GENETIC ANALYSIS OF THE MATERNAL FACTORS CONTROLLING THE SURVIVAL OF TRISOMY 16 MOUSE FETUSES

by

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ABSTRACT

The BxH recombinant inbred strains of mice were used to undertake a genetic analysis of the maternal factors controlling the survival of trisomy 16 fetuses. The data presented indicate that the prevalence of trisomic fetuses on day 15 of gestation varies significantly with the genetic background of the mother. The strain difference in the frequency of trisomy appears to be the result of selective elimination of trisomic fetuses. Various statistical methods to elucidate the genetic architecture of the trait from the recombinant inbred strains data indicate that the number of loci involved in the selection process ranges from one to five. Linkage association with two loci have been found; however, with a low probability level ($p=0.292$).

ABREGE

Une analyse génétique des facteurs maternels contrôllant la survie des foetus trisomiques pour le chromosome 16 a ete entreprise par l'utilisation des lignees murines recombinantes BxH. Les données presentees indiquent que la prévalence de fetus trisomiques observés au quinzième jour de la gestation varie de façon significative selon le bagage génétique de la mère. Les différences de frequence de trisomie observées entre les souches murines seraient le résultat d'une élimination sélective des foetus trisomiques. L'utilisation de diverses méthodes statistiques permettant d'élucider la composition génétique du trait à partir des données obtenues des lignées recombinantes, indique que le nombre de loci impliqués dans le processus de sélection varie de un à cinq. Deux associations avec des loci déja localises ont été découvertes; ceci avec un niveau de probabilité relativement bas (p=0.292).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

Maternal influences on embryonic development have been recognized in many organisms. This is not surprising when it is realized that maternal effects can manifest themselves in a number of ways; through the egg cytoplasm, through the uterine environment and through differential functioning of chromosome regions (imprinting).

The cytoplasm of the zygote is almost exclusively a product of the female gamete; its content is prepared under the direction of the genome of the mother. The zygote is thus a very special cell in which the gene products of one generation coexist with the not-yet functional genome of the next generation. Developmental mutants that produce defective eggs incapable of supporting normal zygotic development can provide valuable material for analyzing the functions of individual egg components.

In higher organisms, the uterine environment can have a substantial influence on growth and survival of the conceptus. In addition, differential imprinting of specific genes during female (as well as male) gametogenesis can lead to quite severe phenotypic manifestations and even to developmental failure.

All these phenomena are ultimately controlled by maternal genes, e.g. genes coding for components of the egg cytoplasm, genes controlling the function of the uterus or genes

modifying (imprinting) the expression of other genes. Therefore, they can be classified as maternal effect mutations because the mutant phenotype of the embryo depends on the genotype of the mother.

In the following pages, an overview of maternal genetic factors influencing early development in different organisms will be presented. It will be seen that in Drosophila, the establishment of body axis in the blastoderm is under the control of maternally derived morphogens. In Caenorhabditis elegans, maternal gene products are mostly involved in the events surrounding the first few cell divisions in the zygote. In amphibia, where the amount of cytoplasm in the egg is much more considerable than in human or mouse, a number of maternal mutations act through the egg cytoplasm to provoke an early arrest in development. Finally, in mouse, evidence of maternal genetic influences on the embryo through the uterine environment, the egg cytoplasm and by imprinting of specific chromosome regions will be reported.

The techniques used to establish a maternal genetic effect in development (embryo transfer, ovary grafts, cross-fostering) are difficult to apply in human; therefore, the extent of maternal effects in human fetal development is unknown. On the other hand, it is evident that chromosomally abnormal conceptions (particularly trisomics) are the object of a drastic in utero selection and that women exist that are at greater risk of producing aneuploid progeny. An

examination of some factors influencing the survival of chromosomally abnormal conceptuses will thus be undertaken.

A model designed for the induction of specific trisomies in the mouse has been used to investigate the factors controlling the survival of aneuploid fetuses. It was established that the survival of trisomy 16 and 19 fetuses is under the control of a small number of maternal genes (Vekemans and Trasler, 1987; Epstein and Vekemans, 1990). The last part of this study is thus an attempt to understand the genetic basis of the factors modulating the survival of trisomy 16 mouse fetuses.

SECTION I: LITERATURE REVIEW

1. MATERNAL GENES AFFECTING EARLY DEVELOPMENT

1A. Drosophila

The earliest events of embryonic development in Drosophila are under the control of the maternal genome. In fact, the Drosophila embryo develops in an egg that contains a large number of maternal gene products. Two hours after fertilization, zygotic development proceeds to produce a blastula containing a cluster of pole cells at the posterior end and a syncitial blastoderm layer with about 3500 nuclei (Rice and Garen, 1975). Following this syncitial period, a transition to a cellular blastoderm, during which a cell membrane is formed around each blastoderm nucleus, occurs in normal embryos. Depending on their location within the egg, the nuclei are exposed to different maternal gene products which trigger the expression of specific set of zygotic genes (Manseau and Schupbach, 1989).

The metameric organization of the Drosophila embryo is generated early during development. Maternal factors set the frame in which the zygotic segmentation and homeotic genes act. Due to the concerted action of the segmentation genes, the appropriate number of segments is established, and homeotic selector genes control the diverse pathways by which each of the segment acquires a unique morphology. The gap genes participate in the complex process of pattern formation by providing a link between the maternal and the zygotic gene

activities: the gap gene patterns are controlled by the maternal factors, and the gap gene activities in turn, control the pattern of zygotic segmentation and homeotic genes (Gaul and Jackle, 1989).

Positional information in the Drosophila embryo requires the activity and correct regulation of about 60 maternal and zygotic genes (Lasko and Ashburner, 1990). The maternally active genes can be grouped into 2 classes according to the phenotypes of embryos from mutant mothers: those which are essential for specifying positional information along the anterior-posterior axis and those that affect the dorsal-ventral fate Both classes of mutations will be considered in turn in order to illuminate the mechanisms involved in establishing positional information and to emphasize the influences of maternal genes on Drosophila development.

Anterior-posterior patterning

Three maternal genes (bicoid, exuperantia and swallow) are necessary for formation of anterior body pattern. Embryos from bicoid (bcd) mutant females lack head and thoracic structures and exhibit abnormalities in the first four abdominal segments (Manseau and Schupbach, 1989). Cytoplasmic transplantation experiments revealed the presence of an activity located at the anterior tip of embryos from wild-type but not from bcd mutant females. This activity, when transplanted to any position along the anterior-posterior axis of a recipient embryo, was able to induce

anterior development and suppress the formation of posterior structures at the site of injection (Driever et al, 1990). Bicoid is thus considered as a morphogen.

The bcd gene is transcribed maternally and bcd mRNAs become localized at the anterior tip of the egg during oogenesis (Berleth et al, 1988). Since the oocyte nucleus is largely transcriptionally inactive, the bcd message, like most maternal messages, is probably produced in the nurse cells and transported into the oocyte. Proper localization of the bcd mRNA also depends on the swallow (swa) and exuperantia (exu) gene products. Loss of exu function, for example, causes a more even distribution of the bcd gene product, and consequently the loss of anterior structures (Winslow et al, 1988). Shortly after fertilization, bcd protein is present in a concentration gradient along the anterior-posterior axis with a maximum at the anterior tip (Driever et al, 1990). The bcd protein contains a homeo domain, suggesting it plays a role in DNA binding and gene regulation. The zygotic gap genes hunchback and Kruppel were shown to be activated by bcd in a concentration-dependent and a site-specific manner (Gaul and Jackle, 1989; Hulskamp et al, 1990).

There are nine maternal genes required for formation of abdominal segments. Injection of cytoplasm from the posterior pole of a wild-type embryo into the abdominal region of posterior group mutant embryos results in extensive

rescue of abdominal segmentation (Nusslein-Volhard et al, 1987). However, an exception to this is the mutation pumilio. Abdominal segmentation can be rescued in pumilio mutant embryos by transplantation of cytoplasm from the pole of the mutant embryo to the region of the prospective abdomen, indicating that pumilio embryos contain rescuing activity but lack the ability to distribute it to the prospective abdominal region (Lehmann and Nusslein-Volhard, 1987). nanos is the only posterior group mutant that does not produce rescuing activity in the nurse cells during oogenesis (cited in Manseau and Schupbach, 1989). This makes nanos the best candidate for encoding the posterior segmentation rescuing activity.

Maternal control of abdominal segmentation is different from maternal control of anterior segmentation. In the anterior of the embryo, the level of maternal bcd is used to direct the formation of structures along the anterior-posterior axis. However, the only function of the maternal effect loci of the posterior group in abdominal segmentation is to repress maternal hunchback in the posterior of the embryo, thereby permitting organization of the posterior segments by zygotic gap genes. For example, if the nanos gene product is not correctly localized to the posterior pole, then this will result in the failure of inactivation of the maternal hunchback product and a consequent failure of activation of the zygotic gap gene knirps (Lasko and

Ashburner, 1990). Moreover, in the absence of both maternal hunchback and posterior group function, normal abdominal segmentation can occur (Manseau and Schupbach, 1989).

All the genes of the posterior group, except nanos and pumilio, are also required for the formation of pole cells (the germ cell progenitors). Synthesis of polar granule components, which are eventually required for the formation of the germ cell lineage, and their localization to the posterior pole of the egg begins to occur during late oogenesis. The fact that localized polar granules are absent in vasa mutants indicates that vasa is a necessary component of polar granules (Lasko and Ashburner, 1990). Based on its homology with RNA helicases (nucleic acid-binding proteins), another role of vasa is to regulate the translation of mRNA (e.g. nanos) important for determination of posterior structures or to provide a permissive environment (RNA secondary structure) for RNA localization via other RNA-binding proteins (Hay et al, 1990). Observations from the phenotypes of oskar, staufen, cappucino and spire mutations suggest that these gene functions are required for posterior localization of vasa during maternal oogenesis. The valois product is thought to function as a cytoskeletal component required throughout the embryo following fertilization whereas the tudor product is thought to be a polar granule component that is required for the assembly of that structure (Hay et al, 1990).

Six loci are required for the correct establishment of the terminal regions of the Drosophila embryo. Homozygous mutant females produce embryos lacking all structures derived from approximately the anterior-most 10% as well as the posterior-most 20% of the blastoderm. Instead, cellular fates that are only assumed by cells in more central positions are expanded to the poles. Mutations at the zygotic locus tailless eliminate a subset of the terminal structures, suggesting that tailless may be one of the zygotic target genes regulated by the terminal group of genes (Weigel et al, 1990).

The torso and l(1)polehole genes have recently been cloned. The former encodes a putative receptor kinase while the latter encodes a protein with sequence similarity to the raf proto-oncogene, which possesses a Ser/Thr kinase domain (Manseau and Schupbach, 1989). This led these authors and Degelmann et al (1990) to speculate that the genes of the terminal group encode members of a phosphorylation cascade. The torso gene product might act as a transmembrane receptor that, upon activation by an extracellular signal and within the correct cellular environment, can phosphorylate and thus activate certain other gene products. These in turn, might activate zygotic genes such as tailless. Two other gene products, fs(1)Nasrat and fs(1)polehole are required for the stability of the vitelline membrane and have specialized functions at the poles which involve direct interaction with

other terminal gene products (Degelmann et al, 1990). The torso protein is uniformly expressed at the surface of the early embryo, but specially activated at the poles. It is synthesized only after fertilization, implying that the activating ligand must be localized in the perivitelline space separating oocyte and follicle cells. One of the terminal class genes, torsolike, has been reported to be synthesized by the follicle cells and is thus a candidate gene for producing a spatially restricted signal molecule in the egg chamber. Degelmann et al (1990) speculated that fs(1)Nasrat and fs(1)polehole might be involved in the retention or stabilization of the torsolike product in the perivitelline space at the egg poles, thus ensuring localized activation of the torso protein.

In summary, each of the three maternal organizing activities (anterior, posterior and terminal) is responsible for the formation of a particular subset of pattern elements in defined regions along the anterior-posterior axis of the egg. However, these domains show considerable overlap. Therefore, interactions between the three maternal activities must occur at some level, since in the absence of either the anterior or the terminal activity, the abdominal pattern elements are formed in the defective area (Winslow et al, 1988). Conversely, when anterior or terminal activity is elevated, pattern elements corresponding to the abdomen are compressed (Driever et al, 1990; Klinger et al, 1988). These

observations demonstrate that anterior and terminal activity negatively modulate the extent and spatial distribution of the abdominal domain, allowing for a certain plasticity in the formation of the anterior-posterior pattern of the embryo.

Dorsal-ventral patterning

Maternal mutations affecting embryonic dorsal-ventral pattern can be separated in two groups. The first group (fs(1)K10 and spindle) are pure maternal effects and affect the polarity of the egg shell. Mutations in the genes of the second group (dorsal, gastrulation defective, easter, mat(3)2 and mat(3)4) cause the total dorsalization of the embryonic pattern as a maternal effect, without affecting the polarity of the egg shell. Of course, a third group of mutants (twist and snail) are zygotic in their function, causing only partial dorsalization of the pattern, and are apparently the last in the temporal series of gene functions.

In the mutant fs(1)K10, homozygous females produce eggs in which the egg shell is less asymmetric in the dorsal-ventral axis, and the appendages are fused and lie ventrally rather than dorsally. Whenever embryos develop from these abnormal egg shells, the embryos are dorsalized (all the cells of the blastoderm behave like the cells normally located in the dorsal positions of the blastoderm) (Price et al, 1989). The female sterile locus spindle produces eggs which appear to be ventralized in shape and which are never fertilized (Anderson

and Nusslein-Volhard, 1984). Identification of these two genes reveals that the dorsal-ventral polarity of the egg case is under genetic control.

The dorsal allelic series, comprised of strong phenotypes in which the embryo is totally dorsalized, with the smooth progression to weaker phenotypes, involve the gradual addition of more ventral structures in the same order in which they appear in the embryonic fate map (Anderson and Nüsslein-Volhard, 1984). This progression reveals that the dorsal gene is involved in the establishment of positional information along a dorsal-ventral gradient. Multiple alleles with a range of phenotypes going from complete dorsalization to progressive addition of ventral structures are also observed for the maternal effect gastrulation defective, easter, mat(3)2 and mat(3)4 (Gans et al, 1975; Anderson and Nüsslein-Volhard, 1984). The fact that the strong phenotypes are indistinguishable implies that these genes are responsible for components of a single, integrated process. The involvement of multiple components, all of which are essential to prevent dorsalization of the embryo, suggests that the gene products are interacting components of a coordinated, self-regulating system of positional information.

The zygotic mutations (twist and snail) cause relatively minor aberrations in the dorsal-ventral pattern of embryos compared to the maternal effect mutations (Simpson, 1983).

Therefore, all the information necessary for the establishment of the embryonic dorsal-ventral pattern is maternally derived. This maternally provided system includes the production of the morphogen, the means of distributing the morphogen in a concentration gradient, and the receptor system which measures the morphogen concentration (Anderson and Nusslein-Volhard, 1984). The zygotic genes may stabilize or enhance this positional information (Simpson, 1983).

1B. CAENORHABDITIS ELEGANS

C.elegans is a self-fertilizing hermaphrodite with one pair of sex chromosomes (XX) and five pairs of autosomes. Males (XO), which are morphologically distinguishable from hermaphrodites, appear in the population at a frequency of 1 in 700 animals (Hirsh et al, 1977). Males mate with hermaphrodites but hermaphrodites do not mate with each other.

By about 10 minutes after fertilization, the C.elegans zygote has formed a vitelline membrane and a chitinous shell and become autonomous; i.e. it can continue normal development if removed from the parent. Embryos are normally laid about 3 hours after fertilization at a 30-cell stage that corresponds approximately to the time of gastrulation. The first-stage larva (L1), 250 um in length, hatches from the egg about 14 hours after fertilization with a total of 546 somatic nuclei and 4 primordial gonadal nuclei (Sulston

and Horvitz, 1977). During the next 35 hours, the animal passes through three more larval stages (L2-L4), separated by 4 molts, and grows to a 1 mm adult with 808 somatic nuclei (Sulston and Horvitz, 1977). The four primordial gonadal nuclei begin to divide a few hours after hatching and proliferate to form the gonad during larval development. In the hermaphrodite, spermatogenesis takes place during the L4 stage to produce about 1500 mature sperm in each of two spermathecae. Mature oocytes appear just before the fourth molt and oogenesis continues for up to 8 days in the adult (Hirsh et al, 1976).

The nematode C. elegans provides a special opportunity for the study of maternal-effect lethal mutations by virtue of its completely described embryonic cell lineage and its strictly determinate and invariant developmental pattern. Genes essential for embryogenesis were first identified in screens for temperature-sensitive lethal mutations following EMS mutagenesis, using 25°C and 16°C as the restrictive and permissive temperatures respectively (Hirsh and Vanderslice, 1976; Wood et al, 1980; Miwa et al, 1980; Cassada et al, 1981; Hirsh et al, 1985). In these screens, between 10% and 28% of the lethals recovered have shown phenotypes of embryonic lethality; that is, homozygous mutant hermaphrodites shifted to nonpermissive temperature as third or fourth-stage larvae self-fertilize to produce embryos that fail to hatch.

