

# THE APPLICATION OF NEW STAINING TECHNIQUES IN THE IDENTIFICATION OF INDIVIDUAL CHROMOSOME PAIRS IN DOMESTIC ANIMALS

## Die Anwendung neuer Färbungstechniken bei der Identifizierung individueller Chromosomenpaare bei Haustieren

## La aplicación de las nuevas técnicas de coloración de pares de cromosomas individuales de animales domésticos

M. HAGELTORN \*

I. GUSTAVSSON \*

Research workers in veterinary cytogenetics have long been confronted with considerable problems in the identification of individual chromosomes in most domestic animals. Often identification problems have rendered the cytogenetic observations almost meaningless; the analysis has produced very crude and even faulty results. This has particularly concerned the analysis of karyotypes consisting exclusively, or almost exclusively, of chromosomes of similar size and morphology such as the canine, equine, caprine, bovine, and ovine karyotypes. In other domestic animals such as the pig, cat, and rabbit, several individual chromosome pairs have been distinguishable using conventional staining and autoradiographic techniques, but in most cases, even in the latter species, it has only been possible to group the pairs according to size and morphology.

To-day, some of the staining techniques available make it possible to carry out faultless identification of all individual chromosomes in the most common domestic animals. The perfection of the new staining techniques is best demonstrated by the fact that even in domestic animals with high chromosome numbers, only minor identification problems are met.

The *Q-banding* technique (CASPERSSON *et al.*, 1971) has been successfully applied to the pig karyotype (GUSTAVSSON *et al.*, 1972, 1973; HAGELTORN and GUSTAVSSON, 1973; HAGELTORN *et al.*, 1973), but would appear less successful for the identification of individual pairs in ruminants. From the different *G-banding* techniques, the technique involving trypsin pre-treatment (SEABRIGHT, 1971) has in our hands given the most reliable and consistent results. The same technique has also been successfully combined with the *Q-banding* technique (HAGELTORN and GUSTAVSSON, 1973). The *C-banding* technique has hardly made any contribution to the solving of identifi-

---

\* Department of Animal Genetics, Nutrition & Hygiene, Royal Veterinary College, S-104, 05 Stockholm 50, Sweden.



FIG. 1

G-banding patterns of the female dog chromosomes. The autosomes have been arranged according to decreasing size

cation problems, but its application has provided interesting information on rearrangements, such as centric fusions (POPESCU, 1973), and has revealed polymorphism of the centromere region (HAGELTORN *et al.*, in prep.) which was previously unknown. The *R-banding* technique has hitherto been very little utilized in chromosome studies of the domestic animals.

Some results of the application of the Q- and G-banding techniques on karyotypes of species with high chromosome numbers are shown in Figs. 1-5. The autosomes of the dog, goat, and sheep chromosomes were arranged as far as possible

