The effect of simple Robertsonian fusions on the fertility of male Collared Lemmings (*Dicrostonyx richardsoni*) from Churchill, Manitoba: applicability of the stasipatric speciation model.

By

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A thesis submitted in conformity with the requirements

for the degree of Master of Science

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To Hanka

Abstract

The effect of simple Robertsonian fusions on the fertility of male Collared Lemmings (*Dicrostonyx richardsoni*) from Churchill, Manitoba: applicability of the stasipatric speciation model.

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Master of Science, 1998

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Stasipatric speciation has been suggested as a mechanism of chromosomal speciation in many taxa, including collared lemmings (*Dicrostonyx*) (White, 1978; Hoffman, 1981; Modi, 1987). In this model of speciation, chromosomal rearrangements can act as primary reproductive barriers due to negative heterosis. Fifteen male *Dicrostonyx richardsoni* were examined to determine the effects of heterozygous Robertsonian (Rb) fusions on fertility. Fertility was measured by testes size, litter size, number of chiasmata and their location, metaphase I pairing success, and spermatid aneuploidy as measured by flow cytometry. None of the fertility assessments demonstrated significant differences between homozygotes and heterozygotes for Rb fusions. These fusions cannot, therefore, induce reproductive isolation. Since a large proportion of *Dicrostonyx* species level-diversity is based on presence or absence of Rb fusions, this study calls into question the current species level taxonomy and the applicability of the stasipatric speciation model to speciation of *Dicrostonyx*.

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CHAPTER 1: General Introduction

The possibility that species of collared lemmings (*Dicrostonyx)* arose by chromosomal speciation was proposed by Hoffmann (1981), Modi (1987) and White (1978). In particular, stasipatric speciation (White et al., 1967) has been cited as the process underlying the generation of species in this group. According to this model, a novel, negatively heterotic chromosomal rearrangement can arise anywhere from within a contiguous population. Given a population structure composed of small, semi-isolated demes, genetic drift can drive the novel rearrangement to fixation in the face of negative selection pressures. After demic fixation, the new rearrangement spreads and comes into contact with the ancestral population where a reproductive barrier is established by means of negative heterosis. Subsequent selection for pre-mating isolation in a tension zone between ancestral and derived populations then completes the establishment of a reproductive barrier, and speciation. Speciation in this scenario is directly caused by chromosomal rearrangements and is rapid. The purpose of this study was to assess the degree of negative heterosis resulting from Robertsonian translocation (Rb) events (centric fusions) in *Dicrostonyx richardsoni* in order to test the central thesis of stasipatry and other chromosomal speciation models that chromosomal rearrangements themselves can directly cause reproductive isolation. The results of this assessment were used to investigate the suitability of the stasipatric speciation model as an explanation for the species level diversity in this genus.

Chromosomal Speciation

Since the neo-Darwinian synthesis of evolutionary thinking in the middle of the twentieth century, chromosomal rearrangements have taken on an increasingly important role in models of speciation. The observation that distinct species tend to possess different chromosomal complements led to the hypothesis that chromosomal changes can directly cause reproductive isolation. Bush (1975, and Bush et al., 1977 suggested that chromosomal rearrangements are the main driving force in mammalian speciation, particularly in primates, rodents and horses. The first chromosomal speciation models proposed (Wallace, 1953; Lewis, 1966; Grant, 1972) were restricted to specific populations under stringent conditions. These models provided an important framework upon which the later more general concepts of chromosomal speciation were built. The first of the more general chromosomal speciation models was the stasipatric model (White et al., 1967; White, 1968, 1978), which broadened the potential applicability of chromosomal speciation to a wide array of taxa. Among others, groups whose evolutionary diversification have been described by this model include: wingless grasshoppers, (Morabinae; White, 1968); iguanid lizards (*Sceloporus grammicus*; White, 1978); the Australian gecko (*Diplodactylus vittattus*; King, 1977); the house mouse, (*Mus musculus*; Redi and Cappana, 1988; King, 1993); and collared lemmings (*Dicrostonyx*; Hoffmann, 1981; Modi, 1978; White, 1978).

The stasipatric model is based on a few general observations across diverse classes and phyla. White (1978) noted that chromosomal rearrangements are very common and one in 500 individuals possess them in almost all species. Most of these rearrangements are deleterious and are quickly eliminated from a population by

selection, but some can be beneficial. Chromosomal rearrangements with a positive selective value when heterozygous are maintained in a population in a stable polymorphic state. These positively heterotic rearrangements do not play a direct role in speciation. In contrast, rearrangements that are selectively disadvantageous when heterozygous, but advantageous when homozygous, are central to White's model. Fitness is often lowered in rearrangement heterozygotes due to complications in pairing and in formation of chiasmata preceding meiotic segregation. Failure to complete normal segregation results in an increase in the frequency of aneuploid gametes and a decrease in fertility (White, 1968,1978; Key, 1968; Lande, 1979; Futuyma and Mayer, 1980; Templeton, 1981; Chandley et al., 1986; Sites and Moritz, 1987; King, 1993; Conn et al., 1998). Although these chromosomal rearrangements are negatively heterotic, they can become fixed in small populations by genetic drift (Lande, 1979). When the population structure is composed of small, semi-isolated demes, novel chromosomal rearrangements can arise and become fixed anywhere within the geographic range of the species. Once fixed in the new, selectively advantageous, homozygous condition, the rearrangement spreads until it encounters the ancestral chromosomal configuration, where the two form a 'tension zone' of hybridization. This zone acts as a partial filter, allowing passage of some genes and restricting others until, eventually, complete reproductive isolation is achieved via selection against formation of heterozygotes. The tension zone moves in space until it reaches the geographic point at which the new homozygote is no longer at a selective advantage relative to the ancestral condition. The stasipatric speciation model, therefore, has two components,

the initial establishment of the new chromosomal rearrangement and secondary contact with the parental population via a moving tension zone.

Despite the potential applicability of White's model, the theory of stasipatric speciation met with considerable opposition (Key, 1968; Futuyma and Mayer, 1980; Templeton, 1981; Nei et al., 1983). The major criticism of the model involves the fixation paradox: the probability of a rearrangement becoming fixed in a population is inversely proportional to its subsequent effectiveness as an isolating mechanism. Extremely small effective population sizes (10 individuals or less) are required for fixation of even moderately heterotic rearrangements ($s = 0.025$) (Hedrick, 1981; Baker and Bickham, 1986; Chesser and Baker, 1986; Baker et al., 1987; Sites and Moritz, 1987; Sites et al., 1988). Moreover, chromosomal heterozygotes must bear a selective disadvantage exceeding 30% to precipitate reproductive isolation between derived and ancestral populations (Barton, 1979; Futuyma and Mayer, 1980; Spirito et al., 1983; Bengsston, 1986; Sites and Moritz, 1987).

Given that extremely small effective population sizes and intense inbreeding are required to fix even a moderately negatively heterotic chromosomal rearrangement by genetic drift (Hedrick, 1981; Baker and Bickham, 1986; Chesser and Baker, 1986; Baker et al., 1987; Sites and Moritz, 1987; Sites et al., 1988), species that could potentially produce daughter species via stasipatric speciation must be composed of small, semi-isolated demes distributed in patches of suitable habitat with limited gene flow among demes (i.e. species with low vagility) (White, 1978). If meiotic drive (the preferential production of one genotype by unequal segregation and/or spermatid competition) favors segregation of the new rearrangement, the stringency of the

requirements for effective population size, genetic drift, and degree of negative selection is relaxed somewhat (Patton, 1967; Lande, 1979; Walsh, 1982).

Dicrostonyx

Richardson's collared lemming, *Dicrostonyx richardsoni*, is ideal for investigation of the meiotic consequences of potentially negatively heterotic rearrangements and their role in speciation. Collared lemmings (*Dicrostonyx*) are highly polymorphic for Rb fusions, and thus highly variable in diploid number over their geographic range. For example, North American collared lemmings vary in diploid number from 2n=28 to 2n=48 (Jarrell and Fredga, 1993; Figure 1). Rausch and Rausch (1972) first suggested that chromosomally distinct populations represented distinct species in *Dicrostonyx*. They divided the samples available to them into five species based largely on differences in numbers of fixed chromosomal rearrangements. Following that approach, anywhere from 2 to 11 species of *Dicrostonyx* are currently recognized worldwide (Hoffmann, 1981; Hall, 1981; Corbet and Hill, 1991; Musser and Carleton, 1993; Jarrell and Fredga, 1993). Of particular interest is the population of *Dicrostonyx richardsoni* from Churchill, Manitoba, which is known to be polymorphic for several independent Rb fusions (Rausch and Rausch, 1972; Malcolm et al., 1986; van Wynsberghe and Engstrom, 1992). Rb translocations are negatively heterotic in some other groups (Cattanach and Moseley, 1973; Capanna et al., 1976; Cattanach, 1978; Gropp et al., 1982; Redi and Capanna, 1988; Garagna et al., 1997; Kingswood et al., 1998), are the only type of rearrangement present in *Dicrostonyx,* and thus have been implicated in speciation of collared lemmings (Hoffmann, 1981; Modi, 1987;

White, 1978). The purpose of this study was to examine the role of Rb fusions as potential isolating mechanisms in this population and, by extension, in the genus *Dicrostonyx* (Figure 2).

Dicrostonyx richardsoni has extensive chromosomal variation with nine separate chromosomes (chromosomes 7, 8, 9, 11, 14, 19, 23, X and Y) involved in independent Rb fusions. *Dicrostonyx richardsoni* also lacks B chromosomes (van Wynsberghe and Engstrom, 1992). These randomly assorting, non-transcribed chromosomes are present in several other populations of North American *Dicrostonyx* (van Wynsberghe and Engstrom, 1992; Borowik and Engstrom, 1993; Engstrom et al., 1993; Berend et al., in press) and can potentially confuse assessment of aneuploidy.

Dicrostonyx richardsoni also fulfills the criterion of extreme population structuring required for stasipatric speciation to occur. In the summer, Richardson's collared lemmings inhabit patches of dry sandy soil and the species occurs in semi-isolated pockets of suitable habitat (Scott and Hansell, 1989). The home range of the species is also very small, with females averaging 0.06 ha in periods of high density and 0.3 ha in periods of low density. The dispersal distance for the high and low density periods is 42 m and 120 m respectively. Male lemmings are somewhat more vagile averaging 0.18 ha for home range at high density with a dispersal distance of 77 m and an average home range of 2.375 ha with 316 m dispersion at periods of low density (Rodgers and Lewis, 1986; Brooks, 1993). Population fluctuations occur in a cyclical fashion in this species with numbers ranging from less than 1 animal per hectare in years of low numbers to 40 animals per hectare at population crests (Shelford, 1943; Brooks and Banks, 1973; Brooks, 1993). These extreme population fluctuations

combined with patchy distribution of demes and episodes of low vagility are conducive to the periodic formation of small demes, inbreeding and genetic drift (Gileva, 1983). Finally, meiotic drive has been suggested as a driving force in genetic divergence within the genus (Gileva, 1987). A breeding experiment on *Dicrostonyx torquatus* demonstrated unequal segregation of the sex chromosomes wherein the Y chromosome was preferentially passed on to offspring relative to the X. *Dicrostonyx richardsoni* is, therefore, an ideal study group to test the stasipatric speciation model.

Meiosis

The central tenet of most chromosomal speciation models is that individual or multiple chromosomal rearrangements cause meiotic aberrations that lead to reduced fertility. This reduction in fertility is usually thought to be caused by the production of aneuploid gametes. As a background to the assessment of aneuploidy, I will review the mechanisms of meiotic division; the biological process that is altered in the reduction of fitness. Due to the complications of investigating meiosis in female mammals (Eichenlaub-Ritter and Winking, 1990; Searle, 1990; Wallace et al., 1992) and the tendency for many fertility effects to appear only in males in mammals (Haldane, 1922; Forejt, 1982) only the spermatogenic cycle was examined in this study.

Reproductively healthy male mammals produce millions of sperm daily (Evenson, 1989). Different cells and biological processes are involved in the transformation of stem cell to mature sperm. The spermatogenetic process occurs in the seminiferous tubules of the testes where two cell types dominate: Sertoli cells for

support and nutrition; and germ cells which undergo spermatogenesis. As an indication of the importance of high productivity, ninety-five percent of all cells found in the testes are germinal cells whose sole purpose is the production of spermatozoa (Clausen et al., 1977).

Spermatogenesis follows the same general pattern in all mammals (Evenson, 1989; Gledhill et al., 1990; Bickham et al., 1994): type A spermatogonia, or stem cells, found on the tubule wall differentiate first into intermediate spermatogonia, and later to type B spermatogonia as internal physiological changes prepare the cell for eventual transformation into mature spermatozoa. The stem cells also undergo continual mitosis to replenish their numbers. This phase represents the first step of the spermatogenetic cycle; spermatogonial proliferation and renewal.

Type B spermatogonia migrate farther away from the tubule wall and become primary spermatocytes at the start of the second step, meiosis. The primary spermatocytes replicate DNA during S-phase until the entire genome has been duplicated (4n) and meiosis commences. There are two separate divisions in the meiotic cycle. The first meiotic division is the reduction division wherein the chromosomal complement is effectively reduced from the diploid to the haploid condition (from 4n to 2n) by pairing and segregation of homologues (Figure 3). It is the disruption of these processes that leads to non-disjunction and increased levels of aneuploidy. All of the primary spermatocytes remain supported by Sertoli cells as they gradually migrate towards the tubule lumen while undergoing the first meiotic division. The second (mitotic) division of meiosis occurs in secondary spermatocytes, cells of similar physiological makeup to primary spermatocytes but with less DNA (2n) and positioned

relatively closer to the center of the tubule. The secondary spermatocytes continue the meiotic division until the duplicated haploid complements are segregated completing genome reduction (2n to 1n) and spermatid cells are produced. The second meiotic cycle is similar to a normal mitotic division and heterozygosity has little, if any, effect on this stage.

The last step of spermatogenesis is the maturation of spermatids to spermatozoa. As spermatids mature from round, to elongating, to elongated spermatids, there is a general loss of RNA and an alteration of the nucleic proteins. Round spermatids contain somatic-like proteins and a relatively high amount of RNA. Elongating spermatids contain a mixture of proteins in the nucleus and less RNA. Elongated spermatids have virtually no RNA and condensed DNA nucleic proteins. When the spermiogenetic phase reaches its conclusion, the elongated spermatids are released into the tubular lumen and transported to the epididimys for final maturation into spermatozoa. The final transformation to spermatozoa involves further nucleic condensation and tail and acrosome formation until the motile sperm cell is produced (Swanson et al., 1981).

Chromosomal rearrangements can affect the meiotic cycle in a number of different ways resulting in breakdown. In particular, Rb fusions (fusion of two acrocentric chromosomes into a single metacentric or the fission of a metacentric chromosome into two acrocentrics; Figure 4; Robertson, 1916) are known to be negatively heterotic in certain mammalian systems (Cattanach and Moseley, 1973; Cattanach 1978; Forejt, 1982; Redi and Capanna 1988; Garagna et al., 1998; Conn et al., 1998; Kingswood et al., 1998). In fusion heterozygotes, when the chromosomes involved pair during meiosis, the derived metacentric chromosome is homologous to

two individual acrocentric chromosomes. During pachytene, the acrocentric chromosomes each pair with one arm of the metacentric chromosome forming a trivalent structure (set of three chromosomes). Problems can occur during this stage if the three elements do not align properly and fail to form complete synaptonemal complexes (White 1978; Wallace and Searle, 1990). Improper formation of the synaptonemal complex can affect formation of chiasmata which in turn can lead to unbalanced segregation at later stages of meiotic division (Miklos, 1974). Complete spermatogenic arrest can also result from incomplete pairing, if the XY bivalent becomes associated with unpaired regions of autosomes (Forejt, 1982). The anaphase I division can also be affected by heterozygosity for Rb fusions. Even if pairing is complete it is much more difficult to ensure that elements of a trivalent will segregate to the proper poles relative to a normal bivalent. The outcome is often mal-segregation resulting in aneuploidy in the haploid gametes derived from these divisions (Eichenlaub-Ritter and Winking, 1990).

Despite these theoretical expectations, many Rb fusions appear to have little effect on fertility of heterozygotes in the wild (Hall and Sellander, 1973; Searle and Beechy, 1982; Baverstock et al., 1983; Searle, 1988; Hale and Greenbaum, 1988; Wallace et al., 1990; Searle, 1990; Mercer et al., 1992; Nachmann, 1992; Reed et al., 1992). Levels of aneuploidy and fertility need to be measured in real systems before making conclusions concerning their potential role in precipitating speciation. Several methods are available to detect, identify, and measure aneuploidy, and other problems, to quantify any reduction in fertility caused by centric fusions.

Techniques for Assessment of Fertility

Fertility can be measured by a variety of cytological techniques. Traditionally, fertility was quantified by measurements such as testis size, breeding records, chiasma and pairing counts, and histological examinations. More recently, measurement of fertility, by the detection of aneuploidy, has been automated using flow cytometry. Each of these cytological techniques was employed in this study and will be briefly reviewed below.

Chiasmata and pairing data. --Rb fusions can affect meiosis in heterozygotes in several ways: difficulties in pairing in heterozygotes; misalignment of centromeres that cause mal-segregation; suppression of cross-overs; and complete interruption of meiosis (Gropp et al., 1982; Burgoyne and Baker, 1984; Searle, 1988; Eichenlaub-Ritter and Winking, 1990). These abnormalities can result either in an increased number of aneuploid gametes and a reduction in fertility, or no gamete production at all and complete sterility. Observations of pairing and counts of chiasmata are performed, respectively, at the pachytene and diplotene stages of meiosis I. These techniques provide information on how the meiotic process is proceeding before the first division of anaphase I (Elder and Pathak, 1980; Eichenlaub-Ritter and Winking, 1990; Wallace and Searle, 1990; Wallace et al., 1991; Johannison and Winking, 1994). Disruption of normal segregation occurs in anaphase I and resulting rates of aneuploidy can be measured via counts of chromosomes in metaphase II cells or by flow cytometry. Proper pairing of homologous chromosomes at prophase I is essential to prevent non-disjunction in the first division (Mittwoch and Mahadevaiah, 1992; King, 1993). Chiasma counts are also important because in chiasmate meiosis, at least one cross-over

is essential in each chromosome pair for the meiotic cycle to proceed normally. Rearrangements that reduce the number of overall cross-overs or alter the position of cross-overs could potentially cause either non-disjunction or complete meiotic arrest.

Testis size. --The size of mammalian testes is positively correlated with fertility, that is, as relative testis size decreases so too does the fertility of the individual (Mahadevaiah et al., 1990; Wallace and Searle, 1990; Mercer et al., 1992). Sperm count and testis mass also are positively correlated with fertility and a relative testis mass of 55% below normal generally indicates sterility (de Boer and de Jong, 1989). Therefore relative testis size and mass are good indications of the reproductive potential of an individual. Large decreases in testis mass and size are expected in individuals with impaired fertility.

Litter size. --One of the most common and direct means of assessing fertility is simply to record the number and condition of offspring produced by a test subject (Gileva, 1987; Long, 1988; Viroux and Bichau, 1992; King, 1993). The relative number of offspring produced is the ultimate measure of reproductive capacity. If an individual contributes to production of healthy offspring in normal quantities then it does not suffer from impaired fertility regardless of the results of other assessments.

Histology. –Histological examinations provide a precise and detailed visual depiction of the spermatogenic process (de Boer and de Jong, 1989) and is one of the most commonly used procedures to evaluate fertility (Ratomponirina et al., 1988; Wallace and Searle, 1990; Mercer et al., 1992; Wallace et al., 1992; Jaafar et al., 1993; Handel et al., 1994; Johannison and Winking, 1994). Histological sectioning provides a view of all stages of the spermatogenic cycle and the relative number of cells present at

each stage. This technique can reveal the stage of the meiotic cycle in which any gross disturbances occur.

Flow Cytometry. --Flow cytometry is a potentially powerful technique for examining aneuploidy but has been applied in relatively few studies (exceptions include Meistrich et al., 1978b; Smith et al., 1984; Smith et al., 1987; McBee and Bickham, 1988). In my study, flow cytometry was used to assess the degree of aneuploidy in sperm cells (referred to as DNA aneuploidy when measured by the flow cytometer: Shankey et al., 1995), by first staining the sperm with a fluorescent dye and then measuring the amount of fluorescence in thousands of cells as they are passed individually through the cytometer. The dye binds stochiometrically to the DNA in each sperm cell and the amount of fluorescence emitted is an accurate measure of the amount of DNA present.

The value of an analytical tool can be measured in terms of its resolution, precision and accuracy. An early application using a flow cytometer descriminated between X and Y bearing sperm (Meistrich et al., 1978), wherein the difference in amount of DNA between the two sub-populations was about 3.4%. Even with the relatively low resolution provided by early machines, the two sperm lines were identifiable. Other experiments demonstrated that cell lines with a difference of 2000 base pairs of DNA could be discriminated (Petty et al., 1995) and cells of a unique subpopulation could be distinguished when present as only 0.2% of the overall sample (Clausen et al., 1978). Today, cell sorters can reliably separate X and Y bearing sperm and even individual chromosomes in large enough quantities for development of genome libraries (Van Dilla et al., 1980; Rabinovitch, 1994). Additionally, where at

least four sub-populations of the spermatogenetic cycle should be definable based on DNA content (Aravindan et al., 1990), flow cytometric analysis actually has revealed seven (Clausen et al., 1977; Evenson, 1989; Gledhill et al., 1990) with three distinct spermatid populations definable based on degree of chromatin condensation: A spermatogonia, B and intermediate spermatogonia, secondary spermatocytes, primary spermatocytes, round spermatids, elongating spermatids, and elongated spermatids.

In a flow cytometric analysis of samples of human testes, no statistical differences were found between repeated samples of the same area, between upper and lower poles of a testis, or between testes (Thorud et al., 1980) . Similarly, Aravindan et al. (1990) found no statistical differences among repeated runs of the same sample. As a precautionary note, however, Otto et al. (1980), found statistically significant differences among repeated runs of the same sample in hamsters. Runs of the same sample on different machines are also notoriously variable.

Finally, diagnostic accuracy has been examined in a number of papers. Benaron et al. (1982) found that percentages of malformed sperm detected by flow cytometry and by microscopy were highly correlated (r=0.99). Frequencies of spermatogenetic cells identified by flow cytometry were similar to those identified by microscopy (Clausen et al., 1977; Pinkel et al., 1982; Evenson, 1989; Gledhill et al., 1990). And finally, spermatogenetic investigations on Arctic foxes showed high correlations between testes size and flow cytometry (Smith et al., 1984) and between histology and flow cytometry (Smith et al., 1987) for assessments of fertility. In my study, flow cytometry was used to detect DNA aneuploidy, the expected result of meiotic mal-

segregation. In *D. richardsoni* the size of the chromosomes involved in fusions is easily large enough to detect elevated levels of aneuploidy in heterozygotes.

Summary

Chromosomal rearrangements such as inversions, translocations and centric fusions can result in speciation because they can act as post-mating reproductive barriers between populations. Heterozygous individuals produced by interbreeding among populations are expected to suffer mal-segregation which leads to the production of aneuploid gametes and therefore, reduced fertility. Once fixed, reduced fertility in heterozygotes produced by hybridization between demes, results in formation of a tension zone between ancestral and derived populations. Selection against heterozygotes eventually leads to establishment of complete reproductive barriers (White, 1978). Hybrids can be sterile purely as a result of the mechanical difficulty in pairing and segregation of heterozygous rearrangements in meiosis, and these rearrangements can play a primary role in speciation (White 1968; Mayr, 1970). King (1987) noted that not all chromosomal changes affect reproduction. Certain rearrangements, such as heterochromatic additions, do not affect meiotic division and therefore, do not affect the fertility of heterozygotes. In other cases, rearrangements that should lead to reduced fertility in heterozygotes do not affect meiosis due to compensatory mechanisms (Hall and Sellander, 1973; Wallace and Searle, 1980; Searle and Beechy, 1982; Baverstock et al., 1983; Hale and Greenbaum, 1986; Searle, 1988; Hale and Greenbaum, 1988; Wallace et al., 1990; Searle, 1990; Mercer et al., 1992; Nachmann, 1992; Reed et al., 1992). In the stasipatric model, only those rearrangements that induce substantial

negative heterosis can potentially act as isolating mechanisms. In North American *Dicrostonyx* Rb fusion rearrangements predominate. For stasipatric speciation to have occurred in *Dicrostonyx*, fertility must be severely reduced in heterozygous carriers of Rb fusions.

The purpose of this study is to examine the effects of Robertsonian fusions on the fertility of male *Dicrostonyx richardsoni* to test the primary assumption of the stasipatric model. Fertility was assessed by examining testes size and mass, breeding records, meiotic pairing and chiasmata counts, histology, and flow cytometry. Each of these techniques provides an independent measure of fertility. In this thesis, results are separated into two chapters. Traditional cytological techniques for examining fertility are presented in Chapter 2; Rates of aneuploidy as measured by flow cytometry is examined in Chapter 3; and conclusions from all assessments are synthesized in Chapter 4.

