The First-Generation Whole-Genome Radiation Hybrid Map in the Horse Identifies Conserved Segments in Human and Mouse Genomes

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A first-generation radiation hybrid (RH) map of the equine (*Equus caballus*) genome was assembled using 92 horse × hamster hybrid cell lines and 730 equine markers. The map is the first comprehensive framework map of the horse that (1) incorporates type I as well as type II markers, (2) integrates synteny, cytogenetic, and meiotic maps into a consensus map, and (3) provides the most detailed genome-wide information to date on the organization and comparative status of the equine genome. The 730 loci (258 type I and 472 type II) included in the final map are clustered in 101 RH groups distributed over all equine autosomes and the X chromosome. The overall marker retention frequency in the panel is ~21%, and the possibility of adding any new marker to the map is ~90%. On average, the mapped markers are distributed every 19 cR (4 Mb) of the equine genome—a significant improvement in resolution over previous maps. With 69 new FISH assignments, a total of 253 cytogenetically mapped loci physically anchor the RH map to various chromosomal segments. Synteny assignments of 39 gene loci complemented the RH mapping of 27 genes. The results added 12 new loci to the horse gene map. Lastly, comparison of the assembly of 447 equine genes (256 linearly ordered RH-mapped and additional 191 FISH-mapped) with the location of draft sequences of their human and mouse orthologs provides the most extensive horse–human and horse–mouse comparative map to date. We expect that the foundation established through this map will significantly facilitate rapid targeted expansion of the horse gene map and consequently, mapping and positional cloning of genes governing traits significant to the equine industry.

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The primary aim of genome analysis in the horse is to generate composite map information for improving equine health, reproduction, and disease resistance. To achieve these goals, comprehensive knowledge of the genome will be instrumental in understanding the molecular causes underlying various equine hereditary disorders, and will be crucial in developing diagnostic and prevention/therapeutics approaches for these conditions. Additionally, other traits of significance such as coat color, etc., can be better addressed by understanding the molecular cause of variation in expression. Recent studies have shown that the mouse cannot be 'taken for granted' as a model animal for all human conditions (Rieder et al. 2000; Heinzerling et al. 2001), and that in some cases, the horse can be a better model (Rieder et al. 2000).

The First International Equine Gene Mapping Workshop (October 1995, Lexington, Kentucky, USA) signaled the beginning of an organized equine genomics program. Significant strides have since been made in expanding the gene map of the horse (*Equus caballus;* ECA, reviewed in Chowdhary and Raudsepp 2000). Currently ~1200 markers have been mapped/assigned to various equine chromosomes using approaches such as synteny analysis (Caetano et al. 1999a,b; Shiue et al. 1999), genetic linkage mapping (Lindgren et al. 1998; Guérin et al. 1999, 2003; Swinburne et al. 2000a), and fluorescent in situ hybridization (FISH; e.g., Raudsepp et al.

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1999; Godard et al. 2000; Lear et al. 2001; Mariat et al. 2001; Milenkovic et al. 2002). Among the recent prominent developments are the generation of preliminary radiation hybrid (RH) maps for chromosomes ECA1 and ECA10 (Kiguwa et al. 2000) and RH and comparative maps for some of the other equine chromosomes (Chowdhary et al. 2002; Raudsepp et al. 2002). Several years ago, a Zoo-FISH-based landmark comparison of the organization of horse and human genomes was provided by Raudsepp et al. (1996). Analyses of a somatic cell hybrid (SCH) panel (Caetano et al. 1999a,b; Shiue et al. 1999) and the use of horse and goat BAC clones as FISH probes on horse metaphase spreads (Godard et al. 2000; Milenkovic et al. 2002) have provided additional information.

With this progress, the current focus of equine genomics is to develop a high-resolution ordered physical map comprising: (1) uniformly distributed highly polymorphic markers and (2) a large set of ESTs or human/mouse orthologs that can provide a comprehensive comparative map. The former fulfills the need for the development of a robust genome scan panel that can help locate gene(s) governing traits of interest, and the latter is critical for candidate gene searches from the highly developed human and mouse genomes. RH cell lines are excellent for this purpose because they readily integrate markers from all sources into a consensus map using efficient and economic PCR-based typing (e.g., Geisler et al. 1999; Hukriede et al. 1999; Van Etten et al. 1999; Watanabe et al. 1999; Band et al. 2000; Murphy et al. 2000; Breen et al. 2001). The power of this approach has recently been exploited in the horse to obtain preliminary physical maps for some of the chromosomes (Kiguwa et al. 2000; Chowdhary et al. 2002; Raudsepp et al. 2002).

Expanding considerably on our earlier work, herein we report the use of a variety of markers—some already mapped using different approaches, and others newly generated—to develop the first physically ordered RH maps for *all* equine chromosomes, except the Y. The aim of this "first-generation RH map" is to integrate data from linkage, synteny, and FISH maps into a single consensus map for each of the chromosomes. The map thus generated will provide the most extensive coverage currently available for the equine genome with a variety of markers. Incorporation of equine orthologs for human genes in the RH map considerably enhances the comparative status of the horse genome in relation to human/mouse genomes. This will not only form the basis for initiating searches for genes of economic value to the equine industry, but will also be valuable in understanding the comparative organization and evolution of this perissodactyl genome in relation to other mammals or vertebrates.

