

Construction of a 5000_{rad} whole-genome radiation hybrid panel in the horse and generation of a comprehensive and comparative map for ECA11

Bhanu P. Chowdhary,^{1,4} Terje Raudsepp,^{1,4} Dee Honeycutt,¹ Elaine K. Owens,² François Piumi,³ Gérard Guérin,³ Tara C. Matise,⁵ Srinivas R. Kata,³ James E. Womack,³ Loren C. Skow¹

¹Department of Veterinary Anatomy & Public Health, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA

²Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA

³INRA, Centre de Recherche de Jouy, Département de Génétique animale Jouy-en-Josas, France

⁴Division of Animal Genetics, The Royal Veterinary and Agricultural University, Grønnegårdsvej 3, 1870, Frederiksberg, Denmark

⁵Rutgers University, Piscataway, New Jersey 08854, USA

Received: 7 June 2001 / Accepted: 4 October 2001

Abstract. A 5000_{rad} whole-genome radiation hybrid (RH) panel was created for the horse. The usefulness of the panel for generating physically ordered maps of individual equine chromosomes was tested by typing 24 markers on horse Chromosome 11 (ECA11). The overall retention of markers on this chromosome was 43.6%. Almost complete retention of two of the typed markers—CA062 and AHT44—clearly indicated the location of thymidine kinase gene on the short arm of ECA11. Seven of the typed markers were FISH mapped to align the RH and cytogenetic maps. With the RH-MAPPER approach, a physically ordered map comprising four linkage groups and incorporating all the markers was obtained. The study provides the first comprehensive map for a horse chromosome that integrates all available mapping data and adds new information that spans the entire length of the equine chromosome. The map clearly underlines the resolving power and utility of the panel and emphasizes the need to have uniformly distributed cytogenetic markers for appropriate alignment of RH map with the chromosome. A comparative status of the ECA11 map in relation to the corresponding human/mouse chromosome is presented.

Introduction

Development of gene maps in a number of farm animal species has clearly underlined the importance and application of maps in approaching genes of interest. During recent years this fact has also been recognized in the horse. Consequently, organized global efforts are being made to generate a gene map in the horse that can help bring improvement in disease resistance and performance traits. The past two years have witnessed a notable expansion of the horse gene map. Currently, the map comprises over 500 loci that are mapped primarily through typing of markers in somatic cell hybrid panel (SCH; Shiue et al. 1999, 2000; Caetano et al. 1999a, 1999b), analysis of pedigree data to detect genetic linkage (Lindgren et al. 1998; Guérin et al. 1999; Swinburne et al. 2000), and cytogenetic localization of markers by fluorescent in situ hybridization (FISH; see for review Chowdhary and Raudsepp 2000; Godard et al. 2000; Mariat et al. 2001). These traditional mapping approaches, however, need to be bolstered through the introduction of new high-throughput methods that can facilitate rapid mapping of a variety of markers.

Radiation hybrid (RH) mapping has emerged as an important

tool for rapid expansion of gene maps in human (Gyapay et al. 1996; Stewart et al. 1997; Olivier et al. 2001), mouse (Flaherty and Herron 1998; Van Etten et al. 1999), rat (Watanabe et al. 1999; Scheetz et al. 2001), pig (Hawken et al. 1999), cattle (Band et al. 2000), cat (Murphy et al. 1999), dog (Priat et al. 1998; Mellersh et al. 2000) and other mammalian species. The versatility of the approach lies in the ability to map all PCR-based markers, irrespective of their polymorphic status. Consequently, primer pairs from markers ranging from microsatellites to nonpolymorphic expressed sequence tagged sites (ESTs) can be used alike on the RH panel to rapidly generate ordered physical maps. Availability of such a resource in the horse is expected to be of great value in augmenting current efforts to improve the status of the map. Recently, production of a 3000_{rad} whole-genome equine radiation hybrid panel was reported (Kiguwa et al. 2000). Typing of markers on the panel resulted in preliminary characterization and creation of RH maps for ECA1 and 10. Concurrent to this work, a whole-genome RH panel with a higher dosage of irradiation exposure (5000_{rad}) was generated by us for the horse (Chowdhary et al. 2001). Herein, we report the construction and use of this panel, in combination with new cytogenetic mapping data, to obtain the first comprehensive map for a horse chromosome.

Materials and methods

Generation of an equine whole-genome RH panel. The equine donor cells (JEW66) used in constructing this panel were from a normal diploid fibroblast culture established from an Arabian male horse (Sonny). Nearly confluent flasks were trypsinized and suspended in Gibco DMEM without supplements. Approximately 10⁷ cells were irradiated at room temperature in 10 ml suspension medium in a T75 flask. A cobalt 60 source delivered 185 rad/min for a total dose of 5000 rad. Attached cells were removed with trypsin, and all cells were suspended in Ca⁺⁺/Mg⁺⁺-free Hanks balanced salt solution (HBSS; pH 8.0) at 10⁶ cells/ml. One-half ml cells (5 × 10⁵ cells) was removed for controls, and 4.5 ml was used for fusion. The recipient Chinese hamster TK⁻ (thymidine kinase-deficient) fibroblast line A23 was kindly provided by David Cox, Stanford University School of Medicine. These cells were also suspended in HBSS at a concentration of 10⁶ cells/ml. An aliquot of 0.7 ml of this suspension was removed for controls, and 9 ml was used for fusion.

