# THE PYRIDOXINE AUXOTROPHS

# OF NEUROSPORA CRASSA

# A GENETIC ANALYSIS OF THE PYRIDOXINE AUXOTROPHS OF NEUROSPORA CRASSA

by

ALAN RADFORD, M.Sc.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

August 1966.

DOCTOR OF PHILOSOPHY (1966)

McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: A Genetic Analysis of the Pyridoxine Auxotrophs of *Neurospora crassa*.

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

SUPERVISOR: Professor S. F. H. Threlkeld

NUMBER OF PAGES: IX, 159

SCOPE AND CONTENTS:

Evidence of gene conversion and inter-allelic complementation from earlier work on the *pdx-1* locus of *Neurospora crassa* suggested that a more detailed analysis of this locus should lead to a better understanding of these genetic phenomena. The work described in this thesis is concerned with the derivation of complementation maps for seven alleles of the *pdx-1* locus, and with a study of recombination between these alleles through the isolation of prototrophs from suitably marked inter-allelic crosses together with the isolation of asci including those exhibiting the phenomenon of gene conversion. In addition, the relationships between the *pdx-1* alleles and a gene, *en-pdx-1*, affecting the excretion of a pigment into the medium on which the *pdx-1* strains were grown, were studied. The effect of desoxypyridoxine hydrochloride, and the extent to which the *pdx-1* mutants respond to various normal forms of the vitamin, were also studied.

## PREFACE

This thesis describes studies undertaken in the Department of Biology, McMaster University, from October 1963 to July 1966. Except where others are specifically mentioned, it consists entirely of my own work. No similar thesis has been submitted at any other university.

My grateful thanks are due to my supervisor Dr. S. F. H. Threlkeld, and to Drs. D. R. McCalla and J. A. D. Zeevaart, for their advice and encouragement throughout the work. Thanks are also due to the Fungal Genetics Stock Center for the donation of stocks, and to the Ontario Department of Education and the National Research Council of Canada for the award of scholarships covering the period of the research reported herein. Finally I wish to thank my typist, Mrs. Dorothy Brown for her patience and understanding.

TABLE OF CONTENTS

FIGURES AND TABLES

FIGURES AND TABLES (cont'd)

# CHAPTER I

# INTRODUCTION

## Part 1. The pdx locus

Biochemical genetics can be said to have started with the revolutionary work of Beadle and Tatum about a quarter of a century ago. The seven pyridoxine mutants of Neurospora crassa, and one of Neurospora sitophila studied and discussed herein were all produced, isolated, and characterised in that pioneer work. These pyridoxine mutants are of interest to both biochemists and geneticists, but during the past twenty-five years have proved to be not the most tractable of Neurospora mutant strains. The extreme leakiness of these strains without pyridoxine supplementation has discouraged researchers, and work on them has been sporadic.

The pyridoxine mutants were first reported in 1941 (Beadle & Tatum 1941), in the first published work on the biochemical genetics of Neurospora when the Neurospora sitophila pdx mutant #299 was described. An attempt was made to utilise this pyridoxine mutant of Neurospora sitophila in a bio-assay technique for pyridoxine in tomato plants (Bonner & Dorland 1943). In the same year, it was reported that the same mutant was able to grow without pyridoxine under certain conditions of pH and nitrogen nutrition (Stokes et al. 1943). In medium buffered with sodium acetate at pH 5.8 or higher, appreciable growth of the mutant occurred. Growth was dependent on an ammonium source being

available, other sources of nitrogen being unsuitable. The pyridoxine concentration in mutant mycelium grown above pH 5.8 with an ammonium ion supply but without pyridoxine was higher than the level found in the same mutant at lower pH with pyridoxine itself in the medium.

Houlahan, Beadle, and Calhoun (Houlahan et al, 1949) published tetrad analysis data of crosses of Neurospora crassa isolate #35405 against the other six Neurospora crassa mutants of the locus. In the cross between the two pdx alleles #35405 and #44204, one ascus from a total of 20 analysed showed that a cross-over event had taken place between the two alleles. In the other five crosses, no inter-allelic recombinations were observed in the small samples of asci analysed. On the basis of this work, allele #44204 was designated the pdx-2 gene, and all six others were grouped together as pdx-1.

Two years later, results of a further investigation of the pH-sensitivity of some of the pdx mutants were published (Strauss 1951). Strauss examined pdx-2 (#44204), and two alleles of pdx-1, #37803 and #44602. The first of these two pdx-1 alleles has an absolute requirement, but the second is pH-sensitive. He confirmed that #44602, like #299 (Stokes et al. 1943) contained pyridoxine when grown at high pH with $(NH_4)^+$ but without pyridoxine. The level of pyridoxine in the mutant grown at high pH with ammonium ions was found to be lower than that in a wild-type strain, and its transaminase activity was consequently also lower. Strauss suggested that the lower pyridoxine concentration in the mutant mycelium might be the result not of a lower synthetic rate but a higher breakdown rate. He also found that the requirement for ammonium ions was absolute and could not be fulfilled by amino nitrogen in the form of amino acids such as methionine.

Work by Mitchell (Mitchell, M.B. 1955a, b, Mitchell, H.K. 1957) on a cross between two pdx-1 alleles, #37803 and #39106 gave the first generally accepted evidence for non-reciprocal recombination. In this cross, pyr-1 was used as the proximal marker, and col-4 as the distal outside marker. Four aberrant asci were recovered from a total number of 988 asci analysed. They were of the following types:

Ascus 1/ + pdxp col A, + + col A, pyr pdxp + a, pyr pdx + a

2/ pyr pdx + a, pyr pdx + a, + + col A, + pdxp col A

3/ + + + a, + pdxp col A, pyr pdx + A, pyr + col A

4/ + pdxp col, + + col, pyr pdx + , pyr pdx +

All aberrant progeny were back-crossed, and the data from these crosses confirmed that the phenotypes were in fact the genotypes in all cases.

Other non-reciprocal meiotic products at this locus were recovered by Threlkeld (Threlkeld 1961), but these aberrant asci occurred at a much higher frequency than those found by Mitchell (Mitchell, M.B. 1955a). Threlkeld's cross concerned alleles #37803 and #44602. Out of 153 asci analysed, 7 gave a segregation of 3 pdx:1 pdxp and two gave 1 pdx: 1 pdxp: 1 $pdx^+$ spore pairs. Although some of these asci were recovered from a cross supplemented with 5 bromo-uracil, later work suggests that 5 BU had no effect on the segregation within these asci. Not only is the frequency of aberrant asci in this system far higher than the frequency recovered by Mitchell for the same locus, but it is also far higher than that observed for other loci in the same and other fungal species, (except possibly in Yeast) - e.g. Aspergillus nidulans (Strickland 1958) - 6 aberrant asci in 1,642;

*Neurospora crassa* *pan-2* (Case & Giles 1958) - 11 in 856; *Sordaria fimicola* (Kitani *et al* 1961, 1962) - 0.12% at the *g* locus; *Ascobolus immersus* (Lissouba *et al* 1962) - approximately 1%.

Radford (Radford 1963) investigated the aberrant asci found by Threlkeld (Threlkeld 1961), and attributed the 3:1 asci to a duplication involving the *pdx-1* locus in the parental strain containing the mutant allele #37803. Recombination in the region of the duplication gave rise to a progeny class which was genetically *pdx* (#37803) - *pdxp* (#44602) and phenotypically *pdx*. This progeny type, on back-crossing to wild-type, gave rise to *pdxp* at a fairly low frequency. The 2 asci below, containing *pdx*$^{+}$ spores, arose from meiotic events in which non-disjunction had occurred, and were pseudo-wild type. One unresolved problem was that the complementation behaviour differed between the strains involving the duplication and those which were pseudo-wild. The aberrant asci were:

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 | pdxp | tryp-4 | leu-2 | |
| " 7 & 8 | pdx | | | |

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 | |

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 & 7 | Failed to germinate | | |
| " | 8 | Wild type | | |

Part 2. The Mechanism of Recombination

Many data on different recombination systems have been collected since the pioneer work of Bridges on Drosophila melanogaster (Bridges 1916), and the field has been generously endowed with hypotheses to explain the phenomenon of recombination in terms of an underlying mechanism.

Since the 1930's, several main hypotheses have been in favour at different times, and for quite a long period of time the two mutually exclusive hypotheses of Darlington and Belling were in vogue. Both hypotheses were based on both genetic and cytological observations.

Belling's hypothesis of "copy-choice" recombination (Belling 1933) postulated that at meiotic prophase, when homologous chromosomes had synapsed, replication of the daughter chromatids proceeded in a linear manner along the parental chromosome. If the two replicating chromatids switched templates at a point during their linear replication, the

cytological result was a chiasma and the genetic result was a crossover. The correlation between genetic crossovers and chromosomal exchange had been established two years before, (Creighton & McClintock 1931; Stern 1931).

Darlington's hypothesis (Darlington 1936) differs from Belling's in that replication proceeds along the length of the parental template chromosomes without switching. Subsequent to replication, breakage occurs at homologous points of homologous chromatids, and cross-rejoining of the broken ends is the mechanism which produces recombination and the cytologically visible chiasmata.

Subsequent work in fungi, such as aberrant ascus segregations (Lindegren 1953; Mitchell 1955; Kitani et al 1962; Lissouba et al 1962) and an apparent excess of 2-strand double crossovers (negative chromatid interference) (Perkins 1956) could all be interpreted on the basis of a copy-choice mechanism of recombination. Ambiguities did occur however, as the aberrant 5:3 ascus segregations in Sordaria (Kitani, Olive, et al 1962) were explained by mis-copying on a semi-conservative replication model, but the 6:2 ascus segregations which predominated in Neurospora and other fungi (Mitchell, M.B. 1955, Lissouba et al 1962) could only really be explained on a conservative DNA replication system.

Supporters of the breakage and rejoining model for recombination based their arguments on autoradiographic cytological studies (Taylor 1958) which showed exchanges of parts of sister chromatids, the fact that a small amount of DNA synthesis occurs at pachytene and may be responsible for rejoining the broken chromatids (Wimber & Prensky 1963), and mainly on the evidence that, in bacteriophage at least, recombination can occur without the simultaneous occurrence of DNA replication (Meselson & Weigle 1961).

Recently attempts have been made to synthesise the apparently conflicting data on recombination into a comprehensive, unified, hypothesis covering both inter- and intra-gene recombination (Whitehouse 1963, Holliday 1964, Whitehouse & Hastings 1965). Although the original "Whitehouse hypothesis" assumed the existence of a DNA continuum (Whitehouse 1963), both the Holliday model (Holliday 1964) and Whitehouse's revised model (Whitehouse & Hastings 1965) do require regions lacking genetic information, i.e. linkers between DNA replication units.

Before discussing the most recent and most satisfactory model for the mechanism of recombination, work on aberrant asci and high resolution analysis of intra-genic crosses will be considered.

Following the pioneer work of Lindegren on "gene conversion", the phenomenon associated with the meiotic process by which one allele at a locus is converted to the other allele at that locus (Lindegren 1953), and the previously discussed work on the pdx-1 locus of Neurospora (Mitchell, M.B. 1955), important work was published by Olive and his co-workers on the related Ascomycete Sordaria fimicola (Kitani et al, 1961, 1962). Their work on the spore color locus "g" showed that aberrant segregation of this gene occurs with a frequency of about 0.12%. Outside morphological markers were used in this work, and it was found that a gene conversion event at the "g" locus was, in the vast majority of cases, associated with a crossover event between these outside markers. 37 Aberrant asci were examined in this work, 23 being of the 5:3 type (20, $5g^+:3g$, 3 $5g:3g^+$), fourteen with 6:2 segregations (all $6g^+:2g$). In addition, one $7g^+:1g$ ascus, and 9 aberrant 4:4 asci, were obtained. (An aberrant 4:4 ascus has an abnormal spore order, not 4:4, 2:2:2:2, or 2:4:2.)

The pan-2 locus of Neurospora crassa has also been shown to be subject to the process of "gene conversion". Early work (Case & Giles 1958) on inter-allele crosses at the locus revealed 11 asci out of a total of 856 examined which showed a wild-type spore pair. Most of these had no corresponding doubly mutant spore pair, and must have been the result of the conversion of one mutant allele to wild-type in one of the products of meiosis. In more recent work on this locus, (Case & Giles 1964), the authors had developed a technique of classifying double mutants at the locus by complementation tests. Thus they were able to carry out three-point crosses within the gene. Among 1457 asci analysed, 11 showed 3:1 ratios with respect to one or more of the pan-2 sites. 6 were aberrant at only one site, one was hybrid at 2 sites, two were aberrant at all three sites within the locus, and two asci had 5:3 segregations. As in the Sordaria aberrants, outside markers showed the usual 2:2 segregations. The aberrant asci are shown below:

3:1 segregations for one allele:-

Ascus 153

ylo + + 72 + tryp A
\+ + + 72 + tryp A
ylo ad 23 + 36 + a
\+ ad + + 36 + a
aberrant 3:1 for 23

Ascus 497

\+ + + 72 + tryp a
\+ + + 72 + tryp a
ylo ad + + 36 + A
ylo ad 23 + 36 + A
aberrant 3:1 for 23

Ascus 614

| | | | | | | |
|---|---|---|---|---|---|---|
| + | + | + | 72 | + | tryp | a |
| + | + | + | 72 | 36 | + | a |
| ylo | ad | 23 | + | 36 | + | A |
| ylo | ad | 23 | + | 36 | tryp | A |

aberrant 1:3 for 36

Ascus 1021

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | 23 | + | 36 | + | a |
| ylo | ad | + | + | 36 | + | a |
| + | + | + | 72 | + | tryp | A |
| + | + | + | 72 | + | tryp | A |

aberrant 3:1 for 23

Ascus 583

| | | | | | | |
|---|---|---|---|---|---|---|
| + | + | + | 72 | + | tryp | a |
| + | + | 23 | + | 36 | tryp | a |
| ylo | ad | 23 | + | + | + | A |
| ylo | ad | 23 | 72 | + | + | A |

aberrant 1:3 for 23

Ascus 545

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | + | 72 | + | tryp | a |
| ylo | ad | 23 | + | 36 | + | A |
| + | + | + | 72 | + | tryp | a |
| + | + | + | + | 36 | + | A |

aberrant 3:1 for 23

3:1 segregation for two alleles:-

Ascus 78

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | + | 23 | + | + | + | A |
| + | + | + | 72 | + | tryp | A |
| ylo | ad | 23 | + | 36 | + | a |
| + | ad | 23 | + | 36 | tryp | a |

aberrant 3:1 for 72

" 1:3 for 23

3:1 segregation for three alleles:-

Ascus 581

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | + | 72 | + | + | a |
| ylo | ad | 23 | + | 36 | + | a |
| + | + | + | 72 | + | tryp | A |
| + | + | + | 72 | + | tryp | A |

aberrant 1:3 for 23

" 3:1 " 72

" 1:3 " 36

Ascus 565

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | 23 | + | 36 | + | A |
| ylo | ad | + | 72 | + | tryp | A |
| + | + | + | 72 | + | tryp | a |
| + | + | + | 72 | + | + | a |

aberrant 1:3 for 23

" 3:1 " 72

" 1:3 " 36

Mixed aberrant segregation

Ascus 43

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | 23 | + 72 + | 36 | + | a |
| ylo | ad | + | + | 36 + | + | a |
| + | + | + | 72 | 36 | tryp | A |
| + | + | 23 | 72 | + | tryp | A |

aberrant 5:3 for 72

" 4:4 " 36

Ascus 529

| | | | | | | |
|---|---|---|---|---|---|---|
| ylo | ad | 23 | + | 36 | tryp | a |
| ylo | ad | + | + + | 36 36 | + | a |
| + | + | + | 72 | + | tryp | A |
| + | + | + | 72 | + | + | A |

aberrant 1:3 for 23

" 3:5 " 72

" 5:3 " 36

This system differs from the "g" locus of Sordaria in that there is no correlation of non-reciprocal recombination with reciprocal recombination between outside markers. 4 of the 6:2 asci show associated reciprocal events, and 5 do not. In the nomenclature of Kapuler (1963), uni-transitional (crossover) events do not predominate over bitransitional ones at this locus. Two other asci with recombination events within

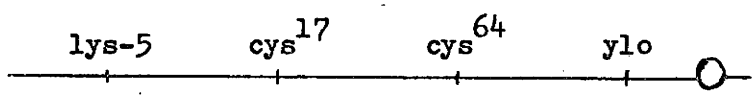
the pan-2 locus were recovered, and each has an apparent reciprocal recombination event between alleles #23 and #72.

Inter-allelic crosses at the cys gene in Neurospora crassa (Stadler & Towe 1963) have produced essentially similar results to those at pan-2 (Case & Giles 1964). 14 asci out of 1651 were aberrant with respect to recombination at the cys locus. Of these 14, 6 were bitransitional, 7 were unitransitional, and 1 had a 5:3 type of segregation.

The cross studied was:-

$$\text{lys-5, cys}^{17} \quad \text{x} \quad \text{cys}^{64}\text{, ylo}$$

where the order of markers, all on the left arm of linkage group VI is:-




The order of the two cys alleles with respect to the outside markers is deduced from the prototroph analysis of the same locus by the same workers (Stadler & Towe 1963).

Ten asci exhibited conversion at cys$^{17}$, and were of the following types:-

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| lys | 17 | + | + | lys | 17 | + | + | lys | 17 | + | + |
| lys | + | + | + | + | + | + | + | lys | + | 64 | + |
| + | + | 64 | ylo | lys | + | 64 | ylo | + | + | + | ylo |
| + | + | 64 | ylo | + | + | 64 | ylo | + | + | 64 | ylo |
| 5 asci | | | | 4 asci | | | | 1 ascus | | | |

Four asci had conversion at cys$^{64}$:-

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| lys | 17 | + | + | lys | 17 | + | + |
| lys | 17 | + | + | + | + | + | + |
| + | + | + | ylo | lys | 17 | + | ylo |
| + | + | 64 | ylo | + | + | 64 | ylo |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| lys | 17 | + | + | lys | 17 | + | + |
| + | 17 | + | + | lys | 17 | + | + |
| + | + | + | ylo | + | + | + 64 | ylo |
| lys | + | 64 | ylo | + | + | 64 | ylo |

The most extensive analysis of aberrant asci has been carried out not on Neurospora, but on the Discomycete Ascomycete, Ascobolus immersus (Lissouba et al 1962). This work was carried out on spore color mutants, and as the contents of individual asci are released together, sets of spores from individual asci were easily examined in large numbers. Despite the fact that these workers used no outside markers for their inter-allelic crosses, the information they obtained was most important to the theory of recombination. Although in many of the mutant gene series studied, reciprocal recombination was found, Lissouba & Rizet's most important data come from series 46. A linear map of the mutants in this series was constructed on the basis of the frequency of occurrence of 6:2 asci in crosses between pairs of alleles. These 6 mutant :2+ ascus types in crosses between two mutants are the sum of both reciprocal and non-reciprocal recombination events. In the conversion 6:2 asci, (those without a pair of doubly mutant spores), the parental allele which

occurred twice in the tetrad was termed the "majority" parent, and that which occurred in only one product of meiosis, the "minority" parent. Nineteen of the 21 possible pairwise crosses among the seven mutants in series 46 show the majority parent to be consistently toward the same end of the linear map. The two exceptions both involve the distal locus to the majority parent end of the series map, and this locus is thought to be in the adjacent cistron. The six remaining crosses in series 46 were interpreted by Rizet on the copy-choice model, still in favour at that time, by postulating that copy-choice errors occurred at random but could only be corrected at the end of the replication unit. This unit of replication was termed the "polaron" by virtue of the polarity of gene conversion within it, and its polarity was thought to be the result of the uni-directional replication of the DNA of which the genetic unit was composed. For data see Figure 1-1.

Rizet's hypothesis explained quite well the unipolar data from series 46, and also the data from a study of the me-2 gene of Neurospora crassa (Murray 1963). Murray's system was based on the classification of majority and minority relationships of prototrophs by outside marker genes, a treatment apparently analogous to Rizet's. To summarise Murray's technique of analysis in a hypothetical cross where the outside markers of the parents are $AB^+$ and $A^+B$, prototrophs from a cross with such outside markers will be $A^+B$, $AB^+$, $A^+B^+$, and AB. The first two classes are parental with respect to outside markers, and the larger of these two classes is the majority parental class. Within the two recombinant classes also, one will predominate over the other and hence be the majority recombinant class.

Figure 1-1

A summary of the data of Lissouba et al (1962) on Ascobolus immersus series 46 (Reproduced from "Fungal Genetics" by Fincham & Day, Blackwell, 1963).

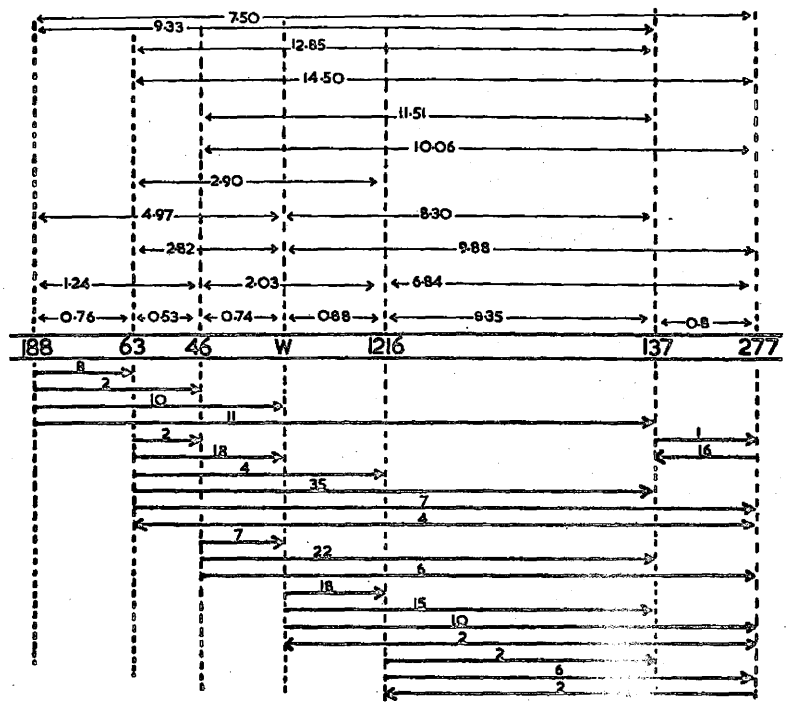



FIG. 28. Two ways of ordering the pale-ascospore *Ascobolus immersus* mutants of series 46. Figures in the map, represented as a double line, refer to mutant isolation number. Distances between sites, shown *above* the map, are given in terms of frequencies per 1,000 of 6 : 2 asci (6 mutant spores : two wild type). The arrows *below* the map indicate relationships of mutants within 6 : 2 asci. Single-ended arrows indicate conversion-type asci and point from the majority to the minority parent. Double-ended arrows indicate reciprocal cross-over asci. The numbers on the arrows are the numbers of asci analysed giving the indicated result. For further discussion, see text. From data of Lissouba *et al.*[255].

Other loci at which the production of prototrophs has been found and studied are hist-1 (Jessop & Catcheside 1965) and hist-5 (Smith 1965). Hist-5 is another example of a unipolar polaron, as determined by analysis of the outside markers of prototrophs. With the exception of one out of fourteen crosses between alleles within the hist-1 locus, this also appears to be unipolar.

The copy-choice hypothesis of Belling, as modified by Lederberg (Lederberg 1955), at least up to and including the work of Meselson and Weigle (Meselson & Weigle 1961), was the most popular hypothesis of recombination among microbial geneticists. However, evidence from other sources has recently lessened its support. Some of the contradictory evidence is valid, but at least some of the commonly quoted evidence against copy-choice is far from unequivocal.

1. Firstly, evidence generally accepted as proving that the DNA double helix replicates semi-conservatively (Meselson & Stahl 1958), is used to exclude the possibility of conservative replication which is an integral part of the copy-choice hypothesis. There is, however, evidence that DNA replicates conservatively (Cavalieri & Rosenberg 1961). Until the technical problem of determining accurately the molecular weight of macromolecules is solved, this dispute cannot be resolved.

2. Work on chromosome replication as studied by autoradiographic techniques gives evidence relevant to the problem. Taylor's work, the generally accepted work on the subject, indicates that chromosome replication, at least at mitosis, is semi-conservative. However, other workers (Plaut & Mazia 1956, LaCoeur & Pelc 1958) found evidence of conservative replication of the chromosomes. The most recent work in this

field, by Marimuthu (Marimuthu & Threlkeld, 1964, 1966; Marimuthu 1966), on the angiosperm Haplopappus gracilis suggests conservative replication at both mitosis and meiosis.

3. Involvement of all four chromatids in crossing over, when only the newly synthesised chromatids would be expected to participate according to the copy-choice hypothesis, is evidence against copy-choice. However, the parental strands may also become involved through subsequent sister-strand exchanges by breakage and rejoining. In support of this is the slightly negative chromatid interference found in some systems. (Perkins 1956, Lindegren & Lindegren 1942, Day & Swizynski.)

4. Copy-choice fails to explain the occurrence of post-meiotic segregation, the occurrence of 5:3 and aberrant 4:4 segregations observed in both Sordaria and Neurospora and also the 5:3, and 7:1 ratios of Ascobolus.

5. Copy choice is not possible as nearly all replication of DNA occurs before the synapsis of homologous chromosomes at Zygotene (Swift 1950). However, the small amount of DNA synthesis at pachytene (Wimber & Prensky 1963) is sufficient to repair lesions caused by a breakage mechanism of recombination.

The above lines of evidence, especially points 4 and 5, and to a lesser extent point 3, lead to the rejection of copy-choice as a likely mechanism of recombination. Whitehouse therefore attempted to formulate a unified hypothesis of recombination based on a system of breakage and rejoining of DNA double helices, based mainly on ascus data.

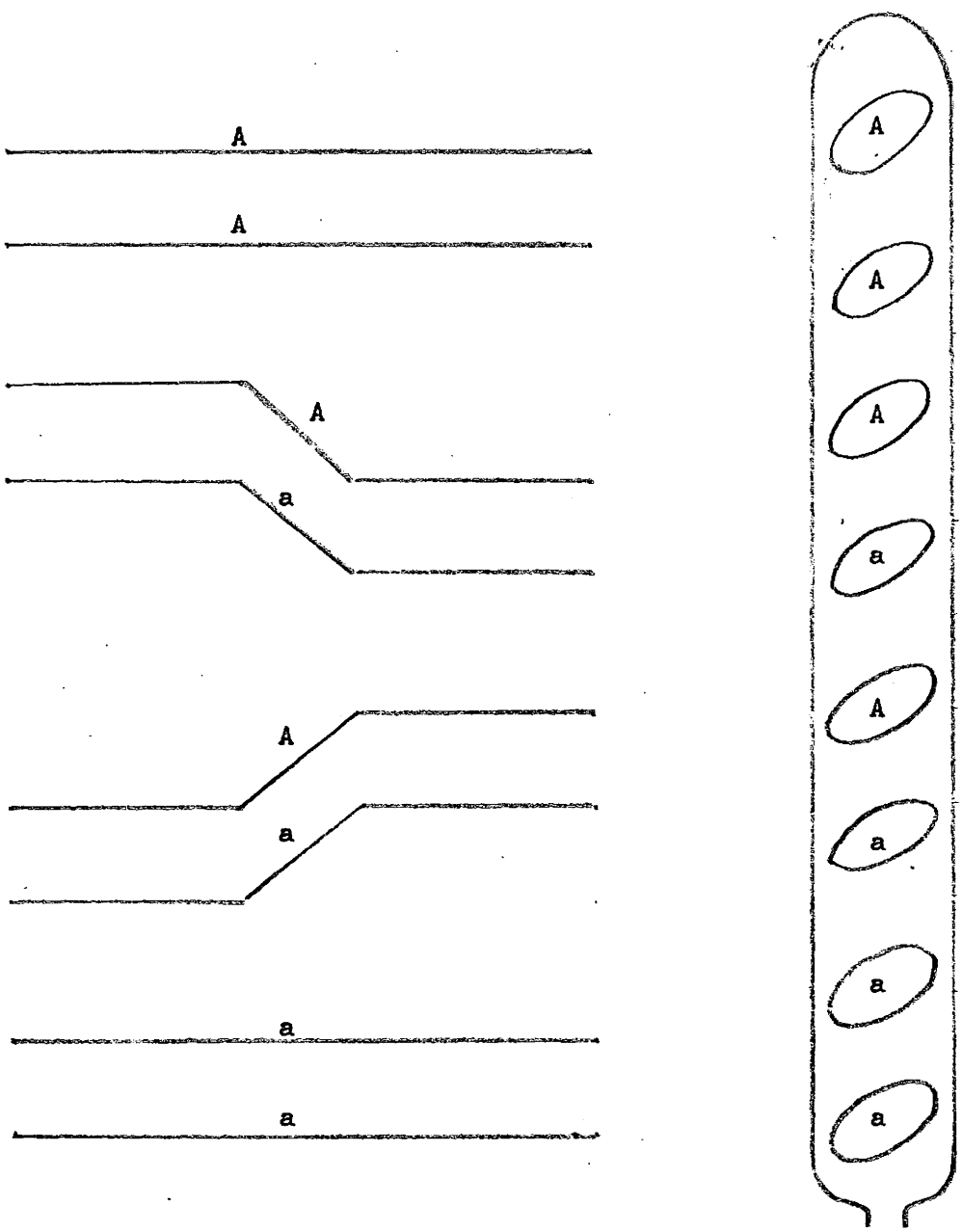
The first model proposed by Whitehouse was based on random breakage events along a continuous DNA double helix (Whitehouse 1963).

If breaks occurred in the honologous double helices, near, but not exactly opposite, each other, and unwinding of the double helices by breakage of the hydrogen bonds occurred for a short distance back from each break, single strand free ends of DNA would be produced. The + and - strands from the side of the break overlapping the break in the other double helix would have complementary base sequence for the region of overlap at the ends of the - and + single strands on the other side of the breakage point on the other double helix. Pairing of these complementary sequences, followed by incorporation of free nucleotides into the single strand parts of the interchange would restore the double helix. Meanwhile the shorter broken ends would be synthesised on the template of the original unbroken strands, and in due course these would unwind and then cross-link. Further synthesis to complete this duplex, and enzymatic dissolution of the remaining parental single strands would complete the recombination. If a heterozygous mutant site were included in the region of interchange, the four chromatids at this site (two of which had taken no part in the recombination, and two of which had been involved) would be as in Figure 1-2.

If this zyogote then completed meiosis, the ordered ascus produced would show an aberrant 4:4 segregation for the hypothetical A locus. This type of segregation occurs in Sordaria, but can be mistaken for spindle overlap at the post-meiotic mitotic nuclear division unless suitable outside markers are included in the cross. In order to account for 5:3 and 6:2 segregation of alleles in Ascomycetes, Whitehouse postulated a repair enzyme which would correct the heterozygosity in the two recombinant DNA double helices. Detection of this heterozygous DNA by an enzyme would be comparatively simple as the non-complementary

Figure 1-2

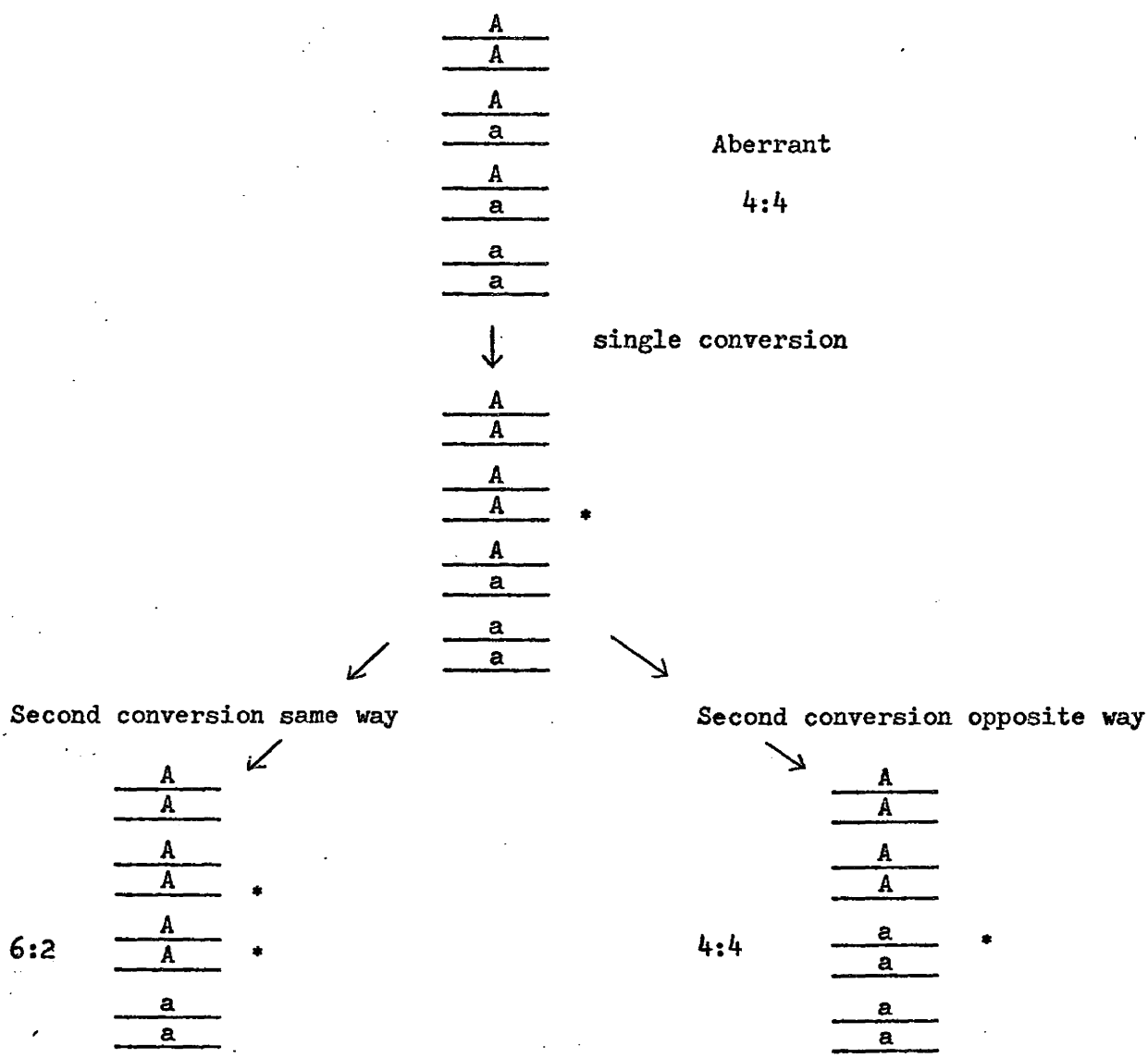
Hybrid DNA resulting from a crossover giving rise to an aberrant 4:4 segregation within the ascus



nucleotide bases would not be cross-hydrogen-bonded but merely held in place by the deoxyribose phosphate DNA backbone. Correction of the heterozygosity in one DNA double helix would produce a 5:3 ratio, while correction in both would produce either a 4:4 or a 6:2 segregation, as shown below in Figure 1-3.

