Localization of the U2 Linkage Group of Horses to ECA 3 Using Chromosome Painting

T. L. Lear and E. Bailey

The U2 linkage group of horses includes the genes albumin (ALB), vitamin D binding protein (GC), mitochondrial glutamate oxaloacetate transaminase 2 (GOT2), and haptoglobin (HP), which are found on two human chromosomes, namely, 4 (HSA 4) and 16 (HSA 16). Likewise, these genes are also found on two different chromosomes in mice, rats, and cattle. Chromosome painting demonstrated that only horse chromosome 3 (ECA 3) hybridized with whole chromosome paints for both HSA 4 and HSA 16. This indicated that the equine U2 linkage group occurs on ECA 3, spanning the centromere. This technique will be useful to study the chromosome rearrangements associated with speciation of the genus Equus.

Chromosome painting has been shown to be useful for identifying homologous chromosome segments among primates (Blakely 1994; Stanyon et al. 1995) and among distantly related mammals (Rettenberger et al. 1995; Scherthan et al. 1994; Solinas-Toldo et al. 1995). Composite, fluorescently labeled DNA probes are prepared from microdissected or flow-sorted chromosomes from one species and hybridized to metaphase chromosome spreads of another species by standard in situ hybridization. By this method, homologous chromosome regions between two species can be identified. Chromosome painting is most useful to identify chromosome homology in species that do not have high-resolution gene maps, as is the case for the domestic horse (Equus caballus).

Among domestic animals, the gene map for the horse is the least well developed. Only five linkage groups have been reported in the domestic horse, of which three have been mapped to chromosomes and only four genes have been mapped using in situ hybridization [reviewed by Sandberg and Andersson (1993)]. The largest linkage group known for horses is designated U2 and has not yet been mapped to a horse chromosome. This linkage group spans approximately 40 cM and includes the genes albumin (ALB), vitamin D binding protein or group-specific component (GC), carboxylesterase (ES), mitochondrial glutamate oxaloacetate transaminase 2 (GOT2), haptoglobin (HP), and the coat color genes roan (RN), tobiano (TO), and chestnut or extension (E) (Andersson and Sandberg 1982; Andersson et al. 1983; Bowling 1987).

The U2 linkage group includes genes that are distributed among at least two linkage groups in several other species. The human gene map shows linkage of ALB and GC on human (Homo sapiens; HSA) chromosome 4 and linkage of GOT2 and HP on HSA 16 (O’Brien and Graves 1991). Likewise, ALB and GC have been mapped to bovine (Bos taurus; BTA) chromosome 6 and to rat (Rattus norvegicus; RNO) chromosome 14, and ALB to mouse (Mus musculus; MMU) chromosome 5, while other mapping studies demonstrated HP and GOT2 to MMU 8 and HP on BTA 18 and RNO 19 [reviewed in O’Brien (1993)].

This information can be used to identify the chromosomal location of the U2 linkage group. The horse chromosome possessing the U2 linkage group should hybridize with paints from both HSA 4 and HSA 16. Here we report using whole chromosome painting probes for HSA 4 and HSA 16 to identify homologous segments on horse chromosomes and determine the chromosomal location of U2.

Materials and Methods

Lymphocytes from four karyotypically normal, unrelated, mixed breed or thoroughbred stallions were purified with Histopaque (Sigma, St. Louis, Missouri) following the manufacturer’s directions and cultured using methods already described (Jacky 1991; Ponce de Leon et al. 1991) with stimulation by pokeweed mitogen and phytohemagglutinin. Slides were stored in light-tight containers under desiccant at -70°C until used. GTG-banded slides were destained and pretreated prior to hybridization as described (Klever et al. 1991; Scherthan et al. 1994). Chromosome identification was based on the standard for the domestic horse (Richer et al. 1990).

Chromosome-specific painting probes for HSA 4 and HSA 16 were purchased from Vyssis, Inc. (Framingham, Massachusetts) and Oncor, Inc. (Gaithersburg, Maryland). Probe preparation, hybridizations, and posthybridization washes were done according to manufacturer’s directions with the following exceptions: (1) GTG-banded slides were denatured individually at 70°C for 2 min. Nonbanded slides were denatured 2–5 minutes at 70°C–73°C. (2) When possible, the probe concentration was double the recommended concentration for each reaction. (3) Slides were hybridized at 37°C for 24–48 h. (4) All posthybridization washes were performed at 37°C.

The slides were counterstained with the propidium iodide or DAPI solutions supplied with the probes. Metaphase spreads were observed with a Zeiss Axioskop fluorescent microscope equipped with single or triple band-pass filters and the unenhanced images were recorded with an Onorotics CCD video camera and Sony 5500 series color printer.

Results and Discussion

Both HSA 4 paints consistently hybridized to the long arms of horses (Equus caballus; ECA) chromosomes ECA 2 and ECA 3 (Figure 1A) except for the centromeric regions. The strong signal intensity made the two areas of hybridization clearly identifiable. Smaller areas of hybridization to other chromosomes were not apparent.

