Non-random inactivation of the X-chromosome in interspecific hybrid voles

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Summary
Expression of X-linked genes for G6PD and GALA in interspecific hybrids between Microtus arvalis, M. subarvalis and M. kirgisorum voles was studied. Quantitative predominance of the enzyme activities of M. arvalis over G6PD activity of M. subarvalis and the GALA activity of M. kirgisorum in the female hybrids was shown. The definitive patterns of these enzyme activities was found on day 6-5 of embryonic development. Non-random inactivation of X-chromosomes derived from M. subarvalis and M. kirgisorum in the interspecific hybrids with M. arvalis is supposed to be the cause of the phenomenon observed. A hypothesis is proposed that there is a connection between the presence of large heterochromatin regions in the X-chromosomes derived from M. subarvalis or M. kirgisorum and the preferential inactivation of these in female hybrids with M. arvalis.

1. Introduction
One of the two X-chromosomes in the somatic cells of female mammals can become genetically inactive early in embryogenesis, and this has been thought to be brought about by random inactivation of either one or the other of the two X's (Lyon, 1961, 1974; Gartler & Andina, 1976; Gartler & Riggs, 1983). While not entirely dismissing the significance of this explanation, non-randomness of X-inactivation has been observed in different situations. In marsupials, the X-chromosome derived from the father is preferentially inactivated, and it is also given preference in the extraembryonic tissues of some eutherian mammals (Richardson et al. 1971; Johnston et al. 1975; Takagi & Sasaki, 1975; West et al. 1977). A situation germane to this point is also provided by the effects of the Xce locus (X chromosome controlling element) of the mouse in the case of non-random inactivation of the X-chromosomes in the cis-position (Cattanach & Isaacson, 1967; Cattanach, 1975; Rastan, 1982a; 1983). Non-randomness of X-inactivation is phenotypically expressed in Xce²/Xce⁰ females as predominance of cell populations with the Xce⁰ carrying X in active state.

Data concerning non-random inactivation of the X-chromosomes in interspecific hybrid voles Microtus arvalis × M. subarvalis and M. arvalis × M. kirgisorum are presented here. To investigate this inactivation, X-linked enzyme markers glucose-6-phosphate dehydrogenase (G6PD) and α-galactosidase (GALA) were used.

2. Materials and methods
Microtus arvalis and M. subarvalis were captured in the environs of Leningrad, and M. kirgisorum in the delta of the Amu Darya river. The animals were maintained in the vivarium of the Zoological Institute (Leningrad). Interspecific hybrid voles were produced by crossing M. arvalis × M. subarvalis and M. arvalis × M. kirgisorum. These interspecific hybrids are sterile (Meyer et al. 1981).

Blood was collected from the upper gums cut with a razor blade while the animals were under ether anaesthesia. Samples of tissues and organs were obtained from sacrificed animals. Tissue homogenates were sonicated three times for 10 s at 4 °C, centrifuged and electrophoresed in 14% starch gels (Zakijan et al. 1984). Visualization of G6PD and GALA activities in the gels was done according to Harris & Hopkinson (1976).
crosses. A survey of organ and tissue G6PD and GALA was performed on 20 females (11 females were from ♀ M. subarvalis × ♂ M. arvalis and 9 from ♀ M. arvalis × ♂ M. subarvalis crosses).

The activity of GALA in the liver, kidney and brain was assayed in 8 ♀ M. kirgisorum × ♂ M. arvalis females.

Specific activity of G6PD in erythrocytes of M. arvalis and M. subarvalis was carried out as described earlier (Serov & Zakijan, 1977).

The sex of the hybrid embryos at early stages of development was determined cytogenetically. Metaphase spreads of the hybrid embryos were obtained as suggested by Baranov (1983). Sumner’s C-band technique was used for staining the chromosomes (Sumner, 1972). The hybrid embryos were used for cytogenetic analysis, and the rest was subjected to electrophoretic analysis.

