Advances in Veterinary Science and Comparative Medicine

*Volume 34*

# Domestic Animal Cytogenetics

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and Comparative Medicine

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# PREFACE

My first introduction to the discipline of cytogenetics was in the summer of 1962 when, as a young veterinarian fresh from a farm animal clinical practice, I reported to Professor John Biggers' laboratory at the University of Pennsylvania to begin a postdoctoral training program in reproductive biology. It was an exciting time. Many pioneering efforts in ova and early embryo culture were under way, and a steady stream of scientists, legendary in this emerging field, were either training in the laboratory or visiting for periods of time. As I had no preconceived ideas about which direction my research should take, Professor Biggers suggested looking at the chromosomes of some of the domestic animals because he felt that the discipline of mammalian cytogenetics was about to come into its own.

After a few trips to the library I had learned, among other things, that there were two fewer human chromosomes since I had taken the basic genetics course at my undergraduate university 8 years ago. Since I was sure that evolution did not occur that rapidly, it was apparent that a major breakthrough in technology had taken place, and that it was now possible to study mammalian chromosomes in a great deal more detail. Not only was the normal human chromosome complement now well defined, but certain developmental defects in children had been linked to specific chromosome abnormalities, and the "Philadelphia chromosome" associated with human chronic granulocytic leukemia had been described by colleagues working in the same building in which our laboratory was located. I decided that the time was right for me to learn more about this field and to begin to apply some of the techniques to domestic animals.

Professor Paul Moorhead, who, with his colleagues, had just published a method for displaying chromosomes after short-term culture of cells obtained from peripheral blood, was located across the street in the Wistar Institute, and he was kind enough to take the time to help me get started. My colleague and good friend, W. C. D. Hare, was also getting started in the chromosome business as part of his research on bovine leukemia. He also shared his ideas and knowledge with me, and before long we were able to establish the laboratory which I still occupy today. Doug Hare's interests eventually took him from the Uni-

versity of Pennsylvania and from a direct association with mammalian chromosomes, but his help and encouragement in my formative years as a scientist will never be forgotten. With the encouragement and support of John Biggers and W. C. D. Hare, I embarked on a study of chromosome abnormalities in pig embryos and of developmental defects associated with reproductive failure, a study which continues to this day.

In the mid 1960s, most of those interested in mammalian cytogenetics in the United States assembled in Gatlinburg, Tennessee for the first of what was to become an annual Mammalian Chromosomes and Somatic Cell Genetics Conference. The discussions were informal and lively, and information was exchanged freely. The pioneer spirit which prevailed must have been similar to the feelings of other early explorers. Regardless of the species involved, interest in new developments was keen.

As the number of workers interested in chromosomes of domestic animals increased, it was necessary to create a new forum for the exchange of ideas. The European workers gathered first in Giessen in 1970, and have continued to meet every other year; their most recent meeting, the 9th European Colloquium on Cytogenetics of Domestic Animals, was held in Toulouse, France in July 1990. Several years later, the North American workers began to meet on years alternate to the European meeting. The 7th North American Colloquium on Domestic Animal Chromosomes and Gene Mapping will convene in Philadelphia in July 1991.

When approached by the editorial staff of Academic Press about this volume, I agreed to serve as editor conditional on the recruitment of appropriate authorities to write the individual chapters. All of the contributors are leading authorities in the field, and all agreed to participate with minimal persuasion.

The purpose of the book is to provide a comprehensive review of the status of domestic animal chromosomes and to serve as an introduction to the literature. Two chapters on gene mapping, a relatively new field in cytogenetics, have been included.

A very special acknowledgment is due my wife, Lynne R. Klunder, who has worked as a colleague in the laboratory, and who has supported and enthusiastically encouraged my efforts in this field for the last 15 years.

