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A Comparative Gene Map of the Horse (*Equus caballus*)

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A comparative gene map of the horse genome composed of 127 loci was assembled based on the new assignment of 68 equine type I loci and on data published previously. PCR primers based on consensus gene sequences conserved across mammalian species were used to amplify markers for assigning 68 equine type I loci to 27 horse synteny groups established previously with a horse-mouse somatic cell hybrid panel (SCHP, UC Davis). This increased the number of coding genes mapped to the horse genome by over 2-fold and allowed refinements of the comparative mapping data available for this species. In conjunction with 57 previous assignments of type I loci to the horse genome map, these data have allowed us to confirm the assignment of 24 equine synteny groups to their respective chromosomes, to provisionally assign nine synteny groups to chromosomes, and to further refine the genetic composition established with Zoo-FISH of two horse chromosomes. The equine type I markers developed in this study provide an important resource for the future development of the horse linkage and physical genome maps.

The rapid progress being made in the development of genetic maps for humans and mice (Hudson et al. 1995; Stewart et al. 1997; Rhodes et al. 1998) has led to a recent boom in the construction of genome maps for a number of domesticated mammalian species of economic importance (Bishop et al. 1994; Archibald et al. 1995; O'Brien et al. 1997a; de Gortari et al. 1998). Many recent technological advances have contributed to the generation of these data which will greatly increase our ability to find and isolate genes that lead to genetic diseases and/or have an effect on economically important production traits of livestock (Cockett et al. 1994; Georges and Andersson 1996; Grobet et al. 1997).

Molecular markers based on repetitive or anonymous DNA sequences (Type II markers, O'Brien et al. 1993) such as microsatellites, Random Amplified Polymorphic DNA (RAPDs), and Amplified Fragment Length Polymorphisms (AFLPs) have been used extensively to saturate the genetic maps of various species because of their technical advantages and high degree of polymorphism (O'Brien et al. 1993; Georges and Andersson 1996; Andersson et al. 1996). Type II markers, however, are seldom informative across mammalian orders and therefore do not allow for the comparison of genetic maps from different species (O'Brien et al. 1993; Georges and Andersson 1996; Andersson et al.

1996). Conversely, molecular markers for functional genes conserved across species (Type I anchor loci, O'Brien et al. 1993) can be used for this purpose.

In conjunction with chromosome painting (Zoo-FISH) studies, mapping studies with type I loci have revealed that genomes from related species have a high degree of synteny conservation (O'Brien et al. 1993; Andersson et al. 1996; Wakefield and Graves 1996). This information forms the basis for comparative genome mapping, a discipline that allows the prospect of using information from highly characterized genomes to study genetic phenomena in map-poor species (O'Brien et al. 1993; Georges and Andersson 1996; Andersson et al. 1996; Wakefield and Graves 1996).

Although the horse genome is not as highly characterized as the genomes of other domestic animals, much progress has been made recently. Somatic cell hybrid (SCH) panels have been used to make synteny assignments of 240 type II markers (Bailey et al. 1995; Shiue et al. 1999), many of which were physically assigned to horse chromosomes by FISH (Sakagami et al. 1995; Tozaki et al. 1995; Breen et al. 1997; Godard et al. 1997, 1998). In addition, the first low-resolution microsatellite-based linkage maps of the horse have been published (Lindgren et al. 1998; Guérin et al. 1999). In the horse, a framework for comparative mapping has been established with chromosome painting studies (Raudsepp et al. 1996, 1997; Rettenberger et al. 1996), FISH assignments of type I loci (Lear et al. 1998a,b,c;

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Godard et al. 1998), and by synteny assignments of type I loci with SCH panels (Williams et al. 1993; Bailey et al. 1995; Caetano et al. 1999a,b). Using human chromosome-specific probes, Zoo-FISH was used to determine that a minimum of 21 chromosomal breaks, followed by the appropriate rearrangements, are necessary to reconstruct the horse karyotype relative to the human karyotype (Raudsepp et al. 1996; Rettenberger et al. 1996; Raudsepp et al. 1997; Chaudhary et al. 1998). However, in addition to these advancements in the equine genome map, a larger group of type I anchor loci needs to be mapped to allow for thorough comparisons with maps from model species and the study of the fine intrachromosomal gene structure of the horse genome.

Despite their value for comparative mapping, the incorporation of type I anchor loci to genome maps of multiple species has not been used extensively because of technical reasons. Large collections of PCR primer sets for type I loci [i.e. universal mammalian–sequence-tagged sites (UM-STs), comparative anchor tagged sequences (CATS)] have been designed to amplify mammalian sequences by taking advantage of public sequence databases (Venta et al. 1996; Lyons et al. 1997). In this report we present the synteny assignment of 68 equine type I loci by analysis of a horse-mouse SCH panel with PCR-based markers generated with universal primers for mammalian genes.

RESULTS

Development of PCR-Based Markers for Equine Type I loci

In addition to amplifying gene-specific fragments, a key feature necessary for the effective use of PCR-based markers in synteny mapping with interspecific SCH panels is the ability to differentiate between fragments amplified from the donor species (i.e. horse) and the murine background in each hybrid cell line. Most of the primers used in this study were designed to anneal to conserved short exon sequences flanking introns, which should therefore result in the amplification of fragments of different sizes and/or sequences in the two species.

A total of 289 previously published primers for mammalian type I loci (Venta et al. 1996; Lyons et al. 1997) were tested for their ability to amplify horse-specific fragments useful for synteny mapping with a SCH panel. PCR conditions were optimized to amplify markers for 42 equine type I loci (Table 1). Digestions of PCR products from mouse and horse using restriction enzymes were used to obtain gene-specific equine markers with an additional 26 primer sets. In one instance (*RB1*), an equine-specific primer was designed (Table 1) based on the sequence obtained from a fragment amplified from horse with primers published pre-

viously for this gene (Venta et al. 1996). The remaining primers amplified no fragments (5 primers), multiple equine fragments (180 primers), or fragments from horse and mouse that could not be differentiated with the techniques used (36 primers).

Sequencing PCR Products

To verify that the PCR products used for making synteny assignments correspond to the expected equine gene homologs, a total of 50 isolated PCR fragments amplified from a thoroughbred horse were cloned and sequenced. The remaining 18 horse PCR products were gel-isolated and partially sequenced by direct sequencing (Table 1). All of the nucleotide sequences obtained were used to search GenBank with the BLAST routine and confirm the identity of 66 of the genes mapped. In two instances (*KRAS2*, *SOD2*), the cloned equine PCR fragment lacked the expected intron sequence, suggesting that the PCR products mapped are pseudogenes.

Many of the primers that amplified horse-specific fragments were designed to target specific genes from large paralogous gene families. Several of the isolated PCR products cloned were found to contain multiple fragments of highly homologous sequences, most likely amplified from different syntenic isoforms of paralogous genes (Table 1). For example, the *MYH6* primers (Lyons et al. 1997) amplified two equine fragments that are 97% identical at the expected exon regions. These equine fragments are highly homologous to the human myosin heavy polypeptide alpha and beta genes (*MYH6* and *MYH7*, respectively), which are closely linked and show 93% sequence identity. Similarly, the primers used to amplify two highly homologous equine fragments from the equine *IL1B* gene (92% identity) were originally designed to amplify *IL1A* (Lyons et al. 1997), two genes that are closely linked in human Chromosome 2. Therefore, although these markers were useful for determining the equine synteny assignment of the respective loci, further characterization will be necessary for determining which gene sequence was amplified from these paralogous gene families in the horse.

