Cytogenetics of Animal Production: Chromosomal Responses to Environmental Stress

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Introduction

The success or failure of any organism must be, in part, a function of the genotype of the individual. Through meiosis, the genotype is transmitted from parent to offspring; in mitosis, from mother cell to daughter cells within an individual. It is remarkable that in the countless cell divisions involved, normality is highly prevalent. Cytogeneticists have always been interested in those factors that affect cell division. By studying deviations from the norm, much can be learned about the structure and behavior of chromosomes, the carriers of the genetic material. Cytogenetic investigations are now commonplace in reproductive inefficiency, oncology, congenital defects and many other research areas. The cytogeneticist does not claim that chromosome errors are the sole basis for these problems but rather that numerical and structural chromosome aberrations may be contributory. It should be noted that chromosome aberrations are not necessarily always deleterious. Certainly we should be interested in those changes that result in reproductive failure or tumor formation, but manipulation of the chromosomes may provide many benefits. Chief among these benefits are the knowledge of what factors affect chromosome structure and behavior, thereby providing information on what environmental hazards to avoid or in the case of rapidly dividing tumor cells, what factors can stop the progression of tumor formation. In the plant world, chromosome manipulation has resulted in a number of common successful plants which offer the advantages of better growth and disease resistance. Efforts along these lines in food animals may offer the same benefits (Shoffner, 1974).

This review will concentrate on chromosome changes as influenced by environmental stress—hyperthermia as well as hypothermia. In hyperthermia, evidence will be presented to show how high ambient temperature affects meiosis and mitosis and may result in reproductive inefficiency as well. In addition, the results of a pilot study using hypothermia to manipulate chromosome number will be presented.

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A Cytogenetic Primer

Cytogenetic terminology often overlaps with anatomy, reproductive physiology and classical genetics but also contains some unique words to describe changes to chromosomes. An effort will be made here to present a short primer on the terminology describing chromosome changes in response to environmental stress. It may be helpful to refer to Table 1 for examples of the numerical chromosome disorders.

First, most animal species we are familiar with are *diploid* or have the 2n chromosome number. This means that the chromosome number in, for example, domestic swine is 38 and is composed of 18 homologous pairs of chromosomes called *autosomes* and one pair of *sex chromosomes*. The boar has the sex chromosomes X and Y while the female has two X chromosomes. The gametes produced by domestic swine normally have 19 chromosomes and are therefore haploid; ova should contain 18 autosomes and the X chromosome whereas spermatozoa should have 18 autosomes and either an X or Y chromosome.

Table 1. Numerical Chromosome Abnormalities

	Chromosome Number in:		
	Mice	*Swine*	*Channel Catfish*
Euploidy			
Haploidy (n)	20	19	29
Diploidy (2n)	40	38	58
Polyploidy			
Triploidy (3n)	60	57	87
Tetraploidy (4n)	80	76	116
Aneuploidy			
Monosomy (2n−1)	39	37	57
Trisomy (2n+1)	41	39	59

There are a number of numerical deviations possible from the diploid or normal complement of chromosomes. First, *euploidy* consists of those cells that have multiples of the base or n number of chromosomes. *Polyploidy* includes those cells with three or more entire sets of chromosomes. *Triploidy* then is the 3n number, *tetraploidy* is the 4n number and so on. Polyploid cells arise through several mechanisms. The production of a diploid (2n) gamete which is fertilized by a nor-

mal haploid (n) gamete would create a triploid embryo. Triploidy in mammalian species is generally lethal with the embryo dying during gestation. Several triploids have survived to term in mice and humans but died shortly after birth due to multiple congential defects. Polyploidy can also arise from *polyspermy* which is the union of a haploid egg with two or more haploid spermatozoa. Another mechanism producing polyploidy is failure of cytokinesis after nuclear division during the first cleavage of a one-cell embryo. This would result in a tetraploid embryo.