Figure 1-3

Correction of sites of heterozygosity in hybrid DNA




* = correction of heterozygosity by enzyme

The early hypothesis of Whitehouse described above gave an explanation of aberrant segregation at a locus, but on the basis of the model all asci with such segregations should also have reciprocal recombination of outside marker genes. As discussed earlier, in work on several loci (Case & Giles 1964, Stadler & Towe 1963) aberrant segregations are just as likely to have no recombination of outside markers as reciprocal events. For this reason it was necessary to postulate the existence in the DNA double helix of fixed points (linkers) to which breakage would be restricted (Whitehouse & Hastings, 1965). Breakage at a linker and unwinding of the double helices in one direction only would produce only reciprocal crossovers between outside marker genes. However, unwinding out from the linker in both directions would produce an interchange of linkers but no overall reciprocal crossover event between outside markers. This is diagrammatically represented in Figure 1-4 below.

## Figure 1-4

A summary of the polaron hybrid DNA model of recombination (Whitehouse and Hastings 1965). (Reproduced from the original paper.)

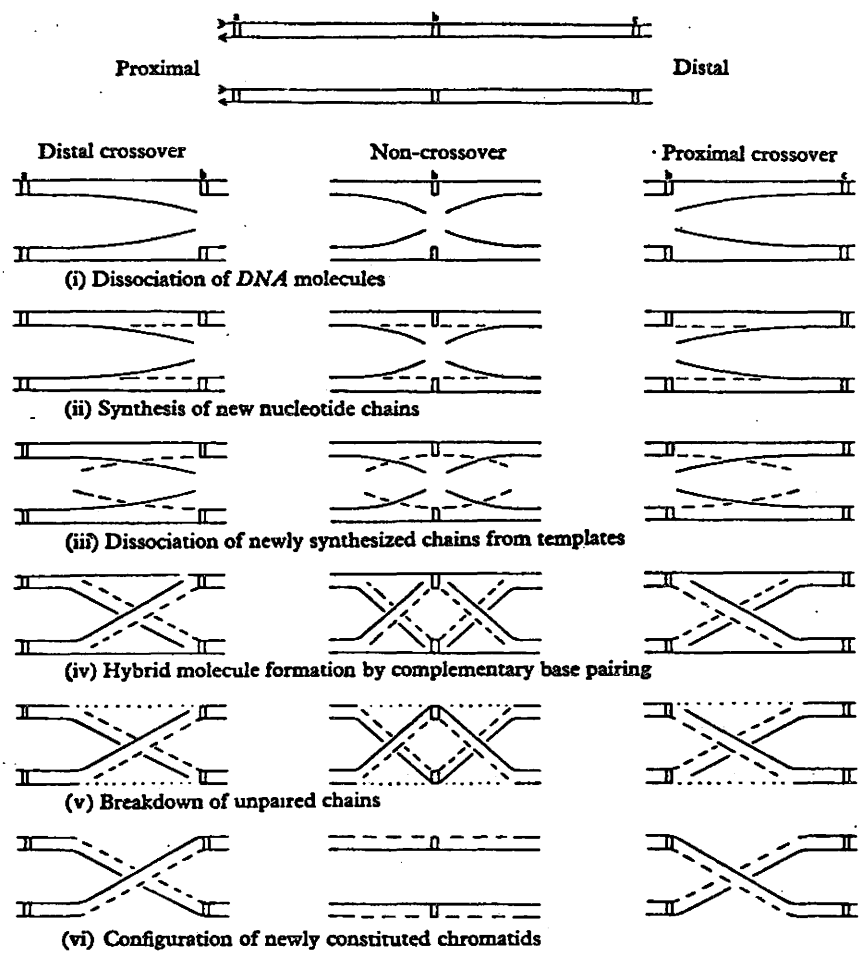



Fig. 1. Diagram to illustrate the polaron hybrid DNA model of genetic recombination. The lines represent the nucleotide chains of two homologous DNA molecules. The orientation of the chains is shown by arrows in the top diagram. The rectangles labelled *a*, *b* and *c* indicate the fixed points of primary breakage from which the dissociation is initiated. The broken lines represent newly-synthesized nucleotide chains, and the dotted lines chains which are breaking down.

The polarity of conversion within a gene is a reflection of the proximity of a mutant site to the breakage point, and hence the frequency with which the mutant site will become involved in the hybrid DNA produced by breakage and interchange of the DNA double helices. Unipolarity suggests potential breakage at only one end of the genetic unit, and hence the likelihood of heterozygosity at a mutant site decreases as the distance of the mutant site from the site of breakage increases. In a bipolar unit, Whitehouse and Hastings postulate two potential breakage points, one at each end of the genetic unit. Whereas a site at the middle of the polaron would infrequently become heterozygous as the unwinding of the double helices would rarely proceed that far from either end, sites at either end would become heterozygous, and hence subject to conversion, more frequently. It is conceivable that breaks may occur at one end of the polaron more frequently than at the other, and hence conversion at the former would be more frequent than at the latter. Such a system would give rise to the type of unequal bipolarity observed by Jessop & Catcheside at hist-1. An alternative explanation of the hist-1 data is that the mutant sites may not be randomly distributed along the length of the polaron.

## Part 3: Complementation

The phenomenon of heterokaryosis has been known to mycologists in many forms of fungi for some time, and the biochemical genetics group of Beadle and Tatum was not slow in realising the importance of this type of mycelial fusion to their embryonic science. Much work had been done in diploids on the nature of dominance of one allele of a gene over another allele of the same gene, but at first such studies were not possible in haploid organisms such as Neurospora crassa.

Beadle & Coonradt (Beadle & Coonradt 1944) realised that in many ways a heterokaryon is analogous to a diploid, and that dominant-recessive relationships could be established between wild-type genes and their auxotrophic mutants. It is still generally true that the heterokaryon and diploid are analogous. However, in the diploid both alleles are in a common nucleus, whereas in a heterokaryon different alleles are contained in separate nuclei. Nuclear localisation of a gene product can, therefore, lead to differences of expression between diploids and heterokaryons in organisms in which both states exist (Pontecorvo 1963). Beadle & Coonradt found, as a result of the common origin of their mutants, that unisexual heterokaryons could be produced at will. They produced a heterokaryon between the strains $pan^-$, $lys^+$ and $pan^+$, $lys^-$. The resultant heterokaryon was found to grow at a rate equal to that of a control, non-mutant, strain. A bisexual heterokaryon containing the same mutant genes (i.e. A, $pan^-$, $lys^+$, a, $pan^+$, $lys^-$) was observed to show slight growth. Growth rate in these experiments was measured on a growth tube containing minimal medium. Two nicotiniless strains of separate origin were combined in a heterokaron, and these too grew at wild-type rate. On the basis of this finding, the two $nic^-$ strains were classified as being non-allelic. Two tryptophan auxotrophs

(10575 and 40008) gave the same result, and crosses in both cases between the alleles gave prototrophic progeny, thus providing further evidence of non-allelism. In other cases, strains with identical biochemical requirements showed no complementation, and this was interpreted as being evidence for allelism.

Later works found it increasingly more difficult to produce heterokaryons, but it was not until mutants from various unrelated strains had become mixed that it was realised that there was a genetic control of heterokaryon compatibility, and that this increasing difficulty was due to heterozygosity at the compatibility loci. This problem, of course, was not encountered by Beadle and Coonradt. Analysis of the control of compatibility revealed that three loci in addition to mating type must be identical before a heterokaryon can be formed (Wilson & Garnjobst 1966). Because of this fact it is common practice now for complementation studies to be undertaken on mutants all derived from the same wild-type strain. This eliminates the necessity of back-crossing and selecting for the desired compatibility homozygosity.

Crosses between three mutants at the pyr-3 locus (37301, 37815), and 45502) were observed to give rise to numerous asci in which not all eight spores matured and germinated (Mitchell et al, 1952). Some apparent pyr-3$^{+}$ isolates from these crosses gave rise to auxotrophic progeny when crossed to a genuine wild-type strain (up to 49% auxotrophs in some cases). Allele #37815 is temperature-sensitive, and in crosses 37301 x 37815 and 37815 x 45502, on back-crossing the apparent wild-type progeny, some of the progeny auxotrophs were temperature-sensitive and some were not. Therefore both mutant alleles may be recovered from an apparent

(or pseudo-) wild-type. A cross was then made between pyr-3 and co (colonial), and asci from this cross were examined. In those asci which contained one or more pseudo-wild spores, at least one other spore pair had aborted. On this basis, it was postulated that pseudo-wild progeny result from non-disjunction in at least one of the anaphases of the meiotic division of the zygote. A pseudo-wild spore therefore is, at least initially, disomic for the chromosome on which the gene under investigation is located.

Pittenger later surveyed a number of different genes for the occurrence of pseudo-wild-types (Pittenger 1954). It will be seen from the summary of Pittenger's findings, below, that these disomics were found in four of the linkage groups known at that time:-

| Linkage group | Cross | | | % PWT's |
|---|---|---|---|---|
| A | al-2(15300), nic(3416) | x | aur(34508), lys(4545) | 0.26% |
| B | cys(84605) | x | cys-2(38401) | 0.10% |
| C | ad(27663) | x | tryp(10575) | 0. |
| D | pdx(35405) | x | pdx(44602) | 0.10% |
| | pdx(35405) | x | pdx(44204) | 1.50% |
| E | inos(37401) | x | inos-t(83201) | |
| | iv-3 | x | iv-1 | |

A number of other crosses failed to give rise to pseudo-wild-types, but it is clear that the phenomenon of pseudo-wilds is far from unusual, and has potential value in testing for heterokaryon complementation between alleles.

In 1956, de Serres investigated the five alleles of ad-3 then available (de Serres, 1956). By using suitable forcing markers he was able to obtain six complementing pairs of alleles out of the ten possible pairs. This divided the mutants into two non-complementing groups, A and B. Mutants 38701, 38709, and 68306 belonged to Group A, and 45601 and 35203 to group B. Any member of group A would, in a compatible background, complement with either of the group B strains. The growth rates of these A + B heterokaryons was as great as the wild-type control strains, and the addition of adenine did not increase the rate on minimal medium. de Serres then derived 24 new ad-3 mutants from the St. Lawrence wild-type strain 74A. Of these, 3 belonged to group A, and 21 to group B. Crosses between any group A strain and any group B strain were found to give rise to prototrophs, at a low rate, by a reciprocal crossover event. Therefore groups A and B had all the characteristics of being separate cistrons, with, however, a combined function in the enzymatic step controlled by the ad-3 gene.

Subsequent work by de Serres on the ad-3 gene revealed that complementation may occur between two mutants, both of which are in the ad-3A group (de Serres 1960). Such heterokaryons, produced with the aid of forcing markers, grew at the same rate as a wild-type strain. Crosses between these mutants, and examination of the outside markers of the prototrophs recovered, showed that recombination within the ad-3A region was not simply reciprocal. Therefore, the ad-3A region was interpreted as a single cistron, and still complementation was possible between certain mutants within that one cistron.

A similar system had been discovered at the pan-2 locus (Case &

Giles, 1960). These workers produced 75 pan-2 mutants, 23 of which were capable of showing complementation. On the basis of complementation behaviour, a linear complementation map consisting of six sections, or complons, was constructed for the locus. A complon is a subdivision of a cistron within which complementation between two mutants cannot occur. A comparison of this map and the recombination map of the gene revealed an overall colinearity. It was found that certain heterokaryons between complementary strains were temperature-sensitive, and it was postulated that the enzyme produced by this cooperative interaction was itself more thermolabile than the wild-type enzyme. It was therefore further postulated that complementation between mutants within the single cistron of the pan-2 gene was due to a cooperative interaction between the two basically similar but differentially defective polypeptide chains produced by the complementing mutants. In the association of these polypeptides to form the active enzyme, the lesion in one chain would be compensated for by the normal part of the polypeptide chain produced by the other mutant.

Catcheside, in the same year, produced a large number of histidine auxotrophs (Catcheside 1960). These mutants were classified into seven different loci, two of which were previously unknown. Within the hist-2, hist-3, and hist-5 loci, the complementation pattern was found to be simply linear. No complementation was found within the 99 mutants at the hist-6 locus, or the 7 at the hist-7 locus, and only one mutant at the hist-4 locus was found. The hist-1 locus appeared to be exceptional in its complementation behaviour in that it proved impossible to draw a complementation map with each mutant represented as a single line. One

mutant at this locus required two defects, in two non-adjacent complons.

Further work has shown this early anomaly in the hist-1 complementation map to be spurious. This work (Jessop & Catcheside, 1965) shows that all the complementing mutants at this locus do in fact show a normal linear arrangement. Recent work on the hist-5 locus has confirmed the linearity of the complementation map found by Catcheside (Catcheside 1960). In the same work, the complementation and recombination maps are compared, and are only found to be colinear if the recombination map is folded back on itself. Smith postulates that this required fold in the recombination map reflects the geometry of the enzyme associated with the hist-5 gene. Complementation would in such a case be produced by cross-bonding, and compensation for lesions in adjacent regions of the polypeptide.

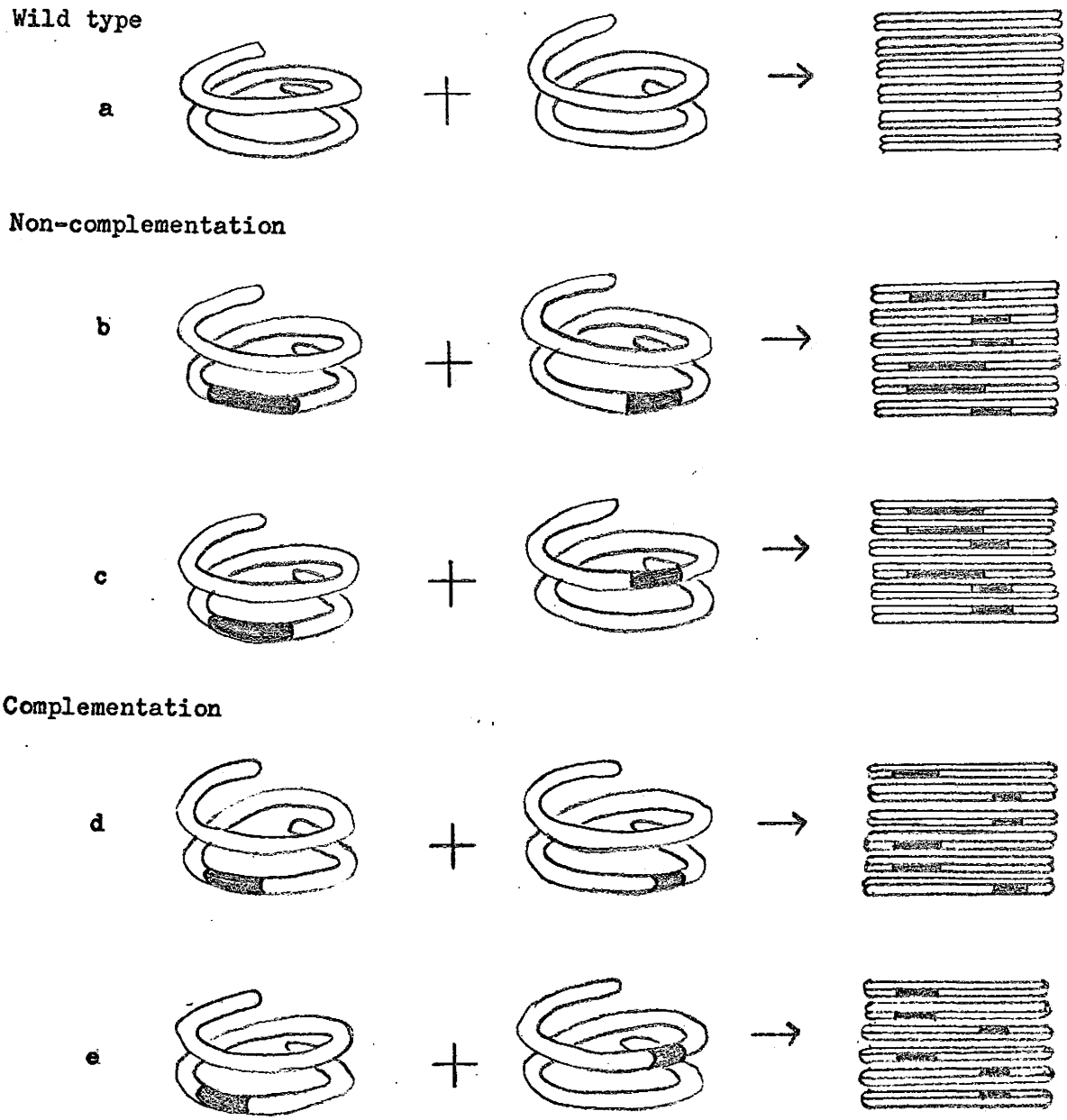
The most complex case of non-colinearity of recombination and complementation maps is that at the ad-8 locus (Ishikawa 1962). It was found that only if the recombination map was arranged in a spiral could colinearity with the complementation map be achieved, and on this basis it was suggested that the polypeptide produced by the ad-8 gene underwent spiralisation to attain its enzymatic activity. This interpretation was explored in detail by Kapuler & Bernstein (Kapuler & Bernstein 1963).

Kapuler & Bernstein suggested a regular layering of like polypeptides to form the homologous active adeylosuccinate synthetase specified by the ad-8 gene, and the stacking, according to their hypothesis, should be such that homologous peptide portions directly adjoin. The overlap of defective regions between or within the spiral polypeptides would inhibit complementation, but complementation would occur where such lesions were not overlapping. This model of intragenic complementation is illustrated in Figure 1-5.

Figure 1-5

A model of intragenic complementation (after Kapuler & Bernstein, 1963).

a) Aggregation of wild-type molecules in a layered stack.
b) Overlapping defects in the same loop.
c) Overlapping defects in different loops.
d) Non-overlapping defects in the same loop.
e) Non-overlapping defects in different loops.



Criticism of the above model of complementation (Kapuler & Bernstein, 1963) has since appeared, on the basis that the model suggested is too specific to be the usual explanation of complementation (Crick & Orgel, 1964). Crick considers that most mutants capable of intra-cistronic complementation are caused by an amino-acid substitution that alters the tertiary configuration of the polypeptide. Such misfolding may be correctible by a polypeptide which is wild-type in this region, a similar concept to that of Kapuler & Bernstein. Crick disagrees, however, with the postulate that a layered stack of like polypeptides is at all common. This criticism also rejects the emphasis on colinearity of genetic and complementation maps, as although the recombination map reflects the physical linearity of the genetic material, more than one mechanism of complementation is possible.

Probably the most thoroughly investigated locus with respect to inter-allelic, or intra-cistronic, complementation is the am locus of Neurospora crassa (Fincham & Coddington, 1963). This locus controls the structure of the enzyme glutamate dehydrogenase. Allels $am^1$ and $am^3$ each produced a mutant form of the enzyme, the former being inactive and the latter having abnormal activity. A heterokaryon between these two mutant alleles produced three types of glutamate dehydrogenase as revealed by chromatographic separation, consisting of $am^1$ and $am^3$ enzyme types, and also an intermediate form which in activity more nearly resembled the wild type enzyme. In vitro production of this third enzyme type (complementation enzyme) is brought about by mixing purified $am^1$ and $am^3$ proteins isolated from separately growing mutants. For in vitro optimum activity of the enzyme, Fincham & Coddington found that the ratio of $am^1$ to $am^3$

protein in the mixture should be 2 or 3:1. From these results they postulated that the complementation enzyme was a multimer, produced in vitro by exchange of polypeptide sub-units between the interacting protein types, and in vivo possibly by random association of newly formed polypeptide sub-units from the two nuclear types.

Part 4: The Pigment Enhancer Mutant and other phenomena associated with by-products of the pdx-1 mutants.

Several mutants of Neurospora have been observed, under certain conditions of environment, to produce large quantities of compounds related in some way to the block in their metabolism. N-acetyl-p-amino-benzoic acid was observed as a green-yellow, water-soluble pigment (Cushing & Reid, 1948, Reid et al, 1952), and poky mutants accumulate large quantities of unsaturated fatty acids (Hardesty & Mitchell, 1963). Perhaps the most obvious of this type of accumulation product is, however, the purple pigment accumulated by ad-3 mutants (Mitchell & Houlahan 1946), and which turns out to be the degradation product of the intermediate metabolite which is on the adenine pathway immediately before the metabolic block. It is characteristic of poky and ad-3 that the accumulation product is produced under all conditions of growth.

In 1953, Martin found that under certain environmental conditions, namely minimal medium + $(NH_4)^+$ (Strauss, 1951) certain isolates of the pH-sensitive pdx-1 allele 44602 produced large amounts of a yellow compound which was excreted into the medium. In a cross giving rise to these pigment-producing strains, only half the pdx-1 progeny did in fact have the yellow phenotype. The yellow colour in the medium was easily distinguished from the slight discoloration of the medium normally produced by pdx-1 mutants on the same medium. Martin found that there appeared to be a gene controlling the pigment production, and it segregated as if closely linked to the mating type locus A/a on linkage group 1 (Catchside, personal communication). Further mapping data on the pigment enhancer data showed that the locus (Y or en-pdx) was situated between mating type

and the centromere, 2.1 crossover units from the former and 4.2 from the latter (Perkins, personal communication). Supplementation with a non-limiting amount of pyridoxine was found by Martin to inhibit the accumulation of the yellow pigment in auxotrophic strains containing the enhancer gene, a clear difference from the unconditional accumulation of the purple pigment by ad-3 mutants.

Allele-specific, fluorescent and other, compounds have been investigated in pdx-1 unenhanced strains (Lunan, personal communication). Lunan studied only five of the pdx-1 alleles, and found that he could differentiate between alleles by quantitative and qualitative differences in their excretion of four phenolic aldehydes. Optimum production of these compounds was again observed in medium containing $(NH_4)^+$ and a limiting concentration of pyridoxine.

## CHAPTER II

## MATERIALS AND METHODS

### MATERIALS

#### Strains

The strains of Neurospora crassa used in this work were as follows:

| Mutant | Isoln.# | F.G.S.C.# | Mating type | Linkage group | Mutagen | Phenotype |
|---|---|---|---|---|---|---|
| arg-3 | 30300 | - | - | IL | U.V. | requires arginine |
| arg-6 | 29997 | - | - | IR | U.V. | " " |
| al-2 | 15300 | - | - | IR | X-rays | albino conidia |
| col-4 | 70007 | 67/486 | A/a | IVR | U.V. | colonial growth |
| cr | - | - | - | IR | Sp. | crisp growth |
| pdx-1 | 35405 | 362 | A | IVR | Sp. | requires pyridoxine |
| pdx-1 | 37803 | 76 | A | IVR | U.V. | " " |
| pdx-1 | 39106 | 370 | A | IVR | U.V. | " " |
| pdx-1 | 39706 | 1185 | A | IVR | U.V. | " " |
| pdx-1 | 44602 | 657 | A | IVR | U.V. | " " |
| pdx-1 | 46904 | 1172 | a | IVR | U.V. | " " |
| pdx-2 | 44204 | 933 | a | IVR | U.V. | " " |
| pyr-1 | H263 | 72/85 | A/a | IVR | X-rays | " cytidine |
| leu-2 | 37501 | - | - | IVR | U.V. | " leucine |
| tryp-4 | Y2198 | - | - | IVR | N-Mustard | " indole |
| en-pdx-1 | K30 | - | - | IL | Sp. | pigment enhancer |
| tryp-2 | 75001 | - | - | VIR | Sp. | requires anthranilic acid |
| ylo | Y30539y | - | - | VIL | Sp. | yellow conidia |

Four wild type strains were used in the course of the work. These were:

| Isolation# | F.G.S.C.# | Mating type | Origin |
|---|---|---|---|
| 1 | 354 | A | Lindegren |
| 25 | 353 | a | Lindegren |
| 74 | 262 | A | St. Lawrence |
| 74-OR8-1 | 532 | a | St. Lawrence |

Certain of the mutants were obtained already combined into multiply marked strains. These were:

| Strain | Genotype | Mating type | Source |
|---|---|---|---|
| 313 | arg-6, al-2 | A | F.G.S.C. |
| 272 | arg-6, al-2 | a | F.G.S.C. |
| H815 | pyr-1, pdx(37803) | A | S.F.H. Threlkeld |
| H823 | pyr-1, pdx(37803) | A | S.F.H. Threlkeld |
| J104 | pdx(44602), tryp-4, leu-2 | a | S.F.H. Threlkeld |
| 639-8 | arg-3, tryp-2, cr, ylo | a | S.F.H. Threlkeld |
| 345 | pdx(39106), col-4 | a | F.G.S.C. |
| 388 | pdx(35405), col-4 | a | F.G.S.C. |
| 404 | pdx(37803), col-4 | A | F.G.S.C. |
| E229 | arg-3, tryp-2, cr, ylo | A | S.F.H. Threlkeld |

The above mutants and strains have been described in the following references:

H815, H823, J104, 639-8, and E229: Threlkeld 1961.

1172: Barratt & Ogata 1964b, 1965a.

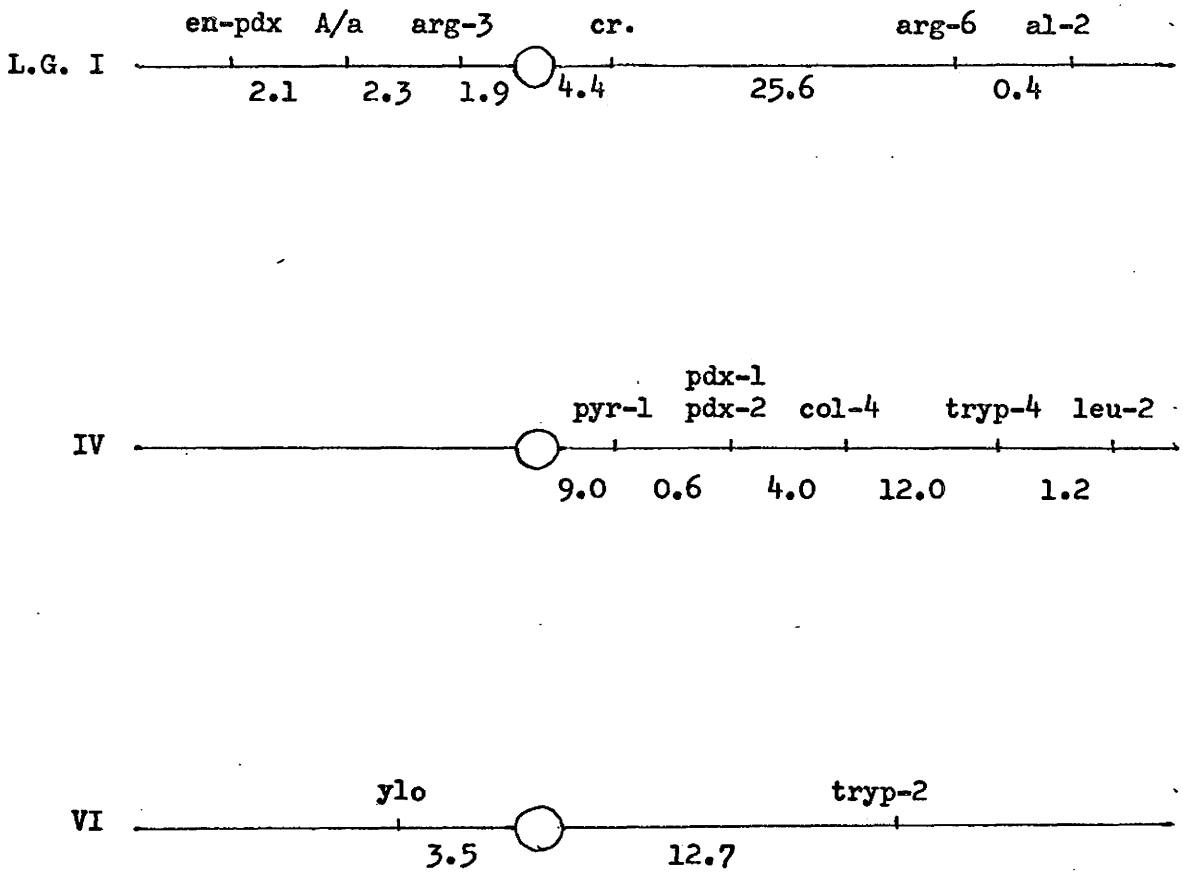
1185: Barratt & Ogata 1965b.

All others: Barratt & Ogata 1964a

The sites of the above mutants on the linkage groups of Neurospora crassa is shown in Figure 2 - 1.

Figure 2-1

The sites of the mutants on the linkage groups.



Culture media

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

1) Medium on which crosses were grown. This medium 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 medium 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 medium contains a limiting concentration of sucrose, and sorbose to induce colonial growth. Isolates were tested for the presence of any biochemical markers by innoculating a series of appropriately supplemented media in 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.
5) Medium on which prototrophs were isolated (Brockman & de Serres 1963)

These media were identical with respect to inorganic salts and biotin, the only essential biochemical for wild type Neurospora.

6) The sixth medium was used for testing the expression of the en-pdx-1 gene, and contained a different salts solution formulation (Vogel 1956), with normal glucose, biotin, and trace elements.

The salts were made up as follows:

4 x salts 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 elements solution:-

| | |
|---|---|
| $Na_2B_4O_7.10H_2O$ | 0.01 gm |
| $CuSO_4.5H_2O$ | 0.10 gm |
| $FePO_4.2H_2O$ | 0.02 gm |
| $MnSO_4.4H_2O$ | 0.02 gm |
| $Na_2MoO_4.2H_2O$ | 0.02 gm |
| $ZnSO_4.7H_2O$ | 2.00 gm |
| Distilled water up to 250 ml | |

Vogel 50 x salts solution:-

| | |
|---|---|
| $Na_3$ citrate | 150 gm |
| $KH_2PO_4$ | 250 gm |
| $NH_4NO_3$ | 100 gm |
| $MgSO_4.7H_2O$ | 10 gm |
| $CaCl_2.2H_2O$ | 5 gm |
| trace elements | 10 ml |
| biotin | 2.5 mg |
| distilled water | 750 ml |
| Chloroform (preservative) | 2 ml |

Where buffering was required, the potassium dihydrogen phosphate in the salt solution was replaced by a phosphate buffer (potassium dihydrogen phosphate - disodium hydrogen phosphate) at the desired pH and at a concentration to give the buffer in the resultant medium a strength of M/15.

The media 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

| | |
|---|---|
| 4 x strength salt solution | 250 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 |

5 - Sorbose medium for prototroph analysis (Brockman & de Serres 1963)

| | |
|---|---|
| 4 x strength salt solution | 250 ml |
| Sorbose | 10 gm |
| Glucose | 0.5 gm |
| Fructose | 0.5 gm |
| Distilled water up to | 1000 ml |

6 - Vogel's Vegetative Medium

| | |
|---|---|
| 50 x salt solution | 20 ml |
| Glucose | 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, and pyridoxine hydrochloride at 10 mg/l.

## METHODS

### Crosses

Crosses were made by innoculating conidia of both parental strains onto suitably supplemented sucrose medium. The innoculated slopes were then incubated at 25°C. until mature perithecia and ascospores were produced.

### Analyses of Crosses

Three methods of analysing the ascospores produced by a cross were used herein. These are ascus analysis, prototroph analysis, and random spore analysis.

1) Random spore analysis.

Mature ascospores were 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 had been found that this dormancy could be broken and germination induced by placing the culture tubes in a water bath at 60°C. for 30 minutes. This heat treatment, in addition to inducing the germination of the ascospores, also had the effect of killing any conidia which might simultaneously have been

transferred to the slopes. After the heat treatment the tubes were incubated at 25°C.

2) 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 was 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 they were subjected to a heat treatment of 60°C for 30 minutes.

3) Prototroph Analysis.

Ascospores were harvested from a cross by adding 5 ml of sterile distilled water to the cross tube, agitating with a "Vortex Junior Mixer", and decanting the spore suspension produced. The spore suspension was then filtered through glass wool to remove perithecia and large mycelial fragments. The spore suspension was made up to 12 ml, or more if the suspension was too concentrated, with distilled water.