The Oncor HSA 16 paint hybridized to both the short arm of ECA 3 and to the long arm of ECA 13 (Figure 1B). The Vysis HSA 16 paint consistently hybridized to ECA 3p, but signal was not observed on ECA 13. Although the signal intensity for the Vysis probe was much less than that for the HSA 4 paint, it was sufficient to identify the hybridization area. No small areas of hybridization to other horse chromosomes were apparent with either paint. These results indicate that ECA 2 and ECA 3 appear to have homologous regions to HSA 4. Since HSA 4 is a submetacentric chromosome, it would be of interest to determine if the different horse chromosomes are hybridizing to DNA from the different arms of HSA 4. HSA 16 is an acrocentric chromosome and it appears that both ECA 3 and ECA 13 are homologous to portions of this chromosome. The gene for hemoglobin alpha maps to horse chromosome 13qter (Oakenfull et al. 1993) and to HSA 16 (Buckley et al. 1988), demonstrating homology of these two chromosome regions and corroborating the chromosome painting results presented here. Since the genes of equine linkage group U2 correspond to genes on HSA 4 and HSA 16 and since ECA 3 is the only horse chromosome showing homology to both, the results of this study indicate that U2 probably occurs on ECA 3 spanning the centromere. Comparable results have also been obtained by Raudsepp et al. (1996) using...
painting probes from other human chromosome libraries.

Hybridization signals were seen only along the chromosome arms and not in the centromeric regions of the horse chromosomes. This was also observed when we used other human chromosome paints (Lear TL, unpublished data). This may be due to the species-specific nature of repetitive DNAs comprising the centromere or to the absence of centromeric sequences in the libraries from which the probes were derived.

The intensity and appearance of the signal varied according to the source of the probe and the type of probe label. The directly labeled probes, purchased from Vysis, Inc., gave a more homogenous signal. The indirectly labeled probes, purchased from Oncor, Inc., gave a more diffuse signal that increased upon signal amplification. Painting probes from these sources always gave similar but not necessarily identical results. The Vysis HSA 16 paint did not hybridize in our system to ECA 13; however, this region was consistently visible when the Oncor HSA 16 paint was used. Clearly the sensitivity of the technique is limited and we cannot exclude small regions of homology to HSA 4 and HSA 16 on other horse chromosomes.

Chromosome painting probes were clearly useful in identifying homologous segments on horse chromosomes. Although humans and horses diverged from a common ancestor 55-65 million years ago (MacFadden 1994), large regions of chromosome homology still exist. Further gene mapping studies using anchor loci are needed to confirm the position of U2.

It is not known whether the U2 linkage group is conserved among all equids. Equid chromosomes have undergone rapid karyotypic evolution (Bush et al. 1977) and chromosome numbers vary from 2N = 66 (NF = 92) in Equus przewalskii to 2N = 32 (NF = 62) in Equus zebra hartmannae. The formation of the U2 linkage group may reflect one of the many putative chromosomal rearrangements that contributed to the evolution of the equids. It would be of interest to determine the degree of U2 conservation among equids to gain a better understanding of how and when this linkage was formed and what significance it might have for the horse and its related species. Chromosome painting would clearly be a powerful tool for determining the number and type of chromosomal rearrangements that have occurred during the evolution of the Equidae.

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References


Black Crystal: A Novel Color Mutant in the American Mink (Mustela vison Schreber)

O. V. Trapezov

Black crystal, a new mutant of coat color pattern occurring in the American mink in the course of selection for domestic behavior, is described. A salient feature of the mutation is the appearance of white guard hairs producing a veil-like covering of the body. In the Black crystal homozygote, coat color is of the Himalayan type. Breeding data demonstrate that the novel color phase is inherited as a monogenic autosomal semidominant trait. The mutant gene is designated as Black crystal and is symbolized by Cr. The Cr gene is not allelic to the multiple-allelic series at the Black cross locus.

Color mutations occurring in minks bred in farms offer promise in the fur industry. Since the beginning of this century 10 dominant and 20 recessive coat color mutations have been revealed in minks bred in captivity (Ness et al. 1988). Most, however, have accumulated in wild minks, hidden as recessives (Belyaev 1959; Castle and Moore 1946; Robinson 1975; Shackelford 1948). Restriction of free mating and inbreeding under commercial farm conditions have resulted in homozygization of these mutations. The de novo arisen genetics changes, as is well known, are extremely rare. To our knowledge, since the beginning of the 1960s, only three semidominant mutations have been recorded in all the mink farms in the world (Ness et al. 1988).

Long-term selection of the silver fox (Vulpes vulpes) and the American mink (Mustela vison) for tame behavior has been carried out at the experimental farm of this Institute of Cytology and Genetics (Novosibirsk, Russia). The idea of these experiments, the results, and elicited correlated responses have been described (Belyaev 1969, 1979; Belyaev and Khvostova 1974; Belyaev and Trut 1981; Trapezov 1987, 1991, 1994; Trut 1988).

Evidence was presented indicating that selection for tame behavior gives rise to new physiological and morphological changes in populations of fur animals (Belyaev et al. 1981). The de novo appearing variations in coat color in the mink population we subjected to domestication are good examples of correlated responses to selection. The suggestion that the occurrence of coat color variation is a correlated response to selection for behavioral traits is justified as follows. First, Black crystal is inherited as an incompletely dominant mutation; the heterozygotes and mutant homozygotes are phenotypically quite different from normal. Consequently, it cannot be hidden by a wild phenotype and made apparent through inbreeding as of...

Table 1. Segregation ratios in crosses of Black crystal with Standard mink

<table>
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<tr>
<th>Crosses</th>
<th>Female</th>
<th>Male</th>
<th>Offspring number</th>
<th>Standard (+/+)</th>
<th>Black crystal (Cr/+)</th>
<th>Himalayan (Cr/+)</th>
<th>χ²</th>
<th>p</th>
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<tbody>
<tr>
<td>Black crystal</td>
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<td>267</td>
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*Pups dying soon after birth.