ENVIRONMENTAL AND GENETIC FACTORS AFFECTING INSTABILITY AT MITOSIS IN *ASPERGILLUS NIDULANS*

by

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A C K N O W L E D G E M E N T S

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G E N E R A L I N T R O D U C T I O N

GENERAL INTRODUCTION

The fidelity of chromosome replication at mitosis is an obvious essential for genetic continuity. Although earlier ideas on the stability of the genetic material have been modified in the light of recent work on DNA repair mechanisms, the effective stability forms the basis of genetics, biology and life itself. Cytology provided a visual understanding of the accuracy of chromosome replication and the Watson-Crick structure of DNA (1953), together with the studies of Meselson & Stahl (1958), gave an explanation in molecular terms. However, there are now many recorded instances of genetic instability in flowering plants, Drosophila, maize and, more recently, microorganisms. Limited explanations of some of these have been given in formal genetic terms (for example, McClintock, 1951; Fincham 1967). But these cases of instability cannot yet be reconciled readily with the known structure of DNA and its mode of replication, and would appear to indicate that effective mutational stability is not the rule for specific chromosomal regions or for specific loci.

The homothallic fungus Aspergillus nidulans has features which make it very suitable for studies on genetic instability. The detection of vegetative variants is facilitated by its flat, colonial growth. In appropriate cases the use of autonomous conidial colour mutants eases detection of mosaic colonies and the isolation of variants. Such isolated variants, the equivalent of somatic tissues in a higher organism, can then be subjected to genetic analysis. In fact, it was these features of A. nidulans which permitted elucidation of the parasexual cycle when diploid strains were first synthesised.

Until about 1966 instability in A. nidulans was explored on two fronts. There was considerable study of variation apparently due to cytoplasmic changes (Jinks, 1954, 1956, 1957). At the chromosomal level there was intensive study of diploids and aneuploids. Diploids undergo mitotic crossing-over (Pontecorvo & Roper, 1953). They also undergo haploidization (Pontecorvo and Roper, 1953; Pontecorvo, Tarr Gloor & Forbes, 1954; Kafer, 1960) in which chromosomes are lost successively until a stable haploid state is reached. The chromosomal events of mitotic crossing-over and haploidization, although in a sense cases of instability, did not fall outside general and classical genetic experience. Stern (1936) had provided a full classical understanding of mitotic crossing-over and haploidization could be regarded as a succession of non-disjunctional events.

The first novel case of mitotic instability was found by Bainbridge & Roper (1966). From certain crosses of morphologically-normal parents two thirds of the progeny were normal and one third had reduced linear growth rate and a characteristic "crinkled" morphology.[1] Ascus analysis showed that 25% of the acrospores were inviable. Examination of the pedigrees showed that in each such cross one parent was chromosomally standard while the other had an apparently non-reciprocal translocation of a segment of linkage group III to linkage group VIII. The two thirds normal progeny were of the two parental types, the inviable ascospores were presumed to lack the relevant segment of linkage group III and

1. Intrinsic to this morphology is a marked mound-shape morphology.

the crinkled progeny were inferred to have this segment in duplicate, one segment being translocated. Confirmation of this duplication was obtained through the entirely unexpected, vegetative instability of crinkled types. After a few days' incubation they produced sectors which, in varying degree, approached wildtype in growth rate and morphology. Such sectors were generally referred to as improved sectors or variants. Cystine-independent, crinkled progeny were obtained from a cross involving the cys2 allele (cystine requirement) which was known to be located on the relevant linkage group III segment. Some individual colonies of this type produced[2] both cystine-independent and cystine-requiring improved sectors. This showed that these crinkled progeny were heterozygous for cys2 and it suggested ways in which the sectors might have arisen. Perhaps a variable part of one or other duplicate segment became inactive during growth; this would lead to phenotypic improvement and, if that segment carried cys2$^+$, to cystine requirement. There would have been analogy with the random inactivation of an X chromosome in female mammals (Lyon, 1961, 1962). Alternatively it was possible that the variants arose by deletion of a variable part of one or other duplicate segment. Refined cytological examination of A. nidulans is at present impossible (Elliott, 1960) but genetic analysis showed the deletion hypothesis to be correct. Improved sectors arose by loss of a variable part of either duplicate segment. Thus, morphological improvement (morphology

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2. In general, the nonmutant region of a colony which gives rise to mutant sectors or variants is referred to as the parental region.

approaching wild-type or normal morphology) and increased (improved) growth rate involved loss of chromosomal material from the duplication.

Further details of this form of instability were shown through the use of a strain whose lineage traces back to a particular culture first studied by Pritchard (1956). Along with a complete linkage group I, this strain carried a segment of linkage group I terminally attached to linkage group II. It had the particular advantage that the duplicated I segment carries the loci of two known nutritional markers (ad20 and bi1) and the locus for yellow conidia. This duplication strain (I duplication strain) was also crinkled but comparatively to a far lesser degree than a III duplication strain (i.e., a strain carrying the duplication of a segment of chromosome III). Such a I duplication strain was also slow growing. The length of the I duplication is not known but studies by Nga and Roper (1968) indicate that it is at least 28 to 30 units in length. The findings with this I duplication strain (Nga & Roper, 1968, 1969; Roper & Nga, 1969) may be summarized as follows. It was vegetatively unstable, producing improved sectors of different types, e.g., yellow sectors (variants); from this and a study of other duplication strains, it seemed likely that all duplication strains are unstable. A quantitatively-haploid or near-haploid genome was reached either in one step or in successive steps; in some cases stability was reached only after deletions, inevitably non-overlapping, from both of the duplicate segments. Loss of chromosomal material occurred by an intrachromosomal process since in no instance did a variant (sector) show altered linkage arrangement of the duplication

segment markers. Discovery of a new class of variants, "browns", (produced by the I duplication strain) suggest possible chromosome mechanisms for these instability processes. Brown variants[3] had deteriorated morphology, were sparsely conidiating, had slightly less than parental growth rate, and frequently showed greatly enhanced instability. In the cases analysed the loci of morphological change and enhanced instability were located on either of the duplicate I segments. The evidence indicated that brown types carried new tandem duplications; further study by Azevedo and Roper (1970) provided additional evidence of this. Unequal sister chromatid exchange or crossing-over within an intrachromosomal loop would explain both deletions and new tandem duplications.

At this stage there remained a critical unresolved problem in relation to the role of the duplication in instability. On the one hand the duplication might merely have permitted expression of the instability; if there were sufficiently frequent, spontaneous deletions <u>throughout</u> the genome, only those from either duplicate segment would yield viable nuclei. Indeed, such types would be not only viable, they would have also a selective advantage. On the other hand, it could be supposed that a duplication provoked (its own) instability. This latter explanation was established by a study of haploid and diploid strains, with and without translocations and duplications. The most important comparisons were of a duplication haploid, a standard diploid and a diploid with a duplicate segment. The standard (or balanced) diploid showed

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3. Brown variants were also produced by the III duplication strains and some brown variants were shown to produce morphologically improved sectors.

only rare sectors which could be fully explained by mitotic crossing-over and haploidization. The duplication diploid (unbalanced diploid) showed ten times as many sectors as did the duplication haploid, although the sectors of the former had less advantage than those of the latter over their respective parents. All, or almost all, of the sectors produced by the duplication diploid were still diploid and arose by deletions from the translocated duplicate segment. A proportion, perhaps about 10%, were, in fact, hypodiploids arising from deletions which extended into the distal end of linkage group II. These studies showed that chromosomal imbalance provoked instability and that the events were confined largely to the segments involved in imbalance.

The study of 'mitotic non-conformity' in A. nidulans has three features of interest. First, it should at least provide information as to the reasons why mitosis can become less than an accurate process. Second, there are striking similarities between mitotic non-conformity and certain cases of genetic instability in other species. Diverse and sometimes complex explanations have been offered for some of these cases. Extension of the parallels between mitotic non-conformity and other instability phenomena might eventually lead to unification and simplification. The third feature is speculative and must be approached cautiously. We may ask whether genetic instability represents only disordered mitosis or whether it might, as a controlled and programmed process, serve a useful purpose in higher organisms. Development in some cases is known to involve selective elimination of genetic material (see Waddington, 1956; Fischberg and Blackler, 1961; Beermann, 1966; Brown, 1969). However, the generally held view (see for

example Ebert, 1965) is that the genome is constant throughout development which proceeds by selective gene activation and inactivation. Though there is no denying that gene activation and gene inactivation play an important role in development, it is possible that many developmental situations ascribed to gene inactivation may in fact be based upon mutations or genetic changes. In this regard, genetic diversity within a mitotic lineage is clear in the case of antibody variations, and it is interesting that recent speculations on the origin of antibody variation (Edelman and Gally, 1967) propose processes also proposed for instability in Aspergillus (Nga & Roper, 1968).

The work described in this thesis was designed to obtain information dealing principally with the first two of the above features - the causation of mitotic infidelity and the parallel with other cases of instability. But it was hoped that it might provide evidence which could be used in constructive thinking on the third feature - the useful role, if any, of mitotic non-conformity in higher organisms.

All previous studies of instability had been made at about 37°C, the optimum temperature for Aspergillus. Apart from some casual observations by Nga & Roper (1968), there was no knowledge of the effects of different temperatures on the processes of instability. A. nidulans grows well over a wide temperature range from 28°C to 42°C and it was proposed to investigate the effects of different temperatures on instability of the III and of the I duplication strains. Since certain mutable genes have been studied at different temperatures there might be a basis for further comparisons. The second project was to try to combine, in a single strain, both the I and III duplications. This might open the way to a study of interactions of various kinds.

perhaps of a type which could be used as a model of programmed genetic change.

MATERIALS AND METHODS

MATERIALS AND METHODS

A. Organism and Strains

Aspergillus nidulans is a homothallic ascomycete; a description of its life cycle has been given by Thom and Raper (1945) and the pioneer genetics of this species was described by Pontecorvo et al. (1953). Figure 1 illustrates the essential features of the organism. In the vegetative cycle, a haploid, uninucleate conidium germinates to give multinucleate septate hyphae. Nuclear division is synchronous and there is a connection between mitotis and septum formation (Clutterbuck, 1965). The septa existant between the multinucleated cells of the hyphae have passages[1] or channels. On the surface of the colony specialised cells (conidiophores) produce primary and secondary sterigmata. The nuclei in the latter undergo repeated divisions and bud off chains of conidia. An important feature of the vegetative cycle is hyphal anastomosis. It occurs readily between branches of one hypha and between different hyphae of one monosporous mycleum; anastomosis can also occur between hyphae of two mycelia of the same or of different strains. In regards to the sexual cycle, meiosis occurs in specialized bodies (perithecia). A perithecium is initiated by two haploid nuclei which undergo conjugate divisions in the ascogenous hyphae. Pairs of nuclei then fuse and the zygotes undergo meiosis, followed by a single mitotic division; the eight (four pairs) haploid ascospores resulting

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1. Though the presence of such passages allows for the continuity of cytoplasm from cell to cell, the movement of nuclei from cell to cell is nevertheless quite restricted (Clutterbuck, 1965).

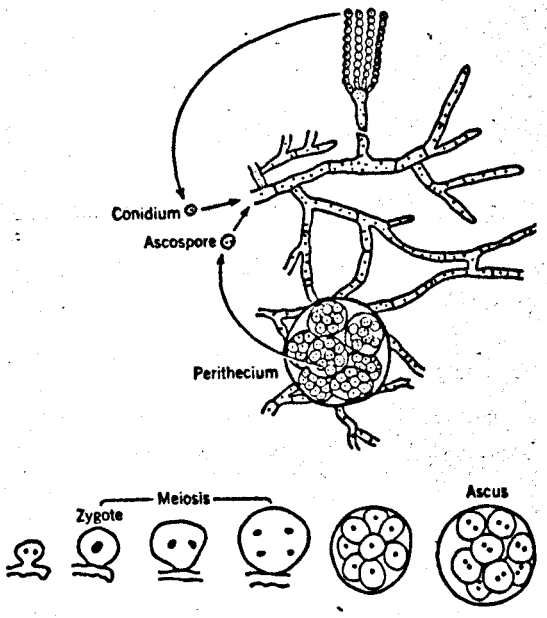


Figure 1. Life cycle of Aspergillus nidulans (After Pontecorvo et al).

from each zygote are held together within a spherical ascus. Each perithecium has up to about 10^5 asci. Since the species is homothallic, a culture derived from a single haploid nucleus is potentially fertile though, during laboratory culture, many strains have become self-sterile. Crossing is achieved via heterokaryosis (p. 18, p. 20).

Most of the strains of A. nidulans used originated from the Glasgow stocks (Pontecorvo, et al, 1953, and Kafer, 1958). The designation of mutant alleles, and the phenotypes they determine are listed in Table A. The chromosomal location of markers used in this work are shown in Figure 2. The genotype of the master strain 'E' is given with the Figure. This master strain, having a marker in each of the eight chromosomes of A. nidulans, is used for the initial location of a new marker (or lethal) to its linkage group via mitotic haploidization. Through a process of elimination, other strains, each having only a few of its chromosomes marked, can also be used for the location of new markers or lethals. Except when otherwise stated all strains were free of translocations and duplications.

Stock cultures of non-duplication strains were maintained at 5°C on complete medium (CM) slopes. Such cultures of stock strains in current use were replaced every six months by new stock cultures through recovery from a low density conidial plating followed by classification tests confirming nutritional genotype.

Colonies of duplication strains not in frequent use were stored at 5°C on sealed plates with very thick CM. Only one colony of a given duplication strain was existant per plate.

TABLE A

List of Mutant Alleles and the phenotypes they determine.

1. Nutritional Mutant Alleles

Symbol	Phenotype (requirement for or dependence on)	Linkage Group (Chromosome)
ad14	adenine	IL
ad20 (or ad8)*	adenine	IR
arg2	arginine	IIIL
bi1	biotin	IR
nic2	nicotinic acid	VL
nic8	nicotinic acid	VII
orn4	ornithine	IVR
paba6 (or paba1)≠	p-aminobenzoic acid	IR
phen2	phenylalanine	IIIR
pro1	proline	IR
pyro4	pyridoxin	IVR
pyro12≠	pyridoxin	-
ribo1	riboflavine	IL
ribo2	riboflavine	VIIIR
s3	thiosulphate	VI
s12	thiosulphate (unable to utilize)	IIIR
facA	acetate	VL
gal1	galactose	IIIL
2. Spore colour	Mutant Alleles	
y	yellow conidia	IR
w3⁹	white conidia	IIL
3. Suppressor	Mutant Alleles	
su1 ad20	suppressor of ad20	IL

*ad8 is an allele of ad20 (Pritchard, 1960).

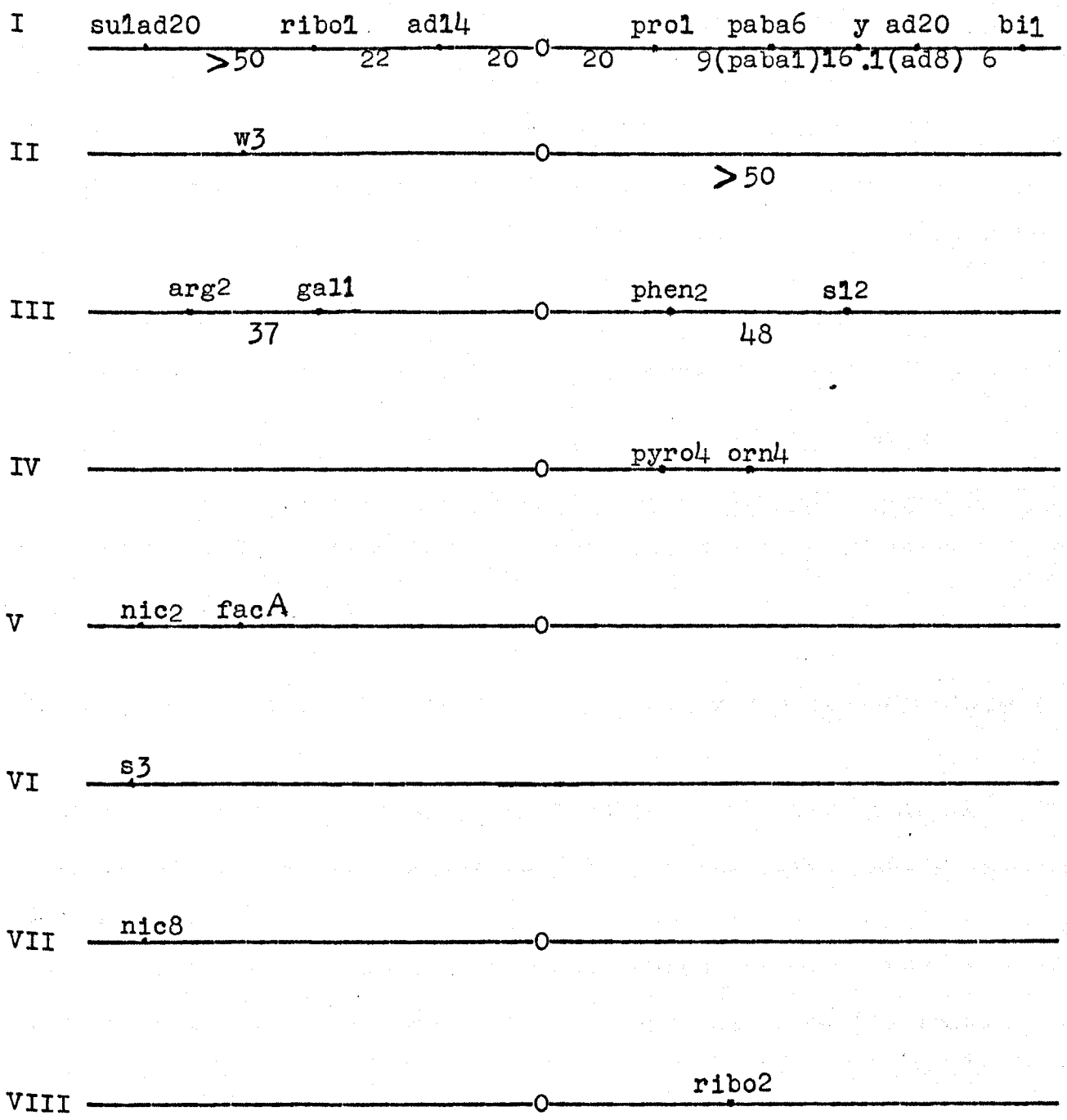
≠paba1 is an allele of paba6 (Roper, Personal Communication).

≠To be located in present study

⁹w3 is epistatic over y^+ (dominant allele of y; y^+, allele for green conidial colour) and y.

FIGURE 2

Linkage Map of *Aspergillus nidulans*



Genotype of Master Strain 'E' (M.S.E.) is as follows:

su1ad20 ad20y; w3; gal1; pyro4; facA; s3; nic8; ribo2

(McCully and Forbes, 1965)

After storage nutritional phenotypes of such stored duplication strains were confirmed through classification tests. Duplication strains in frequent use were also maintained at 5°C on plates with thick CM and were subcultured at monthly intervals or more frequently if the experimental time table dictated it.

Subculturing involved the following procedure: Conidia were taken from the crinkled centre of a given colonial duplication strain and placed in diluted Tween 80 present in a test tube. A uniform solution of conidia in diluted Tween 80 was obtained by an extended agitation of the test tube using a Whirlimixer. Enough conidia were added to the Tween so as to obtain a grey or cloudy solution after three or four minutes of agitation. Using the tip of a nichrome wire, conidia from the solution were inoculated on to each of the centres of a given number of CM plates. Incubation then followed. In this way each CM plate would have (subsequent to incubation) one colony of (made from) the given duplication strain, and thus one would have obtained as many colonies (colonial subcultures) of the given duplication strain as needed. To obtain many colonies of a given normal or translocation haploid strain, the same subculturing technique was employed,[2] the original conidia being taken from the normal or translocation haploid strain growing on a CM slope. When only one colony (colonial subculture) from any specific mutant sector or colony was to be derived, conidia were taken from the middle of the sector or colony in question and placed directly onto the centre of a CM plate without first mixing in Tween.[3]

2.Colonial subcultures of a diploid strain were also obtained using the same method.
3.Care was taken so that only the very end of the needle (wire) was covered with a very thin film of conidia.

Incubation followed incubation. Thus, when one colony (colonial subculture) is made from each of a group of different mutant sectors or different colonies, it is clear that each CM plate would contain (following incubation) only one colony derived from each different sector or colony.

Unless otherwise stated it is understood from what has been said that in this study any colony (colonial subculture) made or derived from a given colony or sector or strain refers to a colony which grows alone on a CM plate from the centrally placed conidial inoculum obtained (directly or indirectly) from such a given colony or sector or strain.

Symbols used for mutant alleles and translocated duplicate segments are illustrated by an example.

I $\frac{\text{y ad20 bi1}}{\text{I-II y ad20 bi1}}$; III $\frac{\text{s12}}{\text{III-VIII s12}}$

designates the genotype: Chromosome I carrying the mutant alleles y, ad20, and bi1; duplicate segment of I translocated to II, carrying the mutant alleles y, ad20, and bi1; chromosome III carrying the mutant allele s12; duplicate segment of III translocated to VIII, carrying the mutant allele s12.

The components making up a heterokaryon are separated by the symbol +. The haploid components of diploid strains are separated by the symbol //.

B. Conidial plating

Conidia were suspended in diluted Tween 80 and chains were broken by agitation with a Whirlimixer. Appropriate dilutions were spread on media so as to give colonies each arising from a single conidium.

C. Media

1) Minimal medium agar (MM)

Sodium nitrate	6g
Potassium chloride	.52g
Magnesium sulphate (7 H20)	.52g
Ferrous sulphate	small crystal
Zinc sulphate	small crystal
Potassium dihydrogen phosphate	1.52g
D-glucose	10g
Agar	15g
Distilled water	1000ml

The pH was adjusted to 6.5 with sodium hydroxide and hydrochloric acid before autoclaving at 10 lb for 10 min.

2) Complete medium (CM)

Minimal medium supplemented as follows:-

	Quantity per litre
Bacteriological Pepton (Oxoid)	2g
Hydrolysed casein (Oxoid)	1.5g
Yeast extract (Oxoid)	0.5g
*Yeast nucleic acid hydrolysate	2.5ml
**Vitamin solution	1.0ml

The pH was adjusted to 6.5 as before.

3) *Yeast nucleic acid hydrolysate

2g of yeast nucleic acid (Oxoid) were hydrolysed in 15ml of N.HCI for 20 min at 100°C. This procedure was repeated with a further 2g of yeast nucleic acid using N.NaOH. The two hydrolysates were mixed, the pH adjusted to 6.0 and the mixture filtered hot. The volume of the solution was adjusted to 40ml. It was kept over chloroform at 5°C.

4) **Vitamin solution

In 100ml water

p-aminobenzoic acid	10mg
pyridoxin HCl	50mg
aneurin	50mg
nicotinamide	100mg
riboflavine	100mg
biotin	0.2mg

The solution was steam sterilised for 10 min. and stored in a light proof bottle at about 5°C.

5) Saline

A 0.89% solution of NaCl.

6) Tween 80

A 0.1% v/v solution in water.

7) Autoclaving

Media were autoclaved at 10 lb for 10 min and stored in the dark at room temperature.

8) Supplement for nutritional mutants

MM supplemented as follows:

Growth factor	Final concentration
Amino acids	
arginine	6.9×10^{-4}M
ornithine	3.8×10^{-4}M
phenylalanine	3.0×10^{-4}M
proline	4.4×10^{-4}M
Nucleotides	
adenine	1.1×10^{-3}M
Vitamins	
biotin	50 ug/l
nicotinic acid	400 ug/l
pyridoxin HCl	200 ug/l
p-aminobenzoic acid	200 ug/l
riboflavine	400 ug/l

Other substances

Sodium thiosulphate	200mg/l

Other substances

Base medium (MM-glucose) supplemented as follows:

Ammonium acetate	12g/l
galactose	10g/l

D. Production of heterokaryons

Pairs of strains used to prepare heterokaryons differed from each other in nutritional requirements or in the combinations thereof. Members of each pair also differed in conidial colour, even though a few differences in colour were expressed as major differences between colour shades, e.g., grass-green as opposed to grey-green.

About 10^6 conidia of each of two strains were inoculated into 3ml of liquid MM containing 2% liquid CM. The CM permitted both the strains to grow a little which is necessary for hyphal anastomosis. The liquid medium containing the conidia was agitated. Incubation followed. After three or four days incubation, the mycelial matt formed on the surface was teased out on solid MM. After three or four days incubation, heterokaryotic growth emerging from the original pieces of mycelium was first sought, and, in most cases, found. The ability to grow well on MM is one of the main characteristics indicating heterokaryotic growth or heterokaryosis. Furthermore, as the two strains differ in conidial colour, the juxtaposition of conidial heads of different colour is an additional and important indicator of heterokaryotic growth.

When a pair of strains carried the same allele for a particular nutritional requirement which the strains shared, appropriately supplemented liquid and solid MM was used and heterokaryotic-growth was maintained by other requirements for which the strains differed.

Heterokaryons were incubated at 37°C; the minimum period of incubation for a given heterokaryon was five days.

E. Isolation of diploids from heterokaryons

The method of Roper (1952) was used to isolate diploids from heterokaryons: millions of conidia from a given heterokaryon were plated on solid MM, or on solid MM supplemented with a nutrient when required, and diploid colonies were noted after three or four days incubation at 37°C.

F. Haploidization

Strains of A. nidulans with diploid nuclei show vegetative segregation of haploids (Pontecorvo and Roper, 1953). The production of haploid segregants results from the loss of chromosomes at successive mitotic divisions so that haploid segregants are derived from diploids through successive degrees of aneuploidy (Kafer, 1960). Crossing-over is not involved (Pontecorvo, Tarr-Gloor and Forbes, 1954) and the members of each chromosome pair segregate independently of each other pair. In other words, genes or markers on a given linkage group will always segregate together and independently of any markers located on another linkage group. The process can be used for the assigning of a gene or lethal or other chromosomal change, such as a translocation, to the relevant linkage group or groups (Forbes, 1959; Kafer, 1962). It can also be used for chromosome substitution.

The technique of Morpurgo (1961) was used to facilitate haploidization studies. From a given diploid colony diploid conidia were inoculated onto CM+DLp-fluoro-phenylalanine (pFA) at a final concentration of about 1/10,000 W/V. Four well spaced inoculations were made per plate. On this medium, the diploid conidia or inocula grow very badly and give very stunted colonies, while normal haploids (haploids lacking a duplication) arising during this period of very stunted growth produce vigorous sectors which are readily isolated.[4] The concentration of pFA used in haploidization selects against haploid segregants carrying a duplication, and though they may occur as very stunted and abnormal sectors they are very difficult to detect except by close examination (Nga and Roper, 1968; Personal Communication).[5] Haploidization was at 37°C.

G. Meiotic Analysis

Heterokaryons were established as described previously, and after three or four days incubation, the dishes of heterokaryons were then sealed with cellulose tape and further incubated for nine days. Such incubation within sealed plates is known to favour perithecia formation. By the completion of this additional period of incubation, mature perithecia were produced. The perithecia were freed from adhering conidia and Hülle cells and crushed on to the wall of tubes containing 2ml saline.

They were screened for hybids by streaking samples of ascospores from individual perithecia on MM (or on MM+ a given

4.Only one sector of each colour was isolated from each colony
5.In general, the concentration of pFA used in haploidization strongly selects against those strains or variants carrying some type of duplication.

nutrient, when required) where only non-parental recombinants grew, i.e., non-parental recombinants either prototrophic or prototrophic save for a given requirement held in common by both parent strains and determined at the same locus. Furthermore, ascospores from individual perithecia were also streaked onto CM and the resulting recombinants were observed. Since any given cross was made between strains in which one had at least one colour marker not shared by the other (or between strains each of a different colour) examination of the colour of the segregants obtained through such streaking allowed one (by way of confirmation) to determine which perithecia were hybrids. Hybrid perithecia are also larger than selfed perithecia and this was also used as a criterion in the initial screening process. Hybrid perithecia were only used for analysis in various experiments.

Random Ascospore Analysis was carried out by plating a sample of ascospores from a hybrid perithecium at low density.

H. Classification of segregants (Nutritional test of segregants)

When necessary, colonies obtained by low density platings of ascospores from a hybrid perithecium were classified to determine their nutritional phenotype (or genotype). In this method, several dishes each containing media lacking, in turn, a single growth factor were made. 26 points in the form of a matrix were marked on the base of each of these plates and conidia from the individual segregants were inoculated onto the medium at the corresponding positions in all the plates. The dishes were then incubated at 37°C for 24 hours and classification for (or determination of) requirements was made. The same

procedure was used for classifying the haploid segregants from diploid strains. For the confirmation of the known phenotype (or genotype) of any strain, a very similar classification test was used.[6]

Where only a certain number of segregants from a cross were to be tested at a given time for one requirement, the following procedure was undertaken: Plates containing media lacking the growth-requirement in question were made. Points in the form of a matrix were marked on the base of these plates and conidia from the individual segregants were inoculated onto the media at positions corresponding to the points. As before, dishes were incubated at 37°C for 24 hours. The results of the test were then noted.

I. Mutant Sectors

Mutant sectors produced by duplication cultures were tested for nutritional requirements according to methods previously described. Wherever possible, mutant sectors classified for nutritional requirements were not in close proximity to other variants so classified. Except when it was not possible, colonial subcultures were made from only these mutant sectors which were not in close proximity to one another.

The period of time in which a given mutant sector emerged was determined by means of the following procedure: Beginning with (at) 48 hours of incubation a line (on the plate) was drawn

6. Six spaced inoculations were made from a given strain onto respective dishes each containing media lacking in twin a given nutrient or growth factor which the strain could not produce, and onto a dish of MM supplemented with all the nutrients for which the given strain was known not to be able to produce. Following 24 hours of incubation at 37°C, the nutritional genotype could be confirmed.

every 24 hours around the perimeter of any growing colony until 48 hours before the end of incubation. As viewed from beneath a plate, the boundary and point of origin of any mutant sector was plainly visible, and by observing the point of origin of a given mutant sector in relation to the drawn lines, one could determine the approximate period in which the sector emerged. This procedure was only employed in those specified experiments where one wanted to determine the periods in which mutant sectors emerged.

J. Temperature

During all experiments temperatures were maintained at 28°C, 36°C, 37°C, 39.5°C, and at 42°C.

Unless otherwise stated, incubation was at 36°C.

P A R T I

PART I

Before undertaking a study of the instability of duplication strains at different temperatures, it was important to carry out control experiments in which haploid non-duplication strains and balanced diploids were grown at different temperatures.

In the first of these control experiments, a group of 60 colonies of the strain y+ bi1 was grown in 42°C, another group of 80 of such colonies was grown at 39.5°C while a third group of 70 such colonies was grown in 36°C. The colonies were left at these temperatures until colony edges were generally 3 to 4mm. from the plate edge (diameter of a plate was approximately 8.6cm). Colonies at the higher temperatures appear to take longer in this growth than those at the lowest temperature. Upon the completion of incubation, it was observed that all colonies at these various temperatures did not display any type of vegetative instability in the form of mutant sectors.

In a second experiment a group of 20 colonies of the strain paba1 y+ ad8 * was grown in 42°C, another group of 20 of such colonies was grown at 36°C while a third group of twenty was grown at 28°C. These colonies were left to grow until their rims were generally 3 to 4mm. from the plate edge. Observations indicated that these colonies did not display any type of vegetative instability in the form of mutant sectors.

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*One must point out that adenine-requiring strains carrying the y+ allele such as paba1 y+ ad8 are of a grey-green or silver-green colour (i.e., have grey-green conidia) whereas adenine-independent strains carrying the y+ allele such as y+ bi are of a grass-green colour (i.e., have grass-green conidia). This observation is useful in later experiments.

In a third experiment ten colonies from the strain y ; s12 pyro4 ; nic2 (strain B) and ten colonies from the strain MSE were grown at 42°C, another group of 10 B and 10 MSE colonies was grown at 36°C, while a third group of 10 B and 10 MSE colonies was grown at 28°C. Upon the termination of incubation when the colonies were again 3 to 4mm. from the plate edge, it was observed that colonies did not display any type of sector.

In a subsequent experiment, one group of colonies of a white strain, J, was grown in 36°C while another group of such was grown at 42°C. Colonies of this strain did not display any type of instability. Strain J, which will be utilized in subsequent experiments, carries a III-VIII non-reciprocal translocation where a terminal segment, at least 47 units in length, of the right arm of chromosome III has been attached to the terminal end of chromosome VIII, a VI-VII reciprocal translocation and the markers w3, bi1, y^+, and pyro12 (Kafer, 1963, Personal Communication; Bainbridge, 1964, 1966). Before this present work, the location of pyro12 was not known. Experiments to be described later in this study established that pyro12 is located on linkage group I distal to ribo1.

By making a synthesis between J and ribo1 ad14 y ; phen 2, a phenotypically green, J // ribo1 ad14 y ; phen 2 diploid was obtained. Fifty colonies from this balanced diploid were grown at 36°C while a second group of 50 of such colonies was grown in 39.5°C. Incubation was until the edges of such colonies were within 3 to 4mm. from the plate edge. Upon the completion of incubation, it was observed that the balanced diploid colonies were stable at mitosis. This would be in accord with the studies

made by Nga and Roper (1969) in which they found that different balanced diploids carrying various translocations are stable at mitosis after growth at about 37°C.

In summary haploid strains not carrying duplications and balanced diploid strains carrying translocations display stability upon growth at different temperatures.

P A R T II

PART II

This part deals with experiments undertaken to study the effect of various temperatures upon the instability of strains carrying a segment of chromosome III in duplicate (III duplication). The first phase of these investigations commence with the derivation of III duplication strains used in the studies and include trial or preliminary experiments. A few crosses between strain J and a strain without translocations were used by Bainbridge to derive crinkled colonies all carrying a segment of chromosome III in duplicate. The reciprocal VI-VII translocation also carried by J has been shown to be not involved in the production of crinkled colonies (Kafer, Personal Communication; Bainbridge, 1964). This would be as expected in as much as colonies carrying a chromosomal segment in duplicate could not be obtained from a cross between a strain only carrying a reciprocal translocation among its chromosomal complement and one without translocations. To obtain crinkled colonies carrying a segment of chromosomes III in duplicate (III duplication) for these experiments, a cross was thus made between

$$\frac{\text{pyro12 } y^{+} \text{ bi1}}{\text{T (III-VIII)}} ; \frac{\text{w3}}{\text{T (VI-VII)}} \quad \text{(J)}$$

and

$$\frac{y}{} ; \frac{s12}{} ; \frac{pyro4}{} ; \frac{nic2}{} \quad \text{(B)}$$

From the cross, 194 normal and 87 crinkled progeny-colonies were obtained, the ratio of normals to crinkled fitting a 2:1 ratio at $P > .05$ as expected. 64 green, 71 yellow and 138 white colonies were also observed and the ratio of greens to yellows to white fitted a 1:1:2 ratio at $P > .05$. All the green

crinkled colonies were of a definite olive-green colour shade while all the green normal colonies were of a grass-green colour shade. This indicates that in colonies carrying the $\underline{y}^+$ and $\underline{w3}^+$ alleles the olive-green colour is endemic to or associated with those colonies carrying a segment of chromosome III in duplicate. This observation is important in subsequent experiments. Inasmuch as the s12 locus is within the translocated portion of chromosome III and is 17 ± 4 units from the point which joins the translocated segment of III to the end of chromosome VIII (Bainbridge, 1964), and inasmuch as such a translocated portion of chromosome III will make up the translocated duplicate segment of the III duplication, then most of the crinkled or III duplication progeny-colonies from the cross B x J would be heterozygous for the s12 locus (i.e., $s12/s12^+$). Classification of a portion of crinkled progeny-strains showed that such colonies in general were phenotypically thiosulphate-independent ($\underline{s}^+$). In this study crinkled colonies carrying the III duplication are collectively referred to as the R strains while their characteristic crinkled morphology is referred to as the Bainbridge morphology or the Bainbridge crinkled morphology.

From each of a random sample of crinkled colonies obtained through this cross, one colony was derived and incubated for a period of 9 days. One group of these colonies was incubated at 42°C, another group at 36°C, while a third group was incubated at 28°C. This was done in order to test the stability of such colonies at various temperatures. The results of this experiment are given in Table 1a. The data suggests a temperature effect on the instability of III duplication strains. Additional colonial subcultures (colonies) were made from each of a random sample of crinkled colonies obtained through a second

T A B L E 1a

First Range of III Duplication Strains Grown at 42°C, 36°C, and 28°C for Nine Days

	Number of Colonies	Number of Improved Sectors	Mean Number of Improved Sectors per colony	Number of Deteriorated Brown Sectors	General Description of Sizes of Parental Regions
Colonies at 42°C	24	25	1.0	13	Larger than those at 36°C and 28°C
Colonies at 36°C	24	149	6.5	0	Larger than those at 28°C
Colonies at 28°C	24	180	7.5	0	.

T A B L E 1b

Second Range of III Duplication Strains Grown at 42°C, 36°C, and 28°C for Nine Days

	Number of Colonies	Number of Improved Sectors	Mean Number of Improved Sectors per colony	Number of Deteriorated Brown Sectors	General Description of Sizes of Parental Regions
Colonies at 42°C	19	22	1.1	9	Larger than those at 36°C and 28°C
Colonies at 36°C	15	93	6.2	2	Larger than those at 28°C
Colonies at 28°C	15	115	7.6	0	.

cross between J and B[1] and incubated for a period of nine days; as in the previous experiment, one group of the colonial subcultures were grown at 42°C, a second group was grown at 36°C, while a third group was grown at 28°C. The results are recorded in Table 1b. The data again suggests that instability is very temperature sensitive. The interpretation of the data from both these experiments has taken account of the fact that during growth a larger parental region would have that many more nuclei on the circumference potentially able to give rise to improved sectors.

Having studied a heterogenous group of III duplication strains under various temperatures, one now turns to a limited study of the effect of various temperatures upon III duplication colonies each derived from an individual conidium (nucleus) of one III duplication strain (one III duplication colony). From the centre of a yellow Bainbridge crinkled colony (Ry), phenotypically pyridoxin-requiring, conidia were obtained and plated out at very low density on several CM plates, taking care that each plate had approximately the same density of spores as any other. One group of these plates was incubated in 42°C, another group in 36°C, while a third was incubated in 28°C. The plates were left for a period of nine days. Upon the completion of incubation, a random but very large sample of colonies (referred to as Ry) was scored for sectors; the parental region diameters of a random sample of colonies were also measured. The results are recorded in Table 2.

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1. From the cross, 152 normal and 67 crinkled colonies were obtained. The segregation of normal to crinkled colonies was in accord with a 2:1 ratio at $P > .05$.

