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THE POSSIBILITY OF CATTLE CHROMOSOME CLASSIFICATION, IDENTIFIED BY G-BANDING PATTERNS

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A trypsin-Giemsa banding technique was carried out in order to identify 30 pairs of cattle chromosomes. All of the 30 pairs displayed characteristic banding patterns. The autosomes were classified into 4 groups, each member of which could be identified on the basis of their banding patterns. Details of the G-banding patterns of each chromosome were described and illustrated both in a karyotypical photograph and a schema. This technique may be valuable in cytogenetical diagnosis of chromosomal abnormalities in cattle.

Since the development of banding techniques, the chromosome constitution of various species has become clarified. In the human, in particular, the standardization of a banding pattern has been completely established (Paris Conference, 1971). On the other hand, in cattle, although the G- (SCHNEDL, EVANS et al. and SCHNEDL & CZAKER) and Q-banding patterns (SCHNEDL and HANSEN[2,3]) have been studied, the order of autosomes has not yet been agreed upon by researchers. In this report, a trypsin-Giemsa banding technique was employed to obtain a new criterion for the identification of each chromosome in cattle.

MATERIALS AND METHODS

Venous blood samples from five clinically normal Holstein cattle (three males and two females) were cultured in Eagle's minimum essential medium with 10 % calf serum for 72 hr, and the cultures were arrested with colchicine for 1.5 hr. Hypotonic treatment and fixation were carried out using a conventional procedure, and the slide preparations were air dried. A Giemsa banding technique which slightly modified the method of WURSTER was employed: these slide preparations were treated with 0.25 % trypsin solution made up of a calcium and magnesium free phosphate buffered saline for 120~180 sec., and were stained with 2 % Giemsa solution diluted with phosphate buffer for 6~8 min. The metaphase cells on the slides were photographed and karyotyped, and then each chromosome was classified according to its banding pattern.

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FIGURE 1 *G-banding patterns in cattle chromosomes*