RESULTS

The RH Map

A total of 901 markers were typed on the 5000rad International Equine Whole Genome RH panel. Of these, 40 markers (~5%) either showed no amplification or were considered 'unreliable' because of inconsistent results, and therefore were discarded from further analysis. An additional 131 markers (14.5%) were considered unlinked at the threshold set for analysis (lod ≥ 7; RHMAP), leaving 730 markers (81%) clustered in 101 RH groups. Of these, 258 represented specific genes/ESTs (type I) and 472 were microsatellites (type II). The final or comprehensive map is comprised of 259 framework markers, around which the remaining 471 markers were positioned.

Estimated Size, Genome Coverage, Marker Distribution, and Retention Frequency

The estimated size of the RH map in this study, summed up over all the chromosomes (except the Y), is 14,587 centiRay (cR). The total map distance for individual chromosomes ranged from 102 (ECA26) to 958 cR (ECA1). Assuming that, like other mammals, the physical size of the equine genome is ~3000 Mb, the map currently provides, on average, one marker every 4 Mb (~19cR) of the equine genome. Further, 40 of the 901 markers could not be typed in the panel, indicating the coverage of the equine genome to be only ~95%. However, considering that a total of 171 markers dropped out of the final analysis, this estimate reduces to ~81%. The latter is certainly an underestimation, because the statistic excludes even those 'unlinked' markers (the group of 131 mentioned above) that are just short of the accepted threshold for inclusion in the RH groups. It is expected that several (if not all) of them will be incorporated in the map as its density improves. Hence, a realistic estimate of the genome coverage in the panel is probably ~90%.

Of the markers incorporated in the RH map, the highest number of markers is located on ECA1 (63 markers; ECA1 is the largest chromosome), whereas only six markers were localized to ECA28 (the lowest number/chromosome). The latter is among the smallest equine chromosomes. However, if viewed in terms of density of markers per unit length of the chromosome, ECA11 appears to be the most densely mapped chromosome (13 markers/unit length), followed by ECA4 and ECA20 (~10 markers/unit length). The corresponding value for ECA1 is 8.9, and for ECA28 is ~3–4. Despite the differences, the entire horse genome is reasonably well covered with markers, especially for a first-generation RH map.

On average, 3–4 RH groups were found on each of the equine chromosomes, with the number ranging from 10 on ECA1 to only one each on, for example, ECA26–ECA29 (Fig. 1, enclosed poster). The relatively large number of RH groups on ECA1 could be attributed to more irradiation-induced breakages expected in large-sized chromosomes than in the smaller ones. The overall retention frequency of the markers in the panel is ~21%, with a range of 11% for ECA1 to 37% for ECA11. Retention of markers was slightly higher than average in the pericentromeric regions of a number of meta- and submetacentric chromosomes (e.g., ECA1–10) and a few acrocentric chromosomes (ECA23). A similar marginally high retention frequency was observed towards the telomeric regions of some chromosomes (e.g., ECA3p, ECA6p, ECA13p, ECA16q, and ECA20q). On ECA11, the overall retention of markers was much higher on the short arm than the long arm, most likely due to the presence of the *TK1* gene on ECA11p, which is preferentially retained in all cell hybrids.

FISH Mapping and Alignment of RH Maps

A total of 69 RH-mapped loci (33 genes and 36 microsatellites) were FISH-mapped. These represent new cytogenetic data on the equine genome. The majority of the loci mapped to ECA1 (19), ECA10 (12), ECA14 (12), and ECA17/21 (5 each), where the number of RH-typed markers is also relatively high. A complete list of all the markers FISH-mapped in this study is presented in Table 1. These localizations, together with published FISH data on 191 markers (see Chowdhary et al. 2002; Milenkovic et al. 2002; Raudsepp et al. 2002), provided 253 'anchors,' 118 type I and 135 type II, that aligned 88 of the

Table 1. Detailed Information on Individual Markers Typed in the Equine Whole-Genome RH Panel