CHAPTER 2: The Role of Robertsonian fusions in speciation of *Dicrostonyx richardsoni* Part I: Traditional cytological techniques

Collared lemmings, *Dicrostonyx*, are extremely variable in diploid number over their geographic range due to Robertsonian (Rb) fusions and the presence of supernumary chromosomes in several populations (van Wynsberghe and Engstrom, 1992; Borowik and Engstrom, 1993). Variation in diploid number has resulted in the recognition of several groups of populations as separate species (Rausch and Rausch, 1972; Musser and Carleton, 1993; Jones et al., 1997), many of which display only minimal morphological divergence. It is not surprising, therefore, that stasipatric speciation has been proposed to explain species-level divergence in *Dicrostonyx*, given that Rb fusions are the primary character distinguishing many of the taxa (White, 1978; Hoffman, 1981; Modi, 1987).

In the stasipatric model of chromosomal speciation (White et al., 1968; White, 1978) negatively heterotic chromosomal rearrangements erect reproductive barriers between derived and ancestral populations. The chromosomal rearrangements themselves act as reproductive barriers because meiotic pairing between the derived and ancestral chromosomal morphologies results in non-disjunction. These rearrangements reduce the fertility of heterozygous carriers sufficiently to impede gene flow between

populations that are fixed for the new chromosomal rearrangement and those that maintain the ancestral conformation. Via selective reinforcement, populations that differ by the chromosomal rearrangement eventually become fully reproductively isolated and represent distinct species (Mayr, 1970).

The critical assumption of stasipatric speciation and many chromosomal speciation models is that individual rearrangements severely depress fertility in heterozygotes. To determine the effects of centric fusions on the reproductive fitness of heterozygotes, I examined a population of *Dicrostonyx richardsoni* from Churchill, Manitoba. The population of *Dicrostonyx* from this locality has extensive chromosomal polymorphism due to centric fusions, with the diploid number ranging from 40 to 46 (Rausch and Rausch, 1972; Malcolm et al., 1986; Van Wynsberghe and Engstrom, 1992; Engstrom et al., 1993). These rearrangements present a useful system for studying the effects of both individual and multiple simple fusions on spermatogenesis and consequently their role in speciation. The purpose of this study was to examine the effects of independent autosomal fusions on male fertility in heterozygotes versus homozygotes, in *D. richardsoni*. These results can be extended to assess the potential contributions of these same rearrangements to speciation in *Dicrostonyx*.

A Robertsonian (Rb) fusion is the joining of two small acrocentric (single-armed) chromosomes into one large metacentric (bi-armed) chromosome. When heterozygous, this rearrangement is likely to be negatively heterotic because the derived chromosome is homologous to two individual acrocentric chromosomes. During the pachytene stage of meiosis, each of the acrocentric chromosomes pair with the homologous arm of the metacentric forming a trivalent structure (Figure 3). Problems

can occur during anaphase I division because it is much more difficult to ensure that elements of a trivalent will segregate to the proper poles relative to a normal bivalent. The outcome is often mal-segregation resulting in aneuploidy in the haploid gametes derived from these divisions. In some empirical studies, Rb fusions have been shown to be negatively heterotic resulting in lower fertility as predicted by the stasipatric speciation model (Cattanach and Moseley, 1973; Cattanach, 1978; Forejt, 1982; Gropp et al., 1982; Chandley et al., 1986; Redi and Capanna, 1988; Conn et al., 1998; Kingswood et al., 1998).

Aneuploidy, and other problems associated with Rb rearrangements, can be detected and measured to quantify the reduction in fertility caused by various fusions. Due to complications in examining female meiosis (Eichenlaub-Ritter and Winking, 1990; Searle, 1990; Wallace et al., 1991) assessments of fertility are usually performed on males. Spermatogenesis is a complex biological process involving three distinct ploidy levels and resulting, ultimately, in independently motile spermatozoa. Due to the number of different cell types and biochemical steps involved, the process is inherently susceptible to perturbations. A small alteration in the initial germ cells by a chromosomal rearrangement can result in greatly distorted mature spermatozoa. Examinations of spermatogenetic cells are often undertaken to determine how fertility is affected by chromosomal rearrangements (Elder and Pathak, 1980; Wallace et al., 1990; Nachmann, 1992; Johannison and Winking, 1994).

Herein, fertility was assessed by five techniques: 1) Pairing configurations and rates of univalency provide information on how the meiotic process is proceeding before the first division of anaphase I (Elder and Pathak, 1980; Gropp et al., 1982;

Burgoyne and Baker, 1984; Searle, 1988; Eichenlaub-Ritter and Winking, 1990; Wallace and Searle, 1990; Wallace et al., 1992; Johannison and Winking, 1994). Proper pairing of homologous chromosomes at prophase I is essential to prevent non-disjunction in the first division (Mittwoch and Mahadevaiah, 1992; King, 1993). 2) Chiasmata numbers and positions also provide information on the progress of meiosis before the first division (Elder and Pathak, 1980; Gropp et al., 1982; Burgoyne and Baker, 1984; Searle, 1988; Eichenlaub-Ritter and Winking, 1990; Wallace and Searle, 1990; Nachmann, 1992; Wallace et al., 1992; Johannison and Winking, 1994). Chiasma counts are important because in chiasmate meiosis, at least one cross-over is essential in each chromosome pair for the meiotic cycle to proceed normally. Rearrangements that reduce the number of overall cross-overs or alter the position of cross-overs could potentially cause either non-disjunction or meiotic arrest. 3) Testes size is frequently used to assess fertility (Smith et al., 1984; Mahadevaiah et al., 1990; Wallace and Searle, 1990; Mercer et al., 1992). The size of mammalian testes is positively correlated with fertility, that is, as relative testes size decreases so too does the fertility of the individual. A relative testis mass of 55% below normal generally indicates sterility (de Boer and de Jong, 1989). 4) The ability to produce offspring is the most direct measure of realized fertility (Gileva, 1987; Long, 1988; Viroux and Bichau, 1992; King, 1993). If a male sires healthy offspring in normal quantities then it does not suffer from impaired fertility regardless of the results of other assessments. 5) Histology provides a precise and detailed visual depiction of the spermatogenic process (de Boer and de Jong, 1989) and is one of the most commonly used procedures to evaluate fertility (Ratomponirina et al., 1988; Wallace and Searle, 1990; Mercer et al., 1992; Wallace et

al., 1992; Jaafar et al., 1993; Handel et al., 1994; Johannison and Winking, 1994). Any major disruptions to the meiotic division would be detected as alterations in the relative number and condition of populations of meiotic cells at various stages. Individuals that are experiencing a reduction in fertility are expected to produce few mature spermatids in the lumen. Individuals suffering from sterility are expected to produce no mature spermatids. This procedure not only allows the detection of meiotic breakdown but it also provides information on which stage of the meiotic cycle is being affected.

Materials and Methods

Lemmings and mitotic characterization --A captive breeding colony, founded by 12 wild caught *Dicrostonyx richardsoni* from Churchill, Manitoba, was maintained in the animal care facility at the University of Toronto (Animal Care Protocol #4711 issued to M.D. Engstrom). G-banding (Seabright, 1971) was performed on mitotic spreads from cultured spleen cells (Robinson and Elder, 1987) to identify individual chromosomal rearrangements. G-bands were obtained for 26 animals; 20 males and 6 females. The chromosome numbering system used was that of Borowik and Engstrom, (1993). G-band karyotypes of two animals from Arviat which have 46 chromosomes with no autosomal rearrangements (van Wynsberghe and Engstrom, 1992) were used as a standard. Bone marrow preparations following Patton (1967) as modified by Robbins and Baker (1981) were also prepared to supplement information provided from the spleen cultures. Tissues and voucher specimens are deposited in the Royal Ontario Museum (Appendix A).

Assessment of fertility --For each of the procedures a one-tailed t-test was used to determine if heterozygotes had significantly depressed fertility relative to homozygotes as predicted by the stasipatric model and other class "A" models of chromosomal speciation (Sites and Moritz, 1987). All of the data sets were normally distributed with equal variance.

Testes were removed following euthanasia. The left testis was weighed, measured and placed in phosphate buffered saline (PBS). For meiotic analysis, the tunica albuginea was cut and removed and the tubules were minced until a fine cell suspension was achieved (1 ml of this suspension was used for the flow cytometry procedure in Chapter 3). Hypotonic KCl (5%) was added to the suspension and incubated for 30 min. After incubation, the cells were fixed in Carnoy's fixative and stored at -4 °C. The right testis was removed and fixed in 10% formalin for histological analysis.

Pairing data and chiasmata counts-- Diakinesis-early metaphase nuclei were examined for 217 cells from 18 different animals. For pairing the following criteria were recorded for each cell (after Nachmann, 1992): the total number of configurations, the number of bivalents, the number of trivalents, the presence of a sex chromosome bivalent, the number and identity of any univalents, and the association of sex chromosome with other meiotic elements.

Chiasmata were counted and scored as proximal, interstitial, terminal or distal according to the following criteria: proximal chiasmata, at centromere without telomere association; interstitial chiasmata, near center of chromosome with cross conformation; terminal chiasmata, telomere to telomere association; distal chiasmata, telomere

bonding when the centromeres are also bonded forming a loop conformation (Figure 5a). All XY bivalents that displayed contact were scored as a single terminal chiasmata. Trivalents were scored as possessing two chiasmata with the identity of the chiasmata based on the conformation displayed by the trivalent (Figure 5b).

The expected numbers of bivalent, trivalent and XY bivalents for each rearrangement complement were calculated assuming complete pairing in all instances. The observed pairing conformations were compared to those expected, to determine if there was an increased frequency of univalency as a result of heterozygosity for centric fusions. Number and location of chiasmata were analyzed for each Rb fusion state. Locations and numbers of chiasmata for all carriers of at least one fusion, whether heterozygous or homozygous (i.e., individuals of 2n=45 or lower) were compared with those of ancestral 2n=46 homozygotes to determine if the presence of Rb fusions significantly affected number and position of chiasmata (proximal, interstitial, terminal, distal, and total number of chiasmata). For each Rb fusion state, 30 cells were examined. The null hypothesis that individuals with no Rb fusions had more chiasmata per location than Rb carriers (both heterozygotes and homozygotes) was tested.

Testes size and breeding data -- Measurements of testes size and mass were grouped for homozygous individuals (N=5) and the means compared to those for pooled heterozygotes (N=11), to test the null hypothesis that homozygotes did not have significantly larger mean testes size and mass than heterozygotes.

The size of litters sired by all of the males in the study that were paired with females were assessed. A total of seven males were paired with females and each sired at least one viable litter. Litter size was recorded as the number of offspring surviving to

the reproductive age of 30 days. Thus both fertility and viability of offspring are taken into account. Data for homozygotes (three individuals which produced 11 litters in total) were pooled and compared to data for heterozygotes (four individuals which produced 13 litters in total), to evaluate the null hypothesis that mean litter sizes of homozygotes were larger that those of heterozygotes. The seven individuals represented the following Rb fusions states: Homozygotes: 2n=46, no rearrangements (N=1); 2n=44, homozygous for a 9.11 fusion (N=2); Heterozygotes: 2n=45, one 7.14 fusion (N=1); 2n=45, one 9.11 fusion (N=1); 2n=44, one 8.23 and one 9.11 fusions (N=2).

Histology -- To determine if any gross histological deficiencies were associated with heterozygosity, tissue sections of testes were analyzed for the following karyotypes: ancestral homozygote, 2n=46, no fusions; single heterozygote, 2n=45, with one fusion (9.11); double heterozygote, 2n=44, for two independent fusions (7.14 and 8.23); 2n=44 homozygous for one fusion (9.11 and 9.11); and 2n=43 triple heterozygote for three independent fusions (7.14, 8.23, and 9.11). Tissue sections of 7 μm from the left testis were prepared and stained with haematoxylin-eosin following Luna (1968). The overall morphology and cell density was recorded for each fusion condition. Additionally, testes sections from a sterile interspecific back-cross (*D. richardsoni* X *D. richardsoni\groenlandicus* hybrid) were analyzed for comparison.

Results and Discussion

Rb fusions -- The colony was polymorphic for centric fusions 7.14, 8.23 and 9.11 and individual lemmings possessed from 0 to 4 rearrangements (Figure 6; Table I). All *D. richardsoni* had the same autosome-sex chromosome fusions (X.19 and Y.19)

relative to the ancestral condition for *Dicrostonyx* (Modi, 1987; Borowik and Engstrom, 1993). Although some female lemmings were G-banded to establish a more complete representation of the range of rearrangements present in the population, only males were used in the assessment of fertility. The lowest diploid number in the sample was 2n=43 in two individuals heterozygous for all three Rb fusions (7.14, 8.23, and 9.11). The highest diploid number was 2n=46 in individuals with no autosomal fusions. This karyotype occurred in two individuals from the laboratory stock and in both wild caught individuals obtained from Arviat, N.W.T. Three males had 2n=44 and were homozygous for the 9.11 fusion. The rearrangements were independent and none shared homologous arms with other fusions (i.e. no fusion was monobrachially homologous with another).

Chromosome Pairing – In 3 of 217 cells examined (1.38%), the X and Y chromosomes failed to pair (Table II). No other univalency was observed. One cell each (3.2 %) with XY univalency was observed for the following individuals: a heterozygote with 2n=45 (9.11), a heterozygote with 2n=44 (7.14, 8.23), and a homozygote with 2n=44 (9.11, 9.11.). These rates were not significantly different than 0 ($X^2 = 0.097$, d.f. = 6). The triple heterozygote (7.14, 8.23, 9.11) displayed no univalency for either autosomal bivalents or the XY sex bivalent.

Chromosome pairing examines fertility impairments that occur before metaphase I. Pairing failure can lead to reduced fertility in four ways when univalents are present: 1) the spindle mechanism can be disturbed; 2) unbalanced gametes can be produced as a result of non-disjunction; 3) abnormal pairing can lead to meiotic arrest (Miklos, 1974); 4) sex chromosome-autosome associations can activate the X and result

in spermatogenic breakdown (Forejt, 1982). Given that univalency was observed in both heterozygotes and homozygotes and was in each individual restricted to a single occurrence, there was no obvious correlation between univalency and the number of Rb rearrangements. Two of the lemmings that did have a cell with unpaired sex chromosomes, the first homozygous for two 9.11 fusions and the second heterozygous for one 9.11 fusion, sired healthy offspring (the first had 11 offspring; the second had 10). In no cells did the XY sex bivalent associate with any other elements. Heterozygosity does not appear to affect chromosome pairing in this population of *D. richardsoni*. Fertility impairments commonly associated with the presence of univalent elements are unlikely to affect heterozygous Rb fusion carriers in this species.

Chiasmata --The number and position of chiasmata not only provides data on the degree of impairment of fertility but also on the timing of this impairment (Elder and Pathak, 1980; Eichenlaub-Ritter and Winking, 1990; Nachmann, 1992). Normal chiasmate meiosis requires at least one chiasma per chromosome for the division to proceed. The position of these chiasmata can also be very important to the success of division. Individuals that suffer a reduction in fertility may have fewer chiasmata per cell at metaphase I and/or show an alteration in the location of chiasmata.

For proximal chiasmata there were no differences between the 2n=46 (ancestral state) and 2n=44 (two 9.11 fusions) homozygous states (Table III). There were significantly fewer proximal chiasmata present in heterozygotes relative to the 2n=46 homozygote; with the exception of the 2n=45 (heterozygous for one 9.11 fusion) state. The fewest proximal chiasmata were present in heterozygotes carrying two or more Rb fusions. For distal and total number of chiasmata there was a positive correlation

between diploid number and number of chiasmata, including a significant difference between the 2n=46 homozygotes and the 2n=44 homozygotes. Terminal chiasmata showed a significant decrease in number associated with all rearrangements possessing two or more Rb fusions, including the 2n=44 homozygous state. There were no consistent trends apparent between homozygous 2n=46 males and males carrying one or more Rb fusions, although carriers of Rb fusions had a higher average number of interstitial chiasmata.

Although these results seem to indicate a positive correlation between the number of chiasmata present in a cell and diploid number, variation in the total number of chiasmata is due largely to the variation in the number of distal chiasmata observed. The distal chiasmata were only scored when a ring structure was visible at diakinesis or metaphase I (Figure 5a). The homozygous 2n=46 condition can form a maximum of 8 ring structures (Figure 7), with bivalents 1, 2, 3, 4, 5, 7, 8, and 9 capable of assuming this conformation; the other chromosomes are constrained to different conformations (bivalent 6 and XY) or are too small to form a ring (bivalents 10-23). Therefore, the 2n=46 state can have a maximum of 8 distal chiasmata. The 2n=45 condition with a single rearrangement can only form 7 ring structures (and 7 distal chiasmata) since the heterozygous trivalent cannot assume a ring conformation (Figure 5b). The same holds for 2n=44 heterozygotes and 2n=43 heterozygotes which can possess a maximum of 6 and 5 distal chiasmata respectively. Without the variation caused by distal chiasmata, there is no significant difference in the total number of chiasmata between homozygotes and heterozygotes (Table IV). The differences in the presence of interstitial chiasmata between homozygotes and heterozygotes is due to the limited number of conformations

available to trivalents. In the 2n=46 homozygous state, interstitial chiasmata are present in chromosomes 21 and 22 and only occasionally in other chromosomes. In Rb heterozygotes, interstitial chiasmata are still present in chromosomes 21 and 22 but they can also be present in trivalents (Figure 5b), giving Rb heterozygotes a higher number of interstitial chiasmata. Based an these results, no change in the total number of chiasmata was induced by increasing heterozygosity for Rb fusions in *D. richardsoni*, when adjusted for the reduction in chiasmata resulting from physical constraints on pairing in heterozygotes.

Testis size and breeding results -- Mean mass of testes in heterozygotes was not significantly smaller than that of homozygotes ($t = -0.98$; $P = 0.18$). Likewise, heterozygotes were not smaller than homozygotes in length ($t= 0.15$; $P = 0.44$) or width ($t = 0.35$; $P = 0.37$) of testes (Table V). Given that no reduction in length, width, or mass was associated with heterozygosity for Rb fusions, there is no evidence of a decrease in fertility based on gross size differences of testes.

There also was no significant difference in mean litter size between homozygous and heterozygous individuals ($t = -1.24$; $P = 0.14$; Table VI). Any significant impairment of fertility in males should result in smaller litter sizes (Gileva, 1987; Ratomponirina et al., 1988; Viroux and Bichau, 1992). If an individual is capable of siring normal numbers of viable, fertile offspring than any apparent reduction in fertility found in other assessments is moot in terms of its effectiveness as a reproductive isolating mechanism. Male *D. richardsoni* possessing one or two heterozygous Rb rearrangements suffer from no apparent loss in fertility as measured by the number of healthy offspring produced.

Histology -- There were no signs of meiotic arrest in any of the transverse sections from *D. richardsoni* regardless of number of rearrangements or degree of heterozygosity. Normal populations of cells in all stages of the spermatogenic cycle up to mature spermatozoa were present in all individuals examined of this species (Figure 8, Table VII). In contrast, the back-cross male used for comparison (*D. richardsoni* x *D. richardsoni/groenlandicus*) had a complete breakdown of the spermatogenic cycle with a near absence of cells from any stage visible in the cross sections. The back-cross male produced no haploid cells (Chapter 3) and was apparently completely sterile. In *D. richardsoni* Rb heterozygosity does not result in any reduction in populations of cells of any stage (in contrast to the condition of the sterile back-cross) let alone the 90% reduction required to induce sterility (Mercer et al., 1992).

The effect of Rb fusions on fertility

In the polymorphic population of *D. richardsoni* from Churchill, Manitoba, there is no evidence of loss of fertility associated with increased heterozygosity for Rb fusions. Animals heterozygous for 0, 1, or 2 rearrangements all produced viable, fertile offspring in similar numbers. Additionally, there was no reduction in fertility associated with increased heterozygosity as measured by chromosome pairing, chiasmata numbers and location, testes size and mass, litter size, and histological analysis. There was no observable negative heterosis associated with single fusions or with multiple independent fusions.

The stasipatric model states that chromosomal rearrangements cause non-disjunction when heterozygous and thereby cause reproductive isolation. The present

study shows that the Rb fusions present in *D. richardsoni* are not negatively heterotic. These fusions cannot, therefore, be responsible for reproductive isolation. Since a large proportion of *Dicrostonyx* species level-diversity is based on presence or absence of Rb fusions, this study calls current species level taxonomy of *Dicrostonyx* into question.

CHAPTER 3: The Role of Robertsonian fusions in the speciation of *Dicrostonyx richardsoni* Part II: Flow Cytometry

Stasipatry has been suggested as a mechanism of speciation in several groups known to be chromosomally variable (Bush et al., 1977; White, 1978; King, 1993). This model of direct chromosomal speciation proposes that novel chromosomal rearrangements can become fixed in small, semi-isolated populations by genetic drift. Fixation can occur anywhere within the geographic range of the species. Subsequent to fixation, demes incorporating novel rearrangement expand from these localized pockets until contact with the parental chromosome race occurs. Once in contact, a hybrid zone is established and gradually, reproductive isolation is achieved by selection against chromosomal heterozygotes. The criteria required for this model of speciation are small population sizes, low vagility and structured demes, all of which lead to genetic drift. Meiotic drive has been suggested as a *deus ex machina* to help overcome the population genetic limitations imposed on this speciation scenario (White, 1978). Stasipatric speciation has been proposed for many taxa (Bush et al., 1977; White, 1978; King, 1993) including *Dicrostonyx* (White, 1978; Hoffman, 1981; Modi, 1987).

In *Dicrostonyx*, diploid numbers of non-supernumerary chromosomes ("A" complement) range from 2n=28 to 2n=48 due to the presence of Robertsonian (Rb) fusions (Rausch and Rausch, 1972; Borowik and Engstrom, 1933; Jarrell and Fredga, 1993).

The genus is currently divided into anywhere from two to nine species in North America (Honacki et al.,1982; Corbet and Hill, 1991; Jarrell and Fredga, 1993; Musser and Carleton, 1993; Jones et al., 1997; Engstrom, 1994; Engstrom et al., in press) based on differences in the number of Rb fusions present among populations and the hypothesized role of these rearrangements in reproductive isolation (Rausch and Rausch, 1972). *Dicrostonyx* also fulfills the population structure criteria required for stasipatric speciation (see Chapter1). Despite the importance placed on Rb fusions as the primary mechanism of speciation in *Dicrostonyx*, there have been no studies to test the effectiveness of these chromosomal rearrangements as reproductive barriers in the genus.

The population of *Dicrostonyx richardsoni* from Churchill, Manitoba, is polymorphic for several Robertsonian fusions (2n=40 to 2n=46, Malcolm et al., 1986; van Wynsberghe and Engstrom, 1992). Nine independent fusions have been identified within this population and individuals are commonly heterozygous for one or more rearrangements (van Wynsberghe and Engstrom, 1992). This species also lacks B chromosomes which, when present, would confuse assessments of aneuploidy due to their random segregation in meiosis. If Rb fusions fail to reduce fertility significantly, the central tenet of the stasipatric model would be falsified (at least for the rearrangements in this population). Although not all rearrangements or populations are necessarily equal with respect to degree of negative heterosis, such a result would nonetheless raise doubt as to the role of these rearrangements in speciation within *Dicrostonyx* as a whole.

The main class of rearrangements in *Dicrostonyx* is centric fusion (Rausch and Rausch, 1972; Gileva, 1987; van Wynsberghe and Engstrom, 1992; Borowik and Engstrom, 1993). When heterozygous, metacentrics derived via fusion must pair with two independent acrocentric chromosomes to form a trivalent structure. Because there is an odd number of chromosomal elements, segregation of trivalents at anaphase I can be problematic, resulting in a higher non-disjunction rate compared to chromosomal homozygotes. Heterozygous centric fusions are known to cause disorders in gametogenesis and consequent reduction in fertility in some taxa (Cattanach and Moseley, 1973; Cattanach, 1978; Forejt, 1982; Gropp and Winking, 1982; Redi and Capanna, 1988; Conn et al., 1998; Kingswood et al., 1998). Non-disjunction can be quantified and measured by cytogenetic techniques. Cytogenetic assessments are more often performed on male subjects due to the difficulty of oogenetic analysis (Eichenlaub-Ritter and Winking, 1990; Searle, 1990; Wallace et al., 1992).