RICHARD A. MCFEELY

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# Introduction

RICHARD A. McFEELY

*Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania*

I. History
II. Chromosomes
III. Chromosome Abnormalities
IV. Genes
V. Molecular Genetics
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## I. History

Cytogenetics is defined as the study of the morphology and behavior of chromosomes. Chromosome studies in domestic animals and man have evolved as a modern scientific discipline in the past three decades, although human chromosomes were first observed in dividing cells by Virchow (1857) over 100 years earlier. The significance of their true nature apparently was not appreciated at that time.

The process of cell division called mitosis, in which chromosomes can be visualized, was described by von Török (1874), and detailed descriptions of human chromosomes were made by Arnold (1879). During the next decade several long treatises amplified the knowledge about cell structure and function. Flemming (1882) introduced the term *chromatin* for the darkly staining portions of the nucleus. That chromatin was the physical basis of inheritance was concluded independently by Weismann (1883), Strasburger (1884), and von Kölliker (1885). Waldeyer (1888), after a lengthy review and discussion of the literature, introduced the term *chromosome* for these chromatin structures.

During the middle of the nineteenth century, the Austrian monk, Gregor Mendel (1866), developed his theories about inheritance, which were published in 1866. However, his concepts were so far ahead of his

time that it was not until the early part of the twentieth century that his ideas were rediscovered and became widely accepted. In 1903, Sutton and Boveri independently formulated the connection between chromosomes and Mendelian inheritance (Peters, 1959).

Through the first half of the twentieth century, geneticists working primarily with plants and insects began to develop a large body of knowledge about chromosome morphology and behavior during cell division. In contrast, relatively less was known about chromosomes of domestic animals and man. Three events stimulated increased interest in mammalian cytogenetics in the years following World War II. The first was the discovery of a sexual dimorphism in the neurons of cats. In 1948 Dr. Ewart G. Bertram, a postgraduate student, was working with Professor Murray Barr on a project involving stimulation of the hypoglossal nerve of cats and subsequent histologic evaluation of the affected neurons. It was noted that in the neurons of some animals, a darkly staining body, which they called a nucleolar satellite, was present in a large number of cells, but was absent from virtually all cells of other animals subjected to the same treatment. Analysis of the records quickly revealed that the structure was present in nerve cells from female cats, but was absent in similar cells from males. Similar findings were reported for nerve cells in humans. The discovery of the sex chromatin body, as it was subsequently named, was published by Barr and Bertram (1949).

The second event was the result of a fortuitous observation of a laboratory mistake. Techniques for culturing mammalian cells *in vitro* were being developed, and these cell cultures provided the scientist with a source of cells undergoing mitosis. The use of colchicine, an alkaloid derived from the autumn crocus, inhibited mitotic spindle formation and arrested cells in metaphase when the chromosomes were maximally condensed and could be most easily identified. However, when viewed through the microscope, the chromosomes were overlapped and identification of individual chromosomes was not possible. In fact, it was not possible to accurately count the number of chromosomes in a cell. Hsu (1952) observed that the chromosomes were separated and could be visualized and counted without difficulty in cells that had been washed, accidently, with a hypotonic solution instead of an isotonic solution prior to fixation. Hypotonic treatment opened the door for the mammalian cytogeneticist.

The third event was the report by Tjio and Levan (1956) that the diploid number of chromosomes in man was 46 rather than 48. Shortly thereafter, the discovery of an extra chromosome associated with Down's syndrome (Lejeune *et al.*, 1959) started a massive search for

other types of chromosomal anomalies. The technique for the display of chromosomes after short-term culture of cells obtained from peripheral blood hastened the process (Moorhead *et al.*, 1960). In the decade that followed, reports of human chromosome abnormalities associated with congenital defects, reproductive disorders, spontaneous abortions, and neoplasia proliferated at an unprecedented rate.

