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THE INFLUENCE OF ELECTROSTATIC AND MAGNETIC FIELDS ON NONDISJUNCTION, CROSSING OVER AND MUTATION IN *DROSOPHILA MELANOGASTER*

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THE INFLUENCE

OF ELECTROSTATIC AND MAGNETIC FIELDS

ON NONDISJUNCTION, CROSSING OVER AND MUTATION

IN DROSOPHILA MELANOGASTER

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CHAPTER I

INTRODUCTION

With the advent of space exploration and continued development of highly specialized weapon and defense systems whose operation in some cases produces high intensity electric fields, and ever increasing electrical power demands by society, man is in turn being subjected to stronger and more varied electric and magnetic fields than ever before. We have known for some time that high energy radiation produces gene mutations and chromosomal aberrations in almost all organisms so it would be of great interest then to find out if lower energy fields such as electrostatic and magnetic fields could affect genetic phenomena. To do this Drosophila melanogaster were subjected to electrostatic and magnetic fields to determine the influence on nondisjunction, crossing over and gene mutation.

Flies were subjected to homogeneous and inhomogeneous electrostatic fields. There is no steady state current in an electrostatic field. A homogeneous field is one which is uniform throughout and the direction of the field is that which a positive charge would move when placed in the field. An inhomogeneous field is one which is not uniform throughout, i.e., the field strength is not the same at every point. If the chromosomes or genes exhibit a net charge or a dipole moment the electrostatic fields may exert a force strong enough to influence their movement, or pairing, or cause gene mutation.

The magnetic field used in this study was homogeneous so the field strength was uniform between the pole faces. Biological material, including chromosomes and genes, show a very weak magnetic property, if any at all, but unless the biological material under test exhibits a net charge there would be no force exerted on it when placed in the homogeneous magnetic field.

Electric Fields

A number of experiments have been done to determine the effect of electric current and electric fields on biological material. A review of the literature, however, indicates that the number of experiments using static electric fields on biological systems is quite small and, what is of special interest, studies using any type of electrical apparatus report results which are not always the same under similar experimental conditions.

This study concerns the effect of electrostatic fields on genetic phenomena in Drosophila melanogaster, and perhaps unfortunately, there are very few studies dealing with any type of electrical energy effect on genetic material of any organism. In those studies which have been done it is difficult to draw any conclusions as to what specifically is affected by the electric current, or fields, or why an effect is produced.

The types of possible effectors used in various studies include electromagnetic waves, alternating current, direct current, and direct current pulses. Electromagnetic waves of various lengths have been produced from wave generators and were suspected of having the ability to produce changes in the biological system similar to the effect produced by x-rays.

In 1930, Hersh and Karrer subjected Drosophila to electric waves of 1.14×10^4 Hertz (Hz). They reported 57 anomalies in 26,125 progeny from the flies exposed to the waves. Of these 57 anomalies they found 5 flies with spotted eyes and indicated that this character was transmitted to the offspring as an inheritable trait.

Some time later Schmitt and Oliver (1933), however, reported negative results when subjecting Drosophila to electric waves of 5×10^5 Hz. They reported that of the flies subjected to the waves, 35 percent remained fertile, or at least were viable and able to produce progeny. There were no visible mutations in the progeny, no translocations were detected and lethal mutations were not found in any greater frequency than in the controls. The number of flies tested was not stated.

Krejevoj (1936) exposed wheat and pea seeds, just beginning to sprout, to ultra-short electric waves (10-11 MHz) for 3-15 minutes and reported some very interesting results in chromosomal activity. He reported that in the pea seedling electric waves produced lagging of the chromosomes in anaphase, the formation of micronuclei, chromosome breaks and fragmentation, translocations, "somatic reduction", and pseudomeiotic division of somatic cells. From the discussion the latter two phenomena refer respectively to somatic pairing of chromosomes in which the chromosomes appear as bivalents and nuclear division followed by no cellular division. In regular meiosis there is one chromosomal replication and two cell divisions. Unfortunately nothing was said about controls in this experiment.

Contrary to the results obtained by Krajevoj, Bitter (1936) found no effect upon subjecting barley seeds to high frequency waves of

approximately 20.7 and 58.8 MHz.

Capacitor plates connected to an alternating current source maintaining low or high voltage have been used as possible effectors. In this type of apparatus charges continually flow into and out of the plates as the voltage is increased or decreased. The current through such a capacitor would have the possibility of producing a heating effect in a living organism placed between the capacitor plates. It would seem that if effects are noted in an organism or biological system subjected to such an apparatus the effect would most likely be due to heating.

In 1930, Horlacher placed Drosophila males between capacitor plates supplied with 33 kV and a frequency of 60 Hz. Some variations were reported but no mutations were produced in the flies. He then increased the voltage to 225 kV at 1.225 MHz and exposed male flies for one minute. A total of 690 crosses were made for the purpose of detecting recessive lethal mutations but none were found. Too few chromosomes were tested to show a true effect.

Quite some time later Heller and Teixeira-Pinto (1959), using a similar apparatus which produced a frequency of 27 MHz (pulses were on the order of 5×10^{-5} sec.) exposed garlic root tips submerged in water to the current and reported that chromosomal aberrations were produced. The aberrations manifest themselves in showing linear shortening, pseudochiasmata, amitotic division and chromosomal bridges. They indicated that most of the effects noted were produced when operating at 80-180 pulses per second. This work lends some support to the study by Krajevoj.

Dormant tobacco seeds were also found to be affected by an electric current. Burk and Nelson (1964) report that the treatment caused reduced seed viability and morphological changes. The latter were not always

found to be associated with chromosomal changes but some were. The current also caused genetic changes because mutants were found in generations following treatment.

A study done by Fedorov and Rogov (1963) indicated that a pulsed current had a bactericidal effect on microbes present in milk. The effect was said to be due to impact and cavitation on the microbes. Along this same line Knoepp, et. al., (1962) found that an alternating current of 99-1000 Hz and with variable voltages produced a lethal effect in some normal and malignant cells. Cells of various types were used but the effect was not consistent on all cells. They indicated that the effects noted were not due to heat but were, in fact, due to the current.

A capacitor connected to a direct current will produce a static electric field since there is no current across the capacitor. Some researchers have supplied direct current pulses to the capacitor plates. A study by Doevenspeck (1961) indicates that direct current pulses have lead to the inactivation of microorganisms.

Some detailed studies by Hamilton and Sale (1967) and Sale and Hamilton (1967, 1968) are of great interest. They subjected suspensions of microorganisms and free cell suspensions to direct current pulses (pulse length of 2-20 microseconds at a rate of 1 pulse per second) of 1-27 kV/cm and found that a bactericidal effect was produced which was not due to heating. They reported that the electric field controlled the degree of kill. To attempt to determine specifically what was being acted upon to produce lethality a variety of systems were checked. They suggested that the field may cause a loss of membrane semipermeability and loss of intracellular material. However, there was no observable damage to the cell

membrane when viewed with an electron microscope. They indicated that the effect on the cell membrane may, however, be localized to a specific area or areas and that the likelihood of obtaining a section of the cell membrane which included the site would be very small when such thin sections were used. In flagellated organisms the loss of motility was not due to loss of flagellae and the field did not appear to inhibit enzyme activities in the cells. Lysis of erythrocytes was also reported when the potential difference across the cell membrane (which was considered to be 10^{-6} cm thick) was approximately 1 volt. It was suggested that lysis may be due to some structural change in the cell membrane caused by the electric fields.

Murr (1963, 1964) subjected grass plants to a homogeneous electrostatic field and found plant growth inhibition and indeed in some cases the effect of the static field was lethal for the plants. Murr suggested that the effect of electric fields on plants may be associated with the accumulation of ionizable salts which may reach a poisonous level and eventually may lead to dehydration of the cell or may alter enzyme activity which could also alter metabolic activity. Priovano (1963) cited in Mihalyfi and Serf (1967) also believes that the effect of an electric field on plants is ionic. He suggests the field causes a displacement of free ions or of those to be released.

Experiments by Mihalyfi and Serf (1967) on catalase activity in seeds subjected to homogeneous and inhomogeneous electrostatic fields of 3-5 kV/cm indicate nonuniform increases in enzyme activity. Cucumber seeds show a uniform increase in catalase activity in homogeneous fields with increasing field strength and exposure time while in maize and peas, activity in seeds exposed at 3 kV/cm and 5 kV/cm for 5 seconds shows a lower catalase activity

than in the controls. However, when exposed at 5 kV/cm for 10 seconds the activity in both seed types was greater than in the controls for each group. Exposure of the seeds to inhomogeneous fields of 3-5 kV/cm produced almost identical results to those obtained when seeds were exposed to homogeneous fields.

Studies in which Drosophila have been subjected to electrostatic fields have been very few. Two such studies were done by Avio and Tarozzi (1956, 1958) in which D. melanogaster were placed in a static field of 125 V/meter and a second group in a Faraday cage which served as the control. Negative results were reported in these studies on the effect of the field on oviposition and egg hatch.

A true effect of electric fields on biological systems seems to be inconclusive and there still seems to be some question on the possible genetic effect in Drosophila subjected to an electrostatic field. As mentioned previously homogeneous and inhomogeneous electrostatic fields are used in this study. If a particle with zero net charge is placed in a homogeneous static field no force is exerted on the particle but a force would be exerted on such a particle in an inhomogeneous static field. A force would be exerted on a charged particle placed in a homogeneous or inhomogeneous field. If Drosophila chromosomes or genes have no net charge no force would be exerted on them in the homogeneous field but if a charge is exhibited then a force would be exerted. The consistency of the fly is not uniform and parts of the fly vary in density and molecular constitution. In addition molecules may exhibit a net charge, therefore, one might expect forces to vary on unlike charged molecules within the organism.

Hill (1968) provides a theoretical approach to the possible mechanism

of the interaction of an electrostatic field and macromolecules. He suggests that the field causes polarization of the macromolecules and that if effect it would be possible that field strengths on the order of 10 kV/cm could cause separation of DNA strands or cause protein chains to shorten. If the theoretical approach of Hill is valid then one might expect genetic phenomena to be affected. The effect may be manifested in a change in regular chromosomal movement, or pairing, or a change in gene mutation rate. The purpose of this study is to attempt to determine such an effect.

Magnetic Fields

Since the late 1800's there have been many scientific publications dealing with possible biological effects of magnetic fields. The value of some work is questioned, however, because improper experimental techniques were used to identify some genetic phenomena, expecially in Drosophila. In some studies completely opposite results have been reported when what appears to be almost identical technique had been used. To say that reported biological effects due to magnetic fields are not uniform would be a slight understatement but this would not overshadow a good quantity of evidence which very much indicates that biological effects of magnetic fields do exist and that in some aspects the effects do show a degree of consistency.

A magnetic field is a region around a current carrying conductor. The unit of magnetic field induction presently being used is the tesla (T). One tesla is equal to one weber/meter^2. Other units of magnetism which have been used are the oersted (Oe) which exerts upon a unit pole a force of 1 dyne and the gauss (G) which is specifically a unit of magnetic induction

(the number of lines of force per unit area). One tesla is equal to 10^4 gauss. One oersted is equal to one gauss when the material in the field is in a vacuum since the field induction is equal to the permeability of the material between the poles times the field strength. The permeability of air at 20^oC is equal to 1.000024 so 1 Oe is approximately equal to 1 G.

Biological effects in homogeneous and inhomogeneous fields have been reported, the effects not always being the same, so it will be necessary to distinguish between the two types of fields as far as biomagnetism is concerned. The specific type of fields used by the researchers whose papers are included in the following literature survey is mentioned here unless it was not noted in the report discussed.

Barnothy (1964a) offers the most detailed information on biomagnetism. He states, "The inhomogeneity of a magnetic field is usually characterized either by the gradient or by the relative variation of the field strength over the volume in which the specimen is confined." The basic physical difference between the two fields is that an inhomogeneous field exerts a force upon substances which are more paramagnetic or diamagnetic than their surroundings, while a homogeneous magnetic field does not exert such a force. In an inhomogeneous magnetic field materials experience a force toward the weaker areas of the field whereas paramagnetic materials are urged toward stronger areas of the field.

Barnothy suggests various physical phenomena which may occur in biological material exposed to magnetic fields. These are: (a) the generation of electromotive force in moving conductors, (b) force exerted upon moving charge carriers, (c) torque exerted on permanent magnetic dipoles and non-spherical para- or diamagnetic materials and (d) force exerted on

permanent magnetic dipoles or para- and diamagnetic substances. Factors (a), (b) and (c) will occur in homogeneous and inhomogeneous fields but factor (d) will only occur in inhomogeneous fields. It may be noted, however, that torque is equal to force times moment arm, therefore, phenomenon (c) and (d) are not basically different.

In a homogeneous field phenomenon (a) is said to lead to the rearrangement of electric charges (current) and in the inhomogeneous field conduction currents would be produced which are also associated with heating.

Phenomenon (b) is suggested to change the motion of ions in electrolytes which leads to an aggregation of substances which would be at variance to their normal distribution while phenomenon (c) is said to lead to changes in biological reactions of para- or diamagnetic material or particulate material such as erythrocytes.

Phenomenon (d) may lead to a displacement of para- and diamagnetic materials which could cause an accumulation of the substance that might change diffusion processes necessary in biological material.

The literature seems to provide evidence that some, but not all, biological material is affected by magnetic fields. In 1929, Leusden, cited by Barnothy (1964c), exposed coliform and staphylococci bacteria to a magnetic field and found no effect on growth of the organisms.

Shortly thereafter Ssawostin (1930) found that exposing plants, with the plants axis perpendicular to the lines of force, to what apparently was a homogeneous field of 20 to 215 micro-tesla (mT) notably increased the growth rate but did not affect growth direction. He further stated that there was no distinct increased growth rate when the lines of force were parallel to the axis of the plants. A report appeared that same year by

Spraque (1930) in which it was indicated that greater variegation, an increase in the number of mosaics and the production of translocations resulted from exposure of maize pollen to a magnetic field. Nothing was said of the field strength or homogeneity.

Sometime later Jennison (1937) exposed various species of bacteria, yeasts and molds grown on solid medium to a homogeneous magnetic field of 0.3 T and reported no effect on colony size, staining properties, spore formation, and pigment production.

The following year Kimball (1938) reported no growth effect in yeast subjected to homogeneous fields of 1.1 T but she did report significant decreases in the growth rate of yeast cultures subjected to weak inhomogeneous fields of 0.4 mT or less.

Lenzi (1940), cited by Becker (1962), published a lengthy paper in which it was reported that fields of 150-170 mT greatly reduced the number of tumor growths when adenocarcinoma was injected into mice and then subjected to the field. After the animals were removed from the field delayed tumor development began at a normal rate at sites where the growth had been inhibited. On the other hand if tumors were allowed to develop before the animals were subjected to the field no decrease in growth was caused by the magnetic fields.

Chevais and Manigault (1942) reportedly had some success in producing recessive lethal mutations and mutations affecting the wings in Drosophila which hatched from eggs subjected to an inhomogeneous magnetic field for 24 hours. They used a permanent magnet having a field strength measured in several million cgs units (the specific values were not given) at the strongest region of the pole gap.

Close and Beischer (1962) also attempted to determine genetic effects in Drosophila subjected to a magnetic field. In their study wild type males were subjected to a 10 T homogeneous magnetic field for 30 minutes. Three days later the males were mated to yellow-Muller-5 females. The purpose of this mating was to detect recessive mutations at the yellow locus of the X chromosome. They found 1 yellow-body female among 250 females from the matings while no yellow-body females were found from non-treated wild type males mated to yellow-Muller5 females. In the same study no significant difference in the sex-ratio of F_1 progeny of the cross just described or of a cross of males and females exposed to a homogeneous field of 10 T for 2 hours was found.

A further study by Beischer (1964) reports no effect on post-exposure development, per cent hatching, or sex-ratio in Drosophila subjected as eggs, larvae, pupae and adults to a 14 T homogeneous field. Steen and Oftedal (1967) reported no effect on egg hatch when subjecting D. melanogaster eggs to a homogeneous field of 160 and 500 mT. While field strength is very much less than that used by Close and Beischer this would tend to give support to the study by Beischer (1964).

Mulay and Mulay (1961, 1964) exposed Drosophila to, probably, inhomogeneous fields of 0.01 to 0.8 T for 1-3 generations. Flies were checked for autosomal and sex-linked recessive and recessive lethal mutations and for dominant lethals. None of any type were found but it was not mentioned how many chromosomes were tested. They stated that visible morphological abnormalities of the wings and eyes did occur in the field. I believe the variations noted may just be normal variations found in cultures due to environmental conditions. In the same studies it was recorded that magnets

of the field strength ranges previously mentioned caused degeneration of Sarcoma-37 mouse tumor cells. A similar study on KB cells in vitro by Butler and Dean (1964) showed inhibited growth of the cells when exposed to a 0.4 T homogeneous field.

In Russia in 1965, Kogen, cited by Presman (1970), reported that Drosophila will collect at the poles of a magnet and that females will thus deposit more eggs near the poles as compared to the space between the poles. This would be expected if, in fact, the flies do collect near the poles. Unfortunately the strength of the magnetic field was not noted.

Based on two generations, Akhmerov, et. at., (1966), cited in Presman (1970), reports inheritable changes induced in Drosophila adults exposed to magnetic fields of 75-110 mT. Treatment resulted in 36% increase in the number of pupae and adult progeny. Also in 1966, Shakhbazov, et. al., also cited in Presman (1970), reported an increase in fecundity in Drosophila females and males mated after being exposed to magnetic fields of 190 mT. No increase in fecundity was noted when exposed females were mated to non-exposed males or when exposed males were mated to non-exposed females. Length of treatment time was not stated.

Levengood (1967) subjected individual D. melanogaster pupae to a magnetic probe which had a field strength of approximately 2.4 T. The field was probably quite inhomogeneous because of the apparatus used. A conical shaped probe from which the field was emitted was placed close to the region of the developing gonads of the pupae. Levengood reports an increased generation time which he said lasted more than 30 generations. He suggests the factor causing the increase involves an epigenetic system transmitted

cytoplasmically by the male flies but almost always not by (treated) female flies. This explanation seems suspect because cytoplasmic inheritance primarily occurs through the female and not through the male parent.

Tegenkamp (1969) also reports some questionable interpretations of data from a study of magnetic effects on Drosophila. He reports a mutant causing withered wing in Drosophila progeny from parents subjected to a field of 52 mT. He suggests this is an autosomal mutant, however, he could not produce a stock of the mutant. After reporting a 3:1 ratio of wild type to mutant (withered wing) flies in the F_2 generation no mutants were found in the F_4 generation. If the mutant was an autosomal recessive it would have to have been produced in both a treated male and a treated female for a single mutant fly to occur in the progeny of the treated parents. He states that there were eleven mutant and 28 wild type F_3 progeny. One would expect by chance alone a mating of a mutant female and a mutant male among the F_3 mutants but he states no mutants were found in the F_4. Likewise some of the wild type progeny would be expected to be heterozygous for the mutant and mating between heterozygotes would also be expected to produce some mutant progeny in the F_4. This was not noted by Tegenkamp but he reported that the mutant reappears in later generations. He also reported significantly greater numbers of female progeny from female flies with the withered wing mutant. He suggested that the irregular sex-ratio is due to a sex-1 linked recessive lethal mutation. He indicated that the standard Muller-5 test for sex-linked recessive lethal mutations is not suitable to detect the mutation reported in his data for he suggested that several DNA replications are necessary for the mutant to express itself in the X chromosome.

This idea would assume the chromosome in Drosophila to be a multistranded DNA structure with only 2 strands active at one time and not always the same 2 strands in the daughter cells. There is no evidence to support this idea. He also reports a mutant causing convergent bands in the dorsal abdominal segment. This pattern occurs in a number of mutants as well as sporadically in wild type stocks and is influenced by environmental factors. Most likely the genetic basis for all of Tegenkamp's abnormal flies was already present in his stocks before he started treating them.

Magrou and Manigault (1946) using inhomogeneous fields in which the strength was given as from 1 x 10^6 to 22 x 10^6 cgs units applied to a site on the plant Pelargonium zonale infected with Bacterium tumefaciens, an organism which causes the crown gall tumor, found that tumor development was almost completely retarded in the highest fields used and only slightly retarded at the lowest field strength. They report a great distinction between the treated groups and the controls.