In one instance (*SST*), the equine PCR product contained fragments with the expected sequence in addition to fragments with completely unrelated sequence, except for the primer binding sites. Primers specific to the anonymous equine fragment were designed (F-5'-TTTCCATGGACTTATTTCCC-3'/R-5'-TCCCTTGT-TACCTGGAGTATG-3'). The PCR product amplified with these primers was only found in the SCH clonal lines where the *SST* product was found, suggesting this unrelated sequence is syntenic with the equine *SST* gene.

SCH Panel Analysis with Equine PCR-based Type I Loci

The genome of the horse consists of 31 autosomes plus

Table 1. PCR Conditions for Amplifying 68 Equine Type I Loci with Universal Primers

Hsa	UCD	Symbol	Locus name	Anl T. °C	MgCl (mM)	R.E.	Size (bp)	Accession no. ^a
1p36.2	2	<i>NPPA</i>	natriuretic peptide precursor A	50	2	<i>HinfI</i>	305	AF134229
1p13.1	5	<i>NGFB</i>	nerve growth factor, β	53	2.5	<i>RsaI</i>	158	AF134228
1q21	5	<i>GBA</i>	glucosidase, β acid	53	2	<i>RsaI</i>	345	AF134220
1q23–q25.1	5	<i>AT3</i>	antithrombin III	55	2	—	187	AF134055
1q31	5	<i>LAMC1</i>	laminin, C-1	50	2	—	~1400	AF134225, 6
2p21	15	<i>SPTBN1^b</i>	spectrin, β , nonerythrocytic 1	50	2	—	975	AF130782
2q12–q14	15	<i>PAX8</i>	paired box homeotic gene 8	55	2	<i>HaeIII</i>	147	AF130778
2q14	15	<i>IL1β^{c,d}</i>	interleukin-1 β	55	1	<i>HindIII</i>	~906	AF130767, 8
2q24–q32	18	<i>CHRNA</i>	cholinergic receptor, nicotinic, α	58	1	—	405	AF130750
2q31–q32	18	<i>NEB</i>	nebulin	56	1	<i>PvuII</i>	~2000	AF130774, 5
2q33–q34	D	<i>CHRNA</i>	cholinergic receptor, nicotinic, γ	60	1	—	264	AF130751
2q34	D	<i>FN1</i>	fibronectin 1	55	2	—	1009	AF130764
3p24.3–p24.2	16	<i>RARB</i>	retonic acid receptor, β	55	3	<i>CfoI</i>	208	AF13424
3p21.33	16	<i>GLB1^b</i>	galactosidase, β -1	50	2	<i>RsaI</i>	429	AF130765
3q21–q24	16	<i>CP</i>	ceruloplasmin	55	2.5	—	~1500	AF134057
3q21–q24	16	<i>RHO^b</i>	rhodopsin	55	2	<i>RsaI</i>	327	AF130780
3q28	19	<i>SST^e</i>	somatostatin	50	1	—	931	AF130783
4q22	3	<i>ADH2</i>	alcohol dehydrogenase 2	52	1	—	295	AF133272
4q22	3	<i>ADH3</i>	alcohol dehydrogenase 3	52	2	—	>2000	AF134056
4q28	2	<i>FGG</i>	fibrinogen, γ polypeptide	53	2	—	~1100	AF134218, 9
5q32–q34	14	<i>ADRB2</i>	adrenergic receptor, β -2	55	2	<i>HinfI</i>	354	AF130746
6p24–p23	20	<i>EDN1^b</i>	endothelin-1	58	2	—	266	AF130760
6q13	20	<i>COL9a1^b</i>	collagen, type IX, α -1	50	2	—	292	AF130755
6q21.1–q23	10	<i>CGA</i>	chorionic gonadotropin, α chain	45	4	—	242	AF130749
6q21–q22.3	10	<i>COL10^b</i>	collagen, type X, α 1	50	2	<i>PvuII</i>	365	AF130754
6q25.3	14	<i>SOD2^{ps9}</i>	superoxide dismutase 2	55	2	—	154	AF130781
7p15–p14	4	<i>TCRG</i>	t cell antigen receptor, γ subunit	42	2.5	<i>HinfI</i>	252	AF134235
7q11.2	13	<i>ELN^b</i>	elastin	58	1	—	263	AF130761
7q21.11	13	<i>GUSB</i>	glucuronidase β	53	2	<i>Sau3AI</i>	382	AF134223
7q36	4	<i>EN2</i>	Engrailed 2	53	2	<i>PvuII</i>	189	AF134060
8q24.2–q24.3	9	<i>TG</i>	thyroglobulin	58	1	—	749	AF130785
9p22	23	<i>IFNA1</i>	interferon, α -1	53	2	<i>PvuII</i>	373	AF135017
9p21	23	<i>IFNB1</i>	interferon, β -1	55	2	<i>HaeIII</i>	450	AF134227
9p13	23	<i>CNTFR</i>	ciliary neurotrophic f. recept.	57	2.5	—	456	AF134058
9q31	25	<i>TXN</i>	thioredoxin	55	2	<i>HinfI</i>	~780	AF134237
9q34	25	<i>GRP78</i>	glucose-regulated protein	55	2	<i>PvuII</i>	~740	AF134222
10p13	29	<i>VIM</i>	vimentin	57	2	—	~1230	AF135018
11p15.5	7	<i>HBB</i>	hemoglobin, β	50	2.5	—	171	AF134224
11p15.3–p15.1	7	<i>PTH</i>	parathyroid hormone	55	2	—	311	AF134233
11cen–q13	12	<i>ADRBK1</i>	adrenergic receptor β kinase I	53	1	—	328	AF134059
11q13	12	<i>CD20^b</i>	CD20 antigen	55	2	—	1007	AF130748
11q23	7	<i>DRD2</i>	dopamine receptor D2	50	2.5	—	~1300	AF134061, 2
11q23.3	7	<i>THY1</i>	Thy-1 T-cell surface antigen	50	2	—	~800	AF134236

(Continued on following page.)

the X and Y sex-chromosomes. Previous work with the UC Davis horse-mouse SCH panel established 33 equine synteny groups. A total of 25 synteny groups were assigned to their respective horse chromosomes by correlation with microsatellites and type I loci mapped previously by FISH (Shiue et al. 1999; Caetano

et al. 1999a), with microsatellites assigned by DNA typing of trisomic horses (Bowling et al. 1997) and with sex-chromosome type I markers (Shiue 1999). Chromosome assignments of synteny groups were made based on a range of one to six physically assigned loci.