JEW66 (4.5 × 10⁶) and A23 (9 × 10⁶) cells were thoroughly mixed, pelleted, and resuspended. One-half ml PEG (polyethylene glycol 1500 in 50% sterile solution; Boehringer Mannheim) was slowly added with constant mixing. After 2 min, 10 ml HBSS (pH 8.0) was also added slowly with gentle mixing. The cells were pelleted and then resuspended in 5 ml HBSS, pH 8.0, for 15 min at 37°C. Each control line was exposed to PEG

Table 1. List of horse Chromosome 11 (ECA11) markers typed in the 5000_{rad} horse × hamster radiation hybrid panel, with the primer sequences and PCR conditions for each of the markers. Where available, FISH locations are presented. Data in bold represent work carried out in this study.

Name	Symbol	Location	Primers 5'–3'	Product size (bp)	MgCl ₂ (mM)	T _a	Reference	Access. No.
Actin, gamma 1	<i>ACTG1</i>	11	F: GCTGAGGTGAGAACATATCCGTGG R: AGTGGGTCTCAAGGCTCGGCC	209	1.5	60	Brandon et al., Unpub.	G62169
Angiotensin I converting enzyme	<i>ACE5</i>	11	—	140	3.0	60	Primers from I. Tammen, our primers	AF130762
Ecotropic viral integration site2A	<i>EV12A</i>	11	F: AACAAACAACAAATCGCTATGC R: CGCTTGCCTACTTGTTTTGA	140	3.0	Touch-down 60		
Fatty acid synthase	<i>FASN</i>	11p14	F: CACCGGGTGAAGGCTGGCACT R: GGTTTAATCGTGGGAGGCTCC	296	1.5	55	Lear et al. 2001	G62204
Growth hormone	<i>GH</i>	11	F: TCAGGATGTGGCGCCTTC R: TGGCGTTCTGGATCGAGTATCT	372	4.0	58	Caetano et al. 1999a	AF097589
Hyperkalemic periodic paralysis	<i>HYPF</i>	11	F: GGTCTTCATCATCGTCTTCACG R: CACAATGGACAGGATGACAACC	118	4.0	58	Caetano et al. 1999a	AF
Myosin, light polypeptide 4	<i>MYLA</i>	11p12.3-p13	F: TGTTTTCGACAAGGAGAGCA R: AGACAGGGTGGATCAGGATG	196	1.5	58	Caetano et al. 1999a our primers	AF130773
Neurofibromin I	<i>NF1</i>	11q12-q13	F: ACAGTGGCCTCATGCACTC R: CTGTGCCTTGTGGAGGATT	221	1.5	58	Caetano et al. 1999a our primers	AF130776
Oligodendrocyte myelin glycoprotein	<i>OMG</i>	11q14	F: AGTGTGTAACTCCTTCCCGCG R: ACATACACAGGGAGAACTGGG	273	1.5	60	Lear et al. 2001	G62156
Tumor protein p53	<i>TP53</i>	11	F: AAGACTCCAGGTAGGAATC R: CAGATTACCACTACCCAGGT	400	2.0	58	Lindgren et al. 2001	X91793
Protein coding sequence	<i>HEST19</i>	11p12	F: GGTCCCAAAGGCCAAACAGTTGG R: CGTAGACAAGGAATCTGAGTCT	150	3.0	Touch-down 60	Godard et al. 2000	
<i>v-erb-a</i> avian erythroblastic leukemia viral oncogene homolog 2-like procollagen-proline	<i>P4HB</i>	11	F: GACGAGCTGACAGCAGAGAA R: AGGACAGCCATCTTACACC	219	1.5	58	Caetano et al. 1999, our primers	AF130777
Microsatellite	<i>AHT44</i>	11p13-p14	F: CCTCCACATGAGGTGCATTT R: GACAATGAGAGAGGCCAACCC	160	2.0	58	Swinburne et al. 2000	AJ271529
Microsatellite	<i>ASB35</i>	11	F: ATGCATGAGCAGAGTGTCTTCC R: TAGTACTTCTCTTAATATAAGC	170	4.0	58	Breen et al. unpib.	X93549
Microsatellite	<i>CA39</i>	11p13-p14	F: GCCCTTCACCGGATTTAATA R: GAACCCAGCACATTATCGGG	188	2.0	58	our primers	U25168
Microsatellite	<i>CA062</i>	11p13-p14	F: AAACCTGAGCACCAGACTC R: GATGGATACTCCTGTAGCA	151–167	2.0	58	Eggleston-Stott et al. 1996	U25170
Microsatellite	<i>CA439</i>	11p13-p14	F: GTTGATGCTCAGAGGAAGGC R: GGTGCACAGTCCACAAGAAA	207	2.0	58	Eggleston-Stott et al. 1999	U67409
Microsatellite	<i>D-8</i>	11p12-p13	F: TTTTGTGTCTCAGGAGTGTG R: AGTCTGATGGTGGAGGAAGG	95–105	3.0	Touch-down 64	Marti et al. 1998	
Microsatellite	<i>HLM2</i>	11q14-q15	F: CCCACCTCCCCATCTCCAACC R: AAGCCAGTTCTCAGCCCCACC	123–137	1.5	58	Vega-Pla et al. 1996	U36494
Microsatellite	<i>LEX068</i>	11p13prox	F: AAATCCGAGCTAAAATGTA R: TAGGAAGATAGGATCACAAGG	154–168	3.0	58	Coogle & Bailey, 1999	
Microsatellite	<i>NV40</i>	11	F: TGGCATCTGAATGGAGAATG R: GATTATGATGCTACAGGGGAAAAG	144–158	3.0	58	Røed et al. 1998	AF056395
Microsatellite	<i>SG13</i>	11q12	F: GGACTAAAGCCCAACATCCAGC R: CTCACCAGTAAGGGTTATGGGGC	162	2.0	58	Godard et al. 1997	U90592
Microsatellite	<i>SG22</i>	11p14	F: GGAATCACTGCCAGCCTG R: GGTGGTTGGGAAAGGGTTGAGGC	112	2.0	58	Godard et al. 1997	U90600
Microsatellite	<i>SG24</i>	11p12	F: CTACCATTGAAGAGGGGTGCC R: GAAACGAGCAGGAAGTGAATCTCC	121	4.0	Touch-down 64	Godard et al. 1997	U90602