Traditionally, the spermatogenetic cycle has been examined using counts of metaphase II cells (Elder and Pathak, 1980; Nachmann, 1992; Wallace et al., 1992), histological sections (Mercer et al., 1992; Jaafar et al., 1993; Handel et al., 1994) and counts of spermatozoa (Searle and Beechey, 1974; Karabinus et al., 1990; Mahadevaiah et al., 1990). While these procedures can be used to examine fertility, the time involved and degree of inherent subjectivity limits their scope. Flow cytometry provides comparable resolution (Clausen et al. 1978; Meistrich et al., 1978a; Gohde et al., 1980; Petty et al., 1995; Holden, 1997;), reproducibility (Thorud et al. 1980; Aravindan et al., 1990) and accuracy (Benaron et al., 1982; Baron et al., 1984; Evenson, 1989) to cytological, histological and microscopic procedures with the advantage of rapid

assessment, much larger sample sizes and objective collection of data (Benaron et al., 1982; Evenson, 1989). In particular, flow cytometry provides a simple measure of rates of aneuploidy (Meistrich et al., 1978b; McBee and Bickham, 1988; Aravindan et al., 1990; Custer et al., 1994) and has been previously applied to assessment of fertility (Meistrich et al., 1978b; Smith et al., 1984; Smith et al., 1987; McBee and Bickham, 1988).

Flow cytometry measures the amount of light given off by fluorescent dye stochiometrically bonded to each cell's DNA. The procedure produces a histogram where each channel corresponds to a specific DNA content and the height of each channel corresponds to the number of cells possessing that amount of DNA (Figure 9). Due to their distinctive DNA contents, populations of aneuploid cells occur in unique channels on the DNA content histogram. When the size of the mal-segregated chromosome is large, aneuploid cells will appear as a separate peak independent of the normal haploid peak. When the size of the mal-segregated chromosome is small, the aneuploid cells are distinguishable as a broadening of the haploid peak (i.e. the peak is relatively platykurtotic) resulting in an increased coefficient of variation (CV) (Vindelov et al., 1983; Mcbee and Bickham, 1988; Benson and Braylan, 1994).

To act as an effective reproductive isolating mechanism, fusion heterozygotes must incur at least a 30% rate of non-disjunction (Barton, 1979; Futuyma and Mayer, 1980; Spirito et al., 1983; Sites and Moritz, 1987). At this level of non-disjunction even aneuploidy for small chromosomal elements would be visible as a broadening of the haploid peak with a corresponding increase in CV, while larger aneuploid elements would be resolvable as distinct peaks (Vindelov et al., 1983; Mcbee and Bickham, 1988;

Benson and Braylan, 1994). Assuming the chromosomes involved in fusions are sufficiently large, if there are no distinct peaks or significant platykurtosis of the haploid peak associated with Rb heterozygosity, then elevated rates of non-disjunction at the level required by the stasipatric model (and most other models) do not occur.

In this study, I investigated the effects of heterozygous Rb fusions on meiosis in *Dicrostonyx richardsoni* by measuring the level of DNA aneuploidy in sperm cells. If these rearrangements are significantly negatively heterotic, then stasipatry remains a potential model of speciation in this group.

Materials and Methods

Size of rearrangements. -- Lengths of chromosomes were measured from 10 standard karyotypes prepared from spleen cultures (Robinson and Elder, 1987) and the relative size of each was calculated (Meistrich at al., 1978). The relative size of individual chromosomes involved in rearrangements was used to estimate positions of expected aneuploid peaks on DNA content histograms. The numbering system of individual chromosomes follows Borowik and Engstrom (1993).

Lemmings. --Lemmings were bred from wild caught *Dicrostonyx richardsoni* from Churchill, Manitoba and kept in a breeding colony. The lemmings were maintained on a regime of 16 hours light, 8 hours darkness. Males were euthanized after reaching reproductive age and if possible after siring at least one litter. Individuals in the colony were polymorphic for three Rb fusions, 7.14, 8.23, and 9.11. Homozygous males (2n=46 and 2n=44) and heterozygotes (2n=45, 44, and 43) for one, two and three

independent fusions were examined. Tissues and specimens were deposited at the Royal Ontario Museum (Appendix A).

Sample preparation. --The left testis was removed from each lemming and placed in phosphate buffered saline (PBS). The tunica albuginea was then removed and the remaining tubules minced until a fine cell suspension was achieved. The solution was transferred to a centrifuge tube and PBS added to make up a volume of 2 ml. The solution was then filtered through 25 µl nylon mesh to remove large particles and agglutinated clumps. Five, 2-µl aliquots of cell suspension were transferred to plastic freezing tubes and 1ml of freezing solution added to each (8.56 g sucrose, 1.18 g citric acid dihydrate, 5 ml Dimethyl Sulphoxide (DMSO), distilled water added to 100 ml and pH adjusted to 7.6; Vindelov et al., 1982). The samples were immersed in liquid nitrogen and stored at -80 °C.

Staining Procedure. --Preliminary runs demonstrated that a minimum amount of disruption and cell agitation produced the best resolution. The staining procedure followed was, therefore, based on Krishan (1975) because this technique involved the least amount of cell manipulation. The stain used was: 7.5 mg Propidium Iodide (PI), 100 mg sodium citrate, 30 µl Nonidet P-40, 5 mg RNase and 100 ml distilled water. The stain was added to the cell suspensions in a darkened room. Cells were stored at -4 °C for 30 min to 60 min in a dark container with mild agitation before analysis.

Flow cytometry. --DNA content of testes cells was analyzed on a Beckton and Dickinson FACSCaliber flow cytometer operated with a 488 nm air cooled argon ion laser. The FL2 channel was used for data collection using 560 SP, 640 LP and 585/42 lenses. The machine was tested each day for linearity with Immuno Check fluorospheres

(EPICS Coulter Corp. Batch 1568). The cytometer was run on the low flow rate setting at high resolution (1024 channels) to maximize sensitivity. The voltage gain was set at 410 placing the haploid peak at the 200 channel to avoid an increase in CV caused by low channel numbers (Vindelov and Christensen, 1990). Cell suspensions were vortexed for 30 sec and filtered through 25 μm nylon mesh before analysis by the flow cytometer. The cells were run and the solution diluted until a flow rate of 100-150 cell/sec was achieved (Dressler and Seamer, 1994). The cells were run for 2 min after dilution to provide time for equilibrium to be achieved in the flow apparatus before data were gathered. All of the samples were run in one session to reduce variability attributable to the machine.

A region was set around the haploid peak ensuring that more than 10 000 cells were collected from the round spermatid region guaranteeing an adequate sample size after removal of debris (Shankey et al., 1995)(debris is composed of cell and DNA fragments bound to dye and is present as a side effect of sample preparation). The CV was calculated for each haploid peak to estimate rates of aneuploidy in round spermatid.

Two separate procedures were used to measure the effect of Rb fusions on the fertility of heterozygotes. The first procedure (MODFIT) was performed to test for the presence of distinct peaks, using the debris removal algorithm in MODFIT LT V2.0 (Verity Software House Inc., 1996). The haploid peak was assigned G0/G1 and the diploid peak G2M with the S-phase component set at 1 using the Synchronization Wizard (Herbert, 1997, pers. comm.). Debris and aggregate algorithms were selected and CVs were recorded for the haploid peak. The MODFIT procedure was included in

the analysis because it is a standardized debris removal algorithm (Shankey et al., 1995).

Because the Modfit software was designed for mitotic analysis, the debris algorithm used is also based on mitosis and cannot account for the condensed spermatid peak to the left of the haploid peak. The algorithm might interpret this condensed region as debris and therefore, overcompensate the removal algorithm, possibly masking DNA aneuploidy in the channels higher than the haploid peak. While this procedure was not used for CV comparisons, the fact that this analysis detected no extra peaks (no DNA aneuploid peaks) in any of the samples run was instructive.

An additional debris removal procedure (FILTER) was performed to detect increases in haploid peak CV, using a DNA histogram from a sterile male as a filter (Figure 10). The sterile male was produced from a back cross between a *D. richardsoni* male and an F_1 female hybrid between *D. groenlandicus* and *D. richardsoni*. The sterile male produced no secondary spermatocytes or spermatozoa (Figure 10ii) and therefore, all events detected in the haploid region were caused by debris. The sterile male histogram provided an empirical view of debris produced by the cell preparation procedure. The histogram from the sterile male was used to subtract debris from all of the study histograms. (Figure 10iii and Figure 10iv). The CV of the haploid peak on a filtered histogram would therefore, be expected to approximate the true CV of round spermatids because of reduced interference of debris. The FILTER debris removal did not mask the presence of DNA aneuploidy in the channels to the immediate right of the round spermatid peak because the number of events subtracted from these are much less than the total number of aneuploid events expected with significant rates of non-

disjunction. For example, the sterile histogram contains roughly 10 events in channels 200 to 220 and 5 events from 220 to 250 (Figure 11). Thirty percent non-disjunction of any chromosomal element from a sample of 5000 cells (the haploid region was gated for 10000 cells but the region contains equal amounts of X and Y sperm present as two distinct subpopulations) would result in 1500 cells containing more than the haploid DNA content and 1500 cells containing less than the haploid amount. Hyperhaploid cells would be located immediately to the right of the haploid peak in a higher channel corresponding to the size of the chromosomal element added to the complement by non-disjunction. Subtracting a maximum of 10 events from the channels to the immediate right of the normal haploid peak would not obscure the presence of such a large population of aneuploid cells.

CVs of the DNA histograms were calculated using CELL QUEST V3.0.1 (Becton and Dickinson, 1996) for the FILTER debris removal procedure by gating the entire haploid peak. This method of gating was chosen to minimize the errors in setting the CV markers. However it does not reflect the lowest CV of the haploid spermatid peak because it includes two distinct subpopulations; X bearing spermatid and Y bearing spermatid, within the marker boundary. For each raw data set analyzed with CELL QUEST, the CV of the haploid round spermatid peaks, diploid peak and tetraploid peak were calculated as well as the DNA index (DI; DI equals the value of a given peak divided by the value of the diploid peak: Dressler and Seamer, 1994) and percentage of cells per ploidy level (Hiddemann et al., 1984) for these peaks. Unmodified DNA histograms for the original data are referred to as RAW (Appendix B).

CVs were grouped into homozygous and heterozygous data sets. The distribution of both the RAW and FILTER data sets deviated significantly from normal (RAW goodness of fit to normal distribution, X^2= 29.83, 4 df; FILTER: X^2 = 17.22, 4 df). Therefore, a non-parametric Mann-Whitney U test was used to determine if the mean CVs between homozygotes and heterozygotes were significantly different. CVs of heterozygous individuals were also compared to the 95% confidence interval of the mean CV for homozygotes. Any CV for a heterozygote exceeding the 95% confidence limit would indicate significantly higher rates of DNA aneuploidy (Otto and Oldiges, 1980; McBee and Bickham, 1988). Due to the presence of a condensed spermatid peak to the left of the haploid peak that obscures subtle events in this region, only positive DNA aneuploidy was assessed, in the channels to the right of the haploid peak (Figure 9). Any non-disjunction or mal-segregation would result in equal numbers of negatively and positively aneuploid cells.

Results and Discussion

Relative chromosome size and DNA aneuploidy. -- Acrocentric chromosomes 7, 8, 9, 11, 14, 19, 23, X and Y were involved in Rb fusions. These chromosomes comprise the following proportions of the total haploid genome: 7 = 4.8 ± 0.5%; 8 = 4.7 ± 0.5%; 9 = 4.6 ± 0.8%; 11 = 3.1 ± 0.3%; 14 = 3.2 ± 0.4%; and 23 = 3.6 ± 0.4% (Table VIII). If non-disjunction of the smallest chromosome involved in a fusion (chromosome 11) can be detected by flow cytometry then all the other possible combinations of DNA

aneuploidy would also be detectable. The Rb fusions X.19 and Y.19 were fixed in this and all other populations *of D. richardsoni* (X.19 = 7.2 ± 0.9 and Y.19 = 2.0 ± 0.4%).

Robertsonian fusions present in the population are 7.14, 8.23, and 9.11, with individual male lemmings heterozygous for from 0 to 3 rearrangements (Table I). The metacentric derived from a 7.14 fusion comprises 8.0 ± 0.6% of the overall DNA content (Table VIII), fusion 8.23 involves 8.3 ± 0.6% of the total DNA, and fusion 9.11 includes 7.7 ± 0.6% of the DNA content. The difference in DNA content between the X and Y spermatid subpopulations is 5.2 ± 1.0%.

Theoretical limitations on the detection of DNA aneuploidy by flow cytometry have been described (Vindelov et al., 1983; Benson and Braylan, 1994). The resolution of two populations with similar DNA content is dependent on the extent of the DNA difference, the degree of non-disjunction, and the CV of the populations. Maximum resolution occurs when non-disjunction is high, the CV is low, and the difference in DNA content of aneuploid and non-aneuploid cells is large. As a rough estimate of the sensitivity of flow cytometry, aneuploid populations can usually be resolved as separate peaks if the DNA difference is greater than twice the value of the peak CV (Vindelov et al., 1983). For DNA differences less than this amount aneuploidy can be detected as a broadening of the peak CV; differences less than 2.1% cannot usually be detected (Vindelov et al., 1983). When the level of non-disjunction is less than 50%, the limitations on detection become more stringent (Benson and Braylan, 1994). The DNA differential required to detect two distinct peaks at 30% non-disjunction (the minimum level of non-disjunction required to produce eventual reproductive isolation under the stasipatric model: Barton, 1979; Futuyma and Mayer, 1980; Spirito et al., 1983; Sites

and Moritz, 1987) are considerably larger than those required at 50% non-disjunction (Benson and Braylan, 1994).

Empirically, the X and Y spermatid populations (50% non-disjunction) were resolved as separate peaks in all of the histograms; Table VIII, Appendix B). If this level of resolution is assumed to be the lowest possible level of resolution for this technique, none of the individual acrocentric chromosomes involved in the Rb fusions in *D. richardsoni* would be resolved as distinct peaks on a DNA content histogram (all individual acrocentrics represent less than 5.5% of the DNA complement and the minimum level of non-disjunction is 30%, not 50%). Nonetheless, even the smallest acrocentric, chromosome 11 at 3.1 ± 0.3% of the overall DNA content, would cause significant broadening of the haploid peak CV if rates of aneuploidy were 30% or more (Benson and Braylan, 1994). Non-disjunction of single metacentric chromosomes (Rb fusion products 7.14, 8.23 and 9.11 all with DNA contents over 7.5%) would all be visible as resolved individual peaks.

DNA content and CV. –Using MODFIT no additional peaks corresponding to aneuploid cell populations were detected in any of the DNA content histograms examined. Comparisons using either RAW or FILTER histograms for 0, 1, 2 and 3 Rb fusion carriers show no distinct aneuploid peaks or statistically significant differences between homozygotes and heterozygotes (Figure 12, Figure 13, Table IX). A Mann-Whitney U test revealed no significant differences between homozygotes (2n=46, no rearrangements; 2n=44, homozygous for a 9.11 fusion) and heterozygotes (2n=45, one 9.11 fusion; 2n=44, 7.14 8.23 fusions; 2n=44 8.23 9.11 fusions; 2n=43, 7.14, 8.23 9.11 fusions) for either of the treatments (RAW, $P = 0.24$; FILTER, $P = 0.11$). In fact, the

mean CV of heterozygotes, was consistently lower than that of homozygotes, although not significantly so (Table IX), in contrast to theoretical expectations. The 95% confidence interval for the CV of the homozygous group was 5.23 ± 0.25% (4.98% - 5.48%) for the RAW treatment, and 5.00 ± 0.29% (4.71% - 5.29%) for the FILTER treatment (Table IX). None of the heterozygote CVs was higher than the upper range value of the homozygotes for either of the debris removal procedures. All Robertsonian fusion states had very similar CVs, DNA indices, and percentage of cells in each ploidy level for the haploid, diploid and tetraploid peaks (Figure 12, Table X). In all the distributions, the round spermatid haploid peak represented approximately 45% of all cells, the diploid peak 9%, and the tetraploid peak 8% of cells. Contrary to expectations of negative heterosis, none of the heterozygotes had significantly elevated CVs relative to homozygotes.

Chromosomal speciation. --The central tenet of the stasipatric speciation model is that heterozygous chromosomal rearrangements cause non-disjunction or other meiotic complications, resulting in increased rates of aneuploidy and significantly reduced fertility. This reduction in fertility acts as a post-mating isolating mechanism between diverging populations. Populations that evolved by stasipatric or other class "A" models of chromosomal speciation (Sites and Moritz, 1987) must, therefore, have increased levels of aneuploidy associated with the presence of chromosomal rearrangements when heterozygous. No increase in DNA aneuploidy in heterozygotes relative to homozygotes was detected by flow cytometry for the Rb fusions present in the study population. In fact, no reduction in fertility was detected in heterozygote Rb fusion carriers in any of the procedures used to assess fertility (see also Chapter 2).

Therefore, the independent autosomal Rb fusions examined herein are not sufficiently negatively heterotic to cause reproductive isolation among populations in *D. richardsoni.*

Other studies investigating the effects of Rb fusions in diverse taxa have come to similar conclusions. In *Holichilus brasiliensis,* simple Rb fusions were found to have no affect on the fertility of heterozygous carriers (Nachmann, 1992). Similarly, Rb fusions did not affect the fertility of heterozygotes in *Sorex araneus* (Wallace and Searle, 1990; Mercer et al., 1992), *Rattus* (Baverstock et al., 1983), *Mus* (Wallace et al., 1990), or *Sceloporus grammicus* (Porter and Sites, 1987; Reed et al., 1992). Thus effectiveness of chromosomal rearrangements as reproductive barriers is likely case and taxon specific. If these results are applicable to other populations of *Dicrostonyx*, stasipatric speciation as proposed by White (1978), Hoffman (1981) and Modi (1987) for this genus seems an unlikely mechanism to explain the origin of species-level diversity. Since much of the recognized diversity of *Dicrostonyx* is based on the presence of chromosomal rearrangements to differentiate species, these results question the validity of species level taxonomy of the genus.

Despite these results, some chromosomal rearrangements could still play a role in speciation of *Dicrostonyx*. In this study, only a sample of independent centric fusions from a single population were analyzed. *D. richardsoni* is known to have a diploid number ranging from 2n=40 to 2n=46 (Engstrom, unpublished data) which suggests that there are three Rb fusions in this population. While the three Rb fusions investigated here do not display any negative heterosis, it is possible that an unknown Rb fusion exists that could be negatively heterotic and play a role in speciation.

Since only independent fusions were present in *D. richardsoni*, another model of chromosomal speciation, the monobrachial homology model (Capanna, 1982; Bickham and Baker, 1988), remains a plausible mechanism of speciation in *Dicrostonyx*. In this model, centric fusions are not independent and one or more metacentrics derived through Rb fusions in independent populations share one but not the other acrocentric arm. For instance, acrocentric **a** might fuse to acrocentric **b** to form a metacentric chromosome **a.b**. In heterozygotes for this Rb fusion a trivalent structure will form at metaphase I. However, if acrocentric **a** fuses to **b** to form **a.b** in one population and acrocentric **a** fuses to acrocentric **c** to form a metacentric **a.c** in another, isolated population, these derived metacentrics will be monobrachially homologous. In a heterozygote formed between the populations, acrocentric **b** will pair with metacentric **a.b**, acrocentric **c** will pair with metacentric **a.c** and **a.b** will pair with **a.c** (Figure 14). When the chromosomes align at metaphase I, instead of forming bivalent pairs, these monobrachially homologous chromosomes would form a chain multivalent made up of four chromosomes. Multivalents have increased rates of mal-segregation relative to a bivalent or trivalent structure, and the expected levels of non-disjunction are correspondingly higher (Capanna, 1982; Bickham and Baker, 1988; Ratamponirina et al., 1988; Mercer et al., 1992). In contrast to the stasipatric model, heterozygotes for simple fusions within an isolated population are not expected to suffer from negative heterosis, whereas monobrachially homologous fusions found in heterozygotes between populations suffer from meiotic breakdown. Therefore, fixation of novel simple rearrangements in individual, small, isolated populations is much more probable.

While no partially homologous fusions were found among individuals of *D. richardsoni*, they do occur among populations of *D. groenlandicus* (Jarrell, 1995; Engstrom, unpublished data). Partial homology of some chromosomal arms also occurs between *D. richardsoni* and other species of *Dicrostonyx*; including the sex chromosomes. A cross between *D. richardsoni* (X.19 Y.19) and *D. groenlandicus* (X.23 Y.23) would result in a minimum of two long chain configurations during meiosis due to the partial homology of the sex chromosomes. These chain conformation are expected to reduce the fertility in the offspring of such a cross, and indeed this occurs (Engstrom, unpublished data). It is unclear at this stage if the sex chromosomes themselves are responsible for the observed reproductive isolation between crosses of *D. richardsoni* and other species of *Dicrostonyx* or if the reproductive isolation is a result of the monobrachial fusions involved in these crosses. Further investigations into monobrachial fusions and their affect on fertility in *Dicrostonyx* may reveal more about the origin of species in this genus. In contrast, there is no evidence that individual, simple Rb fusions reduce fertility in heterozygotes, rendering the stasipatric model of speciation implausible in this genus.

CHAPTER 4: SUMMARY AND CONCLUSIONS

Stasipatric speciation

The cytological assessments performed on male collared lemmings indicate that there is no loss of fertility associated with increased heterozygosity for centric fusions. Animals possessing 0, 1 and 2 chromosomal rearrangements all produced viable, fertile offspring and heterozygous animals displayed no reductions in the other fertility assessments.

There was also no detectable negative heterosis among Rb fusion states. In all of the fertility tests, no negative heterosis was found for single or multiple independent fusions. Metaphase I pairing failure was observed in a single Rb heterozygote (2n=45, with one 9.11 fusion) and in a double Rb heterozygote (2n=44, with a 7.14 and a 8.23 fusion) but univalency was also seen in a Rb homozygote (2n=44, with two 9.11 fusions). Univalency was thus not restricted to heterozygotes and likely was present in these instances due to chance. In no case was the rate of pairing failure significantly different than zero. Despite slight variation among heterozygotes, there were no significant differences among these states for degree of negative heterosis. The principal tenet of chromosomal speciation, that chromosomal rearrangements must be negatively heterotic is falsified for the rearrangements in this population.

One criticism of using polymorphic characters for fertility assessments was expounded by King (1987). He noted that not all chromosomal rearrangements were

negatively heterotic and suggested that only those that could be demonstrated to reduce the fertility of heterozygous carriers could be implicated in the speciation process. Two examples of neutral rearrangements proposed by King were heterochromatic additions and polymorphic rearrangements. By this reasoning then, one could make an a priori conclusion that the Rb fusions present in this one population of *D. richardsoni* could not be in the same class as those involved in chromosomal speciation because they are polymorphic. This reasoning effectively renders the model unfalsifiable: it can only be tested after the fact (not during initial stages when polymorphism first arises) and any negative result is dismissed. By King's somewhat circular argument (1987) only those rearrangements which have become fixed and are demonstrably negatively heterotic can be tested (a posteriori) to see if they are sufficiently negatively heterotic to participate in speciation, despite the fact that any new rearrangement must be present in a population initially as a polymorphism.

Nonetheless, the effect of centric fusions on fertility are critical because it is these rearrangements that have been postulated to drive speciation in this genus (Hoffman, 1981; Modi, 1987; White, 1978). *Dicrostonyx richardsoni* is an excellent test group because it is variable for these rearrangements and, therefore, the effect of both individual and multiple fusions on meiosis can be determined without introducing the confounding variable of crosses between individuals with distinct genetic backgrounds.

While it is possible that only those fusions that become fixed in a population are potential isolating mechanisms (for example *D. groenlandicus* from Coral Harbor have 6 fixed, derived fusions, van Wynsberghe and Engstrom, 1992) preliminary results in hybridization of different chromosomal races suggest otherwise (Engstrom, 1997; pers.

comm.). Crosses among chromosomal races within *D. groenlandicus* that are homozygous for different Rb fusions have no loss in fertility. Crosses of *D. groenlandicus* with *D. richardsoni*, however, produce male offspring that are sterile or suffer from severe reductions in fertility. Even with King's caveat (1987) regarding polymorphism, crosses between populations with different fixed rearrangements should be at least partially sterile, if these rearrangements play a direct role in speciation as envisioned in the stasipatric model (Hoffman, 1981; Modi, 1987). Clearly, at least some autosomal rearrangements, whether fixed or polymorphic, are not sufficient to initiate reproductive isolation in collared lemmings.

Another potential pitfall of using laboratory populations to assess the effects of chromosomal rearrangements on fertility is that individual rearrangements have different effects against distinct genetic backgrounds. One of the classic mammalian examples of apparent chromosomal speciation via incorporation of Rb fusions is the house mouse, *Mus*. Preliminary studies on crosses of laboratory and wild races of mice that carried different Rb fusions (Cattanach and Mosely, 1973; Capanna et al., 1976; Winking and Gropp, 1976; White, 1978; Capanna, 1982) suggested that simple Rb fusions caused sterility in heterozygotes. More recently, Winking (1986) determined that crosses between laboratory and wild races of mice resulted in reductions of fertility in offspring even when there were no Rb fusions present. Further studies (Mittwoch et al., 1990; Wallace et al., 1992; Viroux and Bauchau, 1992) demonstrated that simple Rb heterozygotes in mice suffered no loss in fertility if mice with similar genetic backgrounds were involved (wild crosses). In the present study, all of the crosses made were of laboratory stock derived from a single population of wild caught *D.*

richardsoni. Because all of the lemmings were derived from the same locality, genetic background was controlled. Indeed, if any bias was introduced by crossing distinct local lineages within this meta-population it would have resulted in inflated estimates of negative heterosis. Hence, the results of this study indicating lack of expected effects are, if anything, conservative.