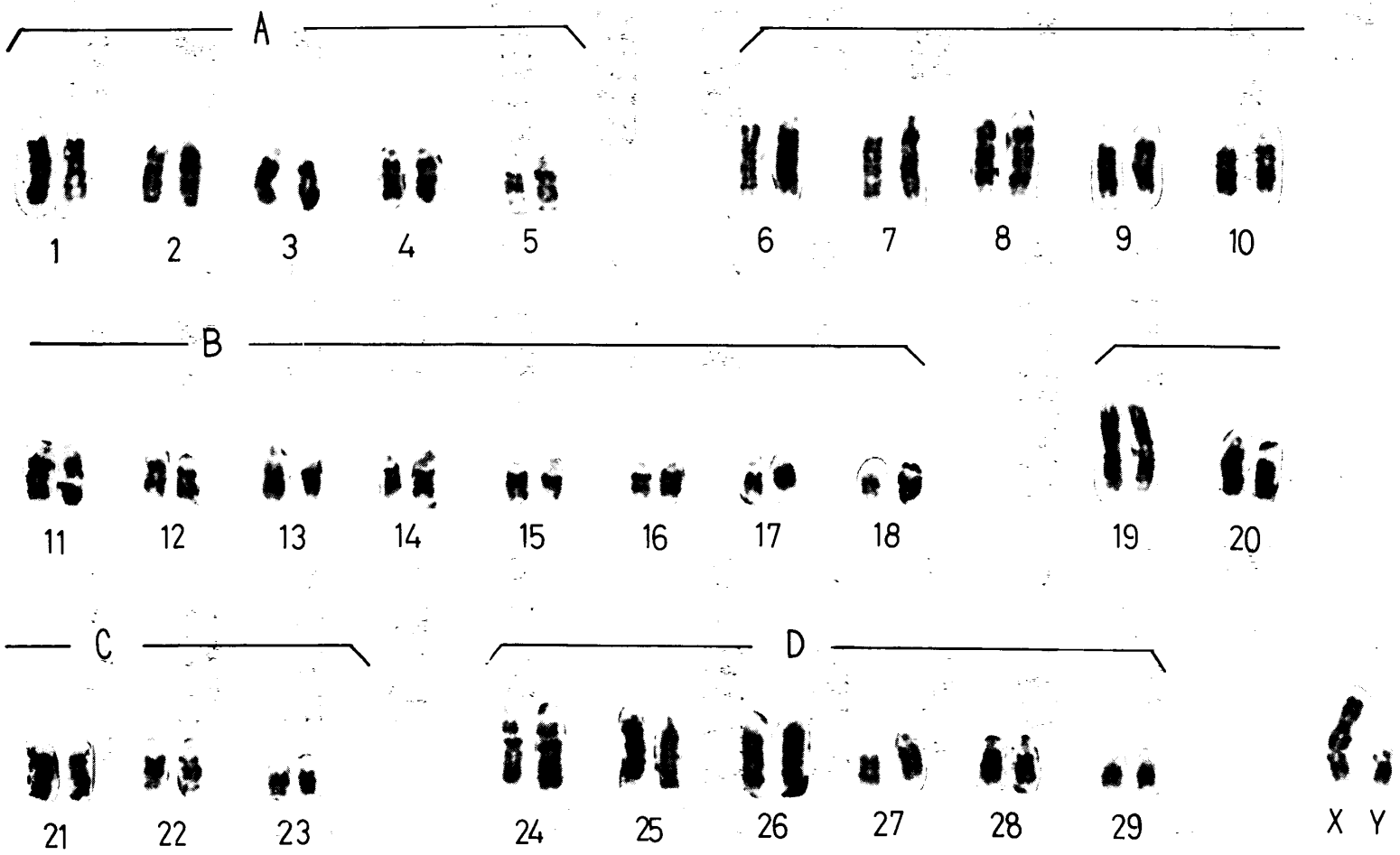
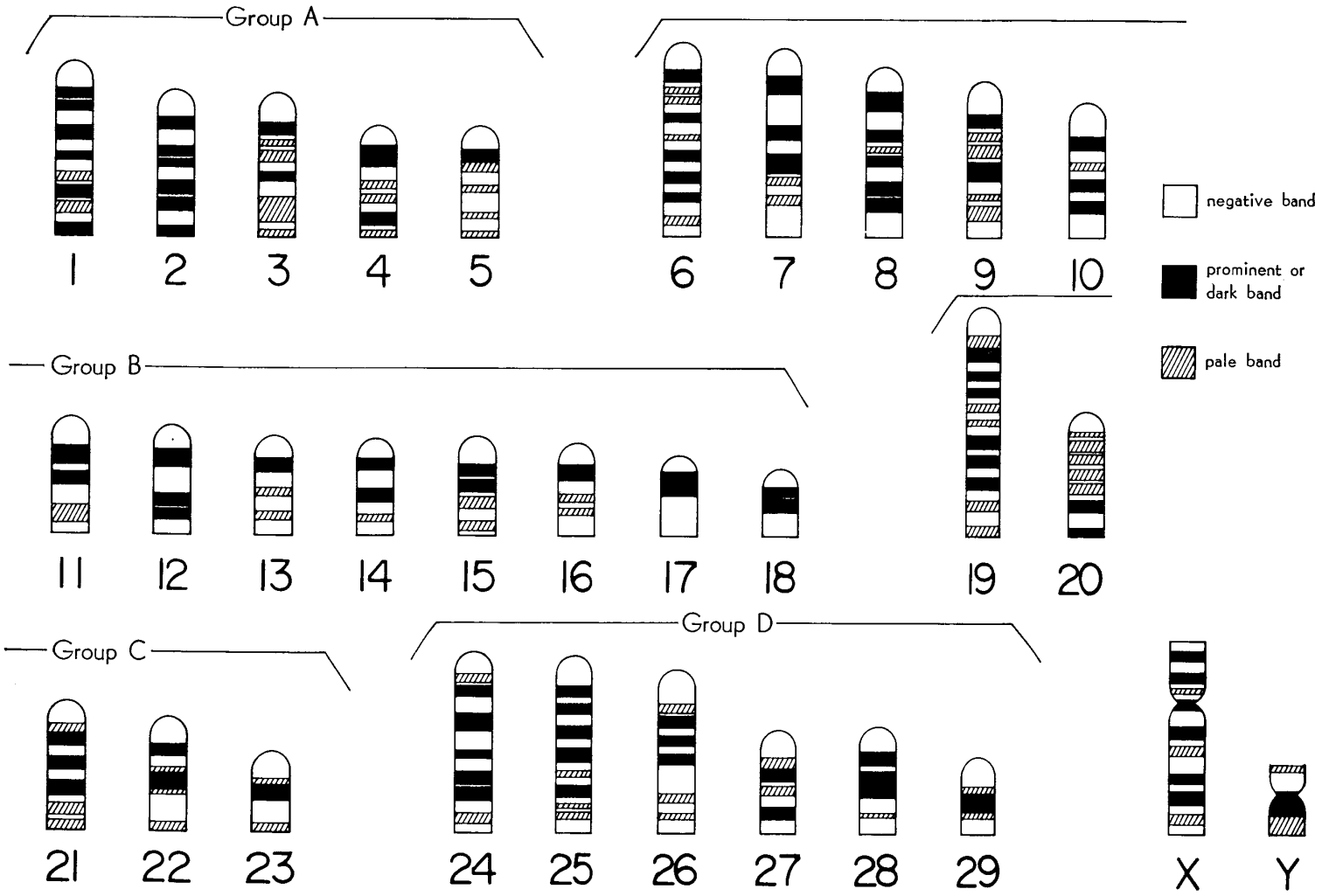