by the same process. The fusion suspension was mixed with Gibco DMEM containing 10% FBS + HAT (Sigma) + 5×10^{-7} M ouabain in a total volume of 90 ml. The resultant mix was incubated at 37°C for 15 min. Ten ml of this mixture was dispensed to each of the nine 100-mm plates (approximately 1.5×10^5 cells/plate). Controls were mixed in the same solution and plated identically. All plates were incubated at 37°C in 5% CO₂. All JEW66 and A23 control cells were dead by day 7. All fusion plates had six to eight or more colonies growing by day 8. From a total of two irradiation-fusion experiments, 168 hybrid cell lines (116 from the first and 52 from the second experiment) were picked and cloned into single wells of 24-well plates containing DMEM, HAT, and ouabain. Cells were subsequently transferred to T25 flasks and maintained on HAT without ouabain. Cells from confluent flasks were divided into cryopreserved seed stocks, to inoculate roller bottles for large-scale culture and to produce a small pellet for DNA extraction and preliminary genotyping. Additionally, 10 cell lines were randomly chosen for cytogenetic analysis to assess chromosome fragmentation and retention of horse DNA (see below under FISH).

DNA from each of the 168 hybrid cell lines was typed with eight markers (*AHT44*, *ASB35*, *CA062*, *CA439*, *D-8*, *SG13*, *SG22*, *SG24*) from

horse Chr 11 (ECA11). This chromosome is anticipated to contain the TK gene, for which the rodent line was deficient. Hybrid lines showing no positive amplification for any of the selected markers were excluded. From the remaining 138 cell lines, the first 93 were chosen for the final panel. The remaining 45 lines were reserved to accommodate any future need for inclusion in the panel.

Three 900-cm² roller bottle cultures from each of the 138 radiation hybrid colonies were grown to produce the final harvest for DNA extraction. On average, 3.5 mg of DNA was obtained from each RH, sufficient for an estimated 70,000 PCR reactions, assuming 50 ng will be required per reaction. The DNA harvest ranged from 1.5 to 7.9 mg.

Genotyping with genome-wide and ECA11 markers. In order to ensure overall representation of the genome in the selected panel, 30 cell lines were randomly selected and typed with 64 primer pairs for markers located on different horse chromosomes (two markers per chromosome). Next, primers were obtained for a total of 24 ECA11 markers (12 genes and 12 microsatellites; Table 1). After optimization, the markers were typed on the panel of 93 hybrid cell lines along with a negative control and horse and

hamster genomic DNA as test controls. For five markers, new primers were constructed because the primer pairs shown in databases did not give clear amplification. Marker names, primers pairs and PCR conditions for ECA11 markers are presented in Table 1.

Analysis of ECA11 typing results. Following two-point linkage analysis, the RH-MAPPER program (Slonim et al. 1997) was used to analyze the typing results for ECA11 markers and to construct a map for the chromosome.

Fluorescent in situ hybridization. To visualize equine chromosomal fragments in the hybrid cells, metaphase preparations were obtained from the first 12 hybrid cell lines by standard procedures. Horse genomic DNA was biotin labeled by using nick translation and was hybridized to the preparations as described elsewhere (Chowdhary et al. 1995).

BAC clones containing markers mapped to ECA11 were isolated as described previously (Godard et al. 2000). Cytogenetic location of the BACs was determined in metaphase spreads obtained from lymphocyte cultures. Approximately 1 μ g DNA from each BAC was biotin labeled (BioNick Labeling System, Life Technologies, Grand Island, NY) and hybridized to chromosomes. FISH, washing, signal amplification/detection, and imaging were carried out according to Chowdhary et al. (1995) and Raudsepp et al. (1999).

