# Characterization of the Domestic Horse (*Equus caballus*) Karyotype Using G- and C-Banding Techniques

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*Summary.* Giemsa banding techniques have been used to identify the chromosomes of the domestic horse.

Cytogenetic studies in the domestic horse (*Equus caballus*, $2n=64$) have been conducted on only a limited scale so far. In early studies on the karyotypes of normal horses[2,3], and of animals showing sex chromosome mosaicism[4–7], chromosome preparations stained only with orcein were used. Application of the G- and C-banding techniques to horse chromosomes has recently enabled us to identify a number of constitutional sex chromosome abnormalities in infertile mares[8]. As it seems likely that further cytogenetic studies on the horse and other equine species will be carried out in the future, we present here a detailed description of the karyotype of *E. caballus* based on G- and C-banding.

*Materials and methods.* Peripheral blood was obtained from 8 mares and a stallion. These samples were cultured using a modification of the whole blood microtechnique[9] and air-dried chromosome preparations made in the standard way.

Giemsa bands (G-bands) were produced by the following procedure. Chromosome preparations were incubated in $2\times$SSC (SSC = 0.15 *M* NaCl: 0.015 *M* trisodium citrate) at 60 °C for 1 h, rinsed in deionized water, dipped in a solution of 1% trypsin in deionized water for 5–30 sec at room temperature, rinsed, stained in 5% Giemsa (Gurr's R66 Giemsa in buffer, pH 6.8) for 5–8 min, rinsed and mounted in DPX (cf. GALLIMORE and RICHARDSON[10]).

Quinacrine bands (Q-bands) were obtained by the standard method employed in this laboratory[11], and C-bands by the barium hydroxide method[12].

*Results and discussion.* The normal diploid chromosome number of *E. caballus* is $2n=64$. The X chromosome is sub metacentric and the second largest chromosome in the complement. The *Y* chromosome is similar in size to the smallest autosomes. In addition there are 26 metacentric or submetacentric autosomes and 36 acrocentric autosomes[2].

*G-banding.* A standard G-banded karyotype of the domestic horse has been established from a study of 13 well-banded metaphase spreads, 12 of which were from the 8 mares. The length of each pair was measured from photographic enlargements and the chromosomes arranged, according to length, within 3 groups, meta- or submetacentrics, acrocentrics and sex chromosomes. Figures 1 and 2 show G-banded karyotypes of the stallion and mare respectively. The centromeric regions, with the exception of chromosome pair 11 and the *Y* chromosome, take up no stain, a feature which the horse karyotype shares with that of certain other species[13].

The banding patterns of many chromosome pairs are distinctive enough to enable them to be identified with reference to Figures 1 and 2, and a detailed description of these pairs was considered unnecessary. Chromosome pairs which have poor banding patterns, however, are described to aid in their identification, and pairs which have similar banding patterns have their distinguishing features described collectively.

a) *Meta- and submetacentrics.* Chromosome 5. Metacentric, the short arm having 2 positive bands, 1 near the centromere and 1 near the distal end of the arm. The long arm stains palely, and 3 vague positive bands can be discerned in clear preparations. These are spaced evenly along the arm, the central band being the broadest.

Chromosomes 6, 7 and 9. These have similar centromere indices and banding patterns. All have a broad positive band in the proximal half of the long arm, but in chromosome 6 this is half-way down the long arm, whereas in

