FROM CHROMOSOME SHAPE TO CHROMOSOME MAPPING: 30 YEARS OF DOMESTIC ANIMAL CYTOGENETICS

DESDE LA FORMA DEL CROMOSOMA AL MAPA CROMOSÓMICO: 30 AÑOS DE CITOGENÉTICA DE LOS ANIMALES DOMÉSTICOS

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SUMMARY

Almost 30 years have now passed since the first chromosomal abnormality in cattle was described by Ingemar Gustavsson. Some years later, in 1970, the first european meeting on cytogenetics of domestic animals was held in Giessen. Since the second edition in 1975, this important meeting have been held every two years. The North American version of this meeting started in 1978 in East Lansing, and arrived to the 9th edition last year in Texas.

The early period of domestic animal cytogenetics was devoted to the description of normal karyotypes of each domestic species, first by conventional Giemsa staining and after 1970, by different banding methods.

In the seventies a great effort was devoted to discover the different chromosomal abnormalities in animals with low fertility and reproduction troubles. In many countries a policy was adopted for the erradication of 1/29 translocation in cattle. The gamete and embryo cytogenetics has been developed in the same period.

During the 4th edition of this meeting in Uppsala in 1980, the first chromosomally assigned genes were reported in domestic animals.

Today, some 120 research groups around the world are involved in gene mapping programmes. On spite of this important development of animal cytogenetics, it is necessary to explore several fields. For example very few knowledges are about the meiosis in domestic animals, the recombination (chiasma) frequency especially in females. Very few has been reported also about the different polymorphisms. Evolutionary studies using the newly developed techniques as FISH and chromosome painting will increase our knowledge about the genome evolution and speciation.

RESUMEN

Han pasado casi 30 años desde la descripción de la primera anomalía cromosómica en la especie bovina por Ingemar Gustavsson. Unos años más tarde, en 1970, se celebró la primera Conferencia Europea de Citogenética en Animales Domésticos en Giessen. Desde la segunda edición en 1975, esta importante conferencia se ha celebrado cada dos años. La versión norteamericana empezó en 1978 en East Lansing y cumplió 9 ediciones el año pasado en Texas.

Los primeros años de estudios citogenéticos en

animales domésticos, se dedicaron a la descripción de los cariotipos normales de cada especie, primero con la colaboración Giemsa convencional y luego, a partir de 1970 con distintos métodos de bandeo.

En los años setenta se produjo un gran impulso para descubrir las distintas anomalías cromosómicas en animales con baja fertilidad y problemas reproductivos. Se adoptó un plan de acción para la erradicación de la translocación 1/29 en bovino. La citogenética de gametos y embriones se desarrolló en el mismo periodo.

En la cuarta edición de los congresos en Uppsala en 1980, se presentaron los primeros genes asignados a cromosomas en animales domésticos.

Hoy, unos 120 investigadores de todo el mundo están involucrados en programas de mapeo genético. A pesar de este importante desarrollo de la citogenética animal, es necesario explorar varios campos. Por ejemplo, sabemos muy poco sobre meiosis en animales domésticos o la frecuencia de recombinación (quiasma) especialmente en hembras.

Poco ha sido publicado sobre los diferentes polimorfismos. Los estudios evolutivos utilizando nuevas tecnologías como FISH y la identificación específica de cromosomas mediante hibridación *in situ* aumentarán nuestro conocimiento de la evolución del genoma humano y la especiación.

INTRODUCTION

Almost 30 years have now passed since the first chromosomal abnormality in cattle was described by J. Gustavsson. Some years later, in 1970, the first european meeting on cytogenetics of domestic animals was held in Giessen. Since the second edition also held in Giessen in 1975 this meeting have been held every two years **(table I)**. The initiative for the first two editions in Giessen was taken by Professor Georg W. Rieck, who is one of the pioneers of this field.

In 1978 the North American workers interested in chromosomes of domestic animals began to meet on years alternate to the European meeting. The last American meeting, the 9th edition, was held in Texas University College Station in 1995 **(table II)**.

The early period of domestic animal cytogenetics was devoted to the detailed description of chromosomes of each domestic species. The main domestic species as the cattle sheep and goat but also the horse are very diffl cult for cytogenetic studies because of their great number of chromosomes and their similar morphology. After 1970, the different banding methods as G, Q, C, R and T-banding have been adopted to domestic animal chromosomes.