TABLE 2

Ry Colonies Grown for Nine Days

	Number of Colonies Observed	Mean Diameter of Parental Region	Number of Improved Sectors	Mean Number of Improved Sectors per colony	Number of Deteriorated Brown Sectors
Colonies at 42°C	73	1.9cm	18	.24	1
Colonies at 36°C	70	2.2cm	161	2.3	0
Colonies at 28°C	66	1.4cm	240	3.6	0

	% of Colonies which Produced Improved Sectors
Colonies at 42°C	24%
Colonies at 36°C	90%
Colonies at 28°C	95.5%

Overall results show greater frequency of improved sectors at lower temperatures, and results at 28°C indicated that the population is homogenous in its temperature response. Thus this latest experiment provides further evidence that a strain carrying a III duplication displays an instability which is temperature sensitive. Moreover, the data would indicate that in a given III duplication strain each nucleus has generally the same capability of responding in the same manner to a given temperature condition. In looking at all the experiments performed thus far, we see that consistently the greatest frequency of improved sectors (or variants) is present at the lower temperatures while the greatest frequency of deteriorated brown sectors or variants is at the highest temperature. It is possible that the very low frequency of improved sectors at 42°C may be due to such sectors being at a less selective advantage at 42°C compared to sectors at 36°C or 28°C. To determine whether differential selection was a factor, an experiment was devised. The rationale and detailed results of the experiment are presented in Appendix A. In summary the experiment indicated that improved sectors at 42°C are not at a less selective advantage compared to sectors at the lower temperatures. All data in this Part and in Part III regarding brown variants suggests that browns are not at a selective advantage at 42°C.

The previous experiments definitely showed that a more detailed investigation along generally the same lines was justified. To this end, two III duplication strains, both $\underline{s}^+$, were isolated by way of colonial subculturing for further study. One of these strains was olive-green (i.e., had olive-green conidia) and was phenotypically biotin-requiring, nicotinic acid-

requiring and pyridoxin-requiring (i.e., bi, nic, and pyro). This strain was referred to as Rg. Rg was heterozygous for the s12 locus as indicated by the fact that two of the colonial subcultures made from Rg each produced a thiosulphate-requiring (s) sector. It was possible that Rg carried both pyro4 and pyro12 or either pyro4 or pyro12. A complementation test[2] was made in order to determine which of the genes determining a requirement for pyridoxin in Rg were carried by this strain. The test indicated that Rg carried both pyro4 and pyro12. Data in Appendix C confirms that Rg carried both pyro4 and pyro12. The data also suggests that pyro12 is on linkage group I. Data in part IV confirms this.

The other strain which was isolated was of a dull white colour shade and like Rg was phenotypically bi, pyro, and nic. This white III duplication strain was referred to as Rw. Because colonial subcultures made from Rw produced some thiosulphate-requiring sectors, it was indicated that Rw was heterozygous for the s12 locus.

The s12 allele present in Rg and Rw would either be carried on the translocated duplicate III segment or on the non-translocated duplicate III segment. As the $s12^{+}$ allele (in J) is $17^{+}_{-}4$ units from the point which joins the translocated III segment to VIII, it is most likely that both strains carry the s12 allele on the non-translocated duplicate III segment.

Nevertheless, in order to be sure on which of the two duplicate III segments the s12 allele is carried in Rg, the first step was to combine Rg with bi1 ; arg2 to make a diploid

2.The complementation test involved the attempted establishment of a $Rg^{+}J$ heterokaryon on MM supplemented with biotin on the one hand and Rg+MSE heterokaryon on MM on the other hand. If Rg had pyro12 it would not form a heterokaryon with J which has pyro12; if Rg had pyro4 it would not form a heterokaryon with MSE which has pyro4; if Rg had both pyro12 and pyro4 it would not form a heterokaryon with J and MSE. It was noted that Rg neither formed a heterokaryon with MSE nor with J.

(diploid A). Such a Rg // bi1 ; arg2 diploid was then haploidized and a portion of the resulting normal haploid segregants[3] were tested for various requirements. The results are presented in Table 3a. If the s12 allele were on the non-translocated duplicate III segment, roughly 50% of the haploid segregants tested would be s, but, if the s12 allele were on the translocated duplicate III segment, all haploid segregants tested would be s^+ as segregants just having / III-VIII s12 would be inviable.

Thus the data in Table 3a indicates that s12 in Rg is located on the non-translocated duplicate III segment. Furthermore it was found that all segregants which were s were also found to be arg^+ while all segregants which were arg were found to be s^+; these two haploid linkage classes could not have (both) been obtained together through the haploidization of a Rg // bi1 ; arg2 diploid had s12 not been on the non-translocated duplicate III segment.

One must also take note of the very small number of nic^+ segregants among those tested. A given marker, e.g., $nic2^+$ should have been distributed among very roughly 50% of the haploid segregants upon haploidization. Except in the case where phen2 segregants do not grow on pFA (Azevedo and Roper, 1967), experience has shown that a situation where such a distribution is not conformed to is extremely rare. The very low number of nic^+ segregants among those tested strongly suggests in this light that a semi-lethal or deleterous factor is in linkage with $nic2^+$.

3. Segregants having a duplication do not grow on the concentration of pFA used in haploidization.

T A B L E 3a

Segregations from the Diploid Rg// bi1 ; arg2 /Diploid A/

Green normal haploid segregants were only produced

Chromosome	III			V	
allele	s^+	s	(All s segregants were arg^+	nic^+	nic
	21	24	whereas all arg segregants	4	34
	arg^+	arg	were s^+)		
	24	21			

T A B L E 3b

Segregations from the Diploid Rg// bi1 ; arg2 /Diploid B/

Green normal haploid segregants were only produced

Chromosome	III			V	
allele	s^+	s	(All s segregants were arg^+	nic^+	nic
	15	15	whereas all arg segregants	0	30
	arg^+	arg	were s^+)		

T A B L E 4

Segregations from the Diploid Rw // bi1 ; arg2

Approximately equal numbers of white and green normal haploid segregants were produced*

Chromosome	III			V	
allele	s^+	s	(All s segregants were arg^+ whereas	nic^+	nic
	22	28	all arg segregants were s^+)	3	35
	arg^+	arg			
	28	22			

*Equal numbers of white and green haploid segregants were tested.

In this regard it was of interest to determine whether a second haploidization from a newly synthesized Rg // bi1 ; arg2 diploid would again result in a very low number of nic2$^+$ segregants among those tested. To this end a second Rg + bi1 ; arg2 heterokaryon was made, from which a second Rg // bi1 ; arg2 diploid (B) was synthesized. The diploid was haploidized and a portion of the resulting haploids were tested for nicotinic acid and thiosulphate requirements. The results are given in Table 3b. The lack of nic$^+$ segregants strongly suggests a lethal in linkage with nic2$^+$.

The present results would thus appear to suggest that linkage group V has been affected while in a Rg // bi1 ; arg2 diploid. The implications of data of this type will be dealt with more fully in the general discussion.

Having located the position of the s12 allele in Rg, one turned to the location of this allele in Rw. To this end, the first step was to synthesize a diploid from Rw and bi1 ; arg2. The Rw // bi1 ; arg2 diploid was haploidized. Approximately one-half of the resulting haploid segregants were white while the rest were green. The lack of yellow haploid segregants indicates that Rw carries the y$^+$ allele. A portion of the haploid segregants were tested for various requirements. The results are recorded in Table 4. The fact that s segregants were obtained indicates that s12 in Rw in located on the non-translocated duplicate III segment. The fact that all arg$^+$ segregants were found to be s while all arg segregants were found to be s$^+$ confirms such a location. The very low number of nic$^+$ segregants among those tested strongly suggests a new semi-lethal in linkage with nic2$^+$.

Now that sl2 had been located in Rg and Rw, one could turn to a more meaningful study of the effects of temperature variation on both of these III duplication strains. One commences first with an investigation of Rg instability.

Colonies made from Rg were incubated for 9 days - one group of Rg colonies was grown at 42°C, another at 36°C, while a third group of such were grown at 28°C. Upon the completion of incubation, Rg colonies grown at 36°C and 28°C displayed the Bainbridge morphology; the parental regions of such colonies were olive-green in colour shade. Rg colonies at 42°C also displayed the Bainbridge morphology though they were of paler olive-green. At 36°C all improved sectors produced by Rg colonies were fuzzy in texture, lacked the Bainbridge morphology and were olive-green in colour. Improved sectors of this type shall be referred to as class one sectors. They will also be referred to as r sectors. Plate 1 shows a Rg colony with class one (or r) sectors. At 28°C the class one or r sectors were present along with a group (class) of sectors which were definitely not olive-green in colour but of a grass-green colour and fairly smooth.

This group of grass-green comparatively smooth sectors was referred to as class two sectors. The fact that class two sectors are very much more similar to a normal green strain, which lacks the III duplication, in colour and general morphology than class one sectors indicates that any given class two sector retains in comparison to any given class one sector far less (if at all) of the original III duplication. In other words, a far greater amount of the genetic or chromosomal material was deleted

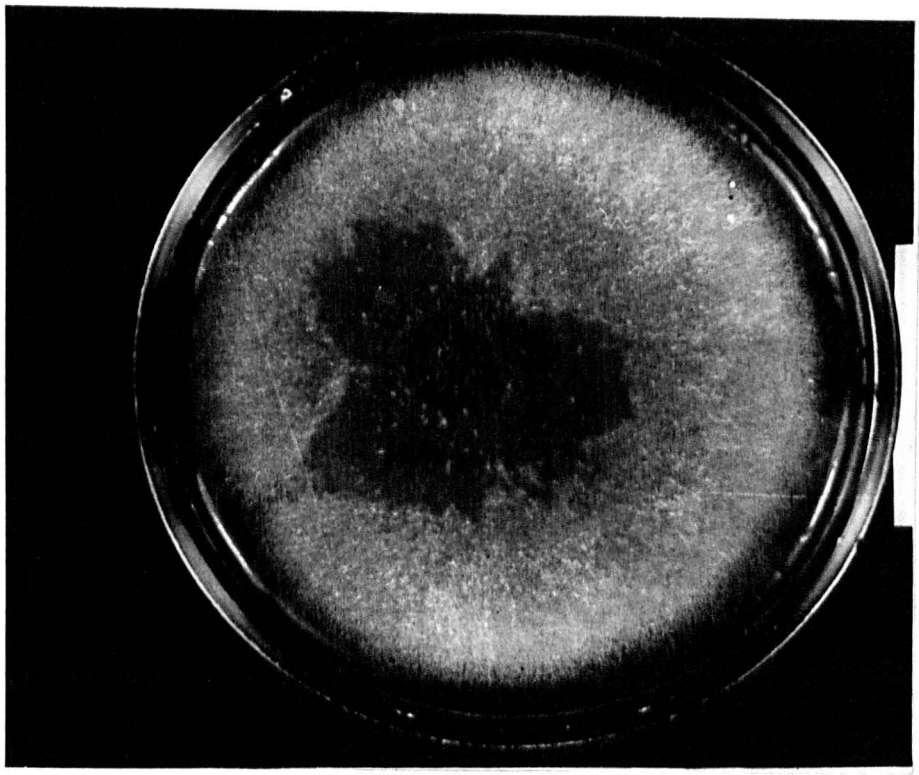

Plate 1. Rg colony with class one sectors at 36°C. Growth period was 9 days.

from the III duplication in the production of a class two sector than was deleted in the formation of a class one sector.

Improved sectors at 42°C could not be divided into any definite categories. These sectors were clearly defined and tended to have more conidia than the parental region; they were also darker than the parental region. A sample of the improved sectors produced at 36°C and 28°C were tested for thiosulphate requirement (dependence) while all improved sectors produced at 42°C were so tested. The results of this test along with other information relating to this last experiment with Rg colonies is recorded in Table 5. The data indicates that in Rg the s12$^+$ region of the translocated duplicate III segment is subject to far more deletionsat 42°C and 28°C than it is at 36°C, while the overall frequency of deletions occurring from the more inclusive III duplication is far greater at 36°C and 28°C than it is when growth is at 42°C.

This means, in other words, that deletional instability in general is far greater at the lower temperatures than at the comparatively higher temperature even though the deletional instability of a specific genetic region of the III duplication is far greater at the highest and lowest temperatures than it is at the intermediate temperature.

As indicated (Table 5 and Appendix A) class two sectors are not at a less selective advantage at 36°C than at 28°C. Therefore, when comparing sectors produced at 28°C and 36°C the presence of all class two sectors at 28°C would suggest that sectors at 28°C are exceedingly more likely to arise with a much larger part of the original III duplication deleted than those arising at 36°C. As 78% of the class two sectors were

T A B L E 5

Rg colonies grown for nine days

	Number of Colonies	Mean diameter of parental region	Number of improved sectors at 42°C	Improved sectors other than those at 42°C: Number of class one sectors	Improved sectors other than those at 42°C: Number of class two sectors
Colonies at 42°C	60	4.2cm	52	•	•
Colonies at 36°C	40	3.3cm	•	265	0
Colonies at 28°C	40	2.2cm	•	231	49

T A B L E 5 (continuation)

	Mean number of improved sectors per colony	Number of improved sectors at 42°C tested for thiosulphate requirement	Number of improved sectors at 42°C found to be s^+	Number of improved sectors at 42°C found to be s^-
Colonies at 42°C	.86	52	28	24
Colonies at 36°C	6.6	.	.	.
Colonies at 28°C	7.0	.	.	.

T A B L E 5 (continuation)

	Number of class one sectors tested for thiosulphate requirement	Number of s^+ class one sectors noted	Number of s^- class one sectors noted	Number of class two sectors tested for thiosulphate requirement
Colonies at 42°C	.	.	.	.
Colonies at 36°C	80	74	6	.
Colonies at 28°C	50	48	2	49

T A B L E 5 (continuation)

	Number of s^+ class two sectors noted	Number of s class two sectors noted	Total number of sectors tested for thiosulphate requirement	Total number of s^+ sectors noted	Total number of s sectors* noted
Colonies at 42°C	.	.	52	28	24
Colonies at 36°C	.	.	80	74	6
Colonies at 28°C	11	38	99	59	40

*Respective s sectors arise as a result of respective deletions which include the $s12^+$ allele on the translocated duplicate III segment.

T A B L E 5 (continuation)

	Number of deteriorated brown sectors
Colonies at 42°C	0
Colonies at 36°C	0
Colonies at 28°C	0

Distribution of improved sectors at each temperature conforms to a Poisson distribution, and this is so for all improved sectors produced by III duplication cultures in subsequent experiments. This allows one to use a particular statistical test[1] whereby one can make a statistical comparison of the different sector means. Using such a test, it was found that at $P > .05$ the means 6.6 and 7.0 are not significantly different from one another but at $P < .01$ are both significantly different from the mean .86. As shown in Appendix A, differential selection is not a contributing factor to the significant difference between particular sector means. Moreover, class two thiosulphate-requiring sectors are not at different temperatures subject to differential selection. (Appendix A).

[1] Described by E. Lehminn (1959).

phenotypically s, this indicates that the production of class two sectors at 28°C entails in the very large majority of cases deletions including the s12$^+$ allele on the translocated segment, where in contrast the production of class one sectors does not entail in most cases deletions which include the s12$^+$ allele.

Deletions including the s12$^+$ allele suggest the possibility that most deletions involve only the translocated duplicate III segment. Bainbridge in his work never determined whether or not this was the case. It was thus decided to determine whether a sample of class one sectors (or r sectors) produced at 36°C generally arose as a result of deletions from the translocated duplicate III segment or whether they generally arose as a result of deletions from the non-translocated duplicate III segment.

To this end a diploid was first synthesized from each of ten independent s$^+$ r sectors and the strain ribo1 ad14 y ; phen2.[4] (Only two clearly separated r sectors from any given Rg colony were used in the synthesis.) Each of the ten r // ribo1 ad14 y ; phen2 diploids were then haploidized and a portion of the haploid segregants were tested for various requirements. The results are given in Tables 6a to 6j. Had the deletions occurred only from (on) the translocated duplicate III segment in the formation of the ten r sectors, all haploid segregants tested and derived from each r // ribo1 ad14 y ; phen2 diploid would each be s phen$^+$ (or s). Had any r sector carried a deletion on the non-translocated duplicate III segment a diploid synthesized from such a sector and ribo1 ad14 y ; phen2 would not give rise to any haploid segregants upon haploidization. This would be so for two reasons:

4. The phen2 locus is not located within the III duplication.

T A B L E 6a

Segregations from the diploid r1 // y ad14 ribo1 ; phen2

Only yellow haploid segregants were produced

Chromosome	I		III			V	
allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
	0	18	0	18	are phen$^+$)	8	10

phen$^+$	phen
18	0

T A B L E 6b

Segregation from the diploid r2 // y ad14 ribo1 ; phen2

Only yellow haploid segregants were produced

Chromosome	I		III			V	
allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
	0	16	0	16	are phen$^+$)	9	7

phen$^+$	phen
16	0

T A B L E 6c

Segregations from the diploid r3 // y ad14 ribo1 ; phen2

No haploid segregants produced

T A B L E 6d

Segregations from the diploid r4 // y ad14 ribo1 ; phen2

All haploids produced were yellows but for one green haploid sector

Chromosome	III			V	
allele	s^+	s	(all s haploids	nic^+	nic
Colour: yellow	0	21	are $phen^+$)	9	12
	$phen^+$	phen			
yellow	21	0			

T A B L E 6e

Segregations from the diploid r5 // y ad14 ribo1 ; phen2

All haploids produced were yellow

Chromosome	I		IIII			V	
allele	$ribo^+$	ribo	s^+	s	(all s haploids	nic^+	nic
	0	18	0	18	are $phen^+$)	8	5
			$phen^+$	phen			
			18	0			

T A B L E 6f

Segregations from the diploid r6 // y ad14 ribo1 ; phen2

All haploids produced were yellows but for one green haploid sectors

Chromosome		I		III			V	
	allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
Colour:	yellow	0	20	0	20	are phen$^+$)	11	9
				phen$^+$	phen			
	yellow			20	0			

T A B L E 6g

Segregations from the diploid r7 // y ad14 ribo1 ; phen2

All haploids produced were yellows

Chromosome	III			V	
allele	s$^+$	s	(all s haploids	nic$^+$	nic
	0	16	are phen$^+$)	6	10
	phen$^+$	phen			
	16	0			

T A B L E 6h

Segregations from the diploid r8 // y ad14 ribo1 ; phen2

All haploids produced were yellows

Chromosome	I		III			V	
allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
	0	14	0	14	are phen$^+$)	5	9
			phen$^+$	phen			
			14	0			

T A B L E 6i

Segregations from the diploid r9 // y ad14 ribo1 ; phen2

All haploids produced were yellows

Chromosome	I		III			V	
allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
	0	15	0	15	are phen$^+$)	9	6
			phen$^+$	phen			
			15	0			

T A B L E 6j

Segregations from the diploid r10 // y ad14 ribo1 ; phen2

All haploids produced were yellows

Chromosome	I		III			V	
allele	ribo$^+$	ribo	s$^+$	s	(all s haploids	nic$^+$	nic
	0	18	0	18	are phen$^+$)		
			phen$^+$	phen			
			18	0			

all normal s haploid segregants would have a lethal in linkage with s12, and all phen segregants (which would be phen s^+) do not grow on pFA. The data thus indicates that 9 out of 10 class r sectors arose at 36°C as a result of deletions having occurred only from (on) the translocated duplicate III segment. By inference each of these nine s^+ r sectors must be heterozygous at the s12 locus.

In view of these results it is very likely that nearly all improved sectors arising from Rg colonies at 36°C do so as a result of deletions occurring only from (on) the translocated duplicate III segment. In other words, it is very likely in view of these results that at 36°C nearly all deletions occur from the translocated duplicate III segment in Rg. Such data coupled with the observations that many sectors at 28°C and 42°C are thiosulphate-requiring increases the probability that most improved sectors arising from Rg at 28°C and 42°C do so as a result of deletions having occurred only from the translocated duplicate III segment. This would mean that there is a likelihood that at 28°C and 42°C most deletions occur from the translocated duplicate III segment in Rg.

It is also of interest to note that haploidization of eight r // ribo1 ad14 y ; phen2 diploids resulted only in yellow haploid segregants being produced, while haploidization of a ninth diploid resulted in the production of only one green haploid segregant, the remaining haploids being yellows. This strongly suggests that in eight of these diploids a recessive lethal arose in linkage with y^+, while in the ninth diploid a recessive semi-lethal is in linkage with y^+. In this connection it is to

be noted that chromosome V did not carry either a recessive lethal or semi-lethal while present in any r// ribo1 ad14 y; phen2 diploid.

Additional Rg colonies were grown for a period of nine days. As before, one group of such colonies were incubated at 42°C, another group of 36°C, while a third group of Rg colonies was incubated at 28°C. The approximate times at which improved sectors arose at 36°C were noted according to methods previously described.

Upon the completion of incubation it was again noted that Rg colonies displayed the Bainbridge morphology and had parental regions which were olive green in colour. All improved sectors produced at 36°C and 28°C were either class one or class two sectors. Improved sectors produced at 42°C could not be placed into any definite category. A sample of sectors produced at each temperature was tested for thiosulphate dependence. The overall results of this latest study of Rg colonies are recorded in Tables 7a and 7b. Again we see that the s12$^+$ region of the translocated duplicate III segment in Rg is subject to far more deletions at 42°C and 28°C than it is at 36°C, while the overall frequency of deletions occurring from the more inclusive III duplication is far greater at 36°C and 28°C than it is when growth is at 42°C. We also note again that sectors at 28°C are exceedingly more likely to arise with a much larger part of the original III duplication deleted than those arising at 36°C. As before, the production of class two sectors is due in a large majority of cases to deletions which include the s12$^+$ allele, while, in contrast the production of class one sectors is not due in most cases to deletions which include the s12$^+$ allele. Table 7b shows that a very large majority of improved sectors

T A B L E 7a

Rg colonies grown for nine days

	Number of colonies	Mean diameter of parental region	Number of improved sectors at 42°C	Improved sectors other than those at 42°C: Number of class one sectors	Improved sectors other than those at 42°C: Number of class two sectors	Mean number of improved sectors per colony
Colonies at 42°C	36	4.3cm	27	.	.	.75
Colonies at 36°C	42	3.2cm	.	267	5	6.4
Colonies at 28°C	36	2.1cm	.	191	65	7.1

T A B L E 7a (continuation)

	Number of improved sectors at 42°C tested for thiosulphate requirement	Number of improved sectors at 42°C found to be s^+	Number of improved sectors at 42°C found to be s^-	Number of class one sectors tested for thiosulphate requirement	Number of s^+ class one sectors noted
Colonies at 42°C	26	14	12		
Colonies at 36°C	.	.	.	53	50
Colonies at 28°C	.	.	.	65	61

T A B L E 7a (continuation)

	Number of s class one sectors noted	Number of class two sectors tested for thiosulphate requirement	Number of s^+ class two sectors noted	Number of s class two sectors noted	Total number of sectors tested for thiosulphate requirement
Colonies at 42°C	•	•	•	•	26
Colonies at 36°C	3	5	4	1	58
Colonies at 28°C	4	65	12	53	130

T A B L E 7a (continuation)

	Total number of s^+ sectors noted	Total number of s^- sectors noted	Number of deteriorated brown sectors
Colonies at 42°C	14	12	0
Colonies at 36°C	54	4	0
Colonies at 28°C	73	57	0

At $P > .05$ the means 6.4 and 7.1 are not significantly different from one another, but at $P < .01$ are both significantly different from the mean .75.

T A B L E 7b

Periods at which improved sectors arose from Rg parental regions during growth at 36°C

	0-48 hours	49-72 hours	73-95 hours	96-120 hours
Number of improved sectors	2	95	141	34

	121-144 hours	145-168 hours	169-216 hours
Number of improved sectors	0	0	0

Total number of sectors which arose during the 0-95 hour period: 238

Total number of sectors which arose during the 96-216 hour period: 34

arose at 36°C during the early period of growth of Rg colonies, thereby indicating that a very large majority of deletions altering the III duplication occur in Rg during early growth at 36°C.

Before continuing with further studies of Rg colonies and their sectors, one turns briefly to an investigation relating to the instability of Rw colonies.

Colonies of Rw were grown for 9 days at 42°C, 36°C, and at 28°C. At the completion of incubation Rw colonies grown at 36°C and 28°C displayed the Bainbridge morphology and had dull white parental regions. Normal white colonies are of bright white colour shade. Rw colonies grown at 42°C also displayed the Bainbridge morphology, parental regions being dull white or gray-white in colour shade. At both 36°C and 28°C two types of improved sectors arose from Rw colonies. One type of improved sector was fuzzy in texture, lacked the Bainbridge morphology and was dull white in colour shade. Improved sectors of this type were analogous to the class one sectors, produced by Rg, and were referred to as class three sectors. The other type of improved sector was comparatively smooth and of a bright white colour shade. Improved sectors of this second type were referred to as class four sectors. Because class four sectors exhibited a more improved morphology than class three sectors and because class four sectors were of a more normal colour shade than class three sectors, any class four sector can be seen as having retained less of the original III duplication than any class three sector. Improved sectors produced at 42°C could not be put into any definite category.

A sample of the improved sectors produced at 36°C and 28°C were tested for thiosulphate dependence (or requirement), while

all improved sectors produced at 42°C were so tested. The results of this test are presented in Table 8 along with other information relating to this experiment with Rw colonies. First, the data in Table 8 clearly indicates that the general deletional instability of the Rw, III duplication strain, as that of the Rg, III duplication strain, is far greater at the lower temperatures of growth than at the comparatively higher temperature of growth.

Specific deletional instability of the s12$^+$ region of the translocated duplicate segment in Rw does not appear from the data to be significantly modified by temperature. The data also suggests that sectors produced by Rw at 28°C are not more likely to arise with a larger part of the original III duplication deleted than those arising at 36°C. In contrast to class three sectors, more class four sectors arise with deletions of the s12$^+$ region. In spite of the specific mutagenic differences between Rw and Rg, the instability behaviour pattern of Rw has still much in common with the instability behaviour pattern of Rg. In view of this, it is possible that most improved sectors produced by Rw colonies arose as a result of deletions having only occurred from the translocated duplicate III segment.

Having considered the instability behaviour of Rw colonies, one now turns to a further study of Rg colonies. It was decided to leave Rg colonies to grow in 42°C for a period of 15 days[5] in view of the possibility that the extended period of incubation would have some relation to or effect on sector frequency or the types of sectors produced. The times at which such improved sectors arose were also noted; a large sample of

5. Growth at 42°C is still possible well after 9 days inasmuch as growth is slow at 42°C.

T A B L E 8

Rw colonies grown for nine days

	Number of colonies	Mean diameter of parental region	Number of improved sectors at 42°C	Improved sectors other than those at 42°C: Number of class three sectors	Improved sectors other than those at 42°C: Number of class four sectors	Mean number of improved sectors per colony	Number of improved sectors at 42°C tested for thiosulphate requirement
Colonies at 42°C	31	4.1 cm	25	.	.	.80	25
Colonies at 36°C	24	3.4 cm	.	163	34	6.7	.
Colonies at 28°C	24	2.2 cm	.	136	42	7.4	.

T A B L E 8 (continuation)

	Number of improved sectors at 42°C found to be s^+	Number of improved sectors at 42°C found to be $\underline{s}$	Number of class three sectors tested for thiosulphate requirement	Number of s^+ class three sectors noted	Number of $\underline{s}$ class three sectors noted	Number of class four sectors tested for thiosulphate requirement
Colonies at 42°C	14	11	.	.	.	.
Colonies at 36°C	.	.	34	32	2	34
Colonies at 28°C	.	.	42	42	0	42

T A B L E 8 (continuation)

	Number of $\underline{s}^+$ class four sectors noted	Number of $\underline{s}$ class four sectors noted	Total number of sectors tested for thiosulphate requirement	Total number of $\underline{s}^+$ sectors noted	Total number of $\underline{s}$ sectors* noted	Number of deteriorated brown sectors
Colonies at 42°C	.	.	25	14	11	0
Colonies at 36°C	16	18	68	48	20	0
Colonies at 28°C	8	34	84	50	34	0

At $P > .05$ the means 6.7 and 7.4 are not significantly different from one another, but at $P < .01$ are both significantly different from the mean .80.

By using the 2 x 2 contingency test, it was found that the ratio of class three to class four sectors at 36°C does not differ significantly (at $P > .05$) from the ratio of class three to class four sectors at 28°C. Using the same test, it was also found that the $\underline{s}^+$ to $\underline{s}$ sector ratio noted at 36°C does not differ significantly (at $P > .05$) from either the $\underline{s}^+$ to $\underline{s}$ sector ratio noted at 42°C or from that noted at 28°C.

*Respective $\underline{s}$ sectors arise as a result of respective deletions which include the $\underline{s12}^+$ allele on the translocated duplicate III segment.

the improved sectors which arose within the two different periods were also tested for thiosulphate dependence. In addition to the preceding, Rg colonies were also grown in 36°C for a period of ten days. The results of these experiments are given in Tables 9a and 9b.

Even though more sectors on average were produced at 42°C upon 15 days of incubation than were produced on average at the same temperature upon nine days of incubation, the data indicates that the III duplication is still subject to far more deletions during growth at 36°C than during growth at 42°C. In contrast to the improved sectors which arise at 36°C from Rg colonies, we see that most improved sectors which arise at 42°C do not do so within the first 120 hours of growth, this suggesting that temperature is at least one factor which conditions the period of growth in which an improved sector will arise. Furthermore, when incubation is at 42°C, the s12$^+$ region is far more prone to deletion during the 0-215 hour growth period than it is during a subsequent period of growth, 216-360 hours, thereby indicating that the susceptibility of this specific region to deletion appears to be conditioned by or related to the period of growth. Nevertheless, this is still in accord with the indication that the s12$^+$ region in Rg is subject to far more deletions at 42°C and 28°C than it is at 36°C.

As temperature is related to the susceptibility of a given region of the III duplication to deletion as well as to the time, type, and overall frequency of deletions involving the III duplication, we have also seen that a second type of mutational activity, the production of brown deteriorated sectors through a process almost certain not to involve deletions, appears to be influenced or conditioned by temperature.

T A B L E 9a

Rg colonies grown at 42°C for a period of fifteen days (360 hours)

Number of colonies	Mean diameter of parental region	Number of improved sectors	Mean number of improved sectors per colony	Number of deteriorated brown sectors
39	6.9 cm	71	1.8	2

Periods during which improved sectors arose

	0-124 hours	125-167 hours	168-215 hours	216-360 hours
Number of improved sectors	9	1	21	40

T A B L E 9a (continuation)

Improved sectors which arose during the 0-215 hour period:		Improved sectors which arose during the 216-360 hour period:	
Number of which tested for thiosulphate requirement		Number of which tested for thiosulphate requirement	
25		27	
Number found to be $\underline{s}^+$	Number found to be $\underline{s}$	Number found to be $\underline{s}^+$	Number found to be $\underline{s}$
15	10	24	3

Using the 2 x 2 contingency test, it was found that at $P < .01$ the ratio of $\underline{s}^+$ to $\underline{s}$ sectors found for one period (0-215 hours) differs significantly from the ratio of $\underline{s}^+$ to $\underline{s}$ sectors found for another period (216-360 hours).

T A B L E 9b

Rg colonies grown at 36°C for a period of ten days

Number of colonies	Mean diameter of parental region	Mean number of improved sectors* per colony	Number of deteriorated brown sectors
35	3.4cm	6.9	0

At $P < .01$ the mean 1.8 (Table 9a) and the mean 6.9 are significantly different from one another.

*Virtually all improved sectors are class one sectors; an extremely large majority of improved sectors arose during the first 95 hours of colony growth.

Specifically, all experiments so far have clearly shown that the production of deteriorated brown sectors is virtually confined to the highest ($42^{o}C$) of these temperatures. Furthermore, Table 10 shows that a group of different genotypes taken collectively has a far greater stimulatory effect upon the frequency of deteriorated brown sectors than that exerted by one particular genotype shared by many colonies. This would indicate that the frequency of deteriorated brown sectors can also be greatly influenced by or modified by the genetic background. In this light, it is possible that a particular genetic background can regulate in an orderly manner certain types of deletional activity. What might be suggestive of this is indicated on P.65 where we saw that the tendency for a specific region to be deleted is related to the period if growth.

T A B L E 10

	Total number of colonies present at 42°C	Total number of deteriorated brown sectors produced at 42°C	Mean number of deteriorated brown sectors per colony at 42°C	Total number of colonies at 36°C
Inclusive range of genetically heterogenous colonies carrying the III duplication (Tables 1a, 1b)	43	22	.51	39
All Rg colonies studied (colonies sharing the same genotype)	135	2	.014	117

T A B L E 10 (continuation)

	Total number of deteriorated brown sectors produced at 36°C	Mean number of deteriorated brown sectors per colony at 36°C	Total number of colonies at 28°C	Total number of deteriorated brown sectors produced at 28°C
Inclusive range of genetically heterogenous colonies carrying the III duplication (Tables 1a, 1b)	2	.05	39	0
All Rg colonies studied (colonies sharing the same genotype)	0	0	76	0

P A R T III

PART III

The investigations of the effect of temperature were further extended by studying the instability behaviour at various temperatures of strains carrying a segment of chromosome I in duplicate (I duplication). It is these further investigations which are to be described in this part.

Work commenced with the I duplication strain (strain P) first studied by Nga and Roper; P was found to have the following genotype, or linkage relationships,

I pro1 paba6 y ad20^{+} bi1^{+} / I-II y^{+}[1] ad20 bi1

and phenotypically P was of a grass-green colour (i.e., had grass-green conidia), was proline and p-aminobenzoic acid dependent (requiring), and compared to III duplication strains had a far less, though distinct, crinkled morphology - the P crinkled morphology. Intrinsic to the crinkled morphology of the I duplication strain, P, was a moundshape morphology, but a moundshape morphology far less pronounced than that intrinsic to the Bainbridge crinkled morphology of the III duplication strains, e.g., Rg.

In the first experiment, P colonies were incubated for a period of 8 days; one group of colonies was grown at 42°C, a second group at 39.5°C, a third group at 36°C, while a fourth group of P colonies were grown at 28°C. At the completion of incubation the colonies were scored for mutant sectors or variants (improved sectors and deteriorated brown sectors) and improved sectors produced at each temperature were tested for adenine and biotin dependence. The overall results from the experiment are recorded in Table 11a.

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1. The recombination frequency between the y^{+} locus and the point where the translocated I segment joins chromosome II is about 9% (Nga and Roper, 1968).

A second series of P colonies were grown at 42°C, 39.5°C, 36°C, and 28°C for a period of eight days. Upon the completion of incubation, colonies were scored for mutant sectors (or variants) and improved sectors produced at each temperature were tested for adenine and biotin dependence. The results of this experiment are recorded in Table 11b. The data in Tables 11a and 11b indicates that the y$^+$ region on the translocated duplicate I segment is subject to significantly more deletions at 39.5°C than at either 36°C or 28°C. With an increase of temperature from 28°C to 36°C, however, there is no significant change in the amount or number of deletions to which y$^+$ region is subject. The more inclusive I duplication is seen at 39.5°C to be subject to significantly more deletions than at either 36°C or 28°C, while the overall frequency of deletions occurring from the I duplication at 36°C is not significantly different from the frequency of such at 28°C.

It would appear in comparison to the other three temperatures that growth at 42°C allows for the least general deletional instability upon eight days of incubation. However, in this respect one must keep in mind that in subsequent experiments where colonies at 42°C and at other temperatures were grown for extended periods of time, overall deletional instability of the I duplication is significantly greater at 42°C than at either 36°C or 28°C, though still significantly less than at 39.5°C.

In regard to the deletional instability of particular genetic regions of the I duplication, the data in Tables 11a and 11b unequivocably shows that deletional instability of the respective ad20$^+$ and bi1$^+$ regions on the non-translocated duplicate I segment is highest at 42°C and that such instability

T A B L E 11a

P colonies grown for a period of eight days

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
Colonies at 42°C	36	4.4cm	11	10	21	47%
Colonies at 39.5°C	36	6.3cm	25	105	130	80%
Colonies at 36°C	36	7.4cm	27	38	65	58.4%
Colonies at 28°C	36	6.1cm	30	28	58	48.2%

*All yellow sectors are improved; respective yellow sectors arise as a result of respective deletions which include the $\underline{y}^+$ allele on the translocated duplicate I segment.

T A B L E 11a (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of y^+ ad^+ bi^+ sectors noted	Number of[≠] y^+ ad bi sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of y ad^+ bi^+ sectors noted
Colonies at 42°C	11	3	8	10	10
Colonies at 39.5°C	25	20	5	75	75
Colonies at 36°C	27	26	1	36	36
Colonies at 28°C	30	30	0	28	28

[≠]Respective y^+ ad bi sectors arise as a result of respective deletions which include the $ad20^+$ and $bi1^+$ alleles on the non-translocated duplicate I segment.

T A B L E 11a (continuation)

	Total number of improved sectors tested for adenine and biotin requirements	Total number of tested improved sectors found to be ad bi sectors	% of yellow and green tested sectors found to be ad bi sectors	% of green improved sectors found to be ad bi sectors	Mean number of yellow sectors per colony	Mean number of improved sectors per colony	Number of deteriorated brown sectors
Colonies at 42°C	21	8	38%	76%	.27	.58	0
Colonies at 39.5°C	100	5	5%	20%	2.9	3.6	0
Colonies at 36°C	63	1	1.5%	3.6%	1.0	1.8	8
Colonies at 28°C	58	0	0%	0%	.77	1.6	0

Distribution of yellow sectors (as well as all improved sectors) at each temperature conforms to a Poisson distribution and this is so for all yellow sectors (as well as for all improved sectors) produced by I duplication cultures in subsequent experiments.

Relating to yellow sectors, the means .77 and 1.0 are not significantly different at $P > .05$ but are each significantly different at $P < .01$ from the mean 2.9; at $P < .01$ the mean .27 is significantly different from the means 1.0 and 2.9; at $P = .01$ the mean .27 is significantly different from the mean .77.

Regarding all improved sectors, the means 1.6 and 1.8 are not significantly different at $P \geq .05$ but are each significantly different at $P < .01$ from the mean 3.6; at $P < .01$ the mean .58 is significantly different from the means 1.6, 1.8, and 3.6.

T A B L E 11a (continuation)

As the data indicates in Appendix A, improved sectors (yellow and green mutant sectors) arising from strain P are not at a greater selective advantage (or are not at a less selective advantage) at any given temperature i.e., variants are not at different temperatures subject to differential selection. The data in all experiments is interpreted in conjunction with this knowledge.

