SOMATIC CHROMOSOMES OF THE HORSE, 
THE DONKEY AND THEIR HYBRIDS, 
THE MULE AND THE HINNY

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Summary. The chromosome complement of the horse, the donkey and their two hybrids has been studied in somatic mitoses obtained by short-term cultures of peripheral blood leucocytes. The diploid number of chromosomes found was: horse sixty-four, donkey sixty-two, mule sixty-three, hinny sixty-three. In addition to the numerical differences between horse and donkey, there exist structural differences which are most noticeable in greater numbers of metacentric chromosomes in the donkey. These differences are reflected in the karyotypes of the hybrids, and partial assortment of parental chromosomes can be accomplished in mitoses of the mule and the hinny. It is possible that these physical differences play a role in synaptic failure during meiosis.

INTRODUCTION

The reason for the notorious sterility of the mule has been investigated on several occasions in the past. One of the earliest students of these hybrids, Wodsedalek (1914, 1916), found in testicular preparations that the horse possessed thirty-seven chromosomes while the mule he studied had fifty-one chromosomes. From these findings, he inferred that the donkey should have sixty-five chromosomes and that the azoospermia of the mule was the result of defective mitosis and meiosis. This investigator found abnormalities of cell division in spermatogonial plates, and disorderly pairing of chromosomes in meiosis was pronounced and was accompanied by cell death. More recently, Makino (1943) described sixty-six chromosomes as the normal equine chromosome complement and this is also the number at present accepted for the donkey (White, 1961). The same author studied the possible cytogenetic reasons for the mule's sterility in testicular preparations of one mule and also determined the chromosome complement of the donkey (Makino, 1955). His preparations showed sixty-six chromosomes in all three animals with at least twelve metacentric members. An unusually large J-shaped chromosome (subterminal centromere) distinguished the donkey, and this element was found in the mule as well. Makino (1955) refers to the work of Leon (1938, cited by Makino, 1955) who found thirty-eight chromosomes in the mule; however, the methods employed by Wodsedalek (1914, 1916) and Leon (1938, cited by Makino, 1955) were judged unsatisfactory by modern standards.
The introduction of newer methods for the preparation of mammalian somatic chromosomes in recent years has led to a revision of the accepted chromosome set in several species. Thus it is now established that man normally possesses forty-six chromosomes instead of forty-eight as was formerly thought; the armadillo of Texas, *Dasypus novemcinctus*, has sixty-four as 2n (Beath, Benirschke & Brownhill, 1962) instead of thirty or sixty as found before; in entirely unrelated studies Rothfels, Axelrad, Siminovitch, McCulloch & Parker (1959) have found that dissociated renal cells of five horses displayed only sixty-four chromosomes in 95% of the mitoses after tissue culture which they considered to be the normal diploid set, etc.

These discrepancies led us to re-investigate the number and structure of the chromosomes of the horse, the donkey and their hybrids, the mule and the hinny.

**METHODS**

Two male horses, three Sicilian donkeys (two males, one female), three mules (two males, one female) and one male hinny (the offspring of a she-ass and a stallion) were available for our studies. Buccal smears taken from all these animals were stained by the Feulgen method with light green as counter-stain in order to detect the possible presence of 'sex chromatin'. Peripheral blood films, stained with Wright's stain, were examined for 'drumsticks' on the lobes of polymorphonuclear leucocytes. Cytological studies were made on peripheral blood cultured according to the method first described by Moorhead, Nowell, Mellman, Battips & Hungerford (1960). The phytohaemagglutinin employed was prepared in one large batch by extracting red beans according to the method of Li & Osgood (1949). The blood was obtained by puncture of the external jugular veins employing a long No. 15 needle. In comparison to human cultures, more heparin was necessary to prevent coagulation in the syringe. We found no other reason to deviate from the techniques employed in human cytogenetic studies, and spreading of the metaphase plates was as successful despite the larger number of chromosomes. The often adverse circumstances at the time of obtaining specimens and the long transport prior to incubation are thought to be responsible for the smaller number of perfectly spread plates than is usual for our human cultures.

**RESULTS**

The horse was found to have a chromosome complement of sixty-four = 2n with a composition similar to that described by Rothfels et al. (1959). The modal number for the donkey clearly lay at sixty-two while that for mule and hinny was sixty-three, the sum of the haploid sets of the parents (Table 1).