Results obtained by Gerencser, et. al., (1961) were, in part, similar to those of Magrou and Manigault. Gerencser reported a retarded growth rate of Serratia marcescens and Staphylococcus aureus exposed to an inhomogeneous magnetic field of up to 1.6 T but the retardation was then followed by a recovery of growth rate. They suggested that mutant strains developed during treatment which were resistant to the effect of the field. Growth rate would then show an increase due to the replication of resistant cells.

Hedrick (1964) also showed a decreased growth rate in S. aureus exposed to a homogeneous field, but no effect was noted in S. lutea or E. coli. He used a field strength of 14 T which was constant or interrupted

but only noted the effect in the constant field. He also subjected S. aureus to a 70 mT homogeneous field in a permanent magnet and found the characteristic clustering was not seen but single isolated cells became evident.

A variety of papers have been published concerning the effects of magnetic fields on plants. Audus (1960) and Audus and Whish (1964) exposed growing root tips to inhomogeneous fields of 0.4-0.5 T and found the growth to curve in the direction of the field. Similar results have been obtained by Krylov and Tarakanova (1960), cited in Presmen (1970), and Krylov (1961). They found greater root and shoot tip growth when the same were pointed toward the earth's south pole or the south pole of a magnet.* They also reported that shoots and roots of various plants, if pointed toward the north pole, would curve toward the south pole during growth. Along this same line more rapid growth of shoots and roots of various grass species exposed to permanent magnets of approximately 0.13 T has been noted by Whish (1963) and Mericle (1964).

Increased cellular division was the effect of magnetic fields noted by Barnothy, et. al., (1956). They reported leukocytosis in mice after exposure to a static homogeneous magnetic field of 0.42 T. However, Eiselein, et. al., (1961) report magnetic field strengths of 0.88-1.4 T have no effect on the white cell count in mice, growth rate and ascites tumor cells while Gross and Smith (1961) indicate a delay in wound healing in mice is produced

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*The earth's "south" pole is actually comparable to the north pole of a magnet because a magnet will align with its south pole pointed to the "south" pole of the earth.

by a magnetic field. Gross (1962) reports that mice immunized against carcinoma cells are more susceptible to injected carcinoma cells when in a magnetic field of 0.4 T.

Barnothy and Sumegi (1969) found an increase in mitotic activity of mouse liver cells exposed to magnetic fields of 0.42 and 0.9 T but not in a magnetic field of 0.22 T. A study by Pumper and Barnothy (1969) shows that a strong magnetic field of 1.4 T increases the growth rate of rabbit myocardium and mouse lung fibroblasts.

Somewhat contrary to the studies just mentioned Cook, Fardon and Nutine (1969) report that respiration in mitotically active tumor cells, embryo and young neonatal tissues was reduced when exposed to magnetic fields of 8 mT or greater but respiration in adult and old neonatal tissue was not reduced. Yeast cells, on the other hand, showed an increase in aerobic respiration upon being subjected to the field.

An interesting experiment by D'Souza, et. al., (1969) has indicated that the exposure of ascites Sarcoma-37 cells to a 0.73 T magnetic field for 1-3 hour periods inhibits DNA synthesis about 18 to 24%. Whether or not the cells showed a lasting inhibition of DNA synthesis was not stated.

Many studies showing a positive biological effect by magnetic fields are convincing but the results are not always uniform. A number of the effects of magnetic fields are to cause cell proliferation, gene mutation, mosaics in plants, and chromosomal translocation, to increase the life span in mice (Barnothy, 1964b), to increase fecundity in Drosophila females but to decrease bacterial cell growth and inhibit DNA synthesis in tumor cells.

What exactly causes the specific effects of magnetic fields on biological material still seems to be not clear. Dorfman (1962), cited in

Presman (1970), offered an hypothesis that macromolecules, which are usually diamagnetic, could exhibit orientation in a magnetic field. If true one could infer from this that it could be possible for DNA molecules and, therefore, chromosomes to be oriented within the cell in a fashion which would be abnormal for the cell. This has the possibility then of producing distinct genetic changes.

Of special interest in this study is the effect of magnetic fields on chromosomes or genetic material. Barnothy (1964d) has presented several possible ways in which magnetic fields could alter the DNA molecule. She indicates that a magnetic field could affect the spin orientation of the hydrogen proton involved in the hydrogen bond of the DNA molecules; that the proton endowed with a magnetic moment could, in an inhomogeneous field, experience an accelerating force which could affect molecular pairing or, more likely, the magnetic field could alter the energy levels of the nucleotide bases thus affecting the stability of the DNA molecule.

With these possible effects in mind the purpose of this study was to determine the effects of magnetic fields on crossing over and gene mutation in D. melanogaster. It was thought that the magnetic field may exert a force on the chromosomes (if they exhibit a net charge) during meiosis and, if so, the effect might be noted by a change in the recombination frequency. It was also thought that if the DNA molecule was affected by the magnetic field the effect might be manifested in sex-linked recessive lethal mutations.

Because all organisms are continually being subjected to more and more power sources which produce electric and magnetic fields of varying intensities it is hoped that this study will contribute to our knowledge

and understanding of the effects of these fields on hereditary material.

A brief summary of some of the effects of electrostatic and magnetic fields on organisms or biological material is shown in Table 1.

Table 1

EFFECTS OF ELECTROSTATIC AND ELECTROMAGNETIC FIELDS ON BIOLOGICAL SYSTEMS

Field	Intensity	Organism or Biological Material	Effect	Reference	
1.14×10^4 Hz	--	Drosophila	Morphological anomalies	Hersh & Karrer	1930
5×10^5 Hz	--	Drosophila	Negative	Schmitt & Oliver	1933
10-100 MHz	--	Wheat & Pea seeds	Chromosome breaks, fragmentation, lagging & translocation	Krajevoj	1936
20.7 & 58.8 MHz	--	Barley seeds	Negative	Bittern	1936
60 Hz	33Kv	Drosophila	No mutations	Horlacher	1930
1.225 MHz	225 kV	"	" "	"	"
27 MHz	--	Garlic root tips	Chromosomal aberrations	Heller & Teixeira-Pinto	1959
39 MHz	2.05 & 5.07 kV/cm	Tobacco seeds	Genetic & morphological changes	Burk & Nelson	1964
"Pulsed"	--	Milk microbes	Bactericidal	Fedorov & Rogov	1963

TABLE 1--Continued

Field	Intensity	Organism or Biological Material	Effect	Reference	
99-1000 Hz	--	Normal & malignant cells	Lethal	Knoepp, et. al.,	1962
"D.C. Pulsed"	--	Microbes	Inactivation	Doeven-speck	1961
"pulsed"	1-27 kV/cm	Microbes & cells	Bactericidal	Hamilton & Sale	1967
Static homogeneous	--	Grass plants	Growth inhibition	Murr & Murr	1963 1964
Static homogeneous	3-5 kV/cm	Seeds	Increase in enzyme activity	Mihalyfi & Serf	1967
Static homogeneous	125 V/m	Drosophila	Negative	Avio & Tarozzi	1956 1958
Constant magnetic	--	Bacteria	Negative	Leusden	1929
Constant magnetic	2.0-215 mT	Plants	Increased growth rate	Ssawostin	1930
--	--	Maize pollen	Translocation & mosaics	Sprague	1938
Homogeneous	0.3 T	Microbes	Negative	Jennison	1937
Homogeneous	1.1 T	Yeast	No growth rate effect	Kimball	1938
Inhomo-geneous	0.4 mT	Yeast	Decreased growth rate	Kimball	1938
--	150-170 mT	Tumor cells	Reduced growth	Lenzi	1940
Inhomo-geneous	--	Drosophila	Mutations	Chevais & Manigault	1942

TABLE 1--Continued

Field	Intensity	Organism or Biological Material	Effect	Reference	
Homogeneous	10 T	Drosophila	Negative	Close & Beischer	1962
Homogeneous	14 T	Drosophila	Negative	Beischer	1964
Homogeneous	160 & 500 mT	Drosophila eggs	Negative	Steen & Oftedal	1967
Homogeneous	0.42 T	White Blood cells (mice)	Leukocytosis	Barnothy	1956
--	0.88-1.4 T	"	Negative	Eiselein	1961
--	--	Mice cells	Delayed wound healing	Gross & Smith	1961
--	0.4 T	Mice	More susceptable to tumor	Gross	1962
--	0.42 & 0.9 T	Mouse liver cells	Increased mitosis	Barnothy & Sumegi	1969
--	1.4 T	Cells	Increased mitosis	Pumper & Barnothy	1969
--	0.73 T	Tumor cells	Inhibition of DNA synthesis	D'Souza	1969
--	--	Mice	Increased life span	Barnothy	1964

CHAPTER II

GENERAL METHODS AND MATERIALS

Drosophila melanogaster stocks were maintained in the laboratory in half-pint bottles on agar, cornmeal, molasses, and Brewer's yeast medium. The medium, used in all crosses, consists of 87 oz (Vol.) water, 15 gm agar, 3 tsp. methylparaben (Heyden) mold inhibitor, 42 gm Brewer's yeast (Nutritional Biochemicals Corp.), 6 oz (Vol.) molasses and 12 oz (Vol.) corn meal. All experimental and control crosses were made in one-half or one-quarter pint bottles, or in 8 dram shell vials as indicated. All control and experimental flies were incubated after treatment at 25 ± 1°C except where indicated.

In all nondisjunction and cross over experiments parent flies were transferred to fresh medium every 2 days or, rarely, every 3 days for a total of three times. The three transfers were labeled as a, b, and c. The parents in the original cross containers were exposed to the experimental field for 1 or 2 days as indicated as a pre-treatment. Eggs deposited by the female were subjected to the field a maximum of one day and larvae a maximum of 2 days during their initial development. The original cross progeny were not included in the data because the gametes had not been subjected to the experimental field during their entire meiotic development. All progeny eclosing from each transfer for a period of 18 days after the parents were placed in the containers were classified and

counted. Parent flies were subjected to the experimental field 7-9 days except where indicated.

In all nondisjunction and cross over experiments where crosses were made in bottles, dry Baker's yeast was sprinkled on the medium and a piece of mold inhibitor treated paper toweling was placed down into the medium. Dry Baker's yeast was added to all vials except where indicated.

The control groups were treated the same as the experimental groups except for not being subjected to the experimental fields. Any exceptions are noted. The controls were placed 1.06 to 1.21 meters from the experimental apparatus.

CHAPTER III

NONDISJUNCTION OF THE SEX CHROMOSOMES

METHODS AND MATERIALS

In all experiments involving primary nondisjunction, $\underline{yw}^a/\underline{yw}^a$ virgin females were mated to $\underline{y}/sc^{8}Y.y^+$ males. The yellow gene (y; 1-1.5) causes a yellow body color and the white-apricot gene (w^a; 1-1.5) causes apricot colored eyes in melanogaster. The $sc^{8}Y.y^+$ chromosome is a modified Y chromosome that carries the y^+ allele on the distal tip of its short arm. This specific chromosome in the males allows one to detect an XXY female in the stock cultures of $\underline{yw}^a/\underline{yw}^a$ females mated to $\underline{yw}^a/sc^{8}Y.y^+$ males. Therefore, it is possible to select only XX females for crossing. An XXY female would produce a higher frequency of exceptional progeny due to secondary nondisjunction as compared with primary nondisjunction in XX females (Bridges, 1916).

The parent stocks were made co-isogenic for chromosomes II and III by the following mating procedure. Virgin $\underline{yw}^a/\underline{yw}^a$ females were collected from a stock carried at the University of Oklahoma and mated to $\underline{Cy}/\underline{Pm}$; $\underline{D}/\underline{Sb}$ males. The dominant mutant genes Curly ($\underline{Cy}$) and Plum ($\underline{Pm}$) are on homologous second chromosomes. The $\underline{Cy}$ gene causes curly wings and is associated with an inversion In(2L)Cy. The $\underline{Pm}$ gene causes deep red eyes and is associated with the inversion $In(2LR)bw^{V1}$. The dominant mutant genes Dichaete ($\underline{D}$) and Stubble ($\underline{Sb}$) are located on homologous third chrom-

osomes. The D gene causes the wings to be extended from the body axis and is associated with the inversion In(eLR)DcxF. The Sb gene causes short, heavy bristles. Each of these four genes is lethal in the homozygous condition. The F_1 $yw^a/++$; Cy/+; D/+ females were individually back-crossed to Cy/Pm; D/Sb males. F_2 yw^a/Y; Cy/Pm; D/Sb males were mated to single F_1 $yw^a/++$; Cy/+; D/+ females. F_3 yw^a/yw^a; Cy/Pm; D/Sb females were mated to their yw^a/Y; Cy/Pm; D/Sb brothers to produce a stock. From the cross of an F_1 $yw^a/++$; Cy/+; D/+ female to an F_2 yw^a/Y; Cy/Pm; D/Sb male, F_3 $yw^a/+$; Cy/+; D/+ females were mated to their F_3 yw^a/Y; Cy/+; D/+ brothers by individual crosses. From this cross an isogenic stock of yw^a/yw^a; +/+; +/+ females and yw^a/Y; +/+; +/+ males was obtained.

To obtain the stock of y/y ♀♀ X $y/sc^8Y.y^+$ ♂♂, Cy/Pm; D/Sb females were mated to $y/sc^8Y.y^+$ males. F_1 y/+; Cy/+; D/+ females were collected. F_1 $+/sc^8Y.y^+$; Cy/+; D/+ males were individually back-crossed to Cy/Pm; D/Sb females. From the backcross progeny $+/sc^8Y.y^+$; Cy/Pm; D/Sb males were individually mated to the F_1 y/+; Cy/+; D/+ females. F_3 $y/sc^8Y.y^+$; Cy/Pm; D/Sb and $+/sc^8Y.y^+$; Cy/Pm; D/Sb males were selected. Both of these males would be wild type in body color. F_3 Cy/Pm; D/Sb females were also collected. One-half of these females would be expected to be heterozygous for the y allele and one-half would be expected to be homozygous wild type for body color. The F_3 males and females were individually mated in vials. The males were removed after five days and kept in individually labeled vials. The females were transferred to fresh vials after 6 days, kept in the transfer vial for 6 days and then discarded. Those vials which produced F_4 y/y; Cy/Pm; D/Sb females were selected and the rest discarded. The F_4 y/y; Cy/Pm; D/Sb females were back-crossed to their fathers for their

fathers would have been $\underline{y}/sc^{8}Y.\underline{y}^{+}$; $\underline{Cy}/\underline{Pm}$; $\underline{D}/\underline{Sb}$. A stock from this cross was made and maintained.

A $\underline{yw}^{a}/\underline{yw}^{a}$; $\underline{Cy}/\underline{Pm}$; $\underline{D}/\underline{Sb}$ female was mated to a $\underline{yw}^{a}/Y$; isogenic male. F_1 $\underline{yw}^{a}/\underline{yw}^{a}$; $\underline{Cy}/+$; $\underline{D}/+$ females were individually mated to $\underline{y}/sc^{8}Y.\underline{y}^{+}$; $\underline{Cy}/\underline{Pm}$; $\underline{D}/\underline{Sb}$ males. The F_2 $\underline{yw}^{a}/\underline{y}+$; $\underline{Cy}/+$; $\underline{D}/+$ females were individually mated to their $\underline{yw}^{a}/sc^{8}Y.\underline{y}^{+}$; $\underline{Cy}/+$; $\underline{D}/+$ brothers. F_3 $\underline{yw}^{a}/\underline{yw}^{a}$; $+/+$; $+/+$ females were mated individually to their $\underline{yw}^{a}/sc^{8}Y.\underline{y}^{+}$; $+/+$; $+/+$ brothers. A stock of yellow, white-apricot females and non-yellow, apricot males was obtained from this cross.

F_3 $\underline{yw}^{a}/\underline{y}+$ females were also individually mated to their $\underline{y}/sc^{8}Y.\underline{y}^{+}$ brothers. One-half of the F_4 female progeny from this cross would be expected to be $\underline{yw}^{a}/\underline{y}+$ and one-half would be expected to be $\underline{y}/\underline{y}$, also one-half of the male progeny would be expected to be $\underline{yw}^{a}/sc^{8}Y.\underline{y}^{+}$ and one-half would be expected to be $\underline{y}/sc^{8}Y.\underline{y}^{+}$. Both types of females would be yellow. A number of these females were individually mated to their $\underline{y}/sc^{8}Y.\underline{y}^{+}$ brothers. After five days the females were transferred to fresh medium. The vials which produced only yellow females and non-yellow, non-apricot males were selected and the others discarded. A stock of $\underline{y}/\underline{y}$ ♀♀ X $\underline{y}/sc^{8}Y.\underline{y}^{+}$ males was made from one of these vials.

The mating procedure outlined above produced co-isogenic stocks of $\underline{yw}^{a}/\underline{yw}^{a}$ ♀♀ X $\underline{yw}^{a}/sc^{8}Y.\underline{y}^{+}$ ♂♂ and $\underline{y}/\underline{y}$ ♀♀ X $\underline{y}/sc^{8}Y.\underline{y}^{+}$ ♂♂. From these stocks $\underline{yw}^{a}/\underline{yw}^{a}$ females and $\underline{y}/sc^{8}Y.\underline{y}^{+}$ males were collected to be used as parents from which nondisjunction in the female and male parents could be followed.

Larvae from the nondisjunction parent stocks were collected and several salivary gland chromosome smears were made periodically to determine

if any of the autosomes or X chromosomes carried inversions which would increase the X chromosome nondisjunction rate. None were identified. In addition stocks from which the female parents for the primary nondisjunction study were collected were watched closely for the production of XXY females which would be non-yellow and apricot eyed. An excess number of these exceptional females would indicate either the presence of an unmarked Y chromosome (or fragment), or inversion heterozygosity, as indicated above, but there were no culture bottles which produced a conspicuously large number of such females.

Experiment 1

Single 2-3 day old $\underline{yw}^a/\underline{yw}^a$ virgin females were placed in individual vials with 2-3 $\underline{y}/sc^{8}Y.y^{+}$ males aged 2-3 days. Males and females were lightly etherized prior to mating in all crosses in the entire study. The vials were placed in a 3 kV/cm, (-) polarity, homogeneous electrostatic field. The parents were pre-treated for one day and subjected to the field for a total of 7-8 days.

In this experiment the controls were not placed in a capacitor like the treatment group but were placed in a metal tray. The cotton plugs were placed well down in the vials just as in the treatment vials. The controls were under the same environmental conditions as the treatment group except for not being subjected to the field and the immediate area around it.

Three separate electric field apparatuses were used in this study. The apparatus used to produce a 3 kV/cm homogeneous electrostatic field consisted of a high voltage D.C. source, which operated at 30 kV when used,

connected to a capacitor made of two aluminum plates 0.317 cm thick, 45.8 cm long and 22.9 cm high arranged parallel to each other (Fig. 1). The capacitor was housed in a wire cage to keep anyone from touching the capacitor plates. The vials were placed between the aluminum plates and arranged in rows without touching the sides of the plates. The fresh transfer vial was placed in the same position between the plates as the original mating vial.

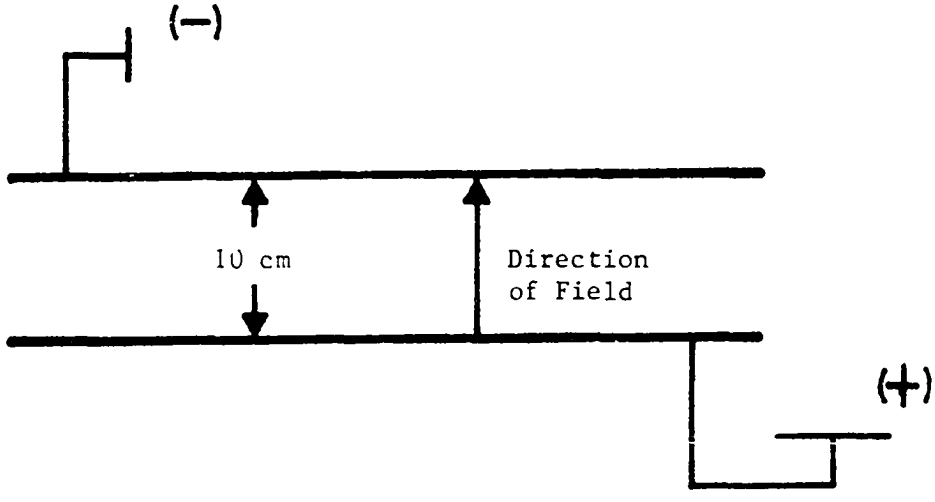

Fig. 1.-- Diagram of apparatus used to produce a 3.0 kV/cm homogeneous, electrostatic field.