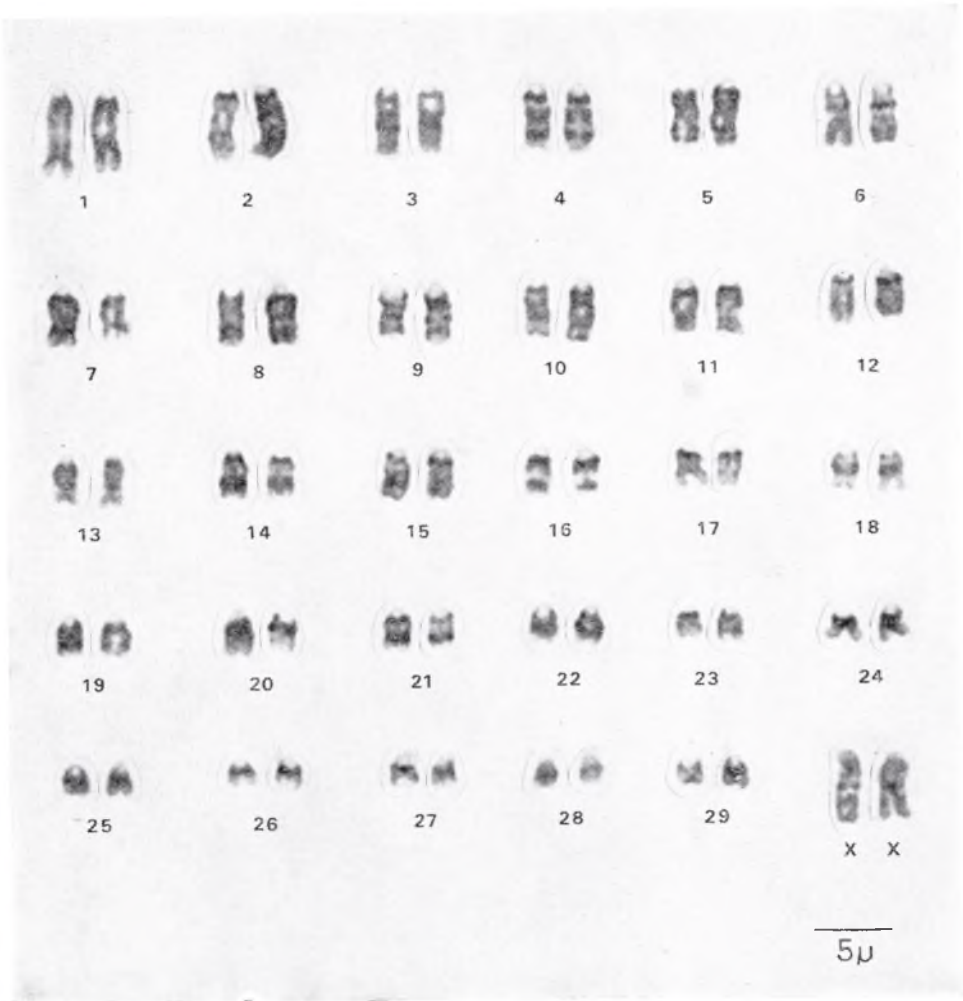


FIG. 2

G-banding patterns of the female goat chromosomes. The autosomes have been arranged according to decreasing size

