

Cytogenetics of donkey chromosomes: nomenclature proposal based on GTG-banded chromosomes and depiction of NORs and telomeric sites

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Abstract

With the expansion of comparative genome analysis across different mammals, there is an increasing need to have well-defined banded karyotypes for the species chosen for investigation. In this context, the steadily growing gene mapping data in the donkey urgently require a framework whereby alignment/comparison of genetic information can be readily made with equids and other mammalian species. Hence a GTG-banded karyotype of the donkey (*Equus asinus*; EAS) is presented, along with schematic drawings and nomenclature of the banded chromosomes. In addition, the most characteristic features of individual chromosomes are described and their relative size estimated. Using the FISH approach, the location of nucleolous organizer regions (NORs) and telomeric repeat sequences (TTAGGG) were detected. Where possible, information on asine chromosomes is supplemented with known/likely equine and human homologues. The study thus primarily aims to provide an appropriate cytogenetic basis for the donkey chromosomes, so that research focused on gene mapping and comparative genomics in this species can be reported under a common format.

Introduction

Cytogenetics of the domestic ass (*Equus asinus*; EAS), or the donkey, has not flourished the way it has in the horse. Early interest in chromosome studies in this species was primarily triggered by the desire to understand the genetic cause of infertility in interspecific hybrids between horse and donkey (Woodsdalek 1914, 1916, Benirschke *et al.* 1962). The correct chromosome number in the donkey ($2n=62$) was first reported by Benirschke *et al.* (1962) and Trujillo *et al.* (1962). Since

then, the karyotypic arrangement of donkey chromosomes has been presented in several ways (Hsu & Benirschke 1967, Eldridge & Blazak 1976, Ryder *et al.* 1978). This is attributed in part to initial disagreement between research groups on the number of meta/submetacentric versus acrocentric chromosomes and later, in part, to preference of individual groups to adhere to personal versions of chromosome arrangements.

A variety of banding techniques (e.g. GTG, RBG, CTG and Ag-NOR) have hitherto been applied to donkey chromosomes (Gustavsson

1978, Ryder *et al.* 1978, Kopp *et al.* 1983, 1986, 1988, Power 1984, Trommershausen-Bowling & Millon 1988). Although this has resulted in correct identification of the homologues, it has been of little help in resolving the disparities in chromosome numbering by different research groups. Consequently, it has also been difficult to correlate even the NOR-bearing chromosomes in different published karyotypes.

The past few years have shown a prolific increase in studies related to comparative organization of closely and distantly related genomes. In this context, recent comparative studies between horse, donkey and humans are worth mentioning (Raudsepp *et al.* 1997, Raudsepp & Chowdhary 1999, Raudsepp *et al.* 1999). In order to systematically expand this work to other equids/Perissodactyls, it is essential to have a well-defined banded karyotype in the donkey, with appropriate nomenclature for individual chromosomes. In the current study, we focus on this aspect and provide relative length, GTG-banded karyotype and salient identification features of individual donkey chromosomes. A basic nomenclature system, which is essential to define cross-species homology, is proposed through schematic drawings of GTG-banded chromosomes. In addition, we use molecular approaches to study the telomeres, and assign the NORs to specific chromosomes.

Materials and methods

Chromosome preparations, G-banding, microscopy and karyotyping

Metaphase chromosome preparations from one male and two female donkeys were obtained using Pokeweed (GibcoBRL) stimulated peripheral blood lymphocyte cultures. The slides were routinely stained with Giemsa (Sigma). Twenty well-spread metaphase spreads at different stages of chromosome condensation were photographed on an AGFA APX25 film under a Leitz Wetzlar transmitted light microscope with the magnification $100 \times 1.25 \times 10$. Following this, the chromosomes were destained and G-banded using trypsin (Seabright 1972). Karyotypes of four rep-

resentative metaphase spreads showing different stages of condensation (prometaphase to metaphase) were prepared.

Chromosome measurements and schematic drawings

Prior to G-banding, images from the four selected metaphases were used for obtaining chromosome measurements. Individual chromosomes were electronically measured using AutoCad (Autodesk AB) software. Measurements on each cell were repeated twice to ensure accuracy. The data were analysed using the SAS software, which provided mean relative lengths and centromeric indices. Schematic drawings of individual donkey chromosomes followed these mean values and the GTG-banding patterns.