The ages of four animals were known: one male mule was 21, one male hinny 35, one gelding horse 3 and one female donkey 8 to 9 years of age.

Buccal smears for sex chromatin proved to be less suitable in attempts at determining sex than peripheral blood smears. While seemingly typical Barr bodies were found in one female mule, there were markedly clumped chromatin particles in the nuclei of the male, and the sexes could not be clearly distinguished
by this means. More systematic studies of this family would be needed to verify the possible sexual dimorphism of buccal mucosal cells. On the other hand, typical drumsticks (Pl. 1, Fig. 1) were found only in the female animals (mule 6% and donkey 4%) while none were seen in the male mule, the horse or the hinny. However, no mare or female hinny was available for study.

**Table 1**

**Distribution of chromosome numbers in total cell counts**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Chromosome No.</th>
<th>Total cells analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>Horse (1)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Donkeys (2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mules (3)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hinny (1)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**Chromosome complement**

The horse

A representative metaphase plate of a male horse is depicted in Pl. 1, Fig. 2. From this, a tentative karyotype is prepared and shown in Pl. 1, Fig. 3. As will be seen, the chromosomes are arranged in four different groups according to their size and the position of the centromere. They are numbered for the purpose of reference only. This classification was adopted after preparation of several karyotypes, including one made from the spread shown by Rothfels et al. (1959), arranging the chromosomes in different positions. The present photograph was chosen because in this spread the chromosomes show least contraction and thus metacentrics are more readily evident. Our preparations show fourteen pairs of metacentric chromosomes, possibly fifteen if one is willing to consider No. 24 as a metacentric chromosome, in addition to the metacentric X-chromosome. This number of twenty-nine or thirty-one metacentrics differs from that of Rothfels et al. (1959) who found only twenty-seven such chromosomes. The difference lies presumably in interpretation of Nos. 21 and 24 which appear acrocentric in some of our other preparations. The degree of colchicine-induced contraction, and borderline resolution, even in phase microscopy are presumably reasons for these different interpretations and we do not wish to press this point further. Also, cultures of tissue cells other than peripheral blood may yield somewhat differing results.

Group A. The eleven pairs of this group are all large metacentric chromosomes and only the first two stand out by their significantly larger size. This difference becomes less impressive as the degree of colchicine-induced contraction increases. Only ten such pairs can be identified in the picture shown by Rothfels et al. (1959). We have considered whether the second member of No. 6 and the first of No. 8 are long acrocentrics with crossed arms. Phase-contrast observation and other spreads, however, led us to place them in this group.
**Group B.** The nine pairs of acrocentrics in this group can be distinguished from those in Group D only in preparations with little contraction. The centromere is terminal or subterminal.

**Group C.** We have placed four pairs of small metacentrics in this group although it can be argued that No. 24 should be considered an acrocentric. Whether or not the apparent stretching of the one arm of No. 23 is an expression of nucleolus organizing activities cannot be decided from our preparations, most of which do not show this feature.

**Group D.** There are seven pairs of small acrocentrics in this group. At least two of these show areas of secondary constriction.

**Sex chromosomes.** The Y-chromosome is not clearly distinguishable from members of Group D. The X-chromosome resembles a large metacentric with submedian centromere. In size it ranks third in our plates.

**The donkey**

A representative metaphase plate and karyotype of a female donkey are shown in Pl. 1, Figs. 4 and 5; the preparations from the males were adequate for counting but less favourable for karyotyping. In addition to the numerical difference from the horse (horse sixty-four, donkey sixty-two), the individual chromosomes have morphological features which distinguish them as a set, from those of the horse. Primarily, the donkey clearly possesses more metacentrics than the horse. In numerous karyotypes prepared by us, the number of metacentric pairs varies between nineteen and twenty-one, excluding the metacentric X-chromosomes. Whether or not nineteen or twenty-one such pairs are distinguished depends on the degree of contraction as well as on the interpretation of chromosomes with nearly terminal centromeres. An especially large metacentric with subterminal centromere as described by Makino (1955) could not be identified here or in the hybrids.

**Group A.** These large metacentrics are most similar to those of the horse. There are two or three distinctly larger members and ten smaller ones. Reservations are held regarding our interpretation of this animal's X-chromosomes (v.i.). The difference from the horse is mainly the addition of one metacentric in this group.

**Group B.** As has been pointed out, there are fewer acrocentrics in the donkey and this is reflected by the presence of only five pairs as opposed to the nine of the horse in this group of large acrocentrics.

