On the Production of Polyploidy with Paradichlorbenzene

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ON THE PRODUCTION OF POLYPLOIDY WITH PARADICHLORBENZENE

By

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Preface

The author wishes to express his gratitude to Dr. E. S. McDougall of the Marquette University Biology Department. His preliminary work on the subject of polyploidy and his suggestions were of great assistance in the preparation of this thesis. To Mr. Leo Massopust of the University Medical School is due a debt of gratitude.

Historical Introduction

Statement of Problem

Allium Cepia—Methods and Materials

Allium Cepia—Experimental Observations and Results

Drosophila Melanogaster—Methods and Materials

Drosophila Melanogaster—Experimental Observations and Results

Summary and Conclusions

Description of Photographs

Bibliography
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These photographs were taken of the actual experimental work in the preparation of the thesis.

Polyploidy has been divided into two classes: (1) condition in plants, (2) condition in animals. It has been supposed that polyploidy is more common in plants than in animals, because of the fact that plants have growing points which, when affected, may affect the entire organism.

Polyploidy may be said to be the result of incomplete nuclear division. (Stewart 1919) Prior to 1919, the general opinion was that polyploidy was the result of the fusion of two nuclei.

Apparently Slinker (1918) was the first man to produce polyploidy artificially in plants. He did this by grafting two species of Solanum. Then, by cutting across the union of the two plants, he found that the adventitious spouts arising therefrom contained polyploid cells.
Historical Introduction

The subject of polyploidy has been of scientific interest for some time. The true nature of the cause of this phenomenon has been disputed ever since its existence was made known. Polyploidy is the condition in which the haploid number of the chromosomes, contained within the nucleus, is multiplied so that there is a higher number of chromosomes present than there is in the typical diploid cell of that particular plant or animal. The diploid number of the chromosomes is known as $2n$, therefore, higher multiples would be referred to as $3n$, $4n$, $8n$ etc. Some plants or animals have one or more cells which contain some multiple of the basic chromosome number other than that typical of the variety. These organisms, which are cellular chimaeras, are called mixoploids.

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Lindstrom (1931) also produced polyploidy by the decapitation method. The doubling of chromosomes took place after young tomato plants with 3-5 leaves were decapitated. A callus formed on the cut stem and sprouts arose from this callus. These sprouts were rooted, and 5-30% developed with double the number of the chromosomes of the seedling. The fertile tetraploids, which were produced, bred true and thus a new tetraploid generation was started.

Sax (1937) discovered that polyploidy could be produced in the Rhoeo by means of temperature changes. He exposed the plants to many artificially produced sudden temperature changes, and discovered that this was as effective as some chemicals in producing polyploidy.

The Tradescantia was the subject of the experiment of Dermen (1937). The stamen hairs of this plant were given particular attention and treatment was varied, as in the experiment of Sax, to provide all possible temperature changes.

Nebel and Ruttle (1937) also noted polyploidy in the Tradescantia. The phenomenon was produced by colchicine and was manifested by the stunting and thickening of the hypocotyl and also by the degeneration of the chromosomes in various cells.

Chemicals have also been used to produce polyploidy. An example of a chemical used is colchicine, which was used by Levan, (1938) on the plants of the diploid Petunia, Allium, and the Colchicum. He found that the soaking of the seeds in the solution was more effective than the treatment with
the colchicine agar. The *Allium* and the *Colchicum* were also treated with acenaphthene, which showed results similar to that of the treatment with colchicine, i.e., the production of C-pairs, cytologically. Externally, he noted unnatural swelling of the apical growing point. There was also a predominance of tetraploidy, with mixoploidy also present. It was in these experiments that C-pairs and their importance in the manifestation of polyploidy were first noted.

Blakeslee and Avery (1938) found various methods of producing polyploidy in plants. Chloral hydrate and Colchicine were used in inducing the doubling of chromosome in *Datura*.