FISH for localizing NORs and telomeric TTAGGG repeats

One μg of mink ribosomal DNA probe (Christensen *et al.* 1996) was nick translated and labelled with bio-14-dATP (Life Technologies) according to manufacturers' instruction. The whole labelled product was vacuum dried together with 10 μg mink genomic DNA and dissolved in 20 μl hybridization mix (50% formamide, $2 \times \text{SSC}$, 10% dextran sulphate). After denaturation the probe was preannealed for 10 min and hybridized to donkey chromosomes. Signal detection was carried out as described earlier (see Raudsepp & Chowdhary 1999). Location of telomeric sequences in the asine genome was detected using Telomere PNA FISH Kit/Cy3 (DAKO) following the manufacturer's protocol.

Microscopy and image analysis of FISH

Hybridization signals were examined under a Leica DMR fluorescent microscope. Images were captured through a Hamamatsu C472-95 digital camera and processed using the ISIS 3 software (MetaSystems). A total of 41 metaphase spreads were analysed to detect the location and number of NORs. The location of telomeric sequences was studied in 30 metaphase spreads from one male and two female individuals.

Results and discussion

Donkey GTG-banded karyotype and ideogram

The GTG-banded karyotype of a male donkey is presented in Figure 1. Autosomes are arranged into two groups as presented earlier (Ryder *et al.* 1978): nineteen pairs of meta- and submetacentrics and eleven pairs of acrocentrics. The sex chromosomes are placed beside the smallest meta- and submetacentric autosomes. Chromosome order and numbering corresponds exactly to the recently published gene mapping and ZOO-FISH data for the donkey (Raudsepp & Chowdhary 1999, Raudsepp *et al.* 1999), and is in close alignment with the relative length estimates for each of the chromosomes (see below). The karyotype, however, differs slightly from that proposed by Ryder *et al.* (1978).

A haploid set of GTG-banded chromosomes from three cells at slightly different levels of condensation are presented in Figure 2. This is expected to facilitate chromosome identification from different cells. Schematic drawings and band numbering follow the principles used in the International system for cytogenetic nomenclature of the domestic horse (ISCNH 1997). Keeping in mind that the nomenclature of donkey chromosomes is primarily needed for chromosome identification and comparative mapping, the number of GTG bands (350) corresponds to medium resolution. Brief description of the most characteristic features of each chromosome arm is provided to assist in chromosome identification.

For the first time, donkey chromosomes are provided with measurements of relative lengths (Figure 2) and centromeric indices which are useful for correlating chromosome lengths in the schematic drawings and also provide a basis for comparison with other closely related species (the equids). The latter might be of interest while comparing the size of homologous chromosomal segments across species with donkey. For example, the donkey X chromosome forms 4.67% of the haploid genome which is close to the estimated 5% for the horse (Stranzinger 1980), as well as several other eutherian mammals (Ohno *et al.* 1964).

Where possible, schematic drawings of donkey chromosomes are supplemented with hitherto

known homologous chromosome(s)/segment(s) from the horse and human karyotypes. These data are based on the available human–horse, horse–donkey and human–donkey ZOO-FISH and comparative gene mapping results (Raudsepp *et al.* 1996, 1997, 1999, Raudsepp & Chowdhary 1999, Raudsepp *et al.* in preparation). Supplementation of the GTG-banded karyotype and the schematic drawings with gene mapping and comparative information will provide a stronger molecular basis for chromosome identification than that available earlier. Based on this consolidation, it will be easier for groups working with equids/Perissodactyls to develop a consensus with regards to chromosome numbering in the donkey.

Nucleolus organizer regions (NORs)

The ribosomal DNA (rDNA) gene clusters were mapped to secondary constriction sites on 8 pairs of acrocentric chromosomes identified as EAS20, 21, 22, 24, 25, 26, 27 and 28 (Figure 2). The aim to use FISH technique for the localization of rDNA in the donkey genome was to investigate whether or not there are extra NOR sites, which, due to being transcriptionally inactive, could not be detected by earlier silver-staining studies (Kopp *et al.* 1988). For example, FISH with rDNA probe in the horse has enabled the detection of an additional NOR-bearing chromosome (ECA27) which did not show any site with the silver staining technique (Deryusheva *et al.* 1997). However, our results did not reveal any additional site. Therefore, the number of NOR-bearing chromosomes remains the same as reported by Kopp *et al.* (1988).