On the whole, a total of 55 genes required for embryogenesis were identified. Information on when these genes act in development has been obtained from parental-effect tests on the temperature-sensitive mutations that define them (Wood et al, 1980; Miwa et al, 1980; Isnenghi et al, 1983). These tests involve experimental elimination of one or more possible sources of the normal gene product, by varying the configuration of the parental and embryonic genotypes and determining the subsequent phenotypic expression of the mutation in the progeny (Vanderslice and Hirsh, 1976). These tests were performed for the 55 embryonic lethal mutations and 4 classes could be distinguished:

1) The 32 mutations in this class show a strict parental effect; that is the homozygous mutant (m/m) self progeny of a heterozygous mutant (m/+) hermaphrodite escape embryonic lethality, and the m/+ outcross progeny of an m/m hermaphrodite mated to a wild-type (+/+) male die as embryos. Therefore, expression of these genes in the maternal parent appears to be both necessary and sufficient for embryonic survival.

2) The 17 mutations in this class show a partial parental effect: m/m self progeny of m/+ hermaphrodites survive, but so do m/+ outcross progeny of m/m hermaphrodites. Therefore, expression of these genes in either the maternal parent or the embryo is sufficient for embryonic survival.

3) Two mutations behaved in a strictly nonmaternal fashion; that is, m/m self progeny of m/m hermaphrodites die as embryos, and m/+ outcross progeny of m/m hermaphrodites survive. Expression of these genes in the embryo is therefore both necessary and sufficient for embryonic survival.

4) Regarding the four genes in this class, neither the m/m self-progeny embryos of m/+ hermaphrodites, nor the m/+ outcross progeny embryos of m/m hermaphrodites survive, indicating that expression of this gene is required both maternally and embryonically for embryonic survival.

These results are consistent with the view that most of the gene products required during embryogenesis are incorporated into the egg during oogenesis in the maternal parent, and furthermore that most of them are produced maternally in sufficient excess that their embryonic production is not required.

More detailed analysis of when these genes act has been carried out for the temperature-sensitive mutations by temperature-shift experiments to determine the time and the duration of the temperature-sensitive period (TSP) leading to embryonic lethality (Wood et al, 1980; Miwa et al, 1980; Hirsh and Vanderslice, 1976; Isnenghi et al, 1985). Interpretations of such experiments is complicated by the uncertainty of whether the TSP defines the time of synthesis or the time of function of the mutant gene product. However,

strict maternal mutants tend to show the earliest TSPs, either before or close to the time of fertilization, and partial maternal mutants show later TSPs, generally during the first half of embryogenesis. The two strict nonmaternal mutants have TSPs during the second half of embryogenesis.

Most of the genes identified by the temperature-sensitive embryonic lethal mutations are also required at stages of the life cycle outside of embryogenesis. This probably reflects the likelihood that many genes identified by temperature-sensitive embryonic lethal mutations encode general metabolic functions, and thus have essential roles in cell viability or proliferation rather than having controlling roles in embryogenesis (Wood et al, 1980; Cassada et al, 1981). Analysis of 29 maternal effect lethal loci from a saturation screen of a region of chromosome II has led to a more precise estimate of the number of pure maternal effect genes in C.elegans as being probably around 12 (Kemphues et al, 1988b). In the set of 55 genes discussed above, only about 14 are possible candidates for exclusively embryonic function. Among these 14 genes, 11 show strict maternal, 3 show partial maternal, and only 1 shows strict nonmaternal requirements for function.

Many of the strict maternal mutants show defects in meiosis or first cleavage; for example, absence of pronuclear fusion or of polar bodies, absence or abnormal position of the first division cleavage, anomalous cytoplasmic streaming,

egg shell defects, abnormal cytoplasmic yolk granules, extra nuclei, endomitosis or arrest at the one-cell stage (Wood et al, 1980; Miwa et al, 1980; Denich et al, 1984; Kemphues et al, 1986). In these, subsequent anomalies may be the result of aberrant chromosome distribution rather than lack of specific factors required for determination of cleavage pattern or cell fates (Wood, 1988). Schierenberg et al (1980) and Denich et al (1984) observed that the mode of expression of most mutants with defects in the timing of embryonic cell divisions is at least maternal sufficient. This led these authors to suggest that the autonomous division rates of the cell lines are preprogrammed in the C.elegans uncleaved egg by maternal genes. Here again, it is not known whether the timing defects are symptomatic or independent of the other defects observed in these mutants.

More extensive analysis of mutations defining two loci, zyg-9 and zyg-11, have shown that these are pure maternal effect genes, expressed specifically during oogenesis and encoding protein products whose functions are required only during embryogenesis (Kemphues et al, 1986). Mutations at both loci have TSPs during the one-cell stage and alter the position of the first cleavage spindle. More specifically, the absence of functional zyg-9 product seems to affect microtubule functions in the one-cell embryo (Kemphues et al, 1986). The existence of a maternally expressed gene necessary for microtubule function is not surprising since

the relatively large size of blastomeres in early embryos requires microtubules of unusual length. Lack of functional zyg-11 gene produces a wide range of effects suggesting that this product may play a role in several cytoplasmic processes (Kemphues et al, 1986). The zyg-11 gene has been sequenced and codes for a 91 kDa protein (Carter et al, 1990). Microinjection of the cloned DNA can rescue zyg-11 mutations. Surprisingly, a transcriptional analysis shows that transcription of the gene is not limited to the female germ-line, despite the strict maternal-effect phenotype of zyg-11 mutations (Carter et al, 1990). Further studies using immunofluorescence staining with antibodies against zyg-11 and Northern blot analysis using RNA from males and hermaphrodites C.elegans of specific developmental stages should clarify these findings.

In addition, Kemphues et al (1988a) have isolated strict maternal effect mutations at four new loci, designated par-1 to par-4 (for partitioning-defective), which may be important in the asymmetric partitioning of cytoplasmic components in the early embryo. These mutations affect only embryogenesis and are characterized by synchronous and usually equal, rather than the normal asynchronous and unequal, early cleavages. These mutants have abnormal positioning of the early mitotic spindles; they fail to localize P granules (the germ-line precursors) properly; they do not produce the intestinal differentiation markers and finally arrest

development as amorphous masses of differentiated cells. Four mutations are incompletely expressed, that is some embryos from homozygous mothers survive and grow to become infertile adults due to the absence of functional germ cells (grandchildless phenotype). The authors proposed that the par genes could encode products required for the specialized functions or distributions of actin microfilaments in the early embryo since actin microfilaments have been previously shown to be required for both P granule localization and the proper positioning of the mitotic spindle in wild-type C.elegans embryos.

A set of dominant maternal effect temperature-sensitive embryonic lethal mutations has been isolated with the goal of identifying either haploinsufficient loci which are required in two normal doses for normal embryonic development or members of multigene families or of multimeric proteins which would not be identified in screens for recessive mutations (Mains et al, 1990). Eight such mutations have been found, representing six loci, one of which appear tc be a loss-of-function mutation in a haploinsufficient locus and two others seem to encode gene products that interact with each other and with products of the zyg-9 locus during early embryonic cleavage.

1C. AMPHIBIA

The pattern of early post-fertilization amphibian development has been well documented as being largely under the control of the maternal genome (Gilbert, 1985). It is, again, regional differences in the egg's cytoplasm which are thought to be responsible for the varied gene expression patterns of the embryo's population of nuclei. There is evidence that specific macromolecules which are synthesized during oogenesis and stored in the egg cytoplasm, participate in early embryogenesis. A summary of the extent to which some maternally derived egg components persist in embryogenesis is presented in Malacinski and Spieth (1979). In the case of histones, sufficient amounts are present in the egg prior to fertilization to supply a major proportion of the requirements of cleavage division up to the blastula stage. For more than a dozen enzymes of intermediary metabolism, catalytically active isozymes which are products of the maternal genome persist through the neural fold stage of development. Populations of mRNA which are synthesized during oogenesis remain functionally active up to the blastula stage, whereas maternal ribosomal RNA persists up to the swimming tadpole stage.

In order to study maternal control on development in amphibia, a number of maternal effect mutations have been recognized in the Mexican axolotl (a neotonous salamander) (Humphrey, 1975), and these will be described in the present

section.

nc: no cleavage mutant

The maternal effect which acts earliest on post-fertilization development is the gene nc (no cleavage). Eggs spawned by females homozygous for the nc, appear to be morphologically normal. Fertilization and egg activation are accompanied by a normal cortical reaction, and meiosis also proceed normally. However, the mitotic apparatus and cytasters never form and cleavage division does not take place (Raff et al, 1976). This mutation appears to be a true maternal effect mutation in that fertilization by a wild-type sperm is incapable of rescuing the mutant phenotype. Furthermore, the nc can be phenocopied by electrically activating unfertilized eggs from normal females (Raff et al, 1976).

Ovary graft experiments have shown that the effect of the nc gene occurs within the ovary itself rather than indirectly through effects on other tissues (Raff et al, 1976). The egg tubulin protein in the nc mutant was analyzed on protein gels and was shown to be present and not altered compared to wild-type (Raff, 1977). The nc phenotype can be partially corrected by injection of microtubules obtained from the tails of sea urchin sperm (Raff et al, 1976). The first cleavage divisions are apparently normal in the animal hemisphere but incomplete in the vegetal region. Later cleavages appear irregular and embryos finally arrest as

partial blastula. Microtubules form in parallel arrays in corrected embryos but do not attach to the kinetochores, and the chromosomes appear fragmented. It was suggested that the defect in nc mutants involves an early event of egg activation (Raff, 1977).

cl: cleavage defect mutant

The maternal effect mutation cl (cleavage defect) which affects the cleavage process during embryogenesis has been first described by Carroll and Van Deusen (1973). The eggs from cl/cl females are fragile and tend to break during spawning or to flatten in the animal pole when the egg's gelatinous capsule is removed. In addition, cytoplasm occasionally appears to leak from the crater-like areas which mark the points of sperm entry. Usually, cleavage is normal only in the animal hemisphere, leaving the entire vegetal region uncleaved. Even eggs left in their jelly capsules rarely complete gastrulation. Therefore, the surface of the egg appears to lack any rigidity, indicating the possibility of a defect in some aspect of the egg cortex (Malacinski and Spieth, 1979).

In eggs that arrest during the first few cleavages, the positions of the succeeding cleavages are evident on the egg surface as a white line at the time when these cleavages would normally occur (Carroll and Van Deusen, 1973). This indicates that the plane of cleavage, at least for the first

six cleavage divisions, are predetermined in the egg and do not rely on spatial orientation derived from preceeding cleavages or the presence of blastomere cell membranes. The ability of cells from the animal pole to survive and differentiate neural structures when grafted into a homologous position in a normal recipient indicates that these cells are viable and that the uncleaved cytoplasm is the most likely cause of the limited development of mutant embryos beyond gastrulation (Carroll and Van Deusen, 1973). These results suggest the existence of an animal-vegetal gradient of the wild-type cl gene product. Such a component probably exists in a high concentration at the animal pole and a low concentration at the vegetal pole. The maternal effect might reduce the low concentration at the vegetal pole sufficiently to render it developmentally inactive. A similar reduction in concentration at the animal pole might still maintain the cl gene product above a threshold concentration (Malacinski and Spieth, 1979).

v: vasodilatation mutant

The maternal effect mutation v (vasodilatation) show variable penetrance of the altered phenotype. Embryos which are homozygous for gene v show reduced growth and vasodilatation. Most of these v/v larvae die, but a small proportion develop to fertile adults (Humphrey, 1962). By raising v/v embryos in a medium which contains high levels of sodium, potassium and calcium, a greatly increased yield of

surviving v/v larvae is obtained. It has been postulated that a deficiency in steroids may be responsible for this apparent defect in either the uptake or utilization of ions (cited in Malacinski and Spieth, 1979).

A variable maternal effect is always seen in the offspring of the few surviving v/v females. Embryos from these homozygous females usually arrest between cleavage stages and the onset of gastrulation. The genotype of the fertilizing sperm can modify the expression of the maternal effect of v. Eggs from v/v females sometimes develop to advanced embryonic stages, but whether they survive these stages depends on their zygotic genotypes. Eggs fertilized by a wild-type (V) sperm often are viable, whereas eggs fertilized by a mutant (v) sperm invariably arrest (Humphrey, 1962).

The arrest of cleavage, or the failure to complete gastrulation seem far removed from the vasodilatation and pronephric enlargement seen at later stages in v/v embryos derived from V/v females. In this latter situation, a defect in the uptake or utilization of certain ions has been postulated. In the case of the early maternal effect, embryos spawned as eggs from a v/v female do not respond as uniformly, or completely, to an increase in ions in their water.

Retardation or cessation of growth may be the common factor linking the maternal effect of gene v with the usual syndrome appearing in the v/v genotype derived from

heterozygous parents. The earliest manifestation of the maternal effect is a cessation of development, be it in cleavage, gastrulation or neurulation. It was suggested further that the vasodilatation and pronephric swelling in all v/v larvae from heterozygous mothers may be a reflection of physiological problems in certain organs due to their delayed growth and differentiation (Humphrey, 1962). In ovary graft experiments, the V/V host produced some eggs which continued to express the mutant phenotype indicating that the maternal effect is not the result of a general metabolic deficiency in the mother. Likewise, the deficiency leading to the arrest of development associated with the maternal effect can be corrected by joining v/v embryos (from v/v mothers) parabiotically to normal embryos, suggesting a diffusible product of the gene V (Humphrey, 1962).

f: fluid imbalance

A. mexicanum embryos which are homozygous for gene f (fluid imbalance) display, by the tail-bud stage, swelling in the suprabranchial regions. Later in development, the swelling diminishes and these embryos survive to sexual maturity. A more complicated picture of the gene f phenotype emerges from an examination of the progeny of a cross in which the female is homozygous for the gene f and the male is heterozygous (f/f female x F/f male). All the offspring of this cross display a fluid imbalance as early as the cleavage stage of embryogenesis with an enlarged blastocoelic cavity

at the late blastula stage. As the progeny of this cross develop to the hatching stage, an interesting segregation of the phenotypic effects is observed. The progeny which are f/f continue to manifest the severe fluid imbalance. Subsequently, organogenesis is retarded and the embryos fail to hatch and die. However, f/f embryos joined in parabiosis with normal embryos show a decrease in the swelling and survive (Humphrey, 1960). The rescue is probably accomplished by the action of the circulatory system of the normal parabiont. On the other hand, embryos heterozygous for f (F/f) which originated from the cross f/f female x F/f male are indistinguishable from homozygotes at the gastrulation stage. During neurulation, however, much of the blastocoel fluid escapes. Later, these heterozygous can be divided into three groups: 1) those that appear normal and survive to adulthood; 2) those that become distended again and die; and 3) those that display intermediate effects: blisters and wrinkled appearance (Humphrey, 1960).

Ovary graft experiments have shown that all the progeny from the cross of an f/f female with an F/f male display the characteristic fluid imbalance even when the eggs matured in a normal host (Humphrey, 1960). Furthermore, the swelling could be diminished somewhat when the embryos were raised in a high osmotic strength mannitol solution. The f/f embryos also showed a diminished influx of sodium (Dunson et al, 1971).

o: ova deficient mutant

Eggs spawned by a female which is homozygous for the gene o (ova deficient) uniformly arrest during gastrulation, regardless of the genotype of the fertilizing sperm. During the late blastula stage, eggs from o/o females show reduction of both cell division and DNA synthesis (Malacinski, 1971). An analysis of the spectrum of newly synthesized proteins in mutant embryos revealed alterations in the pattern of protein synthesis (Malacinski, 1971). By the late gastrula stage, morphogenesis in mutant eggs comes to a complete halt.

Eggs which are spawned by o/o females can be completely rescued by microinjection of normal egg cytoplasm prior to first cleavage (Briggs, 1972). This provided direct proof that the gene o is responsible for a cytoplasmic deficiency. The cytoplasmic component which is effective in correction of the defect is concentrated in the nucleus of the oocyte and has been found in the oocytes' nucleus of a variety of amphibian species (Briggs, 1972). Therefore, genes resembling the gene o might be active in the embryogenesis of a wide variety of species.

Nuclear transplantation experiments have given insight into the mechanism of action of the corrective component (Brother, 1976). Normal early blastula nuclei transplanted into enucleated eggs from an o/o female failed to develop beyond the stage at which arrest usually occurs (gastrulation). Normal mid-blastula or later stage nuclei,

however, were capable of supporting complete development when transferred in enucleated recipient eggs from o/o females. These observations suggest that the corrective component might act by effecting a stable (heritable) change in mid-blastula nuclei.

Each of the axolotl maternal effect mutants act through effects on the egg cytoplasm. In one case, the gene o, a single protein is most probably missing from the cytoplasm of eggs spawned by homozygous females. For the other genes, direct evidence is lacking. Finally, most of these genes appear to be the primary cause of arrest in embryos from mutant females.

1D. MOUSE

In mice, maternal effects may be exerted before fertilization, through the cytoplasm of the egg; during gestation through the environment of the reproductive tract; or after birth, for example via lactation. The effects can be distinguished by a combination of egg or embryo transfer and cross-fostering studies. For example, the number of lumbar vertebrae varies among different inbred strains, and the F_1 progeny of reciprocal crosses tend to resemble the maternal strain (McLaren, 1981). Furthermore, mice developing from reciprocally transferred embryos resembled their foster mothers rather than their genetic mothers with respect to number of lumbar vertebrae, thus establishing that

the maternal effect was exerted through the uterus rather than through the egg cytoplasm (McLaren and Michie, 1958). Embryo transfer experiments have also established that maternal effects on birth weight are exerted during intrauterine life in mice (McLaren, 1979). These maternal effects on birth weight and vertebral type are controlled by genetic factors present in the uterine foster mother. Furthermore, the maternal genotype plays a part in the rate of embryonic survival to term (Barkley and Fitzgerald, 1990), and the physiological basis for the genetic differences in survival between lines of mice resides in the uterus, either as a result of lacking components essential to a high embryo survival, or due to asynchronous development between the embryo and the uterus (Spencer Jenkins and Anderson, 1990).