T A B L E 11b

P colonies grown for a period of eight days

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
Colonies at 42°C	36	4.3cm	13	12	25	48%
Colonies at 39.5°C	36	6.4cm	19	90	109	82.5%
Colonies at 36°C	36	7.3cm	22	40	62	64.5%
Colonies at 28°C	39	6.2cm	31	32	63	50%

*All improved

T A B L E 11b (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of $y^+ ad^+ bi^+$ sectors noted	Number of $y^+ ad\ bi$ sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of $y\ ad^+ bi^+$ sectors noted
Colonies at 42°C	13	4	9	12	12
Colonies at 39.5°C	19	13	6	70	70
Colonies at 36°C	22	20	2	36	36
Colonies at 28°C	31	31	0	32	32

T A B L E 11b (continuation)

	Total number of improved sectors tested for adenine and biotin requirements	Total number of tested improved sectors found to be ad bi sectors	% of yellow and green tested sectors found to be ad bi sectors	% of green improved sectors found to be ad bi sectors	Mean number of yellow sectors per colony	Mean number of improved sectors per colony	Number of deteriorated brown sectors
Colonies at 42°C	25	9	36%	72%	.44	.69	0
Colonies at 39.5°C	89	6	6.7%	31.4%	2.5	3.0	0
Colonies at 36°C	58	2	3.4%	9%	1.1	1.7	5
Colonies at 28°C	63	0	0%	0%	.82	1.6	0

Regarding yellow sectors, the means 1.1 and .82 are not significantly different at $P > .05$ but are each significantly different at $P < .01$ from the mean 2.5. At $P < .01$ the mean .44 is significantly different from the means 2.5 and 1.1; at $P = .01$ the mean .44 is significantly different from the mean .82.

Regarding all improved sectors, the means 1.6 and 1.7 are not significantly different at $P > .05$ but are each significantly different at $P < .01$ from the mean 3.0; at $P < .01$ the mean .69 is significantly different from the means 3.0, 1.7, and 1.6.

of these two regions decreases markedly with a progressive decrease in temperature till a temperature is reached where the ad20$^+$ and bi1$^+$ regions are apparently not subject to deletions (i.e., not instable). It was also noted that irrespective of incubation temperature deletion of the ad20$^+$ region did not occur without deletion of the bi1$^+$ region. Furthermore, it was obvious from the data that at respectively 39°C, 36°C and 28°C the $\underline{y}^+$ region on the translocated duplicate I segment is subject to far more deletions than either the ad20$^+$ region or bi1$^+$ region. At 42°C it is not clear whether or not the $\underline{y}^+$ region is subject to significantly more deletions than either the ad20$^+$ region or bi1$^+$ region. However, as shall be seen shortly, it is obvious that at 42°C the $\underline{y}^+$ region upon extended periods of incubation is subject to significantly more deletions than either the ad20$^+$ region or bi1$^+$ region.

In regard to brown variants or mutants, their production appears to be circumscribed to 36°C.

In a subsequent experiment, P colonies were grown at 42°C and 36°C for a period of nine days. At the end of incubation a sample of yellow and green improved sectors were tested for adenine and biotin dependence. This and other relevant data from the experiment are recorded in Table 12. The data indicates that upon nine days of incubation the frequency of deletions including the $\underline{y}^+$ allele at 42°C is not significantly different from the frequency of such at 36°C. Furthermore, the overall frequency deletions occurring from the I duplication at 42°C is not significantly different from such at 36°C, even though the ad20$^+$ and bi1$^+$ regions at 42°C are each subject to far more deletions than at 36°C.

T A B L E 12

P colonies grown for a period of nine days

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)
Colonies at 42°C	35	4.9cm	17	43	60
Colonies at 36°C	35	8.0cm	26	40	66

*All improved

T A B L E 12 (continuation)

	% of sectors which are yellow sectors	Number of improved green sectors tested for adenine and biotin requirements	Number of $y^+ ad^+ bi^+$ sectors noted	Number of $y^+ ad\ bi$ sectors noted	Number of yellow sectors tested for adenine and biotin requirements
Colonies at 42°C	71.6%	17	6	11	17
Colonies at 36°C	60%	26	24	2	26

T A B L E 12 (continuation)

	Number of y ad$^+$ bi$^+$ sectors noted	Total number of improved sectors tested for adenine and biotin requirements	Total number of tested improved sectors found to be ad bi sectors	% of yellow and green tested sectors found to be ad bi sectors	% of green improved sectors found to be ad bi sectors	Mean number of yellow sectors per colony
Colonies at 42°C	17	34	11	32.3%	64.6%	1.2
Colonies at 36°C	26	52	2	3.8%	7.6%	1.1

T A B L E 12 (continuation)

	Mean number of improved sectors per colony	Number of deteriorated brown sectors
Colonies at 42°C	1.7	0
Colonies at 36°C	1.9	0

TABLE 13

P colonies grown for a period of nine days

	Number of colonies	Mean diameter of parental region	Number of deteriorated brown sectors
Colonies at 42°C	85	5.0cm	6
Colonies at 36°C	82	7.9cm	7
Colonies at 28°C	85	6.9cm	1

T A B L E 14

Periods at which mutant sectors arose from the parental regions of P colonies during growth at 36°C

	0-95 hours	96-120 hours	121-144 hours	145-167 hours
Number of yellow sectors	6	13	15	10
Number of improved green sectors	2	17	10	4

	168-191 hours	192-240 hours
Number of yellow sectors	9	3
Number of improved green sectors	3	2

Total number of yellow sectors which arose during the 0-95 hour growth period = 6

Total number of improved green sectors which arose during the 0-95 hour growth period = 2

Total number of yellow sectors which arose during the 96-240 hour growth period = 50

Total number of improved green sectors which arose during the 96-240 hour growth period = 36

Number of P colonies involved in the experiment = 55

Mean number of yellow sectors* = 1.0

*All are improved sectors

In order to obtain additional data pertaining to the number of deteriorated brown sectors produced at various temperatures, P colonies were grown in 28°C, 36°C, and 42°C for a period of nine days. The results are given in Table 13. Such results would appear to indicate that upon 9 days of incubation the production of brown variants is no longer circumscribed to 36°C. Though this experiment was done with the intention of noting brown variants, observation also revealed that there was an average of approximately one yellow sector per colony at 36°C and 42°C upon nine days incubation, again indicating that upon nine days growth the $\underline{y}^+$ region is subject at 42°C to the same frequency of deletions to which the $\underline{y}^+$ region is subject during growth at 36°C. Though observation of yellow sectors produced at 28°C was not as detailed, a general inspection of such sectors nevertheless revealed that the colonies at 38°C produced roughly one yellow sector per colony.

Before performing additional experiments relating to the instability of P colonies at higher temperatures upon extended periods of incubation, an experiment was undertaken in order to determine the approximate periods of times (growth periods) at which P colonies grown at 36°C gave rise to yellow and green variants (sectors). Fifty-five P colonies were incubated for a period of ten days, and the times at which the mutant sectors arose were noted; it is to be noted that P colonies at 36°C cannot be left longer than ten days insofar as the parental circumferences are very near the edges of the plates upon ten days incubation. The approximate times at which the mutant sectors arose are recorded in Table 14. It is clear from the results that a very large majority of yellow and green mutant sectors arose during the later days of growth. In fact only 10%

of the yellow sectors arose during the 0-95 hour period following inoculation and only 2.6% of the green mutant sectors arose during the same period. In marked contrast the Rg strain (Part II) grown at 36°C gave rise to 88.8% of its improved sectors within the 0-95 hour period following inoculation.

Thus where the P, I duplication, strain at 36°C gives rise to most of its improved sectors during a later period of time, the Rg, III duplication, strain at 36°C in contrast gives rise to a very large majority of its improved sectors during a comparatively earlier period of time. This indicates, therefore, that deletions generally occur from the I duplication (at 36°C) during a later period of growth, while, in contrast, deletions generally occur from the III duplication (at 36°C) during a comparatively earlier period of growth.

Having completed this experiment, it was decided to extend the period of incubation beyond nine days for the P colonies at 42°C and 39.5°C. With this intention, P colonies were grown at 42°C for a period of 12 days and at 39.5°C for a duration of ten days. Furthermore, another group of P colonies were grown at 36°C for ten days and an additional group of P colonies were grown at 28°C for a duration of 12 days.[2] The results are given in Table 15 (also see Plates 2a, 2b, 3a and 3b). The yellow and green improved sectors produced at each temperature were tested for adenine and biotin requirements (or dependence). The results of these tests are also recorded in Table 15. The inclusive data indicates that the frequency of deletions to which the y^+ region is subject does not significantly become augmented with an increase of temperature from 28°C to 36°C.

2. By twelve days incubation, circumferences of colonies at 28°C are very close to edge of plates.

However, with an increase of temperature from 36°C to 39.5°C the frequency of deletions to which the $\underline{y}^+$ region is subject becomes significantly increased; in fact the data makes it clear that the frequency of deletions which include the $\underline{y}^+$ allele is approximately five times greater at 39.5°C than at 36°C upon ten days of incubation. With a further increase of temperature from 39.5°C to 42°C, however, there is a significant decrease in the frequency of deletions to which $\underline{y}^+$ region is subject, even though the $\underline{y}^+$ region at 42°C is subject to significantly more deletions than at either 36°C or 28°C upon extended incubation; in point of fact, upon 12 days of incubation at 42°C, the frequency of deletions which include the $\underline{y}^+$ allele is approximately three times greater at 42°C than at either 36°C or 28°C; or in other words, upon 12 days of incubation at 42°C, the deletional instability of the $\underline{y}^+$ region is approximately three times greater at 42°C than at either 36°C or 28°C.

Furthermore, the data indicates that the overall frequency of deletions occurring from the I duplication does not significantly become augmented with an increase of temperature from 28°C to 36°C; with an increase of temperature from 36°C to 39.5°C, the frequency of deletions occurring from the I duplication becomes significantly increased, while a further increase in temperature from 39.5°C to 42°C is however associated with a significant decrease in the overall frequency of deletions occurring from the I duplication, even though the overall frequency of deletions occurring from the I duplication at 42°C is significantly greater than at either 36°C or 28°C.

In addition, we again see that the frequency of deletions which include the respective $\underline{ad20}^+$ and $\underline{bi1}^+$ alleles is highest at 42°C, and the frequency of deletions which include these two

T A B L E 15

P colonies grown for extended periods

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors*)	% of sectors which are yellow sectors
Colonies at 42°C for a period of 12 days	38	6.7cm	20	122	142	85.9%
Colonies at 39.5°C for a period of 10 days	36	7.6cm	28	185	213	86.8%
Colonies at 36°C for a period of 10 days	40	8.2cm	25	45	70	64.2%
Colonies at 28°C for a period of 12 days	40	8.3cm	23	34	57	59.6%

*All improved

T A B L E 15 (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of $y^+ ad^+ bi^+$ sectors noted	Number of $y^+ ad\ bi$ sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of $y\ ad^+ bi^+$ sectors noted	Total number of sectors tested for adenine and biotin requirements
Colonies at 42°C for a period of 12 days	20	7	13	80	80	100
Colonies at 39.5°C for a period of 10 days	28	21	7	100	100	128
Colonies at 36°C for a period of 10 days	25	23	2	39	39	64
Colonies at 28°C for a period of 12 days	23	23	0	34	34	46

/A white improved sector produced at 39.5°C by a deteriorated brown variant was not included in tabulations

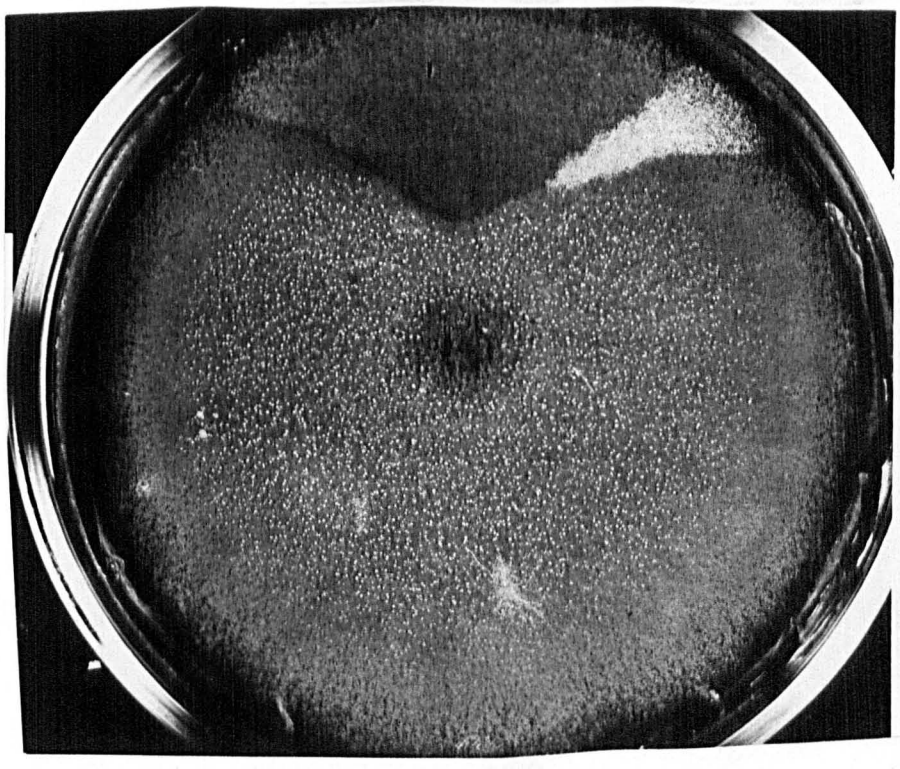
T A B L E 15 (continuation)

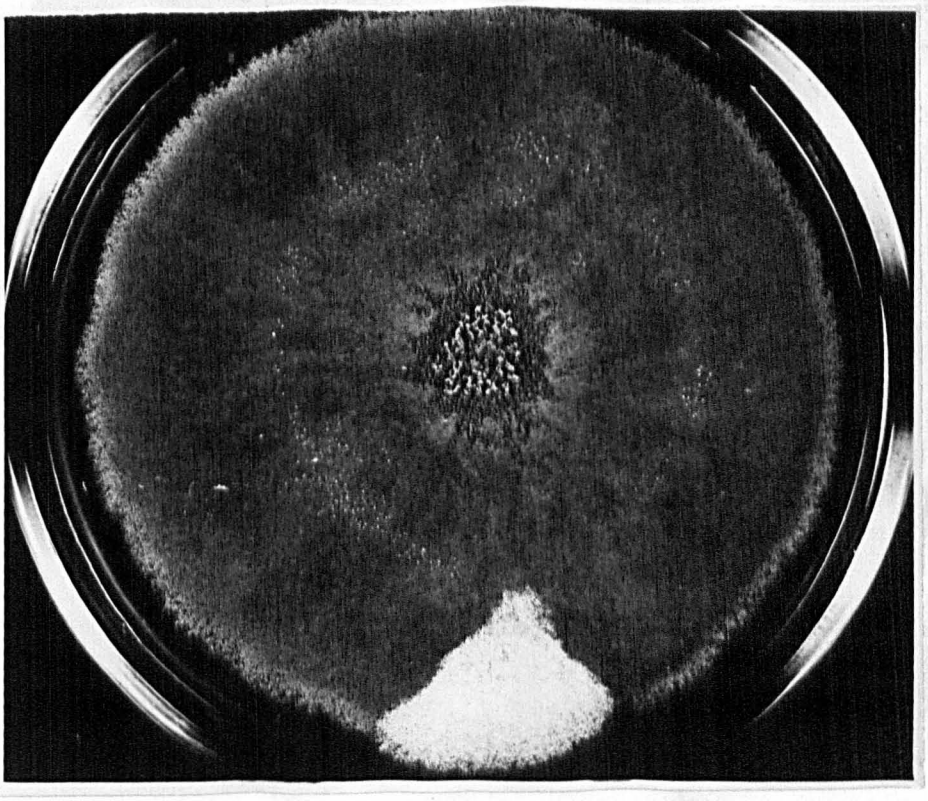
	Total number of tested improved sectors found to be ad bi sectors	% of yellow and green tested sectors found to be ad bi sectors	% of green improved sectors found to be ad bi sectors	Mean number of yellow sectors per colony	Mean number of improved sectors per colony	Number of deteriorated brown sectors
Colonies at 42°C for a period of 12 days	13	13%	65%	3.2	3.7	0
Colonies at 39.5°C for a period of 10 days	7	5.4%	25%	5.1	5.9	2
Colonies at 36°C for a period of 10 days	2	3.1%	8%	1.1	1.7	2
Colonies at 28°C for a period of 12 days	0	0%	0%	.85	1.4	1

T A B L E 15 (continuation)

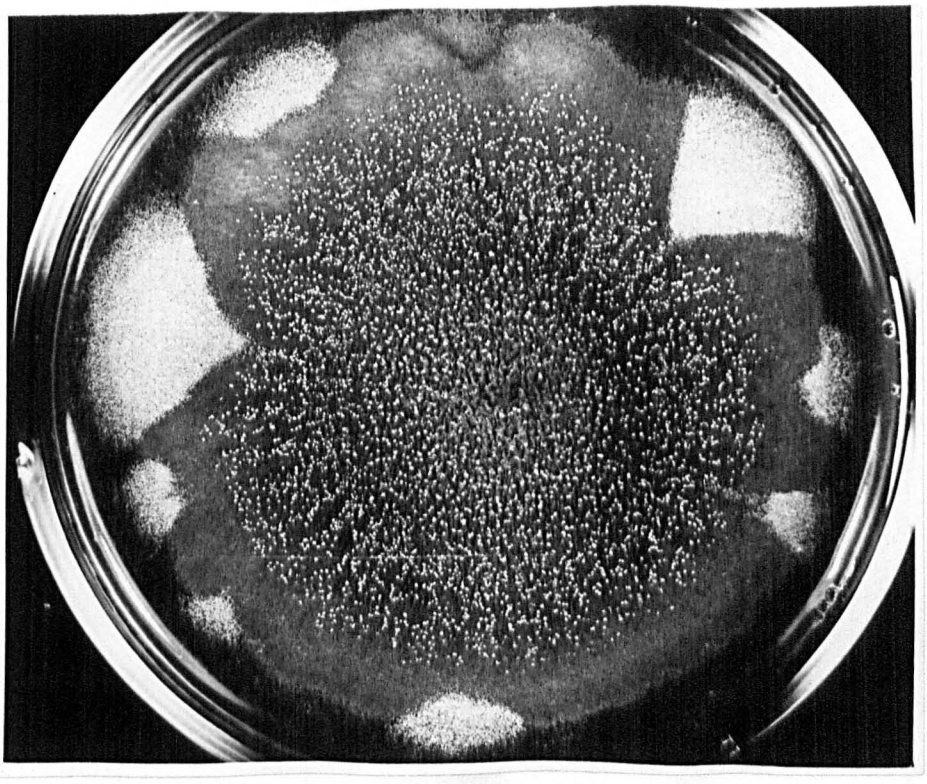
Regarding yellow sectors, the means .85 and 1.1 are not significantly different at $P > .05$ but are each significantly different at $P < .01$ from the mean 5.1. At $P < .01$ the mean 3.2 is significantly different from the mean 5.1; moreover, at $P < .01$ the mean 3.2 is significantly different from the (respective) means 1.1 and .85.

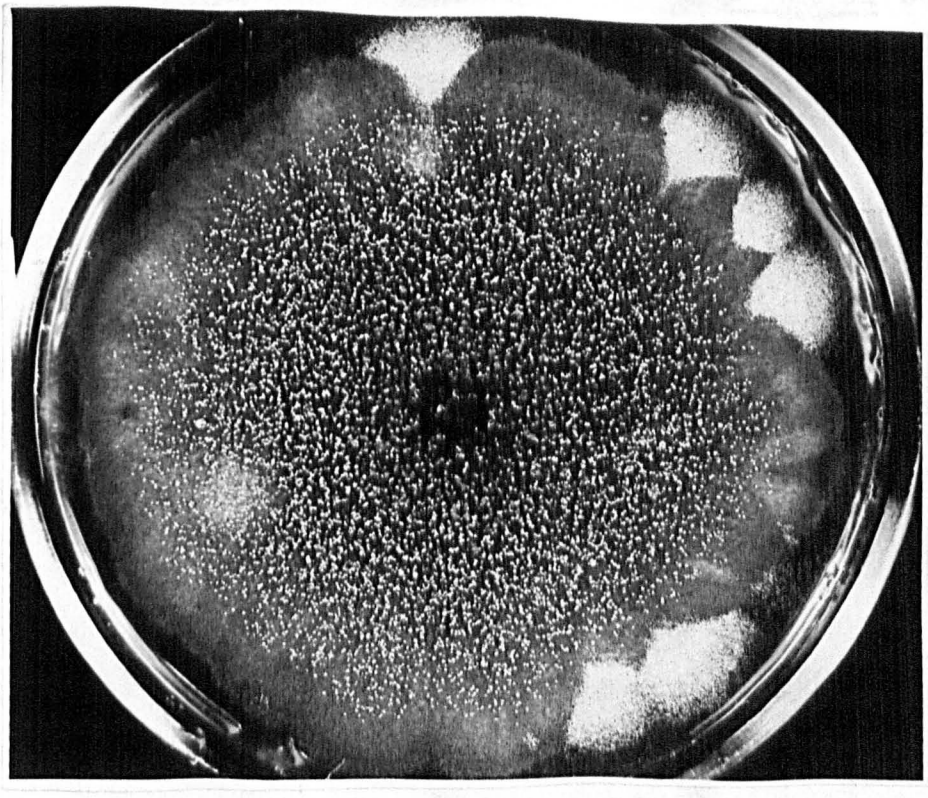
Regarding all improved sectors, the means 1.7 and 1.4 are not significantly different at $P \geq .05$ but are each significantly different at $P < .01$ from the mean 5.9; at $P < .01$ the mean 3.7 is significantly different from the mean 5.9; moreover, at $P < .01$ the mean 3.7 is significantly different from the means 1.4 and 1.7.





Plates 2a and 2b. Two P colonies with improved yellow and green sectors at 36°C. Growth period was 10 days.





Plates 3a and 3b. Two P colonies with improved yellow and green sectors at 39.5°C. Growth period was 10 days.

respective alleles decreases markedly with a progressive decrease in temperature till a temperature is reached (28°C) where the ad20$^+$ and bi1$^+$ regions are apparently not subject to deletion. It was again noted that irrespective of incubation temperature deletion of the ad20$^+$ region did not occur without deletion of the bi1$^+$ region. Furthermore, it was again obvious from the data that at respectively 39.5°C, 36°C and 28°C the y^+ region is subject to far more (significantly more) deletions than either the ad20$^+$ region or bi1$^+$ region. At 42°C it is very clear that upon 12 days of incubation the y^+ region is subject to significantly more deletions than either the ad20$^+$ region or bi1$^+$ region. Moreover, it was at this time that it was finally noted that all y^+ ad bi sectors were of a silver-green or grey-green colour in contrast to y^+ ad$^+$ bi$^+$ sectors which were of a grass-green colour.

Another series of P colonies were grown at 36°C, 39.5°C and 42°C. Specifically colonies at 42°C were incubated for a period of 15 days. By this time colonial circumferences are very near plate edges. Colonies at 39.5°C were incubated for a period of 12 days and by the end of this period of incubation colonial circumferences at this temperature are also very close to plate edges; colonies at 36°C were incubated for a period of ten days. The results are given in Table 16. Improved sectors produced at each temperature were tested for adenine and biotin requirements. The results from these tests are also recorded in Table 16. The data from this latest experiment confirms previous findings. Again we see that with an increase of temperature from 36°C to 39.5°C the frequency of deletions to which the y^+ region is subject becomes significantly increased; in fact the frequency of deletions which include the y^+ allele is five times as great

at 39.5°C than at 36°C. With a further increase of temperature from 39.5°C to 42°C, however, there is a significant decrease in the frequency of deletions to which the $\underline{y}^+$ region is subject, even though the $\underline{y}^+$ region at 42°C is subject to significantly more deletions than at 36°C. In addition, the data indicates that with an increase of temperature from 36°C to 39.5°C the overall frequency of deletions occurring from the I duplication becomes significantly increased, while a further increase in temperature from 39.5°C to 42°C is however associated with a significant decrease in the overall frequency of deletions occurring from the I duplication, even though the overall frequency of deletions occurring from the I duplication at 42°C is significantly greater than at 36°C.

In this latest experiment the deletional instability of the respective $\underline{ad20}^+$ and $\underline{bi1}^+$ regions is highest at 42°C and is again seen to decrease markedly with a progressive decrease in temperature. In accord with previous findings, the latest data also indicates that at 36°C and 39°C the $\underline{y}^+$ region is subject to far more (or significantly more) deletions than either the $\underline{ad20}^+$ region or the $\underline{bi1}^+$ region; at 42°C it is obvious that upon 15 days of incubation the $\underline{y}^+$ region is subject to significantly more deletions than either the $\underline{ad20}^+$ region or $\underline{bi1}^+$ region; or in other words, it is obvious that upon 15 days of incubation at 42°C the $\underline{y}^+$ region manifests significantly more deletional instability than either the $\underline{ad20}^+$ region or $\underline{bi1}^+$ region. Moreover, the latest findings indicate that irrespective of temperature deletion of the $\underline{ad20}^+$ region did not occur without deletion of the $\underline{bi1}^+$ region. In this light, all $\underline{y}^+$ ad bi sectors were seen again to be of a silver-green or grey-green colour while all $\underline{y}^+$ $\underline{ad}^+$ $\underline{bi}^+$ were noted to be of a grass-green colour.

In reviewing all the experiments in which strain P was grown at 42°C, it is quite apparent that the mean frequency of yellow sectors produced at this temperature upon eight days of incubation increases markedly by a factor varying from 6.5 to 11 in a four day period of time subsequent to eight days growth, with no further increases occurring during a subsequent period of incubation. This would suggest that the susceptibility of the y^+_- region to deletion at 42°C is greatly enhanced within a specific four day period of growth time, thereby indicating that the deletion of chromosomal material including the y^+_- allele at 42°C is dependent upon or conditioned by a specific period of growth; such a period of enhanced susceptibility appears to be a condition of growth at high temperature. This is seen readily when one takes note that P colonies grown at 36°C rarely produced yellow sectors after eight days incubation, with the vast majority of yellow sectors being produced during the four to eight days growth period subsequent to inoculation. Furthermore, upon a duration of incubation subsequent to eight days growth, the degree of deletional instability at respectively 36°C and 28°C remained as it was upon eight days of incubation.

Regarding all P colonies grown at 39.5°C, it is clear that the mean frequency of yellow sectors produced at this temperature upon eight days of incubation increases approximately by a factor of .76 within a two day period of time subsequent to eight days incubation, with no further increases occurring by twelve days growth. This may suggest that during this particular two day growth period at 39.5°C the y^+_- region is more susceptible to deletion than during any other two day period.

T A B L E 16

P colonies grown for extended periods

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
Colonies at 42°C for a period of 15 days	38	8.2cm	30	114	144	79.1%
Colonies at 39.5°C for a period of 12 days	40	8.3cm	30	216	246	87.8%
Colonies at 36°C for a period of 10 days	40	8.3cm	26	48	74+ one het. sector/	64%

*All improved

/Improved sector displaying a moisaic of yellow and green conidia.

TABLE 16 (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of y^+ ad^+ bi^+ sectors noted	Number of y^+ ad bi sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of y ad^+ bi^+ sectors noted
Colonies at 42°C for a period of 15 days	30	9	21	95	95
Colonies at 39.5°C for a period of 12 days	30	24	6	130	130
Colonies at 36°C for a period of 10 days	26	24	2	43	43

T A B L E 16 (continuation)

	Total number of improved sectors tested for adenine and biotin requirements	Total number of tested improved sectors found to be ad bi sectors	% of yellow and green tested sectors found to be ad bi sectors	% of green improved sectors found to be ad bi sectors	Mean number of yellow sectors per colony	Mean number of improved sectors per colony	Number of deteriorated brown sectors
Colonies at 42°C for a period of 15 days	125	21	16.8%	70%	3.0	3.7	1
Colonies at 39.5°C for a period of 12 days	160	6	3.7%	20%	5.4	6.1	0
Colonies at 36°C for a period of 10 days	69	2	2.8%	7.6%	1.2	1.8	0

T A B L E 16 (continuation)

Regarding yellow sectors, the means 5.4 and 1.2 are significantly different at $P < .01$; at $P < .01$ the mean 3.2 is significantly different from the mean 5.4; furthermore, at $P < .01$ the mean 3.2 is significantly different from the mean 1.2

Regarding all improved sectors, the mean 6.1 is significantly different from the mean 1.8 at $P < .01$; at $P < .01$ the mean 3.7 is significantly different from the mean 6.1; furthermore, the mean 3.7 is significantly different from the mean 1.8 at $P < .01$.

The production of a white improved sector by a brown variant at 39.5°C is of interest but it is not clear whether this production is related to the presence of duplications. In regard to the production of brown variants, themselves, the data in Table 17 appears to indicate that upon extended periods of growth the production of brown variants by strain P is not circumscribed to any one given temperature, though the data precludes any definite conclusions regarding the relationship of temperature to the degree of such production.

Turning from the production of brown variants to the production of green and yellow mutant sectors by P colonies, the data taken as a whole clearly indicates that deletional instability of the I duplication in P is definitely temperature sensitive or in other words, the degree of deletional instability of the I duplication is conditioned or modified by the temperature of growth, with deletional instability being significantly greater at the two highest growth temperatures, the apogee in deletional instability apparently being at 39.5°C. In this connection, the period of greatest deletional instability in P also appears to be tied in with the temperature of growth.

Moreover, the data may suggest another interesting facet in regards to deletion.

Because the y^+ allele has been shown by the data to be at various temperatures far more prone or susceptible to deletion than either the $ad20^+$ allele or the $bi1^+$ allele, one may suggest that this vast difference is susceptibility is related on the one hand to the fact that the y^+ allele (or region) is located on the translocated segment of the duplication, and on the other, to the fact that the $ad20^+$ and $bi1^+$ alleles (or regions) are located on

T A B L E 17

All P colonies grown for 9, 10, 12 and 15 days

	Total number of colonies	Total number of deteriorated brown sectors	Mean number of deteriorated brown sectors per colony
Colonies at 42°C	198	7	.035
Colonies at 39.5°C	76	2	.026
Colonies at 36°C	198	11	.055
Colonies at 28°C	128	2	.015

the non-translocated segment of the duplication. If such were the case, it would suggest that the location of the y^+ allele (or region) on the translocated segment is an important factor in making the y^+ allele (or region) most subject to deletion at 39.5°C.

To determine whether or not genes located on the translocated segment of the I duplication are far more subject to deletion than those genes positioned on the non-translocated segment of the I duplication, the deletional instability behaviour of a I duplication strain (also investigated by Roper and Nga, 1969) having the linkage relationships

I pro1 paba6 y^+ ad20 bi1 / I-II y ad20$^+$ bi1$^+$

was studied at various temperatures, with results being ultimately compared to the P data. This second I duplication strain, referred to as Q, has the same duplicated segment of chromosome I as does P. However, in strain Q, as can be seen y^+, ad20, and bi1 are all located on the non-translocated segment of the I duplication, whereas y, ad20$^+$ and bi1$^+$ are located on the translocated segment.[3] Q has the same general phenotype as P.

Work commenced with a series of Q colonies being grown at 28°C, 36°C and 39.5°C for a period of eight days. Upon the completion of incubation all yellow and green mutant sectors produced at each temperature were scored; furthermore, all yellow and green mutant sectors, produced at each temperature, were tested for adenine and biotin dependence (requirements). Overall results are recorded in Table 18a. In addition, another series of Q colonies were grown at 28°C, 36°C and 39.5°C for a period of eight days. Upon the completion of incubation, the number of yellow and green mutant sectors produced

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3.As in P, the translocated duplicate I segment is attached to the end of chromosome II.

at each temperature was noted; moreover all yellow and green mutant sectors produced at each temperature were tested for adenine and biotin requirements. Overall results are recorded in Table 18b.

Subsequently, a group of Q colonies was grown at 42°C for a period of eight days. Data relating to these Q colonies is presented in Table 18c. Furthermore a second group of Q colonies was grown at 42°C for a period of eight days. Results relating to this experiment are presented in Table 18d. Reference to data in these two tables will be made shortly.

In regard to the first two experiments with Q colonies the data in Tables 18a and 18b indicates that upon eight days incubation the general deletional instability displayed by Q at 39.5°C is not significantly different from that displayed at 36°C; compared to instability at 36°C and 39.5°C, instability at 28°C is significantly less upon eight days incubation. However, as shall be seen shortly, the general deletional instability behaviour of Q at 39.5°C is far greater than that displayed by Q at 36°C upon an extended period of incubation of Q at these two temperatures.

The Q data of respectively Tables 18a, 18b, 18c and 18d was compared to the P data of respectively Tables 11a and 11b. Such comparisons indicated the following: of the deletions involving the I duplication, the proportion affecting the $\underline{y}^+$ region in the P strain is far greater at any given temperature than the proportion of such in the Q strains. Furthermore, of the deletions involving the I duplication, the proportion affecting the $\underline{ad20}^+$ and $\underline{bi1}^+$ regions in the Q strain is far greater at any given temperatures than the proportion of such in the P strain. The meaning or implication of the foregoing becomes clear: of deletions involving the I duplication, the $\underline{y}^+$, $\underline{bi1}^+$, and $\underline{ad20}^+$

T A B L E 18a

Q colonies grown for a period of eight days

	Number of colonies	Mean diameter of parental regions	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
Colonies at 39.5°C	35	6.3cm	43	0	43	0%
Colonies at 36°C	36	7.4cm	37	3	40	7.5%
Colonies at 28°C	34	6.2cm	12	0	12	0%

*All yellow sectors are improved; respective yellow sectors arise as a result of respective deletions which include the y^+ allele on the non-translocated duplicate I segment.

T A B L E 18a (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of y^+ ad^+ bi^+ sectors noted	Number of y^+ ad bi sectors noted [/]	Number of yellow sectors tested for adenine and biotin requirements	Number of y ad^+ bi^+ sectors noted	Total number of sectors tested for adenine and biotin requirements
Colonies at 39.5°C	43	24	19	.	.	43
Colonies at 36°C	37	25	12	3	3	40
Colonies at 28°C	12	9	3	.	.	12

[/] Respective y^+ ad bi sectors arise as a result of respective deletions which include the $ad20^+$ and $bi1^+$ alleles on the translocated duplicate I segment.

T A B L E 18a (continuation)

	Total number of tested sectors found to be *ad bi* sectors	% of tested sectors found to be *ad bi* sectors	Mean number of yellow sectors per colony	Mean number of *ad bi* sectors per colony	Mean number of improved sectors per colony
Colonies at 39.5°C	19	44.1%	0	.54	1.2
Colonies at 36°C	12	30%	.08	.30	1.1
Colonies at 28°C	3	25%	0	.08	.35

Regarding all improved sectors, it is obvious the means 1.2 and 1.1 are not significantly different from one another; however, at $P < .05$ the means 1.2 and 1.1 are each significantly different from the mean .35.

T A B L E 18b

Q colonies grown for a period of eight days

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors*	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors	Number of improved green sectors tested for adenine and biotin requirements
Colonies at 39.5°C	24	6.2cm	29	3	32	9.3%	29
Colonies at 36°C	27	7.2cm	24	1	25	4%	24
Colonies at 28°C	29	6.1cm	11	1	12	8.3%	11

*All improved

T A B L E 18b (continuation)

	Number of y^+ ad^+ bi^+ sectors noted	Number of y^+ ad bi sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of y ad^+ bi^+ sectors noted	Total number of sectors tested for adenine and biotin requirements
Colonies at 39.5°C	14	15	3	3	32
Colonies at 36°C	18	6	1	1	25
Colonies at 28°C	8	3	1	1	12

T A B L E 18b (continuation)

	Total number of tested sectors found to be ad bi sectors	% of tested sectors found to be ad bi sectors	Mean number of yellow sectors per colony	Mean number of ad bi sectors per colony	Mean number of improved sectors per colony
Colonies at 39.5°C	15	46.8%	.12	.62	1.3
Colonies at 36°C	6	24%	.03	.22	.92
Colonies at 28°C	3	25%	.03	.09	.41

At $P > .05$, the means 1.3 and .92 are not significantly different, but at $P < .05$ are each significantly different from the mean .41.

TABLE 18c

Q colonies at 42°C for a period of eight days

Number of colonies	Mean diameter of parental region	Number of yellow sectors	Number of improved green sectors	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
36	4.5cm	1[#]	11	12	8.3%

TABLE 18d

Q colonies at 42°C for a period of eight days

Number of colonies	Mean diameter of parental region	Number of yellow sectors	Number of improved green sectors	Total number of green and yellow sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
27	4.4cm	0	9	9	0%

[#]Improved

regions are each subject to a far greater proportion of such deletions by being on the translocated segment than by being on the non-translocated segment. The preceding thereby implies that by being on the translocated segment of the I duplication the y^+, $ad20^+$ and $bi1^+$ alleles (or regions) become irrespective of various growth temperatures, extremely more prone to or far more subject to deletion than they would be if they were located on the non-translocated segment. This conclusion can be arrived at from another standpoint: in reviewing the Q data (Tables 18a and 18b) we see that at each of three temperatures the $ad20^+$ and $bi1^+$ regions in Q are each subject to far more deletions than is the y^+ region in the same strain, whereas, in contrast, as we have seen, the y^+ region in strain P is subject at the same three temperatures to far more deletions than either the $ad20^+$ region or $bi1^+$ region in the same strain. Such information clearly leads to the same conclusion. Namely, the presence of the y^+, $ad20^+$, and $bi1^+$ alleles (or regions) on the translocated duplicate I segment, makes them far more subject to deletion than they otherwise would be had they been located on the non-translocated segment.

Having studied the instability behaviour of Q colonies upon eight days incubation, it was decided to study such behaviour upon an extended period of incubation. To this end a group of Q colonies were grown at 36°C for a period of 10 days[4] and another group were grown at 39.5°C for a period of 12 days[5]. All yellow and green mutant sectors produced at each temperature were noted; moreover, all yellow and green mutant sectors produced at the respective temperatures were tested for adenine and biotin

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4. By ten days, colony (parental) circumferences are very near plate edges.
5. By twelve days, colony (parental) circumferences are very near plate edges.

TABLE 19

Q colonies grown for extended periods

	Number of colonies	Mean diameter of parental region	Number of improved green sectors	Number of yellow sectors	Total number of yellow and green sectors (inclusive number of improved sectors)	% of sectors which are yellow sectors
Colonies at 39.5°C for a period of 12 days	38	8.3cm	176	8*	184	4.3%
Colonies at 36°C for a period of 10 days	36	8.3cm	40	0	40	0%

*All improved

T A B L E 19 (continuation)

	Number of improved green sectors tested for adenine and biotin requirements	Number of y^+ ad^+ bi^+ sectors noted	Number of y^+ ad bi sectors noted	Number of yellow sectors tested for adenine and biotin requirements	Number of y ad^+ bi^+ sectors noted
Colonies at 39.5°C for a period of 12 days	176	15	161	8	8
Colonies at 36°C for a period of 10 days	40	29	11	.	.