Results

The retention of the equine chromosome fragments in the hybrid cell lines was determined with two approaches. The first approach involved typing of all the lines with eight randomly chosen ECA11 markers. Based on comparative human data, the *TK* gene is expected to be present on this chromosome, which implies that parts of ECA11 will be preferentially retained in the hybrid lines to compensate for the absence of the gene from the hamster genomic component. Consequently, amplification of any one of the markers is anticipated to be indicative of the retention of equine DNA in the hybrid cell lines. Typing results showed that the retention frequency for markers *CA062* and *AHT44* was 100% and 98.9%, respectively, demonstrating that the *TK* gene is most likely located in close vicinity to these markers. Lines showing no amplification for any of the eight chosen markers were further tested for the presence of other ECA11 markers. Thirty cell lines gave no positives for any of the additional markers and were not retained for future analysis. Thus, beginning with 168 hybrid cell lines, 138 were retained, of which the first 93 were chosen for the final panel.

In the second approach, metaphase spreads obtained from the first 12 hybrid cell lines were subjected to FISH with horse genomic DNA. Ten of these lines gave clear indication for the presence of equine chromosomal fragments (Fig. 1); the remaining two lines showed no specific hybridization signal. The latter were also negative for all ECA11 markers tested above.

In order to verify representation of all equine chromosomes in the final panel, 30 hybrid lines were randomly chosen and typed with 64 genome-wide markers (two per chromosome). Results showed that all the chromosomes were represented in the panel. A preliminary estimate of overall retention frequency (RF) was obtained by randomly typing 10 markers each from chromosomes 3, 4, 10, 14, 20, and X, in addition to the 24 markers typed for ECA11. The average retention frequency for each of these chromosomes was 27.2, 21.7, 20.1, 18.4, 16.7, and 13.9% (haploid), respectively. For ECA11, the RF based on 24 markers was 43.6% (Fig. 2). Thus, the overall retention frequency of markers in the panel averaged around 26%, which can be taken as a predictive estimate for the remaining chromosomes. Distribution of the retention frequency of the ECA11 markers showed that the markers located on the short arm and around the centromere are preferentially retained compared with those located on the long arm, especially towards the telomere. The high retention of markers on the short arm can be attributed to the presence of the *TK* gene in this chromosomal region.

Cytogenetic mapping. To develop an RH map that is aligned at regular intervals along the length of ECA11, we arbitrarily chose markers from regions deficient in cytogenetically mapped markers. BAC clones containing these markers were isolated and FISH mapped to horse chromosomes. BACs containing markers *CA39*, *CA062*, *AHT44*, *CA439*, *LEX068*, and *MYL4* mapped to the short arm (p), while that containing *HLM2* localized to the subterminal part of the long arm (q) (Table 1 and Fig. 3). A reasonably good coverage of the chromosome with well-dispersed cytogenetic markers was thus available to facilitate construction of the RH map.

Generation of an RH map for ECA11. A two-point analysis involving all loci provided four linkage groups comprising 4, 2, 4, and 14 loci each, at lod score 7 (see Fig. 3). The RH-MAPPER (Slonim et al. 1997) program was then used to permute the order of the loci within each of the groups that span 70.5, 3.2, 58.1, and 214.7 centiRays (cR), respectively. The linkage groups were readily oriented relative to each other based on the cytogenetic data available for 15 of the 24 markers. The overall order of the loci thus obtained was in general agreement with the available linkage and cytogenetic maps. It is noteworthy that the position of *HST19* in the RH map does not correspond to that determined previously by in situ hybridization (Godard et al. 2000).

Discussion

Availability of diverse mapping resources is crucial for an organized and rapid expansion of gene map of any species. Despite the progress made in generating equine gene maps during recent years, the number of approaches/resources at hand are still limited. Generation of the 5000_{rad} whole-genome radiation hybrid panel reported here provides the horse geneticist with an additional tool that can be readily exploited to obtain a physically ordered map of all PCR-able markers. The ECA11 comprehensive map obtained in this study clearly reflects the *resolving power* and *utility* of the panel. The average retention of markers in the panel (overall as well as for individual chromosomes) is in reasonable agreement with that reported for a number of other RH panels in different species (Gyapay et al. 1996, human; McCarthy et al. 1997, mouse; Hawken et al. 1999, pig).