STANDARDIZATION MEETINGS

The first international conference for the standardization of banded karyotypes of domestic animals, usually know as *Reading Conference* was held at Reading University in 1976. The Reading standard proposed basic GTG standard karyotypes for the most important domestic animals: cattle, goat, sheep, horse, pig and rabbits. These standards karyotypes have formed the basis for all subsequent karyotypes nomenclatures.

The second international conference on standardization of domestic animal karyotypes was held in Jouy-en-Josas in 1989. The following points have been agreed upon at this conference:

- standard G, Q, R -banded karyotypes for cattle.

- nomenclature (land marks, regions and band numbering) and schematicc representations for G and R -banded cattle chromosomes.

Table I. *European Meetings.* (Congresos europeos).

Year	Place	Organiser	Nb. of participants	Nb. of papers
1970	Giessen	G.W. Rieck	38	20
1975	Giessen	G.W. Rieck	52	36
1977	Jouy-en-Josas	P. Popescu	59	47
1980	Uppsala	I. Gustavsson	71	66
1982	Milano Gargnano	G. Succi	55	54
1984	Zurich	G. Stranzinger	68	69
1986	Warsaw	P. Sysa	?	60
1988	Bristol	S. Long	53	31+Posters
1990	Toulouse	G. Echard	76	44+Posters
1992	Utrecht	A. Bosma	99	56+Posters
1994	Copenhagen	K. Christensen	65	39+Posters

- R -banded karyptypes for sheep and goat.

- comparison of R -banded cattle, goat and sheep chromosomes.

All these standards karyotypes have been published in Cytogenetics and Cell Genetics (ISCNDA, 1989).

In 1995, prior to the 9th North American Colloquium on Domestic Animal Cytogenetics held in College Station Texas a standardization meeting was organised and a new standard was obtained. This new standard correlates the differents previous standards with marker genes mapped on each chromosome (Popescu *et al.*, 1996).

THE CLINICAL CYTOGENETICS. THE 1/29 TRANSLOCATION

The chromosome abnormalities involve the chromosome numbers on the chromosome structure. The chromosome abnormalities are very often associated with developmental anomalies,

Table II. *North American Meetings.* (Congresos norteamericanos).

1978	East Lansing-Michigan
1981	Raleigh-North Carolina
1983	Madison-Wisconsin
1985	Urbana-Illinois
1987	Columbia-Missouri
1989	Purdue University-West Lafayette-Indiana
1991	University of Pensilvania-Philadelphia-Pennsilvania
1993	University of Guelph-Canada
1995	Texas A and M University-College Station-Texas

embryonic death and various levels of infertility.

In 1964, Gustavsson and Rockborn discovered in Sweden the first structural aberration in cattle, the 1/29 robertsonian-like translocation. Since that time this abnormality was identified in 50 breeds distributed over 5 continents **(figure 1)**. The frequency of this abnormality varies consideratly from one breed to another but also according to the size of sample analysed in different countries. This abnormality causes 5-10 p. cent, reduction of fertility by increasing the embryonic death due to formation of unbalanced gametes. Formation of these unbalanced gametes was shown by meiotic studies (Popescu, 1990) and by investigations of the embryos sired by heterozygous parents (Popescu, 1980; King *et al.*, 1981).

An erradication policy towards this abnormality was adopted early in seventies, in different european countries.

In a scientific point of view, the discovery of this abnormality has a very big consequence on the development of the domestic animal cytogenetics. In 1991, in a review Popescu and Pech presented a reference list of 231 scientific paper published on this subject. Today, they are probably around 300 papers devoted to this abnormality. Many cytogenetics laboratories have been created all over the world with the aim of searching for this abnormality.

GENE MAPPING

The first chromosomally assigned genes were reported in domestic species at Uppsala meeting (1980). Since this

Figure 1. *Geographical distribution of 1/29 translocation.* (Distribución geográfica de la translocación 1/29).

time, several national and international programmes were devoted to gene mapping in main domestic animal species. Today, two domestic species present a quite advances physical and genetical map: the pig and cattle. The 1228 markers assigned on pig chromosomes represent

Table III. *Pig Map.* (Mapa porcino).