The 68 markers for equine type I loci we charac-

Table 1. (Continued)

Hsa	UCD	Symbol	Locus name	Anl T. °C	MgCl (mM)	R.E.	Size (bp)	Accession no. ^a
12p12.1	9	<i>KRAS2ps^g</i>	Kirsten murine sarc. 2 viral onc., ps.	58	1	—	329	AF130769
12q13.3	D	<i>PFKM</i>	phosphofructokinase m	60	2	—	~1600	AF134230, 1
12q14	D	<i>IFNG</i>	Interferon γ	45	2	—	325	AF130766
12q24.2	6	<i>TCF1</i>	transcription factor 1	58	1	—	602	AF130784
13q14.1q-14.2	17	<i>RB1^f</i>	retinoblastoma	55	2	—	705	AF130779
14q11.2	1	<i>CHY^b</i>	chymase- mast cell	58	2	—	697	AF130752
14q12	1	<i>MYH6^d</i>	myosin, heavy pol. 6 &/or 7, see text	58	2	<i>RsaI</i>	~650	AF130771, 2
15q22-qter	1	<i>CYP1A2</i>	cytochrome P450, subf. I, polyp. 2	50	2.5	<i>HinfI</i>	278	AF134063
15q26.1	1	<i>FES^b</i>	feline sarcoma virus homologue	58	2	—	510	AF130763
17q11.2	11	<i>EVI2A</i>	ecotropic viral int. site 2A	55	4	—	156	AF130762
17q11.2	11	<i>NF1</i>	neurofibromatosis I	58	2	—	380	AF130776
17q25	11	<i>P4HB</i>	prolyl-4 hydroxylase β	62	2	—	460	AF130777
17	11	<i>MYL4</i>	myosin light chain 4	58	2	—	682	AF130773
18p11.32	6	<i>TS^b</i>	thymidylate synthetase	55	1	—	539	AF130786
19q13	10	<i>CKM^b</i>	creatine kinase, muscle	58	1	—	819	AF130753
20pter-p12	22	<i>PRNP</i>	prion protein	55	1.5	—	191	AF134232
20q13.2	22	<i>GNAS1</i>	guanine nuc. binding protein	50	1.5	<i>RsaI</i>	324	AF134221
20q13.11	22	<i>ADA</i>	adenosine deaminase	50	1.5	—	~1350	AF135790
21q22.3	26	<i>ETS2</i>	avian erythroblastosis virus onc. 2	55	2	<i>HinfI</i>	~1000	AF134064
22q13.1	C	<i>CYP2D^d</i>	cytochrome P450, subfamily IID	58	2	—	980	AF130756- 9
22q13.31-qter	C	<i>ARSA</i>	arylsulfatase A	58	2	—	247	AF130747
Xp11.21	X	<i>ALAS2</i>	Δ -aminolevulinatase synthase	53	2	<i>HaeIII</i>	~800	AF133200
Xq27.1-q27.2	X	<i>F9</i>	coagulation factor IX	55	2	—	453	AF133201
Xq28	X	<i>BGN</i>	biglycan	57	2	<i>HinfI</i>	~710	AF135019, 0
Xq28	X	<i>G6PD</i>	glucose-6-phosphate dehydro.	55	2	<i>HinfI</i>	~750	AF133202

All primers used in this study were published by Lyons and coworkers (1997), with the following exceptions:

^aMultiple accession numbers indicated sequences obtained from the 5' and 3' ends of partially sequenced PCR products, or multiple highly homologous sequences most likely amplified from different syntenic isoforms of paralogous genes (see text).

^bPrimers published by Venta and associates (1996).

^cThe primers used to amplify equine *IL1B* were originally designed to amplify *IL1A* (see text).

^dThese primers amplify multiple equine fragments of different but highly homologous sequences (see text).

^eAmplifies a fragment of unknown sequence in addition to the equine *SST* gene (see text).

^fThe forward primer used to amplify equine *RB1* (5'-TTTGATATCGAAGGGTCTGAC-3') was designed in this study and the reverse primer was as in Venta et al. (1996).

^gThese primers amplify equine sequences lacking expected introns and therefore are thought to be horse pseudogenes from the respective loci.

terized were mapped to 27 different syntenic groups (Table 2). Forty-five of these markers were mapped to 18 syntenic groups that had been assigned previously to specific horse chromosomes. The remaining 23 markers were mapped to 9 syntenic groups which are not presently assigned to equine chromosomes.

DISCUSSION

Sixty-eight markers for equine type I loci were characterized and mapped by SCH panel analysis. These data, combined with 26 type I loci previously assigned with the UC Davis SCH panel (Caetano et al. 1999a,b) and

32 type I loci mapped by other groups working on the characterization of the horse gene map, provide the most comprehensive comparative mapping data currently available for the horse genome (Table 3), when considered in conjunction with chromosome painting studies of the horse karyotype (Raudsepp et al. 1996, 1997; Rettenberger et al. 1996; Chaudhary et al. 1998). These data have allowed us to confirm the assignment of 24 equine syntenic groups to their respective chromosomes, to provisionally assign nine syntenic groups, and to further refine the genetic composition of two horse chromosomes established previously with ZooFISH.

Table 2. Correlation Values of 68 Equine Type I Loci with Markers Previously Assigned to UCD Davis Syntenic Groups and/or Horse Chromosomes