Although the number of workers interested in chromosomes of domestic animals was quite small, there was general interest in the normal chromosome complement of a wide variety of species, and by 1975 an *Atlas of Mammalian Chromosomes* (Hsu and Benirschke, 1967) containing about 500 entries had been published. Reports of chromosome abnormalities associated with developmental defects and reproductive failure in domestic animals began to appear in the literature in 1964. Until the early part of the 1970s, it was still very difficult to identify individual chromosomes, especially in those species in which the morphology was similar and the only discernible difference was the size of the chromosome. The development of techniques using quinacrine mustard initially, and subsequently, a variety of other treatments that allowed differential staining of individual chromosomes, facilitated their identification. The banding patterns also permitted the cytogeneticist to identify minor structural chromosomal rearrangements, which had previously gone undetected. Today, chromosome analysis is frequently requested as part of breeding soundness examinations of valuable stallions and is one of the requirements for importation of certain species into various countries.

Currently, new technology is permitting scientists to investigate the structure and function of individual chromosomes. Single genes have been isolated, purified, and cloned and their chemical sequence has been identified. They have been introduced into cells in tissue culture to study how genes are regulated and have been introduced as foreign genes into other species, where, in some instances, they function as if in their own species. The possibility of using laboratory-purified genes as therapy for some genetic diseases now seems to be technically feasible.

Determining the exact location of each gene on individual chromosomes is known as gene mapping. Today, a major effort is being made to map the entire human genome. This is an extraordinarily ambitious undertaking and can only be accomplished with the efforts of many cooperating laboratories and the expenditure of vast sums of money. As usual, similar studies in domestic animals lag behind these efforts to study the human genome, but many laboratories have made impressive beginnings and there is considerable worldwide interest in such

studies. For gene mapping to proceed, it is necessary to have general acceptance of the standardized karyotype for each species. With this goal in mind, workers on standarization committees for the cow, sheep, goat, and horse met for the Second International Conference for Standardization of Domestic Animal Karyotypes in Jouy en Josas, France in May 1989 to update the standards published as the result of the first standardization meeting held in Reading, England in 1976 (Ford *et al.*, 1980). The standard karyotype for the pig has been published (Gustavsson, 1988) and the results of the Jouy en Josas conference will appear in early 1990.

## II. Chromosomes

Although chromosomes have been studied extensively since the beginning of the century, it has only been in recent years that we have gained detailed knowledge of their structure and function. Chromosomes are best visualized during certain stages of cell division, when they are maximally condensed and can be seen through a light microscope.

Mitotic chromosomes have certain fundamental characteristics. First, the number of chromosomes is constant for all normal animals in a given species (Table I). Second, when the chromosomes are photographed, cut out, and arranged in pairs, the resulting karyotype is identical for all members of that species. Third, with one exception, the chromosomes occur in pairs and are called autosomes. The exception is the sex chromosomes, which are paired in females but are unpaired in

TABLE I

DIPLOID CHROMOSOME NUMBERS

| Species | Number |
|---|---|
| Pig | 38 |
| Cat | 38 |
| Sheep | 54 |
| Cow | 60 |
| Goat | 60 |
| Horse | 64 |
| Dog | 78 |
| Chicken | 78 |