Estimated over 41 metaphase spreads, the number of NORs averaged around 13.5 per cell. Because the FISH approach is expected to detect all rDNA sites, irrespective of their transcriptional activity during the previous interphase, this variation between cells can be attributed to different hybridization/detection efficiency across the slides. Variation was observed also in the signal intensity and frequency between individual chromosome pairs (see Figures 2 & 3). EAS20 and EAS25 tended to show weak FISH signals while EAS21 and EAS24 gave extremely strong signals on one or both homologues in the majority

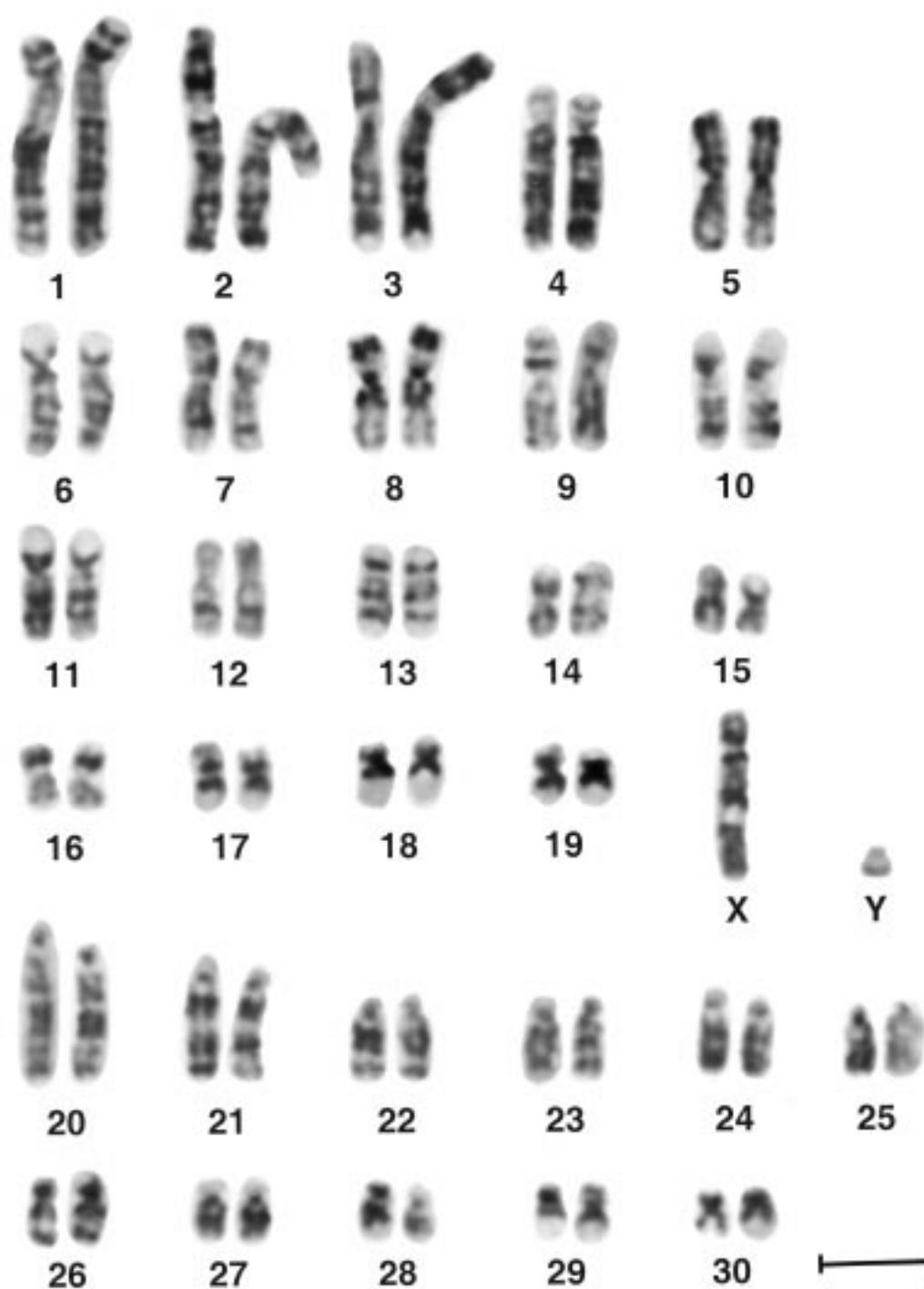


Figure 1. A representative GTG-banded karyotype of a male donkey (*Equus asinus*). The chromosomes are arranged according to their relative lengths and the recently published horse–donkey ZOO-FISH data (Raudsepp *et al.* 1999). Bar: 10 μm.

of cells analysed. It is likely that this polymorphism reflects variation in rDNA copy number on different chromosome pairs but can also be attributed to the specific NOR pattern of the animals studied. It is well known that chromosomal distributions of rDNA, such as the number of chromosomal loci and the number of genes at each locus, vary among populations, individuals and cell types in several eukaryotes (Kurihara *et al.* 1994, Delany & Krupkin 1999, Gallagher *et al.* 1999) including the donkey (Kopp *et al.* 1986, 1988). However, as the aim of the present study was to tag the rDNA sites to specific donkey chromosomes, no expanded polymorphism studies involving more individuals were carried out.