Sass (1938) found that polyploidy could be produced in corn and several small grains by means of ceracon (ethyl mercury phosphate). The abnormal polyploid cells were produced in the axillary bud primordia and other meristematic cells remote from the absorptive region.

Kostoff (1939) used the chemical, ethyl mercury chloride, (CH₃CH₂HgCl) in working on the *Nicotiana vavidovii* and found by experiments similar to those of Levan that polyploidy could be produced by this compound.

Heat alone was used in Cooper's experiment on *Alfalfa*, with a temperature of 42°C (1939).

Aside from heat, there are other methods, such as the reduction of the water content, an experiment performed by Giles (1939) who worked on the *Tradescantia*. His method was to reduce the water content during microsporogenesis in cut inflorescences, thereby affecting the entire plant. Giles observed that the
three vital divisions which took place; chromosomal, nuclear and cell-were disturbed. He concluded that cell division by furrowing often took place.

Greenleaf (1939) used the Tradescantia as the subject for his experiment with indole--3 acetic acid in 1% lanoline (heteroauxin) and found that this hormone treatment was very affective in the production of polyploidy. In this experiment, yeast extract, anhydrous lanoline, and petroleum jelly were used, but they all proved ineffective.

The use of caratin--monochloronaphthalene and carotin mono Bromonophthalen proved affective by Simonet and Guinochet's experiment on various plants. (1939)

Muntzing (1940) of the Swedish Institute also reported the general use of colchicine as good means for the production of polyploidy.

Antirrhinum Majus was the subject of the experiment performed by Thomas Little (1941) who used the chemicals, sanguinarine hydrochloride, colchicine, and lycorine. The results of this experiment was the production of tetraploids and also the presence of the shortened and partially split C-chromosomes.

A study of the problem was made by Miss Ruth Dornfeld (1941) in experiments on the Zea Mays L. with colchicine.

The Linum Usitatissimum was the subject of the experiment by Simonet and Guinochet (1939) who used commercial Paradi-chlorbenzene for the first time to produce polyploidy artificially. The method used in carrying out this experiment was
to subject the seeds of the flax to the fumes of this compound for a short time. The amounts of the compound were varied from .01g. up to 1.0g. and the time of treatment was also varied, then various lengths of time were given for the seeds to recover. These experiments were carried on in the most practical method possible, in closed Petri dishes. After a suitable length of time had elapsed and the seedlings were given a chance to grow to about 0.5 to 0.6cm., the roots were cut off, fixed and imbedded. Navashin fixation fluids were used. The root tips showed a multiplicity of chromosomes in the majority of the cells. Two nucleoles were present instead of one. Anomalies gave birth to daughter cells having an irregular number of chromosomes unlike their mother. At the time of division, observations were made. After one day of treatment, observations were made and the diploid number was found. After two days, the triploid number was found, and then after 3 days of treatment, the triploid number (90 chromosomes) was noted during the metaphase. All of these characteristics were more noticeable after the five day treatment.

In the animal kingdom, an excellent description is given by Darlington (1937) of the polyploidy occurring naturally in the Drosophila Melanogaster. He cites an example of an otherwise diploid parent, in which the ovary is tetraploid. This ovary produces diploid gametes, instead of haploid gametes, which upon union with the normal haploid gamete will produce a triploid individual will be produced. The complexity of this
series is attributed to the 'closed growth system' of animals as opposed to the 'open growth system' of plants.

Another instance of artificially induced polyploidy in the animal kingdom is that produced in the snowy tree cricket by Edith Penfield Beach (1939). Adults were subjected to X-rays. The exposure to the rays caused a reduction in the number of eggs to be laid, it inhibited the cell division in the egg and after the effect of the ray had worn off, it produced polyploidy in the eggs. In the male, after the ray had been applied, abnormal spermatagonia were found.