Other genetically derived maternal effects which have not been analysed by embryo transfer include some effects on the penetrance and expression of genes in progeny. Tabby mice resemble their mothers rather than their fathers with respect to whisker number (Kindred, 1961). For the incompletely penetrant gene Fused, more of the young are affected if the mother is phenotypically normal than if she herself is affected (Green, 1989). For the agouti locus gene, which produces a variable phenotypic effect, ranging from yellow to agouti, the phenotype of the young depends on the genotype of the mother, but not at all on the phenotype of the father (Wolff, 1978). Another matenal effect of the agouti locus

was discovered when it was observed that $\underline{A}^{Y}/\underline{A}^{Y}$ (lethal yellow) mouse embryos develop further in the uterus of an $\underline{A}/\underline{A}$ than in that of an $\underline{A}^{Y}/\underline{A}$ female (Robertson, 1942).

Evidence from the mouse suggests that the maternal gene products are entirely responsible for development up to the two-cell stage, and continue to be involved at least up to the 8-cell stage (McLaren, 1979). This emphasizes the importance, in the control of early development of cytoplasmic components in the egg. Therefore, genes should exist that exert maternal effects through modification of the egg cytoplasm.

The possible role of genetic variation in maternally inherited mitochondria is raised by the results of Verrusio et al (1968). Two inbred strains of mice, if maintained on a particular diet, show a difference in the frequency of cleft palate after treatment with 6-aminonicotinamide. The difference persists in reciprocal crosses, and in the backcross progeny of F_1 females of the two reciprocal types. It was hypothesized that the diet-dependent difference in response to the teratogen is due to a cytoplasmic factor. Since 6-aminonicotinamide forms an inactive nicotinamide adenine dinucleotide analogue that interferes with oxidative phosphorylation in mitochondria, the cytoplasmic factor may well be associated with a genetically determined difference in mitochondria between the two strains (Verrusio et al, 1968).

The mutation ovum mutant (om) represents one instance of a genetic effect exerted through the cytoplasm of the egg. Female mice of the Japanese strain DDK, mated with males of their own strain, have litters of reasonable size, with little embryonic mortality. But if DDK females are mated to other strains, litter size drops significantly and most of the embryos die at about the time of implantation (Wakasugi et al, 1967). The reciprocal crosses are fully fertile. Embryological study revealed that a defect in trophoblast formation was a common feature of the dead embryos (Wakasugi, 1973). Moreover, F_1 embryos from the semi-sterile cross (DDK females to non-DDK males) showed a very low mean cell number and a reduced mitotic index compared with DDK morula (Wakasugi and Morita, 1977).

Ovary grafts from DDK to F_1 females established that the effect was cytoplasmic rather than uterine; that is, it was not the reproductive tract of DDK mothers that was reacting in a hostile manner to the genetically foreign material, but the cytoplasm of the egg (Wakasugi, 1973). It has been suggested that the abnormalities and arrest of development in om/om embryos are due to some incompatibility between DDK cytoplasm and alien spermatozoa, resulting in a detrimental effect on development during cleavage.

To distinguish whether the incompatibility was between the DDK maternal pronucleus or egg cytoplasm, and foreign spermatozoa, zygotic pronuclei from the inviable cross of DDK

females and non-DDK males were transplanted into enucleated non-DDK egg (Mann, 1986). Normal development resulted, indicating that indeed, the incompatibility involved a cytoplasmic component.

From a genetic analysis, Wakasugi (1974) postulated that a single locus or closely linked pair of loci was involved, determining a cytoplasmic factor in the egg, as well as a factor in spermatozoa. The symbols om and OM were given to the DDK gene and its wild-type equivalent, and s, S to the corresponding pair of alleles acting in spermatozoa; om and s being either identical or closely linked. Homozygous om/om and OM/OM females are postulated to produce substances o and O respectively during oogenesis; these are stored in the egg cytoplasm, and interact specifically with the s and S gene or its products to synthesize some substances necessary for the post-implantation development of the embryo. Heterozygous females (om/OM) are presumed to produce o and O in equal amounts, with one or the other interacting irreversibly with the sperm product in any given fertilized egg. Of the four combinations: O/s, O/S, o/s and o/S, the last is usually (but not always) lethal.

To prove whether such an interaction between the o and S products determine the appearance of the embryonic lethality, reciprocal nuclear transplantation between DDK and non-DDK strains were performed (Renard and Babinet, 1986). This experiment revealed that the mortality is due to a

modification of the egg cytoplasm mediated by the male pronucleus, because enucleated cytoplasm of a DDK oocyte after fertilization with non-DDK sperm is very inferior in supporting development, regardless of the genome of the pronuclei subsequently placed into such a cytoplasm. The nature of this effect remains unknown. It has recently been observed that DDK eggs fertilized with non-DDK sperm result in embryos with reduced gap junctional communication between blastomeres (Buehr et al, 1987). However, this defect may be secondary to the initial incompatibility.

Another example of a maternal gene product transmitted through the egg cytoplasm comes from studies of XO mice. Since in normal XX females, both X chromosomes are active during oogenesis, X-coded enzyme activity is halved in eggs produced by XO females, and this relative enzyme deficiency persists to the 8-cell stage (McLaren, 1979). Embryos from XO females have a high failure rate during the preimplantation period, a maternal effect that must be mediated by the egg cytoplasm rather than the oviduct environment, because it is still present under conditions of in vitro culture (Burgoyne and Biggers, 1976).

Nuclear transplantation experiments in mice to create zygotes which contained only maternal or only paternal genetic contributions provided evidence that the maternal and paternal genomes are differently imprinted and that the result of complete uniparental disomy was a failure to

complete embryonic development (McGrath and Solter, 1984a; Surani et al, 1984). The phenomenon of non-complementation in genetic crosses marked by translocation chromosomes represents an attempt to refine which regions of the genome are subject to imprinting (Lyon and Glenister, 1977; Searle and Beechey, 1978,1985; Cattanach and Kirk, 1985).

In animals with reciprocal translocations between nonhomologous chromosomes, normal meiotic disjunction will give rise to normal gametes, as well as those that are genetically unbalanced because they contain duplications or deficiencies of specific distal regions of the chromosomes (frequency=17%) (Searle and Beechey, 1978). Similarly, at a much lower frequency (5%) after adjacent-2 disjunction during meiosis, which involves nondisjunction of centromeric regions of the chromosomes, gametes with duplications or deficiencies of the proximal regions of some chromosomes will be produced (Searle and Beechey, 1985). Furthermore, nondisjunction in heterozygotes for Robertsonian (whole arm) translocations leads to gametes that are disomic or nullisomic for one or the other of the chromosomes involved in the translocation (Cattanach and Kirk, 1985). Therefore, intercrossing of mice carriers of such reciprocal or Robertsonian translocations produces a small number of progeny derived from a gamete carrying a duplicated portion of a chromosome and a gamete in which the complementary chromosome region is deleted. The resulting zygote is

genetically normal in quantitative terms; however, certain parts of the genome are derived from one parent only. If that part of the genome is suitably marked (by a recessive allele) in one parent, it becomes quite easy to score for nondisjunction complementation because this results in a visible, recessive phenotype. If a sufficient number of progeny is obtained and an individual with the recessive phenotype is not observed, one can conclude that the nondisjunction cannot be complemented; that is, that specific regions of the chromosome must be represented in both a paternal and maternal copy.

Studies with Robertsonian translocations have demonstrated that mice that are monoparental for either maternal or paternal chromosomes 1,3,4,5,9,13,14 and 15 are viable and normal. Paternal disomy 6 is also normal (Cattanach, 1986). Moreover, results from matings of reciprocal translocation heterozygotes have added to this array the distal parts of chromosomes 10 and 18 (Searle and Beechey, 1985).

On the other hand, maternal duplication of specific regions of chromosomes 2,6,7,8 and 17 failed to complement the corresponding paternal deficiency and this resulted in nonviable embryos or offspring (reviewed in Searle and Beechey, 1985). However, the reciprocal combination of paternal duplication with corresponding maternal deficiency of the same chromosomal regions produced fully viable animals. An exception to this however, is chromosome 7,

where both maternal duplication/paternal deficiency and maternal deficiency/paternal duplication of the distal region results in lethality. In this case, two imprinted genes are postulated to be present in the same region; one is active only in the paternal genome and the other is active only in the maternal genome (Solter, 1988). The fact that the paternal duplication results in earlier lethality reinforces this statement (Beechey and Searle, 1987a). Therefore, the two imprinted genes would probably be separable by doing identical crosses with other reciprocal translocations involving chromosome 7.

Anomalous phenotypes have also been described in chromosomally balanced mice that are monoparental for certain regions. Cattanach and Kirk (1985) showed that mice disomic for maternally derived chromosome 11 were significantly smaller than their littermates, whereas paternally derived disomic-11 mice were substantially larger. Postnatal growth rates of the small maternal and large paternal disomies appeared similar to that of their normal sibs with the consequence that the size differences remained evident even in adulthood. Neither type of disomy exhibits viability problems and both sexes are fertile (Cattanach, 1986). Further work using mice with reciprocal translocations demonstrated that this effect was due to the proximal region of chromosome 11.

Studies involving a distal region of chromosome 2 also

revealed contrasting phenotypes depending on the parental origin of the chromosomal region (Cattanach and Kirk, 1985). Maternal duplication/paternal deficiency for distal 2 resulted in flat-sided, arch-backed, hypokinetic newborn which failed to suckle effectively and usually died within 24 hours. However, the reciprocal genetic type showed an opposite phenotype: the mice had short, square bodies and flat backs and were hyperkinetic. These often survived for several days but failed to thrive and developed tremor and balance defects.

The contrasting phenotypes (small vs large; hyperkinetic vs hypokinetic) depart from the normal in opposite directions and this suggests excess versus shortage of gene activity. It could be that the level of gene expression differs in the maternal and paternal genes or that the time of action of the genes in the region of interest depend on their parental origin (Cattanach and Kirk, 1985; Solter, 1988). The size phenomena associated with proximal 11 might involve a growth hormone gene, Ames dwarf, which is located in this chromosome region (Green, 1989). By homology with a region of a human chromosome, the proximal chromosome 11 may also contain alleles for other growth hormones, one of which acts on the placenta (Cattanach and Kirk, 1985). However, there is no evidence as yet for hormonal control of fetal growth mediated through the placenta (Solter, 1988). For distal 2, the behavioural problems of the anomalous mice may be associated

with a neurological defect. Therefore, the adenosine deaminase (ADA) deficiency of the wasted mutant whose locus lies in the relevant region of chromosome 2 has been suspected (Cattanach and Kirk, 1985). However, no significant differences have been found in ADA levels between normal and both types of complementation defective mice (Cattanach, 1986).

Studies were also carried out on chromosome 17, especially with respect to a proximal region of chromosome 17 that contains a deletion mutation, the hairpin tail (T^{hp}) which is an allele of the mouse T/t complex (Bennett, 1975). $T^{hp}/+$ heterozygotes die in late gestation if the mutant gene is transmitted from the mother, but are fully viable in both sexes if it comes from the father (Johnson, 1974). Moreover, in matings between $T^{hp}/+$ males and $T^{hp}/+$ females, heterozygous individuals receiving the paternal T^{hp} chromosome live, whereas those receiving the maternal T^{hp} chromosome die (Johnson, 1975). Maternal deficiency/paternal duplication for a region of chromosome 17 that included the t complex was also found, in a number of crosses to give a lower frequency of successful complementation than its reciprocal. The lethality can be suppressed by aggregation of $T^{hp}/+$ and normal embryos, but chimeric females are unable to transmit the T^{hp} gene to viable offspring, indicating preservation of the maternal effect passed through the oocyte (Bennett, 1978).

The observed effect is clearly due to the nucleus, rather than the cytoplasm of $\underline{T}^{hp}/+$ eggs, because transfer of pronuclei to eggs of another strain also resulted in embryonic lethality (McGrath and Solter, 1984b). Similarly, the $\underline{T}^{hp}$ chromosome can be recovered from the mother if both $\underline{T}^{hp}$ and wild-type forms of chromosome 17 are maternally inherited simultaneously through complementary nondisjunction in both parents, indicating again that the lethality was due to a nuclear effect (Winking, 1981).

It has been suggested that the affected region of chromosome 17 contains an "activation centre" that functions only when it is maternally derived and that switches on some structural locus required for normal development in late gestation (Johnson, 1975). Alternatively, it has been suggested that the maternally inherited locus may be preferentially active, whereas the paternal locus is inactivated (McLaren, 1979). Therefore, when the $\underline{T}^{hp}$ deletion is transmitted from the mother, the embryo would be essentially nullisomic for the chromosome region in question. Deficiency mapping has allowed the specific chromosome region responsible, denoted T maternal effect (Tme) to be more precisely identified (Winking and Silver, 1984).

The conclusion from these studies is that maternal and paternal copies of certain chromosome regions in the mouse may function differently during embryonic development. Thus, during germ cell formation, a modification of genetic

information temporarily occurs and permits differential expression of this information according to its parental origin.

1E. CATTLE

The importance of potential cytoplasmic genetic effects on beef cattle performance (birth weight, growth, carcass traits, milk yield, etc.) has been studied (Maurer and Gregory, 1990; Tess and Robison, 1990). Because cytoplasmic elements, including mitochondria, are inherited only from the female parent, important cytoplasmic genetic effects on performance could increase the value of some maternal families or breeds. However, both studies failed to demonstrate that ovum cytoplasm and uterine environment have important effects on performance among beef cattle. Rather, postnatal influences (lactation and other postnatal maternal components) were of primary importance in contributing to maternal effects on growth and carcass characteristics (Maurer and Gregory, 1990).

In sheep, selection for reproductive rate, which is the most important factor for profitability of an enterprise, was shown to be correlated with change in ovulation rate, rather than with embryo survival (Schoenian and Burfening, 1990). Ovulation rate in ewes is under genetic control.

2. EXTENT OF EMBRYONIC SELECTION IN ANEUPLOIDY

Chromosome anomalies and mechanisms of selective elimination of abnormal zygotes are basic phenomena inherent in mammalian reproduction. Both principles are of considerable clinical importance in humans due to the particularly great risk of the human species to disorders of meiotic segregation in the course of gametogenesis as well as of the fertilization process. As a result, high primary rates of different types of chromosome abnormalities occur that are subject to differential postzygotic selection (Boue et al, 1975).

The order of magnitude of the problem in humans is characterized by the fact that chromosome abnormalities are thus far the main cause of prenatal mortality, and trisomy is the most frequent disorder among these abnormalities. In first trimester spontaneous abortions, the observed incidence of chromosome abnormalities of all types is about 60%, but several more frequent autosomal trisomies account together for more than half of this rate (Boué et al, 1985). On the basis of a commonly accepted 15% rate of spontaneous abortions among all conceptus, the proportions of the lethal chromosome aberrations as a whole and of autosomal trisomies in particular, extrapolated to all conceptuses are 9% and 5.4% respectively (Gropp et al, 1983). On the other hand, the frequency of all chromosome aberrations at birth,

including numerical and structural disorders is 0.45%, while that of autosomal trisomy is only 0.18% (Hassold and Jacobs, 1984). Moreover, the trisomic conditions found at birth belong mainly to very few selected types in which survival to or beyond term is possible (trisomy 21, 13 and 18).

The comparison of the extrapolated rates of first trimester losses with the rates of chromosome anomalies in newborns indicates the strong effects of selective elimination of abnormal, and not the least trisomic zygotes. Such comparison also provides possibilities for an approximate estimation of the developmental span of zygotes with specific types of chromosome aberrations. In fact, there is wide variation of indices for chance of survival from the first trimester of prenatal life until newborn age, as 1:7.5 in G trisomy, 1:44 in trisomy 18, 1:200 in trisomy D and 1:infinity in trisomy of chromosome 16 (Gropp, 1981). Thus, it is evident that all autosomal trisomies as well as the XXY condition are associated with an increased likelihood of fetal wastage, but that the degree of in utero selection varies greatly among different trisomies.

What kind of factors would influence the capability of aborting an anomalous fetus? Fetal survival is thought to be determined by both fetal and maternal attributes, acting separately or together (Stein et al, 1975). The rate of loss of anomalous conceptuses can thus be moderated by characteristics of the fetus other than the identified

defect. Selective survival by sex and mosaicism are two such characteristics (Stein et al, 1975). Intrauterine mortality of chromosomally unbalanced zygotes can also be explained by the existence of vulnerable phases of development, in which the consequences of chromosome anomalies become critically expressed (implantation, early organogenesis and late fetal stage) (Gropp, 1981). Byrne et al (1985), in a comparison of anatomic malformations in aborted fetuses and termbirths with the same chromosome anomaly, found that the high rate of loss associated with chromosome anomalies was explained by the failure of such conceptuses to develop beyond the embryonic stage, and not by the presence of more severe malformations than are seen in term births with identical karyotypes.

One point that has emerged from the study of abortuses has been the observation that chromosomally normal women who have produced a chromosomally abnormal abortus or livebirth are at increased risk of producing another one, and this elevated risk is not entirely explained by the increase in maternal age (Boué et al, 1975; Hassold, 1980; Alberman, 1981). This led to the conclusion that there might be individuals or couples at greater risk of producing aneuploid offsprings. Many excellent reviews exist that discuss the maternal age-independent genetic and environmental factors susceptible to be implicated in the etiology of aneuploidy (e.g.: parental HLA antigens, alpha-1-antitrypsin genotype, radiation, chemical agents, ...) (Hassold and Jacobs, 1984; Warburton,

1985; Mikkelsen et al, 1989). These factors can act before or at conception to increase the incidence of meiotic nondisjunction in either parent or later in gestation as a selection process against aneuploid conceptuses. The next section will concentrate on the latter.