Two bottles, each containing 500 ml of Sorbose - Glucose - Fructose Media, one completely supplemented with cytidine and pyridoxine

and the other with cytidine and desoxypyridoxine, both buffered at pH 5, 5, were available, molten, in an incubator at 60°C.

Spore suspension (0.1 ml) was added to the bottle of complete medium, and 10 ml to that of minimal medium. The bottles were then returned to the incubator for 30 minutes to induce germination.

After the heat shock, all the medium from each bottle was poured into petri dishes. The petri dishes were then incubated at 25°C for 3 days. Then, counts were made of the total number of colonies growing on the plates of complete medium, and of the colonies (prototrophs and pseudo-wild types) growing on the minimal medium. Mitchell (Mitchell 1955) had observed that it was possible to distinguish morphologically between prototrophs and pseudo-wild types on sorbose plates because, she stated, the latter grew less densely. In general this was found to be true in crosses between complementary alleles, where three colony classes could often be distinguished between:-

1/ Dense (col-4) prototrophs

2/ Less dense (col-4$^+$) prototrophs

3/ Sparse pseudo-wild types

Where this was so, pseudo-wild types were not included in the count of colonies on medium lacking pyridoxine. However, in other cases there was no distinct break between classes two and three above. In this situation the provisional prototropn count included pseudo-wild types, which were subsequently corrected for by the plating test described later.

A sample of prototrophs was taken, and each colony sampled was sub-cultured on a slope of supplemented glucose medium. Subsequently,

the outside markers of each such prototroph were scored.

4) Conidial Analysis

In order to determine the nature of heterokaryons (pseudo-wild strains), it was necessary to carry out a conidial analysis. In doing so, it was necessary to observe strict asceptic techniques, as no heat treatment, which served 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. The tube was then 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. This suspension (0.1 ml) 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, 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 small amounts of conidia from the cultures under examination.

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.

The tests were carried out in 125 ml Erlenmeyer flasks containing 20 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, counted 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.

Unless otherwise stated, 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, being the duration of the log phase of growth.

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, and dried.

Complementation tests

The test requires that each one of a numbef of isolates of one pdx allele with the appropriate forcing and colour markers is innoculated

with each one of a number of isolates of another pdx allele with the complementary forcing and colour markers. Normally six isolates of each pdx allele were selected if possible, and details of this are given in Chapter V.

In testing a pair of pdx alleles therefore, each isolate of one allele had to be combined with each of the six isolates of the other allele. Therefore there were thirty-six possible combinations to be made. As a check, each isolate was also innoculated by itself, so that if it was at all leaky, a comparison could be made between the growth of the single isolate and that of the possible heterokaryon. Therefore forty-eight tubes were required.

Conidia of the twelve strains were suspended in sterile distilled water, and filtered through glass wool to remove large mycelial fragments. One drop of this suspension was then added to each of the seven tubes along the appropriate row, as shown below (Fig. 2-2). The six isolates of the one allele were innoculated along the rows, and the six of the others innoculated across.

Proof of compatibility and complementation was obtained when a good growth of mycelium with pink conidia developed.

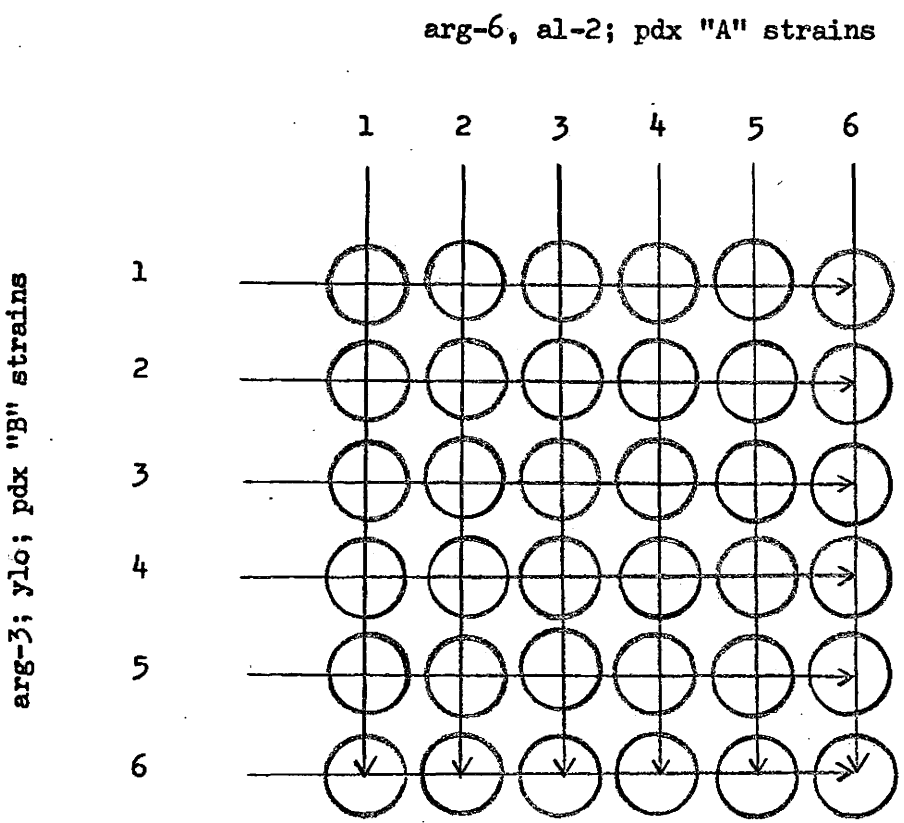
In order to check the heterokaryon compatibility of a pair of strains, they were innoculated onto glucose medium containing 10 mg/l of pyridoxine hydrochloride. When testing for complementation between the pdx alleles, the pyridoxine hydrochloride was replaced by 100 mg/l of desoxypyridoxine hydrochloride.

As there was the risk of contamination in the above technique, suspected complementing combinations were checked by re-innoculating

the parental strains on desoxypyridoxine medium under full aseptic conditions.

Figure 2-2

The heterokaryon test technique



Detection of Pseudo-wild strains

In the high resolution recombination analysis of the pdx-1 locus, when spores from an inter-allelic cross are plated on minimal medium, only those progeny spores which do not require pyridoxine for growth will produce colonies. One type of colony-forming strain is the prototroph, a recombinant strain which has the wild-type homologues of both parental pdx-1 alleles recombined in a single genome. However, in crosses between alleles which are capable of complementation, a second type of colony which is pseudo-wild will be found. The pseudo-wild originates by non-disjunction, producing a disomic which later becomes a haploid heterokaryon, and hence has both parental linkage group IV chromosomes. (Pittenger 1954). The pseudo-wild strain will therefore contain both mutant alleles of the pdx-1 locus, pyr-1 and its wild type homologue, and col-4 and its wild type homologue. As not all conidia produced by a heterokaryon are still heterokaryotic, it is possible by utilising this phenomenon of heterokaryon breakdown to identify pseudo-wild strains. Because of its origin as a disomic, the pseudo-wild is phenotypically wild type not only for the pdx-1 locus, but also for the non-selected outside markers.

When conidia from a prototroph are plated on complete medium (200 to 1000 conidia per plate) all conidia are genotypically identical and produce identical colonies. However, when a pseudo-wild strain is analysed in the same way, three types of colony are produced:-

1) Still heterokaryotic pseudo-wild colonies

2) pyr-1 , $pdx^-$ colonies

3) $pdx^-$, $col\text{-}4^-$ colonies

The first two of these classes are indistinguishable on sorbose plates, but morphology of the third class is unmistakable. Therefore, pseudo-wilds can be detected and a correction may be made in the prototroph frequency and in the outside marker group proportions.

Pigment production by the Enhancer gene

The enhancer gene, in the presence of a mutant allele of the pdx-1 locus, when grown on medium with a growth-limiting concentration of pyridoxine, produces a brown-yellow pigment which is excreted into the medium. The pigment is produced in greatest quantity in medium containing ammonium ion, and under these conditions the pH-sensitive alleles produce it, and grow, even in the complete absence of externally supplied pyridoxine.

For classification, the strains were grown on glucose medium with Vogel salts solution and limiting pyridoxine. For more detailed study of the pigment, the strains were grown in similar liquid medium (50 ml. in a 125 ml. Erlenmeyer flask), filtered, lyophilised, and extracted with methanol.

## CHAPTER III

## THE USE OF THE COMPETITIVE INHIBITOR DESOXYPYRIDOXINE HYDROCHLORIDE IN CLASSIFICATION OF THE PYRIDOXINE MUTANTS

Although the pyridoxine auxotrophs of Neurospora have been in existence over twenty years, since they were first isolated in the pioneering work of Beadle and Tatum (Beadle and Tatum, 1941; Houlahan et al. 1949), they have proved very difficult to work with because of their extreme leakiness, which is of an order far greater than this author has observed in mutants at any other locus.

Table 3-1 shows the growth results of the seven mutants of Neurospora crassa when grown on liquid medium according to the method of Strauss (Strauss 1951).

TABLE 3-1

The effect of supplementation with pyridoxine hydrochloride on the pyridoxine auxotrophs. Growth was for 6 days.

| | Min | $\bar{x}$ | s | +10mg/l Pyridoxine HCl | $\bar{x}$ | s |
|---|---|---|---|---|---|---|
| 74-OR8-1a | 59 mg | 53.5 | 7.7 | 50 mg | 51 | 1.4 |
| | 48 | | | 52 | | |
| 37803 | 10 | 10.0 | 0 | 50 | 49.5 | 0.7 |
| | 10 | | | 49 | | |
| 35405 | 9 | 9.5 | 0.7 | 53 | 48.5 | 6.3 |
| | 10 | | | 44 | | |
| 39106 | 31 | 24.5 | 9.2 | 49 | 50.5 | 2.1 |
| | 18 | | | 52 | | |
| 44602 | 6 | 6.5 | 0.7 | 18 * | 18.0 | 0 |
| | 7 | | | 18 | | |
| 44204 | 11 | 10.0 | 1.4 | 49 | 49.0 | 0 |
| | 9 | | | 49 | | |
| 46904 | 19 | 17.5 | 2.1 | 56 | 53.0 | 4.2 |
| | 16 | | | 50 | | |
| 39706 | 35 | 33.5 | 2.1 | 50 | 50.5 | 0.7 |
| | 32 | | | 51 | | |

*It is noticeable from the above data that allele #44602 exhibited poor growth even on fully supplemented medium. This slower rate of growth has been observed previously (Strauss 1951). Although in this genetic back-

ground (F.G.S.C. #657), which is presumably that in which the mutant was isolated, and also that which Strauss studied, growth is poor, progeny did recover a higher growth rate. Probably, therefore, this is a genetic background effect not associated with the mutant gene itself.

The effect of desoxypyridoxine as a competitive inhibitor of pyridoxine had been observed in work by Glazer on human excretion patterns (Glazer 1951), and it was postulated that its competitive action might well reduce the leakiness of the growth of the mutants, and hence allow more certain classification of mutants. It was also hoped that its activity might make possible the high resolution genetic analysis of the pdx locus.

First it was necessary to determine the effect of desoxypyridoxine on the growth of a wild type strain. Medium was made up with various concentrations of desoxypyridoxine hydrochloride from zero to 200 mg/litre. The results of this experiment are given in Table 3-2.

TABLE 3-2

The effect of desoxypridoxine HCl on the growth of wild-type Neurospora for 6 days.

| | Medium | Wt. | $\bar{x}$ | s |
|---|---|---|---|---|
| 1/ | Minimal | 68 mg<br>69<br>67 | 68 | 1.0 |
| 2/ | + 10 mg/l desoxypyridoxine HCl | 68<br>70<br>66 | 68 | 1.4 |
| 3/ | + 50 mg/l " " | 70<br>68 | 69 | 1.4 |
| 4/ | + 100 mg/l " " | 69<br>72<br>62 | 67 | 5.2 |
| 5/ | + 200 mg/l " " | 70<br>63<br>63 | 65 | 4.1 |

As will be seen, desoxypyridoxine hydrochloride concentrations up to at least 200 mg/litre have no significant effect on the growth of the wild-type strain 74-OR8-1a.

The seven Neurospora crassa pyridoxine auxotrophs were then grown on glucose medium at pH 5 supplemented with desoxypyridoxine hydrochloride at the rate of 100 mg/litre. The results are given below in Table 3-3.

TABLE 3-3

The effect of desoxypyridoxine HCl on the pyridoxine auxotrophs

| Strain | + Desoxypyridoxine | | | Minimal | | |
|---|---|---|---|---|---|---|
| | Wts. | $\bar{x}$ | s | Wts. | $\bar{x}$ | s |
| 37803 | 0 mg | | | 1.2 mg | | |
| | 0 | 0 | 0 | 1.1 | 1.23 | 0.15 |
| | 0 | | | 1.4 | | |
| 35405 | 0 | | | 2.7 | | |
| | 0 | 0 | 0 | 2.6 | 2.57 | 0.15 |
| | 0 | | | 2.4 | | |
| 39106 | 44 | | | 57 | | |
| | 48 | 46.3 | 2.1 | 66 | 63.3 | 5.5 |
| | 47 | | | 67 | | |
| 44602 | 0 | | | 0.7 | | |
| | 0 | 0 | 0 | 0.8 | 0.77 | 0.06 |
| | 0 | | | 0.8 | | |
| 44204 | 0 | | | 2.0 | | |
| | 0 | 0 | 0 | 2.5 | 1.93 | 0.60 |
| | 0 | | | 1.3 | | |
| 46904 | 0 | | | 3.9 | | |
| | 0 | 0 | 0 | 5.1 | 4.1 | 0.92 |
| | 0 | | | 3.3 | | |
| 39706 | 40 | | | 47 | | |
| | 47 | 42.3 | 4.0 | 43 | 48 | 5.6 |
| | 40 | | | 54 | | |

(Growth was for 6 days.)

The extent of growth of the mutants on minimal medium shown above in Table 3-3 is lower than that in Table 3-1. The explanation of this is not known with certainty, but it is possible that iron concentration may be involved, and that minute changes in concentration of this trace element might be the cause of this anomaly (Mitchell 1955).

Mutants 39106 and 39706 in the experiment summarised in Table 3-3 still show considerable leakiness even in the presence of desoxypyridoxine. In experiments on solid sorbose medium, their growth was further reduced, but no numerical estimate of reduction in growth can be given.

The use of desoxypyridoxine at pH 5 definitely assists in making a distinction between mutants at the pdx locus and prototrophs. As Stokes had shown the presence of pyridoxine in pH-sensitive pdx mutants grown in the presence of $(NH_4)^+$ ions at pH 7, it was postulated that such mutants grown under these conditions may, because of their contained pyridoxine, be more resistent to the competitive inhibitory effect of desoxypyridoxine than the non-pH-sensitive, absolute, pdx mutants. To test the hypothesis, the pyridoxine mutants were grown on four media, all at pH 7. The first contained no supplements. The second contained ammonium sulphate at the rate of 1 gm/litre. The third contained ammonium sulphate at the previous rate, and also desoxypyridoxine at the rate of 100 mg/l. The fourth contained 100 mg/l of desoxypyridoxine only.

All pH-sensitive strains were tested, and the results of this experiment are given below in Table 3-4.

TABLE 3-4

The effect of desoxypyridoxine HCl on mutant growth at high pH for 6 days.

| Strain | Minimal | | | $+(NH_4)^+$ | | | $+(NH_4)^+$ and Dpdx | | | +Dpdx | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wts | $\bar{x}$ | s | Wts | $\bar{x}$ | s | Wts | $\bar{x}$ | s | Wts | $\bar{x}$ | s |
| 39106 | 49mg | | | 80mg | | | 83mg | | | 39mg | | |
| | 44 | 45 | 3.6 | 84 | 81 | 2.3 | 81 | 82.7 | 1.6 | 28 | 28.7 | 9.8 |
| | 42 | | | 80 | | | 84 | | | 19 | | |
| 44602 | 1 | | | 18 | | | 30 | | | 0 | | |
| | 1 | 1 | 0 | 25 | 29 | 13.9 | 34 | 28.7 | 6.2 | 0 | 0 | 0 |
| | 1 | | | 45 | | | 22 | | | 0 | | |
| 44204 | 2 | | | 57 | | | 41 | | | 0 | | |
| | 2 | 2.3 | 0.7 | 50 | 52.7 | 3.8 | 53 | 46.7 | 4.8 | 0 | 0 | 0 |
| | 3 | | | 51 | | | 46 | | | 0 | | |
| 46904 | 7 | | | 51 | | | 40 | | | 0 | | |
| | 7 | 7 | 0 | 51 | 51.7 | 1.2 | 36 | 39.7 | 3.5 | 0 | 0 | 0 |
| | 7 | | | 53 | | | 43 | | | 0 | | |
| 39706 | 51 | | | 79 | | | 78 | | | 47 | | |
| | 62 | 59.9 | 7.3 | 67 | 71.7 | 6.4 | 65 | 69.3 | 7.3 | 25 | 40 | 13.0 |
| | 65 | | | 69 | | | 66 | | | 48 | | |

The reason for the growth of strains 39106 and 39706 at such a high rate might be that some nuclei may have reverted in these strains, as reversion is known at this locus (Mitchell, personal communication), although another possible explanation is mentioned on page 55.

To summarise, by using an appropriate rate of supplementation of medium with desoxypyridoxine, classification of the alleles at the pdx locus is greatly facilitated. Table 3-5 summarises these data.

TABLE 3-5

The growth of pdx mutants on different media

| Strain | pH5 | | | | | | pH7 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minimal | | $+(NH_4)^+$ | | +Pdx | | Minimal | | $+(NH_4)^+$ | | +Pdx | |
| | | + Dpine | | + Dpine | | + Dpine | | + Dpine | | + Dpine | | + Dpine |
| 362(35405) | + | - | + | - | +++ | +++ | + | - | + | - | +++ | +++ |
| 76(37803) | + | - | + | - | +++ | +++ | + | - | + | - | +++ | +++ |
| 370(39106) | ++ | + | ++ | + | +++ | +++ | ++ | + | +++ | +++ | +++ | +++ |
| 657(44602) | + | - | + | - | +++ | +++ | + | - | +++ | +++ | +++ | +++ |
| 933(44204 | + | - | + | - | +++ | +++ | + | - | +++ | +++ | +++ | +++ |
| 1172(46904) | + | - | + | - | +++ | +++ | + | - | +++ | +++ | +++ | +++ |
| 1185(39706) | ++ | + | ++ | + | +++ | +++ | ++ | + | +++ | +++ | +++ | +++ |
| 74-OR8-1a | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |

\- indicates no measurable growth

\+ indicates a trace of growth

++ indicates significant growth

+++ indicates full wild type growth rate

Dpine is desoxypyridoxine hydrochloride, added at the rate of 100 mg/liter of medium

# CHAPTER IV

## THE EFFECTS OF DIFFERENT SUPPLEMENTS AND DIFFERENT RATES OF SUPPLEMENTATION ON THE GROWTH OF PYRIDOXINE MUTANTS

Earlier work (Stokes 1943, Strauss 1951) has shown that the pdx-1 mutants respond to supplementation with pyridoxine, pyridoxal, and pyridoxamine. The following forms of the vitamin were used in this work:-

pyridoxine hydrochloride

pyridoxal hydrochloride

pyridoxal phosphate

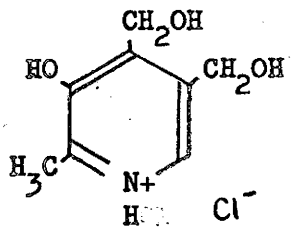
pyridoxamine dihydrochloride

pyridoxamine phosphate hydrochloride

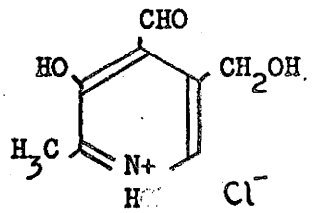
The structural formulae of these five forms are shown in Figure 4-1.

Figure 4-1

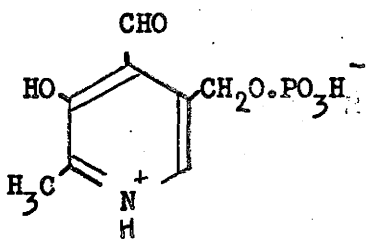
The structural formulae of the different forms of the pyridoxine group



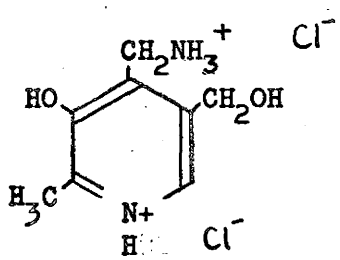
pyridoxine hydrochloride



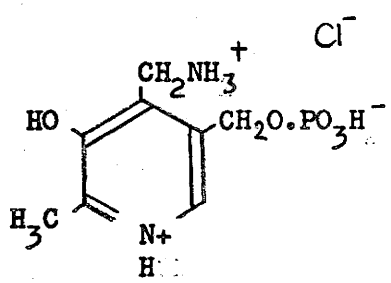
pyridoxal hydrochloride



pyridoxal phosphate



pyridoxamine dihydrochloride



pyridoxamine phosphate hydrochloride

Pyridoxal phosphate and pyridoxamine phosphate are the active forms, each being interconvertible during the vital metabolic process of transamination. Pyridoxine itself also may be extracted from the cell. It was the first form of the vitamin to be isolated. Pyridoxal and pyridoxamine were isolated only after their presence had been inferred from the discrepancies that were constantly observed in bio-assay for the vitamin.

In order to investigate the efficiency of the various forms of the vitamin as supplements to growth of the mutants, the glucose medium was supplemented with equi-molar, non-limiting, quantities of the following supplements:

| | |
|---|---|
| pyridoxine hydrochloride | 20.5 mg/l |
| pyridoxal hydrochloride | 20.3 |
| pyridoxal phosphate | 25.0 |
| pyridoxamine dihydrochloride | 24.5 |
| pyridoxamine phosphate hydrochloride | 29.7 |

In this experiment, to make certain that the pH of the medium did not exceed the level above which the pH-sensitive mutants would become independent of their pyridoxine requirement, a drop of bromo-phenol blue indicator was added to each growth flask. The M/15 buffer was found to be adequate to control the pH.

After inoculation, the flasks were incubated at 25° C for seven days. The results of growth for this period in media containing the various forms of the vitamin are given below in Table 4-2.

TABLE 4-2

The efficiency of the various forms of vitamin $B_6$ as growth stimulants to the pdx mutants.

| | Pyridoxine HCl. | | | Pyridoxal HCl. | | | Pyridoxal P. | | | Pyridoxamine 2 HCl. | | | Pyridoxamine P.HCl. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s |
| 37803 | 79 mg | | | 94 mg | | | 106 mg | | | 75 mg | | | 102 mg | | |
| | 73 | 75 | 3.5 | 71 | 82 | 11.5 | 80 | 96 | 14.0 | 83 | 78 | 4.2 | 92 | 91 | 11.0 |
| | 73 | | | 82 | | | 102 | | | 77 | | | 80 | | |
| 35405 | 79 | | | 70 | | | 88 | | | 90 | | | 95 | | |
| | 64 | 71 | 7.6 | 78 | 71 | 6.6 | 94 | 90 | 3.9 | 82 | 86 | 4.0 | 83 | 93 | 9.2 |
| | 70 | | | 65 | | | 87 | | | 85 | | | 101 | | |
| 39106 | 79 | | | 112 | | | 91 | | | 100 | | | 115 | | |
| | 85 | 82 | 3.5 | 91 | 95 | 15.8 | 111 | 95 | 14.0 | 91 | 96 | 4.7 | 111 | 109 | 6.7 |
| | 82 | | | 81 | | | 84 | | | 98 | | | 102 | | |
| 44602 | 47 | | | 82 | | | 88 | | | 80 | | | 94 | | |
| | 36 | 44 | 6.7 | 72 | 75 | 7.2 | 81 | 87 | 5.1 | 85 | 80 | 5.5 | 42 | 66 | 26.2 |
| | 48 | | | 72 | | | 91 | | | 74 | | | 62 | | |
| 44204 | 46 | | | 49 | | | 53 | | | 68 | | | 63 | | |
| | 53 | 47 | 6.0 | 41 | 46 | 4.2 | 57 | 61 | 11.2 | 65 | 67 | 1.6 | 72 | 70 | 6.3 |
| | 41 | | | 47 | | | 74 | | | 67 | | | 75 | | |

(Growth was for seven days)

TABLE 4-3

The effect of pyridoxine concentration on mutant growth.

| | Minimal | | | 0.01 mg/l | | | 0.1 mg/l | | | 1.0 mg/l | | | 10 mg/l | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s | wts | $\bar{x}$ | s |
| 74-OR8-1a | 59 mg | | | 47 mg | | | 51 mg | | | 48 mg | | | 50 mg | | |
| | 48 | 53.5 | 7.7 | 48 | 47.5 | 0.7 | 50 | 50.5 | 0.7 | 62 | 55 | 9.9 | 52 | 51 | 1.4 |
| 37803 | 10 | | | 30 | | | 47 | | | 55 | | | 50 | | |
| | 10 | 10 | 0 | 44 | 37 | 9.9 | 47 | 47 | 0 | 52 | 53.5 | 2.1 | 49 | 49.5 | 0.7 |
| 35405 | 9 | | | 37 | | | 48 | | | 54 | | | 53 | | |
| | 10 | 9.5 | 0.7 | 34 | 35.5 | 2.1 | 50 | 49 | 1.4 | 51 | 52.5 | 2.1 | 44 | 48.5 | 6.3 |
| 39106 | 31 | | | 30 | | | 47 | | | 60 | | | 49 | | |
| | 18 | 24.5 | 9.2 | 34 | 32 | 2.8 | 51 | 49 | 2.8 | 53 | 56.5 | 4.9 | 52 | 50.5 | 2.1 |
| 44602 | 6 | | | 15 | | | 17 | | | 19 | | | 18 | | |
| | 7 | 6.5 | 0.7 | 15 | 15 | 0 | 20 | 18.5 | 2.1 | 20 | 19.5 | 0.7 | 18 | 8 | 0 |
| 44204 | 11 | | | 35 | | | 54 | | | 53 | | | 49 | | |
| | 9 | 10 | 1.4 | 32 | 33.5 | 2.1 | 42 | 48 | 8.5 | 44 | 48.5 | 6.3 | 49 | 49 | 0 |
| 46904 | 19 | | | 25 | | | 53 | | | 59 | | | 56 | | |
| | 16 | 17.5 | 2.1 | 21 | 23 | 2.8 | 51 | 52 | 1.4 | 60 | 59.5 | 0.7 | 50 | 53 | 4.2 |

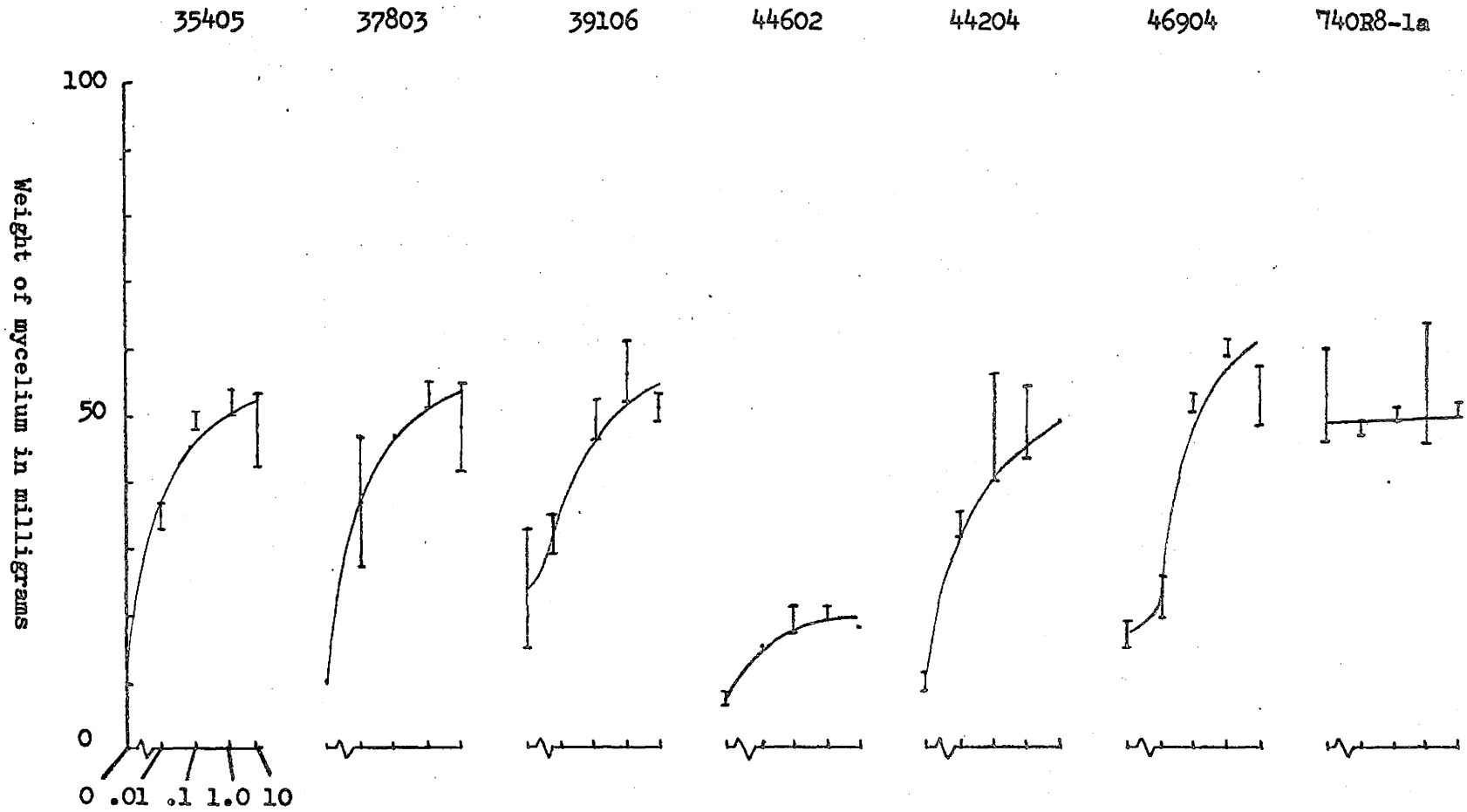
(Growth was for six days)

Certain strains grow fully on concentrations as low as 0.01 mg/l of pyridoxine hydrochloride, and these are #37803 and #35405. Others require at least 0.1 mg/l before a level of growth approaching that of a wild-type strain is achieved, and these strains are #39106, #44204, #46904, #39706 and #44602. It will be seen that the first group consists of the two absolute mutants, whereas the second consists of the pH-sensitive strains.

From these experiments, it is clear that all forms of the vitamin tested enhance the growth of the mutants,

Figure 4-4

The effect of pyridoxine concentration on mutant growth after a growth period of six days.



# CHAPTER V

## COMPLEMENTATION AMONG THE pdx-1 MUTANTS OF NEUROSPORA CRASSA

The control of heterokaryosis in Neurospora crassa is complex, and appears to reflect the heterogeneity associated with the combination of strains derived from multiple sources. Successful heterokaryon formation requires homozygosity of mating type and three other genes, c on the left arm of linkage group II, d on the right arm of II, and e on the left arm of VII. (Wilson & Garnjobst 1966).

As the pyridoxine auxotrophs are still in some cases leaky even when grown on medium containing the competitive inhibitor desoxypyridoxine, use was made of"forcing markers", i.e. genes controlling other biochemical requirements.

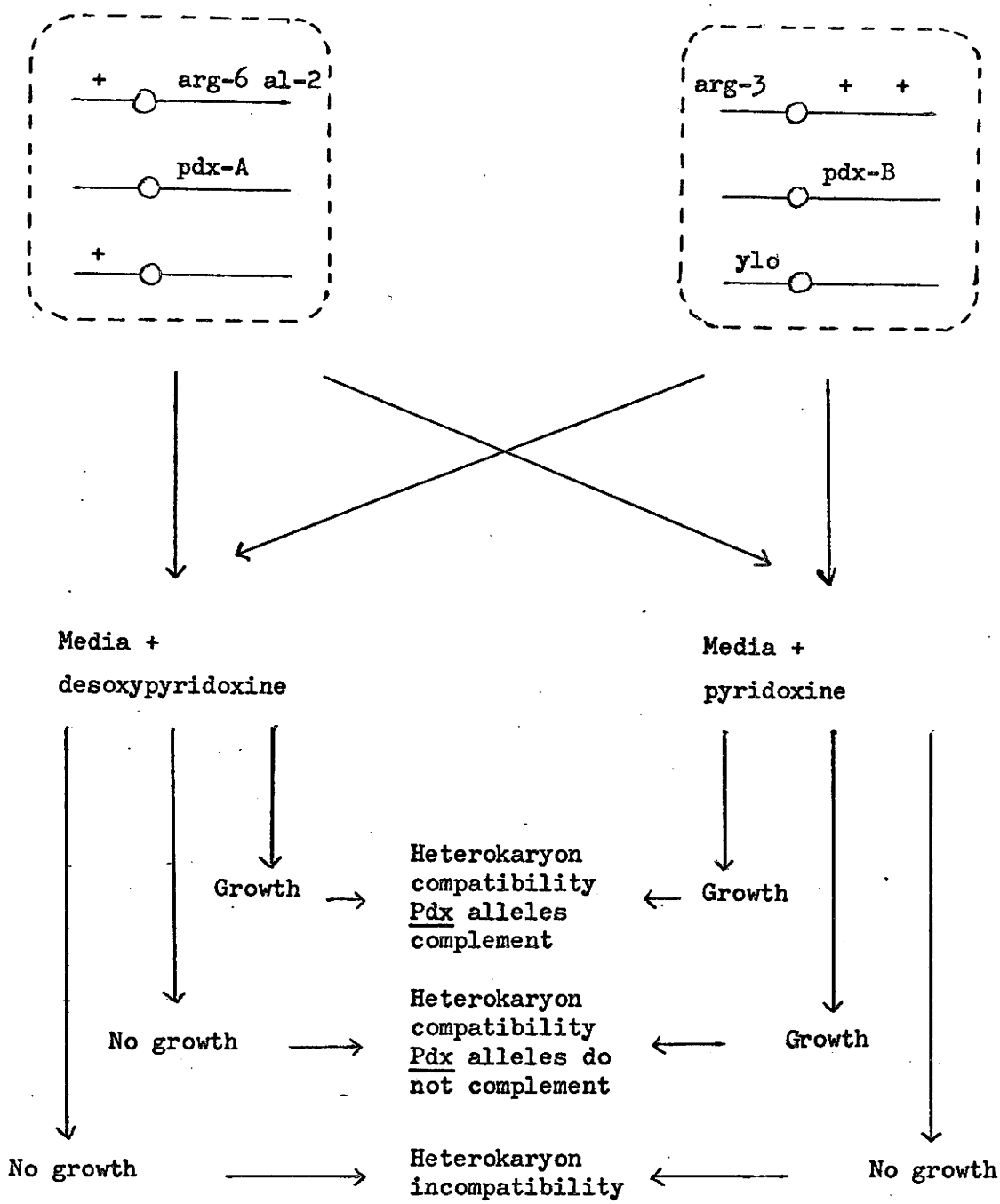
For forcing markers, two separate mutant loci, controlling the requirement of arginine, were utilised, namely arg-3 and arg-6; these loci are on the left and right arms of linkage group I respectively. Also, in order to distinguish between true heterokaryosis and mere cross-feeding of metabolites, conidial colour mutants were incorporated into the strains. These were the albino marker al-2 on the right arm of linkage group I, and the yellow marker ylo-1 on the left arm of linkage group VI.

