# SOME UNUSUAL ASCI

# IN NEUROSPORA CRASSA

# GENETIC ANALYSIS OF SOME UNUSUAL ASCI FROM A CROSS IN NEUROSPORA CRASSA.

by

ALAN RADFORD, B.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science.

McMaster University

September 1963.

MASTER OF SCIENCE (1963)

McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: Genetic Analysis of Some Unusual Asci From a Cross in Neurospora crassa.

AUTHOR: Alan Radford, B.Sc. (The University of Leeds)

SUPERVISOR: Professor S. F. H. Threlkeld.

NUMBER OF PAGES: vii, 118.

SCOPE AND CONTENTS: The investigation was carried out in order to determine the nature of some aberrant asci obtained, at a high frequency, from a single cross in Neurospora crassa. The subsequent investigations shed light on an aspect of pyridoxine metabolism and the nature of its genetic control, as well as showing the existence of a dicentric chromosome and its great instability when present in the disomic condition.

## PREFACE

This thesis describes studies carried out in the Department of Biology, McMaster University, from October 1962 to August 1963. Except where others are specifically mentioned, it consists entirely of my own original work. No similar thesis has been submitted at any other university.

My grateful thanks are due to my supervisor, Dr. S. F. H. Threlkeld for advice and encouragement throughout the work, and also for the donation of the strains of Neurospora upon which this work is founded.

I am also deeply indebted to the Department of Biology, McMaster University, for the award of a Departmental Scholarship covering the period of the research reported herein.

Biology Department,
McMaster University,
August 1963.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

# CHAPTER I

## INTRODUCTION

Among the Eucaryotes (Levine and Ebersold, 1958), or organisms with true nuclei, it has long been realized that the meiotic process, by which the diploid nucleus is reduced to the haploid state, is the key in the production of exchange of genetic material between homologous chromosomes. It is hence the basis for the understanding of the inheritance of all characters of any organism which are under genetic control. More recent work on parasexuality and somatic recombination in Aspergillus (Pontecorvo, 1953) has detracted little from the importance of meiotic recombination.

The process of crossing over to produce genetically reciprocal exchanges between non-sister chromatids was first discussed by Bridges (Bridges, 1916) with respect to Drosophila melanogaster. This organism is a classic genetic research tool from which much important information has been obtained, but in both theory and practice the most perfect tool in the study of recombination in Eukaryotes is the ascomycete fungus Neurospora crassa, together with a few closely related species and genera. In the ascus of Neurospora one has, in order, the four products of a single meiotic event; thus its unique suitability in the study of the process of recombination. Commencing with the work of Dodge, Lindegren, and Beadle and Tatum, Neurospora crassa has been continuously employed in the sophisticated study of recombination processes.

Reciprocal recombination has been thoroughly investigated in an effort to determine the mechanism of replication and crossing over, attempting to resolve the controversy between supporters of copy-choice (Belling, 1933) and breakage and rejoining (most recent work being that of Meselson and Stahl, 1958, and Meselson and Weigle, 1961). However in 1949 another type of recombination, with many problems of an unique nature, was discovered.

Lindegren (Lindegren, 1953) reported examples of tetrads in Saccharomyces which showed not the normal 2:2 segregation for a marker and its wild type allele, but a 3:1 ratio in the products of the tetrad. The term "gene conversion" was coined by Lindegren for the phenomenon which gave rise to these aberrant tetrads. He suggested that the mutant allele could convert one of the wild type alleles of the tetrad into a mutant like itself. The reverse process could also occur, to produce a tetrad of three wild type alleles to one mutant. Lindegren's interpretation of this data was regarded as highly controversial, and was challenged by many other workers. However, in 1955 there was evidence produced from Neurospora which gave valuable support to Lindegren and his hypothesis. (Obviously only in organisms where an individual tetrad can be analysed can one investigate non-reciprocal recombination).

The second piece of evidence in support of "gene conversion" came from the work of M. B. Mitchell (Mitchell, 1955, 1957). This study concerned the pyridoxine requiring pdx-1 locus of Neurospora, the locus considered in the present investigation. There are three mutant alleles at this locus, pdx, pdxp, and pdxq (discussed in more detail in Chapter 2). In a cross between pdx and pdxp, with outside markers also, aberrant tetrads of the type 1 wild type : 3 mutants for the pdx-1 locus were

observed. In Mitchell's investigation, 7 aberrant asci were isolated out of a total of 1200 asci. All aberrant progeny were back-crossed, and the data from these crosses confirmed that the phenotypes were in fact the genotypes in all cases. H. K. Mitchell (Mitchell 1957) sums up the work of M. B. Mitchell (Mitchell 1955) as follows: "On the basis of these experimental findings it is considered here that pdx, pdxp, pdxq, and the corresponding non-mutant locus represent a series of alleles or similar genes at the same locus, in so far as crossing over is concerned. The findings are not consonant with the assumption of a gene cluster or a group of sub-genes, which are separable by crossing over, and they provide conclusive evidence for the existence of the gene conversion process in Neurospora."

The terms in which the analysis by Mitchell of the situation at the pdx-1 locus is discussed sound somewhat unsophisticated only six years later. The fact that a heterokaryon between different mutants at the "same" locus can complement and show pseudo-wild type growth, (Pateman and Fincham 1958, de Serres 1956, 1960) casts doubt on Mitchell's interpretation; when it was found by Strauss (Strauss 1951) and confirmed by the present author that a heterokaryon containing the pdx and pdxp mutants also exhibits pseudo-wild growth. The interpretation of this data in terms of complementation between different cistrons at the polypeptide level shows Mitchell to be incorrect, and that pdx, pdxp, and pdxq are mutants in three separate cistrons of the pdx-1 locus. The fact that the use of selective techniques in analysis of the system is unsatisfactory eliminates the direct proof of this hypothesis, but the mutant loci must be arranged in a linear order within the pdx-1 locus.

This does not however invalidate Mitchell's postulated "gene conversion" as recombination between the cistrons would be most unlikely to be high enough to give the observed frequency of aberrant asci.

Later work, also on Neurospora crassa (Case & Giles, 1958), produced atypical tetrads for the pan-2 locus. 11 asci out of 856 examined showed such aberrant 3:1 segregation. Using different mutants at the same locus, i.e. Mutants in different cistrons within pan-2 locus, Case and Giles were able to demonstrate pseudo-wild type colonies heterokaryotic for two different mutants at this locus. A number of different mutant alleles were examined, and were found to belong to either one of two complementing groups, indicating that at the level of resolution available the pan-2 locus consists of two cistrons. The cistrons were also genetically separable, at a low frequency, by crossing over. The aberrant tetrads were interpreted on the basis of a copy-choice method of DNA replication, with both newly developing strands replicating, over a short region including the pan-2 locus, from the same parental strand. It is in the mechanism of such replication that we find the strongest evidence in favour of the conservative replication of DNA.

In Aspergillus nidulans, another Ascomycete, Strickland overcame many technical difficulties and succeeded in carrying out tetrad analysis. (Strickland, 1958). He completely analysed 1,642 asci, and among these discovered six abnormal ratios which because of the use of outside markers were most unlikely to be due to technical errors. One is almost certainly due to non-reciprocal recombination over a distance of eight map units, and three others all show a 3:1 ratio for $bi^+$ : bi (biotin requiring).

Two other Ascomycetes have also given rise to aberrant asci. The first of these is Sordaria fimicola, a species very closely related to Neurospora crassa. Olive and his co-workers have found both 3:1 and 5:3 ratios in Sordaria asci (Kitani, Olive, and El-Ani, 1961, 1962). The first occur at the rate of approximately 1 in 2000, and can be explained, as can the other examples previously discussed, on the basis of copy-choice and mis-replication. The 5:3 ratios are less frequent, and not found at all in some crosses that do produce 3:1 ratios. In the earliest of the 5:3 ratios reported, 5 wild type : 3 mutant examples out-numbered the reciprocal class by approximately 6:1, which is difficult to explain on the basis of Olive's postulated semi-conservative replication with mis-replication. A far more likely explanation may be found in aneuploidy. More recent examples are more likely to be caused by post-meiotic recombination and segregation.

The last organism in which unequivocal "gene conversion" has been found is another Ascomycete, Ascobolus immersus. In crosses involving mutants in two cistrons for spore colour, some 6 pale mutant:2 wild type spores are found in an ascus. The wild type spores were normal for both mutants; two pale spores were mutant for one cistron, two for the other, and the last two for both. This is an example of perfectly normal reciprocal recombination. In other cases however, in series 46, a double mutant is never recovered, and for any one cross the majority parent is always the same. Mutants in series 46 can be placed in order, and it is found that the majority parent is always on the same side of the minority parent. The average frequency of these polarised non-reciprocal segregations is approximately 1 in 100 (Lissouba et al, 1962).

The observations by Rizet and his fellow workers are consistent with the switch hypothesis, as opposed to any real gene conversion by directed mutation. The Ascobolus data also leads to the conclusion that replication is polarised with respect to "majority-minority" relationships.

The only other organism in which 3:1 ratios have been recovered is Chlamydomonas reinhardi. Following ultra-violet irradiation of one cross, 3 of the 27 tetrads for one locus and 2 out of 8 tetrads for another locus showed 3:1 segregation. In the control, one exceptional segregation was found in 73 tetrads. (Levine and Ebersold, 1958). Levine and Ebersold explain their results in terms of the failure to develop of one product of meiosis, followed by an extra mitosis of one of the other meiotic products. There is one remarkable point in this system, and that is the fact that all of the aberrant tetrads contain a recombination event between the two gene markers under analysis.

Threlkeld (Threlkeld, 1961), working on the pdx-1 locus of Neurospora crassa, the same locus studied by Mitchell (Mitchell, 1955, 1957), also produced some aberrant asci. In one cross, 6 out of 71 asci were aberrant. From the pdx x pdxp cross, 4 asci showed 6 pdx : 2 pdxp segregations, and two produced $pdx^+$ progeny. This cross was grown on medium containing limiting cytidine, but supplemented with the pyrimidine base analogue 5-bromo-uracil. In a cross on normal medium between the same parents, 4 asci out of 52 showed 6 pdx : 2 pdxp segregation, but none gave rise to any $pdx^+$ progeny. There appears to be no significant difference between the results with and without 5-bromo-uracil.

The frequency with which the aberrant asci were found by Threlkeld is far higher than that found in any system of non-reciprocal recombination

yet discussed. In Saccharomyces the proportion of 3:1 tetrads is high also, but Saccharomyces is unusual in several ways, e.g., it has a far higher rate of spontaneous mutation than is known in other organisms.

This, then, is the problem investigated and reported in this thesis; the problem of the cause of this extremely high rate of occurrence of aberrant asci in Neurospora crassa.

## CHAPTER II

## MATERIALS AND METHODS

### MATERIALS

#### Strains

Where wild type strains of Neurospora crassa were used in this work, they were strains 1A and 25a isolated, described, and first used by Lindegren.

The mutant strains utilized were as follows:

| Mutant | Isolation no. | Mutagenic agent | Biochem. requirement |
|---|---|---|---|
| leu-2 | 37501 | U.V. | leucine |
| pdx-1 | 37803 | U.V. | pyridoxine |
| pdxp (pdx-1) | 44602 | U.V. | pyridoxine, or $(NH_4)^+$ at pH > 6. |
| pdxq (pdx-1) | 35405 | U.V. | pyridoxine, or T 30°C. |
| pyr-1 | H 263 | X-rays | cytidine or uridine |
| tryp-4 | Y 2198 | N-mustard | tryptophane or indole |

Most of the above mutants are described in greater detail by Barratt et al. (Barratt et al. 1954). Exceptions are leu-2 (37501) which is described by Perkins (Perkins 1959), and pdxq (St. Lawrence 1956).

The Lindegren wild type strains and all except one of the mutants were supplied by Dr. S. F. H. Threlkeld. The exception was strain pdxq (35405) which was obtained from the Fungal Genetics Stock Centre, at Dartmouth College, New Hampshire.

Again with the exception of pdxq, the mutants were already combined in the aberrant asci to be discussed when obtained by the author.

Culture media

Four types of culture media were used during the course of the investigation. These were:

1) Medium on which crosses were grown. This contained salts solution, sucrose, agar, and the required biochemical supplements.
2) Medium on which ascospores were germinated and on which vegetative cultures were maintained. This differed from medium (1) in having glucose as its organic carbon source instead of sucrose.
3) Medium on which growth rate tests were carried out. This was a liquid glucose medium.
4) Medium on which isolates were tested for their biochemical requirements. This contains a limiting concentration of sucrose, and sorbose to induce colonial growth. Isolates were tested for the presence of any biochemical markers by innoculating series of appropriately supplemented Petri dishes with minute numbers of conidia from each isolate. By inducing colonial growth by the presence of sorbose it is convenient to test up to about 25 isolates on each plate.

Media 1, 2, and 4 were identical with respect to inorganic salts and Biotin, the only essential Neurospora growth factor.

The salts were made up as follows:

4 x strength salt solution:-

| | |
|---|---|
| $KNO_3$ | 4.0 gm |
| $KH_2PO_4$ | 4.0 gm |
| $MgSO_4.7H_2O$ | 2.0 gm |
| $CaCl_2.6H_2O$ | 0.4 gm |
| NaCl | 0.4 gm |
| Biotin | 16 ugm |
| Trace element solution (see below) | 1.0 ml |
| Distilled water up to 1000 ml | |

Trace element solution:-

| | |
|---|---|
| $Na_2B_4O_7.10H_2O$ | 0.01 gm |
| $CuSO_4.5H_2O$ | 0.10 gm |
| $FePO_4.2H_2O$ | 0.02 gm |
| $MuSO_4.4H_2O$ | 0.02 gm |
| $Na_2MoO_4.2H_2O$ | 0.02 gm |
| $ZuSO_4.7H_2O$ | 2.00 gm |
| Distilled water up to 250 ml | |

For the growth rate tests, for convenience, a more concentrated stock salts solution was used. This was the Vogel 50x salts solution (Vogel 1956). The formula is:-

| | |
|---|---|
| $Na_3$citrate | 150 gm |
| $KH_2PO_4$ (anhydrous) | 250 gm |
| $NH_4NO_3$ (anhydrous) | 100 gm |
| $MgSO_4.7H_2O$ | 10 gm |
| $CaCl_2.2H_2O$ | 5 gm |
| Trace elements (as above) | 10 ml |
| Biotin | 2.5 mg |
| Distilled water | 750 ml |
| Chloroform (preservative) | 2 ml |

The media mentioned above were made up as follows:-

1 - Reproductive Medium (Westergaard and Mitchell 1947)

| | |
|---|---|
| 4 x strength salt solution | 250 ml |
| Sucrose | 20 gm |
| Difco Bacto-agar | 15 gm |
| Distilled water up to | 1000 ml |

2 - Vegetative Medium

| | |
|---|---|
| 4 x strength salt solution | 250 ml |
| Glucose | 20 gm |
| Difco Bacto-agar | 15 gm |
| Distilled water up to | 1000 ml |

3 - Liquid Medium

| | |
|---|---|
| 50 x strength salt solution | 20 ml |
| Glucose | 20 gm |
| Distilled water up to | 1000 ml |

4 - Sorbose Medium

| | |
|---|---|
| 4 x strength salt solution | 250 ml |
| Sorbose | 4 gm |
| Sucrose | 2 gm |
| Difco Bacto-agar | 20 gm |
| Distilled water up to | 1000 ml |

Where biochemical supplements were added to media, unless otherwise stated, they were added at the rate of 100 mg/litre, except for indole which was added at half this concentration.

Buffering was required in the determination of the pdx genotype and in the growth rate tests, and this was accomplished by the use of $KH_2PO_4$ and $Na_2HPO_4$.

## METHODS

### Crosses

Two methods were used in the making of crosses on suitably supplemented sucrose medium.

1) The slope was innoculated with conidia and mycelial fragments from both parental strains.
2) One strain was innoculated onto the slope, and 4 to 6 days later a conidial suspension of the other parent was introduced.

In the present work both of these procedures produced the same result. However, if one requires to use a specific strain as the protoperithecial (female) parent, only the second method would be suitable.

The innoculated slopes were incubated at 25°C until mature perithecia and ascospores were produced.

### Analysis of Crosses

There are three common methods of analysing the ascospores produced by a cross. These are ascus analysis, perithecial analysis, and random spore analysis. Although only the latter two methods were used in this work, the starting point of the study was the result of ascus analysis (Threlkeld 1961). So all three methods of analysis will be considered.

1) Random spore analysis.

Ripe ascospores are discharged from mature perithecia onto the

sides of the culture tube. Such discharged ascospores were collected with a sterile wire loop and placed in a drop of sterile distilled water on a cavity slide. A loopful of a suspension of these spores was then spread on a block of plain 4% agar on an ordinary microscope slide. The agar block was then allowed to dry a little before the next stage. On attaining an optimum dryness, of the agar block, single spores were picked from it, under a dissecting microscope, and transferred to a small slope of appropriately supplemented glucose medium. The spores were picked off with a tungsten wire needle sharpened in molten sodium nitrite. Ascospores isolated from a cross are dormant, but it has been found that this dormancy can be broken and germination induced by placing the culture tubes in a water bath at 60°C. for 45 minutes. This heat treatment, in addition to inducing the germination of the ascospores, also had the effect of killing any conidia which had inadvertently been also transferred to the slopes. The tubes were then incubated at 25°C.

2) Perithecial analysis.

Nearly mature perithecia were removed from the cross slope, transferred to a drop of sterile distilled water in a cavity slide, and isolated. Single perithecia were then rolled across a block of 4% agar to remove any adhering conidia and mycelial fragments. The cleaned perithecia were then transferred to fresh blocks of 4% agar, where they were broken open in a drop of sterile distilled water and the ascospores released. Single ascospores were isolated as for random spores above, but as the spores were still immature a heat shock was not given immediately. Instead the tubes were incubated at 25° for 6-10 days so that the spores might mature. By this time the agar in the tubes had

partially dried out, and so in order to ensure good germination a drop or two of sterile distilled water was added to each tube before the heat shock was applied as for random spores. The germinating ascospores were then incubated at 25°.

3) Ascus (Tetrad) Analysis.

"Perithecia were transferred from the cross to a cavity slide, and treated with dilute sodium hypochlorite. Individual perithecia were then transferred to agar blocks and, under a dissecting microscope, broken up with tungsten wire needles so that intact asci were released. The asci were spread out on the agar and left to dry. When the right conditions of dryness were reached each ascus to be dissected was lifted, intact, from the surface of the agar, and placed on another part of the agar away from all stray spores. Thus it may be accepted with reasonable confidence that the set of spores under dissection were in fact derived from one ascus, and that the inclusion of stray spores was unlikely. Each spore of the ascus was then transferred to a small tube.

The spores were left to mature for seven days, after which odd numbered spores were subjected to a heat treatment of 60°C. for 45 minutes. If germination was largely successful, the even-numbered spores were heat-treated within 10 days of isolation." (Threlkeld, 1961).

In order to determine the nature of a possible heterokaryon, i.e., isolation 42-1, it was necessary to carry out a conidial analysis. In doing so it was important to observe strict aseptic techniques, as no heat treatment, which serves as a valuable safeguard in ascospore isolation, could be applied to these isolates. A suspension of conidia and mycelial fragments was made by transferring a loop of innoculum to a

tube of sterile distilled water, followed by the tube being shaken mechanically for 30 minutes. The resulting suspension was then filtered through a pad of sterile cotton wool to remove the mycelial fragments and groups of conidia. The concentration of the resulting suspension of single conidia was then estimated by the use of a hemocytometer slide, and dilutions were made to give approximately 2000 conidia per millilitre. 0.1 ml of this suspension was then transferred to, and spread on, a plate of supplemented sorbose medium. The plate was then incubated at 25°C. for 48 hours. On examination under a dissecting microscope, minute colonies arising from single conidia could be seen. These colonies were then cut out of the plate with tungsten needles, and transferred to small slopes of supplemented glucose medium, and incubated at 25°C.

Determination of genotypes

The biochemical markers were scored by the standard procedure of innoculating series of plates of appropriately supplemented sorbose medium with minute numbers of conidia from the cultures under examination. In later analyses, the leu-2 marker was not scored. It was considered that one distal marker was satisfactory.

Growth rate tests

The growth rate tests were carried out in liquid medium, containing salts, glucose, and supplements where appropriate as in the normal vegetative medium. The agar was omitted. Vogel 50 x strength salt solution was used instead of 4 x salt solution, it being more convenient to use the more concentrated stock solution when dealing with the large volumes of medium involved in these tests.

Whereas in medium based on 4 x salt solution it was necessary to add ammonium sulphate as well as altering the pH with Phosphate buffer in order to determine the pdxp genotype, 50 x salt solution already contained ammonium nitrate at a sufficiently high concentration and it was merely necessary to buffer the pH.

The tests were carried out in 125 ml Erlenmeyer flasks containing 50 ml of liquid medium. Except where otherwise stated, the flasks were each innoculated with approximately 6000 conidia. These were first suspended in sterile distilled water, filtered to remove mycelial fragments, estimated with a hemocytometer, diluted as required, and then used as innoculum. Strauss (Strauss 1951) used small, washed mycelial pads as innoculum to eliminate the possible effects of differential viability of the conidia of different strains, but this method probably introduces even greater potential variations in the selection of the small mycelial pads, no estimate of the weight of which is possible. Strauss also used only 20 ml of medium in the 125 ml flasks, but this practice probably increases the possibility of growth-limiting factors through exhaustion of nutrients or through the accumulation of toxic metabolites.

Unless otherwise stated, in order to minimize the risk of growth-limiting factors arising, the growth experiments were carried out for a period of six days, this period of time being sufficient for the production of a convenient weight of mycelium.

At the end of the experiment, the contents of each flask were filtered through a Whatman No. 1 filter paper and washed well with distilled water. After preliminary drying on a paper towel, the mycelial

pad was transferred from the filter paper to a glass slide, and dried to constant weight in an oven at 65°C. Cooling was carried out in a dessicator prior to the final weighing.

Note on diagrams

In some of the diagrams herein, it proved more convenient to label the gene loci with a single letter instead of the conventional gene symbol (e.g. pyr-1). The key to these is:-

| | | |
|---|---|---|
| c | = | pyr-1 |
| x | = | pdx |
| p | = | pdxp |
| q | = | pdxq |
| t | = | tryp-4 |
| l | = | leu-2 |
| s | = | hypothetical suppressor |

## CHAPTER III

## THE ORIGIN OF THE STRAINS AND ASCI

All nine aberrant asci were the result of a cross between strains H823 and J104 (Threlkeld, 1961).

H823 was genotypically A, pyr-1, pdx-1, both of the biochemical markers being situated on the right arm of Linkage Group IV. The ancestry of strain H823 is:

A, pdx-1 (37803) x a, pyr-1 (H263).

↓

A, pyr-1, pdx-1 (H823 and H815).