Among the asine NOR-bearing chromosomes, EAS27 is the only one for which an equine homologue was hitherto not known (see Figure 2). For others, it is interesting to note that none of the donkey rDNA-carrying chromosomes correspond to those bearing NORs in the horse. NOR studies within other mammalian families, like primates and bovids, indicate that, in some cases, rDNA is located on homologous chromosomes while, in others, they may be located on chromosomes with no correspondence (Dutrillaux 1979, Di Meo *et al.* 1993, Gallagher *et al.* 1999). Contemporary studies within equids/Perissodactyls have yet to be extended to all member species. At least initial indications between horse and donkey point to no correlation – either in number or in location – on chromosomes showing correspondence.

Telomeric (TTAGGG)_n sequences

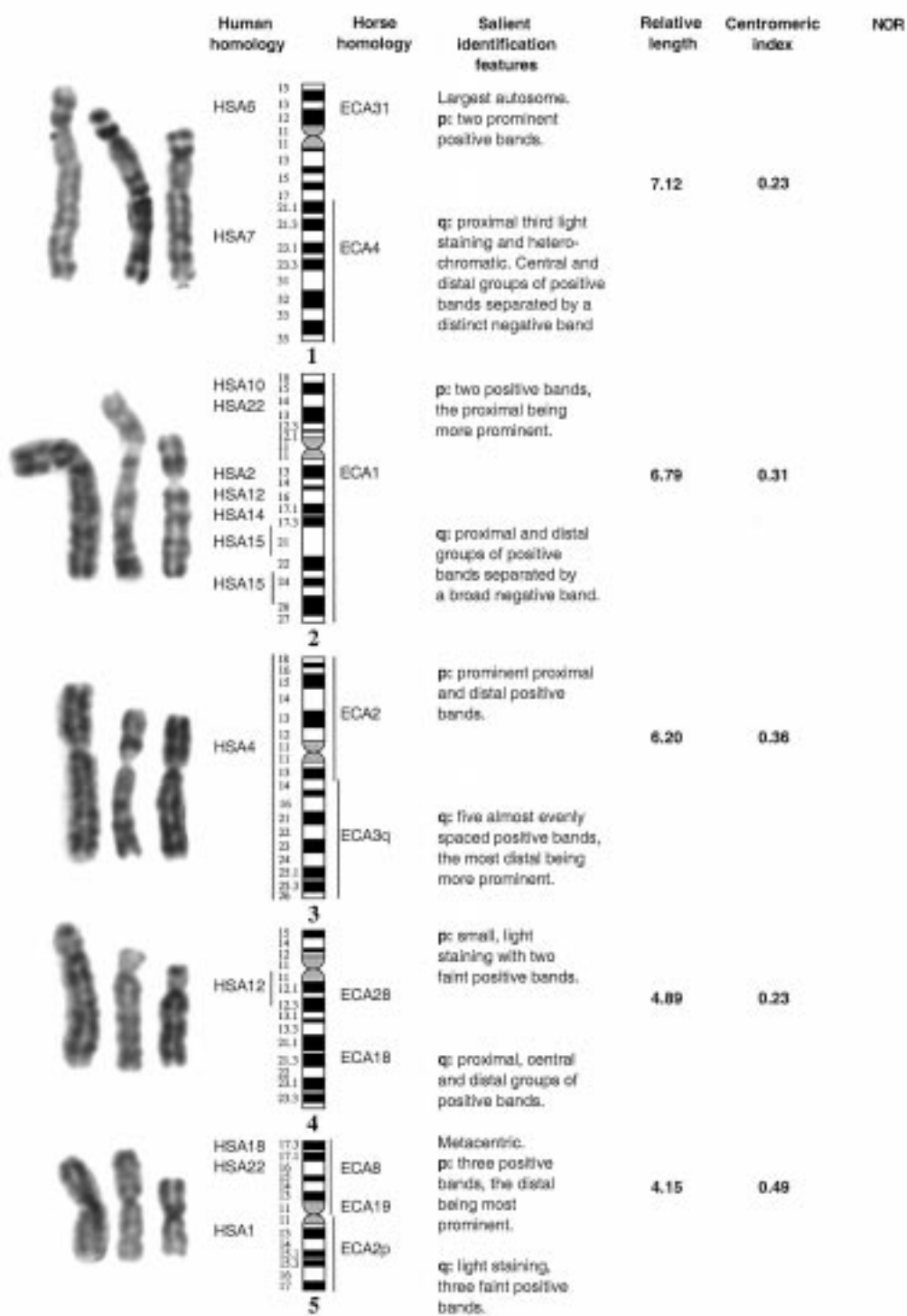
In-situ hybridization with a sensitive telomere-specific synthetic DNA/RNA analogue where the sugar phosphate backbone has been replaced by a neutral peptide/polyamide backbone and

