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Zoo-FISH delineates conserved chromosomal segments in horse and man

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Human chromosome specific libraries (CSLs) were individually applied to equine metaphase chromosomes using the fluorescence *in situ* hybridization (FISH) technique. All CSLs, except Y, showed painting signals on one or several horse chromosomes. In total 43 conserved chromosomal segments were painted. Homoeology could not, however, be detected for some segments of the equine genome. This is most likely related to the very weak signals displayed by some libraries, rather than to the absence of similarity with the human genome. In spite of divergence from the human genome, dated 70–80 million years ago, a fairly high degree of synteny conservation was observed. In seven cases, whole chromosome synteny was detected between the two species. The comparative painting results agreed completely with the limited gene mapping data available in horses, and also enabled us provisionally to assign one linkage group (U2) and one syntenic group (NP, MPI, IDH2) to specific equine chromosomes. Chromosomal assignments of three other syntenic groups are also proposed. The findings of this study will be of significant use in the expansion of the hitherto poorly developed equine gene map.

Key words: chromosome painting, comparative mapping, horse, human, zoo-FISH

Introduction

During the past few years, research in basic domestic animal genetics has centred around developing a better understanding of genomes of various farm animal species. The work is directed primarily at comprehending (i) inheritance and expression of genetic diseases and (ii) variation in economically important traits. The information is intended to be used for a more accurate DNA-based diagnostics approach for inherited disorders, and developing systems for marker-assisted selection for economically important traits. Noteworthy progress has been made in pigs and cattle, and also to some extent in chicken and sheep, where a 30- to 40-fold increase in the number of loci mapped has contributed to significant initial breakthroughs. Important issues such as mapping of inherited disease traits (e.g. the weaver disease in cattle, Georges *et al.* 1993; and malignant hyperthermia in pigs, MacLennan *et al.* 1990) and quantitative trait loci (e.g. fat locus in pigs, Andersson *et al.* 1994) have been successfully addressed during recent years.

Unlike pigs, cattle, sheep and chickens, genome analysis in horses (*Equus caballus*) is not directly needed for the study of production traits. Instead, it is mainly required for the study of the underlying genetics of the numerous inherited disorders, for which no clear diagnostic tools are at hand. Plenty of documented records on genetic disorders in horses are available (Bowling 1992), but little is known at the DNA level about these conditions. To enable analysis of genetic disorders in horses, a basic knowledge of the equine genome, defining its general organization, is essential. Hence, a skeletal physical and genetic linkage map that can act as a platform for future research and expansion has to be developed. A cursory analysis reveals that, in spite of the impetus gained during the past 3 years, the horse gene map is still in an early stage of development. Organized efforts are therefore needed for the expansion of the equine gene map.

Comparative genome analysis has emerged as an important tool, not only in developing a better understanding of genome organization and evolution between different mammalian species, but also in revealing the most conserved segments of their genomes (Edwards 1994, Rettenberger *et al.* 1995a,b, Chowdhary *et al.* 1996). This further enables a rapid expansion of gene maps in 'map-poor' species, e.g. horse, with the help of data available from 'map-rich' species, e.g. humans and mouse. Of the various strategies employed to develop comparative maps, the recently developed cross-species chromosome painting – also referred to as zoo-FISH – provides direct information on similarities between chromosomal segments (see Scherthan *et al.* 1994, Hayes 1995, Rettenberger *et*

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al. 1995a,b, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996, Frönicke *et al.* 1996). In the present study, we use this technique to find conserved segments in the human and equine karyotypes. The results are coupled with available gene mapping information in horses to provide an updated comparative map between the two species. The information will be of specific significance in expanding the hitherto poorly developed equine gene map.

Materials and methods

Chromosome preparation

Horse (*Equus caballus*) chromosome spreads were obtained from pokeweed (Seromed) stimulated peripheral blood lymphocytes according to standard techniques. The preparations were stored at −20°C until use.