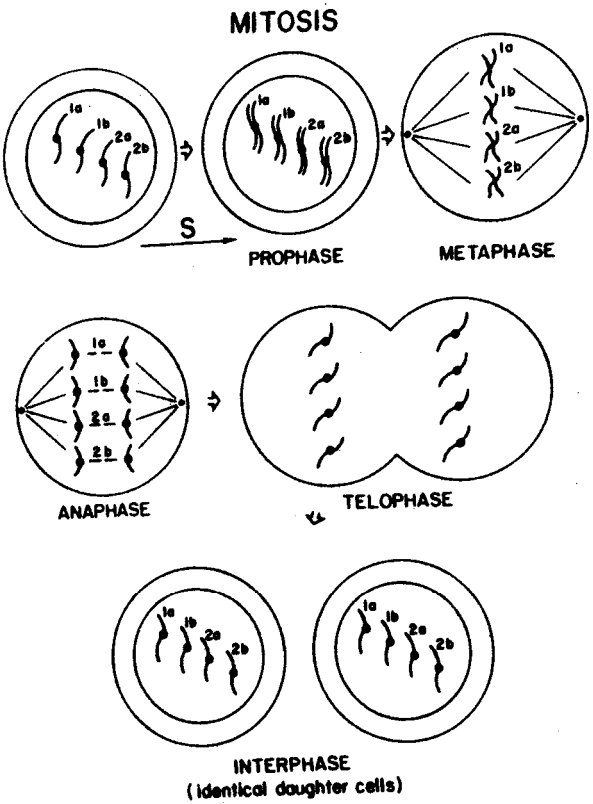


FIG. 1. Mitosis: cell division producing two identical daughter cells, each with an exact duplicate of the nuclear DNA of the parent cell. (From Kelly, T., "Clinical Genetics and Genetic Counseling," 2nd Ed. Year Book Med. Publ., Chicago, Illinois, 1986.)

males. In domestic mammals, the female X chromosome is generally much larger than the male Y chromosome. In birds, the female is the heterogametic sex with ZW sex chromosomes; the male has two Z chromosomes.

Cell division in somatic cells is called mitosis, a process in which two genetically identical cells are produced from a single cell (Fig. 1). The cell cycle can be divided into two phases—interphase and cell division. During interphase, the chromosomes are present as uncoiled molecules of deoxyribonucleic acid (DNA). The interphase portion of the

cell cycle is subdivided into three periods. During G1 the cell carries out its normal metabolic functions under the control of its DNA. Following the G1 period, the cell enters the S period, during which the DNA is replicated, producing an exact copy of itself. Each chromosome then consists of two daughter chromatids. After a short G2 period, cell division begins.

Mitosis consists of four stages. During prophase the DNA molecules begin to spiral, forming coils that are recognizable as chromosomes. The nuclear membrane disappears and the mitotic spindle begins to form as the centrioles move to opposite poles of the cell. The second stage of mitosis is metaphase, when the chromosomes are fully condensed and are clearly visible. It is during late prophase or metaphase that the chromosomes are usually analyzed. At this point, the centromere, which unites the two daughter chromatids, attaches to the mitotic spindle and the chromosomes align along the middle of the cell. During anaphase the centromere divides along the longitudinal axis and the sister chromatids of each chromosome migrate toward opposite poles to become a chromosome of the daughter cell. The final phase of mitosis is called telophase. The chromosomes uncoil and return to their interphase state, the nuclear membrane is reestablished, duplication of the centriole occurs, and the cytoplasm divides, completing the formation of two identical daughter cells. The normal chromosome number for a somatic cell is called the diploid number and may be represented as $2n$.

Meiosis is a type of cell division, restricted to germ cells, in which gametes containing one chromosome from each pair are produced (Fig. 2). Thus, the chromosome number of a gamete is one-half the diploid ($2n$) number and is referred to as the haploid number ($n$). At the moment of fertilization, when a male and female gamete unite to begin the formation of a new individual, the diploid number is restored.

Meiosis is more complex than mitosis and differs in two critical ways. The first involves the exchange of genetic material among homologous chromosomes, a process called *crossing over*. The net effect is that the new chromatids resulting from the exchange now have genes derived from the original maternal and paternal chromosomes. Unlike mitosis, the chromosomes in which crossing over has occurred are not exact copies of the original parental cell, but have new genetic combinations. The second major difference between meiosis and mitosis is that the number of chromosomes in the resulting gametes is reduced from the diploid to the haploid number. Meiosis consists of two cell divisions; meiosis I is a reductional division and meiosis II is an equational division. Both crossing over and the reduction in chromo-