Marker name/ symbol	Marker type or full name	Location horse	Location human	Primers 5′-3′	PCR product size (bp)	$MgCl_2$ (mM)	T_a	References	Accession number
ICA16	microsatellite	1q		**F: TCACTGGGGGGTATATGCAT R: GATCCTACTCCACCTGAAGTGG**	121	3.0	58		AF043200
ICA20	microsatellite	**1q13**		**F: TGGACAAAATGCAAAAGTCA R: TCCACTACACAGGAAAACGAA**	194	1.5	50		AF043202
ICA22	microsatellite	1		**F: TGGGCAAGAGGACAAATAGC R: TGGCAGCCTTGGTTTGTACT**	208	3.0	58		AF043204
ICA24	microsatellite	1		**F: AGATATTGGGGGCGGAAG R: TGGAGGTCAACTGGCTATCTC**	205	3.0	58		AF179211
ICA25	microsatellite	**1q17.3**		**F: TCCAATTTTCCCCAATGGTA R: CTGCATTTTGACAATGGTGG**	206	3.0	58		AF043205
ICA28	microsatellite	1		**F: CCTACGCCCACACTCACATA R: TGGACTTTCTTACCCCCAAA**	213	1.5	58		AF043208
ICA30	microsatellite	**1p16**		**F: TGGGGAGGGGTTGTTCTAG R: GCTCTCCACTGCATGCATAA**	130	3.0	58		AF043209
ICA32	microsatellite	1		**F: AGTTACCAAATGTCGCATTGC R: TTCATCTGTAAAATGGGCAGG**	107	1.5	58		AF043210
ICA40	microsatellite	1		**F: TTGAAAGCCGTGTTTTTGTG R: ACCCCATGTTCTGACAAGGA**	226	1.5	58		AF043213
ICA41	microsatellite	**1q15–q16**		**F: CTGGTCAGGCCTATTACCCA R: AGATATTGGGGGCGGAAG**	245	1.5	58		AF043214
ICA43	microsatellite	**1q17.1–q17.3**		**F: ATGGCATGATTTGCTTCTCC R: TGGAAACAACCTAAATGTCCA**	122	1.5	58		AF043215
ICA44	microsatellite	**1p16**		**F: GGCAGCACACCAAATCAAGT R: TCCTGCAAAACAACAGAGGA**	203	2.0	58		AF043216
AHT021	microsatellite	**1q14–q15**		F: TCCAAGTTGCTGAATGGATC R: ACGGCCTGATTCTCTCTTTG	199–215	2.0	58	Swinburne et al. 1997	
AHT040	microsatellite	**1q15–q16**		F: GCAAGTTCAGCACCTCCCT R: TTTATGACACCTGCTGAGAACG	230	2.0	58	Swinburne et al. 2000[a]	AJ271525
AHT058	microsatellite	1		F: CAGTGATGAGCCGCAAATAG R: TCTACCTATAATCCGCCTCCC	163–198	2.0	58	Swinburne et al. 2003	AJ507675
ANXA2	annexin A2	**1q21.3–q22**	15q21–q22	F: GTGGGGATGACTGAAGCCTGT R: CACTGCCAACTCCTTGGAATGT	234	3.0	58	Fetal cDNA library EST#912	G62155
ANXA7	annexin A7	1	10q21.1–q21.2	**F: GCCGTCCTCACAGTCTTAT R: GAGCTTGACCTCCAGGATA**	856	3.0	58	Testis cDNA library #AM004_F05	
ASB08	microsatellite	1q16–q17		F: GACAACGTGGCAGCTCACTGCC R: GCAAGTAAGCCATATGTGCATGCG	143	3.0	TD64	Breen et al. 1997	X95323
ASB12	microsatellite	1q12–q13		F: TCAGCAATAGAAGCCAGCTCC R: TCCTATGGAGGTGACCTTCCC	170	3.0	65	Breen et al. 1997	X95327
ASB41	microsatellite	1p14		F: AAAGTTCACTTAGTCCTTGG R: CCACCTGTTTGCACTTGC	129–159	3.0	TD64	Irvin et al. 1998, Lear et al. 1999	AF004771
CMA1=CHY	chymase – mast cell	1q	14q11.2	**F: GAATTGCTCCTCTGCAGGTC R: CCTGTAGGTCCCACCTCTGA**	200	1.5	TD60	Caetano et al. 1999[a]	AF130752
COR006	microsatellite	**1q25**		F: GTTCTGCACATCCTGCTCTT R: AGTGCCCTGAAACTGTATGG	187–199	3.0	58	Hopman et al. 1999	AF083449
COR046	microsatellite	**1q17.1**		F: TGTTTGCAAAGATATTGGGG R: ACCTGGTCAGGCCTATTACC	245–259	1.5	55	Ruth et al. 1999	AF108363
COR053	microsatellite	1		F: AATTGACTGTGGAAGCCTTG R: GGCTGAGGAGTAAGCTGAAAG	173–197	3.0	55	Ruth et al. 1999	AF108370
COR059	microsatellite	**1p12.3–p12.2**		F: ATGAATGGCAAATTGAAGGA R: TTCTGTTTAGGGGCTCTTAGG	265–277	3.0	58	Ruth et al. 1999	AF108376

This is a portion of Table 1. Complete table is available as an online supplement. Data shown in bold (horse cytogenetic locations and primer sequences) represent work from the present study. S, new synteny mapped loci; TD, touch-down PCR; T_a, annealing temperature.

101 RH groups to individual chromosomes, thus facilitating their physical placement and orientation. A distribution of the number of anchor markers by chromosome is presented in Table 2. The remaining 13 RH groups were placed using available genetic linkage information (Lindgren et al. 1998; Swinburne et al. 2000a; Guérin et al. 2003) and the RH2pt data for end markers with markers in the adjacent RH groups (Fig. 1, enclosed poster; see linkage groups with white bars).

Synteny Mapping

A total of 39 new equine genes/ESTs were mapped using the UC-Davis somatic hybrid cell panel (see Caetano et al. 1999a; Shiue et al. 1999). The loci were distributed on 20 equine chromosomes. Twenty-seven of these loci are also on the RH map presented here. Except for *HDAC1*, the SCH mapping data for all loci are in agreement with the RH localizations.

Table 2. Detailed Chromosome-Wise Information on Different Parameters of the Horse RH and Comparative Maps

