Chromosome Polymorphism in Japanese Sika,
_Cervus (Sika) nippon_

Yoshiaki OMURA, Yukio FUKUMOTO, and Kazuo OHTAKA

Asa Zoological Park, Asa, Asakita, Hiroshima 731-33, and 1)Radiation Effects
Research Foundation, 5-2 Hijiyama Park, Minami, Hiroshima 730

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ABSTRACT. Chromosome examination by blood culture was conducted on 35 Japanese sika,
_Cervus (Sika) nippon_, revealing that the number of chromosomes varied according to individuals,
such as 65, 66, 67 and 68. This numerical variation depended on the presence or absence of
large metacentric chromosomes and submetacentric chromosomes in the autosomes. It was
possible to observe six karyotypes according to the presence or absence of these two kinds of
chromosomes. Polymorphism of these chromosomes is presumed to be formed when either
large metacentric chromosomes or submetacentric chromosomes are formed following fusion
of four acrocentric chromosomes in the autosomes at the centromere. The fundamental
number was constant, 70 in all cases. This is considered to be due to the so-called Robertsonian
translocation. The common phenomenon observed in all the individuals examined was the
presence of one pair of middle-sized metacentric chromosomes in the autosomes. The X-
chromosome was the largest among the acrocentric chromosomes and the Y-chromosome could
be easily identified as a small submetacentric chromosome.

Detailed reports have been published on chromosome polymorphism in the
cow [6] and sheep [1, 2, 3] among domestic animals, and in the Owl monkey [8, 9,

As for chromosomes of the Japanese sika, _Cervus (Sika) nippon_, no report has
been published, except for a report [10] on two cases of Hokkaido sika, _Cervus
(Sika) hortulorum yesoensis_, according to which the number of chromosomes was
68. There is also another report on polymorphism observed in 11 cases of the
Chinese sika, _Cervus (Sika) hortulorum_ [7], based on the presence or absence of
three chromosomes different in morphology.

The authors conducted a chromosome examination on 35 Japanese sika, and ob-
served six types of polymorphism based on the presence or absence of two chro-
mosomes different in morphology.

MATERIALS AND METHODS

As shown in Table 1, the animals observed in this study consisted of 31 Japa-
nese sika kept in the Asa Zoological Park and Miyazima Park, 4 Wild Japanese sika
brought to the Asa Zoological Park for medical care.

Most of the animals were anesthetized by a shot with a capture gun (succinyl-
choline chloride, 0.1 mg/kg) and some were tied by rope. Five—10 ml of blood
was drawn from the cervical vein of each animal. Chromosome specimens were
prepared according to the method of Sasaki [14]. Chromosome medium 1A
was used as a culture medium.

RESULT

The number of chromosomes of the Japanese sika examined varied individu-
Table 1. Materials and results of chromosome analysis in Japanese sika

<table>
<thead>
<tr>
<th>Chromosome numbers (Karyotype)</th>
<th>Miyazima</th>
<th>Asa zoo</th>
<th>Wild</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>65(2M, ISM)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>66(2M)</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>66(1M, ISM)</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67(1M)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>66(1SM)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>68</td>
<td>2</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

Remarks M=metacentric chromosome
SM=submetacentric chromosome

ally, such as 65, 66, 67 and 68. This variation was attributable to the presence or absence of large metacentric (M) chromosomes and submetacentric (SM) chromosomes in the autosomes. As a result, six different karyotypes were observed as follows.

(1) 2n=68 was observed in two females. The autosomes were composed of 66 acrocentric (A) chromosomes and one pair of middle-sized M-chromosomes, while the sex chromosomes were composed of two X-chromosomes which were the largest among the A-chromosomes (Fig. 1). (2) 2n=67 (ISM type) was observed in four females. The autosomes were composed of 62 A-chromosomes, one pair of middle-sized M-chromosomes and one large SM-chromosome (Fig. 2). (3) 2n=67 (1M type) was observed in two males and three females. The autosomes were composed of 62 A-chromosomes, one pair of middle-sized M-chromosomes and one large M-chromosome. The Y-chromosome could be readily identified as a small SM-chromosome (Fig. 3). (4) 2n=66 (1M, ISM type) was observed in one male. The autosomes were composed of 60 A-chromosomes, one pair of middle-sized M-chromosomes, one large SM-chromosome and one M-chromosome (Fig. 4).

(5) 2n=66 (2M type) was observed in five females and in 16 males. The four wild Japanese sika were all of this type. The autosomes composed of 60 A-chromosomes, one pair of middle-sized M-chromosomes and one pair of large M-chromosomes (Fig. 5). (6) 2n=65 (2M, 1SM type) was observed in two males. The autosomes were composed of 58 A-chromosomes, one pair of middle-sized M-chromosomes, one pair of large M-chromosomes and one large SM-chromosome (Fig. 6).

The characteristic common to the foregoing six types was the presence of one pair of middle-sized M-chromosomes. Although the number of chromosomes thus varied, the fundamental number was consistently 70.

As for the sex chromosomes, the X-chromosome was the largest among the A-chromosomes and the Y-chromosome was a small SM-chromosome, facilitating sex discrimination.

**Discussion**

Genus *Cervus* is, at present, classified into sub-genera. Reports have been published on the chromosome number and the karyotypes of six sub-genera; Hog deer, *Cervus (Hyelaphus) porcinus*, with 68 chromosomes [16]; Chital deer, *Cervus (Axis) axis*, with 66 chromosomes [12]; Barasingha deer, *Cervus (Rucervus) duvauceli*, with 54 chromosomes [16]; Sambir, *Cervus (Rusa) unicolor*, with 64–65 chromosomes [4]; Red deer, *Cervus (Cervus) elaphus*, with 68 chromosomes [5].