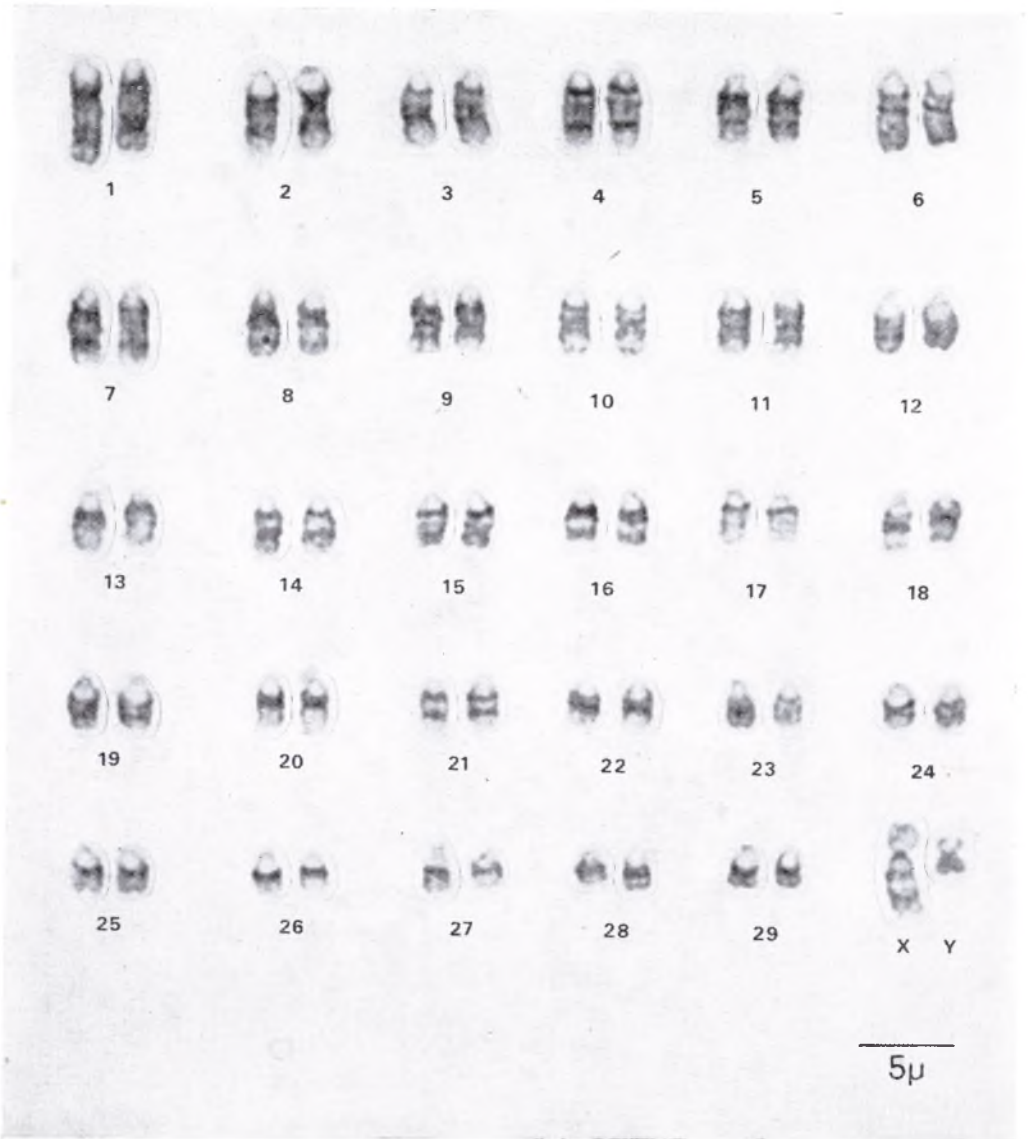


FIG. 3

G-banding patterns of the male cattle chromosomes. The goat karyotype has been used as a standard for the arrangement of the chromosomes

in decreasing order of size, but the arrangement of the cattle chromosomes did not entirely conform to this scheme, since the goat karyotype was used as a standard (EVANS *et al.*, 1973). The horse autosomes were grouped according to

centromere position and the autosomes arranged within the groups in decreasing order of size. The chromosome arrangements in Figs. 1-5 may be regarded as proposals for common systems of arranging chromosomes in future. With the exception of the cat, international agreements for arranging the chromosomes of domestic animals do not yet exist but would be highly desirable.

