

Cytogenetic Variation in Farmed Stock of Dybowski's Sika Deer

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The chromosome constitution of Dybowski's sika deer was studied on the basis of 15 samples obtained from farmed stock maintained in an enclosure. The diploid chromosome number was $2n=68$, $2n=67$ and $2n=66$. The constitutive heterochromatin (C-bands) was located in the centromeric regions of all acrocentric chromosomes. Metacentric chromosomes were C-negative. Chromosomes of three pairs proved to be NORs carriers. The size polymorphism of silver deposits was identified in two animals. A cytogenetic analysis indicated that the farmed stock of Dybowski's sika deer demonstrates considerable variation. The chromosome polymorphism observed may be a valuable marker for the management and preservation of this species.

Key words: Dybowski's sika deer, cytogenetic analysis, chromosome polymorphism.

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The sika deer (*Cervus nippon*) is divided into several subspecies, widespread throughout north-eastern Asia (WHITEHEAD 1993). Some of these, including Dybowski's sika deer, are endangered and placed on the Red List of the International Union for Conservation of Nature and Natural Resources (GROOMBRIDGE 1993; TAMATE *et al.* 2000; BONNET *et al.* 2001). Dybowski's sika deer (*Cervus nippon hortulorum* Tacz., 1864) is the largest subspecies of *Cervus nippon*. This subspecies has been maintained for many years in Russia in farm conditions for the production of velvet. Cytogenetic studies of sika deer revealed substantial intraspecific differentiation in the chromosome number, which ranges from $2n = 64$ to $2n = 68$, depending on the subspecies (GUSTAVSSON & SUNDT 1969; KOULISHER *et al.* 1972; VAN TUINEN *et al.* 1983; BONNET *et al.* 2001). The fundamental number (NF) of chromosome arms is the same for all subspecies (NF = 70). It has been suggested that the centric fusions or translocations have been the predominant means of karyotype evolution in *Cervus nippon* (HERZOG 1987). The present study describes a karyotype analysis performed in order to determine the extent of cytogenetic variation in Dybowski's sika deer stock, founded by a small number of individuals.

Material and Methods

The analysed stock of Dybowski's sika deer was maintained within an enclosure in the Kadzidłowo Wildlife Park. This stock originated from nine founder animals, one stag and 8 hinds, imported from breeding farms in Russia.

Chromosome preparations were obtained from peripheral blood lymphocyte cultures of 15 animals (8 males and 7 females) born in the Kadzidłowo Wildlife Park. The culture and chromosome preparations were made according to standard methods. Chromosome slides were stained according to the routine Giemsa method, while CBG banding was performed according to the technique by SUMMER (1972). The NOR staining was carried out by the rapid colloidal silver method according to HOWELL and BLACK (1980). A minimum of 50 metaphases were analysed for each specimen.

Results and Discussion

The diploid chromosome numbers found in samples obtained from the Dybowski's sika deer farm

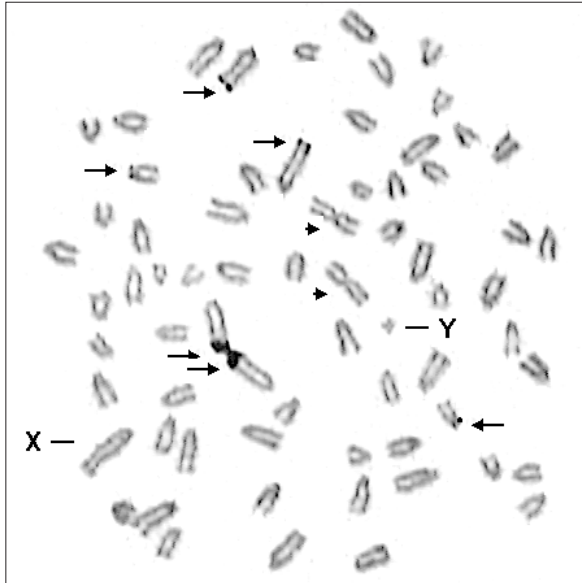


Fig. 1. Silver stained metaphase of a male ($2n=68$) with two metacentric chromosomes (arrowheads). The NORs on six chromosomes, and association between two chromosomes (arrows).