J104 was genotypically a, pdxp, tryp-4, leu-2, these biochemical markers also being on the right arm of Linkage Group IV. Its origin was:

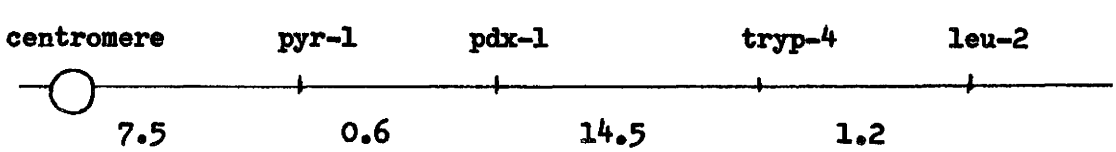
a, pdxp (44602p) x A, tryp-4, leu-2 (37501).

↓

a, pdxp, tryp-4, leu-2 (J104).

The arrangement of these biochemical markers on the right arm of Linkage Group IV has been shown to be as below:

Linkage Group IV




Inter-gene distances (cross-over units).

(Threlkeld, 1961).

The problem of the nature of the pdx and pdxp mutants at the pdx-1 locus, their relationship, and order, if any, is unknown, and their positions in the above diagram of chromosome IV is purely arbitrary (Mitchell, 1957).

Of 123 asci from the above cross which were analysed, 116 showed normal reciprocal recombination and 7 showed non-reciprocal recombination. The non-reciprocal events all concerned the pdx - pdxp locus. The frequency of non-reciprocal recombinant events here is far in excess of that observed by Mitchell in a cross between the same two mutant loci, where 7 out of 1200 asci analysed were aberrant in this way. (Mitchell, M.B. 1955, Mitchell, H.K. 1957).

The aberrant asci studied are:

JL 212

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pyr-1 | pdx | | |
| " | 3 & 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 5 & 6 | pdx | | | |
| " | 7 & 8 | pdxp | tryp-4 | leu-2 | |

JL 213

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pyr-1 | pdx | | |
| " | 3 & 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 5 & 6 | pdx | tryp-4 | leu-2 | |
| " | 7 & 8 | pdxp | | | |

JL 222

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 3 & 4 | pyr-1 | pdx | | |
| " | 5 & 6 | pdxp | tryp-4 | leu-2 | |
| " | 7 | pdx | | | |
| " | 8 | Failed to germinate | | | |

JM 253

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 3 & 4 | pyr-1 | pdx | | |
| " | 5 & 6 | pdx | | | |
| " | 7 & 8 | pdxp | tryp-4 | leu-2 | |

HR 85

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pdxp | tryp-4 | leu-2 | |
| " | 3 & 4 | pdx | | | |
| " | 5 & 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 7 & 8 | pry-1 | pdx | | |

EH 754

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pdxp | tryp-4 | leu-2 | |
| " | 3 & 4 | pdx | | | |
| " | 5 & 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 7 & 8 | pyr-1 | pdx | | |

JD 142

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 & 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| " | 3 & 4 | pyr-1 | pdx | | |
| " | 5 & 6 | pdx | | | |
| " | 7 & 8 | pdxp | tryp-4 | leu-2 | |

Two other abnormal tetrads occurred in the cross H823 x J104, and are shown below:

HK 42

| | | | | | |
|---|---|---|---|---|---|
| Spores | 1 | Wild type | | | |
| " | 2,3 & 4 | Failed to germinate | | | |
| " | 5 & 6 | pdxp | tryp-4 | leu-2 | |
| " | 7 & 8 | pyr-1 | pdx | | |

HX 112

| | | | | |
|---|---|---|---|---|
| Spores | 1 & 2 | pdxp | tryp-4 | leu-2 |
| " | 3 & 4 | pyr-1 | pdx | |
| " | 5 & 6 | Failed to germinate | | |
| " | 7 & 8 | Wild type | | |

It will be seen that the first seven asci all show a 3:1 ratio for pdx:pdxp. One pdxp product in each tetrad has been converted to the expression of a pdx phenotype. This has some of the characteristics of Lindegren's "gene conversion" in that although the ratio is abnormal, the allele expressions are of the parental type.

The last two asci introduce a new phenotype, equivalent in expression to the wild type allele at the locus. Such a situation could possibly arise by reciprocal recombination, but at an extremely low frequency. However, some form of post-genetic complementation may explain this reversion to a wild type of growth.

## CHAPTER IV

## 3:1 RATIOS FOR PDX:PDXP

Due to the polarised nature of the 3:1 ratios in the tetrads from this cross and the extremely high frequency, neither of which were found by Mitchell (Mitchell 1955, 1957), their origin by "gene conversion" can reasonably be rejected.

One situation in which a 3:1 ratio may occur by normal reciprocal processes is that of a cross concerning a biochemically deficient mutant and its suppressor gene. Diagrammatically this is as shown in Figure 1. The situation in Figure 1a concerns unlinked genes, and gives an overall ratio of 3 prototrophs: 1 auxotroph. Such is not the case in the cross being considered. However, if the biochemical gene and its suppressor were both on the homologous linkage group, 3:1 tetrads would only arise where there was a cross-over between the two loci, as in Figure 1b.

Applying this concept to ascus 213, postulating that a pdxp su genotype results in a pdx phenotype, and arbitrarily placing the suppressor of the pdxp phenotype to the left of pyr-1, the meiosis in the ascus would have occurred as in Figure 2.

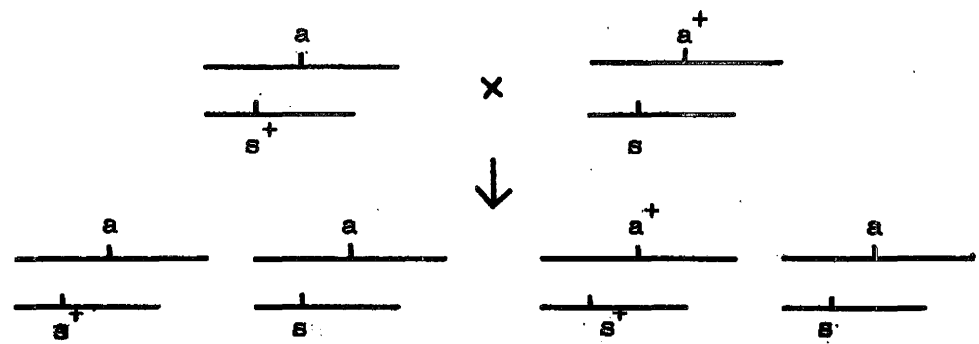
Another possible explanation would be the occurrence of a duplication of the pdx-1 region in H 823, with the resulting meiosis occurring as in Figure 3.

Yet another possibility is unequal crossing over as shown in

Figure 1

A 3:1 ratio from a Mutant-suppressor system

a) Unlinked genes




b) Linked genes

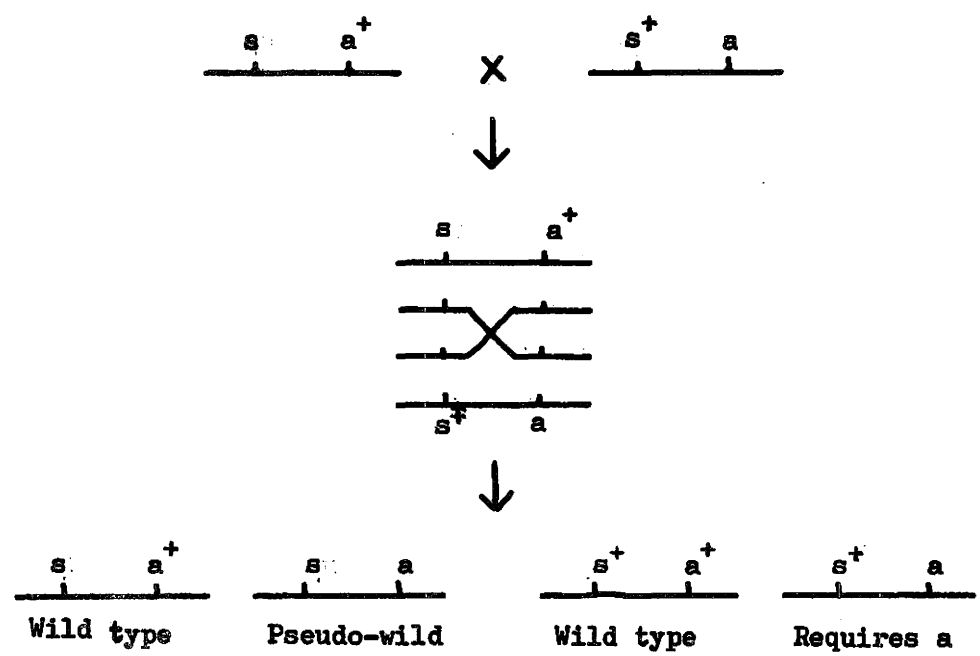


Figure 2

A Mutant-suppressor system applied to Ascus 213.

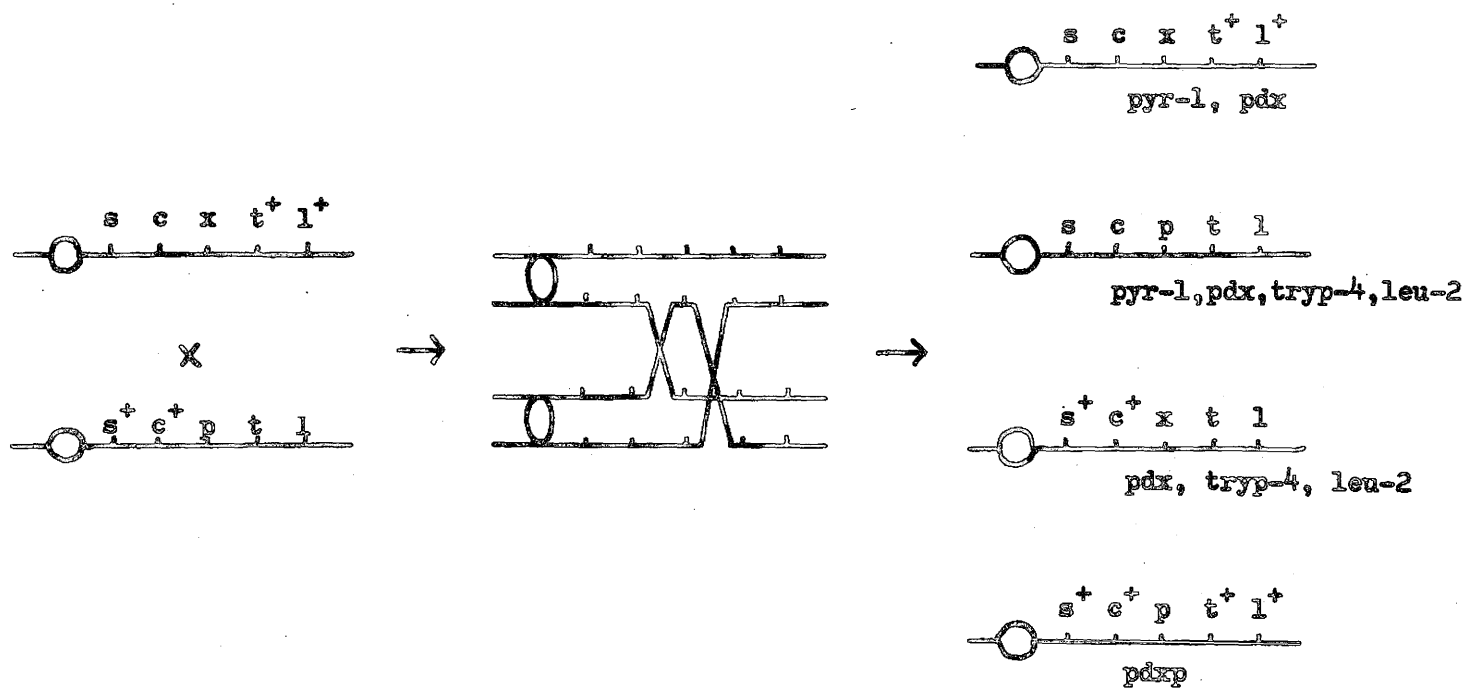


Figure 3

A 3:1 ratio due to a duplication of the pdx region

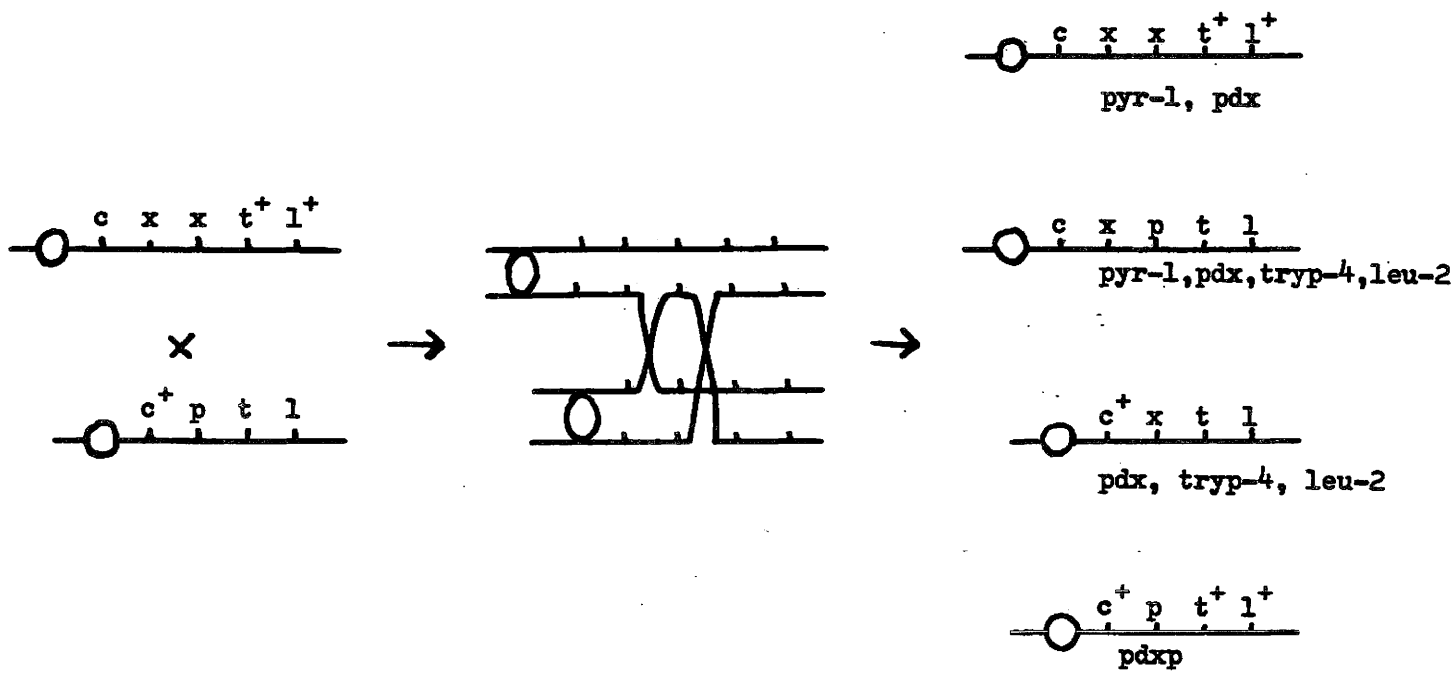


Figure 4. Such frequent unequal crossing over is most unlikely, and this hypothesis will not be considered further.

A fourth possibility could be due to the existence of an aneuploid fragment of chromosome IV in H 823. This is represented in Figure 5.

All except the first of these possibilities assume that where pdx and pdxp are both present, the more extreme mutant pdx phenotype is expressed. If the product of the pdx gene were anabolic, i.e. concerned with the synthesis of pyridoxine this would not be so. This therefore suggests that the gene product is catabolic, i.e. concerned with the removal, or breakdown, of pyridoxine from the system. This will be discussed in greater detail in a later chapter.

To investigate the possibility of the missing pdxp in these three tetrads, the twelve isolates were back-crossed to the appropriate Lindegren wild type strains. On attaining maturity, random spores were isolated from the back-crosses of the tetrad products of ascus HR 85, germinated, and analysed for their biochemical requirements. See Table I. The results were as follows:

85-1 (pdxp) x + produced + and pdxp.

85-4 (pdx) x + " + and pdx.

85-5 (pdx) x + " 8+, 5 pdx, and 2 pdxp.

85-8 (pdx) x + " + and pdx.

This agrees with the possible explanations suggested above, i.e. the concealed pdxp is to be found in the product with all the biochemical markers in these three asci. Therefore the back-crosses

Figure 4

A 3:1 ratio due to unequal crossing over

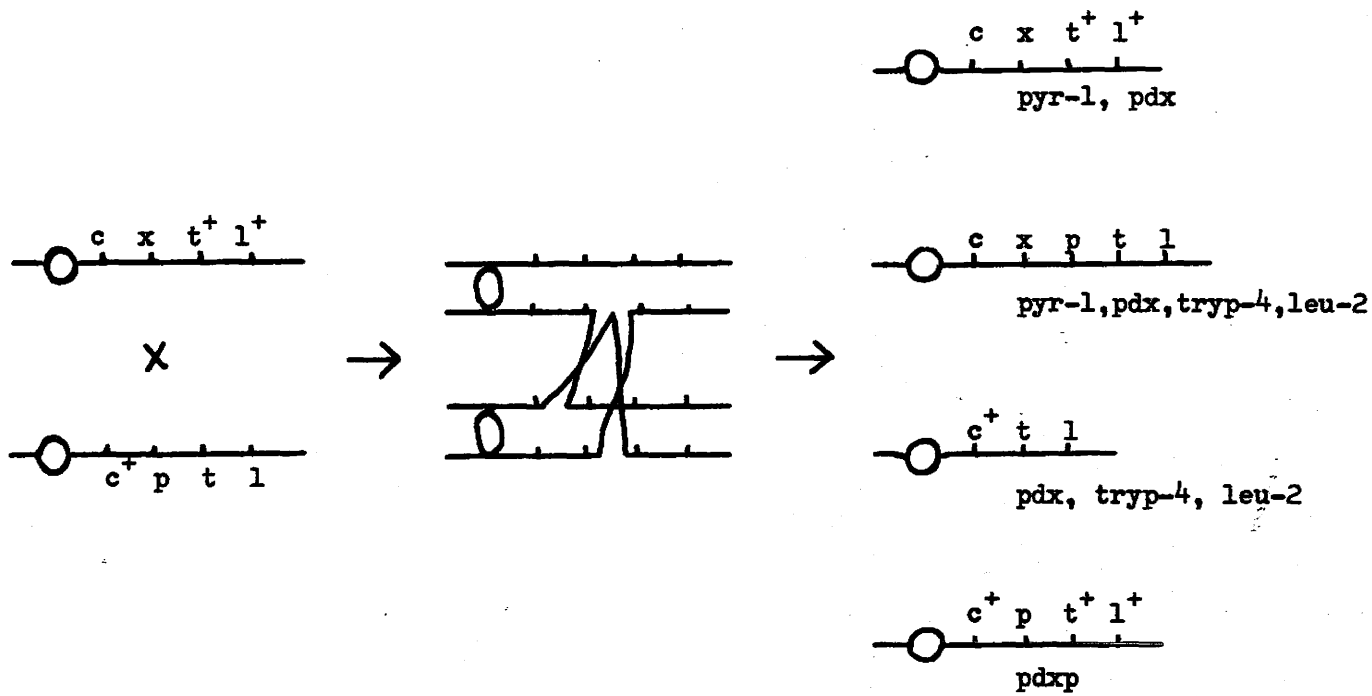
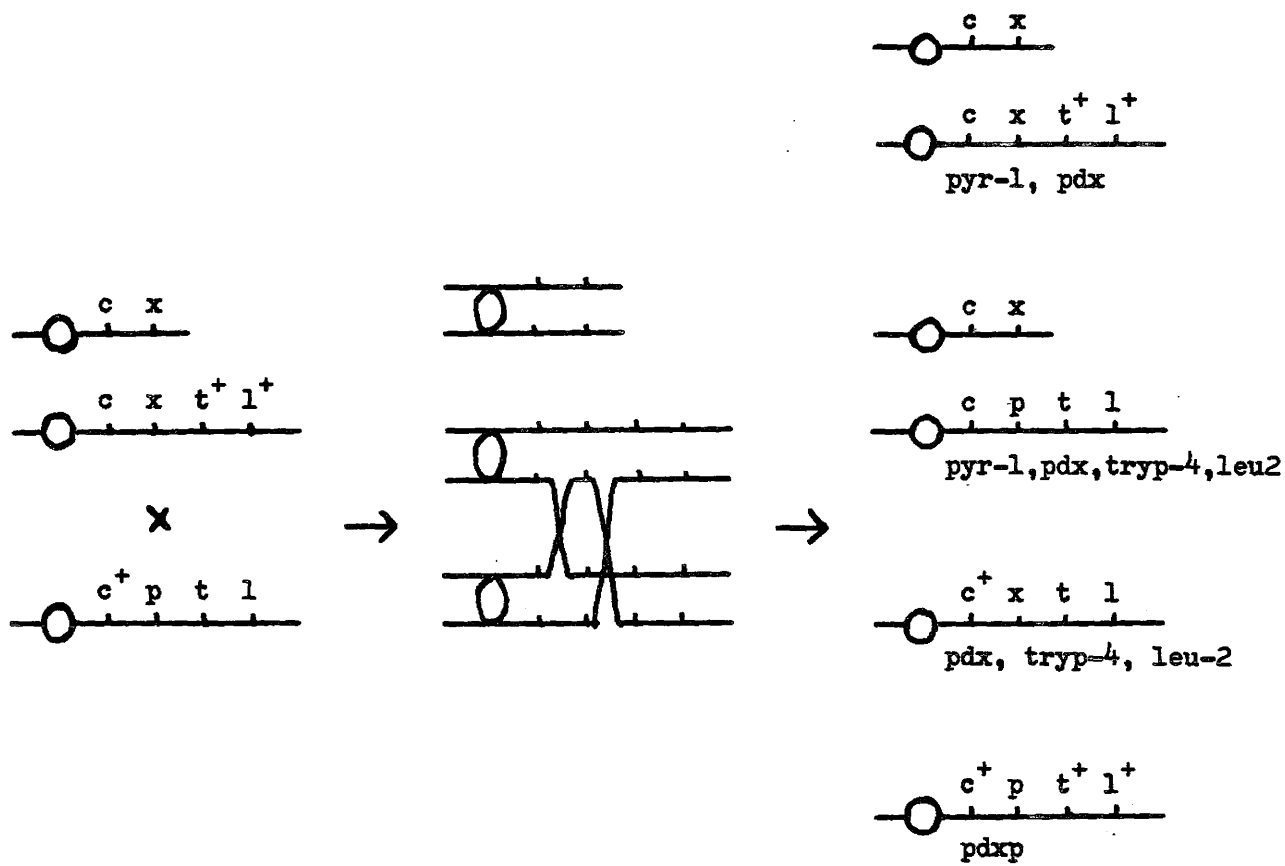


Figure 5

A 3:1 ratio due to Aneuploidy



of 253-1 and 213-3 with Lindegren 1A and 25a respectively were examined. The former gave 9 *pdx*$^+$ : 8 *pdx* : 1 *pdxp*, and the latter gave 11 *pdx*$^+$ : 3 *pdx* : 2 *pdxp*. See Tables 2 and 3 respectively.

