Comparative mapping of oculocutaneous albinism type II (*OCA2*), transient receptor potential cation channel, subfamily M member 1 (*TRPM1*) and two equine microsatellites, *ASB08* and *1CA43*, among four equid species by fluorescence in situ hybridization

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Rationale and significance

The extant members of the family Equidae appear to have diverged from a common ancestor approximately two million years ago (Oakenfull et al., 2000). Despite this recent divergence the family has undergone rapid karyotypic evolution demonstrated by large differences in diploid chromosome number indicating that multiple chromosomal changes and rearrangements have occurred (Bush et al., 1977; Ryder et al., 1978). In order to obtain comparative mapping information and thus, further evolutionary insight into a chromosome region, we FISH mapped horse BAC clones containing genes for *OCA2* and *TRPM1* (Terry et al., 2004), and two other linked markers, *ASB08* and *1CA43*, to the metaphase chromosomes of the domestic horse (*E. caballus*; 2n = 64), and three equid species with diverse chromosome numbers: So-

mali wild ass (*E. africanus somaliensis*; EAF; 2n = 62–64), Burchell's zebra (*E. burchelli*; EBU; 2n = 44) and Hartmann's mountain zebra (*E. zebra hartmannae*; EZH; 2n = 32). These markers were chosen because *ASB08* and *1CA43* were previously shown to be linked to the gene for Appaloosa coat color pattern (*LP*) and we were interested in *OCA2* and *TRPM1* as candidate genes for this trait.

Materials and methods

Horse metaphase chromosomes from lymphocytes were prepared as previously described (Bailey et al., 1997). Exotic equid metaphase chromosomes were prepared at the Zoological Society of San Diego's Conservation and Research for Endangered Species (Kumamoto et al., 1996). The USDA CHORI-241 Equine BAC library (<http://bacpac.chori.org/equine241.htm>) was screened by PCR to obtain clones for *OCA2*, *TRPM1*, *1CA43*, and *ASB08*. Published equine primers were used to screen the library for *1CA43* and *ASB08* (Breen et al., 1997; Swinburne et al., 2000). Primers for *OCA2* and *TRPM1* were designed from human sequence data. Primer sequences and BAC clone identification are shown in Table 1. Clone identity was confirmed by cycle sequencing (ABI3700 Applied Biosystems) and sequences compared by BLAST searches (*OCA2* GenBank accession numbers: DU708591 and DU708592; *TRPM1* GenBank accession numbers: DU708593 and DU708594) or in the case of the microsatellites to published horse sequences (Breen et al., 1997; Swinburne et al., 2000). DNA was prepared from these clones and labeled as previously described (Lear et al., 2001). *ASB08* and *1CA43* were labeled with SpectrumOrange™-dUTP, *TRPM1* with SpectrumGreen™-dUTP and *OCA2* with SpectrumRed™-dUTP. Chromosomes were counterstained with 31.5 ng/ml DAPI III in antifade solution (Vysis, Inc.) and the slides were stored at –20 °C until use. The hybridization results were analyzed using an Axioplan 2 fluorescent microscope (Carl Zeiss, Inc.) equipped with Cytovision/Genus Application Software, Version 3.0 (Applied Imaging Corp.).

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Fig. 1. Comparative FISH mapping of equine BAC clones. (A–D) *ASB08* (SpectrumOrange™-dUTP), *TRPM1* (SpectrumGreen™-dUTP) and *OCA2* (SpectrumRed™-dUTP) hybridized to (A) ECA1q17.1, 17.2, and 17.3, respectively, (B) EAF2q21, (C) EBU2q, and (D) EZH3q. (E–H) *TRPM1* (SpectrumGreen™-dUTP), *OCA2* (SpectrumRed™-dUTP) and *ICA43* (SpectrumOrange™-dUTP) hybridized to (E) ECA1q17.2, 17.3 and 17.3, respectively, (F) EAF2q21, (G) EBU2q, and (H) EZH3q.

Table 1. Primer sequence and equine CHORI241 BAC clone identity for each of the markers mapped in this study

Marker	Primer	Clone identity
<i>TRPM1</i>	TRPM1F 5'-TGTACCAGACCATGTCCAACCCTCTA-3'	117B6
	TRPM1R 5'-TCTTCTGCAGGGAGATGTGCTTTT-3'	
<i>OCA2</i>	OCA2x16F 5'-AGCACGAGATTCACGTCTGG-3'	190I17
	OCA2X16R 5'-TCGGGCCAGCAGGTGCTCCA-3'	
<i>ASB08</i>	ASB08F 5'-GACAACGTGGCAGCTCACTGCC-3'	188D9
	ASB08R 5'-GCAAGTAAGCCATATGTGCATGCG-3'	
<i>ICA43</i>	ICA43F 5'-ATGGCATGATTTGCTTCTCC-3'	95M17
	ICA43R 5'-ATGGAAACAACCTAAATGTCCA-3'	

Results and discussion

The *E. caballus* chromosomes were identified using the international standard nomenclature for horse (ISCNH, 1997). In the horse, *ASB08* and *ICA43* mapped to ECA1q17.1 and ECA1q17.3 as previously described (Breen et al., 1997; Chowdhary et al., 2003), and *TRPM1* and *OCA2* mapped to ECA1q17.2 and ECA1q17.3, respectively (Fig. 1A and E). Interphase spreads allowed us to confirm the gene order of these markers as ECA1q = cen–*ASB08*–*TRPM1*–*OCA2*–*ICA43* (data not shown). This confirmed that both of the candidate genes for appaloosa spotting mapped within the same critical region as *LP*.

Our results indicated the gene order was, in fact, conserved in the three other equid species studied (Fig. 1B–D and F–H). Using the donkey chromosome standard proposed by Raudsepp et al. (2000), in Somali wild ass all four markers mapped to EAF2q21. For Burchell's zebra the four markers

mapped to EBU2q. The Hartmann's zebra chromosomes were identified according to Richard et al. (2001), and these markers mapped to EZH3q.

Mapping these four markers to the chromosomes of three additional equid species has provided comparative mapping information for these species and demonstrated that this region has been conserved despite the rapid karyotypic evolution of this genus.

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