UCD ¹	1	2	3	4	5	D	6	7	8	9	9	10	10	11	12	13	13	14	15	16	16	17	18	19	20	22	23	23	25	26	C	29	32
ECM ²	1	2	3	4	[5]	6	7	[8]	9	9	10	10	11	12	13	13	[14]	15	[16]	[16]	[17]	18	19	20	22	23	23	[25]	26	[28]	[29]	X	
	HMS15 ^{3,4}	ASB13	SGCV18	HMS19	LEX04	LEX06	C3	ASB14	DNAPK	HTG4	ASB6	ASB9	SGCV13	IGF2	VHL161	ASB1	CSF2	HTG6	UM042	I-F8	LEX055	UCDEQ387	ASB7	HTG5	HMS47	SGCV04	CNTFR ⁵	UCDEQ405	A-17	IGFI	VIAS-HB9	LEX003	
CHY	0.91	0.22	0.50	0.30	0.15	0.10	0.26	0.23	0.37	0.56	0.39	0.39	0.39	0.35	0.12	0.32	0.30	0.40	0.42	0.27	0.36	0.10	0.25	0.27	0.02	-0.01	0.03	0.14	0.19	-0.05	0.06	0.46	
CYP1A2	0.84	0.19	0.45	0.33	0.15	0.17	0.28	0.22	0.31	0.52	0.41	0.37	0.38	0.34	0.15	0.32	0.32	0.42	0.40	0.24	0.37	0.10	0.24	0.25	0.03	0.04	0.03	0.08	0.17	-0.05	0.05	0.48	
FES	0.71	0.15	0.34	0.26	0.06	0.06	0.22	0.15	0.32	0.40	0.28	0.32	0.21	0.25	0.17	0.22	0.15	0.32	0.20	0.14	0.30	0.03	0.25	0.10	-0.01	0.02	-0.05	-0.05	0.17	-0.04	0.04	0.37	
MYH6	0.92	0.21	0.49	0.27	0.10	0.06	0.22	0.20	0.31	0.49	0.36	0.37	0.36	0.27	0.12	0.31	0.28	0.37	0.36	0.20	0.35	0.05	0.28	0.20	0.05	-0.01	0.02	0.11	0.20	-0.06	0.02	0.44	
PGG	0.16	0.84	0.08	0.14	0.15	0.07	0.50	0.28	0.10	0.28	0.28	0.27	-0.05	0.06	0.24	0.33	0.01	0.21	0.06	0.05	0.11	0.24	0.29	0.07	0.32	0.12	0.10	0.23	0.16	0.30	0.16	0.01	
NPP4	0.21	0.84	0.20	0.12	0.24	0.15	0.57	0.27	0.08	0.27	0.34	0.29	0.03	0.05	0.18	0.37	0.14	0.31	0.18	0.08	0.24	0.36	0.32	0.14	0.50	0.16	0.14	0.11	0.15	0.53	0.19	0.14	
ADH2	0.35	0.09	0.77	0.32	0.31	0.37	0.24	0.19	0.45	0.50	0.24	0.12	0.35	0.36	0.20	0.25	0.34	0.21	0.25	0.25	-0.13	-0.05	0.18	0.36	0.08	0.20	0.30	0.28	0.23	0.12	0.11	0.25	
ADH3	0.30	0.12	0.73	0.40	0.28	0.35	0.11	0.15	0.40	0.41	0.09	-0.03	0.32	0.32	0.17	0.23	0.26	0.21	0.20	0.17	-0.18	-0.13	0.11	0.31	0.01	0.26	0.31	0.26	0.18	0.09	0.09	0.26	
EN2	0.20	0.02	0.44	0.85	0.25	0.41	0.16	0.21	0.35	0.36	0.15	0.06	0.33	0.65	0.10	0.26	0.32	0.17	0.45	0.46	-0.19	0.04	-0.05	0.47	-0.04	0.45	0.28	0.22	0.17	0.21	0.16	0.39	
TCRG	0.16	0.01	0.38	0.77	0.25	0.33	0.06	0.13	0.29	0.28	0.03	-0.03	0.25	0.46	0.14	0.19	0.21	0.06	0.32	0.37	-0.25	-0.03	0.00	0.39	-0.15	0.29	0.13	0.22	0.18	0.18	-0.03	0.26	
AT3	0.17	0.10	0.38	0.26	0.76	0.11	0.23	0.51	0.36	0.08	0.22	0.17	0.40	0.13	0.05	0.04	0.10	0.45	0.41	0.30	-0.14	0.12	-0.21	0.14	0.05	0.44	0.38	0.34	0.11	0.30	0.48	0.25	
GBA	0.22	0.15	0.45	0.37	0.86	0.21	0.33	0.61	0.40	0.22	0.32	0.17	0.42	0.26	0.00	0.13	0.23	0.46	0.57	0.46	-0.15	0.25	-0.11	0.29	0.10	0.53	0.44	0.37	0.14	0.40	0.47	0.26	
LAMC1	0.17	0.10	0.38	0.26	0.80	0.15	0.23	0.51	0.41	0.12	0.22	0.17	0.44	0.17	0.05	0.04	0.10	0.45	0.45	0.34	-0.14	0.16	-0.17	0.18	0.05	0.47	0.42	0.38	0.15	0.30	0.48	0.25	
NGFB	0.12	0.11	0.43	0.25	0.96	0.19	0.29	0.47	0.32	0.10	0.23	0.04	0.37	0.21	0.06	0.07	0.22	0.37	0.45	0.33	-0.12	0.15	-0.09	0.30	0.16	0.43	0.39	0.25	0.07	0.28	0.38	0.22	
CHRNA3	0.17	0.16	0.33	0.40	0.21	0.88	0.13	0.11	0.33	0.23	0.09	0.00	0.30	0.45	0.15	0.07	0.15	0.15	0.29	0.33	-0.17	0.02	-0.03	0.40	0.15	0.26	0.23	0.19	0.09	0.15	-0.03	0.23	
FN1	0.30	0.16	0.30	0.37	0.32	0.72	0.12	0.09	0.28	0.16	0.13	0.04	0.35	0.45	0.12	0.01	0.30	-0.01	0.36	0.28	-0.04	0.02	-0.09	0.47	-0.09	0.28	0.19	0.18	0.04	0.11	0.11	0.32	
IFNG	0.17	0.10	0.34	0.51	0.16	0.88	0.09	0.10	0.38	0.31	0.06	0.01	0.31	0.49	0.16	0.09	0.18	-0.07	0.33	0.40	-0.22	0.01	-0.04	0.40	-0.22	0.31	0.24	0.24	0.17	0.15	0.07	0.29	
PPKM	0.15	0.11	0.33	0.42	0.10	0.84	0.07	0.06	0.30	0.23	0.12	0.08	0.27	0.50	0.08	-0.03	0.22	-0.10	0.26	0.30	-0.23	-0.09	-0.03	0.44	-0.16	0.27	0.26	0.19	0.13	0.12	0.08	0.22	
DRD2	0.21	0.39	0.20	0.14	0.25	0.05	0.84	0.19	0.11	0.21	0.36	0.25	0.06	0.08	0.08	0.32	0.04	0.35	0.23	0.14	0.19	0.38	0.25	0.13	0.49	0.23	0.21	0.05	0.27	0.27	0.23	0.11	
HBB	0.17	0.41	0.29	0.12	0.24	0.02	0.93	0.22	0.17	0.23	0.54	0.35	0.03	0.14	0.05	0.28	0.08	0.41	0.27	0.18	0.24	0.45	0.19	0.19	0.59	0.25	0.23	0.06	0.24	0.33	0.32	0.14	
PTH	0.18	0.46	0.29	0.12	0.31	0.10	0.95	0.23	0.19	0.22	0.50	0.28	0.07	0.09	0.18	0.31	0.05	0.32	0.24	0.14	0.16	0.40	0.23	0.20	0.57	0.27	0.31	0.17	0.34	0.37	0.28	0.13	
THY1	0.19	0.48	0.30	0.08	0.26	0.05	0.92	0.18	0.15	0.21	0.46	0.33	0.02	0.04	0.13	0.33	0.02	0.33	0.21	0.11	0.20	0.39	0.29	0.17	0.60	0.25	0.26	0.14	0.31	0.32	0.23	0.12	
TCF1	0.21	0.29	0.32	0.22	0.59	0.19	0.21	0.79	0.31	0.21	0.27	0.23	0.11	0.16	0.12	0.13	0.21	0.29	0.19	0.16	-0.23	0.07	-0.01	0.14	0.14	0.34	0.25	0.10	-0.03	0.25	0.45	0.13	
IS	0.18	0.23	0.28	0.21	0.52	0.09	0.19	0.98	0.29	0.25	0.25	0.21	0.07	0.15	-0.04	0.10	-0.01	0.31	0.30	0.27	-0.22	0.11	-0.18	-0.01	0.31	0.13	0.17	-0.12	0.53	0.41	0.11		
TG	0.38	0.09	0.61	0.52	0.36	0.41	0.20	0.38	0.84	0.67	0.25	0.25	0.42	0.56	0.11	0.15	0.08	0.21	0.44	0.49	-0.27	-0.01	0.12	0.33	-0.19	0.26	0.26	0.43	0.43	0.24	0.22	0.26	
KRAS2ps	0.49	0.18	0.42	0.26	0.16	0.12	0.28	0.22	0.64	0.82	0.33	0.31	0.23	0.26	0.20	0.32	-0.11	0.31	0.25	0.30	0.22	0.17	0.32	0.10	-0.02	0.03	0.08	0.33	0.33	0.04	0.11	0.34	
CGA	0.37	0.31	0.24	0.15	0.23	0.06	0.41	0.35	0.25	0.36	0.81	0.75	0.12	0.27	0.02	0.18	0.06	0.35	0.26	0.16	0.14	0.46	0.19	0.18	0.22	0.27	0.19	0.19	0.10	0.50	0.28	0.20	
CKM	0.32	0.17	0.24	0.06	0.21	-0.02	0.46	0.26	0.20	0.27	0.91	0.58	0.12	0.23	0.06	0.18	0.16	0.49	0.26	0.11	0.26	0.42	0.10	0.22	0.31	0.27	0.11	0.05	0.10	0.30	0.37	0.30	
COL10A1	0.36	0.34	0.14	-0.03	0.04	0.08	0.32	0.22	0.14	0.25	0.59	0.78	0.04	0.17	-0.04	0.07	-0.05	0.26	0.15	0.05	0.13	0.27	0.27	0.05	0.14	0.08	0.05	0.07	0.14	0.17	0.13	0.08	
EV2A	0.44	-0.16	0.48	0.43	0.34	0.34	0.07	0.15	0.45	0.20	0.19	0.07	0.85	0.54	0.15	0.09	0.46	0.15	0.47	0.33	-0.06	-0.03	-0.07	0.37	-0.22	0.25	0.26	0.31	0.30	0.05	0.17	0.41	
MYL4	0.38	-0.08	0.45	0.33	0.35	0.33	0.02	0.10	0.47	0.18	0.18	0.11	0.93	0.55	0.17	0.04	0.31	0.18	0.48	0.34	-0.05	-0.08	-0.03	0.37	-0.20	0.25	0.36	0.44	0.39	-0.01	0.15	0.39	
NF1	0.48	-0.05	0.57	0.45	0.43	0.36	0.10	0.23	0.50	0.32	0.26	0.15	0.91	0.63	0.14	0.13	0.45	0.19	0.57	0.45	-0.07	0.01	0.03	0.46	-0.13	0.35	0.36	0.38	0.35	0.07	0.18	0.46	
P4FB	0.46	-0.01	0.43	0.21	0.32	0.26	0.09	0.08	0.47	0.28	0.26	0.14	0.85	0.45	0.28	0.12	0.27	0.21	0.43	0.29	0.11	-0.01	0.00	0.32	-0.12	0.22	0.35	0.35	0.39	-0.02	0.18	0.47	
ADRBK1	0.41	-0.06	0.52	0.50	0.19	0.39	0.02	0.14	0.36	0.20	0.23	0.20	0.62	0.88	0.16	0.19	0.49	0.20	0.58	0.41	-0.09	0.00	-0.14	0.59	-0.12</								