DNA probes and labelling

DNA was prepared from human chromosome specific plasmid libraries (CSLs) (Collins *et al.* 1991) and PCR libraries (Vooijs *et al.* 1993) generously provided by J. Gray, Department of Molecular Cytometry, University of California, San Francisco, CA, USA. The probes were nick translated with biotin-14-dATP (Life Technologies) and/or digoxigenin-11-dUTP (Boehringer Mannheim) according to a standard protocol (BioNick Labeling System). For human chromosome 11, a commercial probe (Oncor, Coatasome 11) was used.

In situ *hybridization, washing and signal detection*

Zoo-FISH was performed primarily as described elsewhere (Scherthan *et al.* 1994, Frönicke *et al.* 1996), but with some modifications. Briefly, in cases in which the hybridization signal was undetectable with the standard protocol (CSL 11; CSL 21; CSL 22), prolonged hybridization time (6–7 days) was used. In the post-hybridization washing, the preparations hybridized with biotin-labelled CSLs were blocked in 5% bovine serum albumin (BSA), in order to reduce background. For each human CSL, at least 15–20 horse metaphase spreads were analysed and the results were confirmed through at least two hybridization experiments. The signals were classified as strong, medium or weak according to visual estimation.

Microscopy and chromosome identification

A Zeiss Axioskop fluorescence microscope equipped with single bandpass filters (Chroma Technology), was used to visualize FITC, rhodamine and DAPI fluorescence. Images were captured and processed using the ISee software (Inovision Corporation, Durham, NC, USA), which controlled a cooled CCD camera (Photometrics PXL, KAF 1400) mounted in a TV-port of a Zeiss Axiovert 135M/TV inverted microscope. The microscope was equipped with a filter wheel with excitation filters, multibandpass mirror and emission filter (Chroma Technology, Brattleboro, VT, USA) to avoid image shift from filter changes. Equine chromosomes were identified by DAPI staining, which produced a G-band like pattern. After capturing images, GTG banding was performed on the same preparations (Chowdhary *et al.* 1996). For chromosome identification the standard karyotype of the domestic horse (Richer *et al.* 1990) was used. Schematic drawings of G-banded horse chromosomes were used for demonstrating segments of equine chromosomes painted by the human CSLs.

Results and discussion

The 24 human CSLs (representing 22 autosomes and the sex chromosomes) used on horse metaphase chromosomes successfully detected conserved chromosomal segments between the two species. Except for the Y-CSL, all other CSLs painted one or several such segments on the horse (*Equus caballus*, ECA) chromosomes. The human Y-CSL did not cross-hybridize with any equine chromosome. Centromeres of equine chromosomes in general did not show cross-hybridization(s). An overlay of the results on an ideogram of G-banded horse chromosomes (Figure 1) shows that a high degree of conservation could be detected for a majority of the chromosomes. However, some autosomes, namely ECA6p, 12, 13p, 27 and 31 could not be painted with any of the CSLs used. For the latter three chromosomes it is also possible that the hybridization signals were very weak, and therefore went unnoticed. A summary of equine chromosomal segments painted by individual human CSLs along with the intensity of signal (strong, medium or weak) observed on each painted segment is presented in Table 1. Some representative partial metaphase spreads showing paints on equine chromosomes are presented in Figure 2. The identification of all equine chromosomes, except ECA28 and 29 was unequivocal. The latter were difficult to identify both through DAPI banding and post-hybridization G-banding. Hence, the suggested homoeologies to these equine chromosomes are tentative until confirmed through other methods or better visible banding patterns.

The human chromosome specific libraries painted a total of 43 conserved chromosomal segments on the horse karyotype. A matrix indicating segmental homoeology between human and horse chromosomes (Figure 3) shows that eight human chromosomes painted only one segment each in horses, whereas another 11 painted two segments each. Further, there were three human chromosomes that showed homoeology to three separate horse chromosomes; HSA12 was the only human chromosome that painted four separate blocks. Conversely, except for ECA1 and 8, which corresponded to six and three different human chromosomes respectively, a majority (22) of equine chromosomes were painted by only one human CSL each. The remaining five equine chromosomes showed correspondence to two different human chromosomes each.