T A B L E 19 (continuation)

	Total number of sectors tested for adenine and biotin requirements	Total number of tested sectors found to be <u>ad bi</u> sectors	% of tested sectors found to be <u>ad bi</u> sectors	Mean number of <u>ad bi</u> sectors per colony	Mean number of yellow sectors per colony	Mean number of improved sectors per colony
Colonies at 39.5°C for a period of 12 days	184	176	95.6%	4.2	.21	4.8
Colonies at 36°C for a period of 10 days	40	11	27.5%	.28	0	1.0

Regarding <u>y^+ ad bi</u> sectors, it is obvious that the means 4.2 and .28 are significantly different. Regarding all improved sectors, the means 4.8 and 1.0 are significantly different at $P < .01$.

<u>y^+ ad bi</u> sectors are not at a greater selective advantage at 39.5°C. (Appendix A).

requirements. Overall results from the experiment are recorded in Table 19. It is clear that upon 12 days of incubation, the general deletional instability at 39.5°C is significantly greater than that at 36°C upon ten days of growth. In fact the general instability at 39.5°C upon 12 days of incubation is approximately 5 times greater than the general instability at 36°C. Under identical temperatures and growth periods, this is seen to be similar to the instability behaviour of strain P. Moreover, in Q, the ad20$^+$ and bi1$^+$ regions at 39.5°C are each subject upon further incubation to significantly more deletions than they are at 36°C; specifically the ad20$^+$ and bi1$^+$ regions at 39.5°C are each upon (within) 12 days of growth subject to a frequency of deletion 15 times as great as that to which the ad20$^+$ and bi1$^+$ regions are each subject at 36°C.

Within the four day period subsequent to eight days growth, the frequency of deletions including the ad20$^+$ and bi1$^+$ alleles at 39.5°C can become greater than it was during the eight day period by a factor ranging from 5.7 to 6.7, suggesting that the ad20$^+$ and bi1$^+$ regions on the translocated segment (in Q) become most susceptible to deletion within a specific four day period of growth at 39.5°C. It is obvious that additional incubation at 36°C is not associated with a further increase in the frequency of deletions involving the ad20$^+$ and bi1$^+$ alleles.

Reviewing the Q data in Table 19 and the P data in Table 16, it is again obvious that the ad20$^+$ and bi1$^+$ regions in Q are each subject at 36°C and 39.5°C to far more deletions than the y$^+$ region in the same strain, whereas in contrast, the y$^+$ region in P is subject at 36°C and 39.5°C to far more deletions than either the ad20$^+$ region or bi1$^+$ region in the same strain. The preceding

again implies, as well as confirms, that by being on the translocated segment of the duplication the $\underline{y}^+$, $\underline{ad20}^+$, and $\underline{bi1}^+$ alleles become at different temperatures far more subject to deletion than they would be if they were located on the non-translocated segment.

More generally, all such comparisons between P and Q data imply that irrespective of various growth temperatures most deletions involve regions of the translocated segment of the I duplication. Thus, in conjunction with other information relating to instability at various temperatures, this would mean that the highest frequency of deletions involving regions of the translocated duplicate I segment occurs at 39.5°C. Information would also indicate that regions of the non-translocated duplicate I segment (at least in P) become progressively more subject to deletions with an increase in temperature.

Moreover, all data relating to the P and Q[6] strains indicates that the $\underline{ad20}^+$ region is always deleted along with the $\underline{bi1}^+$ region, apparently irrespective of duplicate segment and temperature, though, it is clear from the preceding work that general deletional instability of the I duplication is definitely altered (or modified) by temperature, with the I duplication upon extended periods of growth displaying significantly greater deletional instability at temperatures above 36°C.

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6. All $\underline{y}^+$ $\underline{ad}$ $\underline{bi}$ sectors produced by Q were of a grey-green colour shade while all $\underline{y}^+$ $\underline{ad}^+$ $\underline{bi}^+$ sectors produced by Q were of a grass-green colour shade.

P A R T IV

PART IV

In this part, various experiments will be described which deal with the genetic behaviour of haploid cultures with two different duplications; these experiments required related or auxiliary investigations; such investigations as they are important will also be illustrated.

By way of a Rg + P heterkaryon, a cross was made between Rg and P. This cross, Rg x P, was ultimately the source of a double-duplication haploid strain. From the cross,

pyro12 $\frac{y^+\ bi1}{}$; III $\frac{s12}{}$ / III-VIII $\frac{s12^+}{}$

(Rg) X I $\frac{pro1\ paba6\ y\ ad20^+\ bi1^+}{}$ / I-II $\frac{y^+\ ad20\ bi1}{}$

(P), one should obtain the following segregations:

25% of the haploid segregants should have two duplications, i.e., a partial-duplication of chromosome III and a partial-duplication of chromosome I; 25% should have a partial-duplication of chromosome I only; 25% should have a partial-duplication of chromosome III only; and 25% of the haploid segregants should be without a duplication. In other words, 75% ($\frac{3}{4}$) of the haploid segregants (progeny-colonies) should have at least one duplication and hence display a crinkled morphology of some type, whilst approximately 25% of the segregants should be without a duplication and hence smooth and/or flat (of normal morphology). It is expected that among the haploid segregants, progeny-colonies having respectively the Bainbridge and P morphologies should be found. It is also expected that the progeny-colonies having two duplications within a nucleus should be smaller and perhaps be of a more extreme crinkled morphology than those colonies having respectively the P and Bainbridge morphologies.

As expected, the cross between Rg and P yielded four general classes of progeny-colonies. One class was made up of colonies displaying the P morphology; these colonies were of a grass-green colour. Another class was made up of colonies displaying the Bainbridge morphology; green Bainbridge crinkled progeny were olive-green in colour shade. A third class was made up of colonies displaying a normal morphology. And, the fourth class was made up of colonies much smaller than all the others and definitely very crinkled. In fact, their morphology was generally characteristic of the Bainbridge crinkled morphology but the lobes making up the crinkled area or tissue were more compact or tightly knit than those of colonies known to have only the III duplication. This gave such small colonies the appearance of having a pronounced Bainbridge morphology; these colonies were also of a dull colour shade. This last class of colonies was assumed to be made up of double-duplication types.

The total number of colonies noted to have a normal morphology was 119 whilst the total number noted to display a crinkled morphology of any degree or type was 432. The expected number of normal types would be 137 (25% of all progeny) while the expected number of crinkled types would be 414 (75% of all progeny). The observed results conform to the expected at $P > .05$ (the ratio of crinkleds to non-crinkleds fits a 3:1 ratio at $P > .05$).

In regards to colour, it is expected that 75% of all progeny-colonies should be green whilst 25% of all the progeny-colonies should be yellow; in other words the ratio of green to yellow colonies should be in accord with a 3:1 ratio. Of the progeny-colonies, 410 were green while 141 were yellow. At $P > .05$ the ratio of green to yellow colonies is in accord with a 3:1 ratio.

A sample of the progeny-colonies were tested for various requirements. In regards to the thiosulphate phenotype, it is expected that approximately three colonies out of four would be thiosulphate-independent ($\underline{s}^+$) or one-half of the normal colonies should require thiosulphate whilst all Bainbridge type colonies should be thiosulphate-independent. Testing 60 colonies consisting of 30 Bainbridge types and 30 normal colonies, it was found that 50 colonies were $\underline{s}^+$ while 10 were $\underline{s}$. As expected, it was noted that the ratio of $\underline{s}^+$ to $\underline{s}$ colonies fitted a 3:1 ratio at $P > .05$. Regarding the nic phenotype, it is expected that approximately one-half of the progeny-colonies would be nicotinic acid-independent ($\underline{nic}^+$). Testing 70 colonies, it was found that 29 colonies were nic while 41 colonies were $\underline{nic}^+$, the ratio of $\underline{nic}^+$ colonies to nic colonies fitting a 1:1 ratio at $P > .05$. In testing only green colonies for biotin requirement, it is expected that the ratio of biotin-requiring to biotin-independent green colonies (progeny) should be in accord with a 2:1 ratio. From a large group of green progeny-colonies numbered 1 through 93, a sample of 70 colonies were tested for biotin requirement (or dependence). It was noted that the number of bi colonies was 47 while the number of $\underline{bi}^+$ colonies was 23. It was found that the ratio of biotin-requiring to biotin-independent colonies was in accord with a 2:1 ratio at $P > .05$. No putative double-duplication types were as yet tested.

In this group of 93 green colonies just referred to was a group of 9 putative double-duplication types. These, to repeat, were of a pronounced Bainbridge crinkled morphology, dull in colour shade and much smaller than other types of colonies. These nine colonies (numbered respectively 55, 56, 71, 80, 81, 82, 90, 92 and 93) were also tested for biotin requirement. All the

colonies but number 81 were found to be biotin-requiring. Colony number 81 was bi^+ and olive-green in colour like strain Rg. It was very distinct and clearly well spaced from the other colonies on the same plate.

From the cross,

pyro12 $\frac{\quad y^+ \; bi1}{}$; III $\frac{\quad s12}{}$ / III-VIII $\frac{\quad s12^+}{}$

(Rg) X I $\frac{pro1 \; paba6 \; y \; ad20^+ \; bi1^+}{}$ / I-II $\frac{y^+ \; ad20 \; bi1}{}$

6.1

(P), it is evident that haploid products (haploid segregants) which are biotin-independent and green are most likely (a 94% chance, approximately) to have a partial-duplication of chromosome I (the type of I duplication carried by strain P). Since colony number 81 has also the pronounced Bainbridge type morphology and is olive-green in colour as is Rg where the III duplication is completely intact, it was inferred that colony number 81 is a haploid colony (strain) which most likely has a partial-duplication of chromosome III (the type of III duplication carried by strain Rg) and a partial-duplication of chromosome I i.e., a haploid strain most likely having the I and III duplications.

From colony number 81, five inocula were placed separately on the respective centres of each of five CM plates. The plates were left in 36°C for a period of seven days. At the end of seven days incubation five colonies were observed. The parental region of each colony was seen to have the pronounced Bainbridge crinkled morphology and each parental region was also seen to be olive-green. (Such a morphology and colour indicate the presence of the III duplication.) From the parental regions, each colony had given rise to mutant sectors of different types, with mutant sectors of one type also emerging from other variants.

Four types or classes of mutant sectors were produced:

a) class h sectors; these sectors were grass-green in colour, lacked the Bainbridge morphology and were very (highly) improved in terms of growth rate and morphology; such h sectors could be described as being somewhat smooth or of a comparatively even texture; a h sector can be seen as being due to at least a large portion of the III duplication having been lost, for such a large loss would produce a sector having the grass-green or wild-types colour[1]; h sectors emerged from the parental region.

b) class fz sectors; these were virtually identical to the class one sectors produced by Rg; class fz sectors had an increased growth rate, were olive-green in colour and of a fuzzy non-smooth texture, though lacking any definite Bainbridge morphology. The lack of a Bainbridge morphology and the retention of the olive-green colour would indicate that a smaller part of the III duplication had been lost in the production of a given fz sector than in the production of a given h sector; fz sectors emerged directly from the parental region.

c) class ye sectors; these sectors were yellow; in place of a Bainbridge morphology, these sectors displayed a very improved morphology; virtually all of these yellow sectors emerged directly from the class h sectors; an extremely small minority emerged directly from the parental region; ye sectors emerging from the parental region were of a greatly increased growth rate relative to the parental region.

d) class bw sectors; sectors of this type were a-conidiating and of a pinkish-brown colour; the hyphae of these sectors were greatly compressed together into a solid continuous layer, giving such sectors a continuous but uneven surface; compared to the

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1. Another indication of such a major loss would be a sector displaying a highly improved morphology in place of a Bainbridge crinkled morphology. A further indication would be a sector of a highly improved growth rate.

parental region, it appeared that one of these sectors was of an increased growth rate, but this was far from certain; as bw sectors resemble the orthodox brown variants, the mutations which gave rise to bw sectors would very likely be similar to those which gave rise to the orthodox brown variants.

In regards to the five colonial subcultures of colony 81 whose mutant sectors have just been considered, the parental regions of these five colonial subcultures of colony 81 were each tested for biotin requirement, thiosulphate requirement, proline requirement, p-aminobenzoic acid requirement, nicotinic acid requirement, adenine requirement, and pyridoxin requirement. Each of the colonial subcultures was found to be bi$^+$, ad$^+$, s$^+$, pro, paba, pyro, and nic$^+$. Because the colonial subcultures of colony 81 each had an olive-green parental region which displayed the pronounced Bainbridge crinkled morphology, because the colonial subcultures of colony 81 were each bi$^+$ as well as green, and because these colonial subcultures eventually gave rise to yellow sectors, one is able to confirm that colony 81 and its colonial subcultures are cultures (representatives) of a double-duplication haploid strain having the I and III duplications. Individual colonies of this double-duplication strain displaying the same phenotype as that of the five colonial subcultures of colony 81 were referred to as RP81 (or RP81 colonies). Such colonies were regarded as members of the RP81 double-duplication strain.

It is clear that bi1$^+$, bi1, y, and y$^+$ are carried on (or within) the I duplication in RP81. It is very likely that ad20$^+$ and ad20 are also carried on (or within) the I duplication in RP81, for, even after crossing-over and subsequent segregation, over

95% of all green double-duplication progeny obtained from the Rg x P cross would have been heterozygous at the ad20^{+} locus. In fact a later experiment to be described showed that RP81 carries both ad20^{+} and ad20. Had RP81 not in fact been heterozygous at the ad20 locus, the linkage relationships of the alleles carried on (or within) the I duplication would have been different from what they were shown to be (P.130 to P. 138). As will be shown, a forthcoming experiment also demonstrated that RP81 carries both s12 and s12^{+} on (or within) the partial-duplication of chromosome III (the III duplication). Complementation tests indicated that RP81 has the pyro12 allele but not the pyro4 allele. It appears that pyro12 had become in linkage with y through crossing-over in the formation of RP81. As shall be seen shortly, such a situation was confirmed.

Twenty-eight RP81 colonies (colonial subcultures) were derived from an RP81 colony. These colonies had been left at 36^{o}C for a period of 9 days. Being RP81 colonies, the parental region of each colony displayed the pronounced Bainbridge crinkled morphology and was olive-green in colour. It was also noted that the parental regions of a few colonies displayed rill-type structures.

The same four mutant sector classes (types) were produced by this latest group of RP81 colonies, plus three additional mutant sector types or classes. The first of these latter classes (f1) was made up of a few sectors of a very fluffy texture and of increased growth rate. These f1 sectors emerged from the parental region, lacked the Bainbridge morphology, and were made up of a thick mycelium which virtually lacked conidial-heads. The extremely few conidial-heads present were olive-green as observed under the binocular. The second of these three classes (referred

to as or) was made up of one very small yellow sector of a Bainbridge crinkled morphology. This sector was located within the parental region of a RP81 colony. The third of these three classes was made up of sectors referred to as g. g sectors emerged only from h sectors. Such g sectors displayed the same morphology and colour as h sectors but were variants of a further improved growth rate.

In this experiment where RP81 colonies[2] were left at 36°C for 9 days, it was noted that nearly all of the yellow sectors emerged from class h sectors. Of the green sectors produced, general inspection revealed that a large proportion of such sectors was made up of class h sectors emerging from the parental region. Furthermore, it was noted that a portion of the bw sectors were produced by h sectors. Compared to the h sectors, these bw sectors were not of an increased growth rate. Plates 4a and 4b show two nine-day-old RP81 colonies with mutant sectors.

It is important to establish the linkage relationships of the known genes present in RP81. To this end a series of experiments were initiated. The first of these involved a cross between RP81 and ad8 paba1[3]. From this cross, 190 green and 76 yellow progeny-colonies were obtained. (Between 40% and 50% of these progeny-colonies displayed the Bainbridge crinkled morphology, thus further confirming that RP81 carries the III duplication.) In regards to the colour of the progeny obtained from the cross,

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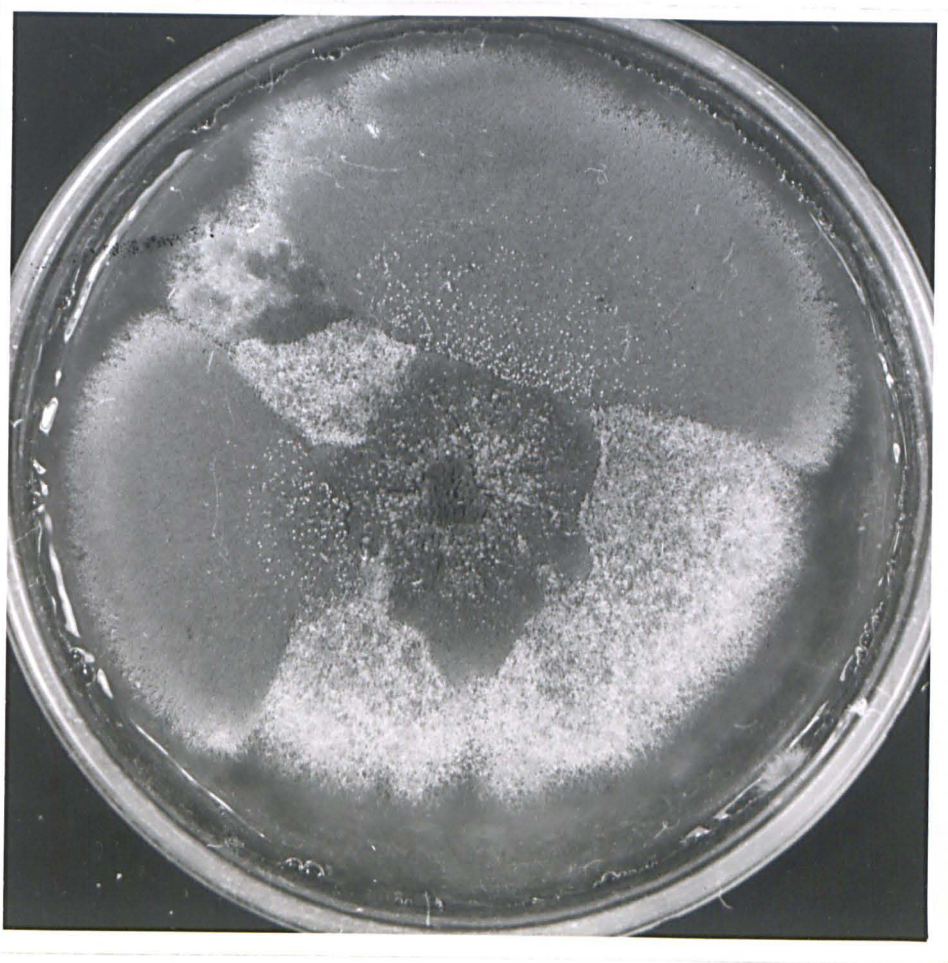
2. The mean diameter of the parental region was 2.5cm.
3. One will recall that ad8 is an allele of ad20.



Plates 4a and 4b. Two RP81 colonies with fz, h, ye, and g sectors at 36°C. Growth period was 9 days.

the ratio of green colonies to yellow colonies was in accord with a 3:1 ratio at $P > .05$. This is what one would expect if the y allele (in RP81) were on the non-translocated segment of the I duplication. If the y allele were on the translocated segment of the I duplication, crossing-over and resultant segregation would have resulted in the proportion of yellow colonies being very much smaller.

Seventy-four yellow colonies were tested for proline requirement. Recall that the pro1 locus resides approximately 25 units proximal to the y locus; the pro1 locus is not within the duplication as one will remember. It was found that 60 yellow colonies were proline-requiring and 14 were proline-independent. In other words, 81% of the tested yellow colonies were proline-requiring. This definite linkage with pro1 again shows that y is on the non-translocated duplicate I segment. Had the y allele been on the translocated duplicate I segment (and the y^+ allele thus on the non-translocated segment) only 50% of the tested yellow colonies would have required proline. As $ad20^+$ is .1 from y, it is inferred that $ad20^+$ is also located on the non-translocated segment of the I duplication. As will be seen very shortly, this is confirmed. One tested 41 of the above yellow colonies for biotin requirement; in this connection recall that the bi1 locus is 6.1 units from the y locus. In testing, it was found that 39 yellow colonies or 95.2% were biotin-independent and 2 (or 4.8%) were biotin-requiring. The bi1 allele is clearly seen to have been contributed by the RP81 parent. If the bi1 allele (in RP81) were on the non-translocated segment of the I duplication, it is expected that an extremely large majority (approximately 94%) of the tested yellow colonies would be biotin-requiring, thus indicating a definite linkage between y and bi1.

The fact that an extremely large majority (95.2%) of the tested yellow colonies were biotin-independent indicates a definite linkage between y and $bi1^+$ and thus implies that $bi1^+$ is on the non-translocated segment of the I duplication, while bi1 must exist in RP81 on the translocated duplicate I segment.

A mutant yellow variant (y1) of RP81 was crossed with bi1 ; orn4. y1 was produced by a h sector; y1 was phenotypically ad^+ bi^+ s^+. From the cross, 133 yellow and 147 green progeny-colonies were obtained. The ratio of yellow to green colonies is in accord with a 1:1 ratio at $P > .05$, and such a segregation is what one would expect with the y allele being on the non-translocated segment. This cross was of interest because in testing 70 normal colonies for thiosulphate requirement (dependence) it was noted that one colony was thiosulphate-requiring (s). This clearly showed that y1 contained the s12 allele as well as the $s12^+$ allele, and thus definitely demonstrated that RP81 carries the s12 and $s12^+$ alleles within the III duplication, though, it did not indicate on which of the two duplicate III segments the s12 allele resides. Furthermore, the fact that y1 is heterozygous at (for) the s12 locus indicates that y1 and the grass-green sector which produced it, retained a portion of the III duplication.

In regards to the location of the s12 allele in RP81, a RP81 // bi1 ; arg2 diploid was synthesized and in turn haploidized. Yellow and green haploid segregants (sectors) were produced and general inspection indicated that approximately equal numbers of yellow and green haploid sectors were present. A sample of the haploid segregants produced by the diploid were tested for thiosulphate, arginine, pyridoxine, adenine and biotin requirements. The results of the tests are given in Table 20.

If the s12 allele in RP81 were on the non-translocated duplicate segment of chromosome III, haploidization of the diploid RP81 // bi1 ; arg2 should produce a class of haploids phenotypically arg^+ s and another phenotypically arg s^+. If the s12 allele in RP81 were on the translocated duplicate III segment, haploidization of the aforesaid diploid should produce a class of haploids phenotypically arg s^+ and another phenotypically arg^+ s^+; in other words, all haploids (tested) ought to be s^+. Thus, the data indicates that in RP81 the s12 allele resides on the translocated segment of the III duplication. The fact that haploidization of this diploid resulted in the production of yellow haploid sectors or segregants clearly confirms that the y allele in RP81 is on the non-translocated segment of the I duplication. If the y allele were on the translocated segment of the I duplication (and the y^+ allele on the non-translocated segment), no yellow haploid sectors will have been produced upon haploidization. Because all the tested yellow haploid segregants were biotin and adenine independent (ad^+ bi^+), it is confirmed that $bi1^+$ and $ad20^+$ are on the non-translocated segment of the I duplication in RP81. This thus confirms that ad20 and bi1 are located on the translocated segment of the I duplication in RP81. The biotin dependence (requirement) of green haploid segregants is as expected, as is their adenine independence. Furthermore, the data first confirms that pyro12 and not pyro4 is present in RP81, and second, definitely confirms that pyro12 is on linkage group I. In RP81, pyro12 is seen to be definitely linked to the y allele, and it is inferred that it has become so as a result of crossing-over when Rg was crossed to P. The fact that RP81 is not phenotypically $pyro^+$ means that the pyro12 allele does not reside within the I duplication but must lie somewhere proximal to the y allele.

T A B L E 20

Segregations from the diploid RP81 // bi1 ; arg2

Chromosome	I						III			
allele	pyro	pyro$^+$	bi	bi$^+$	ad	ad$^+$	s	s$^+$	arg	arg$^+$
Colour										
yellow	22	0	0	22	0	22	0	22	10	12
green	0	23	23	0	0	23	0	23	10	13

By way of a RP81 + ribo1 ad14 y ; phen2 heterokaryon, a RP81 // ribo1 ad14 y ; phen2 diploid was synthesized and in turn haploidized. As expected only yellow haploid segregants were produced. Thirty of these yellow haploid sectors were tested for various requirements. The results are given in Table 21. The fact that all tested yellow haploid segregants were phen$^+$ s$^+$ is additional evidence that the s12 allele in RP81 is on the translocated segment of the III duplication. The presence of phen$^+$ s haploids among the segregants would clearly have meant that the s12 allele in RP81 is located on the non-translocated duplicate III segment. The results suggest a lethal in linkage with paba6. Furthermore, the results not only indicate again that pyro4 is not carried by RP81 but show again that in RP81 pyro12 is in linkage with paba6, thus demonstrating again that pyro12 is on linkage group I.

In order to determine the approximate position occupied by the pyro12 allele on linkage group I, a cross was executed between a h variant produced by RP81 and the normal strain ribo1 ad14 y ; phen2. This grass-green variant, h, had produced a yellow sector thereby indicating that it retained the y allele. It is to be recalled that ad14 and ribo1 are both on the left arm of chromosome I and are approximately 22 units from one another. From the cross, 329 green and 284 yellow progeny-colonies were obtained, the ratio of greens and yellows fitting a 1:1 ratio at $P > .05$ as expected. (The y allele is donated by both the h and ribo1 ad14 y ; phen2 parents.) The recombination frequencies used to localize the position of pyro12 were based on the classification of 60 normal yellow progeny-colonies. It was noted that the recombination frequency (R.F) between the pyro12 and ad14$^+$ loci was 33%, the R.F between the ad14 and ribo1 loci was

T A B L E 21

Segregations from the diploid RP81 // ribo1 ad14 y ; phen2

(only yellow haploid segregants produced)

Chromosome	I						III			
allele	pyro	pyro$^+$	paba	paba$^+$	ribo	ribo$^+$	s	s$^+$	phen	phen$^+$
	0	30	0	30	30	0	0	30	0	30

20%, and the R.F between the pyro12 and ribo1$^+$ loci was 15%. These results clearly place the pyro12 locus approximately 15 units distal to the ribo1 locus with the ribo1 locus between the pyro12 and ad14 loci.

Having established the approximate position of the pyro12 locus, additional information was sought in regards to the linkage relationships within RP81. In this respect a RP81 // MSE diploid was synthesized and in turn haploidized. Upon haploidization only white haploid sectors or segregants were produced. This is as expected because the w3$^+$ allele on linkage group II is also in linkage with the translocated duplicate segment of chromosome I, and thus with the I duplication.

Thirty-two of these white haploid segregants were tested for various requirements; the results are recorded in Table 22. It was expected that all tested haploid segregants would be ribo. This is because the ribo2$^+$ allele on linkage group VIII is also in linkage with the translocated duplicate segment of chromosome III, and thus with the III duplication. The data showed that all tested haploid segregants were ribo. The data also showed that RP81 has a VI-VII translocation; it is evident that this translocation is the reciprocal VI-VII translocation carried by strain J and ultimately contributed to RP81 by way of Rg. Moreover, as Table 22 points out, there is no evidence of any other translocation existant in RP81 other than those which go to make up the two duplications. This would be as expected. In regards to specific loci, the lack of adenine-requiring (ad) and biotin-requiring (bi) haploid segregants among those tested is additional confirmation that the ad20 and bi1 alleles are on the translocated duplicate I segment in RP81. Furthermore, in regards to the s3 and s12 loci, only the s3 allele would be distributed among the

T A B L E 22

Segregations from the diploid RP81 // MSE

(Only white haploid segregants produced)

Chromosome	I		III		I and IV		V		VI	
allele	paba$^+$	paba	gal$^+$	gal	pyro$^+$	pyro	fac$^+$	fac	s$^+$	s
	17	15	18	14	7	25	13	19	19	13

Chromosome	VII		VIII		Translocations
allele	nic$^+$	nic	ribo$^+$	ribo	VI-VII$^{\pm}$
	19	13	0	32	

$^{\pm}$The existence of the VI-VII translocation is demonstrated by the fact that no s nic$^+$ and s$^+$ nic recombinants were found along with s nic and s$^+$ nic$^+$ segregants.

white haploid segregants due to the s12 allele being located on the translocated segment of the III duplication; results presented in Table 22 are in definite accord with this.

Because of the previous studies, the linkage relationships in RP81 are definitely established and can be summarized as follows:

$$\text{I } \underline{\text{pyro12}\text{---o---}\text{pro1 paba6 y ad20}^{+}\text{ bi1}^{+}} \;/\; \text{I-II } \underline{y^{+}\text{ad20 bi1}} \;;$$

$$\text{III } \underline{\quad s12^{+}} \;/\; \text{III-VIII } \underline{\quad s12}$$

T(VI-VII)

Having established the genetic linkage relationships within RP81, one now turns to a more detailed study of the double-duplication strain. Using RP81 conidia, additional colonial cultures were made according to methods earlier described. These colonies were left at 36°C for 9 days. The phenotypic lineages of given sector types produced by the 40 RP81 colonies[4] present following incubation were observed. Also, observations were made as to the periods of time in which different sector types had been produced by the 40 RP81 colonies. The same seven sector classes or types were observed as noted in the earlier experiments with RP81 (P.125 to P.128). Figure 3 indicates the phenotypic lineages of the seven sector classes observed in this latest experiment. A number within a bracket refers to the number of a given sector type produced within a given lineage or to the number of a particular type produced as an end product of a

4. Mean parental diameter was 2.6cm.

given lineage. This indicates the frequency of different sectoring or mutational events. Except g sectors, the times of the occurrence of the various sector types or classes are recorded in Table 23.

With respect to Table 23, the total number of h, fz, and fl sectors or variants which arose 0-95 hours following inoculation was 235 or 88.1% of all h, fz, and fl variants produced, while during the same period the total number of yellow variants (ye and cr variants) which arose was 19, or 16.3% of all yellow variants (sectors) produced. The total number of h, fz, and fl variants which arose 96-216 hours following inoculation was 32 or 11.9% of all h, fz and fl variants produced, while the total number of yellow variants (ye variants) which arose during this period was 97 or 83.7% of all yellow variants produced. In regards to fl and bw variants, all fl variants but one arose before 96 hours, whereas all bw variants arose 96-216 hours following inoculation, with about three-fourths of these having arisen 144-216 hours following inoculation.

During the 0-120 hour period following inoculation, the total number of ye variants or sectors which arose directly from the RP81 parental region was 9. After this period no ye sectors arose from the RP81 parental region. As noted (Fig. 3) only four ye sectors were produced by fz sectors and two ye sectors were produced by fl sectors, with no yellow sectors being produced by g sectors. This means that the remaining ye sectors (98 in total) were produced by (or emerged from) h sectors. Recall that the total number of yellow sectors (ye and or sectors) produced by all RP81 colonies in this experiment (Table 23) equalled 116. It is to be noted that the production of such yellow sectors involved deletions which included the $\underline{y}^+$ allele on the translocated segment of the I duplication.

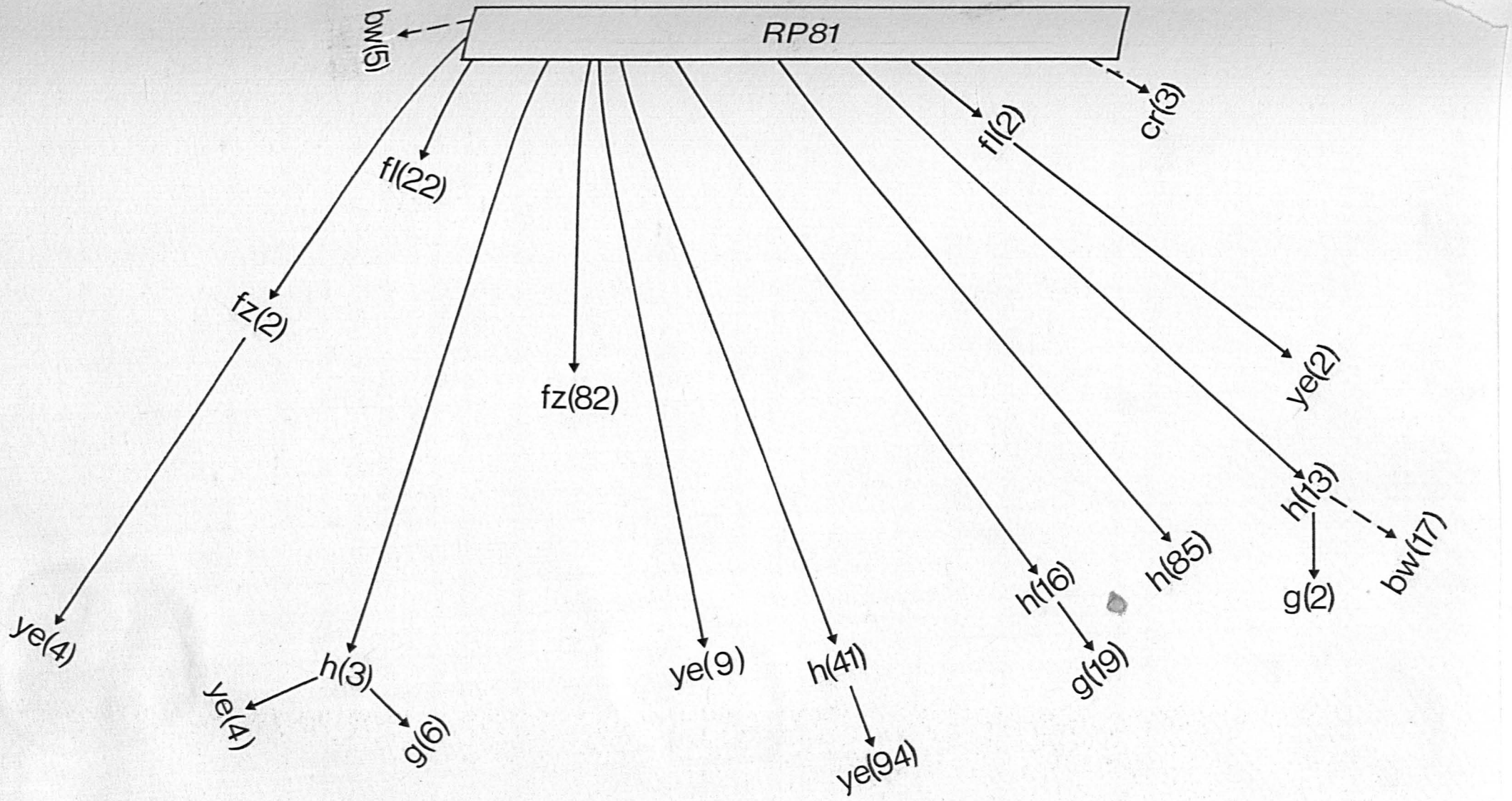


Figure 3. Lineages of mutant sectors from RP81. The symbol ⟶ refers to an improvement or an increase in growth rate in relation to the parental region or in relation to another sector as well as to the lineage of a given sector type. The symbol ⇢ also refers to the lineage of a given sector type but does not indicate whether or not sector is of an increased growth rate.

T A B L E 23

Sectors produced during nine days of incubation by RP81 colonies at 36°C

Period of incubation when sectors arose (hours following inoculation when sectors arose)	No. of h sectors	No. of ye sectors	No. of cr sectors	No. of bw sectors	No. of fl sectors	No. of fz sectors
0- 47 hours	4	0	2	0	6	0
48- 71 hours	65	7	1	0	14	25
72-95 hours	80	9	0	0	3	37
96-120 hours	9	40	0	6	1	22
121-143 hours	0	15	0	0	0	0
144-168 hours	0	35	0	11	0	0
169-216 hours	0	7	0	5	0	0

Total number of RP81 colonies involved in experiment: 40
Total number of yellow sectors or variants (cr and ye variants) produced: 116
Mean number of yellow sectors per colony: 2.9
not including bw variants, RP81 colonies in this experiment produced an average of 10.2 variants per colony
The distribution of yellow variants in this experiment and in all respective experiments but one (Table 25) in Part IV, where yellow variants were produced, was found to be in conformance with a Poisson distribution.

Members of a given sector class which arose during a given period of time were tested for adenine, biotin, and thiosulphate requirements. Only a sample of a given sector class which arose during a given period of time was so tested. The results are recorded in Table 24. Reference to this table will be made shortly.

As the h sectors produced in the experiment (Table 23; Fig. 3) displayed a very improved morphology in place of the Bainbridge crinkled morphology, as they were clearly of a highly improved growth rate, and as they were grass green it is indicated that at least large portions of the III duplication were deleted in their production. Furthermore, as the fz sectors produced in the same experiment were of increased growth rate and lacking the Bainbridge morphology, and as they were very similar to the class one sectors produced by the III duplication strain, Rg, it is indicated that they arose as a result of portions of the III duplication being deleted. Moreover, because the fl sectors, also produced in the experiment, clearly lacked the Bainbridge morphology and were of increased growth rate, it is indicated that they arose as a result of deletions from the III duplication. In this connection it is important to note that the information on P.139 shows that collectively a large majority of these h, fz, and fl sectors (variants) were produced at an early period of time in the growth history of RP81, while in contrast, most yellow sectors (variants) were produced at a comparatively later period of time. This and the manner in which the sectors (variants) were produced would suggest that deletions of, at least, parts of the intact III duplication generally occurred earlier in time than deletions including the y^+ allele on the translocated segment of the I duplication. Such a suggestion is given much more support

by the fact that almost all of the yellow sectors emerged from h, fz, and fl sectors, clearly showing that in sequence nearly all deletions including the $\underline{y}^+$ allele on the translocated segment of the I duplication followed deletions of, at least, portions of the intact III duplication.[5] Furthermore, as an extremely small minority of ye sectors emerged directly from the parental region of RP81, it is evident that deletions including the $\underline{y}^+$ allele on the I duplication were rarely simultaneous to those deletions from the intact III duplication. Moreover, in regards to fl and bw variants, the data suggests that the particular mutations which gave rise to the bw variants generally occurred later in time than those particular mutations which gave rise to fl variants.

Looking at Table 24, we can note that the only two sectors which were biotin-requiring were produced later in time than most of the biotin-independent h, fz, and fl sectors. This would appear to suggest that most of the deletions from the intact III duplication occurred earlier than those deletions including the $\underline{bi1}^+$ allele on the non-translocated segment of the I duplication.

In addition, as part of a very important study to be described in great detail later, 61 green colonies were each derived from each of 61 h sectors which had not given rise to yellow sectors; these h sectors made up a portion of the sectors described on P.139 and noted in Figure 3. Though the 61 green colonies (colonial subcultures) were studied extensively it is enough for now to summarize a small amount of the data regarding these colonies. Each of the 61 colonies had given rise to yellow sectors. This observation points out that the h sectors from which the green

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5.Data on P.128 also provides a clear indication of this.

colonies were derived still retained the y allele as well as the y^+ allele after deletions had occurred from the intact III duplication. Also, the fact that such colonial subcultures of h sectors eventually gave rise to yellow sectors again indicates that deletions including the y^+ allele on the I duplication will eventually follow deletions from the intact III duplication.