Currently, gene maps for different horse chromosomes are being generated by genetic linkage, SCH, and cytogenetic approaches. Except for the partial integration of RH and genetic linkage maps reported for ECA1 and 10 by Kiguwa et al. (2000), very little has as yet been done to integrate different maps into consensus maps for individual equine chromosomes. This is partly attributed to the use of different sets of markers for different mapping approaches. In the present study, we focused on the generation of a consensus map for ECA11. There are three genetic linkage maps available for this chromosome (Lindgren et al. 1998; Guérin et al. 1999; Swinburne et al. 2000) that contain four to nine loci each. Of these, only three loci are cytogenetically mapped. Further, among the syntenic markers on this chromosome (six genes and six microsatellites; Shiue et al. 1999; Caetano et al. 1999a), only two are common to linkage maps, while three are sublocalized to a chromosomal region. This disarray in mapping data makes it difficult to obtain a consensus map for the chromosome. In order to overcome this situation, we typed the 5000_{rad} panel with all available as well as newly generated ECA11 markers. Moreover, the information was supplemented with new cytogenetic mapping data. Consequently, it was possible to align all the maps and obtain a consensus map for the chromosome. The physically ordered map reported here thus represents the first integrated map for an equine chromosome. Most importantly, the map spans almost the entire length of the chromosome. Earlier, carrying out preliminary analysis of the 3000_{rad} RH panel, Kiguwa et al. (2000)

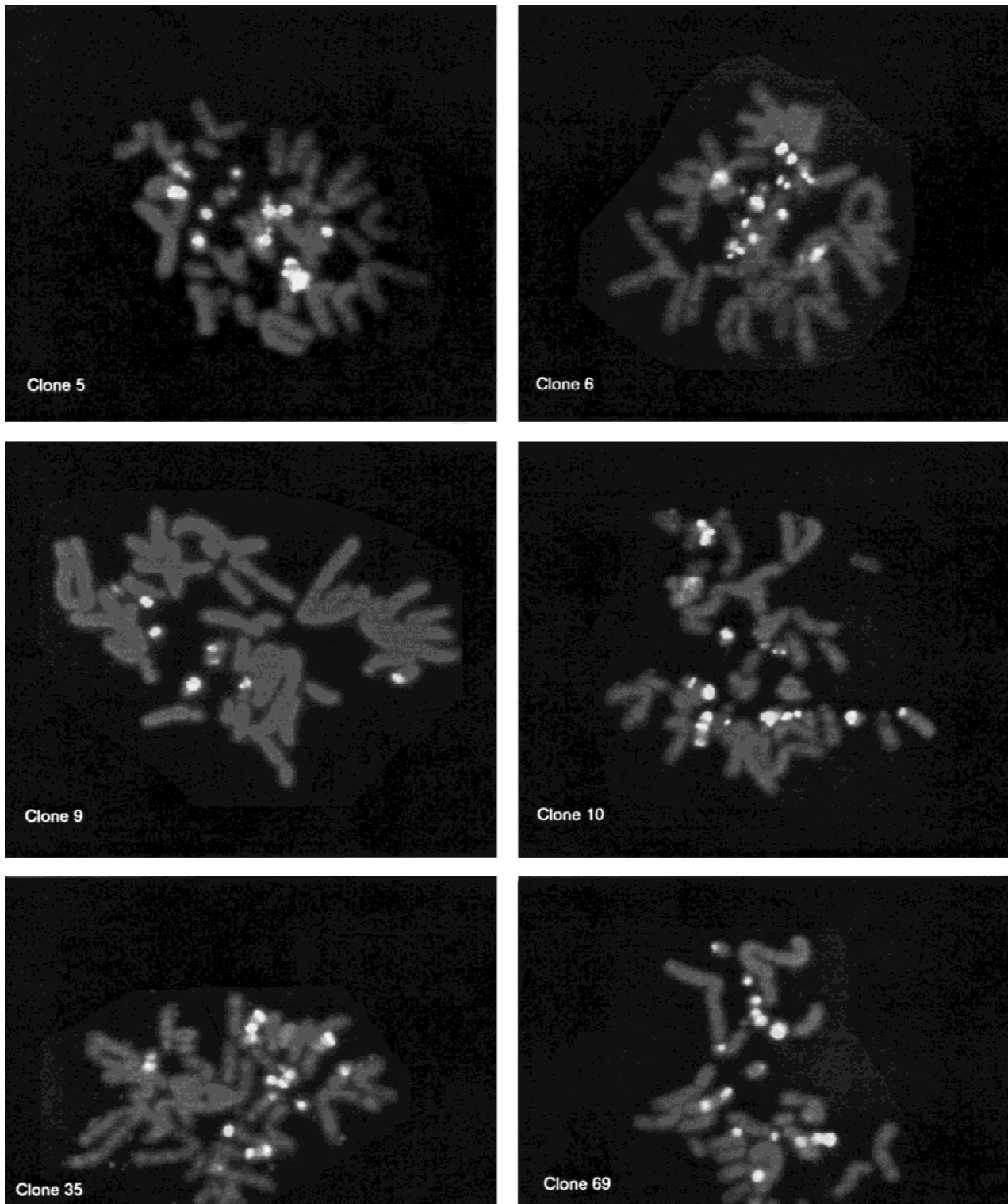


Fig. 1. Representative metaphase spreads showing fragmented horse chromosomes (green/yellow) in a hamster background (red), after hybridization with biotin-labeled DNA from individual hybrid cell lines.

reported maps for ECA1 and 10. Compared with this, the map reported for ECA11 is more informative and comprehensive.