Most of the intrachromosomal rearrangements are considered/assumed to be species specific (Rettenberger *et al.* 1995b). Interchromosomal exchanges/reshuffles in the ancestral mammalian genome, however, are a part of karyotype evolution in different species (Ohno *et al.* 1964, Lundin 1993). The latter phenomenon accounts for variability in chromosome number, shape and size. Painted chromosomal segments of one species by CSLs

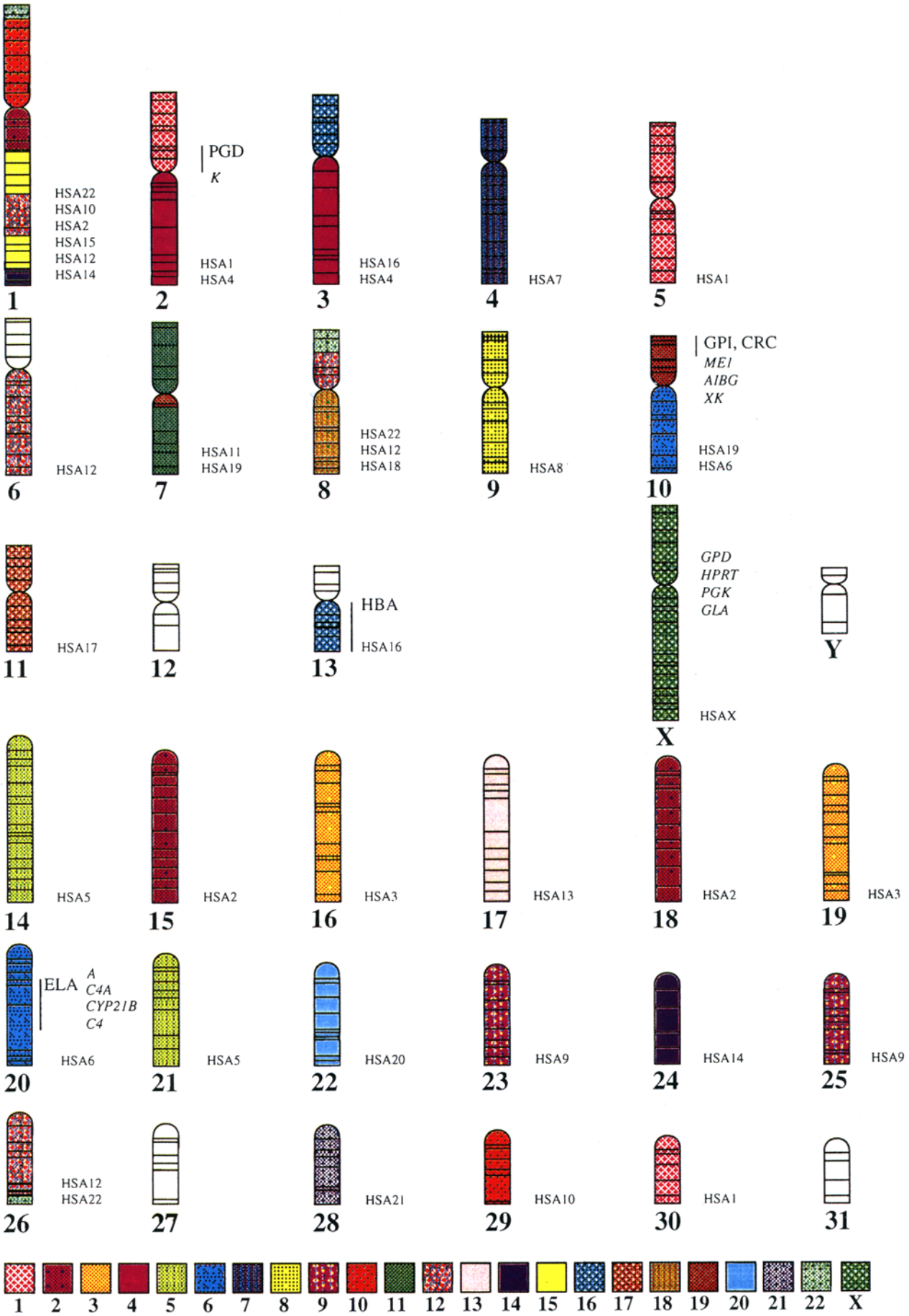

HSA22
HSA10
HSA2
HSA15
HSA12
HSA14
1
PGD
K
HSA1
HSA4
2
HSA16
HSA4
3
HSA7
4
HSA1
5
HSA12
6
HSA11
HSA19
7
HSA22
HSA12
HSA18
8
HSA8
9
GPI, CRC
MEI
AIBG
XK
HSA19
HSA6
10
HSA17
11
12
HBA
HSA16
13
GPD
HPRT
PGK
GLA
HSAX
X
Y
HSA5
14
HSA2
15
HSA3
16
HSA13
17
HSA2
18
HSA3
19
ELA
A
C4A
CYP21B
C4
HSA6
20
HSA5
21
HSA20
22
HSA9
23
HSA14
24
HSA9
25
HSA12
HSA22
26
27
HSA21
28
HSA10
29
HSA1
30
31
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X

Table 1. Conserved chromosomal segments detected on equine metaphase chromosomes after zoo-FISH with individual human chromosome specific libraries (CSLs).

Human CSLs for chromosome no.	Conserved ECA chromosome segments revealed by zoo-FISH
1	**2p** (W), 5(S); 30(W)
2	1q(W); 15(M); 18(M)
3	16(M); 19(S)
4	2q(S); 3q(S)
5	14(S); 21(W)
6	10q(S); **20**(M)
7	4(M)
8	9(M)
9	23(M); 25(W)
10	1p(S); 29(W)
11	7p, q(S)
12	1q(W); 6q(S); 8p(W); 26(M)
13	17(S)
14	1qter (M); 24(S)
15	1q(M)
16	3p(S); **13q**(M)
17	11(S)
18	8q(S)
19	**10p**(S); 7qcent(W)
20	22(M)
21	28(W)
22	1pter(W); 8pter(S); 26ter(M)
X	**X**(S)

Bold and underlined segments represent correspondence between human and equine gene mapping data. Relative signal intensity on horse chromosomes: (S) strong; (M) medium; (W) weak.