The second major class of numerical chromosome disorders is aneuploidy. This includes any cell that is not euploid, and therefore has one or a few chromosomes either present in excess or missing from the diploid number. *Monosomic* (2n − 1) cells have one chromosome missing whereas *trisomic* (2n + 1) cells have one chromosome extra. These aneuploid cells arise from *non-disjunction* of homologous chromosomes during either meiosis or mitosis, so that one of the products of the cell division would have one extra and the other product would have one missing chromosome. Chromosome *lag* can also cause aneuploidy since a chromosome at anaphase of mitosis that hasn't reached one of the poles at karyokinesis may be lost into the cytoplasm instead of being included into one of the nuclei of the daughter cells. It should be noted that alterations to the diploid number are important in that missing or extra genetic material may have a significant influence on the phenotype of the individual.

There are a number of other numerical disorders including mixtures of cell populations and a variety of structural chromosome abnormalities that are not relevant to this discussion. For further information, see a review by Fechheimer (1968).

Hyperthermia and Chromosome Aberrations in Mice

High ambient temperature either alone or in conjunction with high relative humidity is known to have adverse effects on the reproductive efficiency of many mammalian species. Heat stress of males may cause aspermia, oligospermia, or even result in normal appearing spermatozoa that have undergone subtle changes which render them incapable of producing normal zygotes (Cowles, 1965; Ulberg and Burfening, 1967; Burfening et al., 1970). The effects in the female, whether directly or indirectly affecting ovum production, fertilization, or early embryonic development appear to be more complex (Hafez, 1964; Bellve, 1973). However mediated, heat stress can result in altered gamete morphology and function, embryonic mortality and congenital abnormalities. The problem is not restricted to laboratory species such as mice, rats and rabbits but extends to swine and humans. It should be noted that the extent of elevation of body temperature in response to hyperthermic conditions is more pronounced in those species without functional sweat glands.

Some experiments have suggested direct nuclear effects of hyperthermic conditions. Edwards et al. (1974) found nuclear clumping and meiotic delay in guinea-pig embryos subjected to a short period of maternal hyperthermia on day 21 of gestation and suggested that spindle microtuble alterations were present. Reider and Bajer (1977) subjected newt lung cells to hyperthermia and determined that spindle fiber rearrangements resulted in either complete spindle dysfunction or asynchrony of chromosome movement at the anaphase stage of mitosis. Zeuthen (1972) found irreversible damage in mitotic cells as well as mitotic delay in interphase cells of sea-urchin embryos exposed to elevated temperature. In these instances, hyperthermia could be classified as a mutagen, creating effects such as those attributed to radiation, environmental pollutants, chemicals or drugs. Work in this laboratory has focused on identifying the cytological changes produced by hyperthermia in both male and female laboratory mice. Specifically we have been interested in chromosomal changes produced by heat stress that could lead to lowered litter size in polytocous (litter bearing) species.

Our first endeavor to test high ambient temperature as a mutagen (Chrisman and Doolittle, 1979) was to subject 60 pregnant female mice from two different random bred stocks to 34°C and 50% relative humidity (RH) during days 5 and 6 of gestation. Observations of cells from 650 eight-day embryos showed a significant increase in trisomy (cells with 41 instead of 40 chromosomes) in the heat stressed group as compared to the controls maintained at 21°C and 50-70% RH.