The actual heterokaryon test was therefore carried out by inoculating the two strains arg-6, al-2, pdx "A" and arg-3, ylo-1 pdx "B", together on minimal medium to which desoxypyridoxine had been added at

the rate of 100 mg/l. If compatibility existed, the two arginine mutants would complement to give the resulting heterokaryon an *arg*$^+$ phenotype, the *al-2* and *ylo-1* mutants would complement to produce pink pigment in the conidia, and the *pdx* "A" and "B" mutants, if complementing, would allow growth of the mycelium and the expression of the *arg*$^+$ and pink phenotypes. If no growth occurred in a test, the parental strains were re-innoculated onto medium containing pyridoxine instead of desoxypyridoxine. In this case, whether or not the *pdx* alleles complemented, if there was heterokaryon compatibility the arginine alleles would complement to produce growth. Diagrammatically this system is depicted in Figure 5-1.

Figure 5-1

Outline of the heterokaryon complementation test



Having discussed the theory behind the heterokaryon test technique, the practical aspects will now be considered.

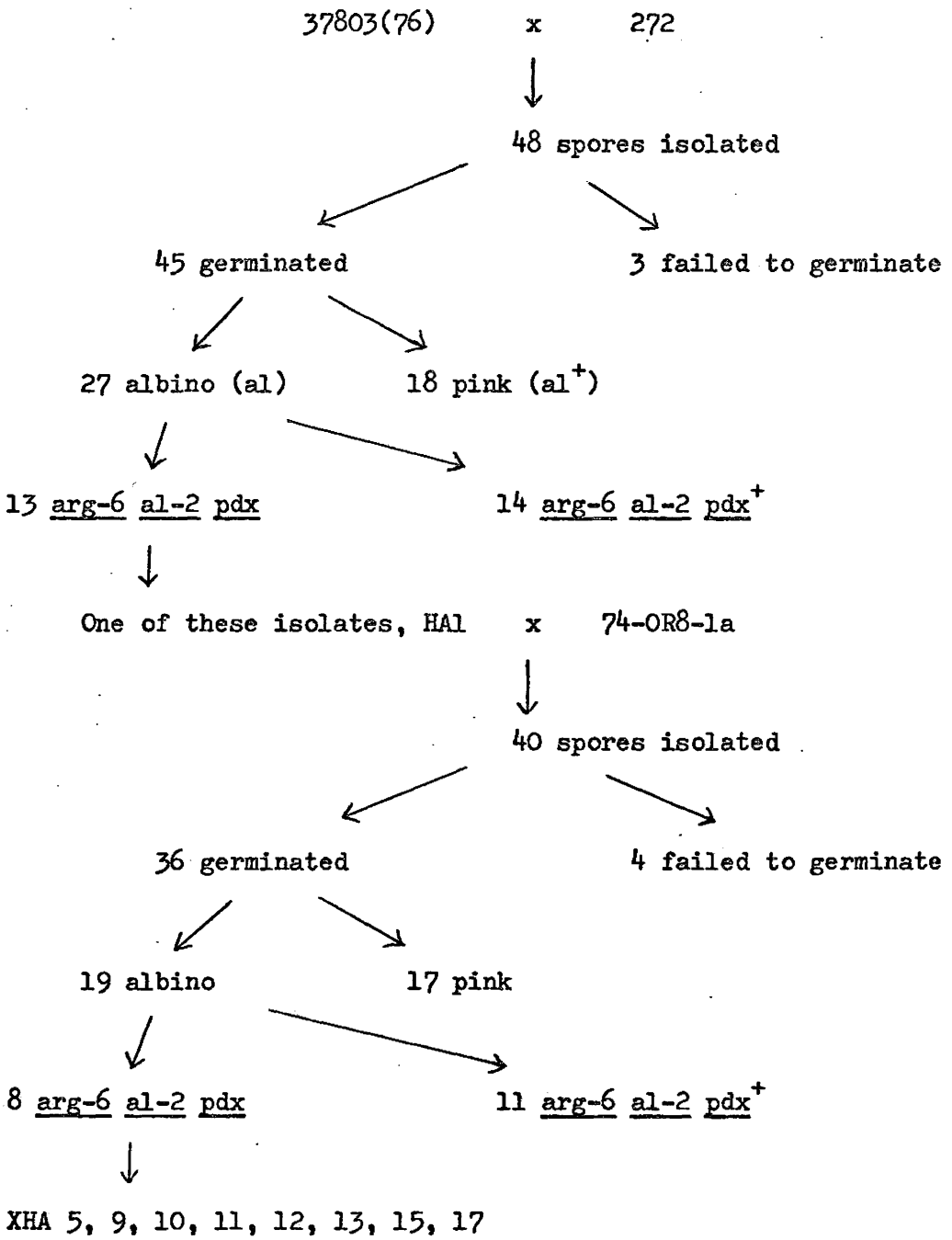
All single pdx strains, as obtained from the Fungal Genetics Stock Center, were crossed to one of two arg-6, al-2 strains from the same source, depending on mating type. These were arg-6, al-2 strains #272, mating type a, and #313, mating type A. The crosses were:-

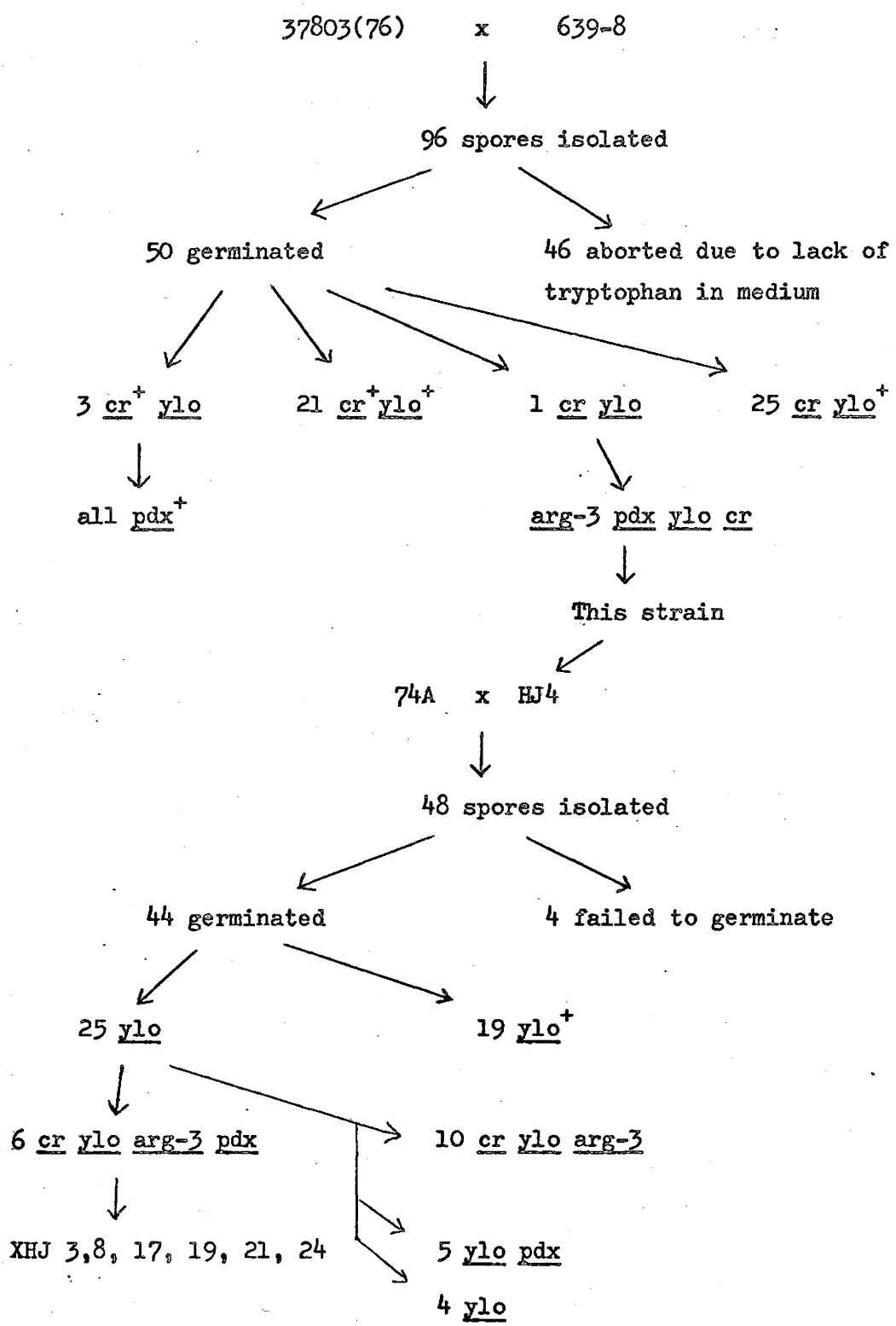
| FGSC# | allele# | m.t | | |
|---|---|---|---|---|
| 76 | 37803 | A | x | 272 a |
| 362 | 35405 | A | x | 272 a |
| 370 | 39106 | A | x | 272 a |
| 657 | 44602 | A | x | 272 a |
| 933 | 44204 | a | x | 313 A |
| 1172 | 46904 | a | x | 313 A |
| 1183 | 39706 | A | x | 272 a |

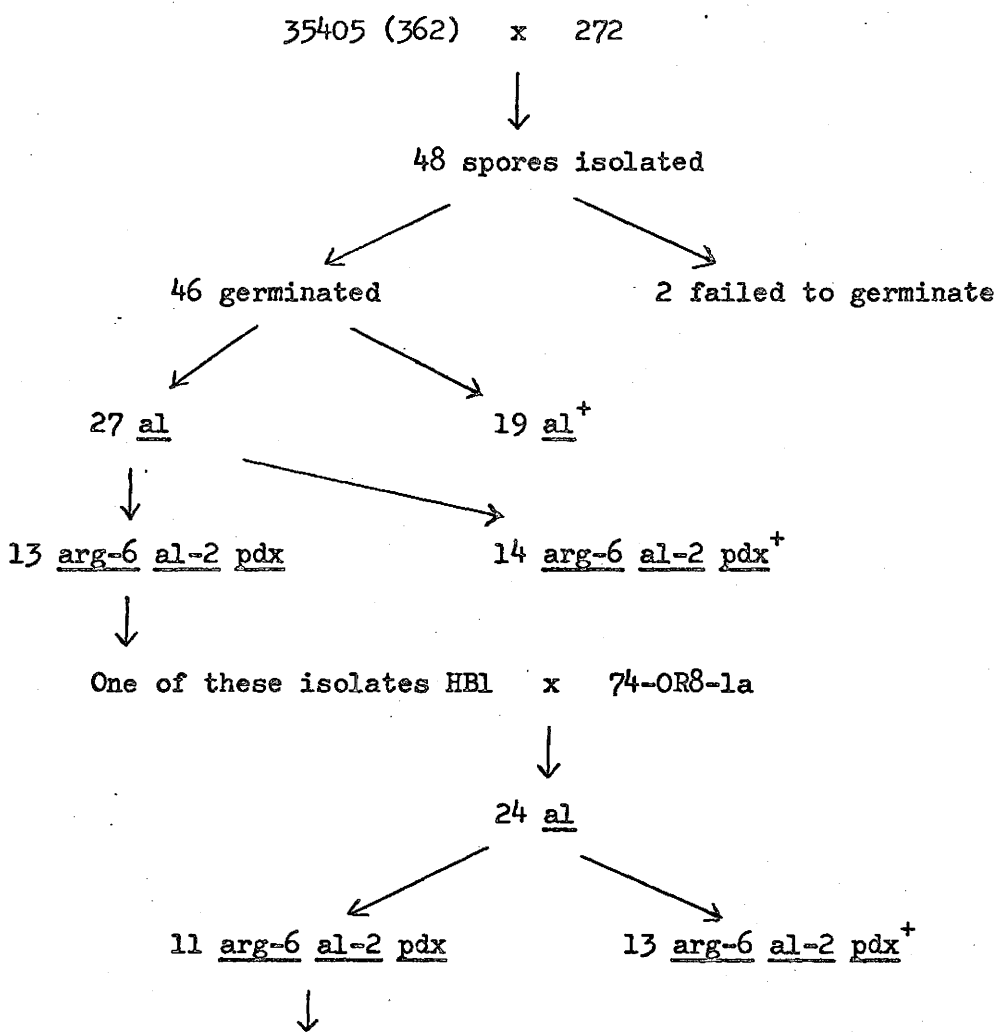
No arg-3, ylo-1 strains were immediately available, but two arg-3, ylo-1; tryp-2, ylo-1 were obtained from Dr. S.F.H. Threlkeld. There were #E229, mating type A, and #639-8, mating type a. The first four pdx strains were also crossed to one of these two strains.

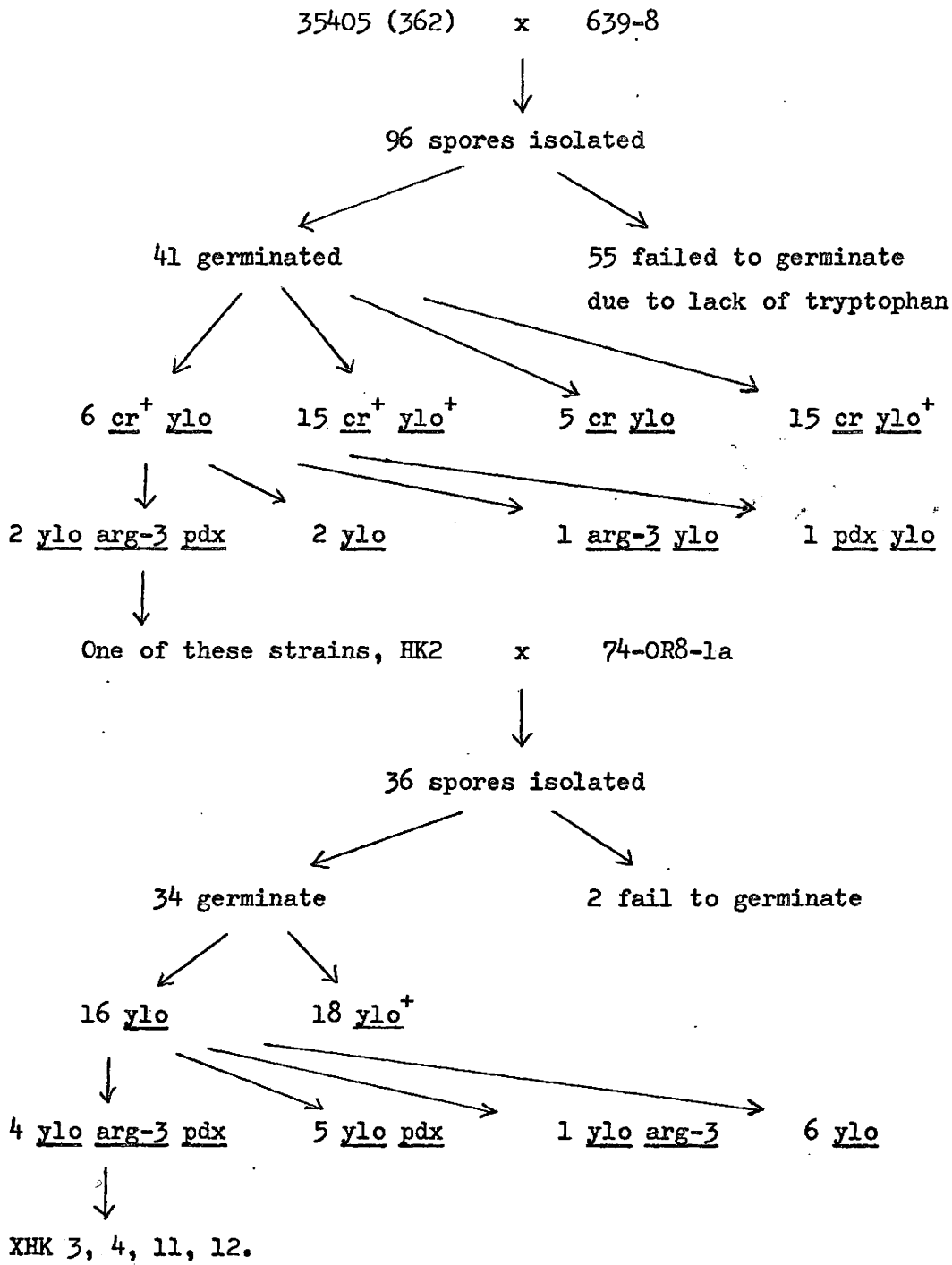
| FGSC# | allele# | m.t. | | |
|---|---|---|---|---|
| 76 | 37803 | A | x | 639.8 a |
| 362 | 35405 | A | x | 639.8 a |
| 370 | 39106 | A | x | 639.8 a |
| 657 | 44602 | A | x | 639.8 a |

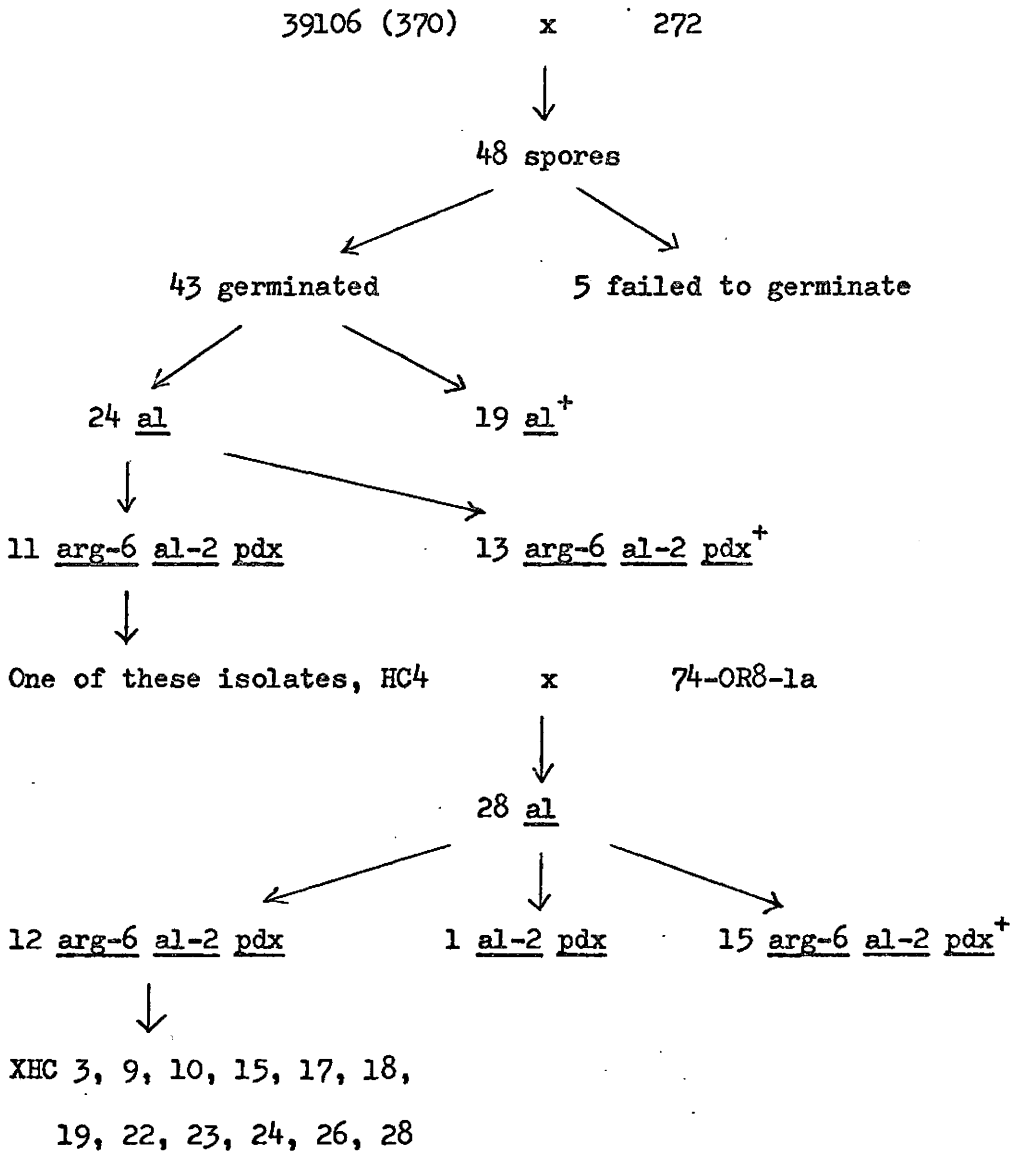
The results of these two series of crosses were as follows:

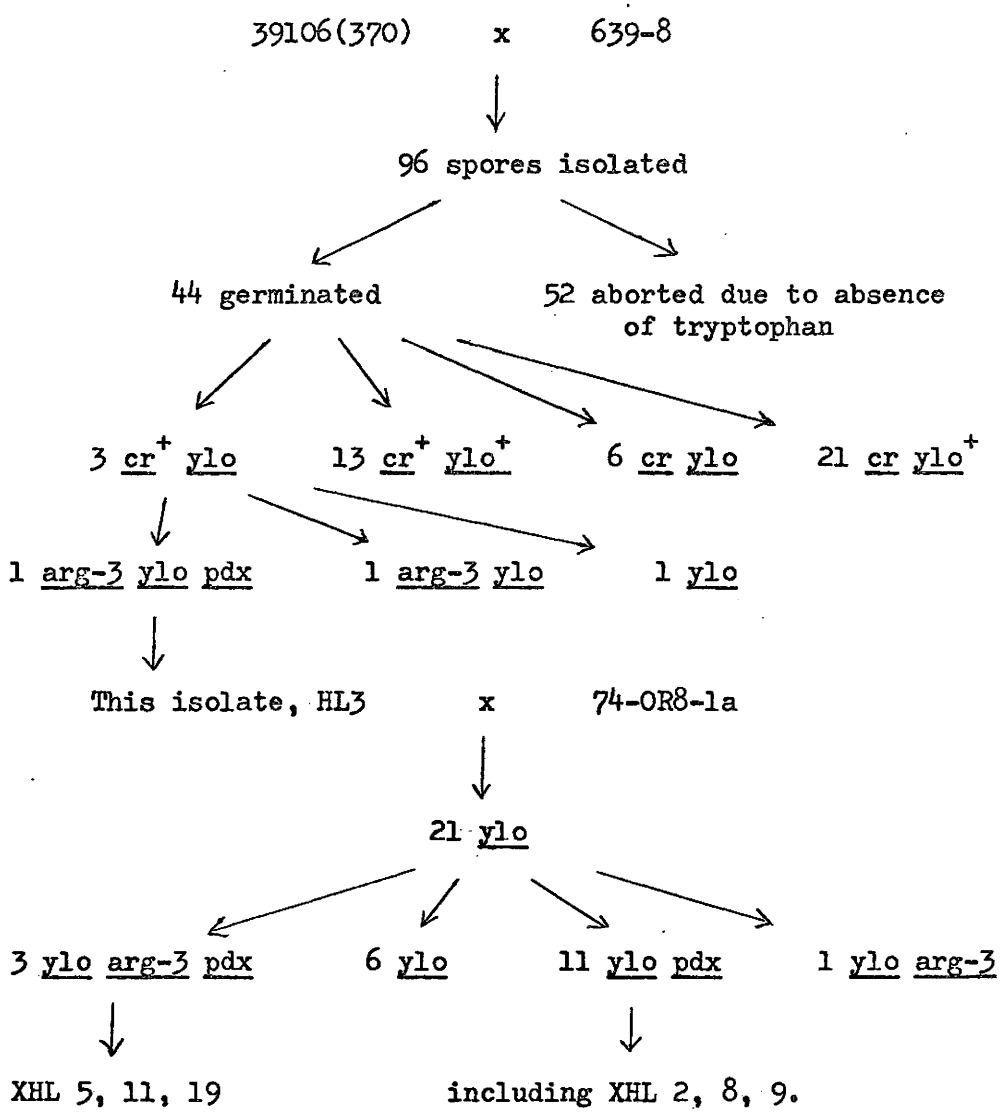

37803(76) x 272
48 spores isolated
45 germinated
3 failed to germinate
27 albino (al)
18 pink (al+)
13 arg-6 al-2 pdx
14 arg-6 al-2 pdx+
One of these isolates, HA1 x 74-OR8-1a
40 spores isolated
36 germinated
4 failed to germinate
19 albino
17 pink
8 arg-6 al-2 pdx
11 arg-6 al-2 pdx+
XHA 5, 9, 10, 11, 12, 13, 15, 17

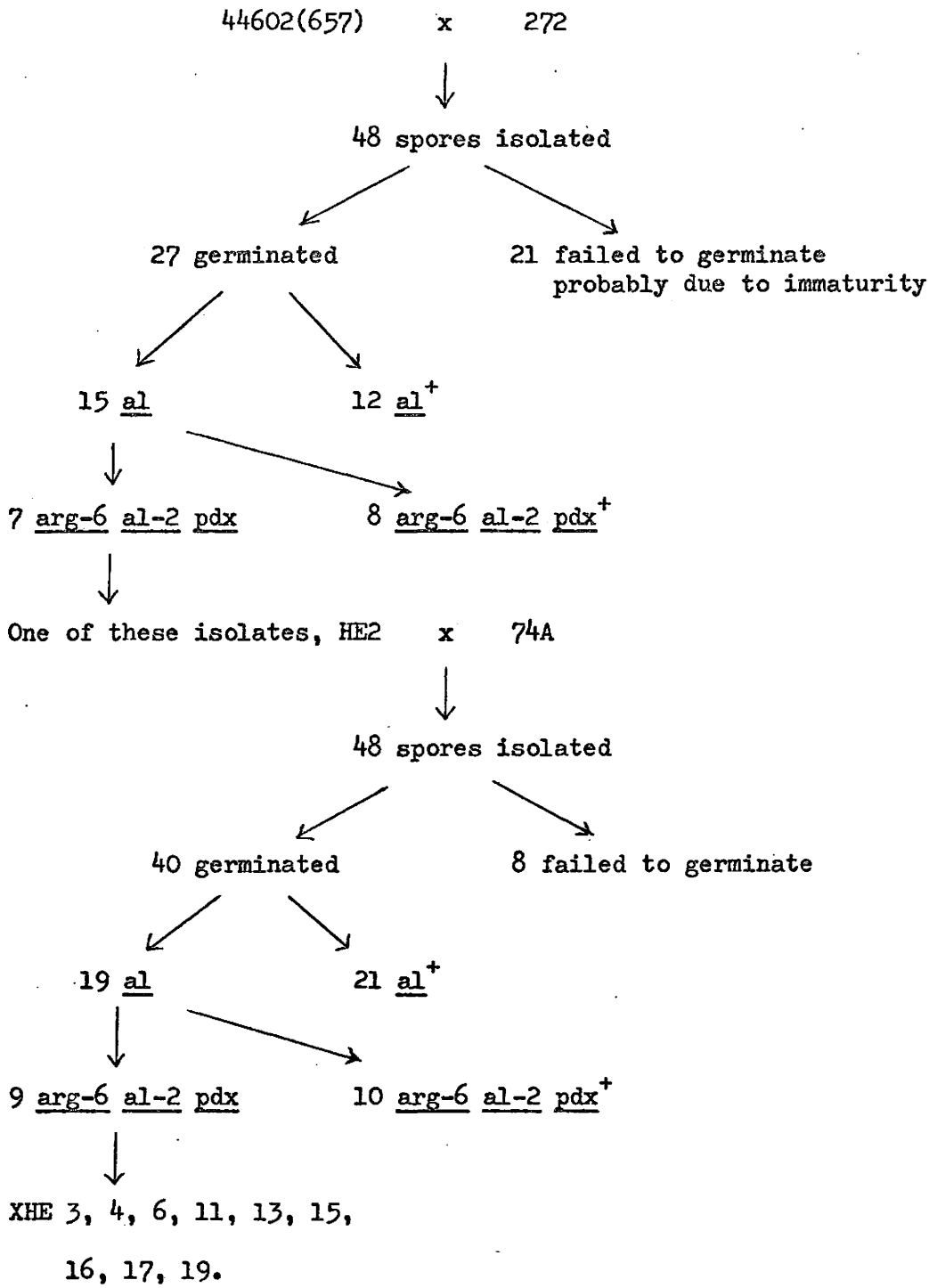

37803(76) x 639-8
96 spores isolated
50 germinated
46 aborted due to lack of tryptophan in medium
3 cr+ ylo
21 cr+ylo+
1 cr ylo
25 cr ylo+
all pdx+
arg-3 pdx ylo cr
This strain
74A x HJ4
48 spores isolated
44 germinated
4 failed to germinate
25 ylo
19 ylo+
6 cr ylo arg-3 pdx
10 cr ylo arg-3
XHJ 3,8, 17, 19, 21, 24
5 ylo pdx
4 ylo

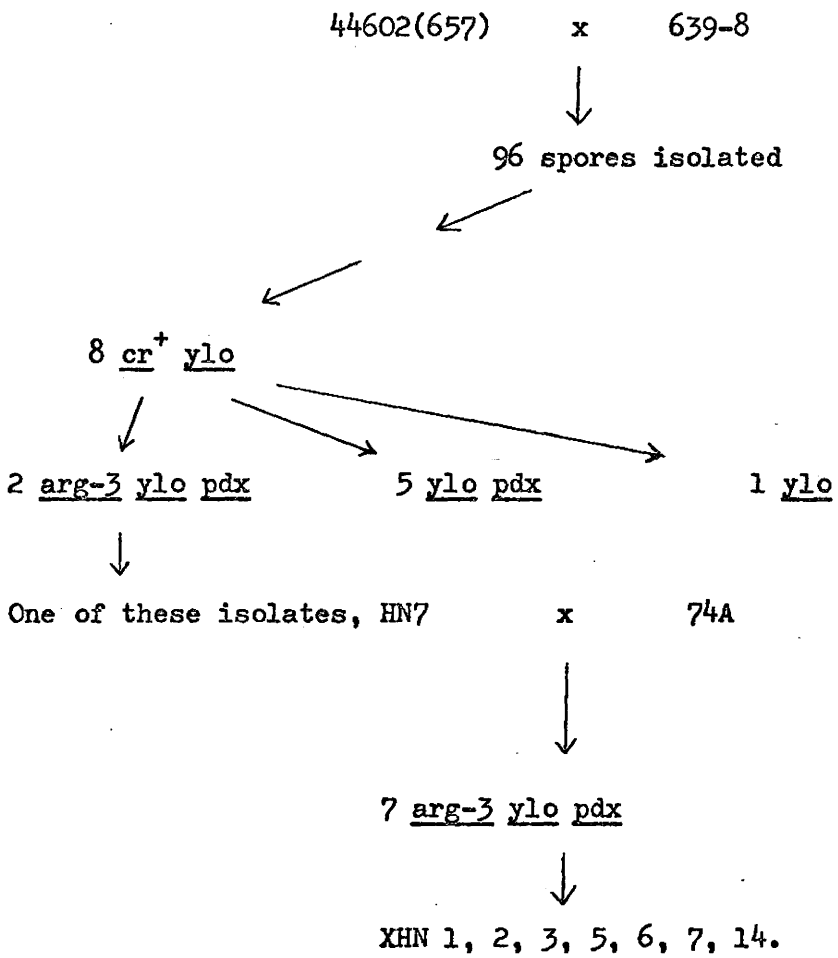

35405 (362) x 272
48 spores isolated
46 germinated
2 failed to germinate
27 al
19 al+
13 arg-6 al-2 pdx
14 arg-6 al-2 pdx+
One of these isolates HB1 x 74-OR8-1a
24 al
11 arg-6 al-2 pdx
13 arg-6 al-2 pdx+
XHB 2, 4, 5, 6, 7, 9, 10, 11, 21, 23, 24.