Phenotypically these colonies were each ad^+, bi^+, and s^+, but it is important to stress that eleven of these colonies each produced a bi green sector, while analysis of four additional colonies established that each of the four, nevertheless, carried the bi1 allele. It is clear that each of the 15 bi^+ h sectors from which these 15 colonies were respectively derived each retained the bi1 allele. Though not all of the colonies were analyzed in order to determine the presence of the bi1 allele, the fact that the four analyzed each proved to have the bi1 allele (as well as $bi1^+$) coupled with the fact that eleven additional colonies each produced a bi sector would clearly imply that it is very likely that the remaining h sectors from which the colonies were derived, as well as all bi^+ h sectors, also retained the bi1 allele. This would indicate that the bi^+ h sectors, which make up virtually all h sectors tested directly or indirectly for biotin requirement (dependence), still retained the bi1 allele after deletions had occurred from the intact III duplication. In so far as a proportion of the colonies derived from many of these bi^+ h sectors gave rise in turn to biotin-requiring sectors, it is definitely indicated that, in general, deletions including the $bi1^+$ allele on the non-translocated segment of I duplication will eventually follow deletions from the intact III duplication. Furthermore, as it appears that all bi^+ h sectors carry the bi1 allele, it is indicated that any deletions including the $bi1^+$ allele

T A B L E 24

Nutritional tests of a group of sectors produced by RP81 colonies at 36°C

Period of incubation when tested sectors arose	No. of h sectors tested for adenine, biotin, and thiosulphate requirements	h *ad+ bi+ s+	h *ad+ bi s+	No. of ye sectors tested for adenine, biotin, and thiosulphate requirements	ye ad+ bi+ s+
0- 47 hours	4	4	0	0	-
48- 71 hours	23	23	0	5	5
72- 95 hours	58	58	0	3	3
96-120 hours	9	7	2	15	15
121-143 hours	0	-	-	14	14
144-168 hours	0	-	-	29	29
169-216 hours	0	-	-	7	7

*The production of bi sectors involved deletions which included the bi1+ allele on the non-translocated segment of the I duplication.

T A B L E 24(continuation)

Period of incubation when tested sectors arose	No. of fz sectors tested for adenine, biotin, and thiosulphate requirements	fz ad^+ bi^+ s^+	No. of fl sectors tested for adenine, biotin, and thiosulphate requirements	fl ad^+ bi^+ s^+
0- 47 hours	0	-	2	2
48-71 hours	0	-	16	16
72- 95 hours	26	26	2	2
96-120 hours	16	16	0	-
121-143 hours	0	-	0	-
144-168 hours	0	-	0	-
169-216 hours	0	-	0	-

T A B L E 24(continuation)

Period of incubation when tested sectors arose	No. of cr sectors tested for adenine, biotin, and thiosulphate requirements	cr ad^+ bi^+ s^+	No. of g sectors tested for adenine, biotin, and thiosulphate requirements	g ad^+ bi^+ s^+	g ad^+ bi^+ s [/]
0- 47 hours	2	2	0	-	-
48- 71 hours	0	-	0	-	-
72- 95 hours	0	-	0	-	-
96-120 hours	0	-	0	-	-
121-143 hours	0	-	0	-	-
144-168 hours	0	-	26	25	1
169-216 hours	0	-	1	0	1

[/] The production of s sectors involved deletions which included $s12^+$ allele on the non-translocated segment of the III duplication.

on the I duplication will, in general, follow deletions from the intact III duplication.

Regarding the $\underline{y}^+$ allele on the I duplication, information relating to colonies obtained from fz sectors provides further evidence that deletions including the $\underline{y}^+$ allele on the I duplication will follow deletions from the III duplication (Appendix E). Moreover, subsequent data relating to the colonies derived from h sectors suggested that during the origination of h sectors the non-translocated duplicate I segment was rarely subject to deletions, clearly indicating that any deletions from the non-translocated duplicate I segment will, in general, occur subsequently to those deletions from the intact III duplication (see P.173).

Data in Table 24 indicates that deletions of the $\underline{s12}^+$ region in the double-duplication haploid strain, RP81, are rare, suggesting that this part or region of the III duplication in RP81 is rarely subject to deletions. Furthermore, it is not clear from the data when in this double-duplication strain deletions of the $\underline{s12}^+$ region of the III duplication occur in relation to deletions of the $\underline{bi1}^+$ and $\underline{y}^+$ regions of the I duplication.

Regarding the sequence of deletions from the I and III duplications, one ought to recall that the III duplication strain, Rg, had generally given rise to improved sectors 0-95 hours following inoculation while the I duplication strain, P, had generally given rise to yellow and green improved sectors 96-240 hours following inoculation. This means, as pointed out in Part III, that deletions from the III duplication in Rg generally occur earlier than those deletions from the I duplication in P. In view of the information relating to RP81 sectors and the derivatives of such sectors (P.139 to P.148), it

would thus appear that with both duplications present in the double-duplication haploid strain deletions from the intact III duplication generally continue to occur before those deletions which include at least specific portions of the I duplication. This would suggest, therefore, that the I, III double-duplication haploid retains in some manner the respective deletional periods of strains P and Rg. Hence, the synthesis of these two independent deletional periods would help to provide the basis of the overall deletional sequence associated with the double-duplication strain. Such a deletional sequence would itself be a deletional programme synthesized with the creation of RP81 (or the RP81 genotype).

In addition to the findings relating to deletional sequence, work connected with the double-duplication strain furnished further findings which were of definite interest. The sub-culturing described on P.138 not only resulted in the 40 RP81 colonies whose sectoring behaviour has been detailed in regards to deletional sequence, but also resulted in the production of six colonies which did not resemble the 40 RP81 colonies. At the end of the 9 day incubation period, these 6 colonies, referred to as im colonies (improved colonies), did not display the characteristic Bainbridge crinkled morphology of RP81 nor the characteristic olive-green colour. The 6 colonies all had the same highly improved morphology and each colony was of a grass-green colour. The im colonies were definitely smaller than P colonies grown for the same period of time but definitely much larger than the parental regions of the RP81 colonies.

Each of the im colonies produced a very high frequency of yellow sectors. These sectors emerged from the peripheral areas of the colonies and were generally spaced evenly from one another. Most of the yellow sectors produced by each colony were approximately equidistant from a given colony centre, thereby indicating that most yellow sectors arose at the same approximate period in time. The production of so many sectors by individual colonies under the particular experimental conditions, e.g., growth at 36°C was clearly not expected and beyond previous experience. In fact im colonies produced on the average exceedingly more yellow sectors at 36°C than any group of P colonies at 36°C (see P data in Part III and im data in Table 25). Table 25 gives a detailed account of the sectoring behaviour of the im colonies. The table among other things shows that most yellow sectors arose 145-167 hours following inoculation, thus confirming that most of the yellow sectors arose at the same approximate period.

The im colonies were certainly still heterozygous for the linkage group I segment (retained the I duplication) as shown by their production of yellow sectors. Furthermore, because the im colonies were of a highly improved morphology and growth rate, and were of a grass-green colour, it is clear that they did not carry the intact III duplication, but a largely altered III duplication. Regarding this changed III duplication, it could be suggested with some confidence that in view of their reduced growth rate in comparison to P colonies, im colonies still carried at least part of the original III duplication. It would be evident in this light that im colonies derived from germinating-RP81-conidia in which alteration of the original (or intact) III duplication had occurred by means of the major loss of chromosomal material from the III duplication.

T A B L E 25

Im colonies at 36°C for nine days

Colony	Total number of yellow sectors produced* by a given colony	Number of yellow sectors which arose 0-71 hours following inoculation	Number of yellow sectors which arose 72-96 hours following inoculation	Number of yellow sectors which arose 97-119 hours following inoculation
im number 1	10	0	1	0
im number 2	10	0	0	0
im number 3	10	0	1	0
im number 4	12	0	0	1
im number 5	15	0	0	1
im number 6	12	0	1	0

*The production of yellow sectors involved deletions which included the $\underline{y}^+$ allele on the translocated segment of the I duplication.

T A B L E 25 (continuation)

Number of yellow sectors which arose 120-144 hours following inoculation	Number of yellow sectors which arose 145-167 hours following inoculation	Number of yellow sectors which arose 168-216 hours following inoculation
0	9	0
0	9	1
0	9	0
0	11	0
1	13	0
0	11	0

T A B L E 25 (continuation)

Total number of yellow sectors* produced by all im colonies	Mean number of yellow sectors per colony	Total number of green improved sectors produced by all im colonies[/]	Mean number of green improved sectors per colony
69	11.5	10	1.6

*It was not determined whether or not yellow sectors produced by im colonies conformed to a Poisson distribution.

[/]Mean diameter of im colonies was 5.6 cm

Like the im colonies at 36°C, the 40 RP81 colonies at 36°C ultimately produced a frequency of yellow sectors higher than anticipated. In fact, the mean number of yellow sectors ultimately produced by the 40 RP81 colonies at 36°C (Table 23) was significantly higher[6] (at $P < .01$) than the mean number of yellow sectors produced by P colonies at 36°C. It is obvious that the mean number of yellow sectors produced by im colonies at 36°C was far higher (significantly higher) than the mean number of yellow sectors ultimately produced by RP81 colonies.

In this regard, it is clear that colonies (RP81) originally having the unaltered III duplication gave rise at 36°C directly or indirectly (by means of h, fz, and fl sectors) on the average to far fewer yellow sectors than did colonies (im) at 36°C originally carrying some type of changed III duplication. This indicates that the original change or alteration of the III duplication in im colonies gave rise to a situation which affected or greatly enhanced the frequency of those deletions including the $\underline{y}^+$ allele on the translocated segment of the I duplication. Because it is indicated that a change or alteration of the III duplication can greatly change the yellow sectoring frequency and thus the frequency of deletions involving the $\underline{y}^+$ allele on the I duplication, then it would follow that the region of the III duplication has some definite effect on the yellow sectoring frequency, and thereby upon the frequency of deletions which include the $\underline{y}^+$ allele on the I duplication. In this connection, the fact that RP81 colonies at 36°C ultimately give rise on the average to significantly more yellow sectors than P colonies at 36°C would indicate that the

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6. It is also to be noted that the 28 RP81 colonies described on P.127 ultimately produced an average of 2.6 yellow sectors per colony at 36°C. This average was significantly higher (at $P < .01$) than the average number of yellow sectors produced by the P strain grown at the same temperature. Not including bw variants the 28 RP81 colonies produced an average of 10.6 variants per colony.

originally unaltered III duplication determines the course which ultimately leads to this significant increase in the frequency of deletions involving the $\underline{y}^+$ allele on the I duplication. Thus, in this light, it would seem that the period at which deletions include the $\underline{y}^+$ allele on the translocated duplicate I segment may also to some extent be affected by processes initially determined by the originally unaltered III duplication of RP81.

As it appears that the changed III duplication is a III duplication of reduced size, it is clear in view of what has been said that as a result of im colonies carrying the smaller or reduced III duplication the $\underline{y}^+$ region on the translocated segment of the I duplication in im colonies is subject to exceedingly more deletions than is the $\underline{y}^+$ region on the translocated duplicate I segment in P colonies, which lack any type of III duplication. Since most of the yellow sectors produced by each of the im colonies arose at a specific period in time, one cannot but suppose that the changed III duplication had produced a situation which defined the specific period in which most of the sectors arose. From this it follows that such a changed III duplication has produced a situation which defined or determined the specific period in which the $\underline{y}^+$ region on the translocated duplicate I segment was subject to deletions. This is further supported by the fact that most of the yellow sectors produced by the I duplication strain (P) did not arise at 36°C 145-167 hours following inoculation but over a less defined or much broader period of time; in general, yellow sectors produced by P colonies at 36°C are not equidistant from any colony centre, further showing that in the I duplication strain deletions including the

y^+ allele are not circumscribed to a specific period of time. Additional consideration of im colonies will be made in conjunction with forthcoming data.

At this point studies were undertaken which dealt with sector derivatives (or colonial subcultures) of class h sectors.[7] As noted earlier, a portion of the h sectors described on P.139 did not give rise to yellow sectors. From each of 61 h sectors included in that portion, inoculations were made on the centres of 61 dishes of CM, with the result that each of 61 CM plates had one inoculum at its centre. The plates were then left at 36°C for a period of 9 days.

Inocula from the h sectors gave rise to 61 grass-green colonies collectively referred to as h colonies, and all smaller than P colonies grown for the same period, though the morphology of h colonies was very improved. A large majority of these h colonies had each given rise to a high number of yellow sectors, one colony producing as many as 33 yellow sectors. In fact, h colonies produced on the average exceedingly more yellow sectors at 36°C than any group of P colonies at 36°C (see data relating to h colonies in Table 26 and P data in Part III). Green sectors, further improved in terms of growth rate, were also produced by the h colonies.

The fact that each of the h colonies produced yellow sectors indicates that h colonies are still heterozygous for the linkage group I segment, and therefore each carries the I duplication. As a number of h colonies were found to be heterozygous at the bi1 locus (Tables 27, 30, 31, 32, and 34), it is confirmed that h colonies carry the I duplication.

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7. Derivatives were also obtained from class ye sectors and class fz sectors (Appendix D and Appendix E).

T A B L E 26

h colonies incubated for nine days at 36°C

h colony	Number of yellow sectors produced* per colony	Diameter of parental region
h32	17	6.0 cm
h33	2	6.6 cm
h34	3	5.1 cm
h35	6	6.5 cm
h36	10	5.8 cm
h37	11	6.5 cm
h38	3	6.5 cm
h39	13	5.1 cm
h40	22	6.1 cm
h41	8	6.0 cm
h42	20	5.7 cm
h43	12	6.4 cm
h44	8	5.4 cm
h45	14	5.5 cm
h46	24	5.9 cm
h47	8	6.4 cm
h48	10	5.0 cm
h49	14	5.5 cm
h50	9	5.9 cm
h51	20	6.0 cm
h52	20	5.0 cm
h53	21	5.0 cm
h54	13	5.5 cm

*The production of yellow sectors involved deletions which included the $\underline{y}^+$ allele on the translocated segment of the I duplication.

T A B L E 26 (continuation)

h colony	Number of yellow sectors produced per colony	Diameter of parental region
h55	8	5.6 cm
h56	14	5.8 cm
h57	9	5.7 cm
h58	15	5.5 cm
h59	8	5.7 cm
h60	5	5.5 cm
h61	13	5.6 cm
h62	26	5.9 cm
h63	14	6.0 cm
h64	20	5.9 cm
h65	13	5.2 cm
h66	8	6.0 cm
h67	9	5.0 cm
h68	10	6.0 cm
h69	18	6.0 cm
h70	11	5.9 cm
h71	11	5.8 cm
h72	9	5.9 cm
h73	20	5.9 cm
h74	13	5.0 cm
h75	6	5.5 cm
h76	4	5.9 cm
h77	12	5.9 cm
h78	26	6.0 cm
h79	10	5.8 cm
h80	7	5.9 cm
h81	33	5.8 cm

T A B L E 26 (continuation)

h colony	Number of yellow sectors produced per colony	Diameter of parental region
h82	14	6.0 cm
h83	19	5.7 cm
h84	9	6.0 cm
h85	7	5.0 cm
h86	7	5.7 cm
h87	13	6.0 cm
h88	31	5.8 cm
h89	21	5.9 cm
h90	12	6.0 cm
h91	32	5.8 cm
h92	19	5.9 cm

Mean number of yellow sectors per colony = 13.5

Mean diameter = 5.7cm

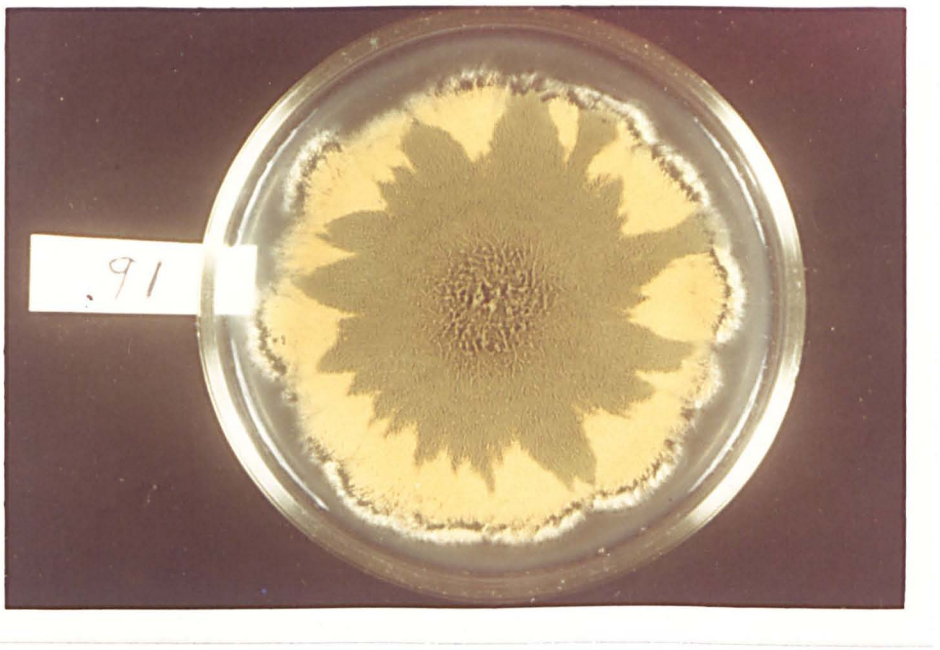
The total number of yellow sectors produced by all h colonies was 824, while the total number of green improved sectors produced by all h colonies was 108; the mean number of green improved sectors per colony was 1.7.

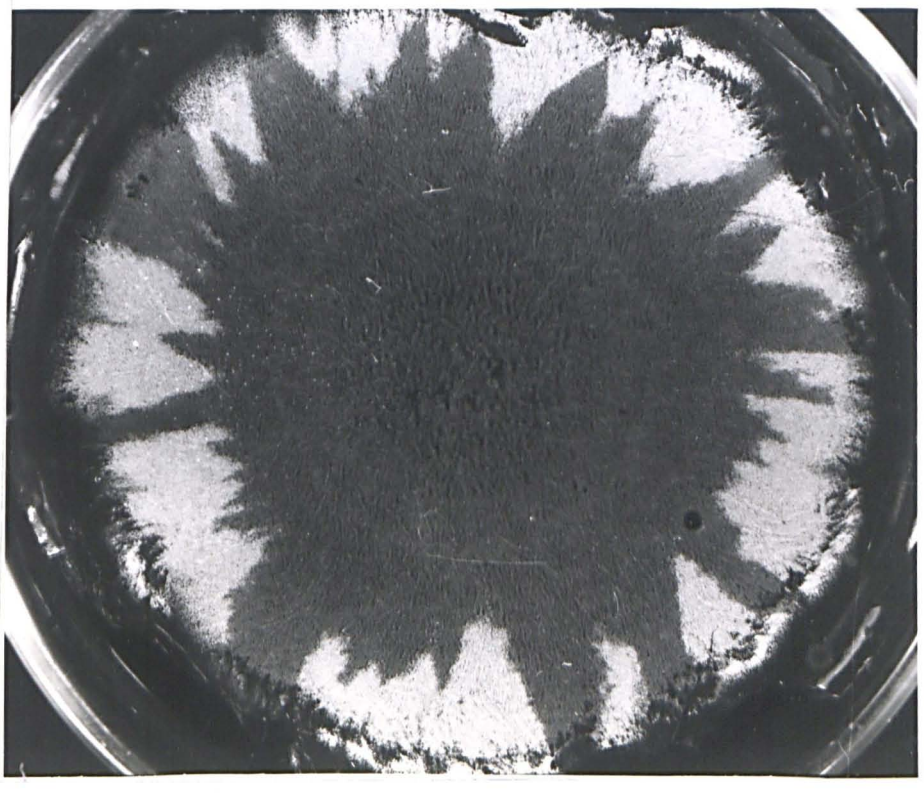
As can be seen in plates 5a and 5b yellow sectors commenced at points approximately equidistant from the respective centres of two h colonies. This was also the case for the remaining h colonies which had produced respectively a large number of yellow sectors, e.g., approximately 6 or more yellow sectors per colony.

All of the h colonies were phenotypically $\underline{ad}^+$, $\underline{bi}^+$, and $\underline{s}^+$. A sample of the sectors produced by the h colonies were tested for adenine, biotin, and thiosulphate requirements. The results of these tests are given in Table 27 and are referred to in connection with other relevant information.

One can see that the general instability behaviour of the h colonies conforms to that of im colonies. As im colonies, h colonies have the changed III duplication, the change having been effected by means of the major loss of chromosomal material from the originally unchanged III duplication, and like the im colonies, h colonies produced on the average a far higher number of yellow sectors than RP81 colonies which carry the intact or unchanged III duplication.

Thus, it is quite definite that a changed or altered III duplication in h colonies is implicated in (or promotes) the general production of a very high frequency of yellow sectors. Being so, it is therefore demonstrated again that a changed III duplication promotes (or is implicated in) an overall, major increase in the frequency (or amount) of those deletions which include the $\underline{y}^+$ allele on the translocated segment of the I duplication. In this connection, it is important to realize the fact that some h colonies produced in comparison to other h colonies less yellow sectors (Table 26) may indicate that the





Plates 5a and 5b. h colonies with yellow sectors at 36°C.
Period of incubation was nine days.

T A B L E 27

Nutritional tests of a group of sectors produced by h colonies at 36°C

Colony	Number of green improved sectors tested for adenine, biotin, and thiosulphate requirements	y^+ ad^+ bi^+ s^+	y^+ ad^+ bi s^+ *	Number of yellow sectors tested for adenine, biotin and thiosulphate requirements	y ad^+ bi^+ s^+
h32	2	1	1	4	4
h33	2	2	0	5	5
h34	4	4	0	2	2
h35	2	1	1	2	2
h36	1	1	0	1	1
h37	2	2	0	0	-
h38	1	1	0	1	1
h39	2	2	0	0	-
h40	3	3	0	3	3
h41	5	5	0	2	2
h42	4	4	0	2	2

*The production of bi sectors involved deletions which included the $bi1^+$ allele on the non-translocated segment of the I duplication.

T A B L E 27 (continuation)

Colony	Number of green improved sectors tested for adenine, biotin, and thiosulphate requirements	y^+ ad^+ bi^+ s^+	y^+ ad^+ bi s^+	Number of yellow sectors tested for adenine, biotin and thiosulphate requirements	y ad^+ bi^+ s^+
h43	3	3	0	1	1
h44	4	4	0	3	3
h45	2	2	0	1	1
h46	2	2	0	3	3
h47	3	3	0	0	-
h48	1	0	1	2	2
h49	2	2	0	1	1
h50	1	1	0	1	1
h51	1	1	0	1	1
h52	1	0	1	1	1
h53	1	1	0	1	1
h54	1	0	1	1	1
h55	1	1	0	1	1

T A B L E 27 (continuation)

Colony	Number of green improved sectors tested for adenine, biotin, and thiosulphate requirements	$y^+ ad^+ bi^+ s^+$	$y^+ ad^+ bi\ s^+$	Number of yellow sectors tested for adenine, biotin and thiosulphate requirements	$y\ ad^+ bi^+ s^+$
h56	1	1	0	1	1
h57	1	1	0	1	1
h58	0	-	-	2	2
h59	1	1	0	1	1
h60	2	2	0	0	-
h61	0	-	-	1	1
h62	2	2	0	2	2
h63	3	3	0	1	1
h64	3	2	1	2	2
h65	1	1	0	0	-
h66	2	1	1	1	1
h67	2	2	0	1	1
h68	1	1	0	1	1
h69	0	-	-	2	2
h70	3	3	0	0	-

T A B L E 27 (continuation)

Colony	Number of green improved sectors tested for adenine, biotin, and thiosulphate requirements	y^+ ad^+ bi^+ s^+	y^+ ad^+ bi s^+	Number of yellow sectors tested for adenine, biotin and thiosulphate requirements	y ad^+ bi^+ s^+
h71	2	2	0	1	1
h72	2	2	0	1	1
h73	2	2	0	1	1
h74	1	1	0	1	1
h75	2	2	0	1	1
h76	2	2	0	1	1
h77	2	2	0	1	1
h78	1	1	0	1	1
h79	1	1	0	1	1
h80	2	2	0	1	1
h81	2	1	1	1	1
h82	1	0	1	1	1
h83	1	0	1	1	1

T A B L E 27 (continuation)

Colony	Number of green improved sectors tested for adenine, biotin, and thiosulphate requirements	y^+ ad^+ bi^+ s^+	y^+ ad^+ bi s^+	Number of yellow sectors tested for adenine, biotin and thiosulphate requirements	y ad^+ bi^+ s^+
h84	1	1	0	1	1
h85	3	3	0	1	1
h86	1	1	0	1	1
h87	2	2	0	4	4
h88	0	-	-	2	2
h89	2	2	0	2	2
h90	2	1	1	4	4
h91	0	-	-	1	1
h92	2	2	0	4	4

specific degree of enhanced the $\underline{y}^+$ region instability as manifested by a colony is conditional upon the type of changed III duplication in a colony.

Regarding the yellow sectors produced by each h colony, one must also consider the fact that yellow sectors were equidistant from the centre of any given h colony which had produced a large number of such sectors. This situation clearly indicates that in the growth history of any one of a large majority of h colonies all of the large number of yellow sectors arose at the same specific period of time, thereby strongly suggesting that the changed III duplication caused the $\underline{y}^+$ region of the I duplication to be generally subject to a very high frequency of deletions according to some type of temporal programme.

Furthermore, in regards to the changed III duplication in h and im colonies, data in Tables 25 and 26 indicates that the major mutagenic influence (effect) of the changed III duplication upon the I duplication is confined to the translocated segment of the I duplication. That is, the major increase in the deletional instability of the I duplication as promoted by the changed III duplication is confined to the translocated duplicate I segment.

Because the mere presence of an unchanged or intact III duplication ultimately stimulates a significant increase in the amount or frequency of deletions including the $\underline{y}^+$ allele on the translocated duplicate I segment, it is most likely that the change or alteration of the III duplication resulted in a large part of the III duplication being deleted away, leaving a comparatively smaller III duplication in h colonies to provoke generally a very great increase in the frequency of deletions involving the $\underline{y}^+$ allele on the I duplication. The fact that some h sectors, phenotypically thiosulphate-independent, produced

thiosulphate-requiring sectors (Table 24) would tend to indicate the presence of a reduced III duplication in h colonies. Also, the data (P.131) referring to the partial (reduced) III duplication in y1 gives additional evidence on which this view is based. Furthermore, the distinctly smaller mean diameter size of h colonies when compared to that of P colonies grown for the same period suggests the existance of an extra duplication in the h colonies. Moreover, as will be seen shortly, additional evidence confirms or corroborates the existance of a reduced III duplication in h colonies.

In regards to the originally unchanged III duplication, nearly all or an extremely large majority of the yellow sectors produced by RP81 were in effect produced by the mutant class h sectors of RP81; the mutations giving rise to the h sectors must have occurred long after the development of hyphae from the original RP81 conidia of the inocula. This would clearly imply that the changed III duplication must at least reside in early growing hyphae in order for such early-hyphae-growth to be able to give rise to colonies, e.g., h and im colonies producing (on the average) a very high frequency of yellow sectors during 9 days of incubation. When the unchanged III duplication is originally in early growing hyphae, it has been shown that colonies, e.g., RP81 colonies will result which produce (on the average) directly or indirectly a comparatively far lower frequency of yellow sectors during the same period of incubation, even though during 9 days of incubation the intact III duplication itself ultimately promotes a situation which enhances the frequency of yellow sectors.

Thus, it becomes apparent that the $\underline{y}^+$ region of the I duplication is ultimately subject to far more deletions during

9 days of incubation in colonies arising from early-hyphal-growth where the changed III duplication is present than in the sectors produced by colonies arising from early-hyphal-growth in which the unchanged or intact III duplication is present, even though the intact III duplication itself ultimately (indirectly) induces during the same period a significant enhancement in the frequency of deletions including the y^+ allele on the I duplication. Insofar as the changed III duplication is in fact a III duplication of reduced size, then it would appear that the particular increase in the frequency of deletions including the y^+ allele on the I duplication within 9 days of incubation ultimately depends on the size of III duplication present during the early stages of hyphal growth. This means that an intact or undiminished III duplication present from very early development ultimately (indirectly) determines a significant increase (enhancement) in the frequency of those deletions involving the y^+ allele on the I duplication during 9 days of incubation, while in contrast, a III duplication of reduced size, and present from very early development, ultimately promotes (stimulates) during the same period of incubation a far greater enhancement in the frequency of those deletions involving the y^+ allele on the I duplication.

It is realized, however, that the situation just described may be related to factors or conditions as yet not apparent. As will be seen, subsequent data does suggest some such factors or conditions and tends to make apparent what they are.

In order to confirm or corroborate the existence of a partial III duplication (reduced III duplication) in h colonies, a group of experiments was undertaken. It was also decided to determine whether or not the change of the original III duplication was due

to the loss (deletion) of chromosomal material from the translocated duplicate III segment; perhaps the change of the original III duplication was due to loss of chromosomal material from the non-translocated duplicate III segment as opposed to the translocated duplicate III segment. Studies were principally undertaken to throw light on this matter, and these are first described.

h88, h81, h32, h51, and h91 were each combined in a diploid with y ; s12 ; pyro4 ; nic2 (strain B). The respective diploids were in turn haploidized, and the haploid segregants[8] were tested for thiosulphate, nicotinic acid, and p-aminobenzoic acid requirements. The results of these tests are given in Tables 28a, 28b, 28c, 28d, and 38e. The presence of thiosulphate-independent segregants among the haploid segregants from each diploid clearly implies or indicates the following: of the two segments making up the III duplication, major deletions occurred only from the translocated duplicate III segment in the production of the h sectors from which the five h colonies were derived and in turn used in the synthesis of the five diploids. Had these deletions occurred only from the non-translocated duplicate III segment each haploid segregant having $s12^+$ would also have a lethal (deletion) in linkage with $s12^+$, and thus, the only haploid segregants present would be thiosulphate-requiring. Hence, it is clear that the changed III duplication residing in each of these five h colonies arose as a result of the loss of chromosomal material from the intact translocated duplicate III segment.

In view of the fact that all of the h colonies tested were shown to come from h sectors each having a changed III

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8. As expected, only yellow haploid segregants were produced.

T A B L E 28a

Segregations from the diploid h88 // B

(Only yellow haploid segregants produced)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
	4	7	6	5	5	6

T A B L E 28b

Segregations from the diploid h32 // B

(Only yellow haploid segregants produced)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
	8	9	5	12	16	1

T A B L E 28c

Segregations from the diploid h51 // B

(Only yellow haploid segregants produced)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
	5	11	6	10	8	8

T A B L E 28d

Segregations from the diploid h81 // B

(Only yellow haploid segregants produced)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
	7	12	9	10	15	4

T A B L E 28e

Segregations from the diploid h91 // B

(Only yellow haploid segregants produced)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
	5	9	4	10	3	11

duplication which arose as a result of the major loss of chromosomal material from the translocated segment of the III duplication, it is most likely that nearly all (if not all) of the remaining h sectors each carried a changed III duplication which arose in the same manner. Thus, it can be inferred with some confidence that the changed III duplication carried by each of the remaining h colonies arose (in virtually all or all cases) as a result of the major loss of chromosomal material from the intact translocated segment of the III duplication. In light of what has been said about h sectors, it is quite likely that loss of chromosomal material from the intact III duplication in RP81 occurs in most (if not all) cases only from the intact translocated duplicate III segment.

From the present studies we again see (Tables 28b, 28d) that haploidization of some diploids obtained by means of a synthesis between a duplication strain and a normal strain resulted in distributions of the nic2 allele which do not conform to general experience; such distributions strongly suggest a semi-lethal (or deleterious factor) in linkage with $nic2^{+}$. Furthermore, in regards to the distribution of the paba6 allele among the haploid segregants, data in Tables 28a, 28b, 28c, 28d, and 28e clearly indicates that in respectively h88, h32, h81, h91, and h51 the non-translocated segment of the I duplication does not carry lethals, and thereby demonstrates that these h colonies each carry a non-translocated duplicate I segment which has never been subject to deletions. In view of the preceding it is very likely that most, if not all of the bi^{+} h sectors, which make up nearly all tested h sectors, each carry a non-translocated duplicate I segment which has not been subject to deletions. From this it would follow that virtually all h colonies carry an intact or

unaltered non-translocated duplicate I segment.

Having shown that the changed III duplication present in respective colonies most likely arose as a result of the loss of chromosomal material from the translocated duplicate III segment, one turns to the group of experiments principally undertaken in order to confirm the existence of a partial or reduced III duplication in h colonies, that is to confirm that the changed III duplication in h colonies is in fact a partial or reduced duplication. These experiments consisted of studies dealing with a series of crosses, and it is these studies which are now described or considered.

The crosses in question were those made between h81 and ad8 paba1, h89 and ad8 paba1, h51 and ad8 paba1, h91 and ad8 paba1, h32 and ad8 paba1, and h88 and ad8 paba1. The results of each cross are given in respectively Tables 29, 30, 31, 32, 33, and 34.

As the data regarding some of these crosses shows that all grey-green segregants (progeny-colonies) tested for adenine requirement were phenotypically adenine-requiring (ad), while all grass-green segregants (progeny-colonies) tested for adenine requirement were phenotypically adenine-independent (ad^+), it is clearly indicated again that a requirement for adenine is connected with a grey-green colour. Because of the extremely small distance between ad8(20) and y^+, because grass-green and grey-green progeny colonies were obtained from each of these 6 crosses, and because all grass-green progeny or segregants derived from each of these 6 crosses must be adenine-independent (as opposed to the necessarily adenine-requiring grey-green progeny), it is inferred that every grass-green segregant or colony obtained from each of these crosses carries the $ad8^+(20)$ and ad8(20) alleles, and thereby each carries at least the I duplication.

As shown in the tables in regards to the results of the tests for thiosulphate requirement, no thiosulphate-requiring (s) recombinants were present among the tested normal progeny from each of these crosses. Had loss of chromosomal material not occurred from the original translocated segment of the III duplication in the production of the changed III duplication carried respectively by h81, h89, h51, h91, h32, and h88, one would have expected approximately 17 ± 4% of the tested normal progeny from each of these crosses to be s recombinants. Because no s recombinants were present among the tested normal progeny from each of these crosses, it is indicated that either s recombinants from each cross respectively carried a lethal (deletion) very closely linked to s12 or that no s recombinants were produced in (through) each cross as a result of the s12 region having been deleted along with other regions of the original translocated III segment in the production of the respective h sectors from which h81, h89, h51, h91, h32 and h88 were obtained. This provides, therefore, additional evidence in the case of h81, h51, h91, h32, and h88 that the changed III duplication in respectively h81, h51, h91, h32, and h88 arose as a result of the loss of chromosomal material from the original translocated duplicate III segment, and in the case of h89, provides the first evidence that the changed III duplication in h89 arose as a result of the loss of chromosomal material from the original translocated duplicate III segment. Moreover, this information relating to the lack of s recombinants among the tested progeny from these crosses may further indicate that h81, h89, h51, h91, h32, and h88 each do not carry the s12 allele.

The fact that biotin-requiring (bi) recombinants were detected among the progeny from each of four of these crosses

T A B L E 29

The cross h81* x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
125 green segregants and 32 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P >.05)	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 155 of these colonies	2 colonies having a Bainbridge crinkled morphology - 1.2% of all progeny obtained displayed the Bainbridge crinkled morphology

(Consisted of grey-green progeny (identical in colour shade to that of the ad8 paba1 parent) and grass-green progeny (identical in colour shade to that of the h81 parent)).

*h81 is heterozygous at the bi1 locus (Table 27)

T A B L E 29 (continuation)

Nutritional tests of progeny obtained

Number of grey-green colonies tested for adenine requirement	Number of grey-green colonies found to be adenine-requiring:	Number of grass-green colonies tested for adenine requirement:	Number of grass-green colonies found to be adenine-independent	Number of yellow and green (grey-green) normal colonies tested for thiosulphate requirement:	Number of yellow and green normal colonies found to be thiosulphate-requiring
30	30	30	30	70	0

T A B L E 30

The cross h89 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
134 green segregants and 46 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at $P > .05$)	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 177 of these colonies	3 colonies having a Bainbridge crinkled morphology - 1.6% of all progeny obtained displayed the Bainbridge crinkled morphology

[Consisted of grey-green progeny (identical in colour shade to that of the ad8 paba1 parent) and grass-green progeny (identical in colour shade to that of the h89 parent)].

T A B L E 30 (continuation)

Nutritional tests of progeny obtained

Number of grey-green colonies tested for adenine requirement	Number of grey-green colonies found to be adenine-requiring	Number of grass-green colonies tested for adenine requirement	Number of grass-green colonies found to be adenine-independent	Number of yellow and green normal colonies tested for biotin requirement	Number of yellow and green normal colonies found to be biotin-requiring	Number of yellow and green normal colonies tested for thiosulphate requirement	Number of yellow and green normal colonies found to be thiosulphate-requiring
40	40	40	40	67	3	67	0

T A B L E 31

The cross h51 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
278 green segregants and 92 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P >.05)	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 344 of these colonies	26 colonies having a Bainbridge crinkled morphology* - 7.0% of all progeny obtained displayed the Bainbridge crinkled morphology

/Generally consisted of grass-green and grey-green colonies/.

*Of the Bainbridge crinkled colonies, some displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny; these colonies of the pronounced Bainbridge crinkled morphology were similar in appearance to colony No. 81, the original colony carrying the I and III duplications.

