Cytogenetics in Animal Production

ABSTRACT

Development of cytological techniques over the last 20 yr enables accurate and detailed observations of the chromosomes of mammals and birds. Various applications for cytogenetic methodology in animal science research are suggested. Much of the pregnancy wastage in farm animals may be caused by abnormal chromosome complements of afflicted zygotes. To reduce embryonic loss, the causal bases of chromosomal abnormalities should be sought. Many therapeutic agents administered to animals, as well as herbicides, pesticides, feed additives, and other products mediate undesirable side effects. Karyological methods are useful to detect chromosomal damage or irregular mitosis or meiosis which are indicators of toxicity and carcinogenic activity.

It is probable that polymorphisms of "banding patterns" of chromosomes are sufficiently widespread to enable identification of individual animals and authentication of pedigree. The study of relationships of animals within populations and among populations also will be enhanced by application of chromosome banding techniques to livestock.

In animal breeding, cytogenetic methodology is applied to test assumptions of theory. New sources of genetic variation may be chromosome rearrangements, duplications, aneuploidy, and euploidy. When applied to cells in culture and combined with modern methods of molecular biology, cytogenetic technique can help discover new loci, assign genes to specific chromosomes, and create genetically modified cell lines which can be introduced into the germ line of animal populations.

INTRODUCTION

An enthusiasm to observe chromosomes has characterized animal geneticists since it was recognized that chromosomes are the vehicles in which the genes reside. The science of cytogenetics developed in the expectation of finding associations between variations of chromosome number, morphology, or behavior and gross anatomical or physiological functions of animals as a whole. It was not until about 1960, however, that techniques became available to enable accurate and detailed observations of chromosomes of mammals and birds. Since that time an increasing number of methods has made possible a detailed analysis not only of the gross genome but of the morphological fine structure of individual chromosomes. When applied to man, cytogenetic methodology has revealed a number of pathological and benign variations and has been incorporated as an integral aspect of diagnostic and clinical medicine, obstetrical and pediatric practice, oncology, and human genetics. Indications of the general acceptance of the importance of karyological studies in medicine and their contribution to the further understanding of human biology are in a review by Ford (32). He estimated that about two million human individuals have been karyotyped at 625 centers in 57 countries.

As a tool, cytogenetics has an equally important contribution to make to the various disciplines involved in the study of ways to enhance more economical production of animal products for human consumption. There are about 20 to 25 laboratories around the world applying modern cytogenetic methodology to problems of animal production. The work from these laboratories is not, for the most part, being integrated into animal production practice.
nor into the broad field of animal science. This paper will suggest applications of cytogenetic studies in various subdisciplines of animal science; the proposed applications are based on known methods and on data from studies with man, domestic fowl, laboratory animals, or with livestock where data are available. In other sections of the paper, arguing by analogy from recent developments in human genetics, ways are suggested in which knowledge of the genetics of livestock might be accumulated more rapidly by application of the methods of cellular genetics and cytogenetics.

APPLICATIONS OF CYTOGENETIC METHODOLOGY IN VETERINARY MEDICINE

Between 3% and 7% of livestock births result in presentation of a late abortion, still birth, or a liveborn young destined for neonatal death. Causes for these losses remain largely unknown, but it reasonably may be supposed that some proportion of them will bear either structural or numerical abnormalities of chromosomes. Surveys of newborn babies at six centers in North America and Europe indicate an incidence of about 5 per 1,000 liveborn babies with chromosomal aberrations, of which about 40% are sex chromosome aneuploids, 20% autosomal aneuploids, and 40% structural rearrangements (27 for review). No similar surveys appear of any of the large livestock although there have been case reports of a number of cattle, sheep, and pigs carrying various chromosomal abnormalities (summary of these in 74). In Ohio more than 4,000 domestic fowl which survived to 3 to 6 wk posthatching have been karyotyped. Although the chicks were from stocks in which the frequency of aberrations in early embryos was 5% to 13%, no aberrations were detected in the samples of hatched chicks. Apparently all afflicted embryos died during incubation. It would be useful to determine the incidence of abnormalities in other species of food-producing animals, to describe the symptoms characterizing each, and to make epidemiological analyses to find the causal factors.