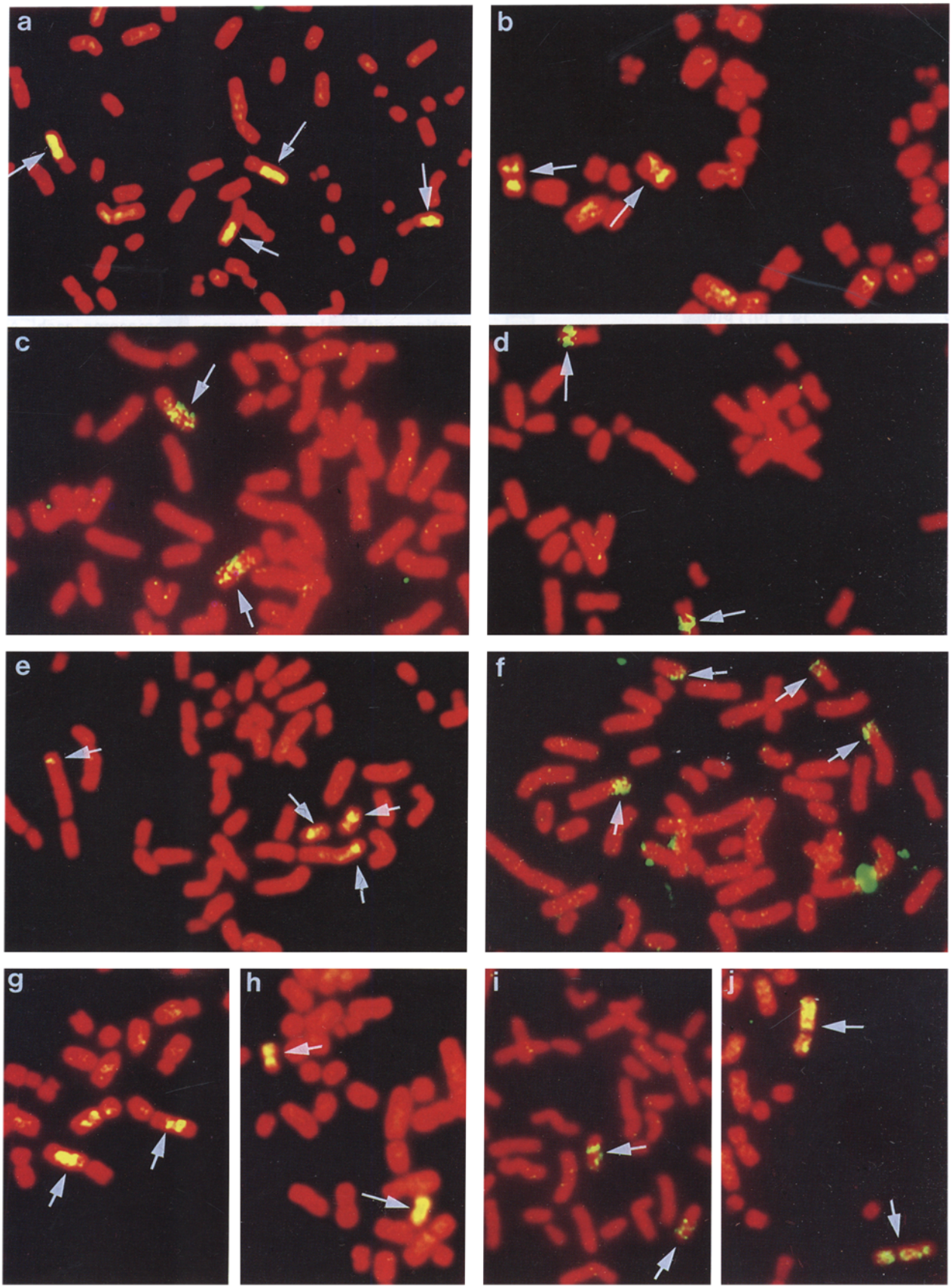
of another species demonstrate this interchromosomal reshuffle in the mammalian genome, and the observations can be used to (i) precisely define interspecies chromosomal homoeologies, (ii) reconstruct the most likely ancestral karyotype with the help of knowledge on conserved segments in different species, and (iii) define the number of breaks needed to convert karyotype structure from one species to another. Data presented in Figure 3 indicate that a total of 20 breaks in the human karyotype, followed by appropriate fusions, are needed to reconstruct the equine karyotype. On the contrary, only seven breaks and a number of fusion rearrangements are needed in the human genome, to recreate the cat karyotype (Rettenberger *et al.* 1995b).