This study led to a second (Chrisman and Baumgartner, 1980) using a newer approach called the micronucleus test (MT; Schmid, 1975; Chrisman and Baumgartner, 1979). Established as a means for screening chemical mutagens, the MT is based on observations that chromosome fragments and/or entire chromosomes separated (lagging) from the main group at anaphase of mitosis tend to be excluded from a daughter cell nucleus at the telophase stage of mitosis. These chromosomes or fragments are often transformed into micronuclei in the cytoplasm of the daughter cell. As this micronucleus formation may occur spontaneously (not under mutagen influence) at a low rate of incidence, a specific cell type known to have just undergone mitosis under mutagen influence is needed. The mammalian erythrocyte offers this opportunity and has the added advantage of not having a main nucleus so micronuclei can be easily observed (Figure 1). After the last mitotic cycle in the bone marrow, mammalian erythroblasts expel their nuclei but retain any micronuclei and take on a different staining ability. These erythroblasts are polychromatic and stain blue for a period of about 24 hours, then become normochromatic or red staining. Therefore, if a bone marrow smear is stained with May-Gruenwald-Giemsa, the polychromatic erythrocytes (blue) can be distinguished from the older, normochromatic erythrocytes (red). In this way a mutagen can be introduced, bone marrow collected and stained and the influence of the mutagen observed. As a normal procedure, micronuclei are also scored in the normochromes as a check on technique error since this class of cells has undergone mitosis before mutagen treatment. Negative and positive controls are also used. Table 2 presents both the experimental design as well as the observations on micronucleated bone marrow cells in an hyperthermia experiment on mice. Analysis of variance and Newman Keuls analysis of means indicated highly significant effects of temperature and chemical treatment on incidence of micronuclei in polychromatic erythrocytes. The positive control, cyclophosphamide (Cytoxan, Mead Johnson, Evansville, IN) is com-

monly used in mutation studies and in cancer chemotherapy, but had not been previously tested with whole-body hyperthermia (Hahn, 1979). There is evidence that chemotherapeutic agents may be more effective if body temperatures or the temperature of the tumor is slightly elevated. The body temperature of these mice (random bred ICR) supposedly rises approximately 1°C in response to the temperature humidity combination imposed in the heat stress, however body temperature was not monitored. Rectal probing in itself is sufficient stress to cause elevated body temperature. Miniature transmitters for monitoring temperature are being tested.

Several other studies on hyperthermia in mice have been completed in this laboratory. Baumgartner and Chrisman (1980) subjected 60 virgin, random-bred ICR females to either 21 ± 2°C and 65 ± 5% RH or 35 ± 1°C and 65 ± 3% RH for 15½ hours following gonadotropin treatment to insure that ovulation could be timed. Pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) were used for follicle stimulation and ovulation, respectively with heat stress begun at the time of HCG injection. All animals were killed and their ova collected at 15½ hours post HCG when the eggs were still located in a cumulous oocyte mass in the proximal oviducts. Heat stressed animals had a significant increase in morphologically abnormal oocytes as shown in Figures 2-3. It appears that the spindle apparatus of the pronucleus-second polar body complex has been displaced toward the center of the oocyte creating not only larger second polar bodies but also multi-polar spindles with the resulting multi-cellular appearance sometimes called oocyte fragmentation.

Garriott and Chrisman (1980) examined the testes of ICR mice subjected to hyperthermia for 2, 3, or 5 days and found

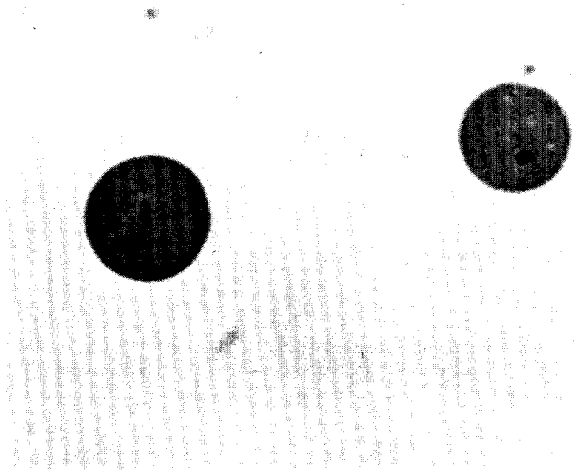

Figure 1. Micronucleated polychromatic erythrocyte (left) from a heat stressed male mouse.