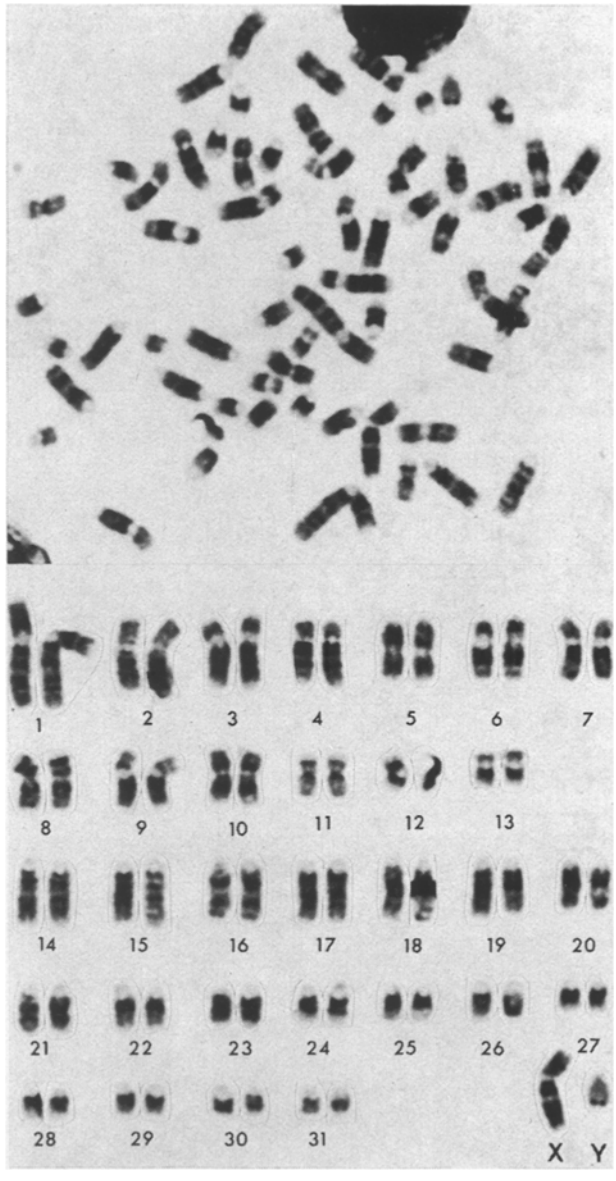


Fig. 1. G-banded metaphase cell and karyotype from a male horse ($2n$ = 64). Note the negatively stained centromeric region on most of the chromosomes.

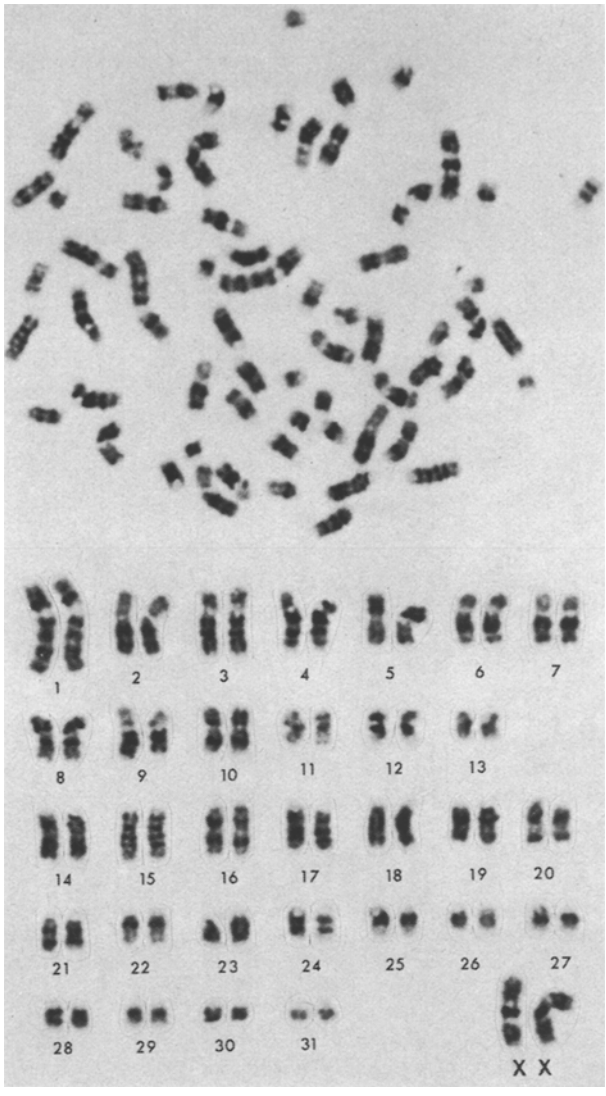


Fig. 2. G-banded metaphase cell and karyotype from a female horse ($2n = 64$).

chromosomes 7 and 9 it is only a third of the way down. These 2 pairs may be distinguished by the presence of a prominent positive band next to the centromeric region in the short arm of chromosome 7.