Cotton plugs, used to stopper the vials, were placed well down into the vials to prevent the cotton strands from shorting across the capacitor plates.

Because of the high voltage on the capacitor plates of the 3 kV/cm field a quantity of ozone was suspected of being produced due to the break-down of air. To reduce the concentration of ozone, if produced, an electric fan was placed next to the capacitor to aid in the circulation of air around

the capacitor. A centigrade thermometer was placed down into one of the vials in the field and as nearly as could be measured the temperature in the 3 kV/cm, homogeneous, or inhomogeneous, electrostatic field was 24 ± 1°C. Control parents were at the same temperature.

Experiment 2

The same procedure as in experiment 1 was used except that the experimental flies were subjected to a 3 kV/cm, inhomogeneous, electrostatic field.

The apparatus used to produce a 3 kV/cm inhomogeneous field utilized the same D.C. voltage source as used to produce the 3 kV/cm, homogeneous field. The capacitor was made of a straight aluminum plate arranged parallel to a corrugated copper plate (Fig. 2). Vials were placed between the plates with no vials touching the capacitor plates.

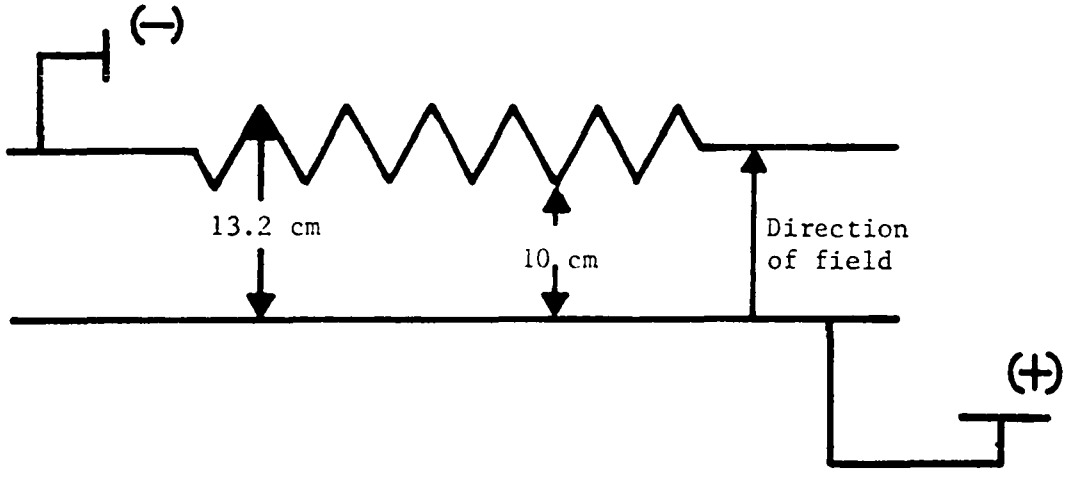

Fig. 2.--Diagram of apparatus used to produce a 3.0 kV/cm inhomogeneous, electrostatic field.

Experiment 3

Four $\underline{yw}^a/\underline{yw}^a$ females per 1/4 pint bottle were mated to 6-8 $\underline{y}/sc^{8}Y.y^{+}$ males aged 2-6 days at the time of mating. Females of differing age groups were tested. Uchida (1962) reported that radiation significantly increased the rate of nondisjunction in aged females. It was thought that the nondisjunction rate in females exposed to the electrostatic field may be age sensitive. The experimental parents were exposed to a 0.3 kV/cm inhomogeneous electrostatic field for a total of 7-8 days.

Figure 3 shows a diagram of the apparatus used to treat flies with a 0.3 kV/cm or a 0.6 kV/cm inhomogeneous electrostatic field. The apparatus consisted of a capacitor made of two aluminum plates 0.317 cm thick, 39.7 cm long and 18.4 cm high, arranged parallel to one another. The bottles or vials were arranged in a single row between the plates and rested on wood or styrofoam to elevate them. Each container was plugged with a one-hole rubber stopper. A piece of 5 mm flint glass tubing 7-8 cm long was placed through the hole. A piece of 14 gauge copper wire, 18-19 cm long, was placed through the glass tubing and immersed into the medium. There was a small air space between the wire and the glass tube lumen. A single piece of 14 gauge copper wire was then attached by alligator clips at right angles to the wire emerging from the bottle or vial. The single wire was connected to the ground terminal and the capacitor plates were connected to the positive terminal. Hereafter this setup will be referred to as a negative (-) polarity field. The direction of the field was reversed by connecting the single wire to the positive terminal and the capacitor plates to the ground terminal. Hereafter this setup will be referred to as a positive (+) polarity field. The power supply was made

by the NJE Corporation and operated at 0-30 D.C. kV at 0-3.0 D.C. milliamperes.

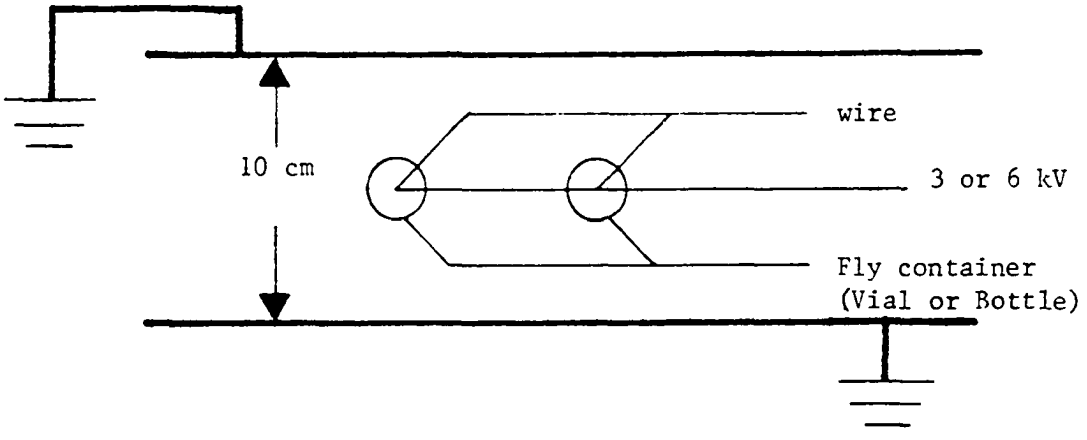

Fig. 3.--Diagram of apparatus used to produce a 0.3 or 0.6 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field.

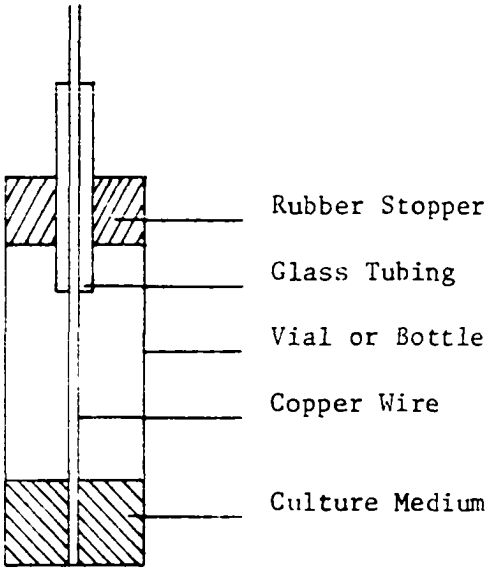

Fig. 3a.--Side view of fly container (vial or bottle).

Experiment 4

Four $\underline{yw}^a/\underline{yw}^a$ females, 3-4 days old were mated in 1/4 pint bottles to 6-8 $y/sc^{8}Y.y^{+}$ males 2-4 days old at time of mating. The experimental parents were subjected to a 0.3 kV/cm inhomogeneous, (+) polarity electrostatic field for a total of 7-8 days. The temperature range in the 0.3 kV/cm field for all experiments was 24 ± 1°C.

RESULTS

Table 2 shows the genotypes and phenotypes of regular and exceptional progeny resulting from primary nondisjunction in $\underline{yw}^a/\underline{yw}^a$ females and $\underline{y}/sc^{8}Y.y^{+}$ males. The yellow and yellow-apricot exceptional males are expected to be sterile if the result of primary nondisjunction since they would have no Y chromosome. These exceptional males were mated to virgin females to determine if they were sterile and if so were recorded as re-

TABLE 2.--Expected gametes and progeny in primary nondisjunction experiment

female \ male	regular		nondisjunction	
	y	$sc^{8}Y.y^{+}$	$y/sc^{8}Y.y^{+}$	0
regular y w^a	y w^a/Y yellow female	y $w^a/sc^{8}Y.y^{+}$ apricot male	y w^a/y/$sc^{8}Y.y^{+}$ wild type female	y w^a/0 yellow, apricot male (sterile)
y w^a/y w^a	triplo-X (dies)	$yw^a/yw^a/sc^{8}Y.y^{+}$ apricot female	triplo-X (dies)	y w^a/y w^a yellow, apricot female
nondisjunction 0	y/0 yellow male (sterile)	lethal	$y/sc^{8}Y.y^{+}$ wild type male	lethal

sulting from primary nondisjunction. All exceptional males were tested for sterility except those which were damaged during counting, over-etherized prior to counting or were lost prior to mating.

Experiment 1

Table 3 shows the number of regular and exceptional progeny produced from $\underline{yw}^a/\underline{yw}^a$ females and $\underline{y}/sc^{8}Y.y^+$ males subjected to a 3 kV/cm homogeneous electrostatic field. A greater number of exceptional progeny were produced from treated and control female parents as compared to the number of exceptional progeny produced from the corresponding male parents. A greater number of exceptional yellow males resulting from primary nondisjunction in the $\underline{yw}^a/\underline{yw}^a$ females and exceptional yellow-apricot males resulting from primary nondisjunction in the $\underline{y}/sc^{8}Y.y^+$ males were found as compared to exceptional apricot females from the female parents and exceptional wild type females from nondisjunction in the male parents. Out of a total of 12334 progeny primary nondisjunction in the treated females

TABLE 3

Number of regular and exceptional progeny from primary nondisjunction in cross of yw^a/yw^a ♀♀ X $y/sc^{8}Y.y^+$ ♂♂. Experimental parents subjected to a 3 kV/cm homogeneous electrostatic field

Treatment	Regular progeny	N	Exceptional progeny from nondisjunction: in ♀♀		in ♂♂	
			w^a♀	y♂	+♀	yw^a♂
3 kV/cm	12304	114	8	16	1	5
Control	7812	52	4	10	2	5

N = number of female parents

resulted in 16 exceptional males and 8 exceptional females while nondisjunction in the treated male parents resulted in 5 exceptional males and 1 exceptional female. Out of a total of 7833 progeny from the control parents there were 10 exceptional males and 4 exceptional females from the female parents and from the male parents there were 5 exceptional males and 2 exceptional females.

The frequency of primary nondisjunction in the treated $\underline{yw}^a/\underline{yw}^a$ females was 0.194% or 1/513 progeny as compared to 0.179% or 1/559 progeny from the control females (Table 4). The frequency of primary nondisjunction in the treated $y/sc^8Y.y^+$ males was 0.048% or 1/2051 progeny as compared to 0.089% or 1/1117 progeny from the control males. The differences in nondisjunction frequencies are statistically nonsignificant.

TABLE 4

Per cent primary nondisjunction. Parents subjected to 3 kV/cm homogeneous, (-) polarity electrostatic field

Parent	Per cent nondisjunction 3 kV/cm	Control	P
♀	0.194	0.179	-
♂	0.048	0.089	-
♀ & ♂	0.243	0.268	-

* indicates significance at .05 level

The per cent nondisjunction in the female and male parents was calculated as follows:

$$\% \text{ nondisj. in } \underset{++}{\varphi\varphi} = \frac{(\#\text{ excep. } w^a \underset{++}{\varphi\varphi} + \#\text{ excep. } y \hat{\sigma}\hat{\sigma})}{\text{Total \# of regular and exceptional progeny}} \times 100$$

$$\% \text{ nondisj. in } \male\male = \frac{(\#\text{ excep. } + \underset{++}{\female\female} + \#\text{ excep. } yw^a\ \male\male)}{\text{Total \# of regular and exceptional progeny}} \times 100$$

Experiment 2

Table 5 shows the total number of regular and exceptional progeny and the number produced per transfer due to primary nondisjunction in parents subjected to a 3 kV/cm inhomogeneous electrostatic field.

TABLE 5

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a $\underset{++}{\female\female}$ X $y/sc^8Y.y^+$ $\male\male$. Parents subjected to 3 kV/cm inhomogeneous electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females $w^a\female$	females $y\male$	males $+\female$	males $yw^a\male$
3 kV/cm	a	41	2688	0	0	1	2
	b	41	2399	0	1	1	2
	c	37	1926	2	3	0	2
	Total	119	7013	2	4	2	6
Control	a	32	2225	0	1	1	2
	b	32	2245	1	2	2	1
	c	28	1460	2	3	0	1
	Total	92	5930	3	6	3	4

N = number of parent females

Out of a total of 7027 progeny, nondisjunction in the treated $\underline{yw}^a/\underline{yw}^a$ female parents resulted in 4 exceptional males and 2 exceptional females while nondisjunction in the treated $\underline{y}/sc^8Y.y^+$ male parents resulted

in 6 exceptional males and 2 exceptional females. Nondisjunction in the control female parents resulted in 6 exceptional males and 3 exceptional females while nondisjunction in the control male parents produced 4 exceptional males and 3 exceptional females out of a total number of 5946 progeny.

Except for transfer c the frequency of nondisjunction in the treated male parents was greater than in the treated female parents while the overall frequency was higher in the control female parents than in the control male parents, however, the nondisjunction frequency was greater in transfers a and b of the control males (Table 6). The nondisjunction rate was lower in the treated female parents compared to the control female

TABLE 6

Per cent primary nondisjunction. Parents subjected to a 3 kV/cm inhomogeneous electrostatic field

Parent	Transfer	Per cent Nondisjunction 3 kV/cm	Control	P
♀	a	0.000	0.044	-
	b	0.041	0.133	-
	c	0.258	0.341	-
	Total	0.085	0.151	-
♂	a	0.111	0.134	-
	b	0.124	0.133	-
	c	0.103	0.068	-
	Total	0.113	0.117	-
♀ & ♂	a	0.111	0.178	-
	b	0.165	0.266	-
	c	0.361	0.409	-
	Total	0.198	0.268	-

* indicates significance at .05 level

parents and only very slightly lower in the treated males compared to the control male parents. The differences in nondisjunction rates were nonsignificant.

Experiment 3

The total number of regular and exceptional progeny due to primary nondisjunction in $\underline{yw}^a/\underline{yw}^a$ females of varying age groups and $\underline{y}/sc^{8}Y.y^{+}$ males 2-6 days old at the time of mating is shown in Table 7.

TABLE 7

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females of varying age groups and $y/sc^{8}Y.y^{+}$ males. Experimental parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field. Combined data from transfers a, b, and c.

Treatment	Parent ♀ age	Total regular progeny	Exceptional progeny from nondisjunction in: females		males	
			w^a♀	y♂	+♀	yw^a♂
0.3 kV/cm	0-8 hr.	4952	3	0	2	6
"	1-2 day	6594	3	7	5	4
"	3-4 "	4686	6	7	0	1
"	5-6 "	4231	5	1	3	2
"	0-6 "	20463	17	15	10	13
Control	0-8 hr.	7452	1	10	3	7
"	1-2 day	13325	5	9	6	11
"	3-4 "	8456	9	15	5	5
"	5-6 "	19006	10	22	5	11
"	0-6 "	48239	25	56	19	34

Parents were subjected to a 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field. Fewer exceptional yellow males than apricot females were produced from treated female parents 0-8 hours old and 5-6 days old at the

time of mating and when subjected to the test field while fewer exceptional yellow-apricot males than wild type exceptional females were produced from treated $\underline{y}/sc^{8}Y.y^{+}$ male parents mated to females 1-2 and 3-4 days old at the time of mating and when exposed to the test field. There was a greater number of exceptional males than exceptional females from treated females 1-2 and 3-4 days old at the time of mating and more exceptional yellow-apricot males than wild type exceptional females from treated male parents mated to females 0-8 hours old and 3-4 days old at the time of mating. The control females of each age group produced a greater number of exceptional yellow males than exceptional apricot females. There was a greater number of exceptional yellow-apricot males than wild type exceptional females from control males mated to the control female parents of every age group tested except females 3-4 days old at the time of mating.

The frequency of primary nondisjunction based on total progeny yield from females of varying ages at the time of mating is shown in Table 8.

TABLE 8

Per cent primary nondisjunction in yw^{a}/yw^{a} female parents of varying age groups and in $y/sc^{8}Y.y^{+}$ male parents 2-6 days old. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field.

Parent ♀ age	% Nondisj. in ♀ 0.3 kV/cm	Control	P	% Nondisj. in ♂ 0.3 kV/cm	Control	P
0-8 hr.	0.060	0.147	-	0.161	0.134	-
1-2 day	0.151	0.104	-	0.136	0.127	-
3-4 "	0.276	0.283	-	0.021	0.118	-
5-6 "	0.141	0.168	-	0.118	0.084	-
0-6 "	0.156	0.167	-	0.112	0.109	-

* indicates significance at .05 level

No significant difference in primary nondisjunction rates in females of each age group is noted. The frequency of nondisjunction in treated females was lowest in 0-8 hour old females and highest in 3-4 day old females. In the control females the primary nondisjunction rate was highest in females 3-4 days old and lowest in females 1-2 days old. The greatest difference in nondisjunction rates was noted in the 0-8 hour old females. Except for male parents mated to females 3-4 days old the rate of nondisjunction was slightly greater in the treated males than in the control males. The difference in rate of nondisjunction in the treated males as compared to the control males was not significant.

Figure 4 shows the relationship of the frequency of primary

Fig. 4.-- Per cent primary nondisjunction in $\underline{y}\ \underline{w}^{a}/\underline{y}\ \underline{w}^{a}$ females of varying ages subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field.

nondisjunction in $\underline{yw}^a/\underline{yw}^a$ females to female age at the time of mating and exposure to the test field. The frequency of nondisjunction was greatest in females 3-4 days old. There is no significant difference between the treated and control females of comparable age groups but the rate of nondisjunction in treated females 3-4 days old is significantly higher than in treated females 0-8 hours old at the time of mating. The rates in the same control groups were not significantly different. The reason for the high nondisjunction rate in females 3-4 days old is not clear. The treated males mated to the treated females 3-4 days old did in fact show the lowest rate of nondisjunction of any treated or control male group, but the males were from 2-6 days old.

Table 9 shows the number of regular and exceptional progeny

TABLE 9

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females 0-8 hours old at the time of mating to $y/sc^8Y.y^+$ males. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females		males	
				$w^a♀$	$y♂$	$+♀$	$yw^a♂$
0.3 kV/cm	a	11	1508	1	0	1	2
	b	10	2169	2	0	0	4
	c	8	1275	0	0	1	0
	Total	29	4952	3	0	2	6
Control	a	9	1571	0	3	0	1
	b	9	3212	0	1	2	4
	c	9	2669	1	6	1	2
	Total	27	7452	1	10	3	7

N = number of bottles

produced per transfer from females 0-8 hours old at the time of mating. No exceptional yellow males were noted in any transfer of the treated female parents. A greater number of exceptional males than exceptional apricot females from nondisjunction in the control female parents was noted. Based on total progeny from the treated female parents the per cent nondisjunction was 0.060% (1/1651) compared to 0.147% (1/679) in the control females. The nondisjunction frequency in the treated males was 0.161% (1/620) and 0.134% (1/746) in the control males (Table 10).

TABLE 10

Per cent primary nondisjunction. yw^a/yw^a females X $y/sc^{8}Y.y^+$ males. Females 0-8 hours old at the time of mating. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field

Parent	Transfer	Per cent Nondisjunction 0.3 kV/cm	Control	P
♀	a	0.066	0.190	-
	b	0.092	0.031	-
	c	0.000	0.261	-
	Total	0.060	0.147	-
♂	a	0.198	0.147	-
	b	0.184	0.186	-
	c	0.078	0.074	-
	Total	0.161	0.134	-
♀ & ♂	a	0.264	0.253	-
	b	0.276	0.217	-
	c	0.078	0.373	-
	Total	0.221	0.281	-

* indicates significance at .05 level

A significantly greater number of regular progeny were produced

per bottle from control parents for each transfer than from treated parents in each transfer (Table 11). Three bottles were lost from the test group due to death of the parents. There seemed to be a tendency for more parents to die in the treatment group than in the control group. This was not always consistent though. The significant difference between the number of regular progeny is very likely due to a loss of some of the test female parents. Paper caps with a glass tube with a wire immersed into the medium were used to plug the control bottles containing females 5-6 days old but the same type rubber stopper described in the methods and materials was used to plug all other control groups as well as the experimental groups. The environment inside the control bottles (with the possible exception of those with paper stoppers) should have been similar to that inside the experimental bottles except for the presence of the field.