which binds to DNA/RNA in a sequence-specific manner produced distinct FISH signals on both ends of all asine chromosomes (Figure 4). The fact that all acrocentric autosomes and the Y chromosome show signals on both chromosome ends, suggests the presence of a small short arm beyond the centromeric DNA in these chromosomes and is in agreement with findings in other vertebrate species (Meyne *et al.* 1990, Reimann *et al.* 1994, de la Seña *et al.* 1995).

The terminal parts of EAS6p and EAS30q showed at least 8–10 times stronger signals as compared with other chromosomes. This observation was consistent in all cells and animals studied. Similar differences in other species have previously been attributed to the presence of a larger number of telomeric repeats in these chromosome ends (Biesmann & Mason 1992). In some cases, the pattern is also considered individual specific, as, for example, in cattle (de la Seña *et al.* 1995). However, in the present study, we saw the same pattern in all the cells of the three individuals examined. It could not be ascertained whether these individuals were related. Hence, more data is needed to ascertain whether the sites on EAS6p and EAS30q are species specific or are subject to polymorphism.

Analysis of more than thirty cells in three individuals showed that, as in the horse (de la Seña *et al.* 1995), no interstitial telomeric sites were detected in the donkey genome. Against the background of suggested rapid karyotype evolution and proposed extensive chromosomal rearrangements within equids (Ryder *et al.* 1978, Power 1984, Wichman *et al.* 1991), this observation is of special interest. The available ZOO-FISH and comparative gene mapping data between the donkey and the horse (Raudsepp & Chowdhary 1999, Raudsepp *et al.* in preparation) indicate that a number of fusion, fission and inversion events have taken place during the

Figure 2 (overleaf). Haploid sets of GTG-banded chromosomes from three cells at different stages of chromosome condensation along with their diagrammatic representation. The chromosomes are arranged such that each column contains chromosomes from the same set. The schematic drawings are provided with band nomenclature, salient identification features, relative length and centromeric index. Protruding structures shown at the centromeres of some of the acrocentric chromosomes represent nucleolus organizer regions (NOR). Intensity and frequency of signal for the NOR probe on each of the chromosomes is indicated in the last column. * denotes extraordinary strong telomeric signals on EAS6p and EAS30q. In order to facilitate chromosome identification and provide comparative information confirmed (bars) and likely human and equine homologues are presented beside the ideograms (see text for details).