Chromosomes 12 and 13. These are very similar in size, but chromosome 12 is metacentric whereas 13 is sumbetacentric. Both have broad positive bands on either side of the centromere but those of chromosome 12 tend to be darker and the negatively staining centromeric region of

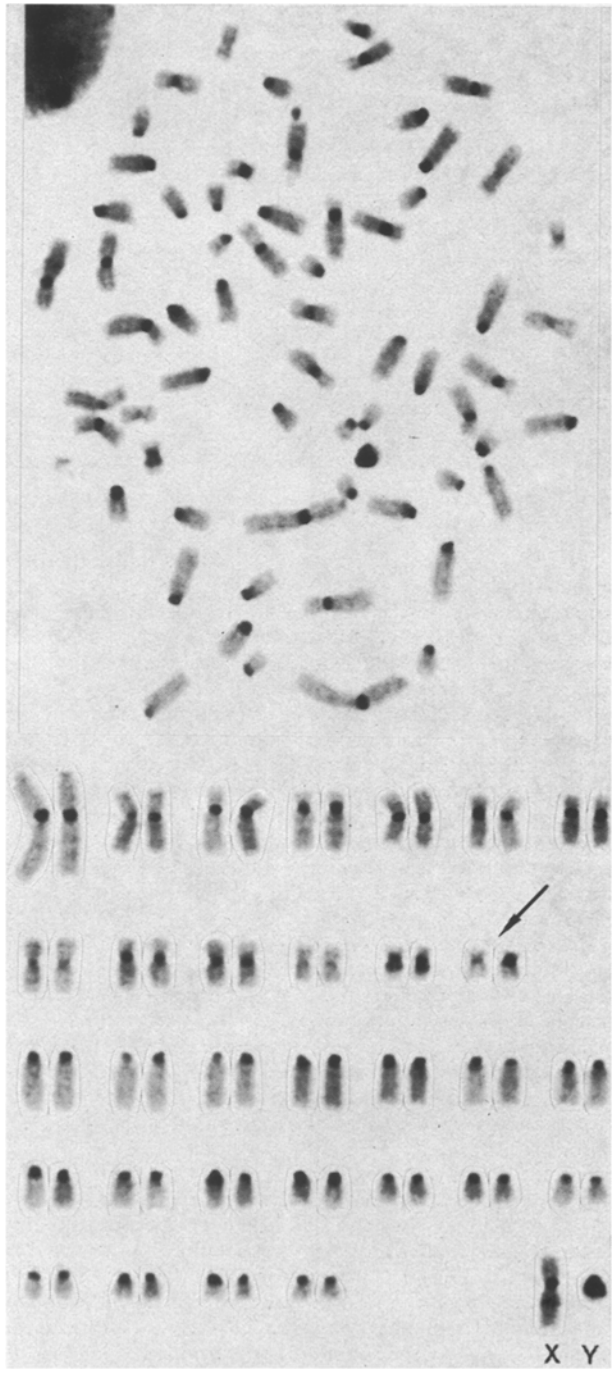


Fig. 3. Horse metaphase chromosomes and karyotype, stained with the C-band technique. All but 2 pairs contain centromeric heterochromatin. Note also that pair 13 is heteromorphic for its heterochromatin content (arrow).

[1] The authors wish to acknowledge the assistance of Mr. PETER ROSSDALE and his veterinary colleagues from Newmarket, Suffolk, for supplying us with the blood samples.

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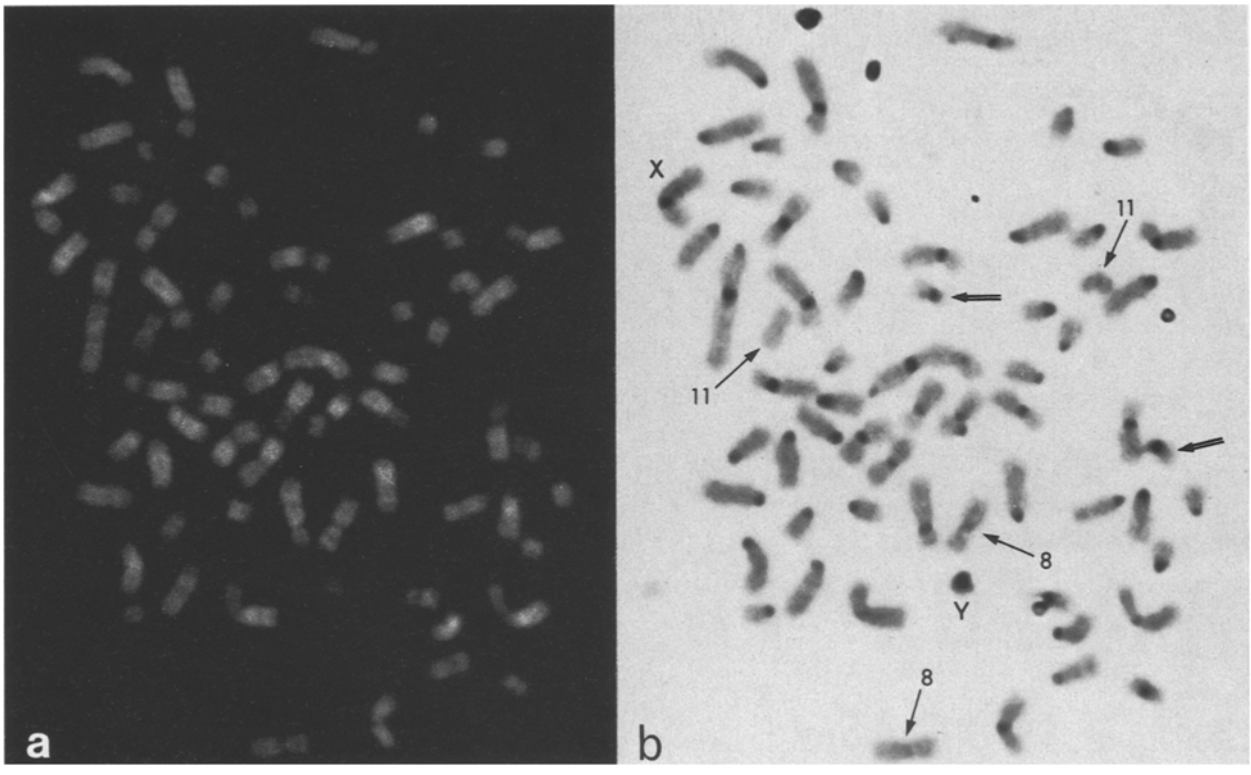