TABLE 11

Comparison of mean number of regular progeny produced per bottle per transfer. yw^a/yw^a female parents 0-8 hours old at time of mating and subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

	0.3 kV/cm		Control		
Transfer	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
a	11	137.0 ± 10.9	9	174.5 ± 13.7	*
b	10	216.9 ± 37.1	9	356.8 ± 28.1	*
c	8	159.3 ± 29.7	9	296.5 ± 13.4	*

* indicates significance at .05 level

N = number of bottles

Based on total data Table 12 shows a greater number of exceptional yellow males than apricot exceptional females from primary nondisjunction in treated and control females. A greater number of exceptional wild type females than yellow-apricot exceptional males resulting via nondisjunction in the test male parents were recorded. The opposite was true concerning the corresponding control male parents.

TABLE 12

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females 1-2 days old at the time of mating to $y/sc^{\delta}Y.y^+$ males. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females $w^a♀$	females $y♂$	males $+♀$	males $yw^a♂$
0.3 kV/cm	a	8	2664	1	3	4	2
	b	8	2424	2	2	1	0
	c	8	1506	0	2	0	2
	Total	24	6594	3	7	5	4
Control	a	17	4950	1	2	2	1
	b	17	5365	2	3	2	6
	c	17	3010	2	4	2	4
	Total	51	13325	5	9	6	11

N = number of bottles

Table 13 shows the per cent of primary nondisjunction in $\underline{yw}^a/\underline{yw}^a$ females 1-2 days old at mating and exposure to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field and the corresponding control female

TABLE 13

Per cent primary nondisjunction in yw^a/yw^a females and $y/sc^8Y.y^+$ males. Females 1-2 days old at time of mating and subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Parent	Transfer	Per cent nondisjunction 0.3 kV/cm	Control	P
♀	a	0.149	0.060	-
	b	0.164	0.092	-
	c	0.132	0.198	-
	Total	0.151	0.104	-
♂	a	0.224	0.060	-
	b	0.041	0.148	-
	c	0.132	0.198	-
	Total	0.136	0.127	-
♀ & ♂	a	0.373	0.121	*
	b	0.205	0.241	-
	c	0.264	0.397	-
	Total	0.287	0.232	-

* indicates significance at .05 level

parents. The differences in nondisjunction rates between the treated and control parents are nonsignificant except for the difference in the combined rate of nondisjunction in transfer a of the treated males and females compared to the rate in control males and females combined. The rate was higher in the treated parents than in the control parents. This is the only group in the entire nondisjunction study which showed a significant difference in the rate of nondisjunction. The total number of regular progeny in transfer a of the treated parents was 2664. It is doubtful that the difference in the rate of nondisjunction was truly due to the effect of the field, however, the cause is not clear. Table 14 shows a comparison of the mean number of regular progeny from the same

parents.

TABLE 14

Comparison of mean number of regular progeny produced per bottle per transfer. yw^a/yw^a female parents 1-2 days old at time of mating and subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

	0.3 kV/cm		Control		
Transfer	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
a	8	333.1 ± 18.0	17	291.1 ± 27.3	-
b	8	303.0 ± 41.9	17	315.5 ± 22.2	-
c	8	188.2 ± 45.7	17	177.0 ± 14.2	-

* indicates significance at .05 level

N = number of bottles

The number of regular and primary exceptions produced per transfer from $\underline{yw}^a/\underline{yw}^a$ females 3-4 days old at mating and exposure to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field and male parents is shown in Table 15.

Primary nondisjunction rates of treated females 3-4 days old at the time of mating and exposure to a 0.3 kV/cm inhomogeneous field and control females are shown in Table 16.

A comparison of the mean number of regular progeny produced from the same female parents is shown in Table 17. A significantly greater number of regular progeny were produced in transfers a and b of the control groups compared to the experimental groups. Because the standard error is so great in transfer c the difference noted is not significant. The large standard error is a result of some bottles having only a very few

flies in transfer c.

TABLE 15

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females 3-4 days old at the time of mating to $y/sc^8Y.y^+$ males. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females		males	
				$w^a♀$	$y♂$	$+♀$	$yw^a♂$
0.3 kV/cm	a	6	2134	1	1	0	1
	b	5	1513	3	5	0	0
	c	5	1039	2	1	0	0
	Total	17	4686	6	7	0	1
Control	a	5	3342	1	0	2	1
	b	5	2819	4	6	1	3
	c	5	2295	4	9	2	1
	Total	15	8456	9	15	5	5

N = number of bottles

Table 18 shows the number of regular and exceptional progeny from primary nondisjunction in females 5-6 days old at time of mating and in corresponding control females. Overall more exceptional females than exceptional males were produced from the treated parents. More exceptional males than exceptional females were produced from the control parents. The rates of primary nondisjunction from the same females and parent males are shown in Table 19. The rate of nondisjunction in the control parents was not significantly different from the rate in the treated parents of any transfer or total group data.

TABLE 16

Per cent primary nondisjunction in yw^a/yw^a females and $y/sc^8Y.y^+$ males. Females 3-4 days old at time of mating and subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Parent	Transfer	Per cent nondisjunction 0.3 kV/cm	Control	P
♀	a	0.093	0.029	-
	b	0.525	0.353	-
	c	0.287	0.283	-
	Total	0.276	0.283	-
♂	a	0.046	0.089	-
	b	0.000	0.141	-
	c	0.000	0.130	-
	Total	0.021	0.118	-
♀ & ♂	a	0.140	0.119	-
	b	0.525	0.494	-
	c	0.287	0.692	-
	Total	0.297	0.400	-

* indicates significance at .05 level

TABLE 17

Comparison of mean number of regular progeny produced per bottle per transfer. yw^a/yw^a female parents 3-4 days old at time of mating and subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

	0.3 kV/cm		Control		
Transfer	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
a	6	355.6 ± 81.8	5	653.8 ± 23.1	*
b	6	252.1 ± 53.5	5	563.8 ± 42.9	*
c	5	207.8 ± 96.7	5	459.0 ± 76.3	-

* indicates significance at .05 level

N = number of bottles

TABLE 18

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females 5-6 days old at the time of mating to $y/sc^8Y.y^+$ males. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females		males	
				$w^a♀$	$y♂$	$+♀$	$yw^a♂$
0.3 kV/cm	a	11	2013	3	1	0	1
	b	11	738	0	0	2	0
	c	11	1580	2	0	1	1
	Total	33	4231	3	1	3	2
Control	a	22	5877	3	3	2	4
	b	22	7082	3	5	3	5
	c	22	6047	4	14	0	2
	Total	66	19006	10	22	5	11

N = number of bottles

The mean number of regular progeny produced per bottle per transfer from females 5-6 days old at mating and the parent males, both of which were subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field, was significantly lower than the regular progeny from the corresponding control parents (Table 20). This again is likely due to the death of some of the treated parents.

In general the nondisjunction rate was higher in female parents than in male parents. This was true for treated female parents of every age group tested except for females 0-8 hours old at mating and subjected to the field. The same was true of the control female parents except for females 1-2 days old at mating. The nondisjunction rate in parent females

TABLE 19

Per cent primary nondisjunction in yw^a/yw^a females and $y/sc^8Y.y^+$ males. Females 5-6 days old at time of mating and subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Parent	Transfer	Per cent nondisjunction 0.3 kV/cm	Control	P
♀	a	0.198	0.101	-
	b	0.000	0.112	-
	c	0.126	0.296	-
	Total	0.141	0.168	-
♂	a	0.049	0.101	-
	b	0.270	0.112	-
	c	0.126	0.033	-
	Total	0.118	0.084	-
♀ & ♂	a	0.247	0.203	-
	b	0.270	0.225	-
	c	0.252	0.329	-
	Total	0.259	0.251	-

* indicates significance at .05 level

TABLE 20

Comparison of mean number of regular progeny produced per bottle per transfer. yw^a/yw^a female parents 5-6 days old at time of mating and subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

	0.3 kV/cm		Control		
Transfer	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
a	11	183.0 ± 25.6	22	266.5 ± 18.1	*
b	11	67.0 ± 17.1	22	321.2 ± 27.8	*
c	11	143.4 ± 43.4	22	274.8 ± 32.9	*

* indicates significance at .05 level

N = number of bottles

1-2, 3-4, and 5-6 days old at mating and subjected to the field was 1/660, 1/316, and 1/706 respectively and 1/952, 1/311, and 1/595 respectively for the control groups. The rate in males mated to 1-2, 3-4, and 5-6 day old females was 1/633, 1/4687, and 1/846 respectively for the experimental groups and 1/785, 1/846, and 1/1188 respectively for the control groups.

Except for female parents 1-2 days old at mating the nondisjunction rate was slightly lower in the experimental than in the control female groups while the nondisjunction rate was higher in the experimental males than in the control male parents except for the control males mated to females 3-4 days of age at mating.

Experiment 4

Table 21 shows the number of regular and exceptional progeny from $\underline{yw}^a/\underline{yw}^a$ females 3-4 days old at mating and $\underline{y}/sc^8Y.y^+$ males 2-4 days old at mating and subjected to a 0.3 kV/cm inhomogeneous, (+) polarity, electrostatic field. A greater number of exceptional yellow males than apricot females, from primary nondisjunction in the females, were produced in each transfer of the control parents. More yellow-apricot exceptional males than wild type females were produced from nondisjunction in the control males. The opposite was true of the treated male parents.

Table 22 shows the differences in the nondisjunction frequencies for the treated and control parents to be nonsignificant, however, based upon the total progeny yield the nondisjunction rate was greater in the experimental females than in the controls while the rate was higher in the control males than in the experimental males. In this experiment the non-

TABLE 21

Number of regular and exceptional progeny from primary nondisjunction in yw^a/yw^a females and $y/sc^8Y.y^+$ males subjected to a 0.3 kV/cm inhomogeneous, (+) polarity, electrostatic field

Treatment	Transfer	N	Regular progeny	Exceptional progeny from nondisjunction in: females		males	
				$w^a♀$	$y♂$	$+♀$	$yw^a♂$
0.3 kV/cm	a	14	2439	0	1	3	3
	b	13	2177	1	5	2	0
	c	10	1390	0	2	0	1
	Total	37	6006	1	8	5	4
Control	a	31	8878	2	3	10	10
	b	31	12588	2	15	5	18
	c	31	8809	1	9	6	9
	Total	93	29825	5	27	21	37

N = number of bottles

disjunction rate was the same in the treated parents, however, the rate was higher in the control male than in the control female parents.

Table 23 again shows a significantly greater number of regular progeny from the control parents as compared to the treated parents.

TABLE 22

Per cent primary nondisjunction in yw^a/yw^a females and $y/sc^{8}Y.y^+$ males. Experimental parents subjected to a 0.3 kV/cm inhomogeneous, (+) polarity, electrostatic field

Parent	Transfer	Per cent nondisjunction 0.3 kV/cm	Control	P
♀	a	0.040	0.056	-
	b	0.274	0.134	-
	c	0.143	0.113	-
	Total	0.149	0.106	-
♂	a	0.245	0.224	-
	b	0.091	0.182	-
	c	0.071	0.169	-
	Total	0.149	0.193	-
♀ & ♂	a	0.286	0.255	-
	b	0.365	0.316	-
	c	0.215	0.282	-
	Total	0.298	0.300	-

* indicates significance at .05 level

TABLE 23

Comparison of mean number of regular progeny produced per bottle per transfer. yw^a/yw^a females mated to $y/sc^{8}Y.y^+$ males. Parents exposed to 0.3 kV/cm inhomogeneous, (+) polarity, electrostatic field

	0.3 kV/cm		Control		
Transfer	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
a	11	137.0 ± 10.9	9	174.5 ± 13.7	*
b	10	216.9 ± 37.1	9	356.8 ± 28.1	*
c	8	159.3 ± 29.7	9	296.5 ± 13.4	*

* indicates significance at .05 level

N = number of bottles

DISCUSSION

Nondisjunction refers to the failure of a pair of homologous chromosomes to segregate during meiosis, thus producing eggs with two or no maternal chromosomes and sperm with two or no paternal chromosomes for the homologs involved.

The phenomenon of nondisjunction (for the X chromosomes of a Drosophila female) as evidenced by the production of patroclinous males and matroclinous females, was first noted by Bridges (1913).

In D. melanogaster the normal female has two X chromosomes and 3 pairs of autosomes while the male has one X and one Y chromosome and 3 pairs of autosomes (Bridges, 1916). Bridges suggested that patroclinous males and matroclinous females from XX and XY parents be called primary exceptions and that nondisjunction producing primary exceptions be called primary nondisjunction.

Primary nondisjunction may produce XXX meta-female zygotes, but these are very difficult to detect since few survive to the imago stage. YO male zygotes may also be produced but this condition is lethal in the early egg stage and adults of this chromosomal constitution are not found.

Exceptional progeny coming from primary exceptional, matroclinous females having an XXY chromosomal constitution are called secondary exceptions (Bridges, 1916) and the type of nondisjunction producing secondary exceptions is called secondary nondisjunction.

Bridges (1916) suggests that nondisjunction is due to the entanglement of paired chromosomes which fail to disengage during the anaphase stage of meiosis. Sturtevant and Beadle (1936) suggested that nondisjunction is due to a failure of metaphase pairing of homologues followed by independent segregation of each unpaired homologue. This would give 1/2 mono-X eggs, 1/4 nullo-X eggs and 1/4 diplo-X eggs. Sandler and Braver (1954) propose a similar method in which one or both unpaired homologues may be lost at metaphase instead of nondisjoining.

Grell (1962a, 1962b) proposed a scheme of two kinds of pairing of the X chromosomes of D. melanogaster females; (1) exchange pairing which precedes crossing over and (2) distributive pairing which precedes disjunction. Exchange pairing occurs only between homologues while distributive pairing occurs between nonhomologous chromosomes. Non-homologous pairing as the mechanism of primary nondisjunction has also been suggested by Sandler and Novitski (1956) and Forbes (1960, 1962). Pre-equational sister chromatid separation with no secondary division of homologous chromatids has also been suggested as a method of primary nondisjunction (Merriam and Frost, 1964).

The recovery of more exceptional males than exceptional females, as a result of primary nondisjunction in the females, was first described by Safir (1920). The excess of exceptional females as a result of primary nondisjunction in both the female and male parents was generally noted in the present study. The cause of this phenomenon is unknown, but Safir indicated there may be some type of elimination of the X chromosomes or a lagging of the paired X chromosomes at the metaphase plate, resulting in all nullo-X eggs.

Sandler and Braver (1954) indicate that one or both unpaired X

chromosomes may be lost at the metaphase plate in oogenesis and this could lead to the recovery of more patroclinous males than matroclinous females.

Experiments by Mavor (1921, 1922, 1924, 1929) were the first to show that x-irradiation increased the frequency of primary nondisjunction. Since that time several workers (Patterson, Brewster and Winchester, 1932; Savhagen, 1961; Strangio, 1961; Uchida, 1962; Zimmering, 1962; Zimmering and Wu, 1963, 1964; Clark and Clark, 1963; Traut, 1964; Day and Grell, 1966; and Grell, 1966a) have demonstrated an increase in nondisjunction in Drosophila by x-irradiation.

Patterson, et. al., (1932) showed that x-rays applied to retained maturing Drosophila eggs increased primary nondisjunction although it has been suggested that a number of the excess exceptional males they found as compared to the exceptional females could have been due to X chromosome loss rather than nondisjunction.

Mavor (1921-1929), however, x-rayed females of varying ages and showed that the effect of x-rays was not limited to retained mature eggs but to eggs in earlier stages of development as well. Further information was added by Uchida (1962) when she reported an increase in the rate of primary nondisjunction in x-rayed D. melanogaster females. The nondisjunction rate appeared to increase as the females were aged after irradiation and prior to mating. Females aged 29 days before mating showed the highest nondisjunction rate whele control females aged 7 days prior to mating showed the highest rate for the control group (although the rate at 7 days was not greatly different from the females younger or older). Data obtained by Uchida in the control group were confirmed by Kelsall (1963) and Roberts (1963) when they reported that the rate of nondisjunction of sex chromosomes

did not increase with increasing parental age of D. melanogaster.

Savhagen (1961) has shown that in young D. melanogaster males irradiated at 0-1 and 3-4 days of age and then mated to virgin females, the frequency of exceptional males and females was greatest in the 8th day after irradiation. The 0-1 day old males did show a higher rate than the 3-4 day old males, however. A similar pattern of spermatogenesis sensitivity was reported by Strangio (1961). It was reported by Savhagen and Strangio that nondisjunctional events exceeded those of exchange events when adult males were irradiated. On the other hand, Zimmering and Wu (1963, 1964) found a higher frequency of X-Y exchange than X-Y nondisjunction in x-rayed D. melanogaster males. Zimmering suggests that the genetic tests used by Savhagen may have been faulty but the tests used by Strangio apparently should have given results similar to theirs.

A correlation between nondisjunction and female age and temperature has also been noted (Hildreth and Ulrichs, 1969, and Tokunaga, 1970). They found that pre-inseminated or post-inseminated females held at 10°C and aged showed a higher rate of nondisjunction than a like group held at 25°C. Unlike previously mentioned studies on x-ray influence and parent age in which more exceptional males than exceptional females are found it was reported by Hildreth and Tokunaga that the ratio of exceptional females and males was 1:1. They suggest that the unequal sex ratio of exceptional progeny obtained in other studies may be due to two types of exceptional males being revovered, those resulting from chromosome loss and those resulting from nondisjunction.

While there is fluctuation in the rate of primary nondisjunction in females of varying age groups subjected to a 0.3 kV/cm inhomogeneous, (-)

polarity, electrostatic field, no significant difference was noted between the control and experimental groups. It can be noted, however, that the highest total rate of nondisjunction for broods a, b and c combined occurred in females 3-4 days old at the time of mating, therefore, eggs from these females were collected when the females were aged from 6-11 days. Transfer b of these same experimental and control females shows the highest nondisjunction rate. The females would have been 7-9 days old at the time eggs were collected for brood (b). This is consistent with the control data of Uchida and the data obtained by Roberts (1963) and Kelsall (1963) which indicated a fluctuation in spontaneous primary nondisjunction rates in non-treated, aged, Drosophila females. Uchida (1962) reported the highest rate of nondisjunction in non-treated females aged 7 days. While the tendency in primary nondisjunction rates in this study is consistent with other studies, the overall rates for transfers or broods a, b and c combined herein (1/361), are somewhat higher than those reported by Uchida (about 1/1400), Kelsall (about 1/2300 exceptions from XX eggs and 1/960 exceptions from nullo-X eggs) and Bridges (about 1/2000), but not as high as reported by Roberts (1963) who used females carrying inversions on the X chromosomes and which were also inversion heterozygotes for chromosomes II and III.

Temperature fluctuation in not believed to greatly affect the nondisjunction rates herein since the control and treated parents were always at the same temperature and at no time were the parents subjected to a temperature lower than 22°C or higher than 27°C.

In view of the relatively high rate of primary nondisjunction in the controls and also in the flies subjected to electrostatic fields, several larvae were taken from the stock cultures periodically and salivary gland

chromosome smears made for the purpose of looking for any chromosome structural aberrations which might increase the rate of primary nondisjunction. At no time were any identified. The stocks from which the parents for crossing were obtained were derived from a single pair of flies for each parental line. If any chromosomal aberrations had been present they would have been in all of the progeny. The presence of inversions, deletions or translocations in the parents can apparently be ruled out, unless any one type was too small to be detected by microscopic examination.