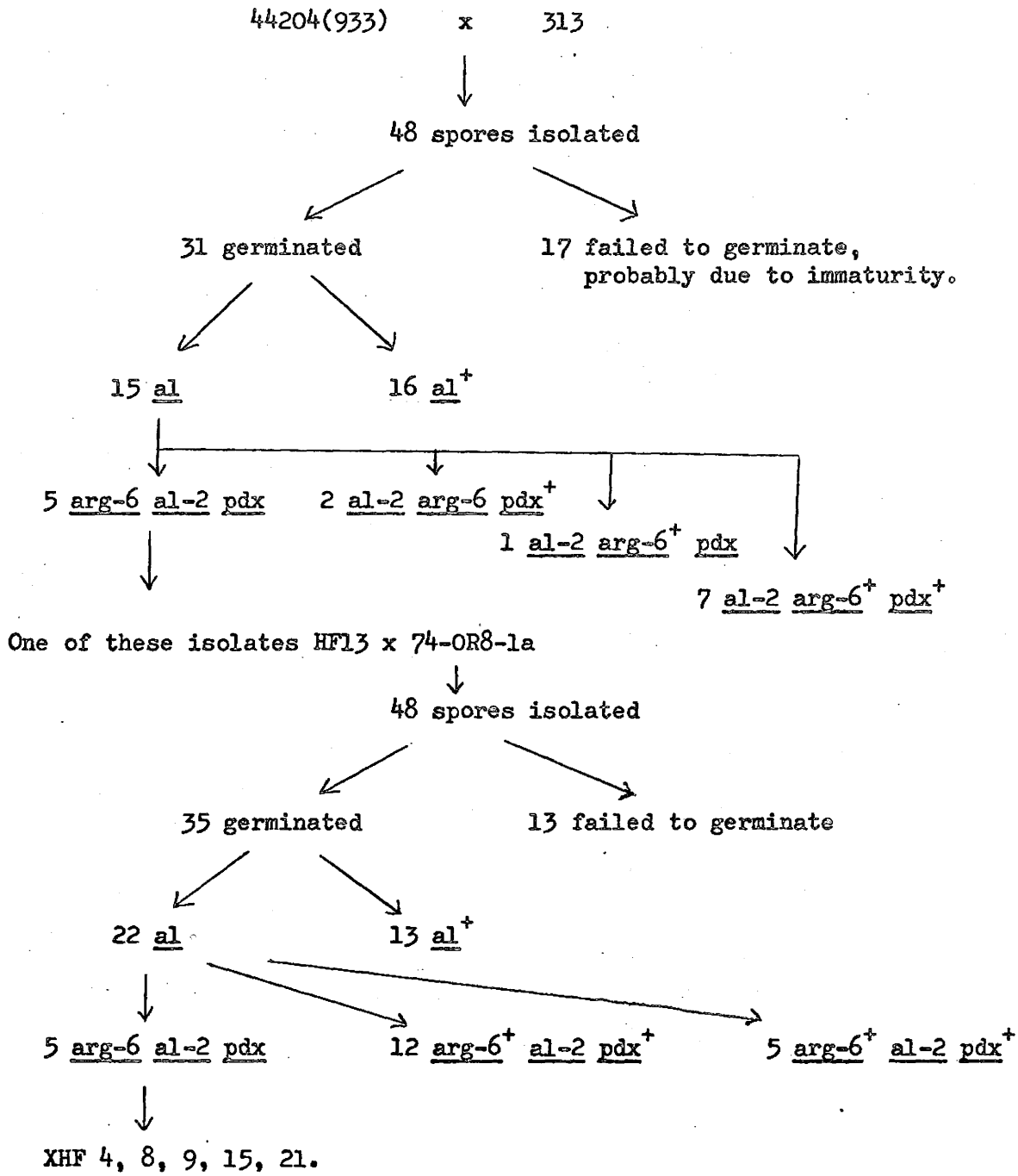

35405 (362) x 639-8
96 spores isolated
41 germinated
55 failed to germinate due to lack of tryptophan
6 cr+ ylo
15 cr+ ylo+
5 cr ylo
15 cr ylo+
2 ylo arg-3 pdx
2 ylo
1 arg-3 ylo
1 pdx ylo
One of these strains, HK2 x 74-OR8-1a
36 spores isolated
34 germinate
2 fail to germinate
16 ylo
18 ylo+
4 ylo arg-3 pdx
5 ylo pdx
1 ylo arg-3
6 ylo
XHK 3, 4, 11, 12.


39106 (370) x 272
48 spores
43 germinated
5 failed to germinate
24 al
19 al+
11 arg-6 al-2 pdx
13 arg-6 al-2 pdx+
One of these isolates, HC4 x 74-OR8-1a
28 al
12 arg-6 al-2 pdx
1 al-2 pdx
15 arg-6 al-2 pdx+
XHC 3, 9, 10, 15, 17, 18,
19, 22, 23, 24, 26, 28

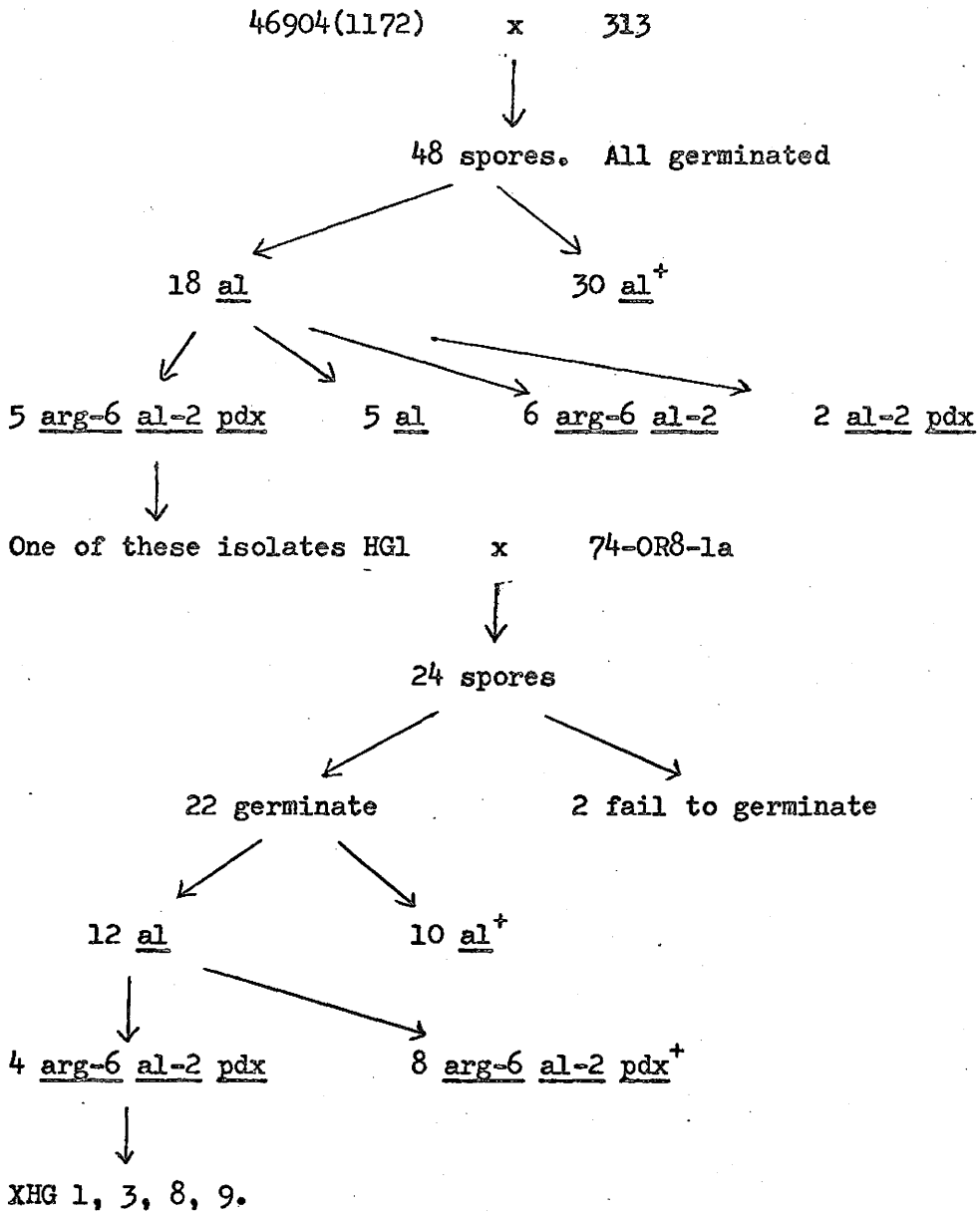

39106(370) x 639-8
96 spores isolated
44 germinated
52 aborted due to absence of tryptophan
3 cr+ ylo
13 cr+ ylo+
6 cr ylo
21 cr ylo+
1 arg-3 ylo pdx
1 arg-3 ylo
1 ylo
This isolate, HL3 x 74-OR8-1a
21 ylo
3 ylo arg-3 pdx
6 ylo
11 ylo pdx
1 ylo arg-3
XHL 5, 11, 19
including XHL 2, 8, 9.

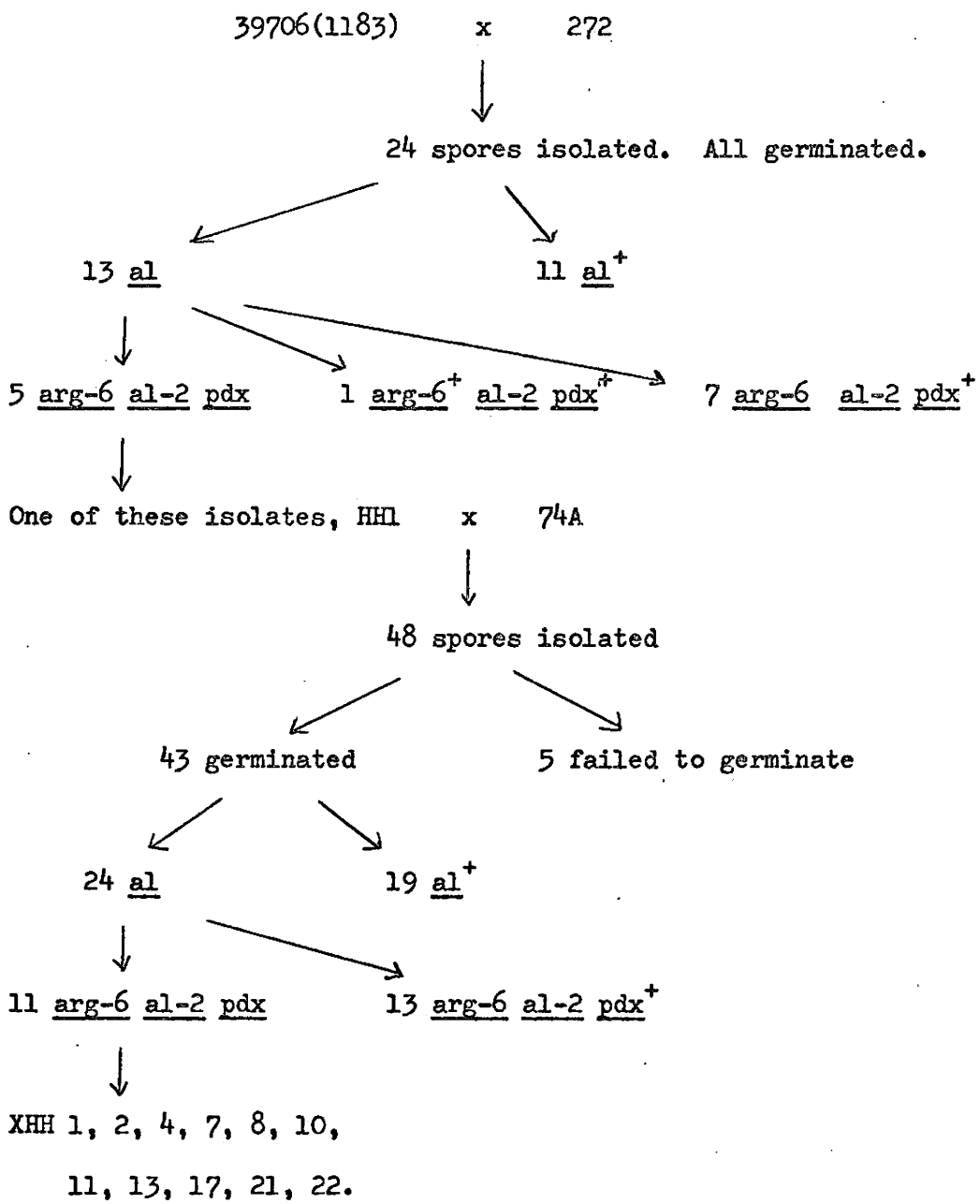

44602(657) x 272
48 spores isolated
27 germinated
21 failed to germinate probably due to immaturity
15 al
12 al+
7 arg-6 al-2 pdx
8 arg-6 al-2 pdx+
One of these isolates, HE2 x 74A
48 spores isolated
40 germinated
8 failed to germinate
19 al
21 al+
9 arg-6 al-2 pdx
10 arg-6 al-2 pdx+
XHE 3, 4, 6, 11, 13, 15,
16, 17, 19.


44602(657) x 639-8
96 spores isolated
8 cr+ ylo
2 arg-3 ylo pdx
5 ylo pdx
1 ylo
One of these isolates, HN7 x 74A
7 arg-3 ylo pdx
XHN 1, 2, 3, 5, 6, 7, 14.




In this cross and 1172 x 313, half of all albino isolates are arginine-independent despite their close linkage on linkage group I. The simplest explanation of these date would be the existence of a suppressor of arg-6, unlinked to any of the known genes in the crosses, in strains 933 and 1172.


46904(1172) x 313
48 spores. All germinated
18 al
30 al+
5 arg-6 al-2 pdx
5 al
6 arg-6 al-2
2 al-2 pdx
One of these isolates HG1 x 74-OR8-1a
24 spores
22 germinate
2 fail to germinate
12 al
10 al+
4 arg-6 al-2 pdx
8 arg-6 al-2 pdx+
XHG 1, 3, 8, 9.


39706(1183) x 272
24 spores isolated. All germinated.
13 al
11 al+
5 arg-6 al-2 pdx
1 arg-6+ al-2 pdx+
7 arg-6 al-2 pdx+
One of these isolates, HH1 x 74A
48 spores isolated
43 germinated
5 failed to germinate
24 al
19 al+
11 arg-6 al-2 pdx
13 arg-6 al-2 pdx+
XHH 1, 2, 4, 7, 8, 10,
11, 13, 17, 21, 22.

From the crosses detailed above, the following strains were selected for use in testing for heterokaryon complementation of the pdx alleles.

| | | | | |
|---|---|---|---|---|
| arg-6 | al-2 | pdx | 37803 | XHA 5, 9, 10, 11, 12, 13 |
| " | " | " | 35405 | XHB 2, 4, 5, 6, 7, 9 |
| " | " | " | 39106 | XHC 3, 9, 10, 15, 17, 18 |
| " | " | " | 44602 | XHE 3, 4, 6, 11, 13, 15 |
| " | " | " | 44204 | XHF 4, 8, 9, 21, 22* |
| " | " | " | 46904 | XHG 1, 3, 8, 9 |
| " | " | " | 39706 | XHH 1, 2, 4, 7, 8, 10 |

| | | | | | |
|---|---|---|---|---|---|
| cr | ylo-1 | arg-3 | pdx | 37803 | XHJ 3, 8, 17, 19, 21, 22 |
| " | " | " | " | 35405 | XHK 3, 4, 11, 12, 2*, 5* |
| " | " | " | " | 39106 | XHL 5, 11, 19, 2*, 8*, 9* |
| " | " | " | " | 44602 | XHN 1, 2, 3, 5, 6, 7 |

*These strains did not have a requirement for arginine, and therefore could not be used to test for heterokaron compatibility in the absence of pdx complementation.

From this first series of tests, the following combinations were found to form heterokaryons which exhibited complementation between the pdx alleles:-

| | | |
|---|---|---|
| XHA 10 (37803) | + | XHL 11 (39106) |
| XHA 9 | + | XHL 2 |
| XHA 10 | + | XHL 9 |
| XHB 2 (35405) | + | XHN 5 (44602) |
| XHC 15 (39106) | + | XHK 2 (35405) |

| | | |
|---|---|---|
| XHE 3 (44602) | + | XHK 2 (35405). |
| XHF 8 (44204) | + | XHK 3 (35405 |
| 9 | + | XHK 3 |
| 22 | + | XHK 3 |
| XHH 8 (39706 | + | XHK 3 (35405 |
| XHH 10 | + | XHK 2 |

Heterokaryon compatibility on medium containing pyridoxine, without complementation between the pdx alleles on medium containing desoxypyridoxine, was observed in the following combinations:-

| | | |
|---|---|---|
| XHC 3 (39106) | + | XHK 3 (35405) |
| XHC 10 | + | XHK 3 |
| XHC 17 | + | XHK 3 |
| XHC 18 | + | XHK 3 |
| XHE 4 (44602) | + | XHK 3 (35405) |
| XHE 6 | + | XHK 3 |
| XHE 11 | + | XHK 3 |
| XHE 3 (44602) | + | XHL 11 (39106) |
| XHF 4 (44204) | + | XHL 11 (39106) |
| XHH 10 (39706) | + | XHL 11 (39106) |

If a strain A is heterokaryon compatible with two other strains B and C, then strain B should be compatible with strain C as compatibility requires the same alleles at the mating type and three other loci (Wilson & Garnjobst 1966). On this basis, it was possible to make further tests without the necessity of isolating and testing more suitable isolates of the pdx alleles.

The pdx alleles described earlier as XHC 15, XHE 3, and XHH 10 all showed compatibility with XHK 2. They were also, on the above basis,

heterokaryon compatible with each other. As all three strains contained the arg-6 allele, it was necessary to innoculate the pairwise combinations of these strains on desoxypyridoxine-glucose medium supplemented with arginine. No complementation occurred between any pair of strains, so therefore the alleles 39106, 39706, and 44602 were not complementary with each other.

XHL 11 was compatible with XHA 10, XHF 4, XHE 3, and XHH 10. In the same manner, as explained above, these strains were also tested for complementation. XHA 10 proved to be complementary to XHE 3 and XHH 10, but the other combinations gave negative results. Therefore 37803 complemented both 44602 and 39706.

XHK 3 was compatible with XHF 8, XHF 9, XHA 11, and XHH 8. On testing these four strains with each other, no complementation was observed between any pair of alleles. The combination XHF 8 - XHH 8 was originally classified as exhibiting complementation (Radford 1965), but on re-synthesising the heterokaryon it was found that the original result could not be duplicated. As both component strains contained the same al-2 and arg-6 marker genes, conidial analysis of the first heterokaryon would not have given much information as to the cause of this discrepancy in results. However, in the light of subsequent studies of reversion at the pdx locus it seems likely that one of the component strains underwent a reversion or suppressor mutation which was selected for in that particular test tube.

Mitchell (Mitchell, M.B., 1955) had observed complementation between alleles 37803 and 39106, and Threlkeld (Threlkeld, 1961) had found complementation between 37803 and 44602. It was also known

(Lunan, 1963) that 46904 complements with both 44204 and 44602. These earlier results are in agreement with the present findings.

Use was then made of strains from other aspects of this study to complete the compatible pairs of alleles tested, using a test technique similar to that described in chapter 2 (i.e. using medium containing desoxypyridoxine). Compatible and complementing pairs of strains found in this experiment are shown below:- e.g.

XHG 8 (*pdx*(46904), *arg-6* *al-2*) - CR 9 (*pdx*(39706), *pyr-1*)

CP 117 (*pdx*(46904), *pyr-1*) - CO 52 (*pdx* (44204) *pyr-1*)

In order to check the degrees of complementation between the various alleles of the *pdx* locus, a quantitative study by means of a liquid medium growth experiment was required. A preliminary experiment indicated that the normal rate of supplementation with the amino acid arginine was limiting to the growth of arginine-requiring heterokaryons (i.e. those heterokaryons in which both nuclear types contained the same *arg* mutant). The experiment was therefore repeated, but with arginine added at the rate of 500 mg/litre. In addition to testing those pairs of strains which had shown qualitative evidence of complementation, certain other pairs of strains which were known to be compatible but which had not shown qualitative complementation were also tested.

The results of the growth experiment on liquid medium are shown below in Tables 5-2 and 5-3. Certain component strains were tested singly, and heterokaryons were both synthesised *de novo* at the beginning of the experiment by double innoculation of the flasks, and by innoculation of conidia from previously established heterokaryotic cultures.

Table 5-2

Heterokaryon-constituent strains tested singly on minimal medium + arginine (500 mg /l).

| Strain | weights of mycelium | | $\bar{x}$ | s |
|---|---|---|---|---|
| 74-OR8-1a | 76mg | 76mg | 76 | 0 |
| XHA 10 | 1 | 2 | 1.5 | 0.7 |
| XHA 11 | 5 | 2 | 3.5 | 2.1 |
| XHB 2 | 5 | 5 | 5.0 | 0 |
| XHC 15 | 5 | 7 | 6.0 | 1.4 |
| XHE 3 | 2 | 1 | 1.5 | 0.7 |
| XHF 4 | 1 | 2 | 1.5 | 0.7 |
| XHF 8 | 9 | 10 | 9.5 | 0.7 |
| XHG 8 | 3 | 3 | 3.0 | 0 |
| XHH 8 | 3 | 4 | 3.5 | 0.7 |
| XHH 10 | 1 | 2 | 1.5 | 0.7 |
| XHJ 3 | 1 | 1 | 1.0 | 0 |
| XHK 2 | 7 | 3 | 5.0 | 2.8 |
| XHK 3 | 4 | 7 | 5.5 | 2.1 |
| XHL 11 | 1 | 4 | 2.5 | 2.1 |
| XHN 2 | 1 | 1 | 1.0 | 0 |
| XHN 5 | 8 | 3 | 5.5 | 3.5 |

(Growth was for six days)

Table 5-3

The growth of heterokaryons on minimal medium + arginine (500 mg/litre).

| Strains | | Weights of mycelium | | $\bar{x}$ | s |
|---|---|---|---|---|---|
| XHA 10 | XHE 3 | 48mg | 40mg | 44 | 5.7 |
| XHA 11 | XHF 8 | 1 | 12 | 6.5 | 7.8 |
| XHA 10 | XHG 3 | 5 | 8 | 6.5 | 2.1 |
| XHA 10 | XHH 10 | 36 | 35 | 35.5 | 0.7 |
| XHA 11 | XHK 3 | 7 | 8 | 7.5 | 0.7 |
| XHA 9 | XHL 2 | 20 | 96 | 93 | 4.2 |
| XHA 10 | XHL 11 | 58 | 52 | 55 | 4.2 |
| XHB 2 | XHN 5 | 23 | 13 | 18 | 7.1 |
| XHC 15 | XHE 3 | 1 | 2 | 1.5 | 0.7 |
| XHC 9 | XHG 1 | 85 | 81 | 83 | 2.8 |
| XHC 9 | XHG 3 | 72 | 74 | 73 | 1.4 |
| XHC 15 | XHH 10 | 4 | 2 | 3.0 | 1.4 |
| XHC 15 | XHK 2 | 94 | 98 | 96 | 2.8 |
| XHE 3 | XHF 4 | 4 | 2 | 3.0 | 1.4 |
| XHE 3 | XHH 10 | 4 | 7 | 5.5 | 2.1 |
| XHE 3 | XHK 2 | 91 | 99 | 95 | 5.7 |
| XHE 3 | XHL 11 | 13 | 3 | 8 | 7.1 |
| XHF 8 | XHH 8 | 3 | 3 | 3 | 0 |
| XHF 8 | XHK 3 | 80 | 66 | 73 | 9.9 |
| XHF 9 | XHK 3 | 85 | 78 | 81.5 | 5.0 |
| XHF 22 | XHK 3 | 79 | 86 | 82.5 | 5.0 |
| XHF 4 | XHL 11 | 5 | 4 | 4.5 | 0.7 |
| XHG 8 | XHJ 3 | 3 | 6 | 4.5 | 2.1 |
| XHG 8 | XHK 3 | 3 | 6 | 4.5 | 2.1 |
| XHG 8 | XHN 2 | 58 | 65 | 61.5 | 5.0 |
| XHH 10 | XHK 2 | 95 | 10 | 87.5 | 10.6 |
| XHG 8 | CR 9 | 62 | 70 | 66 | 5.7 |
| CP 117 | CO 52 | 60 | 49 | 54.5 | 6.3 |

(Growth was for six days)

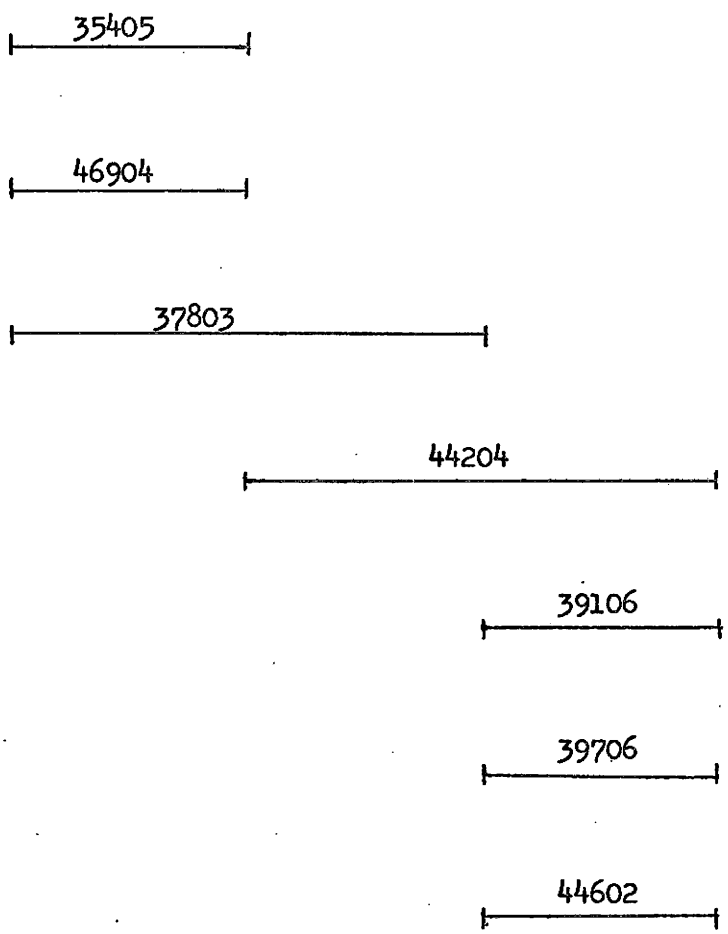
It will be seen that heterokaryotic combinations which do complement each other to give significantly more growth than the sum of the growth of the two constituent strains individually do not all grow at the same rate. Whereas XHA 10 - XHH 10 produced only 35.5 mg. of mycelium after six days, XHC 15 - XHK 2 produced 96 mg.. It was at first thought that this difference in growth may represent different degrees of partial complementation. However, two heterokaryons of the same two alleles in different genetic backgrounds show almost that degree of difference in their growth; XHA 9 - XHL 2 produced a mean of 93 mg., whereas XHA 10 - XHL 11 produced only 55 mg.. This is more suggestive of the growth potential being modified by the genetic background of the constituent alleles.

In the light of the evidence from the quantitative study of complementation regarding the lack of complementation between alleles 44204 and 39706, one modification must be made to the previous complementation map (Radford 1965). Alleles 39106, 39706, and 44602 all have the same complementation pattern. Each will complement with 37803, 35405, and 46904, but none will complement with 44204 or each other. Therefore it is not possible to put these three alleles in a linear order at the distal end of the complementation map. Figure 5-5 represents the complementation characteristics of the locus.

Figure 5-4

The complementation map of the pdx locus

at 25° C



(Lunan, 1964) stated that he obtained heterokaryons between 46904, 44204 or 44602 only when the strains carried the A mating type allele. Theoretically, heterokaryons should also be possible between a mating type strains, provided the alleles at the het loci are the same. In the present work, both A - A and a - a heterokaryons were produced.

An unexpected result was found in the present work when not all compatible combinations of strains of complementing alleles did in fact complement. Examples are XHC (39106) with XHK (35405) and XHE (44602)with XHK (35405). In each of these cases, a - a combinations complemented but A - A combinations did not.

It was also observed in the present work that no XHJ (37803) strains, all of which carried the marker cr, complemented with any other strain.

The striking thing about all of these unexpected results is that they are associated with linkage group I. It is suggestive of a gene, on linkage group I which, in one form,allows the expression of complementary pdx alleles, but in its other form inhibits that expression. It is unlikely that this postulated gene is either cr or mating type, as cr was not involved in Lunan's system, and the phenonenon is not consistently associated with either mating type. It can not be one of the het genes either as these are general in function, and no het gene is on linkage group I. All that can be said on the basis of present knowledge is that there appears to be a gene on linkage group I which, in one form allows the expression of complementation between complementary pdx alleles but in another form prevents

this expression. Another example of this type of phenomenon has been reported, (Woodward 1961).

Finally the division of the seven pdx alleles into the two loci pdx-1 (6 mutants) and pdx-2 (44204) which was postulated, on the basis of recombination data (Houlahan et al 1949) does not seem compatible with the present data. Houlahan et al postulated that pdx-2 is situated on linkage group IV a few crossover units distal to pdx-1. The complementation data, however, suggests that 44204 is situated in the middle of the six alleles of the pdx-1 locus.

As examples have been found of enzymes resulting from inter-allelic complementation having somewhat different physico-chemical characteristics than the enzyme produced by the wild-type gene, and that this may be reflected by the gross growth characteristics of the resultant heterokaryon such as increased temperature-sensitivity (Case & Giles, 1960, Fincham, 1957), the effect of temperature on complementation at the pdx locus was also investigated.

Relative growth of heterokaryons at 25° and 37° was investigated as before, using the technique of growth on liquid medium (Horowitz 1947), for a growth period of four days. This relatively short growth period, terminating in the middle of the log phase of growth (Strauss, 1951) was chosen to maximise any differences in growth rate of different strains. The experiment was carried out in duplicate, and the results are shown below in Table 5-5.

Table 5-5.

The effect of temperature on the growth of pdx heterokaryons - complementing heterokaryons. Growth was for 4 days.

| Strains | Alleles | 25° Wts. | 25° $\bar{x}$ | 25° s | 37° Wts. | 37° $\bar{x}$ | 37° s |
|---|---|---|---|---|---|---|---|
| XHK 2 XHC 15 | 35405 - 39106 | 25 mg.<br>28 | 26.5 | 2.1 | 47 mg.<br>73 | 60 | 18.5 |
| XHK 2 XHH 10 | 35405 - 39706 | 32<br>35 | 33.5 | 2.1 | 46<br>44 | 45 | 1.4 |
| XHK 3 XHF 8 | 35405 - 44204 | 48<br>46 | 47 | 1.4 | 58<br>68 | 63 | 7.1 |
| XHK 2 XHE 3 | 35405 - 44602 | 50<br>41 | 45.5 | 6.4 | 50<br>39 | 44.5 | 7.8 |
| XHA 10 XHL 11 | 37803 - 39106 | 30<br>22 | 26 | 5.7 | 31<br>31 | 31 | 0.0 |
| XHA 10 XHH 10 | 37803 - 39706 | 13<br>12 | 12.5 | 0.7 | 38<br>38 | 38 | 0.0 |
| XHA 10 XHE 3 | 37803 - 44602 | 11<br>20 | 15.5 | 6.4 | 29<br>31 | 30 | 1.4 |
| XHG 3 XHC 9 | 46904 - 39106 | 37<br>30 | 33.5 | 5.0 | 36<br>37 | 36.5 | 0.7 |
| XHG 8 CR 9* | 46904 - 39706 | 35<br>32 | 33.5 | 2.1 | 40<br>35 | 37.5 | 3.5 |
| CG 30* CO 64* | 46904 - 44204 | 20<br>13 | 16.5 | 5.0 | 13<br>19 | 16 | 4.2 |
| CP 162* PE 9* | 46904 - 44602 | 26<br>24 | 25 | 1.4 | 0.0<br>0.0 | | |
| CG 30* PE 9* | 46904 - 44602 | 11<br>10 | 10.5 | 0.7 | 0.0<br>0.0 | | |

* Strains with other outside markers:-

CG 30, *col-4*

CR 9, CO 64, CP 162, *pyr-1*

PE 9, *al-2*

Table 5-5 (cont'd.)

