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# Chromosomal localization of the major histocompatibility complex of the horse (*ELA*) by *in situ* hybridization

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**Abstract.** The first gene assignment to a horse chromosome is reported for equine leucocyte antigen (*ELA*), the major histocompatibility complex of the horse. A cloned DNA sequence derived from a class I gene of the porcine major histocompatibility complex was used as a probe for an *in situ* hybridization experiment. We present the regional localization of *ELA*, using this sequence, to equine chromosome *20q14–q22*.

## Introduction

The equine lymphocyte antigen (*ELA*) system is the major histocompatibility complex (MHC) in the horse. Extensive studies on serological aspects of the *ELA* system have been summarized in four international workshops (Antczak et al. 1986, Bailey et al. 1984, Bernoco et al. 1987, Bull 1983). Molecular genetic techniques have only recently been introduced to localize the portions of horse genome responsible for the *ELA* system (Alexander et al. 1987, Guerin et al. 1987, Halldorsdottir and Davies 1987, Vaiman et al. 1986). These works employ the technique of Southern blotting to detect restriction fragment length polymorphisms in horses using mainly class I and class II MHC probes derived from human and mouse genomes. The chromosomal location of the horse MHC is still unknown. This may be attributed to the difficulty in obtaining good metaphase preparations and identification of banded chromosomes of the horse. Since an extensive nucleotide sequence similarity had been shown between MHC genes from different mammalian species, we undertook to map the equine MHC by *in situ* hybridization using a probe derived from a porcine class I MHC gene. After chromosomally mapping *ELA* on a horse chromosome, we now present the first regional gene assignment in the horse.

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## Material and methods

Metaphase spreads of cultured peripheral lymphocytes stimulated with pokeweed mitogen were obtained from a "Hannoveran" warm blood stallion. Before hybridization, QFQ (quinacrine mustard)-stained metaphases were photographed, and chromosomes were identified on the basis of Reading Conference recommendations (Ford et al. 1980). The chromosome measurements of Hansen (1984) were used for constructing the idiogram. The DNA hybridization was carried out using a SLA class I-specific 4.3 kb *Bam* HI/*Hind* III fragment excised from PD1A, a recombinant pBR322 clone (Singer et al. 1982). The labeled probe was prepared by the process of oligolabeling originally presented by Feinberg and Vogelstein (1983, 1984) and later modified by Lin and co-workers (1985) for using tritiated nucleotides. The specific activity obtained by using three radionucleotides ($^3$H-dATP, $^3$H-dCTP, and $^3$H-TTP) was $9 \times 10^8$ dpm/μg, and the probe was used at a final concentration of 37.5 ng/ml. Hybridization procedure, stringency of washings, and autoradiography were done as described by Fries and co-workers (1986) except that the emulsion-coated slides were developed after 17 days of exposure. The slides were stained with 4% Giemsa diluted in Sørensen's buffer at pH 6.8, and previously Q-banded metaphases were reexamined under the microscope for the location of hybridization sites.

## Results

Sixty-three well-spread and optimally Q-banded metaphases in which the presence of 961 silver grains was noted (an average of 15.2 grains per cell) were analyzed. Only grains in contact with a chromatid were scored. The grains viewed microscopically were marked on the Q-banded photographs taken earlier and used to construct the histogram displayed as Figure 1. Both the homologues of chromosome 20 were labeled in 48 metaphases (76%), whereas only one chromosome 20 showed labeling in ten metaphases. Of the 961 grains scored, 424 (44%) were found on chromosome 20. Among these, 349 grains (82%) were localized in the *20q14–q22* region (Fig. 1, inset). A metaphase plate before and after hybridization is shown in Figure 2a and b. Clustering of grains in the specific region was frequently observed (Fig. 2a). The *ELA* locus, therefore, is most likely located in the *q14–q22* region on chromosome 20.