Flies whose chromosomes are structurally heterozygous show an increase in the rate of primary nondisjunction. Morgan and Sturtevant (1944) reported that females heterozygous for one inversion in the X chromosome and one other in the autosomes produced more primary exceptions than females heterozygous for only an X chromosome inversion. This was confirmed by Cooper et. al., (1955) when they found that several different sex-linked and autosomal inversions increased nondisjunction of the structurally heterozygous X chromosomes. They reported the greatest primary nondisjunction rate is in females which were structurally heterozygous for the sex chromosomes and both major autosomes. These two groups of investigators found equal numbers of exceptional females and males when one X chromosome and one autosome carried inversions while Sturtevant and Beadle (1936) reported that considerably greater numbers of exceptional males than exceptional females were produced from females with certain sex-linked inversions. They suggested that the exceptional males are due to 4 strand double crossing over in the inversion loop of the X chromosomes and also nondisjunction of the X's, thus, since two events could give rise to XO males there would be

a greater number of such males. The females, on the other hand, would be the result of nondisjunction only.

Forbes (1962) suggests the equality of exceptions obtained by Morgan and Sturtevant and Cooper et. al., when working with autosomal inversions might be the result of double crossing over in the X chromosomes of the females occuring in such a low frequency that the number of patroclinous males resulting from this phenomenon was too few to affect the sex ratio observed. Nondisjunction would thus be assumed to be the cause of exceptional males and females and equality of exceptions would be evident. However, in his studies Forbes (1960, 1962) reported a greater number of exceptions when one X and one autosome carried an inversion as compared to females heterozygous for a sex-linked inversion and both autosomes. He reports that the type of sex-linked inversions used influences the number of exceptional progeny and that the number of male exceptions exceeds that of females. He suggests that the excess of exceptional males is due to the frequency of four strand double crossovers in the inversion loop. Since double crossovers in some sex-linked inversions occur more frequently than in others this would explain the difference in the number of exceptional males depending on the type of sex-linked inversion present. Like Cooper et. al., he suggests that nonhomologous pairing leads to primary nondisjunction. The results of Forbes confirm the hypothesis of Sandler and Novitski (1956) and Cooper et. al., (1955) that nonhomologous pairing of chromosomes is the mechanism of primary nondisjunction. This mechanism is again suggested by Grell (1957, 1959) and Oksala (1958) who assumed that nonhomologous pairing took place between the Y chromosome and other autosomes when they found nonrandom assortment between the autosomes and the Y chromo-

some.

Sturtevant (1929) found that an extreme example of X chromosome loss was associated with the claret mutant stock of D. simulans which gave about 50% exceptional sons and 6% exceptional daughters. Sometime later Wald (1936) examined fertilized eggs of claret females and found that at the end of the first meiotic division the chromosomes were widely separated from one another at the poles and that the second meiotic division often did not occur. This gave some cells with less than a haploid set of chromosomes. Lewis and Gencarella (1952) and Davis (1969) also report a genic control of primary nondisjunction and chromosome loss by the influence of the claret-nondisjunctional mutant in D. melanogaster. Davis indicates that nondisjunction of chromosomes in females probably occurs only in meiosis I, never in meiosis II.

A sex-linked mutant, maroon-like, located in the proximal euchromatin of the chromosome causes a high incidence of X chromosome loss and when present with differing genetic backgrounds produces differing levels of X chromosome loss or nondisjunction (Spieler, 1961, 1963; Thompson, 1962). The inducing phenomena act only in females and not in males.

Non-sex-linked genic control of nondisjunction has been reported by Sandler et. al., (1968) when studying a number of meiotic mutants on chromosome II and III found in natural populations of Drosophila. They found mutants which when homozygous resulted in irregular segregation of the sex and fourth chromosomes in females, caused high nondisjunction of the fourth chromosome in males and caused high nondisjunction in the second meiotic division of both sexes. A segretation distorted (SD) gene was also found.

It seems possible, therefore, that the relatively high rate of primary nondisjunction noted in this study could be due to the genetic constitution of the parents. There does seem to be a clear indication that primary nondisjunction was more frequent in female than male parents, each of which had a different genetic constitution and also a different kind of meiosis (there is no crossing over in males), although a higher rate in females was not found in every experiment. Because no inversions were known to be present in either parent this factor can be ruled out, as far as can be determined, as a cause of the increased rate.

Another factor which might be involved is the humidity to which the parent flies were subjected during their mating period. Parents treated with 0.3 kV/cm electrostatic field and also the control groups were mated in bottles which were capped with rubber stoppers. There was every indication that the humidity in the bottles was somewhat higher than when bottles were stoppered with paper caps although the humidity would be even quite high in bottles of the latter type. The humidity variation could be ruled out almost completely because the rate of primary nondisjunction was about the same in parents subjected to a 3 kV/cm electrostatic field which were mated in vials stoppered with cotton plugs (in which the humidity would be comparatively low) as in parents mated in the bottles stoppered with rubber plugs and subjected to a 0.3 kV/cm field.

There is no indication that any force exerted on the parent flies by the electrical field has any effect on the primary nondisjunction rates in females and males.

CONCLUSIONS

The influence of electrostatic fields on primary nondisjunction in D. melanogaster was studied. Results obtained indicate that:

(a) a 3 kV/cm homogeneous or inhomogeneous, (-) polarity, electrostatic field does not affect the rate of primary nondisjunction in males and females;

(b) a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field does not affect the rate of primary nondisjunction in males and females.

CHAPTER IV

NONDISJUNCTION OF THE FOURTH CHROMOSOME OF *DROSOPHILA MELANOGASTER* MALES

METHODS AND MATERIALS

A *Drosophila melanogaster* stock carrying the mutants ci^D (0.0) and ey^D (2.0) located on separate homologs of chromosome four was obtained from Oak Ridge National Laboratory. A stock was made isogenic for chromosomes II and III by the following crosses. ci^D/ey^D virgin females were mated to *Cy/Pm; D/Sb* males. F_1 *Cy/+; D/+;* $ey^D/+$ and *Cy/+; D/+;* $ci^D/+$ females were individually back-crossed to *Cy/Pm; D/Sb* males. F_2 *Cy/Pm; D/Sb;* $ey^D/+$ females were individually mated to F_2 *Cy/+; D/+;* $ci^D/+$ single males. F_3 *Cy/+; D/+;* ci^D/ey^D females were individually mated to F_3 individual males of the same genotype. F_4 ci^D/ey^D males and virgin females were selected and mated to provide an isogenic stock.

Three isogenic wild type females 3 days old were mated in 1/4 pint bottles to 6 ci^D/ey^D isogenic males 6 days old at the time of mating. The experimental flies were subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field for a total of 8-9 days. In this experiment the flies were pre-treated for 2 days prior to the first transfer.

RESULTS

Table 24 shows the results of a study on nondisjunction of the fourth chromosome in D. melanogaster males subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field.

TABLE 24

Frequency of chromosome IV nondisjunction and number of regular and exceptional progeny from nondisjunction in ci^D/ey^D males subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field for 8-9 days

	Regular Progeny			
	(females)		(males)	
Treatment	ci^D	ey^D	ci^D	ey^D
0.3 kV/cm	2517	1924	2271	1834
Control	4599	4089	4265	4045

	Exceptional Progeny			
	(females)		(males)	
Treatment	$ci^D/ey^D/+$	+/0	$ci^D/ey^D/+$	+/0
0.3 kV/cm	1	0	1	4
Control	2	2	1	4

TABLE 24--Continued

Treatment	Total regular progeny	N	Per cent nondisjunction
0.3 kV/cm	8546	70	0.071 (1/1425)
Control	16998	121	0.052 (1/1889)

N = number of bottles (cultures)

Nondisjunction in $\underline{ci}^{D}/\underline{ey}^{D}$ males mated to wild type females would produce exceptional $\underline{ci}^{D}/\underline{ey}^{D}/+$ males and females and $+/0$ exceptional males and females. The former would be triplo-IV progeny and the latter would be haplo-IV progeny. The rate of exceptional progeny combined from treated males was 1/1425 but the rate of triplo-IV exceptions was 1/4274 and the rate of haplo-IV progeny from the treated males was 1/2138. The combined rate of exceptional progeny from the control males was 1/1889. The rate of haplo-IV progeny from the control males was very close (1/2834) to that in experimental males. The rate of triplo-IV progeny from the control males was 1/5667, very close to the experimental rate. The combined rates of nondisjunction of the fourth chromosomes in control and treated males were very similar and the differences in rates were nonsignificant.

It was consistently noted in the treated and control groups that there were fewer progeny carrying the $\underline{ey}^{D}$ mutant than progeny carrying the $\underline{ci}^{D}$ mutant. There were consistently more females than males of each mutant type.

DISCUSSION

Nondisjunction of the autosomes may also occur but aneuploidy for either of the major autosomes of D. melanogaster would result in an inviable zygote. Nondisjunction of the small fourth chromosome may occur, however, and result in viable progeny.

Flies which have only one fourth chromosome (haplo-IV Type) are called "Diminished" (Morgan, Bridges and Sturtevant, 1925) and are characterized by a smaller body size, relatively shorter and more slender bristles, paler body color, darker thorax pattern, larger and rounder eyes but with smaller hairs, reduced or absent aristae and wings slightly spread apart and blunter and cloudier in texture. Flies of this type are less viable and fertile and generally emerge four to five days later than wild type sibs. A nondisjunction event which gives rise to a nullo-IV gamete could also give rise to a diplo-IV gamete which when fertilized by a mono-IV sperm would give rise to a triplo-IV zygote. Flies of this type are distinguished by relatively narrow, pointed wings, of clear texture and held close together, with a dark body color, whose trident pattern is less developed and which have coarser bristles. The viability of triplo-IV flies is apparently comparable to that of diplo-IV flies.

By using males with genetic markers on both homologues of chromosome IV it was possible to distinguish haplo- and triplo-IV progeny in this study.

Morgan et. al., (1925) reported a frequency of production of haplo-IV

gametes of about 1/2000 whereas Hanks (1968) obtained a frequency of about 1/1370. There rates are close to the rates obtained from the control males (1/2834) and from treated males (1/2138) used in this study. The difference in the rates of haplo-IV progeny recorded in this study was not significant though. The frequency of triplo-IV progeny from treated males (1/4274) was very similar to the frequency from control males (1/5667).

Grell (1964) reports that non-cross-over number four chromosomes segregate by distributive pairing depending upon the size of the chromosomes. She found that by adding free X duplications, (ranging in size from "less than or equal to" 0.3 to 3.3 times the length of chromosome four), to the genome of diploid D. melanogaster females there was an increase of nonhomologous pairing of the fours with the X duplication when the duplication was the same size as the four.

Chromosome four marked males were used in this study to insure against crossover chromosome IV gametes being classified as nondisjunction products. Therefore, the rate noted herein is a good indication of the frequency of nondisjunction of the IV's in D. melanogaster (ci^D/ey^D) males. Chromosome movement during meiosis in ci^D/ey^D males does not appear to be influenced by the force produced by an inhomogeneous electrostatic field of 0.3 kV/cm.

CONCLUSIONS

The influence of an electrostatic field on nondisjunction of chromosome IV in D. melanogaster was studied. Results indicate that a 0.3 kV/cm inhomogeneous , (-) polarity, electrostatic field does not affect the rate of nondisjunction of the small fourth chromosome in D. melanogaster males.

CHAPTER V

CROSSING OVER IN THE X CHROMOSOME AND CHROMOSOME II

METHODS AND MATERIALS

A stock of y (1-0.0), w^a (1-1.5), spl (1-3.0), rb (1-7.5) was used to follow crossing over in the distal end of the X chromosome and a stock of m (1-36.1), f (1-56.7), car (1-62.5) was used to follow crossing over in the region more proximal to the centromere of D. melanogaster. The mutants b (2-48.5), cn (2-57.5), c (2-75.5), bw (2-104.5) were used to note crossing over near the centromere and in the right arm of chromosome II.

X chromosome marker stocks were made co-isogenic for chromosomes II and III by the following mating procedure. Canton-S wild type virgin females were mated to Cy/Pm; D/Sb males. F_1 Cy/+; D/+ males were individually mated to Cy/Pm; D/Sb females. F_2 Cy/+; D/+ males and females were selected from an individual vial or bottle and mated to produce a stock of an isogenic wild type stock.

Homozygous y w^a spl rb virgin females were mated to Cy/Pm; D/Sb males. F_1 y w^a spl rb/+ + + +; Cy/+; D/+ females were individually backcrossed. F_2 y w^a spl rb/+ + + +; Cy/Pm; D/Sb females were individually mated to brothers of the same genotype. F_3 y w^a spl rb/y w^a spl rb; Cy/Pm; D/Sb females were mated to their y w^a spl rb/Y; Cy/Pm; D/Sb brothers.

From this cross a stock of y w^a spl rb/y w^a spl rb; Cy/Pm; D/Sb females and y w^a spl rb/Y; Cy/Pm; D/Sb males was obtained. Virgin females from this stock were mated to isogenic wild type males. F_1 y w^a spl rb/+ + + +; Cy/+; D/+ females were mated to brothers of the same genotype. A stock of y w^a spl rb females and males was obtained which was co-isogenic with the wild type stock for chromosomes II and III.

The same mating procedure was used to produce a co-isogenic stock of homozygous m f car stock.

A homozygous stock of b cn c bw, co-isogenic with a wild type stock for chromosme II, was synthesized by the following mating procedure. Virgin b cn c bw females were mated to Cy/Pm; D/Sb males. F_1 b cn c bw/Cy; D/+ females were individually back-crossed to Cy/Pm; D/Sb males. F_2 b cn c bw/Cy; D/+ females were mated to their b cn c bw/Cy; +/Sb brothers. F_3 b cn c bw/ b cn c bw; D/Sb females were individually mated to isogenic wild type males. F_4 b cn c bw/+ + + +; D/+ females were mated to brothers of the same genotype. Homozygous b cn c bw males and females were selected in the F_5 progeny and a stock made.

In all crossover experiments involving mutants on the X chromosome virgin mutant females were collected and mated in 1/2 pint bottles to wild type males. The parents were dumped after 6 days. The F_1 progeny were c collected as they eclosed and were held for 3 days. Females and males were lightly etherized and individual females were placed in vials with 2-3 of their brothers. The F_1 x F_1 parents were subjected to an electrostatic field of (-) or (+) polarity or to a magnetic field as indicated. The magnetic fields were produced by a Harvey-Wells, model HS-1365B, electromagnet with 7.7 cm diameter pole faces. The electromagnet was water cooled and could

operate at 0-65 D.C. amperes and 0-135 D.C. volts. The inter-pole face distance was 3.8 cm.

During the cold weather months the temperature between the pole pieces could be regulated quite well by controlling the water flow around the poles. During the hot weather season the field strength could not be elevated greatly for the water temperature was not low enough to promote sufficient cooling of the pole pieces and increases in temperature between the poles had to be watched very carefully. Temperature ranges during treatment are noted in the appropriate methods and materials or in the results.

Three 8 dram shell vials, stoppered with cotton, were positioned between the poles of the magnet. A piece of styrofoam was cut and holes punched into it so that the three vials were always positioned in the same site after a transfer was made. After initially being placed in the magnetic field the parents were removed only long enough to be transferred to fresh food vials. This was no longer than 3 minutes for each transfer group.

A centigrade thermometer was positioned in the center of the space between the pole pieces of the magnet and a thermometer was positioned above the control group as well.

The parents were pre-treated in the field for two days prior to transfer.

In all cross over experiments involving the second chromosome homozygous mutant virgin females were mated in 1/2 pint bottles to wild type males. The parents were discarded after six days. As the F_1 began to eclose the females were collected as virgins and held for 3 days. Three

day old females were used because preliminary studies showed that the greatest number of progeny resulted from females of this age before mating. In addition if any females were non-virgin, larvae could be detected in the holding vials by at least the end of the third day. If any larvae were noted no females being held in that vial were used. The heterozygous females were lightly etherized and individually mated in vials to 2-3 b cn c bw homozygous males and placed in the treatment field within no more than 30 minutes after mating.

The males came from stock cultures of b cn c bw, from which the original virgin b cn c bw homozygous females were collected.

The same transfer procedure as previously described was followed. After transfer the vials were placed in an incubator at 25 ± 1°C. As the progeny began to eclose classification and counting were carried out on the progeny of each vial.

RESULTS

Crossing Over in the Distal End of the X Chromosome

The number of parental and recombinant types from y w^a spl rb/ + + + + females subjected to a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field are shown in Tables 25 and 26 respectively. There is

TABLE 25

Crossing over in y w^a spl rb/+ + + + females subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

Region	Phenotype	Field ♀♀	Field ♂♂	Control ♀♀	Control ♂♂
E_o	y w^a spl rb	808	766	1264	1276
	+	914	919	1362	1358
E_{1-1}	y	19	24	30	27
	w^a spl rb	17	19	29	20
E_{1-2}	y w^a	15	14	20	12
	spl rb	5	25	16	20
E_{1-3}	y w^a spl	25	34	50	68
	rb	43	39	67	67
	Totals	1846	1840	2838	2848
	Total	3686		5686	

a general tendency that the more mutants the fly carries the fewer the number of progeny of that type. There are some exceptions to this, however, as noted in the data.

Single cross-overs are represented by E_1, doubles by E_2 and triples by E_3. The number following the subscript indicates the cross-over region between the two markers, i.e., E_1-1 represents a single cross-over between the first two markers on the chromosome.

TABLE 26

Crossing over in y w^a spl rb/+ + + + females subjected to a 0.3 kV/cm inhomogeneous, (+) polarity, electrostatic field

Region	Phenotype	Field ♀♀	Field ♂♂	Control ♀♀	Control ♂♂
E_0	y w^a spl rb	446	484	1440	1310
	+	430	453	1500	1530
E_1-1	y	13	12	43	28
	w^a spl rb	13	11	32	31
E_1-2	y w^a	7	1	19	13
	spl rb	10	5	16	19
E_1-3	y w^a spl	33	19	86	74
	rb	29	24	63	61
	Totals	981	1009	3199	3066
	Total	1900		6265	

The crossing over frequency was slightly higher in region 1 (y - w^a) and region 2 (w^a - spl) of females subjected to a (-) polarity field, however, lower in region 3 (spl - rb) when compared to the control

group. The recombination frequency in females subjected to the (+) polarity field was slightly higher in regions 1, 2 and 3 when compared to the control group (Table 27).

TABLE 27

Cross-over values (with standard errors) from y w^a spl rb/+ + + + females subjected to 0.3 kV/cm inhomogeneous electrostatic field

Treatment	N	REGION y - w^a	w^a - spl	spl - rb
(-) Field	9	2.06 ± 0.34	1.58 ± 0.12	3.75 ± 0.43
Control	16	1.84 ± 0.21	1.21 ± 0.18	4.54 ± 0.34
	P	-	-	-
(+) Field	14	2.87 ± 0.67	1.58 ± 0.45	4.73 ± 0.55
Control	26	2.10 ± 0.18	1.41 ± 0.18	4.63 ± 0.25
	P	-	-	-

* indicates significance at .05 level

N = number of females tested

Crossing Over in Proximal Region of X Chromosome

Table 28 shows the recombinant types from m f car/+ + + females subjected to 0.3 kV/cm inhomogeneous, (-) and (+) polarity, electrostatic fields. The mutant markers are in the proximal half of the chromosome, with f and car near the centromere. There are a significantly greater number of females than males in the control group and the group subjected to the (+)

polarity field although a significant difference is not found in the group subjected to the (-) polarity field. There is, as one might expect, a tendency for the number of flies carrying the greatest number of mutants to be less than the number of flies with few mutants.

TABLE 28

Crossing over in m f car/+ + + females subjected to 0.3 kV/cm inhomogeneous, electrostatic fields

		Control		(-) Field		(+) Field	
Region	Phenotype	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
E_0	m f car	857	578	301	288	403	279
	+	990	1050	371	344	441	381
E_1-1	m	228	207	99	83	114	92
	f car	213	179	92	73	102	59
E_1-2	m f	59	60	22	8	18	14
	car	54	69	31	21	27	24
E_2-1,2	m car	1	1	0	1	3	1
	f	2	2	1	0	3	1
	Totals	2404	2146	917	818	1111	851
	Total	4550		1735		1962	

The recombination frequency in region 1 (m - f) was very slightly higher in females subjected to the (-) or (+) polarity field as compared to the control females while in region 2 (f - car) it was lower in the treated females than in the control females (Table 29). None of these differences are significant.