Authors	Number of markers assigned
Ellegren *et al.* (1994)	120
Rohrer *et al.* (1994)	345
Archibald *et al.* (1995) (European Pig Map project)	227
Rohrer *et al.* (1996)	536
Total	1228

the most developped map in domestic animals. The most active gene mapper group was Rohrer's group from USDA. Nebraska who published 345 marker assigned in 1994 and 536 this year **(table III)**. In cattle 877 loci have been reported by Eggen and Fries in 1994 among them 314 coding genes and 563 anonymous loci **(table IV)**.

The physical and genetic map is also in progress in some other domestic species like rabbit and mink. Some 120 research groups around the world are involved today in domestic animals gene mapping programmes.

Table IV. *Cattle gene map.* (Mapa genético bovino).

(Eggen and Fries, oct. 1994)

- 877 *loci*
  (314 coding genes
  and
  563 anonymous *loci*).

Even with the high resohltion technique, the analysis of chromosomes in some domestic species like bovidae species, cattle sheep and goat, is still difficult for the identification of chromosomes. Some gene mappers came to this field without basical knowledges of normal chromosome pattern and morphology. Also, in spite of successive corrections of standard karyotypes some confusions and errors are still present. Because of these reasons, they are some discrepancies in chromosomally assignements of several genes.

An other source of errors in gene mapping, but also in the identifications of chromosomes involved in different abnormalities is the fact that some laboratories are still using the oldest standard, the Reading system. The translation from one standard to an other, based on different banding techniques, may generate errors. For exemple, during the standardization conference in Jouy-en-Josas, a very important group of experts, (more than 30 people) interchanged the chromosome 4 and 6 in cattle translating the Reading standard to ISCNDA (1989). For this reason it is recommended to all cytogeneticists involved in domestic animal species, to use the last nomenclature and numbering standard, even if it is not yet perfect.

NEW CYTOGENETICS TOOLS

During the last few years, the human and animal cytogenetics have benefited

of some new techniques and approaches The most promising technique now is the fluorescent *in situ* hybridization (FISH) with all its variants: multicolor FISH, chromosome painting, ZOO - FISH, PRINS, etc...

The fluorescence *in situ* hybridization technique has expanded rapidly during the past several years especially for gene mapping programmes. But, because of its spatial resolution and sensitivity, this method could be very useful for the detection of numerical and structural abnormalities. By using the repetitive sequences specific for centromeric regions, the fluorescence spots can be counted to determine chromosome copy number for detection of aneuploidies. Chromosome painting with whole chromosome specific probes, provides a valuable tool for analysis of chromosomal rearrangements as such reciprocal translocations (Lengauer *et al.*, 1990).

The hybridization signal produced by FISH is also visible in interphase nuclei with a resolution power increased at least 10 fold as compared with metaphase chromosomes because the chromosomes are very extended. For this reason this new domain called *interphase cytogenetics* play a very important role in ordering DNA probe relative to each other (Lichter *et al.*, 1989). The interphase hybridization technology can be expected to improve our knowledge on interphase nuclei organization and chromosome domains.

By using heterologous chromosome painting or ZOO - FISH we now have the posibility to compare the karyotypes of different domestic animals and to understand their evolution. It is also possible to discerne homologies between human and domestic animal species (Hayes, 1995).

NEED FOR FUTURE DEVELOPMENT

The number of structural and numerical aberrations in domestic species is still low and very little is actually known about chromosomal mutation rate. The numerical aberrations are correlated with the non-disjunction rate. Very little also in known about causes and frequen-cies of nondisjonction rate in domestic species. As it was pointed out in Toulouse meeting by Ingemar Gustavsson (1991),

> *It is surprising that so little has been reported concerning meiosis and chromosomal polymorphism in domestic animals.*

It is very important for the future development of domestic animal cytogenetics to use those new promising tools as FISH and its variants, to study the basic phenomenas as male and female meiosis, the mutation and non-disjunction rate and chromosome polymorphisms.

Some years ago, Professor M. Ferguson Smith wrote in a editorial paper in the American Journal of Human Genetics:

> *Putting the genetics back into cytogenetics.*

Finally in this opening paper of this 12th meeting and to paraphrase Professor M. Fergusson-Smith. I would say:

> *Put the cytogeneticists back to basical cytogenetics.*

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