Broungart and Ott (1941) in work upon the Drosophila used the chemical colchicine. The phenomenon was noted in the brain tissue of this animal. The experiment was originally carried out to affect the salivary gland tissue, in which the chromosomes are of extraordinarily large size, but only the brain tissue was affected. The artificial polyploidy was obtained with best results by the treatment of the eggs with a solution of colchicine. The eggs were subjected for a certain interval to the chemical and then were allowed to recover and develop. It was observed that the brain tissue of animals so treated contained tetraploid cells. This tissue showed another sign of polyploidy, the C-pairs of Levan. These C-pairs were perceived only in the polyploid cells whose division was interrupted at the prophase. These are partially separated chromosomes which cling together at the time when the chromosomes usually separate in the prophase.

A point of interest of which Dobzhansky (1941) takes note, in regard to the general character of naturally occurring
polyploids is the fact that the Ice Age, supposedly, played an important part in the distribution of diploids and tetraploids. He asserts that the tetraploids are hardier individuals, which existed or came about during the glacial period. On the other hand, the diploids, presumed to be weaker, existed as pre-glacial or as interglacial relics. This fact pertains to both animals and plants and would deal with the so-called autopolyploids.

The aim of this experiment was to produce polyploidy in the cells of the plant, *Allium cepa* and in the animal, *Drosophila melanogaster* by means of paradichlorobenzene.

Preliminary work had been done on this problem by Dr. E. S. MacDonald of the M. U. faculty. Buckwheat seeds were treated with paradichlorobenzene to prevent the formation of fungi. The external appearances of polyploidy individuals.

It was, therefore, presumed without further reference to any previous experiments that the commercial "di-chlorioxide" could be used to produce polyploidy. The experiments were conducted upon the *Allium cepa* and attempted in the *Drosophila melanogaster*. This chemical is particularly suited to this type of experiment because of the fact that it produces a gas which penetrates without the necessity of being dissolved in water.
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Methods and Materials

**Allium**

Twenty five onion seeds were kept on moist filter paper in each of six petri dishes. A watch glass containing a measured amount of paradichlorobenzene was placed in each of five of these dishes. The amounts which were placed upon watch glasses, after having been measured to the thousandths of a gram on an analytical balance, were: 1/20g., 1/10g., 1/4g., 1/2g., 1.5g (lots) for four hours and then the chemical was removed. The root tips was allowed to recover and grow for about six days. The period of recovery was one day, then the root tips were cut off, fixed in a fluid, containing three parts absolute alcohol and one part of glacial acetic acid. After this fixation, the root tips were taken out one at a time, and the Aceto-carmine modified technique was on some tips, while the Feulgen technique of smearing was used on others. Another set of seeds was treated with the same concentrations of paradichlorobenzene, with the exception that they were treated for the entire time of the growth of the seedlings. These were also fixed at the appropriate hours of growth, between ten o'clock A.M. and two o'clock P.M., and were given the same treatment with the fixing fluid and the smear technique as those treated for four hours. These sets of experiments were repeated several times for certainty.
Experimental Observations

In the case of the seedlings which were allowed to recover and then grow, it was assumed that if the substance used did produce polyploidy, it would first inhibit the growth and then upon being removed, polyploidy would be produced, as the seedlings continued their growth. The chemical would interrupt the mitosis at certain stages of division and there would be no activity of the nucleus as long as the chemical in certain proportion was present. By means of cytological observation of smear slides it was determined which concentration was the most effective in stopping the nuclear division.

In the case of the roots which were treated for the full time, the exact concentration which produced the inhibition was noted in the following manner: Roots affected by the chemical showed no cell division and those unaffected showed mitosis.