Horse chr.	Size (Mbp)	Size (cR)	No. of RH groups	Loci typed in the RH panel			FISH loci included (*TypeI* + *TypeII*)			Loci used for horse, human and mouse genome comparison
				type I	type II	total	total	this study	anchor loci	
ECA1	204	958.8	10	15	48	63	45	19	26	30
ECA2	135	796.7	4	12	23	35	23	—	11	23
ECA3	132	724.4	3	15	14	29	21	—	11	24
ECA4	120	658.6	6	10	24	34	15	1	10	14
ECA5	111	591.5	3	14	11	25	19	—	7	25
ECA6	108	595.2	2	11	12	23	8	1	4	15
ECA7	105	550.8	3	8	16	24	26	1	8	26
ECA8	105	774.2	3	12	14	26	18	1	6	20
ECA9	99	530.6	3	8	14	22	11	—	3	14
ECA10	96	672.4	5	9	24	33	26	12	21	13
ECA11	72	650.2	5	20	14	34	26	2	17	28
ECA12	63	276.7	2	4	10	14	6	—	3	5
ECA13	60	144.4	4	4	9	13	10	—	5	8
ECA14	120	323.1	4	10	12	22	21	12	14	16
ECA15	111	290.7	4	4	18	22	19	1	8	13
ECA16	108	668.0	4	12	20	32	26	2	13	24
ECA17	102	293.0	3	6	14	20	10	5	7	9
ECA18	102	445.1	3	10	14	24	12	1	8	13
ECA19	96	410.2	3	4	22	26	11	—	6	8
ECA20	87	408.7	3	8	17	25	17	1	5	19
ECA21	81	306.8	3	7	11	18	11	5	8	9
ECA22	72	536.0	3	7	14	21	9	1	6	9
ECA23	69	293.5	3	7	13	20	11	1	7	11
ECA24	63	550.7	2	5	14	19	9	1	6	8
ECA25	60	206.5	2	3	7	10	5	—	1	7
ECA26	63	102.9	1	1	7	8	8	—	3	6
ECA27	57	168.6	1	1	7	8	2	—	2	1
ECA28	54	175.8	1	3	3	6	8	—	3	8
ECA29	51	278.0	1	4	7	11	3	1	2	5
ECA30	48	279.0	2	1	9	10	2	—	1	2
ECA31	45	172.8	1	3	7	10	6	—	4	5
ECAX	153	753.2	4	20	23	43	26	1	17	27
Total	**2952**	**14587.1**	**101**	**258**	**472**	**730**	**470**	**69**	**253**	**447**

The size of individual chromosomes in megabasepairs (Mbp) is deduced from the relative length of the chromosomes (ISCNH 1997) and presuming a genome size of 3000 Mbp. Anchor loci represent cytogenetically mapped type I or type II markers that align RH groups to chromosomes. Total number of loci used for horse-human-mouse genome-wide comparison includes all type I loci mapped by RH analysis and FISH (see text).

FISH data on 12 of the 39 loci (Lear et al. 2001; Mariat et al. 2001; Milenkovic et al. 2002) strongly support the synteny results.

Comparative Mapping

The RH map presented here incorporates a total of 258 type I markers. When compared to the estimated length of the RH map (14,587 cR), the markers are, on average, spaced every 50 cR (or ~10 Mb) of the equine genome. To improve the density of this comparative framework, all previously FISH-mapped equine genes (see Chowdhary et al. 2002; Milenkovic et al. 2002; Raudsepp et al. 2002) were 'placed' in the physically ordered scaffold of the RH type I markers (Fig. 1, enclosed poster; see loci arranged next to colored vertical bars). The 253 'anchors' aligning the RH and cytogenetic maps acted as guides to deduce the *most likely* location of these FISH-mapped genes in the assembly. For example, on ECA31, the FISH location of 'anchor' *AHT34* helped to deduce the likely location of *ESR* as distal to *PCMT1*. Similarly, the location of *IGF2R* was inferred as proximal to *VIP* through the 'anchor' *AHT033*. Accordingly, a plausible order of three RH-mapped (*PLG*, *VIP*, and *PCMT1*) and two previously FISH-mapped genes (*IGF2R* and *ESR*) was derived. This improved the comparative power of the ECA31 map from three to five loci. Following this, a physically ordered collection of a total of 447 equine genes was obtained for comparison with the human and mouse gene maps.

Based on the comparative location of the 447 equine loci, a total of 44 *conserved syntenies* (two or more pairs of homologous genes located on the same chromosome regardless of order; Nadeau and Sankoff 1998; denoted by color bars next to the RH map in Fig. 1, enclosed poster) were identified between the human and the horse genomes. Additionally, eight smaller *homology segments* (one pair of homologous genes in two species; Nadeau and Sankoff 1998) comprising a single locus were found on some chromosomes, for example, ECA1 (*COMT*) and ECA2 (*ACADL*). Five of these segments originate from results compiled from Milenkovic et al. (2002), and two are novel. The latter are based on the mapping of *FABP3* (ECA2q-HSA1) and *ARPC3* (ECA9q-HSA12). Compared to this, the horse and mouse genomes demonstrated a total of 71 conserved syntenies (comprising ≥2 loci; see mouse chromosomal location of equine orthologs in Fig. 1, enclosed

poster) and 41 homology segments (each comprising a single comparatively mapped locus) between the two species. Overall, the horse–human conserved syntenies were larger than the horse–mouse conserved syntenies. Nonetheless, some entire chromosomes or chromosome arms (e.g., ECA17-HSA13-MMU11 and ECA22-HSA20, part of MMU2) were completely conserved among the three species.

In order to produce a refined comparative map, the precise sequence locations of human and mouse orthologs for all the 447 physically arranged equine genes were obtained from the available draft sequence data (http://genome.ucsc.edu; version June 2002 for human and February 2002 for mouse). If sequence locations of a group of human or mouse orthologs indicated conservation of gene order in relation to the derived order of equine genes, the data were clustered in boxes (see Fig. 1, enclosed poster). These clusters, referred to as conserved linkages (maximally contiguous chromosomal region with identical gene content and order; Nadeau and Sankoff 1998), showed a group of genes with similar physical order in horse–human or horse–mouse. In the majority of the cases, clustering highlighted smaller evolutionarily conserved segments within the larger conserved syntenies.