3. FACTORS INFLUENCING THE SURVIVAL OF ANEUPLOID CONCEPTUSES

3A. Maternal age and relaxed selection

Despite many years of inquiry, increasing maternal age remains the only indisputable factor associated with human trisomy. Nevertheless, the biological basis for the exponential increase in risk of trisomy which occurs in women after the age of 30 is far from being understood. One of the hypotheses put forward to explain the relationship differs from all the others in that it assumes the frequency of trisomy is the same at conception across all maternal ages. A decrease in the rate of loss of trisomic conceptions during geatation is assumed to lead to an increased frequency of trisomy among livebirths of older women (Aymé and Lippman-Hand, 1982; Stein et al, 1986).

This hypothesis, termed "relaxed selection", was drawn from two observations. First, several of the cytogenetic studies of the parental origin of Down syndrome observed no difference in mean maternal age between trisomies of paternal and maternal origin, contrary to expectation if the maternal age effect originates in meiosis. Second, Aymé and Lippman-Hand (1982) estimated age-specific rates of in utero selection against trisomic fetuses using published data on spontaneous abortion rate and on the incidence of trisomies in abortions and livebirths. They inferred a decrease in selection against trisomies among older women, which they

suggested, might be due to the weakening with age of a natural screening mechanism. Support for this hypothesis has come from Golbus (1981), who was unable to detect an age-related increase in aneuploidy among mouse oocytes even though an age effect has been clearly demonstrated among mid-gestation embryos (Fabricant and Schneider, 1978). This led Golbus (1981) to suggest that the age effect might be related to decreased selection against aneuploid conceptions instead of increased likelihood of nondisjunction.

The views of Aymé and Lippman-Hand (1982) and Golbus (1981) have not been widely accepted. Carothers (1983) and Warburton et al (1983) have both suggested that methodological flaws in the Aymé and Lippman-Hand study resulted in spurious conclusions regarding the level of in utero selection. Specifically, in their analysis, Aymé and Lippman-Hand (1982) assumed that nondisjunction occurs equally among different chromosomes, that the probability of spontaneous abortion is the same for all trisomies, and that the maternal age effect is similar for all trisomies. However, none of these assumptions is correct (Carothers, 1983). Furthermore, Hook (1983) argued that the relaxed selection model is not consistent with known data. For example, there is no reduction in mean maternal age for trisomy 21 spontaneous abortions in comparison with trisomy 21 livebirths as would be expected, nor is there any evidence of a maternal age effect in translocation Down syndrome. In

addition, Hook (1983) pointed out that several subsequent studies of parental origin in Down syndrome have reported a reduction in mean maternal age associated with cases of paternal origin. Finally, in most studies of metaphase II oocytes of mouse, an obvious effect of maternal age has been observed; therefore questioning the conclusions of Golbus (1981) (reviewed in Dyban and Baranov, 1987).

The fact that spontaneous abortions with viable trisomies show an increase with maternal age is in itself evidence against the simplest form of the relaxed selection hypothesis, which would predict that younger women abort trisomies more often, while older women would tend to keep them until birth. Warburton et al (1986), in an analysis of rate of survival of trisomy 21 for each maternal age class, concluded that, in fact, there is no decrease in rate of selection against trisomy 21 with maternal age. Their data suggested a decrease in survival with increasing maternal age and this was corroborated by the observation that the gestational age at abortion for trisomies 21, 18 and some other chromosome abnormalities, is actually less in older mothers than in younger mothers, or, in other words that for trisomy 21 and 18, the pregnancies which achieved a greater degree of development had a lower mean maternal age.

These data therefore, strongly argue against the relaxed selection hypothesis as presented by Aymé and Lippman-Hand (1982). However, it is possible that all the differences in

selection occur before recognized pregnancy, i.e. between conception and the time a spontaneous abortion can be recognized and karyotyped. Stein et al (1986) suggested that this might occur at the first mitotic division of the embryo, where an extra chromosome might be more efficiently eliminated in younger women.

3B. Placental Mosaicism

Discrepancies between the chromosomal complement in fetal and placental tissues (both cytotrophoblast and villous stroma) have been reported on 2% of pregnancies studied by chorionic villus sampling (CVS) (Mikkelsen, 1985; Simoni et al, 1986). The phenomenon has been coined "confined chorionic mosaicism" (CCM) and some hints on its role in fetal development have been recently collected by Kalousek et al (1987). When mosaicism is tissue limited or confined, its origin can be traced to specific cell lineage(s) and to a specific time at the pre-implantation and early post-implantation period. The existence of CCM with its expression restricted only to the cytotrophoblast, the extraembryonic mesoderm, or both of these lineages, with a complete absence of mosaicism in the embryo reflects the complexity of placental development. The placenta derives from three different lineages: polar trophectoderm, extraembryonic mesoderm and primitive embryonic streak. These will form tertiary villi which are characterized by a

villous core in which primitive capillaries begin to appear. This villous core is surrounded by cytotrophoblast cells and externally by a layer of syncytiotrophoblast (Kalousek, 1988). Furthermore, the primary source of dividing cells for CVS, the cytotrophoblast, has almost all disappeared at late pregnancy, so that at term, it becomes very difficult to confirm the presence of an abnormal cell line identified by CVS (Kalousek et al, 1987).

The effects of CCM on placental function have not been determined yet. In some reports, CCM has been associated with intrauterine death and intrauterine growth retardation (Kalousek and Dill, 1983), while in others, a perfectly normal pregnancy ensued (Mikkelsen, 1985). In type III CCM, the presence of the diploid cell line confined to the cytotrophoblast in nonmosaic aneuploid conceptions has been suggested to facilitate the intrauterine survival of the conceptus. Kalousek et al (1989) showed that all analyzed placentas from live newborns and terminated pregnancies with trisomies 13 and 18 were mosaic, whereas CCM type III has never been reported in spontaneously aborted conceptions. The authors concluded that in 5% of trisomy 13 and 18 conceptuses, the mosaic placenta may facilitate complete embryonic and fetal development through functional compensation provided by diploid cells in the cytotrophoblast, while the remaining 95% of nonmosaic conceptions are spontaneously aborted prior to fetal

viability. No instance of mosaicism in cytotrophoblast of viable gestations with trisomy 21 has been reported (Kalousek et al, 1989). Therefore, trisomy 21 cells produce a less deleterious effect on placental function and a different mechanism for the intrauterine survival of trisomy 21 conceptuses must exist.

The diploid cell line of the trophoblast in viable trisomies 13 and 18 is produced by postzygotic nondisjunction or anaphase lag. If it is assumed that all 5% of viable trisomy 13 and 18 conceptions have CCM, the incidence of nondisjunction/anaphase lag in their cytotrophoblast progenitors would be significantly higher compared with the frequency of CCM seen at CVS in chromosomally normal conceptions, or in cytotrophoblast of viable fetuses with trisomy 21 (Kalousek, 1989). This result suggests that the mechanism of intrauterine survival is specific for each trisomy. The combination of both a pre- and post-zygotic cell division defect in viable trisomy 13 and 18 conceptuses points to the possibility of a genetic predisposition to such events.

In mouse, the relationship between fetus and placenta has been chromosomally investigated by the analysis of trisomy 16-diploid mouse chimeras. Cox et al (1984) described two grossly normal and viable 18-day fetuses that had organs composed of 40% to 80% trisomic cells, but had placentas containing only 10% to 20% trisomic cells. On the other

hand, Gearhart et al (1986a) described three grossly edematous newborn mice that failed to thrive. These had only 15% trisomic cells in their tissues but their placentas were composed of at least 60% trisomic cells. Clearly, the placenta could play a role in the generation of the trisomic phenotype.

Lastly, Bogart and Miyabara (1990) used experimentally reconstructed blastocysts so that some of them had either a trisomic inner cell mass or a trisomic trophectoderm, to examine the effects of a trisomic placenta on the growth and development of a euploid fetus and vice versa. These authors concluded, from the appearance of the fetuses that complete discordance between the chromosomes of the fetus and the placenta is not compatible with survival of the fetus, at least for trisomy 16. However, a trisomic fetus with a chimeric placenta was observed in this same study. The fetus showed typical features of trisomy 16 and was alive at the time of examination (day 17). This situation is analogous to that reported for human trisomy 13 and 18 by Kalousek et al (1989).

3C. Origin of the extra chromosome and imprinting

As explained earlier, studies in the mouse have revealed that transmissible gametic aneuploidy can kill, even when the resultant zygote is fully balanced. Thus, many authors raised the possibility that parental genome per se might have

a harmful effect on development, independent of that generated by the resultant trisomy (Searle and Beechey, 1985; Cattanach, 1986; Hall, 1990). Normal complementation seems to prevail for the whole length of mouse chromosome 16 (the homolog of human chromosome 21). This was demonstrated by the recovery in the expected frequencies, of mice disomic either for the maternal or the paternal chromosome 16 (Berger and Epstein, 1989). Hassold et al (1984) studied the parental origin of human autosomal trisomies and found that most were derived from primary nondisjunction in the female. However, trisomy 21 was more likely to be paternally derived than any of the other autosomal trisomies for which data were available (20% vs 7%) (Hassold and Jacobs, 1984). The authors proposed that this finding might be connected with differences in selection against trisomies of paternal and maternal origin. However, since no evidence was found of an effect of parental origin on gestational age nor of such a difference between liveborn and spontaneously aborted conceptions with trisomy 21, it seems very unlikely that the difference in origin between trisomy 21 and other autosomal trisomies results from differential selection (Hassold et al, 1984).

4. USE OF MOUSE MODEL TO STUDY ANEUPLOIDY

Despite the high frequency of chromosomal anomalies among human conceptuses, very little is known about the direct and indirect consequences of chromosome imbalance In fact, the study of the pathogenesis of human aneuploidies is greatly limited by our inability to investigate developmental phenomena in humans. For this reason, the development of a mouse model for the study of aneuploidy is very appropriate.

This mouse model allows systematic study of the pathogenesis of aneuploid conditions during all stages of embryogenesis and organogenesis. All cells and tissues are accessible to study in vitro and in vivo. Finally, the effects of genetic and environmental factors on the expression of the phenotype of the aneuploid condition can be assessed (Epstein, 1986; Epstein et al, 1984).

The benefit of using a mouse model for human chromosome abnormality is not impaired by the fact that the phenotypes are not the same in human and in mouse. The underlying assumption that the mechanisms by which chromosome imbalance produce developmental disorders are similar in both species is accepted by many authors (Epstein, 1981; Gropp, 1982; Gearhart et al, 1986b). Moreover, it is becoming increasingly clear that homologous chromosomal segments have remained structurally intact between mouse and human; 46 conserved syntenies (presence of two or more genes on the

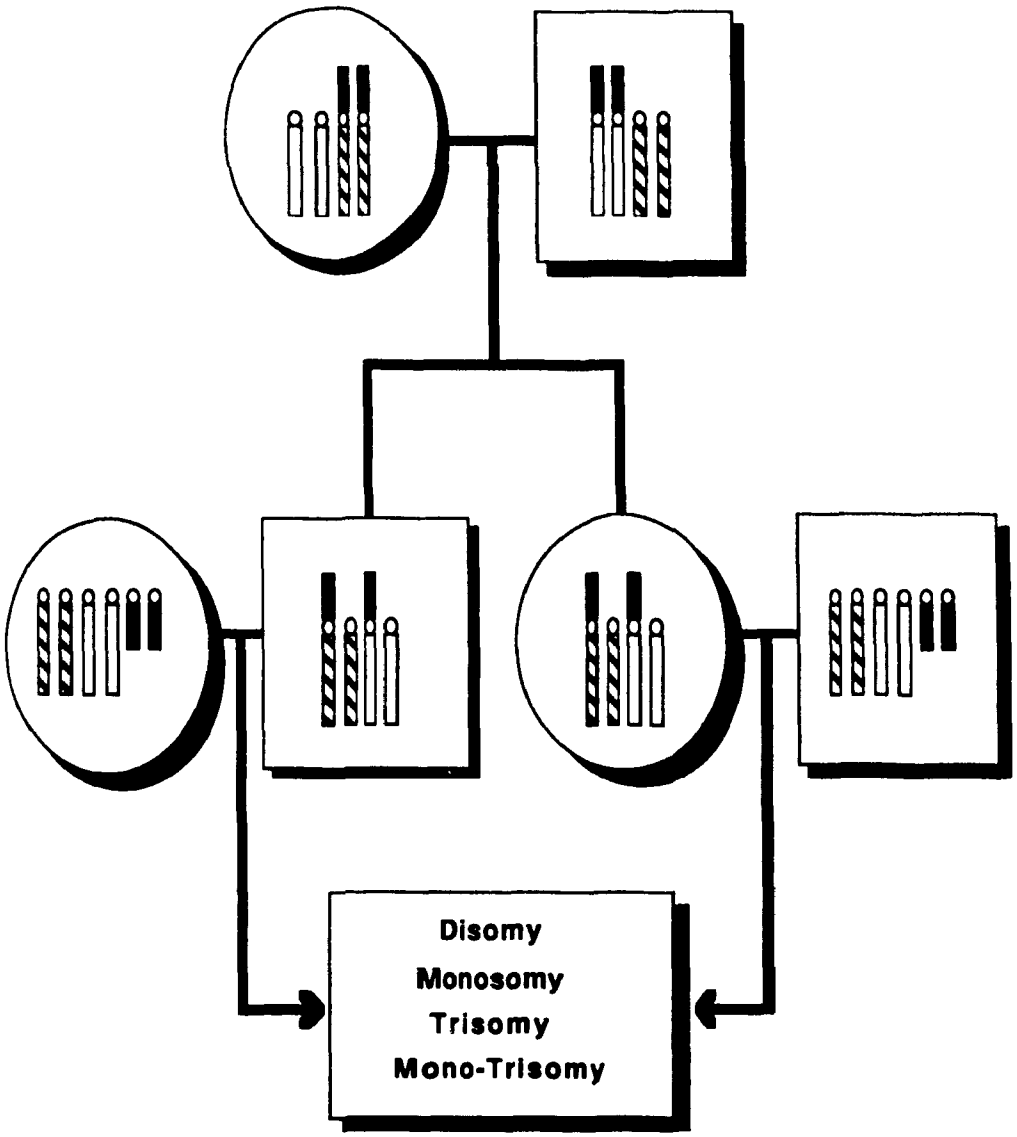
same chromosome) have been established in these two species (Nadeau and Taylor, 1984).

4A. Breeding scheme for the production of aneuploid mice

The breeding design for the experimental induction of specific autosomal trisomies and monosomies is based on the occurrence of and ability to control meiotic nondisjunction in a breeding system with Robertsonian heterozygosity (Gropp et al, 1975). The laboratory mouse (Mus musculus) normally has 19 pairs of acrocentric autosomes and two acrocentric sex chromosomes. Robertsonian (whole-arm) fusion metacentrics are, however, quite common in wild mouse populations from Southern Europe and these can be introduced into laboratory strains by selective breeding procedures (Gropp and Winking, 1981). Mice heterozygous for two metacentric Robertsonian chromosomes with one arm in common (monobrachial homology), are produced from matings of suitable homozygous lines (Fig. 1) (White et al, 1972; Gropp et al, 1975). These heterozygous mice are mated to mice with a normal chromosome complement. In doubly heterozygous animals, a quadrivalent is formed in prophase of meiosis I and nondisjunction produces hypo- and hyperhaploid gametes at high frequency (Fig. 2). Fertilization with gametes from the all acrocentric chromosomes parent result in trisomy and monosomy for the common chromosome arm in the Robertsonian heterozygous parent, as well as in balanced progeny. With

FIGURE 1

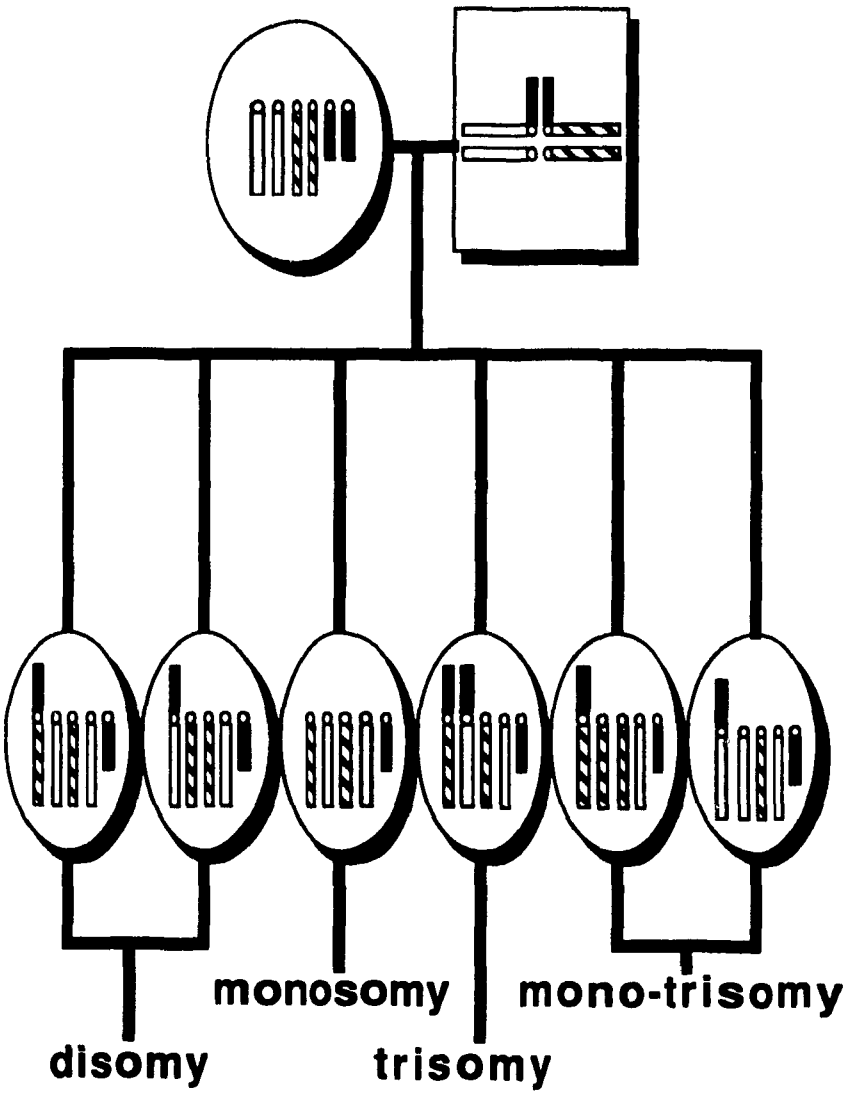
Breeding design for the production of mice doubly heterozygous for Robertsonian translocations with monobrachial homology.