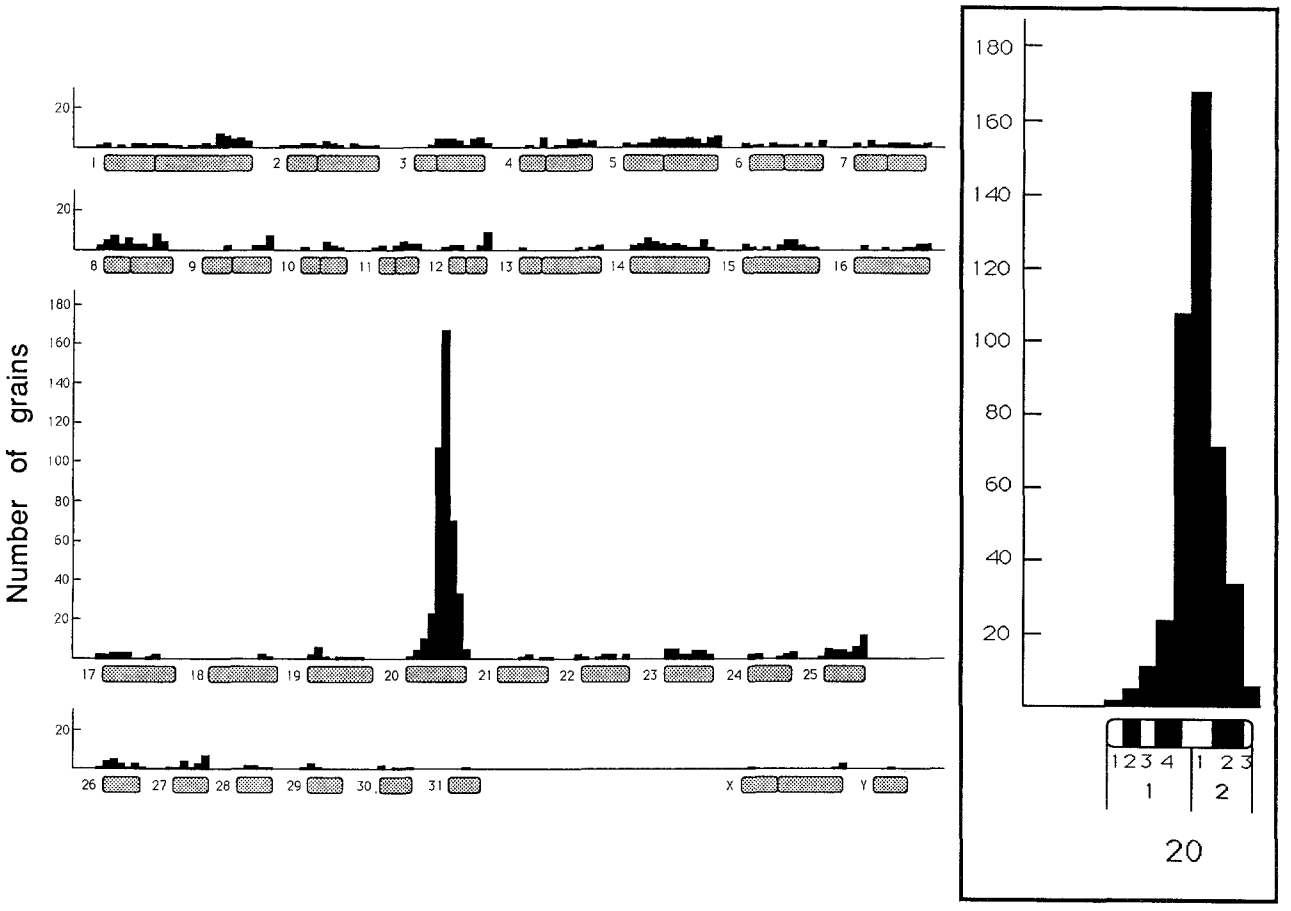


**Fig. 1.** Histogram of silver grain distribution in 63 horse metaphases after hybridization with the radiolabeled porcine MHC probe illustrating the significant hybridization on chromosome 20. *Inset:* distribution of labeled sites on chromosome 20 [schematic Q-banding pattern based on our observations and comparable to the G-banding presented in Reading Conference (Ford et al. 1980)]. Among the 424 grains counted on this chromosome, 349 (82%) were located in the *q14–q22* region