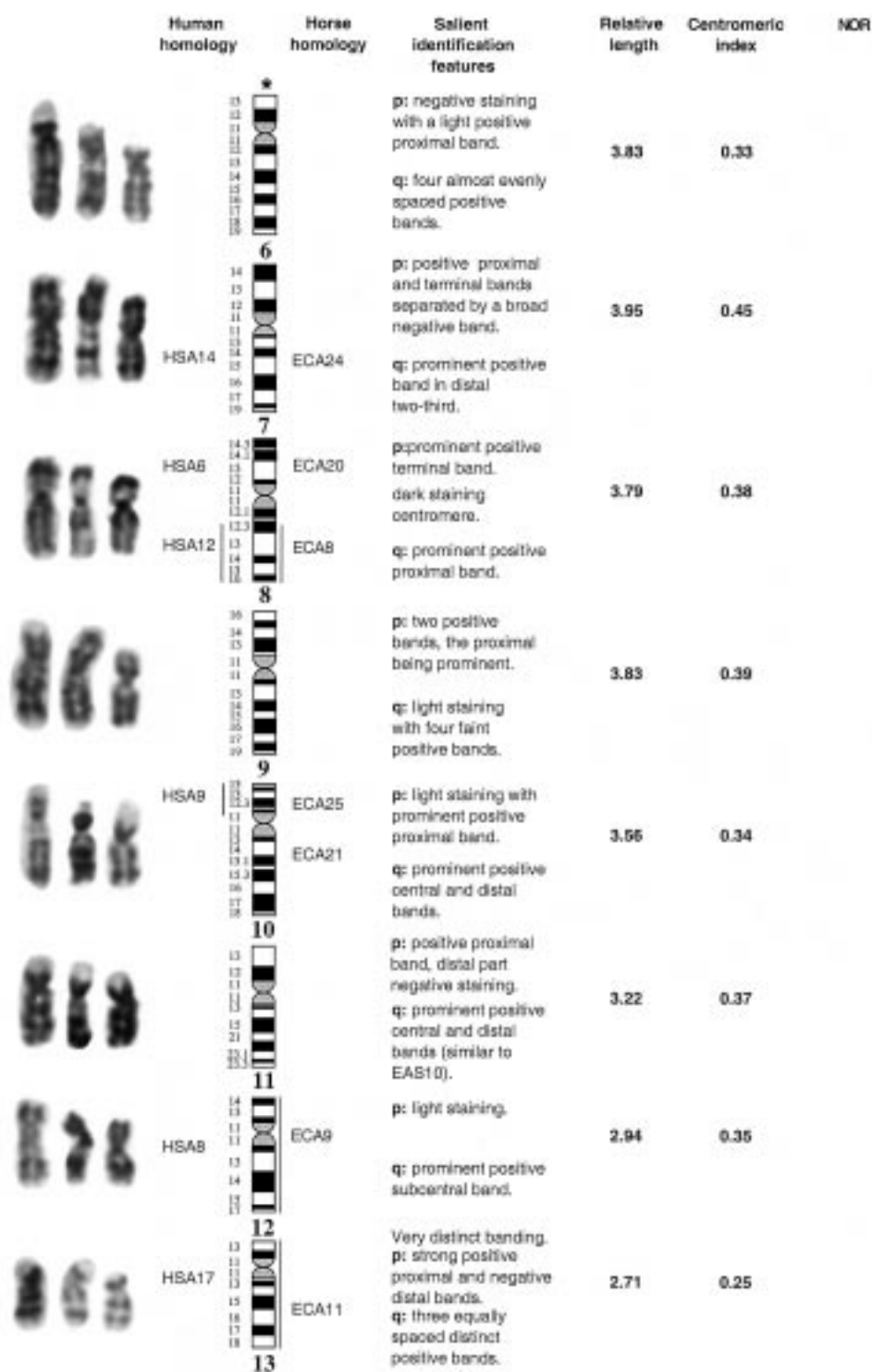









Figure 2 (part 2)