From Jacob (1990).

FIGURE 2

Diagram of chromosome segregation in the progeny from a cross between a male mouse heterozygous for two Robertsonian translocations with monobrachial homology and a wild-type female.



From Jacob (1990)

this mating scheme, it is possible to induce monosomy and trisomy for each of the 19 autosomes of the mouse. The yields range from 14 to 22% for both trisomics and monosomics when determined within two days after fertilization and from 15 to 42% for trisomics alone (the monosomics having died) when determined in midgestation (Epstein, 1985).

4B. General features of mouse trisomies

Although they all survive until at least midgestation, none of the mouse trisomies, except for trisomy 19, survive beyond birth. The times of death appear to cluster in three periods: at about 9-11 days, 13-15 days, and .6 days to term (Gropp, 1981). The length of survival has been shown to depend greatly on the genetic background of the strains used (Gropp and Grohé, 1981; Vekemans and Trasler, 1987; Epstein and Vekemans, 1990). The cause of death seems to be related to poor embryonic growth or to insufficient vascularization of the fetal part of the placenta (Gropp, 1978).

Two other general features observed in all trisomies, though in varying degrees, are growth retardation and hypoplasia resulting in a generalized deficiency of cells. A possible mechanism by which a cell deficiency can arise is through an altered cell cycle. However, studies on murine trisomies 12 and 19 showed no major differences in the cell cycle and in the proliferative capacities of normal and trisomic cells (Gropp et al, 1983). Otherwise, the metabolic

consequences of the triplication of a great number of genes on the chromosome involved may influence the expression of hypoplasia and retardation in trisomy (Gropp, 1978).

Trisomies that allow survival beyond days 14 or 15 of fetal development show usually transient subcutaneous edema of variable intensity (for trisomy 16, see Miyabara et al, 1982). Gropp (1981) attributed this edema to hypoplasia of the fetal placenta, notably that of fetal vasculature. This circulatory deficiency on the fetal side could prevent oxygen and metabolite supply resulting in the edema and subsequent developmental failure.

Finally, a fifth feature of the effect of trisomy on mouse development is the association of specific malformations (of variable severity) with particular trisomies. These malformations are thought to be caused by the effects of local cell deficiency and locally impaired processes of induction in organogenesis rather than as a direct consequence of the triplication of specific loci (Gropp, 1978). In fact, Gropp (1981) proposed three main critical phases that determine the eventual developmental breakdown of aneuploid fetuses: implantation, organogenesis and the fetal period. Monosomics are generally lost during the peri-implantation period (Epstein and Travis, 1979; Baranov, 1983; Magnuson et al, 1985). The origin of gross malformations commonly observed in trisomies, such as neural tube defects, craniofacial anomalies and cardiovascular disorders, falls

into the second critical phase. Each of the organ systems involved relies upon cell proliferation and migration to achieve its final structure. If threshold number of cells are not established for these various organ precursors, significant morphological defects may result, and eventually death.

Furthermore, the phenotypic manifestations of particular trisomies have been shown in a number of study to depend on factors related to the strain background. Miyabara et al (1984) showed that the type and incidence of the cardiovascular anomalies observed in trisomy 16 were dependent on the maternal strain in the cross (the male being carrier of the two Robertsonian translocations). Similar findings on the influence of the maternal strain on the phenotypic expression of various morphological defects have been reported for trisomy 12, 15 and 19 (Gropp and Grohe, 1981; Beechey and Searle, 1987b; Gropp, 1981· White et al, 1972).

4C. The phenotype of trisomy 16 fetuses

The developmental span of trisomy 16 fetuses extends to and even shortly beyond birth. These animals are somewhat smaller than their sibs, by approximately 25% in weight (Epstein et al, 1985). Between days 14 to 17 of gestation, trisomy 16 fetuses display a generalized edema that is almost completely resolved by day 19 (Miyabara et al, 1982). The

eyelids which are closed in normal littermates, remain open in trisomics. In addition, trisomy 16 fetuses show an enlargement of the urinary tract and other renal abnormalities (Miyabara et al, 1982).

Anomalies of the cardiovascular system are present in over 90% of trisomy 16 fetuses (Miyabara et al, 1982; Bacchus et al, 1987). These mainly affect the great vessels (overriding aorta, double outlet right ventricle, complete transposition of the great arteries) and about half of the trisomic fetuses with these lesions also have a common atrioventricular canal (Miyabara et al, 1982). Miyabara et al (1984) noticed that the day when edema begins to appear coincides with the manifestations of cardiovascular anomalies, therefore suggesting that the edema is caused by some circulatory disturbance due to the abnormal cardiovascular morphogenesis. The pathogenesis of common atrioventricular canal has been studied by Miyabara (1990). Since the fusion of the superior and inferior endocardial cushions (the essential event in the morphogenesis of the canals), mediated by migrating mesenchymal cells, fail to occur and the mesenchymal cells do not show impaired proliferative capacity, it was proposed that the migration of mesenchymal cells may be interfered with by increased cell adhesiveness in trisomy 16. This finding should provide good clues on the mechanism by which the same malformation arise in human trisomy 21.

Since trisomy 16 mice do not survive the late fetal period, chimeras have been formed between trisomy 16 and normal mouse embryos to rescue the trisomy 16 cells for postnatal studies (Cox et al, 1984; Gearhart et al, 1986). In both studies, it was seen that trisomy 16 cells were able to populate a variety of tissues, including the brain, in significant quantities. However, a marked deficiency of trisomic cells were found in the blood, spleen, thymus and bone marrow of chimeras, pointing to abnormalities of hematopoietic precursor cells in trisomy 16 conceptuses and justifying their hypoplastic thymus.

Murine trisomy 16 is of special interest because it shares some features with human trisomy 21 (Down syndrome). Comparative gene mapping showed that five genes known to be on human chromosome 21: superoxide dismutase (SOD-1), phosphoribosylglycinamide synthetase (PRGS), a proto-oncogene sequence (ETS-2), the interferon alpha/beta receptor (IFRC) and high-mobility-group chromosomal protein (HMG-14), are syntenic in the mouse and are located on chromosome 16 (Cox et al, 1980; Cox et al, 1981; Watson et al, 1986; Pash et al, 1990). Four of them (SOD-1, PRGS, ETS-2 and HMG-14) have been localized to band q22 of human chromosome 21, the consensus region for the Down syndrome phenotype. Moreover, SOD-1, PRGS and ETS-2 have been mapped to the distal segment (C3->ter) of mouse chromosome 16 (Cox and Epstein, 1985; Reeves et al, 1987). Given these relationships, it seems

reasonable to infer that other homologous loci are also present on the two chromosomes in question. Therefore, the genetic model for trisomy 21, which is based on the production of mice trisomic for chromosome 16, reproduces the imbalance of considerably more than the five loci already mapped.

From descriptive embryology of murine trisomy 16, it is interesting to note that neuronal deficits, thymic hypoplasia and common atrioventricular canal (or endocardial cushion defect) are observed in both conditions. The latter is of particular significance since it is exclusively experienced in human trisomy 21 among chromosome abnormalities (Miyabara, 1990). Similarly, the occurrence of "open eyelid" with its subsequent degeneration of the ocular lens in murine trisomy 16 may be analogous to the lenticular (cataract) anomalies in Down syndrome (Gropp et al, 1983).

In spite of these interesting similarities, there are reasons to believe that the trisomic mouse and human phenotypes may differ significantly from one another. The first one is that certain loci mapped to mouse chromosome 16 are present on human chromosomes other than 21. Furthermore, the genetic length of mouse chromosome 16 is about 35% greater than that of human chromosome 21 (Epstein, 1986). Thus, trisomy for the complete mouse chromosome 16, results in a more extensive genetic imbalance than occurs in human trisomy 21. A more appropriate model could be produced by

using mice carriers of reciprocal translocations; animals can be obtained with a duplication of just the distal part of chromosome 16.

SECTION II: EXPERIMENTATION

1. INTRODUCTION

The incidence of chromosome abnormalities of all types in first trimester abortions is about 60% (Boue et al, 1985). On the other hand, when evaluated at birth, the frequency of chromosome aberrations drops to 0.45% (Hassold and Jacobs, 1984). The difference in the rates of chromosome aberrations in first trimester losses and in newborns is a sign of the strong selection pressure exerted against aneuploid conceptuses.

The survival of trisomic fetuses, and of anomalous conceptions in general, depends upon genetic factors acting in the fetus and in the mother, as well as upon an interaction between these two (Stein et al, 1975). The rate of loss of trisomic fetuses can thus be moderated or enhanced by other factors than those brought about by the trisomic state. An appreciation of this statement comes from the observation that the aborted fetuses with chromosome anomalies do not show more severe malformations than term births with the same karyotypes (Byrne et al, 1985). Therefore, other factors playing a role in fetal demise may reside in the maternal environment or involve a defective interaction between mother and conceptus.

By maintaining the incidence of aneuploidy at conception constant, it is possible to investigate the factors influencing the survival of trisomic fetuses. A mouse model

producing trisomy 16 and 19 has been used to this end, and from it, Vekemans and Trasler (1987) and Epstein and Vekemans (1990) established that the survival of trisomy 16 and 19 fetuses is under the control of maternal genes.

This study reports the use of recombinant inbred (RI) strains of mice to map the maternal gene(s) involved in the strain difference for the frequency of trisomy 16. This approach seemed appropriate both because the data accumulated so far suggest that a small number of genes (perhaps one) are involved and because this genetic approach is cumulative. The incidence of trisomy 16 on day 15 of gestation was determined in a RI set derived from progenitor strains which differed significantly with respect to this trait. The strain distribution pattern of the trait could thus provide some information about the number of genes involved in the strain difference for the frequency of trisomy 16 observed on day 15 of gestation.

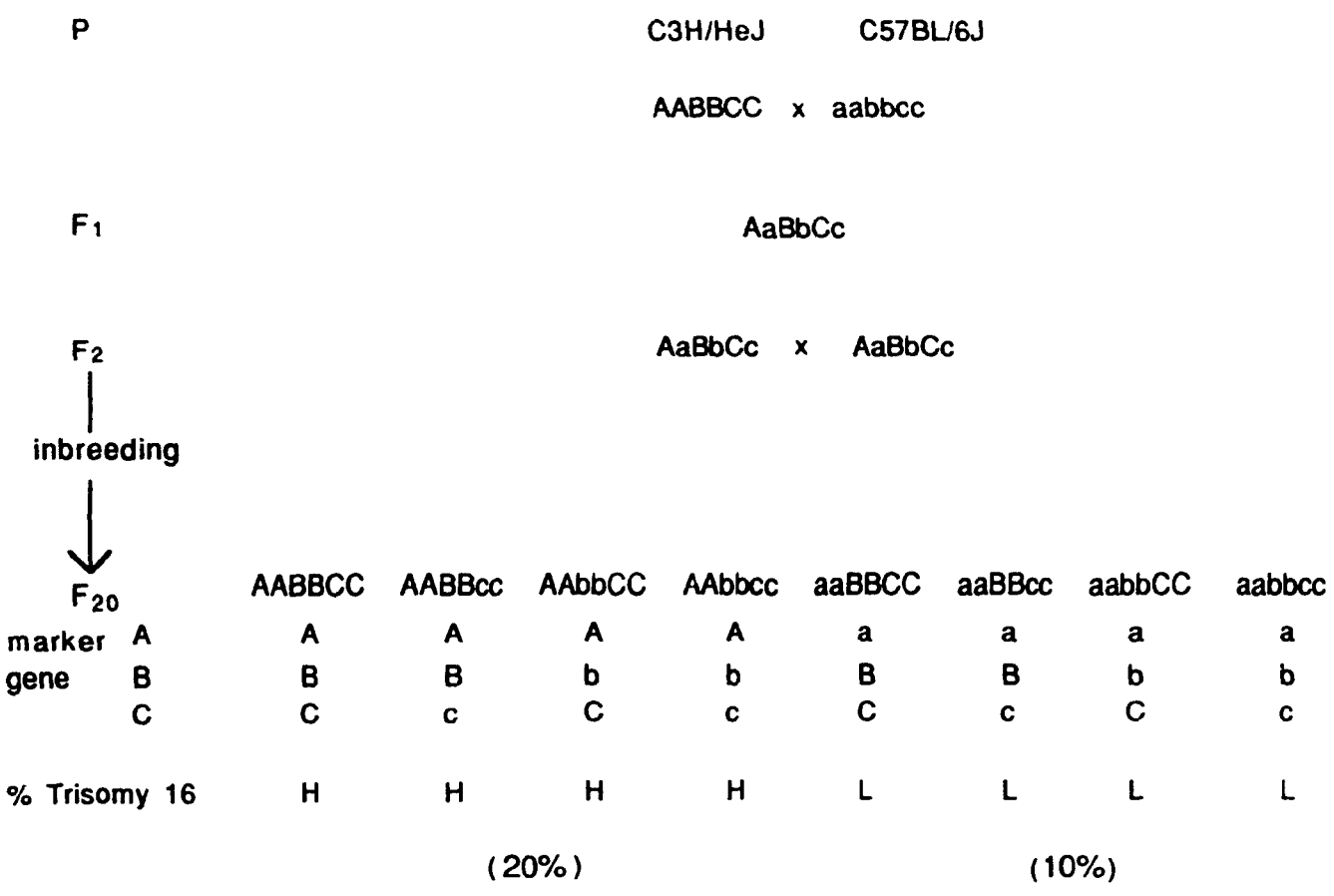
2. MATERIALS AND METHODS

2A. Construction of a RI set

RI strains are constructed by crossing two inbred strains of mice, and by maintaining them independently through sister-brother matings since the F_2 generation (Figure 3) (Bailey, 1971). The new set of inbred strains that are established are a mixture of the two progenitor strain genomes. The obvious difference between the genotypes of the strains in an RI set and individuals in an F_2 generation is that the assorted alleles in the RI strains are all homozygous.

The RI strains thus generated, are then typed with respect to the numerous genetic differences that distinguish the progenitor strains. Each locus has a particular pattern of inheritance called a strain distribution pattern (SDP) as exemplified in figure 3. Because linked loci will tend to become genetically fixed in the same combinations as in one of the parental strains, they will have similar SDPs. Closely matching SDPs between two traits will thus indicate the possibility of linkage. For instance, in figure 3, the frequency of trisomy 16 observed on day 15 of gestation segregates into two groups: high (H) and low (L) in the RI set examined. Thus, the gene involved in this strain difference would be linked to marker A. If more than one locus seems to determine the trait of interest (suggested by

FIGURE 3
Construction of a RI set and example of a strain distribution pattern.



Modified from Vekemans and Biddle (1984).

the observation of at least one RI phenotype differing from that of either progenitor strain), the analysis of the RI strains data still permits the estimation of the size and direction of gene effects (Bailey, 1981).

2B. Breeding scheme

The B x H (C57BL/6J x C3H/HeJ) RI strains established by Dr. B.A. Taylor at the Jackson Laboratory were chosen because there is a significant difference between C57BL/6J and C3H/HeJ in the incidence of trisomy 16 on day 15 of gestation (11.2% and 21.1% respectively, $p<0.05$, G-test of independence) (Epstein and Vekemans, 1990).

Female mice homozygous for the Robertsonian translocation Rb(6.16)24Lub were mated with males homozygous for the Rb(16.17)8Lub translocation (Jackson Laboratory). Hence, the F_1 obtained were heterozygous for two Robertsonian translocations both involving chromosome 16 (Figure 1). To induce trisomy 16, F_1 males (Rb(6.16)/Rb(16.17)) were crossed to females of the 12 BxH RI lines (all acrocentric chromosomes) (Figure 2). All females were virgin at the time of first encounter with the male. Furthermore, maternal age was controlled for by using exclusively females more than 2 months, but less than 6 months of age at the time of conception. All mice were given water ad libitum and were fed Lab Chow (Purina).

Males and females were brought together during the dark period of a 18½ hours light-5½ hours dark cycle. Females

were checked for the presence of a vaginal plug at the onset of the light period. If a plug was present, this was recorded as day 0 of pregnancy and the female was weighed. Fourteen days later, the female was weighed again and palpated. If pregnant, it was put aside for dissection on the following day. Pregnant females were killed on day 15 of gestation by cervical dislocation. The uterus was dissected out and the positions of living and dead fetuses were recorded. Only healthy females bearing three or more living fetuses were included in the data. The number of corpora lutea was counted from the dissected ovaries.