Although the dog karyotype (Fig. 1) demonstrated one of the highest chromo-

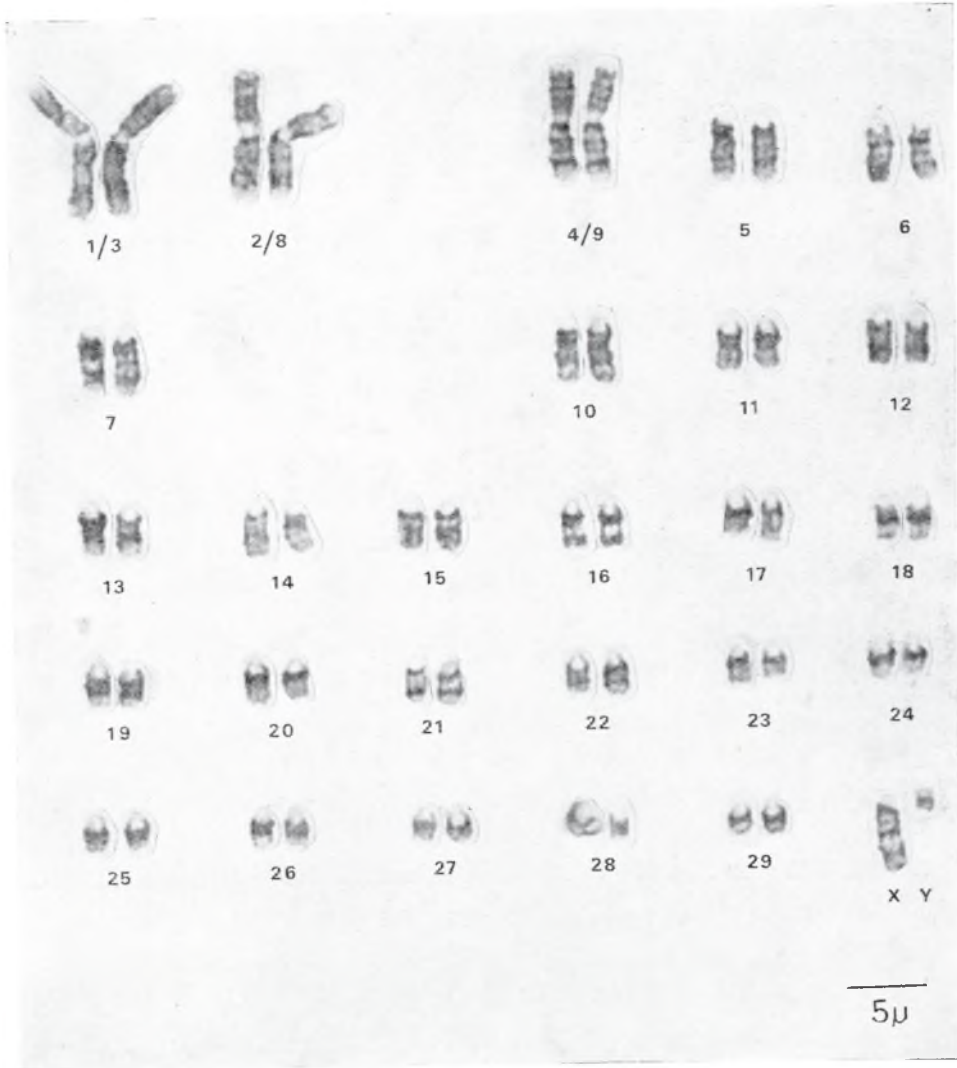


FIG. 4

G-banding patterns of the male sheep chromosomes. The goat karyotype has been used as a standard for the arrangement of the chromosomes

some numbers hitherto known in mammals, most pairs showed very characteristic banding patterns, sometimes with extensive, weakly or strongly stained regions (HAGELTORN and GUSTAVSSON, 1974). Also the horse karyotype demonstrated extensive, strongly or weakly QM-stained regions, which after treatment with trypsin and re-staining with Giemsa were dark or pale, respectively (Fig. 5). It is quite evident that there are extensive homologies in banding patterns of the caprine