Other studies of Rb fusions in mammals have also indicated lack of negative heterosis for naturally occurring rearrangements. Marsh rats (*Holichilus brasiliensis*) are polymorphic for four Rb fusions; both simple and monobrachial (Nachmann, 1992). Cytological studies on this species showed no univalency, no increase in rates of non-disjunction, and no non-disjunction of the X or Y chromosomes for any Rb heterozygotes. As in *D. richardsoni*, Rb fusions did not reduce the fertility of heterozygotes in *H. brasiliensis*. Common shrews (*Sorex araneus*) are also polymorphic for Rb fusions with some populations being fixed for different fusions. Crosses between chromosomal races exhibited no reduction in fertility in simple Rb heterozygotes (Searle, 1990; Wallace and Searle, 1990) and no sterility but a small loss in fertility in monobrachial Rb heterozygotes that formed a chain of 7 chromosomes at metaphase I (Mercer et al., 1992). Goitered gazelles (*Gazella subgutturosa*; Kingswood et al., 1994) and cotton rats (*Sigmodon fulviventer*; Elder and Pathak, 1980) showed no indication of fertility reduction in simple Rb heterozygotes as measured by pairing at diakenisis/metaphase I. Likewise, in crosses between subspecies of *Lemur fulvus*, no negative heterosis was associated with Rb heterozygosity (Ratamponirina et al., 1988). A minor reduction in fertility was detected in certain interspecific crosses and major reductions in fertility were apparent in other interspecific crosses. In each instance of

infertility, monobrachial rearrangements were involved. It was unclear however whether the reduction in fertility was caused by the chromosomal rearrangements or by the different genetic backgrounds of the species hybridized.

In contrast, in species in which Rb fusions do not commonly become established in populations, these fusions often cause partial or complete sterility (i.e. humans, Chandley et al., 1986; Conn et al., 1998). This conundrum harkens back to the well recognized paradox of stasipatric speciation: to be sufficiently negatively heterotic to act as a reproductive barrier to hybridization between populations, a rearrangement must have a large negative selection value when heterozygous. In most cases, such a rearrangement would quickly be eliminated from the population in which it arose. In populations and species (including *Dicrostonyx*) where particular classes of rearrangements commonly become established, they typically have little effect on meiosis of heterozygous carriers (White, 1973; Hale and Greenbaum, 1988), regardless of theoretical expectations.

Collared lemmings (*Dicrostonyx*) are polymorphic for Rb fusions, and vary in diploid number from 2n=28 to 2n=48 in North America (Jarrell and Fredga, 1993). Rausch and Rausch (1972) suggested that at least some of these chromosomally distinct populations represented distinct species. Currently, anywhere from 2 to 11 species of *Dicrostonyx* are currently recognized worldwide based largely on the presence of Rb fusions in different populations (Hoffmann, 1981; Corbet and Hill, 1991; Musser and Carleton, 1993; Jarrell and Fredga, 1993, Engstrom et al., in press). Given that the primary characters used to recognize these species are chromosomal, it is not surprising that chromosomal speciation has been proposed for this genus (Hoffmann, 1981; Modi,

1987; White, 1978). My data indicate, however, that any species recognized based on differences in simple autosomal fusions are suspect, and that species-level taxonomy of this group is likely in need of revision. Thus it seems highly unlikely that differences in individual autosomal Rb fusions between species would result in reproductive isolation in this genus.

Other chromosomal speciation models

The morphology of the sex chromosomes in *D. richardsoni* are distinct from those of any other species of *Dicrostonyx* (Engstrom et al., 1993). *Dicrostonyx richardsoni* has the sex chromosome-autosome fusion X.19 Y.19, *D. hudsonius* has the ancestral euchromatic condition for the genus (Modi, 1987) but has a heterochromatic second arm on the X and Y, and the *D. groenlandicus* species complex has a X.23 Y.23 fusion. Crosses between different chromosomal races/species of the *D. groenlandicus* complex demonstrated that many of the purported species and distinct chromosomal races are interfertile (Engstrom, 1997; pers. comm.). Conversely, crosses between *D. groenlandicus* and *D. richardsoni* produce males that are sterile or suffer from extreme reductions in fertility (Scott and Fisher, 1983; Engstrom, 1997; pers. comm); an effect which continues through the F_2 generation (this study; Engstrom, unpublished data). These breeding results support a hypothesis that the sex chromosome-autosome fusions may play a disproportionate role in speciation. Sex chromosomes play a greater role in sterility and inviability than autosomes (Jablonka and Lamb, 1991) and the effects of X chromosome aberrations on meiotic arrest have been well documented. Whether by X activation that causes deleterious transcription (Forejt, 1982) or X-multivalent associations that halt spermatogenesis (Johannison and Winking, 1994) the sex

chromosomes are often implicated in male sterility. Further breeding studies and investigations into the behavior of the sex chromosomes during species crosses should clarify the role of the sex chromosomes in speciation of this genus. If negative heterosis is found to be the main mechanism of spermatogenesis breakdown then chromosomal speciation may have occurred to generate extant species of *Dicrostonyx* which differ in morphology of sex chromosomes.

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Tables

Table I: Robertsonian fusions in *D. richardsoni* investigated by G-band analysis. Females were not used for assessment of fertility.

Diploid number and Rb state	Lemmings
2n=46, no rearrangements	AR9602, AR9601, CHLB45, CHLB108
2n=45, 7.14	CH9431, CHLB06 (female)
2n=45, 9.11	CHLB30, CHLB66, CHLB83, CHLB84, CHLB87, CHLB109
2n=44, 7.14 8.23	CHLB104
2n=44, 7.14 9.11	CHLB10 (female)
2n=44, 8.23 9.11	CH9402, CHLB02, CHLB57, CHLB19 (female)
2n=44, 9.11 9.11	CH9417, CHLB48, CHLB85
2n=43, 7.14 8.23 9.11	CHLB24, CHLB102
2n=43 7.14 9.11 9.11	CHLB20 (female)
2n=42 7.14 7.14 9.11 9.11	CHLB26 (female)
2n=42 7.14 8.23 9.11 9.11	CHLB05 (female)

* see Appendix A for catalogue numbers

Table II: Number of paired and unpaired chromosomes at early metaphase I in *D. richardsoni.*

		number of pairs (observed/expected)			number of cells with unpaired	
* Rb state	n	autosomal biv	trivalents	XYbivalents	biv / triv	XY
2n=46	30	660/660	0/0	30/30	0	0
2n=45 7.14	33	660/660	33/33	33/33	0	0
2n=45 9.11	31	660/660	33/33	30/31	0	1
2n=44 7.14 8.23	31	558/558	62/62	30/31	0	1
2n=44 8.23 9.11	31	558/558	62/62	31/31	0	0
2n=44 9.11 9.11	31	651/651	0/0	30/31	0	1
2n=43 7.14 8.23	30	496/496	90/90	30/30	0	0
TOTAL	217	4243/4243	280/280	214/217	0/217	3/217

Table III: Chiasma counts and chiasma position for each Robertsonian fusion state (as labelled following the diploid number). The mean count per chiasma position was assessed for significant differences by comparison to the 2n=46 homozygous state (* significant).

* Rb state	n	proximal	interstitial	terminal
2n=46	31	7.032 ± 0.135	2.258 ± 0.139	13.71 ± 0.141
2n=45 7.14	33	6.273 ± 0.170 *	2.818 ± 0.197	13.515 ± 0.185
2n=45 9.11	31	6.742 ± 0.113	2.387 ± 0.144	13.806 ± 0.134
2n=44 7.14 8.23	31	5.355 ± 0.119 *	2.710 ± 0.223	14.968 ± 0.288 *
2n=44 8.23 9.11	31	5.742 ± 0.092 *	2.871 ± 0.206 *	14.387 ± 0.216 *
2n=44 9.11 9.11	31	6.770 ± 0.226	2.226 ± 0.189	13.097 ± 0.229 *
2n=43 7.14 8.23	30	5.200 ± 0.111 *	3.133 ± 0.261 *	14.767 ± 0.252 *

* Rb state	n	distal	total
2n=46	31	6.097 ± 0.117	29.097 ± 0.117
2n=45 7.14	33	5.697± 0.119 *	28.303 ± 0.141 *
2n=45 9.11	31	5.677 ± 0.108 *	28.613 ± 0.110 *
2n=44 7.14 8.23	31	4.452 ± 0.102 *	27.484 ± 0.112 *
2n=44 8.23 9.11	31	5.129 ± 0.101 *	28.129 ± 0.101 *
2n=44 9.11 9.11	31	5.677 ± 0.134 *	27.774 ± 0.129 *
2n=43 7.14 8.23	30	4.433 ± 0.114 *	27.533 ± 0.124 *

Table IV: The number of total chiasmata with the exclusion of distal chiasmata. P-values computed relative to the 2n=46 homozygous state.

	2n=45 7	2n=45 9	2n=44 7 8	2n=44 8 9	2n=44 9 9	2n=43 7 8 9
p values	0.1019	0.3486	0.4819	0.4219	0.0000 **	0.2132

** the homozygous 2n=44 9 9 state is significantly different from the homozygous 2n=46 state because both of the 9.11 metacentrics are joined into a single bivalent structure at metaphase I thereby reducing the total number of chiasmata by 1.

Table V: Mass and size of testes of homozygous and heterozygous Robertsonian fusion carriers. None of the variables were significantly different between heterozygotes and homozygotes.

		Testes data		
Homozygotes	Rb state *	weight (g)	length (mm)	width (mm)
CHLB45	2n=46	0.12	7	5
CHLB108	2n=46	0.09	5	4
CHLB48	2n=44, 9.11 9.11	-	8	6
CHLB85	2n=44, 9.11 9.11	0.08	5	3
CH9417	2n=44, 9.11 9.11	0.13	6	4
Average		0.11	6.20	4.40
Heterozygotes				
CH9431	2n=45, 7.14	-	3	2
CHLB109	2n=45, 9.11	0.13	7	5
CHLB30	2n=45, 9.11	-	8	6
CHLB66	2n=45, 9.11	-	6	4
CHLB83	2n=45, 9.11	0.1	6	4
CHLB84	2n=45, 9.11	0.12	6	4
CHLB87	2n=45, 9.11	0.09	5	3
CH9402	2n=44, 8.23 9.11	-	3	2
CHLB57	2n=44, 8.23 9.11	-	7	5
CHLB104	2n=44, 7.14 8.23	0.14	7	5
CHLB102	2n=43, 7.14 8.23 9.11	0.13	7	5
Average		0.12	5.91	4.09

Table VI: Litter sizes sired by males homozygous and heterozygous for Robertsonian fusion.

		Litter size (# of offspring surviving at 30d)								
Homozygotes	Rb state	Litter #	#2	#3	#4	#5	#6	#7	#8	Average
CHLB45	2n=46	3	1	2	4	4				2.8
CHLB48	2n=44, 9.11 9.11	3								3
CH9417	2n=44, 9.11 9.11	2	2	3	2	2				2.2
Heterozygotes										
CH9431	2n=45, 7.14	6	6	2	3	2	4	3	1	3.4
CHLB30	2n=45, 9.11	2	2	4						2.7
CHLB57	2n=44, 8.23 9.11	2	4							3

Table VII: Cell density calculated from transverse histological sections of seminiferous tubules. There was no significant difference between Robertsonian fusion states.

* Rb state	cell density (± SD)
2n=46	288.00 ± 25.29
2n=44 9.11 9.11	304.00 ± 40.95
2n=45 9.11	309.25 ± 15.56
2n=44 7.14 8.23	299.00 ± 21.29
2n=43 7.14 8.23 9.11	314.75 ± 42.65

t-tests p-values:

* Rb state	2n=46	2n=45 9	2n=44 7 8	2n=44 9 9
2n=45 9.11	0.233			
2n=44 7.14 8.23	0.594	0.222		
2n=44 9.11 9.11	0.476	0.674	0.751	
2n=43 7.14 8.23 9.11	0.486	0.823	0.488	0.75

Table VIII: The relative amount of DNA per chromosome in *D. richardsoni*. The % DNA content represents the relative length of each chromosome averaged from 10 independent karyotypes.

relative measurements				
number	% DNA	SD		
1	7.9	0.5		
2	7.7	0.5		
3	7.1	0.2		
4	6.4	0.5	DNA content of metacentric 7.14	
5	5.9	0.3	7	4.8 ± 0.5
6	5.8	0.4	14	3.2 ± 0.4
7	4.8	0.7	7.14	8.0 ± 0.6
8	4.7	0.5		
9	4.6	0.8	DNA content of metacentric 8.23	
10	4.3	0.5	8	4.7 ± 0.5
11	3.1	0.3	23	3.6 ± 0.4
12	2.9	0.3	8.23	8.3 ± 0.6
13	3.4	0.2		
14	3.2	0.4	DNA content of metacentric 9.11	
15	2.7	0.2	9	4.6 ± 0.8
16	2.4	0.2	11	3.1 ± 0.3
17	2.3	0.3	9.11	7.7 ± 0.6
18	1.9	0.3		
20	1.3	0.2	Multiple rearrangements	
21	2.4	0.4	7.14 8.23	16.3 ± 1.1
22	2.4	0.3	7.14 9.11	15.7 ± 1.2
23	3.6	0.4	8.23 9.11	16.0 ± 1.1
X	7.2	0.8	7.14 8.23 9.	24.0 ± 1.6
Y	2	0.4		
TOTAL	100			

Table IX: Coefficient of variation (CV) calculated from RAW data and the FILTER debris removal procedure (see text). None of the treatments were significantly different between homozygotes and heterozygotes

a) Data used for determination of aneuploidy

		Calculated CVs in %					
		Homozygous				Heterozygous	
Animal	Rb state*	RAW	FILTER	Animal	Rb state*	RAW	FILTER
AR9601	2n=46	5.19	4.78	CHLB30	2n=45 9.11	5.51	5.17
CHLB45	2n=46	5.72	5.57	CHLB83	2n=45 9.11	4.30	3.97
CHLB108	2n=46	5.51	5.41	CHLB84	2n=45 9.11	4.23	3.78
CHLB48	2n=44 9.11 9.11	5.43	5.26	CHLB87	2n=45 9.11	4.23	3.95
CHLB85	2n=44 9.11 9.11	4.31	3.97	CHLB109	2n=45 9.11	5.11	4.62
				CHLB104	2n=44 7.14 8.23	4.90	4.42
				CHLB57	2n=44 8.23 9.11	5.63	5.17
				CHLB102	2n=43 7.14 8.23 9.1	5.34	4.92

b) Mean CVs (CV) and 95% confidence interval (CI)

	Homozygotes		Heterozygotes	
	CV ± SD	95% CI	CV ± SD	95% CI
RAW	5.23 ± 0.25	4.75 - 5.71	4.91 ± 0.21	4.50 - 5.31
FILTER	5.00 ± 0.29	4.43 - 5.56	4.48 ± 0.19	4.11 - 4.86

TableX: The coefficient of variation (CV), DNA index (DI) and percent of cells (%) in each ploidy level before debris removal procedures (RAW).

		Haploid			Diploid			Tetraploid		
Lemming	Rb state *	CV	DI	%	CV	DI	%	CV	DI	%
AR9601	2n=46	5.19	0.51	47.78	3.50	1.00	7.27	1.50	1.96	10.38
CHLB45	2n=46	5.72	0.50	46.32	3.90	1.00	9.75	1.56	1.92	7.77
CHLB108	2n=46	5.51	0.51	44.67	2.63	1.00	9.42	1.43	1.97	8.80
CHLB48	2n=44 9.11 9.11	5.43	0.50	39.57	3.66	1.00	11.58	1.62	1.90	6.47
CHLB85	2n=44 9.11 9.11	4.31	0.50	45.04	2.49	1.00	10.73	1.40	1.96	13.74
RACH30	2n=45 9.11	5.51	0.50	43.11	4.59	1.00	12.22	1.48	1.90	6.11
CHLB83	2n=45 9.11	4.30	0.50	42.13	2.43	1.00	10.64	1.33	1.96	11.84
CHLB84	2n=45 9.11	4.23	0.50	47.06	2.58	1.00	7.87	1.27	1.96	9.56
CHLB87	2n=45 9.11	4.23	0.50	47.21	2.68	1.00	11.52	1.60	1.95	8.85
CHLB109	2n=45 9.11	5.11	0.51	45.57	2.75	1.00	9.14	1.43	1.96	9.51
CHLB104	2n=44 7.14 8.23	4.90	0.50	45.43	2.77	1.00	7.65	1.45	1.97	6.72
CHLB57	2n=44 8.23 9.11	5.63	0.51	46.05	3.29	1.00	11.38	1.66	1.93	9.08
CHLB102	2n=43 7.14 8.23 9.1	5.34	0.50	44.27	2.88	1.00	6.94	1.42	1.97	7.74

Figures

Figure 1: The distribution of Collared Lemmings (*Dicrostonyx*) in North America by cytotypes.

Figure 1

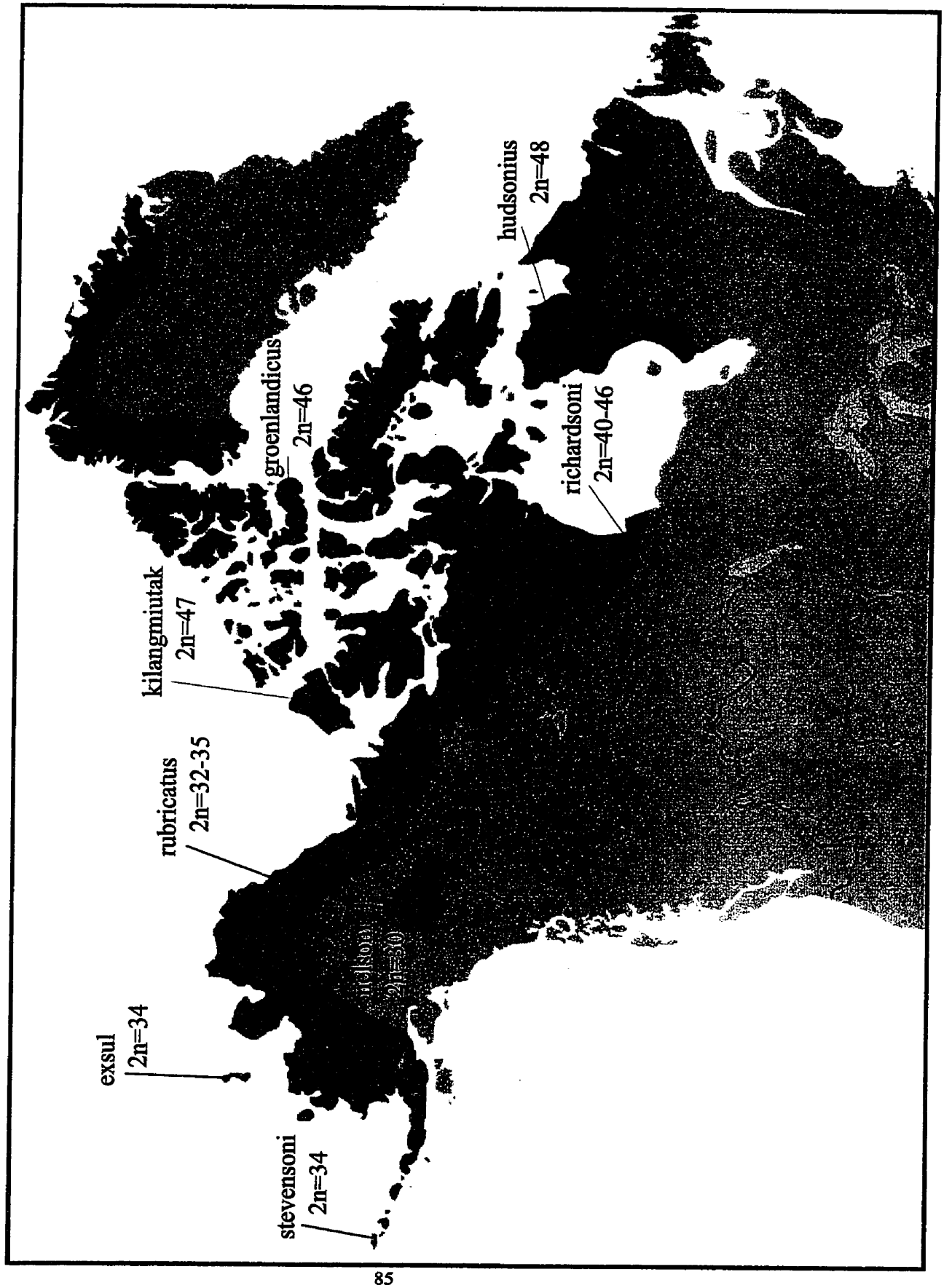

exsul
2n=34
stevensoni
2n=34
rubricatus
2n=32-35
kilangmiutak
2n=47
groenlandicus
2n=46
richardsoni
2n=40-46
hudsonius
2n=48

Figure 2: Species of North American *Dicrostonyx*: i) *D. hudsonius*. ii) *D. groenlandicus*. iii) *D. richardsoni*.

Figure 2

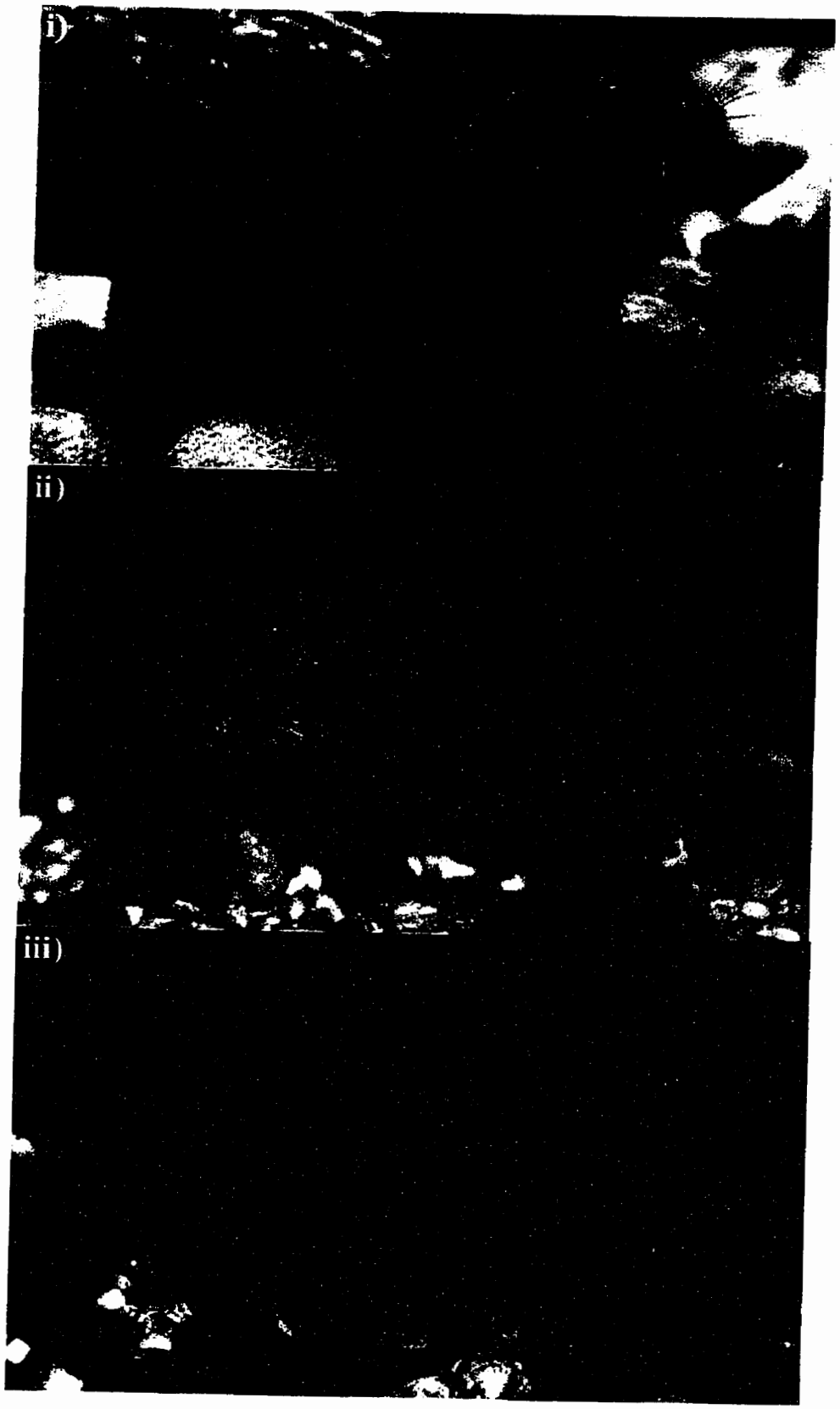


Figure 3: Metaphase I stage of the meiotic division in *D. richardsoni*. Trivalents are indicated by **triv** and the sex chromosome bivalent by **XY**.