In man, use of amniocentesis is now widespread to collect a sample of fetal cells for culturing and subsequent testing for the occurrence of fetal abnormality (58). This procedure, although useful in women at high risk of bearing a baby with abnormality, is expensive and probably has no extensive commercial application in livestock production. In special cases of particularly valuable animals, it may be applicable to test for particular recessive conditions when one or both parents are heterozygous. Fetal sex can be determined at relatively young age in cattle (5); the procedure may have application, therefore, in cases where special matings have been made to produce male young and it is not wished to use an entire gestation to produce a female.

Many therapeutic agents in common use produce chromosome aberrations, and it may be supposed that some of the detrimental side effects of various therapies are indirectly attributable to their effects on chromosomes. Among the physical agents in general use, ionizing radiation, particularly X-rays, produce chromosome breaks and numerical aberrations at therapeutic and diagnostic doses. Elevated temperature also is known, in some circumstances, to interfere with normal cell division. A number of the heavy metals, cytotoxic agents, some antibiotics and hormones, as well as many other compounds used by themselves or in formulations of commonly used drugs, are also damaging (48). Dose-response relationships of most of these agents have not been evaluated carefully in agriculturally useful animals, even though relatively rapid and efficient tests are now available (21).

In man, some of the leukemias, lymphomas, and other malignancies are associated with acquired chromosomal abnormalities (4, 39). In many tumors the chromosomal complement is more variable than in nonmalignant somatic cells. The relationship between the origins and growth of malignant cells and the occurrence of chromosome aberrations is not understood. With the wealth of material available to them, veterinary oncologists and pathologists could make substantial contributions to understanding the relationship.

Counterparts in animals to some rare heritable diseases of man which are characterized by a high frequency of chromosome breaks in somatic cells (33, 78) are not known yet. The more extensive use of karyological techniques in veterinary diagnostic routine may discover similar conditions in one or more species of animal which would not only make available a more suitable model for the study of the phenomenon but also would aid in the classifi-
cation of animal diseases.

In view of the invaluable assistance that cytogenetics has been in elucidating and diagnosing a number of pathological states of man, it is surprising and disappointing that the veterinary profession has not recognized the value of cytogenetic investigations.

**CYTOGENETICS IN LIVESTOCK MANAGEMENT**

As more animals are karyotyped with various techniques to reveal something of the internal structure of chromosomes (16), a number of variant banding patterns no doubt will be detected in livestock as they have in man (66). Some banding pattern variants already have been recorded as intrabreed polymorphisms (71). Banding variations as well as structural polymorphisms of the Y chromosomes (22), and perhaps of the autosomes, will become increasingly useful as genetic markers for identification of animals and verification of parentage. In man the variants appear to be stable, are identified relatively easily, are transmitted in a Mendelian mode, and all "genotypes" are apparent from the "phenotypes" (61). Whether use of chromosomal variants will replace the present system of erythrocyte antigens and serum protein polymorphisms for purposes of individual identification in the animal industry will depend upon the number and frequency of reliable variants found and comparative costs of the two systems.

Animal scientists need not be told of the possible detrimental effects of many of the substances marketed as herbicides, pesticides, feed additives, hormones, and other preparations used to enhance growth or other physiological functions. Among the detrimental properties ascribed to many of such projects are toxicity, carcinogenicity, and mutagenicity (including a propensity to cause chromosome abnormalities). Such matters are of little concern for animals to be marketed at young ages. Two aspects of the situation are of great concern, however. 1) Residues or metabolites with carcinogenic or mutagenic activity will not be permitted for long in animal products sold for food. 2) Young animals which are subsequently to be used for breeding purposes should be exposed minimally. Mutagenic and carcinogenic activity are related to the extent that screening tests for the production of chromosome aberrations are reasonably good indicators of both (49). It is advisable for animal scientists to begin now to consider ways of efficiently and effectively testing for those more subtle properties of products which are to be used in the production of food. Among the means presently available for the task, those which detect chromosome aberrations are the simplest to implement and are recognized as effective indicators of a broad spectrum of adverse effects (28, 67, 77).