These small samples, although showing no statistically valid trends, do clearly demonstrate that the *pdxp* mutant allele is concealed in these apparently *pdx* strains and can be recovered in the progeny of a back-cross. The correctness of the analysis so far was verified when a recovered *pdxp* isolate, from 253-1 x 1A, notably strain B7 was back-crossed again with Lindegren 1A. Of the twenty spores analysed from this cross, 7 were *pdxp*, 12 were wild type, and 1 failed to germinate.

In order to have a sample large enough to be statistically significant for the calculation of recombination data, a further 100 isolates were made from the cross 213-3 x 25a. Eight *pdxp* isolates were recovered from the 98 successful germinations.

Table 1 — 85.5 x 25a

| | |
|---|---|
| B 21 | wild type |
| 22 | " " |
| 23 | " " |
| 24 | pyr-1 pdx tryp-4 leu-2 |
| 25 | wild type |
| 26 | pyr-1 pdx tryp-4 leu-2 |
| 27 | wild type |
| 28 | tryp-4 leu-2 |
| 29 | pyr-1 pdxp tryp-4 leu-2 |
| 30 | pyr-1 pdx tryp-4 leu-2 |
| 31 | " " " " |
| 32 | wild type |
| 33 | failed to germinate |
| 34 | pyr-1 pdx tryp-4 leu-2 |
| 35 | tryp-4 leu-2 |
| 36 | pyr-1 pdxp tryp-4 leu-2 |

BACK-CROSSES:

| Table 2 | 253-1 x 1A |
|---|---|
| B 1 | wild type |
| 2 | pyr-1 pdx tryp-4 leu-2 |
| 3 | " " " " |
| 4 | " " " " |
| 5 | " " " " |
| 6 | " " " " |
| 7 | pyr-1 pdxp |
| 8 | tryp-4 leu-2 |
| 9 | wild type |
| 10 | pyr-1 pdx tryp-4 leu-2 |
| 11 | wild type |
| 12 | wild type |
| 13 | pyr-1 pdx tryp-4 leu-2 |
| 14 | " " " " |
| 15 | tryp-4 leu-2 |
| 16 | wild type |
| 17 | " " |
| 18 | " " |
| 19 & 20 | Failed to germinate |

Table 3 — 213-3 x 25a

| | |
|---|---|
| B 57 | pyr-1 |
| 58 | pyr-1 pdx tryp-4 leu-2 |
| 59 | — |
| 60 | tryp-4 leu-2 |
| 61 | wild type |
| 62 | — |
| 63 | wild type |
| 64 | pyr-1 |
| 65 | tryp-4 leu-2 |
| 66 | — |
| 67 | pyr-1 pdx tryp-4 leu |
| 68 | pyr-1 |
| 69 | — |
| 70 | pyr-1 |
| 71 | tryp-4 leu-2 |
| 72 | pyr-1 pdxp tryp-4 leu-2 |
| 73 | wild type |
| 74 | pdxp tryp-4 leu-2 |
| 75 | wild type |
| 76 | pyr-1 pdx |
| RH 25 | wild type |
| 26 | pdx tryp-4 leu-2 |
| 27 | pyr-1 pdx tryp-4 leu-2 |
| 28 | pyr-1 pdx |
| 29 | pyr-1 pdx tryp-4 leu-2 |
| 30 | pyr-1 pdxp |

Table 3 (cont'd)

| | | |
|---|---|---|
| RH | 31 | wild type |
| | 32 | pyr-1 pdx tryp-4 leu-2 |
| | 33 | wild type |
| | 34 | wild type |
| | 35 | pyr-1 pdx tryp-4 leu-2 |
| | 36 | wild type |
| | 37 | " " |
| | 38 | pdx tryp-4 leu-2 |
| | 39 | pyr-1 pdx tryp-4 leu-2 |
| | 40 | " " " " |
| | 41 | " " " " |
| | 42 | wild type |
| | 43 | pyr-1 pdx tryp-4 leu-2 |
| | 44 | wild type |
| | 45 | pdxp tryp-4 |
| | 46 | wild type |
| | 47 | " " |
| | 48 | — |
| | 49 | pyr-1 pdx tryp-4 leu-2 |
| | 50 | " " " " |
| | 51 | wild type |
| | 52 | pdxp |
| | 53 | pdxp |
| | 54 | wild type |
| | 55 | pyr-1 pdx |

Table 3 (cont'd)

| | |
|---|---|
| RH 56 | pyr-1 pdx |
| 57 | pdx tryp-4 leu-2 |
| 58 | wild type |
| 59 | " " |
| 60 | pyr-1 pdx tryp-4 leu-2 |
| 61 | wild type |
| 62 | pyr-1 pdx tryp-4 leu-2 |
| 63 | " " " " |
| 64 | pyr-1 pdxp |
| 65 | wild type |
| 66 | pyr-1 pdx tryp-4 leu-2 |
| 67 | pyr-1 pdx tryp-4 leu-2 |
| 68 | wild type |
| 69 | " " |
| 70 | " " |
| 71 | " " |
| 72 | pyr-1 pdx tryp-4 leu-2 |
| 73 | " " " " |
| 74 | wild type |
| 75 | pyr-1 pdx tryp-4 leu-2 |
| 76 | pdxp tryp-4 leu-2 |
| 77 | pyr-1 pdx tryp-4 leu-2 |
| 78 | wild type |
| 79 | pyr-1 pdxp |
| 80 | tryp-4 leu-2 |

Table 3 (cont'd)

| | |
|---|---|
| RH 81 | wild type |
| 82 | pdxp tryp-4 leu-2 |
| 83 | pyr-1 pdx tryp-4 leu-2 |
| 84 | wild type |
| 85 | pyr-1 pdx tryp-4 leu-2 |
| 86 | " " " " |
| 87 | " " " " |
| 88 | wild type |
| 89 | pyr-1 pdx tryp-4 leu-2 |
| 90 | " " " " |
| 91 | " " " " |
| 92 | " " " " |
| 93 | " " " " |
| 94 | " " " " |
| 95 | " " " " |
| 96 | tryp-4 leu-2 |
| 97 | pyr-1 pdx tryp-4 leu-2 |
| 98 | " " " " |
| 99 | wild type |
| 100 | pry-1 pdx tryp-4 leu-2 |
| 101 | wild type |
| 102 | " " |
| 103 | pyr-1 pdx tryp-4 leu-2 |
| 104 | " " " " |
| 105 | wild type |

Table 3 (cont'd)

| | |
|---|---|
| RH 106 | pry-1 pdx tryp-4 leu-2 |
| 107 | " " " " |
| 108 | — |
| 109 | pyr-1 pdx tryp-4 leu-2 |
| 110 | wild type |
| 111 | tryp-4 leu-2 |
| 112 | wild type |
| 113 | " " |
| 114 | — |
| 115 | pyr-1 pdx tryp-4 leu-2 |
| 116 | pyr-1 pdx |
| 117 | pdxp tryp-4 leu-2 |
| 118 | pyr-1 pdxp tryp-4 leu-2 |
| 119 | " " " " |
| 120 | pyr-1 pdx leu-2 |
| 121 | pyr-1 pdxp |
| 122 | pyr-1 pdx tryp-4 leu-2 |
| 123 | pyr-1 pdxp |

The twelve classes of progeny recovered in the above isolates, and their frequencies are given in Table 4. The total number of isolates analysed from cross 213-3 x 25a was 112. These are given in Table 3.

Table 4

| | | | | |
|---|---|---|---|---|
| pyr-1 | pdx | tryp-4 | leu-2 | 43 |
| pyr-1$^+$ | pdx$^+$ | tryp-4$^+$ | leu-2$^+$ | 36 |
| pyr-1$^+$ | pdx$^+$ | tryp-4 | leu-2 | 7 |
| pyr-1 | pdx | tryp-4$^+$ | leu-2$^+$ | 6 |
| pyr-1 | pdxp | tryp-4$^+$ | leu-2$^+$ | 5 |
| pyr-1 | pdxp | tryp-4 | leu-2 | 4 |
| pyr-1 | pdx$^+$ | tryp-4$^+$ | leu-2$^+$ | 4 |
| pry-1$^+$ | pdxp | tryp-4$^+$ | leu-2$^+$ | 2 |
| pyr-1$^+$ | pdx | tryp-4 | leu-2 | 2 |
| pyr-1$^+$ | pdxp | tryp-4 | leu-2 | 1 |
| pyr-1$^+$ | pdxp | tryp-4 | leu-2$^+$ | 1 |
| pyr-1 | pdx | tryp-4$^+$ | leu-2 | 1 |

There is some difficulty in the interpretation of the results for the pdx locus, but the cross-over frequencies between the other markers, and hence their distances apart may be easily found and calculated.

If aneuploidy were the cause of the 3:1 ratios, one would expect the aneuploid fragment to be excluded from most of the nuclei in the course of a series of mitotic divisions. As a result of this, mainly pdxp and pdx$^+$ isolates would be expected in the isolates from the back-cross. Any pdx progeny would be due to either residual aneuploidy, or else somatic recombination before the fragment became excluded. Such somatic recombination would have to produce a Linkage

Group IV bearing pyr-1, pdx, tryp-4, leu-2.

Now let us proceed to calculate the recombination frequencies between the markers in this cross, and hence their map positions.

Consider first the case of tryp-4 and leu-2. One crossover between these two loci occurred in the 112 isolates analysed. From this the map distance may be calculated to be:

$$\frac{1 \times 100}{112} = 0.89 \text{ crossover units.}$$

A previous calculation of this distance (Threlkeld, 1961) gave 2 crossover asci out of 71 analysed, equivalent to a map distance of 1.2 c.o.u. Each of these recombinant asci gave rise to 4 recombinant spores out of 8, so for each of these asci, the recombinant spores were 1/2.

There is difficulty in correlating recombinate data from asci with that from random spores, but by conventional practice, one ascus is taken to be equivalent to one random spore.

The recombinants in the asci were 1/2 + 1/2 out of 71 asci.

Applying a contingency table to the data:

| | Recomb. | Non-rec. | Total |
|---|---|---|---|
| Tetrads | 1 | 70 | 71 |
| Random | 1 | 111 | 112 |
| | 2 | 181 | 183 |

$$T \text{ (Tetrads)} = \frac{2 \times 71}{183} = 0.77$$

| | Observed | | Theoretical |
|---|---|---|---|
| | 1 | | 0.77 |
| | 1 | | 1.23 |

$$\chi^2 = \frac{(0.23)^2}{1.23} + \frac{(0.23)^2}{0.77} = \frac{0.56}{1.23} + \frac{.057}{0.77}$$

$$= .045 + .074$$

$$= .119$$

For 1 degree of freedom, $P > 0.70$. Therefore these values do not differ significantly.

Now consider the case of pyr-1 and tryp-4.

The four classes of progeny are:

| | | |
|---|---|---|
| pyr-1$^+$ | tryp-4$^+$ | 38 |
| pyr-1 | tryp-4 | 47 |
| pyr-1$^+$ | tryp-4 | 11 |
| pyr-1 | tryp-4$^+$ | 16 |

The first two classes are the parental types, and the last two are recombinant. From these figures the crossover distance may be calculated:

$$\frac{27 \times 100}{112} = 24 \text{ crossover units}$$

For this interval, Threlkeld (Threlkeld, 1961) obtained 22 recombinant asci out of 71. Applying a contingency table to these data:

| | Recomb. | Non-Recomb. | Total |
|---|---|---|---|
| Tetrads | 11 | 60 | 71 |
| Random | 27 | 85 | 112 |
| | 38 | 145 | 183 |

$$T \text{ (tetrads)} = \frac{38 \times 71}{183} = \underline{14.7}$$

| Observed | Theoretical |
|---|---|
| 11 | 14.7 |
| 27 | 23.3 |
| 60 | 56.3 |
| 85 | 88.7 |

$$\chi^2 = \frac{(3.7)^2}{14.7} + \frac{(3.7)^2}{23.3} + \frac{(3.7)^2}{56.3} + \frac{(3.7)^2}{88.7}$$

$$= 0.92 + 0.58 + 0.24 + 0.15$$

$$= 1.89$$

For one degree of freedom, $P > 0.10$. There is therefore no significant difference between the present data and that of Threlkeld.

Regarding pyr-1 and leu-2, the four progeny classes are:

| | | |
|---|---|---|
| pyr-1$^+$ | leu-2$^+$ | 39 |
| pyr-1 | leu-2 | 48 |
| pyr-1 | leu-2$^+$ | 15 |
| pyr-1$^+$ | leu-2 | 10 |

We must further allow for two apparent double crossovers between these markers in isolates RH 45 and RH 120. Thus the number of crossovers is 29, and the map distance is therefore 26 c.o.u.

Applying a contingency table to the data:

| | Recomb. | Non-Recomb. | Total |
|---|---|---|---|
| Tetrads | 12 | 59 | 71 |
| Random | 29 | 83 | 112 |
| | 41 | 142 | 183 |

$$T \text{ (tetrads)} = \frac{41 \times 71}{183} = 15.9$$

| Observed | Theoretical |
|---|---|
| 12 | 15.9 |
| 29 | 25.1 |
| 59 | 55.1 |
| 83 | 86.9 |

$$X^2 = \frac{(3.9)^2}{15.9} + \frac{(3.9)^2}{25.1} + \frac{(3.9)^2}{55.1} + \frac{(3.9)^2}{86.9}$$

$$= 0.96 + 0.61 + 0.27 + 0.17$$

$$= 2.01$$

For one degree of freedom, $P \geq 0.10$. Therefore, again there is no significant difference between these data.

If we accept the hypothesis that the combined result of pdx and a pdxp mutant in the same locus produces a pdx phenotype, it is possible to score for the presence or absence of the pdxp mutant if we regard any progeny with pdxp phenotype as being $pdx^+$ for the locus homologous for that which was pdx in isolate 213-3. By doing this, it is possible to obtain recombination data, and hence map distances with

reference to this pdx locus.

Considering it first with regard to the pyr-1 locus, the four possible progeny classes are:

| | | | |
|---|---|---|---|
| pyr-1 | pdx | 50 | |
| pyr-1$^+$ | pdx$^+$ or pdxp | 47 | |
| pyr-1$^+$ | pdx | 2 | Recombinants |
| pyr-1 | pdx$^+$ or pdxp | 13 | |

The map distance between pyr-1 and the pdx of 213-3 is therefore:

$$\frac{15 \times 100}{112} = \underline{\underline{13.4 \text{ c.o.u.}}}$$

Threlkeld's data show one crossover out of 71 asci for this region. Once again a contingency table is applied, including Yates' correction:

| Recomb. | Non-Recomb. | Total |
|---|---|---|
| 1 | 70 | 71 |
| 14.5 | 97.5 | 112 |
| 15.5 | 167.5 | 183 |

$$T \text{ (tetrad)} = \frac{15.5 \times 71}{183} = 6.0$$

| Observed | Theoretical |
|---|---|
| 1 | 6 |
| 14.5 | 9.5 |
| 70 | 65 |
| 97.5 | 102.5 |

$$X^2 = \frac{(5)^2}{6} + \frac{(5)^2}{9.5} + \frac{(5)^2}{65} + \frac{(5)^2}{102.5}$$

$$= 4.2 + 2.75 + 0.39 + 0.25$$

$$= \underline{7.57}$$

For one degree of freedom, $P < 0.01$. There is a highly significant difference between these data as shown by the above contingency table, a fact which strongly supports the suggested duplication. A duplication may also explain certain other high recombination frequencies, and possibly also the 2:13 ratio of pyr-1, pdx-1 recombinants.

Thus far, it would appear that the markers are present in the relative positions shown in Figure 6a.

We cannot map the pdxp mutant in the normal manner from the RH series data as we can only score for it when the pdx allele of 213 is replaced by a $pdx^+$ allele in a recombination event. However, we may reasonably postulate that it is in its normal position with respect to pyr-1 from the origin in J 104.

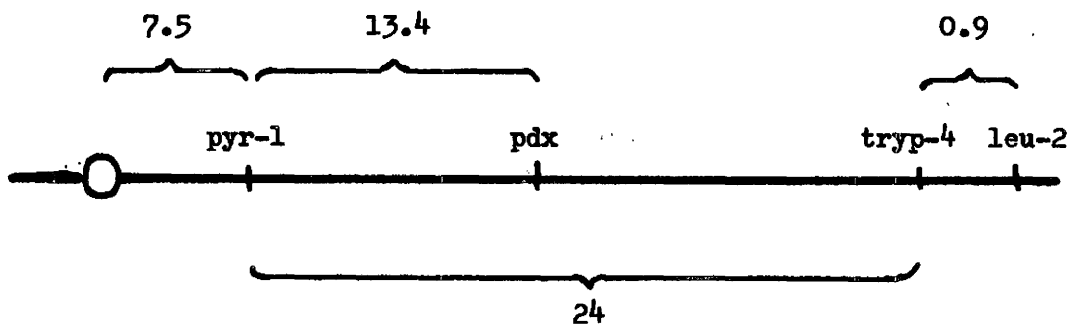
It is also likely that the pyr-1 locus is duplicated as it is closely linked to the pdx locus, and so the second of these should be located 0.6 c.o.u. proximal to the pdx allele. (When mapping any other locus with respect to the pyr-1 allele, one would map it with the pyr-1 locus farthest from that locus).

The modified structure of Linkage Group IV would therefore be as shown in Figure 6b.

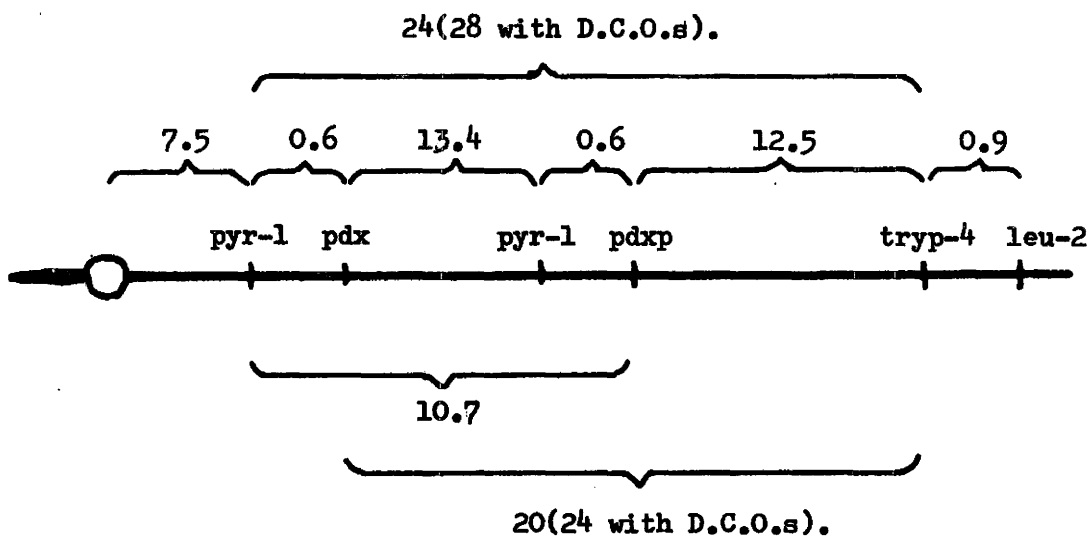
It is possible to obtain an estimate of the distance between pdxp and the other markers by treating the pdxp progeny as one half of reciprocal exchanges. Pdxp was linked to tryp-4 in 213-3, and in

Figure 6

a) Preliminary map of linkage group IV in 213-3




b) Modified linkage map of 213-3



the RH progeny there are seven pdxp isolates no longer in the parental combination. Theoretically there should also be seven reciprocal recombinants, although these are in fact obscured. However, we have 14 recombinants out of 112 for the pdxp - tryp-4 distance, equivalent to a distance of:

$$\frac{14 \times 100}{112} = 12.5 \text{ c.o.u.}$$

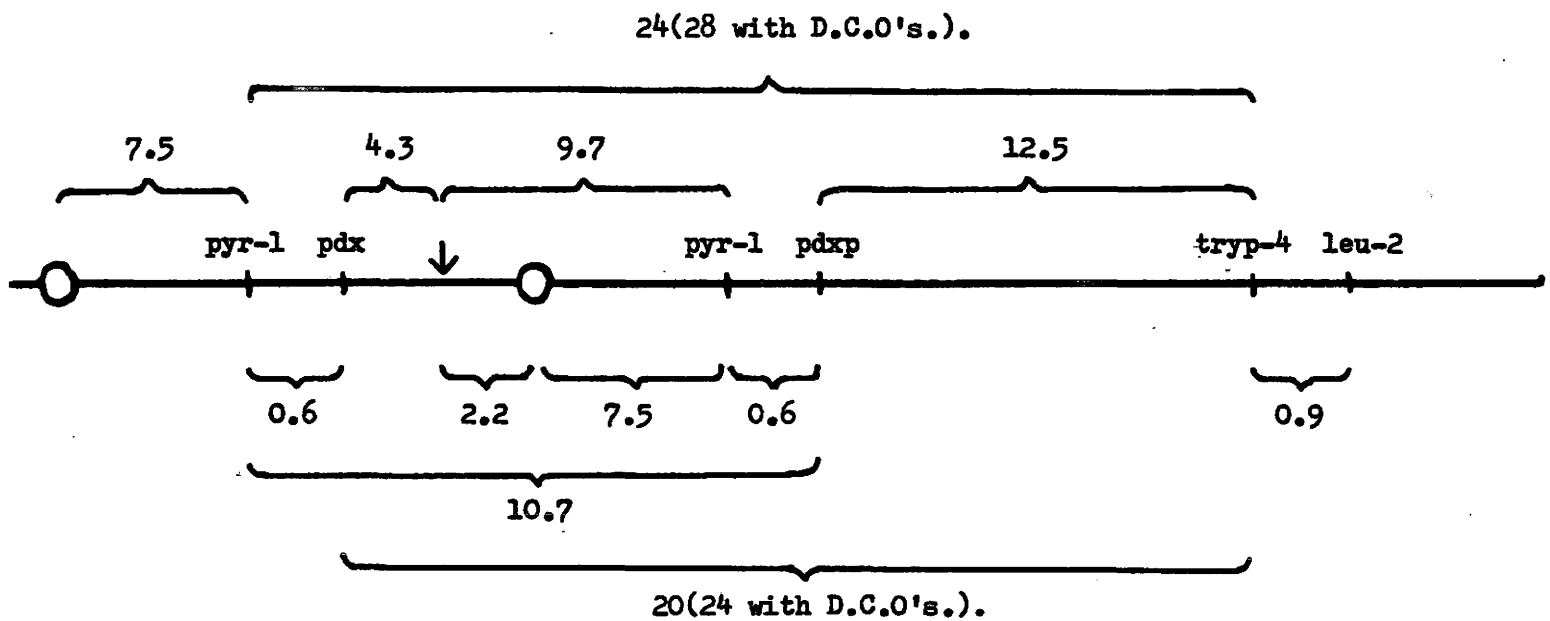
Likewise we can make an approximate map distance of the interval between pdxp and the proximal pyr-1 locus. The visible recombinant is pyr-1$^+$ pdxp of which there are 6. This gives a theoretical number of recombinants of 12. The map distance calculated from this is:

$$\frac{12 \times 100}{112} = \underline{10.7 \text{ c.o.u.}}$$

Although the figures calculated from data involving the pdxp mutant are only one half as accurate as the other distances calculated from the RH isolates, as only one half of the reciprocal recombinants could be classified, these map distances serve as a valuable confirmation of the other data.