Figure 3

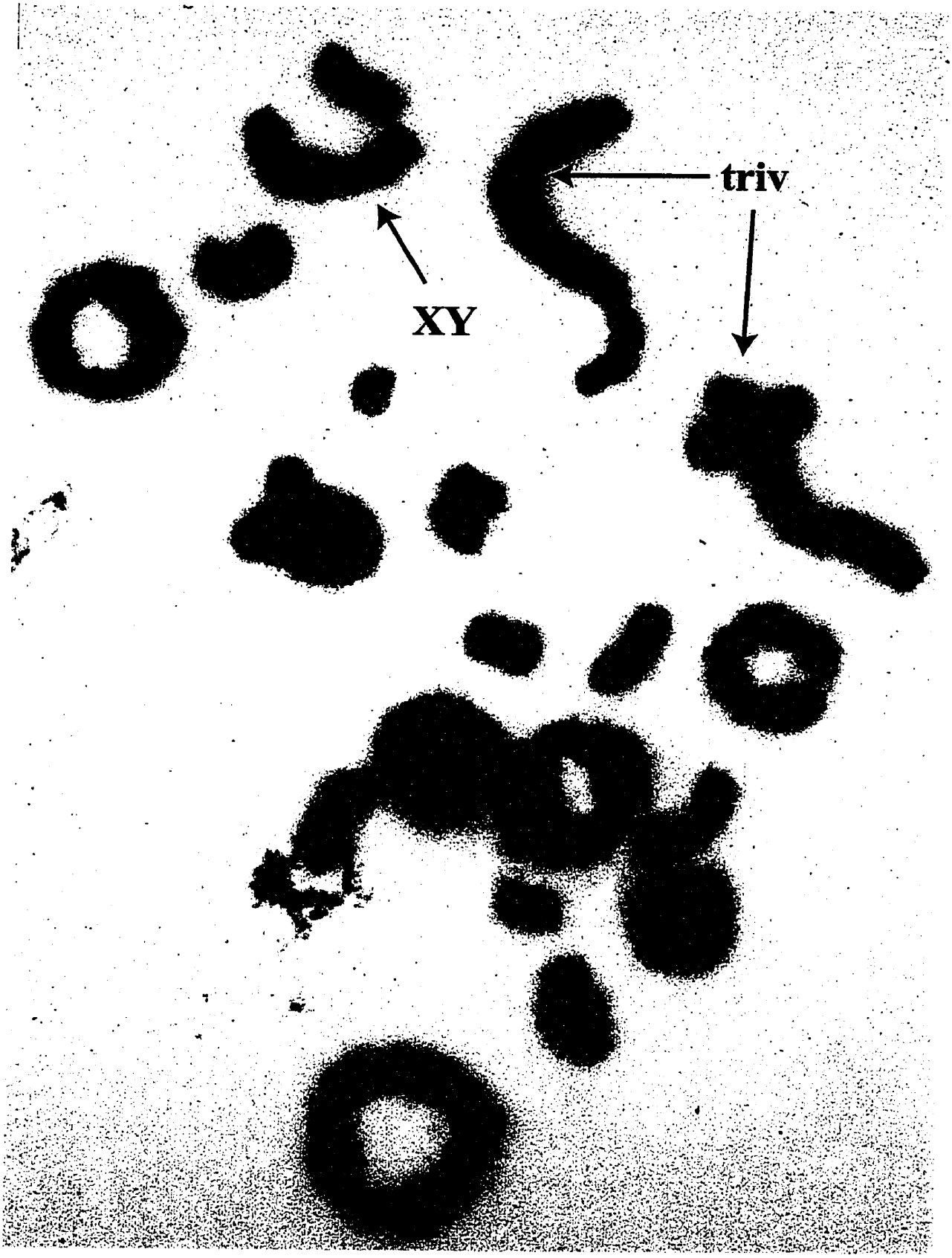


Figure 4: Diagramatic representation of a Robertsonian fusion (Rb). Acrocentric chromosomes a and b fuse to form the metacentric a.b. In the heterozygous condition, there is only one metacentric a.b and a trivalent structure is formed at metaphase I. In the homozygous condition, two metacentrics are formed and a bivalent is present at metaphase I.

Figure 4

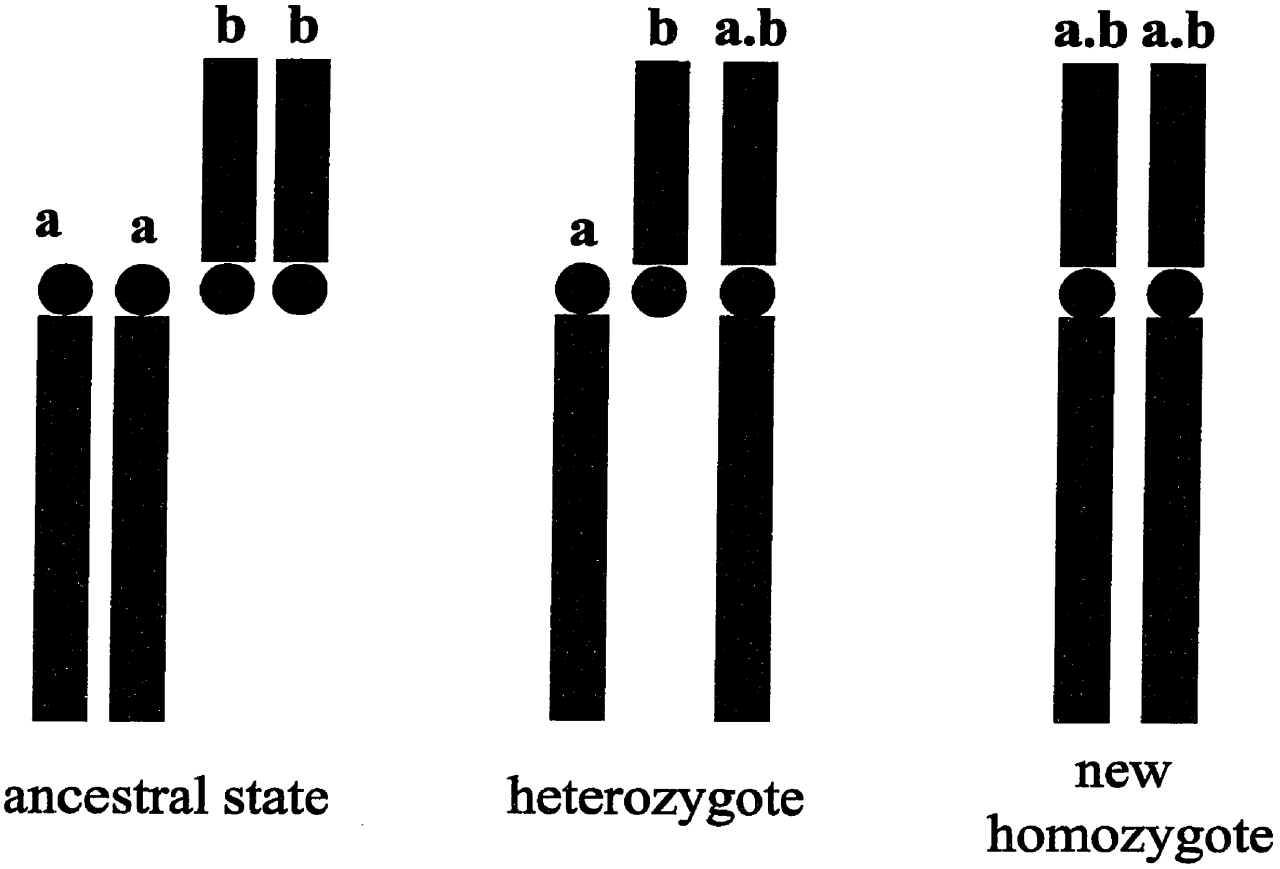

a a
b b
ancestral state
a
b a.b
heterozygote
a.b a.b
new
homozygote

Figure 5: Chiasmata positions and trivalent conformations. a) Chiasmata were scored as proximal, terminal, interstitial, or distal depending on their location (after Nachmann, 1992). b) Trivalents contained two chiasmata and could therefore, assume three unique conformations. Chiasmata were scored based on the conformation of the trivalent structure.

Figure 5

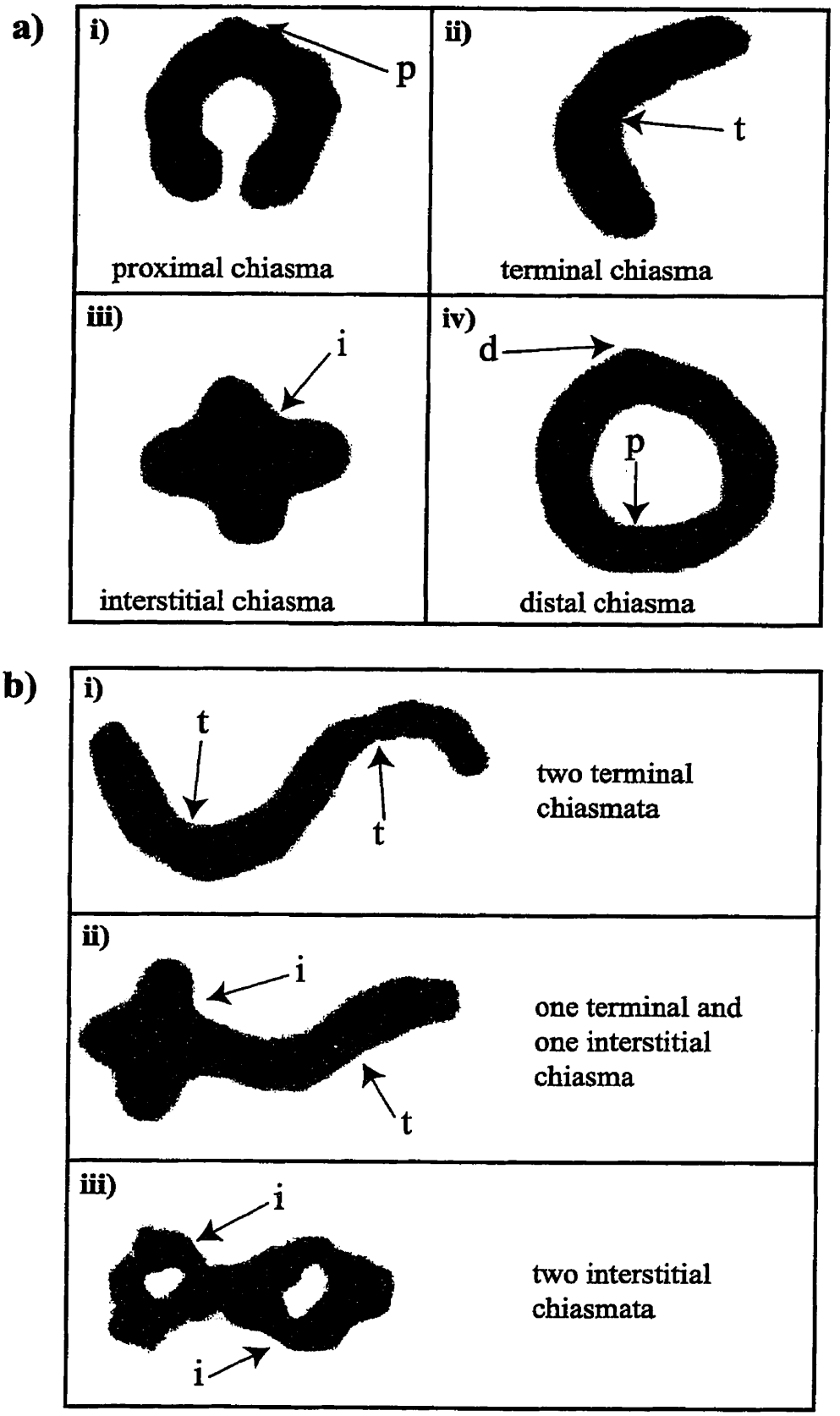

a)
i)
p
proximal chiasma
ii)
t
terminal chiasma
iii)
i
interstitial chiasma
iv)
d
p
distal chiasma
b)
i)
t
t
two terminal chiasmata
ii)
i
t
one terminal and one interstitial chiasma
iii)
i
i
two interstitial chiasmata

Figure 6: G-band karyotypes of *D. richardsoni*. i) 2n=46 with no Rb fusions. This G-band was taken from a D. richardsoni from Arviat, N.W.T. and used as a reference. The majority of the karyotype remains unaltered after a fusion event therefore only the altered chromosomes are shown for each Rb state. ii) 2n=45, heterozygous for one 7.14 fusion. iii) 2n=45, heterozygous for one 9.11 fusion. iv) 2n=44, heterozygous for one 7.14 and one 8.23 fusion. v) 2n=44, heterozygous for one 7.14 and one 9.11 fusion. vi) 2n=44, heterozygous for one 8.23 and one 9.11 fusion. vii) 2n=44, homozygous for 9.11 fusion. viii) 2n=43, heterozygous for one 7.14, one 8.23, and one 9.11 fusion.

Figure 6

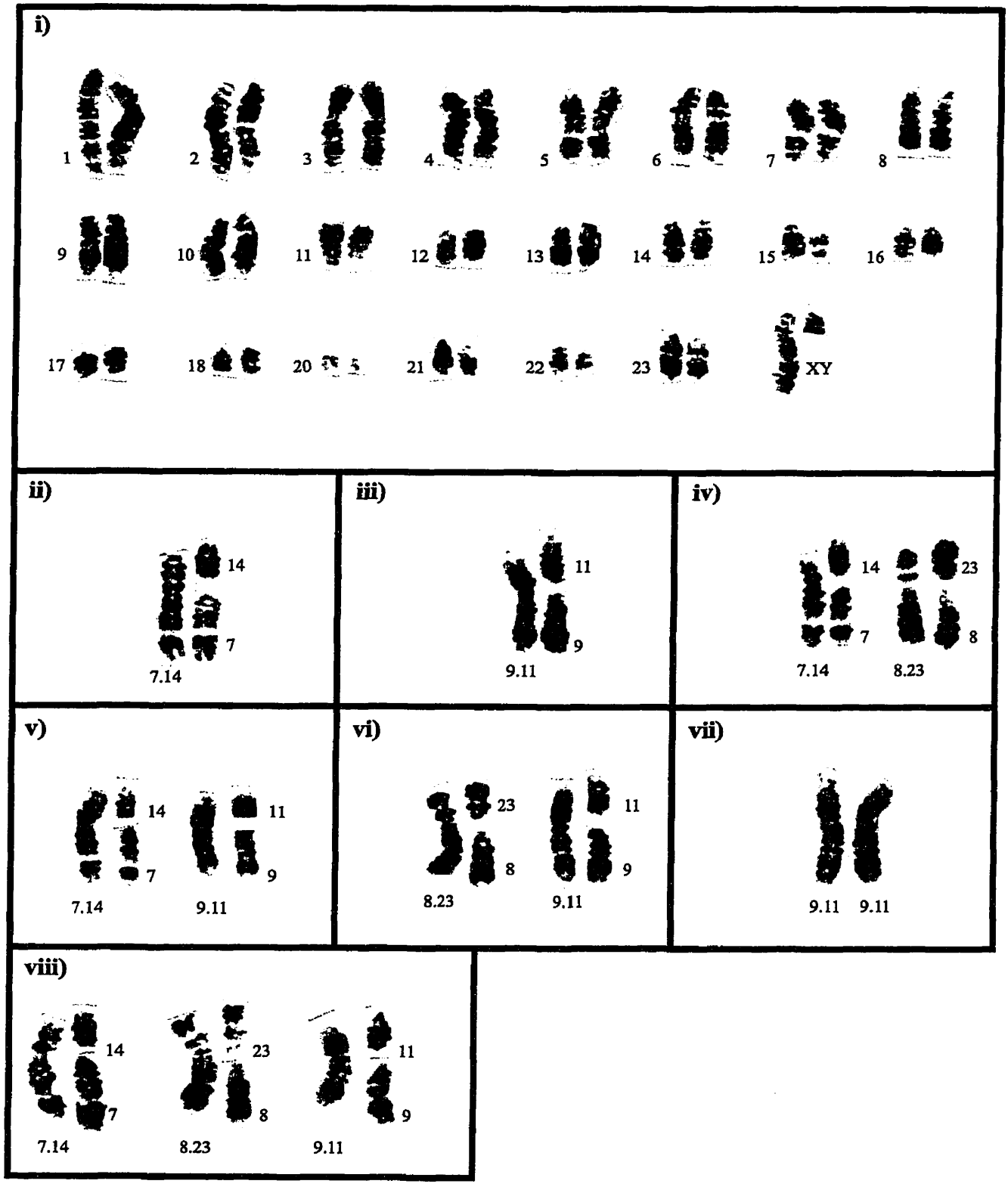

i)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
20
21
22
23
XY
ii)
14
7
7.14
iii)
11
9
9.11
iv)
14
23
7
8
7.14
8.23
v)
14
11
7
9
7.14
9.11
vi)
23
11
8
9
8.23
9.11
vii)
9.11
9.11
viii)
14
23
11
7
8
9
7.14
8.23
9.11

Figure 7: Meiotic karyotypes of *D. richardsoni*. All meiotic structures are bivalents except those labeled **triv** (trivalents) and **XY** (sex chromosomes). All karyotypes were taken from metaphase I cells. i) 2n=46, no Rb fusions. Only bivalents are present. ii) 2n=45, heterozygous for one Rb fusion. iii) 2n=44. Heterozygous for two Rb fusions. iv) 2n=44, homozygous for two fusions. v) 2n=43, heterozygous for three fusions.

Figure 7

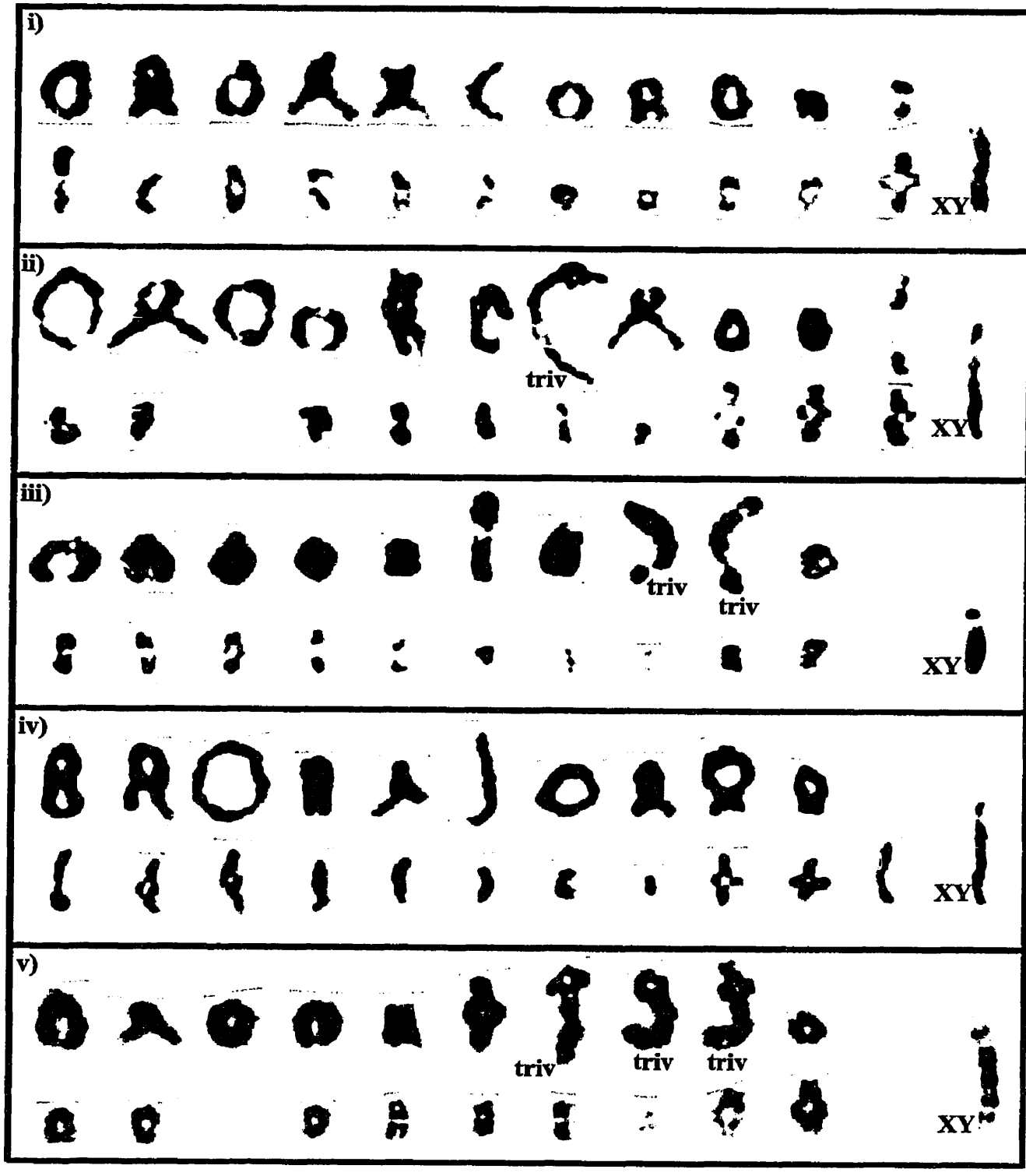


Figure 8: Example of histological sections of testes of *D. richardsoni*. Arrows indicate mature spermatids. i) *D. richardsoni*: 2n=46, homozygote. There are cells from all stages of the spermatogenic cycle and mature spermatid are present. ii) *D. richardsoni*: 2n=44, double heterozygote. There are cells from all stages of the spermatogenic cycle and mature spermatid are present. iii) *D. richardsoni* X *D. richardsoni/groenlandicus* sterile hybrid. There are very few cells present with no secondary spermatocytes or mature spermatids.

Figure 8

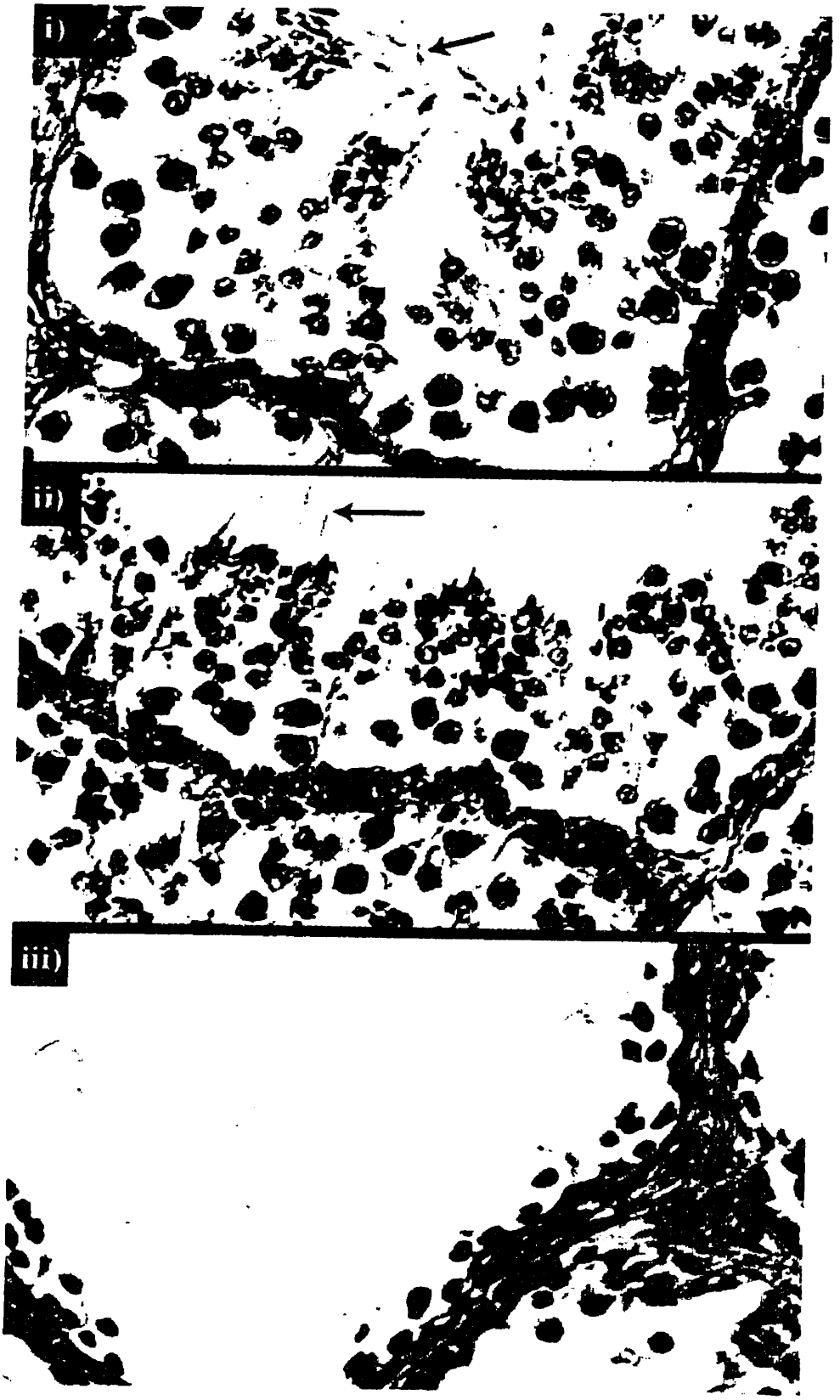


Figure 9: DNA histogram produced by flow cytometry of a heterozygous *D. richardsoni* (CHLB84, 2n=45, 9.11). The haploid peak is located at channel 200, the diploid at channel 400, and the tetraploid at channel 400. The X and Y spermatid subpopulations are clearly resolved into separate peaks. The condensed spermatid peak is located to the left of the haploid peak because mature spermatid are highly condensed and, therefore, do not bind stochiometrically to the florescent dye causing them to appear as though they have less DNA.

