Selecting a mammalian species for the separation of X- and Y-chromosome-bearing spermatozoa

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Summary. All (524) male karyotypes in An Atlas of Mammalian Chromosomes (Hsu & Benirschke, 1967–1977) were visually estimated for the chromatin difference between the X- and Y-chromosome-bearing spermatozoa. After more exact measurements of axial chromatid length for 100 karyotypes, 24 species were found in which the difference between X and Y chromosomes was >6.2%. It is suggested that such species would be the best for attempts to separate the X- and Y-bearing spermatozoa.

Introduction

The separation of X- and Y-chromosome-bearing spermatozoa is aimed at controlling the sex of the offspring. The economic and social benefits of such an accomplishment include selection for females in dairy cattle, ‘balancing’ the sexes in a family with few children, and blocking male transmission of sex-linked genetic diseases, such as haemophilia. Scientific benefits include materials for the study of post-meiotic gene activity, and the ability to maximize population growth, which is limited by the number of mature females.

Sex chromosomes were observed in mammals as early as 1910 (Guyer, 1910). Since then biologists have periodically attempted to fractionate spermatozoa into X- and Y-chromosome-bearing cells. Previous attempts include head length measurements (Zeleny & Faust, 1915), acid or alkaline douching (Unterberger, 1930), electrophoresis of semen (Schroder, 1941), and density centrifugation (Lindahl, 1958; Ericsson, Langevin & Nishino, 1973). However, all of the above separations have been challenged as ineffective (Cole, Waletzky & Shakelford, 1940; Rothschild, 1960; van Duijn, 1961; Nevo, Michaeli & Schindler, 1961; Schilling & Thormaehlen, 1975; see Stolla (1968) for additional references).

Recent attempts to recognize the spermatozoa which give rise to male or female offspring have concentrated on differences between the X and Y chromosomes. Barlow & Vosa (1970) found that the bright, heterochromatic fluorescence of the Y chromosome with quinacrine stain persisted in human spermatozoa. In the worm, Ascaris suum, spermatozoa containing the X complex have 37% more Feulgen-stained DNA (Anisimov, 1973). Sumner, Robinson & Evans (1971) combined fluorescent and Feulgen techniques to infer a 2.7% difference in DNA content between X- and Y-bearing human spermatozoa.

This report identifies mammalian species expected to have a maximal difference in bulk chromat between X- and Y-bearing spermatozoa. The primary source was the work of Hsu & Benirschke (1967–1977) which supplies a large assembly of diverse mammalian karyotypes with paired homologues and labelled sex chromosomes.

Materials and Methods

Study of the work of Hsu & Benirschke (1967–1977) revealed 524 male karyotypes with paired homologues and labelled sex chromosomes. A coarse measurement of every karyotype was first

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taken by visual estimate (Method 1). Those karyotypes showing a large difference were enlarged by projection, and chromosome lengths were then measured by Method 2.

Method 1. Each male karyotype was visually inspected for (1) chromosome number (N), (2) sex chromosome size proportion, i.e. Y/X, and (3) a comparison of the lengths of X and an ‘average’ chromosome, i.e. X/a. The ‘average’ chromosome was chosen from middle-sized chromosomes in the karyotype. The chromosome lengths were measured in millimeters by placing a straight ruler directly on the published picture. The values obtained were used to give a visually estimated difference in chromatin content between the X- and Y-bearing spermatozoa for each species:

\[
\frac{(X/a)(1 - (Y/X))}{N} = \frac{X - Y}{aN} = \text{haploid chromatin content}
\]

Method 2. Projection slides were made from the 100 karyotypes showing the largest chromatin difference by Method 1. The slides of the individual karyotypes were all prepared at the same magnification, and projected to give a total magnification of the chromosomes of 35 000. Metaphase chromosomes generally had one of the appearances shown in Text-fig. 1. A map measurer (Forestry Suppliers, swivel handle, 99 cm, 2141-45240) was used to measure chromatid axial lengths in the projected image. The average (A) of 4 axial lengths of each autosomal pair and axial measurement of the sex chromosomes (X and Y) were combined in the following formula:

\[
\frac{X - Y}{A + X} = \text{chromatin difference} \quad \text{female haploid chromatin content}
\]

Each karyotype was measured twice, and the average measurement used. The measurements disregarded heterochromatic condensation or length distortions from chromosome banding techniques.