Non-complementing heterokaryons.

| Strains | Alleles | 25° Wts. | 25° $\bar{x}$ | 25° s | 37° Wts. | 37° $\bar{x}$ | 37° s |
|---|---|---|---|---|---|---|---|
| XHK 3 XHA 11 | 35405 - 37803 | 8.3 mg.<br>6.0 | 7.2 | 1.6 | 2.8 mg.<br>2.6 | 2.7 | 0.1 |
| XHK 3 XHG 8 | 35405 - 46904 | 4.0<br>2.4 | 3.2 | 1.1 | 1.8<br>1.4 | 1.6 | 0.3 |
| XHA 11 XHF 8 | 37803 - 44204 | 0.5<br>1.0 | 0.8 | 0.4 | 0.2<br>0.4 | 0.3 | 0.1 |
| XHL 11 XHH 10 | 39106 - 39706 | 2.9<br>1.0 | 2.0 | 1.3 | 2.0<br>1.1 | 1.6 | 0.6 |
| XHL 11 XHF 4 | 39106 - 44204 | 0.8<br>2.6 | 1.7 | 1.3 | 0.3<br>0.4 | 0.4 | 0.1 |
| XHL 11 XHE 3 | 39106 - 44602 | 3.4<br>3.8 | 3.6 | 0.3 | 2.7<br>1.0 | 1.9 | 1.2 |
| XHH 8 XHF 8 | 39706 - 44204 | 1.0<br>0.9 | 1.0 | 0.1 | 0.2<br>0.1 | 0.2 | 0.1 |
| XHH 10 XHE 3 | 39706 - 44602 | 3.7<br>8.5 | 6.1 | 3.4 | 0.7<br>1.9 | 1.3 | 1.0 |
| XHF 4 XHE 3 | 44204 - 44602 | 3.2<br>1.6 | 2.4 | 1.1 | 0.4<br>0.5 | 0.5 | 0.1 |
| Wild-type control | 74-OR8-1a | 53.0<br>53.0 | 53.0 | 0.0 | 51.0<br>45.0 | 48.0 | 4.2 |

In the quantitative complementation study, the interpretation of the segments as complons was merely on the basis that a continuum was produced by the overlapping of the alleles. That is to say, regions of common influence occur between adjacent alleles where the fields of influence of the individual alleles overlap. Such a pattern is characteristic of mutants within a cistron, whereas normally a clear break is observed between spheres of influence of mutants in different cistrons. An exception to this general rule is found in an operon system such as hist-3 in Neurospora crassa (Ahmed et al, 1965), for in an operon, the unit of transcription extends over several cistrons and the direction of transcription is constant. Because of this directional transcription, a frame shift mutant or a transcription-stopping mutant prevents normal transcription and translation distally, thus possibly affecting several cistrons and mimicing the complementation pattern of a single cistron. Further evidence of this interpretation may be found in the quantitative complementation data presented in Table 5-5.

The first piece of evidence is the temperature-sensitivity of heterokaryons involving the two alleles 44602 and 46904. ( Similar temperature-sensitive multimeric enzymes and heterokaryons have been found in the am and pan-2 loci, both of which are single cistrons (Case & Giles, 1960, Fincham, 1957)). Other complementing heterokaryons, for example 44204 - 46904 and 37803 - 39106 exhibit a significant reduction in growth at both 25° and 37° compared to wild-type, and 37803 - 39706 shows a much greater increase in growth with the 12° increase in temperatire than any other heterokaryon. Whereas the 44602 - 46904 heterokaryon loses its activity at 37°, the 37803 - 39706 heterokaryon is in fact much more efficient at the higher temperature. Finally, all non-complementing

pairs of alleles show a reduction of their leakiness at the higher temperature compared to their growth at 25°, see Figure 5-6.

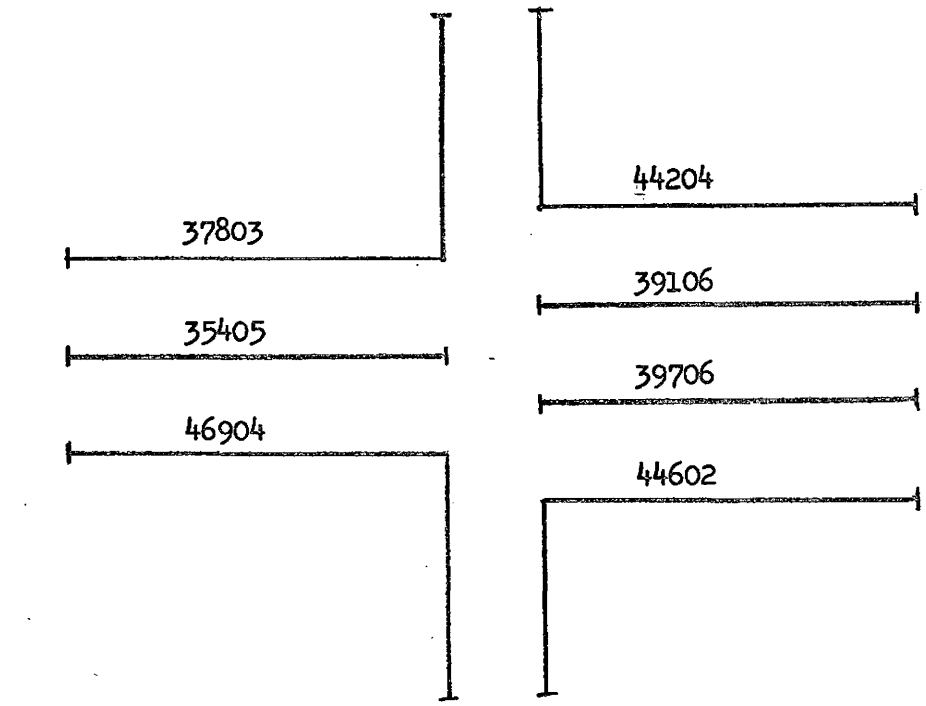
Of all pairwise combinations of alleles tested for complementation, the seven alleles fell into two groups within each group of which no pair of alleles complemented. These two groups were alleles 35405, 37803, 46904, and 39106, 39706, 44204, 44602. Possibly in one of these groups, if two alleles could not complement, three or four together in the same heterokaryon may do so. From the previous heterokaryon tests it was possible to select strains with compatibility, and only these strains were used in the study.

In group A (35405, 37803, and 46904), it had previously been found that XHK 3, XHA 11, and XHG 8 had the necessary homozygosity for the het loci. In group B (39106, 39706, 44204, and 44602) suitable strains were XHL 11, XHH 10, XHF 4, and XHE 3, and within this group three three-component heterokaryons and one four-component heterokaryon could be synthesised and tested for prototrophy. XHL 11 was marked with arg-3 and ylo-1 whereas the other three strains were arg-6, al-2; therefore any heterokaryon between these alleles containing XHL 11 would complement for arginine requirement. The three component system XHH 10, XHF 4, XHE 3 would, however, require supplementation with arginine.

The possible combinations of strains in groups A and B are shown in Figure 5-7.

Figure 5-6

Two representations of the complementation data at 37°



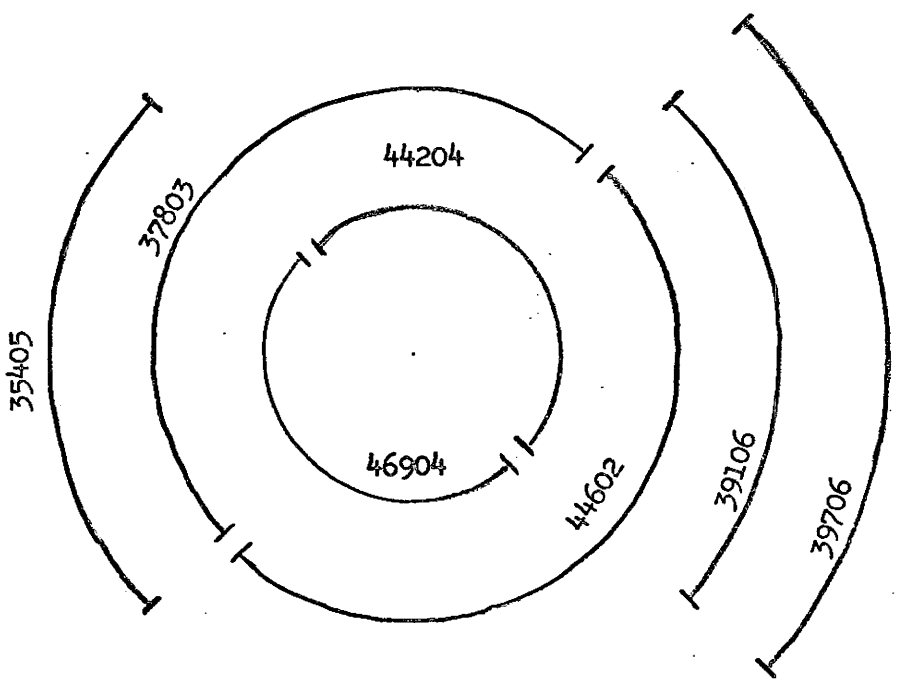



TABLE 5-7

The growth of multiple component pdx heterokaryons after 4 days on liquid minimal medium.

| | | 25° | | | 37° | | |
|---|---|---|---|---|---|---|---|
| Strains | Alleles | Wts. | $\bar{x}$ | s | Wts. | $\bar{x}$ | s |
| XHK 3 | 35405 | 4.4mg | | | 2.2mg | | |
| XHA 11 | 37803 | 4.5 | 4.5 | 0.0 | 1.7 | 2.0 | 0.8 |
| XHG 8 | 46904 | 4.5 | | | 3.1 | | |
| XHL 11 | 39106 | 3.3 | | | 1.0 | | |
| XHH 10 | 39706 | 2.3 | 2.5 | 0.7 | 1.2 | 1.2 | 0.1 |
| XHF 4 | 44204 | 2.0 | | | 1.4 | | |
| XHL 11 | 39106 | 1.8 | | | 1.5 | | |
| XHH 10 | 39706 | 2.0 | 1.9 | 0.0 | 1.2 | 1.3 | 0.2 |
| XHE 3 | 44602 | 1.8 | | | 1.2 | | |
| XHL 11 | 39106 | 1.7 | | | 1.6 | | |
| XHF 4 | 44204 | 2.2 | 2.4 | 0.8 | 1.5 | 1.6 | 0.1 |
| XHE 3 | 44602 | 3.3 | | | 1.8 | | |
| XHH 10 | 39706 | 4.0 | | | 1.8 | | |
| XHF 4 | 44204 | 4.2 | 4.3 | 0.4 | 2.6 | 2.0 | 0.6 |
| XHE 3 | 44602 | 4.8 | | | 1.6 | | |
| XHL 11 | 39106 | 1.9 | | | 1.3 | | |
| XHH 10 | 39706 | 4.0 | 2.4 | 1.4 | 1.0 | 1.1 | 0.2 |
| XHF 4 | 44204 | 1.3 | | | 1.0 | | |
| XHE 3 | 44602 | | | | | | |

Although no stimulation of growth on pyridoxineless medium was observed in the above multiple component heterokaryons over the component strains in isolation (Table 5-2) or the two component heterokaryons (Table 5-3), the negative results shown in Table 5-7 are not readily interpreted. That the active product of the pdx gene appears to be at least dimeric is deducible from the fact that complementation is possible between two different alleles. That complementation has not been found between three alleles that do not complement in pair-wise combination does not eliminate the possible existence of a trimeric or multimeric gene product. A positive result of this test would have strongly suggested that the gene product was at least trimeric, but to prove that the gene product is no more than a dimer is not possible by this method.

## CHAPTER VI

## INTER-ALLELIC RECOMBINATION AT THE PDX-1 LOCUS

Assuming an approximate additivity for inter-gene distances, it is possible to order genes on the basis of recombination frequency. This convention is useful in highly in-bred strains, but because of genetic control of recombination frequencies (Jessop and Catchside, 1965, Griffiths & Threlkeld, 1966), it is of little use for ordering genes in strains where heterozygosity for recombination genes exists. Therefore the conventional three point cross (Maling, 1959), is used to order the genes independently of the rate of recombination itself.

In the ordering of mutant sites within a gene or polaron, not only is the rate of recombination under genetic control (Jessop & Catcheside, 1965) but the very nature of the recombination event is more complex with frequency of recombination between two mutant sites reflecting not simply the distance between the sites, but the polarity of unwinding of the DNA in the polaron and the distance of the proximal mutant site to the breakage point (Whitehouse 1963, Holliday 1964, Whitehouse & Hastings 1965).

In mapping alleles within a gene, with one exception ( Case & Giles 1964), it has been impractical to do more than cross two singly mutant strains for the locus under study. The strains produced for such crosses are marked by two functionally unrelated marker genes, one proximal, and one distal, to the locus under study. These outside

markers are not selected for during prototroph selection at the subject locus, but prototrophs for the subject locus are subsequently analysed for these outside markers.

In the present study of the pdx-1 locus, pyr-1 was used as the proximal marker, and col-4 as the distal. Pyr-1 was not chosen freely but was used as it is the only locus known which is left of the pdx-1 locus on linkage group IV. Col-4 was selected as it was easily classified, and being a morphological mutant did not require plating on sorbose but merely inspection of the sub-culture tube to determine its genotype.

The parental strains for prototroph crosses were produced as follows:-

Allele #37803

H815 (pyr-1, pdx-1) x 74-OR8-1a RJ 1, 2, 3, 4, 5, 6, 7, 8.

404 (pdx-1, col-4) x 74-OR8-1a RA 1, 2, 3, 4, 5.

Allele #35405

362 (pdx-1) x 85 (pyr-1)

CK 105 (pyr-1, pdx-1) x 74-OR8-1a RK 1, 2, 3, 4.

388 (pdx-1, col-4) x 74-OR8-1a RB 1, 2, 3, 4, 5.

Allele #39106

370 (pdx-1) x 85 (pyr-1)

CL 16 (pyr-1, pdx-1) x 74-OR8-1a RL 1, 2.

345 (pdx-1, col-4) x 74-OR8-1a RC 1, 2, 3, 4, 5.

Allele #44602

657 (pdx-1) x 85 (pyr-1)
↓
CN 133 (pyr-1, pdx-1) x 74-OR8-1a → RN 1, 2, 3.

657 (pdx-1) x 67 (col-4)
↓
CE 49 (pdx-1, col-4) x 74-OR8-1a → RE 1, 2, 3, 4, 5, 6.

Allele #44204

933 (pdx-2?) x 72 (pyr-1)
↓
CO 52 (pyr-1, pdx-2?) x 74-OR8-1a → RO 1, 2, 3, 4, 5, 6, 7, 8.

933 (pdx-2?) x 486 (col-4)
↓
CF 47(pdx-2?, col-4) x 74-OR8-1a → RF 1, 2, 3, 4.

Allele #46904

1172 (pdx-1) x 72 (pyr-1)
↓
CP 162 (pyr-1, pdx-1) x 74-OR8-1a → RP 1, 2, 3, 4, 5, 6, 7, 8.

1172 (pdx-1) x 486 (col-4)
↓
CG 5 (pdx-1, col-4) x 74-OR8-1a → RG 1, 2, 3, 4, 5.

Allele #39706

1183 (pdx-1) x 85 (pyr-1)
↓
CR 9 (pyr-1, pdx-1) x 74-OR8-1a → RR 1, 2, 3, 4, 5.

1183 (pdx-1) x 67 (col-4)
↓
CH 6 (pdx-1, col-4) x 74-OR8-1a → RH 1, 2, 3.

From the above "R-series" strains, one pyr-1, pdx-1; A strain, and one pdx-1, col-4; a of each pdx allele, was selected for use in the

high resolution recombination study. These were RA 3, RB 3, RC 3, RE 3, RF 4, RG 3 (poor fertility, later replaced by RG 5), and RH 1 of the colonial type, and RJ 3, RK 2, RL 1, RN 3, RO 1, RP 7, and RR 1 of the pyrimidine-requiring type.

Crosses were made according to the general procedure shown below:-

pyr-1, pdx-x; A x pdx-y, col-4; a

where x and y represent any allele or pair of alleles of the pdx mutant. To eliminate possible cytoplasmic effects, the colonial strain was always used as the protoperithecial parent.

Each colonial pdx strain was crossed to each pyrimidine-requiring pdx strain, giving a total of 49 possible cross combinations. The crosses were numbered according to the scheme shown below in Figure 6-1.

Figure 6-1

Numbering of the inter-allelic crosses

| Pyr-1 Parent | Col-4 Parent: RA 3 | RB 3 | RC 3 | RE 3 | RF 4 | RG 3/5 | RH 1 |
|---|---|---|---|---|---|---|---|
| RJ 3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| RK 2 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| RL 1 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| RN 3 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| RO 1 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
| RP 7 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
| RR 1 | 43 | 44 | 45 | 46 | 47 | 48 | 49 |

Details of the results of these crosses, corrected for pseudo-wild types, are given in Table 6-2.

TABLE 6-2

The results of the second-generation inter-allelic high resolution crosses.

| Cross # | col parent | pyr parent | % viability of spores | total spores treated | total proto-trophs | protos per $10^5$ sp. | Outside Markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 37803 | 37803 | 61% | 144,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 35405 | 37803 | 91% | 570,000 | 2 | 0.35 | 1 | 1 | 0 | 0 |
| 3 | 39106 | 37803 | 98% | 100,000 | 144 | 144 | 50 | 15 | 13 | 12 |
| 3A | 39106 | 37803 | 94% | 14,000 | 17 | 121 | 9 | 1 | 2 | 5 |
| 4 | 44602 | 37803 | 80% | 7,000 | 23 | 329 | 13 | 3 | 0 | 5 |
| 5 | 44204 | 37803 | 90% | 103,000 | 174 | 169 | 49 | 9 | 16 | 11 |
| 6 | 46904 | 37803 | 86% | 68,000 | 3 | 4.41 | 0 | 1 | 1 | 1 |
| 7 | 39706 | 37803 | 95% | 32,000 | 53 | 166 | 14 | 6 | 4 | 32 |
| 8 | 37803 | 35405 | 91% | 800,000 | 9 | 1.13 | 3 | 0 | 1 | 5 |
| 9 | 35405 | 35405 | 94% | 300,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 39106 | 35405 | 88% | 43,000 | 78 | 181 | 43 | 12 | 7 | 11 |
| 11 | 44602 | 35405 | 92% | 31,000 | 20 | 64.5 | 5 | 5 | 3 | 7 |
| 12 | 44204 | 35405 | 86% | 146,000 | 67 | 45.9 | 24 | 22 | 9 | 12 |
| 13 | 46904 | 35405 | 80% | 10,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 39706 | 35405 | | 30,000 | 33 | 110 | 8 | 11 | 9 | 5 |

TABLE 6-2 cont'd

| Cross # | col parent | pyr parent | % viability of spores | total spores treated | total proto-trophs | protos per $10^5$ sp. | Outside Markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|---|
| 15 | — | — | — | — | — | — | — | — | — | — |
| 16 | — | — | — | — | — | — | — | — | — | — |
| 17 | — | — | — | — | — | — | — | — | — | — |
| 18 | — | — | — | — | — | — | — | — | — | — |
| 19 | — | — | — | — | — | — | — | — | — | — |
| 20 | — | — | — | — | — | — | — | — | — | — |
| 21 | — | — | — | — | — | — | — | — | — | — |
| 22 | 37803 | 44602 | 78% | 179,000 | 304 | 170 | 34 | 1 | 55 | 2 |
| 23 | 35405 | 44602 | 95% | 29,000 | 46 | 159 | 19 | 3 | 24 | 0 |
| 24 | 39106 | 44602 | 98% | 38,000 | 12 | 31.6 | 10 | 0 | 2 | 0 |
| 25 | 44602 | 44602 | 94% | 13,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 44204 | 44602 | 98% | 60,000 | 2 | 3.33 | 1 | 0 | 1 | 0 |
| 27 | 46904 | 44602 | 97% | 74,000 | 105 | 142 | 20 | 5 | 15 | 3 |
| 28 | 39706 | 44602 | 79% | 121,000 | 18 | 14.9 | 6 | 0 | 11 | 1 |
| 28A | 39706 | 44602 | 92% | 123,000 | 13 | 10.6 | 5 | 0 | 8 | 0 |
| 29 | 37803 | 44204 | 84% | 62,000 | 155 | 250 | 20 | 0 | 45 | 22 |
| 30 | 35405 | 44204 | 80% | 52,000 | 109 | 210 | 16 | 3 | 48 | 20 |

TABLE 6-2 cont'd

| Cross # | col parent | pyr parent | % viability of spores | total spores treated | total proto-trophs | protos per $10^5$ sp. | Outside Markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|---|
| 31 | 39106 | 44204 | 82% | 89,000 | 67 | 75.3 | 11 | 5 | 11 | 40 |
| 32 | 44602 | 44204 | 76% | 110,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 44204 | 44204 | 77% | 47,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | 46904 | 44204 | 76% | 88,000 | 71 | 80.7 | 14 | 1 | 13 | 10 |
| 35 | 39706 | 44204 | 67% | 11,000 | 3 | 27.3 | 0 | 0 | 2 | 1 |
| 36 | 37803 | 46904 | 88% | 4,680,000 | 39 | 0.802 | 12 | 0 | 25 | 1 |
| 37 | 35405 | 46904 | 93% | 510,000 | 13 | 2.54 | 4 | 0 | 8 | 0 |
| 38 | 39106 | 46904 | 91% | 134,000 | 117 | 87.3 | 72 | 0 | 12 | 3 |
| 39 | 44602 | 46904 | 90% | 8,000 | 8 | 100 | 2 | 2 | 2 | 2 |
| 40 | 44204 | 46904 | 96% | 106,000 | 151 | 142 | 49 | 0 | 14 | 1 |
| 41 | 46904 | 46904 | | N O T | F E R T I L E | | | | | |
| 42 | 39706 | 46904 | 84% | 13,000 | 40 | 308 | 24 | 0 | 16 | 0 |
| 43 | 37803 | 39706 | 77% | 88,000 | 52 | 59.0 | 14 | 2 | 26 | 10 |
| 43A | 37803 | 39706 | 80% | 360,000 | 299 | 83.1 | 27 | 4 | 41 | 22 |
| 44 | 35405 | 39706 | 91% | 210,000 | 92 | 438 | 48 | 1 | 26 | 17 |

TABLE 6-2 cont'd

| Cross # | col parent | pyr parent | % viability of spores | total spores treated | total proto-trophs | protos per $10^9$ sp. | Outside Markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 39106 | 39706 | 94% | 580,000 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | 44602 | 39706 | 70% | 11,000 | 2 | 18.2 | 0 | 1 | 0 | 1 |
| 47 | 44204 | 39706 | 92% | 54,000 | 12 | 22.2 | 3 | 7 | 0 | 2 |
| 48 | 46904 | 39706 | 82% | 17,000 | 23 | 135 | 17 | 2 | 3 | 1 |
| 49 | 39706 | 39706 | 74% | 500,000 | 0 | 0 | 0 | 0 | 0 | 0 |

Several crosses were duplicated to test the reliability of the technical details of prototroph analysis and these duplicate crosses are analysed statistically below to test the reproducibility of the technique.

Crosses 3 and 3A

| prototrophs | colonies on comp. medium | |
|---|---|---|
| 144 | 100 | 244 |
| 17 | 14 | 31 |
| 161 | 114 | 275 |

$X^2$ = 0.181 P > 0.5

| col + | col pyr | + + | + pyr | |
|---|---|---|---|---|
| 50 | 15 | 13 | 12 | 90 |
| 9 | 1 | 2 | 5 | 17 |
| 59 | 16 | 15 | 17 | 107 |

$X^2$ = 3.60 P > 0.3

Crosses 28 and 28A

| prototrophs | colonies | |
|---|---|---|
| 18 | 121 | 139 |
| 13 | 123 | 136 |
| 31 | 244 | 275 |

$X^2$ = 0.769 P 0.3

| col + | col pyr | + + | + pyr | |
|---|---|---|---|---|
| 6 | 0 | 11 | 1 | 18 |
| 5 | 0 | 8 | 0 | 13 |

$X^2$ = 0.781 P > 0.8

Crosses 43 and 43A

| prototrophs | colonies | |
|---|---|---|
| 52 | 88 | 140 |
| 299 | 360 | 659 |
| 351 | 448 | 799 |

$X^2$ = 3.18 P > 0.05

| col + | col pyr | + + | + pyr | |
|---|---|---|---|---|
| 14 | 2 | 26 | 10 | 52 |
| 27 | 4 | 41 | 22 | 94 |
| 41 | 6 | 67 | 32 | 146 |

$X^2$ = 0.599 P > 0.8

As the above pairs of crosses have no significant differences, taking the level of significance as P = 0.05, it is possible to sum the two members of each pair.

If two crosses between a pair of alleles are marked reciprocally with respect to the unselected outside markers pyr-1 and col-4, the four classes of prototrophs should be present in reversed order of size. In other words the major parental class and major recombinant class in one cross should be the minor parental and reciprocal classes in the reciprocal cross. Unfortunately there is no reciprocal to crosses 3 and 3A, as the allele 39106 was too leaky in the pyr-1 background to provide reliable data. However, the other two crosses and their reciprocals can be compared.

Crosses 28, 28A, and 46

| | prototrophs | colonies | |
|---|---|---|---|
| 28 & 28A | 31 | 244 | 275 |
| 46 | 2 | 11 | 13 |
| | 33 | 255 | 288 |

$X^2$ = 0.18 P > 0.5

| | P1 | P2 | R1 | R2 | |
|---|---|---|---|---|---|
| 28 & 28A | 59 | 12 | 15 | 13 | 99 |
| 46 | 1 | 0 | 1 | 0 | 2 |
| | 60 | 12 | 16 | 13 | 101 |

$X^2$ = 0.82 P > 0.9

Crosses 43, 43A, and 7

| | prototrophs | colonies | |
|---|---|---|---|
| 43 & 43A | 351 | 448 | 799 |
| 7 | 56 | 32 | 88 |
| | 407 | 480 | 887 |

$X^2$ = 10.27 P > 0.01

| | P1 | P2 | R1 | R2 | |
|---|---|---|---|---|---|
| 43 & 43A | 41 | 6 | 67 | 32 | 146 |
| 7 | 32 | 4 | 6 | 14 | 56 |
| | 73 | 10 | 73 | 46 | 202 |

$X^2$ = 23.90 P > 0.01

From the above data, it is obvious that there is a highly significant difference between the reciprocal crosses for alleles 37803 and 39706. The results of a statistical analysis of all reciprocal crosses are given in Table 6-3.

TABLE 6-3

A statistical analysis of the prototroph data from reciprocal crosses.

| Crosses | Alleles | Prototroph frequency | | Outside markers | |
|---|---|---|---|---|---|
| | | $X^2$ | P | $X^2$ | P |
| 28, 28A, 46 | 39706, 44602 | 0.18 | $> 0.5$ | 0.82 | $> 0.9$ |
| 43, 43A, 7 | 39706, 37803 | 10.3 | $< 0.01$ | 23.9 | $< 0.01$ |
| 2, 8 | 37803, 35405 | 2.36 | $> 0.1$ | 1.85 | $> 0.3$ |
| 4, 22 | 37803, 44602 | 3.87 | $< 0.05$ | 53.3 | $< 0.01$ |
| 5, 29 | 37803, 44204 | 3.69 | $> 0.05$ | 74.2 | $< 0.01$ |
| 6, 36 | 37803, 46904 | 9.81 | $< 0.01$ | 13.81 | $< 0.01$ |
| 11, 23 | 35405, 44602 | 6.41 | $< 0.02$ | 15.17 | $< 0.01$ |
| 12, 30 | 35405, 44204 | 47.5 | $< 0.01$ | 10.66 | $< 0.02$ |
| 13, 37 | 35405, 46904 | 0.28 | $> 0.5$ | - | - |
| 14, 44 | 35405, 39706 | 10.23 | $< 0.01$ | 28.6 | $< 0.01$ |
| 26, 32 | 44602, 44204 | 3.50 | $> 0.05$ | - | - |
| 27, 39 | 44602, 46904 | 0.47 | $> 0.5$ | 3.98 | $> 0.2$ |
| 34, 40 | 44204, 46904 | 7.9 | $< 0.01$ | 57.3 | $< 0.01$ |
| 35, 47 | 44204, 39706 | 0.09 | $> 0.7$ | 0.71 | $> 0.7$ |
| 42, 48 | 46904, 39706 | 3.20 | $> 0.05$ | 41.2 | $< 0.01$ |

It is clear from the results given in Table 6-3, the results of reciprocal crosses give, in many cases, statistically significant differences from reciprocal results. This unfortunately prevents the meaningful ordering of the mutant sites within the pdx-1 locus.

Crosses between the first-generation strains had been carried out earlier, but the results were incomplete, and no test for detection of pseudo-wild type prototrophs had been devised. The results of those crosses between non-complementing alleles are given below in Table 6-4.

Crosses between the two generations were also made in a few cases, and these results are shown in Table 6-5.

TABLE 6-4

Results of the first generation inter-allelic high resolution crosses.

| Cross | pyr parent | col parent | total spores | total protos | protos per $10^5$ spores | Outside markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|
| 2c | 37803 | 35405 | 19,000 | 4 | 21.1 | 2 | 1 | 0 | 1 |
| 6c | 37803 | 46904 | 29,600 | 38 | 128 | 7 | 17 | 3 | 14 |
| 8c | 35405 | 37803 | 55,300 | 3 | 5.42 | 0 | 0 | 3 | 0 |
| 13c | 35405 | 46904 | 142,100 | 22 | 15.5 | 5 | 12 | 0 | 3 |
| 15c | 37803 | 39106 | 50,000 | 585 | 1170 | 47 | 3 | 0 | 0 |
| 21c | 39106 | 39706 | 20,400 | 10 | 49.0 | 3 | 5 | 0 | 2 |
| 24c | 44602 | 39106 | 69,700 | 423 | 613 | 87 | 5 | 4 | 0 |
| 28c | 44602 | 39706 | 1,000 | 4 | 400 | 0 | 3 | 0 | 1 |
| 29c | 44204 | 37803 | 25,700 | 196 | 762 | 5 | 5 | 44 | 39 |
| 29c | 37803 | 44204 | 1,600 | 4 | 250 | 3 | 1 | 0 | 0 |
| 31c | 44204 | 39106 | 21,000 | 177 | 842 | 76 | 3 | 4 | 13 |
| 32c | 44204 | 44602 | 1,500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35c | 44204 | 39706 | 22,100 | 12 | 54.3 | 0 | 5 | 0 | 3 |
| 36c | 46904 | 37803 | 28,700 | 18 | 62.7 | 6 | 0 | 13 | 0 |
| 37c | 46904 | 35405 | 94,000 | 20 | 21.3 | 2 | 4 | 13 | 1 |
| 45c | 39706 | 39106 | 11,000 | 5 | 45.5 | 0 | 2 | 0 | 2 |
| 46c | 39706 | 44602 | 76,100 | 32 | 42 | 2 | 4 | 7 | 16 |

TABLE 6-5

Results of first and second generation inter-allelic crosses.

| Cross | Col parent | pyr parent | % variability of spores | total spores | total protos | protos per $10^5$ spores | Outside markers col + | col pyr | + + | + pyr |
|---|---|---|---|---|---|---|---|---|---|---|
| 6B | 46904 | 37803 | - | 670,000 | 27 | 4.03 | 9 | 5 | 3 | 8 |
| 13B | 46904 | 35405 | - | 1,090,000 | 29 | 2.66 | 4 | 9 | 2 | 7 |
| 27B | 46904 | 44602 | 90% | 96,000 | 151 | 157 | 36 | 11 | 21 | 10 |
| 34B | 46904 | 44204 | 53% | 69,000 | 148 | 214 | 10 | 3 | 29 | 33 |
| 48B | 46904 | 39706 | 82% | 35,000 | 192 | 549 | 22 | 7 | 28 | 17 |

Analysing the data from reciprocal crosses in the first generation strains, prior to back-crossing to the wild-type strain 74-OR8-1a, the results given in Table 6-6 were obtained.

TABLE 6-6

A statistical analysis of the prototroph data from first generation reciprocal crosses.

| Alleles | Prototroph frequency | | Outside markers | |
|---|---|---|---|---|
| | $X^2$ | P | $X^2$ | P |
| 37803 - 35405 | 3.31 | 0.05 | 3.86 | 0.1 |
| 37803 - 46904 | 5.03 | 0.05 | 7.85 | 0.05 |
| 35405 - 46904 | 0.82 | 0.3 | 7.20 | 0.05 |
| 37803 - 44204 | 3.47 | 0.05 | 1.90 | 0.5 |
| 44602 - 39706 | 20.5 | 0.01 | 6.84 | 0.05 |
| 39106 - 39706 | 0.014 | 0.9 | 8.96 | 0.05 |

As both the first and second generation parental strains show significant differences between reciprocal crosses in many cases, the back-cross to 74-OR8-1a does not seem to have removed any of the variation. If this is so, there should be no differences between the first, first x second, and second generation crosses in those cases in which the second generation strain is a daughter of the first generation strain used in the cross. These cases were analysed, and the results are given in Table 6-7. Also included are similar analyses of crosses with one parent of common origin, and where neither parent is common.

TABLE 6-7

A statistical analysis of crosses between the same alleles in different generations and different genetic backgrounds

| | Crosses | Alleles | Prototroph frequency | | Outside Markers | |
|---|---|---|---|---|---|---|
| | | | $X^2$ | P | $X^2$ | P |
| Part 1. | 2, 2C | 37803 - 35405 | 10.5 | 0.01 | 0.61 | 0.7 |
| | 5, 5C | 37803 - 44204 | 7.02 | 0.01 | 2.02 | 0.5 |
| | 6, 6B, 6C | 37803 - 46904 | 431 | 0.01 | 6.54 | 0.3 |
| | 8, 8C | 35405 - 37803 | 6.50 | 0.02 | 7.99 | 0.05 |
| | 29, 29C | 44204 - 37803 | 71.8 | 0.01 | 18.0 | 0.01 |
| | 35, 35C | 44204 - 39706 | 1.13 | 0.2 | 7.98 | 0.05 |
| | 45, 45C | 39706 - 39106 | 245 | 0.01 | - | - |
| Part 2. | 13, 13C | 35405 - 46904 | 15.3 | 0.01 | - | - |
| | 24, 24C | 44204 - 39106 | 168 | 0.01 | 3.58 | 0.1 |
| | 28, 28A, 28C | 44602 - 39706 | 73.1 | 0.01 | 35.0 | 0.01 |
| | 31, 31C | 44204 - 39106 | 255 | 0.01 | 60.8 | 0.01 |
| | 36, 36C | 46904 - 37803 | 295 | 0.01 | 0.51 | 0.7 |
| | 37, 37C | 46904 - 35405 | 39.8 | 0.01 | 5.11 | 0.9 |
| | 46, 46C | 39706 - 44602 | 14.6 | 0.01 | 1.64 | 0.2 |

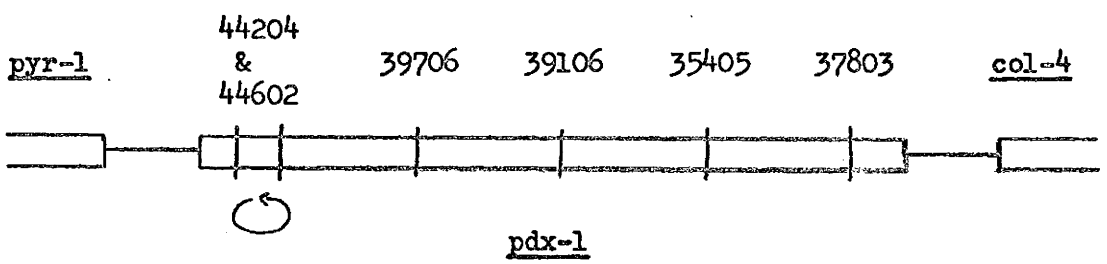
Part 1 contains crosses in which both second generation strains were daughter strains of the first generation with which they are compared.

