16. Karyotype Evolution, Species Differentiation and Environmental Mutagen^{*)}

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The species differentiation or speciation is considered to involve an entire process of evolutionary dichotomy—from the first appearance of genetic isolating mechanism to the final severance of genetic continuity between two populations (White 1978). Important is the understanding of the genetic basis leading to the isolating mechanism. It is now widely accepted that structural rearrangements in chromosomes involving inversions and translocations have played a primary role in the development of many or perhaps even most species. In the process of speciation and evolution two events, one being at the genic level, and the other at the chromosomal level, are to be taken into consideration. The latter event deals primarily with karyotype changes as a result of inversion, translocation, changes of heterochromatin (satellite DNA), and the nucleolus organizer regions (NORs, sites of r-RNA genes).

Evidence favoring the species differentiation at chromosomal level has been available providing recent karyological studies in three mammalian species; the black rats, the Indian spiny mouse and the Indian muntjac. All the species under consideration have widely been distributing in the Indian subcontinent. It seems very likely cytogenetically that their karyological changes in relation to evolution have occurred in southern India. It has been shown that in the black rats, the Ceylonese type rats having $2n=40$ and the Oceanian type rats having $2n=38$ had occurred in the southern India in association with the sequential Robertsonian fusion of certain chromosomes. In the Indian spiny mouse ($2n=26$), the mechanism of tandem fusion between two elements of certain acrocentrics occurring in the house mouse having $2n=40$ seemed to take part in their development. The evolutionary change of the Indian muntjac with $2n=6$ (♀) from the Reeve's muntjac with $2n=46$ has tentatively understood as a sequence of tandem fusion of certain acrocentrics. To know the mechanism of

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such karyotype changes concentratedly occurring in the Indian sub-continent is of utmost biological importance. Theoretically it seems probable that the changes have occurred in association with certain environmental mutagens. Such considerations are to be presented in the present paper on karyotype evolution, species differentiation and environmental mutagen with particular reference to the karyotypic rearrangements in the three species of Indian mammals concerned here.

Accounts on the black rat. A chromosomal survey of the black rats, *Rattus rattus*, in Southwest and Central Asia was conducted by Yosida and his associates (Yosida *et al.* 1974). The investigations have made it possible to clearly define the following three distinct chromosomal types: Asian ($2n=42$), Oceanian ($2n=38$) and Ceylonese types ($2n=40$). They distribute occupying different geographical areas. Based on karyotype analyses, the possible pathways of their karyotype evolution have been favorably interpreted as thus: (1) The Asian type black rats with acrocentric pairs 1 and 9 originally developed in Southeast Asia. From this type the rats with subtelocentric pairs 1 and 9 would arise through the mechanism of pericentric inversion (Fig. 1). Their movement and distribution covered North India and Pakistan. (2) The Ceylonese type rats with $2n=40$ developed through the mechanism of the first Robertsonian fusion occurring between pairs 11 and 12 in the Asian type rats which migrated to South India. (3) The Oceanian type with $2n=38$ made their appearance through the second Robertsonian fusion between pairs 4 and 7 in individuals among them (Fig. 2). They took their distribution widely over South India. (4) The Ceylonese type black rats could be expelled out of India and then they became isolated in Sri Lanka. (5) The Oceanian type black rats took their migration first to Central Asia, then extending towards Europe. (6) Through Europe they took their wide distribution covering the other continents along with the movement of the people. Hybrids between these three chromosomal races have been raised in the laboratory condition (Yosida 1977).

Accounts on the Indian spiny mouse. The karyotype analysis of the Indian spiny mouse, *Mus platythrix*, collected from Mysore, South India, have resulted in that the diploid number is 26, and that all elements are acrocentric in structure (Satya Prakash and Aswathanarayana 1972; Tsuchiya and Yosida 1972; Yosida 1979) (Fig. 3). The comparison of G-banded chromosomes between the spiny mouse ($2n=26$) and the house mouse, *Mus musculus* ($2n=40$), have demonstrated the evidence that certain chromosome pairs of the spiny mouse could be resulted from the tandem fusion of house mouse chromosomes

(Yosida 1979). Noteworthy is the fact that a strong genetic barrier seems to exist between these two species, because viable hybrid specimens have never been produced so far in reciprocal crosses in the laboratory (unpublished). They showed mating behaviour in general between the partners. Very probably *in utero* failure in development may be a cause of hybrid sterility. It appears that these two are distinct species standing at the ultimate stage of speciation.

Figs. 1-4. 1-2: Karyotypes of two geographic types in the black rat (*Rattus rattus*). 1: Asian type with $2n=42$. 2: Oceanian type with $2n=38$. 3: Metaphase chromosomes of the Indian spiny mouse (*Mus platythrix*) with $2n=26$. 4: Metaphase chromosomes of a female Indian muntjac (*Muntiacus muntjak*) with $2n=6$.

