Chromosome Complement: Differences between Equus caballus and Equus przewalskii, Poliakoff

Abstract. *The chromosome number of the domestic horse is 2n = 64; different races have the same complement. The chromosomes of two Przewalski's horses (at Catskill Game Farm, New York), presumably ancestral wild horses from Mongolia, are identical: 2n = 66, with more acrocentric and fewer metacentric elements than the chromosomes of the domestic horse. This apparent difference in karyotype may help resolve the questions of "purity" in the relatively few remaining Przewalski's horses. Moreover, these findings are of interest in relation to the apparent fertility of hybrids between these species.*

We have been studying the chromosome complement of several mammalian species which are known to hybridize, in order to learn more of the reason for the sterility of some of these hybrids. In the course of our studies, we learned that the chromosome number and the "gross" appearance of these elements is similar in those species whose hybrids are apparently fertile (for example, among bears and in dogs crossed with coyotes). On the other hand, it appears that the sterility of the mule and hinny can be accounted for by the gross morphologic dissimilarities between the karyotypes of their parental species, the horse and the donkey (*1*). Numerous descriptions of other hybrids in the horse family (*2*) have led us to a systematic study of the karyotypes of all available members of the genus *Equus* (*3*). The unexpected difference of the chromosome set of the descendants of the feral horse, *Equus przewalskii*, Poliakoff, from that of the domestic horse prompted this report. If confirmed, this finding could be of considerable interest in the characterization of this nearly extinct species.

Przewalski's horse (Fig. 1) was introduced to various zoological gardens of the world at the turn of this century and has been bred by most of these institutions. The history of the existing animals has been reviewed comprehensively by Mohr (*4*) and the genealogy was brought up to date by Volf (*5*). The ancestry of the animals is important.

A large herd of pedigreed animals is kept at the Catskill Game Farm in New York. Four animals were immobilized by means of a Cap-Chur gun, with Anectine (succinyl choline chloride). After shaving the skin, a small piece of tissue was taken from the ear and processed by a tissue culture method (*6*) until a sufficient number of metaphases displaying chromosomes was available for study. Only two animals were studied successfully; cultures of the others were contaminated by fungi, a serious problem when material is obtained in this manner. Attempts to culture blood lymphocytes by conventional methods were unsuccessful. The animals were both males whose names and studbook numbers (*4, 5*) are as follows: Romeo, No. 253; Bertold, No. 171. The results of chromosome studies of these two animals were identical. In No. 253 the cells had been transferred twice after trypsinization and it was therefore deemed necessary to make a detailed study of primary growth of the fibrous tissue culture. This was undertaken exclusively in No. 171 (Table 1).

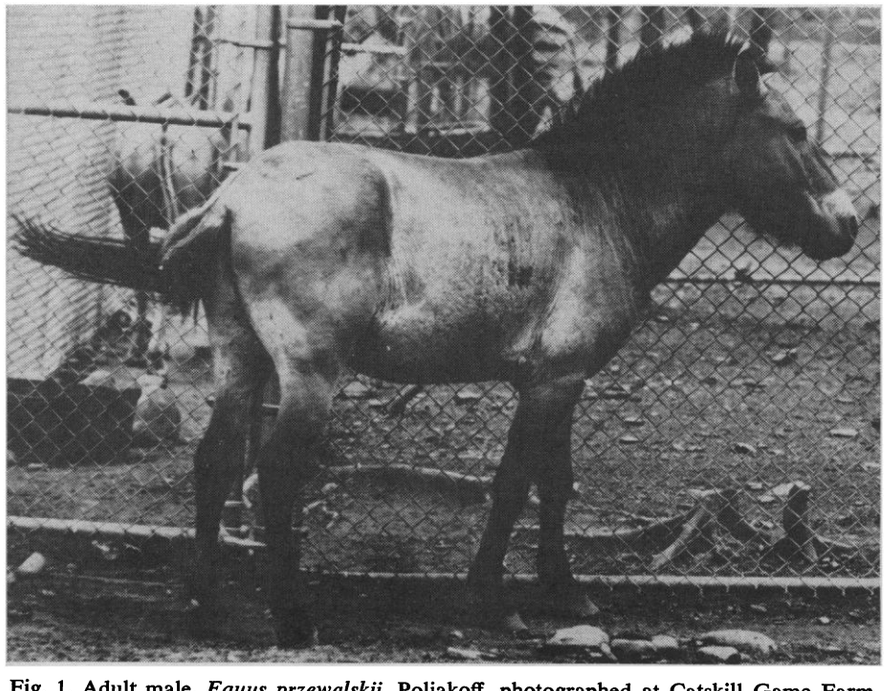

Fig. 1. Adult male, *Equus przewalskii*, Poliakoff, photographed at Catskill Game Farm, New York.

Table 1 shows that the two animals have a complement of $2n = 66$. Moreover, the karyotypes of these two specimens are identical (Fig. 1). This finding is in striking contrast to the diploid number of the domestic horse (*1*). Several studies have shown that in a variety of domesticated horses $2n = 64$; in addition, in all studies on mules and hinnies this number has been indirectly confirmed by the finding of $2n = 63$ for these hybrids; in *E. asinus*, $2n = 62$ (*1, 3*).