A comparison of the pattern of segment conservation among the meta/submetacentric (1–13) and acrocentric (14–31) equine chromosomes with the human chromosomes (Figure 1) shows specific differences. The former group of chromosomes, in addition to whole chromosome conservation with human counterparts (e.g. HSA4, 5 and 9), exhibits breakage in synteny conservation on some pairs (e.g. ECA1, 2, 3, 8, etc.). However, if individual chromosome arms are considered as single units, most of the arms show complete correspondence with a human chromosome, but on some chromosome arms (e.g. 1p, 1q, 8p, etc.), synteny breakages are evident and individual arms show correspondence with two or more human chromosomes. All the acrocentrics on the other hand, except ECA26, are painted as single blocks showing total preservation of synteny conservation with only one human chromosome each. Although minor segments of synteny preservation with the human genome will probably be detected with the expansion of the equine gene map (segments smaller than 5–7 Mb are usually difficult to detect through cross-species chromosome painting; Scherthan *et al.* 1994), the present results suggest that as compared with the meta/submetacentric chromosomes, the latter group of chromosomes has not been subjected to major interchromosomal evolutionary rearrangements. Mapping data on these chromosomes are needed to further verify these observations through comparative analysis with other species.

Of the different human CSLs used in the present study, seven demonstrated total chromosome conservation between horses and man. These one-to-one homoeologies include HSA7/ECA4; HSA8/ECA9; HSA13/ECA17; HSA17/ECA11; HSA20/ECA22; HSA21/ECA28 and HSAX/ECAX (Figure 1). HSA11 is also conserved as a whole chromosome on ECA7, however, the constant thin cross-hybridizing band of HSA19 on this equine chromosome prevents us from concluding this as one-to-one homoeology. A comparison of the four species hitherto zoo-FISH analysed with human CSLs shows that a total of three, four, five and seven chromosome pairs (including the X chromosome) in cattle, pigs, cats and horses respectively, each share complete (one-to-one) correspondence with a human chromosome. A detailed comparative analysis of these conserved chromosomes in different species (B. P. Chowdhary *et al.* in preparation) will assist in the future construction of the ancestral mammalian karyotype.

Conservation of HSA20 as a single equine chromosome corresponds to similar observations in pigs where this human chromosome paints SSC17 (Frönicke *et al.* 1996). Further, conservation of HSA13 and 17 each as a single equine chromosomes provides further support to

Figure 1. Horse ideogram demonstrating segmental conservation with human chromosomes and the status of type I loci (coding sequences) physically mapped in horse (Oakenfull *et al.* 1993, Sandberg & Andersson 1993, Williams *et al.* 1993). Coloured patterns representing individual human (HSA) chromosomes are presented at the bottom. HSA chromosomes showing homoeology to particular equine chromosomes are presented at the bottom right of each chromosome. Vertical bars and symbols of loci adjacent to horse chromosomes show physically mapped genes; loci belonging to the same linkage group are in italic.


a
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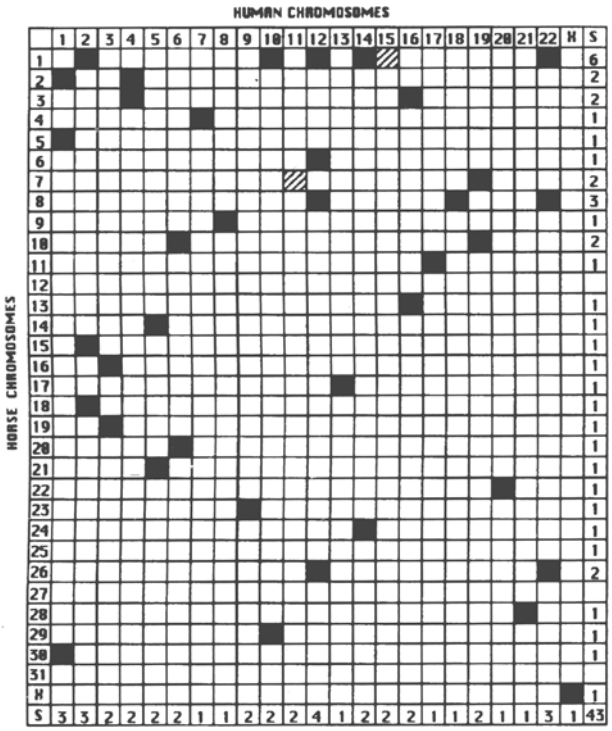