In general, the sub-genera of Japanese sika are classified into three types, namely, Japanese sika, *Cervus (Sika) nippon*, Chinese sika, *Cervus (Sika) hortulorum* and Formosan sika, *Cervus (Sika) taiouanus*.

It has been reported that the number of chromosomes of the Hokkaido sika, which is included in the category of Chi-
nese sika, is 68 [10]. This is in agreement with the karyotype 2n=68 observed in the present study. The polymorphism reported in 11 cases of Chinese sika [7] shows six karyotypes dependent on the presence or absence of three different chromosomes, one middle-sized M-chromosome and two large SM-chromosomes somewhat different in morphology from each other.

In the present observation, all the animals had one pair of middle-sized M-chromosomes. Thus, the observed six karyotypes based on the presence or absence of large M-chromosomes and SM-chromosomes differ from those reported by Gustavsson[7].

In addition to the foregoing six karyotypes, the presence of the following three karyotypes can be assumed theoretically by the way of combination of chromosomes. (1) 2n=64 (2M, 2SM type). The autosomes are composed of 56 A-chromosomes, one pair of middle-sized M-chromosomes, one pari of large M-chromosomes and one pair of large SM-chromosomes. (2) 2n=65 (1M, 2SM type). The autosomes are composed of 58 A-chromosomes, one pair of middle-sized M-chromosomes, one pair of large SM-chromosomes and one large M-chromosome. (3) 2n=66 (2SM type). The autosomes are composed of 60 A-chromosomes, one pair of middle-sized M-chromosomes and one pair of large SM-chromosomes. These expected karyotypes could be detected, if the number of observed cases is increased.

According to Gustavsson [7], the polymorphism in Chinese sika is attributable to a Robertsonian translocation, in which four different A-chromosomes fuse at the centromere to form large M-chromosomes and SM-chromosomes. Bruère [2, 3] has observed a similar Robertsonian translocation in sheep, which shows one to two additional large SM-chromosomes in addition to the normal three pairs of large SM-chromosomes. Thus, the karyotype shows 52–53 chromosomes with the decreased number of A-chromosomes by 2–4. This may be attributable to the fusion of two pairs of A-chromosomes at the centromere to form large M and SM chromosomes. Furthermore, it has been demonstrated that four karyotypes can be produced by mating a normal animal with an animal heterogeneously having one of these translocations [3]. It has also been demonstrated that by mating animals having one or more identical translocations, six karyotypes showing 49–54 chromosomes can be produced and genetic defects such as malformation and decreased reproductive activity are not produced [11]. Such genetic defects have not been observed in the result of mating the animals kept at the Asa Zoological Park, the chromosome number of these animals being 65–68. This may be due to the facts that regardless of variation in the chromosome number, the fundamental number of the chromosomes in all cases is consistently 70 and that there is neither duplication nor loss of genes.

From the results of the present study on the chromosome number and karyotypes of Japanese sika, it is of interest to note that cases of chromosome number of 66 (2M type) are 60% of the animals examined and that the karyotype of all the wild Japanese sika showed this type. Furthermore, chromosome number of 68 resembles the karyotype of Red deer.
and the expected chromosome number of 66 (2SM type) resembles the karyotype of Chital deer. These findings lead to a speculation that the Japanese sika of today have been evolved from those deer which migrated from the south at one period and from those deer which migrated from the north at another period, by the repetitive connection and disconnection of the Japanese archipelagoes with the Asian continent following the rise and fall of the sea level during the glacial and thawing periods.

The present study is restricted to the deer confined to the Chugoku region in Japan, but we expect that important findings can be provided on the evolution of this species by expanding the heretofore reported morphological studies in connection with the cytogenetic studies on the deer living in various areas, particularly in isolated places such as Yaku-shima, Tsushima and Kinkazan.

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References
EXPLANATION OF FIGURES

Fig. 1. 2n=68, female. Metaphase and karyotype of Japanese sika.
Fig. 2. 2n=67 (ISM type), female. Metaphase and karyotype of Japanese sika.
Fig. 3. 2n=67 (1M type), female. Metaphase and karyotype of Japanese sika.
Fig. 4. 2n=66 (1M, ISM type), male. Metaphase and karyotype of Japanese sika.
Fig. 5. 2n=66 (2M type), male. Metaphase and karyotype of Japanese sika.
Fig. 6. 2n=65 (2M, ISM type), male. Metaphase and karyotype of Japanese sika.

要約
ニホンジカにおける染色体多型現象について：尾村嘉昭，福本幸夫，大滝一夫（広島市安佐動
物公園，放射線影響研究所）——35頭のニホンジカについて血液培養により染色体検査を試みたとき
ころ，個体によって65、66、67、68と染色体数に変異があった。この染色体の変異は、常染色体
中に並べたメタセントリック(M)染色体およびサブメタセントリック(SM)染色体の存在に
起因していた。この二つの異形染色体の有無により、6つの核型を観察することができた。このよ
うな染色体の多型現象は、常染色体中の4個のアクロセントリック(A)染色体が不等数部で遅合し
て大型のM染色体をつくるか、SM染色体をつくるかにより、生じたものと思われる。なお、い
ずれの場合も染色体の基本数は70で一定しており、いわゆるRobertson型転座によるものと思わ
れる。すべての検査個体に共通して、常染色体中に中型のM染色体1対が存在した。X染色体は
A染色体中最大で、Y染色体は小型のSM染色体であり、識別は容易であった。