**CYTOGENETICS AND ANIMAL REPRODUCTION**

Sterility, infertility, and embryonic mortality in animals each may be mediated by a chromosomal anomaly. Sterility in males resulting from sex chromosome aneuploidy, particularly XXY, is well recognized in man and occurs in newborn babies with frequencies of about 1 per 1000 (27). Other less frequently occurring anomalies of sex chromosomes have been found as the primary etiological agent of sterility in man (19). Unselected samples of livestock animals have not been surveyed for frequency of aneuploidy of sex chromosomes, but sterile males with the XXY complement have been reported in pigs (10), sheep (14), cattle (74), and pet animals (20, 72). Sterile females with the complement XO have been reported in cattle (74) and in horses (18, 41). Intersexuality accompanied by sex chromosome aneuploidy either in a mosaic state or in all cells of the afflicted animal also has been noted in a number of individual livestock animals. The inclusion of cytogenetic analysis in the diagnostic armamentarium of laboratories working with animal infertility would enable finding primary cause of difficulty in a number of cases which cannot be diagnosed otherwise. In addition, careful study of the anatomy and endocrinological status of animals with sex chromosome anomalies would shed more light on the role of the gonosomes and autosomes in gonadal differentiation, development, and function (80).

Reduced fertility also results from chromosomal aberrations. Structural rearrangements of chromosome segments carried by an animal in the heterozygous state are expected to lead to the production of genetically unbalanced gametes. The ability of such gametes to effect syngamy appears not to be diminished in mice (31) or domestic fowl (91). However, the
resulting zygotes usually have low viability and die as embryos, the manifestation being prolonged estrus cycles, reduced litter size, or lowered hatchability. In man about 2 per 1,000 newborn babies have structural rearrangements, the majority being of the centric fusion type. The reproductive fitness of a sample of such aberrant heterozygotes was about .85 that of normal controls (44), but variation was considerable between the fitness of subsamples bearing different types of aberrations. A number of centric fusions are segregating in populations of cattle, sheep, pigs, and goats; and various wild forms of similar ungulates possess centric fusions in abundance (8, 64, 65, 87). The effect of segregating centric fusions on reproductive performance of domestic animals seems to be variable (11, 12, 13, 35, 36, 37, 73), but even when the reproductive performance of heterozygotes or their progeny was affected detrimentally, the decline was not as great as expected on theoretical grounds. This matter now warrants intensive investigation both for its practical and theoretical importance.

A few spontaneously occurring structural rearrangements have been detected in livestock animals including a reciprocal translocation in pigs (40) and a pericentric inversion in cattle (70). As opposed to the segregating Robertsonian or centric fusions, the reciprocal translocation reduced litter size of the normal sows to which heterzygous boars were mated to about half that of litters by normal boars (2). There will be intense selection against maintenance of spontaneous aberrations in breeding populations of animals, but in some special circumstances it may be advisable to make karyological screenings of males before they are put into widespread service. Cytological techniques enable detection of a high proportion of structural rearrangements without the need for observation of cells at first meiotic division (76).

Heteroploid embryos resulting from errors at meiosis, fertilization, or early cleavage divisions for the most part do not survive and are a contributory factor to the relatively large number of early zygotes that suffer embryonic death. More than 50% of human embryos spontaneously aborted in early pregnancy are chromosomally aberrant (3). Between 8% and 10% of conceptuses are chromosomally abnormal if 15% to 20% of pregnancies are terminated by abortion.

In laboratory animals and domestic fowl, karyological examination of early embryos yields direct estimates of the proportion with heteroploid complements of chromosomes. Perhaps 4% of mouse embryos (52) and 5% of rabbit (29) and Chinese hamster (6) embryos are afflicted. Epidemiological studies with the human data (9, 43) and controlled experiments with laboratory mammals (6 for review) have yielded some understanding of the source of the errors and less about causal factors. The domestic fowl has proved to be a useful model for studying the etiological basis of heteroploid embryos (7, 57, 60, 84). The propensity for heteroploid embryos appears to be heritable in fowl, but nongenetic factors also are involved for some types of heteroploidy. Different lines of birds produce vastly different frequencies of abnormal embryos (1.5% to 13%), and differences between hens within lines also are important. Stocks bearing chromosomal “markers” were prepared and used to determine the parental source of the various types of heteroploidy in chick embryos (30). The direct source for almost all of the aberrant embryos was detected.

