola terrestris,* with a completely different diploid number and chromosome structure, which will be discussed in forthcoming report.

The various elements in male and female chromosome spreads can be classified as follows:

A – 40 autosmomes: 6 pairs of medium and small metacentrics,
2 pairs of small submetacentrics,
12 pairs of acrocentrics of various sizes

B – Two sex chromosomes

Distribution of different sex patterns in Nesokia indica individuals .

Given Number	Sex Chromosome Pattern	No.of Individuals observed	
		Female	Male
1		4	-
2		8	12
3		1	-
4		-	1
5		1	-

Fig. 13

The karyotype of *Nesokia indica*

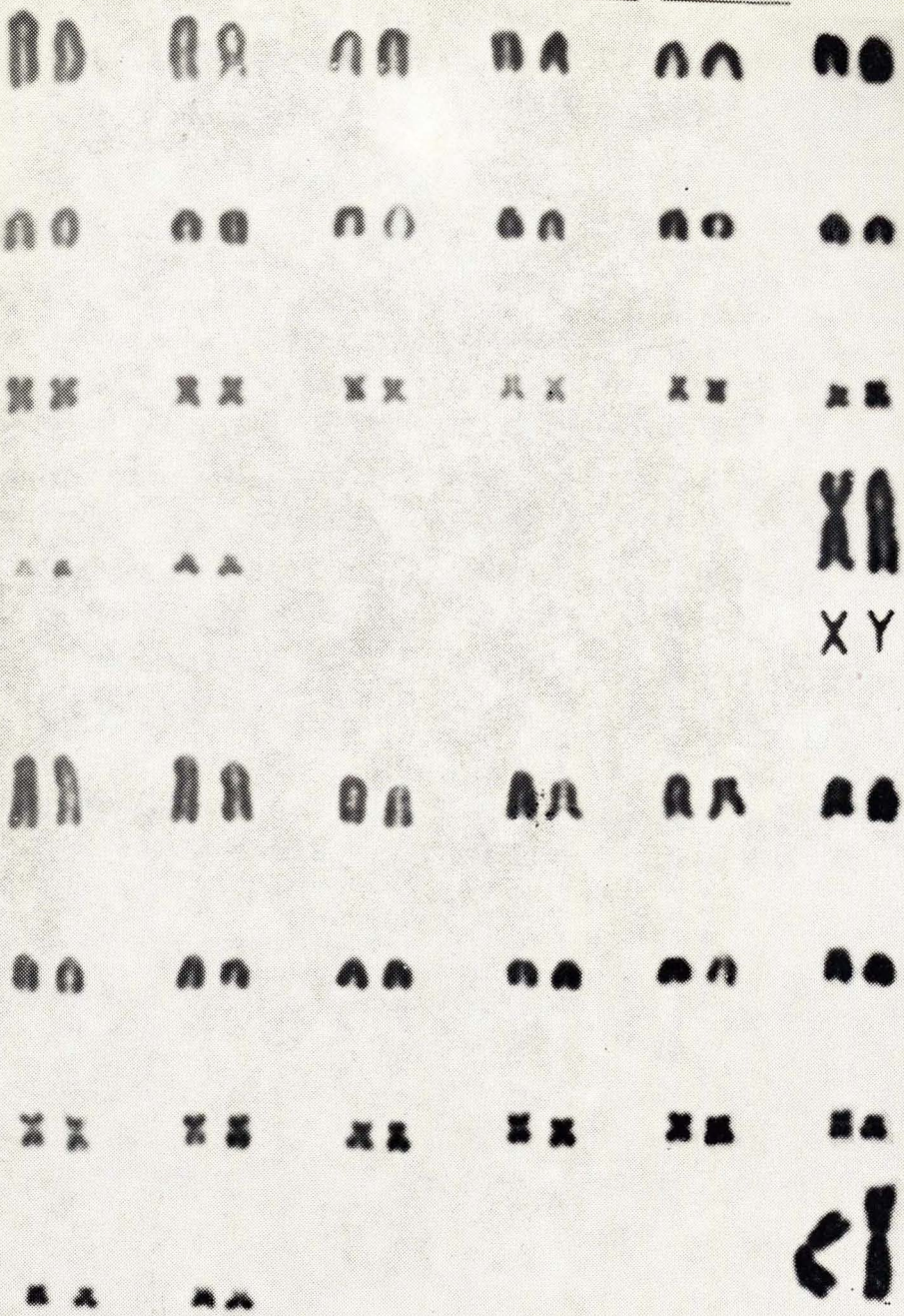

Fig.14

The two largest chromosome in the group, arbitrarily designated as sex elements, were so complex that no comment can at the moment be made in view of their pattern (s).

As demonstrated in the genuine chromosome presentation in Fig. 1, No. 1; 29% of the female rats convey the conventional XX pattern, where both chromosomes appear as metacentrics, and 57% of the females carry one metacentric and one medium sized submetacentric (No.2), exactly the same structure as observed in 12 out of 13 males studied (No. 2). There was one female rat with mosaic sex pattern (No. 3), and another with monosomic sex element, labelled as 41, XO (No. 5).

As it can be noted through inspection of Fig. 13, the X in the males, as well as one of the X's in the females (presumably the functional X) always appear as metacentric chromosome even in the monosomic and in the mosaic individuals.

The Y chromosome, on the other hand, usually appears as a medium submetacentric (No. 2), although in one out of 13 males it undergoes a critical structural change to appear as a large telocentric (No. 4). Due to its characteristic appearance this unique case was included in the karyotype presented.

It is worth mentioning that the 40 autosomes that are demonstrated in the accompanied karyotype, proved to be identical in all 29 rats studied, showing no structural variation from one individual to the other.

Attempts are being made to apply G-banding technique to verify underlying phenomenon, and to detect any probable deletion in the defective XX pair(No. 2), or the presumable inversion in the telocentric Y (No. 4), and the involvement of constitutive heterochromatin (Personal communication with Professor T. C. Hsu), in the sex chromosomes of these rats that live so closely together, and appear so differently from the viewpoint of their sex-chromosome patterns.

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Legends:

Fig. 13. Distribution of different sex chromosome patterns in *Nesokia indica* individuals.

Fig. 14. The karyotypes of *Nesokia indica*.