T A B L E 31 (continuation)

Nutritional tests of progeny obtained

Number of yellow and green normal colonies tested for biotin requirement	Number of yellow and green normal colonies found to be biotin-requiring:	Number of yellow and green normal colonies tested for thiosulphate requirement:	Number of yellow and green normal colonies found to be thiosulphate-requiring:
50	2	55	0

T A B L E 32

The cross h91 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour		Morphological types or classes obtained	
481 green segregants (Consisted of grey-green and grass-green progeny)	183 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P>.05)	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 654 of these colonies	10 colonies having a Bainbridge crinkled morphology* - 1.5% of all progeny obtained displayed the Bainbridge crinkled morphology

*A few of these were of the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 32 (continuation)

Nutritional tests of progeny obtained

Number of yellow and green normal colonies tested for biotin requirement	Number of yellow and green normal colonies found to be biotin-requiring:	Number of yellow and green normal colonies tested for thiosulphate requirement:	Number of yellow and green normal colonies found to be thiosulphate-requiring:
60	3	60	0

TABLE 33

The cross h32* x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour		Morphological types or classes obtained	
287 green segregants and 104 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P >.05)	Generally consisted of grass-green and grey-green progeny	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 383 of these colonies	8 colonies having a Bainbridge crinkled morphology/ - 2.0% of all progeny obtained displayed the Bainbridge crinkled morphology

*h32 is heterozygous at the bi1 locus (Table 27)

/A few of these were of the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 33 (continuation)

Nutritional test of progeny obtained

Number of yellow and green normal colonies tested for thiosulphate requirement:	Number of yellow and green normal colonies found to be thiosulphate-requiring:
60	0

T A B L E 34

The cross h88 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
311 green segregants and 118 yellow segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P > .05)	Normal progeny and mound-shaped progeny with a crinkled morphology resembling that of the P strain: Inclusively, 356 of these colonies	73 colonies having a Bainbridge crinkled morphology* - 17.0% of all progeny obtained displayed the Bainbridge crinkled morphology

/Consisted mostly of grey-green and grass-green progeny/

*Some of these were of the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 34 (continuation)

Nutritional tests of progeny obtained

Number of grey-green colonies tested for adenine requirement	Number of grey-green colonies found to be adenine-requiring:	Number of grass-green colonies tested for adenine requirement	Number of grass-green colonies found to be adenine-independent	Number of yellow and green normal colonies tested for biotin requirement	Number of yellow and green normal colonies found to be biotin-requiring:	Number of yellow and green normal colonies tested for thiosulphate requirement	Number of yellow and green normal colonies found to be thiosulphate-requiring
50	50	50	50	80	4	80	0

(Tables 29, 30, 31 and 32) indicates that h81, h89, h51, and h91 are respectively heterozygous at the bi1 locus. Earlier data (Table 27) indicated that h32 and h88 were respectively heterozygous at the bi1 locus, so the progeny from the two crosses respectively involving these two strains were not tested for biotin requirement.

As shown in Tables 29, 30, 31, 32, and 33, an exceedingly small percentage of the progeny from each of five of these crosses displayed the Bainbridge crinkled morphology[9], e.g., not one percentage being greater than 7%. The (this) exceedingly low frequency of Bainbridge crinkled colonies (colonies of the Bainbridge crinkled morphology) among the progeny from each of these five crosses is in conformance with the loss of chromosomal material having only occurred from the original translocated duplicate III segment in the production of the changed III duplication carried respectively by h81, h89, h51, h91 and h32. A frequency of approximately 33% Bainbridge crinkled colonies among the progeny from each of these five crosses would have been in conformance with the loss of chromosomal material not having occurred from the original translocated duplicate III segment in the production of the changed III duplication carried respectively by h81, h89, h51, h91, and h32. In this connection, the regeneration of an exceedingly small percentage of Bainbridge crinkled colonies through each of these five crosses indicates that a very small proximal section of chromosome III is still attached to chromosome VIII in respectively h81, h89, h51, h91, and h32, after a major loss of chromosomal material had occurred from only the original

9.Plate 6 shows some progeny-colonies from the h88 x ad8 paba1 cross which display the Bainbridge crinkled morphology.

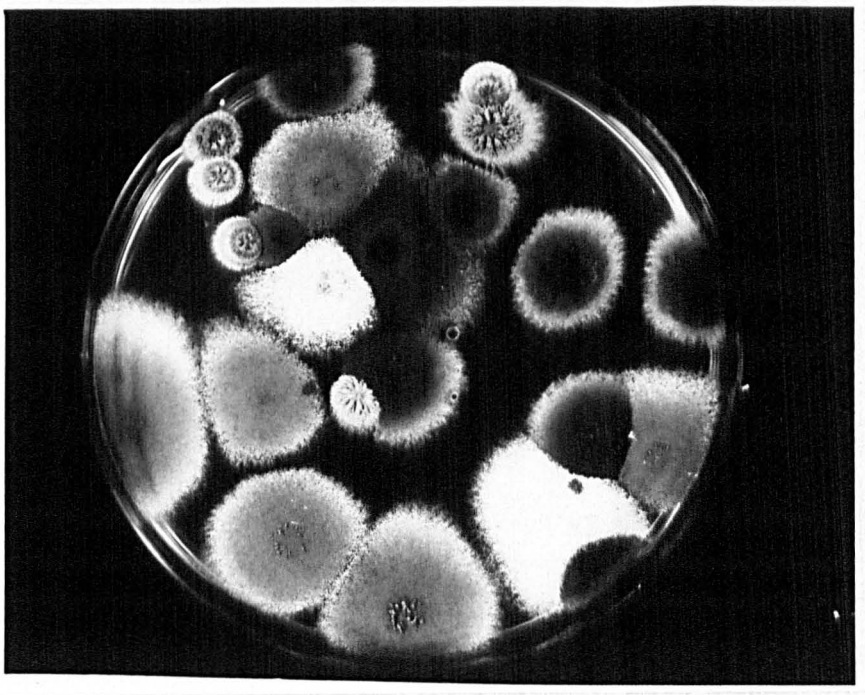

Plate 6. A sample of progeny-colonies from the cross h88 x ad8 paba1

translocated duplicate III segment in the production of the changed III duplication carried respectively by these h cultures, thus, making it clear that h81, h89, h51, h91, and h32 each still carry at least a very small proximal portion of the original III duplication.

Though the frequency of Bainbridge crinkled colonies derived from the h88 x ad8 paba1 cross (Table 34) is much higher than the frequency of such derived, for example, from the h81 x ad8 paba1 cross, the former frequency (of 17%) would still be in conformance with loss of chromosomal material having only occurred from the original translocated duplicate III segment in the production of the changed III duplication carried by h88. The comparatively larger percentage of regenerated Bainbridge crinkled colonies as derived from this cross implies that h88 still carries a section of chromosome III attached to chromosome VIII after loss of chromosomal material from the originally intact translocated duplicate III segment (in RP81), but a section larger than that, for example, present in h81. Thus h88 still retains (at least) a proximal portion of the originally intact III duplication. In this regard, the data pertaining to these crosses would appear to indicate that the proximal portion of the III duplication carried by h51 is larger than that carried by h81 but smaller than that carried by h88.

From a more inclusive point of view, the results from these h x ad8 paba1 crosses enables one to conclude that the change in the III duplication which resulted in the production of the respective h sectors from which h colonies were derived involved a definite reduction in the size of the III duplication. Thus it can be concluded that all h colonies carry a portion of the originally intact III duplication, or in other words, carry a

III duplication of reduced size. Furthermore, the data suggest the possibility that the reduced III duplication carried by h colonies in most (if not all) cases does not have the s12 region, and this may account for the reason why such h colonies did not produce thiosulphate-requiring sectors.

In addition to the crosses just considered, more crosses involving h strains (h colonies derived from the class h variants) were made in order to obtain supplementary information regarding the genetic make up of some of the h strains. The additional crosses in question were those made between h32 and J,[10] h89 and J, h51 and J, h91 and J, and h81 and J. The results of each cross are given in respectively Tables 35, 36, 37, 38 and 39.

The lack of (or apparent lack of) adenine-requiring (ad) recombinants among the progeny from each of these crosses suggests a deletion in respectively h32, h89, h51, h91, and h81 very close to or including the ad20 allele. As shown in the Tables, roughly one-third of the progeny-colonies obtained from each of these particular crosses displayed the Bainbridge crinkled morphology; this was in accord with the loss of chromosomal material having only occurred from the original translocated duplicate III segment in the production of the reduced III duplication carried respectively by h32, h89, h51, h91, and h81. Had loss of chromosomal material occurred only from the original non-translocated duplicate III segment in the production of the reduced III duplication carried respectively by h32, h89, h51, h91, and h81, only an exceedingly small percentage of Bainbridge crinkled colonies would have been present among the progeny from each of these particular crosses.

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10. As one will recall, J is the white, bi1, pyro12 strain carrying the III-VIII non-reciprocal translocation, and the ultimate precusor of RP81.

T A B L E 35

The cross h32 x J

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
171 green segregants, 67 yellow segregants, and 216 white segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P>.05, while the ratio of non-white colonies to white colonies fits a 1:1 ratio at P>.05 as expected)	Normal progeny and mound-shaped progeny with a crinkled morphology similar to that of the P strain: Inclusively, 315 of these colonies	139 colonies having a Bainbridge crinkled morphology* - 30.6% of all progeny obtained displayed the Bainbridge crinkled morphology
	The ratio of non-Bainbridge crinkled colonies to Bainbridge crinkled colonies fits a 2:1 ratio at P>.05	

1. All green normals and all green mound-shaped colonies with a crinkled morphology similar to that of the P strain were of a grass-green colour shade, thereby implying no adenine-requiring colonies to be present among them.

2. Green Bainbridge crinkled colonies were olive-green in colour

* Of the Bainbridge crinkled colonies, a fraction displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 35 (continuation)

Nutritional test of progeny obtained

Number of green and white normal colonies tested for adenine requirement:	Number of green and white normal colonies found to be adenine-independent
100	100

T A B L E 36

The cross h89 x J

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
197 green segregants, 69 yellow segregants, and 251 white segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P>.05, while the ratio of non-white colonies to white colonies fits a 1:1 ratio at P>.05 as expected)	Normal progeny and mound-shaped progeny with a crinkled morphology similar to that of the P strain: Inclusively, 352 of these colonies	165 colonies having a Bainbridge crinkled morphology* - 31.9% of all progeny obtained displayed the Bainbridge crinkled morphology
	The ratio of non-Bainbridge crinkled colonies to Bainbridge crinkled colonies fits a 2:1 ratio at P>.05	

1. All green normals and all green mound-shaped colonies with a crinkled morphology similar to that of the P strain were of a grass-green colour shade, thereby implying no adenine-requiring colonies to be present among them.

2. Green Bainbridge crinkled colonies were olive-green in colour.

*Of the Bainbridge crinkled colonies, a fraction displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 36 (continuation)

Nutritional test of progeny obtained

Number of green normal colonies tested for adenine requirement:	Number of green normal colonies found to be adenine-independent:	Number of white normal colonies tested for adenine requirement:	Number of white normal colonies found to be adenine-independent:
40	40	56	56

T A B L E 37

The cross h51 x J

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
199 green segregants, 56 yellow segregants, and 242 white segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at $P > .05$, while the ratio of non-white colonies to white colonies fits a 1:1 ratio at $P > .05$ as expected)	Normal progeny and mound-shaped progeny with a crinkled morphology similar to that of the P strain: Inclusively, 347 of these colonies	150 colonies having a Bainbridge crinkled morphology* - 30.1% of all progeny obtained displayed the Bainbridge crinkled morphology
	The ratio of non-Bainbridge crinkled colonies to Bainbridge crinkled colonies fits a 2:1 ratio at $P > .05$	

1. All green normals and all green mound-shaped colonies with a crinkled morphology similar to that of the P strain were of a grass-green colour shade, thereby implying no adenine-requiring colonies to be present among them.

2. Green Bainbridge crinkled colonies were olive-green in colour.

* Of the Bainbridge crinkled colonies, a fraction displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 37 (continuation)

Nutritional test of progeny obtained

Number of green normal colonies tested for adenine requirement:	Number of green normal colonies found to be adenine-independent:	Number of white normal colonies tested for adenine requirement:	Number of white normal colonies found to be adenine-independent:
50	50	60	60

T A B L E 38

The cross h91 x J

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
186 green segregants, 77 yellow segregants and 285 white segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P>.05, while the ratio of non-white colonies to white colonies fits a 1:1 ratio at P>.05 as expected)	Normal progeny and mound-shaped progeny with a crinkled morphology similar to that of the P strain: Inclusively, 373 of these colonies.	175 colonies having a Bainbridge crinkled morphology* - 31.5% of all progeny obtained displayed the Bainbridge crinkled morphology.
	The ratio of non-Bainbridge crinkled colonies to Bainbridge crinkled colonies fits a 2:1 ratio at P>.05	

1. All green normals and all green mound-shaped colonies with a crinkled morphology similar to that of the P strain were of a grass-green colour shade thereby implying no adenine-requiring colonies to be present among them.

2. Green Bainbridge crinkled colonies were olive-green in colour.

* Of the Bainbridge crinkled colonies, a fraction displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

T A B L E 38 (continuation)

Nutritional test of progeny obtained

Number of green and white normal colonies tested for adenine requirement:	Number of green and white normal colonies found to be adenine-independent:
100	100

T A B L E 39

The cross h81 x J

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
169 green segregants, 54 yellow segregants and 200 white segregants (as expected the ratio of greens to yellows fits a 3:1 ratio at P>.05, while the ratio of non-white colonies to white colonies fits a 1:1 ratio at P>.05 as expected)	Normal progeny and mound-shaped progeny with a crinkled morphology similar to that of the P strain: Inclusively, 291 of these colonies	132 colonies having a Bainbridge crinkled morphology* - 31.2% of all progeny obtained displayed the Bainbridge crinkled morphology
	The ratio of non-Bainbridge crinkled colonies to Bainbridge crinkled colonies fits a 2:1 ratio at P>.05	

1. All green normals and all green mound-shaped colonies with a crinkled morphology similar to that of the P strain were of a grass-green colour shade, thereby implying no adenine-requiring colonies to be present among them.

2. Green Bainbridge crinkled colonies were olive-green in colour.

*Of the Bainbridge crinkled colonies, a fraction displayed the pronounced Bainbridge crinkled morphology and were much smaller than other progeny.

Though the highly unstable h strain, h91, was crossed respectively with ad8 paba1 and J, it was decided in addition to investigate the haploid segregants of a diploid synthesized from h91 and MSE in order that further information might be obtained regarding the genetic makeup of this unstable strain. The diploid h91 // MSE was thus synthesized and in turn haploidized. A sample of the resulting white haploid segregants were tested for various requirements. The results are recorded in Table 40. As expected h91 is shown to be carrying the VI-VII reciprocal translocation. Other unexpected translocations are not seen to be carried by h91. The extremely low number of fac$^+$ segregants suggests a deleterious factor or semi-lethal in linkage with facA^+ and thus on linkage group V. Lethals or semi-lethals on other linkage groups do not appear to be present. The semi-lethal in linkage with facA^+ would appear to have emerged within the h91 // MSE diploid or during its formation. Moreover, the data in Table 40 does not, apparently, provide any information pertaining to the genetic makeup of h91 which could further explain (or further account for) the very high instability displayed by h91 (or by the other h strains).

Having considered the genetic makeup of h strains, one sought at this point to obtain some genetic information regarding the yellow variants produced by the h strains or h colonies. To this end some crosses were made which involved such yellow variants (sectors) and the strain, ad8 paba1. All yellow sectors studied were phenotypically ad$^+$ bi$^+$.

Specifically, the crosses made were those between y-h81 (yellow variant of h81) and ad8 paba1, y-h91 (yellow variant of h91) and ad8 paba1, y-h51 (yellow variant of h51) and ad8 paba1, and y-h88 (yellow variant of h88) and ad8 paba1. The results

T A B L E 40

Segregations from the diploid h91 // MSE

(Only white haploid segregants produced)

Chromosome	I		III		I and IV		V		VI		VII	
allele	paba	paba$^+$	gal	gal$^+$	pyro	pyro$^+$	fac	fac$^+$	s	s$^+$	nic	nic$^+$
	12	18	13	17	20	10	27	3	11	19	11	19

Chromosome	VIII		Translocations
allele	ribo	ribo$^+$	
	30	0	#VI-VII

#The existance of the VI-VII translocation is demonstrated by the fact that no s nic$^+$ and s$^+$ nic recombinants were found along with s nic and s$^+$ nic$^+$ segregants.

from each of those four crosses are given in respectively Tables 41, 42, 43, and 44.

The existance of at least one Bainbridge crinkled colony among the progeny from each of three of those crosses (Tables 42, 43, and 44) indicates that y-h91, y-h51, and y-h88 still each retain a portion of the original III duplication. The fact that no Bainbridge crinkled colonies were noted among the progeny from the y-h81 x ad8 paba1 cross would suggest that y-h81 either retains a virtually nonexistant reduced III duplication or none at all.

Moreover the presence of at least one biotin-requiring recombinant among the progeny obtained from each of three of those four crosses (Tables 41, 42 and 43) indicates that y-h81, y-h91, and y-h51 are respectively heterozygous at the bi1 locus, as well as indicating that each of these yellow variants arose through a deletion which included the y^+ allele but not the bi1 allele on the translocated duplicate I segment. The lack of biotin-requiring (bi) recombinants among the progeny of the cross where y-h88 was one of the parents (Table 44) would suggest that y-h88 does not carry the bi1 allele or that it carries a deletion exceedingly close to it.

The fact that three out the four tested yellow variants respectively produced by four h colonies (or h strains) were shown to be definitely still carrying the bi1 allele suggests that a very large majority of yellow variants (sectors) produced by h colonies arose through respective deletions which included the y^+ allele but not the bi1 allele. Whereas, in regards to yellow variants produced by the I duplication strain (P) under conditions comparable to those under which yellow variants of h colonies were produced, work by Nga and Roper (1968) suggested

T A B L E 41

The cross y-h81 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained
124 green segregants and 137 yellow segregants (as expected the ratio of greens to yellows fits a 1:1 ratio at $P > .05$)	Normal progeny and mound-shaped progeny with a crinkled morphology either similar to that of the P strain or less marked: Inclusively 261 of these colonies.
All green progeny were grey-green in colour shade, thus indicating that they were all adenine-requiring*	

*This would be as expected insofar as the distance (.1) between the ad20^{+}(8) locus and the y locus is extremely small.

T A B L E 41 (continuation)

Nutritional test of progeny obtained

A sample of yellow and green normal colonies were tested for biotin requirement: of the colonies tested, only one colony was found to be biotin-requiring.

T A B L E 42

The cross y-h91 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
112 green segregants and 119 yellow segregants (as expected the ratio of greens to yellows fits a 1:1 ratio at P >.05)	Normal progeny and mound-shaped progeny with a crinkled morphology either similar to that of the P strain or less marked: Inclusively, 230 of these colonies	1 colony having a Bainbridge crinkled morphology - .4% of all progeny obtained displayed the Bainbridge crinkled morphology
All green progeny were grey-green in colour shade		

T A B L E 42 (continuation)

Nutritional test of progeny obtained

A sample of yellow and green normal colonies were tested for biotin requirement. Of the colonies tested, only one colony was found to be biotin-requiring.

T A B L E 43

The cross y-h51 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
55 green segregants and 67 yellow segregants (as expected the ratio of greens to yellows fits a 1:1 ratio at P>.05)	Normal progeny and mound-shaped progeny with a crinkled morphology either similar to that of the P strain or less marked: Inclusively, 106 of these colonies	6 colonies having a Bainbridge crinkled morphology - 5.3% of all progeny obtained displayed the Bainbridge crinkled morphology
All green progeny were grey-green in colour shade		

T A B L E 43 (continuation)

Nutritional test of progeny obtained

Number of colonies tested for biotin requirement:	Number of colonies found to be biotin-requiring:
112 (all progeny)	1

T A B L E 44

The cross y-h88 x ad8 paba1

Number and colour of segregants obtained: Segregations regarding colour	Morphological types or classes obtained	
82 green segregants and 89 yellow segregants (as expected the ratio of greens to yellows fits a 1:1 ratio at P>.05)	Normal progeny and mound-shaped progeny with a crinkled morphology either similar to that of the P strain or less marked: Inclusively 158 of these colonies	13 colonies having a Bainbridge crinkled morphology - 7.8% of all progeny obtained displayed the Bainbridge crinkled morphology

All green progeny were grey-green in colour shade

T A B L E 44 (continuation)

Nutritional test of progeny obtained

Number of colonies tested for biotin requirement:	Number of colonies found to be biotin-requiring:
171 (all progeny)	0

that most (if not all) of such yellow variants (sectors) of the I duplication strain, P arose as a result of respective deletions which included the y^+ allele and the bi1 allele. It would seem, therefore, that in the production of yellow variants (yellow sectors) the general genetic background held in common by h colonies or h strains makes a region which includes the y^+ allele much more prone to deletion than a region which includes the bi1 allele, where in contrast, the genetic background of the I duplication strain P makes a region which includes the y^+ allele just as prone to deletion as a region which includes the bi1 allele. However, in view of the fact that the changed III duplication significantly enhances the frequency of those deletions involving the y^+ allele, it is likely that the changed III duplication is the major factor in making a region including the y^+ allele far more prone to deletion than a region including bi1 allele.

In regards to the proneness of genetic regions to deletion, it is important to note at this point that all tested green sectors produced by the P strain were either ad bi or ad^+ bi^+, where in comparison, all the tested green sectors produced by RP81 and the h colonies were either ad^+ bi or ad^+ bi^+. (The tested yellow variants of these strains were all ad^+ bi^+, as one will recall). In forthcoming experiments soon to be described, the sectors produced by many colonies (colonial subcultures) of h51 and h88 were found, with one exception, to be either ad^+ bi or ad^+ bi^+; the exception was a ad bi^+ sector, which confirmed the presence of the ad20 allele in RP81. (Tested yellow variants produced by these many colonial subcultures of h51 and h88 were all ad^+ bi^+.) At first this data may appear to suggest that the genetic background of the P strain makes a region including the $ad20^+$ allele just as prone to deletion as a region

including the bi1$^+$ allele, where in contrast, the genetic background held generally in common by the double-duplication strains makes a region including the bi1$^+$ allele exceedingly more prone to deletion than a region including the ad20$^+$ allele.

Before drawing a final conclusion however, one must recall the data (Table 35 through to Table 39) which suggests the very likely presence of a deletion very close to or including the ad20 allele in h colonies (and in h variants). Therefore, it is possible that in the production of green variants (sectors) by h colonies and by the many colonial subcultures of h51 and h88 (previously referred to) deletions including the ad20$^+$ allele had in fact occurred, but by so occurring, each resulted in a small section of the I duplication to be finally deficient for two homozygous segments, thus causing sectors which would have been ad bi to be inviable at their inception. This of course may not have been necessarily the case, for in the production of green variants by h colonies and by the many colonies of h51 and h88, deletions including ad20$^+$ may have not occurred at all. Thus, two conclusions are possible. Furthermore, in regards to the deletion which appears to be very close to the ad20 allele, this may very well have been present in the original RP81 colony (colony No. 81) from which subsequent RP81 colonial subcultures and h sectors were ultimately derived. Hence all h sectors (and h colonies) would be carrying this particular deletion. Therefore, during the origination of any h variant, any deletion of a region including the ad20$^+$ allele might have made a small section of the I duplication deficient for two homozygous segments and thus any ad bi h variants would have been inviable. On the other hand it is possible that during the origination of h variants deletions including the ad20$^+$ allele had not occurred. Thus two conclusions are again possible.

To determine whether or not RP81 carried a deletion very close to the ad20 allele earlier crosses between RP81 and some normal strains, genetically ad^+, had been attempted, but proved unsuccessful inasmuch as no hybrid pericethecia were isolated. The failure to obtain any data in this respect leaves open the possibility that RP81 did not carry a deletion very close to the ad20 allele but that deletions very close to or including the ad20 allele had occurred during the origination of h variants. Such deletions of a very specific part of the translocated duplicate I segment (in a double-duplication strain) would have meant that respective deletions occurred (in a double-duplication strain) which were very close to or which included the ad20 allele but which did not encompass the bi1 allele. Studies by Nga and Roper (1968) suggested that in the P strain respective deletions occur which encompass the bi1 allele as well as the ad20 allele. Such information, assuming that deletions very near (or including) the ad20 allele do occur during the origination of h variants, (sectors) may imply again the differential effect of two generally different genetic backgrounds on the susceptibility of duplication-regions to deletion. As will be seen soon, additional data relating to ad^+ bi and ad^+ bi^+ sectors may also suggest the differential effect of two different genetic backgrounds on the susceptibility of genetic regions to deletion.

At this point, one turns to further studies relating to the sectoring behaviour of h strains. In part, these studies pertain to many colonies (colonial subcultures) respectively made from h88 and h51. It is important to stress that when these colonial subcultures were made h88 and h51 had been stored for approximately

four and one-half months. 40 colonies made from h88 were left at 36°C for ten days. Detailed results are recorded in Table 45. Unlike P colonies and the original h88 colony, but like RP81 colonies, the h88 colonial subcultures (colonies) produced green improved sectors which were clearly defined (or mostly separated) from the parental area and from one another, giving the colonies an uneven serrate appearance. Such a morphology thus appears to be definitely characteristic of colonies carrying both the I duplication and at least a partial III duplication, but not characteristic of colonies carrying only the I duplication.

One should take note of the low frequency of yellow sectors produced by the h88 colonies, comparable to that of the P I duplication strain. Though a definite explanation of this cannot be made, such an extreme reduction of the yellow sector frequency on the part of h strain colonies (subcultures) nevertheless would appear to be due or related to the age of the original h88 colony. As will be pointed out in the general discussion, age has a definite effect upon instability in Drosophila and in maize. Note also the frequency of white sectors. Such a frequency is unprecedented in normal, I duplication, and III duplication strains and may indicate an effect of the h88 genetic makeup upon this particular mutational activity.

The detailed results of the experiment where 39 colonies from h51 were left at 36°C for ten days are given in Table 46. The h51 colonies also produced green improved sectors which were clearly defined (or mostly separated) from the parental area and from one another, giving the colonies an uneven serrate appearance. One again should take note of the low frequency of yellow sectors, comparable to that of the P strain. It would

T A B L E 45

40 h88 colonies (colonial subcultures) at 36°C for ten days

Mean number of yellow sectors per colony	Number of yellow sectors tested for adenine and biotin requirements	Number of $y\ ad^+\ bi^+$ sectors	Number of green sectors tested for adenine and biotin requirements
1.3	40	40	62

Number of $y^+\ ad^+\ bi^+$ sectors	Number of $y^+\ ad^+\ bi$ sectors	Number of $y^+\ ad\ bi^+$ sectors	Number of white sectors	Number of white sectors tested for adenine and biotin requirements	Number of $w\ ad^+\ bi^+$ sectors
58	3	1	8	4	4

appear that such a reduction in the yellow sector frequency is due or related to the age of the original h51 colony.

It was thought that colonies derived respectively from newly generated (or newly produced) h sectors would prove to produce a much higher frequency of yellow sectors than produced by the h88 and h51 colonial subcultures. To this end, 32 RP81 colonial subcultures were made from an RP81 colony (at least eight months old) and left at 36°C for a period of 10 days in order to produce h sectors. Such h sectors were produced as well as yellow sectors. The mean number of yellow sectors was 2.9, this mean being significantly higher at $P<.01$ than the yellow sector mean calculated for P colonies having grown ten days at 36°C. Nearly all yellow sectors (variants) were produced by the h sectors. In view of past experiments, it is extremely likely that these h sectors carried a III duplication of reduced size and it would thus follow that h colonies derived from such sectors would most likely carry the reduced III duplication. One colony from each of 36 of those h sectors numbered h100 to h135 was made and left at 36°C for a period of ten days. Such colonies were respectively numbered h100 to h135. Along with these colonies, 33 P colonies were also grown for the same period. The 36 h colonies (h100 to h135), whose mean parental diameter was 5.5cm,[11] all produced yellow sectors,[12] the average number of yellow sectors per h colony being 3.4, where the 33 P colonies produced an average of 1.2 yellow sectors per colony, the two means being significantly different at $P<.01$. Such a significant difference between the two means thus shows once more that a changed III

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11. Such a mean parental diameter would confirm that such h colonies carry the reduced III duplication.
12. Green improved sectors were also produced.

T A B L E 46

39 h51 colonies (colonial subcultures) at 36°C for ten days

Mean number of yellow sectors per colony	Number of yellow sectors tested for adenine and biotin requirement	Number of y ad+ bi+ sectors	Number of green sectors tested for adenine and biotin requirements
1.4	30	30	38

Number of y+ ad+ bi+ sectors	Number of y+ ad+ bi sectors	Number of white sectors
36	2	1

duplication significantly enhances the frequency of those deletions involving the $\underline{y}^+$ allele on the I duplication.

Colony h123 produced a total of eleven yellow sectors. From this h123 colony forty colonies were made; these colonies were left at 36°C for a period of ten days. These h123 colonies, whose mean parental diameter was 5.2cm, produced an average of 5.3 yellow sectors per colony. Green improved sectors were also produced but no white sectors. Along with these 40 h123 colonies, 40 P colonies were grown for the same period. The 40 P colonies, whose mean parental diameter was 8.2cm, produced an average of 1.1 yellow sectors per colony; at $P < .01$ the mean 5.3 was significantly higher (or greater) than the mean 1.1. Again, we see that a changed III duplication[13] significantly enhances the frequency of those deletions involving the $\underline{y}^+$ allele on the I duplication. One h123 colony, h123-z, is shown in Plate 7. By looking at this colony, it can be inferred that many of the yellow sectors did not arise at any one definite period. This was the case for many of the yellow sectors produced by any given h123 colony. The h123 colonies had green improved sectors (some quite large as in the case of h123-z) which were clearly defined or mostly separated from the parental region and from one another.

In these present or latest experiments, the utilization of newly generated h sectors from the latest RP81 colonies does appear in some manner to be indirectly conducive to a significant enhancement in the frequency of those deletions involving the $\underline{y}^+$ allele, but not to the same major extent as in earlier

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13.As indicated by the data regarding the comparative mean-sizes of P and h123 colonies, this changed III duplication would be a III duplication of reduced size.

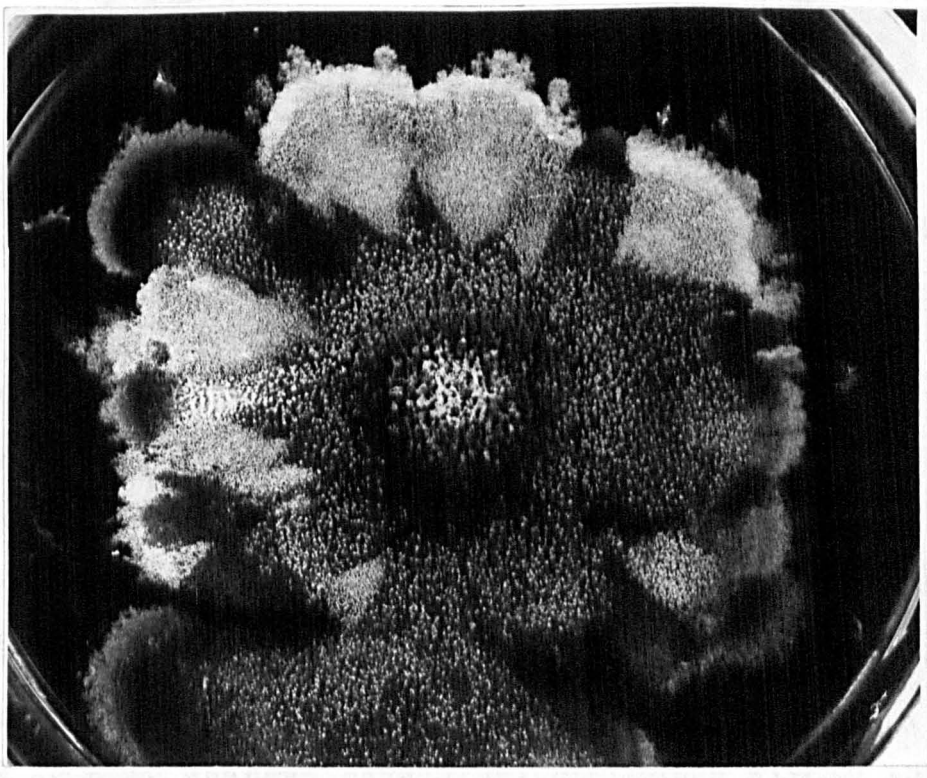

Plate 7. An h123 colony with yellow and green sectors at 36°C. Period of incubation was ten days.

experiments; the far higher enhancement in the frequency of those deletions involving the y^+ allele in these earlier experiments would appear to have been indirectly conditioned by the age of the RP81 colony from which the previous RP81 colonial subcultures and their h sectors were derived. The latest group of RP81 colonies were derived from an RP81 colony considerably older than the one from which earlier RP81 colonies were derived. It would thus appear that the effects of old age were generally maintained through subsequent subcultures. It is not certain how this would be accomplished, but it is very likely that old age involved changes in physiological processes or states within the cytoplasm, and it was these physiological changes which were generally maintained through subculturing. Therefore, the influence of the changed III duplication upon the frequency of those deletions involving the y^+ allele in these latest experiments would have been modified or circumscribed by the effects of such changed physiological states within the cytoplasm, changed physiological states which would have thus been in effect changes in the cytoplasmic environment.[14]

In this connection, the changed physiological processes or states contingent upon old age would have been maintained in the colonial subcultures of h51 and h88, and it would have been specific types of such changed physiological processes which kept (or precluded) the changed III duplication from enhancing the frequency of deletions involving the y^+ allele in these colonies. Thus, it would seem that colony derivatives from newly generated h

14. The differential effect or influence of various cytoplasmic environments or states upon gene action or expression is indicated by a large body of evidence (see Waddington, 1956; Fischberg and Blackler, 1961; Ebert, 1965). Work by Sonneborn, Beale, and Hadorn (see Sinnott et al, 1958; Srb & Owen, 1965) indicates that in Paramecium and Triton particular cytoplasmic states having particular effects upon gene behaviour or action can be transmitted or maintained from cell to cell by means which do not depend on (or are independent of) the transmission of any particular gene or genotype.

sectors maintain other specific types of changed physiological states involved in ageing which, though they do not allow the changed III duplication to induce an extremely large enhancement in the frequency of those deletions involving the $\underline{y}^+$ allele, nevertheless, appear to allow the changed III duplication to enhance the frequency of deletions involving the $\underline{y}^+$ allele to a significant extent. In this connection, the overall physiological situation inherent in and characteristic of newly generated h sectors would appear to have precluded or negated or reversed these further changes in physiological processes involved in ageing which in colony derivatives of very old h sectors (or very old h colonies) might, otherwise, have completely hindered the changed III duplication from enhancing to any significant extent the frequency of those deletions involving the $\underline{y}^+$ allele.

When one compares the average number of yellow sectors produced per colony by the h123 colonies (colonial subcultures) to the average number of yellow sectors produced per colony by RP81 colonies (see Table 23), one finds the two means to be significantly different at $P<.01$. Thus where the intact (or whole) III duplication significantly enhances the frequency of those deletions involving the $\underline{y}^+$ allele on the I duplication, the specific type of changed III duplication residing in the h123 colonies promotes a significantly greater or further enhancement in the frequency of those deletions involving the $\underline{y}^+$ allele on the I duplication. In this connection, however, one should note that colonies h100 to h135 (collectively) produced an average number of yellow sectors (Mean = 3.4) not significantly different at $P>.05$ from the average number of yellow sectors (Mean = 2.9) produced by RP81 colonies (Table 23 and R217). This result may be explained by assuming that many types of changed III

duplication can be readily circumscribed or precluded by the changed physiological processes related to old age from inducing a further enhancement in the frequency of those deletions involving the y^+ allele. Inasmuch as the specific type of changed III duplication in h123 colonies promoted a further enhancement in the frequency of those deletions involving the y^+ allele, it is suggested that the mutagenic action (or effect) of at least one type of changed III duplication is comparatively not as sensitive to the influence of the changed physiological states involved in or related to old age.

Some external environmental situations such as growth temperature may also condition or define the mutagenic effect of a changed III duplication upon the y^+ region of the I duplication. In this connection, it was decided to place h colonies at 39.5°C in order to determine the frequency of yellow sectors produced by h colonies at this temperature as opposed to the frequency of yellow sectors produced by the respective groups of h colonies at 36°C referred to on pages 217 and 219. To this end one colony (colonial subculture) from each of 36 h sectors generated by the RP81 colonies described on P.217 was made and left at 39.5°C for ten days. The same 36 h sectors (h100 to h135) had been used to produce (at 36°C) the 36 h colonies first referred to on P.217. The 36 h colonies at 39.5°C were thus respectively numbered h100 to h135. Upon ten days incubation (at 39.5°C), it was noted that these colonies (mean parental diameter, 5.3cm) produced an average of 13.7 yellow sectors per colony. The colonies were generally of a nonserrate appearance. Along with these h colonies at 39.5°C, 36 P colonies were grown for the same period. These 36 P colonies were also involved in another experiment (Table 15, Part III). As one will recall, these P colonies produced an average of 5.1 yellow sectors per colony. The mean 13.7 was found to be significantly higher at

$P < .01$ than the mean 5.1, thus indicating that the frequency of deletions including the y^+ allele continues to be significantly enhanced at a higher temperature by the changed III duplication. Moreover, it is indicated that the frequency of deletions including the y^+ allele in the h colonies (h strains) is also enhanced by growth at a higher temperature. Yet the data (P.217, 219, 223, 224) would not appear to indicate that the influence of the changed III duplication upon the y^+ region of the I duplication has become more effective in the promotion of deletions of the y^+ region at this higher temperature than at the lower temperature (36°C). Nevertheless, it is important to note that in each of the h colonies grown at this higher temperature, the yellow sectors were equidistant from a given colony centre, thereby indicating that all the yellow sectors of a given colony arose at a specific period. Because of this and the fact that each of the h123 colonies grown at 36°C did not give rise to yellow sectors at a specific period, it would appear that growth of h colonies at a higher temperature aids the changed III duplication to promote deletions of the y^+ region in a controlled and ordered manner.

From one h colony, h126, which had produced many yellow sectors and which had grown at 39.5°C along with the other 35 h colonies in the last experiment, 40 colonies were made and grown at 39.5°C for a period of 12 days. These 40 h126 colonies[15] (mean parental diameter, 6.0cm) produced an average of 21 yellow sectors per colony. In each colony, the yellow sectors were approximately equidistant from the colony centre (see Plate 8). Along with these h126 colonies in 39.5°C, 40 P colonies were grown for the same period; these 40 P colonies were also involved in

15. Colonies were generally nonserrate in appearance.

another experiment (Table 16, Part III). As one will recall, these P colonies produced an average of 5.4 yellow sectors per colony. The mean 21.0 was found to be significantly higher than the mean 5.4 at $P < .01$, thus again indicating that the frequency of deletions including the y^+ allele continues to be significantly enhanced at a higher temperature by the changed III duplication.[16] Besides the 40 h126 colonies grown at 39.5°C, 40 h126 colonies were grown at 36°C for eleven days, these latter colonies also having been derived from the original h126 colony at 39.5°C. The 40 h126 colonies at 36°C produced an average of 3.8 yellow sectors per colony. (These colonies were serrate in appearance.) Information from this latest experiment indicates again that the frequency of deletions including the y^+ allele on the I duplication in h colonies is also enhanced by growth at a higher temperature. Yet the data from this latest experiment together with the data relating to P colonies at 36°C) would appear to indicate again that the influence of the changed III duplication upon the y^+ region of the I duplication has not become more effective in the promotion of deletions of the y^+ region at this higher temperature than at the lower temperature (36°C). The fact that yellow sectors are equidistant from any given h126 colony centre would, in view of data relating to h123 colonies at 36°C, furnish additional evidence that growth of h colonies at a higher temperature aids the changed III duplication to promote deletions of the y^+ region in a controlled and ordered manner.