Within the 44 horse–human conserved syntenies, 84 distinct clusters or conserved linkages of human loci were established where the gene order was conserved between the two species. These conserved linkages included 87% (391/447) of the compared loci. Conversely, the 71 horse–mouse conserved syntenies split into 80 conserved linkages that shared highly conserved gene order with the horse. However, it is noteworthy that, on average, these conserved linkages were smaller than those observed between horse and human, and included only 66% (297/452) of the compared loci. It was observed that for some of the equine chromosomes (e.g., ECA18 and ECA21), the comparative order of the human and mouse orthologs was exceptionally conserved with available equine order. Contrary to this, considerable rearrangements were detected in a few other chromosomes (e.g., ECA7 and ECA16). Lastly, comparison of gene order of the 447 loci across horse, human, and mouse showed 85 clusters of genes that had highly conserved gene order in all three species (Fig. 1, enclosed poster; see yellow horizontal shades across horse–human–mouse gene order). These clusters of conserved linkages were demarcated when the gene order was disrupted between syntenic loci in human or mouse. For example, on ECAXq, the two distal clusters are separated because of gene order disruption in the mouse at *PAK3* and *IGSF1*. Of the 85 clusters, 59 had ~3–10 ordered loci each, and 23 had two loci each that were located close to each other among horse, human, and mouse.

DISCUSSION

This study provides the first whole-genome radiation hybrid map of the equine genome. The map comprises a total of 730 markers that are distributed over 31 equine autosomes and the X chromosome. This represents a greater than twofold expansion over the previously presented linkage (Guérin et al. 1999, 2003; Swinburne et al. 2000a) and comparative (Milenkovic et al. 2002) maps. Although the locus order in the RH map is in general agreement with that reported in published maps (Guérin et al. 1999, 2003; Swinburne et al. 2000a), some differences, mainly attributed to improved resolution, are evident. Examples of these are shown in later sections. Most significantly, for the first time, a true integration of the equine genetic linkage, syntenic, and cytogenetic maps has been achieved, thus producing a map that is, to date, the most comprehensive for this species. Moreover, with greater than 258 physically ordered gene-specific/EST markers, the map is hitherto the most current linearly ordered comparative map of the horse genome.

In terms of number of mapped type I and type II loci, the RH map generated in this study is comparable to contemporary first-generation maps in several species (see Table 3; pig, Hawken et al. 1999; cat, Murphy et al. 2000; dog, Priat et al. 1998; zebrafish, Hukriede et al. 1999). Some of the maps, for example, those for ECA1, 10, 11, 14, 16, and X are exceptionally well covered with markers compared to the current status for these chromosomes in the horse. However, other chromosomes (e.g., ECA12 and ECA23 to ECA30) need new gene-specific markers, and there are still others that require type II polymorphic markers (e.g., ECA28). On the whole, the map is an important foundation upon which a detailed map of the equine genome can be built.

The number of RH groups reported in this study (101) is higher than those reported for first-generation RH maps in other domesticated species such as the dog (57, Priat et al.

Table 3. Comparative Statistics on Major Parameters of Whole-Genome RH Maps (First Generation and Subsequent Maps) in Horse and Other Species

	Number of markers			Resolution		
Species	RF	total	genes/EST	MS/STS		Reference
Human (GB4; 3000rad)	29%	1085	374	711	1.1 Mb	Gyapay et al. 1996
Human (GB3; 10000rad)	16%	10478	3836	6642	240 kb	Stewart et al. 1997
Mouse (T31; 3000rad)	24%	271	—	271	145 kb	McCarthy et al. 1997
Rat (T55; 3000rad)	27%	5255	522	4733	410 kb	Watanabe et al. 1999
Zebrafish (T51; 3000rad)	18%	1275	355	920	350 kb	Geisler et al. 1999
(LN54; 5000rad + 4000rad)	22%	703			500 kb	Hukriede et al. 1999
Dog (5000rad)	21%	400	218	182	630 kb	Priat et al. 1998
Upgraded		1500	320	1078	?	Breen et al. 2001
Pig (7000rad)	29%	757	71	686	145 kb	Hawken et al. 1999
Cattle (5000rad)	22%	1087	768	319	500 kb	Band et al. 2000
(3000rad)	28%	1200	<50	most	?	Williams et al. 2002
Cat (5000rad)	39%	600	424	176	?	Murphy et al. 2000
Horse	22%	733	275	458	~1 Mb	This study

1998) and cattle (61, Band et al. 2000), but lower than those reported in pigs (128, Hawken et al. 1999) and zebrafish (190, Geisler et al. 1999). The average retention frequency of markers in the horse RH panel is similar to the estimates reported for dog, cattle, human, and zebrafish. However, higher estimates have been reported for pig, cat, human, rat, and mouse (see Table 3 for details).

Resolution

The resolution of the panel, often referred to as the shortest physical distance that can be resolved using the panel (McCarthy et al. 1997), is projected as a function of average retention frequency and the total length of the map in cR. In this study, 1 cR corresponds to ~200 kb, implying that the average fragment size of retained horse chromosomes in the panel is around 20 Mb. This divided by the number of hybrid lines (92) × retention frequency (22/100 or .22) suggests a resolution of ~1 Mb for our panel. To our understanding, this estimate is tentative, because actual cR distances are not available for the entire length of all horse chromosomes. Presuming that at least 50 cR separates current RH groups, the 50–60 gaps between different RH groups alone will contribute an additional ~3000cR. This is bound to deflate the current kb/cR estimates and show a lower estimate (better resolution) than is now predicted. Thus, true resolution of the panel will only be known when the density of markers is improved and an enhanced framework map signifying accurate intermarker distances is constructed.