Table 3. Human–Horse Comparative Gene Map Using Synteny, Linkage and in Situ Hybridization (127 Genes)

Hsa ^a	Locus	Locus name	ECA ^b	Method ^c	Bta ^d	Mmu ^e	Fca ^f	Ssc ^g
1p36.2	<i>NPPA</i>	natriuretic peptide precursor, A	2	S	16	4	—	—
1p36.2–p36.13	<i>PGD</i>	phosphogluconate dehydrogenase	2p12	I,L	16	4	C1	6q2.2 q2.5
1p31	<i>PGM1</i>	phosphoglucomutase 1	5	L	3	5	C1	6
1p13.1	<i>NGFB</i>	nerve growth factor, β	5	S	3	3	—	4q1.6–q2.3
1q21	<i>GBA</i>	glucosidase, β, acid	5	S	3	3	—	4
1q23–q25.1	<i>AT3</i>	antithrombin III	5	S	16	1	—	—
1q31	<i>LAMC1</i>	laminin, C1	5	S	16	1	—	—
2p25	<i>ODC1</i>	ornithine decarboxylase	15q27	I	—	12	—	—
2p21	<i>CAD</i>	carbamoylphosphate synthetase	15q25	I	—	1	—	—
2p21	<i>SPTBN1</i>	spectrin, β, nonerythrocytic 1	15	S	—	11	—	—
2q14	<i>IL1B</i>	interleukin 1, β	15	S	2	2	—	3q1.1–q1.4
2q12–q14	<i>PAX8</i>	human paired domain	15	S	—	2	—	—
2q21	<i>LCT</i>	lactase-phlorizin hydrolase	15q21	I	—	1	—	15q1.3
2q32.1	<i>MSTN</i>	myostatin	18	S	2	1	—	—
2q24–q32	<i>CHRNA</i>	cholinergic receptor, nicotin, α	18	S	2	2	—	—
2q31–q32	<i>NEB</i>	nebulin	18	S	2	2	—	—
2q33–34	<i>CHRNA3</i>	cholinergic receptor, nicotin, γ	[6]	S	2	1	—	—
2q34	<i>FN1</i>	fibronectin 1	[6]	S	2	1	—	—
3p24.3–24.2	<i>RARB</i>	retinoic acid receptor, β	16	S	—	14	—	—
3p21.33	<i>GLB1</i>	galactosidase, β, 1	16	S	—	9	B3	—
3q21–q24	<i>RHO</i>	rhodopsin	16	S	22	6	—	—
3q21	<i>TF</i>	transferrin	16q23	S	1	9	—	13q3.1
3q21–q23	<i>LTF</i>	lactotransferrin	16q	I	22	9	—	—
3q21–q24	<i>CP</i>	ceruloplasmin	16	S	1	9	—	13q3.2–q3.3
3q28	<i>SST</i>	somatostatin	19	S	1	16	—	—
4p16.3	<i>FGFR3</i>	fibroblast growth factor receptor	3	S	6	5	—	—
4q11–q13	<i>ALB</i>	albumin	3q14	S,I	6	5	—	8q1.2
4q12	<i>GC</i>	group specific component	3	L	6	5	—	—
4q12	<i>KIT</i>	mast cell growth factor receptor	3q21	L,I	6	5	B1	8p12–p21
4q12	<i>PDGFRA</i>	platelet derived growth factor A	3q21	I, L	6	5	—	8p12
4q22	<i>ADH2</i>	alcohol dehydrogenase 2	3	S	6	3	—	—
4q22	<i>ADH3</i>	alcohol dehydrogenase 3	3	S	6	3	—	8
4q26–q27	<i>IL2</i>	interleukin 2	2	S	17	3	B1	8
4q28	<i>FGG</i>	fibrinogen, γ polyp	2	S	17	3	—	8
5p13	<i>IL7R</i>	interleukin receptor 7	21	S	—	15	—	—
5p13–p12	<i>GHR</i>	growth hormone receptor	21	S	20	15	—	16q1.3–q1.4
5p14	<i>C9</i>	complement component 9	21	S	1	—	—	16q14
5q11–q12	<i>CTLA3</i>	cytotoxic T-1 assoc serine es 3	21	S	—	13	—	—
5q13	<i>HEXB</i>	hexoseaminidase b	14	S	20	13	—	—
5q21–q23	<i>CAMK4</i>	calmodulin depend. Prot. kinase 4	14	S	—	18	—	—
5q31.1	<i>CSF2</i>	colony stimulating factor 2	14	S	7	11	—	5
5q31–q32	<i>SPARC</i>	secreted pro, ac, cyst-rich	14	S	7	11	—	—
5q32–34	<i>ADRB2</i>	adrenergic receptor, β2	14	S	7	18	—	—
5q33.2–q33.3	<i>CSF1R</i>	colony stimulating factor 1 receptor	14	S	7	18	A1	—
6pter–p21.1	<i>CLPS</i>	colipase	20	S	—	17	—	7
6p25–p24	<i>F13</i>	coagulation factor 13, A	20q13	L,I	23	—	—	4
6p21.2	<i>PIM1</i>	Pim-1 oncogene	20q24	I	—	17	B2	—
6p21	<i>HLA</i>	major histocompatibility complex	20q	L,I,S	23	17	B2	7
6p21.3	<i>CYP21</i>	cytochrome P450	20	L	23	17	—	7
6p21.3	<i>C4</i>	complement component 4	20	L	23	17	—	7
6p21.3	<i>TNFA</i>	tumor necrosis factor, A	20	S	23	17	—	7p1.1–q1.1
6q13	<i>COL9A1</i>	collagen type IX, α-1	20q24	S	—	1	—	—
6q24–p23	<i>EDN1</i>	endothelin 1	20	S	—	13	—	7p1.3–p1.2
6q12	<i>ME1</i>	malic enzyme	10q12	S,I	9	9	B2	1
6q21.1–q23	<i>CGA</i>	glycoprotein hormone, α chain	10	S	9	4	—	1
6q21–q22.3	<i>COL10A1</i>	collagen, type X, α 1	10	S	—	10	—	—
6q25.1	<i>ESR</i>	estrogen receptor	31q15	S	9	6	—	1p2.5–p2.4
6q26	<i>PLG</i>	plasminogen	31	S	—	17	—	—