In order to gain some additional information relating to the instability of h colonies at 39.5°C, a sample of the yellow and

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16. Data from a cross soon to be described indicated that this changed III duplication in h126 colonies is a III duplication of reduced size.

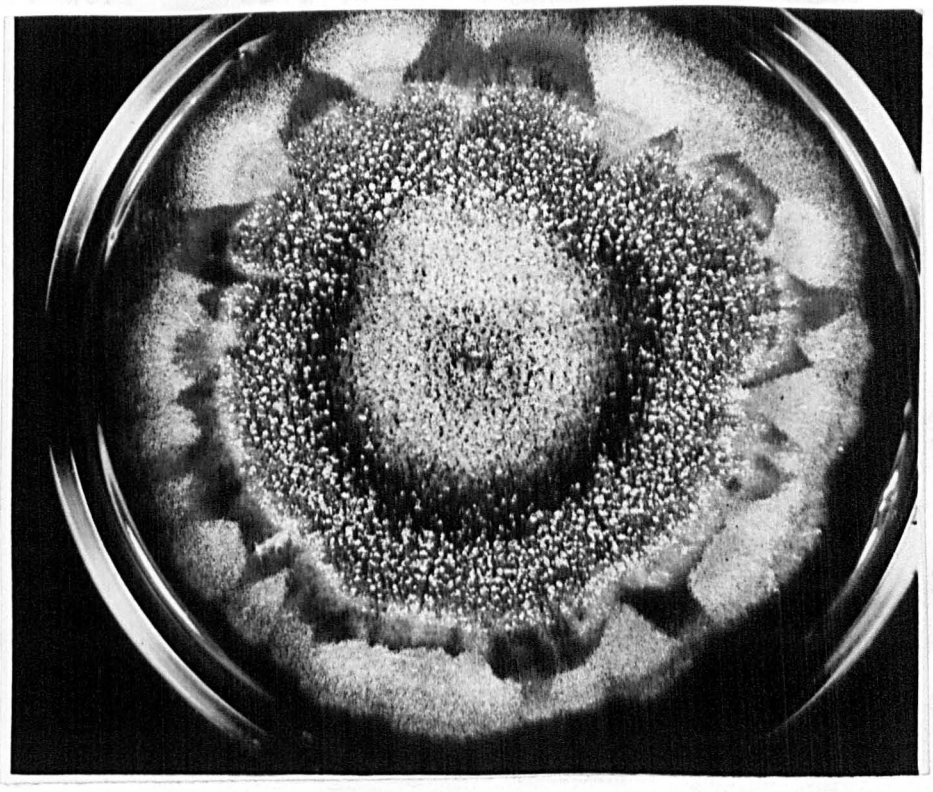

Plate 8. An h126 colony with yellow sectors at 39.5°C. Period of incubation was twelve days.

green sectors produced by the h126 colonies were tested for adenine and biotin requirements. In testing 60 yellow sectors and 39 green improved sectors, it was found that all yellow sectors were $\underline{ad^+\ bi^+}$, while 14 green sectors were $\underline{ad^+\ bi}$ and 25 were $\underline{ad^+\ bi^+}$. As all tested sectors produced by the h126 colonies at 39.5°C were either $\underline{ad^+\ bi}$ or $\underline{ad^+\ bi^+}$, and as all tested sectors produced by the P colonies at different temperatures (including 39.5°C) were either $\underline{ad\ bi}$ or $\underline{ad^+\ bi^+}$, it is again suggested that the particular genetic background (held in common by h colonies) makes a region including the $\underline{bi1^+}$ allele far more prone to deletion than a region including the $\underline{ad20^+}$ allele, where in contrast, the genetic background of the P strain makes a region including the $\underline{ad20^+}$ allele just as prone to deletion as a region including the $\underline{bi}$ allele. However, as pointed out earlier, there may be alternative reasons for h colonies (or the double duplication strains) producing $\underline{ad^+\ bi}$ sectors in place of $\underline{ad\ bi}$ sectors.

At this point, one turns to investigate the instability behaviour (or sectoring behaviour) of cultures derived (directly or indirectly) from single and double duplication bearing progeny-colonies obtained from the h x J and h x $\underline{ad8\ paba1}$ crosses considered earlier, as well as the instability behaviour of cultures derived from single and double duplication bearing progeny obtained from a cross between h126 and MSE. A portion of the progeny-colonies obtained from the h x J and h x $\underline{ad8\ paba1}$ crosses would individually carry both the I duplication and the intact III duplication.

Some progeny-colonies from the cross h91 x J were used to begin the study. When the study began, these progeny-colonies were approximately seven weeks old. From each of ten very small, pronounced Bainbridge crinkled colonies, all of an olive-green colour, one inoculum per CM plate was made. Plates were left at 36°C for 9 days. The resulting 10 colonies each had a parental region which displayed the Bainbridge crinkled morphology and which was olive-green in colour; from the parental region of each colony emerged grass-green sectors which were comparatively smooth in morphology; these were identical to the h sectors, and for this reason, they will be referred to as h2 sectors. Though some fz type sectors were noted, no fl and bw type sectors were observed. The h2 sectors of three of these colonies had produced yellow sectors. Testing the parental regions of these three colonies for biotin, adenine, proline, and p-aminobenzoic acid requirements revealed that the three colonies were each $\underline{bi}^+$, $\underline{ad}^+$, $\underline{paba}$, and $\underline{pro}$. Thus it was clear that each of these three colonies carried both the III and I duplications and were each heterozygous at the $\underline{y}^+$, $\underline{ad20}^+$, and $\underline{bi1}^+$ loci.

From one of these three colonies, R^2P-6, 40 colonial subcultures were made; these were left at 36°C for a period of 10 days. All of the resulting R^2P-6 colonies displayed the Bainbridge crinkled morphology and were olive-green. The 40 R^2P-6 colonies produced grass-green sectors (h2 sectors), as well as olive-green fuzzy sectors and yellow sectors; one bw type sector was also produced. The yellow sectors generally emerged from the h2 sectors. The average number of yellow sectors per colony was 2.7. This mean was significantly higher at $P < .01$ than the average number of yellow sectors produced by P colonies grown at 36°C for ten days, thereby indicating that the III

duplication carried by R^2P-6 continues in this second generation to have a significant effect upon the frequency of those deletions which include the y^+ allele on the I duplication. From each of 40 h2 sectors, one colony was made; these colonies were left at 36°C for a period of 10 days. The 40 colonies produced an average of 3.3 yellow sectors per colony, this average being significantly higher at $P<.01$ than the average number of yellow sectors produced by P colonies grown at 36°C for a period of 10 days.

From one colony, h2-15, which had produced 12 yellow sectors, 40 colonial subcultures were made; these were left at 36°C for 10 days. It was observed that these 40 h2-15 colonies produced an average of 3.7 yellow sectors per colony.[17] At $P<.05$ this mean was significantly higher than the average number of yellow sectors produced by the R^2P-6 colonies. Thus the particular type of changed III duplication carried by h2-15 colonies can be inferred to stimulate or promote significantly more deletions of the y^+ region than the unchanged III duplication carried by R^2P-6, though the closeness of the two averages may imply that the changed III duplication in h2-15 may <u>not always</u> stimulate more deletions of the y^+ region. It is important to note in this connection that this further enhancement of y^+ region instability by a changed III duplication in h2-15 was not to the same extent as that first promoted by a changed III duplication in the h strains obtained from RP81 (Table 26). This difference may have been related to the age of the original R^2P-6 colony or to the fact that the changed III duplication in h2-15 does not have a RP81 genetic background. The age of the h parent involved in the cross may also have been a factor.

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17. Yellow sectors were not equidistant from any given colony centre.

Forty h2-15 colonies grown at 39.5°C for 12 days produced an average of 15.1 yellow sectors per colony. (Yellow sectors were equidistant from each given colony centre). The frequency of deletions which include the y^+ allele in h2-15 colonies is inferred to be also enhanced by growth at a higher temperature. Yet the data would not appear to indicate that the influence of the changed III duplication in h2-15 (a second generation h strain) upon the y^+ region has become more effective in the promotion of deletions of the y^+ region at this higher temperature than at the lower temperature.

In order to obtain some additional data pertaining to the sectoring behaviour of cultures derived from F1 duplication bearing progeny, attention was turned to the duplication bearing progeny obtained from the cross h88 x ad8 paba1. (The progeny obtained from this cross were approximately three months in age at this point.) As one will recall, this cross had produced green mound-shaped colonies (progeny) with a crinkled morphology similar to that of the P strain. A portion of these mound-shaped colonies with a crinkled morphology similar to that of the P strain were grass-green while the rest were grey-green. The former were noted to be ad^+, and thus it is inferred they carry at least the I duplication (heterozygous at the y^+ locus). Approximately, one-half of these former colonies should also carry the reduced III duplication of the h88 parent. One colony (colonial subculture) was derived from each of 55 of these ad^+ grass-green mound-shaped colonies, and left at 36°C for 9 days. It was found that 30 of the resulting colonies displayed the general P strain type morphology while 25 of the remaining colonies did not display the P type morphology; in contrast, these latter colonies, like, for example, the h88 and h123

colonies (colonial subcultures), had a very irregular (serrate) peripheral region as a result of green improved sectors being mostly separated from one another and from the parental region. Because of this, it is inferred that these 25 colonies carry the reduced III duplication of the h88 parent along with the I duplication. The segregation of these two morphological classes or types is in accord with a 1:1 ratio at $P > .05$. This is as anticipated, for one expects 50% of the colonies to be carrying both the reduced III duplication and the I duplication, while 50% of the colonies are expected to be carrying only the I duplication. A portion of colonies in each morphological class referred to produced yellow sectors, but not one of such colonies produced a high frequency of yellow sectors: no significant or definite conclusions could be drawn from this, other than the tentative conclusion that the effects of the age of the F1 progeny were somehow implicated.

To gain definite or more significant information regarding the frequency of yellow sectors produced by F1 colonies in each of the morphological classes just referred to, studies were made of the instability behaviour of cultures derived from duplication bearing progeny obtained from the cross h126 x MSE. In preparation for this cross, 40 colonial subcultures were made from one of the h126 colonies grown at 39.5°C (P.224) and all left in 39.5°C for a period of 12 days. At the time of this further subculturing, the h126 colony providing the conidia was almost three and one-half month old. The latest group of 40 h126 colonies at 39.5°C produced an average of 16.3 yellow sectors per colony, at $P < .01$, this mean being significantly greater than the mean number of yellow sectors produced by P colonies grown at the same temperature for 12 days. It is

possible that had this latest group of h126 colonies been derived from a much older h126 colony specific changes in physiological states would have precluded the changed III duplication in the latest h126 colonies from significantly enhancing the frequency of those deletions including the $\underline{y}^+$ allele. However, it is possible that in this latest (or newly generated) group of h126 colonies such changed physiological processes were already present, but the particular type of changed III duplication in h126 colonies was not precluded by such processes from significantly enhancing the frequency of those deletions involving the $\underline{y}^+$ allele.

From one of these newly produced h126 colonies which had given rise to 22 yellow sectors, conidia were immediately taken to commence the first stage of the cross h126 x MSE. The purpose of the preparation just referred to was to use conidia from a newly generated h126 colony (subculture) which had produced a high number of yellow sectors at 39.5°C, and which made up a group of cultures in which the changed III duplication was shown to be still able to significantly enhance $\underline{y}^+$ region instability. From the cross, h126 x MSE,[18] 191 green, 201 yellow, and 381 white progeny-colonies were obtained, the ratio of greens to yellows to whites fitting a 1:1:2 ratio at $P > .05$ as expected. (The segregations of other markers are given in Table 47.) In regards to morphology this cross resulted in the production of mound-shaped progeny-colonies with a P type crinkled morphology, and normal colonies. In addition, 32 (or 4.1%) of the progeny-colonies displayed the Bainbridge crinkled morphology. This very low frequency of Bainbridge crinkled colonies among the progeny indicates that h126 retains at least a very small proximal portion of the III duplication.

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18. Recall that MSE carries $\underline{y}$, $\underline{ad20}$ and $\underline{su1\ ad20}$.

T A B L E 47

Segregations from the cross h126 x MSE

(12 green, 13 yellow, and 25 white segregants tested)

Chromosome	I and IV		III		V		VI		VIII	
allele	pyro	pyro$^+$	gal	gal$^+$	fac	fac$^+$	s	s$^+$	ribo	ribo$^+$
	38	12	24	26	20	30	21	29	26	24

Not one of the green colonies produced through the cross were of the normal morphology; also, not one of the green colonies were of a grey-green colour shade. With the exception of the green Bainbridge crinkled colonies, which were olive-green in colour shade, all green colonies were grass-green in colour shade; the fact that these colonies were grass-green would indicate that they were all $\underline{ad}^+$; in fact, a test of 75 of these grass-green colonies for adenine requirement confirmed that they were $\underline{ad}^+$. These results indicate that the h126 parent carried a recessive lethal (or deletion) exceedingly close to the ad20 and $\underline{y}^+$ alleles on the translocated duplicate I segment and that all green segregants which inherited this deletion or lethal through recombination without the I duplication were inviable. Therefore, these results indicate that all green mound-shaped colonies (with a P type crinkled morphology) must carry at least the I duplication where the $\underline{y}^+$ locus is heterozygous.[19] A portion of these colonies would also be heterozygous at the ad20 locus, while the remaining portion would be homozygous at the ad20 locus but phenotypically $\underline{ad}^+$ as a result of the su1 ad20 allele being present on the left arm of chromosome I, at least 50 units from the y allele (in MSE).

From each of 76, four-day-old, green mound-shaped colonies (with a P type crinkled morphology) obtained from this cross, one colonial subculture was made and left at 36°C for a period of 10 days. At the finish of incubation, 34 of the colonial subcultures displayed the same morphology as the I duplication strain, P. These colonial subcultures were termed class A colonies. 42 of the remaining colonial subcultures displayed a very irregular morphology giving these colonial subcultures an uneven serrate appearance such as the h88 colonies. These 42

19.Subsequent data would confirm this.

colonial subcultures were referred to as class B colonies. It was inferred that class B colonies carried both the I duplication and the reduced III duplication whereas class A colonies carried only the I duplication. As in earlier crosses, it is expected that the ratio of green mound-shaped progeny-colonies carrying both the I duplication and reduced III duplication to those green mound-shaped progeny carrying only the I duplication should fit a 1:1 ratio. The ratio of class B to class A colonies should thus fit a 1:1 ratio. At $P > .05$ the ratio of class B to class A colonies does fit a 1:1 ratio.

Class A colonies produced an average of .90 yellow sectors per colony (comparable to that of P colonies at 36°C), while class B colonies produced an average of 2.3 yellow sectors per colony. At $P < .01$ these two means were found to be significantly different. To confirm that class B colonies produce on average significantly more yellow sectors than class A colonies, a colony (colonial subculture) from each of the 34 class A colonies and from each of 34 class B colonies was made and left at 39.5°C for a period of 12 days. The 34 class A colonies left at 39.5°C produced an average of 5.5 yellow sectors per colony (comparable to that of P colonies at 39.5°C), whereas the 34 B colonies left at this same temperature produced an average of 9.8 yellow sectors per colony.[20] At $P < .01$ it was found that these two means were significantly different, again showing that class B colonies produced on the average significantly more yellow sectors than class A colonies.

The preceding results clearly indicate that the presence of the reduced III duplication in haploid segregants (progeny-colonies) carrying the I duplication significantly enhances the frequency of those deletions which include the $\underline{y}^+$ allele on the

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20. Every class A and class B colony at 39.5°C produced yellow sectors.

I duplication. It is appreciated that the degree of enhancement of y^+ region instability by a reduced III duplication in F1 progeny was far less than in h cultures of the parental generation. It is possible that this smaller enhancement is related (to some extent at least) to the transmission of the reduced III duplication to a second generation. It is known in this connection that the instability of a gene in Drosophila virilis decreases markedly in succeeding generations (Demerec, 1928).

At any rate there is no doubt that a reduced III duplication in Aspergillus does enhance the frequency of those deletions which include the y^+ allele on the I duplication. In this regard, it is possible that a small section of the I duplication closely linked to y^+ had been altered in some manner during the origination of h sectors (and thereby would be present in h colonies) as a condition (prerequisite) to the promotion by the reduced III duplication of an enhanced y^+ region instability. If this had been the situation, the data just considered makes it quite clear that such an altered section of the I duplication would not have been able to influence or affect the frequency of those deletions involving the y^+ allele without the presence of the reduced III duplication.

GENERAL DISCUSSION

GENERAL DISCUSSION

The following points summarize the essential findings which have emerged, from the work described in this thesis, concerning chromosomal instability in Aspergillus nidulans. First, in relation to the effects of temperature on chromosomal instability, we have seen that in cultures of A. nidulans carrying either the I or III duplications the overall frequency of deletions from either duplication is dependent upon the temperature of growth. The instability of cultures carrying the III duplication responds to temperature differently from the manner in which the instability of I duplication bearing cultures so responds. With respect to cultures of A. nidulans carrying the III duplication, the overall frequency of deletions from the III duplication is greatly enhanced by low temperatures though (in a particular strain at least) a particular region of the III duplication, the $s12^{+}$ region of the translocated duplicate III segment, is subject to far less deletions at an intermediate temperature than at either a higher or lower temperature. With respect to cultures carrying the I duplication, it is clear that the overall frequency of deletions from the I duplication is markedly enhanced by high temperatures. A temperature of 39.5°C appears to enhance the overall frequency of deletions from the I duplication to the greatest extent. In regards to the deletion of particular regions of the translocated duplicate I segment, such as the y^{+} region, the frequency of deletions encompassing or involving particular regions of the translocated duplicate I segment is markedly enhanced by high temperatures. The frequency of such deletions involving (or encompassing) regions of the translocated duplicate I segment is apparently enhanced to the greatest extent

by a temperature of 39.5°C. In regards to regions on the non-translocated duplicate I segment, an increase in temperature progressively enhances the frequency of those deletions to which such regions are subject.

At various temperatures, the translocated duplicate I segment is subject to far more deletions than is the non-translocated duplicate I segment. In the case of the III duplication, it is strongly suggested that the translocated duplicate III segment is subject to far more deletions than is the non-translocated duplicate III segment. Thus, the frequency of deletions to which a genetic region of a duplication is subject not only depends upon the temperature of growth but also depends upon which of the two homologous, duplicate segments carries the genetic region in question. In the case of cultures carrying the III duplication, deletions from the III duplication, and thus apparently deletions from the translocated duplicate III segment, generally occur later in time at 42°C than at 36°C, while at 36°C the III duplication - and thus apparently the translocated duplicate III segment - is subject to deletions at a much earlier time in growth than is the I duplication, or the translocated duplicate I segment. At 42°C we have seen that the $\underline{s12}^{+}$ region of the III duplication and the $\underline{y}^{+}$ region of the I duplication are each far more susceptible to deletion during a specific period of growth than during any other period, thus suggesting that at 42°C deletion of each of these two regions conforms to some type of internal programme. The period of enhanced susceptibility in the case of cultures carrying the I duplication is dependent upon growth at high temperature. At 39.5°C respective regions of the translocated duplicate I segment are far more susceptible to deletion during a late period of growth

than during any other period, thus suggesting that at 39.5°C the deletion of these respective regions conforms to some type of internal programme. This period of enhanced susceptibility is dependent on growth at a temperature at least 3.5°C above 36°C. In regards to cultures carrying the I duplication, it appears in general that the period of greatest instability is tied in with the temperature of growth.

The temperature-effects upon instability that have been considered concern a single environmental variable and may be contrasted with the findings in Part IV of this thesis, in which the instability of Aspergillus nidulans cultures carrying a double-duplication has been studied. Again, in summary, the following essential findings emerged. Incorporation of the I duplication and the intact III duplication in one strain resulted in a strain whose instability (at 36°C) was greater than either the instability manifested by the I duplication strains or that manifested by the III duplication strains. Deletions from the III and I duplications in such a double-duplication strain generally resulted in variants which in terms of colour and morphology were similar to those collectively produced by the cultures carrying the single duplications. Deletions from the intact III duplication generally preceded deletions from specific sections of the I duplication. Nearly all deletions including the y^+ allele on the I duplication occurred eventually after a loss of part of the III duplication.

What is of great interest in this connection is that an intact III duplication present from very early growth in double-duplication cultures can indirectly enhance within a particular period of growth the frequency of deletions which include the

$\underline{y}^+$ allele on the translocated segment of the I duplication, while during the same period, a III duplication of reduced size, and present from very early growth in double-duplication cultures, can eventually promote (at a particular later time) a far greater enhancement in the frequency of deletions which include the $\underline{y}^+$ allele on the translocated segment of the I duplication. Hence, the frequency of deletions to which the $\underline{y}^+$ region of the I duplication is subject during a given period, in a double-duplication strain, ultimately depends upon the size of III duplication which is present from very early growth. In view of what has been said, and in view of other information regarding double-duplication cultures, the following can thus be concluded to be the case: the presence of an intact III duplication during early growth will eventually preclude the $\underline{y}^+$ region of the I duplication from being subject to an extremely high frequency of deletions within a particular period of growth, while late during a subsequent period of growth, an earlier created III duplication of reduced size would determine that the $\underline{y}^+$ region of the I duplication be subject to such an extremely high frequency of deletions. In other words, <u>before</u> the $\underline{y}^+$ region of the I duplication can <u>eventually</u> be subject to an extremely high frequency of deletions in cultures carrying two duplications at 36°C, the intact III duplication must (by way of the major loss of chromosomal material from translocated duplicate III segment) be transformed into a III duplication of reduced size. Only through such a transformation is a particular duplication created which can eventually promote an extremely high frequency of deletions from a second duplication. It is important to stress that the reduced III duplication so created determines that the $\underline{y}^+$ region of the I duplication be subject to

an extremely high frequency of deletions at a particular stage or time in colony development, thereby indicating that the reduced III duplication has a programmed or regulatory effect on the I duplication. What is also of interest in this connection is that the major mutagenic effect (or influence) of the reduced III duplication upon the I duplication is confined to the translocated segment of the I duplication. That is, the major increase in the deletional instability of the I duplication as promoted by the reduced III duplication is confined to the translocated duplicate I segment. Moreover, according to some data in Part IV, it is quite likely that the $\underline{y^+}$ region of the translocated duplicate I segment is far more under the mutagenic influence of the reduced III duplication than is the bi1 region of the translocated duplicate I segment.

In regards to the induction of deletions in Aspergillus cultures carrying single duplications, one will recall that the duplications (chromosomal imbalances) in such cultures provoke their own deletional instability. That is, in cultures carrying single duplications, the underlying cause or regulation behind the induction of deletions from the single duplications in such cultures are the respective duplications themselves. In double-duplication cultures, on the other hand, where the reduced III duplication is present, the I duplication largely loses its ability to direct or control deletions from itself; it appears that the reduced III duplication takes over this function. That is, in a double-duplication strain where the reduced III duplication is present, it appears that the I duplication loses (to a large extent) its self-directing-mutagenic-capability, inasmuch as its capability of directing or provoking its own deletional instability becomes largely subsumed under the control of a second duplication, the reduced III duplication. It is as if such control represents

some kind of bond or tie between the two duplications. What is of interest here in regards to these two duplications is that we have one genetic-entity on the chromosomal level (as opposed to a single gene) programming or determining or directing an extremely high frequency of deletions from a second genetic-entity on the chromosomal level, or from another point of view, we have one chromosomal imbalance causing or inducing a second chromosomal imbalance to become extremely instable.

Before this present study, all information indicated that a chromosomal imbalance rarely provoked deletions outside the particular segments involved in the imbalance. This was based on a study of diploid strains with an extra chromosome segment. In these diploid strains with the chromosomal imbalance, deletions were confined almost exclusively to the chromosome segments present in triplicate (Nga and Roper, 1969, Roper and Nga, 1969). When deletions were not confined to these chromosome segments present in triplicate (the triplication), deletions affected (or included) a chromosomal region adjoining (or contiguously linked to) the triplication - chromosomal imbalance - in question. In this connection, the present study (Part II and Appendix B) showed (strongly suggested) that lethals and semi-lethals were in almost every case linked to either chromosomes I or V derived from 13 diploids formed between a III duplication strain (or a strain carrying a large part of the III duplication) and a normal haploid strain, while other studies showed (Azevado and Roper, 1967; Nga, 1967) that all chromosomes but one derived from 185 diploids formed between haploid strains not carrying any type of duplication[1] were found to be free of lethals and semi-lethals. This would suggest that a

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[1] 35 of these haploids carried translocations.

segment of chromosome III present in triplicate (a III triplication) can (at times) also provoke mutations to occur on either chromosomes I or V (in diploids), and some information in Part IV, taken in conjunction with the control-data would appear to provide further evidence of a III triplication having a mutagenic effect on chromosome V. As the mutations just referred to are collectively lethals and semi-lethals, it is very likely that the lethals are deletions while the semi-lethals are probably extremely small deletions. In view of what has been said, this would mean that a III triplication could (at times) indirectly induce deletions within either chromosomes I or V. Whether or not such lethals and semi-lethals are in fact deletions, a III triplication would appear, nevertheless, to have a mutagenic effect or influence upon chromosomes not linked to this III triplication, and in this respect would appear to be like the reduced III duplication, where such a reduced III duplication has a strong mutagenic effect or influence upon a chromosome segment not linked to this reduced III duplication. Hence, as well as a chromosomal imbalance in the form of the reduced III duplication in a double-duplication haploid being able to induce mutations (deletions) in a chromosome segment not linked to such a chromosomal imbalance, it would also appear that a chromosomal imbalance in the form of the III triplication in a diploid can induce (provoke) mutations (or deletions) in some chromosomes not linked to such a chromosomal imbalance.

In regards to the mutagenic effect that a reduced III duplication has upon the I duplication, information in a section of Part IV suggested that age has an indirect or ultimate influence on the mutagenic relationships between the reduced III duplication and the I duplication. While such information as a whole suggested that age (or agedness) can indirectly affect the

reduced III duplication's ability to induce deletions from the I duplication, some of this information also provided further confirmation that a reduced III duplication can determine that the y^+ region of the I duplication be subject to a high frequency of deletions. That is, some of this information further confirmed that a reduced III duplication can greatly enhance (at 36°C) the frequency of deletions from the I duplication. At a temperature above 36°C, it was seen that the reduced III duplication continues to enhance the frequency of those deletions which include the y^+ allele on the I duplication, and that at this higher temperature, it seems that the reduced III promotes deletions from the I duplication according to some programme. Though growth at this higher temperature also had a definite effect upon I duplication-instability in double-duplication cultures, the data did not appear to indicate that the influence of the reduced III duplication upon the I duplication had become more effective in the promotion of deletions from the I duplication at this higher temperature than at the lower temperature. In double-duplication cultures of the F1 generation, the reduced III duplication is seen to continue to have a mutagenic effect or influence on the I duplication. That is, in F1 generation-double-duplication cultures, the reduced III duplication is seen to continue to enhance the frequency of deletions which include the y^+ allele on the I duplication, again underlying the fact that the frequency of deletions which occur from one duplication can be governed or controlled by a second duplication.

In general terms, the findings of the present work indicated that genotype and environment (in this case temperature) interact to determine the degree, scope, period, and pattern of chromosomal instability in A. nidulans. Clearly, the ultimate

aim must be to explain all these findings in molecular terms; however, at this time, we are far from being in a position to do so. For the present, it may be profitable to explore possible similarities between the above effects observed in Aspergillus and effects observed, under a variety of circumstances, in other organisms. A range of parallels can be found, and the present discussion will deal with some of these.

Like instability in A. nidulans, instability (or mutability) in other organisms is governed or affected by environmental and genetic factors. In two higher plants, Portulaca and Antirrhinum, the instability of pigment genes is markedly enhanced by growth at lower temperatures (Beale and Fabergé, 1941; Harrison and Fincham, 1964). In Antirrhinum, the pigment allele, pal^{rec}, becomes very unstable under certain temperature-conditions and in certain genetic backgrounds. Under other temperature-conditions, and in other genetic backgrounds, the same allele can be relatively stable. With respect to the effect of genetic factors on mutation, Ives (1950) showed that in certain lines of Drosophila melanogaster there is a gene, hi, located on chromosome III which affects the mutation rate of many genes, but not all of them with equal frequency. It increases the general mutation rate up to ten times the normal rate. Whether hi acts as a dominant or a recessive appears to depend on the genotype within which it is acting. Moreover, in some lines, its effect on the mutation rate falls of rapidly to, or close to, the normal mutation rate as the males grow old, thereby indicating that old age can greatly curb or depress the mutagenic action of hi. With regards to the effect of age on mutation, Demerec (1928) showed that in Drosophila virilis the instability of a particular gene in the female is markedly

reduced if the female is of old age, thus indicating that old age can markedly reduce or depress the instability of this particular gene in Drosophila virilis. In regards to the effect of temperature on mutation in Drosophila, high temperature also increases the mutation rate of genes (Muller, 1928; Timofieff-Ressovsky, 1935; and Plough, 1941), and in this connection high temperature has a definite influence upon the somatic instability in cultures derived from hybrids between Nicotiana langdorffii and Nicotiana sanderae (Sand, 1957). In these cultures, two alleles, v_s and v_S, which affect flower colour, are unstable. v_S determines the production of pigment; v_s inhibits the production of pigment. These unstable alleles undergo reversible changes between their alternative forms. The effect of higher temperature is to increase the rate of change from v_S to v_s but to decrease the reverse change. What is also of interest here in regards to instability in Aspergillus is that the change from v_S to v_s occurs during a particular period in development, suggesting some type of internal regulation. In this connection, Demerec (1931) showed that the lavender-alpha gene in Delphinium had a high mutability during early and late ontogeny, and a very low mutability during the mid-stages of ontogeny; in Delphinium, this would suggest an internal regulation of mutability or instability.

A most spectacular case of gene instability has been studied in maize by Rhoades (1941) and is quite relevant to the instability of Aspergillus cultures carrying the two duplications. The dominant allele of a gene, A1, in maize, gives rise to a purple colouration in various parts of the plant, whereas the recessive allele, a1, gives green plants. Under normal conditions both alleles mutate very infrequently. But in plants

which carry the dominant gene Dt (Dotted, located in a different linkage group from that which carries the locus A1-a1), the gene a1 mutates to A1 so frequently that the plant has green leaves streaked with purple and seeds speckled with purple dots. The gene Dt has no other known effects apart from making the gene a1 highly unstable. The mutational stability of the allele A1 is not affected by Dt. Furthermore, in the Dt-a1 system, a1 instability is markedly affected by differences in temperature.

What is of extreme interest in view of the double-duplication system in Aspergillus is the discovery by McClintock (1951) of a two-factor system in maize which determines the frequent induction of chromosome deletions. One genetic factor of this system, Ds, can by moving from one location in the chromosome complement to another induce deletions from various chromosomes. However, Ds will only induce deletions throughout the genome if a second genetic factor, Ac, is present somewhere in the genome; the particular location of Ac is not relevant. If Ac is absent from the nucleus, or genome, Ds will not induce deletions wherever it may be located, and no new positions of Ds activity appear. In effect Ac determines that Ds cause frequent deletions throughout the maize genome by having Ds move from one chromosome region to another. The release of Ds from a given chromosome region results in (or is connected to) the deletion of that region. According to McClintock, the Ac element will determine Ds to induce deletions (at given regions) in accord with a temporal-programme. What is also of interest in regards to Ac is that Ac can also cause five genes (not linked to Ac) to become highly unstable. In the absence of Ac, these genes are stable. When Ac is present, according to McClintock, Ac will have frequent mutations (changes) occur at these respective loci

in accord with a temporal-programme. In other words, frequent mutations at these respective loci are Ac-controlled or Ac-determined. There is also strong evidence that Ac causes frequent mutations (changes) at these loci by frequently directing the Ds factor to be inserted and removed from these loci. Subsequent work by McClintock (1956, 1961, 1965) and work by Peterson (1960) showed that regulated-instability of many genes (loci) in maize is based upon the presence of different controlling elements such as Dt and Ac directing Ds-type factors to be inserted and removed from such genes (or from regions adjacent to such genes) according to some temporal-programme. The instability in maize based upon these two-part mutagenic-systems is also, like the instability in Aspergillus, affected by temperature differences and the age of the tissue.

The phenotypic-mosaicism resulting from instability in higher plants bears a striking similarity to position-effect variegation (P-EV) in Drosophila, and in this connection, there is a striking parallel between the phenotypic mosaicism resulting from deletional instability in Aspergillus and P-EV. The preceding might suggest an underlying connection between the instability in higher plants, instability in Aspergillus, and P-EV. Moreover, as the incidence (or degree) of P-EV in Drosophila,[2] the degree of instability in higher plants, and the degree of deletional instability in Aspergillus are respectively affected by temperature differences, there might be further suggestion that there exists an underlying connection between the instability in Aspergillus, instability in higher

2.Temperature differences affect the incidence or degree of P-EV in Drosophila (Gowen and Gay, 1933a; Noujdin, 1945; Shultz, 1956).

plants, and P-EV. The many parallels (referred to recently) between the instability-situations in higher plants and the instability-situations in Aspergillus may in fact show that there at least exists more of a basis for believing that an underlying connection exists between instability in Aspergillus and instability in higher plants. In order to further explore the possibility of such an underlying connection between instability in Aspergillus, position-effect variegation in Drosophila, and instability in higher plants, and perhaps arrive at some idea of what such an underlying connection might be, it is best at this point to first consider the phenomenon of position-effect variegation in relation to instability in A. nidulans.

As one will recall, P-EV may arise following transposition of an euchromatic segment to a region of heterochromatin. The effect has been studied very thoroughly in Drosophila (see Lewis, 1950; Baker, 1968) and such variegation is attributed to suppression by heterochromatin in some cell lineages of certain of the genes in the translocated chromatin. In Drosophila a single individual may show complex variegation involving the effects of one and more than one gene. This spreading effect (Muller, 1930; Demerec and Slizynska, 1937) is interpreted as polorized suppression extending from the original heterochromatin to a variable number of euchromatic genes on the translocated segment. The generally held view is that such suppression occurs through progessive heterochromatization extending from the original heterochromatin to a variable number of genes on the translocated segment. Morphologically, in larval salivary gland chromosomes, a section of the transposed euchromatin in the P-EV system does take on a heterochromatic appearance in various cells (Caspersson and Schultz, 1938; Prokofieva-

Belgovskaya, 1941), thus providing cytological evidence that P-EV is effected by way of the heterochromatization of transposed euchromatic regions. In this connection, work by Hartmann-Goldstein (1967) also provides strong evidence that P-Ev is effected by way of the heterochromatization of regions of the transposed euchromatin. In P-EV the expression of the untranslocated genes is rarely affected and the phenomenon thus shows a type of cis-trans effect. Deletional instability in the duplication bearing cultures of A. nidulans also shows a cis-trans effect insofar as most deletions only involve regions of the translocated duplicate segment in such cultures. This would thus point to another strong similarity between the two phenomena of P-Ev and deletional instability. The fact that both P-EV and deletional instability are affected by temperature differences would point to still another similarity between the two phenomena.

Because the mutant sectors in P-EV are clonal (Baker, 1967, 1968), the suppression of genes within somatic cell lineages as manifested by P-EV must be irreversible. In view of the parallels between deletional instability in Aspergillus and P-EV, deletions of the induced heterochromatin on the translocated chromosome segment in Drosophila would explain the irreversibility of suppression, and thereby the irreversible variegation of somatic tissue in Drosophila. In this connection, the work of Burns and Gerstel (1967), which they do not interpret as P-EV, is relevant. In a Nicotiana hybrid, a block of heterochromatin (in somatic tissue) showed mitotic instability and was subject to deletions of variable size; variegation resulted when the deletion included a flower colour locus in the contiguous euchromatin. In the cases described as P-Ev, and in other, possibly related situations, there is no

definite evidence that the inactivated genes are still present. Yet genes susceptible to variegation usually show germinal stability (Baker, 1968). If variegation stems from the deletional instability of heterochromatin, as appears likely in view of what has been said, then the germ line may be protected, since a chromosome region need not be heterochromatic in all tissues (Lima-de-Faria and Jaworska, 1968; Priest, 1968). A variation of such protection operates in, for example, Cyclops (a copepod), in which there is an interstitial loss of heterochromatin in somatic cells during embryogenisis, while the chromosomes in the primordial germ cells remain unchanged, heterochromatin being present in germ line cells at all stages of development (Beermann, 1966). In the coccids (homopteran insects), a somewhat different situation with respect to heterochromatin prevails, but nevertheless provides further support for the view that deletion (or elimination) of induced heterochromatin can be the basis of P-EV.

> "The heterochromatin in the coccids... is restricted to the male sex; it is formed anew in each generation during early embryogeny and discarded at the completion of meiosis. The first definite analysis of a coccid chromosome system of this sort was made by Schrader (1929) who observed that one entire chromosome set became heterochromatic during the early development of the male and remained so throughout his life. Meiosis was modified so that the heterochromatic set was segregated from the euchromatic set during the second meiotic mitosis. Only the euchromatic set proceeded to form sperm; the heterochromatic derivative slowly degenerated."[3]

Like in the case of Cyclops, the dissolution or elimination of heterochromatin in coccids appears to be under a developmental control; the cytoplasmic or physiological environment of the heterochromatin would appear to play an important role in such control.[4] In regards to P-EV, it would seem, in view of what

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3. Brown, 1969.
4. In fact, it is known that the cytoplasm plays a regulatory role in development (Waddington, 1956; Fischberg and Blackler, 1961; Ebert, 1965).

has been said, that the translocated genes in the germinal tissue of Drosophila are not susceptible to variegation - as they are in somatic tissue - because the particular cytoplasmic or physiological environment inherent in the germinal tissue of Drosophila precludes the heterochromatization and subsequent deletion of regions including such genes in the germinal tissue.