Figure 7

Duplication map of 213-3



There now faces us the problem of the homology of the genetic material in the region between the proximal pdx locus and the distal pyr-1 locus of 213-3. This region has a length of 13.4 crossover units. Depending on the nature and origin of this segment, there are two possible positions for synapsis.

If the material is proximal to the distal of the two pyr-1 loci, synapsis and possible crossover formation could occur as in Figure 8a. The progeny from such a recombinant event would be as shown in Figure 8b.

If the material originated distally to the proximal of the two pyr-1 loci, synapsis would occur as shown, with possible recombination, in Figure 9a. The progeny would be as in Figure 9b.

By comparing the frequencies of progeny of the first and second types, and disregarding the tryp-4 locus, we may obtain an estimate of the make-up of this segment of genetic material. The progeny classes and relative frequencies are:

| | |
|---|---|
| pyr-1$^+$, pdxp | 4 |
| pyr-1, pdxp | 9 |

Therefore, of the total length of the interval,

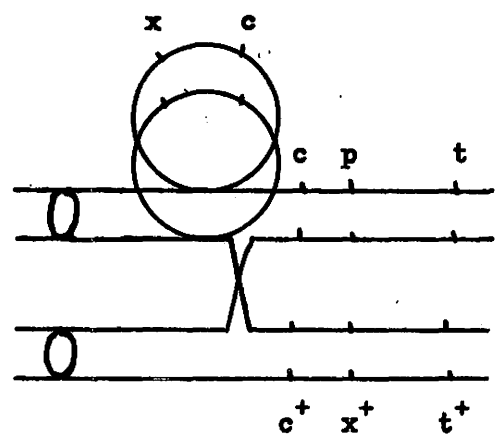
$$\frac{9}{13} \times 14 = 9.7 \text{ c.o.u.}$$

originates as being normally proximal to the distal pyr-1 locus. The remaining portion of 4.3 c.o.u. originates as distal to the proximal pyr-1 locus.

It therefore seems likely that the centromere, being normally only 7.5 c.o.u. proximal to pyr-1 is itself duplicated.

Figure 8

a) Synapsis and possible crossovers if duplication is proximal to the distal of the two pyr-1 loci.




b) Recombinant progeny for Figure 8a.

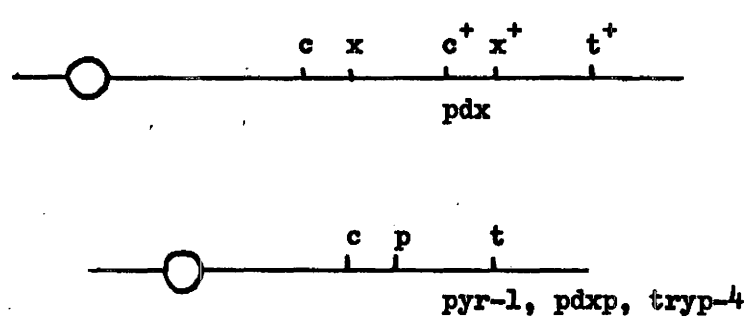
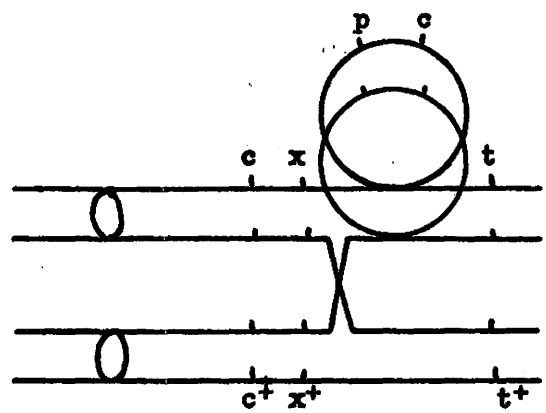
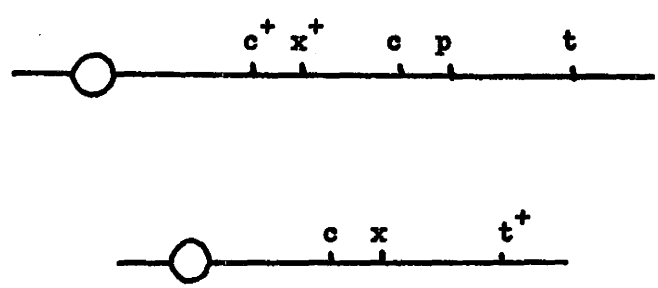


Figure 9

a) Synapsis and possible crossovers if the duplication is distal to the proximal of the two pyr-1 loci.




b) Crossover progeny from Figure 9a



A check on the data from the 213-3 back-cross may be carried out on the back-cross of 253-1 with pdxq. 253-1 is phenotypically pyr-1, pdx, tryp-4, and was shown by a previous back-cross against Lindegren wild type strain 1A to produce some pdxp progeny.

One hundred successfully germinated isolates were analysed (RT 1-100). The phenotypes are given in Table 5.

| Table 5 | 253-1 x pdxq | | |
|---|---|---|---|
| RT 1 | pyr-1 | pdx | tryp-4 |
| 2 | pyr-1 | pdx | tryp-4 |
| 3 | pdxq | | |
| 4 | pdxp | | |
| 5 | pdxq | tryp-4 | |
| 6 | pdxq | | |
| 7 | pdxq | | |
| 8 | pdxq | | |
| 9 | pyr-1 | pdx | tryp-4 |
| 10 | pdxp | tryp-4 | |
| 11 | pdxq | | |
| 12 | pyr-1 | pdx | tryp-4 |
| 13 | pdxq | | |
| 14 | pdxq | | |
| 15 | pyr-1 | pdx | tryp-4 |
| 16 | pdxq | | |
| 17 | pyr-1 | pdx | tryp-4 |
| 18 | pdxq | | |
| 19 | pyr-1 | pdx | tryp-4 |
| 20 | pyr-1 | pdxq | tryp-4 |
| 1 | pyr-1 | pdx | tryp-4 |
| 2 | pdxp | | |
| 3 | pdxq | | |
| 4 | pdxq | | |
| 5 | pyr-1 | pdxq | |

Table 5 (cont'd)

| | | | | |
|---|---|---|---|---|
| RT | 6 | pyr-1 | pdx | tryp-4 |
| | 7 | pyr-1 | pdx | tryp-4 |
| | 8 | pdxq | | |
| | 29 | pdxq | | |
| | 30 | pdxq | tryp-4 | |
| | 1 | pdxq | | |
| | 2 | pdxq | | |
| | 3 | pyr-1 | pdx | tryp-4 |
| | 4 | pyr-1 | pdxq | |
| | 5 | pdxq | | |
| | 6 | pdxq | | |
| | 7 | pyr-1 | pdx | tryp-4 |
| | 8 | pdxq | | |
| | 9 | pyr-1 | pdx | tryp-4 |
| | 40 | pyr-1 | pdx | tryp-4 |
| | 1 | pyr-1 | pdx | tryp-4 |
| | 2 | pyr-1 | pdx | tryp-4 |
| | 3 | pyr-1 | pdx | tryp-4 |
| | 4 | pdxq | | |
| | 5 | pdxq | | |
| | 6 | pyr-1 | pdxp | tryp-4 |
| | 7 | pdxq | | |
| | 8 | pdxq | | |
| | 9 | pyr-1 | pdxp | |
| | 50 | pyr-1 | pdx | tryp-4 |
| | 1 | pyr-1 | pdx | tryp-4 |

Table 5 (cont'd)

| | | | |
|---|---|---|---|
| RT 2 | pyr-1 | pdx | tryp-4 |
| 3 | pdxq | | |
| 4 | pyr-1 | pdxq | tryp-4 |
| 5 | pdxq | | |
| 6 | pdxq | | |
| 7 | pdxq | | |
| 8 | pyr-1 | pdx | tryp-4 |
| 9 | pyr-1 | pdx | tryp-4 |
| 10 | pdxq | | |
| 1 | pyr-1 | pdx | tryp-4 |
| 2 | pdxq | | |
| 3 | pdxq | | |
| 4 | pyr-1 | pdxq | |
| 5 | pyr-1 | pdx | tryp-4 |
| 6 | pyr-1 | pdxq | |
| 67 | pyr-1 | pdxq | |
| 8 | pyr-1 | pdx | tryp-4 |
| 9 | pyr-1 | pdx | tryp-4 |
| 70 | pyr-1 | pdx | tryp-4 |
| 1 | pdxp | | |
| 2 | pyr-1 | pdxp | |
| 3 | pyr-1 | pdx | tryp-4 |
| 4 | pyr-1 | pdx | tryp-4 |
| 5 | pdxp | | |
| 6 | pyr-1 | pdx | tryp-4 |

Table 5 (cont'd)

| | | | |
|---|---|---|---|
| RT 7 | pdxq | | |
| 8 | pyr-1 | pdx | tryp-4 |
| 9 | pyr-1 | pdxq | tryp-4 |
| 80 | pdxq | | |
| 1 | pyr-1 | pdx | tryp-4 |
| 2 | pyr-1 | pdx | tryp-4 |
| 3 | pdxq | | |
| 4 | pyr-1 | pdx | |
| 5 | pdxq | | |
| 6 | pdxq | | |
| 7 | pyr-1 | pdx | tryp-4 |
| 8 | pyr-1 | pdx | |
| 9 | pyr-1 | pdx | |
| 90 | pyr-1 | pdx | tryp-4 |
| 1 | pyr-1 | pdxq | tryp-4 |
| 2 | pyr-1 | pdx | tryp-4 |
| 3 | pdxq | | |
| 4 | pyr-1 | pdx | tryp-4 |
| 5 | pyr-1 | pdx | tryp-4 |
| 6 | pyr-1 | pdx | tryp-4 |
| 7 | pyr-1 | pdx | tryp-4 |
| 8 | pyr-1 | pdx | tryp-4 |
| 9 | pdxq | tryp-4 | |
| 100 | pyr-1 | pdxq | |

The progeny classes and their percentages are given in Table 6.

Table 6

| | | | |
|---|---|---|---|
| + | pdxq | + | 36 |
| pyr-1 | pdx | tryp-4 | 41 |
| + | pdxq | tryp-4 | 3 |
| pyr-1 | pdxq | tryp-4 | 4 |
| + | pdxp | + | 4 |
| pyr-1 | pdxp | + | 2 |
| + | pdxp | tryp-4 | 1 |
| pyr-1 | pdxp | + | 5 |
| pyr-1 | pdx | + | 3 |
| pyr-1 | pdxp | tryp-4 | 1 |

From the data in Table 6, one may calculate, as before, the map distances between the loci. The distance between pyr-1 and pdx is 12 units. For pdxp and tryp-4 it is also 12 crossover units. For pyr-1 to pdxp the distance is 10 units. These values all agree closely with the distances and relative positions calculated from 213-3 x 25a.

As has previously been stated, recombination values involving the same two markers in Neurospora have been known to vary considerably with the genetic background of any specific cross. However, in this case, despite the widely differing genetic constitutions of 25a and pdxq these values are remarkably constant, and may be taken as evidence for the postulated duplication.

Again in Table 6 the phenotype involving the recombination pyr-1, pdx is significantly less frequent than its apparent reciprocal.

## CHAPTER V

## SECOND GENERATION BACK-CROSSES FROM 213-3.

In order to obtain further information on the location of the loci discussed in the previous chapter, a back-cross was carried out on a pyr-1, pdx isolate recovered from the RH series (from 213-3 x 25a), namely RH 79. RH 79 was crossed with pdxq (35405, F.G.S.C. #362), and 100 successfully germinated isolates were analysed. The germination in this cross was 95%.

Any recombination events in the region of a possible aberration would produce deletions and thus lower the viability of the progeny. The fact that the viability of the spores from this cross was as high as any found in this whole investigation certainly indicates no abnormalities of this type. Also, the growth of all isolates on supplemented medium was vigorous, a fact which would again suggest that RH 79 is normal since otherwise a deletion might cause less vigorous growth.

The progeny classes and numbers from RH 79 x pdxq are:

| | | |
|---|---|---|
| pyr-1 | pdx | 48 |
| pyr-1$^+$ | pdxq | 50 |
| pyr-1 | pdxq | 2 |
| pyr-1$^+$ | pdx | 0 |

The distance between the two loci may be calculated as:

$$\frac{2 \times 100}{100} = 2 \text{ crossover units}$$

Two previous estimates of this interval have been that of Threlkeld, and that obtained from cross 213-3 x 25a. The former estimate was 0.6 c.o.u., and the latter 13.4 c.o.u..

Comparing first the current data with those obtained by Threlkeld for recombination within this interval:

| | Recomb. | Non-recomb. | Total |
|---|---|---|---|
| Tetrad | 0.5 | 70.5 | 71 |
| RH 79 x w.t. | 2 | 98 | 100 |
| | 2.5 | 168.5 | 171 |

$$T \text{ (tetrads)} = \frac{2.5 \times 71}{171} = 0.98$$

| Observed | Theoretical |
|---|---|
| 1 | 0.98 |
| 2 | 1.52 |
| 70 | 70.02 |
| 98 | 98.48 |

$X^2 \simeq 0.0006$ . $P > 0.98$

In the above case therefore there is no significant difference. Yates' correction was not applied as this would have further minimised any possible difference.

A comparison with the data from 213-3 x 25a, including Yates' correction this time to minimise what appears to be a large difference, shows:

| | Recomb. | Non-recomb. | Total |
|---|---|---|---|
| 213-3 | 14.5 | 97.5 | 112 |
| RH 79 | 2.5 | 97.5 | 100 |
| | 17 | 195 | 212 |

$$T\ (213\text{-}3) = \frac{17 \times 112}{212}$$

$$= 9.2$$

| Observed | Theoretical |
|---|---|
| 14.5 | 9.2 |
| 2.5 | 7.8 |
| 97.5 | 102.8 |
| 97.5 | 92.2 |

$X^2 = 7.5$ $\quad P < 0.01$

Therefore, although this back-cross data differs significantly from the parental data, it does not differ from the control data of Threlkeld. It is not conceivable that the pyr-1 locus could move with respect to the pdx locus in one generation and then revert to its former position in the next generation. It in fact suggests the duplication of the pyr-1 locus, with one on each side of the pdx. (One locus being 0.6 units away, and the other 13.4 units away.) If the pyr-1 gene was anabolic in function, a recombination event with respect to either outside marker would give the pyrimidine-independent pyr-1, pdx, pyr-1$^+$. In scoring for such recombination, the distance calculated would approximate to the distance between the pdx locus and the more distant of the two pyr-1 loci. If the duplication including the more distant of the

two pyr-1 loci is deleted, any subsequent cross would only give the original low level of recombination between these markers. This lends strong support to the hypothesis of the duplication of both pyr-1 and pdx-1 loci.

Conclusive proof of the duplicated pyr-1 locus should be available from possible progeny of 213-3 x 25a which have had the pyr-1 and pdx alleles distal to tryp-4 replaced by their respective wild type alleles. Such a recombinant would be genotypically:

pyr-1$^{+}$, pdx$^{+}$, pyr-1, pdxp, tryp-4

and phenotypically:

pyr-1$^{+}$, pdxp, tryp-4.

Isolate RH 45 is of this phenotype, and was back-crossed to wild type. Of the 38 progeny from this back-cross which were analysed, 5 were auxotrophic for pyrimidine.

The origin of RH 79 is illustrated in Figure 10, and RH 45 in Figure 11.

Figure 10

The origin of RH 79.

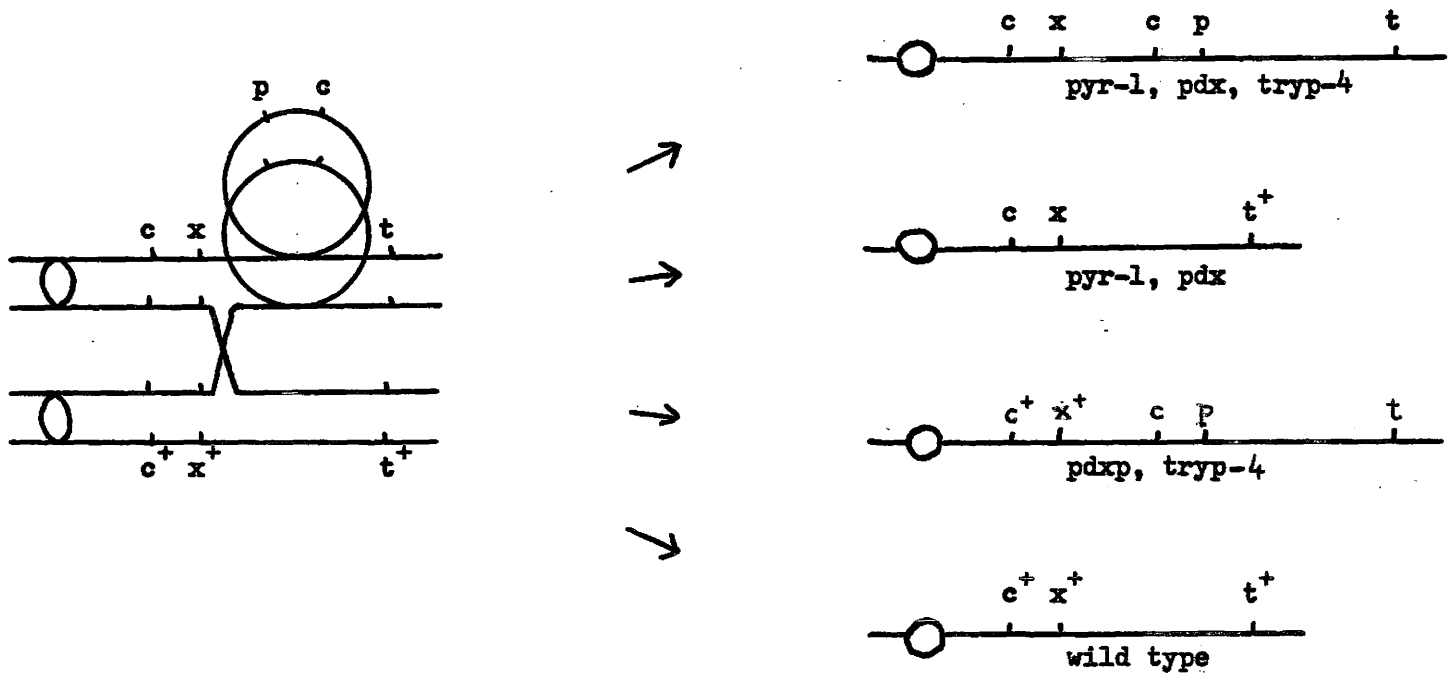
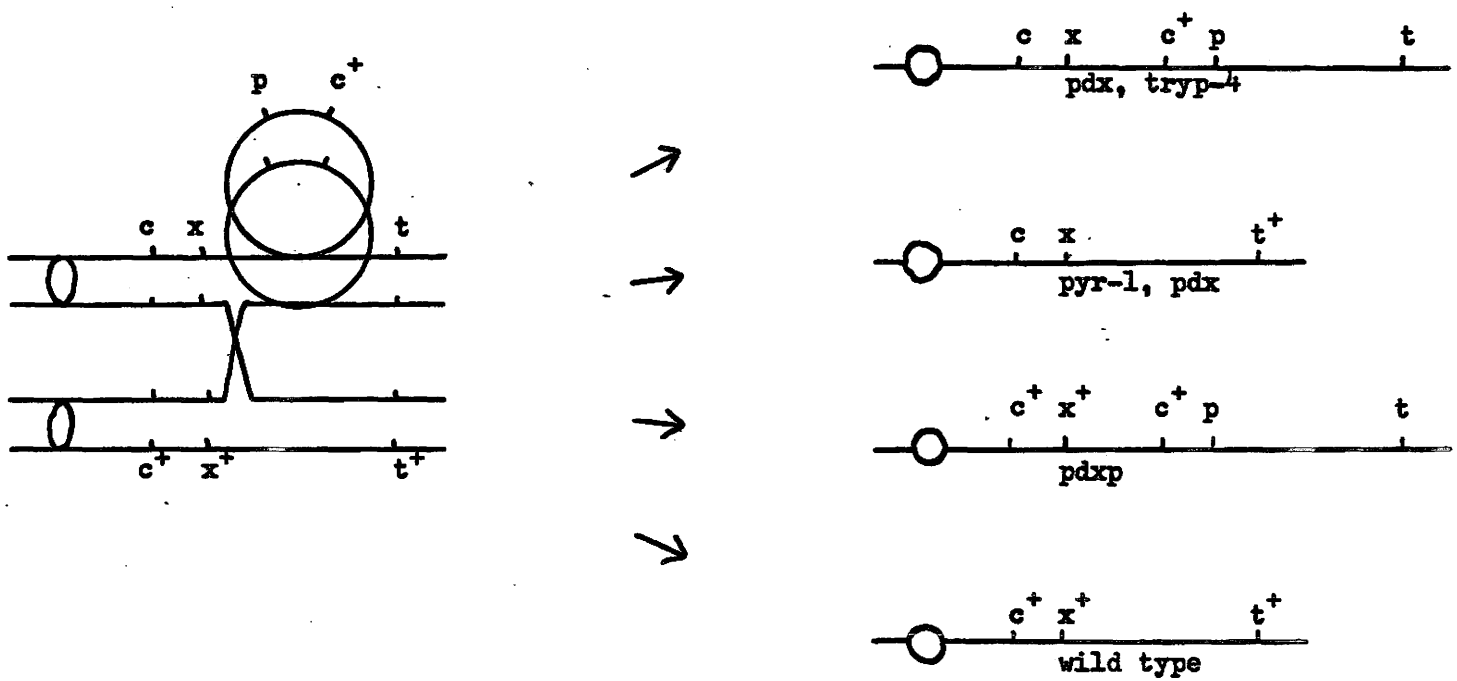


Figure 11

The origin of pyr-1 from pyr-1$^+$



Another test-cross to pdxq was carried out with isolate RH 27, which was analysed as having a pyr-1, pdx, tryp-4, leu-2 phenotype. RH 27 showed rather poor growth, a characteristic associated also with 85-5, 213-3, and 253-1, and usually indicative of the duplicated but phenotypically obscured pyr-1, pdxp segment. The growth characteristics will be considered in detail in a later chapter.

100 single spore isolates from this cross were analysed, without differentiating between pdx and pdxq. The germination was 81%, and the genotypes of the isolates are given in Table 7.