TABLE 29

Cross-over values (with standard errors) from m f car/+ + + females. Electrostatic field of 0.3 kV/cm

Treatment	N	REGION m - f	f - car
(-) Field	8	20.12 ± 0.62	5.02 ± 0.47
(+) Field	8	19.33 ± 1.26	4.78 ± 0.63
Control	18	18.33 ± 0.70	5.22 ± 0.37
	P	-	-

* indicates significance at .05 level

N = number of females tested

Crossing Over in Chromosome II

The number of parental and recombinant type progeny resulting from crossing over in chromosome II of b cn c bw/+ + + + females subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field is shown in Table 30. As noted in the previous experiments there is a general tendency that the more mutants the fly is carrying the fewer the number of that type of progeny recovered. This, in fact, was consistently found in the cross-over studies. It can be noted in Table 30 that certain recombinant phenotypes show a greater number of progeny than their complementary phenotypes.

The recombination frequency in region 1 (b - cn) was slightly higher for the treated than for the control females, however, the recombination frequency was somewhat lower in region 2 (cn - c) and region 3 (c - bw) of

the treated females as compared to the controls (Table 31). The cross-over values obtained here are not very discrepant from the standard values as given in Lindsley and Grell (1967).

TABLE 30

Crossing over in b cn c bw/+ + + + females subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field

		Field		Control	
Region	Phenotype	♀♀	♂♂	♀♀	♂♂
E_0	b cn c bw	119	123	403	388
	+	163	171	429	479
E_1-1	b	11	16	31	22
	cn c bw	4	2	19	26
E_1-2	b cn	44	42	98	114
	c bw	43	46	169	158
E_1-3	b cn c	58	75	242	187
	bw	79	81	199	223
E_2-1,2	b c bw	4	2	7	12
	cn	5	1	11	10
E_2-1,3	b bw	3	3	14	18
	cn c	2	5	2	1
E_2-2,3	b cn bw	5	4	11	18
	c	9	12	93	79
E_3-1,2,3	b c	1	3	10	3
	cn bw	0	0	0	0
	Totals	550	586	1738	1738
	Total	1136		3476	

The number of double cross-over progeny (curved) from the control

group was very high. Although great care was taken when classifying the flies, this large number is most likely a classification error.

It is possible that a somatic cross-over occurred between cn and c which could have given a clone of cells which were c bw/+ +. If these cells became part of the ovaries of the female than a single cross-over between c and bw could have given gametes carrying only c or bw. This would increase the number of c progeny but would also increase the number of bw progeny. The number of bw progeny is not in excess though.

TABLE 31

Cross-over values (with standard errors) from b cn c bw/+ + + + females subjected to a 0.3 kV/cm inhomogeneous electrostatic field

		REGION		
Treatment	N	b - cn	cn - c	c - bw
(-) Field	8	6.02 ± 0.43	19.68 ± 0.91	29.94 ± 0.63
Control	17	4.91 ± 0.36	22.07 ± 0.61	30.34 ± 0.60
	P	-	-	-

* indicates significance at .05 level

N = number of females tested

Magnetic Field Effect on Crossing Over in the Distal Tip of X Chromosome

Table 32 shows the number of parental and recombinant types from y w^a spl rb/+ + + + females subjected to a 0.7366 T magnetic field. There is a significantly greater number of female progeny than males from the parents subjected to the magnetic field. While there are more female

progeny than males in the control group the difference is not significant. The very great difference in the number of parental type progeny from the treated parents should be noted. There were 593 wild type females compared to 445 wild type males and 472 parental type mutant females compared to 388 parental type males. It is this group which leads to the sex ratio differences. The differences in the cross-over progeny are not great enough to affect the total sex ratio.

TABLE 32

Crossing over in y w^a spl rb/+ + + + females subjected to a 0.7366 T magnetic field

		Field		Control	
Region	Phenotype	♀♀	♂♂	♀♀	♂♂
E_0	y w^a spl rb	472	388	1359	1382
	+	593	445	1798	1681
E_1-1	y	14	12	33	27
	w^a spl rb	4	5	35	26
E_1-2	y w^a	6	4	24	23
	spl rb	6	9	21	20
E_1-3	y w^a spl	24	18	56	44
	rb	24	13	67	61
	Totals	1143	894	3384	3264
	Total	2037		6648	

The frequency of crossing over between y - w^a and w^a - spl was very slightly lower in the treated females than in the control females, but slightly higher between spl - rb in the control females compared to the

treated females (Table 33). The recombination frequency in region 1 (y - w^a) in the treated and control females is slightly higher than the standard value of this region. On the other hand the recombination frequency in region 2 (w^a - spl) is slightly lower in the treated and control females than the standard value and somewhat lower in region 3 (spl - rb) of both the treated and control group. Recombination frequencies in regions 1, 2 and 3 of y w^a spl rb/+ + + + females obtained in this study do not differ significantly when comparing experimentals with controls.

TABLE 33

Cross-over values (with standard errors) from y w^a spl rb/+ + + + females subjected to a 0.7366 T magnetic field

Treatment	N	REGION y - w^a	w^a - spl	spl - rb
0.7366 T	6	1.72 ± 0.43	1.27 ± 0.25	3.88 ± 0.30
Control	18	1.80 ± 0.18	1.32 ± 0.25	3.25 ± 0.23
	P	-	-	-

* indicates significance at .05 level

N = number of females tested

Magnetic Field Effect on Crossing Over in Proximal Half of X Chromosome

The results of the influence of a magnetic field on crossing over in the proximal half of the X chromosome are shown in Table 34. Heterozygous m f car/+ + + females were subjected to a 1.1366 T mangetic field.

The frequency of recombination between m - f was very slightly greater in the experimental females than in the controls (Table 35). The recombination frequency between f - car was significantly lower in the treated females than in the control females. Because so few experimental data could be collected it is questionable how meaningful the results are.

TABLE 34

Crossing over in m f car/+ + + females subjected to a 1.1366 T magnetic field

Region	Phenotype	Field		Control	
		♀♀	♂♂	♀♀	♂♂
E_0	m f car	201	198	1160	1113
	+	191	220	1320	1311
E_1-1	m	52	63	375	375
	f car	47	74	351	389
E_1-2	m f	8	5	84	76
	car	8	15	109	99
E_2-1,2	m car	2	0	6	2
	f	0	2	3	4
	Totals	509	577	3408	3369
	Total	1086		6777	

TABLE 35

Cross-over values (with standard errors) from m f car/+ + + females subjected to a 1.1366 T magnetic field

Treatment	N	REGION m - f	f - car
1.1366 T	5	22.29 ± 2.11	3.67 ± 1.04
Control	26	22.02 ± 0.64	5.72 ± 0.25
	P	-	*

* indicates significance at .05 level

N = number of females tested

Magnetic Field Effect on Crossing Over in Chromosome II

Table 36 shows the number of parental and recombinant types from b cn c bw/+ + + + females mated to homozygous mutant recessive males and subjected to a 1.24 T magnetic field. There is a significantly greater number of females than males from parents subjected to the magnetic field. While there are more female offspring than male offspring from the control parents the difference is not significant.

A 1.24 T magnetic field produced no significant difference in the recombination frequency of markers located on chromosome II of D. melanogaster (Table 37). The recombination frequency between cn - c was somewhat lower in the treated females than in the control females. The recombination frequency between b - cn was 5.78% in the treated females and 5.84% in the control females, both of which are lower than the standard 9% between b - cn.

TABLE 36

Crossing over in b cn c bw/+ + + + females subjected to a 1.24 T magnetic field

Region	Phenotype	Field ♀♀	Field ♂♂	Control ♀♀	Control ♂♂
E_0	b cn c bw	150	109	1233	1171
	+	163	164	1654	1629
E_1-1	b	15	12	108	99
	cn c bw	6	9	84	76
E_1-2	b cn	44	34	374	378
	c bw	49	42	500	560
E_1-3	b cn c	80	69	664	639
	bw	95	70	732	758
E_2-1,2	b c bw	2	0	32	20
	cn	3	4	32	21
E_2-1,3	b bw	7	2	62	50
	cn c	2	3	36	35
E_2-2,3	b cn bw	2	2	43	59
	c	7	11	95	63
E_3-1,2,3	b c	0	0	3	1
	cn bw	1	0	1	3
	Totals	626	531	5653	5462
	Total	1157		11115	

TABLE 37

Cross-over values (with standard errors) from b cn c bw/+ + + + females subjected to a 1.24 T magnetic field

		REGION		
Treatment	N	b - cn	cn - c	c - bw
1.24 T	6	5.78 ± 0.68	17.22 ± 1.83	29.10 ± 1.89
Control	42	5.84 ± 0.30	19.01 ± 0.52	29.33 ± 0.55
	P	-	-	-

* indicates significance at .05 level

N = number of females tested

DISCUSSION

Several factors such as temperature (Plough, 1917, 1921; Thompson, 1964; Grell, 1966b), maternal age (Bridges, 1927), radiation (Whittinghill, 1937, 1955; Zimmering, 1958, 1962; Zimmering and Wu, 1963; Merriam and Frost, 1964, and Roberts, 1969), chemicals (Suzuki, 1965a, 1965b), nutritional effects (Nell, 1941; Levine, 1955), genotypic effect (Gowen and Gowen, 1922; Levine and Levine, 1955; Lawrence, 1958, and Hinton, 1966), inter- and intra-chromosomal effects (Steinberg, 1936; Sturtevant and Beadle, 1936; Brown, 1940; Schultz and Redfield, 1951; Oksala, 1957, 1958; Roberts, 1962, and Suzuki, 1963), and cytoplasm effect (Thoday and Boam, 1956) have been found to alter the recombination rate in chromosomes of Drosophila. This would indicate that recombination is a rather sensitive phenomenon. Therefore, if an electrostatic or magnetic field of the strength used in this study affects genetic material one would likely see a change in recombination rates. There is, however, no indication that the electrostatic or magnetic fields have any effect on recombination in the distal or proximal section of the X chromosome or in the centromere region and right arm of chromosome II of D. melanogaster females.

In one particular instance, however, a significantly lower recombination rate resulted between the f - car markers in m f car/+ + + females subjected to a 1.1366 T magnetic field. The difference could possibly be due to the crowded conditions in the vials and not due to the field itself.

It should be noted also that the data were collected from only 5 treated females and the standard error is quite high in this group of data.

Cross-over values obtained in this study were in general close to the standard values (Lindsley and Grell, 1967). The observed recombination value between black and cinnabar on chromosome II shows a reduced value (when compared with the standard figure) in both control and treated females.

CONCLUSIONS

The influence of electrostatic and magnetic fields on crossing over in D. melanogaster females was studied. Results indicate that:

(a) crossing over in the distal tip of the X chromosome is not affected by a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic or a 0.7366 T magnetic field:

(b) crossing over in the proximal half of the X chromosome is not affected by a 0.3 kV/cm inhomogeneous, (-) or (+) polarity electrostatic field. A 1.1366 T magnetic field appears to possibly reduce the frequency of crossing over between the markers forked and carnation on the X chromosome but does not affect crossing over in other proximal regions tested:

(c) crossing over in the right arm and near the centromere of chromosome II is not affected by a 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field or by a 1.24 T magnetic field.

CHAPTER VI

THE INFLUENCE OF ELECTROSTATIC AND MAGNETIC FIELDS ON THE PRODUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS

METHODS AND MATERIALS

To determine the influence of electrostatic and magnetic fields on the production of sex-linked recessive lethal mutations, the standard Muller-5 test was done. Wild type males 4-6 days of age were collected. About 100-150 males were placed in each of several vials. The experimental males were subjected to a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field or a 0.9266 T homogeneous magnetic field for 24 hours. Upon removal from the field the males were lightly etherized. Ten to fifteen treated or control males were mated in 1/2 pint bottles to 10-12 $\underline{y}\ \underline{w}^a\ \underline{B}$ (Muller-5) virgin females. Forty-eight hours after mating, the males and females were lightly etherized and the males discarded. The females were placed back into the culture bottles for an additional four days and then discarded. Controls were treated the same except for exposure to the experimental field.

Single F_1 $\underline{y}\ \underline{w}^a\ \underline{B}/+\ +\ +$ females were mated to 2-3 $\underline{y}\ \underline{w}^a\ \underline{B}/Y$ F_1 males in un-yeasted vials. The $F_1 \times F_1$ cross was maintained at 25 ± 1°C. Upon eclosion of the F_2 progeny the vials were checked for the presence of wild

type males. The absence of wild type males indicates the possible presence of a sex-linked recessive lethal mutation. All vials which did not have wild type males were re-checked by collecting $\underline{y}\ \underline{w}^{a}\ \underline{B}/+\ +\ +$ females from the vial and mating them to their $\underline{y}\ \underline{w}^{a}\ \underline{B}/Y$ brothers. If no wild type males appeared from the re-tested females the vial was scored as a recessive lethal chromosome. To determine the frequency of sex-linked recessive lethals the number of chromosomes carrying a sex-linked recessive lethal was divided into the total number of chromosomes tested. A single mating represents a single X chromosome tested.

Stocks of the mutants were lost before the recessive mutations could be localized.

RESULTS

Table 38 shows the results of a standard Muller-5 sex-linked recessive lethal test for males subjected to a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field, or a 0.9266 T homogeneous magnetic field for 24 hours prior to mating. Of 4761 chromosomes tested from males subjected to the (-) field there were 5 sex-linked recessive lethals observed. Of 4888 chromosomes from control males 4 recessive lethals were found. Of 4802 chromosomes from males subjected to the (+) field there were 4 recessive lethals produced compared to 5 in 4920 chromosomes from corresponding control males. Seven sex-linked recessive lethals were found in 5119 chromosomes subjected to the magnetic field. There were 4 recessive lethals in 4447 chromosomes tested in the corresponding control group. The mutation rates ranged from 1/731 in males subjected to the magnetic field, (corresponding control, 1/1112), to 1/1200 in males subjected to the (+) polarity field, (corresponding controls, 1/985).

TABLE 38

Standard Muller-5 test for the production of sex-linked recessive lethal mutations in males subjected for 24 hours to a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field, or a 0.9266 T homogeneous magnetic field

Treatment	Number of chromosomes tested	Number of recessive lethals	Per cent lethals
0.3 kV/cm (-)	4761	5	0.122 (1/952)
Control	4888	4	0.083 (1/1222)
0.3 kV/cm (+)	4802	4	0.083 (1/1200)
Control	4920	5	0.101 (1/985)
0.9266 T	5119	7	0.136 (1/731)
Control	4447	4	0.089 (1/1112)

DISCUSSION

Many of the same extraneous factors which affect crossing over in Drosophila also influence the mutation rate. Radiation (Muller, 1927; Altenburg, 1934; Catcheside, 1948; De Mazar Barnett, 1963; Kang et. al., 1963; Schouten, 1963; and Mukherjee and Suzuki, 1964), chemicals (Auerbach and Robson, 1946; Bauty and Freese, 1960; Kaplan et. al., 1965; Takahashi and Suzuki, 1966; Alderson and Khan, 1967; and Suzuki et. al., 1967), adult fly ageing (Bateman, 1954), and temperature (Plough and Ives, 1934, 1935; Plough, 1935; and Edmondson and Meyer, 1952) have all been shown to induce recessive lethal mutations in D. melanogaster. There is no indication in this study, however, that a 0.3 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field or a 0.9266 T homogeneous magnetic field increases or decreases the frequency of sex-linked recessive lethal mutations in D. melanogaster males.

Because of the length of time the males were exposed to the field, gametes could have been exposed as functional spermatozoa, spermatids or spermatocytes (Lefevre and Jonsson, 1964). Fahmy and Fahmy (1964) indicated that post-meiotic stages (sperm and spermatids) are used by D. melanogaster males during the first 9 days after irradiation. Because males were allowed to mate with females for 2 days after being subjected to the experimental fields used in this study it is suggested that chromosomes tested in the study were present in spermatids or functional spermatozoa when subjected to the treatment field.

The mutation rate was slightly higher in males subjected to the (-) polarity field and in males subjected to the magnetic field than in control males but the differences are not significant.

The spontaneous mutation rate for sex-linked recessive lethals ranges from about 0.1 per cent (Timofeeff-Ressovsky and Zimmer, 1939) to 0.4 per cent (Traut, 1963).

The mutation rate obtained in this study from the treated and control males was within the spontaneous sex-linked recessive mutation rate range noted by Timofeeff-Ressovsky and Traut.

The negative results obtained using magnetic fields are in accord with those reported by Close and Beischer (1962) and Beischer (1964). There was no noticeable increase in the number of deformities in Drosophila subjected to magnetic fields as was found by Mulay and Mulay (1964) who used magnetic fields of .3 and .44 T.

The magnetic field results reported here suggest that the magnetic field does not affect the spin orientation of protons in the DNA molecule, and that protons do not experience any force (Barnothy, 1964) to effect changes in the molecule.

CONCLUSIONS

Inhomogeneous electrostatic fields of 0.3 kV/cm, (-) or (+) polarity, end a homogeneous magnetic field of 0.9266 T do not significantly affect the rate of sex-linked recessive lethal mutations in D. melanogaster males.

CHAPTER VII

THE INFLUENCE OF ELECTROSTATIC AND MAGNETIC FIELDS ON EGG LAYING, EGG HATCH AND ECLOSION

METHODS AND MATERIALS

Egg Laying and Eclosion

To determine the influence of the electrostatic field on egg laying by D. melanogaster, y w^a/y w^a virgin females 3-4 days old were individually mated in vials to 2-3 y/sc^8Y.y^+ males. After a one day pre-treatment in a 0.3 kV/cm inhomogeneous, (-) polarity field, the parents were transferred every 24 hours, for four days, to fresh medium vials. The temperature in the control vials and in the experimental vials during treatment was 24 ± 1°C. Egg counts were done on each vial after the parents were transferred to fresh vials. After the egg count the vials were maintained in an incubator at 25 ± 1°C.

The average number of eggs laid per day by females subjected to a 0.3 kV/cm inhomogeneous, (-) polarity electrostatic field was compared to the control females by the t-test. The per cent eclosion between the experimental group and control group was also compared by the t-test. The per cent eclosion was calculated by dividing the total number of progeny eclosing per vial by the number of eggs deposited by the female.

$$\% \text{ eclosion} = \frac{\text{Total progeny eclosing per vial}}{\text{Number of eggs deposited per vial}} \times 100$$

Egg Hatch

Wild type females were collected and from 80-100 females were placed in each of several 1/2 pint bottles containing fresh, yeasted cornmeal medium. Females remained in the bottles for 24-48 hours. Females were then lightly etherized and 20 females were placed in each of several vials containing fresh cornmeal medium. Females were left in the vials for 1 hour then discarded or transferred to fresh vials or bottles. Immediately upon removal of the females the eggs which were deposited were counted. Vials containing the eggs were placed in a 0.3 kV/cm or 0.6 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field, or in a magnetic field, within 15 minutes after the egg count. Egg hatch counts were begun 20 hours after the egg counts were done and continued every hour for 3 hours. The accumulated average per cent egg hatch for the specific time periods in hours of the treated eggs was compared to the controls by the t-test.

The number of eggs hatching during the specified counting time intervals was determined by counting the total number of empty egg cases. The accumulated per cent egg hatch for any specific time in hours was calculated from the following formula.

$$\text{Per cent egg hatch} = \frac{\text{Number of empty egg cases}}{\text{Total number of eggs deposited per female per vial}} \times 100$$

RESULTS

Egg Laying and Eclosion

The mean number of eggs deposited per day by single $\underline{y}\ \underline{w}^{a}/\underline{y}\ \underline{w}^{a}$ females is shown in Table 39. The experimental females 3-4 days old at mating were subjected to a 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field. There was a steady decrease in the number of eggs deposited after transfer a of both the treated and control groups. No significant difference in the number of progeny developing from the eggs deposited is noted (Table 40).

TABLE 39

Comparison of the mean number of eggs deposited per day per vial by y w^{a}/y w^{a} females singly mated to y/$sc^{8}Y.y^{+}$ males. Parents subjected to 0.3 kV/cm inhomogeneous, (-) polarity, electrostatic field. Parents transferred every 24 hours

		Mean number of eggs deposited Field	Control		
Transfer	N	$\overline{Y}$	$\overline{Y}$	N	P
a	8	42.2 ± 2.38	39.2 ± 1.74	18	-
b	8	30.8 ± 2.75	26.8 ± 1.91	18	-
c	8	25.5 ± 2.26	22.2 ± 0.80	18	-
d	8	22.8 ± 2.81	19.2 ± 1.84	18	-

* indicates significance at .05 level

$\overline{Y}$ = mean and its standard error

TABLE 40

Comparison of mean per cent of progeny eclosing per vial per transfer from eggs deposited by same females listed in Table 39

Transfer	N	Mean per cent of progeny Field $\bar{Y}$	Control $\bar{Y}$	N	P
a	8	66.0 ± 8.92	58.7 ± 6.51	18	-
b	8	66.5 ± 8.07	73.6 ± 2.71	18	-
c	8	61.8 ±10.36	61.9 ± 6.60	18	-
d	8	69.3 ± 9.33	54.0 ± 7.73	18	-

* indicates significance at .05 level

$\bar{Y}$ = mean and its standard error

Egg Hatch

Table 41 shows the percentage hatch of eggs deposited by wild type (Canton-S) females and subjected to a 0.3 kV/cm or 0.6 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field for a period of 23 hours after the eggs were laid. More than 75% egg hatch occurred between 20 and 22 hours after being subjected to the fields or as controls. Figures 5 and 6 show the relationship of the accumulated per cent egg hatch to time after egg count. There is no significant difference between eggs subjected to the fields when compared to the control group.