FIGURE 2 *Schema of G-banding patterns in cattle chromosomes*



Results

The trypsin-Giemsa banding technique showed the characteristic banding patterns of all the pairs of cattle chromosomes on properly stained preparations. In some improper preparations, however, a clear demonstration of the banding patterns could not be obtained, because the bands were too close to each other, or the chromosomes were bent or overlapped. These preparations were not suitable for the karyotype analysis. For the classification of autosomes, two markers were adopted : the intensity of the first band just below the centromere, and the presence or absence of a band at the distal end of the chromosome arm. Figure 1 shows a cattle chromosome karyotype arranged according to this method, and figure 2 is a schematic representation of each chromosome.

a) Autosomes

Group A (5 pairs): The first positive band just below the centromere is most prominent, and the other positive band is observed in the distal end of the chromosome arm.

No. 1 Eight bands are observed. Proximal two are sometimes shown as one broad, prominent band.

No. 2 Six bands are observed. The band at the distal end is very evident.

No. 3 Seven bands are observed. The fourth band located in the central region of the chromosome is conspicuous.

No. 4 The first band is very broad and prominent, and the next four are narrow and close to each other in the middle portion through the end of the chromosome.

No. 5 Five bands, with the exception of the first, are stained pale and the intensity of the staining decreases towards the distal end.

Group B (13 pairs): The first positive band just below the centromere is most prominent, and no positive band is observed at the distal end of the chromosome arm.

No. 6 Nine bands dispose continuously, the fifth and ninth are stained paler than the others.

No. 7 A broad negative region includes two narrow bands, and five other bands are observed, three of them often appearing as a single broad band.

No. 8 The first band is wide and prominent. Three bands at the middle region, and two bands at the distal region, respectively are observed.

No. 9 Similar to No. 3, but no band is observed at the distal end and is more darkly stained all over the chromosome.

No. 10 Four bands are observed, the second band is stained more faintly than the others.

No. 11 Three broad bands are noticeable.

No. 12 The first band is broad and prominent. The other two bands at the

distal third are sometimes observed as a single prominent band.