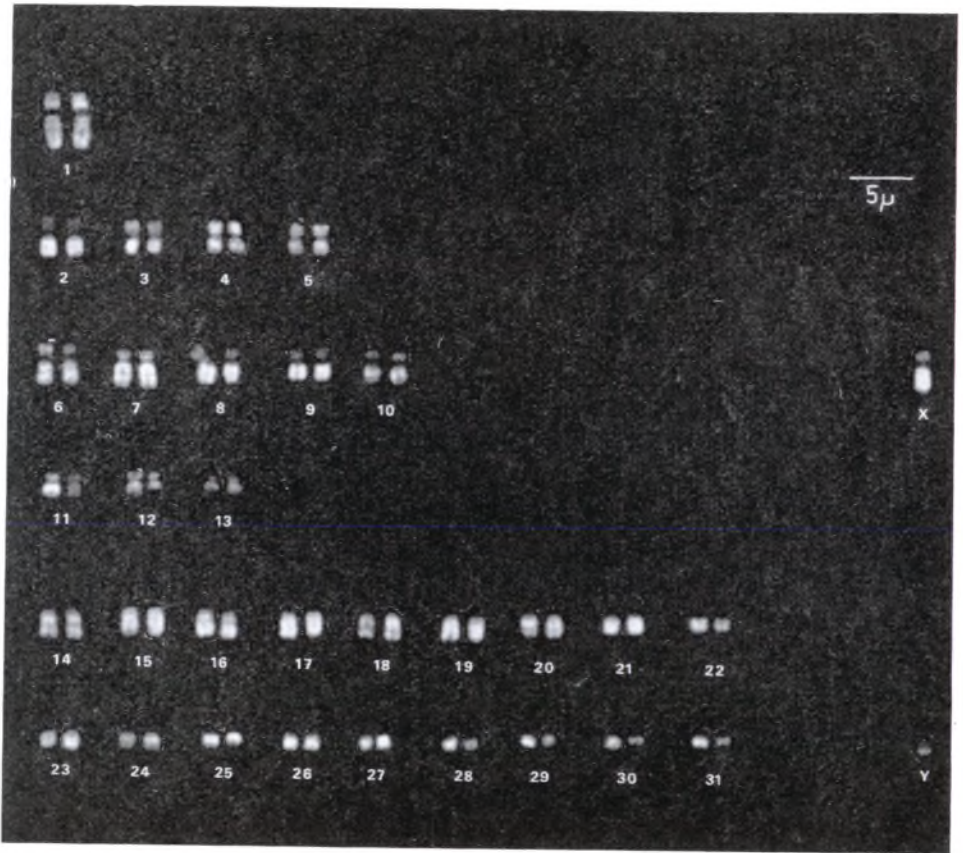


FIG. 5a

Q-banding patterns of the male horse chromosomes

(Fig. 2), bovine (Fig. 3), and ovine (Fig. 4) karyotypes (EVANS *et al.*, 1973; GUSTAVSSON and HAGELTORN, 1974) and the centromeres of the autosomes showed a uniformity in appearance when compared to the centromeres of the chromosomes of the dog and horse karyotypes. For the detailed karyotype description the reader is referred to the publications given in the reference list.

By the application of new staining techniques, possibly combined with photoelectric recordings (CASPERSSON *et al.*, 1970), it is thus possible for every laboratory working with domestic animals to carry out highly detailed chromosome analyses, even in karyotypes containing a high number of chromosomes with similar morpho-

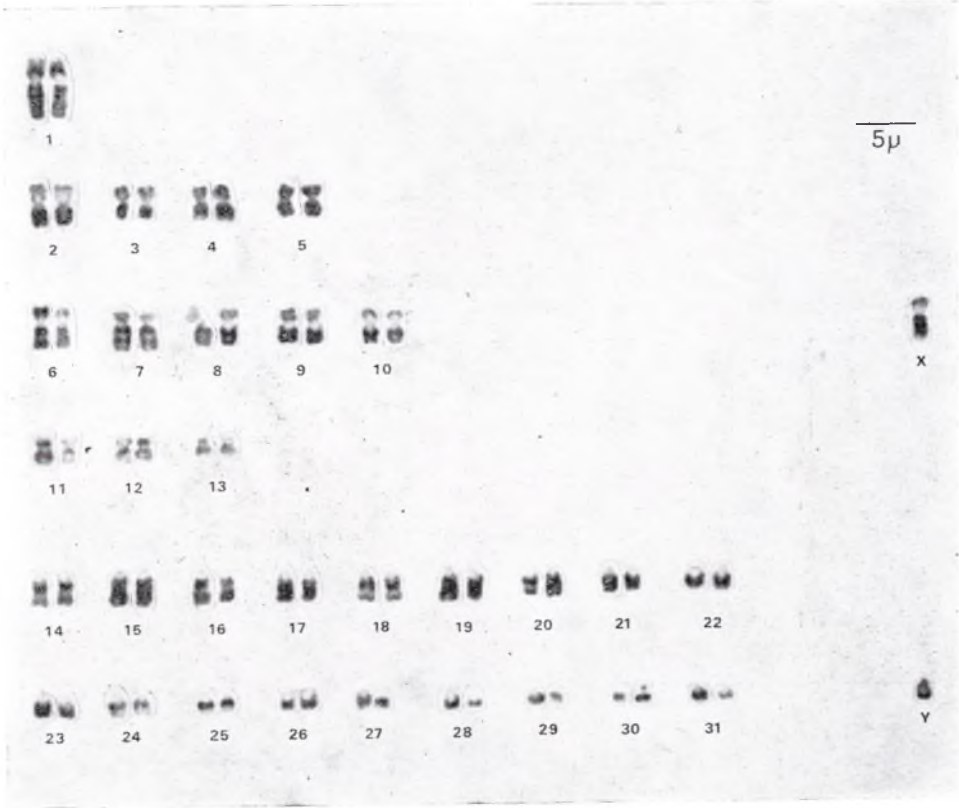


FIG. 5 *b*

G-banding patterns of the male horse chromosomes in fig. 5 *a* after trypsin treatment and re-staining with Giemsa

logy. Such studies are of the utmost importance in the detailed description of chromosome aberrations and evaluation of their phenotypic effects, the detection of cryptosubstructural rearrangements, and the establishment of chromosome homologies.