Little work has dealt with the incidence of chromosomal anomalies in early livestock embryos. McFeely (53) found 10% of preimplantation pig embryos carried one or another chromosome aberration in a sample of less than 100. The incidence in a similarly small sample of sheep embryos was low (50). The high rate of embryo mortality in livestock is a source of great loss to producers. It is urgent to determine the extent to which chromosomal anomalies are a direct cause of the loss and, if important, to find ways to reduce them substantially. The problem is costly and the tools are at hand.

**CYTOGENETICS AND ANIMAL BREEDING**

There are three main areas in which cytogenetics can contribute to the theory and practice of animal breeding. Much of animal breeding is based on assumptions for which critical tests heretofore have not been available; cytogenetic techniques can be adapted to test the validity of some of these assumptions. Second, new sources of genetic variation may be made available to animal breeders by cyto-
geneticists. Third, the large number of chromosome markers segregating in animal populations enables accurate assessments of the relationship among animals within populations and determination of relationships of populations, one to another.

Exceptions are known to almost all laws of genetics. In the absence of any specific knowledge about which exceptions occur in particular groups of animals, it is usual to presume that none do. With special “marker” chromosomes, either those spontaneously occurring or experimentally produced (91, 93), one can test critically in each species for regularly occurring exceptions. Such tests have been made in the domestic fowl for the occurrence of uniparental inheritance with the finding that gynogenesis was entirely nonexistent, but, surprisingly, one embryo was derived from an androgenetic origin (30).

Segregation of homologous chromosomes was regular in both cocks and hens, but a marked propensity for chromosome lagging (and subsequent loss) was established in hens heterozygous for translocations (25). Spermatozoa bearing complementary and unequal products of segregation are not always equally viable (or fertile). This finding, if it is not just a special case limited to the particular system, would have important implications for population genetics. It is feasible now also to test the reasonable assumption that inheritance of cytoplasmic elements is largely maternal (88). Experiments of this type are now underway with domestic fowl as a test system.

Development of techniques for detailed observations of the meiotic divisions in mammals and birds (26) enables estimates of the amount of crossing over in spermatocytes (69) and oocytes (45). Variation among cocks in frequency of chiasmata has been noted (68), and it is suspected that a relatively large portion is under genetic control. Heritability of chiasma frequency in the mouse is about .5 (23). By determining what factors influence recombination frequency, the characteristic could be altered to suit the requirements of the breeder.

The genome of diploid animals may be manipulated to an even greater extent. The number of linkage groups could be increased or decreased over a wide range. By combining into a single stock a series of structural rearrangements, chosen from the pool of such rearrangements available for every class of farm animal but especially for the chicken, animals could be created in which the numbers of linkages were increased by two to three times that in the base population; conversely, in theory at least, the number of chromosomes may be reduced materially in number by combining Robertsonian fusions in homozygous form into single stocks (13). This or a similar mechanism occurred during the process of speciation of the genus Mus in the Alps and Pennines. Populations are found with up to nine pairs of metacentric chromosomes and a 2n = 22. Mus musculus ordinarily has no metacentrics, and 2n = 40 (15).

Polyploidy is widespread among flowering plants and has been exploited advantageously by plant breeders. Triploid and tetraploid embryos occur spontaneously in man, laboratory mammals, pigs, fowl, and probably other livestock. They can be produced experimentally in mammals (82) and birds (89). Although both conditions are ordinarily lethal, triploid and tetraploid chicks have been hatched and reared (1, 79), and tetraploid mouse embryos survive beyond midterm of pregnancy (82, 83). Perhaps appropriately designed selection procedures would be effective in increasing the survival of these potentially interesting conditions. Equally interesting and potentially valuable would be the availability of haploid animals. Haploid chick embryos occur regularly, and haploid mouse embryos have been produced experimentally by eliminating or inactivating one pronucleus in a fertilized egg (46, 85); they also have occurred spontaneously (47). Doubling of chromosomes of haploid eggs to produce homozygous diploid zygotes has been accomplished in the mouse (51).

Less extreme alterations of the genome of animals can be manipulated by combining in single animals different translocations that carry the same chromosome segment. By such techniques, zygotes with duplications or deletions can be produced and examined (34, 90). Such manipulations are possible in fowl, and stocks suitable for similar work can be produced in pigs (93).