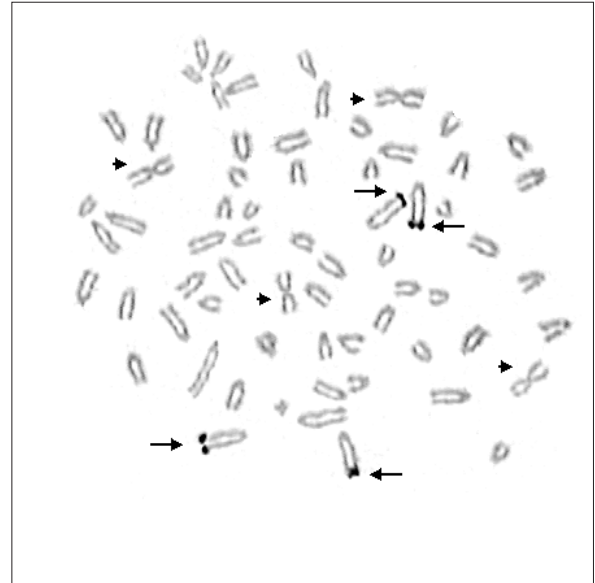


Fig. 2. Silver stained metaphase of a male ($2n=67$) with three metacentric chromosomes (arrowheads). The NORs on five chromosomes (arrows).

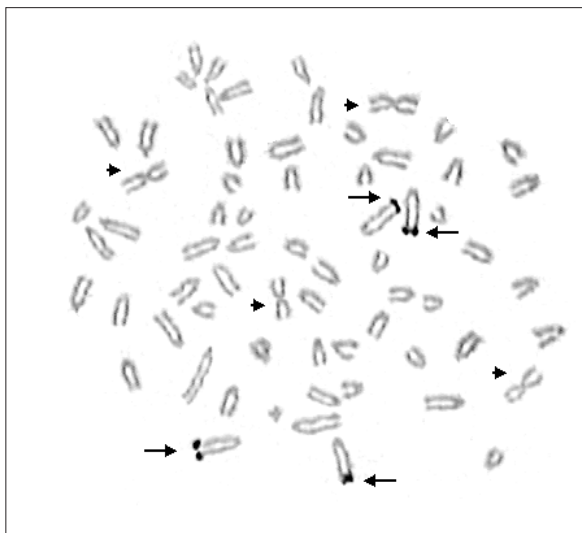


Fig. 3. Silver stained metaphase of a male ($2n=66$) with four metacentric chromosomes (arrowheads). The NORs on four chromosomes (arrows).

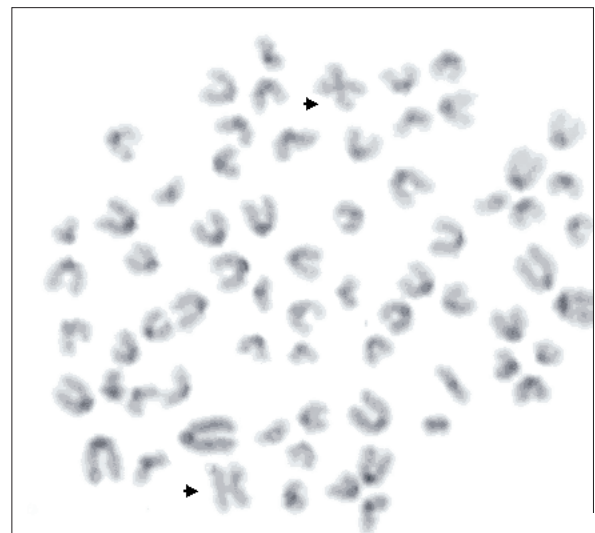


Fig. 4. C-banded metaphase spread. Two metacentric chromosomes without distinct heterochromatin (arrows).