	Human homology	Horse homology	Salient identification features	Relative length	Centromeric index	NOR
	HSA16p 13 11 11 14 15	ECA13 14	p: light staining with positive proximal band q: light staining with broad proximal and small terminal positive bands.	2.06	0.41	
	13 11 11 12 15	ECA15 15	Banding not clear. p: pericentromeric positive band. q: almost the whole arm dark staining.	1.95	0.50	
	HSA1 11 11 13 14 15	ECA5 16	p: dark staining. q: light staining with a small positive terminal band.	1.98	0.43	
	HSA11 14 17 11 12 13	ECA12 17	p: prominent dark pericentromeric band. q: broad positive proximal band, distal half negative staining.	1.98	0.46	
	13 12 11 12 15	ECA18 18	p: dark staining with light terminal band. q: dark pericentromeric band, distal two thirds negative staining.	1.93	0.34	
	HSA2 13 12 13	ECA8p 19	p: dark staining. q: proximal half dark, distal half light staining.	1.57	0.43	
	HSA11 HSA19 13 12 13 14 15 16 18 20.1 20.2 20.3 20.4 20.5 20.6 20.7 20.8 20.9 21.1 21.2 21.3 21.4 21.5 21.6 21.7 21.8 21.9 22.1 22.2 22.3 22.4 22.5 22.6 22.7 22.8 22.9 23.1 23.2 23.3 23.4 23.5 23.6 23.7 23.8 23.9 24.1 24.2 24.3 24.4 24.5 24.6 24.7 24.8 24.9 25.1 25.2 25.3 25.4 25.5 25.6 25.7 25.8 25.9 26.1 26.2 26.3 26.4 26.5 26.6 26.7 26.8 26.9 27.1 27.2 27.3 27.4 27.5 27.6 27.7 27.8 27.9 28.1 28.2 28.3 28.4 28.5 28.6 28.7 28.8 28.9 29.1 29.2 29.3 29.4 29.5 29.6 29.7 29.8 29.9 30.1 30.2 30.3 30.4 30.5 30.6 30.7 30.8 30.9 31.1 31.2 31.3 31.4 31.5 31.6 31.7 31.8 31.9 32.1 32.2 32.3 32.4 32.5 32.6 32.7 32.8 32.9 33.1 33.2 33.3 33.4 33.5 33.6 33.7 33.8 33.9 34.1 34.2 34.3 34.4 34.5 34.6 34.7 34.8 34.9 35.1 35.2 35.3 35.4 35.5 35.6 35.7 35.8 35.9 36.1 36.2 36.3 36.4 36.5 36.6 36.7 36.8 36.9 37.1 37.2 37.3 37.4 37.5 37.6 37.7 37.8 37.9 38.1 38.2 38.3 38.4 38.5 38.6 38.7 38.8 38.9 39.1 39.2 39.3 39.4 39.5 39.6 39.7 39.8 39.9 40.1 40.2 40.3 40.4 40.5 40.6 40.7 40.8 40.9 41.1 41.2 41.3 41.4 41.5 41.6 41.7 41.8 41.9 42.1 42.2 42.3 42.4 42.5 42.6 42.7 42.8 42.9 43.1 43.2 43.3 43.4 43.5 43.6 43.7 43.8 43.9 44.1 44.2 44.3 44.4 44.5 44.6 44.7 44.8 44.9 45.1 45.2 45.3 45.4 45.5 45.6 45.7 45.8 45.9 46.1 46.2 46.3 46.4 46.5 46.6 46.7 46.8 46.9 47.1 47.2 47.3 47.4 47.5 47.6 47.7 47.8 47.9 48.1 48.2 48.3 48.4 48.5 48.6 48.7 48.8 48.9 49.1 49.2 49.3 49.4 49.5 49.6 49.7 49.8 49.9 50.1 50.2 50.3 50.4 50.5 50.6 50.7 50.8 50.9 51.1 51.2 51.3 51.4 51.5 51.6 51.7 51.8 51.9 52.1 52.2 52.3 52.4 52.5 52.6 52.7 52.8 52.9 53.1 53.2 53.3 53.4 53.5 53.6 53.7 53.8 53.9 54.1 54.2 54.3 54.4 54.5 54.6 54.7 54.8 54.9 55.1 55.2 55.3 55.4 55.5 55.6 55.7 55.8 55.9 56.1 56.2 56.3 56.4 56.5 56.6 56.7 56.8 56.9 57.1 57.2 57.3 57.4 57.5 57.6 57.7 57.8 57.9 58.1 58.2 58.3 58.4 58.5 58.6 58.7 58.8 58.9 59.1 59.2 59.3 59.4 59.5 59.6 59.7 59.8 59.9 60.1 60.2 60.3 60.4 60.5 60.6 60.7 60.8 60.9 61.1 61.2 61.3 61.4 61.5 61.6 61.7 61.8 61.9 62.1 62.2 62.3 62.4 62.5 62.6 62.7 62.8 62.9 63.1 63.2 63.3 63.4 63.5 63.6 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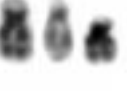













	Human homology	Horse homology	Salient identification features	Relative length	Centromeric index	NOR
	HSA9	 23	q: three equally spaced positive bands - proximal, central and distal.	2.63		
	HSA6	 24	q: positive proximal and distal bands, subcentral negative band.	2.48		strong hyb signal in 100% of the cells, with 83% showing on both homologues.
	HSA1	 25	q: banding not distinct, three faint positive bands.	2.29		weak hyb signal in 54% of the cells, with 15% showing on both homologues.
	HSA10q	 26	q: prominent dark proximal and small terminal bands separated by a distinct negative band.	2.17		strong hyb signal in 100% of the cells, with 70% showing on both homologues.
		 27	q: prominent dark central band.	1.94		strong hyb signal in 100% of the cells, with 73% showing on both homologues.
	HSA16q	 28	q: dark staining with negative pericentromeric and terminal bands.	1.81		strong hyb signal in 100% of the cells, with 80% showing on both homologues.
		 29	q: proximal half dark, distal half light staining.	1.67		
		 30	Smallest autosome. q: dark staining, without distinct banding pattern.	1.45		
	HSAX	 X	p: prominent dark proximal band, q: three dark proximal bands, prominent negative central area, distal part faint with no clear bands.	4.67	0.21	
		 Y	Smallest chromosome. q: light staining with dark terminal band.	1.32		

Figure 2 (part 4)