Table 3. (Continued)

Hsa ^a	Locus	Locus name	ECA ^b	Method ^c	Bta ^d	Mmu ^e	Fca ^f	Ssc ^g
7p15–p14	<i>TCRG</i>	T-cell receptor, γ subunit	4	S	4	13	A2	—
7q11.2	<i>ELN</i>	elastin	13	S	25	5	—	—
7q11.23–q21	<i>GUSB</i>	glucuronidase, β	13	S	25	5	E3	—
7q31.3	<i>LEP</i>	leptin	4	S	4	6	—	18
7q36	<i>EN2</i>	Engrailed 2	4	S	—	5	—	—
8q11	<i>DNAPK</i>	DNA protein kinase, catalytic sub.	9p12	S	—	16	—	—
8q24	<i>TG</i>	thyroglobulin	9	S	14	15	—	—
9p22	<i>IFNA1</i>	interferon, α -1	23	S	8	4	—	1
9p21	<i>IFNB1</i>	interferon, β -1	23	S	8	4	—	1
9p13	<i>CNTFR</i>	ciliary neurotrophic factor receptor	23	S	8q21	4	—	—
9q31	<i>TXN</i>	thioredoxin	25	S	8	4	—	—
9q33	<i>GRP78</i>	glucose-regulated protein	25	S	11	2	—	1q2.12q2.13
10p13	<i>VIM</i>	vimentin	29	S	13	2	—	10
11p15.3–p15.1	<i>PTH</i>	parathyroid hormone	7	S	15	7	—	2
11p15.5	<i>HBB</i>	hemoglobin β	7	S	15	7	D1	—
11p15	<i>TUB</i>	Tubby	7	S	—	7	—	—
11p15.5	<i>IGF2</i>	insulin-like growth factor 2	12q14	S,I	29	7	—	—
11q12	<i>CD20</i>	CD20 antigen	12	S	—	19	—	—
11cen-q13	<i>ADRBK1</i>	adrenergic receptor β kinase 1	12	S	—	19	—	—
11q22	<i>PR</i>	progesterone receptor	7p16	I	—	9	—	—
11q23.3	<i>THY1</i>	Thy-1 T-cell surface antigen	7	S	15	9	—	—
11q23	<i>DRD2</i>	dopamine receptor D2	7	S	—	9	—	—
12p12.2–p12.1	<i>LDHB</i>	lactate dehydrogenase B	[28]	S	5	6	B4	5 q12
12q13.3	<i>PFKM</i>	phosphofructokinase m	[6]	S	5	—	—	—
12q21	<i>PEPB</i>	peptidase B	[28]	S	5	10	B4	5
12q22–q24.1	<i>IGF1</i>	insulin-like growth factor 1	[28]	S	5	10	—	5q2.5
12q24	<i>IFNG</i>	interferon γ	[6]	S	5	10	—	5p1.1–q1.1
12q24.2	<i>TCF1</i>	transcription factor 1	[8]	S	—	5	—	—
13q14.1–q14.2	<i>RB1</i>	retinoblastoma	17	S	12	14	—	—
14q11	<i>MYH6</i>	myosin, heavy 6	1	S	—	14	—	—
14q11.2	<i>CHY</i>	Chymase, mast cell	1	S	—	11	—	—
14q13.1	<i>NP</i>	nucleoside phosphorylase	1q26	S,I	10	14	B3	7q2.1–q2.2
14q32.1	<i>PI/AAT</i>	protease inhibitor/AAT	24q15	L,I	7	12	—	7q2.4–q2.6
15q22–qter	<i>MPI</i>	mannose phosphate isomerase	1	S	21	9	B3	7
15q22–qter	<i>CYP1A2</i>	cytochrome P450, subf. I, polyp. 2	1	S	—	9	—	—
15q25–q26	<i>IGF1R</i>	insulin-1 growth factor 1 receptor	1	S	21	7	—	1q1.7–q1.2
15q26.1	<i>IDH2</i>	isocitrate dehydrogenase 2	1	S	—	7	B3	—
15q26.1	<i>FES</i>	feline sarcoma virus homologue	1	S	21	7	B3	—
16pter–p13.3	<i>HBA</i>	hemoglobin A	13q	I,L	—	11	—	—
16p24.3	<i>MC1R</i>	melanocortin receptor	3p12	L,S,I	18	8	—	—
16q13–q22.1	<i>CEST</i>	carboxylesterase	3	L	—	8	—	—
16q21	<i>GOT2</i>	glutamate oxaloact. transam., mito	3p15	I,L	—	8	—	—
16q22.1	<i>HP</i>	haptoglobin	3	L	18	8	—	—
17q11.2	<i>EVI2A</i>	ecotropic viral integration site2A	11	S	—	11	—	—
17q11.2	<i>NF1</i>	neurofibromatosis 1	11	S	19	11	—	—
17q21	<i>MYL4</i>	myosin, light chain 4	11	S	19	11	—	—
17q22–q24	<i>GH</i>	growth hormone	11	S	19	11	—	12p1.2–p1.5
17q23.1–q25.3	<i>HYPP</i>	hyperkalemic periodic paralysis	11	S	—	11	—	—
17q25	<i>P4HB</i>	prolyl-4 hydroxylase β	11	S	19	11	—	—

(Continued on following page.)