Fig. 4. Male horse cell which was Q-banded prior to C-banding to identify the pairs which lack centromeric heterochromatin. The figure shows these to be pairs 8 and 11. Note that the homologues of pair 13 (double arrows) have the same heterochromatin content in this individual.

this chromosome is larger. These chromosome pairs may be unequivocally distinguished from one another by C-banding, as chromosome 12 has an interstitial C-band on its long arm (see below and Figures 3 and 4).

b) *Acrocentric chromosomes.* Chromosomes 14 and 15. Chromosome 14 has a negative band halfway down the arm, and distal to this it stains darkly. 3 positive bands may usually be resolved in this region. Proximal to the negative band the chromosome stains palely with 3 dark, narrow bands. Chromosome 15 also has a series of narrow positive bands down its length, but these only number 5, and are evenly spaced on a uniform pale staining background.

Chromosome 17. There is a negative band a third of the way down the arm. 2 narrow positive bands may be resolved proximally to this, and 2 broad positive bands distally.

Chromosome 18. There is 1 narrow positive band immediately below the centromeric region and 2 broader ones in the middle of the chromosome. Distal to this is a negative band, which in turn is bounded by a broad positive band.

Chromosomes 19 and 20. Both have positive bands a third of the way down their arms, with broad negative bands distal to these, the tips of the chromosomes being dark. However, these features are all more pronounced in chromosome 20 and it is noticeably smaller.

Chromosome 23. This chromosome has a poor banding pattern. The distal half of the long arm stains darkly as does a narrow region round the centromere.

Chromosomes 25 and 27. These both stain dark proximally and pale distally. The dark region may be resolved into 2 bands which are both the same size in chromosome 27. The more proximal band in chromosome 25 is the same size as these, but the more distal is smaller.

Chromosome 26. This chromosome stains almost uniformly, there being suggestions of positive bands in the middle of the arm and at the distal tip of the chromosomes.

Chromosomes 28–31. Chromosome 28 has 2 positive bands, while chromosome 29 has only one, which is proximal to the centromere. Chromosome 30 has 1 distinct positive band at its distal tip, and chromosome 31 stains palely, having a narrow positive band just below the centromeric region.

c) *Sex chromosomes.* The short arm of the submetacentric *X* chromosome has 2 narrow positive bands, one at the centromeric region and the other halfway along its length. The long arm has a broad intensely stained band just below the centromeric region, distal to which is a broad negative band, which stains positively with C-banding (see below). The distal half of this arm stains moderately, 3 narrow positive bands being resolved.

The banding pattern of the acrocentric *Y* chromosome is very distinctive, the whole chromosome staining a uniform pale colour, except for the tips of the long arms which stain darkly.

*C-banding.* Most of the chromosomes in the complement show a discrete block of heterochromatin at their centromeric region when stained with the C-band technique (Figure 3), and these blocks correspond to the negatively stained areas found at the centromeres with G-banding. There are some chromosomes, however, that deviate from this simple generalization, and to identify these, Q-banding was carried out prior to the C-band procedure (Figure 4). Among the autosomes, 1 metacentric pair (No. 11) has no demonstrable C-band, and another (No. 8) has only the suggestion of one. Some chromosomes, on the other hand, have extra centromeric heterochromatin. The *Y*

chromosome, for example, consists almost entirely of constitutive heterochromatin while chromosome 12 and the *X* chromosome both possess interstitial C-bands halfway along their long arms, and chromosome 1 has a small C-band at the distal tip of the short arm. These variations in constitutive heterochromatin content provide an additional means of identifying these chromosome pairs.