Table 7

Progeny from RH 27 x pdxq

| | | | | |
|---|---|---|---|---|
| RR 1 | pyr-1 | pdx | tryp-4 | leu-2 |
| 2 | pdx | | | |
| 3 | pdx | | | |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pyr-1 | pdx | tryp-4 | leu-2 |
| 6 | pdx | | | |
| 7 | pdx | | | |
| 8 | pyr-1 | pdx | tryp-4 | leu-2 |
| 9 | pdx | | | |
| 10 | pdx | | | |
| 11 | pdx | | | |
| 12 | pyr-1 | pdx | tryp-4 | leu-2 |
| 13 | pdx | | | |

Table 7 (cont'd)

| | | | | |
|---|---|---|---|---|
| RH 14 | pyr-1 | pdx | tryp-4 | leu-2 |
| 15 | pyr-1 | pdx | tryp-4 | |
| 16 | pdx | tryp-4 | leu-2 | |
| 17 | pdx | | | |
| 18 | pdx | tryp-4 | leu-2 | |
| 19 | pyr-1 | pdx | tryp-4 | leu-2 |
| 20 | pdx | tryp-4 | leu-2 | |
| 1 | pdx | | | |
| 2 | pdx | tryp-4 | leu-2 | |
| 3 | pyr-1 | pdx | tryp-4 | leu-2 |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pdx | | | |
| 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| 7 | pyr-1 | pdx | tryp-4 | leu-2 |
| 8 | pyr-1 | pdx | tryp-4 | leu-2 |
| 9 | pyr-1 | pdx | tryp-4 | leu-2 |
| 30 | pdx | | | |
| 1 | pdx | | | |
| 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| 3 | pyr-1 | pdx | tryp-4 | leu-2 |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pyr-1 | pdx | tryp-4 | leu-2 |
| 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| 7 | pyr-1 | pdx | | |
| 8 | pdx | | | |

Table 7 (cont'd)

| | | | | |
|---|---|---|---|---|
| RH 9 | pyr-1 | pdx | | |
| 40 | pyr-1 | pdx | | |
| 1 | pyr-1 | pdx | | |
| 2 | pdx | | | |
| 3 | pyr-1 | pdx | tryp-4 | leu-2 |
| 4 | pyr-1 | pdx | | |
| 5 | pdx | | | |
| 6 | pdx | | | |
| 7 | pyr-1 | pdx | | |
| 8 | pdx | | | |
| 9 | pdx | | | |
| 50 | pdx | | | |
| 1 | pdx | tryp-4 | leu-2 | |
| 52 | pyr-1 | pdx | tryp-4 | leu-2 |
| 3 | pdx | tryp-4 | leu-2 | |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pdx | | | |
| 6 | pdx | | | |
| 7 | pdx | | | |
| 8 | pdx | tryp-4 | leu-2 | |
| 9 | pdx | | | |
| 60 | pyr-1 | pdx | tryp-4 | leu-2 |
| 1 | pdx | | | |
| 2 | pdx | | | |
| 3 | pdx | | | |
| 4 | pdx | | | |

Table 7 (cont'd)

| | | | | |
|---|---|---|---|---|
| RH 5 | pyr-1 | pdx | tryp-4 | leu-2 |
| 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| 7 | pyr-1 | pdx | tryp-4 | leu-2 |
| 8 | pdx | tryp-4 | leu-2 | |
| 9 | pyr-1 | pdx | | |
| 70 | pyr-1 | pdx | tryp-4 | leu-2 |
| 1 | pyr-1 | pdx | tryp-4 | leu-2 |
| 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| 3 | pyr-1 | pdx | | |
| 4 | pyr-1 | pdxp | tryp-4 | leu-2 |
| 5 | pyr-1 | pdxp | tryp-4 | leu-2 |
| 6 | pdx | | | |
| 7 | pdx | tryp-4 | leu-2 | |
| 8 | pdx | | | |
| 9 | pyr-1 | pdxp | tryp-4 | leu-2 |
| 80 | pyr-1 | pdx | tryp-4 | leu-2 |
| 1 | pyr-1 | pdx | tryp-4 | leu-2 |
| 2 | pdx | | | |
| 3 | pyr-1 | pdx | tryp-4 | leu-2 |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pyr-1 | pdx | | |
| 6 | pdx | | | |
| 7 | pdx | | | |
| 8 | pyr-1 | pdx | tryp-4 | leu-2 |

Table 7 (cont'd)

| | | | | |
|---|---|---|---|---|
| RM 9 | pdx | tryp-4 | leu-2 | |
| 90 | pyr-1 | pdx | tryp-4 | leu-2 |
| 1 | pyr-1 | pdx | tryp-4 | leu-2 |
| 2 | pyr-1 | pdx | tryp-4 | leu-2 |
| 3 | pdx | | | |
| 4 | pyr-1 | pdx | tryp-4 | leu-2 |
| 5 | pyr-1 | pdx | | |
| 6 | pyr-1 | pdx | tryp-4 | leu-2 |
| 7 | pyr-1 | pdx | tryp-4 | leu-2 |
| 8 | pdx | | | |
| 9 | pdx | | | |
| 100 | pdx | tryp-4 | leu-2 | |

From the above data, it will be seen that three pdxp progeny were recovered. Therefore RM 27 contains the duplicated, parental type Linkage Group IV.

However, less pdxp progeny were recovered here than in 213-3 x 25a. Applying a contingency table:

| | Recomb. | Non-recomb. | Total |
|---|---|---|---|
| RM 27 | 3 | 97 | 100 |
| 213-3 | 13 | 99 | 112 |
| | 16 | 196 | 212 |

$$T\ (RM\ 27) = \frac{16 \times 100}{212} = 7.5$$

| Observed | Theoretical |
|---|---|
| 3 | 7.5 |
| 13 | 8.5 |
| 97 | 92.5 |
| 99 | 104.5 |

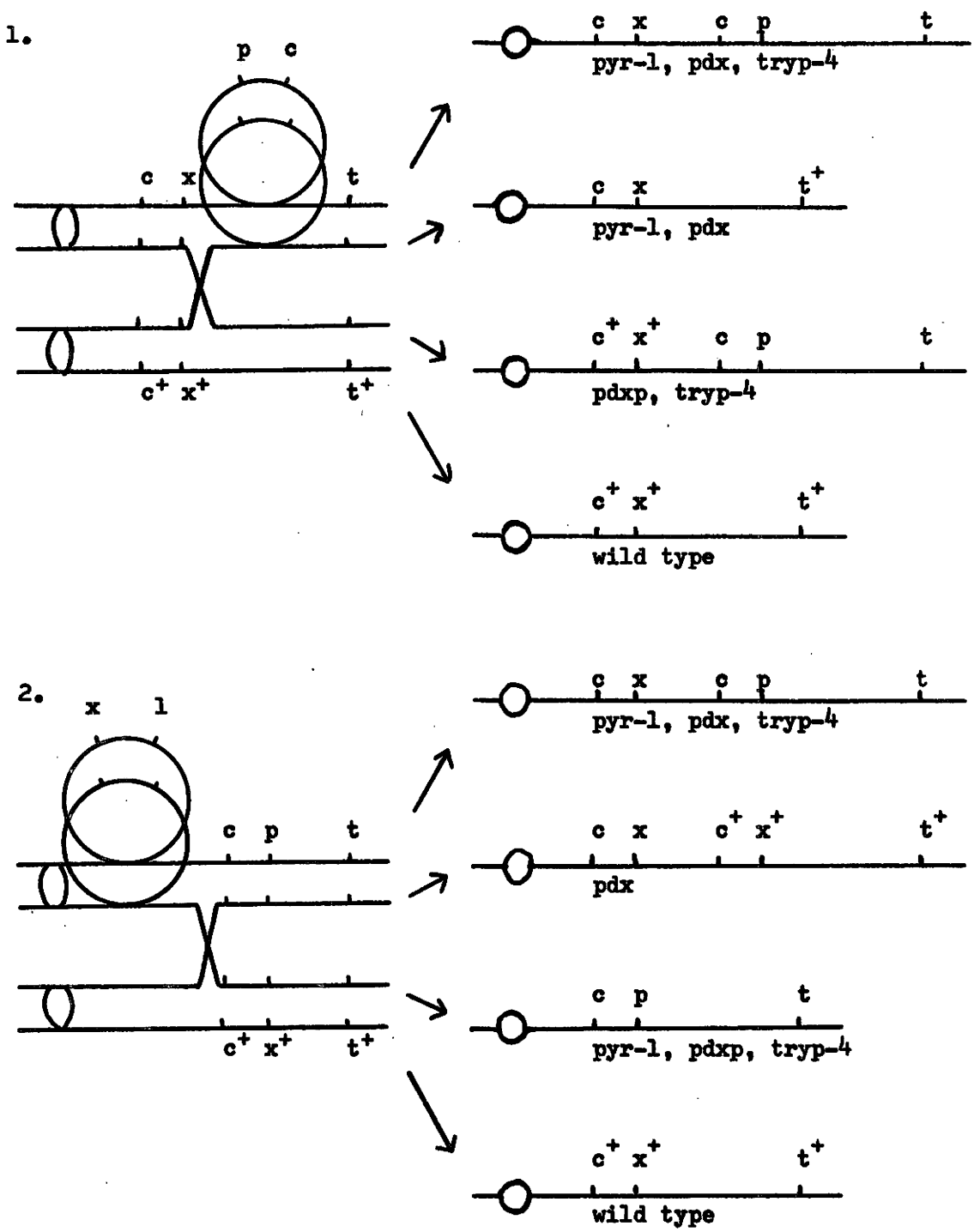
$\chi^2 = 5.6$ $\quad P < 0.02$

Therefore here we have a significant difference. The explanation is that from pdx it was possible to obtain pdxp progeny in two ways from 213-3, and these are shown in Figure 12. Of the equivalent progeny classes from the RH 27 x pdxq cross, only the seventh would express the pdxp phenotype. In the third class of progeny, (pyr-1$^+$, pdxq, pyr-1, pdxp, tryp-4) the joint effect of the pdxp and pdxq alleles in duplication would appear to be the expression of the pdx phenotype.

The data from these three back-crosses tend to corroborate those from the two original crosses, between 213-3 x 25a and 253-1 x pdxq, with regard to the postulated duplicated segment including a second centromere and the pyr-1 and pdx-1 loci.

Figure 12

The origin of pdxp progeny from pdx



## CHAPTER VI

## ASCI 42 AND 112. ANEUPLOIDY AND HETEROKARYOSIS

The remaining two aberrant tetrads were also recovered from the cross between H 823 and J 104, but they were taken from a cross grown on Sucrose medium containing a limiting amount of cytidine (40 mg/l) but supplemented with 100 mg/l of the pyrimidine base analogue 5-bromo-uracil.

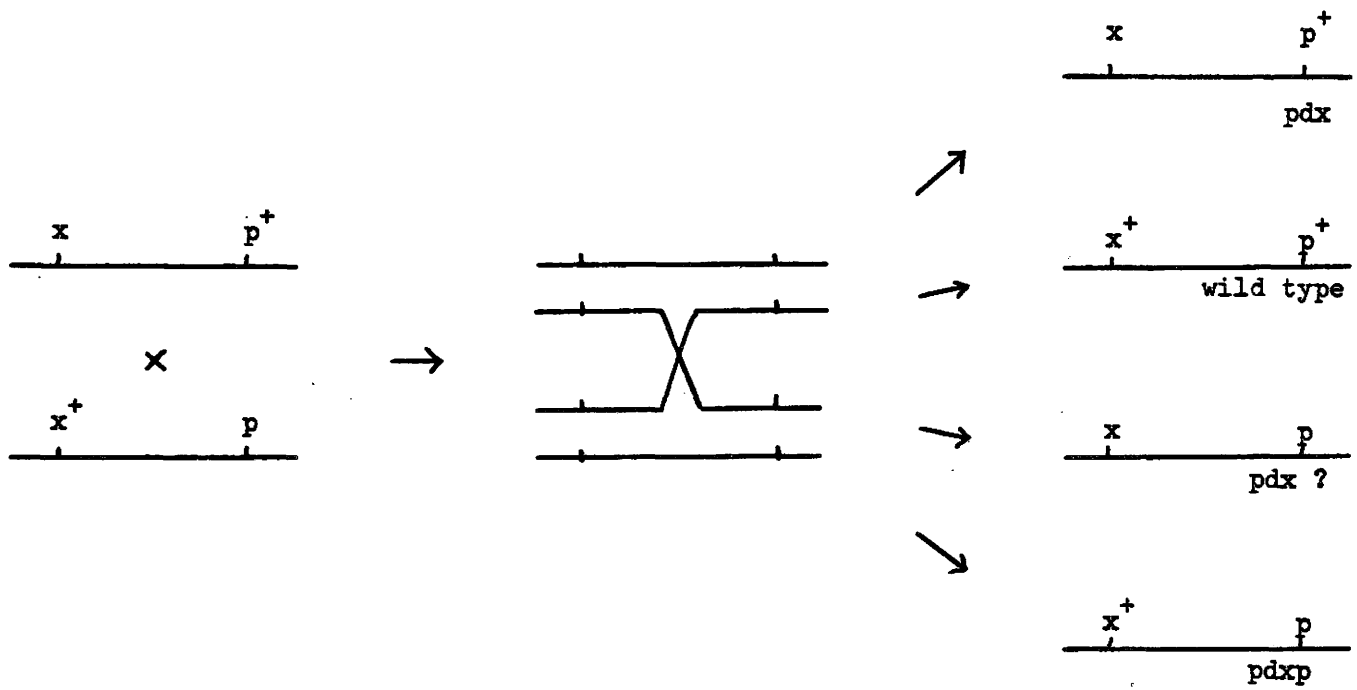
The two asci of this type were HK 42 and HX 112. Both asci produced similar tetrads:

| | | | |
|---|---|---|---|
| HK 42 | Spore | 1 | wild type |
| | " | 2,3,4 | failed to germinate |
| | " | 5,6 | pyr-1, pdx |
| | " | 7,8 | pdxp, tryp-4, leu-2 |

It will be observed that spore 1 is apparently wild type, while spores 2, 3, and 4 failed to germinate. Furthermore, all markers show second division segregation. Mitchell (Mitchell 1955, 1957), although reporting the recovery of rare wild type isolates in a cross of this type, considered the two mutants as different alleles at the same locus. A reciprocal recombination event at meiosis could, in such a cross, give rise to progeny wild type for pdx as shown in Figure 13a. It is unlikely that such is the explanation in this case however, since no example of genetic recombination between pdx and pdxp has ever

Figure 13

Intra-locus (inter-cistron) recombination giving rise to $pdx^+$ progeny.



been reported previously. The occurrence of two such asci in such a small sample arising from this mechanism is therefore highly improbable.

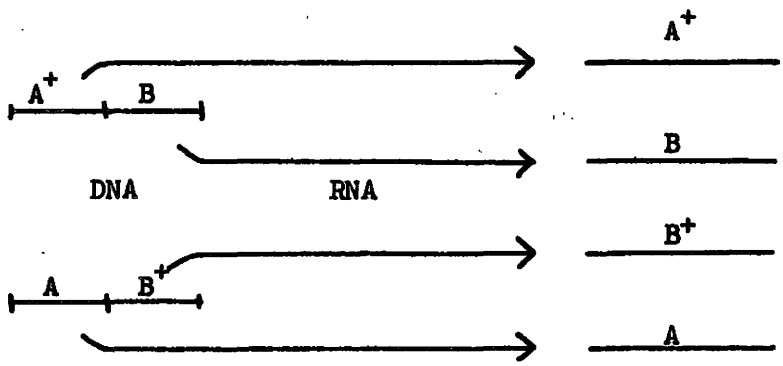
De Serres (de Serres, 1960), working on the ad-3 locus in Neurospora crassa, has found two sub-units of the locus, 3a and 3b. A mutation in either one of these sub-units results in an adenine auxotroph. However, if a heterokaryon is made between an ad-3a mutant and an ad-3b mutant, the resultant heterokaryon is "pseudo-wild", i.e. it no longer requires adenine for growth. A similar situation of two cistrons as sub-units was found in T-2 phage concerning the A and B cistrons in the R 2 locus (Benzer, 1955, 1957). The explanation suggested for the "pseudo-wild" phenotypes in ad-3 and probably in pdx is the mechanism of complementation at the polypeptide chain level. The two cistrons of ad-3 each produce a polypeptide chain via RNA. The two polypeptide chain types combine with each other in a specific spatial configuration to produce a functional enzyme, when both 3A and 3B are non-mutant. An ad-3A mutant has one damaged chain, and hence the final enzyme is non-functional. The situation is similar for ad-3B. In a heterokaryon between the two, the situation is shown in Figure 14a.

Either A chain may combine with either B chain to form an enzyme. The possibilities are, therefore, as in Figure 14b.

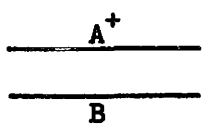
Applying this to the present situation, where the two mutant loci are present in the same mycelium they may complement at the polypeptide chain level to produce a normally functioning enzyme. Such a situation could occur in aneuploidy, where there is an extra chromosome in each nucleus, or in a heterokaryon, where two nuclear types, one pdx and the other pdxp are present in the same mycelium.

Figure 14

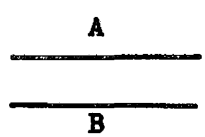
a) Heterokaryotic production of polypeptide chains



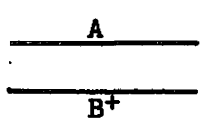

b) Possible combinations of polypeptide chains to form enzyme



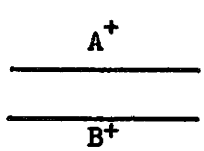

Non-functional




Non-functional




Non-functional




Functional enzyme

How can these two aberrant asci be interpreted in the light of aneuploidy or heterokaryosis? It is not likely that the original condition of our wild types could have been heterokaryotic, as ascospores of Neurospora crassa are originally uninucleate. Aneuploidy is however possible. Remembering the duplication in H 823 already inferred from the previously considered aberrant asci, the meiosis in ascus HK 42 would have occurred as shown in Figure 15.

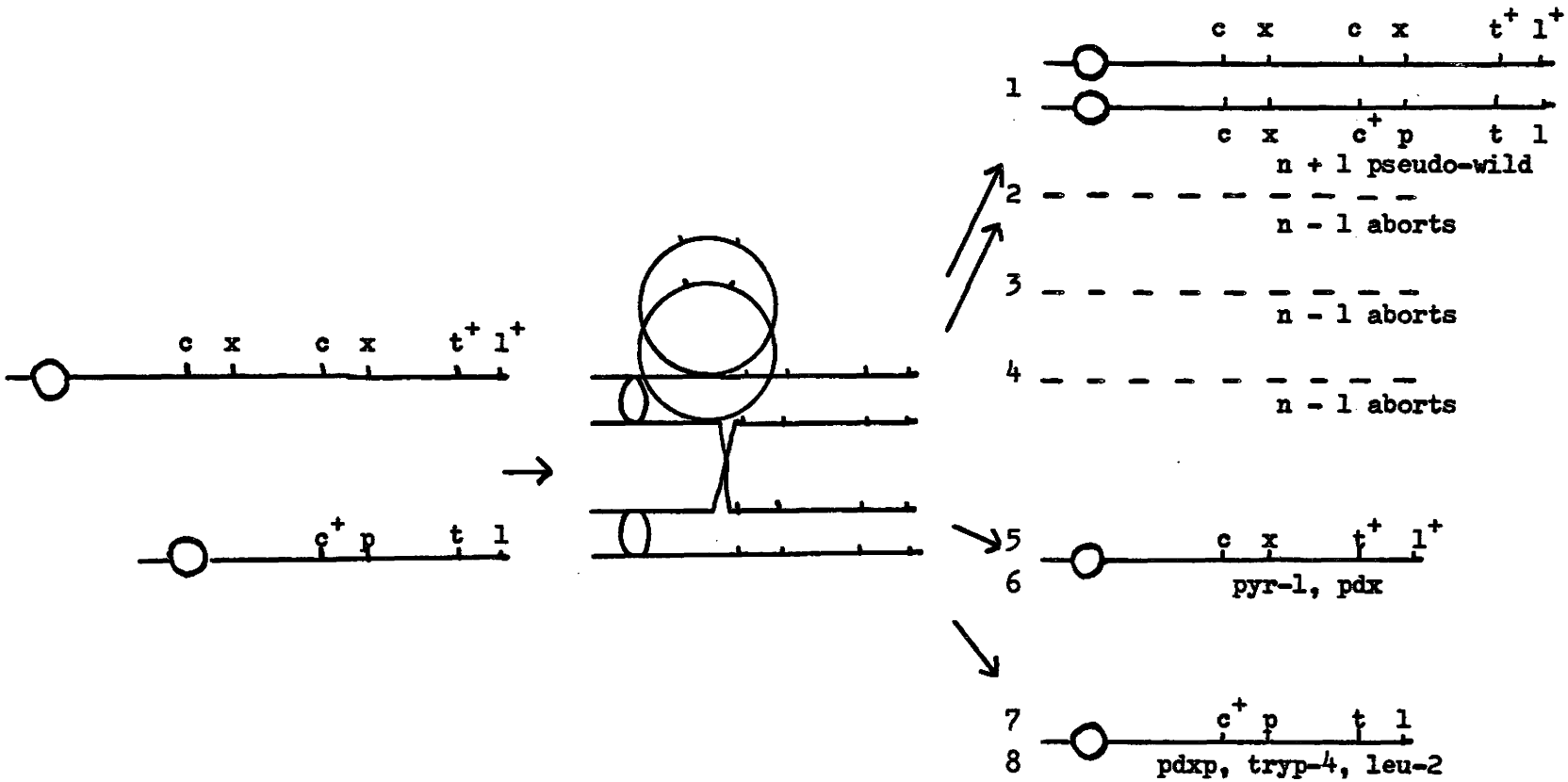
As even single pdx and pdxp mutants show leaky growth, the block to their metabolism would not be absolute. Pittenger (1954) found that the disomic (n + 1) condition in Neurospora is unstable, and that pseudo-wild aneuploids of this type become heterokaryotic in the course of mitotic divisions by the loss of the extra chromosome (exclusion in a micronucleus and eventual loss). Further work (Pittenger 1958) on this disomic instability showed that haploidization occurred with great rapidity, even occurring by the end of the second post-meiotic division in a significant number of cases.

To determine the situation in 42-1, after a period of vegetative growth, with respect to this problem of aneuploidy or heterokaryosis, further investigations were undertaken, both on conidial isolates of 42-1 and by ascospore analysis of a cross between 42-1 and the Lindegren wild type strain 25a.

In the back-cross 42-1 x 25a, analysis of the ascospores of single perithecia was undertaken. The normal situation in Neurospora is that an individual perithecium is the product of only one male and one female nucleus. Exceptions to this have been found (Weijer, 1960), but these are rare. In any case, if one parent is (+) and the other

Figure 15

Meiosis in Ascus HK 42 showing non-disjunction.



an (A,B) heterokaryon, the spores recovered from individual perithecia should be either (A and +) or (B and +). If the situation found by Weijer occurs, some perithecia may contain all three spore types, but as long as some (A and +) and (B and +) perithecia occur, this is a clear demonstration of the heterokaryotic nature of one of the parents in this cross.