Table 3. (Continued)

Hsa ^a	Locus	Locus name	ECA ^b	Method ^c	Bta ^d	Mmu ^e	Fca ^f	Ssc ^g
18p11.32	<i>TS</i>	thymidylate synthetase	[8]	S	24	5	—	—
19p13.3–p13.2	<i>C3</i>	complement component 3	7pter	I,S	—	17	—	2p17–p14
19cen–q13.2	<i>A1BG</i>	β globulin, A1	10	L	—	—	—	—
19q13.1	<i>GPI</i>	glucose phosphate isomerase	10pter	I	18	7	—	6q12
19q13	<i>CRC</i>	calcium release channel	10pter	I	18	—	—	6q12
19q13	<i>CKM</i>	creatine kinase, muscle	10	S	—	7	—	—
20pterp12	<i>PRNP</i>	prion protein	22	S	13	2	—	—
20q11.2	<i>GHRH</i>	growth hormone releasing hormone	22	S	13	2	—	—
20q11.2	<i>ASP</i>	agouti signaling protein	22q15	S,I	—	2	—	—
20q13.11	<i>ADA</i>	adenosine deaminase	22	S	13	2	A3	—
20q13.2	<i>GNAS1</i>	guanine nucleotide-binding protein	22	S	13	2	—	—
21q22.3	<i>MX1</i>	myxovirus resistance	26q17	I	1	16	—	13
21q22.3	<i>ETS2</i>	avian erythroblastosis virus onc. 2	26q17	I,S	1	16	C2	—
22q13.31–qter	<i>ARSA</i>	arylsulfatase A	[28]	S	—	15	—	—
22q13.1	<i>CYP2D</i>	cytochrome P450, subfamily IID	[28]	S	5	15	—	—
Xp11.21	<i>ALAS2</i>	anemia, hereditary sideroblastic	X	S	—	X	—	—
Xq27.1–q27.2	<i>F9</i>	coagulation factor 9	X	S	X	X	X	X
Xq28	<i>G6PD</i>	glucose 6 phosphate dehydrogenase	X	S	X	X	X	X
Xq28	<i>BGN</i>	biglycan	X	S	—	X	—	—
Xq28	<i>F18</i>	factor 18	Xq29	S,I	—	—	—	—

^aData retrieved from Online Mendelian Inheritance in Man (OMIM), Johns Hopkins University, Baltimore, MD (March 1999). Full references are available at <http://www3.ncbi.nlm.nih.gov/omim/>.

^bECA assignment as in Shiue et al. (1999). In bold, physically assigned markers.

^cMethod: (I) In situ hybridization; (L) linkage; (S) syntenic. Full references available at <http://www.vgl.ucdavis.edu/horse>.

^dData retrieved from Bovmap Database, INRA, Laboratoire de Génétique Biochimique et de Cytogénétique de Jouy-en-Josas (March 1999). Full references are available at <http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl> and The Cattle Mapping Database (ARKDB), Roslin Institute, UK (March 1999). Full references available at <http://www.ri.bbsrc.ac.uk/cgi-bin/arkdb/browsers/browser.sh?species=cattle>

^eData retrieved from Mouse Genome Informatics (MGI) Resource, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine (March 1999). Full references are available at <http://www.informatics.jax.org/>.

^fData retrieved from O'brien et al. (1997b).

^gData retrieved from PiGBASE, Roslin Institute, Edinburgh, UK (May 1998). Full references are available at <http://www.ri.bbsrc.ac.uk/pigmap/pigbase/pigbase.html>.

Provisional Assignment of Equine Synteny Groups by Comparative Mapping

The lack of markers physically mapped to certain horse chromosomes has not allowed for the assignment of all 33 established equine synteny groups to their respective chromosomes. Nonetheless, the consideration of the synteny assignments of the equine type I loci we mapped and the other markers mapped with the UC Davis SCH panel, in conjunction with the data obtained by Zoo-FISH studies of the horse karyotype have allowed the tentative assignment of nine equine synteny groups.

A total of four equine type I loci were mapped to synteny group UCD5 (*AT3*, *GBA*, *LAMC1*, *NGFB*). The corresponding human homologs of these genes have been mapped to human Chromosome 1 (Table 1). Raudsepp et al. (1996) reported that painting probes from *Hsa1*, hybridize to three horse chromosomes (*ECA2*, 5, and 30). The previous assignment of UCD

synteny groups 2 and 30 to *ECA2* and *ECA30*, respectively (Shiue et al. 1999), therefore suggests that UCD5 is located on horse chromosome 5.

Four equine genes were assigned to UCD synteny group D (Table 2). The human homologs of equine *FNI* and *CHRNA* have been mapped to *Hsa2*, and the homologs of *PFKM* and *IFNG* have been mapped to *Hsa12*. Furthermore, we have recently assigned *PAX3* (*Hsa2q35*) and *PMEL17* (*Hsa12q13*) to this synteny group and physically mapped *PMEL17* to *ECA6* by FISH (A.T. Bowling et al., unpubl. observations). The q arm of horse Chromosome 6 was shown to hybridize to painting probes from *Hsa12*, whereas the p arm did not hybridize to probes from any human chromosome (Raudsepp et al. 1996). Therefore, the assignment of UCD-D to horse Chromosome 6 is in agreement with ZooFISH studies of the horse karyotype and suggests that the p arm of *ECA6* contains material orthologous to *Hsa2*.

We had previously assigned UCD6 to *ECA6* (Shiue et al. 1999) based on the assignment of a microsatellite (ASB14) by FISH (Breen et al. 1997), but this assignment has now been withdrawn (M. Breen, pers. comm.). Two horse genes, *TCF1* (*Hsa12*) and *TS* (*Hsa18*), were assigned to UCD6 (Table 2). Therefore, the consideration that *ECA8* is the only horse chromosome to hybridize to painting probes from human Chromosomes 12 and 18 (Raudsepp et al. 1996), and that no synteny groups have been assigned to this horse chromosome, suggests that UCD6 should be tentatively assigned to *ECA8*.

Painting probes from human Chromosomes 3, 9, 10, and 13 were shown to hybridize exclusively to horse Chromosomes 16 and 19, 23 and 25, 1 and 29, and 17, respectively (Raudsepp et al. 1996). Equine genes with homologs located on these human chromosomes were assigned to UCD synteny groups 16 and 19, 23 and 25, 29, and 17, respectively (Table 2). The consideration that synteny groups UCD1, UCD19, and UCD23 were assigned respectively to *ECA1*, *ECA19*, and *ECA23* (Shiue et al. 1999), suggests the provisional assignment of UCD synteny groups 16, 17, 25, and 29 to *ECA16*, *ECA17*, *ECA25*, and *ECA29*, respectively. The assignment of equine *ADRB2* (*Hsa5*) to synteny group UCD14 is in agreement with the tentative assignment of this synteny group to horse Chromosome 14 as proposed by Caetano et al. (1999b).