The ECA11 map presented in this report comprises 12 genes and 12 microsatellite markers. All the genes (except *HEST19*) mapped to this chromosome are located on human Chr 17 (HSA17), which is in agreement with the Zoo-FISH results earlier reported by us (Raudsepp et al. 1996). A comparison of the cyto-

genetic and RH maps of the 11 genes between horse and humans shows a general conservation of gene order with minor intra-segmental rearrangements (Fig. 3). Extending this comparison to mouse shows that data available on seven of the mouse loci also demonstrate broad conservation of gene order (last section of Fig. 3). It is, however, evident that, in relation to ECA11, the human (and mouse) chromosome has a reverse orientation. It is expected

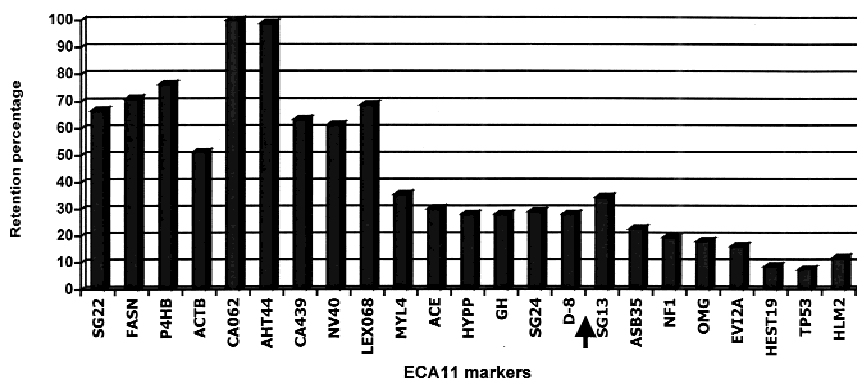


Fig. 2. Retention frequency of markers from ECA11. In total, 14 markers are located on the p-arm (SG22 to D-8), with 8 on the long arm (SG13-HLM2). Arrow represents the centromere. Retention frequency was distinguishably higher on the p-arm than on the q-arm because horse chromosome fragments containing the thymidine kinase (*TK*) gene were preferentially retained when fused with TK⁺ hamster cell line. The overall retention frequency of the 24 markers typed on the panel was ~43%.

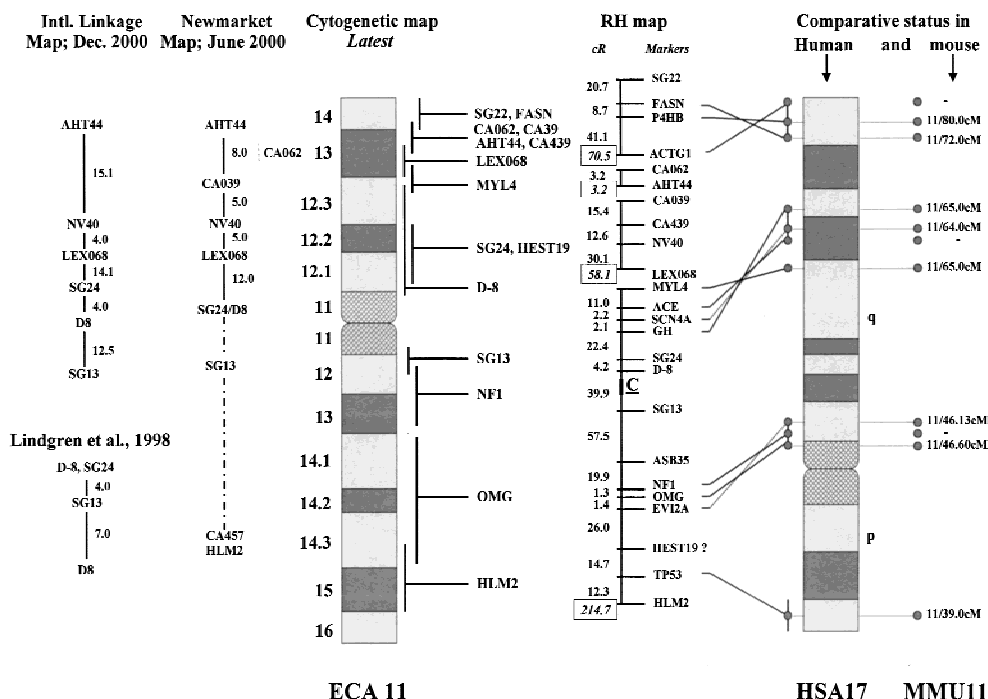


Fig. 3. A comprehensive RH map of horse Chr 11 (ECA11). Three recently published linkage maps are on the left of the updated cytogetic map. To the right of this is the RH map generated in this study. A two-point linkage analysis provided four linkage groups at lod-score 7. Physical order of loci within each linkage group was obtained with the RH-MAPPER program. Centiray (cR) distance between markers and total span of each linkage group (in box) are presented beside the map. The linkage groups are oriented according to available cytogetic data. A comparative map

between horse and human (HSA; right) shows that despite minor intra-segmental rearrangements, the gene order on ECA11 and HSA17 is generally conserved. This pattern is also maintained in the mouse (MMU; last column). The human and mouse mapping data were obtained from <http://www.ncbi.nlm.nih.gov/genemap/query.cgi> and http://www.informatics.jax.org/searches/marker_form.shtml. '?' besides *HEST19* represents disagreement with FISH data reported earlier (Godard et al. 2000).

that when more comparative markers are mapped between the two species, additional intrachromosomal rearrangements in gene order will be detected. In a similar comparison between HSA17 and the cattle homolog (BTA19), three inverted syntenic blocks were detected between the two chromosomes (Yang and Womack 1998), despite whole-chromosome synteny conservation observed between them.