Part 2 contains crosses in which only one strain is a daughter, the other being a daughter of a first generation sister strain.

Although the frequencies of outside marker classes of prototrophs are very variable, it is possible, on the basis of the R1 prototroph class, to construct a tentative map of the pdx-1 locus. The likely positions of six of the pdx alleles are shown below in figure 6-8. The seventh allele, 46904, defies any attempt to deduce its location from the experimental data. Because of the variability in recombination frequencies between alleles in the different generations, no frequencies are given in the figure.

Figure 6-8

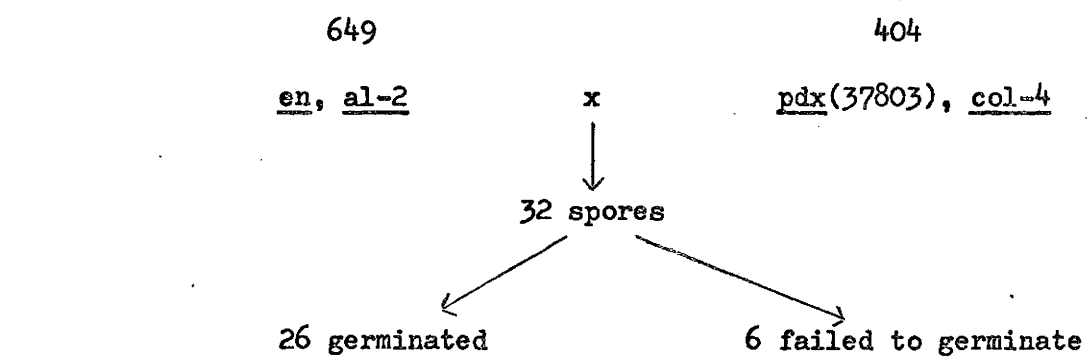
A tentative recombination map of the pdx-1 locus.



## CHAPTER VII

## THE EFFECT OF THE EN-PDX-1 GENE ON THE PYRIDOXINE AUXOTROPHS

The strain, en-pdx-1 (K30), al-2, was obtained from the Fungal Genetics Stock Center, and this was crossed to all seven pdx-1 alleles. Most of the pdx-1 strains used in these crosses were either marked proximally by pyr-1 or distally by col-4. Details of the crosses, and their results, are shown below, scored not only for al-2, pdx-1, and either pyr-1 or col-4, but also for en-pdx-1, which was found to enhance yellow pigment production on medium supplemented with ammonium sulfate.




6 al-2, en; pdx, col-4

2 pdx, col-4

2 en; pdx, col+4

8 al-2

8 +

649 x 362

en, al-2 pdx(35405)

12 spores

9 germinated 3 failed to germinate

2 al-2, en, pdx

1 en, pdx

6 al-2

649 370

en, al-2 x pdx(39106)

32 spores

31 germinated 1 failed to germinate

6 al-2, en; pdx

4 en; pdx

5 pdx

5 al-2

11 +

649 x CN 133

en, al-2 x pdx(44602), pyr-1

32 spores

21 germinated — 11 failed to germinate

2 al-2, en; pdx, pyr-1

2 en; pdx, pyr-1

6 al-2; pdx, pyr-1

6 pdx; pyr-1

1 al-2

4 +

649 x CO 64

en, al-2 x pdx(44204), pyr-1

32 spores

27 germinated — 5 failed to germinate

1 al-2, en; pdx, pyr-1

2 en; pdx, pyr-1

5 al-2; pdx, pyr-1

2 pdx, pyr-1

7 al-2

10 +

649 CG 49

en, al-2 x pdx(46904), col-4

32 spores

18 germinated 14 failed to germinate

1 en; pdx, col-4

7 pdx, col-4

6 al-2

4 +

649 1183

en, al-2 x pdx(39706)

32 spores

15 germinated 17 failed to germinate

3 al-2, en; pdx

2 al-2; pdx

4 al-2

4 +

The above classifications of the enhancer gene were carried out on Westergaard and Mitchell's glucose medium supplemented with ammonium sulfate. Pigment was produced only after increasing the ammonium sulfate concentration from the normal rate of 1 gm/l (Strauss 1951) to 5 gm/l. This confirmed an observation of Martin (Catcheside, personal communication) that the yellow pigment of the enhanced pdx mutant is produced above a threshold concentration of $(NH_4)^+$ ions. Subsequently it was found that the pigment was produced also on medium made with Vogel salt solution, which contains a high concentration of ammonium nitrate as a normal constituent. For both Strauss' medium with ammonium sulfate and Vogel's medium, the addition of a non-limiting concentration of pyridoxine in all cases inhibited the production of the yellow pigment in the medium.

The effect of heterokaryon complementation on pigment enhancement was next investigated. While back-crossing the enhanced strains in order to introduce some degree of homozygosity for the compatibility loci, the heterokaryon strains described in Chapter 5 were sub-cultured on Vogel's medium without pyridoxine to see if the enhancer gene, having no known selective advantage or disadvantage, already existed in stocks suitable for the experiment outlined above. Fortunately, pigment enhancement was widespread, and subject to proof of allelism of this enhancer with en-pdx-1 (K 30), all single pdx-1 alleles and heterokaryon compatible combinations were tested on Vogel's glucose medium supplemented with arginine and cytidine. The results of this experiment are shown in Table 7-1.

TABLE 7-1

The effect of heterokaryon complementation on pigment enhancement.

(x = enhancement.)

| Strain A | Allele A | Strain B | Allele B | Enhancement of Strain A | Enhancement of Strain B | Enhancement of Strain H/K AB | Heterokaryon complementation |
|---|---|---|---|---|---|---|---|
| XHC 15 | 39106 | XHK 2 | 35404 | ✓ | ✓ | x | ✓ |
| XHA 11 | 37803 | XHK 3 | 35405 | ✓ | ✓ | ✓ | x |
| XHH 10 | 39706 | XHK 2 | 35405 | x | ✓ | x | ✓ |
| XHA 13 | 37803 | XHG 3 | 46904 | ✓ | x | ✓ | ✓ |
| XHA 10 | 37803 | XHH 10 | 39706 | ✓ | ✓ | x | ✓ |
| XHG 8 | 46904 | XHK 3 | 35405 | ✓ | ✓ | ✓ | x |
| XHA 10 | 37803 | XHL 11 | 39106 | x | ✓ | x | ✓ |
| XHG 8 | 46904 | CR 9 | 39706 | x | ✓ | x | ✓ |
| XHE 3 | 44602 | XHF 4 | 44204 | x | x | x | ✓ |
| CP 162 | 46904 | PE 9 | 44602 | ✓ | x | x | ✓ |
| XHA 11 | 37803 | XHF 8 | 44204 | ✓ | ✓ | ✓ | x |
| XHH 10 | 39706 | XHL 11 | 39106 | x | ✓ | ✓ | x |
| XHE 3 | 44602 | XHK 2 | 35405 | x | ✓ | x | ✓ |
| XHF 4 | 44204 | XHL 11 | 39106 | x | ✓ | ✓ | x |
| XHA 10 | 37803 | XHE 3 | 44602 | x | x | x | ✓ |
| XHE 3 | 44602 | XHH 10 | 39706 | x | x | x | x |
| XHC 9 | 39106 | XHG 3 | 46904 | x | x | x | ✓ |
| CG 30 | 46904 | CO 64 | 44204 | ✓ | x | x | ✓ |
| XHE 3 | 44602 | XHL 11 | 39106 | x | ✓ | ✓ | x |
| XHF 8 | 44204 | XHH 8 | 39706 | ✓ | ✓ | ✓ | x |
| CG 30 | 46904 | PE 9 | 44602 | ✓ | x | x | ✓ |

To summarise the data given above in Table 7-1, considering first complementing alleles of the pdx-1 locus,

| Strain A | | Strain B | Heterokaryon |
|---|---|---|---|
| en | + | en | no pigment |
| en | + | + | no pigment |
| + | + | + | no pigment |

In other words the complementation between the two pdx-1 alleles is having a similar effect to the addition of non-limiting pyridoxine resulting in the suppression of production of the yellow pigment. In the tests between compatible but non-complementing strains,

| | | | |
|---|---|---|---|
| en | + | en | pigment |
| en | + | + | pigment |
| + | + | + | no pigment. |

This, therefore, indicates that the en allele is dominant over the $en^+$ form of the gene, and so, in the presence of a pdx-1 mutant, produces the pigment.

A similar, but less extensive, experiment was carried out with the enhanced pdx-1 strains derived from the type mutant K 30, and the results are given below in Table 7-2.

TABLE 7-2

The effect of heterokaryon complementation on the pigment enhancer K 30.

| Strain A | Allele A | Strain B | Allele B | Enhancement of Strain A | Enhancement of Strain B | Enhancement of H/K AB | Heterokaryon complementation |
|---|---|---|---|---|---|---|---|
| PA 1 | 37803 | PE 5 | 44602 | ✓ | ✓ | x | ✓ |
| PA 25 | 37803 | PE 4 | 44602 | ✓ | ✓ | x | ✓ |
| PA 12 | 37803 | PH 6 | 39706 | ✓ | ✓ | ✓ | x |
| PA 21 | 37803 | PH 6 | 39706 | ✓ | ✓ | ✓ | x |
| PA 1 | 37803 | PC 26 | 39106 | ✓ | ✓ | x | ✓ |
| PA 4 | 37803 | PC 26 | 39106 | ✓ | ✓ | x | ✓ |
| PA 9 | 37803 | PC 29 | 39106 | ✓ | ✓ | ✓ | x |
| PE 5 | 44602 | PC 9 | 39106 | ✓ | ✓ | ✓ | x |
| PE 5 | 44602 | PH 6 | 39706 | ✓ | ✓ | ✓ | x |
| PE 8 | 44602 | PC 28 | 39106 | ✓ | ✓ | ✓ | x |
| PA 12 | 37803 | PE 8 | 44602 | ✓ | ✓ | x | ✓ |

In addition to supporting the results shown in Table 7-1, Table 7-2 shows one additional fact; that when two enhanced strains with complementary alleles but heterokaryon incompatibility are combined, the pigment is still produced. Therefore, the $\underline{en}^{-}$ allele is still dominant over $\underline{en}^{+}$, and the suppression of pigment enhancement can only be brought about by the joint product of complementing pdx-1 alleles from the two nuclear types in a common cytoplasm.

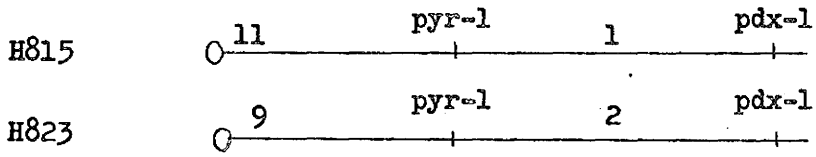
## CHAPTER VIII

## FURTHER DATA ON ABERRANT SEGREGATION AT THE PDX-1 LOCUS

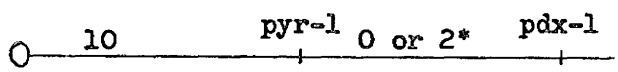
In earlier work on this topic, summarised in Chapter I, the occurrence of 3:1 ratios in asci from crosses between certain pdx-1 strains was reported (Threlkeld 1961, Radford et al 1965). The crosses involved strain J104, a pdx(44602), pH-sensitive strain, and H815 and H823, two pdx (37803) strains. Whereas in asci from H815 x J104 all segregations were normal, 7 asci out of a total of 123 from H823 x J104 had 3 pdx(37803): 1 pdx(44602) segregations. All aberrant asci from this and subsequent crosses described were checked by back-crossing to verify the classifications. It was further found that a low frequency (approx. 1%) of pdx(44602) progeny could be recovered from the back-cross of one pdx(37803) product of each aberrant meiosis.

Because of the apparently abnormally high frequency of occurrence of these aberrant asci, an answer was sought in the form of a postulated duplication, in strain H823, of that region of linkage group IV containing the pdx-1 locus. Further investigations of the aberrant behaviour of H823 were undertaken, and the results are described below.

As aberrant asci were observed only in the cross H823 x J104, and not in H815 x J104, both H823 and H815 were crossed to the "Lindegren" wild-type strain 25a, and fifty asci from each cross were analysed. H815 and H823 were both pyr-1, pdx-1, and the results of their crosses showed no abnormal segregations for either locus. The map distances calculated from two crosses were:-




The supposed abnormality in H823, therefore, was not observed at least at the same frequency as with J104, when crossed with 25a. H823 was therefore crossed to another pdx(44602) strain, HE4, an intermediate strain in the production of the strains described in Chapter V. In the cross H823 x HE4, thirty asci were isolated and analysed, and all loci except the pdx-1 locus segregated normally. In the case of pdx-1 however, two asci were of the 3 pdx:1 pdxp type. Except for the aberrant asci, there were no cross-overs between pyr-1 and pdx-1. The map distances calculated from the data from this cross, from completely analysable tetrads, were:-




(*depending on whether the aberrant asci are taken as having recombined in this region or not.)

The two aberrant asci are shown in Figure 8-1.

Figure 8-1

The aberrant asci from the cross H823 (pyr-1, pdx(37803)) by HE4 (arg-6, al-2; pdx(44602)).

| | | | | | |
|---|---|---|---|---|---|
| Ascus 102 - | Spores 1 and 2 | + | + | pyr-1 | pdx(37803) |
| | 3 and 4 | arg-6 | al-2 | pyr-1 | pdx(37803) |
| | 6 | + | + | + | pdx(44602) |
| | 7 and 8 | arg-6 | al-2 | + | pdx(37803) |
| | (Spore 5 failed to germinate) | | | | |
| Ascus 112 - | Spores 1 and 2 | arg-6 | al-2 | pyr-1 | pdx(37803) |
| | 3 and 4 | + | + | pyr-1 | pdx(37803) |
| | 5 and 6 | + | + | + | pdx(37803) |
| | 7 and 8 | + | + | + | pdx(44602) |

It therefore appeared that H823 gave rise to aberrant segregation when crossed to either of two pdx(44602) strains used, but no abnormal asci were found in a sample of 50 examined from a cross with the wild type strain 25a.

H823 was then crossed to a third allele of the pdx-1 locus, #39106, marked in this cross with the morphological mutant col-4 several crossover units distal to pdx-1 on linkage group IV. Fifty asci from this cross, H823 x 345, and twenty-three from an identical cross, were isolated and analysed. In the fifty asci, two asci had 3 pdx(37803): 1 pdx(39106) segregations, and two had 2 pdx(37803); 1 pdx(39106): 1 $pdx^+$ spore pairs. The identical cross had one aberrant in 17, a 3 pdx(37803): 1 pdx(39106) type. These five asci are shown in Figure 8-2. Asci 59 and 302 show recombination between the outside markers associated with the conversion event at the pdx-1 locus, but in the other three asci there is no overall recombination. A sixth ascus, although showing a normal 2 pdx(37803): 2 pdx(39106), is rare in that it has first division segregations for both pyr-1 and col-4 but a second division segregation for pdx-1. It thus appears to have been produced by a two-strand double crossover within the region pyr-1 - col-4 (Ascus 81, Figure 8-2) The ordering of spores in this ascus has been reconstructed, and may not be correct, but the tetratype segregation is unambiguous.

Figure 8-2

Aberrant asci from the cross H823 x 345.

| | | | | |
|---|---|---|---|---|
| Ascus 59 | Spores 1 | + | pdx(39106)p | col-4 |
| | 3 and 4 | pyr-1 | + | col-4 |
| | 5 and 6 | pyr-1 | pdx(37803) | + |
| | 8 | + | pdx(37803) | + |
| | (Spores 2 and 7 failed to germinate) | | | |
| Ascus 72 | Spores 1 and 2 | pyr-1 | pdx(37803) | + |
| | 3 and 4 | pyr-1 | pdx(37803) | + |
| | 5 | + | pdx(37803) | col-4 |
| | 7 and 8 | + | pdx(39106)p | col-4 |
| | (Spore 6 failed to germinate) | | | |
| Ascus 88 | Spores 2 | + | pdx(39106)p | col-4 |
| | 3 and 4 | + | + | col-4 |
| | 5 and 6 | pyr-1 | pdx(37803) | + |
| | 7 and 8 | pyr-1 | pdx(37803) | + |
| | (Spore 1 failed to germinate) | | | |
| Ascus 97 | Spores 1 and 2 | pyr-1 | pdx(37803) | + |
| | 3 | pyr-1 | pdx(37803) | + |
| | 6 | + | pdx(37803) | col-4 |
| | 8 | + | pdx(39106)p | col-4 |
| | (Spores 4, 5, and 7 failed to germinate) | | | |
| Ascus 302 | Spores 1 and 2 | + | pdx(37803) | col-4 |
| | 3 and 4 | pyr-1 | pdx(37803) | + |
| | 5 and 6 | + | pdx(37803) | + |
| | 7 and 8 | pyr-1 | pdx(39106)p | col-4 |
| Ascus 81 | Spores 1 and 2 | pyr-1 | pdx(37803) | + |
| | 3 and 4 | + | pdx(39106)p | col-4 |
| | 5 and 6 | pyr-1 | pdx(39106)p | + |
| | 7 | + | pdx(37803) | col-4 |
| | (Spore 8 failed to germinate) | | | |

(p = pH-sensitive)

# CHAPTER IX

## DISCUSSION

### Part 1. Growth characteristics of the mutants.

The results of the experiments reported in Chapter IV show that all seven pyridoxine auxotrophs studied respond quite vigourously to the supplements pyridoxine, pyridoxal, and pyridoxamine when most supplements are provided as either phosphates or hydrochlorides.

By comparing the results of the growth of the various allelic strains on minimal medium from Chapters III and IV with those of the same strains in different backgrounds from Chapter V, it is obvious that the extent of leakiness of an allele is not constant, but varies with the genetic background found in particular strains. In original F.G.S.C. strains, Alleles 39106 and 39706 are the most leaky, 37803, 35405, 44602, and 44204 show only 30% of the growth of 39106 and 39706, and 46904 is intermediate. However, in the arg-6, al-2 and arg-3; ylo strains, although overall leakiness is less than in the original strains, the most leaky strains contain 44204, 39106, and 35405. That leakiness is not associated with the marker genes involved is clear from a comparison of two arg-6, al-2; pdx(44204) strains. One of these has 2% of wild-type growth, but the other has 12%.

The vitamin analogue desoxypyridoxine (Glazer et al 1951) is clearly effective in competing with the active form of the vitamin,

pyridoxal phosphate. The competitive effect is concentration-dependent, as a concentration of desoxypyridoxine which reduces leaky growth of the mutants to a negligible level has no effect on the growth of a wild-type strain or on an auxotroph also supplemented with an adequate concentration of pyridoxine. The enzyme responsible for the presence of the vitamin, and which is specified by the pdx-1 gene, under normal circumstances in a mutant produces a limiting amount of the vitamin (Stokes et al 1943, Strauss 1951) which can be isolated from the mycelium and assayed. At high pH, in the presence of a certain ammonia concentration, the mutant enzyme of the pH-sensitive alleles at least partially regains its non-mutant characteristics, and under these conditions becomes considerably more resistant to competitive inhibition. The restored growth seen by the mutants under these conditions is not due to breakdown of the inhibitor, as its efficiency in inhibiting growth of the non-pH-sensitive mutants is unimpaired (Radford 1965). The use of desoxypyridoxine certainly aids in classifying the pdx mutants, and without its use the high resolution genetic analysis of the locus would not have been possible.

Part 2. Complementation.

Mutants at any locus are capable of complementation provided that the active enzyme is at least a polypeptide dimer, and the fact that complementation is possible at the pdx-1 locus therefore indicates that the gene product of pdx-1 undergoes association with another product of the same locus. The mutant abnormality in each polypeptide stabilises the non-overlapping abnormality in its partner (Fincham &

Pateman 1957). If such cross-stabilisation and complementation is possible between two polypeptides in a hybrid dimer, then there must be a similar potential in higher multimers for complementation between three or more alleles, no pair of which are capable of complementing each other. This experiment was tried using the available mutants, but the results were negative. This does not eliminate the possibility of the active gene product of pdx-1 being a higher multimer, as the negative results may merely show that the three or four allele combinations tested had a "lesion region" in common.

Complementation hybrid enzymes at several loci have been shown to differ from true wild-type enzyme in their physico-chemical properties, and the commonest and most easily detected difference is the increased thermo-lability of hybrids. Also temperature-sensitive heterokaryons have been described for the pan-2 locus in Neurospora crassa (Case & Giles 1960), and in analogous systems in Bacteriophage T4 (Edgar et al 1964). In the present work, only one pair of alleles showed a detectable increase in thermolability, this being 46904-44602 which complemented at 25°C but failed to do so at 37°.

At most loci at which complementation has been investigated, it has been found that most alleles are incapable of intra-locus complementation with any other alleles; this work has been summarised in Chapter I. However, at the pdx-1 locus, all alleles are capable of complementation with at least two other alleles. It has been previously found that mutants capable of complementation tend to be leaky on minimal medium (de Serres 1956, 1960), presumably showing that the mutation has not resulted in the production of a drastically altered polypeptide. The fact that all seven

pdx alleles were UV-induced, whereas many mutants at other loci were induced with ionising radiation, may mean that the pdx mutants complement because none has a major structural change, whereas others have gross abnormalities which are incapable of cross-correction.

Some loci, upon close analysis by complementation, have proved to consist of at least two cistrons, e.g. ad-3 (de Serres 1956), and these show a definite discontinuity in their complementation maps. Others show a continuously overlapping complementation map, and are interpreted as single cistrons, e.g. pan-2 (Case & Giles 1960). Still others have a continuously overlapping complementation map, but consist of two or more cistrons which are transcribed together, e.g. hist-3 (Ahmed et al 1964). A feature of this third type of map is the polarity type of mutant, probably a deletion or duplication mutant causing a frame shift, resulting in abnormality all along the distal part of the polypeptide chain. An alternative explanation is that incomplete polypeptides are translated (Sarabhai et al 1964). Because the mutant affects the whole distal part of the chain, it is incapable of cross-correcting another mutant in that region. It maps therefore to the end of the functional unit, sometimes through several cistrons. The pdx-1 locus has no discontinuity, and therefore does not consist of two separately transcribed but functionally associated cistrons. It may be a single cistron, or it may be of the last type, a complex with several functions, transcribed as a unit. Of the seven pdx-1 alleles, only two occupy more than one complon at both temperatures, and each of these two occupy a region extending to an end of the map. These two alleles, 37803 and 44204, extend to opposite ends, and as polarity mutants all extend to the same end, only one of these two alleles

may possibly be such a polarity mutant. However, as the overlap between 37803 and 44204 is also spanned by 46904 and 44602, and these two alleles form a temperature sensitive heterokaryon (characteristic of complementation between alleles within a single cistron), it is probable that pdx-1 consists of a single cistron.

Early work on the pdx-1 locus (Houlahan et al, 1949) resulted in 44204 being separated from pdx-1 as a separate locus, pdx-2. This differentiation was made on the basis of one recombinant ascus, and on the basis of the present work is considered not a valid reason for the division of the alleles. Allele 44204 clearly maps, on the basis of the complementation data, in the pdx-1 locus.

Although a number of loci have been examined with respect to their complementation behaviour, most loci in which only small numbers of alleles have been studied show linearity in the complementation map, (Fincham 1966). Certain loci have been found in which the pattern has been non-linear, but some of these have since been found to be spurious (e.g. Catcheside 1960). Unequivocal examples of non-linearity are Schizosaccharomyces pombe ad-6 (Leupold & Gutz, 1965) and Neurospora crassa ad-8 (Ishikawa, 1962). At 25°C the pdx-1 locus data are consistent with a linear map, but at 37°, taking into consideration the temperature sensitivity of the 46904-44602 allele pair, the map cannot be constructed in a simple linear manner. Figure 5-6 shows the simplest representation of the data at the higher temperature. On a simple interpretation of complementation in a dimeric protein, the explanation of this situation is difficult.

Even if one postulates the type of scheme shown in Figure 1-5 (Kapuler & Bernstein 1963), this model cannot be made to fit the pdx-1 data. However, Crick and Orgels' model (Fincham 1966) shown in Figure 9-1

could provide an explanation of the pdx-1 data. The model assumes that the active enzyme is a dimer with normally identical folded polypeptide chains. If the two polypeptide chains composing the dimer were completely reversed, the dimer from a single mutant would probably be self-correcting. Therefore at least some homologous regions of the two chains must be adjacent to prevent such self correction, and for this reason, the model of Crick and Orgel has only bilateral symmetry. On this model, it is possible to postulate that mutant sites in the single chain parts of the chain have no adjacent chain to stabilise them, and hence they would not be capable of complementation.

What are the reasons why an altered polypeptide chain may be mutant? Firstly, one can conceive of an amino acid change in the region of an active, substrate or coenzyme, binding site. The second main type may well still have normal active sites, but the mutant may cause a configurational change which results in those active sites not having the correct spatial relationship. Mutants of this second type may, for example have a cystene residue replaced by another amino acid, and thus be incapable of forming a disulfide bond required to stabilise the tertiary or even quaternary configuration of the polypeptide chain.

Temperature-sensitivity of the heterokaryon is more likely to be a reflection of conformation rather than an actual mutation in the active site itself. Therefore it may be postulated that alleles 46904 and 44602, or the regions affected by those alleles, are adjacent in the dimer, and are cross bonded in some way at 25° but that at 37° the cross-corrected mutant dimer bonding is unstable. The dissociation of the two previously adjacent regions causes the correct spatial relationship of active sites to be disturbed, thus inactivating the enzyme. All five other pdx-1 alleles may

either be temperature-insensitive configurational, or actually in the active sites.

Part 3. The En-pdx-1 gene.

The enhancer gene produces a yellow pigment in the presence of a mutant pdx-1 gene under certain environmental conditions. The conditions for expression are firstly a fairly high pH (the critical pH has not been determined) and the presence of free ammonia above a certain threshold concentration (approximately 3.5 gm/liter ammonium sulfate (Catcheside, personal communication)). Probably it requires the presence of free ammonia, and that only at high pH can the ion concentration be sufficiently high. In support of this possibility, there is the fact that high pH without externally supplied ammonium ions is not favorable for pigment production. As the yellow pigment only accumulates in pdx-1 mutants in the absence of externally supplied, non-limiting, concentrations of pyridoxine, it is clearly not an intermediate metabolite in the pyridoxine pathway (cf. ad-3). Probably the yellow pigment is an intermediate metabolite in a pathway that has no relation to pyridoxine production. As pigment synthesis is depressed by pdx, it may be postulated that the enzyme which would normally convert it into a colorless compound requires the co-enzymic function of pyridoxal phosphate. Thus the supply of a non-limiting concentration of pyridoxine to this reaction from a $pdx^+$ strain, a complementing heterokaryon, or from the medium, results in no production of the yellow pigment. As the unenhanced strains do not produce the yellow pigment under any circumstances and are recessive to the enhanced strains in heterokaryons, it seems likely that the un-enhanced strains

lack the ability to produce the pigment and therefore the pathway is probably operative only at high pH with ammonia. The alternative, that the enhanced strains fail to metabolise the yellow pigment, is unlikely as one would, in this case, expect the enhancer gene to be recessive. Its recessiveness would be due to the fact that the un-enhanced component strain would have the enzymes to break down the accummulation product. In view of its dominance over the unenhanced state, and in order to abide by the conventions of genetic nomenclature, the enhanced pigment production allele of the enhancer gene should be capitalised to "En-pdx-1".

Part 4. The production of prototrophs from inter-allelic crosses.

It has been known since the early work on the pdx-1 locus that prototrophs could be recovered from inter-allelic crosses (Houldham et al 1949, Mitchell 1955), and it has been found that in some cases the prototroph frequency may be as high as 1%.

From the high-resolution data, although ordering of alleles is impossible, several facts are evident. The first of these is that the frequency of prototrophs in the first generation crosses is, in all except one case, significantly higher than frequencies in the second-generation crosses (after the back-crosses to St. Lawrence wild-type 74-OR8-1a), this fact being reflected by the significant differences shown in Table 6-6. Two reasons for this difference in prototroph frequencies are possible, and the first of these is that there is an overall background effect brought in with the St. Lawrence back-cross, and that this gives an overall reduction in recombination frequencies. Recombination rates in several map regions have been observed to decrease

as the amount of the St. Lawrence genome in the strains increases (de Serres, personal communication). The alternative is that there is a gene controlling recombination at the pdx-1 locus in strain 74-OR8-1a, and that this gene is introduced via the back-cross. The allele of the gene introduced would cause low recombination, and would be dominant as a significant majority of second generation crosses have a significantly lower frequency of prototrophs. A similar type of gene is rec-1, affecting hist-1 (Jessop & Catcheside, 1965). At present it is not possible to distinguish between these alternatives.

Although it was hoped that the inter-allelic crosses would provide the data from which a locus recombination map could be derived, the data obtained show sufficient discrepancies that a map derived from them is tentative. However, it is useful to consider the possible causes of the anomalies. The first of these possibilities is that the technique was not such as to give reliable results, but this is unlikely as duplicate crosses between the same two strains, made and analysed at different times and on different batches of media, give consistently reproducible results. The alternative hypothesis, and that which seems to be the most likely, is that the variability between results from reciprocal crosses is inherent in the strains.

The inter-relationships of wild-type strains of Neurospora crassa are shown in Figure 9-1.

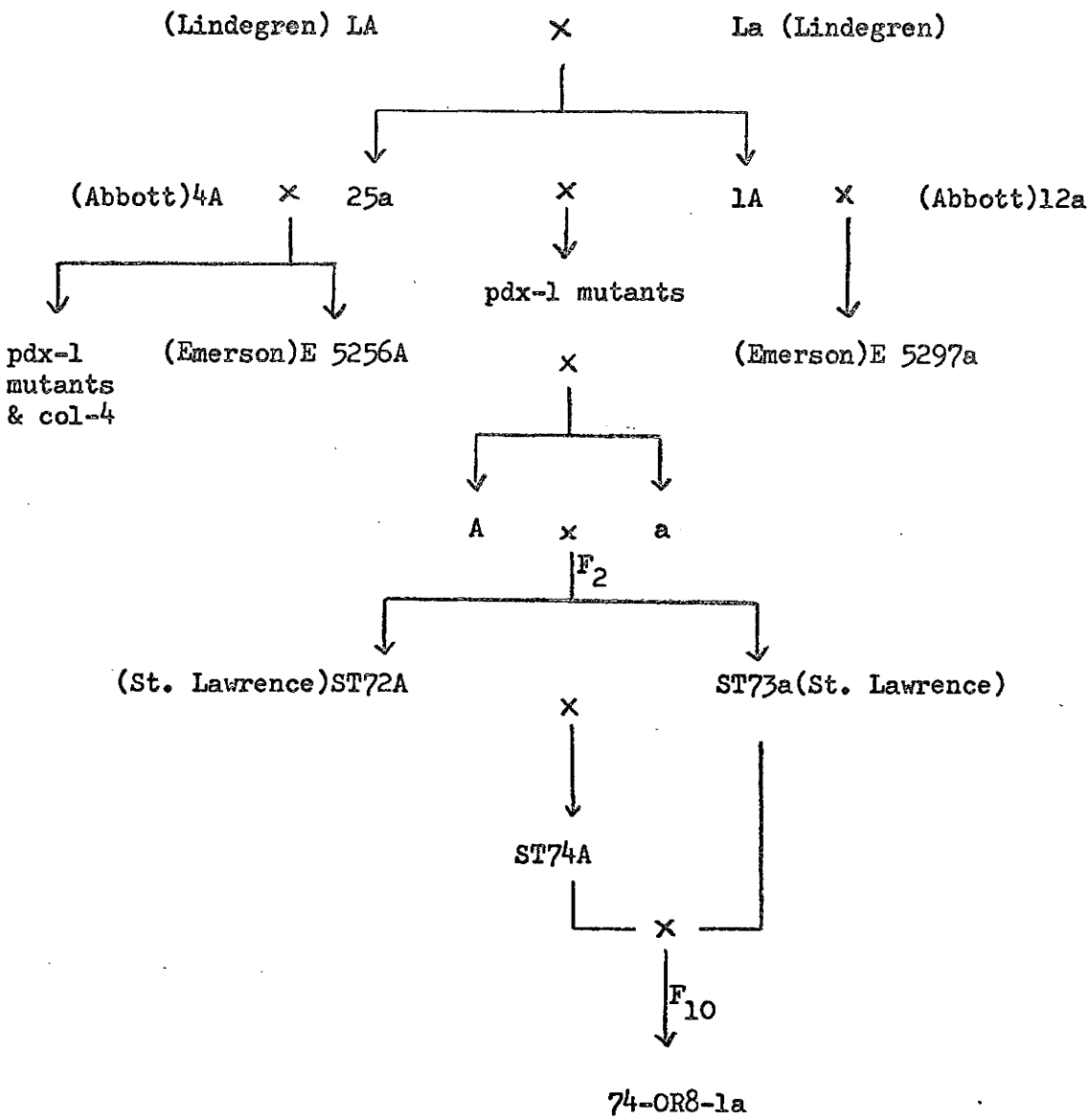



Figure 9-1

The inter-relationships of strains used in the production of strains used in inter-allelic crosses.