The time point in which spermatozoa were identified in the vaginal smears was accepted as gestation onset. The day when spermatozoa were found to be present in the smears was referred to as day 0 of gestation.

3. Results and discussion

Fig. 1 presents starch gel electrophoretic patterns of G6PD in the erythrocytes of M. arvalis, M. subarvalis and their F1 hybrids. The patterns of G6PD obtained from female hybrids are represented by the parental forms of the enzyme, and those from male hybrids by the maternal one only.

Analysis of the patterns yielded by GALA gave the same results in the interspecific hybrids (Figs. 2, 3). Thus evidence was obtained that the genes for G6PD and GALA are located in the X-chromosome in M. arvalis, M. subarvalis and M. kirgisorum.

There was an obvious predominance of the M. arvalis form of G6PD in the patterns obtained from erythrocytes, liver, kidney, spleen, heart and skeletal muscles, brain and lung in the hybrid females (Fig. 4). A hemizygous (or almost hemizygous) expression of the Gpd gene of M. arvalis derivation occurred in the liver, heart and skeletal muscles in the majority of M. arvalis × M. subarvalis females (Fig. 4). This was consistently observed for GALA in those organs where it was identifiable. It should be noted that the phenotypic expression of M. arvalis and M. subarvalis G6PD and GALA in the hybrid females was unrelated to the direction of the crosses. Whichever of the two species were used as maternal or paternal parent, the phenotypic expression was the same.

The question was raised whether the varying expression of the X G6PD may be related to the different specific activities of the enzyme in M. arvalis and M. subarvalis. The measured specific activities of G6PD in erythrocytes were found to be almost equal in the two vole species (75 mU/mg of protein for M. arvalis, and 68 mU/mg of protein for M. subarvalis).

There was the possibility that the different phenotypic expression of the parental genes for G6PD and GALA in the M. arvalis × M. subarvalis females may have resulted from different rates of embryonic...
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Development of the parental species. Comparative data on the developmental rates in *M. arvalis*, *M. subarvalis* and mouse embryos (6.5, 7.5, 11 and 17 days after fertilization). The time course for the two vole species was not different, but the developmental events occurred 12 hrs earlier in mice. This is consistent with Šterba (1976) who has shown an acceleration of 12 h in the embryonic development as causes of the differently expressed parental genes for G6PD and GALA in the *M. arvalis* × *M. subarvalis* females.

We sought to find an explanation for the preferential inactivation of the *M. subarvalis* X-chromosome or, perhaps, developmental selective advantage of cells with an *M. arvalis* X over those with an *M. subarvalis* X active. The observed G6PD and GALA patterns were analysed electrophoretically in embryos 6.5, 7.5–8, 9, 10 and 17 days old. No differences were observed between G6PD and GALA patterns in 6.5-day-old *M. arvalis* × *M. subarvalis* hybrid female embryos and those in adult hybrid females (Fig. 5, 6). In the mouse the X-chromosomes are inactivated completely at 5.5 days after fertilization (Rastan, 1982a). If it is assumed that the timing is the same in voles (also it may be assumed from embryological data mentioned above) then, probably, no significant elimination of cells with an active *M. subarvalis* X could have been feasible during this span of time (5.5–6.5 days). Thus, the data allow us to conclude that non-random inactivation of the parental X-chromosomes in *M. arvalis* × *M. subarvalis* females is the most likely cause of the observed preferential expression of the marker genes for G6PD and GALA of the X-chromosome derived from *M. arvalis*.