Table 2. Bone Marrow Observations in Mice Subjected to Hyperthermia[a]

Temperature	*Chemical treatment*	*Micronucleated polychromatic erythrocytes (%)*	*Micronucleated normochromatic erythrocytes (%)*
21.1 ± 2	none	2.69	1.94
35 ± 1	none	20.88	5.71
21.1 ± 2	NaCl	4.88	1.57
35 ± 1	NaCl	20.31	4.38
21.1 ± 2	Cyclophosphamide	23.50	5.09
35 ± 1	Cyclophosphamide	75.44	11.35

[a]Each treatment represented by 8 animals (4 male and 4 female), 2000 total polychromes and 2040-2550 normochromes observed per animal. All values are treatment cell means. Relative humidity was 65 ± 5% for control temperature and 65 ± 3% for high temperature.

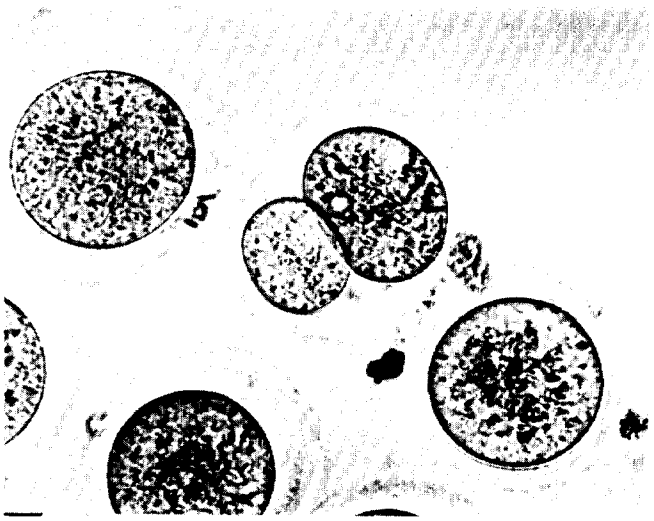

Figure 2. Normal and large polar body unfertilized mouse oocytes from a heat stressed female.

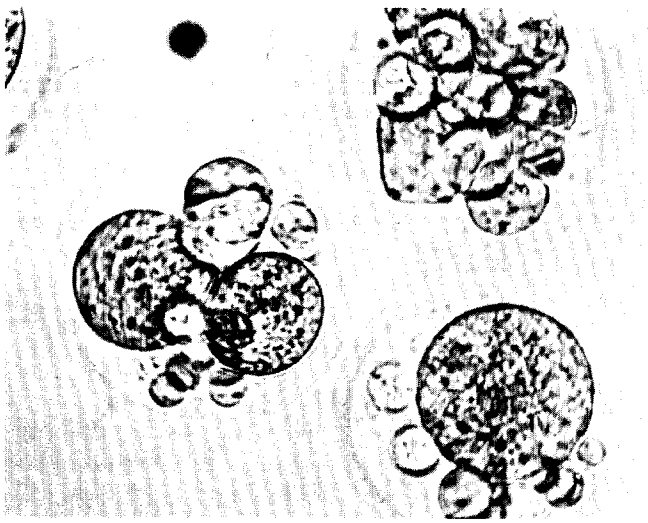

Figure 3. Multicellular (fragmented) unfertilized mouse oocytes from a heat stressed female.

a significant increase in the incidence of X and Y univalents at diakinesis-metaphase I of meiosis (Figure 4). The X and Y chromosomes of the mouse are ordinarily associated in an end to end bivalent, a configuration thought to be necessary for the progression of spermatogenesis. X and Y univalents have also been found in a follow-up study along with obvious testicular histological changes (Chrisman, unpublished).

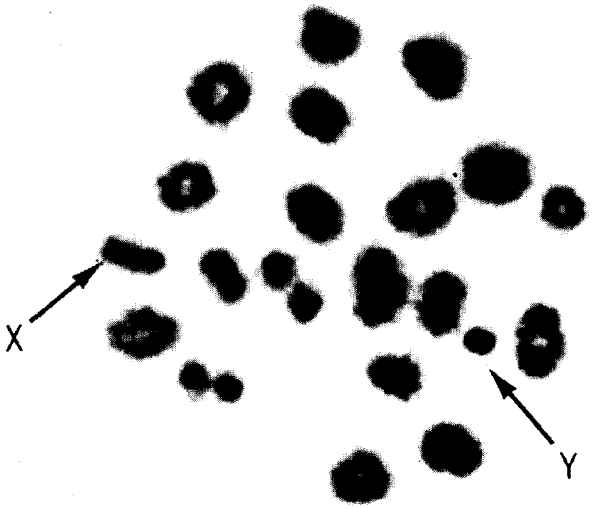

Figure 4. X-Y bivalent dissociation in a diakinesis-metaphase I spermatocyte of a heat stressed male mouse. The X and Y chromosomes should be in an end-to-end association.