Characterization of the 5000_{rad} panel with ECA11 markers clearly underlines the utility of the panel to generate individual chromosome maps in the horse. The resolving power of the panel is reflected in that the RH map could readily provide physical order for a group of loci clustered on the same band on the cytogetic map. The ECA11-HSA17 comparative map generated in this study is expected to form the basis for a parallel radiation hybrid map (Yang and Womack 1998) between the two chromosomes. It is thus expected that the panel will find significant use in rapid expansion of the gene map in the horse. One of the potential

uses will be large-scale mapping of ESTs generated from a number of cDNA libraries recently produced in the horse. This, together with recently constructed BAC libraries, is expected to form the basis for a physically ordered contig map of the equine genome that will augment ongoing efforts to identify genes related to disease and performance traits in this species.

Acknowledgment. This work was supported by funds from Link Endowment (B.P. Chowdhary, L.C. Skow), The Danish Agricultural and Veterinary Research Council (B.P. Chowdhary, T. Raudsepp), NRICGP/USDA Grant 2000-03510 (L.C. Skow), and NIH grant R01-HG01691 (T.C. Matise).

References

Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW et al. (2000) An ordered comparative map of the cattle and human genomes. *Genome Res* 10, 1359-1368

- Caetano AR, Shiue YL, Lyons LA, O'Brien SJ, Laughlin TF et al. (1999a) A comparative gene map of the horse (*Equus caballus*). *Genome Res* 9, 1239–1249
- Caetano AR, Lyons LA, Laughlin TF, O'Brien SJ, Murray JD et al. (1999b) Equine synteny mapping of comparative anchor tagged sequences (CATS) from human Chromosome 5. *Mamm Genome* 10, 1082–1084
- Chowdhary BP, Raudsepp T (2000) Cytogenetics and physical gene maps. In *The Genetics of the Horse*, AT Bowling, A Ruvinsky (eds.). (Wallingford, Oxon: CAB International), pp 171–242
- Chowdhary BP, de la Seña C, Harbitz I, Eriksson L, Gustavsson I (1995) FISH on metaphase and interphase chromosomes demonstrates the physical order of the genes for GPI, CRC, and LIPE in pigs. *Cytogenet Cell Genet* 71, 175–178
- Chowdhary BP, Raudsepp T, Womack JE, Skow LC (2001) Construction of basic radiation hybrid maps for five equine chromosomes. *Proceedings: IX International Plant and Animal Conference*, Jan. 13–17, San Diego, USA, p 213
- Coogle L, Bailey E (1999) Equine dinucleotide repeat loci LEX064 through LEX070. *Anim Genet* 30, 71–72
- Eggleston-Stott ML, DelValle A, Bowling AT, Bautista M, Zahorchak R, Malj W (1996) Four equine dinucleotide repeats at microsatellite loci UCDEQ5, UCDEQ14, UCDEQ46 and UCDEQ62. *Anim Genet* 27, 129
- Eggleston-Stott ML, DelValle A, Bautista M, Dileanis S, Wictum E (1999) Twelve equine dinucleotide repeats at microsatellite loci UCDEQ304, UCDEQ380, UCDEQ387, UCDEQ411, UCDEQ439, UCDEQ440, UCDEQ455, UCDEQ457, UCDEQ464, UCDEQ465, UCDEQ482 and UCDEQ497. *Anim Genet* 30, 69–70
- Flaherty L, Herron B (1998) The new kid on the block—a whole genome mouse radiation hybrid panel. *Mamm Genome* 9, 417–418
- Godard S, Vaiman D, Oustry A, Nocart M, Bertaud M et al. (1997) Characterization, genetic and physical mapping analysis of 36 horse plasmid and cosmid-derived microsatellites. *Mamm Genome* 8, 745–750
- Godard S, Vaiman A, Schibler L, Mariat D, Vaiman D et al. (2000) Cytogenetic localization of 44 new coding sequences in the horse. *Mamm Genome* 11, 1093–1097
- Guérin G, Bailey E, Bernoco D, Anderson I, Antczak DF et al. (1999) Report of the International Equine Gene Mapping Workshop: male linkage map. *Anim Genet* 30, 341–354
- Gyapay G, Schmitt K, Fizames C, Jones H, Vega-Czarny N et al. (1996) A radiation hybrid map of the human genome. *Hum Mol Genet* 5, 339–346
- Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A et al. (1999) A first-generation porcine whole-genome radiation hybrid map. *Mamm Genome* 10, 824–830