**Group C.** At least seven, if not nine, pairs of small metacentrics fall into this group. In order to arrive at a karyotype most resembling that of the horse, admittedly an arbitrary choice, only seven pairs have been placed here. The nature of Nos. 28 and 29 in Group D is debatable. This preparation, as well as some others, suggests a metacentric position of the centromere of these elements in addition.

**Group D.** In this group, the remaining six pairs of chromosomes are placed which are predominantly small acrocentrics although reservations exist for Nos. 28 and 29 as discussed above.

**Sex chromosomes.** The Y-chromosome of the male is a small acrocentric member and indistinguishable from the smaller elements in Group D; possibly it is the
Fig. 1. Polymorphonuclear leucocyte of female mule with 'drumstick' attached to mid-portion of nucleus. (Wright stain, ×2000).
Fig. 2. Metaphase plate of male horse containing sixty-four individual elements. The small black circle to the left of the centre is an artefact in the photographic lens. (Orcein, ×2000).
Fig. 3. Karyotype of male horse prepared from cell shown in Fig. 2.
Fig. 4. Metaphase plate of female donkey. Two small chromosomes at the edge of an adjacent nucleus are marked by white arrows. (Orcein, ×2000).
Fig. 5. Karyotype of female donkey prepared from cell shown in Fig. 4.

(Facing p. 322)
Fig. 6. Metaphase plate of male mule. (Orcein, ×2000).
Fig. 7. Karyotype of male mule prepared from cell shown in Fig. 6.
Fig. 8. Metaphase plate of male hinny. (Orcein, ×2000).
Fig. 9. Karyotype of male hinny prepared from cell shown in Fig. 8.

(Facing p. 323)
smallest of all, as seen in the mule (Pl. 2, Figs. 6 and 7). We suggest that the
X-chromosome, clearly a large metacentric, may be the third largest chromo-
some (see also the hinny, Pl. 2, Figs. 8 and 9). However, the preparations of
the male donkeys are not adequate for the clear separation of this chromosome
from No. 3.

The mule

Representative metaphase plate and karyotype of a male mule are presented
in Pl. 2, Figs. 6 and 7. There was no doubt that this animal possessed sixty-
three chromosomes in the blood cells cultured in this study. Numerous en-
larged photographs were prepared in an attempt to pair the possibly homologous
chromosomes. No consistently satisfactory arrangement could be found and it
was impossible to distinguish unequivocally all of the horse-derived chromo-
somes from those derived from the donkey parent. Comparisons with spreads of
horse and donkey, showing similar degrees of contraction, led to a tentative
separation of the individual chromosomes. In the karyotype (Pl. 2, Fig. 7), the
top row of each group represents those chromosomes which are thought to be
of maternal (horse) origin, while the bottom row is thought to come from the
paternal donkey. It is apparent that a number of chromosomes find no suitable,
structurally homologous, partner (the genetic homology of any of these pairs of
course remains completely unexplored). Even if one assumed Nos. H17 to 20 to
represent two homologous pairs and Nos. D23 and 24 another, major discrep-
ancies would still remain in the metacentrics which are clearly unpairable.
In addition there remains, of course, one isolated horse-derived element, the
sixty-third member. We feel certain about the Y-chromosome and also about
the position of the large metacentric X.

In three preparations of the mule, there were associations of two acrocentric
chromosomes (Groups B/D and D/D) which were identical with the ‘satellite-
associations’ seen in man (Ferguson-Smith & Handmaker, 1961). Delicate
bridges extended from one chromosome to another; however, typical satellites
were never observed although thin orcein-stained extensions can be seen on the
short arms of No. H12 in Pl. 2, Fig. 7 and they were occasionally seen in other
preparations from this animal.

