56. Chromosome Studies on the Hare and the Rabbit^{*)}

By Mrudula J. DAVE,^{**)} Nobuo TAKAGI,^{***)} Hidetsune OISHI,^{****)}
and Yasumoto KIKUCHI^{****)}

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Since the latter half of the 19th century, the evolutionary relationship between the hare and the rabbit has been a subject of special interest as well as of utmost discussion as "Leporiden-problem" in the fields of biology and animal husbandry. The classical literature relating to this subject deals with morphological studies and hybridization experiments. Yamane and Egashira (1924) undertook artificial insemination experiments between the two species which resulted in failure. Tateishi (1936) and Makino (1944) examined the chromosomes in the hare and the rabbit, and established a clear-cut difference in chromosome number between the two species. Karyotype analyses have, however, remained unsatisfactory. Recent development of the tissue culture method for chromosome preparation has facilitated a reliable and precise analysis of the chromosomes in mammals. The reinvestigations with current techniques have thus been required on the chromosomes of mammals which had been studied with the classical sectioning method. Several domestic animals have been reinvestigated chromosomally by current methods (Awa *et al.* 1959, Nakanishi and Mizutani 1959, Nakanishi 1960, Sasaki and Makino 1962, Sasaki and Sasaki 1962, Makino *et al.* 1962, 1963, Shioda and Sasaki 1962, Nakanishi *et al.* 1964, Muramoto and Makino 1965). According to the advice of Professor Makino, the authors undertook a revised study of the chromosomes in the hare native to Hokkaido (*Lepus timidus ainu* Barrett-Hamilton) and the rabbit (*Lepus cuniculus* L.) employing the current tissue culture techniques.

Materials and methods: Chromosome preparations were made from a male and a female hares and two male domestic rabbits. Small pieces of the lung, kidney, spleen or liver were excised under the aseptic condition and explanted in plasma clots or grown in monolayer employing the technique recommended by Youngner (1954). One to two weeks after the commencement of cultivation, cells were exposed to

^{*)} Contribution no. 687 from the Zoological Institute, Faculty of Science, Hokkaido University.

^{**)} The Rockefeller Foundation Scholar at Makino Laboratory, Sapporo. Permanent address: Indian Cancer Research Centre, Parel, Bombay 12, India.

^{***)} Zoological Institute, Faculty of Science, Hokkaido University.

^{****)} National Institute of Genetics, Mishima.

colchicine for 6 to 12 hours. After harvesting with 0.2 per cent trypsin, cells were treated with water, fixed in Carnoy's fixative and air-dried on slides. Attempts to cultivate leucocytes were made in both species applying a slightly modified technique usually adopted successfully for human leucocytes (Moorhead *et al.* 1960). All cultured leucocytes derived from the hares failed to yield any mitosis, but two out of four rabbit cultures were successful. They were harvested on the 5th day of cultivation after 5 hours colchicine treatment, and slide preparations were made in the same manner as described above.

Results and discussion. Chromosomes of the hare: Tateishi (1936) and Makino (1944, 1952) are only authors who reported chromosomes of the hare. The former studied chromosomes of the Formosan hare (*Lepus formosus*) in germ cells and reported that the number of chromosomes was 48 in $2n$ and 24 in n . The latter author described 24 bivalents at the 1st meiotic metaphase of *Lepus gichiganus ainu* (= *Lepus timidus ainu*). They failed to analyse in detail the karyotype in the two species, on account of ill-delineated chromosomes caused by the classical method. The present study was carried out on the basis of 56 metaphase plates from a female and 30 from a male. Careful counting made it clear that the diploid chromosome number in somatic cells in culture was 48 in both sexes. Chromosomes were aligned into 23 apparent homologous pairs of autosomes and a pair of sex chromosomes. Twenty-three pairs of autosomes were tentatively divided into three main groups according to the location of the centromere. Group 1 consists of 8 pairs of metacentric or submetacentric chromosomes in order of their decreasing lengths. All of the 8 pairs were identified unequivocally according to the size and the position of the centromere. Group 2 comprises of 8 pairs of subtelocentrics which can be identified with some degree of certainty. The remaining 7 pairs represented by acrocentric elements are categorised in group 3. Four (16-19) of them are almost similar in both size and morphology, while each of 3 pairs (21-23) was readily identified as a medium-sized metacentric element, being slightly unequal in arm ratio. In general morphology, the X is hardly distinguishable from no. 5 chromosomes.

Chromosomes of the rabbit: Painter (1926) was the first to describe the chromosomes of the rabbit and found the number of chromosomes to be 44 in $2n$. The same number was confirmed later by other authors (Tateishi 1936, Makino 1944, 1952). Recently, Melander (1956) observed 44 chromosomes in squashed embryonic lung cells of both sexes. The results were substantiated in cultured cells.

by Clausen and Syvertson (1962), Srinivasan and Harding (1963) and McMichael *et al.* (1963). The present study confirmed the above results based on 82 metaphase cells, the vast majority of them showing 44 in $2n$. Apparently 44 chromosomes consist of 21 pairs of autosomes and a pair of sex chromosomes. In reference to the position of the centromere as arranged in the hare, the diploid complement of the rabbit was

Figs. 1-3. Karyotype analyses. 1, a male hare. 2, a female hare.
3, a male rabbit.

classified into three main groups, group 1 containing 13 pairs of meta- or submetacentric chromosomes, group 2, 4 pairs of subtelocentrics and group 3 consisting of 4 pairs of acrocentrics. Seven (nos. 1, 2, 3, 4, 5, 12, 13) out of 13 pairs of chromosomes from group 1 are rather easily identifiable according to the size and position of the centromere, but it is difficult to identify the remaining 6 pairs. Four pairs of subtelocentrics are recognizable from each other. The smallest two pairs of chromosomes in group 3 (20-21) are clearly identifiable, although the longer two pairs (18-19) are hardly distinguishable from one another. The X was identified as a relatively large submetacentric chromosome, very similar to no. 3 chromosomes in size and morphology. The Y is telocentric and is almost of the same size as no. 21 and 22 chromosomes.

The X chromosome identified here apparently differs in both size and arm ratio from that of Melander (1956), Clausen and Syvertson (1962) and McMichael *et al.* (1963) which has been described as a small submetacentric element. Further, Clausen and Syvertson (1962) described 14 pairs of meta- or submetacentric chromosomes belonging to group 1, and 3 pairs of acrocentrics in group 3. This study revealed the presence of 13 pairs of chromosomes in group 1 and 4 pairs of acrocentrics in group 3. At the present status of knowledge, it is impossible to say whether the difference in karyotypes of the reported cases has originated from chromosomal polymorphism or is mere technical artifact.

Chromosomal difference between the hare and the rabbit revealed in the present study with the current technique is very striking: it is difficult to interpret the difference by simple structural rearrangements of chromosomes. It is most likely, in the light of cytological evidence, that the hare and the rabbit are not phylogenetically so akin as the external appearance suggests. Through insemination experiments Yamane and Egashira (1924) reached the conclusion that the hare and the rabbit were isolated both physiologically and reproductively.

The rabbit has long been described as *Lepus cuniculus*. Recent systematists, however, are of opinion that the domestic rabbit is not a member of the genus *Lepus*, but is a variety of the wild rabbit native to England, *Oryctolagus cuniculus*. Cytological findings together with the results of insemination experiments greatly favor the above view that the domestic rabbit should be placed in the genus *Oryctolagus*.

Summary: Chromosome studies carried out in the hare and the rabbit revealed that the former strikingly differs from the latter

chromosomally, suggesting no close phylogenetical kinship between them.

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