Hypothermia and Channel Catfish Chromosomes

Lowell (1979) has reviewed the advantages and problems of aquaculture in the United States, including ventures in the cultivation of channel catfish, crayfish, trout, salmon, and freshwater shrimp. Channel catfish, *Ictalurus punctatus*, are particularly well adapted to commercial farming practices. They are able to thrive in densely populated ponds alone or with other species of fish (as long as there is sufficient dissolved oxygen), are rapid growing, efficient feed converters and present an attractive balance sheet for profit. A one hectare pond in the sunbelt region of the United States can yield 5000 kg of catfish in the fall from fingerlings stocked the previous spring. With an average profit of $0.24 per kilogram, this one hectare pond has the potential of making $800-1200 profit.

Although subjected to natural selection pressures for centuries, and more recent limited artificial selection for growth rate by catfish farmers, channel catfish present obvious opportunities for genetic manipulation. From studies on other species of fish subjected to various environmental manipulations (Valenti, 1975; Purdom, 1976), it was theorized that a triploid catfish could be created by cold shocking fertilized eggs. The normal progression of events leading to the formation of normal diploid and triploid embryos is shown in Figure 5. The second polar body has the haploid number of chromosmes (n = 29) as does the female pronucleus. Syngamy of the female pronucleus with a normal haploid sperm

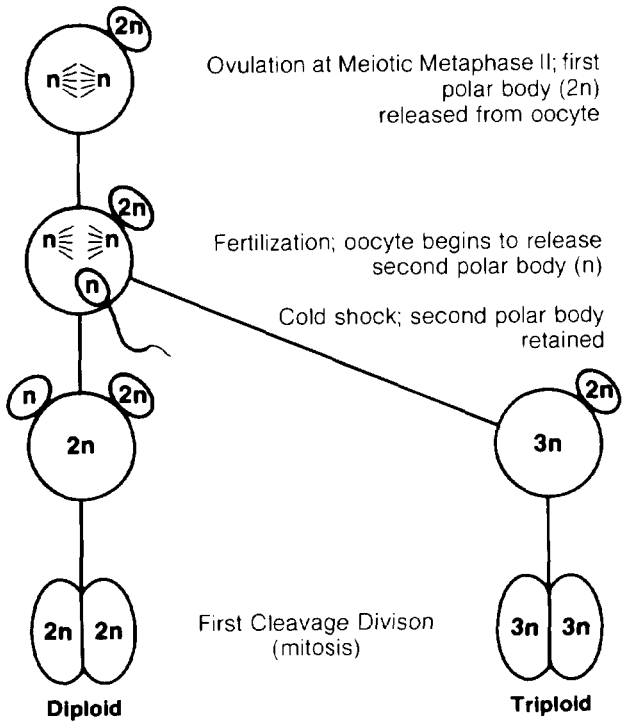

Figure 5. Sequence of fertilization events leading to diploidy and triploidy in channel catfish embryos.

creates a diploid (2n = 58) normal catfish embryo. If the second polar body is retained then a diploid egg fertilized by a normal spermatozoan gives rise to a triploid (3n = 87) embryo. As mentioned previously, the triploid condition is invariably lethal in mammals but apparently is a viable condition in lower vertebrates probably due to the fact that catfish do not have sex chromosomes.