Figure 3. Matrix showing the distribution of conserved chromosomal segments between horse (left) and man (top). Black squares represent a single equine chromosomal segment painted by the corresponding human CSL. Striped squares indicate painting of the same equine chromosome in two distinct segments by the same human CSL. The bottom row shows the number of segments painted by individual human CSLs; the rightmost column shows the number of human chromosomes contributing to conservation with individual equine chromosomes. For ECA12, 27 and 31, no correspondence with human CSLs could be detected in the present work.

earlier reports that consider these chromosomes as ancestral (Scherthan *et al.* 1994, Hayes 1995, Rettenberger *et al.* 1995a,b, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996, Frönicke *et al.* 1996). Exception to synteny conservation of HSA17 is found in gorilla, where this chromosome corresponds to parts of chromosomes 4 and 19 (Wienberg *et al.* 1990). It is very likely that this disruption is a more recent evolutionary event in gorilla, especially in the light of conservation observed in a number of other species. With regard to the painting homoeology between HSA13 and ECA17, it is interesting to note that the two chromosomes share similar morphology, replication patterns and even fragile sites in corresponding bands (Rønne 1992). The conservation observed between HSA21 and ECA28 is also of significance as the human chromosome is considered to be of ancestral nature (see Stanyon *et al.* 1995). Detection of a similar trend in horses further strengthens this contention. Lastly, complete correspondence between HSAX and ECAX observed in this study is also in close accordance with the results of comparative studies (banding, gene mapping and painting) on mammalian sex chromosomes (see O'Brien *et al.* 1993, Morizot 1994, Rettenberger *et al.* 1995b, Chowdhary *et al.* 1996).

The horse gene map is one of the most poorly developed among those of farm animals. A total of about 70 loci (35 coding sequences) has so far been mapped, of which five gene loci have been localized by *in situ* hybridization. As far as is known, 13 syntenic/linkage groups, comprising 52 loci, have also been established (Sandberg & Andersson 1993, Williams *et al.* 1993, Bailey *et al.* 1995). This limited information is insufficient to make broad comparisons between the painting and gene mapping results. Nevertheless, the available gene mapping data on four equine chromosomes (ECA2, 10, 13 and 20) accurately fit with the painting results we observed (Figure 1, Table 1). It is, however, expected, that as in other species (see Chowdhary *et al.* 1996), 'misfits' between the two sets of observations will be noticed as more specific genes are mapped in horses.

The comparative painting data observed in the present study enable us to predict the location of some of the unassigned linkage/syntenic groups. The nucleoside phosphorylase (NP), monophosphate isomerase (MPI) and isocitrate dehydrogenase 2 (IDH2) loci are syntenic in horses (Williams *et al.* 1993). The former locus maps to HSA14, whereas the last two map to HSA15. The two human chromosomes show correspondence with ECA1q, to which the syntenic group can be provisionally assigned. Further, the equine linkage group 2 comprising ALB, E, GC, ES, GOT2, RN, TO and HP loci (for detail of locus names see Sandberg & Andersson 1993) has not been assigned to any chromosome. Most of these loci map either to HSA4 or HSA16. The only equine chromosome painted with this combination of human chromosomes is ECA3, to which we tentatively assign linkage group 2. In horses, two syntenic groups comprising LDHB-PEPB-IGF1 and ADA-PEPC loci were recently reported (Williams *et al.* 1993). In man, these two groups of loci are located on HSA12 and HSA1 respectively. HSA12 paints four (ECA1, 6, 8, 26) and HSA1 three (ECA2, 5, 30) different equine chromosomes, owing to which precise prediction of the chromosomal localization of these two syntenic groups in horses is not possible. However, the results of comparative painting, allow us to reduce the number of candidate horse chromosomes for these syntenic groups from 31 pairs to the above specified four and three chromosome pairs respectively. Although well supported with the comparative painting data, these predictions will have to be verified in the