2C. Cytogenetics

The fetuses were cleared of their membranes. The liver was cut out and incubated for 2½ hours in 2-3 mls of Minimum Essential Medium (Flow) containing 15% fetal calf serum and colcemid (Gibco) (final concentration 0.1 ug/ml) at 37°C in a 5% carbon dioxide atmosphere. Remains of the fetus and its placenta were immersed in Bouin for one week, and then stored in 70% ethanol.

Standard cytogenetic technique was applied in order to karyotype the fetus from liver tissues. That is, after the incubation in colcemid, the liver pieces were transferred to 0.5% KCL at 37°C for 45 minutes and then into freshly prepared cold fixative (3 methanol:1 acetic acid) for at least an hour at 4°C. Next the fixative was removed and the

tissue was covered with 60% acetic acid. After four minutes, the liver pieces were crushed using a pasteur pipette and dropped on slides placed on an inclined slide warmer heated to 40°C. Two slides of each fetus were prepared in every case. The following day, the slides were stained in a 4% Giemsa (Gibco) solution (in phosphate buffer; pH=6.4) for 10 minutes.

Slides were screened on a Leitz compound microscope. At least three metaphase spreads were counted for each fetus and the position on the slide was recorded. Trisomic fetuses were readily identifiable by the presence of two Robertsonian translocations (38 acrocentric and two submetacentric chromosomes or 41 chromosome arms) (Figure 4). Diploid fetuses displayed 38 acrocentric chromosomes and one Robertsonian translocation (either Rb(6.16) or Rb(16.17)); with 40 chromosome arms in total (Figure 4). Only complete litters where every fetus could be karyotyped were used in the data analysis.

Trisomy 16 fetuses present with a generalized edema on day 15 of gestation (Figure 4). This morphologic criterion is a reliable marker for the identification of trisomic fetuses (Miyabara et al, 1982; Gearhart et al, 1986b; Epstein and Vekemans, 1990). In this study, the presence of an edema in a trisomic fetus had a sensitivity evaluated to 98% and a specificity of 97% (Haynes, 1981). Therefore, trisomic fetuses were identified on the basis of this characteristic,

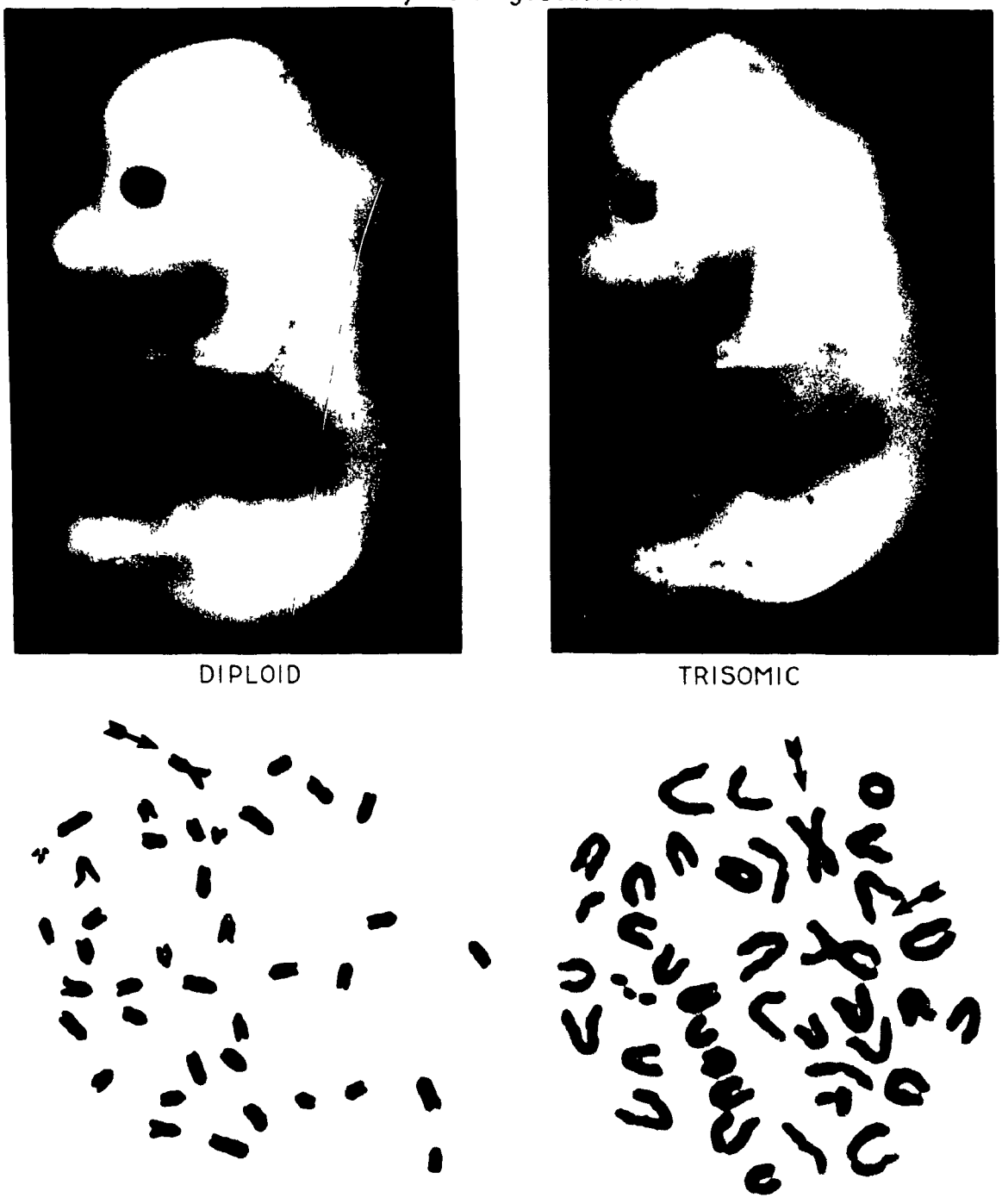
but in every case, the trisomic state was confirmed by cytogenetic analysis.

2D. Statistical analysis

Heterogeneity G-tests (Sokal and Rohlf, 1981; p. 722) were computed to test for significant differences in the frequency of trisomy 16, as well as in pre- and post-implantation losses between mouse strains. Because significant heterogeneities between female strains were detected, a further test was carried out to determine whether there are homogeneous groups of mouse strains with respect to the three aforementionned parameters (Sokal and Rohlf, 1981; p.728). Since all possible comparisons were tested for, the constant critical G value for 13 degrees of freedom and a probability level of 0.05 is 22.362.

Regressions and correlations were performed using the Statview 512+™ program on a MacIntosh SE personal computer. The arcsine transformation was used in the correlations because it is considered particularly appropriate for percentages (Sokal and Rohlf, 1981; p.386).

FIGURE 4
Phenotypes and karyotypes from normal and trisomy 16 mouse fetuses observed on day 15 of gestation.



3. RESULTS

3A. Frequency of trisomy 16

The prevalence of living trisomy 16 fetuses on day 15 of gestation in C57BL/6J and C3H/HeJ inbred females mated to Rb(6.16)/Rb(16.17) males has been determined reviously (Epstein and Vekemans, 1990). A significant difference in the frequency of trisomy 16 between the two female strains was observed (G_H =5.32, d.f.=1, p<0.023) (Table 1). Therefore, the prevalence of trisomy 16 in the females of the RI strains derived from these two inbred strains was investigated, using the same male as the aforementionned study.

A total of 808 fetuses (682 diploid, 126 trisomic) were examined from the twelve crosses (Table 1). Using an heterogeneity G-test (Sokal and Rohlf, 1981), a statistically significant difference was found in the frequency of trisomy 16 on day 15 of gestation between the BxH RI female strains (G_H=25.62, d.f.=11, p<0.01).

In order to determine whether the prevalence of trisomy 16 clusters into high (around 20%) and low (around 10%) groups, as in Epstein and Vekemans (1990) (see also Vekemans and Trasler (1987) for trisomy 19), a G-test for the determination of homogeneous groups was performed on the RI and progenitor strains data (Sokal and Rohlf, 1981). The result of this test indeed shows segregation of the 14 female

TABLE 1

Prevalence of trisomy 16 on day 15 of gestation in females of the BxH recombinant inbred strains and their progenitors mated to Rb(6 16)/Rb(16.17) males.

G-test	STRAIN	FETUSES EXAMINED		FREQUENCY OF TRISOMY (%)
		NORMAL	TRISOMIC	
*	B X H 2	41	2	4 7
*	B x H 19	86	10	10 4
*	#C57BL/6J	127	16	11.2
* *	B x H 9	97	14	12 6
* *	B x H 10	86	13	13 1
* *	B x H 14	44	7	13.7
*	B x H 7	74	12	14.0
* *	B x H 3	53	9	14.5
* *	B x H 11	29	5	14.7
* *	B x H 8	63	14	18 2
* *	#C3H/HeJ	116	31	21.1
* *	B x H 4	51	14	21 5
* *	B x H 6	37	12	24 5
*	B x H 12	21	14	40.0

Data from Epstein and Vekemans (1990)

strains into two classes (G=30.97, d.f.=13, $p<0.005$) with respect to the frequency of trisomy 16 (Table 1; aligned asteriks show the delimitation of the groups). The overlap between the high and low groups can be explained by the small sample size of fetuses obtained from some strains due to their poor health or scarcity. In fact, a sample size of 150 would have been necessary for each strain to establish a significant difference ($p<0.05$) between 10% and 20% (Vekemans and Trasler, 1987).

3B. Influence of other factors

A variety of factors were examined in order to assess their influence on the strain difference for the frequency of trisomy 16 on day 15 of gestation. The factors investigated were: maternal age at conception, number of ova shed (represented as corpora lutea) per pregnant female and number of implants per pregnant female. A regression procedure was performed in order to determine whether these independent variables significantly influence the proportion of trisomic fetuses in a litter. The frequency of trisomy 16 was transformed to arcsine and the value 1/4n (n=number of living fetuses in a litter) was used in lieu of zero values (Bartlett, 1947).

The procedure was run for each of the 12 crosses involving the RI strains (Table 2). In most cases, the independent variables had no significant influences on the frequency of

TABLE 2

Regression analysis of three independent variables on the prevalence of trisomy 16 in the B x H recombinant inbred female mice.

FEMALE STRAIN	VARIABLES					
	MATERNAL AGE		CORPORA LUTEA		IMPLANTS	
	F-value	p-value	F-value	p-value	F-value	p-value
B X H 2	0.073	0.793	3 062	0.118	1.427	0.267
B X H 3	1 168	0.303	0.111	0.745	0.121	0.734
B X H 4	5 137	0.045*	0.210	0.655	0.550	0.474
B X H 6	3.868	0.085	0.299	0.599	0.032	0.862
B X H 7	0.255	0.622	3.137	0.098	0.701	0.417
B X H 8	0.0001	0.993	0 216	0.649	0.915	0.354
B X H 9	1.231	0.279	1.648	0.213	0.006	0.937
B X H 10	0.234	0.634	0.019	0.892	3.677	0.070
B X H 11	0.703	0.434	0.174	0.691	0.623	0.460
B X H 12	1.523	0.272	0.001	0.972	0.016	0.904
B X H 14	0.200	0.665	0.332	0.578	0 155	0.703
B X H 19	0.532	0 476	8.139	0.012 *	5.040	0.039 *

* significant at the 0 05 probability level.

trisomy 16 fetuses. However, the proportion of trisomic fetuses was shown to depend significantly on the age of the female in the B x H 4 strain. Furthermore, the number of ova shed and the number of implanted embryos significantly influenced the prevalence of trisomy 16 in the BxH 19 females. However, since a significant influence of one variable over many strains was not observed, it seems very unlikely that the three factors examined have a direct influence on the prevalence of trisomy 16 on day 15 of gestation.

In addition, a correlation analysis between the mean number of corpora lutea per litter for each of the twelve RI strains and the frequency of trisomy 16 (arcsine) turned out not to be significant ($r=-0.559$, $d.f.=10$, $p>0.05$) (Table 3). Similar results were obtained when the mean number of implants per litter and the mean number of moles per litter were compared to the frequency of trisomy 16 ($r=-0.217$, $p=0.50$ and $r=-0.293$, $p>0.20$ respectively). Therefore, these findings further suggest that the number of eggs shed and the mean litter size have no influence on the prevalence of trisomy 16.

Since the correlation between mean number of corpora lutea and prevalence of trisomy 16 was of borderline significance, a further statistical test was carried out. The number of corpora lutea for each strain was ranked from high to low and the litters were divided into two subgroups. For each

TABLE 3

Corpora lutea/litter, implants/litter and moles/litter in the B x H female mice mated to Rb(6.16)/Rb(16.17) males

STRAIN	NUMBER OF LITTERS	CORPORA LUTEA/ LITTER	IMPLANTS/ LITTER	MOLES/ LITTER
B X H 2	10	10.90	7.70	3.10
B X H 3	13	10.46	6.31	1.54
B X H 4	13	8.92	7.23	2 00
B X H 6	10	8.00	7.20	2.20
B X H 7	16	8.25	6.63	1 19
B X H 8	17	8.18	6.47	1.88
B X H 9	24	10.21	8.71	3.88
B X H 10	22	7.91	6.64	2.05
B X H 11	8	7.88	6.50	2 13
B X H 12	7	8.14	6.86	1.86
B X H 14	11	8.46	5.64	0.91
B X H 19	18	11.78	8.00	2 33

strain, the frequency of trisomy was calculated for the high and low groups and compared by a G-test (Table 4). Only one strain (BxH 19) displayed a statistically significant difference in the frequency of trisomy 16 between high and low corpora lutea subgroups; therefore, indicating that the prevalence of trisomy in that strain is dependent upon a heterogeneous group of corpora lutea. This is not surprising since the same strain revealed a statistically significant regression of mean number of corpora lutea per litter on the proportion of trisomic fetuses. It should be noted that strain BxH 19 has the highest mean number of corpora lutea per litter of the 12 BxH RI strains (Table 3).

3C. Pre-implantation loss

The pre-implantation loss is calculated by dividing the number of nonimplanted embryos (corpora lutea minus implants) by the total number of ova shed. Table 5 lists the frequency of pre-implantation loss for the 12 RI strains and their progenitors. These frequencies were compared using a G-test for homogeneity (G_H=85.74, d.f.=13, $p<0.001$) and shown to cluster into two groups; the overlap between the two groups being attributed to small sample size (Table 5; aligned asterisks show the delimitation of the groups). Since the prevalence of trisomy 16 in the 14 mouse strains has been previously shown to separate in two groups by the same test (Table 1), a correlation analysis between the frequencies

TABLE 4

Comparison between the frequency of trisomy 16 in litters ranked into high and low groups for the number of corpora lutea.

STRAIN	MEAN # CORPORA LUTEA		FREQUENCY TRISOMY 16		PROB. LEVEL
	HIGH GROUP	LOW GROUP	HIGH GROUP	LOW GROUP	
B x H 2	14.2 (5)	7.6 (5)	0 0	11.1	> 0.05
B x H 3	11.6 (5)	9.8 (8)	13.6	13.0	>0.50
B x H 4	10.8 (8)	7.8 (5)	37.5	22.2	>0.50
B x H 6	9.4 (5)	6.6 (5)	28.0	20.8	>0 50
B x H 7	10.3 (4)	7.6 (12)	7.4	17.0	>0.10
B x H 8	9.7 (7)	7.1 (10)	15.6	20.0	>0.50
B x H 9	11.8 (10)	9.1 (14)	17.7	8.3	> 0.05
B x H 10	8.6 (8)	6.8 (14)	14.1	10.7	>0.50
B x H 11	9.3 (4)	6.5 (4)	12.5	16.6	>0.50
B x H 12	9.5 (2)	7.6 (5)	33.3	43.5	>0.50
B x H 14	10.0 (4)	7.6 (7)	20.0	9.7	>0.10
B x H 19	13.9 (10)	9.1 (8)	3.5	21.1	< 0.01 *

Number in parentheses refers to the number of litters examined.

TABLE 5

Pre-implantation loss and prevalence of trisomy 16 in females of the BxH recombinant inbred strains mated to Rb(6.16)/Rb(16.17) males.

G-test	STRAIN	CORPORA LUTEA	IMPLANTS	PRE-IMPL LOSS (%)	FREQUENCY OF TRISOMY (%)
•	**B x H 6**	80	72	10.0	24.5
•	**#C57BL/6J**	227	201	11.5	11.2
•	**B x H 9**	245	209	14.7	12.6
•	**B x H 12**	57	48	15.8	40.0
•	**B x H 10**	174	146	16.1	13.1
•	**#C3H/HeJ**	232	194	16.4	21.1
•	**B x H 11**	63	52	17.5	14.7
•	**B x H 4**	116	94	19.0	21.5
• •	**B x H 7**	132	106	19.7	14.0
• •	**B x H 8**	139	110	20.9	18.2
• •	**B x H 2**	109	77	29.4	4.7
•	**B x H 19**	212	144	32.1	10.4
•	**B x H 14**	93	62	33.3	13.7
•	**B x H 3**	136	82	39.7	14.5

Data from Epstein and Vekemans (1990).

(arcsine) of pre-implantation loss and the frequencies (arcsine) of trisomy 16 was executed and proved not to be significant ($r=-0.407$, $d.f.=12$, $p>0.10$).