Table 1

Distribution of the chromosome number and AgNORs in farmed stock of Dybowski's sika deer

| Karyotype | Number of animals | | Number of metaphases | AgNORs | |
|-----------|-------------------|-------|----------------------|-------------------------|-----------------|
| | Females | Males | | Mean number (\pm SD) | Minimum-maximum |
| $2n = 68$ | 3 | 4 | 350 | 4.00 ± 0.85 | 2-6 |
| $2n = 67$ | 3 | 2 | 250 | 3.94 ± 0.95 | 2-5 |
| $2n = 66$ | 1 | 2 | 150 | 3.68 ± 1.07 | 2-5 |
| Total | 7 | 8 | 750 | 3.92 ± 0.92 | 2-6 |

stock amounted to $2n = 68$, 67 and 66. The distribution of karyotypes in the cytogenetically analysed group of animals is shown in Table 1. The results obtained indicate that seven individuals (4 males + 3 females) with chromosome number $2n = 68$, consisting of 32 acrocentric and one metacentric autosome pair, a large acrocentric X and a small metacentric Y (Fig. 1), comprise a majority within the group of animals examined. In two males and three females the chromosome number amounted to $2n = 67$ and comprised three metacentric chromosomes in complement (Fig. 2). The chromosome number observed for the final three individuals, two males and one female, was $2n = 66$, and contained two pairs of metacentric chromosomes (Fig. 3). Similar karyotype variants of a centric fusion type were found in a population of the Manchurian sika deer (GUSTAVSSON & SUNDT 1969), while in a population of Vietnamese sika deer (*Cervus nippon pseudaxis*) all individuals showed an identical karyotype $2n = 66$ (BONNET *et al.* 2001). Also, a cytogenetical analysis of a group of *Cervus nippon* Temminck showed the karyotype to be identical, possibly as result of a small founder population from which they were derived (VAN TUINEN *et al.* 1993). Several authors have reported that a chromosome rearrangement of the centric fusion type may reduce the fertility of heterozygotes (GUSTAVSSON 1969; REFSDAL 1976; ŚWITOŃSKI 1980; KINGSWOOD *et al.* 1998). However the effect of chromosome heterozygosity on reproductive performance may vary within and also between species (JASZCZAK *et al.* 1987; LONG 1988; KINGSWOOD *et al.* 1994).

C- banding shows that the constitutive heterochromatin in Dybowski's sika deer is located at the centromeric region of all the acrocentric chromosomes (Fig. 4). The metacentric chromosomes appear to be C- negative, similarly as in other species of this family (GOLDONI *et al.* 1984; RUBINI & FONTANA 1988; WESSMAN & GRIPENBERG 1993). The absence of C-band heterochromatin in the centromeric region of metacentric chromosomes may be indicative of the fusion origin of these chromosomes (HSU 1973).

The distribution of nucleolus organising regions (NORs), as revealed by the silver-stained metaphase cell, shows the presence of six NOR-bearing chromosomes. In these specimens, NORs are located terminally on the two longest chromosome pairs and on one of the smaller acrocentrics between pairs 15-17 (Figs 1,2,3).

However, other authors have noted the presence of NORs in sika deer (VAN TUINEN *et al.* 1993) and also in red deer (GOLDONI *et al.* 1994) only on the two longest autosomal pairs. The quantity of

silver deposit in a cell and mean number of NORs in animals with different numbers of chromosomes are presented in Table 1. The mean number of AgNOR within the karyotypic groups of animals varied from 4.00 ± 0.85 to 3.68 ± 1.07 , but this difference was not significant statistically. Size polymorphism of silver deposits on the chromosomes was identified in two animals (Fig. 2). The Ag-NOR pattern, determined by the number of Ag-stained NORs and the size of Ag-spots in the cell, depends on the variation of expression of rRNA genes or on the variation in the number of copies of these genes (HUBBELL 1985)

Associations between the NOR bearing chromosomes (Fig. 1) were observed in about 30 per cent of cells.

The results obtained indicate that the farmed stock of Dybowski's sika deer preserve extensive cytogenetic variation, despite the small population size. The chromosome polymorphism observed in this subspecies may constitute valuable information for the management and preservation of Dybowski's sika deer.

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