Equine gene homologs mapping to human Chromosomes 12 and 22 were assigned to UCD synteny group C. Three horse chromosomes (*ECA1*, *ECA8*, and *ECA28*) have been shown to have blocks orthologous to *Hsa12* and *Hsa22* (Raudsepp et al. 1996). As mentioned previously, UCD1 has been assigned to *ECA1* (Shiue et al. 1999) and we have tentatively assigned UCD6 to *ECA8*. Therefore, the most consistent explanation of the current data is the provisional assignment of UCD6 to horse Chromosome 28.

In view of the described data, *ECA27* is the only horse chromosome without an assigned or provisionally assigned synteny group. UCDA is the only established synteny group that has not been assigned to a horse chromosome. Therefore, the tentative assignment of UCDA to horse Chromosome 27 is reasonable, given the current data.

In addition, the assignments of type I loci to horse synteny groups have allowed two refinements of the human-horse comparative data produced with in situ hybridization experiments. The q arm of *ECA13* was shown to be orthologous to *Hsa16*, whereas the orthology of the p arm of this horse chromosome was not determined (Raudsepp et al. 1996). The human homologs of two equine genes (*ELN* and *GUSB*) assigned to *ECA13* have been mapped to *Hsa7*, which therefore suggests that the p arm of this horse chromosome contains material orthologous to human Chromosome 7.

Similarly, the orthology of the p arm of *ECA6* could not be determined (Raudsepp et al. 1996) but the assignment of equine genes with homologs located on *Hsa2* to *ECA6* (UCDD), suggests that the p arm of this horse chromosome contains material orthologous to human Chromosome 2. Physical mapping of these equine genes mapped by synteny will be necessary to confirm these inferences.

Application of Universal Primers for Type I Loci

Although the published universal primers used for generating type I equine markers were highly useful for producing a first generation comparative map of the horse genome with the techniques used, additional markers could be readily developed from a few of the remaining primers tested. A total of 36 of the primers tested amplified candidate fragments for equine type I loci which could not be amplified in the somatic cell hybrids, while mouse-specific bands were amplified instead, and therefore could not be used for synteny mapping. We speculate that small deviations of the equine gene sequence from the consensus at the primer binding sites caused the observed preferential amplification of mouse products. Further characterization by cloning and sequencing of horse-specific PCR products from these primers, followed by redesigning of horse-specific primers, could result in additional markers for equine type I loci useful for synteny mapping.

It is likely that the observed amplification of no fragments, multiple fragments, and anonymous equine sequences by a number of the primers used in this study may have been caused by deviations of the equine gene sequences from the consensus sequences used for designing the primers. In addition, expansion of intron sequences within targeted gene regions to sizes beyond the amplification range of Taq DNA polymerase may also have caused the observed results. In these instances, markers for equine type I loci may be developed by redesigning new universal primers to target other intron regions of the respective genes using the reported strategies (Venta et al. 1996; Lyons et al. 1997).

General Considerations

Because synteny mapping by SCH panel analysis does not allow for the precise determination of the relative order and distance between syntenic markers, further work is needed to integrate the markers from this study into the International Equine Linkage Map (Guérin et al. 1999). Intraspecific sequence variation at the intron sequences amplified in most of the markers we mapped can be used to produce genetic markers useful for linkage mapping studies with pedigreed families (Lyons et al. 1997), and therefore order these genes relative to each other and to other markers, in the horse linkage

map. In addition, dinucleotide repeat sequences found in the intron regions of a few of the equine PCR products we sequenced are currently being characterized (Caetano 1999) and may be useful in linkage studies as markers for the respective loci. The integration of type I loci into the horse linkage map will be useful for further refining the comparative map of the horse and revealing existing intrachromosomal rearrangements which may have occurred during the evolution of the horse genome.

The assignment of synteny groups to horse chromosomes has been highly dependent on the physical mapping of type I and II markers by FISH. Additional physical mapping data is needed to confirm proposed assignments, ratify provisional assignments, and moreover, to proceed with the establishment of a basic framework map of the horse genome. The small average size of the PCR-based type I markers we characterized precludes their direct use as probes in *in situ* hybridizations to metaphase chromosome spreads. The technical requirements of current FISH procedures demand larger DNA probes, which can be isolated from available horse BAC libraries (Godard et al. 1998) with the equine markers we characterized. In addition to their use in physical mapping, the isolated large-insert genomic clones may also be used for isolating microsatellite sequences associated with the respective equine genes, which could then be used in linkage studies.

Although type I gene homologs from all human chromosomes have been assigned to the horse genome map (Table 3), no equine type I loci have been mapped to horse Chromosomes 27 and 30. Zoo-FISH was used to show that *ECA30* hybridizes to DNA fragments from human Chromosome 1 (Raudsepp et al. 1996) but the homeology of *ECA27* could not be determined with this technique. A continued effort to develop and map markers for equine type I loci will be necessary to fill the gaps in the horse-human comparative map and to continue its refinement.

METHODS

Optimization of PCR Amplification Parameters

The methods utilized for optimizing PCR conditions to amplify horse-specific fragments with each primer set for use in synteny mapping with a horse-mouse SCH panel have been described previously (Caetano et al. 1999a). Optimal conditions for amplifying 68 equine type I loci are indicated in Table 1. Horse and mouse PCR products of the same length were subjected to a panel of 15 restriction enzymes (not shown), in the buffer and temperature conditions recommended by the suppliers, prior to electrophoresis. Restriction enzymes selected to distinguish these PCR products from the two species are indicated in Table 1.

Sequencing

Plugs containing DNA fragments amplified from a thorough-

bred horse with each of the primer sets selected for mapping were taken from agarose gels with glass Pasteur pipettes and incubated at room temperature in 75 μ l of low TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) overnight. This solution was used as template for a PCR reaction under the conditions used to amplify the original product. An aliquot from this PCR reaction was quantified in an agarose gel and the remaining was used for cloning (TA Cloning Kit, Invitrogen). A minimum of 3 clones from each fragment was sequenced with ABI Prism sequencing kits and sequencing products were analyzed with an ABI 377 automated sequencer. Large PCR fragments that could not be readily cloned with the techniques used were isolated from agarose gels with the QIAEXII kit (Qiagen) and sequenced by direct sequencing. A few of the type I markers characterized were not completely sequenced because of the presence of extensive intron sequences which could not be used for sequence comparisons with other species. Each sequence obtained was subjected to BLAST searches of GenBank at the National Center for Biotechnology Information internet server (<http://www.ncbi.nlm.nih.gov/>). Sequences from 68 equine genes were submitted to GenBank.

Somatic Cell Hybrid Panel Analysis

The establishment of the UC Davis SCH panel has been described (Shiue et al. 1999). DNA from the same 108 horse-mouse heterohybridoma cell lines used in previous studies (Shiue et al. 1999; Caetano et al. 1999a,b) were used in this study. DNA from each cell line was amplified with each of the 68 primer sets (Table 1) and scored for the presence or absence of horse-specific fragments after electrophoresis. Amplification products obtained from each hybrid cell line with 26 primer sets were digested with the respective restriction enzymes prior to electrophoresis (Table 1). Correlation coefficients were calculated between all of the markers in the UC Davis SCH panel database and each of the 68 loci studied. A correlation value ≥ 0.70 (Table 2) was accepted as evidence for synteny between two markers (Chevalet and Corpet 1986).

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