3D. Post-implantation loss

The post-implantation loss was obtained by dividing the number of resorptions and late deaths by the total number of implants. The frequencies of post-implantation loss in the RI and progenitor strains were shown to differ significantly by a G-test for heterogeneity ($G_H=50.22$, $d.f.=13$, $p<0.001$) (Table 6). In fact, the 14 strains again formed two groups by an homogeneity G-test (aligned asterisks in Table 6 show the delimitation of the two groups). The overlap between the two groups may be due to the small number of fetuses sampled from some strains. No significant correlation was found either between pre- and post-implantation losses, both transformed to arcsine ($r=0.210$, $d.f.=12$, $p>0.20$) or between post-implantation loss frequencies (arcsine) and prevalence of trisomy 16 (arcsine) observed on day 15 of gestation ($r=-0.330$, $d.f.=12$, $p>0.20$). Additionally, a non-significant inverse correlation exists between the mean number of dead fetuses per litter and the arcsine of the percent trisomy ($r=-0.341$, $d.f.=12$, $p>0.20$) (Figure 5).

TABLE 6

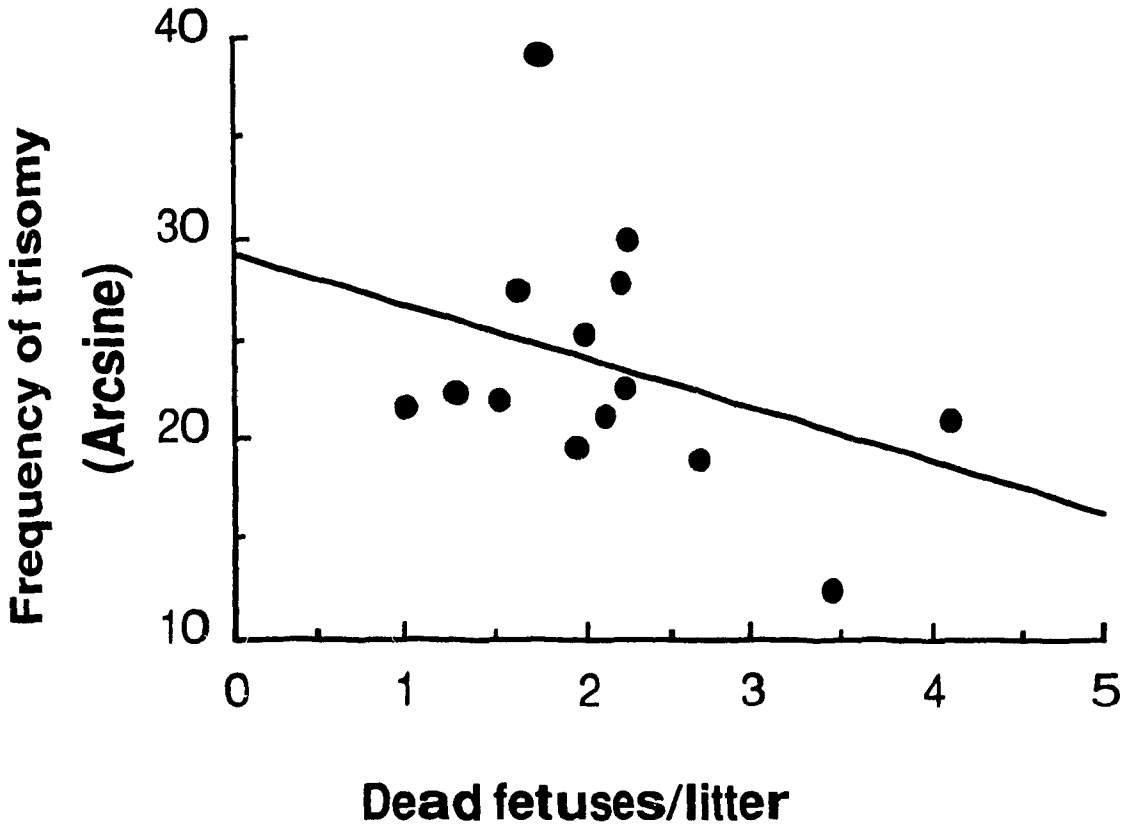
Post-implantation loss and prevalence of trisomy 16 in females of the B x H recombinant inbred strains mated to Rb(6.16)/Rb(16.17) males.

G-test	STRAIN	IMPLANTS	MOLES	POST-IMPL. LOSS (%)	FREQUENCY OF TRISOMY (%)
*	**B X H 14**	62	10	17.7	13.7
*	**B X H 7**	106	19	18.9	14.0
*	**#C3H/HeJ**	194	44	24.2	21.1
*	**B x H 3**	82	20	24.4	14.5
*	**B x H 12**	48	13	27.1	40.0
*	**#C57BL/6J**	201	54	28.9	11.2
*	**B x H 8**	110	32	30.0	18.2
*	**B x H 4**	94	26	30.9	21.5
*	**B x H 6**	72	22	31.9	24.5
*	**B x H 10**	146	45	32.2	13.1
*	**B x H 19**	144	42	33.3	10.4
*	**B x H 11**	52	17	34.6	14.7
*	**B x H 2**	77	31	44.2	4.7
*	**B x H 9**	209	93	46.9	12.6

Data from Epstein and Vekemans (1990)

FIGURE 5

Correlation between fetal loss and frequency of trisomy 16 in the BxH females mated to Rb(6.16)/ Rb(16.17) males.



4. DISCUSSION

From previous work in this laboratory (Vekemans and Trasler, 1987; Epstein and Vekemans, 1990), it was shown that the prevalence of both trisomy 16 and 19 fetuses observed on day 15 of gestation (plug day=0) varies significantly with the genetic background of the female parent. This conclusion was reached through the observation of a significant difference in the frequency of trisomy 16 or 19 between genetically unrelated female mice mated to males heterozygous for two Robertsonian translocations with monobrachial homology (either Rb(6.16)/Rb(16.17) or Rb(5.19)/Rb(9.19)). However, when the reciprocal crosses were analysed, this strain difference was not seen.

The expected frequency of trisomy 19 on day 15 of gestation has been extrapolated from a study of metaphase II preparations from sperm of Rb(5.19)/Rb(9.19) males (White et al, 1972). According to Vekemans and Trasler 1987) who used the same male to induce aneuploidy, the incidence of trisomy 19 on day 15 of gestation was predicted to be at least 20%. Similarly, Zackowski and Martin-DeLeon (1989), by karyotyping first-cleavage stage embryos produced by mating normal females with Rb(6.16)/Rb(16.17) males, evaluated at 22% the incidence of trisomy 16.

In both studies of the prevalence of trisomy 16 and 19 on day 15 of gestation (Vekemans and Trasler, 1987; Epstein and

Vekemans, 1990), the female strains segregated into two significantly different groups with respect to that character. The first group displayed a frequency of trisomy of about 20%, very close to the expected value. The second group presented with a frequency of trisomy of about 10%, thereby giving a hint that the maternal genetic factors controlling the frequency of trisomy 16 and 19 act through a selection process.

The observation of a negative correlation between the number of moles per litter and the frequency of trisomy in both studies further substantiated the hypothesis of a selective elimination of trisomic fetuses. Finally, further evidence for the selection mechanism came from the evaluation of the rate of loss of trisomy 16 conceptuses (Epstein and Vekemans, 1990). The ability to select against a trisomy 16 conceptus was shown to be improved in the mouse strains with a low prevalence of trisomy 16, compared to those mouse strains with a high prevalence of trisomy 16.

In order to determine when during gestation this process is taking place, chromosomal analysis of blastocyst-stage embryos aneuploid for chromosome 16 and 19 was undertaken (Jacob,1990). No significant strain differences were found, therefore, indicating that the maternal genetic factors influencing the survival of trisomy 16 and 19 embryos was not functional before implantation.

Comparison of the prevalence of trisomy 16 and 19 on day 15 of gestation in the same female strains revealed a striking observation. Where one would expect that the maternal genetic process that selects against fetuses with trisomy 19 is present and functional in the same mother in reaction to a fetus with trisomy 16, it was, in fact, found that the maternal gene(s) controlling the selection mechanism is/are specific for the particular trisomy involved (Epstein and Vekemans, 1990)

All these findings prompted us to genetically characterize the maternal factors controlling the selection process against fetuses trisomic for chromosome 16 with the use of RI strains. The prevalence of trisomy 16 on day 15 of gestaion was assessed in each of the 12 BxH RI strains (C57BL/6J x C3H/HeJ) and this frequency was seen to differ significantly with the genetic background of the female strain (Table 1). The results from this study are highly comparable to that of Epstein and Vekemans (1990), since the same male was used (Rb(6.16)8Lub/Rb(16.17)24Lub) and mice were subjected to similar environmental conditions (food, light/dark cycle, temperature, humidity,...); therefore, it is legitimate to conclude that the strain difference in the frequency of trisomy 16 on day 15 of gestation is determined in a significant manner by maternal gene(s).

The prevalence of trisomy 16 on day 15 of gestation is a function of the incidence of that defect at conception and of

the probability that the abnormal fetuses survive until the time of observation (Stein et al, 1975). It is highly unlikely that the significantly increased frequency of day 15 trisomic fetuses in some strains is dependent upon an increased incidence of hyperhaploid sperm. In this study, the male genotype was kept constant; therefore, ensuring that genetically equivalent sperm complements were supplied to all females. Moreover, Zackowski and Martin-DeLeon (1989) have found no differences in the fertilizing capabilities of the meiotic segregation products from germ cells of the Rb(6.16)/Rb(16.17) males. This finding provides evidence that no prezygotic selection is occurring against hypo- or hyperhaploid spermatozoa.

Once it was established that the incidence of trisomy 16 at conception was constant, a variety of factors that could potentially be related to the prevalence of trisomy 16 observed in a strain, were investigated. One of these, maternal age at conception, was examined because it is a well-known risk factor in the etiology of trisomy in human. However, one should take note that, in this study, maternal age was controlled for by using female mice between two to six months of age. A second factor examined was the number of corpora lutea which could be associated with pre-implantation selection, i.e. aneuploid embryos being at a disadvantage over euploid ones in the process of implantation when the number of ova shed is large. Thirdly, the influence

of the number of implants on the prevalence of trisomy was looked at, because trisomic embryos could again be at a disadvantage in larger litters due to the limited supply of nutrients available (post-implantation selection).

The effect of these three variables on the proportion of trisomic fetuses in a litter was investigated by means of a regression procedure (Table 2). In only three comparisons out of the possible 36 were there significant effects detected. In addition, the significant effects involved the three variables in turn on the frequency of trisomy 16 in two female strains. Had there been a significant influence of one factor observed over many strains, then this would have been interpreted as conclusive evidence of its direct relationship with the frequency of trisomy 16. In conclusion, in this study, the influences of maternal age, number of corpora lutea per pregnant female and number of implants per pregnant female on the prevalence of trisomy 16 fetuses are considered negligible. Epstein and Vekemans (1990) found no influences of either of these three variables on the frequency of trisomy 16 in the progenitor strains.

Dividing each strain into high and low subgroups with respect to the number of corpora lutea and subsequently testing the independence of the frequencies of trisomy calculated from both groups yielded only one significant difference (Table 4). The prevalence of trisomy 16 in the BxH 19 strain seemed to be correlated with some heterogeneity

in the number of corpora lutea. However, since this does not seem to be a general trend, either in the RI strains or their progenitors, it is therefore assumed that the number of ova shed affect in a very minor way the prevalence of trisomy 16. It should also be noted that the number of litters for each group in table 4 is rather small.

Correlation analyses between the mean number of corpora lutea, the mean number of implants, the mean number of moles per litter and the frequency of trisomy 16 at day 15 were performed and found not to be statistically significant. The correlation analysis is taken as a more representative comparison between female strains since it uses the frequency of trisomy for the strain instead of the proportion of trisomic fetuses in a litter. These last results seem to confirm that the number of eggs shed and the litter size have no influences on the prevalence of trisomy 16.

Since the incidence of trisomy at conception is constant and none of the factors studied exert an influence, over all female strains, on the prevalence of trisomy 16 on day 15 of gestation, the possibility that a pre-implantation selection mechanism could explain the strain difference in the frequency of trisomy was examined. Such conclusion could not be reached from previous studies (Vekemans and Trasler, 1987; Epstein and Vekemans, 1990). However, the pre-implantation loss, as it is evaluated on day 15 of gestation, does not take into account the occurrence of unfertilized eggs in the

female mouse and can only be considered an estimate of the true pre-implantation loss. In this study, the 12 RI strains and their progenitors segregated into two groups with respect to their pre-implantation losses (Table 5). Nevertheless, a significant correlation was not found between the pre-implantation loss and the frequency of trisomy 16 in the 14 strains. A very recent study of blastocyst chromosome complements produced by a breeding design for the induction of aneuploidy for chromosome 16 and 19 revealed no significant variation with the female background in the frequency of trisomy (Jacob, 1990). In conclusion, all these findings indicate that preferential loss of trisomic embryos (at least for trisomy 16 and 19) is not occurring before implantation.

Better evidence is available for a post-implantation selection process occurring against trisomic fetuses. The frequencies of post-implantation losses among the 14 mouse strains formed two groups (Table 6). Negative, but non-significant correlations were found between post-implantation loss and the frequency of trisomy 16 and between the number of dead fetuses per litter and trisomy 16 prevalence (Figure 5). The same negative correlations have also been found by Vekemans and Trasler (1987) and Epstein and Vekemans (1990). Therefore, the findings from this study confirm that the maternal gene(s) responsible for the strain variation control(s) a selection process against trisomic

fetuses. The lack of statistical significance of the correlations is probably explained by the fact that the post-implantation loss, as measured on day 15 of gestation, is comprised of both euploid and aneuploid deaths, therefore, constituting an heterogeneous group.

A more accurate determination of the proportion of dead trisomic fetuses can be derived from a model published by Stein et al (1975), for the distribution, among spontaneous abortions and births, of conceptuses with and without anomalies. From this model, the equation shown at the bottom of Table 7 can be inferred, and states that the prevalence of trisomy at a given time in gestation is equal to the expected number of living trisomic conceptuses over the total number of living conceptuses (both trisomic and diploid).

The assumptions necessary to fit this model have been reviewed elsewhere (Epstein and Vekemans, 1990). The incidence of trisomy 16 at conception (p) is approximated to 20% (Zackowski and Martin-DeLeon, 1989; White et al, 1972). The probability that a normal fetus will die (r_n) can be determined by first calculating the proportion of moles and late deaths that are trisomics. This is done by multiplying the number of corpora lutea by the expected incidence of trisomy at conception and by substracting from the value thus obtained, the number of living trisomics. The remainder of the fetal loss will be considered as diploid conceptions. It then becomes easy to evaluate r_n by dividing the number of

diploid moles by the total number of diploid conceptuses (living and dead).

Using the aforementioned equation, it is possible to compute r_a (the probability that a trisomic fetus will die) for each of the female strains of mouse examined (Table 7). Substantial differences in the rate of loss of trisomic fetuses are present. Generally, female strains with a low frequency of trisomy 16, have a high rate of spontaneous loss of trisomic fetuses; whereas, female strains with a high prevalence of trisomic fetuses, have a low r_a; i.e. their ability to selectively eliminate aneuploid fetuses is decreased. This is illustrated in Figure 6, which indeed shows a negative correlation between frequency of trisomy 16 and r_a ($r=-0.972$, d.f.=12, $p< 0.001$).

The length of survival of aneuploid conceptuses has been observed by many authors to depend on genetic factors. For example, differential survival, as well as severity of malformations, have been shown to vary drastically with the maternal genetic background in trisomy 12 (Gropp and Grohe, 1981). More specifically, longest development and survival seemed to be related to the occurrence of exencephaly. Similar to the situation with trisomy 12, longer survival of trisomy 18 fetuses was observed in a particular female mouse strain and in association with cleft palate and ventricular septal defect (cited in Epstein, 1986).

TABLE 7

Values of rn and ra for the BxH females and their progenitors mated to Rb(6.16)/Rb(16.17) males.