In the case of the roots which were treated for four hours polyploidy was found to be produced in the dishes containing 1/4, 1/2, and 1.5 grams. The root tips elongated in inverse proportion to the amount of paradichlorobenzene used. Externally, the root tips manifested a shortening and stunting in the concentrations of 1/4 and 1/2 gms. The concentration of 1/10 and 1/20 gms. showed no external signs of polyploidy. The roots treated for the full length of time of growth, showed a shortening and stunting in the concentrations of 1/4 and 1/2 gms. more noticeable than that of the four hour treatment, while these which were treated with 1/10 and 1/20 showed little or no stunting at all.
The polyploidy was manifested by abnormal chromosomal numbers in the metaphase (Fig. 1, l.5g.), Lagging chromosomal (Fig. 2, ½g.) "dumbell" shaped nuclei. (Fig.3, l.5g), three nucleoles instead of two(Fig.4, 1/4g) and clumping of chromosomes. The abnormal chromosome number was not prevalent in all nuclei of these affected root tips. There were diploid cells present. (Fig. 6, Fig.7)

**Drosophila**

The *Drosophila* was chosen as the animal upon which to perform the experiment that was effective upon plants because the animal's salivary gland chromosomes are very large.

The *Drosophila Melanogaster* adults were placed into 1/2 pint milk bottles containing banana agar. Each bottle contained about twenty adult flies. The treatment was carried on for three days after the adults were placed in the bottles in order to give the eggs time to be laid. The treatment performed was as follows: a small vial containing varying amounts of para-dichlorbenzene were suspended in the bottle for different lengths of time. The amounts were: 1/8g., 1/4g., 1/0g., and 1.5g and the lengths of time were: 2hrs. 4hrs. 6hrs. 24hrs. and three days. The experiment was duplicated in the case of each concentration and each time, and it was repeated for verification. As soon as the larvae appeared, and grew large enough, they were removed, decapitated, and the salivary glands were smeared in aceto-carmine.
Experimental Observations

The larvae developed as follows: Judging from the controls there should have been about one hundred larvae appearing after a week in each bottle. However, in the bottle containing 1/8g. there were approximately ten larvae; the 1/4 grams bottle contained about one larva. Those larvae which grew did not show microscopic signs of polyploidy. However, where larger amounts were used, they scarcely appeared, if at all. (Table I). It is possible that if they had grown they would have manifested polyploidy, but there was no immediate evidence that polyploidy was produced. The external effect that was produced was that the larvae were small and undernourished and exhibited very little motion. The adults in all cases died. In the smaller concentrations comparatively large larvae developed. However, none of the larvae developed into pupae and within seven days they had all died.
Summary and Conclusions

The results of these experiments may be summed up as follows:

1. Polyploidy is manifested in onion root tips through the use of paradichlorobenzene and may be demonstrated through Aceto-carmine and Feulgen smears.

2. Polyploidy was manifested in the onion root tips through the use of paradichlorobenzene, by shortening and stunting of tips.

3. Paradichlorobenzene may be used for the production of polyploidy in plants and possibly in animals.

4. The use of this chemical has a very definite effect upon Drosophila and in this experiment only the gross result was noted.

5. In relation to the amount of paradichlorobenzene, the number of larvae produced was inversely proportional i.e. the greater the amount of chemical the smaller the number, and the weaker the larvae produced.
Description of Photographs 1200 X Magnification

Figure I
A dividing polyploid cell in the midst of a group of cells which are in the resting condition.

Figure II
A dividing cell showing the lagging chromosome affected by the chemical. A typical diploid cell is also present on the picture.

Figure III
A dividing polyploid cell surrounded by resting cells.

Figure IV
Cells in the resting condition containing three nucleoles. The normal cells containing two nucleoles are also present. Three nucleoles are indicative of a polyploid condition.

Figure V
A cell in the anaphase stage showing clumped chromosomes, indicative of a polyploid condition.

Figure VI
A typical diploid metaphase stage. A cell in the resting stage is also shown.

Figure VII
A typical diploid metaphase stage. A cell in the resting stage is also shown.
The size of the dose is proportional to the number of larvae; the time of the dosage had no apparent effect in this experiment; the amount was the only factor having an effect.
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