## Discussion

Recent genetic studies of the equine genome have revealed that, as in other higher vertebrates, the *ELA* system consists of class I and class II antigens, the genes for which are tightly linked within the equine MHC. Employing the Southern blot hybridization technique on horse DNA using class I and class II MHC probes derived from mouse, Alexander and co-workers (1987) demonstrated that the *ELA* system consists of more than 20 class I entities and that the genes of the two families are highly polymorphic. Additional parallel studies of the *ELA* system were performed using human MHC class I, II, and III probes (Guerin et al. 1987, Vaiman et al. 1986) and human MHC class II probes (Halldorsdottir and Davies 1987). Cross hybridization of human probes with horse genome in Southern blotting was reported. Our present investigation on the equine MHC by *in situ* hybridization using a porcine class I probe clearly shows the hybridization on chromosome 20. Previously, the same probe was used for physical mapping of MHC in swine (Rabin et al. 1985) and cattle (Fries et al. 1986). The results of the present investigation strongly support the contention that, as in other mammals, the *ELA* system has maintained a high level of nucleotide sequence conservation. It is, however, necessary to determine the localization of different MHC class II probes on horse chromosomes to obtain additional support for the above contention. Since no equine MHC genes have yet been cloned or sequenced, confirmation of the assignment of the *ELA* system using a homologous probe is not presently possible.

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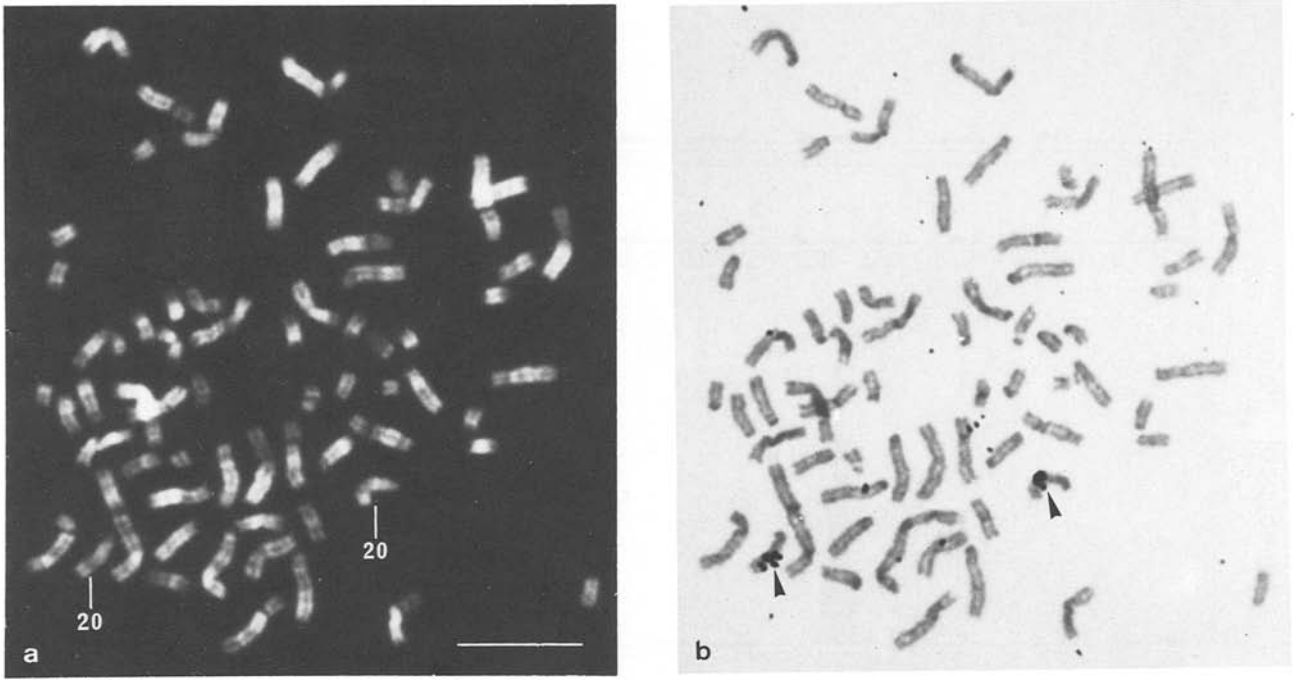


**Fig. 2a** and **b.** A representative horse metaphase spread before (**a**) and after (**b**) the hybridization with radiolabeled probe. (**a**) QFQ-stained metaphase for the identification of chromosomes; (**b**) sequential Giemsa staining after hybridization and autoradiography. Clusters of silver grains can be seen on specific sites on the homologous chromosomes 20 (*arrow heads*). *Bar* represents 10 μm

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