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CHROMOSOMAL ABNORMALITIES IN RATS AFTER BAMBOO FLOWERING IN MANIPUR, INDIA

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ABSTRACT

The famine and bamboo flowering are closely related in North-East India. Bamboo flowering causes population explosion of the rat population. The cytological analysis of metaphase from bone marrow cells through karyotype, C-band and Ag-NOR show chromosomal changes like monosomy, trisomy, robertsonian fusion etc. besides $2n=24$. How the exploded population gets stabilized is a big question. One hypothesis has been put forward through the chromosomal changes leading to the death or sterility of some or many individuals but the natural or normal population remains more or less constant, as maintained by the unaffected individuals left behind.

Key Words: Bamboo Flowering, Rats, Population Explosion, Chromosomal Abnormalities

INTRODUCTION

Bamboo has the peculiarity of flowering and seedling at the end of a very long vegetative growth phase, the length of which is considered to be species specific (John & Mascrenhas 1994). The flowering cycle of *Bambusa tulda*, *Dendrocalamus longipathus* and *Melocanna bambusoides* has been reported the cause of the famines known as “Mautam” and “ Thingtam” in Mizoram (Pathak & Kumar 2004). The flowering in Mizoram had caused four famines-1815, 1863, 1911 and 1959 (Jeeva et al. 2009). Manipur also faces such famines which are not much harmful as reported in Mizoram. People in the north-east India and elsewhere in the world believe that bamboo flowering is the harbinger of famine. The seed-shed attracts seed predators, mostly rats (species of *Mus* and *Rattus*) (John & Nadgauda 2002). In the abundance of food supply, the number of rat increases tremendously and when the seeds germinate they migrate to other places and cause famine thereby invading the crops (Jeeva et al. 2009). On the other hand, when rats feed on bamboo seeds, they become sexually active to such an extend that each female gives birth to as many as 800 offspring during the bamboo flowering season which lasts for 60 months (Kaikho 2004). It is for the first time we report on chromosomal changes in rats due to bamboo flowering.

MATERIALS AND METHODS

Thirty three live rats (20 males and 13 females) of *Rattus rattus bullocki* (Fig.1) Roonwal were collected from Tamenlong district of Manipur state located at 24°52'N 93°33'E at the altitude of 1280 m MSL. In Tamenglong district of Manipur at that time *Dendrocalamus hemiltonii* was flowering from 1997 to 2007. Approval of the Institutional Ethics Committee (IEC) was obtained for using live animals and protocols of the IEC were followed throughout the study. Metaphase chromosomes were prepared from five males and five females using standard colchicine hypotonic spreading technique. The chromosomes were stained with Giemsa or for C-bands (Sumner 1972) or for Ag-NORs (Good pasture & Bloom 1975). Chromosome number and morphology were recorded from 500 Giemsa –stained metaphases from each specimen directly under 100X bright field optics of Leica Dialux microscope as well as from photographs of selected cells. C-band and Ag-NOR expression pattern were examined in 500 metaphases each and

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variant forms were recorded. The types of chromosome and karyotype were done according to Yosida 1983.

RESULTS AND DISCUSSION

We examined 500 mitotic chromosome spreads under microscope and 100 well spread photographs of each specimen of the *Rattus r. bullocki*. Giemsa –stained normal metaphase showed a consistent karyotype consisting of $2n = 42 = 12$ acrocentric autosome pairs (#1 to 9, 11 to 13) and 1 subtelocentric autosome pairs (# 10) + 7 metacentric autosome pair (# 14 to 20) + acrocentric X and Y as reported (Dhananjay & Bhagirath 2005) (Fig. 2). The abnormal chromosome plate included the monosomy in chromosome #13 and subtelocentrics due to pericentric inversion (Fig.3), and other trisomy in chromosome # 13. The other abnormal cells were subtelocentric and one robertsonian fusion in chromosome #11 and trisomy in chromosome # 8 (Fig.4). The important finding included the occurrence of dot or microchromosomes like chromosome fragments besides $2n=42$ (Fig.5). The Ag-NORs were frequently associated between homologous and nonhomologous chromosomes (Fig.6). The normal chromosome showed centromeric heterochromatin (Fig.7).