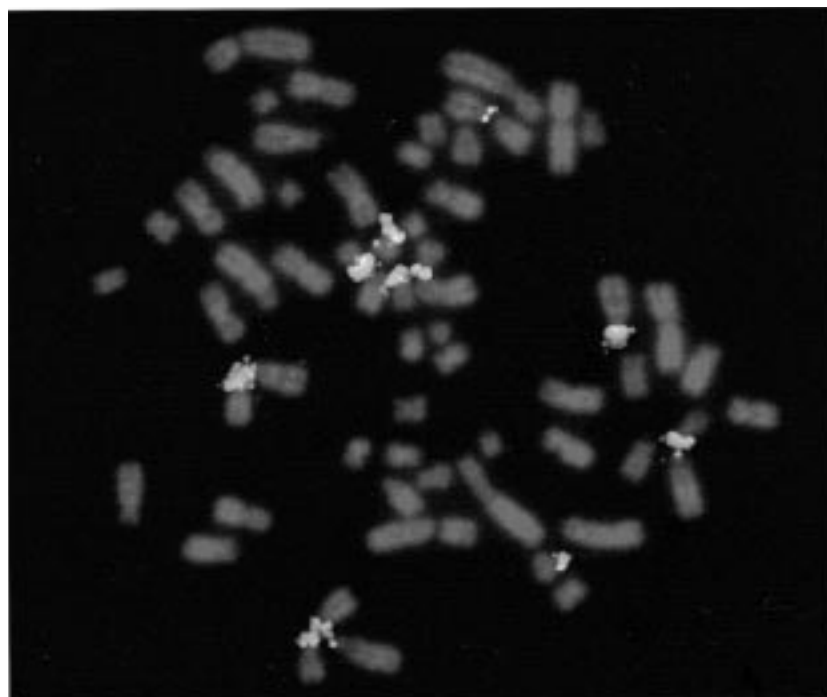


Figure 3. A donkey metaphase spread after hybridization with mink rDNA probe. Distinct signals are seen on 15 acrocentric chromosomes. Several nucleolar associations are evident and variation of rDNA signal intensity between different chromosomes is visible.

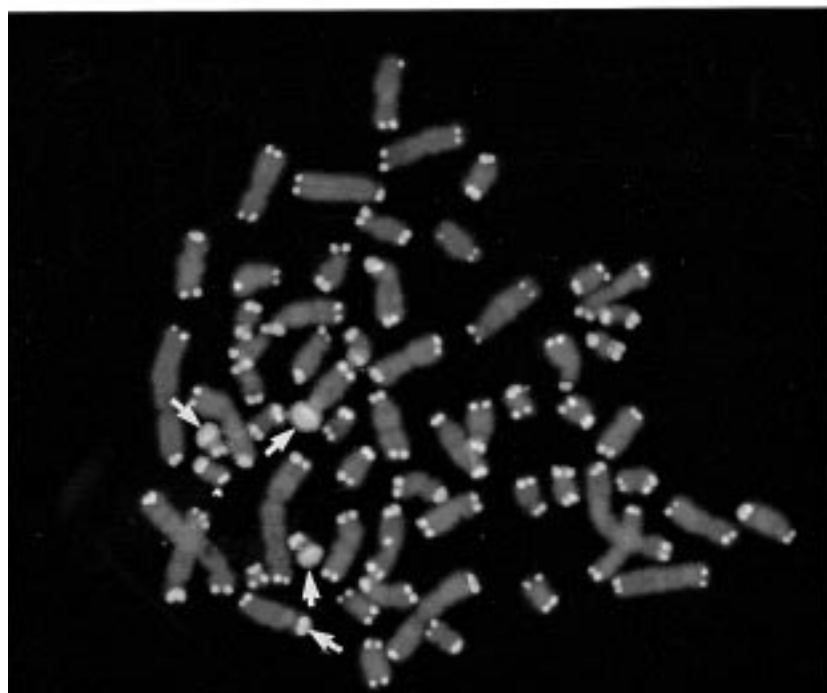


Figure 4. A donkey metaphase spread after hybridization with telomeric (TTAGGG)_n repeat probe. Typical FISH signals are seen at the end of all chromosomes/arms. Arrows indicate exceptionally strong hybridization signals on EAS6p and EAS30q.

evolution of the two karyotypes. The absence of intercalary or pericentromeric telomere signals in the two species leads us to assume that either the telomeric repeats at these sites have been lost or they have decreased to a number that is not detectable by *in-situ* hybridization (see Schubert *et al.* 1992).

Concluding remarks

The information presented herein is expected to be of common use to groups interested in carrying out cytogenetic and gene mapping studies in the donkey. The suggested karyotype and ideogram, fully supported by the available ZOO-FISH and gene mapping data, will be useful for groups involved with comparative genomics and karyotype evolution in equids and other Perissodactyls to adopt a common donkey chromosome nomenclature, which in turn will facilitate putting all the data into a single framework. Such an exercise is essential before new data cause discrepancies. In due course, the donkey karyotype can be further strengthened with chromosome arm-specific probes from the horse.

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