A number of morphologically variant chromosomes segregate in animal (24) and human populations (61). It is presumed that most of such variants are transmitted in a
Mendelian mode and do not affect the fitness of their bearers. Some, such as variation in length of the Y chromosome and size of secondary constrictions, are ascertained easily in preparations stained by any of the standard methods for the display of metaphase chromosomes. Another category, comprising variations in “banding patterns” of individual chromosomes, is detected only when special methods of treating and staining the preparations are applied. In either case, the relative abundance of variants, the regularity of their inheritance, and the ability to distinguish the heterozygotes enable use of variants to determine the degree of relationship of animals within populations and the genetic distances separating distinct populations. The system can be applied to augment similar studies with erythrocyte antigens, blood serum enzyme, or other protein polymorphisms that more traditionally have been used for such work. Specifically the chromosome variants are suited well to determine the degree of migration from one population to another, the phylogenetic relationships among populations, and the origins of more recently derived populations from preexisting ones. Cytotaxonomic methods recently have been applied successfully to establish the order of evolutionary divergence of caprids and ovids from their presumed common ancestor and the ancestral groups from which breeds of sheep and goats were derived (62, 63, 64, 65).

The main obstacle to cytological mapping of genes in livestock is paucity of knowledge of well defined loci for structural genes. Techniques for their identification and characterization have been developed for application to human genetics (54) and can be adapted readily for use with large animals. It is puzzling why so little of such work is in progress.

The relative ease with which cell lines can be established and maintained in culture has opened a new area of investigation, cellular genetics (55). From work with cultured mammalian cell lines, many structural genes have been identified, their activities and interactions elucidated, and mechanisms of their control have been discovered. Of particular interest is the phenomenon of cell hybridization whereby cells containing the genomes of different species are formed, isolated, and induced to form new cell lines. Ordinarily, in such lines the chromosomes of one species are eliminated differentially and randomly so that sublines are evolved containing only one (or a few) of the
chromosomes of the species of interest. Genetic activity of the sole remaining chromosome of interest then is monitored, and genes whose products are found are located on the specific chromosome. Genes whose activity is not required ordinarily for metabolism of cells in culture can be induced to translate and transcribe. Human-mouse cell lines have been used widely in work of this nature for the cytological localization of human genes (75 for review). Similar techniques would not be difficult to devise which would enable the discovery and mapping of genes in domestic animals.

The discovery of haploid cells, derived from supernumerary spermatozoa, in chick embryos (30) raises the possibility that haploid cell lines might be established and maintained in culture. These would be extremely valuable because the activity of recessive genes, being haploid, would be expressed as well as that of dominant ones. Thus, the way is opened for a genotypic analysis of animals without the need for progeny testing. At the more basic level, haploid cell lines would provide a bacteria-like system for study of mutagenesis and isolation of mutant genes in a vertebrate, homeothermic system.

Other recent developments enable direct genetic alteration of cells in culture and transfer of cells in culture into mouse embryos where they are differentiated into germ cells and become a part of the pool of germ cells of the host animal (59). The fact that such manipulations are confined to the mouse and to cell lines derived from undifferentiated cells of teratoma does not detract from the potential of such work for the alteration of the genotypes of higher forms of animal. It behooves animal geneticists and breeders to begin to take a more active interest in developments of this kind.

One wonders if we have become too canalized in the way we think about animal improvement. When the theory and practice of animal breeding were being formulated, agricultural research workers were in the forefront of much of biological research. Once useful methods were established and accepted by industry, research interest seems to have centered upon making refinements of the methods rather than leaving that task to industry and again turning to explorations at the knowledge horizon. The cost of this diversion of interest is that agricultural science no longer is engaged largely in search for new knowledge and, to a great extent, is not even aware of potentially useful discoveries in the biological sciences.

The inclusion of cytogeneticists into the pool of scientists engaged in service to animal production will not suffice to reverse the pull toward insularity, although cytogenetics does have something to offer toward the more efficient production of animal products. Perhaps what is needed most is an interchange of personnel between animal science and other areas of biology. For this to occur we must do two things. First, we must assure that our graduate students are educated sufficiently to find positions outside agriculture. Second, we must be much more willing to bring into our ranks young faculty members who have taken their degrees in areas other than agriculture. They can bring to us some fresh insights into old problems and, perhaps more important, the ability and knowledge to undertake research in those areas which are promising for animal science but which few of us agriculturalists are prepared well enough to delve into.

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