Leaving this abstract situation, if our postulated aneuploid pseudo-wild has become heterokaryotic, the possible crosses in individual perithecia will be

$$\left[\text{pyr} \quad \text{pdx} \qquad\qquad \text{x} \quad +\right]$$

$$\left[\text{pdx} \ (\text{pdxp}) \ \text{tryp} \ \text{leu} \quad \text{x} \quad +\right]$$

$$\left[\begin{array}{llll} \text{pyr} & \text{pdx} & \text{x} & + \\ \text{pdx} \ (\text{pdxp}) & \text{tryp} \ \text{leu} & \text{x} & + \end{array}\right]$$

Existing evidence excludes the possibility that the nuclear types (*pyr-1 pdx*) and (*pdxq* (*pdxp*) *tryp-4 leu-2*) be co-parents, as each of these nuclear types is of *A* mating type.

Ten perithecia were analysed, and sixteen spores were isolated from each. The results are given in Table 9.

## Table 9

Perithecial analysis of 42.1

| | | | | |
|---|---|---|---|---|
| Perithecium 1. | Germination 62.5% | | | |
| Spore 1 | + | pdx | tryp | leu |
| 2 | + | + | + | + |
| 3 | + | + | + | + |
| 4 | + | pdxp | tryp | leu |
| 5 | + | pdxp | + | + |
| 6 | + | pdx | tryp | leu |
| 7 | + | + | + | + |
| 8 | + | pdxp | tryp | leu |
| 9 | + | pdxp | + | + |
| 10 | + | pdxp | tryp | leu |
| Perithecium 2. | Germination 56% | | | |
| Spore 1 | + | + | + | + |
| 2 | + | + | + | + |
| 3 | + | + | + | + |
| 4 | + | + | + | + |
| 5 | pyr | + | + | + |
| 6 | + | + | + | + |
| 7 | pyr | pdx | + | + |
| 8 | pyr | + | + | + |
| 9 | pyr | pdx | + | + |

Table 9 (cont'd)

| | | | | |
|---|---|---|---|---|
| Perithecium 3. | Germination 75% | | | |
| Spore 1 | + | + | + | + |
| 2 | + | + | + | + |
| 3 | + | + | + | + |
| 4 | + | + | + | + |
| 5 | + | + | + | + |
| 6 | + | pdxp | tryp | leu |
| 7 | + | + | + | + |
| 8 | + | + | + | + |
| 9 | + | + | + | + |
| 10 | + | pdxp | tryp | leu |
| 11 | + | + | + | + |
| 12 | + | + | tryp | leu |
| Perithecium 4. | Germination 75% | | | |
| Spore 1 | + | pdx | + | + |
| 2 | + | + | + | + |
| 3 | pyr | pdx | + | + |
| 4 | pyr | pdx | + | + |
| 5 | pyr | pdx | + | + |
| 6 | pyr | pdx | + | + |
| 7 | + | + | + | + |
| 8 | + | + | + | + |
| 9 | pyr | pdx | + | + |
| 10 | pyr | pdx | + | + |
| 11 | + | + | + | + |
| 12 | pyr | pdx | + | + |

Table 9 (cont'd)

| | | | | |
|---|---|---|---|---|
| Perithecium 5. | Germination 75% | | | |
| Spore 1 | + | pdxp | tryp | leu |
| 2 | + | pdxp | tryp | leu |
| 3 | + | pdxp | tryp | leu |
| 4 | + | + | + | + |
| 5 | + | pdxp | tryp | leu |
| 6 | + | + | + | + |
| 7 | + | + | + | + |
| 8 | + | pdxp | tryp | leu |
| 9 | + | + | tryp | leu |
| 10 | + | pdxp | tryp | leu |
| 11 | + | pdxp | tryp | leu |
| 12 | + | pdxp | + | + |
| Perithecium 6. | Germination 94% | | | |
| Spore 1 | pyr | pdx | + | + |
| 2 | pyr | pdx | + | + |
| 3 | pyr | pdx | + | + |
| 4 | pyr | pdx | tryp | leu |
| 5 | + | + | + | + |
| 6 | pyr | pdx | + | + |
| 7 | pyr | pdx | + | + |
| 8 | pyr | + | + | + |
| 9 | + | + | tryp | leu |
| 10 | pyr | pdx | + | + |
| 11 | + | + | + | + |

Table 9 (cont'd)

| | | | | |
|---|---|---|---|---|
| Perithecium 6. | Germination 94% | | | |
| Spore 12 | + | + | + | + |
| 13 | pyr | pdx | + | + |
| 14 | + | + | + | + |
| 15 | pyr | pdx | tryp | leu |
| Perithecium 7. | Germination 81% | | | |
| Spore 1 | pyr | pdx | + | + |
| 2 | + | + | + | + |
| 3 | pyr | pdx | + | + |
| 4 | pyr | pdx | + | + |
| 5 | pyr | + | + | + |
| 6 | pyr | pdx | + | + |
| 7 | pyr | pdx | + | + |
| 8 | pyr | pdx | + | + |
| 9 | + | + | + | + |
| 10 | + | pdx | + | + |
| 11 | + | + | + | + |
| 12 | + | pdx | + | + |
| 13 | + | + | + | + |
| Perithecium 8. | Germination 69% | | | |
| Spore 1 | + | + | + | + |
| 2 | + | pdx | tryp | leu |
| 3 | + | + | tryp | leu |
| 4 | + | pdx | tryp | leu |
| 5 | + | pdx | tryp | leu |

Table 9 (cont'd)

| | | | | |
|---|---|---|---|---|
| Perithecium 8 | Germination 69% | | | |
| Spore 6 | + | + | + | + |
| 7 | + | pdx | tryp | leu |
| 8 | + | pdx | tryp | leu |
| 9 | + | + | + | + |
| 10 | + | pdx | + | + |
| 11 | + | pdx | tryp | leu |
| Perithecium 9. | Germination 75% | | | |
| Spore 1 | pyr | pdx | + | + |
| 2 | pyr | pdx | + | + |
| 3 | pyr | pdx | + | + |
| 4 | pyr | pdx | + | + |
| 5 | + | + | + | + |
| 6 | ——— | | | |
| 7 | pyr | pdx | + | + |
| 8 | + | + | + | + |
| 9 | + | + | + | + |
| 10 | + | + | + | + |
| 11 | + | + | + | + |
| 12 | pyr | pdx | + | + |
| 13 | pyr | pdx | + | + |
| Perithecium 10. | Germination 56% | | | |
| Spore 1 | + | pdx | + | + |
| 2 | + | + | + | + |
| 3 | + | + | + | + |
| 4 | pyr | + | + | + |

Table 9 (cont'd)

| | | | | |
|---|---|---|---|---|
| Perithecium 10. | Germination 56% | | | |
| Spore 5 | pyr | pdx | + | + |
| 6 | pyr | pdx | + | + |
| 7 | pyr | pdx | + | + |
| 8 | + | + | + | + |
| 9 | pyr | pdx | + | + |

In perithecia P2, 4, 7, 9, and 10, we clearly have a straightforward cross between 25a and a nucleus containing only one chromosome IV of the two which must originally have been present in ascospore 42-1 to give that spore, on germination or shortly afterward, its complementation which was the cause of its pseudo-wild phenotype.

Perithecia 3 and 5 give rise to pdxp, tryp, leu, progeny, the original chromosome of J 104. However in the meiosis of ascus 42, a duplication was probably introduced into this chromosome. The pdx-pdxp duplication, as seen from the other aberrant asci, gives rise to an extreme pdx phenotype. A perithecium involving this type of chromosome from ascus 42 is P 8.

The other perithecia may involve aneuploid nuclei, more than two nuclei, or nuclei produced by somatic recombination between the disomic chromosome IV in the aneuploid nuclear type before the extra chromosome was lost to give rise to the heterokaryotic state. Somatic recombination is common in Aspergillus, another ascomycete (Pontecorvo 1953, 1956, 1958), and has been reported in Neurospora crassa (Pittenger, 1963), and Penicillium. (Pontecorvo & Sermonti, 1954).

Single conidial isolates of 42-1 were made and their phenotypes analysed. The deduction of their nuclear genotypes is not possible in many cases as each conidium contains several nuclei, and heterokaryotic complementation may occur to continue the pseudo-wild type phenotype. However, in some conidia the nuclei will not be balanced, but will all be of one type or not all loci will complement. In such cases, auxotrophic colonies may develop from the conidial isolates.

Of 126 single conidial isolates, the following types and numbers of progeny were recovered, and are shown in Table 10.

Table 10

Conidial analysis of isolate 42-1.

| | | | | |
|---|---|---|---|---|
| 65 | "wild type" | | | |
| 2 | pdx | tryp-4 | leu-2 | |
| 8 | pdxp | tryp-4 | leu-2 | |
| 31 | pyr-1 | pdx | | |
| 5 | pyr-1 | pdxp | | |
| 2 | pyr-1 | pdxp | tryp-4 | leu-2 |
| 2 | pyr-1 | | tryp-4 | leu-2 |
| 1 | pyr-1 | | | |
| 7 | | | tryp-4 | leu-2 |
| 1 | | pdx | | |
| 2 | | pdxp | | |

If somatic recombination is in fact occurring, some of the 65 wild type progeny will contain recombinant chromosomes with wild type genotypes for at least pyr-1, tryp-4, leu-2, but the majority

will be heterokaryous relying for their pseudo-wild type phenotypes on inter-nuclear complementation.

However, the complementation between the postulated pdx and pdxp cistrons of the pyridoxine locus that is necessary in a recombinant chromosome with the duplicated segment from H 823 seems to contradict the extreme pdx phenotype observed in 213-3, 253-1, and 85.5.

One of the conidial isolates, C1, which was phenotypically pseudo-wild, was itself subjected to conidial analysis. Of 24 single conidial isolates, 14 were pseudo-wild, 9 were pyr-1, pdx, and 1 was pdx. The simplest explanation for these data would be that conidium C1 contained three types of nuclei:

| | | | | |
|---|---|---|---|---|
| 1 | pyr-1 | pdx | tryp-4$^+$ | leu-2$^+$ |
| 2 | pyr-1$^+$ | pdxp | tryp-4 | leu-2 |
| 3 | pyr-1$^+$ | pdx | tryp-4$^+$ | leu-2$^+$ |

Nuclear type 1 by itself gives a pyr-1, pdx phenotype, a heterokaryon between 1 and 3 gives a pdx phenotype, and a heterokaryon of either 1 or 3 with 2 gives a pseudo-wild phenotype. As type 2 is never recovered alone, this may be only a non-viable fragment consisting of the right arm, the left arm having been detached as discussed later, and then excluded from the daughter nucleus. A nucleus including only the right arm fragment of chromosome IV would not be viable in isolation but could only exist in heterokaryotic combination with another nuclear type including a complete chromosome IV.

It seems clear therefore that isolate 42-1 arose as an aneuploid, disomic for chromosome IV. By somatic recombination events during early

mitotic divisions after germination, a range of chromosome IV types arose. Ultimately, one or other of the disomic chromosome IV was excluded from the daughter nuclei and eliminated. The resulting nuclear types, alone and in various heterokaryotic combinations then could give rise to the range of phenotypes found in the conidial isolates of 42-1. The perithecial analyses confirm the variation in the nuclear phenotypes in this obvious heterokaryon.

To investigate further the heterokaryotic nature of isolate 42-1 and its subsequent conidial isolates, three of the pseudo-wild conidial isolates, C1, C2 and C3 were subjected to the same type of analysis.

The results are given below in Tables 11, 12, 13, and 14.

Table 11

Conidial isolates from C1

| | | | |
|---|---|---|---|
| CA 1 | + | + | + (tryp) |
| 2 | + | + | + |
| 3 | pyr-1 | pdx | + |
| 4 | pyr-1 | pdx | + |
| 5 | + | pdx | + |
| 6 | pyr-1 | pdx | + |
| 7 | pyr-1 | pdx | + |
| 8 | pyr-1 | pdx | + |
| 9 | + | + | + |
| 10 | pyr-1 | pdx | + |
| 11 | + | + | + |
| 12 | pyr-1 | pdx | + |

Table 11 (cont'd)

| | | | | |
|---|---|---|---|---|
| CA 13 | pyr-1 | pdx | + | (tryp) |
| 14 | + | + | + | |
| 15 | + | + | + | |
| 16 | + | + | + | |
| 17 | + | + | + | |
| 18 | + | + | + | |
| 19 | + | + | + | |
| 20 | + | + | + | |
| 21 | + | + | + | |
| 22 | + | + | + | |
| 23 | pyr-1 | pdx | + | |
| 24 | + | + | + | |

Table 12

Conidial isolates from C1

| | | | |
|---|---|---|---|
| CB 1 | pyr-1 | pdx | + |
| 2 | + | + | + |
| 3 | + | + | + |
| 4 | pyr-1 | pdx | + |
| 5 | + | + | + |
| 6 | pyr-1 | pdx | + |
| 7 | pyr-1 | pdx | + |
| 8 | pyr-1 | pdx | + |
| 9 | + | + | + |
| 10 | + | + | + |

Table 12 (cont'd)

| | | | |
|---|---|---|---|
| CB 11 | pyr-1 | pdx | + |
| 12 | + | + | + |
| 13 | + | pdx | + |
| 14 | pyr-1 | pdx | + |
| 15 | + | + | + |
| 16 | + | + | + |
| 17 | + | pdx | + |
| 18 | + | + | + |
| 19 | + | + | + |

Table 13

Series CC from isolate C2

| | | | |
|---|---|---|---|
| CC 1 | + | + | + |
| 2 | + | pdxp | tryp-4 |
| 3 | + | + | + |
| 4 | + | pdxp | tryp-4 |
| 5 | + | + | + |
| 6 | + | pdxp | tryp-4 |
| 7 | pyr-1 | pdx | + |
| 8 | + | pdxp | + |
| 9 | + | + | + |
| 10 | + | + | + |
| 11 | ——— | ——— | ——— |
| 12 | + | + | + |

Table 13 (cont'd)

| | | | |
|---|---|---|---|
| CC 13 | pyr-1 | pdx | + |
| 14 | + | pdxp | tryp-4 |
| 15 | + | pdxp | tryp-4 |
| 16 | + | + | + |
| 17 | + | + | + |
| 18 | + | pdxp | tryp-4 |
| 19 | + | + | + |

Table 14

Series CD from isolate C3

| | | | |
|---|---|---|---|
| CD 1 | + | + | + |
| 2 | pyr-1 | pdxp | + |
| 3 | + | pdxp | + |
| 4 | + | pdxp | tryp-4 |
| 5 | + | + | + |
| 6 | pyr-1 | pdxp | + |
| 7 | + | + | + |
| 8 | + | + | + |
| 9 | + | + | tryp-4 |
| 10 | + | pdxp | tryp-4 |
| 11 | pyr-1 | pdxp | + |
| 12 | + | + | + |
| 13 | + | + | + |
| 14 | pyr-1 | pdxp | tryp-4 |

Table 14 (cont'd)

| | | | |
|---|---|---|---|
| CD 15 | + | + | + |
| 16 | + | + | + |
| 17 | + | + | + |
| 18 | + | + | + |
| 19 | + | + | + |

The meiotic process in ascus HK 42 giving rise to the originally aneuploid and non-heterokaryotic isolate 42-1 has already been considered. However, the cause of non-disjunction in this ascus has not yet been discussed.

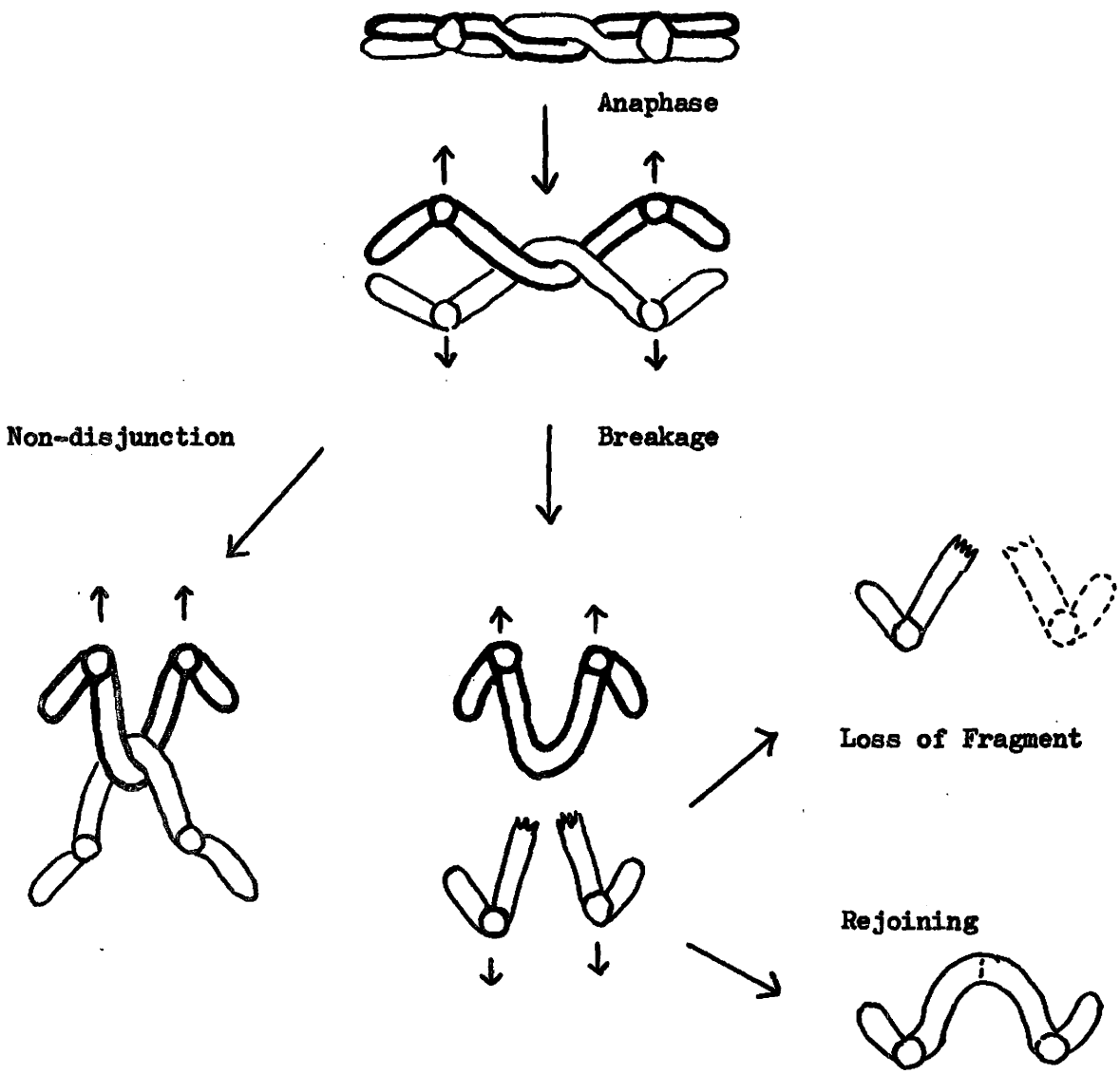
Although there is a low spontaneous rate of non-disjunction in many organisms, in the cross between H 823 and J 104 this rate is far higher and another, abnormal, cause must be found. Based on the previously proposed "duplication" model, this and certain other phenomena of the cross may be explained.

Figure 16 shows how, if both of a homologous pair of chromosomes have the suggested duplication, the duplication including a second centromere, the chromosomes may become inter-twined and both be pulled to the same pole. In ascus 42, it will be recalled, both chromosomes containing the duplication moved to the same pole in the first anaphase of meiosis. The situation depicted in Figure 16 shows the happenings at the second division of Meiosis.

Normally the chromosome pair would not be rotated, and so one of the homologous pair would be pulled unhindered to each pole of the division. In a few other cases however, the homologues would be rotated between the centromeres through an angle approximating 90° and the

Figure 16

The consequences of spindle rotation through 180°

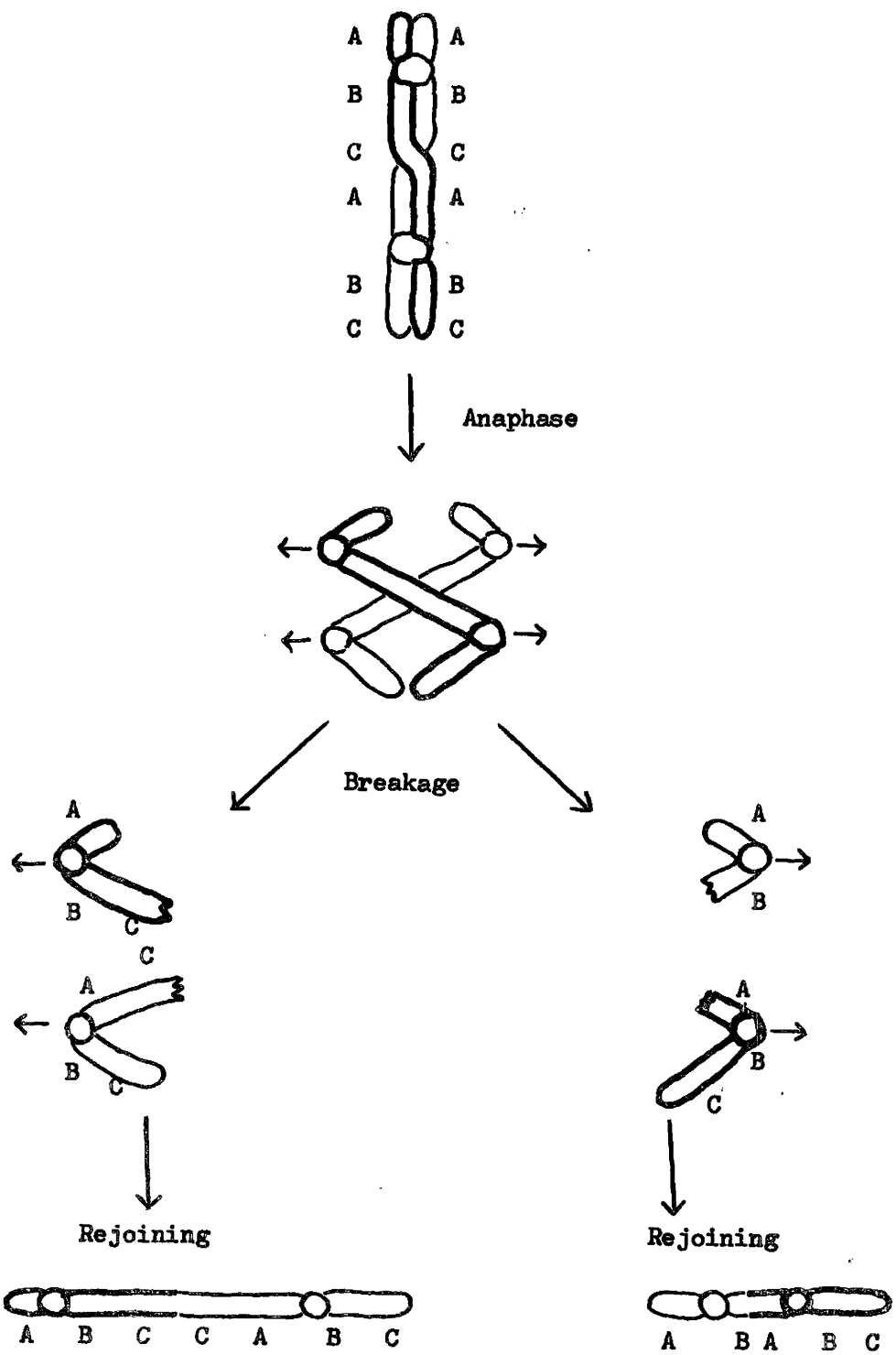


situation depicted in Figure 17 may arise. Both chromosomes will probably break, and rejoin with the complementary part of the homologue. As the breaks will almost certainly not occur at the same site in each homologue, duplications and deletions within the original duplication will occur. This did not occur in the meiotic second division of ascus 42, but this is more relevant to subsequent mitotic divisions of isolate 42-1 before haploidisation of the nuclei giving rise to the heterokaryon. One or more breakage-rejoining cycles could have taken place in mitotic divisions of the aneuploid, until in some nuclei a large part of the region between the duplicated centromeres on chromosome IV would have been deleted. This could happen to one of the two homologues, i.e. pyr-1, pdx, pyr-1, pdx, tryp-4$^+$, leu-2$^+$ without any observed effect on the phenotype. If however one deleted the inter-centromere region from the other homologue, i.e. pyr-1, pdx, pyr-1$^+$, pdxp, tryp-4, leu-2, the result would be pyr-1$^+$, pdxp, tryp-4, leu-2. This would result in a change of phenotype with respect to pyridoxine metabolism, from pdx to pdxp.