The rodent rats are common in North-east India. Some of them are endemic as well as indigenous species. As the name is concerned bamboo flowering is always accompanied. This is the relationship between rat population and bamboo flowering/ seeding. Many incidents of bamboo flowering are recorded in North-east India especially in Manipur and Mizoram. The bamboo seeds are high in protein and carbohydrates but of less lipids content. The diets/ aphrodisiacs may enhance the rate of reproduction and then migrate to adjacent areas invading crops and cause famine. But one question that comes to our mind is “what has happened to the exploded population”. The scarcity of food may lead to reduction in number due to starvation cannibalism or migration. No reports of rat death due to starvation are found as they are omnivorous. Cannibalism occurs when there is no food but there is food for them. The last option is migration. The famines that occurred in 1911 in Mizoram didn't cause famine in Manipur or neighboring states. So it is just a local phenomenon. Thus, the last option/ hypothesis is the damage of the chromosomal level to lead either sterility or morphological change that which causes the death of some or many individuals to stabilize the existing species population. The nullisomy or monosomy may arise due to non-disjunction of homologues in anaphase stage. They are primary characteristics in plants. Hence, the chemicals present in bamboo may affect the cell division. The chemicals like cyanogens, phytic acid etc are carcinogenic in higher amount and might have switched on the gene responsible for sex hormones. The chief phytochemicals present in all the bamboo species are phenolic, terpenoids and alkaloids (Sarita et al. 2008). The protection afforded by phenolics against mammalian herbivores may be based on their bitter taste, offensive odour, toxicity or on slowing down digestion of food material (Freeland & Janzen, 1940; Palo & Robbins, 1991). Iason and Waterman (1988) found less phenolic compound containing plants in the stomach of reproducing females of mountain hares (*Lepus timidus*) compound to non reproducing females, thus suggesting that phenolic compounds may have deleterious effects on the reproduction and growth of mammals. Other compound present in bamboo is phytosterol. The phytosterol thus obtained can be used for the manufacture of steroidal drugs after microbial conversion into 1,4- androstadiene-3,17-dione using *Anthrobacter oxidans* (Srivastave, 1990). The microchromosome like chromosome might be late replicating DNA fragments that were synthesized and unable to form chromosomes. This is a rare and very important result, however it cannot be explain until now. The rat *Rattus rattus tistae* also shows abnormal morphology and Y testicular dysgenesis (Bhagirath & Dhananjay, 2005) was unfortunately collected from Tamenglong where *D. hemiltonii* was flowering/ seedling. To sum up all the observations, chemicals present in the bamboo seeds whether of *Melocona* or *Dendrocalamus* contain that are potent enough to cause changes at micro and macrolevel of genetic material. It is up to us to detect chemicals and potentiality of the chemicals in the bamboo seeds. The result of X^2 test on the presence of subtelocentric in the chromosome pairs No. 1, 3, 5,7, 9 and 13 in the bamboo flowering shows that it was statistically highly significant (Table No. 2). The polymorphism of chromosome is occurring in nature spontaneously in chromosome no. 1, 9 and 13 in *Rattus rattus* (Yosida

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1983) and the causes might be physical, chemical or radiations. But such a high polymorphism in such a little population is an extra-ordinary phenomenon. These results are significant in particular areas. These suggest the flowering of bamboo and eating bamboo seeds by the rats cause some abnormal chromosomal structure in the rats and mainly occur in #1, 3, 5, 9 and 10 besides 7 and 13.



Figure 1:

Figure 1. The two species of rat trapped from bamboo flowering areas

A. *Rattus rattus bullocki*

B. *Rattus rattus tistae*

The special feature of *R. rattus tistae* included abnormal skull size big muzzle and more prominently the hardened testis

The scale is 10 micrometer



Figure 2:

Figure 2. The normal chromosome complement of the species with $2n = 42$ and all the autosome except #10 were telocentric and #14 to 20 were small metacentrics, XY were also telocentric. #10 was telocentric (arrowed).

The scale is 10 micrometer

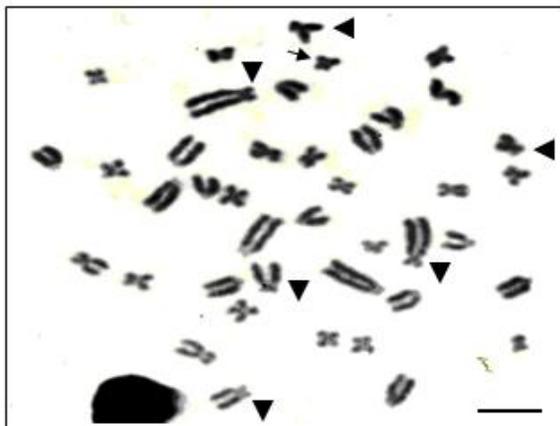


Figure 3:

Figure 3. The abnormal metaphase plate with monosomy #13 and subtelocentric, $2n=41$ and most of autosomes were subtelocentric (arrowed). The scale is 10 micrometer

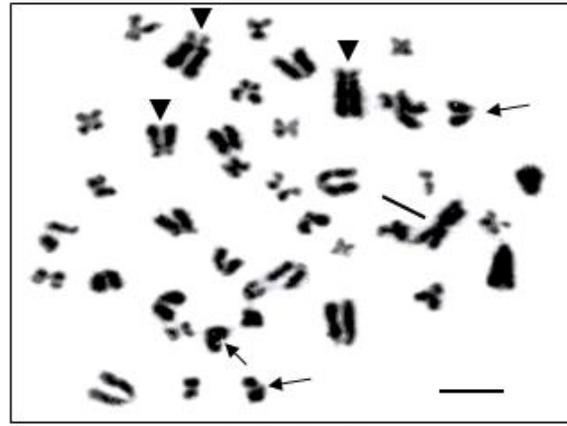


Figure 4:

Figure 4. The abnormal metaphase plate with trisomy #12 (arrowed), $2n=43$, subtelocentric (arrow head) and robertsonian fusion #11 (lined). The scale is 10 micrometer

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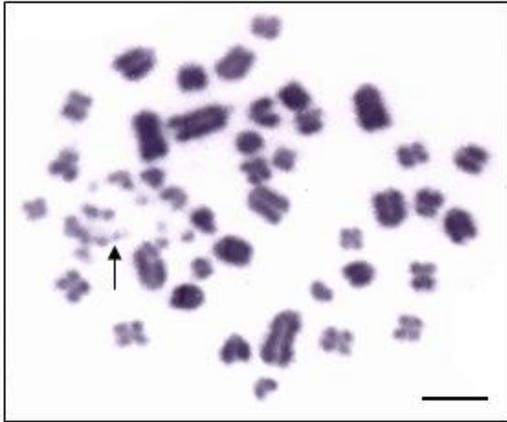


Figure 5:

Figure 5. The abnormal metaphase plate fragmented or micro-chromosomes (Arrowed) besides the normal chromosome compliments.

The scale is 10 micrometer

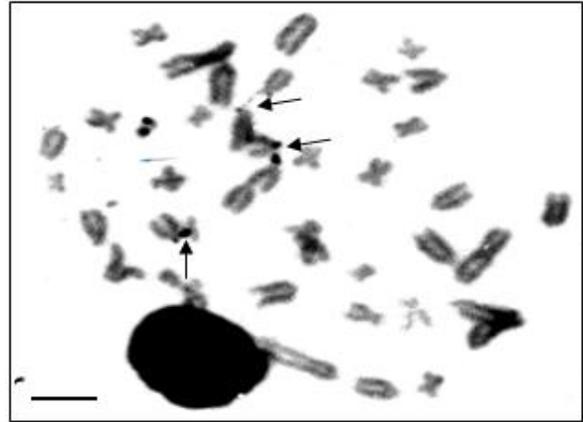


Figure 6:

Figure 6. The Ag- NOR of *R.r. bullocki* on #3,9,11 and 12 exhibited NOR associations in between homologous and non homologous chromosomes (arrowed).

The scale is 10 micrometer

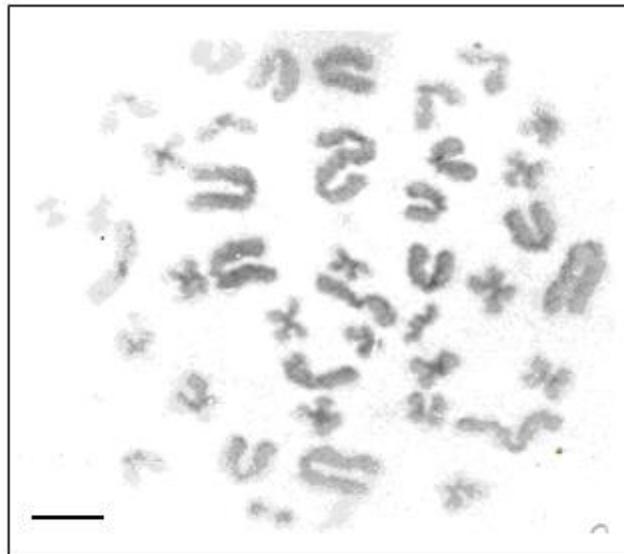


Figure 7:

Figure 7. The C-band metaphase plate of *R.r. bullocki* showing centromeric heterochromatin

The scale is 10 micrometer

Whether such changes in the chromosome caused by the chemicals from the bamboo seeds are reversible or irreversible is yet to be answered. The extracts fermented bamboo shoots which are the main food items of Manipur show dose dependent on mouse. The chromosomal aberration types found in plant extract mice were similar to those caused by physical, chemical agents. Any change in the chromosomal change effect the behavior and nature of the organism. In due course of time such organisms will slowly isolate from the main bulk and become totally isolated leading to the formation of fairly beautiful new species with different chromosome complement. In other view due to the change in karyotype, some of them become sterile and vanished as the time comes. The increase in the heterochromatic regions in this

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species/population might link to changes in the gene sequences due to heterochromatinization or by pericentric inversion. Obviously the chemicals in bamboo may lead to changes in chromosomal as well as in the DNA sequences. The occurrence of Rb fusion shows that the chemical might cause nicking of DNA and fusion at the centric region but the cut segment cannot be detected. In general rats show maximum polymorphic chromosomes due to consanguineous marriage and chemicals of surroundings. If all the above facts hold true, then the evolution of new species regarding the rats is enhanced due to fruiting of bamboo obviously it is yet another magnificent force that causes speedy evolution of the rat species particularly for *R. r. bullocki* to branch out the family tree in future. Further intense work should be carried out to ascertain whether the menace in fact is a really precious boon to the evolutionary mechanism of rats and other species "or" a mechanism that changes the cytochemistry leads to death of individuals.

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