Accounts on the Indian muntjac. The Indian muntjac, *Muntiacus muntjak*, distributing in Indian subcontinent is outstanding by the lowest chromosome number in mammals so far reported [$2n \text{ ♂} = 7 (XY_1Y_2)$, $2n \text{ ♀} = 6 (XX)$] (Fig. 4) (Wurster and Benirschke 1970). In contrast, the Reeve's muntjac, *M. reevesi*, showing a wide distribution through Southeast Asia is quite similar in general morphology to the Indian muntjac, while its diploid number is 46, all elements being acrocentrics similarly to the other artiodactyle karyotype. These

two species are known to have been hybridized with allegedly fertile offspring (Gray 1954).

A suggestion has been made that the chromosomes of the Indian muntjac would be derived by sequential tandem fusion of several chromosomes of the original *reevesi* muntjac. There are cases in early record of successful hybridization between *M. muntjac* and *M. reevesi* (Zuckerman 1953). In view of the extreme karyotypic difference, White (1978) claimed that the hybrids, sometimes fertile, should be regarded as unreliable.

According to Kato *et al.* (1974), the karyotype of a female Indian muntjac revealed that homologous pairs 1 and 3 were heteromorphic with respect to size of the secondary constriction and centromeric constitutive heterochromatin. This leads to suspect a high frequency of chromosomal polymorphism in the natural population of this species.

Note on environmental mutagens in relation to karyotype changes. Three examples mentioned above clearly indicate that the karyotype evolution, after a long duration, ultimately leads to species differentiation. Evidence is interesting showing that the evolutionary events concerned here are those occurring in the Indian subcontinent, especially in South India. Then a question arises as to factor or factors in India, particularly environmental, which influenced the germ cells to cause the karyotype evolution leading to species differentiation.

Since the pioneering work of Muller in 1927 it has been known that mutations could be induced in animals and plants by means of X-rays, and then that other radiations like γ -rays emitted by radioactive substances or the cosmic rays from the outer space exert a similar effect. Natural radiations have been known to arise from two sources; cosmic rays entering the atmosphere from the outer space and radioactive materials in the earth's crust. These materials were already present when the earth was formed, or in the case of short lived radioisotopes, they have been continuously produced by radioactive decay or nuclear reactions. According to the variation in content of natural radioactive elements in the soil and the underlying rock, the intensity of external radiation and the level of radioactive intake by animals and plants vary from place to place. Practically all the natural environmental radiations of terrestrial origin are known to be due to radionuclids from uranium, thorium series and potassium series.

In the laboratory, it is comparatively simple to investigate the immediate genetic effects of large doses of radiations administered acutely. The long term effects of small doses of radiations administered at a low dose rate over many generations are far complex at least with mammals having long life span. Structural changes of the

chromosomes under low doses of radiation for many generations would possibly be raised resulting in karyotype change and consequent species differentiation.

There exist a few areas in the world which embrace a high natural radioactivity. One of these areas is said to occur in the Kerala State of South India.*¹ The carrier of the radioactivity is known to be black monazite sand in the coast, which is derived from igneous rock-minerals containing thorium phosphate. Thorium is known as a radioactive element carrying a very long life (1.39×10^{10} years). This type of monazite sand is known to occur in various concentrations in other parts of South India including coastal areas of the Bay of Bengal like Gopalpur in Orissa State. Gruneberg (1964) surveyed Quilon area of Kerala State during from 1961 to 1962 and undertook the skeletal and dental measurements of the black rats in order to know genetic variance if existed. He reported that if the black rats were laboratory animals amenable to experimentation, the level of genetic variation in the strip (radioactive area) and control areas could be assessed by their response to selection. Although their findings in the field gave no positive indication on skeletal and dental measurements of the rats living in the strip, they did not rule out the possibility of induced mutation beyond the reach of their method. Here, we may say that any cytogenetic analysis and breeding experiments should be conducted in those rats.

The above discussion on the karyotype evolution in the black rats, Indian spiny mouse and Indian muntjac makes it possible to tentatively state that India, particularly South India, might be the platform where cytogenetically unfavourable events such as chromosomal breakages, fusion, inversion and translocation had taken place, probably under the natural mutagens among which radioactive monazite seems to be prominent. Thus, South India seems to be the hot bed of macro-mutation probably due to the following two reasons: One is that geologically the land is very old, and the other is that radioactive monazite sands which emits γ -radiations at a low dose are still present in some southern parts of the Indian subcontinent. Further cytogenetic studies should be demanded on mammalian fauna and flora in the above areas for decided statements.

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*¹) Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, New York, 1966.

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