At the same time as deletion occurs in some chromosomes giving rise to a more normal chromosome type, duplications must arise in others to produce, in effect, triplications. Nuclei containing such triplicated chromosomes may well be metabolically unbalanced, and therefore subject to negative selection pressure. The "deleted" chromosomes would therefore probably become predominant in the mycelium. The data from conidial analysis of 42-1 are in agreement with this proposition, as the pdxp, tryp-4, leu-2 phenotype is clearly the most frequent non-parental type. It is more frequent in fact than its precursor, indicating the great instability of the disomic condition with dicentric

Figure 17

Spindle rotation through 90°



chromosomes.

Let us now consider the phenotypes of conidial isolates from 42-1, and their origin, in detail.

Two isolates were phenotypically pdx, tryp-4, leu-2. This is the expression of the genotype pyr-1, pdx, pyr-1$^+$, pdxp, tryp-4, leu-2, a parental type which is obviously very unstable.

Eight isolates were phenotypically pyr-1$^+$, pdxp, tryp-4, leu-2. This is derived from the parental type above by the loss of a segment between the centromeres which evidently contains the pdx gene and also pyr-1.

Thirty-one were phenotypically pyr-1, pdx. These could be either the other parental type pyr-1, pdx, pyr-1, pdx, tryp-4$^+$, leu-2$^+$, or the type derived from this by deletion of a segment between the centromeres and genotypically pyr-1, pdx, tryp-4$^+$, leu-2$^+$.

One pyr-1 was recovered. Although this could arise by somatic recombination processes, one would expect it to be the theoretically more common heterokaryon between pyr-1, pdx, pyr-1, pdx, tryp-4$^+$, leu-2$^+$ (or pyr-1, pdx, tryp-4$^+$, leu-2$^+$)and a postulated linkage group containing pyr-1 pdxp with either mutant or prototrophic alleles at the tryp-4 and leu-2 loci.

To obtain the five pyr-1, pdxp, and two pyr-1, pdxp, tryp-4, leu-2 phenotypes, one must turn from heterokaryotic complementation, or inter-centromere deletions, to the normal type of somatic recombination investigated by Pittinger (Pittinger and Coyle, 1963). This is necessary as one must delete a segment of genetic material outside the centromere. This would delete entirely the original

duplication, reconstituting a perfectly normal linkage group IV. Whether the recombination is between the two homologues, or within one linkage group it is not possible to say. However, this would produce pyr-1, pdxp, tryp-4, leu-2. Another cross-over between the pdxp and tryp-4 loci would complete the formation of the recombinant pyr-1, pdxp, tryp-4$^+$, leu-2$^+$ as in Figure 18.

The seven tryp-4, leu-2, isolates are probably heterokaryons between pyr-1+, pdx, tryp-4, leu-2, and pyr-1$^+$, pdxp, tryp-4, leu-2. The first of the pair may be replaced by pyr-1, pdx, tryp-4, leu-2.

The single pyr-1 isolate could be produced by a heterokaryon between pyr-1, pdx, tryp-4$^+$, leu-2$^+$ and pyr-1, pdxp, tryp-4$^+$, leu-2$^+$.

The two pyr-1, tryp-4, leu-2 isolates are probably produced by complementation at the pdx locus between pyr-1, pdx, tryp-4, leu-2 and pyr-1, pdxp, tryp-4, leu-2.

A pyr-1, pdx, tryp-4, leu-2 phenotype might well be the result of a recombination event between the original homologues. This is illustrated in Figure 19. Although such an isolate was not recovered, its existence is postulated from evidence presented in the previous paragraph.

One pdx isolate was recovered. This could have arisen by recombination, although it is more likely to be a heterokaron between pyr-1, pdx, tryp-4$^+$, leu-2$^+$ and pyr-1$^+$, pdx, tryp-4, leu-2.

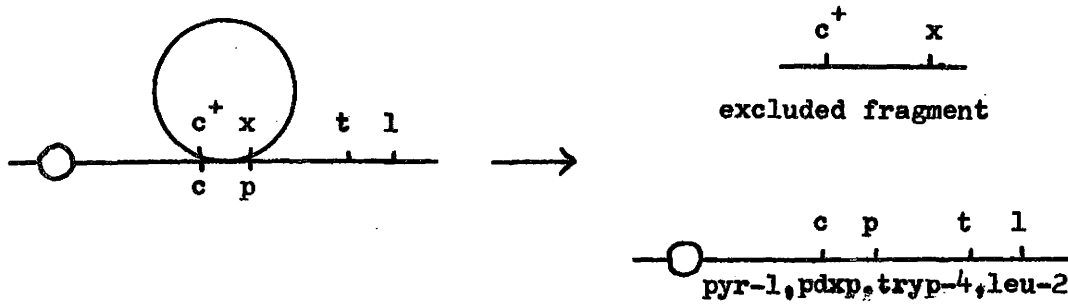
Likewise, the two pdxp isolates are probably heterokaryotic for pyr-1, pdxp, tryp-4$^+$, leu-2$^+$ and pyr-1$^+$, pdxp, tryp-4, leu-2.

Finally, sixty-five isolates were phenotypically wild type. These could arise from various complementations between two or more auxotrophic nuclear types.

Figure 18

The origin of pyr-1, pdxp, tryp-4, leu-2 in 42-1

a) Somatic recombination within a single linkage group




b) Somatic recombination between the disomic pair.

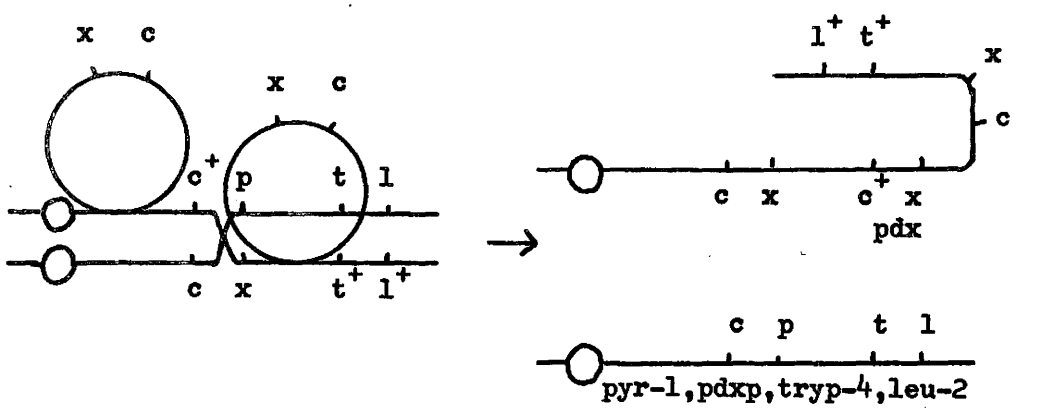
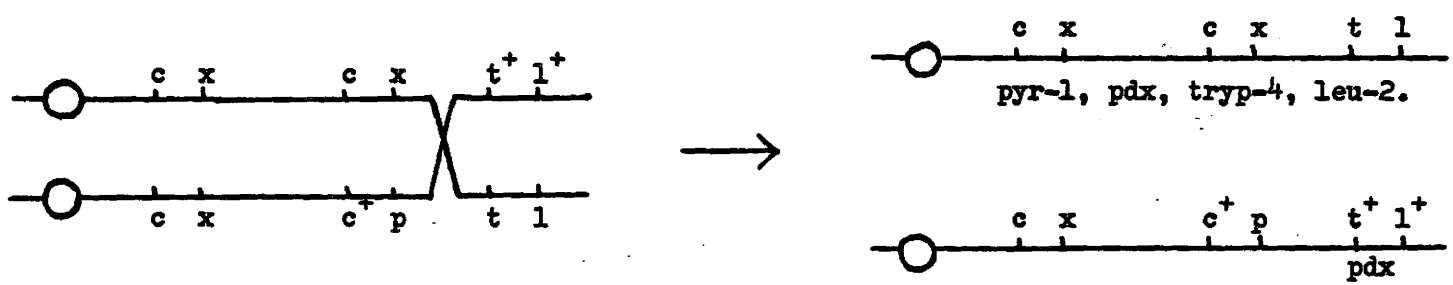


Figure 19

The origin of pyr-1, pdx, tryp-4, leu-2, by somatic recombination.



This hypothesis appears to give a reasonable explanation for the data from the conidial, and perithecial analysis of 42-1.

## CHAPTER VII

## LIQUID CULTURE GROWTH TESTS OF SINGLE AND DOUBLE MUTANTS

The purpose of these growth tests was to attempt to cast further light on the nature of the mutants, both singly and in combination.

The strains used in the growth rate tests were:

| Strain | Genotype | Phenotype |
|---|---|---|
| 25a | $pdx^+$ | $pdx^+$ |
| 85-4 | pdx | pdx |
| 213-7 | pdxp | pdxp |
| F.G.S.C.#362 | pdxq | pdxq |
| 213-3 | pdx-pdxp | pdx |
| 42-1 | pdx-pdxp | $pdx^+$ |

As stated in Chapter II, approximately 6000 conidia per flask was the normal amount of innoculum used. No correction was made for the possible differential viabilities of the conidia of the various test strains, as the calibration experiments described in Tables 15 and 16 indicated that a ten-fold difference in the amount of viable innoculum gave no significant variation in the total weight of mycelium produced.

In the first calibration experiment, shown in Table 15, a suspension of $\underline{pdx}^+$ conidia of arbitrary concentration was prepared in sterile distilled water. This suspension was innoculated, in

varying amounts, into flasks containing glucose minimal medium:

Table 15

The effect of innoculum on growth

| Replicates | 1 drop | 3 drops | 10 drops |
|---|---|---|---|
| A | 161.1 mg | 160.4 mg | 161.3 mg |
| B | 182.0 " | 159.7 " | 150.6 " |
| C | 163.0 " | 155.3 " | 140.5 " |
| D | 168.9 " | 162.3 " | 168.0 " |
| $\bar{X}$ | 168.8 mg | 159.4 mg | 155.1 mg |
| S | 9.5 " | 3.0 " | 12.0 " |

In the above table, pyridoxine was not the factor limiting the growth of the mycelium. Therefore, a further calibration experiment was carried out using the auxotrophic pdx strain 85-4 as innoculum. In this case the amount of pyridoxine available should be the limiting factor. The results of this experiment are given below in Table 16.

Table 16

The effect of innoculum on growth

| Replicates | 1 drop | 3 drops | 10 drops |
|---|---|---|---|
| A | 10.5 mg | 7.7 mg | 15.6 mg |
| B | 15.3 " | 7.1 " | 6.2 " |
| C | 9.4 " | 6.5 " | 10.1 " |
| D | 6.3 " | 11.3 " | 11.8 " |
| $\bar{X}$ | 10.4 mg | 8.2 mg | 10.9 mg |
| S | 3.7 " | 2.2 " | 3.9 " |

It will be seen that in neither of these experiments is there a significant difference between the three sets of data. In the second experiment one might have expected that the amount of growth would be directly proportional to the amount of pyridoxine in the system; as every effort was made to keep the medium and apparatus free from pyridoxine, the amount of pyridoxine in the system should be proportional to the amount of innoculum. However, the amount of growth was clearly not proportional to the amount of conidia. Either the conidia of an auxotroph contain no pyridoxine, or else some other factor is limiting to growth. As there was some growth observed in the auxotrophic mutants in minimal medium, it is reasonable to suppose that the system was not completely free from pyridoxine. If pyridoxine itself was not the absolute limiting factor, one observation which may have some bearing on the situation is that all pyridoxine auxotrophs produce discoloration in the medium in which they are grown. This is possibly indicative of breakdown of the carbohydrate source, but may also indicate the accumulation of toxic products of the abortive metabolism of the mutants in the absence of an external source of pyridoxine.

On the strength of the above observations, it was decided that further experiments could be undertaken without the necessity of estimating the conidial viability of the strains, and hence the exact numbers of viable conidia innoculated into each flask.

A series of growth tests were then carried out by standard procedures to determine the normal growth characteristics of the single mutants and the wild type strain 25a. The results of several such tests are combined in Table 17.

Table 17

Basic growth tests of single mutants

| | Glucose minimal | G.M. + 100 mg/l. pdx | G.M. + $(NH_4)^+$ at pH7 |
|---|---|---|---|
| pdx$^+$ | 161.9, 185.6 | 189.9, 192.3 | 158.9, 155.3 |
| | 170.7, 145.4 | 147.5, 160.1 | 157.2, 173.3 |
| | 225.2, 224.0 | 185.6, 173.2 | |
| | Replicates 6 | Replicates 6 | Replicates 4 |
| | $\bar{x}$ 185.5 | $\bar{x}$ 174.8 | $\bar{x}$ 161.2 |
| | s 33.0 | s 17.8 | s 8.2 |
| pdx | 5.4, 3.9 | 192.9, 183.2 | 4.3, 1.2 |
| | 10.7, 7.2 | 189.5, 194.8 | 2.3, 1.7 |
| | 7.7, 6.6 | 210.0, 191.7 | |
| | Replicates 6 | Replicates 6 | Replicates 4 |
| | $\bar{x}$ 6.9 | $\bar{x}$ 193.7 | $\bar{x}$ 2.4 |
| | s 2.6 | s 9.2 | s 1.4 |
| pdxp | 17.5, 13.4 | 180.6, 162.2 | 116.2, 110.0 |
| | 11.0, 14.6 | 191.3, 190.8 | 96.4, 113.2 |
| | 19.7, 16.8 | 218.4, 205.2 | |
| | Replicates 6 | Replicates 6 | Replicates 4 |
| | $\bar{x}$ 15.5 | $\bar{x}$ 191.4 | $\bar{x}$ 109.0 |
| | s 3.1 | s 19.5 | s 8.8 |

Table 17 (cont'd)

| | Glucose minimal | G.M.+100 mg/l. pdx | G.M.+$(NH_4)^+$ at pH7 |
|---|---|---|---|
| pdxq | 8.5, 12.7 | 181.5, 188.0 | 10.0, 12.7 |
| | 15.8, 10.6 | 197.2, 191.0 | 12.0, 20.1 |
| | 16.4, 17.6 | 185.0, 210.2 | |
| | Replicates 6 | Replicates 6 | Replicates 4 |
| | - | - | - |
| | X 13.6 | X 192.1 | X 13.7 |
| | S 5.4 | S 13.6 | S 4.3 |

(All weights above are expressed in milligrams).

From the data in the preceding table, it will be seen that there is no significant difference between the growth of pdx$^+$ on pH5 glucose minimal medium with or without pyridoxine, and pdx, pdxp, and pdxq on medium supplemented with pyridoxine. This is what one would expect when supplementing the auxotrophs with a sufficiently high concentration of the required biochemical.

The growth rates of unsupplemented pdxp and pdxq mutants do not differ significantly from each other, although both are significantly higher than the rate of growth of a pdx mutant on unsupplemented medium.

The optimum growth rate at pH7, as shown by 25a, is slightly less than it would be at pH5.5. However, this slight difference has little or no significance. The growth of pdxp at this high pH is far higher than at pH5.5, but is still significantly lower than wild type growth. The probable reason for this below optimum growth was dis-

covered when the pH of the medium was checked at the end of the test period, and was found to have dropped to approximately pH 5-5.5. Thus, for a period at the beginning of the test the mutant pdxp would have grown at a wild-type rate. However, once the increasing acidity caused the threshold concentration of ammonium ions (Strauss 1951) to fall below the effective level, only the leaky mutant rate of growth would occur. This increase in acidity of the medium during the course of a six day test was found to be a characteristic feature of all strains tested. Also at pH 7, the leaky growth of pdx is further reduced from the level achieved at pH 5.5, but pdxq attains the same rate at both 5.5 and 7.

Clearly therefore, pdx, pdxp, and pdxq have distinctly different growth characteristics and are different mutants. They may be mutants in three separate cistrons of the pdx-1 locus, they may be mutants at three different positions in the same cistron, or they may be a combination of these possibilities. As high resolution techniques of genetic analysis cannot be satisfactorily employed for these mutants, recombination studies cannot resolve this problem. However, the fact that pdx and pdxp mutants in a heterokaryon can give a pseudo-wild type of growth indicates that at least the pdx and pdxp mutants are not situated in the same cistron of the pdx-1 locus.

The growth characteristics of the combined mutants were now investigated in the same manner used for the single mutants. The strains studied were:

213-3, a pdx-pdxp duplication

42-1, a pdx-pdxp heterokaryon

The results are given in Table 18.

Table 18

Growth tests on double mutants.

| | G.M. pH5.5 | G.M. pH5.5 + pdx | G.M. pH7 + $(NH_4)^+$ |
|---|---|---|---|
| 213-3 | 7.3, 4.3 | 38.8, 30.9 | |
| | 6.5, 4.4 | 46.2, 34.8 | Insufficient |
| | Replicates 4 | Replicates 4 | to weigh |
| | $\bar{X}$ 5.6 | $\bar{X}$ 37.7 | |
| | S 1.5 | S 6.5 | |
| 42-1 | 91.4, 142.6 | 133.6, 150.3 | 112.9, 113.4 |
| | 159.1, 178.0 | 147.8, 143.6 | 113.6, 128.7 |
| | Replicates 4 | Replicates 4 | Replicates 4 |
| | $\bar{X}$ 142.8 | $\bar{X}$ 143.8 | $\bar{X}$ 117.2 |
| | S 37.0 | S 7.4 | S 7.8 |

From Tables 17 and 18 we may see that the double (duplicated) mutant grows less than either of the single mutants included within itself, and that on supplemented medium it grows much less than the supplemented single mutants. This suggests that the pdx and pdxp mutants are catabolic (i.e. these mutants are auxotrophic not because they fail to synthesise pyridoxine but because they actively break down the pyridoxine synthesised by an unidentified gene at another

locus).

If the mutants were anabolic, one would expect the less extreme of the two mutants present in 213-3 to determine the phenotype, (i.e. the pdx mutant requires pyridoxine under all known conditions, but at pH7 the pdxp mutant does not. Therefore at pH7 the strain should express a wild-type phenotype.).

However, with catabolic mutants, both break down pyridoxine, and the sum breakdown is greater than that occurring in either single mutant. Thus the growth rate on unsupplemented medium will be less than either single mutant. At pH7 the pdxp gene will no longer catabolise pyridoxine, but the pdx gene, as may be seen from the data in Table 17, becomes even more efficient at catabolism. Moreover, the amount of pyridoxine normally sufficient to compensate for catabolism in a single mutant is probably still limiting in this case due to the greater catabolic potential of the duplicated mutant loci. Whereas a higher pyridoxine supplement for a single mutant should not further increase the growth, in the case of 213-3 100 mg/l of pyridoxine may still be limiting and a higher rate of supplementation may produce more growth. In order to investigate this possibility further, the growth rates of 213-3 and a normal pdx mutant on medium supplemented with 100 mg/l. and 200 mg/l. of pyridoxine were compared. The results are given in Table 19.

Table 19

The effect of pyridoxine concentration on 213-3 and pdx.

| | 100 mg/l. | 200 mg/l. |
|---|---|---|
| 213-3 | 62.1, 25.3 | 125.0, 120.2 |
| | 45.6, 34.8 | 99.0, 125.8 |
| | Replicates 4 | Replicates 4 |
| | $\bar{X}$ 42.0 | $\bar{X}$ 117.5 |
| | S 15.8 | S 12.5 |
| pdx | 205.6, 183.5 | 185.9, 174.6 |
| | 188.1, 191.2 | 193.1, 182.2 |
| | Replicates 4 | Replicates 4 |
| | $\bar{X}$ 192.1 | $\bar{X}$ 184.0 |
| | S 9.5 | S 7.7 |

In view of the above results, one may conclude that the mutants at the pdx-1 locus are in fact catabolic.

How is it now possible to explain the phenotypic difference between 213-3 and 42-1 (See table 18) both of which contain both pdx and pdxp mutants? 213-3 has both catabolic mutants as well as, presumably, an unknown locus controlling pyridoxine biosynthesis in each nucleus. The heterokaron 42-1 has two such anabolic loci for the same catabolic potential as 213-3. The unsupplemented 42-1 must therefore, even after breakdown of some pyridoxine, still have sufficient of this co-enzyme to bring about a pseudo-wild type of growth.

Although the original linkage group IV pair in 42-1 are postulated as having been as shown in Figure 20a we know from the conidial analysis of 42-1 that the postulated dicentric nature of the chromosomes has caused considerable recombination within and between these two linkage groups. Although the first type does not show probable deletions by changes in phenotype, we know that pdxp, tryp, leu progeny are far more common than pdx, tryp, leu. The former would presumably be as in Figure 20b by exclusion of the duplicated pyr-1, pdx segment. Thus nearly 50% of nuclei will be single pdxp mutants, and probably most of those in the other 50% will be single pdx mutants.

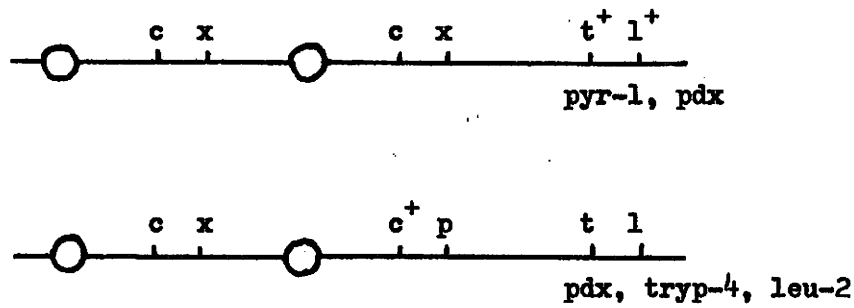
This would give us the constitution of 42-1 that we must require for a pseudo-wild type phenotype.