The hinny

A representative metaphase plate and its karyotype are shown in Pl. 2,
Figs. 8 and 9. While the diploid number was again sixty-three, there were
difficulties similar to those encountered with the mule material in attempts to
distinguish between paternal (horse) from maternal (donkey) chromosomes.
With the exception of the sex chromosome, therefore, the suggested karyotype
of this animal (Pl. 2, Fig. 9) is similar to that of the mule (Pl. 2, Fig. 7). In the
hinny, the identification of the X-chromosome is less definitely correct because
of the relative inadequacy of our contracted preparations of male donkeys,
which do not allow a definite identification of the X-chromosome except to
place it in Group A.
DISCUSSION

The studies here reported show not only a difference in number of chromosomes between the horse and donkey, but also marked structural differences in the individual somatic chromosomes of these animals. This is at variance with the most recent publication on the chromosome morphology of the horse, donkey and mule (Makino, 1955). This author studied the chromosomes in testicular preparations and found a modal number of sixty-six = 2n for all three animals and suggested that an unusually large J-shaped chromosome could serve as a marker for a donkey-derived element. The only report on somatic chromosomes of the horse available to us is that by Rothfels et al. (1959) who first indicated a modal number of sixty-four = 2n. The only figure presented by these investigators, an excellent metaphase plate from kidney cells in tissue culture from a male horse, shows twenty-seven metacentric structures, one being the X-chromosome. Karyotypes, that we prepared from photographs of this plate, agree with our results except for our finding of one additional metacentric pair. In the karyotypes prepared from peripheral blood cultures of the male donkey, we found thirty-nine clearly metacentric chromosomes, and possibly four others with a more terminal centromere which were less readily distinguished from the small acrocentric elements. In our preparations, the donkey had consistently a complement of sixty-two = 2n which was further supported by the finding of sixty-three chromosomes in both types of hybrids. As would be expected, it is not possible to arrange the chromosome complement of these hybrids in homologous pairs. The structural differences, most marked in the smaller members, allow the separation only of some chromosomes as having a paternal and others as having a maternal origin. The structural qualities are more similar in the first groups and true homology may actually exist as suggested by the synapsis of a few pairs in meioses observed by Wodsedalek (1916). Further studies of these phenomena need to be undertaken to support such possible homologous arrangement. Guided only by length and centromere position we construe the karyotypes shown here as tentative only. Those members which are presumably derived from the horse are labeled H, those from the donkey D.

The reasons for hybrid sterility are varied; White (1954) has summarized much of the available data and points out that very little is known about the exact causes in mammals. Various factors control sterility in hybrids of other forms, some of which are related to structural differences of the chromosome sets while others are genic in nature. The paucity of data on mammalian hybrid testicular preparations and the large number of chromosomes not yet satisfactorily enumerated, let alone structurally characterized, make it impossible at this time to assign a definitive mechanism. The disturbance in spermatogenesis appears to be most pronounced in the first meiotic prophase and it is possible that the widely divergent chromosome structure could account for synaptic failure. However, any of the other mechanisms elaborated in White's (1954) review, and such factors as those considered in the report of their interesting studies of carp-tuna hybrids by Makino, Ojima & Matsui (1958) may play a role. From the few direct studies of mule's testes (similar investigations on hinnies could not be located), azoospermia appears to result from degeneration of
spermatocytes. The testicular tubules are consequently remarkably hypocellular and have an atrophic appearance. Despite this, however, mating proceeds frequently. We have not found any descriptions of the structure or the ovary of female hybrids of these species and it appears to be unknown whether ovulation occurs in the mule or hinny in a manner comparable to their parents. Nevertheless, occasional reports of pregnancies in female mules have appeared. Thus, Smith (1930) depicts the mother and her mule-like foal and Anderson (1930) describes three such instances. The photograph of the mule in this latter report is indistinguishable to us from that of a horse, a recurrent problem which is inherent in attempts at the unequivocal diagnosis of hybrids and which has been pointed out by others. Sole reliance is usually placed on the breeding records which are, however, rarely positively established. The foals of such pregnancies are alleged to be more horse-like and Anderson (1930) suggests that exclusion of one parental set of chromosomes (the donkey's) during oogenesis could be the mechanism leading to the occasional mare mule fertility. The cytological events for this, taking place during meiosis, cannot be defined. They are at present entirely speculative and based entirely on the horse-like qualities of the offspring. Craft (1938) calls eleven reports of allegedly pregnant mules and one of a pregnant hinny from the literature. He indicates, however, that several cases are clearly doubtful and emphasizes the need for criteria of hybridity (see also Huskins, 1929). It is apparent that further reports of pregnancies in mules or hinnies will need to be supported by chromosome studies if the present findings can be confirmed by others.

During the progress of these studies, Trujillo, Stenius, Christian & Ohno (1961) have briefly reported their findings on studies of the chromosomes of the horse, donkey and mule. While minor differences exist in the interpretation of certain chromosomes, their results are in essential agreement with those reported here.

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REFERENCES