No. 13 A series of three narrow bands is observed.

No. 14 Similar to No. 13, three of the bands are uniform, and the space between the second and the third is small.

No. 15 Three bands are observed. The distal two are sometimes pale and the first band is often divided into two bands.

No. 16 Similar to No. 15, but each band is narrower.

No. 17 Two bands are adjacent to the centromere.

No. 18 This is probably the smallest chromosome with two prominent bands in the middle region.

Group C (5 pairs): The first positive band is not prominent, and the other positive band is observed at the distal end of the chromosome arm.

No. 19 This is the longest autosome with ten, sometimes eleven bands. The middle region is stained pale.

No. 20 As a whole, this is stained darkly and has five bands. The fourth is the most prominent.

No. 21 Five bands are observed. The third one is located in the central region of the chromosome and is the most prominent.

No. 22 The most prominent band is located in the central region. The distal band is narrow and stained faintly.

No. 23 The middle of the three bands is the most prominent and broad.

Group D (6 pairs): The first band is not prominent and no positive band is observed at the distal end of the chromosome arm.

No. 24 The six, sometimes seven, bands are observed. The most conspicuous band is located in one third of the chromosome arm region. This chromosome is, as a whole, stained rather darkly from the middle to the end.

No. 25 A series of seven uniform bands similar to No. 6 were noted, but the middle band is stronger stained than in No. 6.

No. 26 The six distinct bands are observed. The fourth is most prominent.

No. 27 Four bands are noted. The proximal two are broad and faint, so that sometimes they are recognized as a single band.

No. 28 The proximal half of the chromosome is stained darkly with three bands, while the distal half has only a faint band.

No. 29 This is stained rather pale and has two narrow bands in the middle portion of the chromosome.

b) Sex chromosomes

X chromosome: This is easily recognized as a longer submetacentric chromosome. The short arm possesses two bands, but sometimes an additional faint, narrow band is observed near the centromere. In the long arm, a broad unstained region is noticeable

in the middle portion. There are two bands above that region, and three bands below. The centromeric region of this chromosome, contrary to the autosomes, is strongly stained.

Y chromosome: This is recognized as a smaller metacentric chromosome. The shorter arm which is presumably stained pale, has a faint band at the distal tip, while the longer arm is, as a whole, stained darkly, and has an indistinct narrow band in the central portion. The centromeric region is strongly stained as in the X chromosome.

Discussion

The trypsin-Giemsa banding technique employed in the present study shows characteristic banding patterns in cattle chromosomes as well as in those of the human (Sumner et al. and Wang & Fedroff), the mouse (Wurster) and the cat (Wurster-Hill & Gray).

In autosomes, the centromeric region was not stained prominently. The staining ability of this region was less than that of other regions in the chromosome arm. The centromeric region seemed to lose its staining ability at an early stage of trypsin treatment. On the other hand, in the sex chromosomes, the centromeric region was the most sensitive of all of the bands. These facts agreed with results reported by Schnedl and Evans et al. However, Hansen, employing the quinacrine fluorescence staining in cattle, reported that no fluorescent material was observed in the centromeric region in both the autosomes and the sex chromosomes. Such discordance could not be explained from the results of this work.

In order to identify each chromosome for karyotype analysis in cattle, the number, intensity, width and disposition of each band, as well as the size of chromosomes should be considered. According to Hansen, cattle chromosomes and preparation by the Q-band staining technique frequently varied more than 10% in length among each homologous pair. Such variations may be caused by some physical factors during fixation or the spreading of the chromosomes on a slide. The fact suggests the uncertainty of karyotype analysis on the basis of chromosome length alone in cattle. Therefore, it can be said that banding patterns are necessary for identification of each chromosome. Although sex chromosome abnormalities can be easily detected in cattle, autosomal abnormalities such as aneuploidy, translocation, delation, or inversion may be difficult by the routine method of karyotype analysis. Thus, the G-banding technique will give an exact diagnosis of these autosomal abnormalities.

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