#### SUMMARY

The perfection of the new staining techniques is demonstrated by the identification of individual chromosome pairs in the canine, equine, caprine, bovine, and

ovine karyotypes. These domestic animals are characterized by high diploid chromosome numbers and chromosomes of similar morphology. Arrangement systems are proposed for each species. Utilization of the new staining techniques makes possible very detailed analyses of the normal chromosomes, accurate descriptions of structural rearrangements, detection of cryptosubstructural deviations, and the establishment of chromosome homologies.

## ZUSAMMENFASSUNG

Die Perfektion der neuen Färbungstechniken zeigt sich bei der Unterscheidung individueller Chromosomenpaare in den Karyotypen des Hundes, des Pferdes, der Ziege, des Rindes und des Schafes. Kennzeichnend für diese Haustiere sind hohe diploide Chromosomenzahlen und Chromosomen ähnlicher Morphologie. Anordnungssysteme werden für jede einzelne Art vorgeschlagen. Die Anwendung der neuen Färbungstechniken ermöglicht eine sehr detaillierte Analyse normaler Chromosomen, genaue Beschreibung struktureller Veränderungen, Entdeckung kryptosubstruktureller Abweichungen und die Feststellung von Chromosomenhomologien.

## SUMARIO

La perfección de las nuevas técnicas de coloración queda demostrada por la distinción de pares de cromosomas individuales en los cariotipos canino, equino, caprino, bovino y ovino. Los animales domésticos se caracterizan por un número elevado de cromosomas diploides y de cromosomas de morfología similar. Sistemas de ordenación son propuestos para cada especie. La utilización de las nuevas técnicas de coloración permite análisis muy detallados de los cromosomas normales, descripciones precisas de los cambios en los órdenes estructurales, detectar desviaciones criptoestructurales y establecer homologías de cromosomas.

## LITERATURE CITED

- CASPERSSON, T.; LOMAKKA, G., and ZECH, L. (1971): The 24 fluorescence patterns of the human metaphase chromosomes - distinguishing characters and variability. *Hereditas*, 67:89-102.
- CASPERSSON, T.; ZECH, L.; JOHANSSON, C., and MODEST, E. J. (1970): Identification of human chromosomes by DNA-binding fluorescent agent. *Chromosoma*, 30:215-227.
- EVANS, H. J.; BUCKAND, R. A., and SUMMER, A. T. (1973): Chromosome homology and heterochromatin in goat, sheep and ox studied by banding techniques. *Chromosoma*, 42:383-402.
- GUSTAVSSON, I., and HAGELTORN, M. (1974): Apparent homologies in G-banding patterns of cattle, goat and sheep chromosomes. *Hereditas*. (In press.)
- GUSTAVSSON, I.; HAGELTORN, M.; JOHANSSON, C., and ZECH, L. (1972): Identification of the pig chromosomes by the quinacrine mustard fluorescence technique. *Exp. Cell. Res.*, 70:471-474.
- GUSTAVSSON, I.; HAGELTORN, M.; ZECH, L., and REILAND, S. (1973): Identification of the chromosomes in a centric fusion/fission polymorphic system of the pig (*Sus scrofa* L.). *Hereditas*, 75:153-155.
- HAGELTORN, M., and GUSTAVSSON, I. (1973): Giemsa staining patterns for identification of the pig mitotic chromosomes. *Hereditas*, 75:144-146.



- HAGELTORN, M., and GUSTAVSSON, I. (1974): Identification of individual chromosomes in the dog by a Giemsa staining technique. *Hereditas*. (In press.)
- HAGELTORN, M.; GUSTAVSSON, I., and ZECH, L. (1973): The Q- and G-banding patterns of a t(11 p<sup>+</sup>; 15 q<sup>-</sup>) in the domestic pig. *Hereditas*, 75:147-151.
- POPESCU, C. P. (1973): L'hétérochromatine constitutive dans le caryotype bovin normal et anormal. *Ann. Génét.*, 16:183-188.
- SEABRIGHT, M. (1971): A rapid banding technique for human chromosomes. *Lancet*, II/1971: 971-972.