- Kiguwa SL, Hextall P, Smith AL, Critcher R, Swinburne J et al. (2000) A horse whole-genome-radiation hybrid panel: chromosome 1 and 10 preliminary maps. *Mamm Genome* 11, 803–805
- Lear TL, Brandon R, Piumi F, Terry R, Guerin G et al. (2001) Mapping of 31 horse genes in BACs by FISH. *Chromosome Res* 9, 261–262
- Lindgren G, Sandberg K, Persson H, Marklund S, Breen M et al. (1998) A primary male autosomal linkage map of the horse genome. *Genome Res* 8, 951–966
- Lindgren G, Breen M, Godard S, Bowling A, Murray J et al. (2001) Mapping of 13 horse genes by fluorescence in-situ hybridization (FISH) and somatic cell hybrid analysis. *Chromosome Res* 9, 53–59
- Mariat D, Oustry-Vaiman A, Cribiu EP, Raudsepp T, Chowdhary BP et al. (2001) Isolation, characterization and FISH assignments of horse BAC clones containing type I and II markers. *Cytogenet Cell Genet* 92, 144–148
- Marti E, Breen M, Fischer P, Swinburne J, Binns MM (1998) Six new cosmid derived and physically mapped equine dinucleotide repeat microsatellites. *Anim Genet* 29, 236–238
- McCarthy LC, Terrett J, Davis ME, Knights CJ, Smith AL et al. (1997) A first-generation whole genome-radiation hybrid map spanning the mouse genome. *Genome Res* 7, 1153–1161
- Mellersh CS, Hitte C, Richman M, Vignaux F, Priat C et al. (2000) An integrated linkage-radiation hybrid map of the canine genome. *Mamm Genome* 11, 120–130
- Murphy WJ, Menotti-Raymond M, Lyons LA, Thompson MA, O'Brien SJ (1999) Development of a feline whole genome radiation hybrid panel and comparative mapping of human chromosome 12 and 22 loci. *Genomics* 57, 1–8
- Olivier M, Aggarwal A, Allen J, Almendras AA, Bajorek ES et al. (2001) A high-resolution radiation hybrid map of the human genome draft sequence. *Science* 291, 1298–1302
- Priat C, Hitte C, Vignaux F, Renier C, Jiang Z et al. (1998) A whole-genome radiation hybrid map of the dog genome. *Genomics* 54, 361–378
- Raudsepp T, Frönicke L, Scherthan H, Gustavsson I, Chowdhary BP (1996) Zoo-FISH delineates conserved chromosomal segments in horse and man. *Chromosome Res* 6, 333–335
- Raudsepp T, Kijas J, Godard S, Guerin G, Andersson L et al. (1999) Comparison of horse chromosome 3 with donkey and human chromosomes by cross-species painting and heterologous FISH mapping. *Mamm Genome* 10, 277–282
- Roed KH, Midthjell L, Bjornstad G (1998) Eight new equine dinucleotide repeat microsatellites at the NVHEQ26, NVHEQ29, NVHEQ31, NVHEQ40, NVHEQ43, NVHEQ90, NVHEQ98 and NVHEQ100 loci. *Anim Genet* 29, 470
- Scheetz TE, Raymond MR, Nishimura DY, McClain A, Roberts C et al. (2001) Generation of a high-density rat est map. *Genome Res* 11, 497–502
- Shiue YL, Bickel LA, Caetano AR, Millon LV, Clark RS et al. (1999) A synteny map of the horse genome comprised of 240 microsatellite and RAPD markers. *Anim Genet* 30, 1–9
- Shiue Y-L, Millon LV, Skow LC, Honeycutt D, Murray JD et al. (2000) Synteny and regional marker order assignment of 26 type I and microsatellite markers to the horse X- and Y-chromosomes. *Chromosome Res* 8, 45–55
- Slonim D, Kruglyak L, Stein L, Lander E (1997) Building human genome maps with radiation hybrids. *J Comput Biol* 4, 487–504
- Stewart EA, McKusick KB, Aggarwal A, Bajorek E, Brady S et al. (1997) An STS-based radiation hybrid map of the human genome. *Genome Res* 7, 422–433
- Swinburne J, Gerstenberg C, Breen M, Aldridge V, Lockhart L et al. (2000) First comprehensive low-density horse linkage map based on two 3-generation, full-sibling, cross-bred horse reference families. *Genomics* 66, 123–134
- Van Etten WJ, Steen RG, Nguyen H, Castle AB, Slonim DK et al. (1999) Radiation hybrid map of the mouse genome. *Nat Genet* 22, 384–387
- Vega-Pla JL, Garrido JJ, Dorado G, de Andres-Cara DF (1996) Three new polymorphic equine microsatellites: HLM2, HLM3, HLM5. *Anim Genet* 27, 215
- Watanabe TK, Bihoreau MT, McCarthy LC, Kiguwa SL, Hishigaki H et al. (1999) A radiation hybrid map of the rat genome containing 5,255 markers. *Nat Genet* 22, 27–36
- Yang YP, Womack JE (1998) Parallel radiation hybrid mapping: a powerful tool for high-resolution genomic comparison. *Genome Res* 8, 731–736