From an overall point of view, it would appear from the preceding information that deletion or elimination of chromosomal material bears a fundamental relationship to heterochromatization (or heterochromatin). More specifically, it is probable, in light of previous information regarding heterochromatization and deletion (or elimination) of chromosomal material, that heterochromatization of given chromosomal regions must occur (in various organisms) before such regions could be subject to deletion or elimination or dissolution. In other words, heterochromatization, under given cytoplasmic or physiological conditions, and in various organisms, will probably lead to the deletion or elimination of the chromosomal material which had become heterochromatic. That is, heterochromatization is probably a necessary precondition to the occurrence of deletional (or eliminative) processes in various organisms. Though there is no cytological evidence of heterochromatin in Aspergillus, it is nevertheless probable, in light of what has been related, that heterochromatization of the regions of the duplications in Aspergillus forms the basis of the frequent deletions from those duplications. A duplication would provoke (induce) heterochromatization within a section or sections of itself and the induced-heterochromatin would subsequently undergo

deletion.[5] A commonality between instability in Aspergillus, P-EV in Drosophila and loss of chromatin in other organisms could thus be heterochromatization of chromosomal material leading to deletion or elimination of such chromosomal material. Though Nga and Roper (1968) proposed that a form of crossing-over within intrachromosomal loops could explain the mechanism of deletion in Aspergillus, this need not preclude the view that (in different organisms) heterochromatization could be a necessary precursor to an intrachromosomal cross-over process resulting in the deletion or elimination of chromosomal material. Such a connection between heterochromatization and a form of crossing-over within intrachromosomal loops would seem quite feasible inasmuch as heterochromatization involves intense coiling of chromosome regions. In fact Beermann (1966) proposed that the loss of heterochromatin in Cyclops occurred through the formation of intrachromosomal loops.

In the same way that the physiological or cytoplasmic environment in the somatic cells of Cyclops appears to allow the deletion of heterochromatin in such cells, the physiological or cytoplasmic environment inherent in the somatic or vegetative cells of Aspergillus-duplication-cultures could be seen as allowing the deletion of induced-heterochromatin in such cells; thus the incidence of heterochromatization could be seen as being synomonous to the incidence of deletions. The incidence (frequency) of induced-heterochromatin in duplication-cultures, and thereby the incidence of resulting-deletions, could, in light of earlier information, depend not only on the type of duplication underlying such heterochromatization and deletion,

5. Heterochromatization being mostly confined to the translocated segment of a duplication would explain why deletions are generally (mostly) confined to regions of the translocated duplicate segment.

but on the overall interaction between genotype and environment. The effects of temperature upon instability in Aspergillus might thus be seen as an indirect temperature (environmental) influence on the incidence of heterochromatization. This would appear to be especially the case insofar as the incidence of heterochromatization in the P-EV system of Drosophila is, like the variegation itself, affected by temperature differences[6] (Shultz, 1941; Prokofieva-Belgovskaya, 1947; Hartmann-Goldstein, 1967). In regards to the effects of temperature upon Aspergillus-instability, the particular frequency (incidence) with which regions of a duplication would become heterochromatic, and thereby the particular frequency of resulting deletions which would encompass such regions, could be seen as depending (in effect) on the particular pattern or continuum of specific physiological processes allowed to exist by a particular temperature of growth. Therefore, in accord with this line of thinking, and taking I duplication cultures as an example, the particular continuum of specific physiological or cytoplasmic processes allowed by a temperature of 39.5°C could be seen to permit (in effect) the induction of the highest incidence of heterochromatization as far as I duplication regions are generally concerned, and as a necessary consequence the highest incidence of deletions. The particular continua (or patterns) of physiological or cytoplasmic processes allowed respectively by temperatures either lower or higher than 39.5°C could be seen (in effect) to be only conducive to the induction of a lower incidence of deletions. The particular pattern (or continuum) of physiological or cytoplasmic processes allowed by a temperature of 42°C could be seen, however, to be far more conducive to or

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6. That temperature differences affect the incidence of heterochromatization in the P-EV system would have to be the case if temperature differences affect the incidence or degree of P-EV.

permit the induction of a far higher incidence of heterochromatization of I duplication regions - and thereby the induction of a far higher incidence of deletions - than the particular continua of physiological or cytoplasmic processes allowed respectively by temperatures of 36°C and 28°C.

In this connection, one will recall that the y^+ region of the I duplication is at 42°C most susceptible to deletion during a particular period of growth. Keeping with present thinking, this could be explained in terms of the influence of changing physiological or cytoplasmic processes upon heterochromatization during growth at 42°C. Before this particular period of growth, the physiological or cytoplasmic processes could be such as to inhibit heterochromatization of the y^+ region - and thereby the deletion thereof - from occurring with a high incidence, while such physiological or cytoplasmic processes could change (as a result of a genotypic-environmental interaction) through time or through growth so as to become of such a nature during a particular period of growth that they allow heterochromatization of the y^+ region - and thereby the deletion thereof - to occur with a high incidence. The fact that the *s12*$^+$ region of the III duplication is at 42°C most susceptible to deletion during a particular period of growth could also be explained in terms of the differential influence of changing physiological or cytoplasmic processes upon heterochromatization during growth at 42°C.

In general, physiological or cytoplasmic processes in *Aspergillus*-duplication-cultures could be seen as affecting the incidence of deletions by way of affecting the incidence of heterochromatization. The pattern of such physiological or cytoplasmic processes could be seen as being changed by alterating

either the environmental or genetic conditions, with the eventual consequence being that the incidence of deletions from a duplication would be changed, as well. We have seen how changing the genetic conditions through the doubling of the number of duplications can enhance the frequency of deletions from one of those duplications. This of course leads into the question of why the addition (or introduction) of a second duplication into a genotype already having a duplication can increase the deletional instability of the first duplication. In terms of the probable connection between heterochromatization and deletion, the bare framework of one possible explaination may be erected:

Within double-duplication cultures, the intact III duplication could, by way of provoking (inducing) the heterochromatization of its own sections, also increase indirectly the incidence of heterochromatization of the $\underline{y}^+$ region of the I duplication, and thereby increase the incidence of deletions from a section of the translocated duplicate I segment. In comparison, the reduced III duplication could, by way of provoking the heterochromatization of its own regions, also increase to a far greater degree than the intact III duplication, the incidence of heterochromatization of the $\underline{y}^+$ region of the I duplication, and thereby increase to a far greater degree than the intact III duplication, the incidence of deletions from a section of the translocated duplicate I segment. In effect, either heterochromatization of sections of the intact III duplication or heterochromatization of regions of the reduced III duplication could be seen as playing an essential role in regulating the incidence of heterochromatization - and thereby the incidence of deletions - of the $\underline{y}^+$ region of the I duplication.

Through the heterochromatization of its sections, the intact III duplication could be seen as determining, for a particular period of growth, a particular concatenation (or nexus) of physiological processes, which, before the end of such a particular period of growth, could increase to a small though significant degree, the incidence of heterochromatization of the y^+ region, and thereby increase to a small though significant degree, the incidence of deletions from a section of the translocated duplicate I segment. Subsequent to this period of growth, the concatenation of physiological processes will have become changed (through an overall environmental-genotypic interaction), in such a way that an earlier created reduced III duplication - and a major factor in the development of the changed concaternation of physiological processes - would eventually be allowed at a particular growth stage to heterochromatize its own regions, and in so doing, greatly enhance the incidence of heterochromatization of the y^+ region of the I duplication - and thereby, greatly enhance, at a particular time, the incidence of deletions from a section of the I duplication.

Important observations regarding P-EV in Drosophila would appear to demonstrate the feasibility of the hypothesis (or explanation) just considered. One knows that the incidence or degree of P-EV - and thus the incidence of heterochromatization - can be influenced by a heterochromatic Y chromosome. In almost all cases the addition of a heterochromatic Y chromosome depresses the incidence or degree of P-EV (see Gowen and Gay, 1933b; Baker, 1968), and thereby depresses the degree or incidence of heterochromatization. However,

> "there is one long-known exception to this general rule, the light (lt) locus of D. melanogaster. As pointed out by Shultz (1936) the effect of /heterochromatic/ Y chromosomes is diametrically opposed to the usual situation: addition of /heterochromatic/ Y's enhances the mutant tissue in the light-variegated eyes whereas the deletion of /heterochromatic/ Y's suppresses the mutant tissue. It has been critically shown (Baker and Rein, 1962) that this is indeed an opposite effect. One and the same /heterochromatic/ Y chromosome, or fragments thereof, was introduced by single individual males into a genotype which would show lt variegation and then into two genotypes which would exhibit white variegation. Quantitative measurements were made of the pteridines responsible for the drosopterin pigments in the variegated eyes. The results were unequivocal: the same /heterochromatic/ Y fragments that were most effective in suppressing the mutant tissue in the white variegated genotypes were the ones most effective in enhancing the mutant phenotype in the light system,"7

and thus the heterochromatic Y elements most effective in enhancing the degree or incidence or amount of heterochromatization in the light P-EV system.

These observations might also suggest the feasibility of another hypothesis (or explanation) regarding the manner by which one duplication in Aspergillus can enhance the deletional instability of a second duplication. Considering such a hypothesis, the following picture comes into view. Through the heterochromatization of its sections, the intact III duplication could again be seen as determining for a particular period of growth a particular concatenation of physiological processes, which before the end of such a particular period of growth, could increase to a small though significant degree, the incidence of heterochromatization of the y^{+} region, and thereby increase to a small though significant degree the incidence of deletions from a section of the translocated duplicate I segment. However, during the period, when the III duplication becomes

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7. Baker, 1968.

greatly reduced in size as a result of the loss of chromosomal material, a very small, unincorporated heterochromatic-segment originating from the once-intact III duplication would be seen as being inserted next to the y^+_- region of the I duplication,[8] in the same manner in which F factors and transducing viruses may be incorporated into bacterial chromosomes (see Hayes, 1968). Subsequent to this period of growth, the concatenation of physiological processes will have become changed through an overall environmental-genotypic interaction, in which the reduced III duplication will have played a major role. As a result of this change, the reduced III duplication (the remainder of the III duplication) would at a particular stage in late growth be allowed to heterochromatize its own regions, and in so doing, cause the heterochromatic-III-segment inserted next to the y^+_- region to trigger with a very high incidence the heterochromatization - and thereby the deletion - of the y^+_- region, with the deletion of the y^+_- region being accompanied by the deletion of the inserted III-segment. Thus, in effect, long after a very small segment from the once-intact III duplication becomes inserted next to the y^+_- region of the I duplication, heterochromatization of regions of the reduced III duplication could be seen to induce with a very high incidence the release (deletion) of this segment along with some I duplication genes joined to it. The release (deletion) of such a thread of genetic material would be similar or analogous to the release of a transducing phage from a bacterial chromosome.

In this connection, the release of the Ds factor in maize from various chromosome regions results in (or is connected to) the deletions of those chromosome regions. The release of such a

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8. Work by Azevado and Roper (1970) in fact revealed probable transposable elements in Aspergillus nidulans.

factor, as one will recall, is triggered by or is controlled by a second factor, Ac. In the same way that Ac can have Ds induce frequent deletions from various chromosomes in maize, the reduced III duplication (in Aspergillus) could be seen as having the very small heterochromatic-III-segment induce a high frequency of deletions from (a section of) the I duplication. Therefore, in this respect, Ac in maize becomes analogous to the reduced III duplication in Aspergillus, and the Ds factor in maize becomes analogous to the very small heterochromatic-III-segment in Aspergillus. Even if such a heterochromatic-III-segment does not exist, the reduced III duplication could, in general, still be seen as being analogous to Ac in maize, inasmuch as it can evoke a very high frequency of deletions from a chromosome section not linked to it. The reduced III duplication could thus be regarded as being a complex controlling-element in Aspergillus, but a complex controlling-element whose regulation of the instability of the unlinked genetic factor, the I duplication, probably involves heterochromatization of the element's regions. For that matter, the intact III duplication could also be regarded as being a controlling-element, like Ac, but it would be seen as being a complex controlling-element far less effective than the reduced III duplication, but one which would eventually give rise through its own instability to the far more effective controlling-element.

If heterochromatization of regions of an Ac-like element in the double-duplication system of Aspergillus does in fact play an essential role in regulating the likely heterochromatic-based instability of a second genetic element (factor), one might ask whether or not heterochromatization or heterochromatin plays some role in the instability systems in maize, such as in the Ac-Ds

system. According to McClintock (1951), various observations, such as the Rhoade's Dt controlling-element being within a heterochromatic region, combined with many experiments indicate that the controlling-elements responsible for determining or regulating the various types of instability in maize are heterochromatic, and that the particular heterochromatic nature of these elements, such as Ac and Ds, plays a major role in enabling these elements to determine or regulate instability in maize. According to McClintock, the controlling-elements were derived from a heterochromatic source through a common process in which the breakage-fusion-bridge-cycle was directly involved. Through this process, the organization of heterochromatic chromosome-sections was changed, giving rise to elements whose reorganized-heterochromatin gave them the capability of determining various types (forms) of instability in maize. Thus, heterochromatization would be seen as being involved in - or playing an essential role - the determination of the various types of instability in maize, such as the frequent induction of deletions from different chromosomes. Inasmuch as the instability of chromosome-regions and loci in maize embodies the insertion and release (deletion) of genetic material, it is quite likely that instability in maize (in various cases) is the direct result of cross-over-type processes like those that must be involved in the incorporation and release of transducing viruses and F factors from bacterial chromosomes. If this happens to be the case, then the heterochromatin of the controlling-elements in maize would be seen as playing a critical role in determining - or regulating - such cross-over-type processes.

At any rate, now that it is clear that heterochromatin is involved in - or plays an essential role in - the determination of instability in maize, it would seem feasible that it also plays

a role in the determination of instability in other higher plants, such as Antirrhinum and Nicotiana. In fact, Smith and Sand (1957) proposed that instability at the v locus in Nicotiana is associated with cell heterochromatin. Because Fincham (1967) proposed that the high instability of the pal^{rec} allele in Antirrhinum is due to intrachromosomal cross-over processes in which the exision and insertion of genetic material is involved, one might also suggest that the induction of such intrachromosomal cross-over processes is dependent on or determined by heterochromatization or heterochromatin. In fact, the instability of pigment genes in such higher plants as Nicotiana and Delphinium might very well be due to intrachromosomal cross-over processes (like those proposed by Fincham) whose induction is dependent on or determined by heterochromatization or heterochromatin. One will recall in this connection how in Nicotiana a block of heterochromatin undergoes deletions, and the heterochromatin could very well be seen as undergoing such deletions as a result of intrachromosomal cross-over processes being induced (or evoked) by the heterochromatin itself. This of course brings us back to Aspergillus in which the occurrence of frequent deletions has been regarded to be the result of intrachromosomal cross-over processes determinedor regulated by heterochromatization or heterochromatin. In view of what has been said, an underlying connection between instability in Aspergillus, P-EV in Drosophila, and instability in higher plants can feasibly be seen as being heterochromatin or heterochromatization. In other words, it could be seen that common to instability in higher plants, P-EV in Drosophila, and instability in Aspergillus are mutational-type or cross-over-type processes whose induction is dependent upon the presence or creation of heterochromatin.

In regards to the initiation of instability, Mampel (1943, 1945, 1946) described a case in Drosophila pseudoobscura which is quite relevant to what has been said. In this Drosophila case, a heterochromatic element appeared to be associated with the initiation of instability at another locus. In this connection, it is feasible that the hi mutator in Drosophila melanogaster (referred to earlier) is heterochromatic and that it is its heterochromatic properties which gives it the capability to induce a high frequency of mutations at other loci. From an overall point of view, various types of instabilities or mutabilities in a number of different organisms might very well be associated with or dependent upon heterochromatin. Not only might heterochromatin be involved in various types of instability but it might also play a role in development in higher animals.

What role in fact might heterochromatin or heterochromatization play in development? To best answer this question, one should first recall that in development the cytoplasm has a regulatory role. In the case of Ascaris (a nematode), one knows that chromosome intergrity in development depends upon the particular cytoplasm (cytoplasmic environment) in which nuclei exist.

> "From the two-cell stage onwards, the chromosomes in most cells [of Ascaris] break up into small fragments, each provided with only one centromere and the distal centromere-less ends (which are heterochromatic) are thrown out of the nucleus into the cytoplasm. Only in that lineage of cells which eventually gives rise to the germ cells do the chromosomes retain their original configuration...Boveri, who was the first to describe this process of chromosome diminution, showed by a study of abnormal cleavages in dispermic and centrifuged eggs that the retention of the original [chromosome] structure is dependent on the type of cytoplasm into which the nucleus moves."9

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9. Waddington, 1956.

In the development of the gall midge, it is known (see Fischberg and Blackler, 1961) that a certain kind of cytoplasm allows particular chromosomes to be eliminated (by way of dissolution) from the somatic-cell lineage, while the particular cytoplasm of the germ cells prevents the elimination (dissolution) of such chromosomes in the germ line, and in so doing prevents irreversible nuclear changes from occurring in the germ tissue. In various species of the frog, one knows through the experiments of Briggs, King, Gurdon, Fischberg, and Blackler (again see Fischberg and Blackler, 1961) that somatic nuclei as opposed to germ-cell nuclei, change during development or differentiation. They seem to lose their totipotentiality and become more limited in their ability to promote normal development. Experiments by some of these researchers indicated that the nuclear changes due to natural differentiation in the frog are relatively stable and of a heritable nature. Such irreversible nuclear changes occur in the somatic cells of this complex organism only after a certain stage or period during development; the same nuclear changes occur in different cells of a developing tissue during different periods. In view of what has been said in regards to Ascaris, Nicotiana (P.250), the gall midge, and Cyclops, it is likely that the particular cytoplasmic processes or factors in the somatic cells of the frog determine or allow such irreversible nuclear changes on the chromosomal level, and that such nuclear changes in the frog involve deletion of genetic material, likely preceded out of necessity by heterochromatization of such genetic material. Just as a particular cytoplasm can prevent either chromosome dissolution or chromosome diminution in germ-line cells of certain organisms, it is likely that the cytoplasm of the germ cells in other, more complex organisms, such as the frog, plays a

comparable role in protecting or preventing nuclei or chromosomes in the germ cells of such complex organisms from undergoing irreversible changes, changes which would cost these germ cells their totipotentiality. According to Fischberg and Blackler, there is in fact some direct evidence that the cytoplasm in the germ cells of the frog prevents the induction of irreversible nuclear (or chromosomal) changes. In general, it is quite likely that irreversible chromosomal changes occur in somatic tissues as an aspect or feature of development in various kinds of organisms, and that the occurrence of such irreversible chromosomal changes probably involves first heterochromatization and then a mutational process - such as a deletional process - triggered by heterochromatization.

Insofar as the frequent induction of chromosomal or genetic changes could be seen as being an important aspect of development (or differentiation) in complex organisms, then most (if not all) of the instability-systems that have been considered may be basically related to the developmental-situations which promote those chromosomal changes during differentiation. This would be especially so in view of the likelihood that the occurrence of genetic changes as an aspect or feature of development in complex organisms and the generation of various instabilities are dependent upon the presence or creation of heterochromatin. In fact, many of the instabilities that have been considered may be regarded as being representative of early, though imperfect, forms of developmental processes which promote (in simpler organisms) types of differentiation through the induction of frequent genetic or chromosomal changes, such as deletions.

The double-duplication instability-system in Aspergillus would be a very good example of an early type of developmental-system which, in a programmed manner, was able to determine a type of differentiation through the induction of irreversible chromosomal changes in somatic (vegetative) tissue. This instability-system, which by necessity probably involves heterochromatin or heterochromatization, owes its ultimate origin to basic chromosomal reorganizations in the genome as exemplified by the existance of chromosome imbalances (or non-tandum duplications of genetic material) in a haploid genome. Many other types of chromosomal reorganization in other types of organisms may have been directly associated with - or may have resulted in - instability-systems based upon heterochromatin, systems which could represent the first forms of developmental-systems that effect types of differentiation through the induction of chromosomal or genetic changes. We have seen in maize how chromosomal reorganizations ultimately resulted in heterochromatin-dependent instability-systems, and these systems would also be other examples of early forms of developmental-systems which effect types of differentiation through the induction of chromosomal or genetic changes.

Considering what has been said, then, particular chromosomal reorganizations in the distant past may very well have resulted in heterochromatin-dependent instability-systems, and various combinations of these instability-systems may have in turn evolved into complex developmental-systems based upon the regulated induction of chromosomal or genetic changes, changes (mutations) which would have been dependent upon heterochromatization. In other words, various heterochromatin-dependent instability-systems, or more ultimately, particular chromosomal reorganizations, may have provided in the distant past the basis for the evolution

of complex types of developmental-systems in which heterochromatin-induced genetic changes of various types would play a major role. The very rate of such an evolution may have been greatly affected by the instability-systems in question, insofar as it is certain that such instability-systems would have also determined a very high degree of genetic and phenotypic variability over numerous generations. In general, instability-systems of various types could be seen as being factors of prime importance in evolution because of their capability for generating a very high degree of variability within populations.

A P P E N D I X

Appendix A

Differential selection of improved sectors at different temperatures means or indicates that sectors arising at temperature A have greater selective advantage over their parent (duplication-strain which gives rise to mutant sectors), at temperature A, than do temperature B sectors (sectors arising at temperature B) over their parent at temperature B. Being so, then differences in selective pressures would be reflected in the growth-rates of parents (parental-colonies) and sectors (colonial subcultures of improved sectors - sector-colonies) at the different temperatures. That is, differences in selective pressures would be reflected or expressed in the following terms: each of the ratios of sector-colony-growth-rates to parental-colony-growth-rates found for temperature A would in given sequence be greatly (significantly) different from each of the sequential ratios of sector-colony-growth-rates to parental-colony-growth-rates found for temperature B. In other words, each of such ratios calculated for temperature A and in turn based on a particular incubation period would in given sequence be significantly larger (greater) than each of such consecutive ratios calculated for temperature B. If differential selection was not operating, each of such ratios calculated for temperature A and based in turn on a particular incubation period would not in given sequence be significantly different from each of such consecutive ratios calculated for temperature B.

For example:

let $\frac{\text{Mean diameter of sector-colonies/one day incubation}}{\text{Mean diameter of parental-colonies/one day incubation}}$,

ratio, at temperature A be found to equal a_1;

let $\frac{\text{Mean diameter of sector-colonies/one day incubation}}{\text{Mean diameter of parental-colonies/one day incubation}}$,

ratio, at temperature B be found to equal b_1;

let $\frac{\text{Mean diameter of sector-colonies/two days incubation}}{\text{Mean diameter of parental-colonies/two days incubation}}$,

ratio, at temperature A be found to equal a_2;

let $\frac{\text{Mean diameter of sector-colonies/two days incubation}}{\text{Mean diameter of parental-colonies/two days incubation}}$,

ratio, at temperature B be found to equal b_2;

...let $\frac{\text{Mean diameter of sector-colonies/n days incubation}}{\text{Mean diameter of parental-colonies/n days incubation}}$,

ratio, at temperature A be found to equal a_n;

let $\frac{\text{Mean diameter of sector-colonies/n days incubation}}{\text{Mean diameter of parental-colonies/n days incubation}}$,

ratio, at temperature B be found to equal b_n.

Then if, a_1 is not significantly different from b_1, a_2 is not significantly different from $b_2 \ldots a_n$ is not significantly different from b_n, one can conclude that sectors arising at temperatures A and B are not subject to differential selection, i.e., sectors arising at temperature A are not at a greater selective advantage - or are not at a less selective advantage - than sectors arising at temperature B.

If however, a_1 is significantly greater than b_1, a_2 is significantly greater than $b_2 \ldots a_n$ is significantly greater than b_n, one can conclude that sectors arising at temperature A are at a greater selective advantage than sectors arising at temperature B.

The previous rationale (or method) was used as a means to determine whether improved sectors arising from any duplication strain are subject at different temperatures to differential selection (differential selection pressures): Tables a to d.

Data in the Tables clearly indicates that improved sectors (of any examined type) arising from any of the four given duplication strains are not subject at different temperatures to differential selection, i.e., improved sectors (of any examined type) arising at any given temperature from any of the four given duplication strains are not at a greater selective advantage - or are not at a less selective advantage - than (compared to) improved sectors arising at any other temperature.

In general, such data would indicate that improved sectors of any type arising from any duplication strain are not subject at different temperatures to differential selection.

T A B L E a

42°C:	Ry	v	$\frac{v}{Ry}$	36°C:	Ry*	v	$\frac{v}{Ry}$	28°C:	Ry/	v	$\frac{v}{Ry}$
Mean diameter:	$(\bar{M}1)$	$(\bar{M}2)$	$\left[\frac{\bar{M}2}{\bar{M}1}\right]$		$(\bar{M}3)$	$(\bar{M}4)$	$\left[\frac{\bar{M}4}{\bar{M}3}\right]$		$(\bar{M}5)$	$(\bar{M}6)$	$\left[\frac{\bar{M}6}{\bar{M}5}\right]$
One day incubation	.3cm	.7cm	2.33		.4cm	.8cm	2.00		.3cm	.6cm	2.00
Two days incubation	.9cm	1.4cm	1.55		1.2cm	1.8cm	1.50		.8cm	1.3cm	1.62
Three days incubation	1.5cm	2.2cm	1.46		2.1cm	3.0cm	1.42		1.2cm	1.8cm	1.50
Four days incubation	1.9cm	2.8cm	1.47		3.3cm	4.4cm	1.33		1.7cm	2.4cm	1.41
Five days incubation	2.4cm	3.4cm	1.41		.	.	.		2.2cm	3.2cm	1.45

Ry = colonies of (from) the III duplication strain, Ry: sixteen colonies at each given temperature.

v = colonies of (from) variants (improved sectors) produced by Ry: at each given temperature, two colonies from each of eight different v sectors.

*No discernible parental region after four days incubation.

/No discernible parental region after five days incubation.

T A B L E b

	42°C:Rg	r	$\frac{r}{Rg}$	36°C:	Rg*	r	$\frac{r}{Rg}$	28°C:	Rg✓	r	$\frac{r}{Rg}$
Mean diameter:	($\bar{M}7$)	($\bar{M}8$)	$\left[\frac{\bar{M}8}{\bar{M}7}\right]$		($\bar{M}9$)	($\bar{M}10$)	$\left[\frac{\bar{M}10}{\bar{M}9}\right]$		($\bar{M}11$)	($\bar{M}12$)	$\left[\frac{\bar{M}12}{\bar{M}11}\right]$
One day incubation	.3cm	.4cm	1.33		.5cm	.7cm	1.40		.3cm	.4cm	1.33
Two days incubation	.9cm	1.1cm	1.22		1.3cm	1.6cm	1.23		.8cm	1.0cm	1.25
Three days incubation	1.4cm	1.8cm	1.28		2.3cm	2.9cm	1.26		1.2cm	1.5cm	1.25
Four days incubation	2.0cm	2.6cm	1.30		3.2cm	4.1cm	1.28		1.6cm	2.1cm	1.31
Five days incubation	2.5cm	3.2cm	1.28		.	.	.		2.2cm	2.8cm	1.27

Rg = colonies of (from) the III duplication strain, Rg: sixteen colonies at each given temperature.

r = colonies of (from) class one, thiosulphate-independent sectors produced by Rg: at each given temperature, two colonies from each of eight different r sectors.

*No discernible parental region after four days incubation.

✓No discernible parental region after five days incubation.

T A B L E b (continuation)

42°C:	Rg	s	$\frac{s}{Rg}$	36°C:	Rg	s	$\frac{s}{Rg}$	28°C:	Rg	s	$\frac{s}{Rg}$
Mean diameter:	($\bar{M}7$)	($\bar{M}13$)	$\left[\frac{\bar{M}13}{\bar{M}7}\right]$		($\bar{M}9$)	($\bar{M}14$)	$\left[\frac{\bar{M}14}{\bar{M}9}\right]$		($\bar{M}11$)	($\bar{M}15$)	$\left[\frac{\bar{M}15}{\bar{M}11}\right]$
One day incubation	.3cm	.5cm	1.66		.5cm	.8cm	1.60		.3cm	.5cm	1.66
Two days incubation	.9cm	1.4cm	1.55		1.3cm	2.1cm	1.61		.8cm	1.3cm	1.62
Three days incubation	1.4cm	2.3cm	1.64		2.3cm	3.6cm	1.56		1.2cm	1.9cm	1.58
Four days incubation	2.0cm	3.0cm	1.50		3.2cm	5.2cm	1.62		1.6cm	2.6cm	1.62
Five days incubation	2.5cm	3.8cm	1.52		.	.	.		2.2cm	3.6cm	1.63

s = colonies of (from) class two, thiosulphate-requiring sectors produced by Rg: at each given temperature, two colonies from each of eight different class two, thiosulphate-requiring sectors.

T A B L E c

42°C Mean diameter:	P ($\bar{M}16$)	y ($\bar{M}17$)	y^+ ad bi ($\bar{M}18$)	$\frac{y}{P}$ $\left[\frac{\bar{M}17}{\bar{M}16}\right]$	$\frac{y^+ \text{ ad bi}}{P}$ $\left[\frac{\bar{M}18}{\bar{M}16}\right]$
Two days incubation	.7cm	.9cm	1.2cm	1.28	1.71
Three days incubation	1.3cm	1.8cm	2.0cm	1.38	1.53
Four days incubation	1.8cm	2.3cm	2.6cm	1.27	1.44
Five days incubation	2.3cm	3.0cm	3.2cm	1.30	1.39
Six days incubation	3.0cm	3.7cm	3.8cm	1.23	1.26
Seven days incubation	3.6cm	4.4cm	4.5cm	1.22	1.24

P = colonies of (from) the I duplication strain, P: sixteen colonies at each given temperature.

T A B L E c (continuation)

39.5°C: Mean diameter:	P ($\bar{M}19$)	y ($\bar{M}20$)	$\frac{y}{P}$ $\left[\frac{\bar{M}20}{\bar{M}19}\right]$	36°C:	P ($\bar{M}21$)	y ($\bar{M}22$)	$\frac{y}{P}$ $\left[\frac{\bar{M}22}{\bar{M}21}\right]$
Two days incubation	1.3cm	1.5cm	1.15		1.5cm	1.9cm	1.26
Three days incubation	2.2cm	2.7cm	1.22		2.6cm	3.3cm	1.26
Four days incubation	3.3cm	4.1cm	1.24		3.8cm	4.7cm	1.23
Five days incubation	4.3cm	5.4cm	1.25		4.7cm	6.2cm	1.31
Six days incubation	5.1cm	6.3cm	1.23		5.6cm	7.4cm	1.32
Seven days incubation	5.8cm	7.4cm	1.27		6.6cm	8.2cm	1.24

y = colonies of (from) yellow sectors produced by P: at respectively 42°C, 39.5°C and 36°C, two colonies from each of eight different y sectors.

T A B L E c (continuation)

28°C: Mean diameter:	P ($\overline{M}23$)	$\frac{y^+ \text{ ad bi}}{(\overline{M}24)}$	$\frac{y^+ \text{ ad bi}}{P}$ $\left[\frac{\overline{M}24}{\overline{M}23}\right]$
Two days incubation	.8cm	1.4cm	1.75
Three days incubation	1.8cm	2.9cm	1.61
Four days incubation	2.8cm	4.0cm	1.42
Five days incubation	3.7cm	5.2cm	1.40
Six days incubation	4.5cm	6.3cm	1.40
Seven days incubation	5.3cm	7.5cm	1.41

$\underline{y^+ \text{ ad bi}}$ = colonies of (from) $\underline{y^+ \text{ ad bi}}$ sectors produced by P: at respectively 42°C and 28°C, two colonies from each of eight different $\underline{y^+ \text{ ad bi}}$ sectors.

TABLE d

39.5°C:	Q	y^+ ad bi	$\frac{y^+ \text{ ad bi}}{Q}$	36°C:	Q	y^+ ad bi	$\frac{y^+ \text{ ad bi}}{Q}$
Mean diameter:	($\bar{M}25$)	($\bar{M}26$)	$\left[\frac{\bar{M}26}{\bar{M}25}\right]$		($\bar{M}27$)	($\bar{M}28$)	$\left[\frac{\bar{M}28}{\bar{M}27}\right]$
Two days incubation	1.5cm	1.7cm	1.13		1.7cm	2.0cm	1.17
Three days incubation	2.5cm	2.9cm	1.16		2.7cm	3.4cm	1.25
Four days incubation	3.5cm	4.2cm	1.20		3.8cm	4.8cm	1.26
Five days incubation	4.4cm	5.5cm	1.25		4.6cm	6.2cm	1.34
Six days incubation	5.2cm	6.3cm	1.21		5.7cm	7.5cm	1.31
Seven days incubation	5.8cm	7.3cm	1.25		6.5cm	8.2cm	1.26

Q = colonies of (from) the I duplication strain, Q: sixteen colonies at each temperature.

y^+ ad bi = colonies of (from) y^+ ad bi sectors produced by Q: at each temperature, two colonies from each of eight different y^+ ad bi sectors.

Appendix B

A Rg // MSE diploid was synthesized from Rg and MSE. Such a Rg // MSE diploid was then haploidized and a portion of the resulting normal haploid segregants were tested for various requirements. Results from the tests are included in Table e. The overall data in Table e indicates that linkage groups I, II, III, V, VI, VII, and VIII have not been overtly affected while in a Rg // MSE diploid or during its formation. No data is available to allow one to determine whether or not linkage IV has been overtly affected in such a diploid.

T A B L E e

Segregations from the diploid: Rg // M.S.E.

Yellow, green, and white normal haploid segregants were obtained: the number of yellow segregants was roughly equal to the number of green segregants while the number of white segregants was roughly equal to the total number of yellow and green segregants.

Chromosome	I		III		V		V and VII		VI and III		VIII	
allele	bi$^+$	bi	gal$^+$	gal	fac$^+$	fac	nic$^+$	nic	s$^+$	s	ribo$^+$ *	ribo
Colour:												
green	0	8	5	3	3	5	2	6	1	7	0	8
yellow	8	0	5	3	4	4	1	7	0	8	0	8
white	6	8	7	7	8	6	3	11	4	10	0	14

*No ribo$^+$ segregants are expected inasmuch as all segregants carrying the III duplication would be ribo$^+$.

Appendix C

Experiments were undertaken in order to briefly study the haploid segregants derived from diploids produced as a result of a synthesis between Rg and P, a I duplication strain. Strain P had the following genetic relationships:

I pro1 paba1 y ad20^{+} bi1^{+} / I-II y^{+} ad20 bi1

A Rg // P diploid was synthesized from Rg and P by way of the first of three Rg + P heterokaryons successively made. This Rg // P diploid (I) was haploidized and as a result, green and yellow haploid sectors were produced; general inspection revealed that the green and yellow haploid segregants were produced in approximately equal numbers. This clearly showed that linkage group I was not overtly affected while in this Rg // P diploid or during its formation.

By way of a second heterokaryon (II) a second Rg // P diploid (II) was synthesized from Rg and P and in turn haploidized, with the result that approximately equal numbers of green and yellow haploid sectors or segregants were produced. Thirty-one haploid sectors were tested for various requirements. The results are given in Table f. It is clear that linkage groups I, III, and V have not been overtly affected while in this Rg // P diploid or during its formation.

By way of a third heterokaryon a third Rg // P diploid (III) was synthesized from Rg and P and in turn haploidized with the result that approximately equal numbers of green and yellow haploid sectors were obtained. Some of these were also tested for various requirements; the results are given in Table g. The results from this haploidization again indicate that linkage groups I, III, and V have not been overtly affected while in a Rg // P

diploid or during its formation. Regarding Table g, the distribution of the pyro phenotype and the pyro^{+} phenotype among the tested yellow haploid segregants fits a 1:1 ratio at $P > .05$. No such distribution is found among the tested green haploids: these green haploids were all pyrodoxin-requiring. This would indicate that pyro12 is in linkage with y^{+} and paba6^{+} in Rg, and thereby on linkage group I. Experiments in part IV confirm the existance of pyro12 on linkage group I.

T A B L E f

Segregations from the diploid Rg // P (II)

Chromosome	I		III		V	
allele	paba	paba$^+$	s	s$^+$	nic	nic$^+$
Colour:						
green	0	19	11	8	11	8
yellow	12	0	5	7	7	5

T A B L E g

Segregations from the diploid Rg // P (III)

Chromosome	I		I and IV		III		V	
allele	paba	paba$^+$	pyro	pyro$^+$	s	s$^+$	nic	nic$^+$
Colour:								
green	0	19	19	0	8	11	7	12
yellow	22	0	14	8	9	13	10	12

The fact that the ratio of pyro to pyro$^+$ haploid segregants fits a 3:1 ratio at $P > .05$ would indicate that Rg carries both pyro4 and pyro12.

Appendix D

From each of 15 class ye sectors produced (indirectly) by RP81 (P.138, Fig. 3), one colony was derived and incubated for a period of nine days. It was observed that each of the resulting 15 yellow colonies had produced improved sectors.

Appendix E

An extremely large portion (almost all) of the fz sectors produced by a group of RP81 colonies (P.138, Fig.3) did not give rise to yellow sectors. From each of 16 fz sectors included in that portion, inoculations were made on the centres of 16 CM plates, with the result that each of 16 CM plates had one inoculum at its centre. The plates were then placed in 36°C for a period of nine days.

Inocula from the fz sectors gave rise to 16 colonies having an olive-green parental region which was fuzzy. It was noted that comparatively smooth, grass-green sectors (class h sectors) emerged from the parental region and that many of these grass-green sectors had in turn produced the only yellow sectors present, indicating that deletions including the $\underline{y}^+$ allele eventually followed a series of deletions from the III duplication; this series began with the formation of the fz sectors and ended in the formation of the class h sectors which emerged from the colonial cultures derived from the fz sectors.

R E F E R E N C E S

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S U M M A R Y

Environmental and Genetic Factors Affecting Instability at Mitosis in *Aspergillus nidulans*

Previous work has shown that strains of *Aspergillus nidulans* with a chromosome segment in duplicate (one in normal position, one translocated to another chromosome) are unstable. Deletions occur from either duplicate segment. The present work has shown that most deletions occur from the translocated duplicate segment. Furthermore, it has been found that the overall frequency of deletions from a duplication is dependent upon the temperature of growth. The overall frequency of deletions from a chromosome III duplication is greatly enhanced by low temperatures, while the overall frequency of deletions from a chromosome I duplication is markedly enhanced by high temperatures. A temperature of 39.5°C appears to enhance the overall frequency of deletions from the I duplication to the greatest extent.

When the I duplication and the III duplication are together in a haploid, deletions from the intact III duplication generally precede deletions from particular sections of the I duplication. Furthermore, the III duplication can enhance to some (but not major) extent the frequency of deletions from the I duplication. After the III duplication becomes reduced in size as a result of the loss of chromosomal material from the translocated duplicate III segment, such a reduced III duplication can greatly enhance the frequency of deletions from the I duplication. In other words, a III duplication of reduced size can promote far more deletions from the I duplication than can the intact III duplication. The major increase in the deletional instability of the I duplication as promoted by the reduced III duplication is confined to the translocated duplicate I segment. The reduced III duplication can induce deletions from a section of the translocated duplicate I

segment in accord with a temporal programme. Furthermore, a particular region of the I duplication is far more under the mutagenic influence of the reduced III duplication than another region.

This and other information shows that a chromosomal imbalance either in the form of a duplication in a haploid or a triplication in a diploid can cause mutations or deletions within chromosomal regions not linked to the chromosomal imbalance in question. In effect, a chromosomal imbalance can promote deletions or mutations outside itself.

Comparisons with other cases of instability are made and a common underlying connection is proposed. A manner in which instability may be related to development is also proposed.

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