Cytogenetic Alignment of RH Maps

While generating RH maps, accurate alignment of RH groups to specific chromosomal regions is crucial. This alignment becomes even more significant if there is limited support from the meiotic maps. The currently available meiotic maps in the horse (Newmarket: Swinburne et al. 2000a; International: Guérin et al. 1999, 2003) are comprised of about 325 markers, of which only 85 have been cytogenetically mapped (on average 2–3 markers/chromosome). The RH map presented here marks a threefold increase in the number of markers, with a total of 253 of the typed markers aligned by FISH, and 69 markers reported for the first time. This significantly facilitated the placement and orientation of different RH groups, and provided validation for the suggested order. Although the cytogenetic alignment of the RH map is fairly uniform along the length of the majority of the chromosomes, some chromosomes (e.g., ECA25–ECA30) and chromosomal regions (e.g., middle part of ECA6, ECA7, ECA10, and ECA17) require additional FISH markers.

FISH localizations of some of the loci reported earlier do not agree with the proposed RH order. For example, assignment of microsatellite *AHT27* to ECA12q (Swinburne et al. 2000b) does not agree with the RH markers assigned to this arm. Instead, the locus shows close linkage with microsatellites *SG10* and *RKJ12*, of which the former is FISH-mapped to the short arm. It is therefore likely that due to misidentification of the arm (common for small metacentric chromosomes), *AHT27* was allocated to the long arm. By moving the locus to a corresponding position on the short arm, the FISH and RH maps align perfectly. Whether a similar situation also exists for *MT2A* on ECA13p should be verified, because the human ortholog for this equine gene is on HSA16, which shares homology with the long arm of the chromosome and not the short arm (Raudsepp et al. 1996). Localization of *F11* is discussed in greater detail in the Comparative Map section below.

Integration of Different Maps: A Step Towards a Consensus Map

The RH map presented in this study represents the first organized effort aimed at genome-wide integration of the syntenic, cytogenetic, and meiotic maps of the horse into a single consensus map. Until now, the synteny mapping approach in the horse (Caetano et al. 1999a; Shiue et al. 1999) has been instrumental in assigning markers to specific equine chromosomes, whereas meiotic linkage mapping (Newmarket: Swinburne et al. 2000a; International: Guérin et al. 1999, 2003) has played an important role in identifying and ordering linked markers. However, because very few linked markers are cytogenetically aligned, it is difficult to ascertain the precise physical span of individual linkage groups on the chromosomes. Earlier attempts to improve this status were restricted to ECA1 and ECA10 on a preliminary level (Kiguwa et al. 2000) and ECA11 (Chowdhary et al. 2002) and ECAX (Raudsepp et al. 2002) on a more extended level. Thus, for the first time, ~340 meiotic, 395 syntenic, and >400 cytogenetic markers are integrated, resulting in consensus maps for each of the equine chromosomes. These maps will be extremely useful in devising strategies to close in on genes governing traits of interest in the horse.

Improved Resolution Over Previous Linkage, Cytogenetic, and RH Maps

The RH map presented herein helps resolve the order of a number of markers clustered at one location in the reported cytogenetic (Godard et al. 2000; Mariat et al. 2001; Milenkovic et al. 2002; this study) and linkage maps (Lindgren et al. 1998; Swinburne et al. 2000a; Guérin et al. 1999, 2003). For example, the RH map linearly orders markers that are FISH-mapped to overlapping bands (e.g., *COR46-LEX58-TJP1-1CA43* on ECA1p) or even to the same band of the chromosome (e.g., *1CA44-1CA30* and *CA487-VHL134* on ECA1p and *COR006-HMS7* on ECA1q). Likewise, compared to previously published linkage (Swinburne et al. 2000a) and RH (Kiguwa et al. 2000) maps, the current RH map of, for example, ECA1 resolves and rearranges the order of a number of markers clustered at the proximal (e.g., *1CA44-ASB41-1CA30*) and distal ends (e.g., *1CA16*, *HMS7*, *COR006*, and *1CA40*) of the chromosome (see Fig. 1, enclosed poster). Similarly, the RH map readily resolves the order of meiotic map markers *UCD304-HMS05-HTG15* clustered on ECA5 (Swinburne et al. 2000a), and assigns them to the proximal part of ECA5q—which could not be done previously.

The first-generation RH map shows marked improvement over the recently published 3000rad preliminary RH maps of ECA1 and ECA10 (Kiguwa et al. 2000). On both chromosomes, the total number of mapped markers is almost doubled, new polymorphic markers are included, and several new FISH localizations have been added. With regard to the very recently published maps of ECA11 (Chowdhary et al. 2002) and ECAX (Raudsepp et al. 2002), the new maps show reasonable improvement. Major contributions include the addition of eight genes/ESTs to ECA11 and four new genes and five microsatellite markers to ECAX. Except for minor flips involving closely linked loci, the overall order between the new and the previous RH maps is preserved on both chromosomes.

The Comparative Map

Together with the 258 linearly ordered type I loci in the RH map, the compilation of all the mapped equine genes provided a total of 447 gene-based markers that facilitated the generation of the most comprehensive comparative map between horse and human to date, and the first detailed comparative map between horse and mouse. This represents ~185 more genes compared to the most recent horse–human comparative map (Milenkovic et al. 2002). Although the assembly of the equine genes does not provide precise linear order, it does offer a reliable working framework for comparison. Within this framework, no drastic locus order changes are expected, especially within the conserved syntenic blocks, because the overall order is strongly supported with 256 linearly arranged RH-mapped loci and the 191 moderately well positioned FISH markers.