Figure 2. Partial equine metaphase spreads showing (arrows) painting sites with human (HSA) chromosome specific library (CSL) probes. The CSL used and the equine (ECA) chromosomal arm segments painted are as follows: HSA4/ECA2q, 3q (**a**); HSA8/ECA9 (**b**); HSA13/ECA17 (**c**); HSA18/ECA8q (**d**); HSA14/ECA1qter, 24 (**e**); HSA16/ECA3p, 13q (**f**); HSA15/ECA1q (**g**); HSA17/ECA11 (**h**); HSA20/ECA22 (**i**); HSAX/ECAX (**j**).

future with physical mapping data. Further, it was also interesting to note that two of the nucleolus organizer region (NOR)-bearing human chromosomes (HSA21 and 22) corresponded in part to two equine chromosomes (ECA28 and 1p respectively), known to have NORs (Richer *et al.* 1990). Although it is too early to classify this correspondence either as conservation or a mere coincidence, the analogy is worth noting.

Although the present zoo-FISH experiment demonstrates conserved segments for most of the human and horse chromosomes, there are certain regions of the horse genome for which the human homoeologies are not known (see Figure 1). The available CSLs failed to give any specific hybridization signal at these sites. It is highly unlikely that the segments represent regions with no correspondence to the human genome. Like human CSL11, which did not cross-hybridize satisfactorily with the equine chromosomes (and we had to use a commercial library to get results), it is probable that libraries corresponding to these regions did not demonstrate detectable signals. It is unclear, however, which of the libraries are showing this phenomenon.

The majority of evolutionary changes in mammalian karyotypes are thought to be limited to regional rearrangements (translocations) (Lundin 1993, Edwards 1994, Lyons *et al.* 1994, Morizot 1994) or to intrachromosomal events (Hayes 1995, see Chowdhary *et al.* 1996). Karyotype evolution in equids, however, is known to have occurred at a relatively rapid rate (on an evolutionary time scale), resulting in complex and interspecific chromosomal rearrangements (Bradley & Wichman 1994). The findings of the present investigation show that the number of painted segments of human CSLs in horses are not much different from those observed in cattle and pigs (Rettenberger *et al.* 1995a, Solinas-Toldo *et al.* 1995). A large majority of equine chromosomes (22) are painted by only one human chromosome each. The painting results are thus in accordance with the idea that during the evolution of mammalian genomes, chromosomal regions have moved around in fairly large pieces with strong conservation of close to moderate linkages (Ohno *et al.* 1964, Lundin 1993).

Mammalian radiation occurred during the Paleocene, approximately 70–100 million years (Myrs) ago, and the evolutionary distance between man and the horse is considered to be about 70 Myrs (Lyons *et al.* 1994). The results of the present zoo-FISH, where the majority of equine chromosomes showed homoeology to human chromosomes, strongly support the idea of long-term evolutionary conservation of the mammalian genome (Morizot 1994). The recently published studies on similar cross-species chromosomal conservation of man and pig (Rettenberger *et al.* 1995a, Frönicke *et al.* 1996), man and cattle (Hayes 1995, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996), man and cat (Rettenberger *et al.* 1995b) and even between species with an evolutionary distance greater than 80 Myrs (man and fin whale; Scherthan *et al.* 1994) gives evidence that the zoo-FISH technique is an efficient tool for revealing evolutionarily conserved chromosomal segments between distantly related mammalian species. This can be of vital importance in the transfer of genetic information from man and mouse to less densely mapped genomes, e.g. horses. Hence, the findings of the present study bear special significance, because the zoo-FISH-based homoeology information can be utilized for a rapid and organized development of a type I marker (defined genes; see O'Brien *et al.* 1993) equine gene map. The development of a comparative gene map in horse can be beneficial in finding answers for some of the inherited equine disorders that are analogous to similar human conditions.

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