If recombination occurs to the same extent in a monosomic such as 213-3 as it has done in 42-1, one would expect 213-3 to lose the duplication containing pdx and revert to a pdxp phenotype.

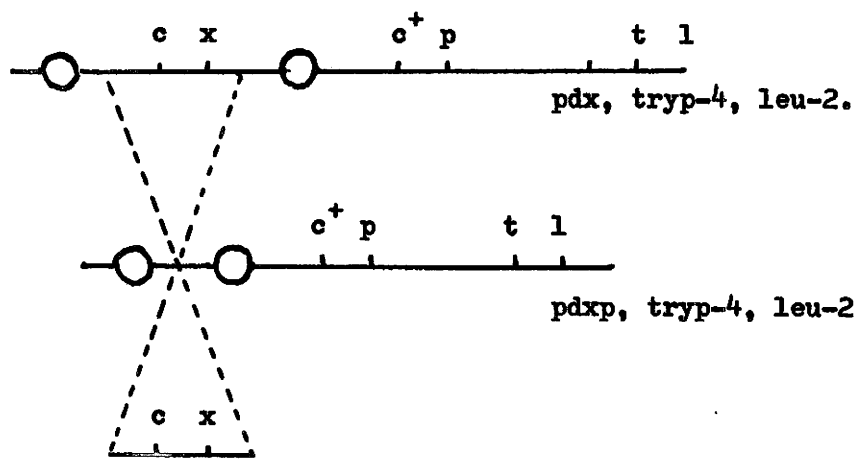
However, if a high rate of recombination only occurs when the isolate is still disomic for chromosome IV, 42-1 could become heterokaryotic and pseudo-wild, whereas 213-3 would remain basically duplicated (pdx-pdxp) and phenotypically extreme pdx.

Figure 20

a) The original disomic chromosome pair in 42-1.




b) The origin of pdxp from a pdx phenotype



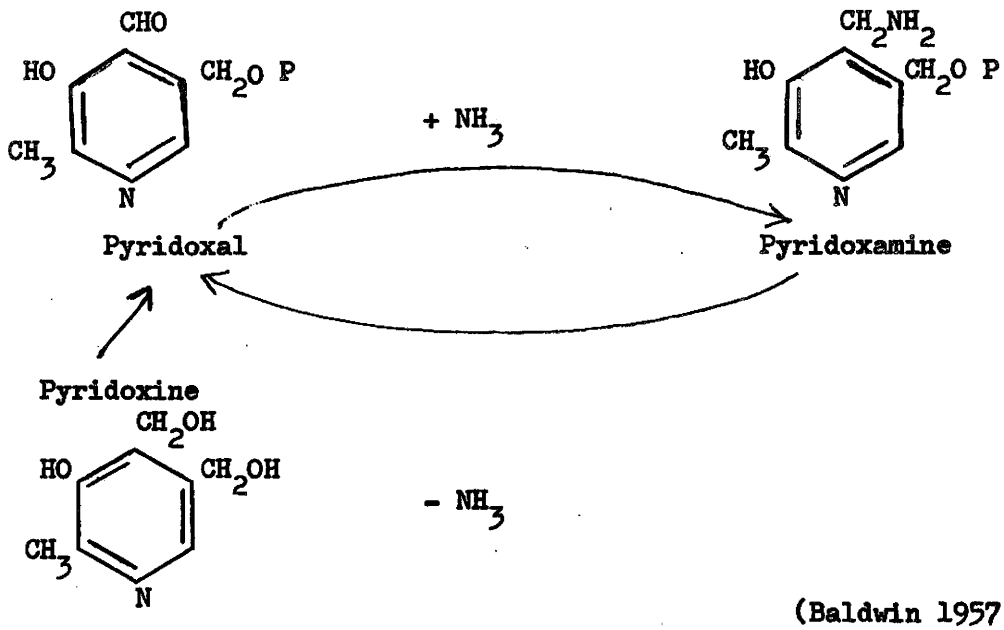
# CHAPTER VIII

## DISCUSSION

Throughout this work, it has been suggested on more than a few occasions, on the basis of evidence from several different crosses and other experiments, that the cause of the original 3:1 tetrads is the existence of a duplication in linkage group IV of strain H823. This has also been invoked as the ultimate cause for the wild-type isolates obtained from the other two asci.

First of all, it was shown that in each of the 3:1 tetrads there was an apparent pdx isolate which in a back-cross gave rise to a small number of pdxp progeny. It was invariably observed that these apparent pdx isolates were less leaky mutants than normal pdx isolates. This growth difference was investigated more fully in Chapter VII, when it was observed that not only was unsupplemented growth of pdx(pdxp) isolates less than that of normal pdx mutants, but that a higher rate of pyridoxine supplementation was required to restore the strains to a normal wild-type growth rate. This evidence led to the formulation of the hypothesis that mutants at the pdx-1 locus were catabolic in nature. The normal function of the wild-type allele at this locus, if indeed it has one, is not known. The fact that a pdx and a pdxp mutant complement in a heterokaryon may be due to polypeptide chain complementation (Pateman & Fincham 1958) may or may not be relevant in this case. Perhaps an alternative is that both

auxotrophs are mutants in the same or different cistrons, and that each catabolises pyridoxine pathway intermediates at separate steps in its metabolism. The pathway is:




(Baldwin 1957)

The enzymes for which pyridoxine is a coenzyme are A-a-decarboxylase, Threonine deaminase, and Transaminase. The coenzyme is in fact pyridoxal phosphate. Possibly the pdx and pdxp mutant loci products have affinities for different metabolites in this pathway, the metabolites having some similarities and some differences. A constant part of the catabolic product could attach to a common feature of all the metabolites, but the specificity for attack would be determined by the differences between the mutants. It is impossible on the basis of the present evidence to give a conclusive answer on this point.

The recombination data basically show a tendency to an increase in recombination between the outside markers of the region under consideration. Most important however, is the approximately ten-fold increase in recombination between pyr-1 and pdx in some

strains, and its reversion to normal in RH79. Although the overall increase may be explained by differences in genetic background of the strains involved, it is difficult to see how the pyr-1, pdx-1 data can be so explained. The simplest explanation is the postulated duplication of the pyr-1 locus as well as the pdx-1 locus, the latter of which is of course shown by the recovery of pdxp progeny from a pdx parent. The duplication of the pyr-1 locus is confirmed by the fact that pyr-1 progeny may be recovered from a cross between two prototrophs (RH45 x w.t.).

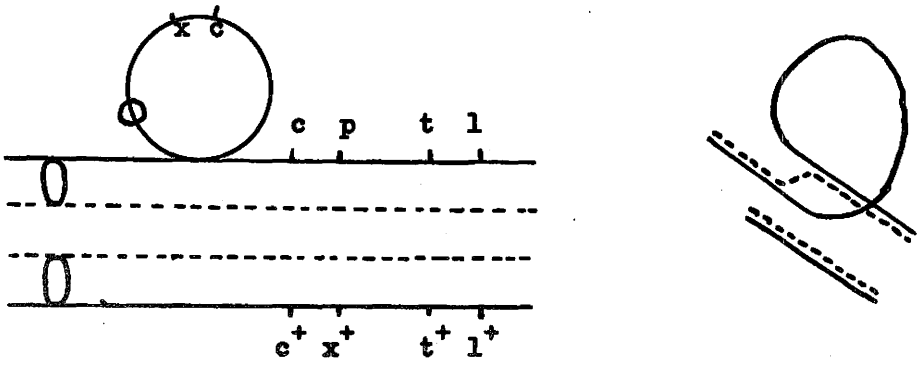
Recombination values involving duplications have been studied by a number of workers, especially in Drosophila (Bridges 1935). Duplication and other chromosomal aberrations occur spontaneously at low frequencies, and the frequency may be increased by certain physical or chemical agents such as X-rays (Muller 1927). Very small tandem duplications, such as the bar eye situation in Drosophila (Sturtevant 1928) have no effect on the recombination frequencies between outside genes. Somewhat larger duplications in which the extra genetic material cannot be so easily accommodated such as those in the X of Drossophila (Dobzhansky 1934), cause a decrease in recombination due to distortions in the synapsing chromosomes, resulting in incomplete synapsis in the region of the duplication. In trisomics, where the "duplication" is free from distortions within the chromosome, there is no decrease observed in recombination frequencies. In fact for tandem duplications involving the centromere there is a 1.4x increase. (Bridges & Anderson 1925, Redfield 1930, 1932). In the present situation the postulated duplication

is of considerable length, and may well be sufficiently long to form a comparatively strain-free loop somewhat as shown in several of the diagrams. Recombination within the loop would not be possible, but would be normal between the regions of the duplicated chromosome synapsed alongside its normal homologue. If recombination between homologous regions of the same chromosome at the origin of the loop also are possible, the recombination frequencies between outside markers may even be increased, although not to the value one would expect between synapsed chromosomes both of which contain the duplication. The possibility of recombination between homologous regions of the same chromosome, or its equivalent (see Figure 21a and b respectively) may also explain the excess of pdxp and pdx$^+$ over pdx progeny in the back-crosses of duplicated strains. In a pdx(pdxp) strain, if the region containing the pdxp was deleted in this way the phenotype would remain as pdx, but if the pdx region was deleted instead, the phenotype would change from pdx to pdxp. By reducing the number of pdx loci in this way, the numbers of possible pyr-1$^+$pdx recombinants would also be reduced (e.g. 2 pyr-1$^+$, pdx isolates and 13 reciprocals in cross 213-3 x 25a). This would not give a reduction in recombinants, as what should have been pyr-1$^+$pdx would become pyr-1$^+$ pdxp, also a recombinant type. Another possible answer to the low pdx progeny is that pdx(pdxp) phenotypic types have poorer growth, and may well have lower viability than normal pdx isolates. In this case, the recombination frequency, and hence the map distance, would be higher than estimated from the available data.

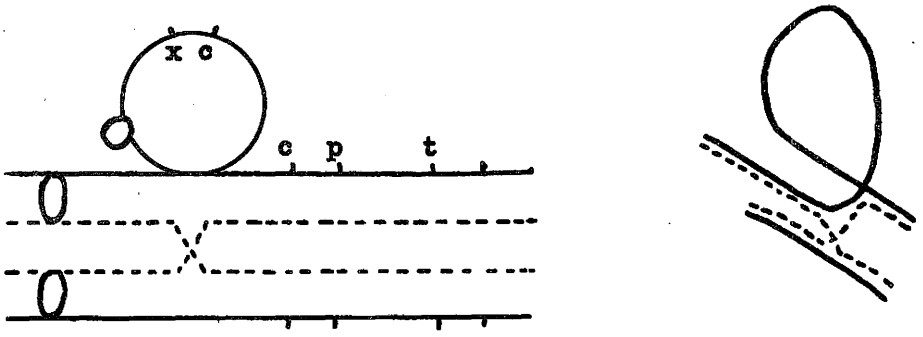
Figure 21

The possible loss of the pdx locus from the system, resulting in an excess of pdxp.

a) Single crossover between homologous regions of the same linkage group, eliminating the duplication from the new replicate




b) Double crossover eliminating the duplicated segment



From the various analyses of the pseudo-wild isolate HK 42-1, it was soon obvious that the cause of the prototrophy was heterokaryosis. A closer look at these analyses showed somatic recombination, but at a far higher rate than that known previously in aneuploid-heterokaryon studies on Neurospora crassa by Pittenger (Pittenger 1963). A probable answer to the cause of most of this somatic recombination was found in the postulated inclusion of the centromere in the duplication resulting in a dicentric chromosome, in fact a pair of dicentric homologues, in the original aneuploid. The possibility of the duplication including the centromere had already been suggested on the basis of recombination data from the pdx(pdxp) back-crosses.

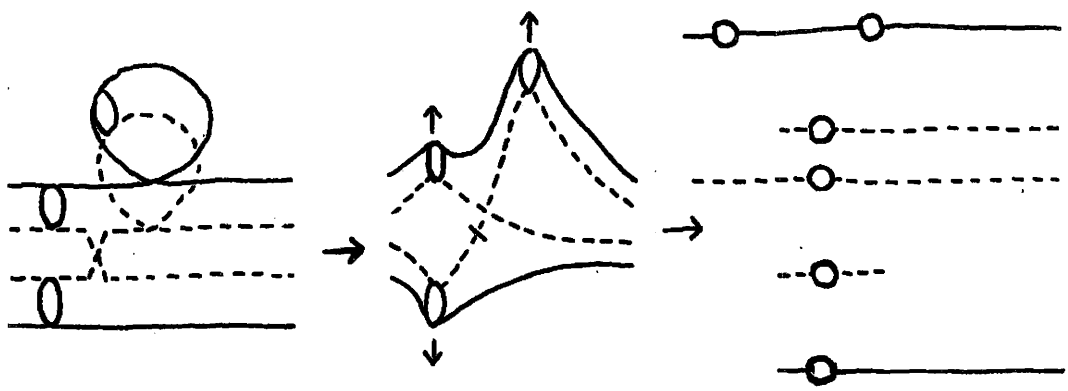
The duplication, especially of the centromere, gives a ready explanation of the low viability observed in all crosses which involved the duplication. Figure 22 shows how non-disjunction or deletions at meiosis within the developing ascus could give non-viable spores. Figure 22a involves a cross-over between the centromere and the duplication loop, and would result in two spores in the ascus aborting. 22b shows the dicentric being pulled in to both poles at the first meiotic anaphase and could result in non-disjunction or breakage and deletion. The probable result would be the abortion of four spores in the ascus.

As the whole of this work has really been founded on the postulated existence of the duplication of a region of linkage group IV in strain H 823, or at least in a significant proportion of nuclei in that strain, we must finally consider the problem of the origin of the duplication. The parental strains were pdx-1 (37801) and pyr-1

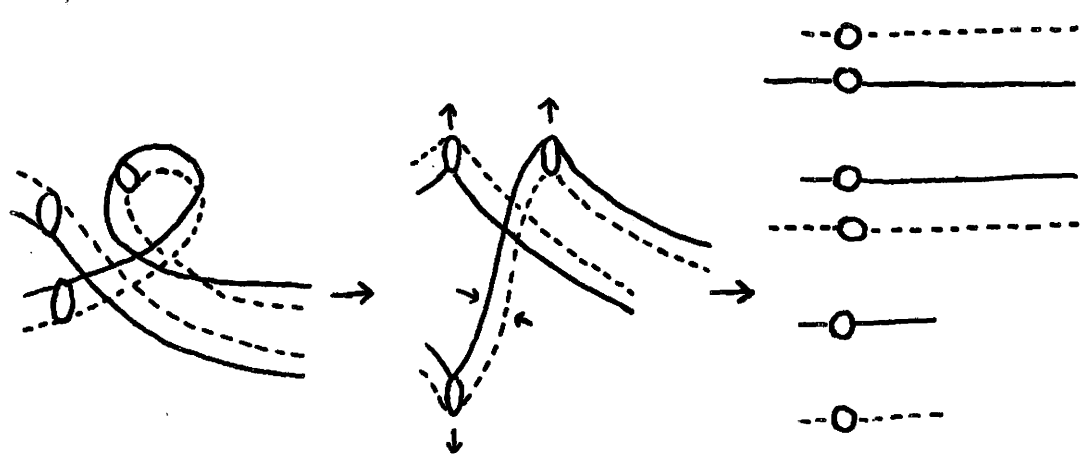
Figure 22

Two possible causes of low viability

a) Cross-over between the centromere and the duplication loop, causing breakage, or perhaps non-disjunction.



b) Twin centromeres moving to opposite poles.



(H 263). Two isolates from this cross, both with a pyr-1 pdx phenotype, have been involved in this study; these are H 815 and H 823. H 815 appears perfectly normal, producing no 3:1 tetrads when crossed to J 104 or any other unusual results; throughout the recombination studies, values from H 815 were used as a control. H 823 produced 3:1 and other aberrant tetrads and gave a significantly higher rate of recombination over some intervals, which led to the conclusion that a duplication was present. If H 823 had been isolated in ascus analysis and the reciprocal products of that meiosis were known, the origin of H 823 would be far easier to determine than in the present case of just a random spore isolate. The simplest of the possible explanations originates with an unequal somatic recombination event in an aneuploid nucleus soon after the meiosis from which the suggested aneuploid spore H 823 was produced. Haploidisation of nuclei of this clone would give rise to two nuclear types, one with the duplication and one with the corresponding deletion. The type containing the deletion would be non-viable in isolation, but may have survived for a short time in the heterokaron. However, the absence of a centromere in linkage group IV in these nuclei would soon lead to these becoming nullisomic for the linkage group, and hence becoming even more extremely non-viable. Some nuclei of the original aneuploid type would lose the disomic without the unequal somatic recombination which gave rise to the duplication. Thus H 823 is probably a heterokaron of nuclei with and without the duplication of the centromere-pyr-1 - pdx-1 region.

## BIBLIOGRAPHY.


Baldwin, E., 1957. Dynamic Aspects of Biochemistry, 3rd Edition, Cambridge University Press.

Belling, J., 1933. Crossing over and gene rearrangement in flowering plants. Genetics 18:388-413.

Benzer, S., 1955. Fine structure of a genetic region in Bacteriophage. Proc. Nath. Acad. Sci. U.S. 41:344-354.

Benzer, S., 1957. The elementary units of heredity. In:- The Chemical Basis of Heredity (ed. W.D. McElroy and B. Glass). Baltimore, Johns Hopkins Press.

Bridges, C.B., 1916. Non-disjunction as a proof of the chromosome theory of heredity. Genetics 1:107-163.

Bridges, C.B., 1935. Salivary chromosome maps. Jour. Heredity 26:60-64.

Bridges, C.B., and Anderson, E.G., 1925. Crossing over in the X-chromosome of triploid females of Drosophila melanogaster. Genetics 10:418-441.

Case, M.E., and Giles, N.H., 1958. Recombination mechanisms at the pan-2 locus in Neurospora crassa. Cold Spring Harbor Symposia Quant. Biol., 23:119-135.

de Serres, F.J., 1956. Studies with purple adenine mutants in Neurospora crassa. I. Structural and functional complexity in the ad-3 region. Genetics 41:668.

de Serres, F.J., 1960. IV. Lack of complementation between different ad-3A mutants in heterokaryons and pseudo-wild types. Genetics 45:555.

Dobzhansky, T.H., 1934. Studies on chromosome conjugation - II. The relation between crossing over and disjunction of chromosomes. Zeitschr. ind. Abstam. Vererbungsl. 68:134-162.

Kitani, Y., Olive, L.S., and El-Ani, A.S., 1961. Transreplication and crossing over in Sordaria fimicola. Science, 134:668

Kitani, Y., Olive, L.S., and El-Ani, A.S., 1962. Genetics of Sordaria fimicola V. Aberrant segregation at the "g" locus. Amer. Jour. Bot. 49:697-706.

Levine, R.P., and Ebersold, W.T., 1958. Gene recombination in Chlamydomonas reinhardi. Cold Spring Harbor Symposia Quant. Biol. 23:101-109.

Lindegren, C.C., 1953. Gene conversion in Saccharomyces. J. Genetics 51:625-637.

Lissouba, P., 1960. Mis en evidence d'une unité génetique polarisée et essai d'analyse d'un cas d'interférence négative. Ann. des Sc. Nat. Bot. 12:644.

Lissouba, P., Mousseau, J., Rizet, G., and Rossignot, J.L., 1962. Fine structure of genes in the ascomycete Ascobolus immersus. Advanc. Genet. 11:343-380.

Maling, B., 1959. Linkage data for group IV markers in Neurospora, Genetics 44:1215-1220.

Meselson, M., and Stahl, F.W., 1958. The replication of DNA in Escherichia coli. Proc. Natl. Acad. Sci. U.S. 44:671-682.

Meselson, M., and Weigle, J., 1961. Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Natl. Acad. Sci. U.S. 47:857.

Mitchell, H.K., 1957. Crossing over and gene conversion in Neurospora, in:- The Chemical Basis of Heredity (ed. W.D. McElroy and B. Glass). pp. 94-113. Baltimore, Johns Hopkins Press.

Mitchell, M.B., 1955. Aberrant recombination of pyridoxine mutants of Neurospora. Proc. Natl. Acad. Sci. U.S. 41:215-220.

Mitchell, M.B., 1955. Further evidence of aberrant recombination in Neurospora. Proc. Natl. Acad. Sci. U.S., 41:935-937.

Muller, H.J., 1927. Artificial transmutation of genes. Science 66:84-87.

Pateman, J.A., and Fincham, J.R.S., 1958. Gene-enzyme relationships at the "am" locus in Neurospora crassa. Heredity, 12:317

Pittenger, T.H., 1954. The general incidence of pseudo-wild types in Neurospora. Genetics 39:326.

Pittenger, T.H., 1958. Mitotic instability of pseudo-wild types in Neurospora. Proc. 10th Int. Cong. Genet. 2:218-219.

Pittenger, T.H., and Coyle, M.B., 1963. Somatic recombination in pseudo-wild type aultures of Neurospora crassa. Proc. Natl. Acad. Sci. U.S. 49:445-451.

Pontecorvo, G., 1956. The parasexual cycle in fungi. Annu. Rev. Microbiol. 10:393.

Pontecorvo, G. and Kafer, E., 1958. Genetic analysis by means of mitotic recombination. Advanc. Genet. 9:71.

Pontecorvo, G., Roper, J.A., and Forbes, E., 1953. Genetic recombination without sexual reproduction in Aspergillus niger. J. Gen. Microbiol. 8:198.

Pontecorvo, G., and Sermonti, G., 1954. Parasexual recombination in Penicillium chrysogenum. J. Gen. Microbiol. 11:94.

Redfield, H., 1930. Crossing over in the third chromosomes of triploids of Drosophila melanogaster. Genetics 15:205-252.

Redfield, H., 1932. A comparison of triploid and diploid crossing over in chromosome II of Drosophila melanogaster. Genetics 17:137-152.

Rizet, G., Lissouba, P., et Mousseau, J., 1960. Les mutations d'ascospore chez l'ascomycete Ascobolus immersus et l'analyse de la structure fine des genes. Bull. Soc. Franc. Physiol. Veg. 6:175.

Strauss, B.S., 1951. Studies on the $B_6$ requiring, pH-sensitive mutants of Neurospora crassa. Archiv. Biochem. 30:292-305.

Strickland, W.N., 1958. Abnormal tetrads in Aspergillus nidulans. Proc. Roy. Soc. B 148:533-542.

Sturtevant, A.H., 1928. A further study of the s-called mutation at the Bar locus of Drosophila. Genetics 13:401-409.

Threlkeld, S.F.H., 1961. Some studies of genetic recombination in Neurospora. Ph.D. dissertation, Cambridge University.

Vogel, H.J., 1956. A convenient growth medium for Neurospora. (Medium N). In Microbial Genetics Bulletin No. 13 (ed. E.M. Witkin). Carnegie Institute of Washington.

Weijer, J. & Dowding, E.C., 1960. Nuclear exchange in a heterokaryon of Neurospora crassa. Can. Jour. Genet. Cytol. 2:336-343.

Westergaard, M. and Mitchell, H.K., 1947. A synthetic medium favouring sexual reproduction. Amer. J. Bot. 34:573-577.