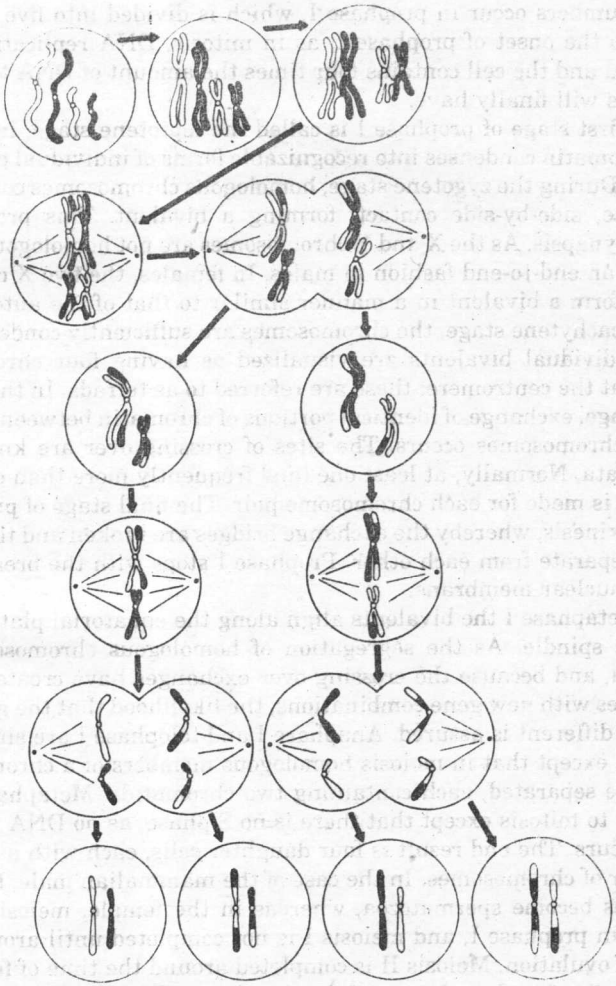

FIG. 2. Meiosis: cell division of germ cells producing haploid gametes. Note that crossing over and reduction of the diploid chromosome number occur in the first meiotic division. (From Kelly, T., "Clinical Genetics and Genetic Counseling," 2nd Ed. Year Book Med. Publ., Chicago, Illinois, 1986.)

some numbers occur in prophase I, which is divided into five stages. Prior to the onset of prophase I, as in mitosis, DNA replication has occurred and the cell contains four times the amount of DNA that the gametes will finally have.

The first stage of prophase I is called the leptotene stage, in which the chromatin condenses into recognizable forms of individual chromosomes. During the zygotene stage, homologous chromosomes come into intimate, side-by-side contact, forming a bivalent. This process is called synapsis. As the X and Y chromosomes are not homologous, they pair in an end-to-end fashion in males. In females, the two X chromosomes form a bivalent in a manner similar to that of the autosomes. In the pachytene stage, the chromosomes are sufficiently condensed so that individual bivalents are visualized as having four chromatids joined at the centromere; these are referred to as tetrads. In the diplotene stage, exchange of identical portions of chromatin between homologous chromosomes occurs. The sites of crossing over are known as chiasmata. Normally, at least one (and frequently more than one) exchange is made for each chromosome pair. The final stage of prophase I is diakinesis, whereby the exchange bridges are broken and the bivalents separate from each other. Prophase I stops with the breakdown of the nuclear membrane.

In metaphase I the bivalents align along the equatorial plate of the meiotic spindle. As the segregation of homologous chromosomes is random, and because the crossing over exchanges have created chromosomes with new gene combinations, the likelihood that the gametes will be different is assured. Anaphase I and telophase I are similar to mitosis except that in meiosis homologous members of a chromosome pair are separated, each containing two chromatids. Metaphase II is similar to mitosis except that there is no S phase, as no DNA replication occurs. The end result is four daughter cells, each with a haploid number of chromosomes. In the case of the mammalian male, the four gametes become spermatozoa, whereas in the female, meiosis is arrested in prophase I, and meiosis I is not completed until around the time of ovulation. Meiosis II is completed around the time of fertilization. In the female only one of the daughter cells becomes the ovum, while one is shed as a diploid polar body at the end of meiosis I and the second is a haploid polar body shed at the completion of meiosis II.

Basically, the techniques used for the display of mammalian chromosomes begin with a source of rapidly dividing cells. These cells can be harvested from cell culture preparations or obtained directly from tissues such as bone marrow, testes, or certain rapidly growing tumors in which cell division is occurring at a high rate. When peripheral