The coarse estimates of Method 1 were correlated for accuracy with the direct measurements of Method 2. The difference between the average coarse estimate and the average direct measurement is a correction factor. The error between this adjusted estimate and the measured value was recorded for each of the 100 karyotypes, and a standard deviation was calculated.

Two additional male karyotypes of *Microtus oregoni* from Ohno (1967) were photographed, projected and measured, in order to test the reproducibility of measurements using the karyotypes shown in the *Atlas* (Hsu & Benirschke, 1967–1977).

**Text-fig. 1.** Homologous metaphase chromosome types: (a) metacentric, (b) telocentric and (c) globular. Chromatid axes are labelled 1, 2, 3 and 4.
Results

Altogether 524 male karyotypes of different species were assigned coarse estimates for chromatin differences between X- and Y-bearing spermatozoa by Method 1 (Text-fig. 2). The average difference in chromatin content was 3.9% (s.d. 1.4%).

Measurements by Method 2 gave estimates for the difference in chromatin between X- and Y-bearing spermatozoa approximately 0.5% less than those obtained by Method 1. When all estimates by Method 1 are lowered by 0.5% (correction factor), the predictions differed from the measured lengths (Method 2) with a standard deviation of 0.8%. Since all of the karyotypes estimated to have more than 4.8% difference in Method 1 were measured by Method 2, it can be stated with 99% certainty ($z = 2.33$, one-tailed test) that none of the unmeasured karyotypes would have had a difference in length measurement of >6.2% (upper 99% confidence limit). The 24 species which do have a chromatin difference of >6.2% between the X- and Y-bearing spermatozoa are given in Table 1.

When chromosome length measurements for Microtus oregoni were compared, values for Y/(A + Y) were 0.08761, 0.09165 and 0.08515 for karyotypes from Hsu & Benirschke (1967–1977) and Ohno (1967) (somatic cells) and Ohno (1967) (testicular cells) respectively; these values are within 0.7% of each other. (The X chromosome is not carried by spermatozoa of this species, and the sperm chromatin is therefore A + O or A + Y.)

Discussion

An Atlas of Mammalian Chromosomes (Hsu & Benirschke, 1967–1977) testifies to the generality and diversity of heteromorphic sex chromosomes throughout the Mammalia. The sex
Table 1. A list of the 24 mammalian species which have the greatest difference in chromatin content (Method 2, see text) in the X- and Y-chromosome-bearing spermatozoa

<table>
<thead>
<tr>
<th>Latin name (common name)</th>
<th>Haploid number</th>
<th>(X - Y)/(A + X)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apodemus sylvaticus</em> (Old World field mouse)</td>
<td>24</td>
<td>0.09253</td>
</tr>
<tr>
<td><em>Psammomys obesus</em> (fat sand rat)*</td>
<td>24</td>
<td>0.09101</td>
</tr>
<tr>
<td><em>Desmodus rotundus</em> (vampire bat)</td>
<td>17</td>
<td>0.08895</td>
</tr>
<tr>
<td><em>Microtus oregoni</em> (Oregon vole)†</td>
<td>9</td>
<td>0.08761</td>
</tr>
<tr>
<td><em>Hyposigmodus monstrosus</em> (hammer-headed bat)</td>
<td>18</td>
<td>0.08688</td>
</tr>
<tr>
<td><em>Macropus rufus</em> (red kangaroo)</td>
<td>10</td>
<td>0.08405</td>
</tr>
<tr>
<td><em>Hydrochoeris hydrochaeris</em> (capeybara)</td>
<td>33</td>
<td>0.08073</td>
</tr>
<tr>
<td><em>Reithrodontomys fulvescens</em> (harvest mouse)*</td>
<td>25</td>
<td>0.07985</td>
</tr>
<tr>
<td><em>Aotus trivirgatus</em> (owl monkey)*</td>
<td>27</td>
<td>0.07954</td>
</tr>
<tr>
<td><em>Myopus schisticolor</em> (wood lemming)</td>
<td>16</td>
<td>0.07616</td>
</tr>
<tr>
<td><em>Mesoprylla macconelli</em> (ectophylla bat)</td>
<td>11</td>
<td>0.07577</td>
</tr>
<tr>
<td><em>Oryzomyx palustris</em> (rice bat)</td>
<td>28</td>
<td>0.07382</td>
</tr>
<tr>
<td><em>Onatra zibethica</em> (muskrat)</td>
<td>27</td>
<td>0.07342</td>
</tr>
<tr>
<td><em>Dolicophilus patagonum</em> (mara)</td>
<td>32</td>
<td>0.07124</td>
</tr>
<tr>
<td><em>Balaebopera physalus</em> (fin whale)</td>
<td>22</td>
<td>0.07007</td>
</tr>
<tr>
<td><em>Chinchilla laniger</em> (chinchilla)</td>
<td>32</td>
<td>0.06919</td>
</tr>
<tr>
<td><em>Gazella leptoceros</em> (gazelle)</td>
<td>16</td>
<td>0.06848</td>
</tr>
<tr>
<td><em>Eguus zebra hartmannae</em> (zebra)</td>
<td>16</td>
<td>0.06725</td>
</tr>
<tr>
<td><em>Perognathus intermedius</em> (desert pocket mouse)</td>
<td>23</td>
<td>0.06643</td>
</tr>
<tr>
<td><em>Pedetes capensis</em> (springhaas)</td>
<td>19</td>
<td>0.06468</td>
</tr>
<tr>
<td><em>Muntiacus muntjak</em> (muntjac)</td>
<td>3</td>
<td>0.06466</td>
</tr>
<tr>
<td><em>Phodopus sungorus</em> (dwarf hamster)</td>
<td>14</td>
<td>0.06460</td>
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<tr>
<td><em>Mesocrictetus brandtii</em> (Azarbaidjan hamster)</td>
<td>21</td>
<td>0.06454</td>
</tr>
<tr>
<td><em>Cheirogaleus major</em> (dwarf lemur)</td>
<td>33</td>
<td>0.06321</td>
</tr>
</tbody>
</table>