Enumeration of autosomal metacentric as opposed to acrocentric chromosomes also reveals differences which follow the expectations. Thus, *E. caballus* has 30 metacentric or submetacentric autosomes and 32 acrocentric autosomes (Fig. 2). *Equus przewalskii* has 26 metacentric or submetacentric autosomes and 38 acrocentric autosomes. In this study, all those elements in which a short arm is not clearly visible are counted as acrocentric (Fig. 2). The sex chromosomes are essentially identical. Some autosomes are specifically identifiable and homologies are suggested; however, the arrangement in Fig. 2 must remain arbitrary until more numerous studies are made and workers in this field can arrange an acceptable karyotype useful for all Equidae.

There has been much discussion concerning the "purity" of the presently extant Przewalski's horse (*4*), and Stecher (*7*) has presented morphologic

Table 1. Distribution of chromosome numbers in analyzed metaphases for two Przewalski's horses (karyotypes in parentheses).

Chromosome numbers				
63	64	65	66	67
		Animal No. 253		
0	1	3	30(6)	1
		Animal No. 171		
1	3	6	35(4)	1

evidence from skeletal studies of museum specimens which suggests that some of these animals may have had domestic horse blood introduced in their ancestors. Indeed, there is some concern over our inability to decide now whether the animals originally imported were not already partially hybridized with wild-running domesticated Mongolian horses. These aspects are fully discussed by Mohr (*4*). Inasmuch as these animals may proceed toward extinction, it would appear urgent to investigate the chromosome complement of the remaining specimens. Moreover, since the pedigree of most animals is not well known it may be possible to reconstruct, after such studies, whether indeed the original Przewalski's horse had the suggested

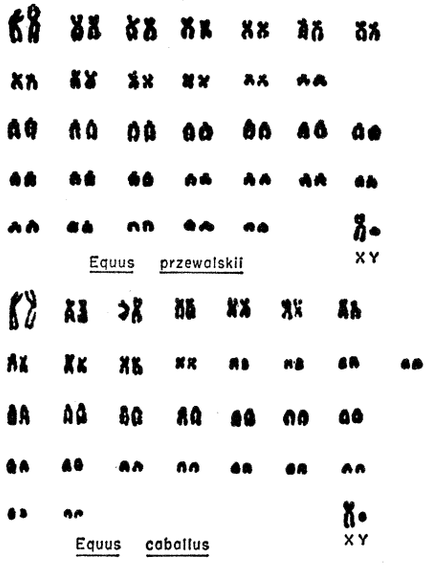

Fig. 2. (Top) Karyotype of metaphase from Romeo (No. 253). There are 66 chromosomes. The chromosomes in the first two rows are considered to be metacentric in this context although, clearly, the last pair could also be considered acrocentric. (Bottom) Karyotype of metaphase from a culture of testis cells from a domestic horse ($2n = 64$). The chromosomes in the first two rows are considered metacentric; however, the last pair may validly be construed as acrocentric.

karyotype or whether it was even more "primitive" and became what has been presented here by a process of hybridization. The pedigree of the animals described here is well substantiated (*4, 5*); they are direct descendants of the original imports and have not been hybridized with domestic horses after capture.

Another interesting aspect of our finding is that hybrids between the Przewalski's horse and various domestic horses have been reported by Iwanoff (*8*) and Lotsy (*9*); others have been referred to by Mohr (*4*). These hybrids are said to be fully fertile (*8, 9*). This is in contrast to the infertility of mules in whom meiotic pairing is impossible because of the extensive rearrangement of chromosomes of the parental species. The reported fertility of the horse hybrids suggests a much simpler type of chromosome rearrangement which is capable of allowing synapsis. Bender and Chu (*10*) have discussed the implications of such potential hybrids for primates (in which they are not known to have occurred) and have drawn attention to the desirability of studying meiotic figures in such instances. By such analysis, possible homologs could be identified, and most autosomes of these two species would have to be homologous to enable meiosis to proceed. Also, this might allow identification of those elements whose robertsonian centric fusion presumably led to the creation of new metacentric autosomes during the reduction of chromosome number in the possible evolutionary step from *Equus przewalskii* to *Equus caballus*. This mechanism of evolution in certain families has been investigated by Bender and Chu (*10*) and is also discussed by Hamerton *et al.* (*11*). In the equines discussed here it may be possible to study this process in greater detail.

Note added in proof: While this manuscript was in press we were able to obtain satisfactory preparations by short-term lymphocyte cultures on Belle (No. 163) and Marcia (No. 263). Both have a complement of $2n = 66$.

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Hybrid Antibody Molecules with Allotypically Different L-Polypeptide Chains

Abstract. *Isolated L-chains with either the A4 or the A5 allotypic markers from rabbit antibodies to 2,4-dinitrophenyl determinant recombine at random with antibody H-chains in the presence or abscence of the hapten to produce hybrid precipitating antibody molecules.*

Normal rabbit γ_2-globulin is composed of two H- and two L-polypeptide chains (*1*). The allelic allotypic markers of the *b*-locus, designated A4 and A5, are present only on the L-chains (*2*). Molecules of γ_2-globulin as they are produced in vivo carry either the A4 or the A5 allotypic marker on each L-chain in animals phenotypically heterozygous in respect to these markers, and no detectable molecules are synthesized in such animals that have one L-chain carrying the A4 marker and one L-chain carrying the A5 marker (*3*). The γ_2-globulin molecules can readily be reformed from the constituent H- and L-polypeptide chains (*4, 5*). Furthermore, when antigen is present during recombination of the polypeptide chains, specific antibody H- and L-chains preferentially reassociate, resulting in a higher percentage of functioning antibody molecules than in the absence of the antigen (*5*). Our experiments were designed to determine whether the presence of antigen during recombination of antibody H- and L-