Of considerable significance is the mapping of the first equine gene (*F11*) to ECA27. This helped infer likely correspondence of the latter with part of HSA4. Earlier comparative studies (Raudsepp et al. 1996; Caetano et al. 1999a; Godard et al. 2000; Lear et al. 2001; Mariat et al. 2001; Milenkovic et al. 2002) could not detect equivalence of this chromosome with any of the human or mouse chromosomes. It is noteworthy that using a goat BAC clone, Milenkovic et al. (2002) FISH-mapped *F11* to ECA3. However, we found the locus to be tightly linked to the RH group assigned to ECA27. Further, most of the loci in the RH group are also in the genetic linkage group assigned to this chromosome (Swinburne et al. 2000a). Thus it seems that the goat BAC used by Milenkovic et al. (2002) resulted in an erroneous assignment of the locus to ECA3. Sequencing of the PCR product obtained during RH typing further verified the locus to be indeed *F11*.

The overall number of *major* horse–human conserved syntenies reported in the present study is in close agreement with earlier Zoo-FISH findings (Raudsepp et al. 1996). However, the figure is less than half of the 113 conserved segments reported in the two species by Milenkovic et al. (2002). This large discordance is *primarily* related to the way the segments were counted in the two studies. Schibler et al. (1998), Band et al. (2000), Pinton et al. (2000), and Milenkovic et al. (2002) divided large conserved syntenies into smaller segments on the basis of *preserved or disrupted chromosome band order* of the mapped loci in the human. This resulted in a significantly higher count of conserved segments in all four species (goat, 107; cattle, 105; pig, 84; horse, 113). Contrary to this, Watanabe et al. (1999) disrupted conserved syntenies only when linearly arranged rat orthologs were from different human/mouse chromosomes, an approach followed in the present study. This explains why, for example, with only 12 mapped markers each on ECA11 and ECAX, Milenkovic et al. (2002) show 6–7 conserved segments for individual human counterparts (HSA17 and HSAX, respectively), compared to only one shown in the present study despite twice the amount of data. However, if the 84 horse–human conserved linkages we observed are compared with the number of conserved segments reported in different livestock species (Schibler et al. 1998; Band et al. 2000; Pinton et al. 2000; Milenkovic et al. 2002), our estimates are at the lower end of the spectrum.

The most striking genome conservation between horse and human was seen on ECA4, 5, 17, 18, 21, 25, and 31 and their human counterparts (see Fig. 1, enclosed poster), where the derived equine gene order closely corresponded to the order reported in the draft sequence of the human genome. A similar situation was also observed on ECA3p, ECA6p, and ECA14. However, of greatest significance was the striking conservation of gene order among ECA3p, ECA6p, and ECA22 genes and their human and mouse homologs: HSA16q/part MMU8, HSA2q/part MMU1, and HSA20/MMU2, respectively. Incidentally, HSA20 represents one of the most conserved mammalian autosomes (Chowdhary et al. 1998; Haig 1999). Similarly, genomic segments corresponding to HSA16q and HSA2q are considered evolutionarily highly conserved (Chowdhary et al. 1998; http://www.informatics.jax.org/menus/homology_menu.shtml, Murphy et al. 2000). Detection of conserved gene order for these segments in the horse is novel and reiterates their ancestral status. Contrary to this, gene order within some of the large conserved syntenies in the horse (e.g., entire ECA16 corresponds to HSA3p or 3q) was found to be considerably rearranged in relation to the observed order of the same genes in human and mouse. This is evident from the greater number of conserved linkages for this chromosome compared to those seen on other chromosomes (see Fig. 1, enclosed poster).

The identification of 85 clusters of loci demonstrating gene order conservation (conserved linkages) across horse, human, and mouse (yellow-shaded regions, Fig. 1, enclosed poster) was extremely significant. Basically, these clusters are core blocks within the conserved syntenies, where the gene order is reasonably well preserved across evolutionarily diverged species. Hence it is tempting to speculate that these segments represent potentially the most conserved genomic regions of the ancestor common to horse, human, and mouse. Discovery of these conserved linkages provides a quick comparative overview of smaller genomic blocks shared among the three species and signifies an important advance in accurate alignment of the three genomes.

Overview

The RH and comparative map presented here is the most elaborate and dense map in the horse produced to date. Among farm animals, the map ranks fifth after cattle, dog, chicken and pig. Translating the quote of Flaherty and Herron (1998) for the mouse RH map, the horse map is the "new kid on the block". This new comprehensive and integrated map will enable equine geneticists to perform a range of studies at a resolution not available earlier. Because the map is linearly ordered and reasonably well aligned along the length of individual equine chromosomes, its overall ability to facilitate accurate mapping of markers/traits of interest is greatly enhanced. Moreover, with the largest assembly of type I markers in the horse, the alignment of the horse genome with human and mouse genomes is markedly refined. This will facilitate the use of the highly developed human and mouse gene maps in searching for candidate gene(s) implicated in various inherited conditions important to the equine industry. In evolutionary terms, the findings are significant in providing upgraded comparative information on the genome organization of an additional mammalian order—the Perissodactyla, for which such detailed information was not available earlier.