* Errors have been found in these karyotypes since their publication in An Atlas of Mammalian Chromosomes.
† The difference actually measured was Y/(A + Y).

Chromosomes segregate during spermiogenesis in mammals. Since the X chromosome is usually larger than the Y chromosome, 'female' spermatozoa should have more chromatin than 'male' spermatozoa.

The desire to distinguish the X- and Y-bearing spermatozoa has been directed towards species of social and economic value, e.g. man and cattle (Leuchtenberger & Leuchtenberger, 1958) and pig (Stolla, 1968). However, these species have only small chromatin differences (2-8, 4-2 and 3-6% respectively). The best method now available for estimating the chromatin difference between X- and Y-bearing spermatozoa is measurement of chromosome lengths in the electron microscope using testicular synaptonemal complexes (Moses, 1977a, b; Moses, Slatton, Gambling & Starmer, 1977; Solari & Ashley, 1977). At synapsis the chromosomes are decondensed and of uniform thickness throughout. Homologous chromosomes and the sex chromosomes are unequivocally identified by chiasmata. In the electron microscope there is also increased resolution permitting more accurate measurement of chromosome length. Electron microscopy of testicular synaptonemal complexes could be used to verify the results in Table 1.

In this chromosome study, length measurements were preferred to area measurements or volume reconstruction in order to minimize error. Typically, a chromatin is about 3-5 μm long and 0-6 μm wide. Since the resolution of the light microscope is at best 0-2 μm (Ruthmann, 1970), error inherent in an area or volume estimate far exceeds the error of length measurement. The main sources of error in the present study stem from assumptions about the karyotypes. First, estimating bulk chromatin by chromosome length measurement assumes that chromatids are cylinders of uniform diameter and density. Calculation of the chromatin difference between X and Y spermatozoa depends much upon the length of the Y chromosome. Mammalian Y...
Mammalian sex chromosomes

chromosomes, however, are typically heterochromatic and condensed. Secondly, the calculations assume correct pairing of homologues and proper identification of the sex chromosomes. The karyotypes for Psammomys obesus, Reithrodontomys fulvescens and Aotus trivirgatus listed in the Atlas are now known to have misidentified chromosomes (Solari & Ashley, 1977; see Errata in Vol. 6, and Vol. 9 folio 298 in the Atlas). Both the error of non-uniform chromosome density and chromosome misidentification are avoided by electron microscopy of testicular synaptonemal complexes.

I commend T. C. Hsu and K. Benirschke for compiling their monumental Atlas of Mammalian Chromosomes. I thank Professor J. H. Sinclair, Indiana University, for guidance with this project and supplying the necessary materials, and Professor M. Ellinger and Professor J. Martan, Southern Illinois University, for constructive criticism.

References


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