The temperature in the control vials and experimental vials during the treatment period in the 0.3 kV/cm or 0.6 kV/cm electrostatic field was 24 ± 1°C. Any temperature variation which did occur was the same in the experimental group and the control group.

No suitable results for the influence of a homogeneous magnetic

field on egg hatch could be obtained due to temperature fluctuation between the pole pieces of the magnet. This experiment was done during the summer and the water temperature was not low enough to keep the temperature down in the magnetic field. The temperature in the field was in the range of 25 ± 1°C while the temperature of the control groups was 22.5 ± 1°C. A temperature range this great did seem to greatly influence egg hatch time, the egg hatch time for the experimental groups being much less than for the control groups.

TABLE 41

PER CENT EGG HATCH. EGGS SUBJECTED TO EXPERIMENTAL INHOMOGENEOUS ELECTROSTATIC FIELD

Treatment	Hours after egg count 20	21	22	23	# eggs
Control	8.3 ± 0.92	43.7 ± 1.85	85.5 ± 2.24	90.3 ± 1.81	327
.3 kV/cm (-)	4.8 ± 0.90	41.9 ± 1.02	84.6 ± 1.08	90.1 ± 1.56	405
.3 kV/cm (+)	6.5 ± 0.88	44.5 ± 0.80	86.8 ± 1.42	91.1 ± 0.84	344
Control	8.7 ± 0.76	41.5 ± 2.32	84.2 ± 2.02	89.5 ± 1.35	738
.6 kV/cm (-)	6.4 ± 0.60	43.0 ± 1.32	87.8 ± 1.27	93.0 ± 0.88	761
.6 kV/cm (+)	6.3 ± 0.93	42.4 ± 1.75	86.8 ± 2.36	92.2 ± 1.41	519

In mean per cent ± standard error

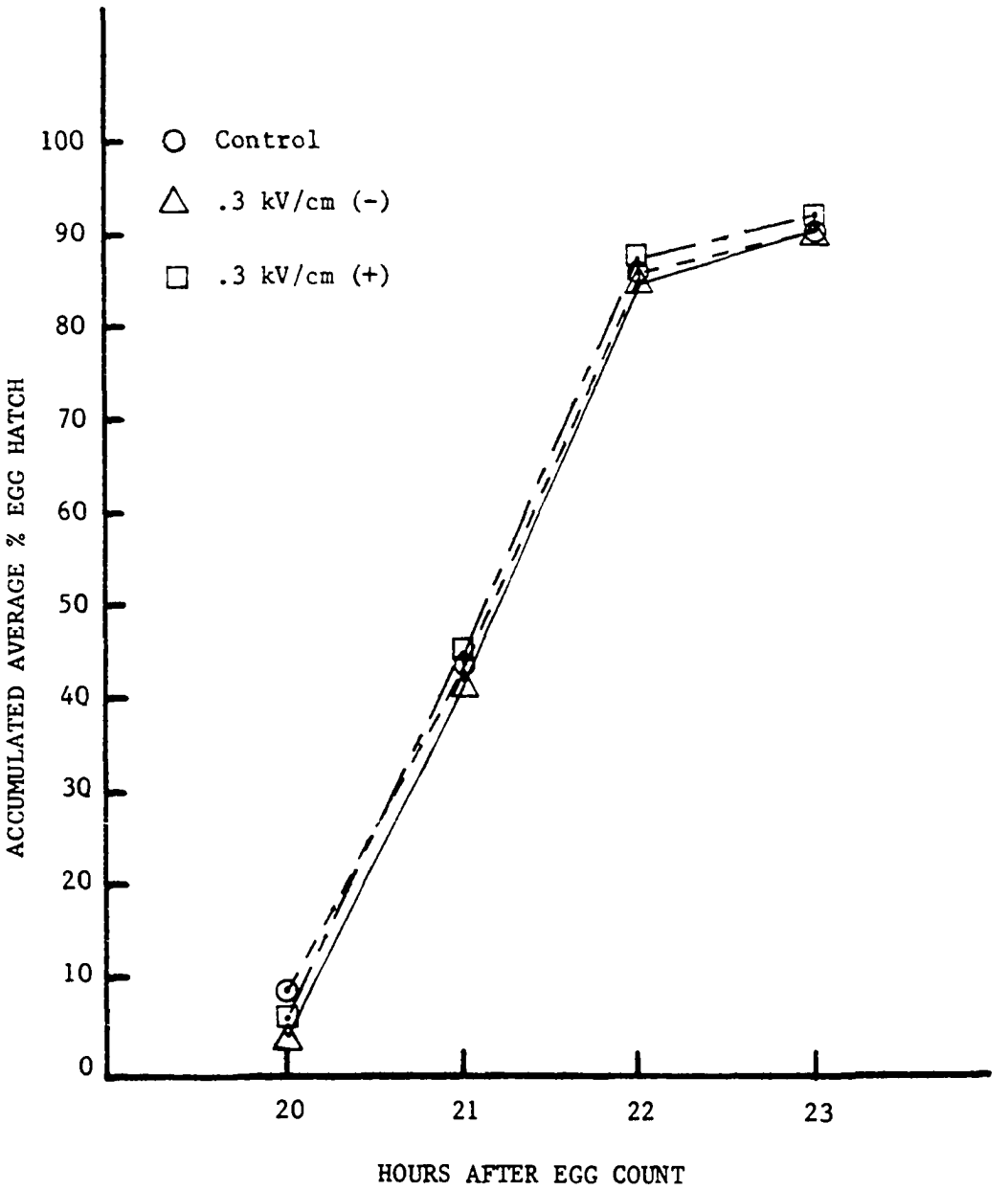

Fig. 5.—Accumulated egg hatch frequencies of eggs subjected to 0.3 kV/cm inhomogeneous electrostatic field.

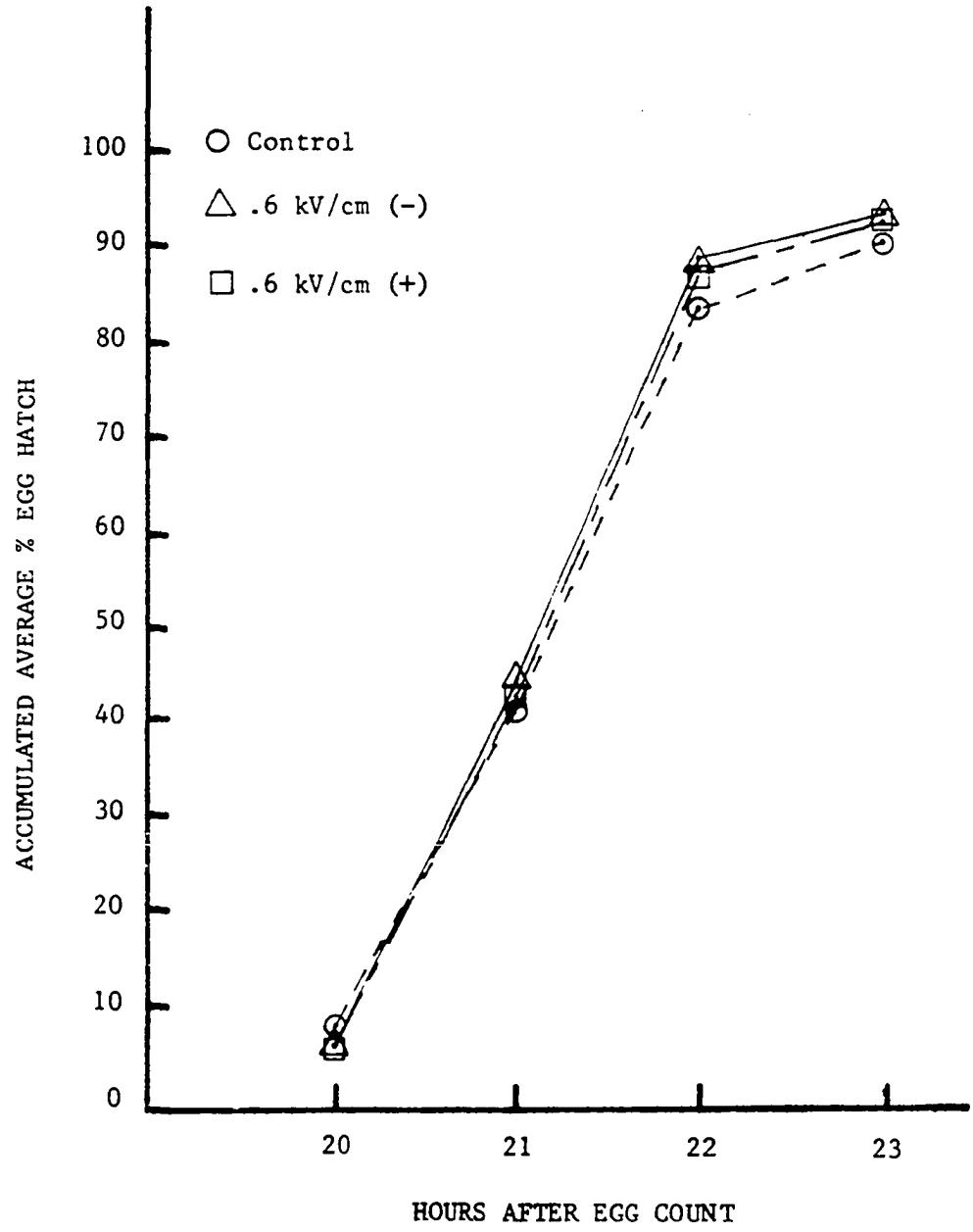

Fig. 6.--Accumulated egg hatch frequencies of eggs subjected to 0.6 kV/cm inhomogeneous electrostatic field.

DISCUSSION

The mean number of progeny eclosing in the control group is quite low. The percent eclosion in wild type D. melanogaster is normally in excess of 90%. Some of the eggs may not have been fertilized or the genotype of the parents may affect zygote development. There is no indication that the electrostatic field affects the frequency of eclosion since the control and experimental values are very similar.

In several, but not all of the individual primary nondisjunction experiments, the electrostatic field seemed to have a lethal effect on some of the parents. A tendency for a significantly lower number of regular progeny from $\underline{y}\ \underline{w}^{a}/\underline{y}\ \underline{w}^{a}$ females and $\underline{y}/sc^{8}Y.y^{+}$ males used to study primary nondisjunction and subjected to the electrostatic field was noted. The difference was thought to be due to the death of one or more of the parent females. However, the seemingly lethal effect was not noted when individual females were mated in vials and subjected to a 0.3 kV/cm electrostatic field. There was no evidence that fewer eggs were laid in the field or that fewer progeny eclosed or that a noticeable number of the female parents died while being subjected to the field. In addition there was no difference in the per cent egg hatch of eggs subjected to 0.3 and 0.6 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic fields. From about 78 to 80% of the control group of eggs and also those subjected to the 0.3 kV/cm fields hatched between 21 and 22 hours after laying and about 75 to

80% of the control eggs and those subjected to the 0.6 kV/cm fields hatched between 21 and 22 hours after laying. This is in close agreement with the control and experimental data obtained by Steen and Oftedal (1967) who subjected eggs to magnetic fields and obtained negative results. Avio and Tarozzi (1956, 1958) also noted no difference in oviposition rates and egg hatch rates of D. melanogaster. They used a field gradient of 125 V/m which is lower than used in this study.

CONCLUSIONS

Egg laying rates, the frequency of egg hatching or eclosion is not affected by a 0.3 or 0.6 kV/cm inhomogeneous, (-) or (+) polarity, electrostatic field.

CHAPTER VIII

THE INFLUENCE OF A MAGNETIC FIELD ON DEVELOPMENT TIME AND PROGENY YIELD

METHODS AND MATERIALS

The effect of the magnetic field upon development time of progeny and progeny yield from a cross of single, virgin y w^a spl rb/+ + + + females mated in vials to y w^a spl rb/Y males and single wild type virgin females, 0-8 hours old, mated to wild type males, was studied. The experimental parents were subjected to a 0.7366 T magnetic field. Parents were pre-treated for two days. A single transfer of the y w^a spl rb/+ + + + females and male parents was made after the pre-treatment period. The parents were therefore subjected to the field for a total of 4 days. Transfer procedure for the wild type cross was the same as nondisjunction and cross-over studies. Eggs deposited by the females could have been subjected to the field a minimum of about 1 minute and a maximum of about 1 day and larvae could have been subjected a maximum of about 1 day. After transfer the vials were maintained at 25 ± 1°C. The time at which progeny began to eclose was noted for each vial. Progeny counts were done periodically on each vial. The accumulated average number of progeny eclosing per time unit was calculated for the experimental and control groups and compared by the t-test.

RESULTS

The accumulated average number of progeny eclosing per vial was significantly less from the treated y w^a spl rb parents when compared to the controls, beginning at 25 hours after initial eclosion (Table 42).

Figure 7 shows the relationship between time and the accumulated average number of progeny eclosing from y w^a spl rb/+ + + + females individually mated in vials to y w^a spl rb/Y males and subjected to a 0.7366 T homogeneous, magnetic field for two days. Control progeny began to eclose 21 hours before the progeny from treated parents.

The progeny from wild type females 0-8 hours old at mating to 1-2 day old wild type males at the time of mating, subjected to a 0.7366 T homogeneous magnetic field, began to eclose less than five hours after the control group for transfer a (Table 43). Except for the progeny counts taken 27 and 33 hours after initial eclosion there was no significant difference in the accumulated average number of progeny per vial. Progeny counts for up to 99 hours after initial eclosion are shown. The relationship between the mean number of flies eclosing and the number of hours after initial eclosion for transfers a, b, and c of wild type females individually mated to wild type males is shown in Figures 8, 9, and 10 respectively. Table 43 also shows the accumulated average number of flies eclosing for transfer b of the wild type parents. Counts up to 96 hours after initial eclosion are shown. As in transfer a, the progeny from the treated parents

began to eclose less than five hours after initial eclosion of progeny from the control parents. No significant difference in the accumulated average number of flies eclosing per vial is noted for transfer b. The progeny from the treated wild type parents in transfer c began to eclose 9 hours later than the progeny from the control parents (Table 43). Except for the 14 hour count there was no significant difference in the accumulated average number of flies eclosing per vial from the treated and control parents. Counts up to 93 hours after initial eclosion are shown.

TABLE 42

The accumulated average number of progeny produced per vial from a cross of y w^a spl rb/+ + + + ♀ x y w^a spl rb/Y ♂♂ subjected to a 0.7366 T magnetic field for 2 days. Counts taken on the hours indicated after first progeny began to eclose

Hours after initial eclosion	Control N	Control $\bar{Y}$ ± S.E.	Field N	Field $\bar{Y}$ ± S.E.	P
0	3	0.3 ± 0.3	3	0	-
2	3	0.3 ± 0.3	3	0	-
4	3	0.6 ± 0.6	3	0	-
6	3	0.6 ± 0.6	3	0	-
9	3	0.6 ± 0.6	3	0	-
11	3	0.6 ± 0.6	3	0	-
13	3	1.6 ± 1.2	3	0	-
15	3	3.0 ± 1.1	3	0	-
21	3	7.0 ± 2.3	3	1.6 ± 0.8	-
23	3	10.3 ± 2.6	3	3.3 ± 0.6	-
25	3	13.0 ± 3.0	3	4.6 ± 0.3	*
28	3	16.6 ± 2.6	3	6.3 ± 0.6	*
30	3	18.0 ± 2.0	3	7.6 ± 1.4	*
32	3	18.0 ± 2.0	3	8.0 ± 1.7	*
34	3	18.6 ± 1.8	3	10.0 ± 2.0	*
45	3	22.0 ± 1.1	3	11.3 ± 1.4	*
47	3	23.6 ± 1.4	3	13.3 ± 0.6	*
49	3	29.6 ± 1.8	3	15.0 ± 0.5	*
51	3	32.3 ± 1.7	3	16.3 ± 0.3	*
53	3	39.3 ± 2.3	3	18.6 ± 0.6	*
55	3	40.6 ± 2.4	3	20.3 ± 0.8	*
57	3	41.3 ± 2.7	3	21.3 ± 1.4	*
59	3	42.0 ± 2.5	3	23.0 ± 1.7	*
69	3	48.0 ± 3.2	3	27.6 ± 2.0	*
80	3	71.3 ± 1.8	3	47.0 ± 4.0	*
94	3	85.6 ± 1.4	3	57.3 ± 1.4	*

* indicates significance at .05 level

N = number of vials or females

$\bar{Y}$ = mean and its standard error

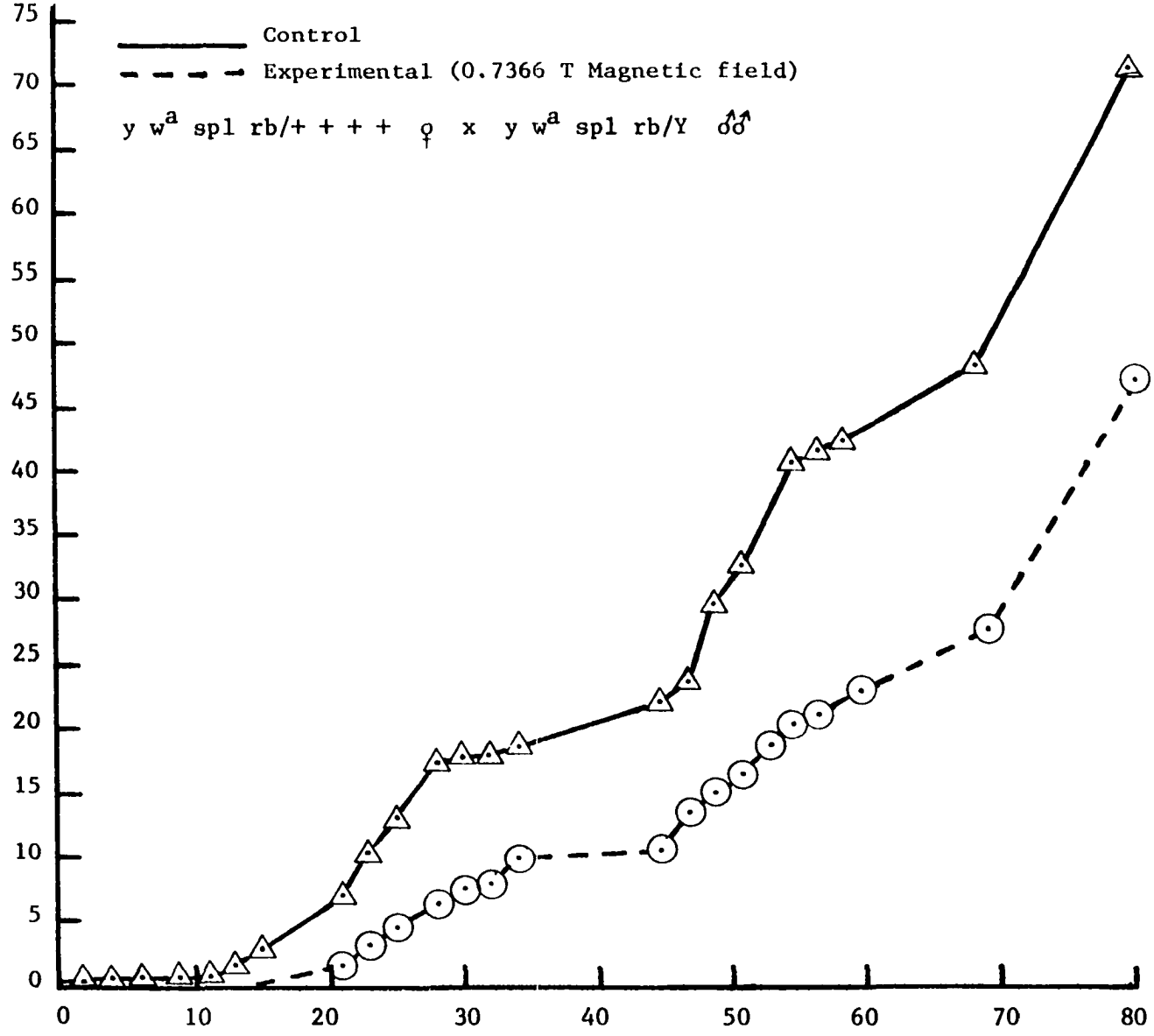

Fig. 7.--Accumulated average number of flies per vial.