Figure 9

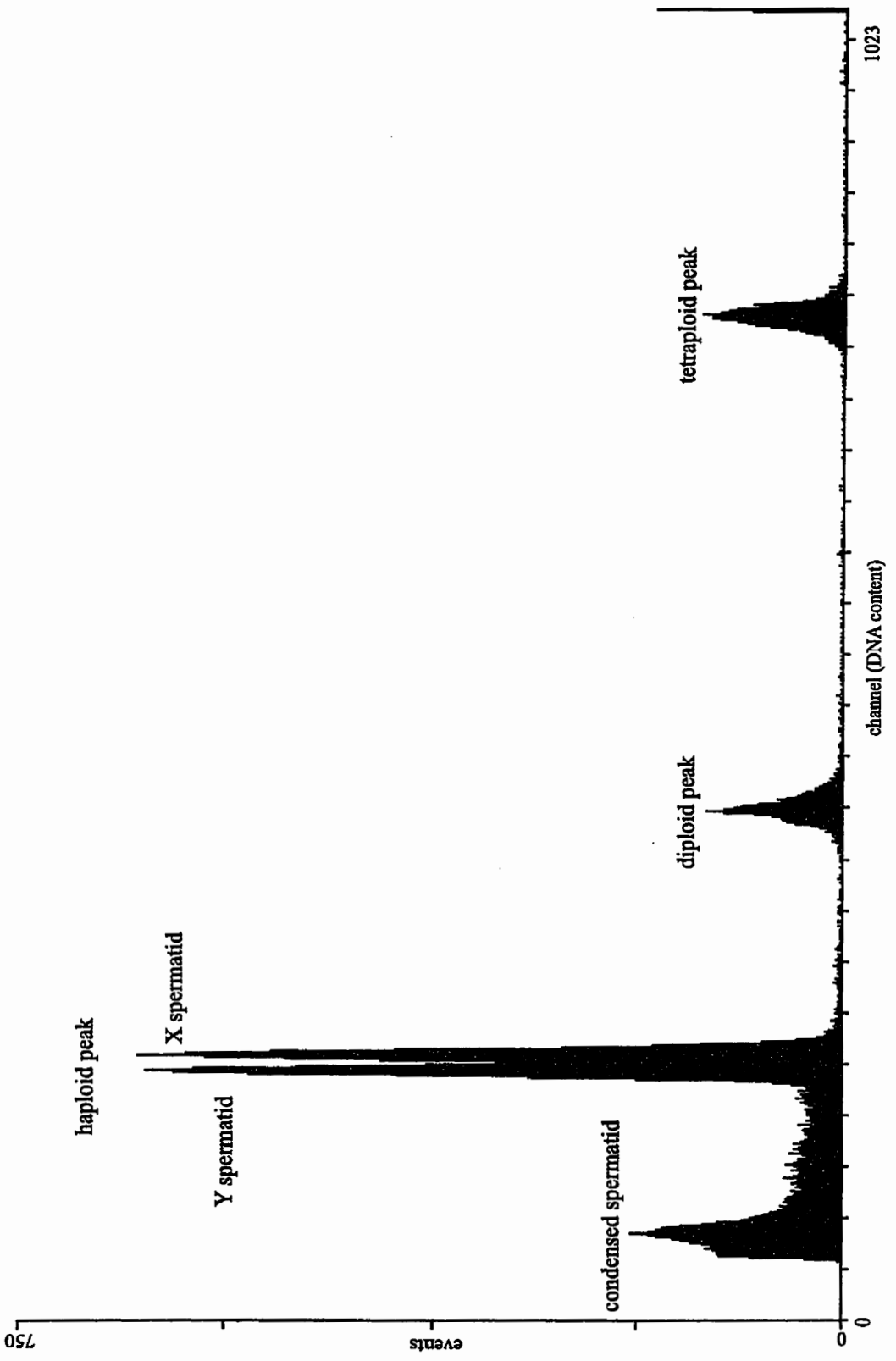

haploid peak
X spermatid
Y spermatid
condensed spermatid
diploid peak
tetraploid peak
events
channel (DNA content)
750
0
0
1023

Figure 10: The FILTER debris removal procedure. i) DNA histogram of 2n=45, 9.11 Rb heterozygote. CV of the haploid peak=4.23. ii) DNA histogram of *D. richardsoni* X *D. richardsoni/groenlandicus* hybrid. There are almost no cells in the haploid region. iii) Histogram ii) superimposed on histogram i). iv) The FILTER histogram produced by subtracting histogram ii) from histogram i). CV of the haploid peak =3.78.

Figure 10

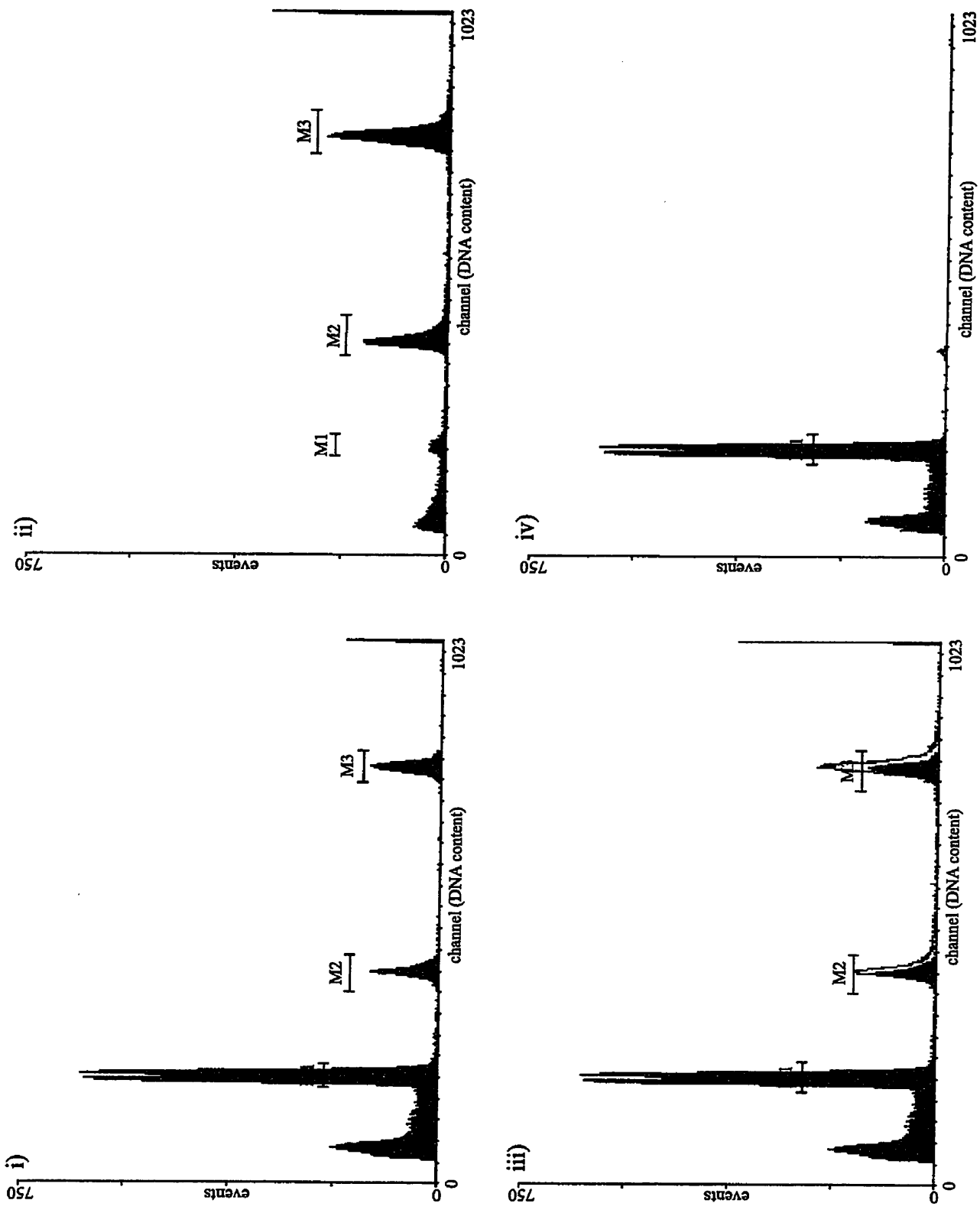
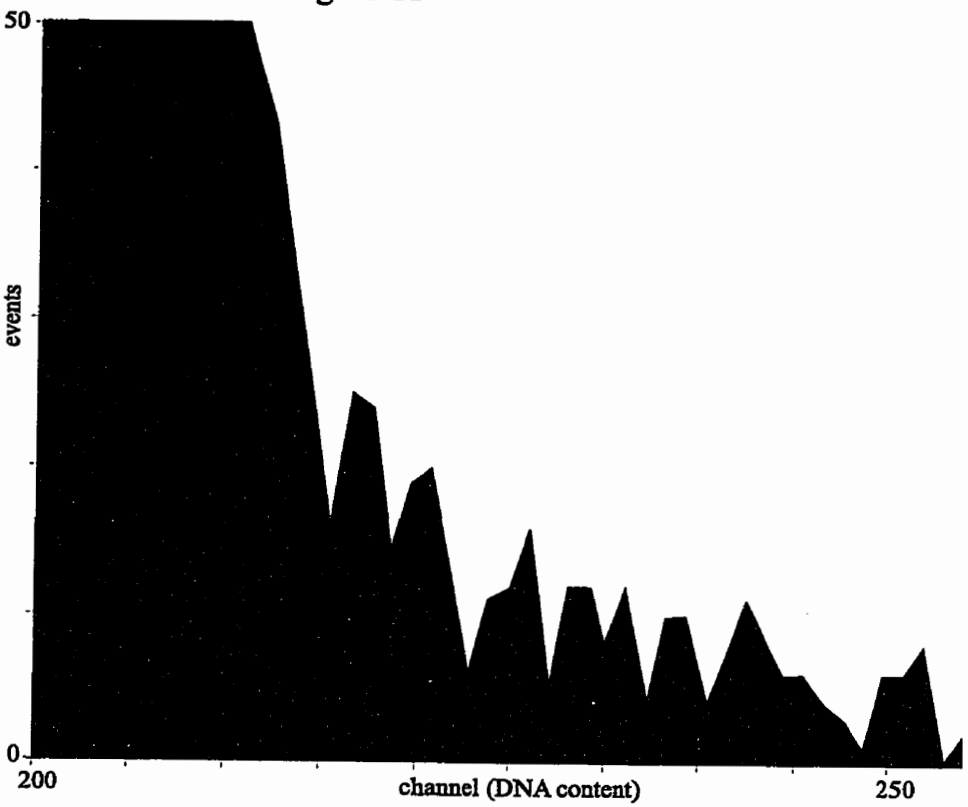


Figure 11: Magnification of channels 200 to 250, the region to the right of the haploid peak. i) 2n=45, 9.11 Rb heterozygote. The X spermatid peak is found up to channel 220. Channels 200 to 250 contain debris, averaging about 10 cells per channel. ii) *D. richardsoni* X *D. richardsoni/groenlandicus* hybrid. The majority of cells in the histogram are due to debris (there is a slight peak in channels 200 to 215 corresponding to the X spermatid subpopulation). Channels 220 to 250 contain about 5 cells per channel.

Figure 11

i)



ii)

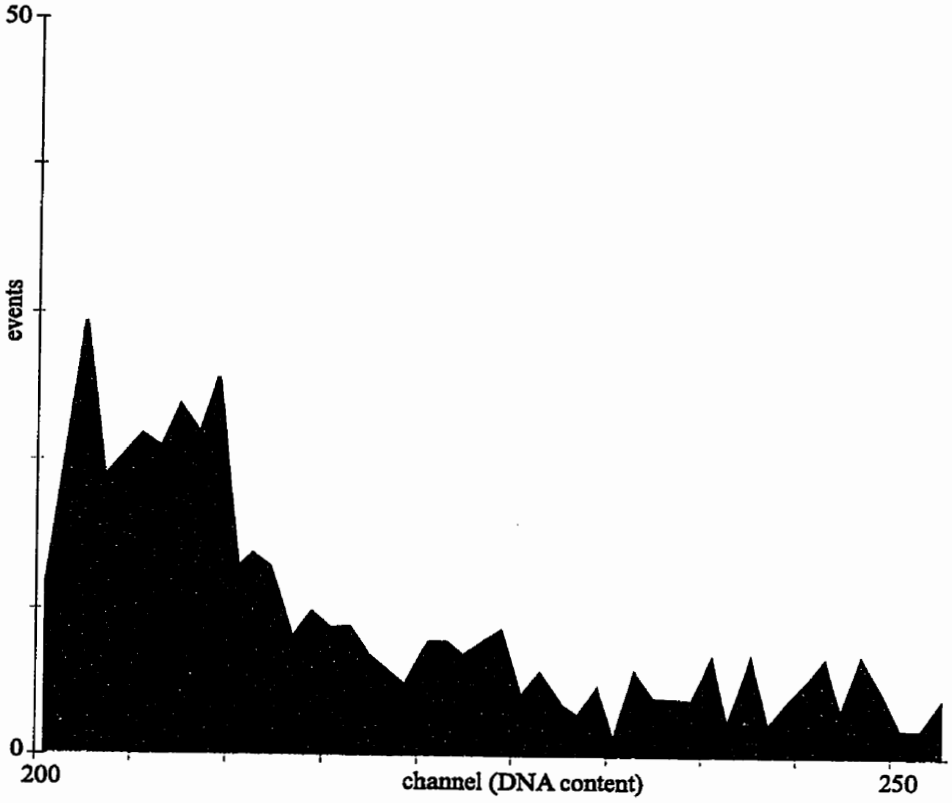


Figure 12: RAW DNA histograms. i) CHLB108, 2n=46, no Rb fusions. CV=5.51. ii) CHLB84, 2n=45, 9.11 Rb heterozygote. CV=4.23. iii) CHLB104, 2n=44, 7.14, 8.23 Rb heterozygote. CV=4.90. iv) CHLB102, 7.14, 8.23, 9.11, Rb heterozygote. CV=5.34.

Figure 12

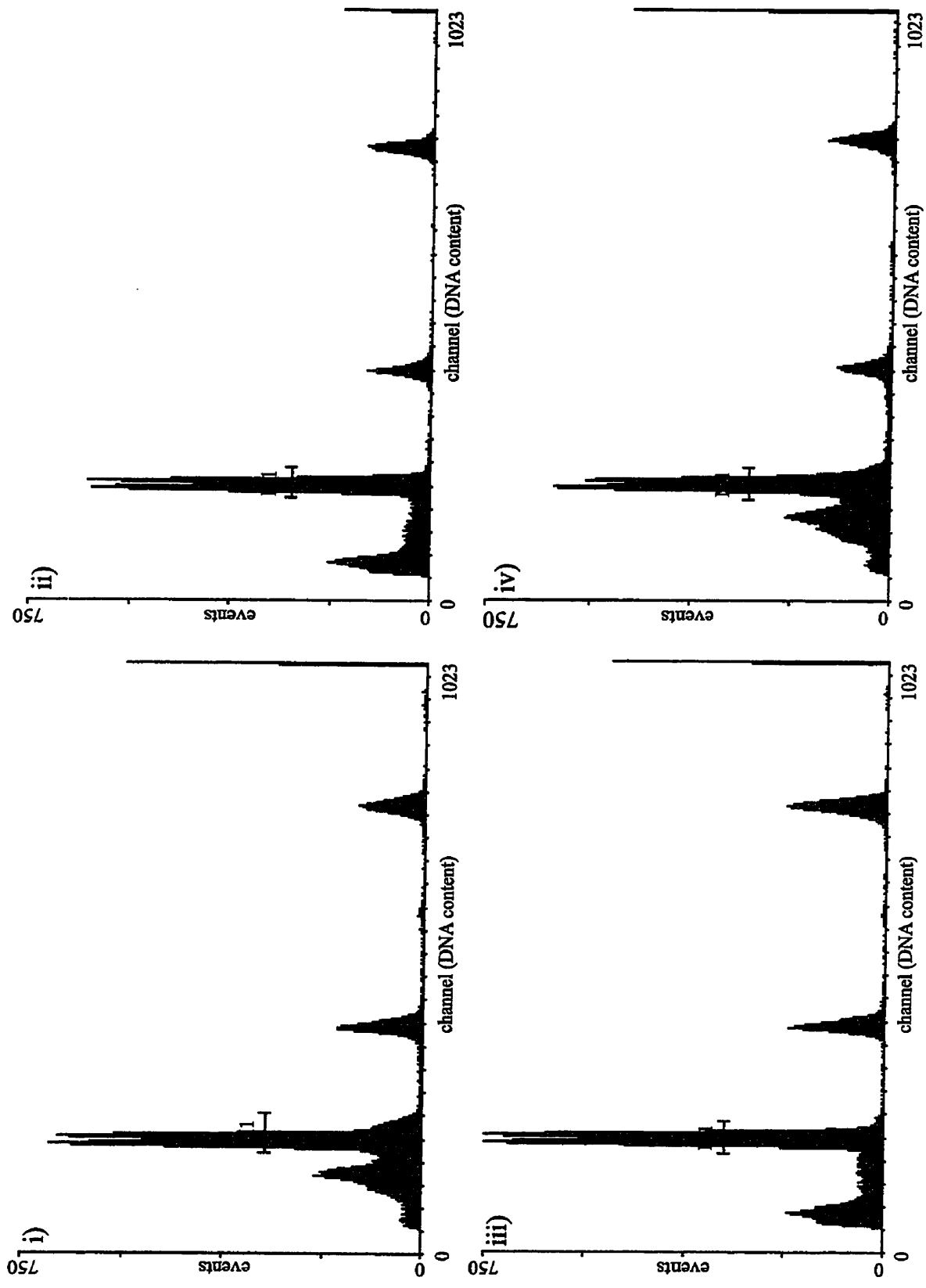


Figure 13: FILTER DNA histograms. i) CHLB108, 2n=46, no Rb fusions. CV=5.41. ii) CHLB84, 2n=45, 9.11 Rb heterozygote. CV=3.78. iii) CHLB104, 2n=44, 7.14, 8.23 Rb heterozygote. CV=4.42. iv) CHLB102, 7.14, 8.23, 9.11, Rb heterozygote. CV=4.92.

Figure 13

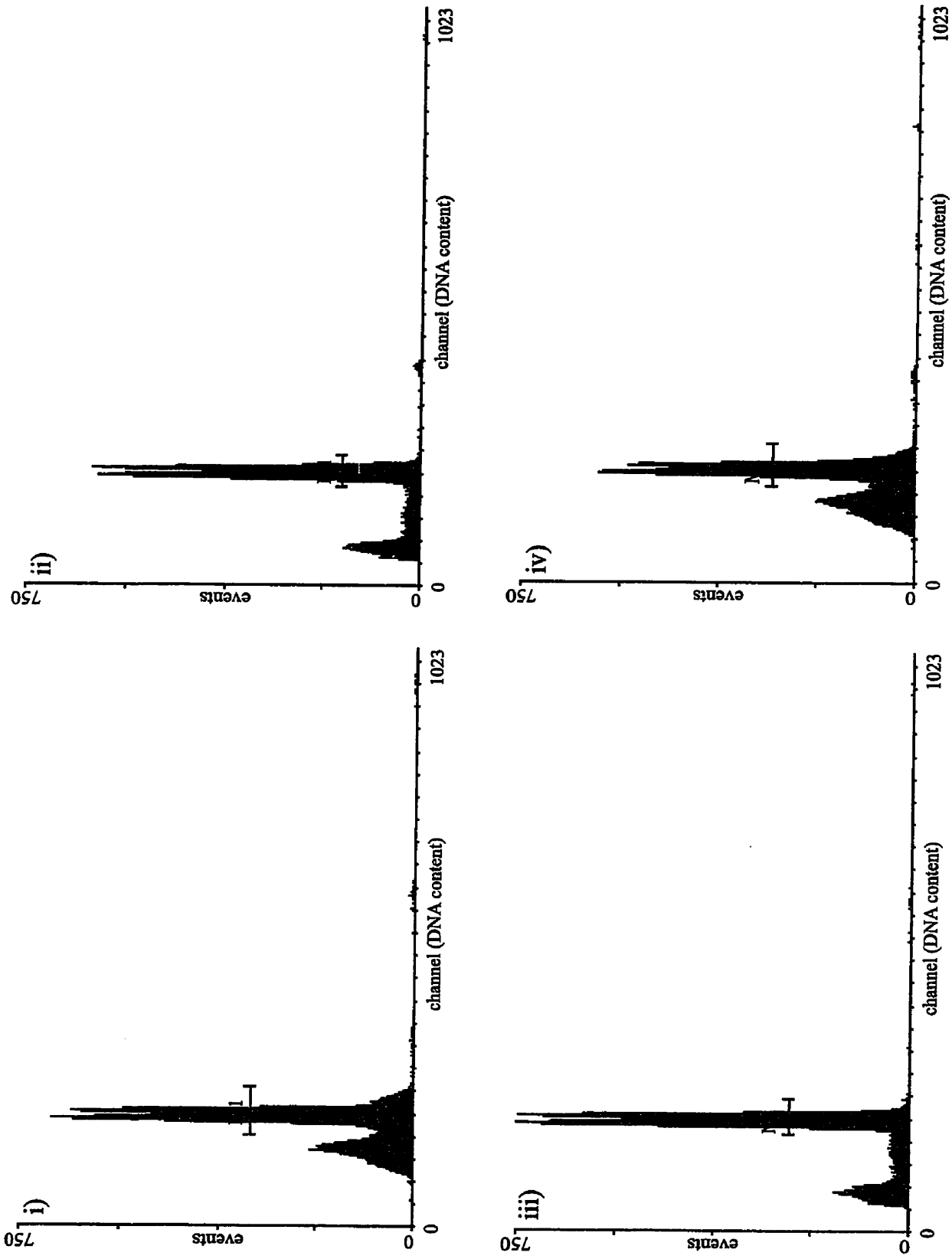
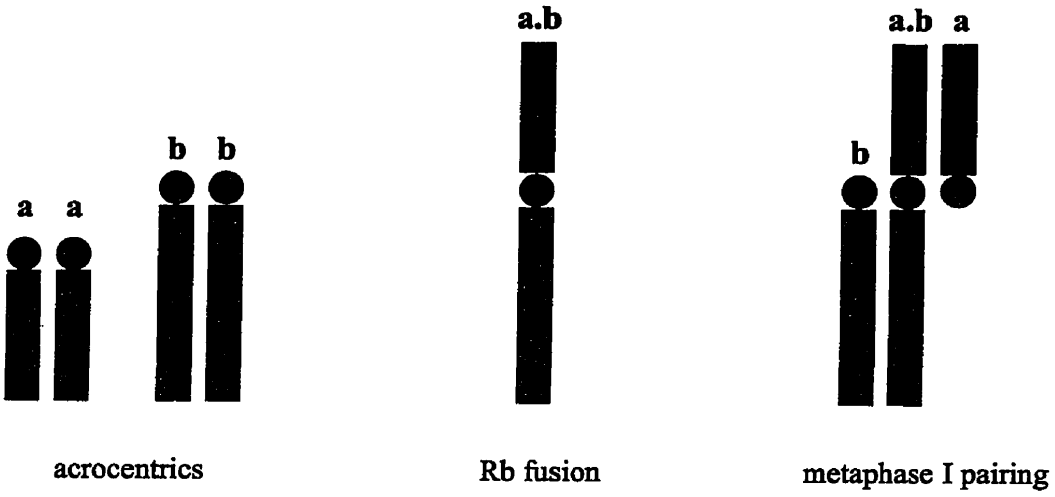