It is also common to find the C-bands differ in size between homologues. This is particularly true of chromosome pair No. 13, which showed a heteromorphism in at least 4 out of the 9 animals studied (e.g. Figure 3). More work is required to determine exactly how widespread this polymorphism is, and how much other variation there might be in the horse karyotype.

## The Phylogenetic Status of Phyllomedusine Frogs (Hylidae) as Evidenced from Immunological Studies of their Serum Albumins[1]

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*Summary.* Based on immunological comparisons of the serum albumins of phyllomedusine frogs with both hyline and bufonid species, it is suggested that phyllomedusine frogs be erected to familial status within the superfamily Bufonoidea.

The Neotropical leaf frogs, Phyllomedusinae, consist of three genera; *Phyllomedusa* (31 species), *Agalychnis* (8 species), and the monotypic *Pachymedusa*. These frogs constitute a distinct phyletic line in the family Hylidae. All frogs tested in this subfamily are unique among other hylids in possessing large amounts of powerful bradykinin-like and physalaemin-like polypeptides in their skin[3]. All species in this subfamily are also characterized by having vertical pupils, diploid chromosome complements of 26, arboreality, and moderately ossified skulls (with or without the dermis co-ossified with the skull). All members deposit their eggs on vegetation over water, into which the hatchling tadpoles drop. The aquatic tadpoles are unique in having a sinistral spiracle lying ventrally on the midline[4]. Consideration of the above characteristics led DUELLMAN[5] to recognize these three genera as a separate hylid subfamily. DUELLMAN's decision has been confirmed by recent biochemical studies which have shown that at least some phyllomedusines, and no other hylids, excrete uric acid rather than ammonia and urea as do most anurans[6]. Additionally, further studies on phyletic affinities of phyllomedusine frogs have suggested these frogs may be closely related to at least some of the Australian hylids (*Litoria*). 2 of 5 species of *Litoria* tested were found to possess large fibrous melanosomes, containing a novel red pigment, which were formerly identified as unique to Neotropical leaf frogs[7].

My interest in phyllomedusine frogs arose during studies of albumin evolution in the anuran superfamily Bufonoidea[8]. These studies with albumins of both hylid and bufonid species suggested that the phyllomedusine frogs should be erected as a proper family, perhaps intermediate between the Hylidae and the Bufonidae. Indeed, earlier serological studies by CEI[9], involving precipitin tests with short term antisera to whole serum, led him to suggest the Phyllomedusinae might represent an independent phyletic branch arizing from some undifferentiated Hylid-Bufonoid stock.

*Materials and methods.* Antisera to pure albumin from a single specimen of *Phyllomedusa trinitatus*[10] was made in 4 male New Zealand white rabbits over a three-month immunization schedule. The individual antisera were tested for purity and pooled according to published procedures[11]. As sources of albumin, plasma or skeletal muscle preserved in a phenoxyethanol solution[12] were used. Microcomplement fixation studies with the albumins of representatives of all three phyllomedusine genera as well as with other hylid and bufonid species were performed. Results are reported as immunological distance units. For anuran albumins 1 unit of immunological distance between 2 species represents roughly one amino acid difference in the albumins of these 2 species[13].

*Results and discussion.* The Table summarizes the results of tests with antiserum to *P. trinitatus* albumin. All available species of *Phyllomedusa* form one immunological cluster with a range of 0–61 units. This is the order of magnitude seen between species of *Gastrotheca* (Hylidae: Amphignathodontinae), between North American *Hyla* species (Hylidae: Hylinae), and between species of *Bufo* (Bufonidae)[8]. *Pachymedusa dachnicolor* is of interest since, when first described by COPE in 1864, the species was placed in the genus *Agalychnis*. It led a spotty history of transfer from *Agalychnis* to *Phyllomedusa* until 1968 when DUELLMAN proposed its independent generic status. Immunologically *Pachymedusa* albumin appears more distant from *P. trinitatus* than any species of *Phyllomedusa* but not quite as distant as both species of *Agalychnis* available for this study. Thus at the molecular level *Pachymedusa*'s generic status is also justified. The

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