Finally, with the availability of an informative RH panel to the equine gene-mapping community worldwide, and the installation of an instant two-point linkage output Web interface (installation in progress), investigators in any country will be able to readily map any locus of interest to the equine genome. This will certainly speed up the rapid determination of likely locations of candidate genes, and facilitate positional cloning.

METHODS

Markers for RH Mapping

A total of 901 markers chosen from a variety of sources were used in this study. Primer pairs for the majority of the markers were available either from databases (HorseBase: http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl?BASE=horse, http://roslin.thearkdb.org/; NCBI: http://www.ncbi.nlm.nih.gov) or from published papers and personal resources (Dr. R. Brandon, University of Queensland, Australia). ESTs were generated from an equine testis cDNA library (kindly provided by Dr. N. Ing, Texas A&M University, Texas). Sequencing of 3360 clones yielded 2090 high-quality sequences. Both ends of each sequence were compared against dbEST and databases of GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) to produce 1732 equine sequences with significant hits (90% or more sequence similarity) to human genes or ESTs. Redundant sequences, especially those representing mitochondrial DNA or genome-wide gene families such as ribosomal RNA, were discarded. On the basis of human orthologs and horse-human Zoo-FISH data (Raudsepp et al. 1996), the sequences were classified into groups that would most likely map to a specific horse chromosome. Primer pairs were designed in the 3′UTR, using Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) to produce a PCR amplification product of 150–300 bp in size. This yielded 76 primer pairs, of which 28 were typed on the RH panel. Lastly, primer pairs were designed for two putative equine orthologs using alignments of human, mouse, and other mammalian sequences to identify conserved regions. A chromosome-wise list of all the markers included in this study, along with details on origin/source, symbols, primer sequences, PCR conditions, and references is presented in Table 1.

RH Panel Typing and Analysis

A 5000rad whole-genome RH panel comprised of 92 hybrid cell lines was typed by PCR as described (Chowdhary et al. 2002). PCR conditions for each of the primer pairs were optimized so that only horse-specific amplification products were obtained. All markers were typed in duplicate along with horse, hamster, and a 'no-DNA' control. PCR products were resolved on 2.5% agarose gels (containing 0.25µg/mL ethidium bromide) and scored manually.

Initially markers were assigned to groups on the basis of confirmed mapping data (linkage, synteny, cytogenetic, and Zoo-FISH). Using RHMAPPER software (Slonim et al. 1997), markers with unknown locations were assigned to individual chromosomes at lod $\geq$ 11.0. Following this, RHMAP 3.0 software (Boehnke 1992; Lunetta et al. 1995) was used for all analyses. RH groups within individual chromosomes were obtained at lod $\geq$ 7.0 using the RH2PT program. Frameworks were obtained at lod 3 (1000:1), and a comprehensive map was built by placing the remaining markers in relation to the framework map. RH groups with no framework markers were ordered at a threshold of lower than 1000:1. Orientation of multiple RH groups on a single chromosome was accomplished using cytogenetic and linkage data and 2pt lod score values of the end marker with markers in adjacent groups. RH groups with no cytogenetic alignment were placed solely on the basis of linkage data (Lindgren et al. 1998; Swinburne et al. 2000a; Guérin et al. 1999, 2003).

BAC Library Screening and Fluorescent In Situ Hybridization (FISH)

The INRA equine BAC library was screened by PCR for 78 markers (37 genes and 41 microsatellites) as described in detail elsewhere (see Milenkovic et al. 2002). Briefly, PCR primers for individual markers were used to identify positive clones. These clones were grown overnight, and DNA was isolated from each of them. Approximately 1 µg DNA from each of the BACs was biotin-labeled, dissolved in 20 µL hybridization mix (50% formamide, 2 × SSC, 10% dextran sulfate), and hybridized to horse metaphase spreads. The signals were detected with FITC-conjugated antibodies, and chromosomes were counterstained with DAPI. Hybridization results were examined and analyzed using a Zeiss Axioplan2 fluorescent microscope and Cytovision/Genus application software version 2.7 (Applied Imaging).

Synteny Mapping

The UC-Davis somatic cell hybrid synteny panel is described elsewhere (Caetano et al. 1999a,b; Shiue et al. 1999). A modified panel representing a more unique set of 70 clones (vs. the 108 hybrid clones present in the original panel) was used. A total of 39 equine genes/ESTs were synteny-mapped. Primer pairs for 33 ESTs were from a horse fetal cDNA library (60-day whole fetus; Dr. R. Brandon, University of Queensland, Australia). Primers for six genes were generated in the following way: CATS (Lyons et al. 1997) or universal primers (Venta et al. 1996) were used to amplify horse-specific PCR product. The PCR product was sequenced, and horse-specific primers were then designed. Details on gene names, primer pairs, PCR conditions, etc. are provided in Table 1. Following typing of the panel by PCR, the products were run on a 2% agarose gel, and the results were scored as + or −. Correlation coefficients were calculated between all markers as described (Caetano et al. 1999a,b). A correlation value of 0.70 was accepted as evidence for synteny between two markers (Chevalet and Corpet 1986).

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The First-Generation Whole-Genome Radiation Hybrid Map in the Horse Identifies Conserved Segments in Human and Mouse Genomes

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Figure 1 (enclosed as a poster in the April issue) in the above article was printed incorrectly. The centromeres were missing in the G-banded ideograms of each chromosome. In every case, a representation of the centromere should have appeared at band 11. We apologize for any confusion this may have caused.

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The paper that follows is being reprinted to correct a printing error (color was omitted) in the previous issue.

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