TABLE 43

Accumulated average number of progeny produced per vial from a cross of a wild type female to wild type males. Females were 8 hours old and males 1-2 days old at time of mating. Experimental group subjected to a 0.7366 T magnetic field for a total of 7 days. Parents transferred to fresh medium vials every 2 days after a 2 day pre-treatment. Progeny counts made as indicated after first progeny began to eclose

Hours after initial eclosion	TRANSFER a				
	Control		Field		
	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
0	6	0.6 ± 0.3	3	0	-
5	6	3.3 ± 1.7	3	0.3 ± 0.3	-
10	6	5.0 ± 1.9	3	1.6 ± 1.2	-
22	6	11.5 ± 2.4	3	6.6 ± 2.0	-
27	6	27.5 ± 2.9	3	19.0 ± 1.0	*
33	6	34.1 ± 3.2	3	23.6 ± 2.3	*
46	6	42.3 ± 3.9	3	31.6 ± 6.6	-
51	6	53.8 ± 4.6	3	40.3 ±11.4	-
56	6	57.8 ± 4.9	3	41.3 ±11.6	-
70	6	62.8 ± 6.7	3	43.0 ±12.1	-
80	6	69.0 ± 8.4	3	44.0 ±12.1	-
94	6	70.0 ± 8.5	3	45.0 ±12.1	-
99	6	70.6 ± 8.6	3	45.6 ±12.7	-
		TRANSFER b			
0	5	1.4 ± 0.5	3	0	-
5	5	6.0 ± 2.7	3	0.3 ± 0.3	-
10	5	10.4 ± 2.7	3	3.0 ± 2.0	-
24	5	15.4 ± 2.8	3	5.0 ± 3.6	-
34	5	29.2 ± 4.9	3	13.6 ± 5.8	-
48	5	36.8 ± 6.8	3	19.6 ± 5.3	-
53	5	44.6 ± 8.4	3	23.3 ± 5.6	-
58	5	50.2 ± 9.2	3	25.3 ± 5.3	-
72	5	54.2 ±10.5	3	30.0 ± 6.9	-
81	5	65.4 ±12.8	3	35.3 ±10.7	-
96	5	71.0 ±14.2	3	38.6 ±13.5	-

TABLE 43—Continued

Hours after initial eclosion	TRANSFER b				
	Control		Field		
	N	$\bar{Y}$ ± S.E.	N	$\bar{Y}$ ± S.E.	P
0	4	1.0 ± 0.7	3	0	-
14	4	5.2 ± 0.6	3	1.3 ± 0.8	*
23	4	18.5 ± 3.2	3	10.0 ± 5.5	-
38	4	26.0 ± 3.4	3	20.3 ±10.5	-
69	4	48.7 ± 6.6	3	40.6 ±19.1	-
86	4	58.5 ±10.9	3	47.3 ±24.6	-
93	4	66.0 ±14.2	3	49.6 ±26.9	-

* indicates significance at .05 level

N = number of vials or females

$\bar{Y}$ = mean and its standard error

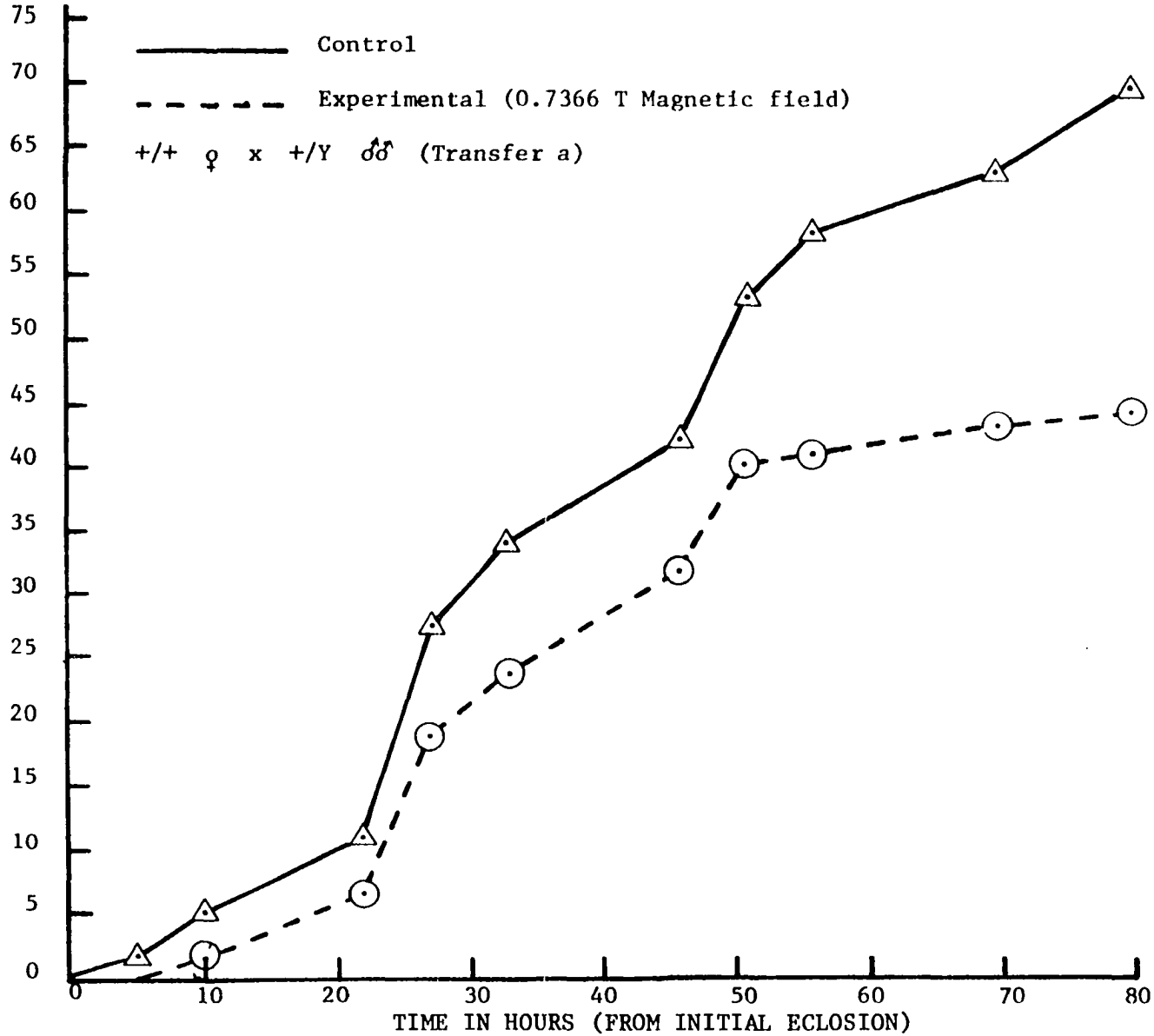

Fig. 8.--Accumulated average number of flies per vial.

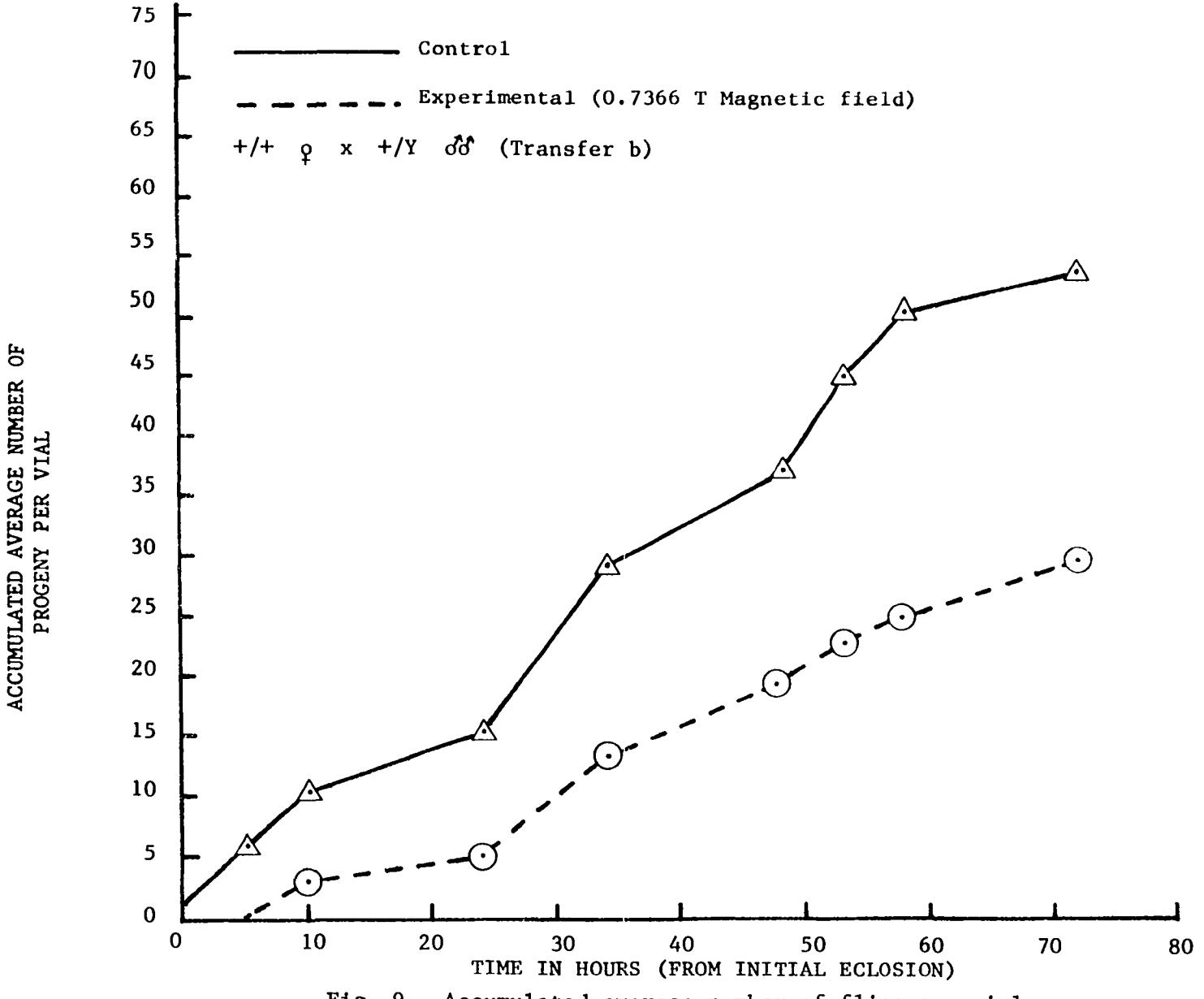

Fig. 9.--Accumulated average number of flies per vial.

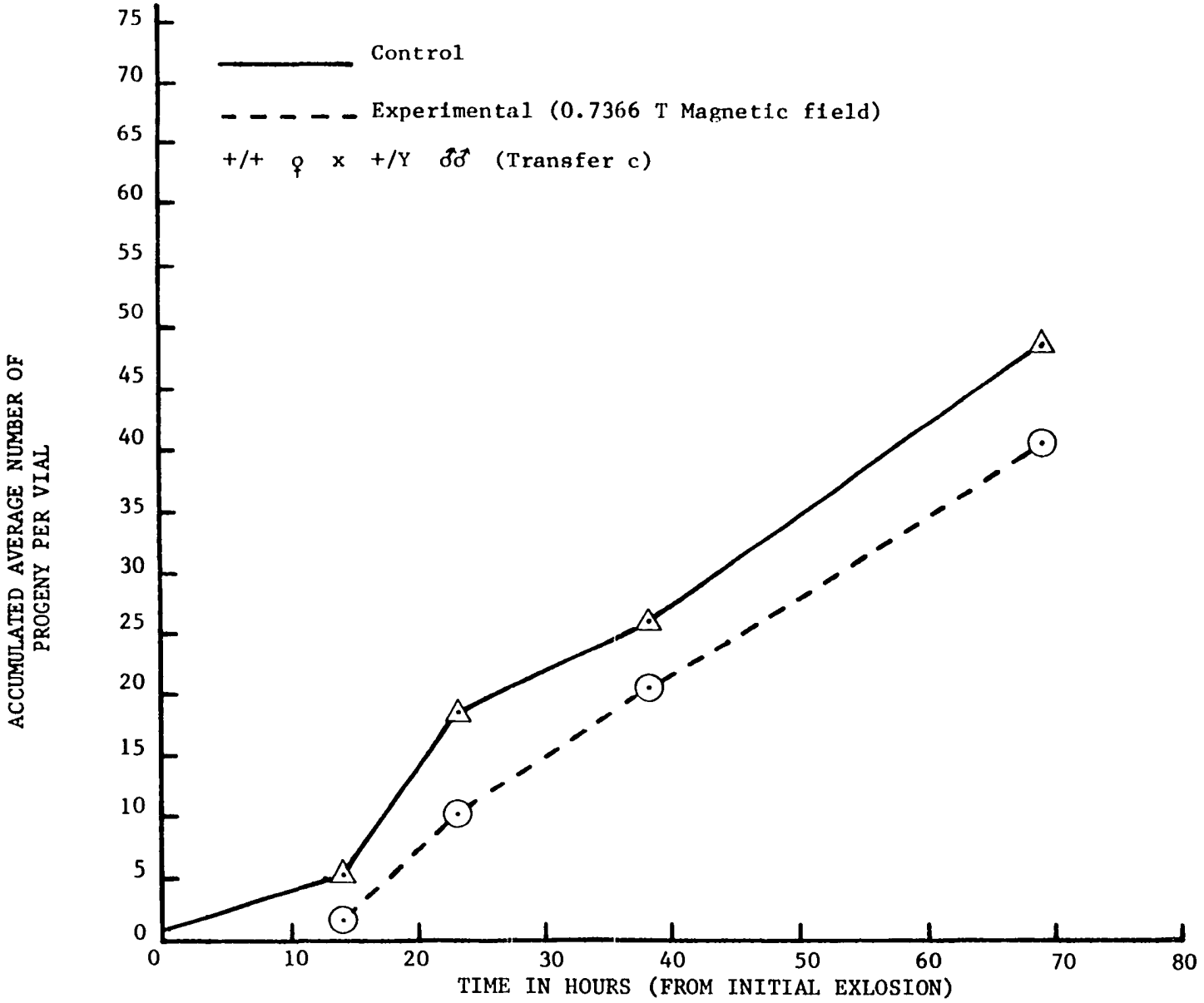

Fig. 10.--Accumulated average number of flies per vial.

DISCUSSION

There seems to be no clear cut evidence that the magnetic field does in fact reduce progeny yield. The data from heterozygous mutant females mated to mutant males is contradictory to the data from wild type flies. The number of flies tested could be important. There were 3 heterozygous females mated to hemizygous mutant males in the experimental and control groups while there were nine sets of wild type parents to begin the experiment, 3 in the experimental group and 6 in the control. One control female was lost in transfer b and another in transfer c. None were lost in the experimental groups of either cross.

Because so few data could be collected and because the standard error values are so high in transfers b and c of the wild type cross it is difficult to draw any specific conclusions as to the influence of the magnetic field.

The activity of parent flies was observed while in the magnetic field and the position of eggs deposited while the females were in the field was noted. There appeared to be no large-scale differences in behavior patterns. The overall movement of the parents in the field did appear to be slightly less than that of the control parents. There appeared to be no difference in the position of eggs deposited or orientation by the treated parents compared to the control parents although a statistical analysis was not performed on these phenomena.

Temperature fluctuation in the experimental and control groups was not more than 0.5°C, therefore, it appears that this factor would not influence the difference noted in progeny yield.

It does appear, however, that the magnetic field does increase the development time for eggs laid in the magnetic field. If in fact the development time is delayed the effect of the field would be on eggs or larvae less than 2 days old. Any effect might be due to reduced enzyme activity as suggested by Levengood (1967). Some evidence has been presented, however, which suggests that a magnetic field increases or reactivates enzyme activity. Cook and Smith (1964) and Wiley (1964) found *in vitro* activity of trypsin to increase upon being subjected to a magnetic field.

CONCLUSIONS

A 0.7366 T homogeneous, magnetic field may cause some increase in the development time of *D. melanogaster*.

APPENDIX I

STATISTICAL METHODS

To compare the nondisjunction rates between the experimental and control groups a 2 x 2 contingency test with Yates correction factor was performed on the data.

The standard error for mean values was obtained from

$$S.E. = \sqrt{\frac{\Sigma Y^2 - \bar{Y}\Sigma Y}{N(N-1)}}$$

(1) for calculations with the number of regular progeny produced from the parents in nondisjunction studies where:

Y = the number of regular progeny produced per vial (per female) or per bottle for each transfer or total cultures

$\bar{Y}$ = the average number of regular progeny produced from all vials (all females) or all bottles for each transfer or total combined data of each group

N = the number of vials (females) or bottles for each transfer or total group data;

(2) for determining the influence of the electrostatic field on egg laying and progeny yield:

Y = the number of eggs laid per vial per female for each transfer, or the frequency of progeny eclosing per vial

$\overline{Y}$ = the average number of eggs deposited per female per vial for each transfer, or the average frequency of progeny yield in all vials of each group for each transfer

N = the number of vials (female parents) for each group for each transfer;

(3) for determining the influence of the electrostatic field on egg hatch:

Y = the frequency of egg hatch per vial for specified time

$\overline{Y}$ = the average frequency of egg hatch for all vials of each group

N = the number of vials (female parents) for each group;

(4) for cross-over values in specified regions:

Y = the cross-over frequency from each vial or female

$\overline{Y}$ = the average frequency of crossing over from total data or from all vials (female parents) for each group

N = the number of vials (female parents) for each group;

(5) for calculating the effect of a magnetic field on progeny yield:

Y = the total number of progeny eclosing per vial at time of count for each group

$\overline{Y}$ = the average number of progeny eclosing from all vials for each group at time of count

N = the total number of vials (female parents) for each group for each transfer or total group number.

Comparisons of the number of regular progeny produced per transfer from parents used in nondisjunction studies, the number of eggs laid per female per day in an electrostatic field, the frequency of progeny eclosing per vial which had been subjected to the electrostatic field, the frequency

of egg hatch per time, the cross-over values obtained from all transfer cultures for each specific cross and group, and the progeny yield per vial from parents subjected to a magnetic field were done by the t-test. The values for $\underline{t}$ were obtained from

$$\underline{t} = \frac{(\bar{Y}_1 - \bar{Y}_2)}{\sqrt{\left[\frac{(N_1 - 1)s_1^2 + (N_2 - 1)s_2^2}{N_1 + N_2 - 2}\right]\left(\frac{N_1 + N_2}{N_1 N_2}\right)}}$$

where: $\bar{Y}_1$ = the mean value of the appropriate experimental data

$\bar{Y}_2$ = the mean value of the appropriate control data

s_1^2 = the variance of the experimental data obtained from

$$s_1^2 = \frac{\sum(Y_1 - \bar{Y}_1)^2}{N_1 - 1}$$

s_2^2 = the variance of the control data obtained form

$$s_2^2 = \frac{\sum(Y_2 - \bar{Y}_2)^2}{N_2 - 1}$$

N_1 = the number of vials (females) or bottles for specific transfer or total group number for experimental crosses

N_2 = the number of vials (females) or bottles for specific transfer or total group number for control crosses.

All data computations were done with the Olivetti Underwood Programma 101 desk computer and the IBM 350 computer. A canned Olivetti program

(code 520) for a t-test between two means was used for some comparisons. IBM 350 library programs from the Triangle Research Computation Center (TUCC) in Chapel Hill, North Carolina, were utilized for the majority of computations. Library programs used were (a) LOAD(UNPRDT)PUBLIC; this program computes the means, standard errors of the mean difference, and t statistic for two groups of observations (unpaired); (b) LOAD(CHISQR) PUBLIC; this program computes the chi-square statistic (with or without Yates correction factor) for a contingency table.

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