The triploid condition, observed to cause an increase in cell size in some species (Valenti, 1975; Purdom, 1976) should be an advantage to growth, particularly in conjunction with the fact that most triploids are known to be sterile. Sterility would allow for the directing of energy to growth rather than to reproductive development. With these thoughts in mind, William R. Wolters and Dr. George S. Libey of the Purdue Department of Forestry and Natural Resources in collaboration with the cytogenetics section of the Department of Animal Sciences embarked on an effort to create triploid channel catfish.

Gravid female channel catfish were injected with carp pituitary extract to induce ovulation. Eggs were handstripped from the females and covered with sperm from freshly macerated testes (Dupree et al., 1969). Table 3 presents the design and results of the cold shock experiment. Control eggs were maintained at 27°C while those eggs to be cold shocked were exposed to 5°C for 1, 1.5, 2, or 3 hours beginning five minutes after fertilization. Cold shocking for more than one hour proved to be fatal to the zygotes. Although fertilization rate was high in all groups, hatching success in the first group was quite low due to a fungal infection of the egg mass. In the second group, both control and one hour cold shock groups exhibited good hatching percentages.

Table 3. Results of Hypothermic Treatment of Channel Catfish Eggs

	Cold shock duration (hours)	No. eggs	Fertil. success (%)	Hatch. success (%)	Incidence diploidy (%)	Incidence triploidy (%)	Wt. at 8 months (g)	Feed conversion (S.E.M.)
Female 1	0	300	91.6	1.3[a]	100	0	—	—
	1.0	104	98.1	4.8[a]	0	100	—	—
	2.0	125	98.4	0	—	—	—	—
	3.0	165	95.8	0	—	—	—	—
Female 2	0	100	98.0	89.0	100	0	87.8 ± (.202)	1.3
	1.0	107	100.0	79.0	0	100	92.9 ± (.182)[b]	1.19
	1.5	106	99.1	0	—	—	—	—

[a]Low hatching success due to fungal invasion of egg mass.
[b]Significantly different from control ($P < .001$); n = 72 diploids and 72 triploids.

Chromosomal analyses of surviving fingerlings was performed using kidney tissue (Hollenbeck and Chrisman. 1981) and peripheral blood lymphocyte cultures (Wolters, Chrisman and Libey, 1981) on 10 control and 10 cold-shocked specimens. All controls analyzed were found to be diploid (2n = 58) while all cold-shocked fish were found to be triploid (3n = 87). A photomicrograph of a mitotic metaphase triploid cell is shown in Figure 6. The 95% confidence interval for the level of triploidy in the 1.0 hour cold-shocked group was 0.6915, 1.000 from the Clopper-Pearson procedure (Hollander and Wolfe, 1973). All remaining control and cold-shocked fish are being observed for growth rate and feed efficiency. Cold-shocking on a larger scale is planned for the 1980 spawning season.

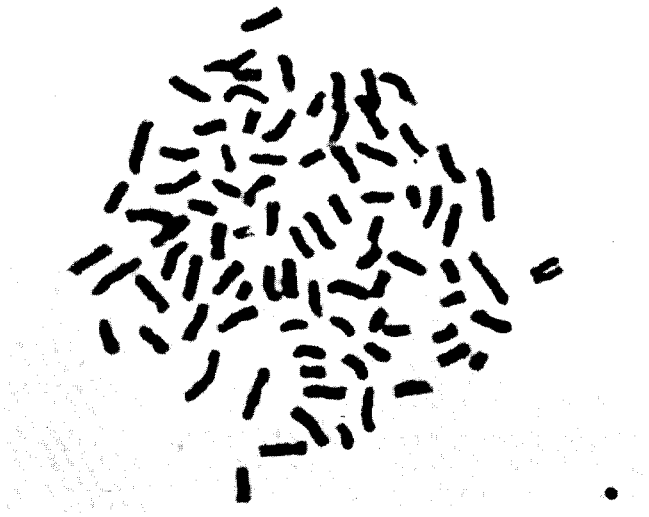

Figure 6. Triploid (3n = 87) mitotic metaphase plate from culture of a channel catfish lymphocyte.

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