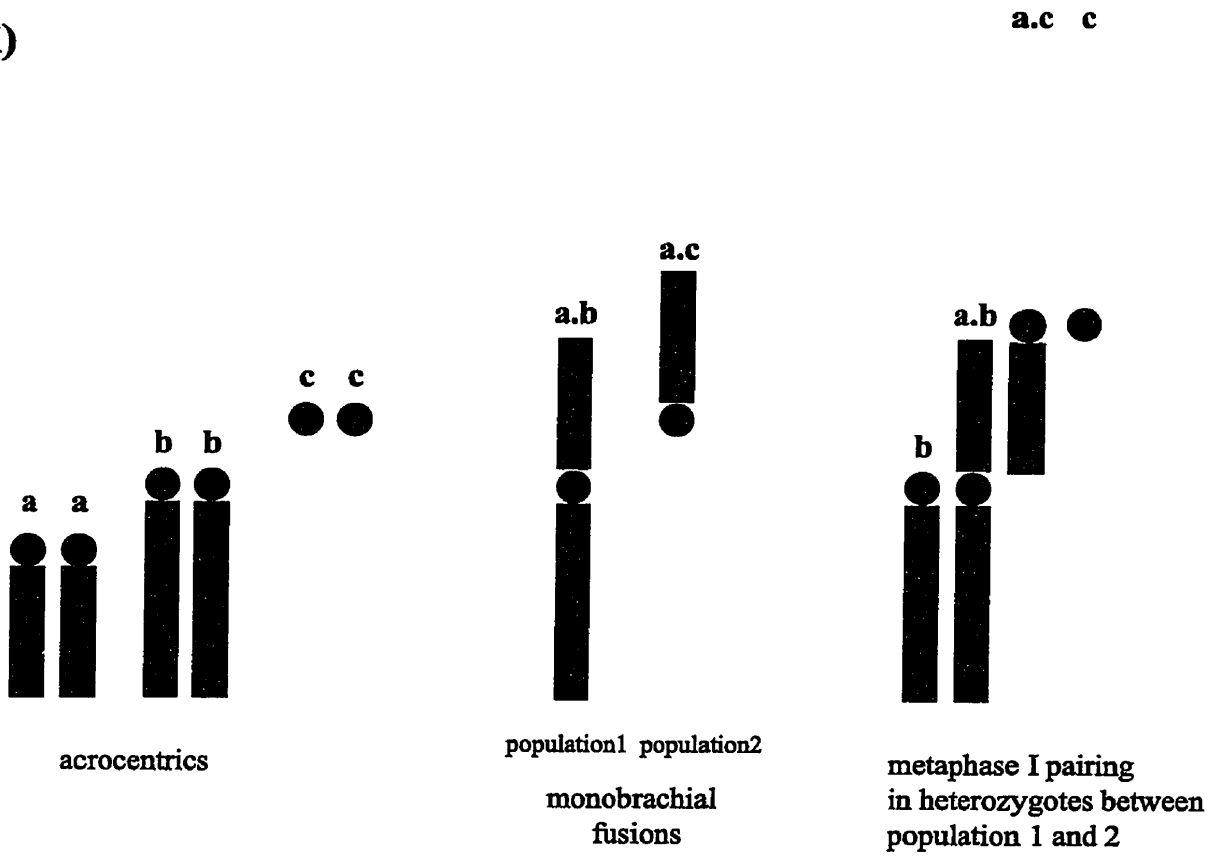
Figure 14: Diagramatic representation of Robertsonian and Monobrachially homologous fusions. i) Simple Rb fusion. **a** fuses to **b** to form metacentric **a.b**. At metaphase I, a trivalent is formed. ii) Monobrachially homologous fusion. **a** fuses to **b** to form metacentric **a.b** in popultion 1, and **a** fuses to **c** to form metacentric **a.c**. in population 2. Subsequent interbreeding between these populations results in heterozygotes for metacentrics which are monobrachially homologous. In these heterozygotes, at metaphase I, a four chromosome chain is formed.

Figure 14

i)



ii)



Appendix A

Specimens examined. In the following list, colony designation is the identification number in the breeding colony and ROM number is the catalogue number of the voucher specimen in the Royal Ontario Museum. Letters in the colony designation indicate the locality from which parental stock were obtained (*D. richardsoni*: AR=Arviat, N.W.T.; CH=Churchill, Manitoba; RA=Rankin Inlet, N.W.T.; RACH=Laboratory stock derived from a cross of Rankin Inlet and Churchill. *D. groenlandicus*: PP=Pearce Point, N.W.T. Interspecific hybrids: CH X PP= F1 hybrid, *D. richardsoni* (Churchill) X *D. groenlandicus* (Pearce Point); CH X CP= F2 backcross, *D. richardsoni* (Churchill) *X D. richardsoni/D. groenlandicus* hybrid.

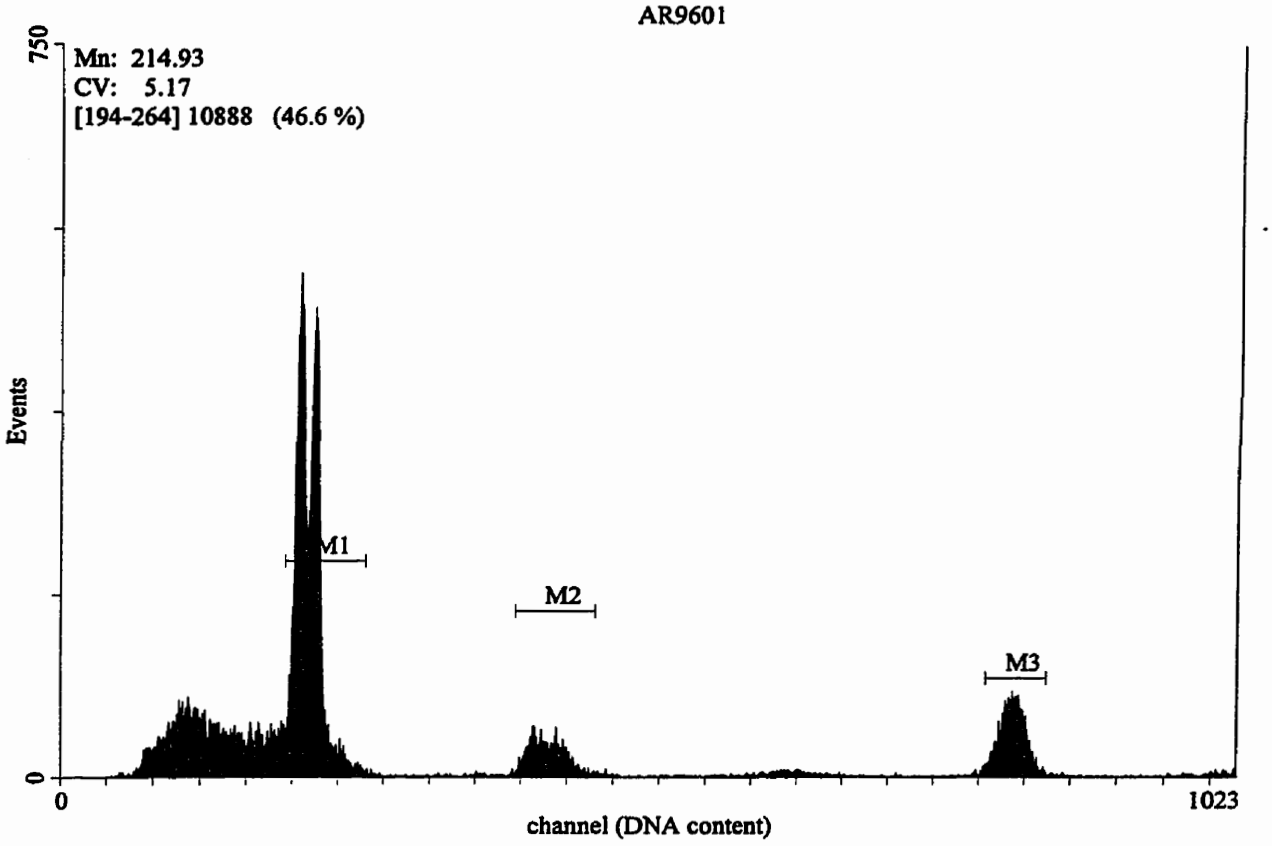
Appendix A

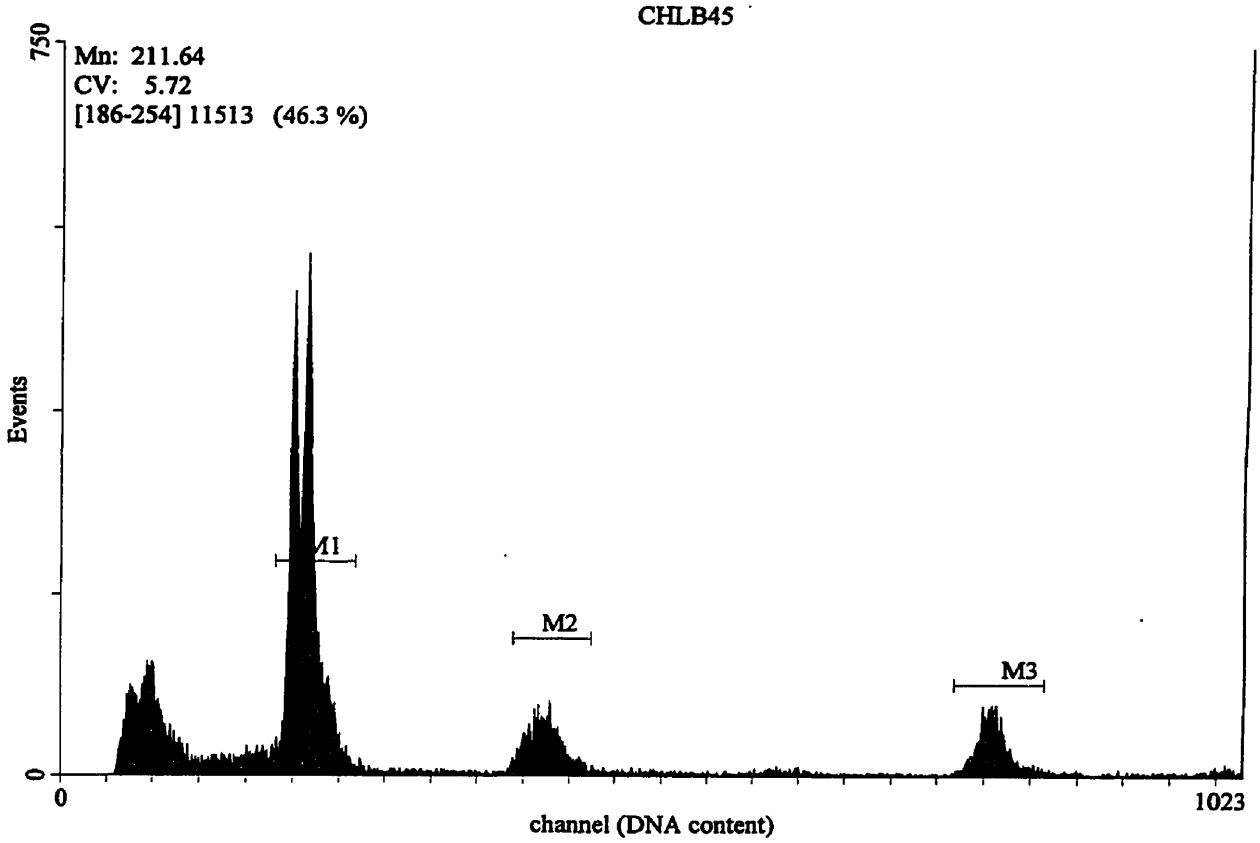
Dicrostonyx richardsoni used in investigation:

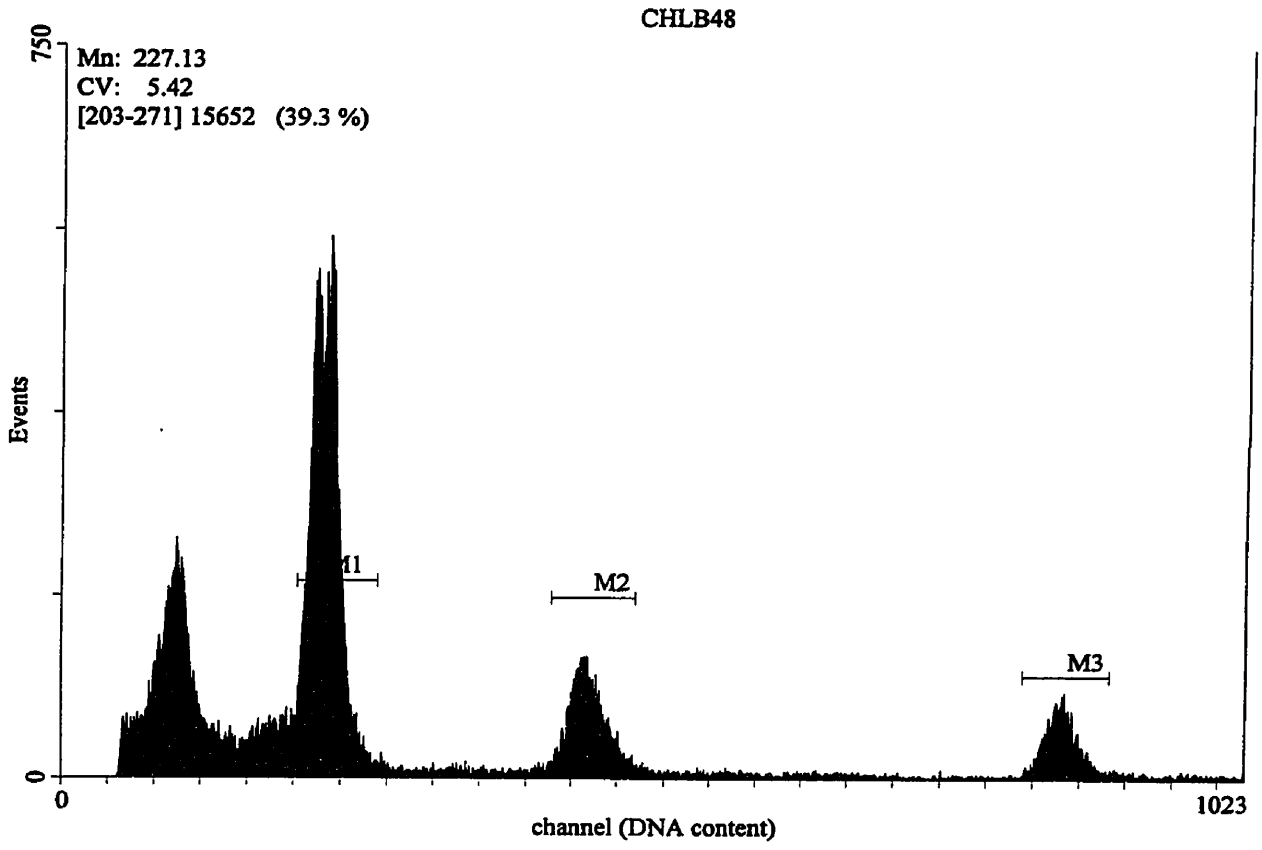
colony number	R.O.M. collection numbe	sex	Robertsonian state
AR9601	106210	M	2n=46
AR9602	106211	M	2n=46
CHLB02	103941	M	2n=44, 8.23 9.11
CHLB05	104086	F	2n=42, 7.14 8.23 9.11 9.11
CHLB06	104087	F	2n=45, 7.14
CHLB10	104088	F	2n=44, 7.14 9.11
CHLB18	103972	M	2n=45, 7.14
CHLB19	105082	F	2n=44, 8.23 9.11
CHLB20	105081	F	2n=43, 7.14 9.11 9.11
CHLB24	105083	M	2n=43, 7.14 8.23 9.11
CHLB26	105390	F	2n=43, 7.14 9.11 9.11
CHLB45	105856	M	2n=46
CHLB48	105857	M	2n=44, 9.11 9.11
CHLB57	105858	M	2n=44, 8.23 9.11
CHLB66	106279	M	2n=45, 9.11
CHLB83	106288	M	2n=45, 9.11
CHLB84	106289	M	2n=45, 9.11
CHLB85	106280	M	2n=44, 9.11 9.11
CHLB87	106281	M	2n=45, 9.11
CHLB102	106310	M	2n=43, 7.14 8.23 9.11
CHLB104	106314	M	2n=44, 7.14 8.23
CHLB108	106311	M	2n=46
CHLB109	106312	M	2n=45, 9.11
CH9402	103388	M	2n=44, 8.23 9.11
CH9417	105435	M	2n=44, 9.11 9.11
CH9431	105436	M	2n=445, 7.14
RACH12	102858	F	2n=45
RACH22	102860	M	2n=44
RACH30	105114	M	2n=44, 9.11
Hybrid lemmings			
CH X CP 02	106274	M	
CH X PP 02	106872	M	
CH X PP 10	106225	M	

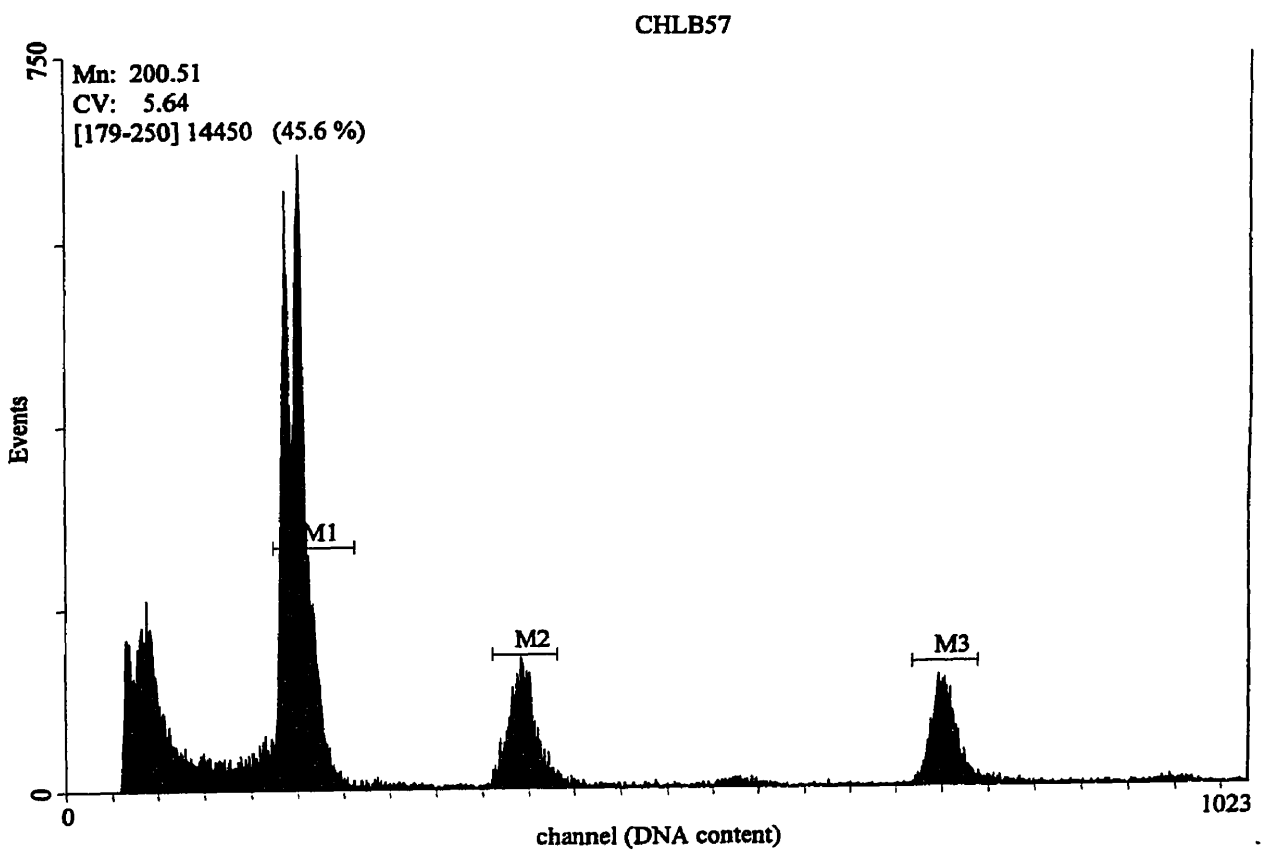
Appendix B

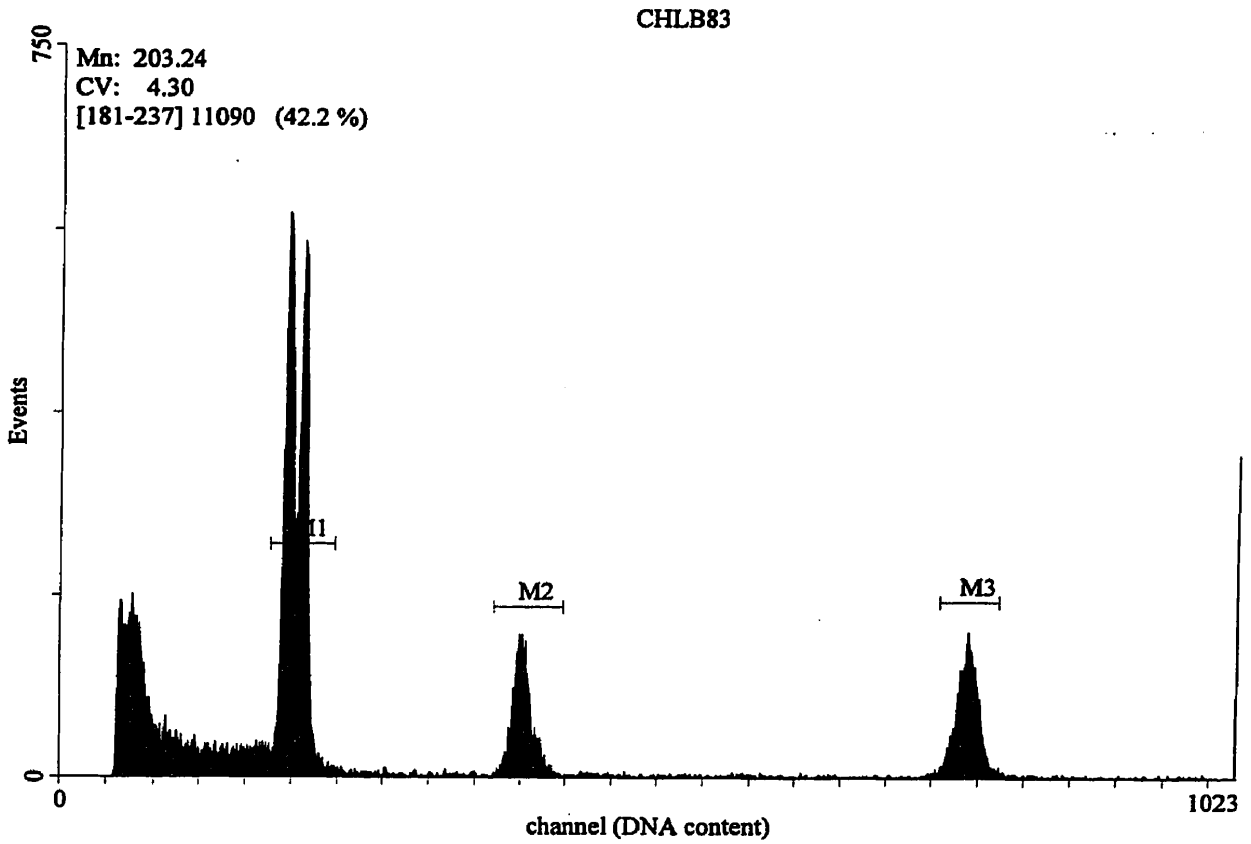
RAW DNA histograms for all *Dicrostonyx richardsoni* examined by flow cytometry.