The next question posed was whether the selective inactivation of the *M. subarvalis* X-chromosome, which was observed in *M. arvalis* × *M. subarvalis* females, may be related to the influence of a locus analogous to the *Xce* known to cause non-randon inactivation of the X-chromosomes in mice (Cattanach & Isaacson, 1967; Cattanach, 1975; Rastan, 1982a, b; 1983). This appeared plausible in view of the fact that initial non-random X-inactivation is the result of the influence exerted by the *Xce* locus rather than of cell selection following the inactivation of the X (Rastan, 1982a). The sterility of the vole hybrids, however, led our explanation to an impasse here. The involvement of a controlling locus of the *Xce* type in female hybrid voles could not be ruled out. It is possible that interspecific variations at the *Xce* locus considered here may be broader than intraspecific ones. In hybrids between the horse and donkey and in those between the silver and polar foxes, however, non-random inactivation of the parental X does not occur (Serov et al. 1978a, b).

**Fig. 4.** Electrophoretic G6PD patterns from organs and tissues of adult female hybrids from ♀ *M. subarvalis* × ♂ *M. arvalis* crosses (3–18) and their parental species (1, 2). 1, *M. subarvalis*; 2, *M. arvalis*; G6PD in organs; 3, 4, liver; 5, 6, skeletal muscles; 7, 8, heart muscle; 9, 10, brain; 11, 12, lung; 13, 14, spleen; 15, 16, kidney; 17, 18, haemolysates. Hb haemoglobin.

**Fig. 5.** Starch gel electrophoresis showing the expression of G6PD in 7.5 to 8-day-old *M. arvalis* × *M. subarvalis* embryos. 1, 2, male embryos; 3–5, female embryos; 6–8, *M. subarvalis* and *M. arvalis* and a 1:1 mixture of *M. subarvalis* and *M. arvalis* haemolysates, respectively. Hb, haemoglobin.

**Fig. 6.** Electrophoretic GALA patterns from 7.5 to 8-day-old embryos from the *M. subarvalis* × *M. arvalis* cross: females (3, 5), and males (6–8) and parental vole species: *M. subarvalis* (1) and *M. arvalis* (2).
The structural differences between *M. arvalis* and *M. subarvalis* X-chromosomes merit consideration (Fig. 7). In *M. subarvalis*, the X-chromosome is large-sized due to the addition of heterochromatin material (C-positive), which is located in the telomeric region. In *M. arvalis*, there are no detectable heterochromatin regions in the X-chromosome (Fig. 7). With this difference in mind, the non-random X-inactivation in reciprocal hybrid females from *M. arvalis* x *M. subarvalis* crosses may be related to the presence of a large block of heterochromatin in the X-chromosome of *M. subarvalis*.

Hybrids from *M. kirgisorum* x *M. arvalis* crosses were used to see if we were correct in the latter conclusion. These hybrids are advantageous because of the specific distribution of heterochromatin in X-chromosomes of *M. arvalis* and *M. kirgisorum* (Fig. 7). It can be seen that the heterochromatin region is located in the precentromeric region of X in *M. kirgisorum*, and Fig. 3 shows an obvious quantitatively predominant of *M. arvalis* over *M. kirgisorum* GALA in the patterns obtained from *M. kirgisorum* x *M. arvalis* crosses.

Thus, another hybrid pair supported the suggestion that the observed non-random X-inactivation in the interspecific hybrid voles is related more to the amount and distribution of heterochromatin in the parental X’s than to any other cause contemplated so far.

The influence of heterochromatin on X-inactivation does not seem to be at all of a ubiquitous kind. The donkey X-chromosome has a block of interstitial heterochromatin which is similar to the one in the horse X-chromosome. However, the horse X-chromosome has a block of heterochromatin in the precentromeric region not observed in the donkey X (Graphodatsky & Radjabli, 1981). Despite these differences, the X-chromosomes are randomly inactivated in female mules (Serov et al. 1978). Thus, the differences in the presumed influence of heterochromatin on X-inactivation in the interspecific vole hybrids may be related to the differences in heterochromatin distribution in the X’s from one species to another.

The idea of the non-random inactivation of the X-chromosome being related to the amount of heterochromatin on the X has the lure of a speculation and, as such, it requires experimental queries. Other explanations involving elimination, cell selection cannot be dismissed.

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