STRAIN	FREQUENCY OF TRISOMY (%)	rn (%)	ra (%)
B x H 2	4.7	25.4	85.3
B x H 19	10.4	15.4	60.7
#C57BL/6J	11.2	18 6	60.0
B x H 9	12.6	39.4	65.1
B x H 10	13 1	22.7	53.4
B x H 14	13.7	0.0	36.5
B x H 7	14.0	7.0	39.4
B x H 3	14.5	3.3	34.4
B x H 11	14.7	26.4	49.3
B x H 8	18.2	23.4	31.8
#C3H/HeJ	21.1	17.5	16.0
B x H 4	21.5	28.0	21.1
B x H 6	24.5	33.9	14.2
B x H 12	40.0	42.6	0

Data from Epstein and Vekemans (1990).

$$F = \frac{Xp(1-ra)}{Xp(1-ra) + X(1-p)(1-rn)}$$

F: prevalence of trisomy on day 15

X: number of fetuses examined

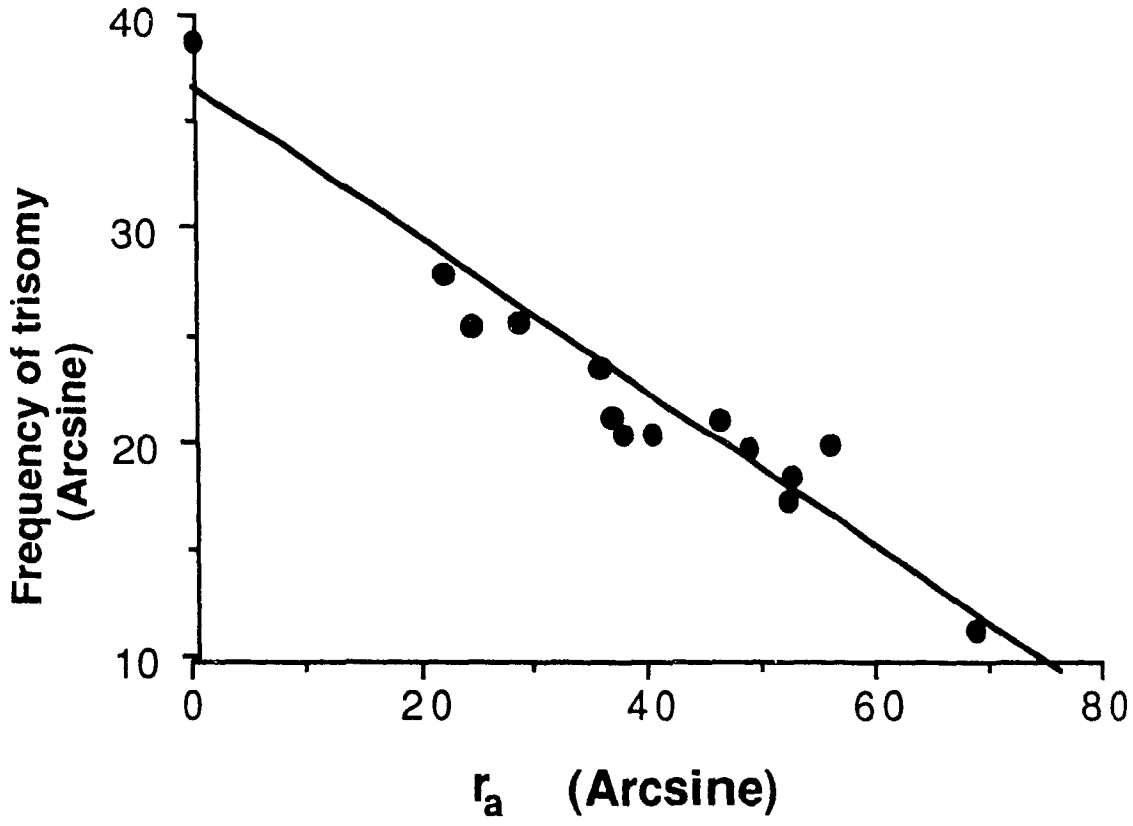
p: incidence of trisomy at conception (20%)

ra: probability that a trisomic fetus will die

rn: probability that a diploid fetus will die

FIGURE 6

Correlation between rate of loss of trisomic fetuses and frequency of trisomy 16.



Miyabara et al (1984), in their study of trisomy 16, also observed differences in the prevalence of trisomics with respect to the maternal strains studied, with, as in Epstein and Vekemans (1990), an higher prevalence in the C3H/He than in the C57BL/6 strain. Bacchus et al (1987) were the first to notice an inverse correlation between resorption rate and frequency of trisomy 16 and therefore, suggested that a selection process aiming at the elimination of monosomy and trisomy 16 embryos was occurring.

The genetic control of the time of death of embryos by maternal genes seem to apply also to monosomy as suggested by the data of Magnuson et al (1985) on monosomy 19, with the lethal period beginning later in the C57BL/6J strain background.

The ultimate goal of RI strains is to take advantage of strain differences to determine the number and position of the genes involved in this variation. This strategy is particularly useful for traits measured as frequency because a large number of genetically identical individuals can be investigated.

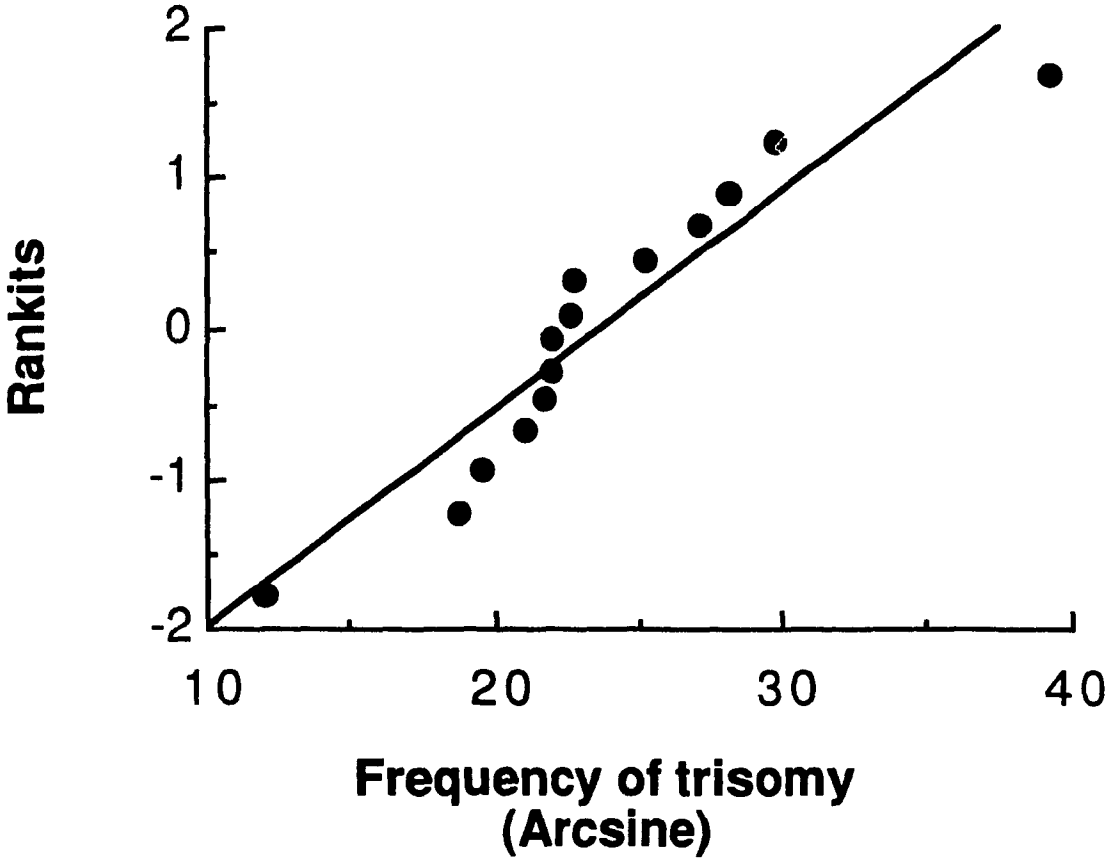
The results of testing females of the 12 BxH RI lines for the frequency of trisomy 16 on day 15 of gestation are shown in Table 1. From this table, it can be seen that the highest and lowest frequencies fall outside the progenitor levels, suggesting genetic complexity (more than one locus). But as mentioned previously, a sample size of 150 would have been

necessary for each strain to detect a statistically significant difference between 10% and 20%. Unfortunately, this could not be attained in a reasonable amount of time. Nevertheless, a number of statistical methods have been devised to help in the determination of the number of phenotypic groups.

One such method is a G-test for homogeneity which is performed on the ranked frequency of trisomy 16 in the RI strains and their progenitors (Sokal and Rohlf, 1981). When this is done, it is seen that the fourteen RI strains form two groups (Table 1). Some questions arise as to which group the strains located in the area of overlap belong to. By increasing the number of fetuses examined from these strains, the problem might be resolved. Then, it will be found that these RI strains either join one of the two groups, or form a separate group with an intermediate frequency of trisomy 16, suggesting in the former, responsibility of one maternal locus in the selection process, or in the latter, involvement of many loci.

A rankit analysis which can be used to determine the type of frequency distribution formed by the data (Sokal and Rohlf, 1981) indicates that the distribution of frequencies of trisomy 16 is bimodal (Figure 7). This suggests that a small number of loci (perhaps one) might be involved in the selection process. The intersection of rankit 0 with the fitted line yields the mean. In the data, the mean frequency

FIGURE 7
Rankit analysis of the frequency of trisomy 16 in females of the BxH recombinant inbred strains mated to Rb(6.16)/Rb(16.17) males.



of trisomy 16 in the 14 strains was calculated as 16.1%, i.e. falling just between the BxH 11 and BxH 8 strains in table 1. This mean value can help to establish a strain distribution pattern for the RI strains: strains with prevalence of trisomy above the mean being labelled high and strains with frequencies of trisomy below the mean termed low.

It is also possible to generate a strain distribution pattern for the BxH lines based on the 95% confidence intervals for the prevalence of trisomy in the parental strains. Using this methodology, each strain could be assigned to one of the two classes (high or low) based on their observed prevalence of trisomy 16 (Table 8). However, it should be noted that the 95% confidence intervals of the BxH strains trisomy frequencies was not taken into account. Coincidentally, the strain distribution pattern obtained by this method corresponds perfectly to the one obtained from the rankit analysis, further confirming that one or very few loci are responsible for the strain differences in the selection process.

One further method, first derived by Wright (1952) but then modified to account for inbreeding of the RI strains, can be used to estimate the minimum number of loci that determine a trait (Taylor, 1976; Mather and Jinks, 1982). It is based on the variance observed between the means of the strains in a RI set. The equation is set up as follows:

$$L=D^2/4V$$

TABLE 8

Strain distribution pattern for the BxH recombinant inbred strains based on the 95% confidence intervals for the frequency of trisomy 16 in the progenitor strains.

STRAIN	FREQUENCY OF TRISOMY (%)	95% CI	SDP
B X H 2	4.7	0.6-15.8	LOW
B X H 19	10.4	5.1-18.3	LOW
#C57BL/6J	**11.2**	**6.5-17.2**	**LOW**
B X H 9	12.6	7.2-20.5	LOW
B X H 10	13.1	7.2-21.4	LOW
B X H 14	13.7	5.7-26.3	LOW
B X H 7	14.0	7.4-23.1	LOW
B X H 3	14.5	6.9-25.8	LOW
B X H 11	14.7	5.0-31.1	LOW
B X H 8	18.2	10.3-28.6	HIGH
C3H/HeJ	**21.1**	**14.8-28.4**	**HIGH**
B x H 4	21.5	12.3-33.5	HIGH
B x H 6	24.5	13.3-38.9	HIGH
B x H 12	40.0	23.9-57.9	HIGH

Data from Epstein and Vekemans (1990).

where D is the difference between the highest and lowest means and V is the genetic variance between the means of all RI strains. The number of factors segregating in the BxH crosses estimated in this manner is 4.6. This method is based upon four assumptions (Mather and Jinks, 1982): 1) that the highest and lowest means included in the calculation represent the most extreme genetic combinations possible, 2) that the loci have equal effects on the trait, 3) that they are unlinked, and finally 4) that there is absence of epistasis. If these assumptions do not hold, and there is no proof that they do here, the number of effective loci will always be underestimated.

In summary, it has been found that anywhere from one to five genes might be implicated in the selection process occurring against trisomy 16 fetuses in the mouse. A more precise estimate of the number of loci involved could be attained by increasing the data already available for the BxH RI strains or by performing an identical study with another RI set in which the progenitors differ significantly for the prevalence of trisomy.

With the strain distribution pattern shown in Table 8, two possibilities of linkage have been proposed. First, the genetic factor selecting against trisomy 16 fetuses could be linked to the Emv-2 (ecotropic murine leukemia virus) locus on the distal part of mouse chromosome 8. Second, linkage to Mtv-6 (mouse mammary tumor virus) was detected on proximal

chromosome 16. However, in both cases two recombinants were found (strains BxH 7 and 12 for Emv-2, strains BxH 11 and 19 for Mtv-6) which yields a probability of linkage of the gene of interest to the marker locus of only 0.292 (Neumann, 1990).

An interesting association has been found between female strains that are selective against trisomy 16 fetuses and the fast allele of the pre-implantation embryo development (Ped) gene (Jacob, 1990). However, the same relationship could not be substantiated in the mouse strains selecting against trisomy 19 fetuses. The Ped gene which controls the rate of embryonic cleavage before implantation, manifests itself in two allelic forms, fast and slow, and maps to the Q region of the H-2 complex (Warner et al, 1988). The aforementioned relationship suggested that the Ped antigen could trigger the maternal response against trisomy 16 fetuses. However, when the strain distribution pattern for the Ped gene in the BxH RI strains was compared to their respective frequencies of trisomy, 6 discordants were observed, thereby questioning the possible involvement of the Ped gene in the differential survival of trisomic fetuses (Table 9).

The role of the placenta in the selective elimination of trisomic fetuses should be investigated because of its location at the interface between mother and conceptus. Indeed, it has already been shown that the development of the placenta is markedly affected by the trisomic genome.

TABLE 9

Observed and expected trisomy 16 response based on the Ped gene segregation in the BxH recombinant inbred strains.

STRAIN	H-2 ALLELE	TRISOMY 16 EXPECTED	TRISOMY 16 OBSERVED *
C57BL/6J	b (fast Ped)	LOW	LOW
C3H/HeJ	k (slow Ped)	HIGH	HIGH
B xH 2	k	HIGH	LOW
B x H 3	k	HIGH	LOW
B x H 4	b	LOW	HIGH
B x H 6	k	HIGH	HIGH
B x H 7	k	HIGH	LOW
B x H 8	b	LOW	HIGH
B x H 9	b	LOW	LOW
B x H 10	b	LOW	LOW
B x H 11	b	LOW	LOW
B x H 12	k	HIGH	HIGH
B x H 14	k	HIGH	LOW
B x H 19	b	LOW	LOW

* based on data from table 8.

Reduction in vascularization of the fetal side of the placenta and in placental weight have been observed for both trisomy 16 and 19 (Miyabara et al, 1982; Kornguth et al, 1987; Gropp, 1981) and this insufficiency of the placenta is thought to be a determinant factor in the late developmental failure of trisomic fetuses (Gropp, 1978). Furthermore, studies of chimeras (Trisomy 16<->Diploid) have emphasized the importance of a predominant population of normal cells in the placenta for the viability of the fetus, until late gestation (Cox et al, 1984; Gearhart et al, 1986a; Bogart and Miyabara, 1990).

It is possible that the maternal genetic background influences placental development in such a way that the maternal part of the placenta is better at maintaining the aneuploid fetuses in some strains of mothers, but not in others. Along the same line, the underdevelopment of the junctional zone of the placenta in trisomy 16 fetuses, which is the interface between maternal and fetal tissue has been proposed to have a relationship with the increased gene dosage for alpha- and beta-interferon cell surface receptors (Bersu et al, 1989). The level of H-2 expression on cells of the junctional zone increases after exposure to interferon. It is possible that the trisomy 16 fetuses with their inherent increased expression of interferon receptors are more at risk for a maternal allogeneic immune response. One can then speculate that the strain difference in the

selection process against trisomic fetuses resides in one of the loci controlling the maternal immune response toward the fetal "allograft".

This mechanism, extrapolated to all mouse trisomies, could mean that some maternal immunologic factor would interact with a gene involved in a cell surface receptor or in antigen formation which would be located on the specific triplicated chromosome, and this interaction would trigger a selection process. Therefore, the induction of partial trisomy with male mice carrying reciprocal translocations could be used to examine whether the maternal selection process needs the entire triplicated chromosome to occur, or just part of it.

A further genetic tool is available to elucidate the genetic architecture of the selection process: recombinant congenic strains. These are built by two generation backcrossing between two strains differing with respect to a trait, and perpetuation by sister-brother matings (Démant and Hart, 1986). Therefore, each recombinant congenic strain carries a small portion of the genome of one strain, on the genetic background of the second strain. In this system, unlinked genes controlling the same trait become separated in different recombinant congenic strains and can be studied individually.

Another approach to the investigation of the components of the selection process would be to alter some environmental factors such that the effect of a major gene would be

enhanced or masked. As it has already been observed that feeding the female mice a particular diet (Purina Mouse Chow) improves the selection process while providing them with another type of food (Purina Rat Chow) renders the selection process ineffective (Vekemans, 1989), the study of the frequency of trisomy 16 in the BxH strains fed with a different diet could be undertaken.

5. CONCLUSION

In this study, recombinant inbred strains of mice have been used to map the maternal gene(s) involved in the strain difference for the frequency of trisomy 16. Results concur with those of others in that selective elimination of trisomic fetuses, controlled by maternal gene(s), is occurring. This conclusion was reached by the observation of a negative correlation between the frequency of trisomy 16 and the number of dead fetuses per litter. A general tendency for strains with a low prevalence of trisomy 16 to exhibit a higher calculated rate of loss of trisomic fetuses is further evidence for the existence of a selection mechanism.

From the prevalence of trisomy 16 in the BxH lines, a variety of methods can be used to estimate the number of loci involved in the selection process against trisomic fetuses. Implementation of these method sets the number of segregation factors to a minimum of 1 and a maximum of 5.

A potential strain distribution pattern could be derived from the RI set data. This suggested linkage to either Emv-2 on chromosome 8 or Mtv-6 on chromosome 16. However, due to the presence of two discordant strains, the probability of linkage is very low (0.292). The proposed association between the maternal genetic factors eliminating trisomy 16 conceptuses and the Ped gene seems not to hold in the present

study.

Because the RI strains approach is cumulative, and the data suggest that a small number of genes are involved, this study will eventually contribute to the description of the genetic make-up of the trait. If one makes use of the conservation of syntenic genes between mouse and human, these murine data will shed some light on the nature of the selection process occurring against trisomy 21 in human and will help in the identification of individuals genetically at risk for giving birth to an aneuploid offspring.

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