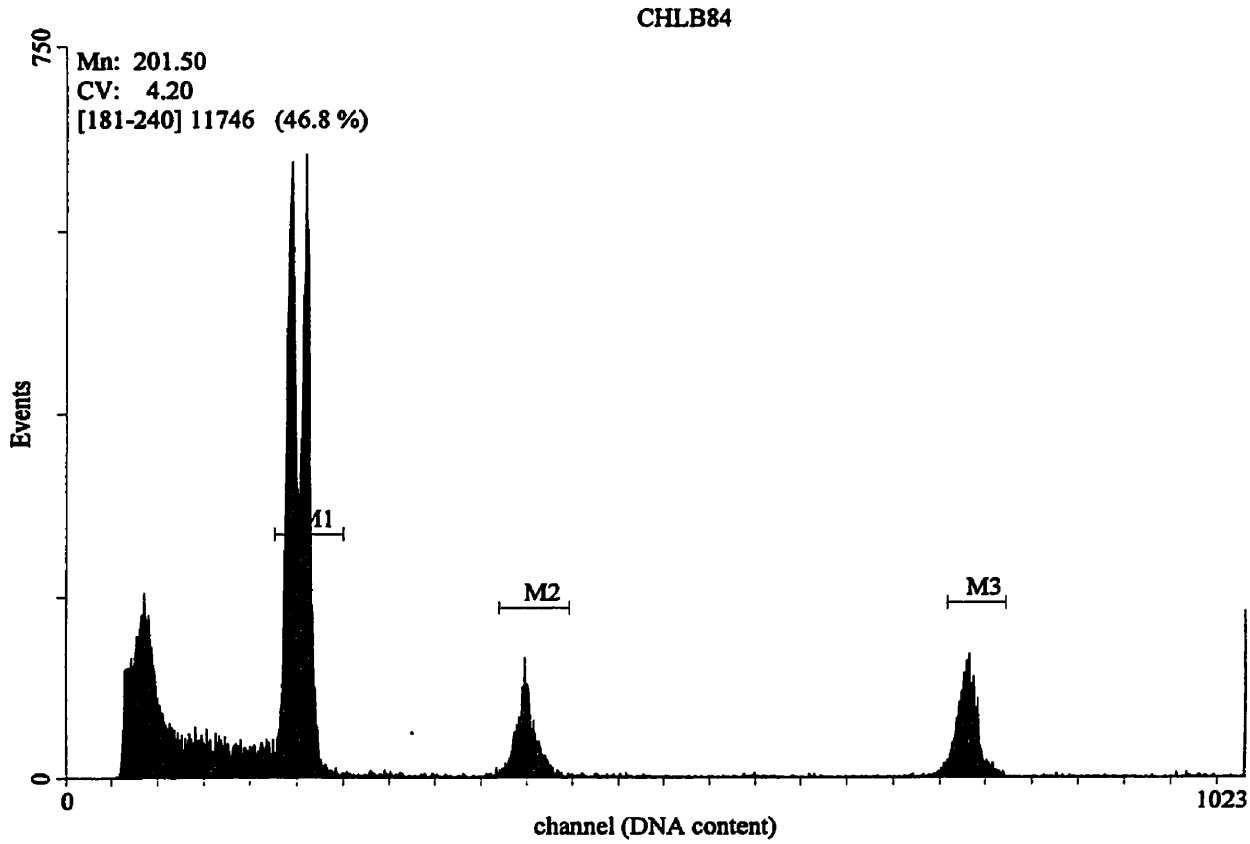

AR9601
Mn: 214.93
CV: 5.17
[194-264] 10888 (46.6 %)
750
0
Events
M1
M2
M3
0
1023
channel (DNA content)

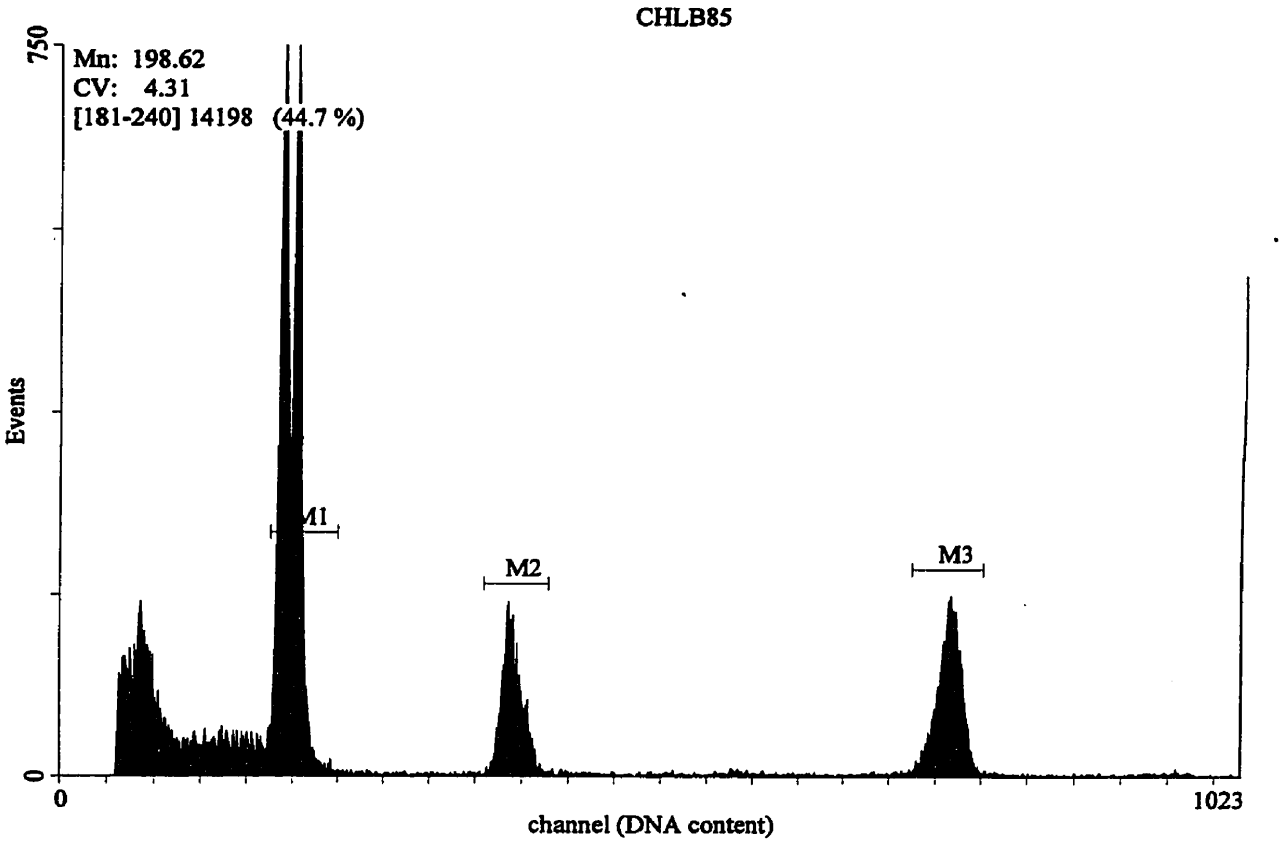

CHLB45
Mn: 211.64
CV: 5.72
[186-254] 11513 (46.3 %)
750
0
Events
M1
M2
M3
0
1023
channel (DNA content)

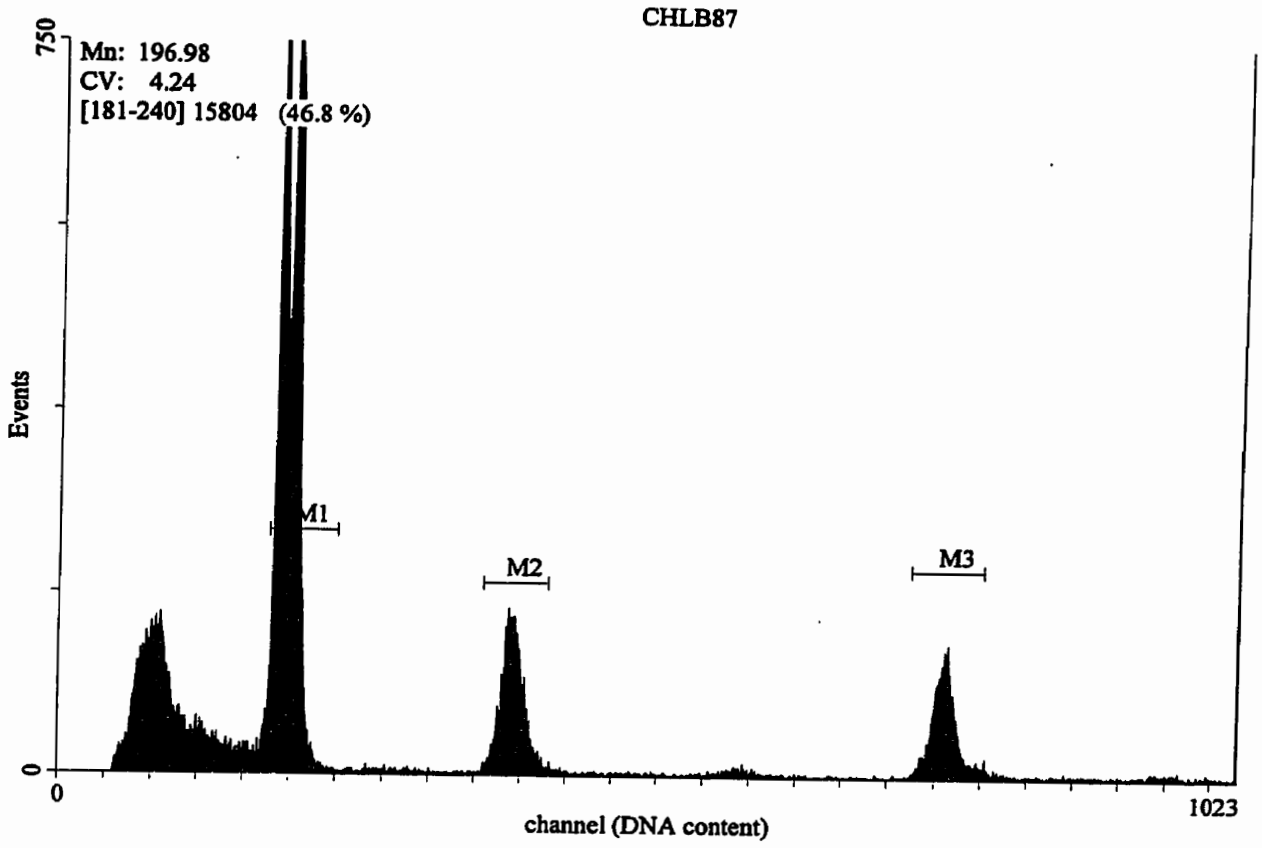

CHLB48
Mn: 227.13
CV: 5.42
[203-271] 15652 (39.3 %)
750
0
Events
M1
M2
M3
0
1023
channel (DNA content)

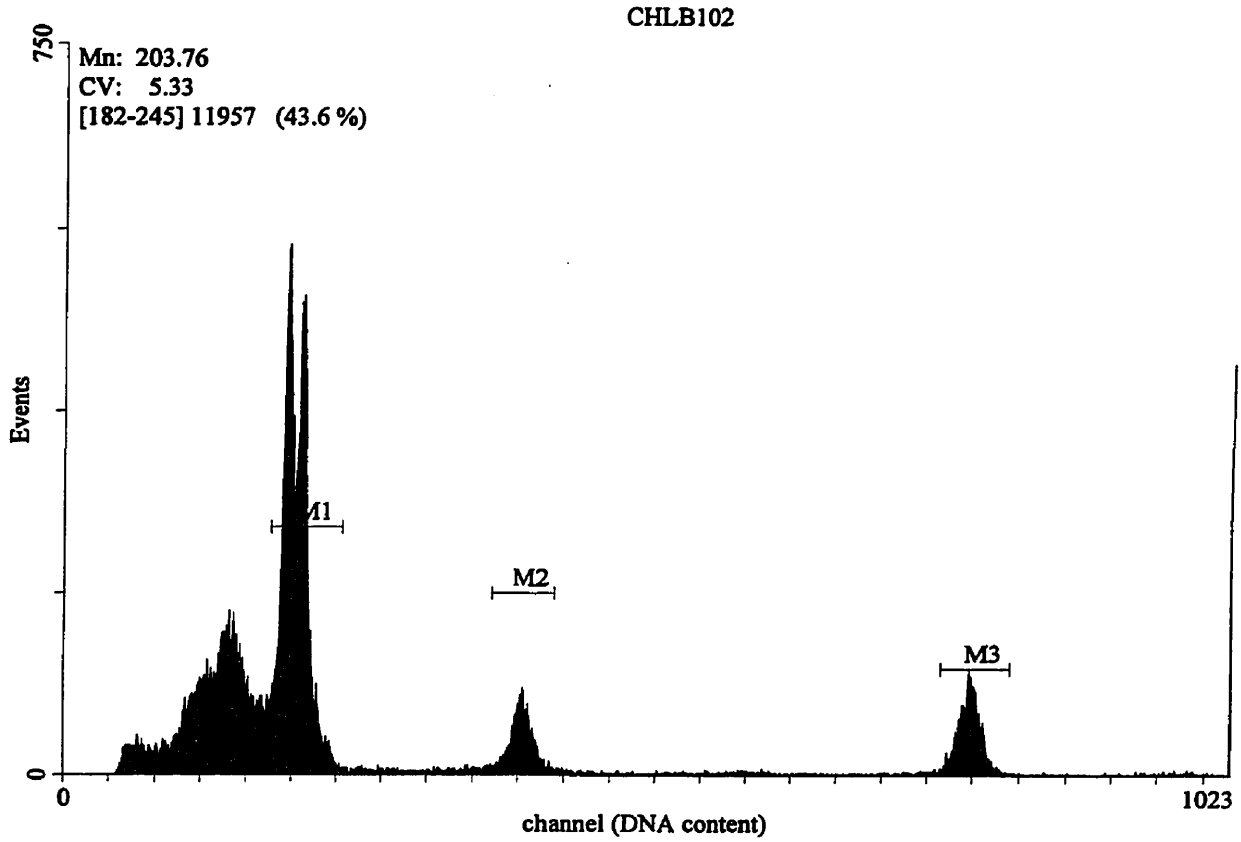

CHLB57
750
Mn: 200.51
CV: 5.64
[179-250] 14450 (45.6 %)
Events
M1
M2
M3
0
0
1023
channel (DNA content)

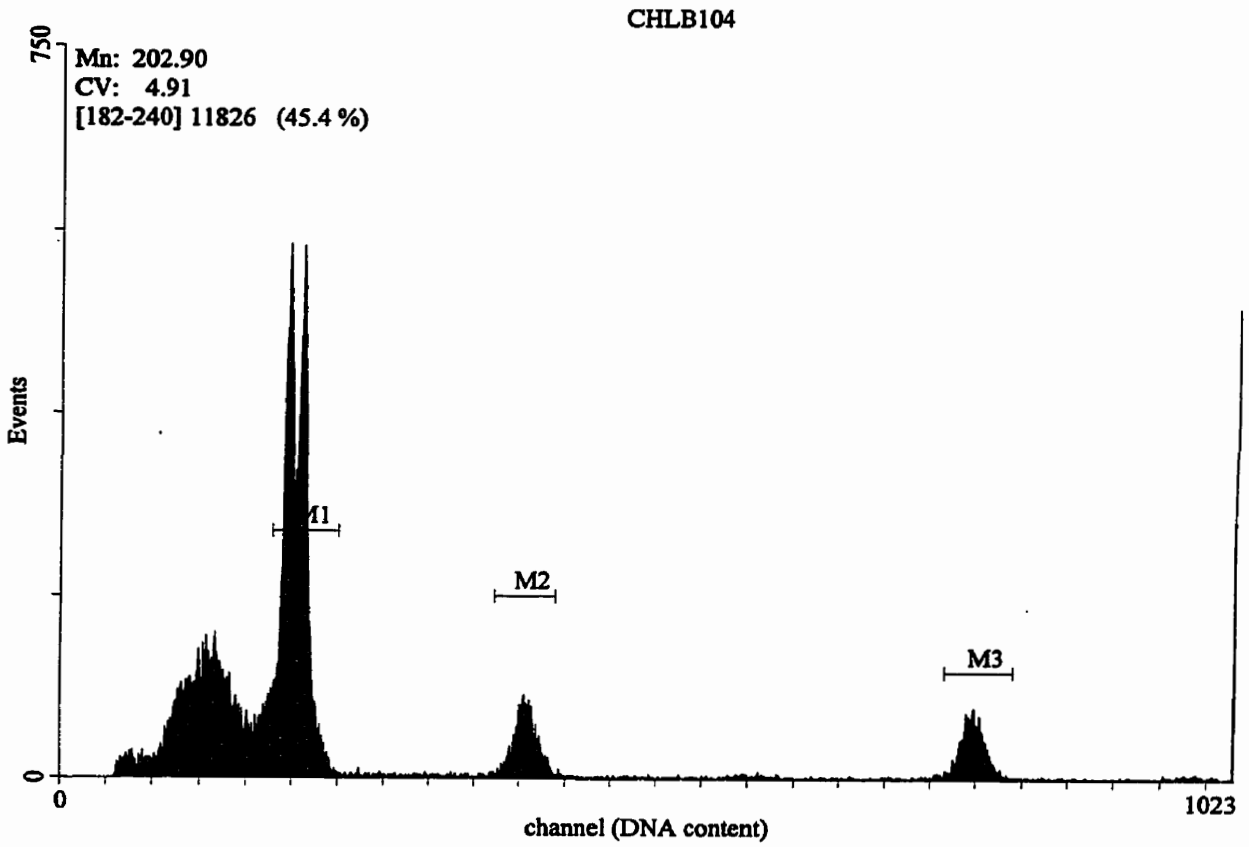

CHLB83
Mn: 203.24
CV: 4.30
[181-237] 11090 (42.2 %)
750
Events
0
M1
M2
M3
0
1023
channel (DNA content)

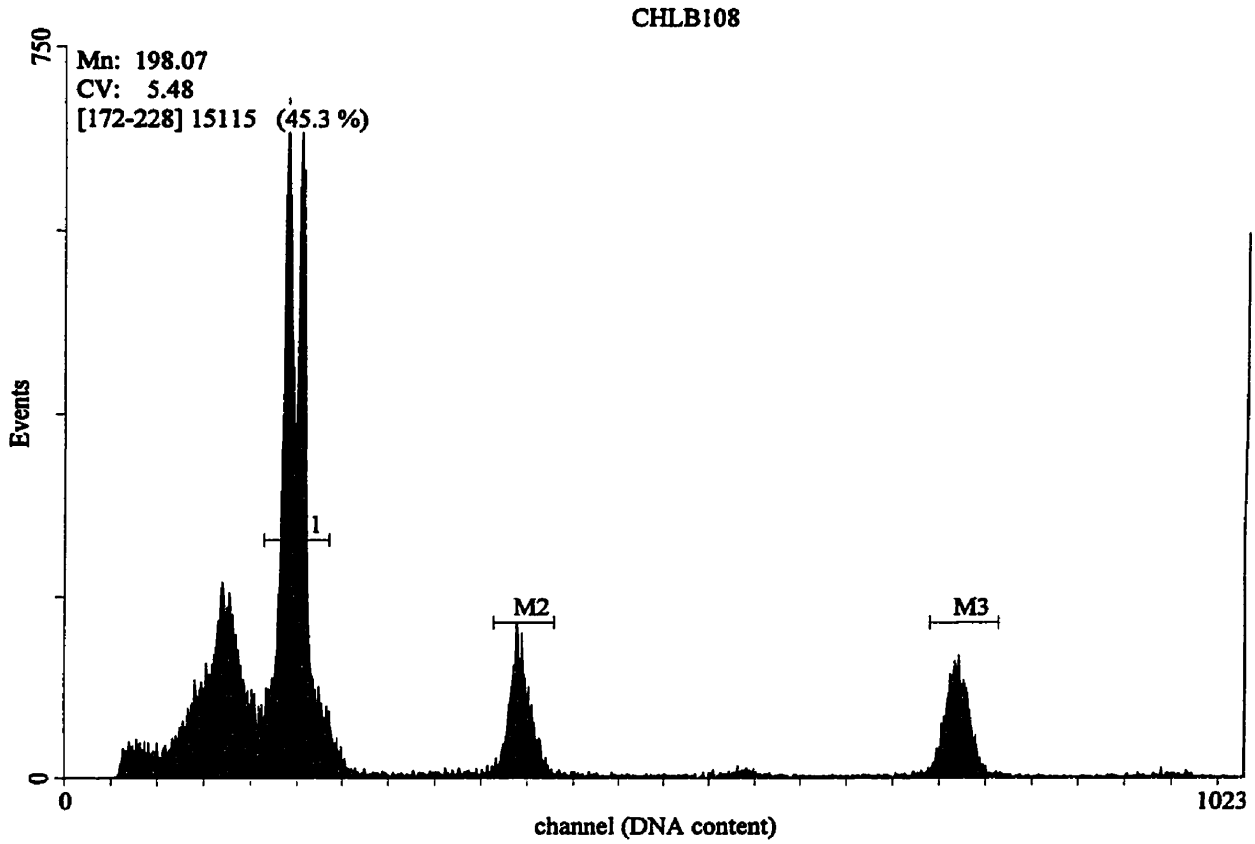

CHLB84
Mn: 201.50
CV: 4.20
[181-240] 11746 (46.8 %)
750
0
Events
M1
M2
M3
0
1023
channel (DNA content)

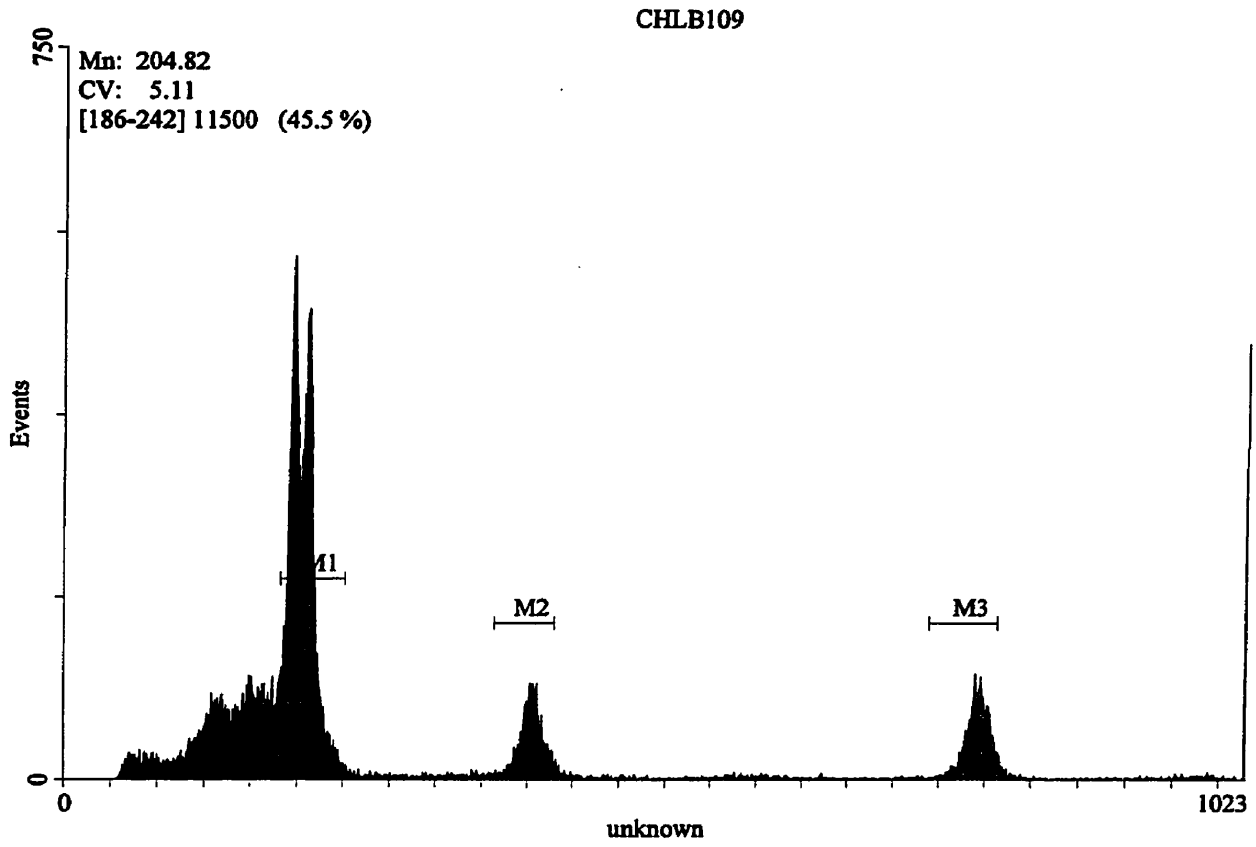

CHLB85
Mn: 198.62
CV: 4.31
[181-240] 14198 (44.7 %)
750
Events
0
M1
M2
M3
0
1023
channel (DNA content)


CHLB87
Mn: 196.98
CV: 4.24
[181-240] 15804 (46.8 %)
750
Events
0
M1
M2
M3
0
1023
channel (DNA content)


CHLB102
Mn: 203.76
CV: 5.33
[182-245] 11957 (43.6 %)
750
0
Events
M1
M2
M3
0
1023
channel (DNA content)


CHLB104
750
Mn: 202.90
CV: 4.91
[182-240] 11826 (45.4 %)
Events
M1
M2
M3
0
0
1023
channel (DNA content)


CHLB108
Mn: 198.07
CV: 5.48
[172-228] 15115 (45.3 %)
750
Events
0
1
M2
M3
0
1023
channel (DNA content)


CHLB109
Mn: 204.82
CV: 5.11
[186-242] 11500 (45.5 %)
750
Events
0
M1
M2
M3
0
1023
unknown