The Abbott and Lindegren strains shown in Figure 9-1 are of independent origin, and are known to have a great deal of structural heterozygosity (Barratt 1954, Frost 1955, Stadler 1956, Frost 1961, Lavigne & Frost 1964).

Alleles 37803, 35405, 39106, and 39706 were induced by UV-irradiation of dry conidia of the Lindegren strain 1A, which were then used to fertilize protoperithecia of Lindegren 25a. From this cross, mutant ascospores were then isolated, and it is likely that these mutant alleles will still be homologous with the parental strain 1A for the pdx-1 region of linkage group IV (Beadle & Tatum 1945).

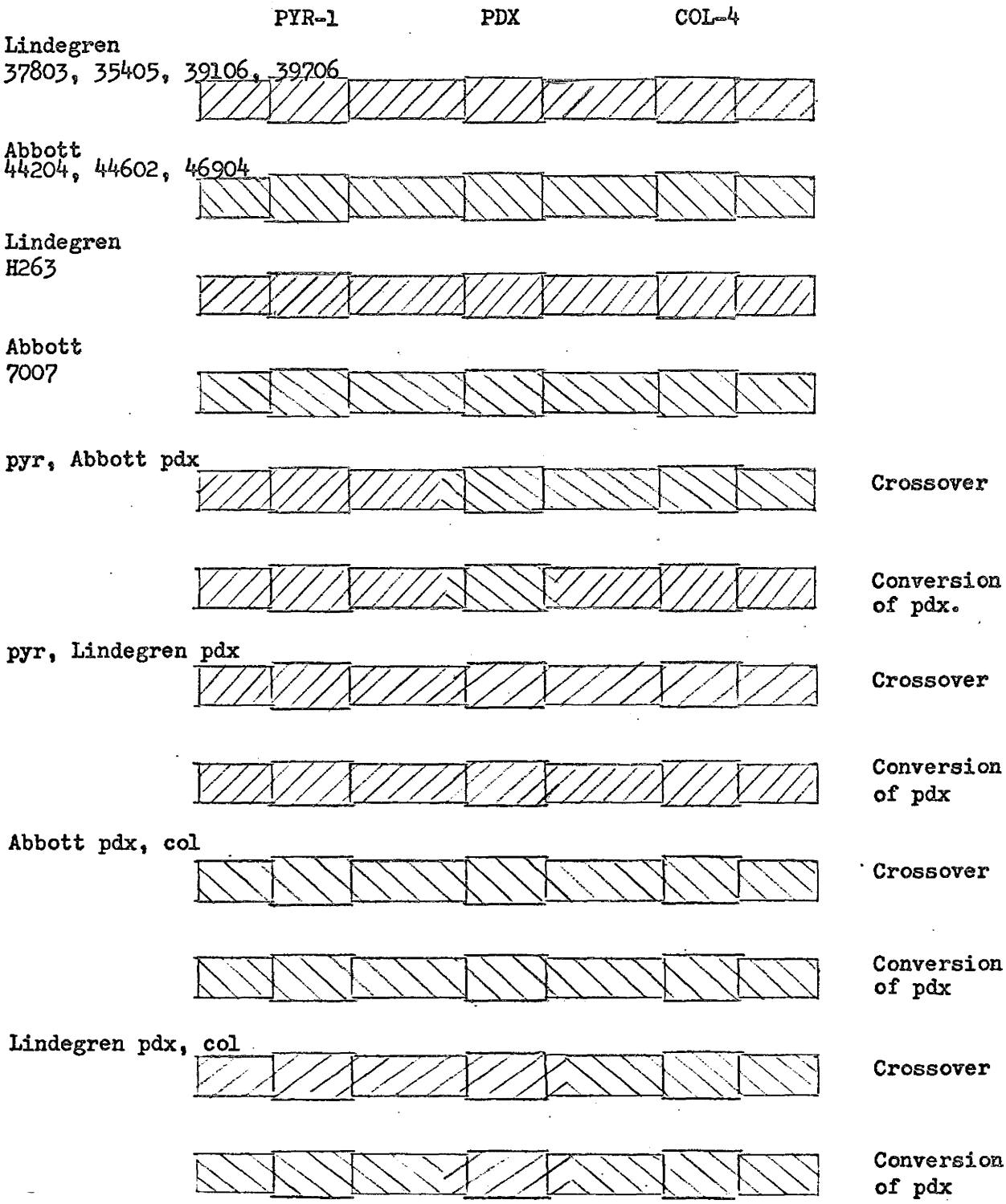
Alleles 44204, 44602, and 46904 were induced in dry conidia of the Abbott strain 4A, which were then used to conidiate Lindegren 25a protoperithecia. The three mutants were found in the ascospores produced, and probably still have Abbott homologies for the pdx-1 region of the left arm of linkage group IV (Beadle & Tatum 1945).

The col-4 mutant 70007 was produced in a similar manner to alleles 44204, 44602, and 46904, (Beadle & Tatum 1945), and is therefore likely to have Abbott homologies for linkage group IV also. The pyr-1 mutant H263 was induced by X-irradiation of a Lindegren wild-type (Perkins, personal communication).

On the basis of the strain homologies deduced above, the strains used in the inter-allele crosses described in Chapter VI are likely to have genetic backgrounds similar to those shown in Figure 9-2.

Figure 9-2

The wild-type homologies of the pdx-1, col-4 and pyr-1, pdx-1 strains.



The Abbott-Lindegren heterozygosity is involved intimately in the pyr-1 to col-4 region under study, and even repeated back-crosses would not remove this heterozygosity as one selects for non-crossover types, and stability, in this region in such back-crosses. Even repeated back-crosses of single mutants followed by recombining the markers would be unlikely to correct the heterozygosity, as the chances of obtaining a crossover close enough to the marker to remove all heterozygous material are remote.

Part 5. Aberrant asci at the pdx-1 locus.

In the cross between the two pdx-1 alleles 37803 and 39106, five aberrant asci, (described in the last chapter) were recovered from a total of seventy-one asci analysed. Of these, for allele 37803, three asci involved 3 37803:1 + ratios, but none showed the reverse of this type. Two other asci involved non-reciprocity at the 39106 allele, and both were of the 1 39106:3 + type. Other data, on a similar cross between the same two alleles (Mitchell 1955) detected 2 aberrant segregations of 37803, and 3 of 39106 in approximately 600 asci. All five asci involved conversion to the 1 mutant : 3 + type, but owing to the selective technique by which these were detected, a non-reciprocal 3 mutant : 1 + segregation would not be detected.

The related crosses involving the alleles 37803 and 44602 showed nine aberrant asci out of about 153 asci examined. Thus on the lines of the Whitehouse hypothesis, seven involved hybridity at 37803 and conversion of the hybrid DNA to the mutant. No abnormal results at allele 44602 were observed. (Threlkeld 1961, and Chapter VIII.)

A previous hypothesis to explain the results from the cross involving alleles 37803 and 44602 (strains H823 and J104) involved a postulated duplication of the pdx locus in strain H823 (Radford & Threlkeld 1965). This is now thought to be unlikely, as prototroph frequencies from inter-allelic crosses are as high as the frequency of aberrant asci that the duplication hypothesis sought to explain. Also, the fact that prototrophs were found at all in asci from a cross between H823 and allele 39106 tends to exclude this possibility. Non-disjunction, which was also previously interpreted on the basis of a duplication, is now considered likely to be due to the non-disjunction gene (Barratt 1954) from Abbott strains which are ancestors of both H823 and J104. It is therefore probable that the aberrant asci found at the pdx locus are in fact due to inter-allelic recombination.

Thus from the above results it appears that, using an interpretation based on the hypothesis of Whitehouse (Whitehouse & Hastings 1965), 37803 and 39106 become involved in hybrid DNA with approximately equal frequency, but 44602 becomes involved in such hybridity with a significantly lower frequency. From the Whitehouse hypothesis, postulating that involvement in hybrid DNA reflects proximity to the breakage point in the DNA (assuming unwinding of the DNA double helix unwinds equally frequently in each direction), alleles 37803 and 39106 are approximately the same distance from a breakage point. 44602 is significantly further from a breakage point, as it was not found to be hybrid in eleven asci in which 37803 was hybrid. From high resolution recombination, and complementation, data, 37803 is near one end of

the pdx-1 locus, and unwinding of the DNA double helix may proceed in either direction.

Part 6. A possible recombination gene affecting pdx-1.

The postulated duplication in the pdx(37803) strain H823 (Radford 1963, Radford & Threlkeld 1965), giving "conversion" at a rate comparable to that in the inter-allele cross prototroph analysis, and its polarity of conversion being much the same as at several other alleles at other loci, no longer seems likely. Therefore the proposed explanation of recombination rate differences is also questionable, requiring another mechanism to explain the observation that strain H823 gave rise to nine aberrant asci out of 153, whereas H815 gave none in a comparable sample. Catcheside has found two genes controlling recombination rates at specific loci (Jessop & Catcheside 1965, Catcheside, personal communication), and these so-called rec genes have been found to cause up to a ten-fold difference in prototroph frequencies. It is considered not unlikely that the significant differences in rates of aberrant asci at the pdx-1 locus are due to a similar recombination gene, as yet of unknown location.

Part 7. A general consideration of gene conversion in the light of the current recombination theory of Whitehouse.

Mutations may be classified into two main sub-divisions, aberrations, and point mutations. The first class is composed of duplications and deletions of one to many base pairs. The point mutation class consists of base pair transitions, transverions, and transition-transversions.

a) i. Considering first the mutant type which is a duplication, unwinding of the mutant and wild-type double helices will unwind normally following breakage, and will commence cross-annealing from the broken end. The annealing will continue to the region of the duplication, where the non-complementary bases will not anneal. If the original unwinding proceeded past the duplication, it will now probably re-anneal with its original partner strand back to the duplication, which will take the form of a projecting loop. Two chromatids out of the four will thus contain a DNase-susceptible single-strand loop. Therefore, with nothing more than the DNase which is essential for other steps in the Whitehouse hypothesis, with the removal of the duplication, both hybrid strands will be corrected to the wild-type nucleotide sequence. It is, however known that in bacteria, UV-sensitivity is associated with a lack of recombination, and this is interpreted as being due to recombination and UV repair having a common mechanism (Clark & Margiules 1965).

The UV-repair mechanism invoked is that of thymine-dimer excision, and lateral dimers give loops very similar to the loops in hybrid DNA postulated above. Evidence from fungi (Holliday 1966) shows a similar, although not absolute, correlation of UV-sensitivity and recombination. Therefore, postulating that the thymine dimer excision enzyme also affects recombination, its likely mode of action is to break the short strand in a duplication hybrid loop, allow the loop to open out, and allow complementary nucleotides to fill in the single

strand gap. By this mechanism, both hybrid strands (chromatids) convert to homozygosity for the duplication mutant. Thus relative rates of 3:1 and 1:3 ratios would then depend on the balance between DNase and the loop-expanding enzyme.

ii. If the mutant is due to a deletion of several nucleotides, the wild-type nucleotide sequence corresponding to the deletion will form the loop. Therefore the correction to mutant or wild-type will be the reverse of the above case.

b) Single base pair changes can be transitions, transverions, or transition-transversions, and for the purposes of this discussion only mutations at a single base pair, which in the wild type state is A-T, will be considered.

i. Let us consider first a transition mutant at the A-T base pair to G-C. Hybridity at this site will reassort the bases to A-C and G-T, both being purine pyrimidine pairs, neither pair of which will cross band, but neither pair of which will cause any major distortion in the double helix. If this hybridity can be detected, the detecting enzyme is unlikely to be the same as that which detects major distortions such as the loops described above. If the hybridity is detected, there is potential for preferential conversion in both strands, but if it is not, aberrant 4:4 asci would be produced. Correction, if it occurs, may go to:-

1/ A-T and A-T (both +, 3+:1-)

2/ A-T and G-C (one + and one -, 2+:2-)

3/ G-C and A-T (one + and one -, 2+:2-)

4/ G-C and G-C (both mutant, 1+:3-)

ii. If the mutation is a transversion of A-T to T-A, hybridity will give A-A and T-T base pairs.. The A-A strand, consisting of two purines, will be larger than a normal purine-pyrimidine base pair, and will cause some distortion of the DNA backbone. However, in the T-T strand, the two small pyrimidines will be accommodated in the double helix without distorting the structure. Maybe the A-A distortion can be detected by the same enzyme that detects thymine dimers and the postulated duplication loops, but the T-T hybridity can only be detected by a second enzyme, probably that which detects non-complementary purine-pyrimidine pairs.

If hybridity at A-A only is detected, correction will be randomly to A-T or T-A as there is, assuming no influence of adjacent bases, no potential on which preferential correction may act. Correction to A-T will give a 5+:3- ratio, whereas correction to T-A will give a 3+:5- ratio. Correction of the T-T but not the A-A strand, again with no preference, will give similar results.

If hybridity in both strands is detected and corrected, and corrected randomly, the possible results are:-

1/ A-T and A-T (both +, 3+:1-)

2/ A-T and T-A (one + and one -, 2+:2-)

3/ T-A and T-A (both -, 1+:3-)

4/ T-A and A-T (one + and one -, 2+:2-)

iii. The third type of point mutation is a transition-transversion of A-T to C-G. The hybrid DNA is therefore A-G and C-T, the first pair being a distorted purine-purine pair, and the second an undistorted pyrimidine-pyrimidine.

If hybridity of A-G only is detected, correction will be either to A-T or C-G, but possibly with a potential preference to one of these. Correction to A-T gives a 5+:3- ratio, but correction to C-G gives 3+:5-. Correction of the C-G but not A-T, again with a potential preference, will give essentially similar results.

If hybridity in both strands is detected, correction of both can give:-

1/ A-T and A-T (both +, 3+:1-)

2/ C-G and C-G (both -, 1+:3-)

3/ A-T and C-G (one + and one -, 2+:2-)

4/ C-G and A-T (one + and one -, 2+:2-)

If A-G $\rightarrow$ A-T and C-T $\rightarrow$ A-T preferentially, there will be a large class 1. If A-G $\rightarrow$ C-G and C-G $\rightarrow$ C-G preferentially, there will be a large class 2. If A-G $\rightarrow$ A-T and C-T $\rightarrow$ C-G preferentially, there will be a large class 3. If A-G $\rightarrow$ C-G and C-T $\rightarrow$ A-T preferentially, there will be a large class 4.

All the above cases assume that equal lengths of hybrid DNA are formed in the two strands involved. Such may not be so, and if this is the case, it will result in a further increase in the frequency of 5+:3- and 3+:5- types.

In all the types of mutation discussed above, only one, that of transversion, has no strand differences on which preferential conversion can act. Very little data has been published in which samples have been large enough to show significance in conversion direction, but possibly pan(72) and pan(36) (Case & Giles, 1964), pan(3) (Case & Giles, 1958) and pdx(39106) are the mutants most likely to be transverions, for the data show equivalence of 3:1 and 1:3 types for these alleles. This is based on the fact that for pan(72) there are one 6:2 and three 2:6 asci, pan(36) has produced two 6:2 and one 2:6 ratios, and pan(3) with two 6:2 and three 2:6 ratios.

Part 9. Prototroph production from inter-allelic crosses.

According to current theory (Whitehouse & Hastings 1965, Holliday 1964), the production of a prototroph from an inter-allelic cross requires firstly the breakage of the DNA double helices in two chromatids, unwinding of these two double helices to involve at least one mutant allele site, cross-annealing, and finally conversion of the hybrid mutant site in the chromatid which is wild-type for the other allele. The hypothesis of ordering alleles in loci by majority-minority relationships (Lissouba et al, 1962) as modified for Neurospora (Murray 1963) is based upon the assumption that conversion of a hybrid site in either direction is a random event. That equal frequency of conversion in either direction is a characteristic of some alleles is beyond doubt,

e.g. pan-2(3) (Case & Giles, 1958), pan-2(72) and pan-2(36) (Case & Giles, 1964) and pdx(39106). However, the majority of alleles studied show significant inequality in conversion, e.g. Sordaria Fimicola "g" (Kitani et al, 1962), Ascobolus immersus w-62 and w-6 (Emersson, 1966), cys-17 and cys-64 (Stadler & Towe, 1963), pan-2(5) (Case & Giles, 1958), and pdx(37803).

It is known, from the asci reported in Chapter VIII, that alleles 37803 and 39106 become involved in hybrid DNA with approximately equal frequency (7 times for 37803 and five for 39106). However, all seven 37803 hybrids were converted to the mutant, hence giving rise to no prototrophs. In the cross RJ3 by RC3 (pyr-1, pdx(37803) x pdx(39106), col-4), of the non-crossover prototrophs $P_1$ and $P_2$, 50 were col-4 and twelve were pyr-1. Presumably the pyr-1 types were derived from breakage leading to non-crossover hybridity at the 37803 allele, whereas the col-4 prototrophs were from breakage and non-crossover hybridity at 39106. Assuming that half the 39106 hybrids became wild-type, there were approximately 100 39106 hybrids in the sample, half of which became corrected to 39106. Hybridity at 39106 and 37803 being of approximately equal frequency, there were also 100 37803 hybrids. However, only twelve of these hybrids became prototrophs, the other eighty-eight being converted back to 37803. The ratio of twelve wild-type convertants to eighty-eight mutants does not differ significantly from the ratio of seven conversions to mutant and none to wild-type found for allele 37803 in the cross H823 x J104.

## CONCLUSIONS

1. The complementation data indicate that the seven pyridoxine auxotrophs are all alleles of the same locus, or functional unit, pdx-1.

2. On the basis of complementation differences between 25° and 37°, the pdx-1 locus consists of a single cistron.

3. The pdx-1 gene, in terms of the Whitehouse hypothesis, undergoes recombination as a unit. DNA breakage and unwinding can occur at both ends of that unit, as alleles 37803 and 39106 can each become involved in hybrid DNA independently of the other.

4. Prototrophs from inter-allelic crosses are produced by a mechanism of non-reciprocal recombination, but background heterozygosity modifies the prototroph class frequencies.

5. The production of a high rate of aberrant asci in crosses involving strain H823 appears to be due to normal gene conversion, although the earlier duplication hypothesis cannot be entirely eliminated. The high rate is compatible with the results of a number of prototroph analyses, and the polarity of conversion probably reflects the preferred direction of conversion of allele 37803.

6. There is a marked genetic background effect on the rate of recombination, reflected in the higher rate of gene conversion in H823 as compared to H815, and suggesting a gene similar to that affecting the recombination rate at hist-1.

7. The En-pdx-1 gene controls the production of a yellow metabolite, and its further metabolism is dependent on the product of the pdx-1 gene.

8. The accumulation product of En-pdx-1 may be on an unrelated metabolic pathway requiring the coenzymic activity of pyridoxal phosphate, although the pigment being a pyridoxine percursor accumulating before the pdx-1 blockage, and inhibited under certain circumstances by feed-back, cannot be ruled out.

9. The En-pdx-1 allele, on the basis of its behaviour in heterokaryons, is dominant over its wild-type, non-pigment-producing allele.

10. The vitamin analogue desoxypyridoxine hydrochloride inhibits leaky growth of all seven pdx mutants at low pH, but is only active against the non-pH-sensitive mutants at pH 7 in the presence of free ammonia. Without this analogue, much of the work described herein could not have been accomplished.

# BIBLIOGRAPHY


1. Ahmed, A., Case, M.E., & Giles, N.H., 1965.
The nature of complementation among mutants in the hist-3 region of Neurospora crassa. Brookhaven Symp. in Biol., 17 : 53-65.

2. Barratt, R.W., 1954.
A word of caution: genetic and cytological effects of Abbott stocks in Neurospora crassa. Microbial Genet. Bull. 11 : 5-8.

3. _____, 1962.
Origin of important wild-type stocks of Neurospora crassa. Neurospora Newsl. 2 : 24-25.

4. Barratt, R.W., & Ogata, W.N., 1964.
Neurospora stock list - second revision. Neurospora Newsletter, 5 : 24-82.

5. _____, 1964.
Neurospora stock list - second revision - first supplement. Neurospora Newsletter, 6 : 29-40.

6. _____, 1965.
Neurospora stock list - second revision - second supplement. Neurospora Newsletter, 7 : 29-31.

7. _____, 1965.
Neurospora stock list - second revision - third supplement. Neurospora Newsletter, 8 : 29-32.

8. Beadle, G.W., & Coonradt, V.L., 1944.
Heterokaryosis in Neurospora crassa. Genetics, 29 : 291-308.

9. Beadle, G.W., and Tatum, E.L., 1941.
Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. U.S., 27 : 499-506.

10. _____, 1945.
Neurospora.II. Methods of producing and detecting nutations concerned with biochemical requirements. Amer. J. Bot., 32 : 678-686.

11. Belling, J., 1933.
Crossing over and gene rearrangement in flowering plants. Genetics, 18 : 388-411.

12. Bonner, J., & Dorland, R., 1943.
Experiments on the application of Neurospora sitophila to the assay of pyridoxin in tomato plants. Arch. Biochem., 2 : 451-462.

13. Bridges, C.B., 1916.
Non-disjunction as proof of the chromosome theory of inheritance. Genetics, 1 : 1-52 and 107-163.

14. Brockman, H.E., & de Serres, F.J., 1963.
Induction of ad-3 mutants of Neurospora crassa by 2-amino-purine. Genetics, 48 : 597-604.

15. Case, M.E., and Giles, N.H., 1958.
Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in Neurospora crassa. Proc. Natl. Acad. Sci. U.S., 44 : 378-390.

16. _____, 1960.
Comparative complementation and genetic maps of the pan-2 locus in Neurospora crassa. Proc. Natl. Acad. Sci. U.S., 46 : 659-676.

17. _____, 1964.
Allelic recombination in Neurospora: Tetrad analysis of a three point cross within the pan-2 locus. Genetics, 49 : 529-540.

18. Catcheside, D.G., 1960.
Complementation among histidine mutants of Neurospora crassa. Proc. R. Soc. B., 153 : 179-194.

19. Cavalieri, L.F., & Rosenberg, B.H., 1961.
The replication of DNA. Biophysical J., 1 : 317-351.

20. Clark, A.J., & Marquiles, A.D., 1965.
Isolation and characterisation of recombination-deficient mutants of Escherichia coli K 12. Proc. Natl. Acad. Sci. U.S., 53 : 451-459.

21. Creighton, H.B., & McClintock, B., 1931.
A correlation of cytological and genetical crossing over in Zea mays. Proc. Natl. Acad. Sci. U.S., 17 : 492-497.

22. Crick, F.H.C., & Orgel, L.E., 1964.
The theory of inter-allelic complementation. J.Mol. Biol. 8 : 161-165.

23. Cushing, J.E., & Reid, E.B., 1948.
Pigment production by Neurospora crassa in the presence of para-aminobenzoic acid. J. Bacteriol., 55 : 551-554.

24. Darlington, C.D., 1936.
The external mechanics of the chromosomes. Proc. R. Soc. B., 121 : 264-319.

25. Dempsey, W.B., 1965.
Control of pyridoxine biosynthesis in Escherichia coli.
J. Bacteriol. 90 : 431-437.

26. de Serres, F.J., 1956.
Studies with purple adenine mutants in Neurospora crassa I.
Genetics, 41: 668-676.

27. _____, 1960.
Studies with purple adenine mutants in Neurospora crassa IV.
Genetics 45: 555-566.

28. Edgar, R.S., Denhardt, G.H., & Epstein, R.H., 1964.
A comparative genetic study of conditional lethal mutations of bacteriophage T4D. Genetics 49 : 635-648.

29. Emerson, S., 1966.
Quantitative implications of the DNA-repair model of gene conversion. Genetics, 53 : 475-485.

30. Fincham, J.R.S., 1957.
A modified glutamic acid dehydrogenase as a result of gene mutation in Neurospora crassa. Biochem. J., 65 : 721-728.

31. Fincham, J.R.S., & Coddington, A., 1963.
Complementation at the am locus of Neurospora crassa.
J. Mol. Biol., 6 : 361-373.

32. Fincham, J.R.S., & Day, P.R., 1963.
Fungal genetics. Blackwell, Oxford.

33. Fincham, J.R.S., & Pateman, J.A., 1957.
Formation of an enzyme through complementary action of mutant 'alleles' in separate nuclei of a heterokaryon.
Nature 179 : 741-742.

34. Frost, L.C., 1955.
A possible interpretation of the cytogenetic effects of Abbott stocks in Neurospora crassa. Microbial Genet. Bull. 12 : 7-9.

35. _____, 1961.
Heterogeneity in recombination frequencies in Neurospora crassa. Genet. Res., 2 : 43-62.

36. Glazer, H.S., Fichter, B., Mueller, J.F., & Vilter, R.W., 1951.
The effect of pyridoxine deficiency induced by desoxypyridoxine in human subjects upon the excretion of $N^1$-methylnicotinamide after tryptophan administration. Arch. Biochem., 32 : 424-427.

37. Griffiths, A.J.F., & Threlkeld, S.F.H., 1966.
Internuclear effects on prototroph frequencies of some crosses in Neurospora crassa. Genetics 54 : 77-87.

38. Hardesty, B.A., & Mitchell, H.K., 1963.
The accumulation of free fatty acids in poky, a maternally inherited mutant of Neurospora crassa. Arch. Biochem. Biophys., 100 : 330-334.

39. Holliday, R., 1964.
A mechanism for gene conversion in fungi. Genet. Res., 5 : 282-304.

40. _____, 1966.
Abstracts of Annual Meeting of the Genetical Society.

41. Horowitz, N.H., 1947.
Methionine synthesis in Neurospora. The isolation of cystathionine. J. Biol. Chem., 171 : 255-264.

42. Houlahan, M.B., Beadle, G.W., & Calhoun, H.G., 1949.
Linkage studies with biochemical mutants of Neurospora crassa. Genetics, 34 : 493-507.

43. Ishikawa, T., 1962.
Genetic studies of ad-8 mutants of Neurospora crassa II. Interallelic complementation at the ad-8 locus. Genetics, 47 : 1755-1771.

44. Jessop, A.P., & Catcheside, D.G., 1965.
Interallelic recombination at the hist-1 locus in Neurospora crassa and its genetic control. Heredity 20 : 237-256.

45. Kafer, E., 1958.
an 8 chromosome map of Aspergillus nidulans. Advan. Genet., 9 : 105-156.

46. Kapuler, A.M., & Bernstein, H., 1963.
A molecular model for an enzyme based on a correlation between the genetic and complementation maps of the locus specifying an enzyme. J. Mol. Biol., 6 : 443-451.

47. Kitani, Y., Olive, L.S., & El-Ani, A.S., 1961.
Transreplication and crossing over in Sordaria fimicola. Science, 134 : 668-669.

48. _____, 1962.
Genetics of Sordaria fimicola V. Aberrant segregation at the g locus. Am. J. Bot., 49 : 697-706.

49. LaCour, L.F., & Pelc, S.R., 1958.
Effect of colchicine on the utilisation of labelled thymidine during chromosomal reproduction. Nature, 182 : 506-508.

50. Lavigne, S. & Frost, L.C., 1964.
Recombination frequency and wild-type ancestry in Linkage group I of Neurospora crassa. Genet. Res., 5 : 366-378

51. Lederberg, J., 1955.
Recombination mechanisms in bacteria. J. Cell. Comp. Physiol. 45, suppl. 2 : 75-107.

52. Leupold, U., & Gutz, H., 1965.
Genetic fine structure in Shizosaccharomyces. Genetics Today 2 : 31.

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

54. Lindegren, C.C., & Lindegren, G., 1942.
Locally specific patterns of chromatid and chromosome interference in Neurospora. Genetics, 27 : 1-24.

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

56. Lunan, K.D., & West, C.A., 1963.
The isolation of $C^{14}$ labelled pyridoxamine from Candida utilis. Arch. Biochem. Biophys. 101 : 261-268.

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

58. Marimuthu, K.M., 1966.
The mode of replication of the chromosomes of Haplopappus gracilis at mitosis and meiosis. Ph.D. Thesis, McMaster University.

59. Marimuthu, K.M., & Threlkeld, S.H.F., 1964.
Tetrad analysis by autoradiography. Nature, 203 : 101-102.

60. _____, 1966.
The mode of replication of the chromosomes of Haplopappus gracilis at meiosis. Can. J. Genet. Cytol.

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

62. Meselson, M., and Weigle, J.J., 1961.
Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Natl. Acad. Sci. U.S., 47 : 857-868.

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

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

65. _____, 1955.
Further evidence of aberrant recombination in Neurospora. Proc. Natl. Acad. Sci. U.S., 41 : 939-937.

66. Mitchell, H.K., & Houlahan, M.B., 1946.
Adenine-requiring mutants of Neurospora crassa. Federation Proc. 5 : 370-375.

67. Mitchell, M.B., Pittenger, T.H., & Mitchell, H.K., 1952.
Pseudo-wild types in Neurospora crassa. Proc. Natl. Acad. Sci. U.S., 38 : 569-580.

68. Mortimer, R.K., & Hawthorne, D.C., 1966.
Genetic mapping in Saccharomyces. Genetics 53 : 165-173.

69. Murray, N.E., 1963.
Polarized recombination and fine structure within the me-2 gene of Neurospora crassa. Genetics 48 : 1163-1183.

70. Perkins, D.D., 1956.
Crossing over a multiply marked chromosome arm of Neurospora. Microbial Genet. Bull. 13 : 22-23.

71. Pittenger, T.H., 1954.
The general incidence of pseudo-wild types in Neurospora crassa. Genetics, 39 : 326-342.

72. Plaut, W., & Mazia, D., 1956.
The distribution of newly synthesised DNA in mitotic division. J. Biophysic. & Biochem. Cytol., 2 : 573-587.

73. Pontecorvo, G., 1963.
The Leeuwenhoek Lecture: Microbial genetics: retrospect and prospect. Proc. R. Soc. B., 158 : 1-23.

74. Radford, A., 1963.
Genetic analysis of some unusual asci from a cross in Neurospora crassa. M.Sc. Thesis, McMaster University.

75. _____, 1965.
Heterokaryon complementation among the pyridoxine auxotrophs of Neurospora crassa. Can. J. Genet. Cytol., 7 : 472-477.

76. Radford, A., & Threlkeld, S.F.H., 1965.
Some unusual 3:1 asci in Neurospora crassa. Can. J. Genet. Cytol., 7 : 357.

77. Reid, E.B., Pritchett, E.G., & Cushing, J.E., 1952.
Compounds produced by Neurospora crassa in the presence of toxic concentrations of p-aminobenzoic acid. J. Biol. Chem., 199 : 443-450.

78. Rodwell, V.W., Volcani, B.E., Ikawa, M., & Snell, E.E., 1958.
Bacterial oxidation of vitamin $B_6$. J. Biol. Chem., 233 : 1548-1554.

79. Sarabhai, A.S., Stretton, A.O.W., Brenner, S., & Bolle, A., 1964.
Colinearity of the gene with the polypeptide chain. Nature 201, 13.

80. Smith, B.R., 1965.
Interallelic recombination at the hist-5 locus in Neurospora crassa. Heredity, 20 : 257-276.

81. Stadler, D.R., 1956.
A map of linkage group VI of Neurospora crassa. Genetics 41 : 528-543.

82. Stadler, D.R., & Towe, A.M., 1963.
Recombinations of allelic cysteine mutants in Neurospora. Genetics, 48 : 1323-1344.

83. Stern, C., 1931.
Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Factorenaustauschs. Biol. Zbl., 51 : 547-587.

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

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

86. Stokes, J.L., Foster, J.W., & Woodward, C.R., 1943.
Synthesis of pyridoxine by a "pyridoxinless" X-ray mutant of Neurospora sitophila. Arch. Biochem., 2 : 235-245.

87. Swift, H.H., 1950.
The desoxyribose nucleic acid content of animal nuclei. Physiol. Zool. 23 : 169-198.

88. Taylor, J.H., 1958.
Sister chromatid exchanges in tritium-labelled chromosomes. Genetics, 43 : 515-529.

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

90. Vogel, H.J., 1956.
A convenient growth medium for Neurospora (Medium N). Microbial Genet. Bull. 13 : 42-43.

91. Westergaard, M., & Mitchell, H.K., 1947.
Neurospora V. A Synthetic medium favouring sexual reproduction. Am. J. Bot., 34 : 573-577.

92. Whitehouse, H.L.K., 1963.
A theory of crossing-over by means of hybrid deoxyribonucleic acid. Nature, 199 : 1034-1040.

93. Whitehouse, H.L.K., & Hastings, P.J., 1965.
The analysis of genetic recombination on the polaron hybrid DNA model. Genet. Res., 6 : 27-92.

94. Wilson, J.F., & Garnjobst, L., 1966.
A new incompatibility locus in Neurospora crassa. Genetics, 53 : 621-631.

95. Wimber, D.E., & Prensky, W., 1963.
Autoradiography with meiotic chromosomes of the male newt (Triturus viridescens) using $H^3$-thymidine. Genetics, 48 : 1731-1738.

96. Woodward, V.W., 1962.
Complementation and recombination among pyr-3 heteroalleles of Neurospora crassa. Proc. Natl. Acad. Sci. U.S., 48 : 348-356.